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Etude des activités anti-inflammatoire, antioxydante et screening par chromatographie gazeuse couplée à la spectrométrie de masse d'extraits éthanoliques de trois fabacées du Bénin : isolement de molécules bioactives.

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Sommaire

Résumé

Face à la résurgence de pathologies infectieuses, notre étude a porté sur le potentiel thérapeutique de *Dialium guineense*, *Parkia biglobosa* et *Tamarindus indica* afin de rechercher des molécules bioactives pouvant contrer l'antibiorésistance ou ses corolaires. L'état de l'art des molécules bioactives des trois plantes a montré que de nombreuses familles de composés sont identifiées dans différents organes aériens. Toutefois le lien avec les activités biologiques reste non élucidé. Ensuite, nous avons évalué quelques activités biologiques des extraits éthanoliques ou hydro-éthanoliques des feuilles, fruits et écorces. Avec de bons taux de viabilité cellulaires, les extraits de *D. guineense* (écore) ainsi que ceux de *P. biglobosa* (feuilles) et *T. indica* (écorce) ont des ratios d'activités anti-inflammatoires de 458,2 ; 161 et 174,6 respectivement. Ces valeurs sont supérieures à celle de la dexaméthasone utilisée comme témoin. Le test KRL a montré une activité antiradicalaire dose dépendante dans la gamme de 0 à 20mg/L. In vitro, 1g de chacun des extraits susmentionnés présente une capacité antioxydante respectivement équivalente à 1585 ; 2092 ; 5071 et 2246 mg de Trolox. Les extraits ont par la suite été analysés par GC-MS révélant pour la première fois la présence de lupéol et de sitostérol dans l'écorce de *D. guineense*. Enfin, l'étude nutritionnelle des trois fruits révèle, à travers les fortes teneurs en nutriments (80% de sucre pour *D. guineense*), leur possible contribution à la lutte contre la malnutrition au Bénin et la nécessité d'œuvrer à leur conservation.
Mots-clés : Pathologies infectieuses, antibiorésistance, anti-inflammatoire, antioxydant, molécules bioactives

Summary

In a context of infectious diseases resurgence, our study focused on therapeutic potential of Dialium guineense, Parkia biglobosa and Tamarindus indica in order to search for bioactive molecules that can counter antibiotic resistance or its corollaries. The state of the art of on bioactive molecules from the three plants has shown that many families of compounds are identified in different aerial organs. However, the link with biological activities remains unclear. Next, we evaluated some biological activities of ethanolic or hydroethanolic extracts of leaves, fruits and bark. With good cell viability levels, extracts of D. guineense (bark) as well as those of P. biglobosa (leaves) and T. indica (bark) have anti-inflammatory activity ratios of 458.2; 161 and 174.6 respectively. These values are higher than that of dexamethasone used as positive control. The KRL test showed dose-dependent antiradical activity in the range of 0 to 20mg / L. In vitro, 1 g of each of the above-mentioned extracts has an antioxidant capacity respectively equivalent to 1585 ; 2092 ; 5071 and 2246 mg of Trolox. The extracts were then analyzed by GC-MS revealing for the first time the presence of lupeol and sitosterol in the bark of D. guineense. Finally, the nutritional study of the three fruits reveals, through the high levels of nutrients (80% sugar for D. guineense), their possible contribution to fight malnutrition in Benin and the need of their conservation.

Keywords : Infectious diseases, antimicrobial resistance, anti-inflammatory, antioxidant, bioactive molecules

I- INTRODUCTION

Le désir de jouvence et de santé est une quête aussi vieille que l'humanité. L'homme s'évertue donc depuis des millénaires à maîtriser son vieillissement et les diverses affections dont il est l'objet. En Afrique, plus de quatre-vingts pour cent de la population a recours exclusivement aux plantes comme sources de traitement [1]. Cette pratique traditionnelle a existé depuis des millénaires sur toute la surface du globe. Au Bénin, elle perdure pour les principales raisons suivantes :

-les revenus très bas des populations limitant leur accès aux soins (le système de sécurité sociale étant absent dans la plupart des pays africains),

-la rareté de structures hospitalières et du personnel médical (surtout en milieu rural),

-la satisfaction éprouvée par les populations recourant aux plantes médicinales.

Un meilleur accès des populations africaines au système de soin ainsi qu'une disponibilité accrue de médicaments à portée de leurs bourses impacteraient donc positivement leur bien-être physique et psychique et partant, leur longévité. Dans cette optique, l'isolement de principes actifs des plantes médicinales et la formulation de phyto-médicaments améliorés sont devenus des défis majeurs. En outre, les enjeux mondiaux actuels de moindre consommation d'énergie et de production réduites de déchets ou sous-produits confèrent une place de choix aux substances naturelles contrairement aux molécules de synthèse [2].

Ceci est d'autant plus intéressant qu'il est à noter depuis une décennie, une résurgence des maladies infectieuses liée à l'apparition de germes résistants. L'accroissement du trafic mondial ainsi que l'usage abusif des antibiotiques en médecine humaine et vétérinaire, en ont fait un problème de santé publique à l'échelle mondiale [3]. Ainsi, selon le 'National Institute of Health', les maladies infectieuses sont la deuxième cause de mortalité dans le monde [4]. Elles sont étroitement liées aux inflammations qui les favorisent [5] ou en constituent un symptôme [6]. Au-delà de traiter la principale cause par des antibiotiques, une prise en charge efficace des pathologies infectieuses implique donc un traitement anti-inflammatoire. La recherche de nouveaux antibiotiques et celle d'anti-inflammatoires devraient donc se faire concomitamment. Mais force est de constater que face aux multiples difficultés génomiques, bio-informatiques et chimiques entravant la découverte de nouveaux antibiotiques [7], les firmes pharmaceutiques préfèrent investir dans des pathologies dites ''stables'' et donc plus rentables. Au nombre de celles-ci, le vieillissement occupe de plus en plus une place prépondérante.

Bien qu'étant un processus naturel, le vieillissement peut être accéléré par le stress oxydatif. L'implication de ce dernier dans le développement de plusieurs pathologies (diabète, cancer, maladies cardiovasculaires) ayant été mis en exergue [8], la recherche de molécules au pouvoir antioxydant dépasse dorénavant le seul cadre anti-âge de la cosmétique. Par leur caractère renouvelable, les substances naturelles occupent une place de choix dans cette recherche. Enfin, au Bénin la prévalence de la malnutrition, surtout celle infantile, est préoccupante [9,10,11]. En fragilisant le système immunitaire, elle est très propice à la survenue de pathologies infectieuses [12,13].

Dans ce contexte, en vue d'étudier le potentiel thérapeutique de *Dialium guineense willd., Parkia biglobosa (Jacq.) R. Br. ex Benth.* et *Tamarindus indica L.* (trois plantes médicinales du Bénin), notre travail de thèse a porté sur l'activité biologique d'extraits de leurs organes aériens (écorces, feuilles, et fruits). Le choix de ces trois césalpiniacées repose d'une part sur leur récurrence dans notre enquête ethnobotanique comme remèdes anti-infectieux ou anti-inflammatoire et d'autre part, sur la rareté de molécules actives décrites. A cela s'ajoute leur surexploitation : elles figurent parmi les dix espèces végétales menacées de disparition au Bénin [14,15]. En effet, aux difficultés naturelles de germination de leurs graines liées à des dormances trop longues, s'ajoutent l'usage de jeunes plants de *Dialium guineense* comme habitacle ou fourrage en pisciculture de lagune et l'utilisation du tronc de ces trois plantes comme bois d'œuvre ou dans la production de charbon [16].

Le présent manuscrit de thèse débute par une revue de littérature publiée présentant les trois plantes, leurs usages traditionnels ainsi que les travaux antérieurs portant sur leurs activités biologiques. S'en suit une série de trois articles publiés sur leurs effets anti-inflammatoire, antioxydant et leurs valeur nutritionnelle. Enfin, une conclusion suivie de perspectives clôt le manuscrit.

II- Etat de l'art sur les molécules bioactives de *D. guineense, P. biglobosa* et *T. indica*

Article 1 : revue de littérature

Senankpon Martial Gnansounou, Samy Iskandar, Maxime Robin, Jean-Michel Brunel, Edwige Dahouenon and Philippe Piccerelle. *Dialium guineense Willd. Parkia biglobosa (Jacq.) R. Br. Ex Benth*. And *Tamarindus indica L*.: Review of known and synergetic bioactive compounds. Journal of Medicinal Plants Studies 2018 ; 6(3) : 103-111.

L'humanité est tributaire des végétaux à maintes égards. Ainsi, pour se soigner et se nourrir par exemple, elle puise dans la végétation. La phytothérapie est donc universelle et très ancienne. Toutefois, la connaissance des molécules bioactives issues des plantes est récente. Elle fut rendue possible grâce, entre autres, aux avancées de la chimie et de la microbiologie. Les firmes pharmaceutiques, se basant sur ces outils devenus très performants, ont réussi à isoler ou synthétiser des principes actifs. Toutefois, le coût de ces derniers les rend inaccessibles pour une grande partie de la population mondiale. La médecine traditionnelle (notamment la phytothérapie) demeure alors le premier recours dans les pays en voie de développement. C'est le cas au Bénin où les feuilles et écorces de *Dialium guineense*, *Parkia biglobosa* et *Tamarindus indica* (trois Fabacées) sont très utilisées comme remède contre les infections et leurs symptômes (inflammation, fièvre etc.). L'objectif de cette revue est de faire une description botanique des trois plantes, de présenter leur intérêt pharmacologique et de faire un inventaire de leurs molécules actives connues.

La plupart des publications recensées rapporte une activité antimicrobienne d'extraits de *Dialium guineense Willd*. Cependant, aucune molécule n'est identifiée comme responsable de cette activité. L'anthraquinone trouvée dans l'extrait d'écorces [17]est capable de former un complexe irréversible avec les acides alpha aminés nucléophiles conduisant à une inactivation des protéines enzymatiques contenant ces acides aminés [18]. Les auteurs supposent que cette propriété est responsable de l'activité antimicrobienne. D'autres travaux lient l'activité antimicrobienne à la présence, de flavonoïdes, de tanins et de saponines [19,20]. La nature exacte des composés actifs reste néanmoins inconnue. Enfin, deux composés ont été clairement identifiés chez cette plante mais leur action est hors du champ antimicrobien. Ainsi, le précocène I [21] est insecticide et la glycoprotéine appelée DigL [22] a été mise en évidence pour son effet d'hémagglutination.

Les molécules (lupéol et catéchine) identifiées à partir d'extraits de *Parkia biglobosa* (*Jacq.*) *R. Br. Ex Benth* n'ont aucun rapport avec les propriétés antimicrobiennes rapportées [23,24,25]. Le lupéol isolé des feuilles [26] stimulerait la fonction des cellules β et augmenterait la sécrétion d'insuline en inhibant l'α-glucosidase et l'α-amylase. En ce qui concerne la catéchine également isolée des feuilles, cette molécule est supposée être impliquée dans des mécanismes susceptibles d'augmenter les teneurs en thiol cellulaire dans des conditions de stress oxydant [27,28].

La bibliographie révèle que peu de molécules ont été décrites chez *Tamarindus indica L*. L'alpha-tocophérol a été isolé à partir d'extrait éthanoliques des graines [29]. C'est un antioxydant bien connu sous le nom de vitamine E [30,31]. Une protéine isolée de cette plante a été identifiée comme étant inhibiteur de protéase de type Kunitz [32]. La troisième molécule rapportée de *Tamarindus indica L*. est le tamarindienal ou (3E) -2,5-dioxo-3-hexénal. C'est elle qui donnerait le goût amer des fruits du tamarin. Aucune activité biologique intéressante ne lui est attribuée.

Malgré la multitude de travaux mettant en évidence leurs activités pharmacologiques, peu de molécules actives ont donc été décrites. Ce manque de connaissances sur les composés bioactifs des trois plantes ne permet pas une analyse objective de leur utilisation contre les maladies infectieuses par les guérisseurs traditionnels du Bénin. Nous pensons alors qu'il serait intéressant de tester leurs extraits, de sélectionner les plus efficaces pour essayer d'en déterminer la composition.

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Dialium guineense Willd. *Parkia biglobosa* (Jacq.) R. Br. Ex Benth. and *Tamarindus indica* L.: Review of known and synergetic bioactive compounds

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Abstract
Native from Africa, *Dialium guineense* Willd. *Parkia biglobosa* R. Br. Ex Benth. And *Tamarindus indica L.* grow in tropical regions, and are very widespread in West Africa, particularly in Benin. They are simultaneously used in the traditional healing of infectious diseases but there is a lack on their bioactive compounds description. This work places a special emphasis on inventory of known bioactives compounds from the three plants and their mechanism of action in order to identify the need for further research. It emerges from this work that numerous studies confirm the biological activities of the plants extracts. Nevertheless, few bioactive molecules are described as well as the mode of action of the active extracts.

Keywords: *Dialium guineense* Willd. *Parkia biglobosa* (Jacq.) R. Br ex Benth. *Tamarindus indica* L. pharmacology, bioactive compound

1. Introduction
Because of their diet and medical care, humans are largely dependent on plants (Uusiku *et al.* 2010) [76]. In Africa, for example, more than eighty percent of the population relies exclusively on plants for healing (World Health Organization, 2013) [79]. While it is true that this practice has existed for thousands of years on the whole surface of the globe, it persists in Benin. This mainly because of the satisfaction of the populations resorting to medicinal plants. Therefore, there is a real culture of traditional medicine in this country, the first resort of eighty percent of the population (Mosnier *et al.* 2006) [53]. The low income of the majority of the population limiting access to modern care (Minot and Daniels, 2005) [50], the lack of social security system and scarcity of hospital facilities or medical staff (especially in rural areas) largely contribute to this situation. Nevertheless, the absence of side effects and low toxicity are worldwide arguments in favor of herbal medicine (Cowan, 1999) [18]; (Iwu *et al.* 1999) [36]. In this context, the Ministry of Health of this country, like many others in Africa, has assigned itself the task of training, promoting and integrating traditional healers into its national health system. At the same time, antibiotic resistance has become a public health problem worldwide (Ventola, 2015) [77], so finding new bioactive molecules has become essential. Plants play an important role in this new battle because their secondary metabolites (papaverine, berberine, curcumin, etc.) have already shown interesting activity both in anti-infectious therapy, and against metabolic or cardiovascular pathologies (Nokta *et al.* 1993) [54]; (Hayashi *et al.* 2007) [31]; (Wang *et al.* 2010) [78]; (Kamatou *et al.* 2006) [38].

Our ethnobotanical inquiry in three medicinal plants markets, and with ten traditional healers revealed a recurrence of *Tamarindus indica* L. *Parkia biglobosa* (Jacq.) R. Br. Ex Benth. And Dalium guineense Willd. As well-known and often combined traditional medicines against microbial infections. The available knowledge on these plants was searched using the key words *Dialium guineense* Willd. *Parkia biglobosa* (Jacq.) R. Br. Ex Benth., *and Tamarindus indica L.* in 'Google scholar', 'NCBI', 'Springer Link' and 'Web of Science' databases. We formaly identified them at the Benin National herbarium where specimen are deposed under Voucher numbers AA 6727/HNB, AA 6728/HNB and AA 6729/HNB respectively.

Their aerial parts are associated in the healing of infectious diseases. This supposed they could contain synergistic compounds and it appears necessary to make the inventory of their known bioactive molecules in order to support this assumption.

Surprisingly, due to their over-exploitation (timber, coal...), or their difficult regeneration linked to a long dormancy (Faustin *et al*. 2013) [25], we also notify that those three plants appear in the top ten plants threatened with extinction in Benin (Lykke, 2000) [44] (Eyog Matig *et al*. 2001) [24] (Meregini, 2005) [47] (Smagadi, 2005) [67] (Ewedje and Tandjiekpon, 2011) [23]. In order to understand why they are combinated by traditionl healers and to contribute to their safeguarding, this work aims to make an inventory of their known molecules and identify the needs for pharmacological and phytochemical research.

2. Botanic aspects of Fabaceae and known antimicrobials from this family

Dialium guineense Willd. *Parkia biglobosa* (Jacq.) R. Br. Ex Benth. And *Tamarindus indica L.* are three plants of the Fabaceae family. Still known as Leguminosae (vegetables), Fabaceae, by the number of species (about 19,500), and genera (751), constitute one of the most represented families of the subphylum Angiosperms or Magnoliophyta (Group and others, 2013) [29]. They have conquered various habitats with varied morphologies ranging from vines to shrubs or trees. An almost exclusive feature of this family is the fixation of atmospheric nitrogen by symbiosis with soil rhizobia. This family identified and described by Adanson then by Jussieu (Adanson, 1763) [1]; (Jussieu, 1789) [37] was subdivided into three subfamilies on the basis of morphological homology criteria: Caesalpinioideae, Mimosoideae, and Papilionoideae. Advances in molecular biology, including sequencing of ribosomal genes (Soltis *et al*. 1997) [68], chloroplasts genes (Savolainen *et al*. 2000) [66], nuclear or mitochondrial genes (Qiu *et al*. 2005) [61] have allowed evolution of taxonomy to become more precise. Thus, a new classification by the 'Legume Phylogeny Working Group' (LPWG) in 2017 reports six subfamilies. Mimosoideae are now divided into four subfamilies: Duparquetioideae, Cercidioideae, Detarioideae (represented by *Tamarindus indica L.*) and Dialioideae of which *Dialium guineense* Willd. Is part (Azani *et al*. 2017) [12]. The phylogenetic tree of the Fabaceae is now as shown below (Fig. 1).

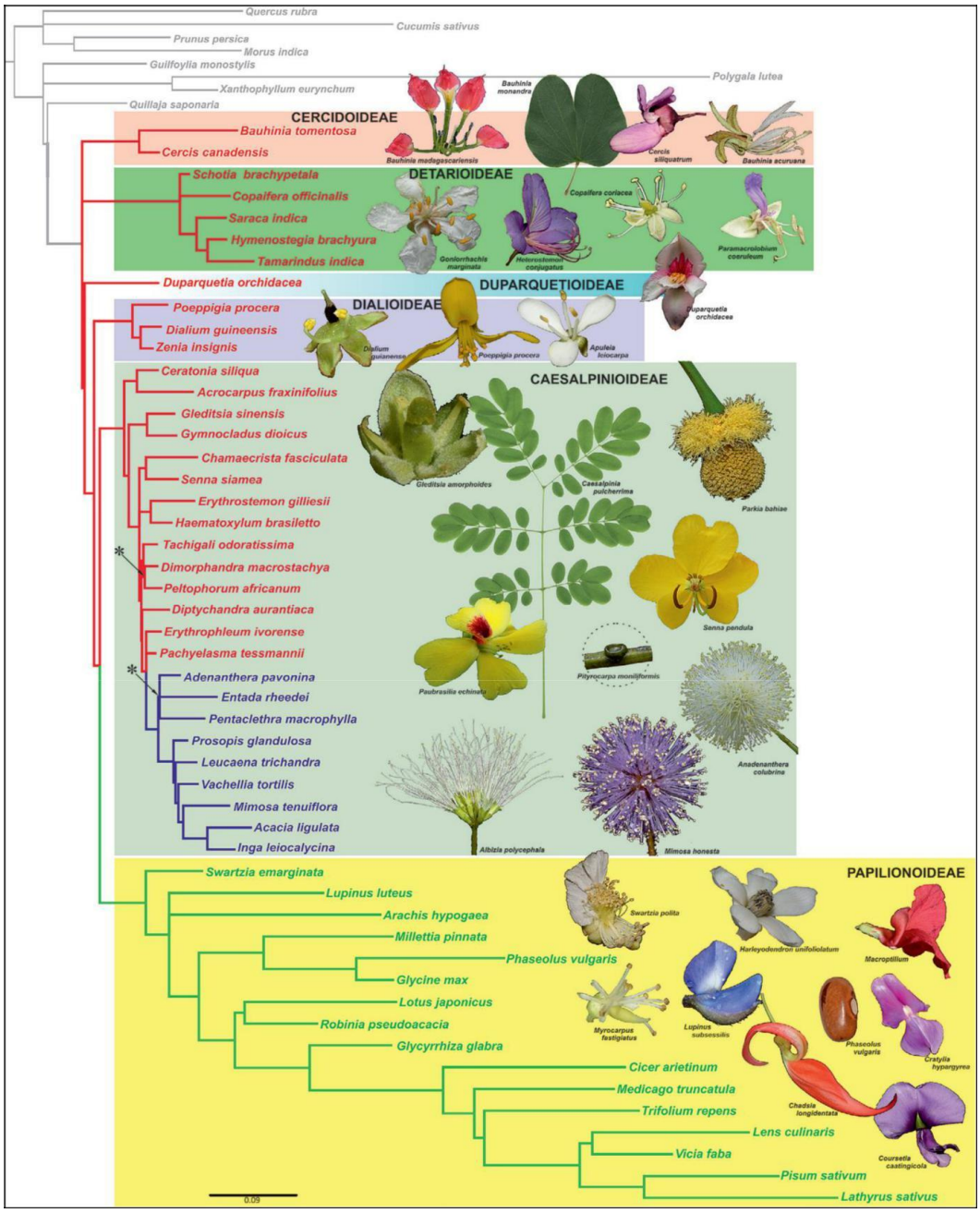

Fig 1: Phylogeny and classification of Fabaceae (Source: LPWG 2017)

Fabaceae are an important source of low-cost protein for many populations in developing countries, as opposed to meat

and fish products (Balogun and Fetuga, 1986) [13]. Their use in traditional medicine is widespread (Rosado-Vallado *et al*. 2000) [64]; (Koné *et al*. 2004) [40]; (Ma *et al*. 2011) [45]; (Rahman and Parvin, 2014) [63]. Furthermore, some antibacterial compounds have already been isolated from this family (Dzoyem *et al*. 2017) [20]. It is then reasonable to expect the same from those three plants.

2.1. Dialium guineense Willd: botanic, uses and bioactive compounds

Dialium guineense Willd. Belongs to the subfamily of Dialioideae. It is a plant from the regions of tropical Africa, but it is exceptionally present in Sao Tome and Principe. It grows on land left fallow and in dry or dense forests, as well as forest galleries, from Senegal to southern Nigeria. It is noteworthy that, in Benin, this plant is present from the coast to the southern region. Its general morphology is illustrated in Figure 2.

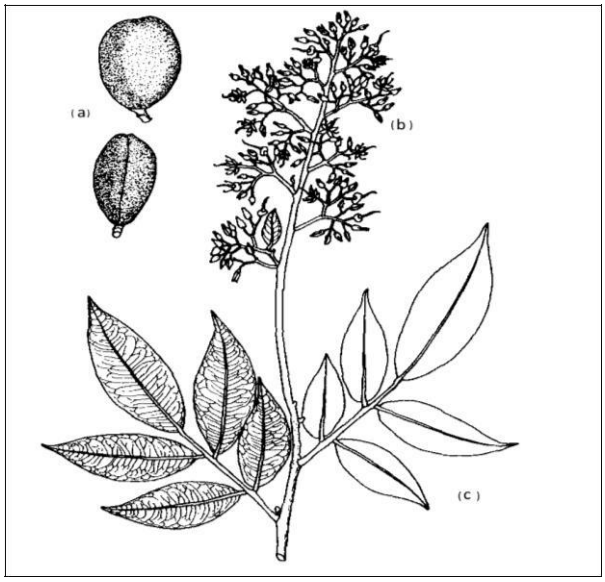

Fig 2: (a) fruit, (b) inflorescence, (c) leaves of *Dialium guineense* Willd. (Drawings by Messrs C.Z. Gbéhou and E.-E. Ewedje, Flore analytique du Bénin).

Roots, leaves and bark of the plant are used in the treatment of malaria, coughs, bronchitis, diarrhea, palpitations, dysmenorrhea, ulcer, anemia, hemorrhoids ("Adjanohoun *et al*. 1989 [4]. Banque de données de médecine traditionnelle et pharmacopée. Paris. 124 p. ISBN: 92-9028-146-4," n.d.) (Odukoya *et al*. 1996) [5] (Bero *et al*. 2009) [16]. They are also used for contraception as well as to regulate menstruation. In Nigeria, women use the leaves against genital infections, and to improve lactation whereas twigs are used as native toothbrushes to protect against tooth decay and dental plaque (Bero *et al*. 2009) [16]; (Okwu and Ekeke, 2003) [56]; (Okwu and Ekeke, 2003) [56]; (Lokonon *et al*. 2013) [43]; (Akinpelu *et al*. 2011) [7].

Rich in nutrients (Arogba *et al*. 1994); (Gnansounou *et al*, 2014); (Ayessou *et al*. 2014), black tamarind (*Dialium guineense* Willd.) fruits are widely consumed in Benin. The tree, by its atmospheric nitrogen fixation, contributes to fertilization of the soil whereas leaves and branches are used in lagoon fish culture as abode and fodder (Ewedje and Tandjiekpon, 2011) [23]. On the other hand, the wood is also used in house or attics constructions, traditional tool handles, and charcoal making or as firewood (Lokonon *et al*. 2013) [43].

Table 1: Summary of known molecules from *Dialium guineense* Willd.

Organs	Extraction Solvent (s)	Biological proven activity	Family/Molecules	Active molecules isolated	Reference (s)
Fruit (pulp)	Water	Molluscicide	Saponins	Glycosides triterpenoids	[30]
	Ethanol	detoxification	Flavonoids, phenolic compounds	N.D.	[77]
Bark (stem or root)	Methanol + Water (maceration)	Antibacterial (*S. aureus*)	Flavonoids, alkaloids, tannins and Saponins	N.D.	[41]
	Ethanol	Antimicrobial	Alkaloids, Flavonoids, tannins and Saponins.	Anthraquinone[a]	[38, 78]
leaves	Hydrodistillation Water (maceration)	Antibacterial (*S. aureus*)	Flavonoids, alkaloids, tannins and Saponins	N.D.	[41]
	Ethanol	Antibacterial (*S. aureus*) Antiviral (Herpes type 1)	Flavonoids, alkaloids, tannins and Saponins	N.D.	[41, 79, 40]
	Methanol/Water	Antimicrobial (vibrio)	Flavonoids, alkaloids, tannins, glycosides cardiac, steroids and Saponins	N.D.	[34]
	Methanol	Antimicrobial/Antioxidant	Compounds phenolic	N.D.	[80]
Seeds	Hydrodistillation	Allatocidal	Precocene I (78.8%) β-caryophyllene (5.3%) Valencene (1.4%) cadalene (1%)	Precocene I[a]	[22]
	Tampon Tris-HCl	hemagglutination	Lectin	Glycoprotein DigL	[43]

N.D = Not determined
a= Structure on Fig. 5 below

Most of work listed in Table 1 above have proven the antimicrobial activity of *Dialium guineense* Willd. Extracts.

However, none of them have isolated the active compound. Concerning anthraquinone found in bark extract (Olajubu *et al.* 2012), this latter is able to form an irreversible complex with nucleophilic amino acids leading to an inactivation of the enzymatic proteins containing these specific amino acids (Stern *et al*. 1996). This property is supposed by the authors to be responsible for the antimicrobial activity, but no experiments were reported to date to support their assumptions. Thus, different teams linked the antimicrobial activity to the presence of chemical entities issued from alkaloids, flavonoids, tannins and saponins ("Agassounon *et al*. 2001. Evaluation des activités cytotoxique, antivirale, antibactérienne et antifongique de six plantes," n.d.)) (Akinpelu *et al*. 2011) [7]; (Orji, 2012) [58] but the exact nature of the active compounds remain unknown. Finally, only two compounds were clearly identified from this plant and out of the claimed antimicrobial domain: precocene I isolated (Essien *et al*. 2007) [22] for its allatocidal effect and a glycoprotein named DigL highlighted (Bari *et al*. 2013) [14] for its hemagglutination effect.

2.2 *Parkia biglobosa* (Jacq.) R. Br. Ex Benth: botanic, uses and bioactive compounds

P. biglobosa (Jacq.) R. Br. Ex Benth. Is a multi-year Mimosoideae (Hopkins, 1983) [33]. It is a tree ten to fifteen meters high with a spreading crown. Globular, the flowers are red or orange (Fig. 3) and bloom from December to January, and from February to March (Akoegninou, 2006) [8]. Concerning its geographical location, this tree is present in tropical Africa between 3° and 15° north.

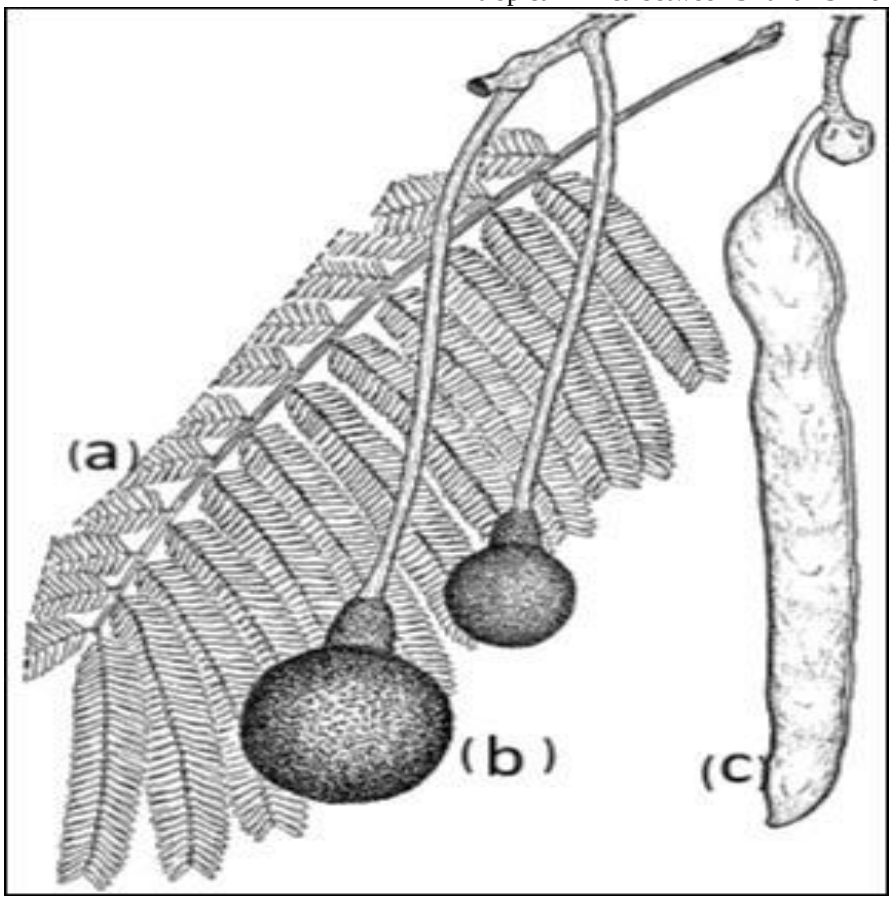

Fig 3: (a) leaves, (b) inflorescence (c) fruit of *Parkia biglobosa* (Jacq.) *R. Br Ex Benth.* (Redrawn and adapted from M.M. Spitteler) / http://database.prota.org/PROTAhtml/Parkia % 20biglobosa_En.htm)

Parkia biglobosa (Jacq.) R. Br. Ex Benth occupies the fifth position among the most used plants in traditional Beninese medicine (Eyog Matig *et al*. 2001) [24]. Its fruits are consumed by humans, and its leaves are used as livestock fodder (Sabiiti and Cobbina, 1992) [65]. The seeds, by fermentation, are used to prepare a very nourishing local mustard used whith different parts of the plant against high blood pressure, hemorrhoids, dermatoses, diabetes, snake venom etc. (Assane *et al*. 1993). For example, the word in Hausa (the language widely spoken in West Africa) for *P. biglobosa* (Jacq.) R. Br. Ex Benth. Is "Dawa Dawa". This word, borrowed from Swahili, refers to the term 'Dawa', used for any plant-based medication ("Swahili-English Dictionary," n.d.).

Like many fruit trees, *P. biglobosa* (Jacq.) R. Br. Ex Benth. Plays an important role in feeding both humans and livestock. It is also used as fuel (Sabiiti and Cobbina, 1992) [65]; (Ræbild *et al*. 2011) [62]. Wild animals, including chimpanzees, feed on them, and disperse seeds (Kunz and Linsenmair, 2007) [42]. The tree is known to farmers to improve soil fertility, and to protect them from erosion (Bayala *et al*. 2007) [15]. Bark and pods are respectively used as dyeing and pottery colorant (Ouédraogo, 1995) [59]. Despite this important role, the regeneration of the species is insufficient (Koura *et al*. 2013) [41].

Table 2: Summary of known molecules from *Parkia biglobosa* (Jacq.) R. Br. Ex Benth.

Organs	Extraction Solvent(s)	Biological proven activity	Family/Molecules	Active molecules isolated	References
Fruit (pulp)	Acetone	Antioxidant	Polyphenols	N.D.	[81]
Bark (root or stem)	Water	Antioxidant Antibacterial Cicatrizing	Polyphenols	N.D. E.O.	[82, 54, 83, 59]
	Ethanol	Antioxidant Antibacterial Cicatrizing	N.D.	N.D.	[56, 59]
	Methanol + Water	Anti- snake venom	N.D.	N.D.	[84]
	Water + ethanol	Antibacterial macrophages stimulation	Sterols, triterpenes, polyphenols Polysaccharides	N.D. Polysaccharides	[54 55, 85]
	Methanol + Dichloromethane	Antioxidant	Proanthocyanins	Procyanidines, prodelphinidines and they glucuronic derivatives	[86]
	Methanol	Hepatoprotective Antibacterial	Polyphenols	N.D. N.D.	[87, 88]
	Methanol + Water	Antioxidant, synergy with antibiotics	Polyphenols	N.D.	[89, 90]
Leaves	Water	Antioxidant Antibacterial Cicatrizing	N.D.	N.D.	[59]
	Butanol	Antidiabetic	-	Lupeol[a]	[57]
	Ethanol	Antioxidant Antibacterial Cicatrizing	N.D.	N.D.	[59]
	Methanol	Neuroprotection	Polyphenols	Catechin[a]	[58]
	Water + ethanol	Vasorelaxation	Procyanidines	N.D.	[91]
Seeds	Water	Antidiabetic, hypoglycemic and antihypertensive	N.D.	N.D.	[92, 93]
	Methanol	Antidiabetic and hypoglycemic	N.D.	N.D.	[92]
	Sulfate ammonium	Antinociceptif Anti-inflammatory	Lectins	Lectin 4MQ0[*]	[94]
	Extraction des proteins	Antioxidant	Protein	E. O.	[95]
	Ethanol/ Ether petroleum	Pesticide	Polyphenols	N.D.	[96]

***** Access code RCSB Protein Databank E.O =Elucidation On going
N.D. = Not determined a= Structure on Fig. 5 below

As summarized in Table 2, molecules identified from *Parkia biglobosa* (Jacq.) R. Br. Ex Benth. Have no relation with its antimicrobial properties assertained by some authors. For instance, (Millogo-Kone *et al.* 2007) [48] (Millogo-Kone *et al*. 2008) [49] (Adetutu *et al*. 2012) [2] concluded that this plant might contain antibacterial molecules but not clearly identified them until now. On the other hand, lupeol isolated from the leaves' butanol extract is known to stimulates β-cell function and increases insulin secretion by inhibiting α-glucosidase and α-amylase in non-competitive and uncompetitive inhibition patterns respectively (Ibrahim *et al*. 2016) [34]. Concerning catechin obtained from leaves methanol extract, this compound is supposed to be involved in mechanisms capable of boosting cellular thiol contents under conditions of oxidative stress (Komolafe *et al*. 2014) [39] (Grønhaug *et al*. 2008) [59].

2.3 *Tamarindus indica* L.

Tamarindus indica L. is a well known Detarioideae native to tropical Africa where it grows wild. Its name (date of India) derives from Arabic (Tamar Hindi) and refers to India from where it was introduced in Arabia. In Woloff (language widely spoken in Senegal), it is called "Dakkhar" and could be origin of the city name ''Dakar'' (Tignokpa *et al*. 1986) [74]. It grows very well in semi-humid or arid regions (Meher *et al*. 2014) [46]. It is a large tree up to 25 meters tall (Tariq *et al*. 2013) [73]. The fruit is a pod containing a seed surrounded by fiber and an acidulous pulp widely used as a condiment (Akoegninou, 2006) [8].

The laxative and carminative effects of *Tamarindus indica L*. fruits are well known. Other organs are used in the treatment of a wide range of pathologies such as toothaches (Tapsoba and Deschamps, 2006) [72], bacterial infections, malaria (Doughari, 2006) [19], snake or insect bites, wound healing (Tignokpa *et al*. 1986) [74], conjunctivitis, diabetes (Buchholz and Melzig, 2016) [17], hypertension, etc. (Havinga *et al*. 2010) [30]. The fruits are also antipyretic (Iwu, 2014) [35], antiscorbutic, hepatoprotective, and used in the treatment of biliary disorder (Morton and Dowling, 1987) [51]. (Tapsoba and Deschamps, 2006) [72] Fruit is both a food (consumed as a drink) and a spice (Hines and Eckman, 1993) [32] (Tsuda *et al*. 1994) [75]. It's subject of intense international trade ("Morton Julia F. 1958 [52] The Tamarind It's food medecinal and industrial uses Florida State Horticultural Society.pdf," n.d.). It is also noteworthy that the tree is useful for the protection of traditional African habitats that are generally fragile typically against wind erosion of soils (Ebifa-Othieno *et al*. 2017) [21].

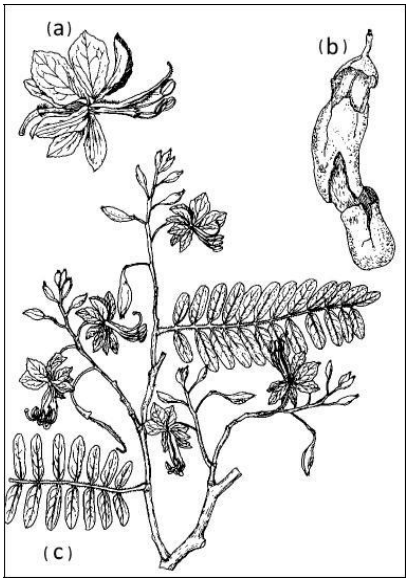

Fig 4: (a) Flower, (b) fruits and (c) leaves of *Tamarindus indica L.* (Illustration: Busson 1965, Flore analytique du Bénin)

Table 3: Summary of known molecules from *Tamarindus indica L.*

Organs	Extraction Solvent(s)	Biological proven activity	Family/Molecules	active molecules isolated	References
Fruit (pulp)	Water	Antibacterial Antioxidant	Flavonoids, Alkaloids, Tannins, Glycosides cyanogenic and Anthraquinones	N.D.	[97, 98, 99, 100]
	Methanol	Antibacterial Anti-inflammatory Lipid-lowering	Polyphenols, sterols, triterpenes, Alkaloids and tannins	N.D.	[101, 102, 103, 104, 100]
	Ethanol	Antidiabetic Antibacterial Hepatoprotective	Flavonoids, Alkaloids, Tannins, Glycosides cyanogenic and Anthraquinones	N.D.	[97, 99, 105, 106]
	Hexane	Antibacterial	Alkaloids and tannins	N.D.	[102]
	Acetone	Antibacterial	Phenols and Flavones	N.D.	[101]
Bark (stem or root)	Water	Antibacterial	Polyphenols, Sesquiterpenes, Alkaloids Phlobatamines Tannins, Glycosides cyanogenic and Anthraquinones	N.D.	[64, 99]
	Methanol	Antibacterial	Polyphenols, Sesquiterpenes, Alkaloids and Phlobatamines	N.D.	[64]
	Acetone	Antibacterial	Polyphenols, Sesquiterpenes, Alkaloids and Phlobatamines	N.D.	[64]
	Ethanol	Antibacterial	Flavonoids, Alkaloids, Tannins, Glycosides cyanogenic and Anthraquinones	N.D.	[99, 107]
Leaves	Water	Antibacterial	Polyphenols, Sesquiterpenes, Alkaloids and Phlobatamines	N.D.	[64, 99, 100]
	Methanol	Antibacterial Antioxidant	Polyphenols, Sesquiterpenes, Alkaloids and Phlobatamines	N.D.	[64, 100, 108]
	Acetone	Antibacterial	Polyphenols, Sesquiterpenes, Alkaloids and Phlobatamines	N.D.	[64]
	Ethanol	Antibacterial	Flavonoids, Alkaloids, Tannins, Glycosides cyanogenic and Anthraquinones	N.D.	[99]
Seeds	Water	Antioxidant Antidiabetic Cicatrizing	Polyphenols Glycosides Sugars	N.D.	[98, 109, 110]
	Ethanol	Growth factor (antibiotic) Antioxidant Cicatrizing	Carbohydrates Compounds phenolic unsaturated fatty acids	Tocopherol[a]	[73, 111, 109]
	Tampon de lyse A	proteinase inhibitor	Protein	Kunitz type proteinase inhibitor	[76, 109]
	Methanol	Cicatrizing	N.D.	N.D.	[109]
	Tampon phosphate saline	Cicatrizing	Alkaloids Tannins Saponins	N.D.	[109]

N.D = Not determined
a=Structure on Fig. 5 below

Table 3 above show that few molecules are well describe from *Tamarindus indica* and have no direct relation whith its antiacterial activity. Alpha tocopherol was isolated from seeds ethanol extract (Aengwanich *et al*. 2009) [5] and is a well-known antioxidant compound ((Gordon, 1990) [27] (Suzuki *et al*. 1993) [70]. A protein isolated from this plant has been identified as a Kunitz type proteinase inhibitor (Patil *et al*. 2009) [60]. The third reported molecule from *Tamarindus indica L.* is tamarindienal or (3*E*)-2, 5-Dioxo-3- hexenal: the bitter principle of the fruits but untit now no interesting biological activity was related to this molecule.

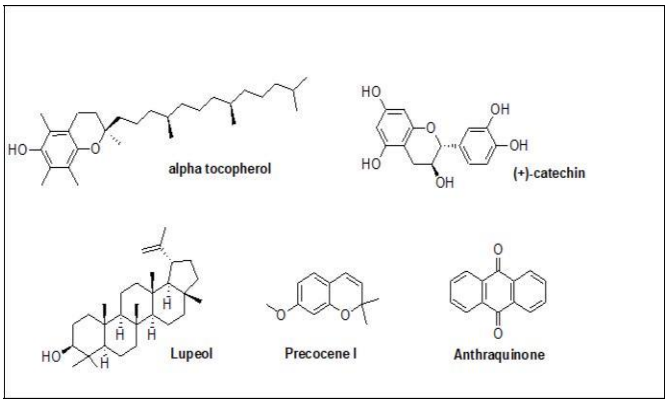

Fig 5: Structures of known molecules from the three plants

Conclusion

Although present work highlights numerous studies on the pharmacological activities of the three plants that have been tested and proven, there is no antimicrobial active molecule described. This lack of knownildge about the bioactive compounds of the three plants don't allow an objective study of their combinated use against infectiuous deseases by Benin traditional healers. We then think it would be interresting to test the extracts together, to select the more efficient combinaison and fractionate it. Antibiotic resistance proving to be a global health priority, this could be a promising way in the search for new antibacterial molecules.

Reference

1. Adanson M. Familles des plantes. Par M. Adanson. Vincent, Paris, 1763. https://doi.org/10.5962/bhl.title.271
2. Adetutu A, Morgan WA, Corcoran O, Chimezie F. Antibacterial activity and *in vitro* cytotoxicity of extracts and fractions of *Parkia biglobosa* (Jacq.) Benth. Stem bark and Ageratum conyzoides Linn. Leaves. Environ. Toxicol. Pharmacol. 2012; 34:478-483.
3. https://doi.org/10.1016/j.etap.2012.06.008
4. Adjanohoun. Banque de données de médecine traditionnelle et pharmacopée. Paris, 1989, 124. ISBN: 92-9028-146-4, n.d.
5. Aengwanich W, Suttajit M, Srikhun T, Boonsorn T. others. Antibiotic effect of polyphenolic compound extracted from tamarind (*Tamarindus indica* L.) seed

coat on productive performance of broilers. Int J Poult Sci. 2009; 8:749-751.
6. Agassounon. Evaluation des activités cytotoxique, antivirale, antibactérienne et antifongique de six plantes, n.d, 2001.
7. Akinpelu DA, Olaniyi AT, Mayowa AO, Olayinka AA. Anthony OI. Anti-Vibrio and preliminary phytochemical characteristics of crude methanolic extracts of the leaves of *Dialium guineense* (Wild). J Med. Plants Res. 2011; 5:2398-2404.
8. Akoegninou A. (Ed.), Flore analytique du Benin, Wageningen University papers. Backhuys, Wageningen, 2006.
9. Arogba SS, Ajiboro AA, Odukwe IJ. A physico-chemical study of nigerian velvet tamarind (*Dialium guineense* L) fruit. J Sci. Food Agric. 1994; 66:533-534.
10. Assane M, Baba Moussa R, Bassene E, Sere A. [Antihypertensive action of Parkia biglobosa+(Jacq) Benth seeds in the rat]. Dakar Med. 1993; 38:49-54.
11. Ayessou NC, Ndiaye C, Cissé M, Gueye M, Sakho M, Dornier M. Nutrient composition and nutritional potential of wild fruit Dialium guineense. J Food Compos. Anal. 2014; 34:186-191. https://doi.org/10.1016/j.jfca.2014.01.002
12. Azani N, Babineau M, Bailey CD, Banks H, Barbosa A, Pinto RB.A new subfamily classification of the Leguminosae based on a taxonomically comprehensive phylogeny-The Legume Phylogeny Working Group (LPWG). Taxon. 2017; 66:44-77. https://doi.org/10.12705/661.3
13. Balogun AM, Fetuga BL. Chemical composition of some underexploited leguminous crop seeds in Nigeria. J Agric. Food Chem. 1986; 34:189-192. https://doi.org/10.1021/jf00068a008
14. Bari AU, Silva HC, Silva MTL, Pereira Júnior FN, Cajazeiras JB, Sampaio AH *et al*. Purification and partial characterization of a new mannose/glucose-specific lectin from *Dialium guineense* Willd seeds that exhibits toxic effect: Purification of a Lectin from *Dialium Guineense* Willd Seeds. J Mol. Recognit. 2013; 26:351-356. https://doi.org/10.1002/jmr.2279
15. Bayala J, Balesdent J, Marol C, Zapata F, Teklehaimanot Z, Ouedraogo SJ. Relative contribution of trees and crops to soil carbon content in a parkland system in Burkina Faso using variations in natural 13C abundance. Nutr. Cycl. Agroecosystems. 2007; 76:193-201. https://doi.org/10.1007/s10705-005-1547-1
16. Bero J, Ganfon H, Jonville MC, Frédérich M, Gbaguidi F, DeMol P *et al*. *In vitro* antiplasmodial activity of plants used in Benin in traditional medicine to treat malaria. J Ethnopharmacol. 2009; 122:439-444. https://doi.org/10.1016/j.jep.2009.02.004
17. Buchholz T, Melzig MF. Medicinal Plants Traditionally Used for Treatment of Obesity and Diabetes Mellitus-Screening for Pancreatic Lipase and α-Amylase Inhibition. Phytother. Res. PTR. 2016; 30:260-266. https://doi.org/10.1002/ptr.5525
18. Cowan MM. Plant products as antimicrobial agents. Clin. Microbiol. Rev. 1999; 12:564-582.
19. Doughari JH. Antimicrobial activity of *Tamarindus indica* Linn. Trop. J Pharm. Res. 2006; 5:597-603.
20. Dzoyem JP, Melong R, Tsamo AT, Tchinda AT, Kapche DGWF, Ngadjui BT *et al*. Cytotoxicity, antimicrobial and antioxidant activity of eight compounds isolated from Entada abyssinica (Fabaceae). BMC Res. Notes, 2017, 10. https://doi.org/10.1186/s13104-017-2441-z
21. Ebifa-Othieno E, Mugisha A, Nyeko P, Kabasa JD. Knowledge, attitudes and practices in tamarind (*Tamarindus indica* L.) use and conservation in Eastern Uganda. J Ethnobiol. Ethnomedicine, 2017, 13. https://doi.org/10.1186/s13002-016-0133-8
22. Essien E, Ogunwande IA, Ogunbinu AkO, Flamini G, Cioni PL. Analysis of the Essential Oil of *Dialium guineense* Willd. J Essent. Oil Res. 2007; 19:545-547. https://doi.org/10.1080/10412905.2007.9699327
23. Ewedje EEB, Tandjiekpon A. Velvet tamarind (Dialium guineense), 2011.
24. Eyog Matig O, Adjanohoun E, de Souza S, Sinsin B. Programme de Resources Genetiques Forestieres en Afrique au Sud du Sahara (Programme Saforgen). Bioversity International, 2001.
25. Faustin AY, Djègo JG, Sinsin B. Capacité de germination de *Dialium guineense* Willd (fabaceae) une espèce agroforestière. J Appl. Biosci. 2013; 62:4566-4581.
26. Gnansounou. Proximate composition and micronutrient potentials of *Dialium guineense* wild growing in Benin. Int. Food Res. J. 2014, 25:36-42.
27. Gordon MH. The Mechanism of Antioxidant Action *in Vitro*, in: Hudson BJF. (Ed.), Food Antioxidants. Springer Netherlands, Dordrecht, 1990, 1-18. https://doi.org/10.1007/978-94-009-0753-9_1
28. Grønhaug TE, Glaeserud S, Skogsrud M, Ballo N, Bah S, Diallo D, Paulsen BS. Ethnopharmacological survey of six medicinal plants from Mali, West-Africa. J Ethnobiol. Ethnomedicine. 2008; 4:26. https://doi.org/10.1186/1746-4269-4-26
29. Group LPW. Others, Legume phylogeny and classification in the 21st century: progress, prospects and lessons for other species-rich clades. Taxon. 2013; 62:217-248.
30. Havinga RM, Hartl A, Putscher J, Prehsler S, Buchmann C, Vogl CR. *Tamarindus indica* L. (Fabaceae): Patterns of use in traditional African medicine. J Ethnopharmacol. 2010; 127:573-588. https://doi.org/10.1016/j.jep.2009.11.028
31. Hayashi K, Minoda K, Nagaoka Y, Hayashi T, Uesato S. Antiviral activity of berberine and related compounds against human cytomegalovirus. Bioorg. Med. Chem. Lett. 2007; 17:1562-1564. https://doi.org/10.1016/j.bmcl.2006.12.085
32. Hines DA, Eckman K. Indigenous multipurpose trees of Tanzania: uses and economic benefits for people, 1993.
33. Hopkins HC. The taxonomy, reproductive biology and economic potential of Parkia (Leguminosae: Mimosoideae) in Africa and Madagascar. Bot. J Linn. Soc. 1983; 87:135-167.
34. Ibrahim MA, Habila JD, Koorbanally NA, Islam MS. Butanol fraction of *Parkia biglobosa* (Jacq.) G. Don leaves enhance pancreatic β-cell functions, stimulates insulin secretion and ameliorates other type 2 diabetes-associated complications in rats. J Ethnopharmacol. 2016; 183:103-111. https://doi.org/10.1016/j.jep.2016.02.018
35. Iwu MM. Handbook of African medicinal plants, 2. ed. ed. CRC Press, Boca Raton, FL, 2014.
36. Iwu MM, Duncan AR, Okunji CO. New antimicrobials of plant origin. Perspect. New Crops New Uses ASHS Press Alex. VA. 1999; 457-462.
37. Jussieu AL. de, Genera plantarum secundum ordines

naturales disposita, juxta methodum in Horto Regio Parisiensi exaratum, anno M.DCC. LXXIV. Apud Viduam Herissant ET Theophilum Barrois, Parisiis, 1789. https://doi.org/10.5962/bhl.title.7762
38. Kamatou GP, Van Zyl RL, Van Vuuren SF, Viljoen AM, Figueiredo AC, Barroso JG *et al*. Chemical Composition, Leaf Trichome Types and Biological Activities of the Essential Oils of Four Related Salvia Species Indigenous to Southern Africa. J Essent. Oil Res, 2006, 18.
39. Komolafe K, Olaleye TM, Seeger RL, Carvalho FB, Boligon AA, Athayde ML *et al. Parkia biglobosa* improves mitochondrial functioning and protects against neurotoxic agents in rat brain hippocampal slices. BioMed Res. Int, 2014, 326290. https://doi.org/10.1155/2014/326290
40. Koné W, Atindehou KK, Terreaux C, Hostettmann K, Traoré D, Dosso M. Traditional medicine in North Côte-d'Ivoire: screening of 50 medicinal plants for antibacterial activity. J Ethnopharmacol. 2004; 93:43-49. https://doi.org/10.1016/j.jep.2004.03.006
41. Koura K, Dissou E, Ganglo J. Caractérisation écologique et structurale des parcs à néré [*Parkia biglobosa* (Jacq.) R. Br. Ex G. Don] du département de la Donga au Nord-Ouest du Bénin. Int. J Biol. Chem. Sci, 2013, 7. https://doi.org/10.4314/ijbcs.v7i2.27
42. Kunz BK, Linsenmair KE. Changes in Baboon Feeding Behavior: Maturity-dependent Fruit and Seed Size Selection within a Food Plant Species. Int. J Primatol. 2007; 28:819-835. https://doi.org/10.1007/s10764-007-9160-6
43. Lokonon BE, Bonou WN, Kassa B, Azihou AF, Assogbadjo E, Glelekakai R. Structural and ethnobotanical characterization of velvet tamarind (*Dialium guineense* Willd), a multipurpose tree species. Agron. Afr. 2013; 25:121-131.
44. Lykke AM. Local perceptions of vegetation change and priorities for conservation of woody-savanna vegetation in Senegal. J Environ. Manage. 2000; 59:107-120. https://doi.org/10.1006/jema. 2000.0336
45. Ma X, Zheng C, Hu C, Rahman K, Qin L. The genus Desmodium (Fabaceae)-traditional uses in Chinese medicine, phytochemistry and pharmacology. J Ethnopharmacol. 2011; 138:314-332. https://doi.org/10.1016/j.jep.2011.09.053
46. Meher B, Dash DK, Roy A. A review on: Phytochemistry, pharmacology and traditional uses of *Tamarindus indica* L. World J Pharm Pharm. Sci. 2014; 3:229-240.
47. Meregini AOA. Some endangered plants producing edible fruits and seeds in Southeastern Nigeria. Fruits. 2005; 60:211-220. https://doi.org/10.1051/fruits:2005028
48. Millogo-Kone H, Guissou I, Nacoulma O, Traore AS. Antimicrobial effects of the stem bark extracts of *Parkia biglobosa* (Jacq.) Benth. On Shigellae. Afr. J Tradit. Complement. Altern. Med. Ajtcam. 2007; 4:392-396.
49. Millogo-Kone H, Guissou IP, Nacoulma O, Traore AS. Comparative study of leaf and stem bark extracts of *Parkia biglobosa* against enterobacteria. Afr. J Tradit. Complement. Altern. Med. Ajtcam. 2008; 5:238-243.
50. Minot N, Daniels L. Impact of global cotton markets on rural poverty in Benin. Agric. Econ. 2005; 33:453-466. https://doi.org/10.1111/j.1574-0864.2005.00415.x
51. Morton JF, Dowling CF. Fruits of warm climates. JF. Morton, Miami, FL, 1987.
52. Morton Julia F. The Tamarind It's food medecinal and industrial uses Florida State Horticultural Society. pdf, n.d, 1958.
53. Mosnier C, Van De Moortel C, Ridier A, Kephaliacos C, Daniel K. Others, Conditionnalités des aides directes: impact de la mise en oeuvre de certaines BCAE dans des exploitations d'élevage et de grandes cultures, 2006.
54. Nokta M, Albrecht T, Pollard R. Papaverine hydrochloride: effects on HIV replication and T-lymphocyte cell function. Immunopharmacology. 1993; 26:181-185. https://doi.org/10.1016/0162-3109 (93)90010-N
55. Odukoya OA, Houghton PJ, Adelusi A, Omogbai EKI, Sanderson L, Whitfield PJ. Molluscicidal triterpenoid glycosides of Dialium guineense. J Nat. Prod. 1996; 59:632-634.
56. Okwu DE, Ekeke O. Phytochemical screening and mineral composition of chewing sticks in South Eastern Nigeria. Glob. J Pure Appl. Sci, 2003, 9. https://doi.org/10.4314/gjpas.v9i2.15962
57. Olajubu F, Akpan I, Ojo DA, Oluwalana S. Antimicrobial potential of *Dialium guineense* (Wild.) stem bark on some clinical isolates in Nigeria. Int. J Appl. Basic Med. Res. 2012; 2:58-62. https://doi.org/10.4103/2229-516X.96811
58. Orji. Antibacterial Activities of Crude Leaf and Bark Extracts of "Icheku" *Dialium guineense* on Bacterial Isolates from Bronchitis Patients. IOSR J Pharm. Biol. Sci. 2012; 1:21-25. https://doi.org/10.9790/3008-0132125
59. Ouédraogo AS. *Parkia biglobosa* (Leguminosae) en Afrique de l'Ouest: biosystématique et amélioration. [sn], 1995.
60. Patil DN, Preeti Chaudhry A, Sharma AK, Tomar S, Kumar P. Purification, crystallization and preliminary crystallographic studies of a Kunitz-type proteinase inhibitor from tamarind (*Tamarindus indica*) seeds. Acta Crystallograph. Sect. F Struct. Biol. Cryst. Commun. 2009; 65:736-738. https://doi.org/10.1107/S1744309109023495
61. Qiu Y, Dombrovska O, Lee J, Li L, Whitlock BA, Bernasconi-Quadroni F *et al*. Phylogenetic Analyses of Basal Angiosperms Based on Nine Plastid, Mitochondrial, and Nuclear Genes. Int. J Plant Sci. 2005; 166:815-842. https://doi.org/10.1086/431800
62. Ræbild A, Larsen AS, Jensen JS, Ouedraogo M, De Groote S, Van Damme P *et al*. Advances in domestication of indigenous fruit trees in the West African Sahel. New For. 2011; 41:297-315. https://doi.org/10.1007/s11056-010-9237-5
63. Rahman AM, Parvin MIA. Study of medicinal uses on Fabaceae family at Rajshahi, Bangladesh. Res. Plant Sci. 2014; 2:6-8.
64. Rosado-Vallado M, Brito-Loeza W, Mena-Rejón G, Quintero-Marmol E, Flores-Guido J Antimicrobial activity of Fabaceae species used in Yucatan traditional medicine. Fitoterapia. 2000; 71:570-573. https://doi.org/10.1016/S0367-326X (00)00200-8
65. Sabiiti EN, Cobbina J. Parkia biglobosa: a potential multipurpose fodder tree legume in West Africa. Int. Tree Crops J. 1992; 7:113-139. https://doi.org/10.1080/01435698.1992.9752911
66. Savolainen V, Fay MF, Albach DC, Backlund A, Van

Der Bank M, Cameron KM *et al*. Phylogeny of the Eudicots: A Nearly Complete Familial Analysis Based on rbcL Gene Sequences. Kew Bull. 2000; 55:257. https://doi.org/10.2307/4115644

67. Smagadi A. Accès aux ressources génétiques et partage des avantages découlant de leur exploitation: la Convention sur la diversité biologique et le système mondial de la FAO. Rev. Eur. Droit Environ. 2005; 9:255-273.
68. Soltis DE, Soltis PS, Nickrent DL, Johnson LA, Hahn WJ, Hoot SB *et al*. Angiosperm Phylogeny Inferred from 18S Ribosomal DNA Sequences. Ann. Mo. Bot. Gard. 1997; 84:1 https://doi.org/10.2307/2399952
69. Stern JL, Hagerman AE, Steinberg PD, Mason PK. Phlorotannin-protein interactions. J Chem. Ecol. 1996; 22:1877-1899.
70. Suzuki YJ, Tsuchiya M, Wassall SR, Choo YM, Govil G, Kagan VE *et al*. Structural and dynamic membrane properties of. Alpha-tocopherol and. alpha-tocotrienol: Implication to the molecular mechanism of their antioxidant potency. Biochemistry (Mosc.). 1993; 32:10692-10699.
71. Swahili-English Dictionary [WWW Document], n.d. URL. http://www.goswahili.org/dictionary/ (accessed 9.26.17).
72. Tapsoba H, Deschamps JP. Use of medicinal plants for the treatment of oral diseases in Burkina Faso. J Ethnopharmacol. 2006; 104:68-78. https://doi.org/10.1016/j.jep.2005.08.047
73. Tariq M, Chaudhary SS, Rahman KH, Zaman R, Shaikh I. Tamarindus Indica: An Overview. J Biol. Sci. Opin. 2013; 1:128-131. https://doi.org/10.7897/2321-6328.01220
74. Tignokpa M, Laurens A, Mboup S, Sylla O. Plantes medicinales populaires des marches de Dakar (Senegal). Int. J Crude Drug Res. 1986; 24:75-80.
75. Tsuda T, Watanabe M, Ohshima K, Yamamoto A, Kawakishi S, Osawa T. Antioxidative components isolated from the seed of tamarind (*Tamarindus indica* L.). J Agric. Food Chem. 1994; 42:2671-2674.
76. Uusiku NP, Oelofse A, Duodu KG, Bester MJ, Faber M. Nutritional value of leafy vegetables of sub-Saharan Africa and their potential contribution to human health: A review. J. Food Compos. Anal. 2010; 23:499-509. https://doi.org/10.1016/j.jfca.2010.05.002
77. Ventola CL. The antibiotic resistance crisis: part 1: causes and threats. Pharm. Ther. 2015; 40:277.
78. Wang Z, Xue Y, Liu Y. The modulation of protein kinase A and heat shock protein 70 is involved in the reversible increase of blood–brain tumor barrier permeability induced by papaverine. Brain Res. Bull. 2010; 83:367-373. https://doi.org/10.1016/j.brainresbull.2010.08.009
79. World Health Organization (Ed.). WHO traditional medicine strategy. 2014-2023. World Health Organization, Geneva, 2013.

III- Compositions et activités biologiques des fruits, feuilles et écorces de *D. guineense, P. biglobosa* et *T. indica*

Article 2

Gnansounou, S. M., Noudogbessi, J. P., Yehouenou, B., Gbaguidi, A. N.M., Dovonon, L., Aina, M. P., Ahissou, H. and Sohounhloue, D. Proximate Composition and Micronutrient Potentials of Dialium guineense Wild growing in Benin. International Food Research Journal 21(4) : 1603-1607 (2014).

La présente publication est notre première étude réalisée sur *Dialium guineense willd.*, une espèce connue dans le domaine alimentaire et dans les pratiques thérapeutiques traditionnelles locales.

Dialium guineense willd. pousse exclusivement en Afrique tropicale. Les fruits de cet arbre sont consommés par les populations depuis des siècles. Ils constituent donc une source historique de nutriments en Afrique de l'Ouest et particulièrement au Bénin. Cette étude a consisté en une série de dosages d'éléments nutritifs de la pulpe par des méthodes standard de l'AOAC (Association of Official Analytical Chemists). Comme la plupart des fruits, il apparaît que la pulpe du fruit de *D. guineense* est riche en minéraux avec des valeurs intéressantes pour l'iode (04,34 ± 0,12 mg / 100 g), le fer (14,75 ± 0,25 mg / 100 g), le calcium (70,14 ± 10 mg / 100 g) et de potassium (366 ± 0,26 mg / 100 g). La pulpe renferme 80% de glucides, 4,5% de vitamine C et 9,21% de lipides. La teneur en protéines est exceptionnelle (6,12%) car les fruits n'en sont pas une source habituelle. Cette enquête visait à fournir des données sur la composition de la pulpe de fruit pour des utilisations diététiques et socio-économiques judicieuses.

L'abondance de sucres dans la pulpe de fruits *Dialium guineense* en fait un bon fournisseur de ce nutriment rapidement utilisable par les cellules. Ses teneurs élevées en minéraux majeurs (calcium, sodium, magnésium, potassium) et en minéraux mineurs (iode, fer) encouragent sa consommation pour pallier les problèmes de carence en minéraux.

Ces résultats ainsi que la bibliographie très fournie sur les propriétés médicinales de *Dialium guineense willd.* ont suscité notre intérêt pour la poursuite de recherches sur cette plante et deux autres de la même famille : *Parkia biglobosa* et *Tamarindus indica L*. Notre choix est motivé par les similitudes de ces trois plantes. Elles sont de la même famille botanique (caesalpiniacea), leurs fruits sont comestibles et les parties aériennes sont très utilisées en médecine traditionnelle. Enfin, elles figurent sur la liste des dix espèces ligneuses alimentaires prioritaires du Bénin car surexploitées et donc menacées d'extinction. Notre travail vise alors à étudier le potentiel de ces plantes afin de contribuer à leur sauvegarde.

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Proximate composition and micronutrient potentials of *Dialium guineense* wild growing in Benin

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Abstract

Dialium guineense wild is a tree growing exclusively in tropical Africa. Fruits from this tree are consumed by populations since many centuries. So it takes part to satisfy their nutritional needs and display an historical role as nutrients provider. Nutrient composition of the fresh fruit pulps was determined using standard methods of AOAC and those of European norms. Like the other fruits, it emerges that the fruit pulp is rich in minerals with interesting values for iodine (04.34 ± 0.12 mg/100 g), iron (14.75 ± 0.25 mg/100 g), calcium (70.14 ± 10 mg/100 g) and potassium (366 ± 0.26 mg/100 g). Bio-molecules analyzes have shown a high content in total carbohydrates (80 ± 1.8 g/100 g) and crud lipid (09.21 ± 0.50 g/100 g).Crud proteins content is (6.12 ± 0.33 g/100 g). Ascorbic acid was found in the pulp (4.5 ± 0.01 mg/100 g) with 9.36
(c) 1.36 g/100 g moisture content. This investigation sought to provide data on the fruit pulp composition for judicious dietetic and socio-economic uses.

Introduction

According to FAO's evaluations, close to 870 millions people were underfed in 2010-2012 (FAO, 2012).The malnutrition by deficiency in micronutrients ("hidden hunger") that touches about 2 billions of people (either more than 30% of the world population), has heavy consequences concerning public health. Minerals particularly have a prime importance in human body metabolism so their deficiency or excess are both harmful (Hashmi *et al.*, 2007). Fruits are known as excellent source of mineral and vitamins (Nahar *et al.*, 1990) and may permit to increase rural population food quality (Kunhlein, 1989). *Dialium guineense* with English name velvet tamarind or black velvet is one of the most consumed wild fruit in tropical Africa. The tree grows to about 20 at 30 m in height, low-branching, rarely straight, bearing a compact densely leafy crown but is often shrubby (Okegbile and Taiwo, 1990; Akoègninou 2006). In Benin, the plant is called "assonswen''among the Fon and "awin'' by Yorubas. The fruit pulp is red with astringent flavour and is eaten raw when dry by man and animals (Matsuda, 2006). Some works have been reported on the fruit in Nigeria (Achoba *et al.*, 1992; Arogba *et al.*, 1994; Ubbaonu *et al*., 2005; Adepoju, 2009) and Ghana (Ofosu *et al*., 2013).There is limited or no information in the literature about the proximate composition of *Dialium guineense* wild growing in Benin where this plant is endangered (Meregini, 2005). It is therefore the aim of this study to corroborate its historic nutrient provider role by chemical analyzes in view to widen rural populace mineral source and preserve the plant.

Material and Methods

Sample collection and identification

The fruits of *Dialium guineense* have been harvested at the stage of maturity in Tankpè, Akassato and Glo from January to March 2013 while ripe fruits were available. Several samplings have been made to some weeks of interval. Samples from these three localities were submitted to the same analyzes in view to compare their results. Voucher specimen was deposited at the Benin National Herbarium for reference under the number AA 6462 / HNB. They have been kept at the laboratory in a dry aerated environment. Analyzes have been made on the pulp obtained before every manipulation by ridding the fruit of its coat and seed.

Chemical analyses

The fruit pulp from each locality has been analyzed in triplicate for moisture (AOAC 2003, Method: 950.46B), crude protein (AOAC 2003, Method: 990.03), crude lipid (AOAC 2003, Method: 920.39) and ash (AOAC 2003, Method: 942.05). Total carbohydrate was obtained using colorimetric

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method of Dubois *et al.* (1956) by adding phenol and sulfuric acid and readings taken on jenway RS 252 spectrophotometer. After calcination in a Nabertherm C 290 furnace (600°C) in crucibles made of china, the ashes have been diluted in hydrochloric acid (3 mol/l). Sodium and potassium have been measured out by spectrometry of flame with a Varian SpectrAA-110 apparatus. The measures have been preceded of cesium chloride's (5%) addition. They took place respectively under steam-powered lamp of sodium or potassium and were compared with absorption of standards of these minerals. A molecular absorption spectrophotometer portable HACH DR /2400 permitted readings at 240 nm and 265 nm of the contents in total iodine and total iron respectively. DPD Total iodine reagent cat 14064-99 lot A 0257 from HACH PRMACHEM USA was added 3 minutes before iodine's measure. Ferrover reagent lot A 1159 from HACH PERMACHEM has been used for iron measure. Complexometry with EDTA (Ethylene Diamine Tetra acetic Acid) served for dosage of calcium (NF T 90-016, August 1984) and magnesium (NF T 90-003, August 1984). Indicators of calcium ions (calver cat 917-99 lot A O313 HACH PERMACHEM USA) and magnesium (black eriochrome T) permitted to follow the reaction. Vitamin C in the pulp was determined by reverse titration with sodium thiosulphate and iodine using thiodene as indicator.

Statistical analysis

Data from three independent replicate trials were subjected to statistical analysis using Statistica version 6.0. Differences between means were tested using Z-test.

Results and Discussion

The mean elemental composition of the fruit pulp showed presence of calcium, sodium, magnesium, iron, iodine and potassium in varying quantities (Table 1 and 2). Because of the proximity of these localities, a great difference doesn't appear in the fruits composition. However, the fruits reaped at Tankpè contained the most elevated values for iron and sodium while those of Akassato had the best contents in iodine and potassium. Calcium, magnesium and vitamin C levels were higher in samples from Glo. Phosphorus, zinc, manganese and copper contents were reported to be low (Adepoju, 2009).The same work showed that Dialium guineense had low moisture because it becomes dry during maturation.

Some considerable variations occur in food composition because of their biologic origin. These

Table 1. Minerals and vitamin C composition of Dialium guineense (D. g.) growing in Akassato,Glo and Tankpè

	D.g. of Akassato	D.g. of Glo	*D.g.* of Tankpè
Vitamin C [mg]	04.3±0.04b	04.6±0.02a	04.6±0.00a
Calcium [mg]	60.12±11a	80.16±12b	70.14±8c
Magnesium [mg]	25.20± 5a	36.42± 6.5a	30.90± 5.3a
Iron [mg]	14.80± 0.35a	14.35±0.2a	15.10± 0.2a
Iodine [mg]	04.50± 0.15a	04.25±0.12 a	04.34± 0.1 a
Sodium [mg]	75.10±0.04 a	76.00±0.16 a	76.92±0.07 b
Potassium [mg]	368.11±0.23 b	364.98±0.25 a	364.91±0.3 a

•n = average of three independent samples, analyzed in triplicate. Means in the same line followed by different letters are significantly different ($p < 0.05$)

variations are the fact of endogenous factors (degree of maturity, genetic influence) or exogenous ones like temperature, sunshine and nature of soils (Paul and Southgate, 1988). However, despite a divergence of water and minerals contents, our results were globally in agreement with those published by Adepoju (2009) and Ofosu (2013) on Nigeria's *Dialium guineense* and those of Ghana. Obviously, with 09.36% of moisture, median value between those reported (04% - 17.1%) (Arogba *et al.*, 1994; Ubbaonu *et al.*, 2005; Adepoju, 2009), the pulp of the studied fruit here couldn't be considered as refreshing in comparison with those consumed fluently (mangoes, oranges, pineapple, bananas).

In spite of the relatively weak value of crud proteins contained in the pulp (06.12%), the fruits of *Dialium guineense* are richer in proteins than the wild berries from British Colombia (Kuhnlein,1989), orange, strawberry and melon (Anon, 1960). This fruit therefore wear a particular aspect because fruits are generally not considered like sources of proteins (Edem *et al.*, 1984; Ishola *et al.*, 1990). This value was within the range (8.3% and 6.3%) stated in literature (Arogba *et al.*, 1994; Adepoju, 2009).

The high level of total sugars (80%) was in the range of the values published early and explains the fruit sweetened taste. The sugars measured out here regrouped digestible carbohydrates as well as the food fibers little or nearly not energizing. A weak rate of these fibers (0.6%) being returned by Adepoju, the quasi-totality of sugars would then be digestible. The fruit is therefore indicated for children and people with hypoglycemia. The diabetics, on the other hand, should avoid some or limit the consumption (Table 3 and 4).

Vitamin C content of the pulp was close to those (1.8 - 6.2 mg/100 g) returned in the literature (Wu Leung, 1968; Eremosele, 1991). It places the fruit of *D. guineense* to the rank of good sources of vitamin C and its consumption can contribute to satisfy the journal requirement that is 90 to110 mg (Marieb, 1999). The importance of this vitamin resides otherwise in its antioxidant power (Vodjani *et al.*, 2000; Laight *et al.*, 2000; Masaki *et al.*, 2010) inhibiting the ominous effect of free radicals on the DNA. It is indispensable for iron absorption; cloves repair and blood vessels

Table 2. Average of vitamin C and minerals of D.g. growing in Benin

Dialium guineense wild growing in Benin	
Vitamin C [mg]	04.5±(0.01)
Iodine	04.34±0.12
Iron	14.75±0.25
Magnesium	30.84±(5.6)
Calcium [mg]	70.14 ±(10)
Potassium	366±0.26

Table 3. Proximate composition of *D. guineense* growing in Akassato, Glo and Tankpè (g /100 g edible portion of fruit pulp)*

	D.g. of Akassato	D.g. of Glo	*D.g.* of Tankpè
Moisture	7.17± 1.4b	11.13 ± 1.38a	09.8±1.6 a
Ashes	1.5 ±0.04 a	1.72 ±0.05 b	1.89±0.06c
Total carbohydrate [g]	78.4 ±3 a	81±1 a	79.7±1.4 a
Crude Lipids [g]	8.66 ±0.4 a	9.66±0.2 a	09.33±0.30a
Crude Proteines [g]	6.17 ± 0.46 a	5.77 ± 0.3 a	06.43±0.23 a

*n = average of three independent samples, analyzed in triplicate. Means in the same line followed by different letters are significantly different ($p < 0.05$)

Table 4. Average of ashes, moisture and bio-molecules of D.g. growing in Benin

Dialium guineense wild growing in Benin	
Mosture	09.36±1.36
Ashes	01.70±0.05
Total carbohydrate	80±1.8
crude lipid	09.21±0.50
Crude protein	06.12±0.33

formation via collagen synthesis. Several works established its beneficial action in the treatment or prevention of rheumatism, type 2 diabetes (Canter *et al*., 2007; Afkhami-Ardekani *et al*., 2007) and of the cardiovascular illnesses (Cook *et al*., 2007; Shinke *et al*., 2007). A food poor in C vitamin is associated to fatigue (Suh *et al*., 2012) and immunodeficiency (Harakesh *et al*., 1990).

The pulp had interesting contents in calcium and magnesium but lower than to those of the literature (Adepoju, 2009; Ofosu, 2013). Calcium participates to ossification, to the muscular contraction and to the blood coagulation (Wardlaw *et al*., 2012). As for magnesium, it cooperates with calcium to the muscular contraction and blood coagulation. It is a cofactor for several enzymes (Rude *et al*., 2010). A magnesium deficiency conducted to neurological or neuromuscular disorder (Shils *et al*., 1988).

Iron content was 14.75 ± 0.25 mg/100 g.This value three times superior to the one found by Adepoju makes of the fruit a good means of preventive struggle against anemia. The pulp iron absorption is more efficient because of the presence of vitamin C (Derman *et al*., 1980; Halberg *et al*., 1987; Siegenberg *et al*., 1991; Cook *et al*., 1977). In spite of its major biologic role, few values appear in the literature about the pulp iodine content. The value found in the present survey is 04.34 ± 0.12 mg /100 gs of pulp. Iodine deficiency leads to physiological mess with trouble of cerebral functions (Benmiloud *et al*., 1983; Hetzel *et al*., 1983; Delange *et al*., 1994; Fischer *et al*., 1998).

The values obtained for sodium and potassium were respectively 76 ± 0.09 and 366 ± 0.26 mg/100 g. They were in agreement with those of the literature. The effects of potassium against the arterial hypertension were reported (Ophir *et al*., 1987; Siani *et al*., 1987; Girmin *et al*., 1990; Young *et al*., 1995; Whleton *et al*., 1997; Suter, 1998). Besides the endogenous and exogenous biologic conditions that could explain the divergences of contents of our samples with those of the Nigeria and Ghana, the methods of analyses used influenced by their sharpness on the different results.

Conclusion

The abundant presence of sugars in the pulp of *Dialium guineense* fruits makes of it a good supplier of this nutriment quickly usable by cells. Its high contents in major minerals (calcium, sodium, magnesium, potassium) as well as that in minor minerals (iodine, iron) open the way for use in order to palliate mineral deficiency problems. Diabetics and obese should avoid this fruit while children and pregnant women need some.

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References

Achoba , I. I., Choba , J. A., Lori, J. A., Elegbede, J. A. and Kagbu, J. A.1992. Nutrient composition of black (African) velvet tamarind seed and pulp from Nigeria. Journal of Food Biochemistry 16(4):229-233.

Adepoju, O. T. 2009. Proximate composition and micronutrient potentials of three locally available wild fruits in Nigeria. African Journal of Agricultural Research 4(9): 887-892.

Afkhami-Ardekani, M. and Shojaoddiny-Ardekani, A. 2007. Effect of vitamin C on blood glucose, serum lipids and serum insulin in type 2 diabetes patients. Indian Journal of Medical Research 126 (5): 471-4.

Akoègninou, A., van der Burg, W. J. and van der Maesen, L. J. G.2006. Flore analytique du Benin. Cotonou and Leiden: Backhuys Publisher.

Anon.1960.Food composition tables. Rome: FAO. AOAC. 2003. Official Methods of analysis of AOAC.17th edn. Arlington, VA: Association of Official Analytical Chemists (AOAC) International.

Arogba, S. S, Ajiboro, A. and Odukwe, I.1994.A physico-chemical study of Nigeria velvet Tamarind (*Dialium guineense*) fruit. Journal of the Science of Food and Agriculture 66(4): 533-534.

Benmiloud M., Bachtarzi H. and Chaouki M.B. (1983) Public health and nutritional aspects of endemic goitre and cretinism in Africa. In: Delange F. and Ahluwalia R. Cassava toxicity and thyroid. p.50. Research and Public Health Issues, IDRC, Ottawa.

Canter, P.H.,Wider, B. and Ernst, E. 2007. The antioxidant vitamins A, C, E and selenium in the treatment of arthritis: a systematic review of randomized clinical trials. Rheumatology (Oxford) 46(8): 1223-1233.

Cook, J. D. and Monsen, E. R.1977. Vitamin C, the common cold and iron absorption. American Journal of Clinical Nutrition 30 (2): 235-241.

Cook, N. R., Albert, C. M., Gaziano, J. M., Zaharris, E., MacFadyen, J., Danielson, E., Buring, J. E. and Manson, J. E.2007. A randomized factorial trial of vitamins C and E and beta carotene in the secondary prevention of cardiovascular events in women: results from the Women's Antioxidant Cardiovascular Study. Archives of Internal Medecine 167(15): 1610-8.

Delange, F. 1994. The disorders induced by iodine deficiency. Thyroid 4 (1): 107-128.

Derman, D. P. 1980.Importance of ascorbic acid in the absorption of iron from infant foods. Scandinavian Journal of Haematology 25 (3): 193-201.

Dubois, M.,Gilles, D. A., Hamilton, J. K., Rebers, P. A. and Smith, F. 1956. Colorimetric methods for determination of sugar and related substances. Analytical Chemistry 28 (3): 350-356.

Edem, D. O, Eka O.U. and Ifon, E.T.1984. Chemical Evaluation of the Value of the fruit of African Star apple (*Chrisysophyllum albidum*). Food chemistry 14: 303-311.

Eremosele,I. C., Eremosele, C. O., and Kuzhkuzha, D. M. 1991. Evaluation of mineral element and ascorbic acid contents in fruits of some wild plants. Plant Foods for human Nutrition41(2): 151-154.

FAO. 2012. The state of food insecurity in the world. Rome, ITALY.

Fisher, D. A. and Delange, F. M.1998.Thyroid hormone and iodine requirements in man during brain development. In: Iodine in Pregnancy. Stanbury, J. B., Delange, F., Dunn, J. T., and Pandav, C. S.editors. New Delhi, Oxford University Press Publication.

Grimm, R. H., Neaton, J. D. and Elmer, P. J.1990.The influence of oral potassium chloride on blood pressure on hypertensive men on a low sodium diet. New England Journal of Medecine 322(9): 569-574.

Hallberg, L., Brune, M.and Rossander-Hultén, L. S. 1987. Is there a physiological role of vitamin C in iron absorption? Annals of the New York Academy of Sciences 498(1): 324-332.

Harakeh, S., Jariwalla, R. and Pauling, L. 1990. Suppression of human immunodeficiency virus replication by ascorbate in chronically and acutely infected cells. Proceedings of the National Academy of Sciences USA 87(18): 7245-7249.

Hashmi, D. R., Ismail, S.and Shaikh, G. H. 2007. Assessment of the level of trace metals in commonly edible vegetables locally available in the markets of Karachi city. Pakistan Journal of Botany 39(3): 747-751.

Hetzel, B. S.1983. Iodine deficiency disorders (IDD) and their eradication. Lancet. 322 (8359): 1126-1129.

Ishola , M.M., Agbadji, E. B. and Agbadji, A. S. 1990. A chemical study of *Tamarindus indica* (Tsamiya) fruits grow in Nigeria. Journal of the Science of Food and Agriculture 51(1): 141-143.

Kuhnlein, H.V. 1989. Nutrient values in indigenous wild berries used by the Nuxalk people of Bela Coola, British Colombia. Journal of Food Composition and Analysis 2:28-36

Laight, D. W., Carrier, M. J., Anggard, E. E. 2000. Antioxidants, diabetes and endothelial dysfunction. Cardiovascular Research 47: 457-464

Marieb E. N. 1999. Anatomie et Physiologie Humaines (Traduction de la 4e édition américaine) Editions du Renouveau Pédagogique inc. ISBN DBU 2 -8041 - 3219-6.

Masaki, H.2010. Role of antioxidants in the skin:anti-aging effects. Journal of Dermatological Science 58(2):85-90.DOI 101016.

Matsuda, R.2006. Feeding ecology of the Mona monkey (*Cercopithecus mona*) in a seasonally dry flooded forest in the Dahomey gap. Dept. of Anthropology, Graduate Center, City University of New York, New York, NY, 10016, (USA IPS Conference 2006 (Abstracts).

Meregini, A. O.2005. Some endangered plants producing edible fruits and seed in southeastern Nigeria. Fruit 60: 211-220.

Nahar, N., Rahman, S. and Mosiihuzzaman, M.1990. Analysis of carbohydrates in seven edible fruit of Bengladesh. Journal of Science of Food and Agriculture 51:185-192.

Ofosu, D. O., Opata, N. S., Gyampo, O. and Odamtten, G.T. 2013. Research Journal of Applied Sciences, Engineering and Technology 6(19): 3536-3539.

Okegbile, E. O. and Taiwo, E. O. 1990. Nutritional potentials of velvet tamarind (*Dialium guineense* Wild). Nigerian Food Journal 8: 115-121.

Ophir, O., Peer, G. and Gilad, J. 1983. Low blood pressure in vegetarians: the possible role of potassium. American Journal of Clinical Nutrition 37: 755-762.

Paul, A. A. and Southgate, D. A. T. 1988. The composition of foods. McCance and Widdowson. Elsevier, North Holland Biomedical Press, London, 418pp.

Rude, R. K. 2010. Magnesium. Encyclopedia of Dietary Supplements 2nd ed. New York, Informa Healthcare.527- 537.

Shils, M. E. 1988. Magesium in health and disease. Annual Review of Nutrition 8: 429-460.

Shinke, T., Shite, J., Takaoka, H., Hata, K., Inoue, N., Yoshikawa, R., Matsumoto, H., Masai, H., Watanabe, S., Ozawa, T., Otake, H., Matsumoto, D., Hirata, K. and Yokoyama, M. 2007. Vitamin C restores the contractile response to dobutamine and improves myocardial efficiency in patients with HF. American Heart Journal 154(4): 645.

Siani, A., Stazullo, P. and Russo l. 1987.Controlled trial of long-term oral potassium supplements in patients

with mild hypertension. British Medical Journal 294 (6585): 1453-1456.

Siegenberg, D. 1991 Ascorbic acid prevents the dose-dependent inhibitory effects of polyphenols and phytates on non heme-iron absorption. American Journal of Clinical Nutrition 53 (2): 537-541.

Suh, S. Y., Bae, W. K., Ahn, H. Y., Choi, S. E., Jung, G. C. and Yeom, C. H. 2012. Intravenous Vitamin C administration reduces fatigue in office workers: a double-blind randomized controlled trial. Nutrition Journal 11: 7

Suter, P. 1998. Potassium and hypertension. Nutrition Reviews 56 (5):151-153.

Ubbaonu, C. N., Onuegbo, N. C., Banigo, E. O. I. and Uzoma, A.2005. Physico-chemical changes in velvet tamarind (*Dialium guineense* wild) during fruit development and ripening. Nigerian Food Journal 23:133-138.

Vojdani, A., Bazargan, M., Vojdani, E. and Wright, J. 2000. New evidence for antioxidant properties of vitamin C. Cancer Detection and Prevention 24(6): 508-23.

Wardlaw, G., Smith, A.2012. Contemporary Nutrition, a functional approach. 3rd edn.Publisher: Mc Graw-Hiller Science /Engenering/ Math.

Whleton, P. K. and Cutler, J. A. 1997. Effects of oral potassium on blood presure: meta-analysis of randomized controled clinical trials. Journal of American Medical Association 277(20): 1624-1632.

Wu, L. 1968. Food composition table for use in Africa. FAO, Rome, Italy.

Young, D.B., Lin H. and McCabe R. D.1995. Potassium's cardiovascular protective mechanism. American Journal of Physiology 268(4 pt 2): 835-837.

Article 3

Ahodegnon k. Donatien, Gnansounou Martial, Bogninou G. S. Reine, Kanfon Estelle, Chabi Bienvenue, Agbangnan Dossa C. Pascal, Anago A. Eugénie, Ahoussi Edwige, Wotto Valentin and Sohounhloue C. K. Dominique. Biochemical Profile and Antioxidant Activity of Parkia biglobosa and Tamarindus indica Fruits Acclimated in Benin. Int. J. Adv. Res.2018 ; 6(11), 702-711.

La santé de l'enfant est une problématique récurrente en en Afrique subsaharienne. Les taux de mortalité infantile élevés [33] ne s'expliquent plus seulement par les maladies infectieuses. La malnutrition y concourt énormément [13]. Au Bénin, malgré sa mise en exergue depuis plusieurs décennies, elle demeure une préoccupation d'actualité [34]. Ainsi, selon l'Enquête Démographique et Sanitaire de 2006, plus de deux enfants sur cinq âgés de 0 à 5ans accuse un retard de croissance.

Les ressources naturelles du pays sont pourtant nombreuses et pourraient fournir des ingrédients pour la formulation de compléments alimentaires à coût réduit. Dans cette optique, ce travail à consister dans un premier temps à l'évaluation du pouvoir nutritionnel des fruits de Parkia biglobosa et Tamarindus indica. Ensuite, nous avons étudié le pouvoir antioxydant des extraits hydro éthanoliques des fruits.

Pour combattre de l'anémie ferriprive, ces fruits pourraient présenter un grand intérêt pour les enfants et les femmes en raison de leurs teneurs élevées en fer (56,75 ± 1,77 µg/g pour *P. biglobosa* et 38,10 ± 0,28 µg/g pour *T. indica*). La présence de vitamine C (0,32 mg/g pour le néré et 0,21 mg/g respectivement) favorise l'absorption du fer[35]. Cette étude est parfaitement en accord avec nos précédents travaux sur les fruits de *D. guineense willd.*

Le criblage phytochimique des deux fruits indique la présence majoritaire de tanins catéchiques, de flavonoïdes libres et d'anthraquinones. L'activité antiradicalaire des extraits a été déterminée par piégeage de radicaux libre (ici le DPPH) en se référant à la quercétine comme témoin. L'activité antiradicalaire des fruits est inférieure à celle de la quercétine et pourrait s'expliquer par leur faible teneur en composés phénoliques connus pour piéger les radicaux libres [36].

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RESEARCH ARTICLE

BIOCHEMICAL PROFILE AND ANTIOXIDANT ACTIVITY OF PARKIA BIGLOBOSA AND TAMARINDUS INDICA FRUITS ACCLIMATED IN BENIN.

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Abstract

This work allowed us to determine the biochemical profile and antioxidant potential of the fru it pulp of *Parkia biglobosa* and *Tamarindus indica* in order to better understand their nutritional and med icinal quality and their value. Following the determination of metabolites by phytochemical screening and determination of phenolic compounds, the antiradical capacity of the extracts was evaluated on the basis of their reactivity with a free radical, stable in solution, 1,1-DiPhenyl-2-Picry lHydrazyl (DPPH). Fro m our study, it appears that the pulps of these fruits are rich in vitamin C and minerals with high levels in the fruits of *Parkia biglobosa*. The fruit of *Tamarindus indica* has the highest protein content (05.39 ± 0.001%) while the fruit of *Parkia biglobosa* has the highest levels of total sugars (3.34 ± 0.01%) and lip ids (23.25 ± 0.01%). Phytochemical screening revealed the presence of tannins, flavonoids, anthraquinones, coumarins, mucilages and saponosides in our samples. The plant material investigated shows high levels of phenolic compounds (total phenol compounds, total flavonoids and condensed tannins) respectively 2.14 ± 0.01 mg EA G / g MS; 8.31 ± 0.57 mg EQ / g DM and 2.60 ± 0.28 mg EC / g DM for *Tamarindus indica* versus 2.00 ± 0.01 mg EA G / g DM , 6.16 ± 0.40 mg EQ / g DMand 0.51 ± 0.01 EC mg / g DMfor *Parkia biglobosa*. This work has provided us with data on the composition of fruit pulp for nutritional, d ietary and socio-economic uses.

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Introduction :

Selon les évaluations de l'organisation des Nation -Unies pour l'Alimentation et l'Agriculture (FA O), près de 795 millions de personnes, soit une personne sur neuf, sont considérées comme étant chroniquement sous -alimentées entre 2014 et 2015 (FA O, 2015). La malnutrition par carence en micronutriments « faim cachée » qui touche environ 2 milliards de personnes, soit plus de 30% de la population mondiale, a de lourdes conséquences sur la santé publique. Les minéraux ont une importance primordiale dans le métabolisme du corps humain, de sorte que leur carence aussi bien que leur excès sont tous deux nocifs (Hashmi *et al*., 2007). Les fruits sont connus comme d'excellentes sources de minéraux, de vitamines, de glucides, de composés phénoliques, ou d'antioxydants (Nahar *et al.,* 1990) et peuvent contribuer à une amélioration qualitative de la santé des populations (Kuhnlein, 1989). Au nombre des arbres fruitiers sauvages de l'Afrique tropicale figurent le *Parkia biglobosa* communément appelé néré en français puis ahwatin en fongbé (langue parlée au sud du Bénin) et le *Tamarindus indica* dont le nom français est tamarinier et jevivit in en fongbé (Humphry *et al*., 1993 ; Koné *et al*., 2002). D'une hauteur de 15 à 20 mètres, le néré est une plante pérenne dont la cime est étalée en forme de parasol et ses inflorescences ressemblent à de remarquables pompons rouges se détachant sur un fond de feuillage vert foncé. L'arbre offre des gousses passant successivement du vert au marron foncé agencées par grappes et suspendues à l'extrémité d'un long pédoncule. A l'intérieur de chaque gousse sont logées des graines marron -claires entourées d'une pulpe jaune safran. D'une très grande longévité, le tamarinier, est originaire d'Afrique avec une hauteur de 20 à 25 m. C'est un arbre au tronc large mais court ; d'un port dense qui affiche un feuillage à grandes feuilles persistantes. Ses fruits comestibles sont des gousses bosselées larges et épaisses qui abritent en leur sein des graines enrobées d'une pulpe fibreuse (Akoègninou *et al.,* 2006). Ces arbres ou arbustes sauvages ou semi-sauvages sont menacés par la déforestation en vue d'emblaver de grandes surfaces pour l'agriculture et par une urbanisation galopante (Herzog et *al*., 1994). Cette pression conduit à l'amenuisement des sources de nutriments avec un impact négatif sur la biodiversité du continent d'où la nécessité de corroborer leur importance alimentaire historique et médicinale par l'étude de leurs compositions.

De nombreuses propriétés médicinales et nutritionnelles sont attribuées aux racines, feuilles, écorces et fruits (graines et pulpe) de *Parkia biglobosa* et *Tamarindus indica.* Certaines de ces propriétés ont été abordées dans des études antérieures (Essien *et al*., 2007 ; David *et al*., 2011), mais peu d'informations ont été rapportées dans la littérature sur la composition de la pulpe du néré et du tamarinier au Bénin où cette plante est en danger (Meregini, 2005). C'est dans ce contexte que cette étude a été initiée et a pour objectif de déterminer le profil biochimique et le potentiel antioxydant de la pulpe des fruits de ces deux plantes en vue d'une meilleure connaissance de leurs qualités nutritionnelle et médicinale afin de contribuer à leur valorisation.

Matériel et Méthodes :

Matériel

Le matériel végétal est constitué de la pulpe des fruits de *Parkia biglobosa* et *Tamarindus indica* récoltés au stade de maturité à Cobly -centre dans le Département de l'Atacora. Plusieurs échantillonnages ont été réalisés à quelques semaines d'intervalle. Les fruits ont ensuite été conservés au laboratoire à une température de 25°C. Ces fruits ont été débarrassés de leurs péricarpes et de leurs graines et les analyses ont porté sur la pulpe.

Méthodes

Analyse nutritionnelle

La teneur en eau et en composés volatils :

Elle a été déterminée suivant la méthode de l'AOAC, (2003) par séchage à l'étuve à 105°C de 100 g de pulpe de chaque échantillon suivi de leur refroidissement au dessiccateur après 90 minutes d'étuvage. La teneur en eau a été déterminée par la formule suivante :

$$TE\ (\%) = 100 \times [m_{éch}-(m_f-m_0) / m_{éch}].$$

TE :

Teneur en Eau et composés volatils ; $m_{éch}$: masse de l'échantillon ; m_f : masse finale ; m_0 : masse initiale. Les cendres (résidus obtenus après incinération) ont été obtenues par carbonisation puis incinération de 25 g de pulpe de chaque échantillon au four (Nabertherm C 290) à 600°C pendant 6 heures dans des creusets adaptés, (AOAC, 2003). La teneur en cendres a été calculée par la formule suivante :

$$TCB\ \% = 100 \times [(m_1-m_0) / m_{éch}]$$

TCB: Teneur en Cendres Brutes; $m_{éch}$: masse de l'échantillon (g); m_1: masse du creuset contenant les cendres (g); m_0: masse du creuset vide (g).

Détermination de la composition minérale :

La cendre obtenue après incinération a été digérée dans l'HNO_3 et dans l'HCl puis filtrée. Le calcium et le magnésium ont été déterminés par complexométrie à l'EDTA (Ethylene DiamineTetra acetic Acid) tandis que l'iode et le fer ont été quantifiés par spectrophotométrie UV-Visible. Les longueurs d'onde utilisées ont été respectivement pour l'iode et le fer 530 n m et 510 n m. Les ions potassium et sodium ont été dosés par spectrométrie d'absorption atomique à flamme sur un appareil Varian Spectr AA -110. Ces dosages ont été fait conformément à la norme NFT 90-016 d'Août 1984.

Les protéines ont été déterminées par la méthode de Kjeldahl (AOAC 2003), tandis que les teneurs en lipides ont été déterminées par la méthode de l'AOA C, (2003). La méthode de Dubois *et al.,* (1956) (Dansi *et al*.,2008) utilisant le phénol et l'acide sulfurique concentré a été utilisée pour le dosage des sucres totaux ; les densités optiques sont lues au spectrophotomètre (Jenway RS 252) à 485 n m.

La vitamine C quant à elle a été dosée par iodométrie indirecte en présence de l'empois d'amidon.

Criblage phytochimique

Il consiste à réaliser une analyse chimique qualitative basée sur des réactions de coloration ou de précipitation plus ou moins spécifiques à chaque classe de principes actifs. Les groupes chimiques recherchés sont entre autres : les alcaloïdes, les composés phénoliques (flavonoïdes, anthocyanes, tanins, …), les saponosides, les coumarines, les mucilages, les sucres réducteurs.

Flavonoïdes :

L'identification des flavonoïdes a été réalisée par le test à la cyanidine (Bruneton, 1999).

Tanins :

Les tanins ont été mis en exergue par le test de Stiasny (Soro *et al*., 2009).

Saponosides :

Les saponosides ont été déterminés par le test de mousse ; degré de dilution d'un décocté aqueux donnant une mousse persistante après agitation (Bruneton, 1993 ; Dohou etal.,2003).

Composés phénoliques :

La détermination des composés phénoliques a été faite par la réaction au perchlorure ferrique (Bru neton, 1993).

Alcaloïdes :

Ils ont été identifiés par le test de Mayer et confirmés par le test de Bouchardat (N'Guessan *et al*, 2009).

Anthraquinones :

Les anthraquinones ont été identifiées par le test de Borntrager (Dohou *et al*., 2003 ; Rizk, 1982).

Mucilages :

L'obtention d'un précipité floconneux à partir d'un décocté mis en présence d'éther éthylique indique la présence des mucilages (Traoré, 2010).

Coumarines :

Elles ont été mises en évidence par la fluorescence à l'UV à 365n m (Soro *et al*., 2009).

Préparation de l'extrait hydroéthanolique

Pour quantifier les composés phénoliques, l'extraction solide -liquide a été réalisée par macération ; le solvant utilisé est un mélange d'eau et d'éthanol (v/v : 50/50). 10 g du matériel végétal séché ont été pesés puis mélangés avec un volume de 100 mL du solvant. Le mélange est maintenu sous agitation magnétique pendant 24 heures à température ambiante. La solution obtenue est ensuite filtrée sur papier filtre (Wattman N°1 de diamètre 0,16 mm) sous vide. Le filtrat a été ensuite récupéré et l'opération a été répétée 3 fois (soit 72 heures d'extraction au total) mais avec 50

mL du solvant dès le deuxième jour. Le volume total du filtrat est concentré sous vide à 60°C à l'aide d'un évaporateur rotatif de type Heidolph. L'extrait sec a été ensuite récupéré, pesé, étiqueté et conservé à +4°C jusqu'à l'utilisation. Le rendement (R) d'extraction est calculé par la formule ci -dessous :

Dosage des composés phénoliques

L'extrait hydroéthanolique a été soumis au dosage colorimétrique par spectrophotométrie UV-Visib le pour quantifier les composés phénoliques.

Composés phénoliques totaux

Ils ont été dosés au réactif de Folin-Ciocalteu (Wong *et al*., 2006 ; Siddhuraju *et al*., 2002). Le réactif de Folin utilisé est constitué d'un mélange d'acide phosphotungstique et phosphomolybdique qui est réduit, lors de l'oxydation des phénols en mélange d'oxydes bleus de tungstène et de molybdène (Ribereau -Gayon, 1968). L'absorbance a été mesurée au spectrophotomètre (JENWA Y 50/ 60 Hz) à 765 n m. L'acide gallique a été utilisé comme référence et la teneur en composés phénoliques totaux dans l'extrait a été exprimée en mg équivalent d'acide gallique par gramme de matière sèche.

Flavonoïdes totaux

Ils ont été quantifiés par la méthode du trichlorure d'aluminium ($AlCl_3$). Cette technique est basée sur la formation du complexe flavonoïde-aluminium qui a une absorption maximale à 500 n m (Enujiugha, 2010 ; Agbangnan et al., 2012).

Tanins condensés

Le dosage des tanins condensés a été réalisé par la méthode à la vanilline sulfurique (Agbangnan *et al*., 2012 ; Xu *et al*., 2007). Le principe de ce dosage est basé sur la fixation du groupe aldéhydique de la vanilline sur le carbone en position 6 du cycle A de la catéchine pour former un complexe chromophore de couleur rouge qui absorbe à 510 nm.

Evaluation de l'activité antiradicalaire

L'activité antiradicalaire a été évaluée suivant la méthode au DPPH (Dohou *et al*., 2003). Le principe de cette méthode est basé sur la mesure du pourcentage de piégeage des radicaux libres d'une solution de DPPH. Ce piégeage est visualisé par la disparition de la couleur violette du DPPH. Les cuves sont laissées à l'obscurité pendant une heure et les absorbances ont été mesurées à 517 n m (Brand -Willams *et al*., 1995 ; Agbangnan *et al*., 2013). Le pourcentage de piégeage a été calculé suivant la formule :

$$P = [(A_{bl} - A_{éch})/A_{bl}] \times 100.$$

3. Pourcentage de piégeage;

A_{bl}: Absorbance du blanc;

$A_{éch}$: Absorbance de l'échantillon.

Résultats et Discussion :

Analyse nutritionnelle

Les résultats de l'analyse nutritionnelle (tableau x 1 et 2) de la pulpe des fruits de *Parkia biglobosa* et *Tamarindus indica* montrent que l'eau, les macronutriments (lipides, protéines et sucres totaux) et les micronutriments (vitamine C, Ca, Mg, Fe, I, K, Na) sont présents dans les fruits investigués.

Ainsi les résultats obtenus révèlent de faibles teneurs en eau au niveau de la pulpe des fruits de *Parkia biglobosa* (7,17 ± 0,14) % et *Tamarindus indica* (5,09 ± 0,14) %. Cette faible teneur en eau constatée permet une meilleure conservation de ces fruits. Les pulpes des fruits objet de la présente étude ne sauraient être considérées comme rafraîchissantes en comparaison avec ceux consommés couramment (mangues, oranges, ananas, bananes…) (Arogba *et al*., 1994 ; Ubbaonu *et al*., 2005 ; Odhav *et al*., 2007).

Les teneurs en lipides, protéines et sucres totaux de la pulpe des fruits de *Parkia biglobosa* et *Tamarindus indica* sont reportées dans le tableau 1.

Tableau 1: Teneurs en lipides, protéines et sucres totaux de la pulpe des fruits de *Parkia biglobosa* et *Tamarindus indica*

	P. biglobosa	*T. indica*
Teneur en lipides (%)	23,25 ± 0,01	17,02 ± 0,01
Teneur en protéines (%)	04,20±0,14	05,39± 0,001
Teneur en sucres totaux (%)	03,34 ± 0,01	01,37 ± 0,01

Le tableau 1 montre que la pulpe du fruit de *Parkia biglobosa* a les plus fortes teneurs en lipides et sucres totaux comparativement à la pulpe de fruit de *Tamarindus indica* qui est plus riche en protéines.

Des variations considérables sont observées dans la composition en macronutriments des différents fruits en raison de leur origine biologique. Ces variations sont le fait de facteurs endogènes (degré de maturité, influence génétique) ou exogènes (température, ensoleillement, nature des sols) (Paul *et al*., 1988). Cependant, ces résultats sont proches de ceux publiés par Samina *et al.,* (2008) Canuto *et al*., (2010), Compaoré *et al*., (2011), Omojola *et al.,* (2011), Omojola *et al*., (2011) sur *Tamarindus indica* et *Parkia biglobosa.*

Les teneurs en vitamine C et minéraux sont reportées dans le tableau 2.

Tableau 2 : Teneurs en vitamine C et minéraux

	P. biglobosa	*T. indica*
Teneur en vitamine C (mg/g)	00,32 ± 0,01	00,21 ± 0,01
Teneur en calcium (mg/g)	02,01 ± 0,01	06,41 ± 0,01
Teneur en magnésium (mg/g)	01,21 ± 0,01	00,49 ± 0,01
Teneur en fer (µg/g)	56,75 ± 1,77	38,10 ± 0,28
Teneur en iode (µg/g)	00,30 ± 0,01	03,90 ± 0,14
Teneur en potassium (g/L)	175,60 ± 0,01	159,40 ± 0,01
Teneur en sodium (mg/L)	00,90 ± 0,01	00,80 ± 0,01

Les résultats du tableau 2 révèlent que les pulpes de ces deux fruits renferment toutes, de la vitamine C et des minéraux (Ca, Mg, Fe, I, K, Na). La pulpe des fruits de *Parkia biglobosa* contient majoritairement la vitamine C, le magnésium, le fer, le potassium et le sodium tandis que la pulpe des fruits de *Tamarindus indica* contient majoritairement le calcium et l'iode.

Les teneurs en vitamine C de nos échantillons (0,32 mg/g pour le néré et 0,21 mg/g pour le tamarin) étant largement supérieures à celles rapportées dans la littérature (Campos *et al*., 2009 ; ICRAF, 2007), ceci place les fruits de *Parkia biglobosa* et *Tamarindus indica* au rang des bonnes sources de vitamine C (Omo jola *et al.,* 2011). L'importance de cette vitamine réside entre-autre dans son pouvoir antioxydant inhibant l'effet néfaste des radicaux libres (Vojdani *et al*., 1999 ; Laight *et al*., 2000). Elle est indispensable à l'absorption du fer, à la réparation tissulaire et la formation des vaisseaux sanguins via la synthèse de collagène.

Les teneurs en calcium de nos échantillons (néré : 02,01 ± 0,01mg/g ; tamarinier : 06,41 ± 0,01mg/g) sont légèrement supérieures à celles obtenues par l'équipe de Omojola en 2011(néré : 01,16 ± 0,005 mg/g; tamarin : 04,65 ± 0,04 mg/g) (Almeida et al., 2009; Omojola *et al.,* 2011). Les taux de magnésium obtenus au terme de cette étude sont inférieurs à ceux d 'Almeida *et al.,* (2009). Compte tenu de sa participation à l'ossification, à la contraction musculaire et à la coagulation du sang (Trumbo *et al*., 2001)**,**

le calcium occupe une place essentielle dans la diète humaine. Son AJR est de 1 g par jour chez l'adulte. Le magnésium quant à lui coopère avec le calcium à la contraction musculaire et à la coagulation sanguine (Brandon *et al*.,1996).

La pulpe des fruits analysés présente un grand intérêt pour les enfants et les femmes en raison de leurs teneurs en fer qui est de (56,75 ± 1,77 µg/g) pour *Parkia biglobosa* et (38,10 ± 0,28 µg/g) pour *Tamarindus indica* ; valeurs relativement proches de celles trouvées par l'équipe de Almeida (2009) (néré : 58,96 ± 0,90 µg/g et tamarin : 39,03 6.0,17 µg/g). Ces fruits peuvent aider à la prévention de l'anémie ferriprive. L'absorption du fer contenu dans les pulpes est plus efficace en raison de la présence de vitamine C (Hallberg *et a*l., 1987 ; Siegenberg *et al.,* 1991).

Les valeurs obtenues pour le sodium et le potassium sont respectivement de (0,90±0,01 mg/g et 175,60 ± 0,14 mg/g) pour *Parkia biglobosa* puis (0,80 ± 0,01 mg/g et 159,40 ± 0,14 mg/g) pour *Tamarindus indica*. Ces résultats sont inférieurs à ceux publiés par Omo jola *et al.,* (2011) pour ce qui concerne les teneurs en potassium (tamarin : 72,03 ± 1,30 mg/g et néré : 90,77 ± 1,32 mg/g) (Almeida *et al*., 2009 ; Omo jola *et al*., 2011).

Outre les conditions biologiques endogènes et exogènes qui expliquent les divergences de teneurs des échantillons, les méthodes d'analyses utilisées pourraient influer sur les différents résultats.

Criblage Phytochimique

Les résultats du criblage phytochimique de la pulpe des fruits de *Parkia biglobosa* et *Tamarindus indica* sont reportés dans le tableau 3.

Tableau 3 : Métabolites identifiés dans les fruits de Parkia biglobosa et Tamarindus indica

Familles de composés recherchées		***Parkia biglobosa***	***Tamarindus indica***
Tanins	Tanins catéchiques	+	+
	Tanins galliques	-	-
Flavonoïdes	Anthocyanes	-	-
	Leucoanthocyanes	+	+
	Flavonoïdes libres	+	+
Mucilages		-	+
Alcaloïdes		-	-
Sucres réducteurs		-	-
Quinones libres		-	-
Anthraquinones combinés	O – hétérosides	-	+
	O – hétérosides à génine réduite	+	-
	C – hétérosides	-	-
Coumarines		-	+
Saponosides		-	+

- : absence, + : présence

Le criblage phytochimique de la pulpe des deux fruits (*Parkia biglobosa* et *Tamarindus indica*), indique majoritairement la présence des tanins catéchiques, des flavonoïdes libres et des anthraquinones. Par contre, les anthocyanes, les alcaloïdes, les sucres réducteurs, les quinones libres, sont absents dans la pulpe de ces deux fruits. Ces résultats sont en accord avec ceux de Pietta *et al.,* (2001) et De Caluwé *et al.,* (2010) qui ont montrés respectivement que la pulpe de *Parkia biglobosa* comporte des tanins et celle de *Tamarindus indica* renferme également des tanins et des mucilages. De même, les résultats de Robarks *et al*., (1999) et de Bhadoriya *et al.* (Bhadoriya *et al*., (2012) signalent respectivement la présence de flavonoïdes dans les fruits du néré et du tamarin et la présence de mucilage et d'anthraquinones dans la pulpe du fruit de *Tamarindus indica.* Ces résultats sont confirmés par Udoli *et al*., (2008) selon qui la pulpe de *Parkia biglobosa* contient en plus de ceux cités par les auteurs précédents, les anthraquinones. De même, Paula *et al*., ont révélé la présence des coumarines et saponosides dans la pulpe du fruit de *Tamarindus indica* (Paula *et al*., 2009).

Dosage des composés phénoliques

Le tableau 4 présente les teneurs en composés phénoliques des extraits hydroéthanoliques de la pulpe des fruits de *Parkia biglobosa* et *Tamarindus indica*.

On note une forte teneur en flavonoïdes totaux (6,16 mg/g) suivie d'une teneur moyenne en composés phénoliques totaux (2,00 mg/g) et une teneur relativement faible en tanins condensés (0,51 mg/g) dans l'extrait hydroéthanolique de la pulpe de *Parkia biglobosa*. Ces résultats sont confirmés par les travaux de Marinova *et al*., (2005) et ceux de Meda *et al.,* (2005).

L'extrait hydroéthanolique de *Tamarindus indica* par contre est majoritairement riche en FVT (8,31 mg/g) suivi des TC (2,60 mg/g) et des CPT (2,14 mg/g). Ces résultats sont en accord avec ceux de Sudjaroen *et al.* (2005) ; Quan *et al*.,., (2007) et Ayoola *et al.,* (2008) qui ont identifiés la présence des FVT en forte proportion dans la pulpe de *Tamarindus indica* suivi des TC et des CPT.

Tableau 4 : Teneurs en composés phénoliques des extraits hydroéthanoliques de la pulpe des fruits de Parkia biglobosa et Tamarindus indica.

Echantillon (pulpe)	Composés phénoliques totaux (mg/g)	Flavonoïdes totaux (mg/g)	Tanins condensés (mg/g)
Parkia biglobosa	2,00	6,16	0,51
Tamarindus indica	2,14	8,31	2,60

Activité antiradicalaire

L'activité antiradicalaire des extraits a été déterminée en se référant à la quercétine, un antioxydant de référence (CI_{50} = 0,1 mg/mL). Tous les extraits ont présenté un pouvoir antiradicalaire plus faible que celui du composé de référence (Tableau5). Il ressort donc de l'analyse de ce tableau que les extraits hydroéthanoliques des échantillons sont moins actifs que la quercétine. Ceci pourrait s'expliquer par les faibles teneurs en composés phénoliques détectées dans les extraits hydroéthanoliques des échantillons. En effet Diallo a révélé en 2005 que les composés phénoliques sont des piégeurs de radicaux libres (Diallo, 2005). Ces résultats sont en accord avec ceux de Compaoré *et al.,* (2011) et Lim *et al,.* (2007).

Tableau 5 : Activité antiradicalaire des extraits hydroéthanoliques de la pulpe des fruits de P. biglobosa et T. indica

Echantillon	CI_{50} (mg/mL)	PAR
Parkia biglobosa	18	0,05
Tamarindus indica	15	0,06
Quercétine	0,1	10

PAR (Pouvoir antiradicalaire) = 1/ CI_{50}.

Conclusion :

Le présent travail qui a consisté en l'investigation de la valeur nutritionnelle et médicinale de la pulpe des fruits de *Parkia biglobosa* et *Tamarindus indica* acclimatés au Bénin a permis de constater leurs richesses en divers métabolites tant primaires que secondaires. Cette étude a révélé des teneurs très intéressantes aussi bien en minéraux (calcium, sodium, magnésium, potassium, iode et fer) qu'en sucres permettant de palier aux problèmes de déficience minérale et d'hypoglycémie.

Il ressort également de cette étude que les pulpes des deux fruits renferment toutes, des tanins catéchiques, des flavonoïdes libres et des leucoanthocyanes.

Ces fruits sont donc recommandés dans les régimes alimentaires en vue de lutter contre la malnutrition et dans la prise en charge de certaines maladies (maladies cardiovasculaires, certains types de cancer etc.)

References :

21. Agbangnan P., Noudogbessi P. J., Chrostowska C. A., Tachon A. C., Fouquet E. and Sohounhloue K. C. D. (2013): "Phenolic compound of benin's red *sorghum* and their antioxidant properties", *Asian J. Pharm Clin. Res*., vol. 6, no. 2, pp. 277-280.

38. Agbangnan P., Tachon C., Bon in C., Chrostowka A., Fouquet E. and Sohounhloue K. C. D. (2012): "phytochemical study of a tinctorial plant of benin traditional pharmacopoeia: The red sorghum (*Sorghum caudatum*) of Benin,"*Scientific Study & Research,* vol. 13, no. 2, pp. 121-135.
39. Akoègninou A., van der Burg J. W. and van der Maesen G. J. L. (2006): "Flore analytique du Benin. Cotonou and Leiden", *Backhuys Publisher*.
40. Almeida B. M. M., Sousa P. H. M. D., Fonseca M. L., Magalhães C. E. C., Lopes M. D. F. G. and Lemos T. L. G. D. (2009): "Evaluation of macro and micro -mineral content in tropical fruits cultivated in the northeast of Brazil"*Food Science and Technology (Campinas),* vol. 29, no. 3, pp.581-586.
41. Arogba S. S., A jiboro A. A. and Odukwe I. J. (1994): "A physico -chemical study of nigerian velvet tamarind (*Dialium guineense L*) fru it", *Journal of the Science of Food and Agriculture*, vol. 66, no. 4, pp. 533-534.
42. Ayoola A. G., Fo lawewo A. D., Adesegun S. A., Abioro O. O., Adepoju -Bello A. A. and Coker H. A. B. (2008): "Phytochemical and antio xidant screening of some p lants of Apocynaceae from South West Nigeria", *African Journal of Plant Science*, vol. 2, no. 10, pp. 124-128.
43. Bhadoriya S. S., M ishra V., Raut S., Ganeshpurkar A. and Jain S. K. (2012): "Anti-inflammatory and antinociceptive activities of a hydroethanolic ext ract of *Tamarindus indica* leaves", *Scientia pharmaceutica*, vol. 80, no. 3, 685.
44. Brandon N. A. and Hill D. R.(1996): List of books and journals in allied health, *Bulletin of the Medical Library Association*, vol. 84, no. 3, pp. 289.
45. Brand-Willams W., Cuvelier E. M. and Beret E. M. (1995): "Use of a free radical method to evaluate antioxidant activity," *Lebensm. Wiss.U. Technol*, pp. 25-30.
46. Bruneton J. (1999):Pharmacognosie, Phytochimie, Plantes medicinales, *Technique & Documentation, 3ème éd.* Lavoisier, Paris.
47. Bruneton J. (1993): Pharmacognosie, phytochimie, Plantes médicinales, *Tec et Doc., 2e éd.*Lavoisier, Paris, pp. 915.
48. Campos M. F., Ribeiro S. M. R., Della Lucia C. M., Pinheiro -Sant'Ana H. M. and Stringheta P. C. (2009): "Optimization of methodology to analyze ascorbic and dehydroascorbic acid in vegetables", *Química Nova*, vol. 32, no. 1, pp. 87-91.
49. Canuto B. A. G., Xav ier O. A. A., Neves L. C. and Benassi M. D. T. (2010): "Caracterização físico -química de polpas de frutos da Amazônia e sua correlação com a atividade anti-radical livre" . *Revista Brasileira de Fruticultura*, vol. 32, no. 4, pp. 1196-1205.
50. Co mpaoré R. W., Nikièma P. A., Bassolé H. I. N., Savadogo A. and Mouecoucou J. (2011): "Chemical composition and antioxidative properties of seeds of *Moringa oleifera* and pulps of *Parkia biglobosa* and *Adansonia digitata* commonly used in food fortification in Burkina Faso", *Current Research Journal of Biological Sciences*, vol.3, no.1, pp. 64-72.
51. Dansi A., Adjatin A., Adoukonou-Sagbadja H., Faladé V., Yedo monhan H., Odou D. and Dossou B. (2008): "Traditional leafy vegetables and their use in the Benin Republic", *Genetic Resources and Crop Evolution*, vol.55, no. 8, pp. 1239-1256.
52. David A. A., Olaniy i T. A., Mayowa O. A., Olayinka A. A. and Anthony I. O. (2011): "Anti-Vib rio and preliminary phytochemical characteristics of crude methanolic extracts of the leaves of *Dialium guineense* (Wild)", *Journal of Medicinal Plants Research,* vol. 5, no. 11, pp. 2398-2404.
53. De Caluwé E., Halamová K. and Van Damme P. (2010): "*Tamarindus indicaL.*: a review of traditional uses, phytochemistry and pharmacology", *Afrika focus*, vol. 23, no. 1, pp. 53-83.
54. Diallo M. A. (2005): Etude des plantes médicinales de niafunke (région Tombouctou) Phytochimie et pharmacologie de *Maerua crassifolia Forsk* (Capparidacée) [These de Doctorat]. Université de Bamako. p 125.
55. Dohou N., Yamn i K., Tahrouch S., Hassani L. M. I., Bodoc A. and GmiraN. (2003):"Screening phytochimique d'une endemique Ibero-marocain, *Thymelaea lytroides*," *Bull. Soc. Pharm.* Bordeau x, pp. 61-78.
56. Enujiugha N. V. (2010):"The Antioxidant and Free Rad ical -Scavenging Capacity of Phenolics fro m African Locust Bean Seeds (*Parkia biglobosa*)," *Advances in Food Sciences*, vol. 32, no. 2, pp. 88-93.
57. Essien E., Ogunwande A. I., Ogunbinu O. A., Flamin i G. and Cioni L. P. (2007): "Analysis of the essential oil of *Dialium guineense Willd"*,*Journal of Essential Oil Research, vol.* 19, no. 6, pp. 545-547.
58. FAO (2015): "The state of food insecurity in the world", *Rome, ITALY*.
59. Hallberg L., Brune M. and Rossander-Hulthén A. N. E. L. (1987) "Is there a physiological role of vitamin C in iron absorption" *Annals of the New York Academy of Sciences*, vol. 498, no. 1, pp. 324-332.
60. Hashmi R. D., Ismail S. and Shaikh H. G. (2007): "Assessment of the level of trace metals in co mmon ly edible vegetables locally availab le in the markets of Karach i city", *Pakistan Journal of Botany, vol. 39, no. 3, pp.* 747-751.

25. Herzog F., Farah Z. and A mado R.(1994): “Co mposition and consumption of gathered wild fru its in the

52. Hu mphry M. C., Clegg S. M., Keen L. C., and Grivetti E. L. (1993): “Food diversity and drought survival. The Hausa examp le”,*International Journal of Food Sciences and Nutrition* , vol. 44, no. 1, pp. 1-16.
53. ICRAF, World Agroforestry Centre, ICRAF Agroforestry Tree Database (visited on 31.01.2007): *Ta marindus indica L*., URL http:// www.worldagroforestrycentre.org.
54. Koné M., Atindehou K., Téré H. and Traoré D. (2002):Quelques plantes médicinales utilisées en pédiatrie traditionnelle dans la région de Ferkessédougou (Côte d’Ivoire). *Bioterre*, 30, pp. 30-36.
55. Kuhnlein V. H. (1989): “Nutrient values in indigenous wild berries used by the Nu xalk people of Bela Coola, British Colo mbia”, *Journal of Food Composition and Analysis*, 2, pp. 28-36.
30. Laight D. W., Carrier M. J. and Änggård M. J. (2000): “Antio xidants , diabetes and endothelial dysfunction”, *Cardiovascular research*, vol. 47, no. 3, pp. 457-464.
67. Lim Y. Y., Lim T. T. and Tee J. J. (2007): “Antio xidant properties of several tropical fruits: A comparative study”, *Food chemistry*, vol. 103, no. 3, pp.1003-1008.
68. Marinova D., Ribarova F. and Atanassova M. (2005): “Total phenolics and total flavonoids in Bulgarian fruits and vegetables“, *Journal of the university of chemical technology and metallurgy* , vol. 40, no. 3, pp. 255-260.
69. Meda A., Lamien C. E., Ro mito M., M illogo J. and Nacoulma O. G. (2005): “Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their rad ical scavenging activity”, *Food chemistry*, vol. 91, no. 3, pp. 571-577.
70. Meregini O. A. (2005): “So me endangered plants producing edible fruits and seed in southeastern Nigeria”, *Fruit*, 60, pp. 211-220.
71. N’Guessan K., Kad ja B., Zirih i G. N., Traore D. and Ake-Assi L. (2009): "Screening phytochimique de quelques plantes médicinales ivoiriennes utilisées en pays Krobou (Agboville, Côte-d’Ivoire)," *Sciences & Nature*, vol. 6, no. 1, pp. 1-15.
72. Nahar N., Rah man N. and Mosiihuzzaman M. (1990): “Analysis of carbohydrates in seven edible fru it of Bengladesh”, *Journal of Science of Food and Agriculture*, 51, pp. 185-192.
73. Odhav B., Beekru m S., Akula U. S. and Baijnath H. (2007): “Preliminary assessment of nutritional value of traditional leafy vegetables in KwaZulu -Natal, South Africa”, *Journal of Food Composition and Analysis*, vol. 20, no. 5, pp. 430-435.
74. Omojo la O. M., Afolayan M. O., Adebiyi A. B., Orijajogun J. O., Tho mas S. A. and Ihegwuagu N. E. (2011): “Further physicochemical characterizat ion of *Parkia biglobosa* (Jacq.) Benth fruit pulp as a mineral supplement”, *African Journal of Biotechnology*, vol. 10, no. 75, pp. 17285-17264.
75. Paul A. A. and D. A. T. (1988): “Southgate In McCance and Widdowson’s The composition of foods”, *Elsevier, North Holland Biomedical Press*, London, pp 418.
76. Paula S. F., Kabeya L. M., Kanashiro A., de Figueiredo A. S., A. Azzolini E. C., Uyemura S. A. and Lucisano - Valim Y. M. (2009): “Modulation of human neutrophil oxidative metabolism and degranulation by extract of *Tamarindus indica L*. fru it pulp”, *Food and Chemical Toxicology*, vol. 47, no. 1, pp. 163-170.
77. Quan P. T., Hang T. V., Ha N. H. and Giang B. L. (2007): “Total polyphenols, total catechins content and DPPH free radical scavenger activity of several types of Vietnam co mmercial green tea”, *Science & Technology Development*, vol. 10, no. 10.
78. Ribereau-Gayon P. (1968): Les co mposés phénoliques des végétaux, *Editions Dunod*, Paris, pp. 254.
79. Rice-Evans C. (2001): “Flavonoid antio xidants”, *Current medicinal chemistry*, vol. 8, no. 7, pp. 797-807.
80. Rizk M. A. (1982):"Constituents of plants growing in Qatar," *Fitoterapia*, vol. 52, no. 2, pp. 35-42.
81. Robards K., Pren zler P. D., Tucker G., Swatsitang P. and Glover W. (1999): “Phenolic co mpounds and their role in o xidative processes in fruits”, *Food chemistry*, vol. 66, no. 4, pp. 401-436.
82. Siddhuraju S. P. P., Mohan P. S., and Becker K. (2002): "Studies on the Antioxidant Activity of Indian Laburnu m (*Cassia fistula* L.), a Preliminary Assessment of Crude Ext racts from Stem Bark, Leaves, Flowers and Fruit Pu lp," *Journal of Food Chemistry*, vol. 79, no. 1, pp. 61-67.
83. Siegenberg D., Baynes R. D., Bothwell T. H., Macfarlane B. J., Lamparelli R. D., Car N. G. and Mayet F. (1991): “Ascorbic acid prevents the dose-dependent inhibitory effects of polyphenols and phytates on nonheme-iron absorption”, *The American journal of clinical nutrition* , vol. 53, no. 2, pp. 537-541.
84. Soro Y. T., Traore F., Datte Y. J. and Nene-Bi S. A. (2009): "Activité antipyrétique de l’extrait aqueux de *Ximenia americana*," *Phytothérapie*, pp. 297-303.
85. Sudjaroen Y., Haubner R., Würtele G., Hull W. E., Erben G., Sp iegelhalder B. and Owen R. W. (2005): “Isolation and structure elucidation of phenolic antio xidants fro m Tamarind (*Tamarindus indica L*.) seeds and pericarp”, *Food and Chemical Toxicology*, vol. 43, no. 11, pp. 1673-1682.

79. Traoré F. (2010):"Proposition de formulation d'un sirop antipaludique à base de *Argemone mexicana L. papaveraceae*", Thèse de doctorat, Faculté de Médecine, de Pharmacie et d'Odonto -Stomatologie du Mali, Université de Bamako, pp. 95.
80. Tru mbo P., Yates, S. Schlicker A. A. and Poos M. (2001): "Dietary reference intakes: vitamin A, vitamin K, arsenic, boron, chromiu m, copper, iodine, iron, manganese, mo lybdenum, nickel, silicon, vanadiu m, and zinc", *Journal of the American Dietetic Association,* vol. 101, no. 3, pp.294-301.
81. Ubbaonu N. C., Onuegbu N. C., Ban igo E. O. I. and Uzo ma A. (2005): "Physico -chemical changes in velvet tamarind (*Dialium guineense Wild*) during fru it develop ment and ripening", *Nigerian Food Journal*, vol. 23, no. 1, pp. 133-138.
82. Udobi E. C., Onaolapo J. A. and Agunu A. (2008): "Antibacterial activit ies and bioactive components of the aqueous fraction of the stem bark of *Parkia bigblobosa* (JACQ) (Mimosaceae)", *Nigerian J. Pharm. Sc*, 7, pp. 49-55.
83. Vo jdani A., Bazargan M., Vo jdani E. and Wright J. (1999): "New ev idence for antio xidant properties of vitamin C", *Cancer detection and prevention*, vol. 24, no. 6, pp. 508-523.
84. Wong C. C., Li H. B., Cheng K. W. and Chen F. (2006):"A systematic survey of antioxidant ac tivity of 30 Chinese medicinal p lants using the ferric reducing antio xidant power assay," *Food Chem.*, 97, pp. 705-711.
85. Xu B. J. and Chang S. K. C. (2007): "A co mparative study on phenolic profiles and antioxidant activit ies of legumes as affected by extraction solvents," *Journal of Food Science*, vol. 72, no. 2, pp. 160-161.

Article 4

Senankpon Martial Gnansounou, Samy Iskandar, Lydia Abou, Maxime Robin, Carole DI Giorgio, Edwige Dahouenon Ahoussi, Philippe Piccerelle and Dominique Koko Sonhounhloue. GC-MS Screening and Evaluation of the Anti-inflammatory and Antioxidant Activities of Ethanolic Leaves and Stem Barks Extracts from *Dialium guineense* Willd*, Parkia biglobosa* (Jacq.) R. Br. ex Benth. and *Tamarindus indica* L. Journal of Pharmacognosy and Phytochemistry 2019; 8(1): 295-301.

Bien qu'étant un processus naturel, le vieillissement peut être accéléré par le stress oxydatif. Ce dernier est étroitement lié aux processus inflammatoires [37,38]. Induite par les infections microbiennes, les allergènes, les radiations, les xénobiotiques etc. [39,40] la réponse inflammatoire peut être aiguë ou chronique. Généralement bénéfique pour l'hôte, l'inflammation aiguë est une étape initiale médiée par l'activation du système immunitaire inné. Si elle dure longtemps, la deuxième étape de l'inflammation ou inflammation chronique s'installe et peut conduire à diverses pathologies chroniques, y compris le cancer [41]. En effet, les leucocytes recrutés sur le site de la lésion induisent une stimulation du métabolisme oxydatif (cytochrome P450 des mitochondries, peroxysomes) d'où une libération et une accumulation accrue d'espèces réactives de l'oxygène [42,43]. La défense de l'organisme humain contre la toxicité de ces radicaux libres est assurée par des enzymes comme la superoxyde dismutase, la catalase et la glutathion peroxydase [44]. Pourtant, il arrive qu'un déséquilibre s'installe entre la production de radicaux libres et le système antioxydant de l'organisme en faveur des radicaux libres : c'est le stress oxydatif.

Les espèces réactives de l'oxygène ont normalement un rôle bénéfique comme messagers secondaires capables de réguler l'apoptose ou d'activer des facteurs de transcription. Elles ne deviennent toxiques que si leur concentration s'accroît à cause du stress oxydatif. Pour éviter le stress oxydatif, un apport suffisant d'antioxydants est donc nécessaire. Ces derniers agissent en piégeant les radicaux libres évitant ainsi l'oxydation de l'ADN, des lipides et des glucides.

Les inflammations et le stress oxydatif étant mis en cause dans des cas de diabète, de cancers ou de maladies cardiovasculaires [45,46,47], la recherche d'antioxydants et d'anti-inflammatoires dépasse désormais le cadre anti-âge ou cosmétique. En Afrique et dans de nombreux pays à revenu faible ou intermédiaire, les connaissances traditionnelles basées sur les plantes médicinales sont utilisées pour lutter contre le stress oxydatif et l'inflammation [48].
Dans un second temps, notre étude a donc porté sur les activités anti-inflammatoire et antioxydante des feuilles et écorces de *D. guineense* Willd*, P. biglobosa* (Jacq.) R. Br. ex Benth. et *T. indica* L. Nous avons ensuite, dans l'optique de formulation galénique à usage cutanée, estimé la phototoxicité des extraits et en avons évalué les compositions chimiques.

Le test KRL (Kit Radicaux Libres) fut utilisé pour déterminer l'activité antioxydante de nos extraits. Ce test permet d'évaluer la résistance globale du sang standardisé de cheval soumis à une attaque radicalaire. Les défenses antioxydantes intra- et extra-cellulaires contribuent au maintien de l'intégrité membranaire et des fonctions cellulaires jusqu'à la lyse des cellules

sanguines. Le KRT permet donc une mesure dynamique du potentiel global de défense antiradicalaire d'un individu. Il permet également de déterminer in vitro, dans des conditions biologiques, la capacité "antioxydante" ou l'action "pro-oxydante" d'un composé. Ainsi, en ajoutant au milieu un composé à action antiradicalaire, il y a augmentation du potentiel global de défense contre l'agression radicalaire d'un sang témoin (sang de cheval standardisé). Au contraire en ajoutant un composé à action pro-radicalaire, il y a diminution la capacité antiradicalaire globale du sang témoin. La résistance globale du sang témoin à l'attaque radicalaire en présence ou non du produit est exprimée par le temps au bout duquel 50% des cellules sanguines sont lysées (T1/2 en minutes). L'efficacité antiradicalaire des produits est alors exprimée en pourcentage du potentiel global de défense antiradicalaire du sang témoin (%T1/2 du sang témoin). Les résultats sont standardisés en équivalents Trolox (analogue hydrosoluble de la vitamine E) et/ou en équivalents Acide gallique (acide phénolique).

Le test anti-inflammatoire in vitro utilisé dans la présente étude repose sur la capacité des macrophages à générer une forte réponse inflammatoire lorsqu'ils sont stimulés par des antigènes. Des macrophages immortalisés de souris (lignée cellulaire RAW 264.7) ont été stimulés par une LPS (endotoxine) d'*Escherichia coli* et exposés aux extraits à tester pendant 24 heures. À la fin de la période d'incubation, la production de NO est évaluée indirectement en mesurant l'accumulation de nitrite / nitrate (produits finaux stables de l'oxydation du NO) dans le milieu de culture à l'aide d'une méthode spectrophotométrique basée sur la réaction de Griess. La dexaméthasone fut utilisée comme contrôle positif.

Parallèlement à l'évaluation de la libération de NO, la viabilité cellulaire a été mesurée pour valider le test. Le réactif colorant vital WST-1 a été utilisé pour mesurer la respiration mitochondriale des cellules.

Les extraits de *Dialium guineense* (feuilles et tige), des feuilles de *Parkia biglobosa* et des écorces de *Tamarindus indica* ont exercé une activité anti-inflammatoire supérieure à celle de la dexaméthasone. L'écorce de Dialium guineense a montré la meilleure activité anti-inflammatoire et contient du sitostérol et de l'acétate de lupéol, molécules sont connues pour leur activité anti-inflammatoire intéressante [49,50]. Ces molécules sont rapportées ici pour la première fois dans *D. guineense*. En synergie avec les composés phénoliques contenus dans cet extrait [20,51], elles pourraient expliquer nos résultats et confirmer la pertinence de l'utilisation de *Dialium guineense* par les guérisseurs traditionnels. Toutefois, la phototoxicité de cet extrait rend son utilisation hypothétique dans la formulation d'anti-inflammatoires à usage cutané. En revanche, les feuilles

de cette même plante ne sont pas photo-toxiques mais ont une activité anti-inflammatoire réduite de moitié par rapport aux écorces. Elles ont une teneur plus élevée en sitostérol (3,5%) et en acétate de lupéol (1,7%), ce qui semblent jouer un rôle clé dans la phototoxicité. En effet ces molécules sont également plus abondantes dans les feuilles de *P. biglobosa* (2,02% et 4,41%) et dans l'écorce de *T. indica* (0,80% et 1,53% respectivement).

Les résultats du test KRL (Kit Radicaux Libres) indiquent que tous les extraits testés ont une activité antiradicalaire dose- dépendante. À 20 mg / L, les feuilles de *Parkia biglobosa* ainsi que les feuilles et écorces de *Tamarindus indica* ont des résultats remarquables. Ils ont augmenté la résistance à une attaque radicalaire du sang témoin à 109.48, 265.36 et 117.55% respectivement. En outre, ces extraits se sont révélés tout au moins aussi efficaces que le Trolox et l'acide gallique. L'extrait d'écorce de *Tamarindus indica* est particulièrement intéressant. Cinq fois plus actif que le Trolox et 2,5 fois plus actif que l'acide gallique, il contient du lupéol (0,83%), de l'amyrine (0,65%) et de l'acétate de lupéol (1,53%). Ces molécules ont déjà été rapportées dans la plante[51] et leur propriété de piégeage des radicaux libres est bien connue [52,53,54].

L'extrait des feuilles de Parkia biglobosa a une activité antioxydante de 109,48% selon le test KRL. Il contient de la lupénone (2,59%), de la β-amyrine (1,05%), du sitostérol (2,02%) et de l'acétate de lupéol (4,41), tous connus pour leur activité anti-inflammatoire ou antioxydante. Le sitostérol et l'acétate de lupéol sont décrits ici pour la première fois dans la plante. Ils semblent contribuer aux activités anti-inflammatoire et antioxydante de cet extrait telles que rapportés par plusieurs auteurs [49,55].

Les composés chimiques identifiés par GC-MS dans les extraits l'ont été par comparaison directe (avec une correspondance d'au moins 80%) des temps de rétention et des données spectrales de masse avec ceux des bibliothèques de l'Institut national de la normalisation et de la technologie (NIST) et de WILLEY.

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GC-MS screening and evaluation of the anti-inflammatory and antioxidant activities of ethanolic leaves and stem barks extracts from *Dialium guineense* Willd*, Parkia biglobosa* (Jacq.) R. Br. ex Benth. and *Tamarindus indica* L

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Abstract
Inflammation has been shown to be greatly involved in the degenerative processes of human skin such as photo aging and atopy. Reduction of low-grade inflammatory reactions by topical products may be necessary in case of skin aggression, to obtain an optimal wound healing and restore the physiological balance of human skin. Present work evaluated the antioxidant, anti-inflammatory and phototoxic properties of ethanolic extracts from leaves and stem barks of *Dialium guineense* Willd., *Parkia biglobosa* (Jacq.) *R. Br. Ex Benth. and Tamarindus indica* L. The antioxidant power of the extracts, measured *in vitro* by the KRL method, showed that each gram of D. guineense bark extract, *P. biglobosa* leaves extract and T. indica bark and leaves extracts has an antioxidant capacity equivalent to 1585, 2092, 5071 and 2246 mg of Trolox respectively. Simultaneously with their actions on cell viability, the anti-inflammatory activity of the extracts was monitored measuring their effects on NO production by mouse macrophages submitted to LPS from E. coli to determine the anti-inflammatory ratio of each extract. The bark and leaves of *D. guineense* Willd., the leaves of *P. biglobosa (Jacq.) R. Br. Ex Benth.* and the bark of *T. indica L*. have anti-inflammatory ratios from 161 to 458.2, whereas Dexamethasone (positive control) has a ratio of 37.87. The *in vitro* 3T3 NRU test was used on mouse fibroblasts to determine the phototoxicity of the six extracts. Only *D. guineense* Willd. stem bark was photo-toxic with a photo-irritation factor greater than 5 (PIF = 8.39). Our study report some molecules such as lupeol, amaryn, sitosterol for the first time in the extracts and shows that the use of these plants in traditional medicine is justified.

Keywords: anti-inflammatory, antioxidant, GC-MS, ethanolic extracts, lupeol, sitosterol, stigmasterol

Introduction
Living beings have two major common enemies: aging and diseases. Although a natural process, aging can be accelerated by oxidative stress which seems closely related to inflammatory processes [1, 2]. Inflammation is the set of defense mechanisms by which the body recognize, destroy and eliminate all foreign substances. Inflammatory reaction sometimes exceeds its objectives leading to deleterious effects. It then needs to be managed. On the other hand, oxidative stress (due to increase of oxygen reactive species) is linked to many pathologies [3, 2, 1]. As implication of oxidative stress and inflammation in occurrence of several pathologies (diabetes, cancer, cardiovascular diseases) was highlighted [3-5] search for molecules with antioxidant and anti-inflammatory power now goes beyond the anti-aging or cosmetic framework. Therefore, during two past decades, scientific research focused on them worldwide. In Africa and many low and middle income countries, traditional knowledge based on medicinal plants is used to fight against oxidative stress and inflammation [6]. Resort to this form of medicine have been linked to cultural and economic reasons [7, 8]. However, we note a worldwide increase of medicinal plants use [9, 10] because of their renewable character and association with no side effects. It is then important to investigate the effectiveness of those medicines. This is the purpose of this study focused on three plants recurrent in Benin traditional medicine: *Dialium guineense* Willd., *Parkia biglobosa* (Jacq.) *R. Br. Ex Benth., and Tamarindus indica* L.. Valorization and safeguarding of those threatened plants in Benin [11, 12] and their recurrence in our ethnobotanical survey justify our choice on them. They belong to the Fabaceae family which is well know for its medicinal purposes [13-16].

Their aerial organs are widely used in the treatment of various infectious diseases in Benin. Because inflammatory reaction may occur in response to infections, natural compounds having both activities would be very interesting.

D. guineense is a dialioideae exclusively present in regions of tropical Africa and exceptionally in Sao Tome et Principe. It grows mainly in the wild as a shrub on land left fallow and in dry or dense forests, as well as forest galleries, from Senegal to southern Nigeria. In Benin, it is present from the coast to the southern region. Antibiotic properties are attributed to its roots, leaves and barks used alone or in combination with other plants in the treatment of malaria, coughs, bronchitis, diarrhea, palpitations, dysmenorrhea, ulcer, anemia, hemorrhoids [17, 18]. Concerning *P. biglobosa*, it is a Mimosoideae [19] present in tropical Africa between 3 ° and 15 ° North. It grows in tropical regions with high rainfall but also in arid environments. In Benin, it is an almost ubiquitous tree occuping the fifth position among the most used plants in traditional medicine [20]. Finaly, *T. indica* is a detarioideae up to 25 meters tall [21] growing very well in semi-humid or arid regions [22]. With well kwon laxative and carminative effects, its organs are used in the treatment of a wide range of pathologies such as toothaches [23], bacterial infections, malaria [24].

Material and Methods

Leaves and bark of the plants were collected in three localities of Benin (Abomey-Calavi, Cotonou and Parakou). Samples were formally identified at the Benin National Herbarium under the voucher numbers AA 6727 / HNB, AA 6728 / HNB and AA 6729/ HNB for *Dialium guineense Willd*., *Parkia biglobosa* (Jacq.) *R. Br. Ex Benth*., and *Tamarindus indica L.* respectively.

RAW 264.7 macrophage assay has been used to monitor the inhibitory effects of the extracts on the low-grade inflammatory cascade (RAW 264.7, Sigma-Aldrich, N° P6110401, Lot. 09I006). Phototoxicity was assessed using Balb /c 3T3 mouse fibroblasts (3T3-L1) (ATCC, United States, ATCC® CL-173™, N° P6110401, Lot. 09I006). DMEM (Dulbecco's Minimum Essential Medium) with stable L-glutamine supplemented with Penicillin 100 IU/ml, streptomycin 100 µg/ml, and 10% of inactivated calf serum served as culture medium. It was freshly prepared with a pH of 7.2. Standardized horse blood was used for KRL test. All test materials were diluted in dimethyl sulfoxide (DMSO, Sigma-Aldrich).

Anti-inflammatory test and cell viability

This *in vitro* anti-inflammatory assay is based on the ability of macrophages to generate a strong inflammatory response when stimulated with antigens. Mouse immortalized macrophages (RAW 264.7 cell line) were stimulated by *E. coli* LPS and exposed to the extracts for 24 hours. At the end of the incubation period, NO production was evaluated indirectly by measuring the accumulation of nitrite/nitrate in the culture medium using a spectrophotometric method based on the Griess reaction. Negative and positive controls were DMSO and Dexamethasone (1, 5, 10, 50 and 100 µM) respectively. Cell mitochondrial respiration measurement allowed to assess cell viability. Inhibition of NO release and inhibition of cell viability were expressed as percentages as compared to the negative controls:

Percentage of NO release (%) = 100 x ($OD_{test\ well} - OD_{blank}$) / $OD_{control} - OD_{blank}$

Percentage of Cell viability (%) = 100 x ($OD_{test\ well} - OD_{blank}$) / $OD_{control} - OD_{blank}$

The concentrations of the test material causing respectively a 50% decrease of NO release ($IC_{50\text{-NO release}}$) and a 50% decrease of cell viability ($IC_{50\text{-cell viability}}$) were calculated using the software Table curve Version 2.0. The anti-inflammatory ratio corresponded to the ratio between the anti-inflammatory activity and the toxicity. It was expressed as follows: Anti-inflammatory ratio = $IC_{50\text{-cell viability}}$ / $IC_{50\text{-NO release}}$

Phototoxicity and cytotoxicity measurement

The *in vitro* 3T3 NRU phototoxicity test was used to evaluate phototoxicity of the six ethanolic extracts measuring relative reduction in viability of cells exposed to them in presence of light versus absence of light. The Photo-Irritation-Factor (PIF) was calculated with concentrations (obtain by the software Phototox Version 2.0.) of the test material causing a 50% release of the preloaded Neutral Red without irradiation (IC_{50} -Irr) and with irradiation (IC_{50} +Irr) as compared to the control culture using the following formula:

PIF = IC_{50} (-Irr) / IC_{50} (+Irr)

On the other hand, the degree of membrane damage (i.e. the increase of released Neutral Red) was measured by a fluorescence-luminescence reader Infinite M200 Pro (TECAN). The Optical Density (OD) of each well was read at 540 nm. The results obtained for wells treated with the test material were compared to those of untreated control wells (HBSS, 100% viability) and converted to percentage values. Neutral Red desorbed solution serves as blank.

The percentages of cell viability were calculated as:

$$\text{Viability (\%)} = \frac{\text{Mean OD of test wells} - \text{mean OD of blanks}}{\text{Mean OD of negative control} - \text{mean OD of blanks}}$$

Kit Radicaux Libres (KRL) test

Evaluation of the overall resistance of standardized horse blood submitted to oxygen reactive species attack was made in absence versus presence of our extracts. The overall resistance of the control blood to the radical attack in the presence or absence of our products was expressed by the time at which 50% of the blood cells were lysed (T1 / 2 in minutes). The antiradical efficacy of the products was then expressed as a percentage of overall potential for antiradical defense of the control blood (% T1 / 2 of the control blood). The results were standardized in Trolox equivalents (water soluble analogue of vitamin E) and in equivalents Gallic acid (phenolic acid).

Gas chromatography and mass spectrometry analysis

100µl of each filtered extract were dried with nitrogen, then 100µl of BSTFA were added and the whole is incubated at 70 °C for 20 min. 2µl were injected for GC-MS analysis. The analyses were carried with a GC-MS apparatus consisting of a chromatograph Shiwazu QP, a mass spectrometer GCMS-QP2010S equipped with an electronic impact ion source and a quadrupole analyzer type, a GC Solution acquisition software as well as banks (NIST, WILEY). The compounds were separated using a DB-1MS capillary column (30 m x 0.25 x mm internal diameter x 0.25 µm film thickness from JW Scientific) which temperature limits were between -60 °C and 350 °C. The mobile phase was helium. Pressure in the column

was 3psi and the flow rate was 1.59 mL /min. The injector temperature (Inlet) was 280 °C, interface temperature was 280 °C, the source and the quadrupole at 150 °C. Temperature gradient was programmed as follows: 1 min at 60 °C, then increasing from 100 to 260 °C at 4 ° / min, then 30 min at 260 °C. The total elution time was 82 min. We focus on compounds with high retention time (>60min) in this work.

Results and Discussion

Results of anti-inflammatory activities and phototoxicity are reported in Tables 1&2 below.

Table 1: Anti-inflammatory, cytotoxic activity and anti-inflammatory ratio of the extracts

	NO release IC_{50} (µM or µg/mL)	Cell viability IC_{50} (µM or µg/mL)	Anti-inflammatory ratio
Dialium guineense barks	0.15 ± 0.01	68.74	458.2
Dialium guineense leaves	0.22 ± 0.05	43.17 ± 8.2	196.2
Parkia biglobosa barks	77.78 ± 9.4	>100	>1.3
Parkia biglobosa leaves	0.62 ± 0.08	>100	>161
Tamarindus indica barks	0.28 ± 0.02	48.91 ± 10.1	174.6
Tamarindus indica leaves	>100	>100	-
Dexamethasone	4.31 ± 1.45 µM	163.22 ± 24.96 µM	37.87

Extracts of *Dialium guineense* (both leave and stem), those of leaves of *Parkia biglobosa* and barks of *Tamarindus indica* exert higher anti-inflammatory activity than Dexamethasone, the positive control (Fig. 1). Barks of *Dialium guineense* extract showed the best anti-inflammatory activity (Table 1). Sitosterol (0.77%) and lupeol acetate (0.55%) are triterpenoids we found and reported for the first time in this extract. Those two molecules have been reported to have interesting anti-inflammatory activity [25, 26]. In association with phenolic compounds contained in this extract [27-29] they could explain our result and support the use of *Dialium guineense* by traditional healers. This extract has a high photo-irritation factor (Table 2). Therefore, it appears suitable to investigate the three others as anti-inflammatory drugs for cutaneous use. The leaves of this plant for example have half of the anti-inflammatory activity compared to the barks but is not phototoxic. It has higher tenor of sitosterol (3.5%) and lupeol acetate (1.7'%) which seems to play a key role in phototoxicity as they are also more abundant in leaves of *P. biglobosa* (2.02% and 4.41%) and barks of *T. indica* (0.80% and 1.53%) (Tables 7&8).

Table 2: Phototoxicity results

	Toxicity in non-irradiated cells IC_{50}(-irr) (µM)	Toxicity in irradiated cells IC_{50}(+irr) (µM)	PIF	Phototoxicity
Dialium guineense barks	75.31 ± 8.54	8.97 ± 1.54	8.39	phototoxic
Dialium guineense leaves	47.56 ± 9.64	36.87 ± 4.21	1.28	Not phototoxic
Parkia biglobosa barks	130.85 ± 9.21	202.78 ± 9.47	0.64	Not phototoxic
Parkia biglobosa leaves	123.61 ± 2.21	66.33 ± 1.34	1.86	Not phototoxic
Tamarindus indica barks	53.64 ± 8.88	49.94 ± 9.54	1.07	Not phototoxic
Tamarindus indica leaves	8.76 ± 9.47	>100	-	Not phototoxic
Chlorpromazine	46.33 ± 2.96	1.04 ± 0.25	44.54	phototoxic

-irr: not irradiated, +irr: irradiated

It clearly appears on Figure 1 that, despite its great anti-inflammatory activity, only *Dialium guineense* barks extract exerted phototoxicity in our study. Leaves extract of this plant have lower anti-inflammatory activity than barks but are not phototoxic. This can be due to the difference of abundance in terpenoids like sitosterol (0.78% vs trace) and lupenone (0.42% vs trace). *Parkia biglobosa* leaves and *Tamarindus indica* barks extracts also aren't phototoxic and have higher amount of sitosterol, lupeol or lupenone than Dialium guineense barks extract.

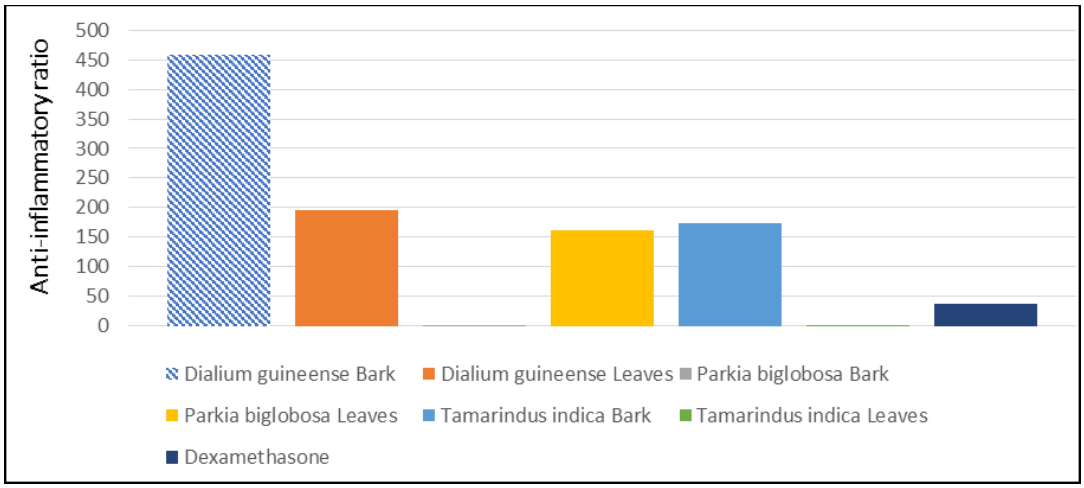

Fig 1: Anti-inflammatory activity and phototoxicity of ethanolic extracts

Table 3: antioxidant activity of the extracts

Concentrations (mg/L) *	% increase of half-hemolysis time ($T_{1/2}$) of control blood					
	Dialium guineense		*Parkia biglobosa*		*Tamarindus indica*	
	Barks	leaves	Barks	leaves	Barks	leaves
0	0.00	0.00	0.00	0.00	0.00	0.00
1	5.64	3.64	6.94	7.30	21.87	10.93
2	15.85	5.39	9.83	14.08	38.19	20.34
5	36.70	13.63	19.65	30.63	73.14	45.10
10	61.33	20.98	30.72	59.86	118.05	75.95
20	82.93	36.80	47.83	109.48	265.36	117.55
50	149.18	73.47	106.94	266.33	451.18	224.66
100	438.64	85.30	277.53	181.70	342.87	338.91

* Concentration in mg of extract per liter of reaction medium

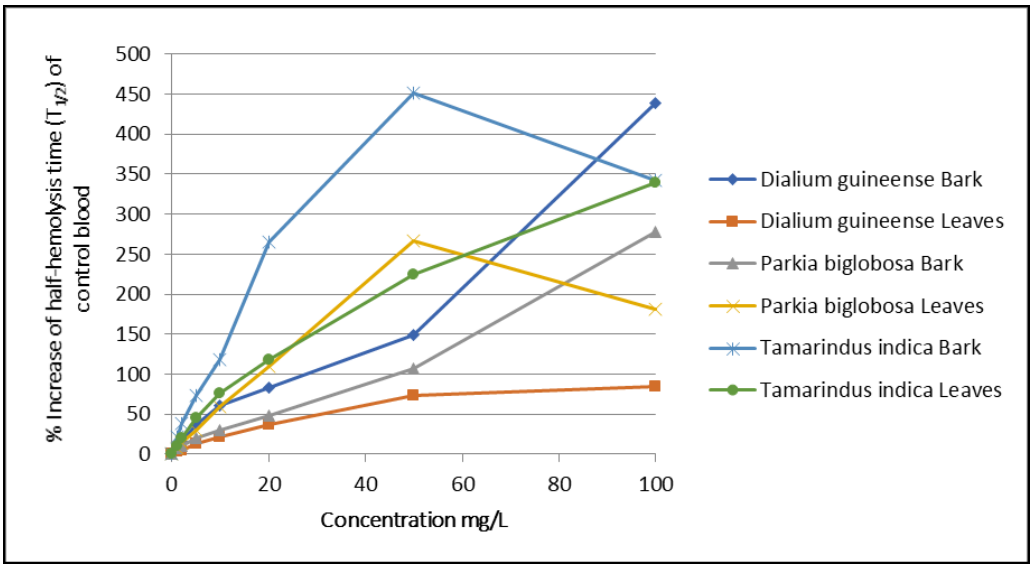

Fig 2: Antioxidant activity of plant extracts

KRL test results indicate that all the tested plant extracts have a dose-dependent antiradical capacity (Fig. 2) and this antioxidant power is strongly increasing in a concentration range of 0 to 20 mg/L (Table 3). At 20 mg/L, leaves of *Parkia biglobosa*, leaves and barks of *Tamarindus indica* extracts have remarkable results. They increased the resistance of the control blood to a radical attack up to 109.48, 265.36 and 117.55% respectively (Table 3). Also, those extracts were at least as effective as Trolox and gallic acid.

Table 4: Anti-radical power of extracts vs Trolox (Equivalent Trolox mg / g product)

Concentrations (mg/L) *	Antioxidant activity of plant extracts of Benin: Equivalents mg of Trolox/g of extract					
	Dialium guineense		*Parkia biglobosa*		*Tamarindus indica*	
	Barks	leaves	Barks	leaves	Barks	leaves
0	0.00	0.00	0.00	0.00	0.00	0.00
1	2157,55	1391,50	2654,27	2789,66	8360,74	4177,09
2	3028,74	1029,43	1879,17	2689,96	7297,49	3887,81
5	2805,47	1041,60	1502,12	2341,37	5591,19	3447,83
10	2344,19	802,04	1174,09	2287,81	4512,03	2902,86
20	1584,90	703,19	914,09	2092,13	5071,12	2246,38
50	1140,39	561,58	817,49	2035,83	3448,87	1717,37
100	1676,51	326,03	1060,73	694,48	1310,49	1295,33

Table 5: Anti-radical power of extracts vs Gallic acid (Equivalent Trolox mg / g product)

Concentrations (mg/L) *	Antioxidant activity of plant extracts of Benin: Equivalents mg of Gallic acid/g of extract					
	Dialium guineense		*Parkia biglobosa*		*Tamarindus indica*	
	Barks	leaves	Barks	leaves	Barks	leaves
0	0.00	0.00	0.00	0.00	0.00	0.00
1	1027,26	662,53	1263,76	1328,22	3980,74	1988,81
2	1442,05	490,14	894,72	1280,75	3474,50	1851,08
5	1335,75	495,93	715,19	1114,78	2662,09	1641,59
10	1116,12	381,87	559,01	1089,28	2148,28	1382,12
20	754,61	334,80	435,22	996,11	2414,48	1069,55
50	542,96	267,38	389,23	969,31	1642,08	817,68
100	798,22	155,23	505,04	330,66	623,96	616,74

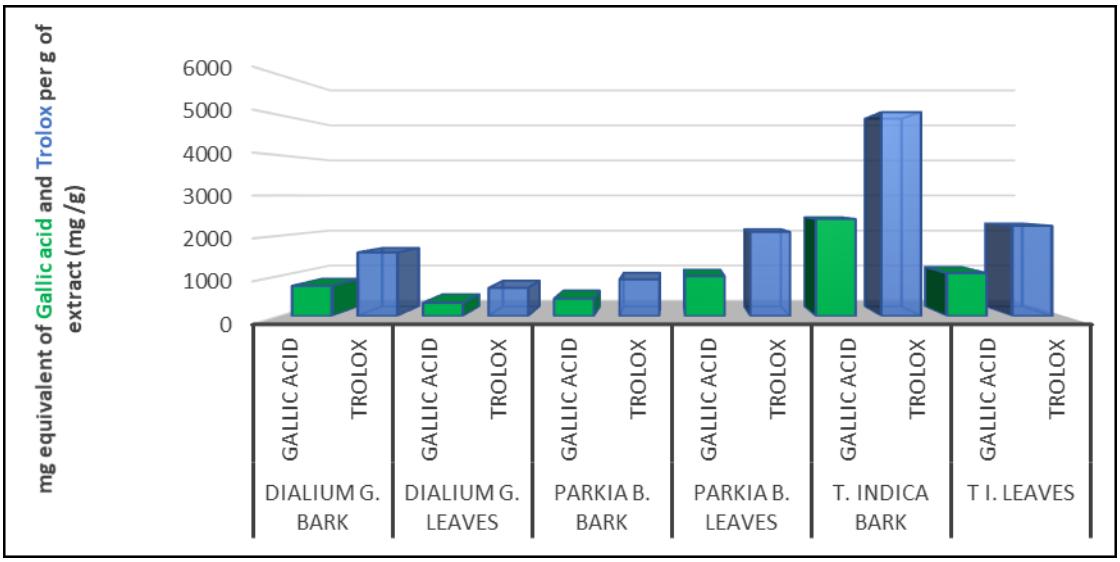

Fig 3: Comparison of the antioxidant power of the extracts with Gallic acid and Trolox at a concentration of 20 mg/L

As shown in Fig. 3, *Tamarindus indica*' s barks ethanolic extract is particularly interesting: it is five times more active than Trolox (Table4) and 2.5 times more active than gallic acid (Table 5). Lupeol (0.83%), amyrin (0.65%) and lupeol acetate (1.53%) were detected in this extract by GC-MS analysis (Table 8). Those molecules were reported in the plant [30]and their free radical scavenging property is well known [31-33]. Leaves extract of this plant also showed high free radical scavenging activity [34, 35].

Chemical compounds identified by GC-MS in the extracts are summarized below. Identification was based on direct comparison and at least 80% matching of the retention times and mass spectral data with those in the National Institute of Standards and Technology (NIST) and WILLEY libraries. DPPH free radical scavenging test of the methanolic extract of *Dialium guineense* leaves showed a concentration-dependent antioxidant activity supposed to be due to the phenolics compounds [36]. Leaves ethanolic extracts of *Parkia biglobosa* showed an antioxidant activity of 109.48% according to the KRL test. It contains lupenone (2.59%), β-Amyrin (1.05%), Sitosterol (2.02%) and lupeol acetate (4.41) all known for biologic activities. Other studies reported similar antiradical activity of the barks according to Diphenyl Picryl Phenyl Hydrazine (DPPH) test: 87.68% for aqueous extract [37].

Table 6: Triterpenoids identified in *Dialium guineense* by GC-MS

		Leaves	Stem-barks
RT	**Compounds**	5.92 %	1.48 %
61.774	Free Sitosterol	0.78	trace
62.337-62.442	Stigmasterol TMS	0.26	0.2
63.086	Lupenone	0.42	-
63.794-63.873	Lupeol	trace	trace
64.603-64.838	Sitosterol TMS	2.72	0.77
66.108-66.433	Lupeol acetate	1.74	0.51

RT: Retention time

Sitosterol and lupeol acetate are reported here for the first time in those extracts. Despite their low amount, we suppose they contribute to the anti-inflammatory role and the antioxidant activities of the extracts as reported by several papers [38, 25, 33].

Table 7: Triterpenoids identified in *Parkia Biglobosa* by GC-MS

		Leaves	Stem-barks
RT	**Compounds**	**10.44%**	**7.31%**
60.665	Taraxerone	-	**0.66**
62.372-62.442	Stigmasterol TMS	0.37	0.72
62.883-63.086	Lupenone	2.59	1.14
63.840-63.873	Lupeol	trace	trace
64.457	beta-Amyrin TMS	1.05	-
64.636	beta Sitosterol	-	1.18
64.838	Sitosterol TMS	2.02	-
66.196-66.433	Lupeol acetate	4.41	3.61

Beta-Amyrin is present in *Parkia Biglobosa* leaves extract which is as active as gallic acid and two times more active than Trolox (Fig. 3). It is well kwon for its antioxidative and anti-inflammatory activities [39, 40].

Table 8: Triterpenoids identified in Tamarindus indica by GC-MS

		Leaves	Stem-barks
RT	**Compounds**	**4.04%**	**3.81%**
62.25	**Alpha-Amyrin TMS**	-	**0.23**
62.316 & 62.385	Stigmasterol TMS	trace	trace
62.869 & 63.023	Lupenone	1.75	trace
63.817	Lupeol	-	0.83
64.345	beta-Amyrin TMS	-	0.42
64.612	beta-Sitosterol	-	0.80
64.724	Sitosterol TMS	1.07	-
66.138 & 66.219	Lupeol acetate	1.22	1.53

Lupeol, beta amyrin and beta sitosterol could to be involved in the good antioxidant and anti-inflammatory activity of Tamarindus indica barks extract. This extract is five times more active than Trolox and two times more active than gallic acid (Fig 3).

Conclusion

In the present study, ethanolic extracts of leaves and barks of *Dialium guineense*, *Parkia biglobosa* and *Tamarindus indica*, showed interesting antioxidant and anti-inflammatory properties. Lupeol acetate and Sitosterol were reported for the first time in *Dialium guineense* barks. Lupenone, amyrin, stigmasterol and taraxenone were also identified in the extracts and could explain their biological activity. The amounts of those compounds and their synergy also seem to play a key role in the biological activities. Except barks

extracts of *Dialium guineense* which is phototoxic, the other extracts can be investigate as cosmetic agents.

Abbreviations
DMSO: Dimethyl sulfoxide
DPPH: Diphenyl Picryl Phenyl Hydrazine
KRL: Kit Radicaux Libres
LPS: Lipopolysaccharide
PIF: Photo-Irritation-Factor

Declarations

- **Ethics approval and consent to participate:** Not applicable.
- **Consent to publish:** Not applicable
- **Availability of data and materials:** All data generated or analyzed during this study are included in this published article (However, raw data are available from the corresponding author on reasonable request).
- **Competing interests:** "The authors declare that they have no competing interests."
- **Funding:** France and Benin governments
- Authors' Contributions

S.M.G. is corresponding author, PhD student working on the subject. He carried out most of the test and wrote this manuscript. S. A. preformed KRL tests, figures and tables. L. A. make GC-MS analyzes. C.D.G. performed anti-inflammatory tests. M.R. make extractions and assisted L.A for GC-MS analyzes. E.D.A. is PhD supervisor and direct and correct this manuscript. P.P. is co- Supervisor of thesis. He also direct and correct the manuscript. D.C.S is member of thesis committee and he correct the manuscript.

All authors read and approved the final manuscript for submission. They have no conflict of interest to declare.

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References

1. Salzano S. *et al.* Linkage of inflammation and oxidative stress via release of glutathionylated peroxiredoxin-2, which acts as a danger signal," Proc. Natl. Acad. Sci., 2014; 111(33):12157-12162.
2. Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. "Oxidative stress, inflammation, and cancer: How are they linked?," Free Radic. Biol. Med. 2010; 49(11):1603-1616
3. Khansari N, Shakiba Y, Mahmoudi M. "Chronic Inflammation and Oxidative Stress as a Major Cause of Age- Related Diseases and Cancer," Recent Pat. Inflamm. Allergy Drug Discov. 2009; 3(1):73-80.
4. Eiró N, Vizoso FJ. Inflammation and cancer," World J. Gastrointest. Surg. 2012; 4(3):62-72.
5. Golbidi S, Badran M, Laher I. Antioxidant and anti-inflammatory effects of exercise in diabetic patients," *Exp. Diabetes Res.*, 2012; 941868, 2012.
6. Adebayo SA, Dzoyem JP, Shai LJ, Eloff JN. "The anti-inflammatory and antioxidant activity of 25 plant species used traditionally to treat pain in southern African," BMC Complement. Altern. Med. 2015; 15:159
7. Sato A. "Revealing the popularity of traditional medicine in light of multiple recourses and outcome measurements from a user's perspective in Ghana, Health Policy Plan. 2012; 27(8):625-637.
8. Oyebode O, Kandala NB, Chilton PJ, Lilford RJ. Use of traditional medicine in middle-income countries: a WHO-SAGE study," Health Policy Plan. 2016; 31(8):984-991.
9. Tilburt JC, Kaptchuk TJ. Herbal medicine research and global health: an ethical analysis," Bull. World Health Organ. 2008; 86(8):594-599.
10. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety, Front. Pharmacol. 2014; 4:177.
11. Meregini AOA. Some endangered plants producing edible fruits and seeds in Southeastern Nigeria," Fruits, 2005; 60(3):211-220.
12. Lykke AM. Local perceptions of vegetation change and priorities for conservation of woody-savanna vegetation in Senegal," J Environ. Manage. 2000; 59(2):107-120.
13. Rosado-Vallado M, Brito-Loeza W, Mena-Rejón G, Quintero-Marmol E, Flores-Guido J. "Antimicrobial activity of Fabaceae species used in Yucatan traditional medicine," Fitoterapia. 2000; 71(5):570-573.
14. Koné W, Atindehou KK, Terreaux C, Hostettmann K, Traoré D, Dosso M. Traditional medicine in North Côte-d'Ivoire: screening of 50 medicinal plants for antibacterial activity, J. Ethnopharmacol. 2004; 93(1):43-49.
15. Ma X, Zheng C, Hu C, Rahman K, Qin L. "The genus Desmodium (Fabaceae)-traditional uses in Chinese medicine, phytochemistry and pharmacology, J. Ethnopharmacol., 2011; 138(2):314-332.
16. Rahman AM, Parvin MIA. Study of medicinal uses on Fabaceae family at Rajshahi, Bangladesh," Res. Plant Sci. 2014; 2(1):6-8.
17. Odukoya OA, Houghton PJ, Adelusi A, Omogbai EKI, Sanderson L, Whitfield PJ. Molluscicidal triterpenoid glycosides of Dialium guineense, J. Nat. Prod. 1996; 59(6):632-634.
18. Bero J, *et al.*, *In vitro* antiplasmodial activity of plants used in Benin in traditional medicine to treat malaria," J. Ethnopharmacol. 2009; 122(3):439-444.
19. Hopkins HC. The taxonomy, reproductive biology and economicpotentialofParkia(Leguminosae: Mimosoideae) in Africa and Madagascar," Bot. J. Linn. Soc. 1983; 87(2):135-167.
20. Eyog Matig O, Adjanohoun E, de Souza S, Sinsin B. Programme de Resources Genetiques Forestieres en Afrique au Sud du Sahara (*Programme saforgen*). Bioversity International, 2001.
21. Tariq M, Chaudhary SS, Rahman K, Zaman HR, Shaikh I. Tamarindus Indica: AN Overview," J Biol. Sci. Opin., 2013; 1(2):128-131.
22. Meher B, Dash DK, Roy A. A review on: Phytochemistry, pharmacology and traditional uses of Tamarindus indica L," World J Pharm Pharm. Sci. 2014; 3(10):229-240.
23. Tapsoba H, Deschamps JP. Use of medicinal plants for the treatment of oral diseases in Burkina Faso," J. Ethnopharmacol. 2006; 104(1, 2):68-78.
24. Doughari JH. Antimicrobial activity of Tamarindus indica Linn," Trop. J. Pharm. Res. 2006; 5(2):597-603.
25. Loizou S, Lekakis I, Chrousos GP, Moutsatsou P. "β-Sitosterol exhibits anti-inflammatory activity in human aortic endothelial cells," Mol. Nutr. Food Res. 2010; 54(4):551-558.
26. Lucetti DL, *et al.*, "Anti-inflammatory effects and possible mechanism of action of lupeol acetate isolated

from *Himatanthus drasticus* (Mart.) Plumel," J. Inflamm. 2010; 7(1):60.
27. Orji. "Antibacterial Activities of Crude Leaf and Bark Extracts of 'Icheku' Dialium guineense on Bacterial Isolates from Bronchitis Patients," IOSR J. Pharm. Biol. Sci., 2012; 1(3):21-25.
28. Olajubu F, Akpan I, Ojo DA, Oluwalana S. Antimicrobial potential of *Dialium guineense* (Wild.) stem bark on some clinical isolates in Nigeria," Int. J. Appl. Basic Med. Res. 2012; 2(1):58-62.
29. Obonga W, Nnadi C, Agbo M, Kenechukwu F, Nwodo U. Distribution of Methicillin-Resistant Staphylococcus aureus (MRSA) in Apparently Healthy Population and its Susceptibility to Saponins from *Dialium guineense*," Br. J. Pharm. Res. 2014; 4(18):2200-2209.
30. Jain A, Bhadoriya S, Ganeshpurkar A, Narwaria J, Rai G. Tamarindus indica : Extent of explored potential," Pharmacogn. Rev., 2011; 5(9):73.
31. Santiago LA, Mayor ABR. Lupeol: An antioxidant triterpene in Ficus pseudopalma Blanco (Moraceae)," Asian Pac. J. Trop. Biomed. 2014; 4(2):109-118.
32. Tchimene MK, Nwaehujor CO, Ezenwali M, Okoli CC, Iwu M. "Free Radical Scavenging Activity of Lupeol Isolated from the Methanol Leaf Extract of Crateva adansonii Oliv. (Capparidaceae)," 2016; 8(3):8
33. Parvez MK, Alam P, Arbab AH, Al-Dosari MS, Alhowiriny TA, Alqasoumi SI. "Analysis of antioxidative and antiviral biomarkers β-amyrin, β-sitosterol, lupeol, ursolic acid in Guiera senegalensis leaves extract by validated HPTLC methods," Saudi Pharm. J. 2018.
34. Razali N, Abdul Aziz A, Lim CY, Mat Junit S. "Investigation into the effects of antioxidant-rich extract of Tamarindus indica leaf on antioxidant enzyme activities, oxidative stress and gene expression profiles in HepG2 cells," Peer J, 2015; 3:e1292.
35. Razali N, Mat Junit S, Ariffin A, Ramli NSF, Abdul Aziz A. Polyphenols from the extract and fraction of T. indica seeds protected HepG2 cells against oxidative stress," BMC Complement. Altern. Med., 2015; 15:438.
36. Ogu GI, Ezeadila J, Ehiobu JM. Antioxidant and antimicrobial activities of *Dialium guineense* (Willd) leaf extract," Pharm Pharmacol Res. 2013; 1(1):1-7.
37. Olabinri BM, Adetutu A, Olaleye MT, Oluwafunsho BO, Oyeniyi OO. A study of the antioxidative potentials of acetone and aqueous extracts of Parkia biglobosa and Tetracarpidium conophorum stem barks *in vitro*," Int. J. Med. Med. Sci. 2013; 5(8):368-373.

38. Awad AB, Burr AT, Fink CS. Effect of resveratrol and β-sitosterol in combination on reactive oxygen species and prostaglandin release by PC-3 cells," Prostaglandins Leukot. Essent. Fatty Acids, 2005; 72(3):219-226.
39. Hernndez L, Palazon J, Navarro-Oca A. "The Pentacyclic Triterpenes α, β-amyrins: A Review of Sources and Biological Activities," in Phytochemicals - A Global Perspective of Their Role in Nutrition and Health, V. Rao, Ed. In Tech, 2012.
40. Krishnan K, Mathew LE, Vijayalakshmi NR, Helen A. "Anti-inflammatory potential of β-amyrin, a triterpenoid isolated from Costus igneus," Inflammopharmacology, 2014; 22(6):373-385.

IV- Conclusion Générale et perspectives

Notre travail de thèse a porté sur l'étude des activités anti-inflammatoire, antioxydante et le screening par chromatographie gazeuse couplée à la spectrométrie de masse d'extraits éthanoliques de trois fabacées du Bénin : Dialium guineense willd., *Parkia biglobosa* (Jacq.) R. Br. ex Benth. et *Tamarindus indica* L. Ces trois plantes médicinales sont utilisées au Bénin et dans la sous- région ouest-africaine dans le traitement de plusieurs pathologies notamment les infections. Leurs fruits étant très consommés, nous avons également évalué le potentiel nutritionnel de ces derniers en déterminant leurs compositions en nutriments.

La revue de littérature que nous avons réalisée et publiée sur les trois plantes met en exergue de nombreuses études rapportant leurs activités pharmacologiques. Toutefois, si les activités antimicrobienne, antioxydante, anti-venin, hépato-protectrice, antidiabétique, antihypertenseur ou anti-inflammatoire sont souvent rapportées, peu de molécules actives ont été décrites à partir de ces plantes d'où l'intérêt de notre investigation.

Dans ce contexte, nous avons simultanément évalué les potentiels nutritionnel et thérapeutiques de *D. guineense*, *P. biglobosa* et *T. indica.* Ainsi, les fruits de P. biglobosa et T. indica ont été soumis à une extraction solide/liquide par macération dans un mélange eau/éthanol (v/v). Le criblage phytochimique de ces extraits a révélé majoritairement la présence des tanins catéchiques, des flavonoïdes libres et des anthraquinones. Les anthocyanes, les alcaloïdes, les sucres réducteurs, les quinones libres, sont au contraire absents dans la pulpe des deux fruits. Le fruit de *T. indica* est riche en flavonoïdes totaux (8,31 mg/g) et a des teneurs moyennes en tanins condensés (2,60 mg/g) et composés phénoliques totaux (2,14 mg/g).

Les deux extraits hydroéthanoliques de fruits ont présenté une activité antiradicalaire sur le DPPH moindre que celle de la quercétine. Cela pourrait s'expliqué par leur teneur faible en composés phénoliques connus pour être des piégeurs de radicaux libres. A l'opposé, les extraits éthanoliques des feuilles et écorces de *P. biglobosa* et *T. indica* testés par la méthode KRL ont montré des activités antioxydantes égales ou supérieures à celles du Trolox et de l'acide gallique. L'extrait éthanolique d'écorces de *Tamarindus indica* est particulièrement intéressant : il est cinq fois plus actif que le Trolox et 2,5 fois plus actif que l'acide gallique.

Le test des macrophages de souris a permis de révéler une activité anti-inflammatoire des extraits éthanoliques. *D. guineense* (feuilles et écorce), *P. biglobosa*

(feuilles) et *T. indica* (écorce) ont exercé une activité anti-inflammatoire supérieure à celle de la dexaméthasone. L'extrait d'écorce de *Dialium guineense* a montré la meilleure activité anti-inflammatoire avec un ratio de 458. Le sitostérol et l'acétate de lupéol ont été identifiés et rapportés pour la première fois chez cette espèce grâce à la chromatographie en phase gazeuse couplé à la spectrométrie de masse.

Le test 3T3 NRU a permis de montrer que la quasi-totalité des extraits était non phototoxique. Il est donc possible d'envisager leur formulation pour usage topique pour la protection cutanée.

Enfin, les trois fruits ont des teneurs intéressantes en nutriments (notamment en fer, iode et vitamine C). Ils pourraient donc servir pour la mise au pont de compléments alimentaires pour enfants malnutris.

En perspective du présent travail, nous envisageons de :

- Réaliser un fractionnement bioguidé de l'extrait d'écorce de T. indica ayant montré des activités anti-inflammatoire et antioxydante supérieures à celles des molécules témoins ;
- Poursuivre l'étude phytochimique des trois plantes afin d'identifier les métabolites responsables de l'activité antimicrobienne clamée par la médecine traditionnelle ;
- Aller vers une optimisation de l'extraction avec de nouvelles méthodes pouvant éventuellement améliorer l'efficacité des extraits ou augmenter les teneurs des molécules identifiées ;
- Tester l'effet booster des extraits sur des antibiotiques de référence ;
- Evaluer l'effet conservateur de l'extrait d'écorce de *T. indica* en cosmétologie.

V-Références

[1] World Health Organization, “WHO traditional medicine strategy. 2014-2023. http://apps.who.int/iris/handle/10665/67313,” p. 78, 2013.

[2] Z. Kibou, N. Cheikh, and N. Choukchou-braham, “A new route for the synthesis of 2-aminopyridines,” *Chem. Chem. Eng. Biotechnol. Food Ind.*, vol. 12, no. 2, pp. 121–126, 2011.

[3] WHO, “OMS. Rapport du Secrétariat. Soixante-huitième assemblée mondiale de la Santé. Résistance aux antimicrobiens. http://apps.who.int/gb/ebwha/pdf_files/WHA68/A68_20-fr.pdf,” pp. 1–20, 2015.

[4] A. S. Fauci, “Infectious Diseases : Considerations for the 21st Century,” *Clin. Infect. Dis.*, vol. 32, no. 5, pp. 675–685, 2001.

[5] N. M. Moutsopoulos and P. N. Madianos, “Low-Grade Inflammation in Chronic Infectious Diseases,” *Ann. N. Y. Acad. Sci.*, vol. 1088, no. 1, pp. 251–264, 2006.

[6] G. H. Cassell, “Infectious Causes of Chronic Inflammatory Diseases and Cancer,” *Emerg. Infect. Dis.*, vol. 4, no. 3, pp. 475–487, 1998.

[7] A. Manuscript and M. Communities, “Mining the Metabiome: Identifying Novel Natural Products from Microbial Communities,” *Chem. Biol.*, vol. 21, no. 9, pp. 1211–1223, 2014.

[8] J. O. DEFRAIGNE, “Un mécanisme physiopathologique central à l’origine des complications du diabète?,” *Rev Med Liege*, vol. 60, no. 5–6, pp. 472–478, 2005.

[9] E. S. Ta, “Aspects épidémiologiques de la malnutrition Infanto-juvénile à Cotonou (Bénin),” *Med Trop*, vol. 181, no. 1, pp. 177–181, 2006.

[10] J. M. Flora J Chadare, , Yann E Madode, Nadia Fanou-Fogny, A. R. Kindossi, Juvencio O Ayosso, Hermann S Honfo, Polycarpe Kayode, and J. D. H. Linnemann, “Indigenous food ingredients for complementary food formulations to combat infant malnutrition in Benin: a review,” *J. Sci. Food Agric.*, vol. 98, no. 2, 2018.

[11] R. E. Black *et al.*, “Maternal and child undernutrition and overweight in low-income and middle-income countries,” *Lancet*, vol. 382, no. 9890, pp. 427–451, 2013.

[12] A. Pradyumna, F. Egal, and J. Utzinger, “Sustainable food systems, health and infectious diseases: concerns and opportunities,” *Acta Trop.*, vol. 191, pp. 172–177, 2019.

[13] E. Villamor, L. Misegades, M. R. Fataki, R. L. Mbise, and W. W. Fawzi, “Child mortality in relation to HIV infection , nutritional status , and socio-economic background,” *Int. J. Epidemiol.*, vol. 34, no. 1, pp. 61–68, 2004.

[14] O. E. Matig, E. Adjanohoun, and B. Sinsin, *Compte rendu de la première réunion du Réseau «Espèces Ligneuses Médicinales». Programme de ressources génétiques forestières en Afrique au sud du Sahara. FAO*. 1999.

[15] Apollos O.A. MEREGINI, “Some endangered plants producing edible fruits and seeds in Southeastern Nigeria,” *Fruits*, vol. 60, no. 3, pp. 211–220, 2005.

[16] A. Ewedje, Eben-Ezer BK; Tanjiekpon, “Velvet tamarind (Dialium guineense). http://citarea.cita-aragon.es/citarea/handle/10532/1684,” 2011.

[17] F. A. Olajubu, I. Akpan, D. A. Ojo, and S. A. Oluwalana, “Antimicrobial potential of Dialium guineense (Wild .) stem bark on some clinical isolates in Nigeria,” *Int. J. Appl. Basic Med. Res.*, vol. 2, no. 1, pp. 58–62, 2012.

[18] S. J. . H. A. E. . S. P. D. . M. P. K. Lewis, “Phlorotannin-protein interactions,” *J. Chem. Ecol.*, vol. 22, no. 10, pp. 1877–1899, 1995.

[19] A. A. David, A. T. Olaniyi, A. O. Mayowa, A. A. Olayinka, and O. I. Anthony, “Anti-Vibrio and preliminary phytochemical characteristics of crude methanolic extracts of the leaves of Dialium guineense (Wild),” *J. Med. Plants Res.*, vol. 5, no. 11, pp. 2398–2404, 2011.

[20] E. . Orji, J.O., Alo, M.N., Anyim, C. and Okonkwo, “Antibacterial Activities of Crude Leaf and Bark Extracts of ‘ Icheku ’ Dialium guineense on Bacterial Isolates from Bronchitis Patients,” *J. Pharm. Biol. Sci.*, vol. 1, no. 3, pp. 21–25, 2012.

[21] E. Essien, I. A. Ogunwande, A. O. Ogunbinu, G. Flamini, P. L. Cioni, and E. Essien, “Analysis of the Essential Oil of Dialium guineense Willd,” *J. Essent. Oil Res.*, vol. 19, no. 6, pp. 545–547, 2007.

[22] A. U. Bari *et al.*, “Purification and partial characterization of a new mannose / glucose-speci fi c lectin from Dialium guineense Willd seeds that exhibits toxic effect,” *J. Mol. Recognit.*, vol. 26, no. 8, pp. 351–356, 2013.

[23] T. A. S. Millogo-Kone H. Guissou I.P., Nacoulma O., “Antimicrobial effects of the stem bark extracts of Parkia biglobosa (jacq.)Benth. on Shigellae,” *African J. Tradit. Complement. Altern. Med.*, vol. 4, no. 4, pp. 392–396, 2007.

[24] T. A. S. Millogo-Kone H., Guissou I.P., Nacoulma O., “Comparative study of leaf and stem bark extracts of Parkia biglobosa against enterobacteria.,” *African J. Tradit. Complement. Altern. Med.*, vol. 5, no. 3, pp. 238–243, 2008.

[25] A. Adetutu, W. A. Morgan, O. Corcoran, and F. Chimezie, “Antibacterial activity and in vitro cytotoxicity of extracts and fractions of Parkia biglobosa (Jacq .) Benth . stem bark and Ageratum conyzoides Linn . leaves,” *Environ. Toxicol. Pharmacol.*, vol. 34, no. 2, pp. 478–483, 2012.

[26] M. A. Ibrahim, J. D. Habila, N. A. Koorbanally, and S. Islam, “Butanol fraction of Parkia biglobosa (Jacq.) G. Don leaves enhance β-cell functions, stimulates insulin secretion and ameliorates other type 2 diabetes-associated complications in rat,” *J. Ethnopharmacol.*, vol. 183, pp. 103–111, 2016.

[27] K. Komolafe, T. M. Olaleye, O. I. Omotuyi, A. A. Boligon, M. L. Athayde, and A. A. Akindahunsi, “In Vitro Antioxidant Activity and Effect of Parkia biglobosa Bark Extract on Mitochondrial Redox Status,” *J. Acupunct. Meridian Stud.*, vol. 7, no. 4, pp. 202–210, 2014.

[28] T. E. Grønhaug *et al.*, “Ethnopharmacological survey of six medicinal plants from Mali ,” *J. Ethnobiol. Ethnomed.*, vol. 4, no. 1, p. 261, 2008.

[29] W. Aengwanich, M. Suttajit, T. Srikhun, T. Boonsorn, and M. District, “Antibiotic Effect of Polyphenolic Compound Extracted from Tamarind (Tamarindus indica L .) Seed Coat on Productive Performance of Broilers,” *Int. J. Poult. Sci.*, vol. 8, no. 8, pp. 749–751, 2009.

[30] M. H. Gordon, “The mechanism of antioxidant action in vitro,” no. 1, pp. 1–18, 1990.

[31] G. G. Yuichiro J. Suzuki, Masahiko Tsuchiya, f Stephen R. Wassal, Yuen M. Choo and and L. P. Valerian E. Kagan, “Structural and Dynamic Membrane Properties of a-Tocopherol and a-Tocotrienol: Implication to the Molecular Mechanism of Their Antioxidant Potency?,” *Biochemistry*, vol. 32, no. 40, pp. 10692–10699, 1993.

[32] D. N. Patil and A. K. Sharma, “Purification and partial characterization of a new mannose/glucose-specific lectin from Dialium guineense Willd seeds that exhibits toxic effect: Purification of a lectin from Dialium guineense willd seeds,” *J. Mol. Recognit.*, vol. 26, no. 8, pp. 736–738, 2009.

[33] K. Hill, D. You, M. Inoue, M. Z. Oestergaard, and T. Advisory, “Child Mortality Estimation : Accelerated Progress in Reducing Global Child Mortality , 1990 – 2010,” *PLoS Med.*, vol. 9, no. 8, p. e1001303, 2012.

[34] H. Alaofè, J. Burney, R. Naylor, and D. Taren, “Prevalence of anaemia , deficiencies of iron and vitamin A and their determinants in rural women and young children : a cross-sectional study in Kalalé district of northern Benin,” *Public Health Nutr.*, vol. 20, no. 7, pp. 1203–1213, 2017.

[35] L. Hallberg, M. Brune, and L. Rossander-hulthen, “Is There a Physiological Role of Vitamin C in Iron Absorption ?,” *Ann. N. Y. Acad. Sci.*, vol. 498, no. 1, pp. 324–332, 1987.

[36] D. M. A., “Etude des plantes médicinales de niafunke (région Tombouctou) Phytochimie et pharmacologie de Maerua crassifolia Forsk (Capparidacée) [These de Doctorat].Université de Bamako, p.125,” 2005.

[37] S. Salzano, P. Checconi, E. Hanschmann, C. Horst, and L. D. Bowler, “Linkage of inflammation and oxidative stress via release of glutathionylated peroxiredoxin-2 , which acts as a danger signal,” *Proc. Natl. Acad. Sci.*, vol. 11, no. 33, pp. 12157–12162, 2014.

[38] and B. B. A. Simone Reuter, Subash C. Gupta, Madan M. Chaturvedi, “Oxidative stress, inflammation, and cancer: How are they linked?,” *Free Radic Biol Med.*, vol. 49, no. 11, pp. 1603–1616, 2010.

[39] B. B. Aggarwal, R. V Vijayalekshmi, and B. Sung, “Targeting Inflammatory Pathways for Prevention and Therapy of Cancer : Short-Term Friend , Long-Term Foe argeting Inflammatory Pathways for Prevention and Therapy of,” *Clin. Cancer Res.*, vol. 15, no. 2, pp. 425–430.

[40] A. J. Schetter, N. H. H. Heegaard, and C. C. Harris, “Inflammation and cancer : interweaving microRNA , free radical , cytokine and p53 pathways,” *Carcinogenesis*, vol. 31, no. 1, pp. 37–49, 2010.

[41] W. Lin and M. Karin, “A cytokine-mediated link between innate immunity , inflammation , and cancer,” *J. Clin. Invest.*, vol. 117, no. 5, pp. 1175–1183, 2007.

[42] L. M. Coussens and Z. Werb, “Inflammation and cancer,” *Nature*, vol. 420, no. 6917, pp. 860–867, 2002.

[43] S. P. Hussain, L. J. Hofseth, and C. C. Harris, “Radical causes of cances,” *Nat. Rev. Cancer*, vol. 3, no. 4, pp. 276–285, 2003.

[44] J. M. M. and F. Sánchez-Jiménez, “Antioxidant enzymes and their implications in pathophysiologic processes,” *Front. Biosci.*, vol. 4, pp. 339–345, 1999.

[45] M. Kanshari, Nemat;Shakiba, Yodallah;Mahmoudi, “Chronic Inflammation and Oxidative Stress as a Major Cause of Age- Related Diseases and Cancer,” *Recent Pat. Inflamm. Allergy Drug Discov.*, vol. 3, no. 1, pp. 73–80, 2009.

[46] N. Eiró and F. J. Vizoso, “Inflammation and cancer,” *World J. Gastrointest. Surg.*, vol. 4, no. 3, pp. 62–72, 2012.

[47] S. Golbidi, M. Badran, and I. Laher, “Antioxidant and Anti-Inflammatory Effects of Exercise in,” *Exp. Diabetes Res.*, vol. 2012, 2012.

[48] S. A. Adebayo, J. P. Dzoyem, L. J. Shai, and J. N. Eloff, “The anti-inflammatory and antioxidant activity of 25 plant species used traditionally to treat pain in southern African,” *BMC Complement. Altern. Med.*, vol. 15, no. 1, p. 159, 2015.

[49] S. Loizou, I. Lekakis, G. P. Chrousos, and P. Moutsatsou, “b -Sitosterol exhibits anti-inflammatory activity in human aortic endothelial cells,” *Mol. Nutr. Food Res.*, vol. 54, no. 4, pp. 551–558, 2010.

[50] D. L. Lucetti *et al.*, “Anti-inflammatory effects and possible mechanism of action of lupeol acetate isolated from Himatanthus drasticus (Mart .) Plumel,” *J. Inflamm.*, vol. 7, no. 1, p. 60, 2010.

[51] W. O. Obonga, C. O. Nnadi, M. O. Agbo, and F. C. Kenechukwu, “Distribution of Methicillin-Resistant Staphylococcus aureus (MRSA) in Apparently Healthy Population and its Susceptibility to Saponins from Dialium guineense,” *Br. J. Pharm. Res.*, vol. 4, no. 18, pp. 2200–2209, 2014.

[52] A. B. R. M. Librado A Santiago, “Lupeol: An antioxidant triterpene in Ficus pseudopalma Blanco (Moraceae),” *Asian Pac. J. Trop. Biomed.*, vol. 4, no. 2, pp. 109–118, 2014.

[53] M. K. Tchimene, C. O. Nwaehujor, M. Ezenwali, C. C. Okoli, and M. Iwu, “Free Radical Scavenging Activity of Lupeol Isolated from the Methanol Leaf Extract of Crateva adansonii Oliv . (Capparidaceae),” *Int. J. Pharmacogn. Phytochem. Res.*, vol. 8, no. 3, pp. 419–426, 2016.

[54] M. K. Parvez, P. Alam, A. H. Arbab, M. S. Al-dosari, T. A. Alhowiriny, and S. I. Alqasoumi, “Analysis of antioxidative and antiviral biomarkers b -amyrin , b -sitosterol , lupeol , ursolic acid in Guiera senegalensis leaves extract by validated HPTLC methods,” *Saudi Pharm. J.*, vol. 26, no. 5, pp. 685–693, 2018.

[55] C. S. F. Atif B. Awad, Andrew T. Burr, “Effect of resveratrol and b -sitosterol in combination on reactive oxygen species and prostaglandin release by PC-3 cells,” *Prostaglandins. Leukot. Essent. Fatty Acids*, vol. 72, no. 3, pp. 219–226, 2005.

VI – Annexes : Travaux collaboratifs sur les molécules bioactives naturelles

Article 5

Gbohaïda Virginie, Agbangnan D. C. Pascal, Nonviho Guévara, Gnansounou Martial, Bothon F. T. Diane, Bogninou G. S. Reine, Avlessi Félicien and Sohounhloué C. K. Dominique. Chemical Study and Evaluation of the Influence of two Physical Parameters on Polyphenols Extraction from *Carapa procera* Leaves.

Plusieurs études scientifiques ont examiné les composés bioactifs de plantes médicinales et ont montré que les polyphénols étaient leurs métabolites secondaires les plus importants. En effet, ils sont connus pour leurs propriétés antioxydantes, anti-inflammatoires, antifongiques, antivirales et anticancéreuses. Le présent travail a porté sur la cinétique d'extraction de polyphénols à partir des feuilles d'une plante médicinale du Bénin. Nous avons évalué l'influence de deux paramètres physiques (taille des particules et température) sur la cinétique d'extraction à l'éthanol des feuilles de *Carapa procera*. Cette plante est connue dans la pharmacopée traditionnelle du Bénin pour le traitement d'affections diverses.

Le criblage phytochimique de l'extrait éthanolique a révélé la présence de plusieurs métabolites secondaires tels que les saponines, coumarines, sucres réducteurs, anthraquinones et polyphénols combinés (anthocyanines, flavonoïdes et tanins catéchiques) à 62.28 mg/g. Son activité antiradicalaire (IC50 = 0,16 mg / mL) est proche de celui de la quercétine (IC50 = 0,1 mg / mL). L'évaluation de l'influence de la granulométrie a révélé que le solvant d'extraction diffuse plus facilement à l'intérieur des petites particules pour extraire les polyphénols molécules.

Considérant le coût élevé d'une l'application de la température et le risque de désintégration des composés extraits, 50 ° C pourrait être selon nos résultats, la température optimale pour un meilleur rendement d'extraction des polyphénols à partir des feuilles de *Carapa procera*.

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CHEMICAL STUDY AND EVALUATION OF THE INFLUENCE OF TWO PHYSICAL PARAMETERS ON POLYPHENOLS EXTRACTION FROM *CARAPA PROCERA* LEAVES.

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ABSTRACT

Background: Various specific biochemical properties of polyphenols are known and their study increasingly attracts the attention of scientists and researchers all over the world. But their extraction from the plant matrix is the major obstacle to their availability. **Objective:** Present work was carried out to study the extraction kinetic of bioactive polyphenols. **Material and Methods:** We evaluated the influence of two physical parameters, (particle size and temperature) on the bioactive polyphenols extraction kinetic from *Carapa procera* leaves. This plant is more known in Benin's traditional pharmacopoeia for the treatment of common affections. **Results:** The phytochemical screening revealed the presence of several secondary metabolites such as saponins,coumarins, reducing sugars, combined anthraquinones and polyphenols (anthocyanins, catechic tannins and flavonoids). *Carapa procera* leaves contain very few leucoanthocyanins, proteins and alkaloids, while the mucilages and Gallic tannins aren't detected. The ethanolic extraction on *Carapa procera* leaves gave a yield of 17.8% and a phenolic compounds content of 62.28 $mg.g^{-1}$ withanaverage antiradical power (IC_{50} = 0.16mg/mL) close to one of

quercetin (IC_{50} = 0.1mg/mL). The granulometry's influence evaluation revealed that the extraction solvent diffuses more easily inside the small particles for extracting polyphenols molecules. Considering the high cost implicated by the temperature application and the disintegration risk of the compounds extracted, 50°C might be according to our results, the optimal temperature for better extraction yield of polyphenols from *Carapa procera* leaves.

Conclusion: Physical parameters influence revealed that the extraction solvent diffuses more easily inside the small particles at 50°C to extract polyphenol molecules.

KEYWORDS: phytochemical screening, polyphenols, extraction, granulometry, temperature.

INTRODUCTION

Fromthe Meliaceae family, *Carapa procera* is a large tree reaching 30 to 35 m high and 1m in diameter. Belonging to the family of Meliaceae its young foliage is red. The paripinnate leaves covering 8-16 leaflets end with an aborted leaf bud. The accumulation of spine ground is characteristic of the species. The stem bark tends to flake in rectangular plates. Fruit ripening requires one year and is in open capsule containing large pyramidal seeds dispersed by rodents. Its wood has a pleasant smell typical of Meliaceae.[1] Originally from the west coast of tropical Africa, this plant is found in South America in Brazil, on the Guyana Shield, in West Africa and Central Africa from Senegal to Angola.[2] It is usually used in African villages in the development of local treatments such as malaria, skin diseases,.[3] Despite the many virtues recognized to this plant in traditional medicine, very little data have been reported in the literature regarding its phenolic composition and anti-radical activity.

The extraction of its secondary metabolites has never been subject of scientific investigation. In addition, thousands of scientific studies have examined the bioactive compounds from medicinal plants and found that polyphenols are their most important secondary metabolites.[4] Indeed, they are known for their antioxidant, anti-inflammatory, antifungal, antiviral and anticancer properties.[5] The antioxidants in our diet are, for the most, polyphenols. Over two hundred studies were conducted on the effect of plant consumption on health. Most of them showed a decrease of the risk factor for many diseases (heart, lung, colon, stomach, kidney, prostate and breast cancer.). Polyphenols having antioxidant activity are increasingly studied. Indeed, oxidation is a widely spread as well in food (lipid oxidation) and physiological (oxidative stress) phenomenon. Due to their antioxidant properties, polyphenols have the ability to scavenge free radicals, which are generated continuously by the body or formed in response to attacks from our environment (smoking, pollutants, infections.) and are, for the most, the base of the reduction of our perishable foodstuffs life.

But extraction is the most important step in the production and characterization of active ingredients from plant material. It is influenced by the extraction method selected according

to phytochemical compounds investigated. Several factors such as pH, temperature, amount of material to the volume of solvent, time intervals, particle size, number and steps of individual extraction, play an important role in this process.[6] Phenolic compounds are heat sensitive, so it is urgent to find suitable methods for making available these active principles. Thus, the main objective of this study is to evaluate the influence of the particle size and temperature on the kinetic of polyphenols extraction from *Carapa procera* leaves. But first, it is important to identify secondary metabolites present in the plant and assess its phenolic content as well as antioxidant properties of its polyphenols.

MATERIAL AND METHODS

Plant material

The plant material used in the present study is constituted of *Carapa procera*'s leaves collected at "Sakété" in the department of Plateau in Benin. After drying at the laboratory temperature (20°C±5°C) for stabilization of their mass and to avoid risk of polyphenols oxidation, the samples were reduced in powder to facilitate solvent penetration.

Methods

We used three different mesh sieves (300 µm, 600 µm and 1.18 mm) for particle size influence and a thermostat bath of refluxing equipped with thermocouple for temperature control during the extraction.

Large families of chemical compounds were detected according to the methods reported by Houghton.[7] For determining the yield and the phenolic compounds content, the solid-liquid extraction was performed by soaking in ethanol (95°) with a ratio of 5%. 50 g of dried plant material were mixed with 500 mL of solvent. The mixture is maintained under magnetic stirring for 24 hours at room temperature.[8] The solution obtained was filtered through Whatman N°1 paper (0.16 mm in diameter) under reduced pressure. The filtrate was then recovered and the operation was repeated 3 times (72 hours total extraction) with 250 mL of solvent from the second day. The total volume of the filtrate was concentrated under vacuum at 60°C on a rotavapor. The dry extract was then collected, weighed, labeled and stored at 4°C until use. The extraction yield was calculated using the following formula:

$$\mathbf{Y = [(M_{ext})/ M_{hd}] \times 100}.$$

Y: yield (%); Mext: extract mass; M_{hd}: herbal drug mass.

To quantify the phenolic compounds, the ethanol extract was assayed by colorimetric UV-Visible spectrophotometry; total polyphenols were measured by the Folin-Ciocalteu,[9] the total flavonoids by aluminium trichloride,[10] the condensed tannins by sulfuric vanillin[11] and anthocyanins by sodium sulphite.[12] The antiradical activity was determined by DPPH (2,2-diphenyl-1-pycrilhydrazil) method reported by Agbangnan *et al*.[13] The percentage of free radical scavenging DPPH was calculated using the formula:

$IP = [(A_{bl} - A_s)/A_{bl}] \times 100$.

IP:Inhibition percentage; A_{bl}: absorbance of the blank; A_s: absorbance of the sample.

For extraction kinetic study, the influence of two very critical physical parameters (size and temperature) was evaluated. Colorimetric method based on the use of UV-Visible spectrophotometer was used to monitor the polyphenols extraction kinetic from the plant material. 2g of the crushed (particle size 300 µm, 600µm or 1.18 mm) are macerated in 200 mL of distilled water. For the influence of the temperature, 5 different temperatures, 25°C to 125°C with a step of 25°C, were used. The polyphenols' diffusion in distilled water was observed through the color change of the medium over time, according to the particle size and temperature. Samples were then taken every 10 min until 1hour and polyphenols were quantified on colorimeter after filtration and adequate dilution (1/5). Distilled water was the reference solvent used as blank.[6]

RESULTS AND DISCUSSION

Phytochemical screening

Various secondary metabolites were identified in the plant by a series of reactions of precipitation and colouring more or less specific to each class of plant active ingredients. The results of phytochemical screening of *Carapa procera* leaves reported in table1 revealed the presence of saponins, anthocyanins, catechic tannins, flavonoids, coumarins, reducing sugars and combined anthraquinones. This organ of the plant contains very few leucoanthocyanins, proteins and alkaloids while the mucilage and Gallic tannins are not detected. Our results are similar to those of Adjè who identified in the leaves of *Carapa procera* the presence of chemical compounds such as anthocyanins, flavonols and phenolic acids.[14] Also, the investigations of Ononga *et al.* revealed in *Carapa procera* plant the presence of flavonoids, saponins and alkaloids. Tannins, quinones, steroids and terpenoids aren't detected.[15] According to the literature, environmental factors influence the production of secondary

metabolites in plants. The differences observed could be related to the fact that samples were collected in different regions and at different times of the year.[16]

Table 1: Metabolites identified in *Carapa procera* leaves

Class of substances		Results
Tannins	Total tannins	+
	Cathechic tannins	+
	Gallic tannins	-
Flavonoids	Anthocyanins	+
	Free flavonoids	+
	Leucoanthocyanins	±
Mucilage		-
Alkaloids		±
Sterols and terpenes		±
Proteins		±
Reducing sugars		+
Free quinones		-
Combined anthraquinones	O-heterosids	-
	Reduced genine O-heterosids	+
	C-heterosids	+
Coumarins		+
Saponins	Foam index	105

+ : Present ; ± : Trace; - : absent.

Phenolic content

The aqueous extract of *Carapa procera* leaves has shown an extraction yield of 17.8%. The results of quantitative analyses of phenolic compounds content in the extract of *Carapa procera* leaves are reported in Fig 1. It appears that the *Carapa procera* leaves extract is very rich in total polyphenols (62.28mg Gallic Acid equivalent/g of dry matter) and has an average content of condensed tannins (24.49 mg Catechin equivalent/g dry matter) and flavonoids (37.61 mg Catechin equivalent/g dry matter). It should be noted that this organ of the plant contains very few anthocyanins (1.92 mg Cyanidin equivalent/g dry matter). The investigations of Adje on *Carapa procera* leaves indicated yields of 5 to 10% powders of polyphenolic extracts (5.1-27.2 mg GAeq.g^{-1}) obtained by atomization.[14] The differences between our results and those of Bothon may be related to methods of extraction and quantification, which are two factors that may affect the phenolic content of plants.[17]

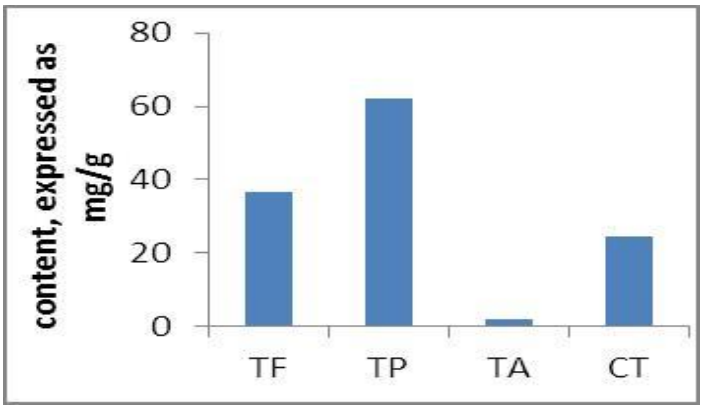

Fig 1: Phenolic composition of *Carapa procera* leaves extract

TF: total flavonoids; TP: total polyphenols; TA: total anthocyanins; CT: condensed tannins

Antiradical activity

DPPH is the best, easiest and widely used method for testing preliminary free radical scavenging activity of a compound or a plant extract.[16] The DPPH test provides information on the reactivity of the test compounds with a stable free radical. DPPH gives a strong absorption band at 517 nm in visible region. When the odd electron becomes paired off in the presence of a free radical scavenger, the absorption reduces and the DPPH solution colour changes from deep violet to light yellow. The degree of reduction in absorbance measurement is indicative of the radical scavenging (antioxidant) power of the extract. The radical scavenging activity of *Carapa procera* leaves' ethanolic extract was determined by DPPH method using quercetin as reference antioxidant. *Carapa procera*'s leaves ethanolic extract had an average antiradical power (IC_{50} = 0.16 mg/mL) close to one of quercetin (IC_{50} = 0.1 mg/mL). Adjè in his investigations, has also notified the antioxidant capacity determined by DPPH method of *Carapa procera* leaves.[14] We note a correlation between antiradical activity of our extracts and their phenolic content. This observation corroborates those already made earlier by Medoatinsa *and al*.[18] Several studies support the antiradical activity by the presence of total polyphenols.[19] The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralising free radicals, quenching singlet and triplet oxygen, or decomposing peroxides.[16]

Kinetic study

The absorption curves of aqueous extracts of *Carapa procera* leaves (Fig 2) revealed two peaks in the Ultra-Violet rang (240 nm and 279 nm) corresponding to the absorption of polyphenols in general.[20] Moreover, the perfect overlapping of curves recorded as a function

of time shows that the extraction of phenolic compounds remain the same regardless of the extraction time.

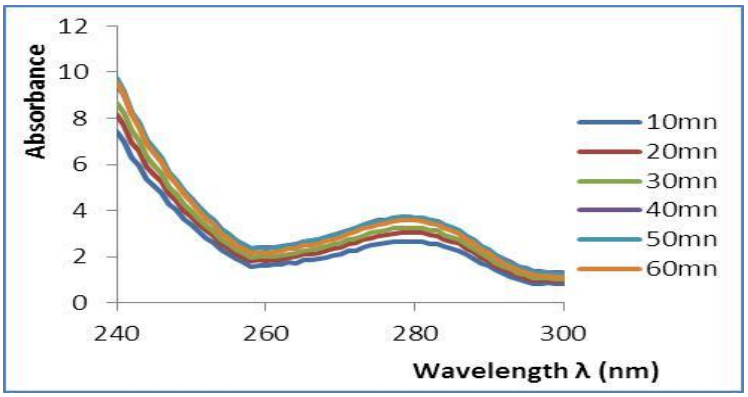

Fig 2: UV spectra of aqueous extracts from *Carapa procera* leaves

The graph TPI (Total Polyphenols Index) versus time (Fig 3) reflecting changes in the amount of polyphenols extracted versus time shows an increasing trend with a steep slope during the first 20 minutes. In addition, average growth was observed between 20 and 40 minutes. After 40 minutes, the extraction rate does not evolve practically.

In general, at first stage, the TPI increased fast, followed by a slow increment and then remained practically constant till the end of the process. This asymptotic behavior was found previously by other authors.[21, 22, 23]

Most of the phenolic compounds were therefore transferred from the vegetable matrix to the solvent during the first 20 minutes and 40 minutes would be sufficient for an almost complete extraction of the polyphenols from the leaves of *Carapa procera*. However, the extraction time can be extended up to 60 minutes without extracted compounds degradation risk.

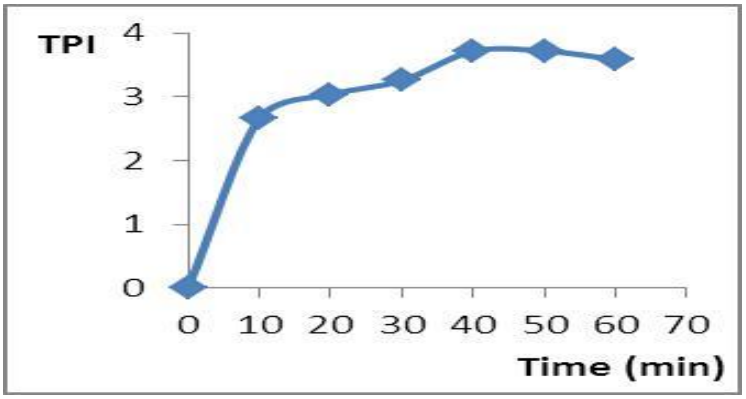

Fig 3: Polyphenol extraction kinetic of *Carapa procera* leaves

Influence of particle size

The results of the influence of particle size on the polyphenols extraction kinetic from *Carapa procera* leaves are shown in the graph of Fig 4. There was a high extraction rate of the particle size of 300 microns. While the other two sizes, we didn't observe a significant difference in the evolution of the extraction rate over time. This had be explained by the fact that the solvent diffuses more easily inside the small particles to extract polyphenols molecules. This confirms our previous results according to which, with the fine particles, chemical compounds are more easily transferred from the plant material to the extraction solvent.[8] Penchev, in his work on bioactive products also notified the significant influence of particle size on the extraction rate in the sense that, the best result is obtained with small particles, this due to their larger specific surface.[6] The finer particles thus have a greater solid-solvent contact surface.

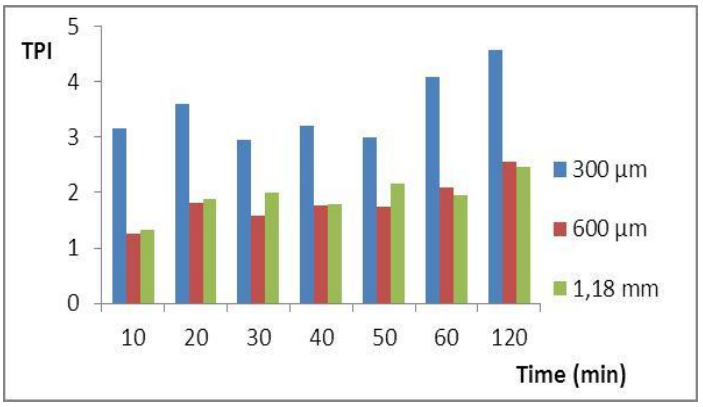

Fig 4: Influence of granulometry on the polyphenols extraction kinetic from *Carapa procera* leaves

Effect of temperature

The influence of temperature on the polyphenols extraction kinetic from *Carapa procera* leaves was carried out on the powder of particle size 300 microns to give the best extraction efficiency in consideration of particle size influence. The results obtained are shown in the graph of Fig 5. The shift range of the temperature used (25°C to 125°C) of the envelope values starting from room temperature (25°C) and up to above the boiling point of the solvent (100°C). The analysis of this graph showed that extraction rate of polyphenols increases with temperature up to 100°C and fall beyond. 100°C would be the maximum temperature tolerable by the extracted molecules would deteriorate beyond. Moreover, we noted that the polyphenols quantity extracted from the leaves of *Carapa procera* in 10 min at 50°C (IPT =

5.72) was higher than that obtained after 120 min at 25°C (IPT = 4.57). But beyond 50°C, the increase of the extraction yield in function of time is low indeed null.

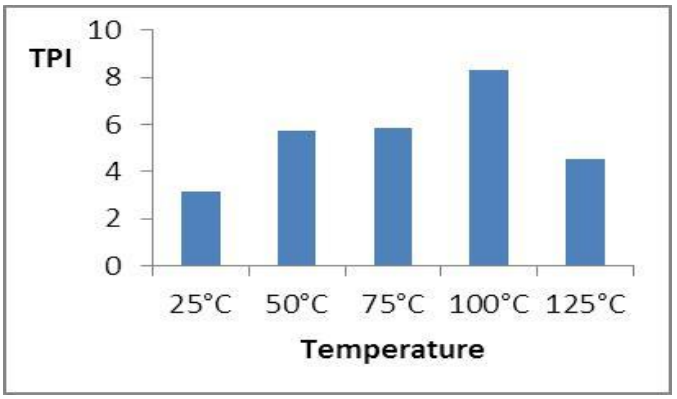

Fig 5: Influence of temperature on the kinetic of extraction of polyphenols from *Carapa procera* leaves

Considering the cost it would take to increase the temperature and the risk of degradation of the extracted compounds, 50°C would be according to our results, the optimal temperature for proper extraction of phenolic compounds from the leaves of *Carapa procera*. These results are consistent with those of Penchev *et al.* showed that the extraction rate increases with temperature and 50°C is the optimal temperature, since maintaining this temperature is more economic in terms of energy consumption.[24] The work of Penchev has also shown that 60°C is a reasonable value for temperature, preserving, on the one hand the active components of the plant with a thermal destruction and, secondly, ensuring intensive kinetic regime.[6] Agbangnan *et al.* have shown that increasing the temperature affects positively the extraction of polyphenols without necessarily offset the cost that would result from the application of this temperature increase.[20]

CONCLUSION

This study revealed the importance of controlling the extraction conditions such as temperature and particle size to obtain an extract with the highest polyphenol content with best antioxidant activity. The extraction solvent diffuses more easily within small particles to extract polyphenol molecules. 50°C is the optimum temperature for better extraction yield of polyphenols from the leaves of *Carapa procera*.

REFERENCES

(d) Caventou E. Deuxième Mémoire sur les végétaux des familles Méliacées et Cédrélacées: Du *Carapa Touloucouna (senegalensis)*, Impr. E. Thunot, Paris: 1859.

(c) Office National des Forêts. Guide de reconnaissance des arbres de Guyane - 120 essences décrites, ONF Guyane (FR), 2004; 374p.

(d) Clarke R. Some further remarks on the economical and medical uses of the oil commonly called Expired croupee on the Gold Coast, touloucouna at the Gambia and Senegal and kundah at Sierra Leone. The Pharmaceutical Journal, 1860; 3p.

(e) Autran JC. Le guide de phytothérapie créole: Se soigner par les plantes créoles, Ile Réunion, édition Orphie: 2010.

(f) Hamza OJM, van den Bout-van CJP, Matee MIN, Moshi MJ, Mikx FHM, Selemani HO, Mbwambo ZH, Van der Ven AJAM, Verweij PE. Antifungal activity of some Tanzanian plants used traditionally for the treatment of fungal infections. Journal of Ethnopharmacology, 2006; 108(1): 124-32.

(g) Penchev PI. Etude des procédés d'extraction et de purification de produits bioactifs à partir de plantes par couplage de techniques séparatives à basses et hautes pressions. Thèse de doctorat, Université de Toulouse, France, 2010; 239p.

(h) Houghton PJ, Raman A. Laboratory handbook for the fractionation of natural extracts London. Chapman and all: 1998.

(i) Gbohaïda V, Mèdoatinsa SE, Nonviho G, Bogninou-Agbidinoukoun GSR, Agbangnan DCP et Sohounhloué DCK. Etude chimique et évaluation de l'Influence de la granulométrie sur la cinétique d'extraction des polyphénols naturels de *Pterocarpus erinaceus* acclimaté au Bénin. International Journal of Innovation and Applied Studies, 2015; 12(2): 325-33.

(j) Wong CC, Li HB, Cheng KW, Chen F. A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay. Food Chem, 2006; 97: 705-11.

(k) Enujiugha VN. The Antioxidant and Free Radical Scavenging Capacity of Phenolics-from African Locust Bean Seeds (*Parkia biglobosa*). Advances in Food Sciences, 2010; 32(2): 88-93.

(l) Xu BJ and Chang SKC. A Comparative Study on Phenolic Profiles and Antioxidant Activities of Legumes as Affected by Extraction Solvents," *Journal of Food Science*, 2007; 72(2): 160-1.

(m) Ribereau-Gayon P, Stone street E. Bulletin de la Société Chimique de France, 1965; 9: 2649-52.

4. Agbangnan DCP, Noudogbessi JP, Chrostowska A, Tachon C, Fouquet E and Sohounhloue DCK. Phenolic compound of Benin's red *sorghum* and their antioxidant properties, Asian J. Pharm Clin. Res., 2013; 6(2): 277-80.
5. Adje FA. Production par procédés membranaires couplés d'extraits polyphénoliques de *Carapa procera*, *Delonix regia* et *Hibiscus sabdariffa*. Détermination des structures moléculaires et d'activités antioxydantes. Thèse de doctorat, Université Paul Cezanne (Aix-Marseille), 2009; 420 p.
6. Onanga M, Ekouya E, Ouabonzi A, Itoua CB. Etudes ethnobotanique, pharmacologique et chimique des plantes utilisées dans le traitement des dermatoses "Mwandza". Pharm Méd Trad Afr, 1997; 9: 85-93.
7. Bothon FTD, Moustapha M, Bogninou GS, Agbangnan DCP, Yehouenou B, Medoatinsa SE, Noudogbessi JP, Avlessi F and Sohounhloué DCK. Chemical Characterization and Biological Activities of *Newbouldia laevis* and P*terocarpus Santalinoides* Leaves. Bull. Env. Pharmacol. Life Sci., 2014; 3(11): 09-15.
8. Lee KW, Kim YJ, Lee HJ, Lee CY. Cocao has more phenolic phytochemicals and a higher antioxidant capacity than teas and red wine. Journal of agricultural and Food chemistry, 2003; 51(25): 7292-5.
9. Medoatinsa SE, Agbangnan DCP, Atchade PS, Lagnika L, Gbohaida V, Bothon FTD, Ahissou H, Sohounhloue DCK. Radical Scavenging and Antiplasmodial Activity of *Polygonum senegalense* of Benin. Int. J. Pharm. Phytopharmacol. Res., 2014; 4(1): 13-7.
10. Hasan SMR, Hossain MM, Akter R, Jamila MM, Mazumder MEH, Rahman S. DPPH free radical scavenging activity of some Bangladeshi medicinal plants. J Med Plants Res., 2009; 3(11): 875-79.
11. Agbangnan DCP, Tachon C, Bonin C, Chrostowka A, Fouquet E, Sohounhloué DCK. Phytochemical study of a tinctorial plant of Benin traditional pharmacopoeia: the red sorghum (*Sorghum caudatum*) of Benin. Scientific Study & Research, 2012; 13(2): 121-35.
12. Bucic-Kojic A, Planinic M, Tomas S, Bilic M and Velic D. Study of solid-liquid extraction kinetics of total polyphenols from grape seeds. Journal of Food Engineering, 2006; 8: 236-42.
13. Sánchez M, Sineiro, J and Núñez MJ. Extraction of polyphenols from white distilled grape pomace: Optimization and modeling. Bioresource Technology, 2008; 99: 1311-18.
14. Librán CM, Mayor L, Garcia-Castello EM, Vidal-Brotons D. Polyphenol extraction from grape wastes: Solvent and pH effect. Agricultural Sciences, 2013; 4(09): 56-62.

7. Penchev PI, Angelov G, Condoret JS. Extraction des agents antioxydants (acide rosmarinique) à partir de la mélisse *(Melissa officinalis* L.). Revue de Génie Industriel, 2010; 5: 115-23.

Article 6

Samy Iskandar, Martial Senankpon Gnansounou, Maxime Robin, Jean Lorquin, Carole Di Giorgio and Philippe Piccerelle. Antioxidant, Anti-inflammatory and Neuroprotective Activities of a Plant Extract Derived from Traditional Chinese Medicine: SuHeXiang Wan (AT000). Chemistry of Advanced Materials 3(2) (2018) 36-59.

Alzheimer est une maladie neurodégénérative incurable des tissus cérébraux qui entraîne une perte progressive et irréversible des fonctions mentales et en particulier de la mémoire. C'est la cause la plus fréquente de démence chez l'homme.

Dans ce travail, Nous avons étudié les activités antioxydantes, anti-inflammatoires et neuroprotectrices d'une préparation nommée AT000 modifiée d'une recette (SuHeXiang Wan) utilisée en médecine traditionnelle chinoise pour le traitement de l'épilepsie et des convulsions. La synergie de *Dryobalanops aromatica* et *Saussurea lappa* (deux parmi les neuf plantes de la formule) dont l'utilisation dans les compléments alimentaires est considérée comme controversée, a été évaluée pour la première fois. L'activité antioxydante de l'extrait a été évaluée par les tests au DPPH et à l'ORAC (Oxygen Radical Absorbance Capacity). L'activité anti-inflammatoire a été déterminée en mesurant la capacité des macrophages à générer une forte réponse inflammatoire lorsqu'ils sont stimulés par des antigènes, induisant une libération de monoxyde d'azote (NO). L'efficacité de l'extrait sur l'atténuation des déficits d'apprentissage induits par l'Aβ25-35 (mémoire de travail spatiale : alternance spontanée dans le labyrinthe en Y et mémoire à long terme contextuelle : test d'évitement passif a été évaluée *in vivo* chez la souris sept jours après l'administration du peptide. L'impact sur la peroxydation des lipides dans l'hippocampe, un indice de stress oxydatif, a également été évalué.

L'extrait AT000 a montré une forte activité antioxydante à 2mg/mL, 10mg/mL et 301774 équivalents de Trolox selon les tests DPPH et ORAC respectivement. Le traitement par AT000 après 21 jours a permis de réduire de manière dose-dépendante les déficits induits par Aβ25-35, avec une prévention significative à la dose la plus élevée testée (250 mg/kg/jour) sur les paramètres d'alternance spontanée, de latence intermédiaire et de latence d'échappement. Le traitement AT000 de 21 jours, a atténué en fonction de la dose l'augmentation de la peroxydation des lipides, induite par Aβ25-35 avec un blocage significatif et complet aux doses les plus élevées testées. Des expériences synergiques ont montré que la présence de *Dryobalanops aromatica* et de *Saussurea lappa* est cruciale pour obtenir un effet neuroprotecteur. Selon ces résultats, AT000 pourrait être un composé candidat pour le développement de médicaments destinés à la prévention et au traitement de la maladie d'Alzheimer.

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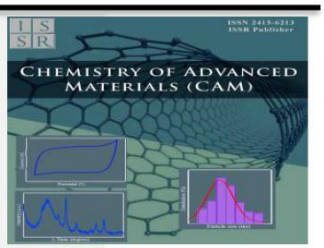

Original paper

Antioxidant, Anti-inflammatory and Neuroprotective Activities of a Plant Extract Derived from Traditional Chinese Medicine: SuHeXiang Wan (AT000)

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Abstract

In this paper, we present SuHeXiang Wan, a medicine used in traditional Chinese medicine for the treatment of epilepsy and convulsions. We investigated the antioxidant, anti-inflammatory and neuroprotective activities of the same treatment designated as AT000. The synergy of the two plants, *Dryobalanops aromatica* and *Saussurea lappa*, of which the use in alimentary supplements is considered controversial, was evaluated for the first time. The antioxidant activity of the extract was assessed by DPPH and ORAC tests while the anti-inflammatory activity was determined by measuring the capacity of macrophages to generate a strong inflammatory response when stimulated with antigens, inducing NO release. The extract efficacy on the attenuation of the $A\beta_{25-35}$-induced learning deficits (spatial working memory: spontaneous alternation in the Y-maze and contextual long-term memory: passive avoidance test) was evaluated *in vivo* in mice seven days after the peptide administration. The impact on lipid peroxidation in the hippocampus, an index of oxidative

stress, was also evaluated. AT000 extract showed a strong antioxidant activity at 2 mg/mL, 10 mg/mL and 301774 Trolox equivalents according to the DPPH and ORAC tests respectively. The 21-days AT000 treatment dose-dependently alleviated $A\beta_{25-35}$-induced deficits, with significant prevention at the highest dose tested (250 mg/Kg/day) on the spontaneous alternation, step-through latency and escape latency parameter. 21-days AT000 treatment dose-dependently attenuated also $A\beta_{25-35}$-induced increase lipid peroxidation, with a significant and complete blockade at the highest doses tested. Synergistic experiments showed that the presence of *Dryobalanops aromatica* and *Saussurea lappa* is crucial to obtain a neuroprotective effect. According to these results, AT000 could be a candidate compound in the development of therapeutic drugs for the prevention and treatment of Alzheimer's disease.

1. Introduction

Alzheimer's disease (AD) is an incurable neurodegenerative disease of cerebral tissue that leads to progressive and irreversible loss of mental functions and especially memory. It is the most common cause of dementia in humans [1]. AD is characterized by two primary lesions: senile plaques which are toxic extracellular aggregates of amyloid peptides and neurofibrillary tangles which are intracellular aggregates formed of pairs of helical filaments due to the abnormal formation of hyperphosphorylated tau protein masses [2]. The clinical symptoms of this disease include memory loss [3], especially recent events in the early phases of the disease and deterioration of other cognitive functions that interfere with mood, reasoning, judgment and language [4, 5].

From a histopathological point of view, abnormal cleavage by beta-secretase of amyloid precursor protein leads to the extracellular accumulation of neurotoxic peptide $A\beta_{42}$ generating senile plaques which compress neurons (n) Oligomeric peptides Aβ play a significant role in mediating neurotoxicity and leading to AD [7]. They provoke strong alterations of plasticity mechanisms leading to a decline in memory [8, 9].

Oligomers of Aβ peptides vary in length between 40 and 43 amino acids. $A\beta_{40}$ and $A\beta_{42}$ are the most abundant species found in AD brains [10], however only the $A\beta_{42}$ fragment forms fibrillar deposits readily [11]. Another toxic minor fragment $A\beta_{25-35}$ has also been identified and used widely in research [12, 13]. Aβ oligomers interact with the intracellular organelles responsible for the regulation of calcium homeostasis leading to oxidative stress and causing neuronal apoptosis 15. Aβ peptides can also provoke an inflammatory response in the brain and cytokine production by active astrocytes and microglia [15].

Several studies have shown the presence of lipids and proteins oxidation products in the tissues of Alzheimer's patients after death [16]. Further studies suggested the presence of a link between increased oxidative stress promoted by Aβ and the presence of senile plaques [17].

Despite scientific advances in this field, at present, there is no effective treatment to inhibit the progression of the disease and stop the cognitive decline. Since brain lesions (amyloid plaques and

neurofibrillary degeneration) trigger a decrease in the neurotransmitter acetylcholine that allows neurons to communicate. Drug treatment options are currently limited to acetylcholine esterase inhibitors. Anticholinesterasics include three different molecules that have been rigorously tested and have proven to be effective in mild-to-moderate forms of the disease: Donepezil, Galantamine and Rivastigmine [18]. These treatments help to stabilize the disease, however; they do not reverse it nor cure it. In addition, these molecules can interact with a wide range of drugs and their consumption is associated with numerous side effects [19].

In non-drug therapies, to alleviate the symptoms of AD and improve cognitive functions, plants were used. The advantage of medicinal plants is their richness in compounds, which can act synergistically with other compounds from the same or another plant. plant-derived molecules can also boost the activity of the constituents or neutralize the toxic effects of compounds from other plant species [20]. Today, traditional Chinese medicine is a potential alternative to drug treatments. By testing different combinations of plants having potential neuroprotective activities, several studies in this field have shown that plant extracts derived from traditional Chinese medicine help fight against the symptoms of neurodegenerative diseases such as AD [21-24].

SuXeHiang Wan, an extract of 9 plants that have been used in the treatment of epilepsy and convulsions, has demonstrated a sedative and anticonvulsant effect as well as inhibitory effects on the central nervous system following the inhalation of its Essential oil [24]. In mice, the essential oil, SuXeHiang Wan, was shown to attenuate the amyloid beta-induced alteration of memory by inhibiting the phosphorylation of tau protein [25]. This extract contains an unlisted plant in the French Pharmacopoeia (*Dryobalanops aromatica*) and another plant which its roots are declared in mutual recognition in the list B of French pharmacopoeia (*Saussurea lappa*). In the present study, the same extract designated as AT000 was evaluated for its antioxidant, anti-inflammatory and cytotoxic activities. We also investigated whether the oral administration of AT000 mixture can alleviate the pathology induced in mice injected intracerebroventricularly (i.c.v) with oligomeric amyloid-β_{25-35} peptide ($A\beta_{25-35}$). In addition, this study evaluated for the first time the synergistic effect of *Dryobalanops aromatica* and *Saussurea lappa in vivo*.

8. Materials and Methods

2.1 Materials

All medicinal plants were purchased from GIVAUDAN Shanghai-China. 2,2-diphenyl-1-picrylhydrazyl radicals (DPPH), N,O-Bis (trimethylsilyl) trifluoroacetamide (BSTFA), phosphate buffer, fluorescein, 2,2'-azobis (2-amidino-propane) dihydrochloride (AAPH), dimethyl sulfoxide (DMSO), Propyl gallate, Ascorbic acid, Griess modified reagent, Dexamethasone, RAW 264.7 cells, and Lipopolysaccharides (LPS) of *E.coli*, were purchased from Sigma-Aldrich (St Louis USA). Trolox was obtained from Calbiochem (San Diego, United States), Dulbecco's modified Eagle Minimum Essential Medium (DMEM), Penicillin/streptomycin and inactivated calf serum were purchased from PAN BIOTECH. $A\beta_{25-35}$ and scrambled amyloid-β protein (25-35) Sc.Aβ were purchased from Polypeptides (France).

2.2 Preparation of plant extract

AT000 was prepared as described previously with some modifications [24]. A total of 175 g of the mixture of *Liquidambar orientalis* (20 g),

Saussurea lappa (20 g), *Aquilaria agallocha* (15 g), *Santalum album* (20 g), *Boswellia carteri* (20 g), *Eugenia caryophyllata* (20 g), *Cyperus rotundus* (20 g), *Styrax benzoin* (20 g), and *Dryobalanops aromatica* (20 g), was pulverized and extracted once with 10 vol. of water/ethanol mixture (70:30, v/v) at 80-85 °C with reflux condenser for 3 hours. The extract was then vacuum filtered using a Büchner flask and evaporated using a rotary evaporator at 60 °C to give a sticky brown oil. To evaluate the impact of *Dryobalanops aromatica* and *Saussurea lappa*, we made 4 different extracts designed as AT formulations in the following manner: AT001: *Dryobalanops aromatica*

AT002: *Dryobalanops aromatica* + *Saussurea lappa*

AT007: AT000 without *Dryobalanops aromatica* and *Saussurea lappa*

AT008: AT000 without *Dryobalanops aromatica*

2.3 Determination of radical scavenging activity The radical scavenging activity of the extract was determined by measuring its ability to trap the stable free radical, DPPH as described by Brand-Williams *et al.* with some modifications [26]. Briefly, 0.05 mM solution of DPPH˙ was prepared in methanol, and 2.9 mL of this solution was added to 0.1 mL extract solution in methanol at different concentrations (from 0.2 to 10 mg/mL). The reaction mixture was stirred at room temperature in a dark chamber for 30 minutes, and the absorbance was recorded at 517 nm using a UV-Vis spectrophotometer (Ultrospec 3000 pro). Control was prepared by adding 2.9 mL of the DPPH solution (0.05 mM) to 0.1 mL of methanol. IC_{50} values, which represent the concentration of the extract that causes neutralization of 50% of the DPPH radicals, were calculated from the percentage inhibition (PI, %) versus concentration curve as Eq. (1). The inhibition of free radicals by DPPH (%) was calculated using the following equation:

$$PI\ (\%) = [1-(A_1/A_0)] \times 100 \quad (1)$$

Where A_0 is the absorbance of the control reaction and A_1 is the absorbance in the presence of the sample. Trolox, ascorbic acid and propyl gallate were used as positive controls. Measurements were performed in triplicate, and the corresponding standard deviation was calculated.

2.4 Oxygen Radical Absorbance Capacity Assay

The oxygen radical absorbance capacity (ORAC) assay was assessed in microplates as described by Ou *et al.* with some modifications 22. The plant extract, AAPH, and fluorescein were diluted in 100 mM potassium phosphate buffer (pH 7.4). 25µL of each extract (0.94-7.5 µg/mL) or phosphate buffer (blank) were mixed with 150 µL of fluorescein solution (8.21×10^{-5} mM) and incubated for 15 min at 37 °C. A volume of 25 µL of AAPH solution (153 mM final concentration) was added, and fluorescence was immediately monitored using an Infinite M200 TECAN plate fluorimeter at 2-min intervals for 90 min. the excitation and emission wavelengths used are 485 nm and 530 nm respectively. A calibration curve was performed with Trolox (0.4−12.5 µM) as standard. The ORAC values were calculated using the neat AUCs and expressed as µmol of Trolox equivalent per 100g of extract (µmol TE/100g). Trolox was used as a control standard.

2.5 Cell culture

Mouse macrophages cells (RAW 264.7) were maintained in DMEM with stable L-glutamine supplemented with Penicillin 100 IU/mL, streptomycin 100 µg/mL and 10% of inactivated calf serum at pH 7.2, freshly prepared, stored no longer than 3 weeks.

2.6 Anti-inflammatory and cytotoxicity assays

In vitro anti-inflammatory assay was determined by measuring the capacity of macrophages to generate a robust inflammatory response when stimulated with antigens, inducing NO release [28]. Cells were seeded into 48-well tissue culture plates at a concentration of 1.10^5 cells/mL (200 µL/well) for 24 hours at 37 °C (5% CO_2). Then the culture medium was replaced by 200 µL of medium containing the appropriate concentrations of AT000 extract, and cells were incubated at 37 °C in a humidified atmosphere of 5% CO_2/90% air for one hour. At the end of the incubation period, pro-inflammatory LPS from *E. coli* were added to cell cultures (1 µg/mL), and cells were incubated under the same conditions for 24 hours. NO release was evaluated indirectly by measuring the accumulation of nitrite/nitrate, the stable end-products of NO oxidation, in the culture supernatant by the Griess reaction. 100 µL of the supernatants were transferred into the wells of a 96-well tissue culture plate, and 100 µL of the Griess modified reagent were added to each well. After 15-min at room temperature, the Optical Density (OD) of each well was read at 540 nm by a fluorescence-luminescence reader Infinite M200 Pro (TECAN). The results obtained for wells treated with AT000 extract were compared to those of untreated control wells (DMSO, 100% viability) and converted to percentage values.

In parallel to the assessment of NO release, cell viability was measured to validate the assay. The WST-1 vital dye reagent was used to measure cell mitochondrial respiration. For this purpose, the culture medium was decanted, and 100 µL of WST-1 reagent (1/10 dilution) was added to each well. After a 30-min incubation period at 37 °C (5% CO_2), the Optical Density (OD) of each well was read at 450 nm by a fluorescence-luminescence reader Infinite M200 Pro (TECAN). The results obtained for wells treated with AT000 extract were compared to those of untreated control wells (DMSO, 100% viability) and converted to percentage values. Experiments were performed in duplicate and dexamethasone was used as a positive control at the concentrations of 1, 5, 10, 50 and 100 µM.

Inhibition of NO release and inhibition of cell viability were expressed as percentages as compared to the negative controls:

$$PI\ (\%) = 100 \times (OD_{test\ well} - OD_{blank})/(OD_{control} - OD_{blank}) \quad (2)$$

The concentrations of the extract causing respectively a 50% decrease of NO release ($IC_{50\text{-}NO\text{-}R}$) and a 50% decrease of cell viability ($IC_{50\text{-}cell\text{-}V}$) were calculated through non-linear regression analysis using software TableCurve Version 2.0. The anti-inflammatory ratio corresponded to the ratio between the anti-inflammatory activity and the toxicity. It was expressed as follows:

$$\text{Anti-inflammatory ratio} = IC_{50\text{-}cell\text{-}V}/IC_{50\text{-}NO\text{-}R} \quad (3)$$

2.7 Animal studies

Male Swiss mice, six weeks old, weighing 30-35 g, from JANVIER (Saint Berthevin, France), were kept for housing. Experiments took place within the animal facility building of the University of Montpellier 2 (CECEMA, Office of Veterinary Services agreement # B- 34-172-23) for experiments 1-2 and in Amylgen (Regional Directorate of Food, Agriculture and Forest of Languedoc-Roussillon, agreement #A 34-169-002 from May 02, 2014) for experiments 3. Animals were housed in groups with access to food and water *ad libitum*, except during behavioral experiments. They were kept in a temperature and humidity controlled animal facility on a 12 h/12 h light/dark cycle (lights off at 07:00 pm). Mice were numbered by marking their tail using permanent

markers. All animal procedures were conducted in strict adherence to the European Union directive of September 22, 2010 (2010/63/UE).

2.7.1 Drugs and administration procedures

The homogeneous oligomeric preparation of $A\beta_{25-35}$ peptide and scrambled $A\beta_{25-35}$ peptide (Sc.Aβ) was performed according to the AMYLGEN's owned procedure. The preparations were dissolved in distilled sterile water at a concentration of 3 mg/mL and stored at -20 °C until use. Before injection, peptides were aggregated by incubation at 37 °C for 4 days. Each mouse was anesthetized with isoflurane 2.5% and injected i.c.v. with $A\beta_{25-35}$ peptide (9 nmol/mouse) or Sc.Aβ peptide (9 nmol/mouse), in a final volume of 3 µL per mouse, according to the previously described method [29-33].

Two administration procedures were examined. In experiment 1, AT000 was administered *per os* (p.o.) by gavage twice-a-day (b.i.d.), starting on the same day as $A\beta_{25-35}$ and lasting until day 7 when the animals' behaviors were examined (7-days injection). In experiment 2, AT000 was administered per os by force-feeding twice-a-day (b.i.d.), starting 14 days before $A\beta_{25-35}$ injection and lasting until day 7 when the animals' behaviors were examined (21-days injection). Finally, a third experiment was performed to analyze the impact of the two controversial plants in the extract: *Dryobalanops aromatica* and *Saussurea lappa*. This last experiment was performed at the active dose of the 21-days injection procedure.

2.7.2 Animals and treatment groups

61. Two hundred and forty (240) male Swiss mice (30-35 g) were used.

62. Twenty (20) groups of animals were constituted in the following manner:

Experiment 1 (groups 1-5):

56. On day 0, Sc.Aβ or $A\beta_{25-35}$-amyloid peptide was injected i.c.v. at 10:00 am.

57. Between day 0 and day 9, AT000 or the vehicle 1 solution (DMSO 5% in water) was administered p.o. by force-feeding b.i.d., at 09:00 am and 05:00 pm. AT000 was administered only once at 09:00 am on day 9.

Experiment 2 (groups 6-10):

58. Between day -14 and day 9, AT000 or the vehicle 1 solution (DMSO 5% in water) was administered p.o. by force-feeding b.i.d., at 09:00 am and 05:00 pm. AT000 was administered only once at 09:00 am on day 9.

59. On day 0, Sc.Aβ or $A\beta_{25-35}$-amyloid peptide was injected i.c.v. at 10:00 am.

Experiment 3 (groups 11-20):

60. On day 0, Sc.Aβ or $A\beta_{25-35}$-amyloid peptide was injected i.c.v. at 10:00 am.

61. Between day -14 and day 9, AT000, AT001, AT002, AT007, AT008 or the vehicle solutions (vehicle 1 = DMSO 5% in water; vehicle 2 = DMSO 10% in grapeseed oil) was administered p.o. by force-feeding b.i.d., at 09:00 am and 05:00 pm.

Then, for all groups:

62. On day 7, all animals were tested for the spontaneous alternation performance in the Y-maze test, an index of spatial working memory.

63. On day 8 and 9, the contextual long-term memory of the animals was assessed using the step-through type passive avoidance procedure.

64. On day 9, immediately after the retention session, animals were euthanized by decapitation and the hippocampus and cortex dissected out. Lipid peroxidation in the hippocampus was analyzed.

2.7.3 Formulation preparation

All solutions were freshly prepared twice a day before each injection as listed in Table 1. No

stock solution was prepared. Solutions were prepared from an initial concentrated solution in DMSO 100%. Then dilution was done in distilled water. Final DMSO concentration was 5%.

Groups 1, 2, 6, 7, 11, 12: vehicle 1 solution (DMSO 5% in water).

Groups 3, 8: AT000 6.2 mg/mL in vehicle 1 solution (corresponding to 37.5 mg/kg b.i.d. or 62.5 mg/kg/day).

Groups 4, 9: AT000 12.5 mg/mL in vehicle 1 solution (corresponding to 62.5 mg/kg b.i.d. or 125 mg/kg/day).

Groups 5, 10: AT000 25 mg/mL in vehicle 1 solution (corresponding to 125 mg/kg b.i.d. or 250 mg/kg/day).

Groups 14, 15: vehicle 5 solution (DMSO 10% in grapeseed oil plus emulsifier).

Group 16: AT000 25 mg/mL in vehicle 2 solution (corresponding to 125 mg/kg b.i.d. or 250 mg/kg/day).

Group 17: AT001 25 mg/mL in vehicle 2 solution (corresponding to 125 mg/kg b.i.d. or 250 mg/kg/day).

Table 1. Groups of animals, treatment period and number of mice used for different experiments.

Experiment		**Groups of animals**	**Number of days**	**Number of mice**
Experiment 1	1	Sc.Aβ + vehicle 1	7	12
	2	$A\beta_{25-35}$ + vehicle 1	7	12
	3	$A\beta_{25-35}$ + AT000: 62.5 mg/kg/day	7	12
	4	$A\beta_{25-35}$ + AT000: 125 mg/kg/day	7	12
	5	$A\beta_{25-35}$ + AT000: 250 mg/kg/day	7	12
Experiment 2	6	Sc.Aβ + vehicle 1	21	12
	7	$A\beta_{25-35}$ + vehicle 1	21	12
	8	$A\beta_{25-35}$ + AT000: 62.5 mg/kg/day	21	12
	9	$A\beta_{25-35}$ + AT000: 125 mg/kg/day	21	12
	10	$A\beta_{25-35}$ + AT000: 250 mg/kg/day	21	12
Experiment 3	11	Sc.Aβ + vehicle 1	21	12
	12	$A\beta_{25-35}$ + vehicle 1	21	12
	13	$A\beta_{25-35}$ + AT000 (in vehicle 1): 250 mg/kg/day	21	12
	14	Sc.Aβ + vehicle 2	21	12
	15	$A\beta_{25-35}$ + vehicle 2	21	12
	16	$A\beta_{25-35}$ + AT000 (in vehicle 2): 250 mg/kg/day	21	12
	17	$A\beta_{25-35}$ + AT001: 250 mg/kg/day	21	12
	18	$A\beta_{25-35}$ + AT002: 250 mg/kg/day	21	12
	19	$A\beta_{25-35}$ + AT007: 250 mg/kg/day	21	12
	20	$A\beta_{25-35}$ + AT008: 250 mg/kg/day	21	12

Group 18: AT002 25 mg/mL in vehicle 2 solution (corresponding to 125 mg/kg b.i.d. or 250 mg/kg/day).

Group 19: AT007 25 mg/mL in vehicle 2 solution (corresponding to 125 mg/kg b.i.d. or 250 mg/kg/day).

Group 20: AT008 25 mg/mL in vehicle 2 solution (corresponding to 125 mg/kg b.i.d. or 250 mg/kg/day).

2.7.4 Euthanization

At the end of the passive avoidance retention session, on day 9, animals were euthanized by decapitation. The hippocampi and frontal cortex were removed. One hippocampus of n=6 animals per group was used to measure lipid peroxidation levels in tissue extracts. The other hippocampus and the cortex were kept at -80 °C awaiting further analysis.

2.7.5 Spontaneous alternation performances

On day 7, all animals were tested for spontaneous alternation performance in the Y-maze, an index of spatial working memory. The Y-maze was made of grey polyvinylchloride. Each arm was 40 cm long, 13 cm high, 3 cm wide at the bottom, 10 cm wide at the top, and converging at an equal angle. Each mouse was placed at the end of one arm and allowed to move freely through the maze during an 8-min session. The series of arm entries, including possible returns into the same arm, was checked visually. An entry was defined as the penetration of the hind paws of the animal at least 2 cm into the arm. An alternation was defined as entries into all three arms on consecutive occasions. The number of maximum alternations was the total number of arm entries minus two and the percentage of alternation was calculated as: actual alternations/maximum alternations) x 100. Parameters included the percentage of alternation (memory index) and the total number of arm entries (exploration index) [29-33].

2.7.6 Passive avoidance test

The apparatus is a two-compartment (15 x 20 x 15 cm high) box, one illuminated with white polyvinylchloride walls and the other darkened with black polyvinylchloride walls and a grid floor. A guillotine door separates each compartment. A 60 W lamp positioned 40 cm above the apparatus lights up the white compartment during the experiment. Scrambled foot shocks (0.3 mA for 3
86. were delivered to the grid floor using a shock generator scrambler (Lafayette Instruments, Lafayette, USA). The guillotine door was initially closed during the training session. On day 8, during the training session, each mouse was placed in the white compartment. After 5 s, the door was raised. When the mouse entered the darkened compartment and placed all its paws on the grid
floor, the door was closed and the foot shock delivered for 3 s. The step-through latency, that is, the latency spent to enter the darkened compartment, and the number of vocalizations was recorded. The retention test was carried out 24 h after training, on day 9. Each mouse was placed in the white compartment again. After 5 s, the door was raised. The step-through and escape latencies (corresponding to the re-exit from the darkened compartment) were recorded up to 300 s [31-33].

Animals that showed all latencies during the training and retention session lower than 10 s were considered as having failed to respond to the procedure and were discarded from the calculations. In this study, no animal was discarded accordingly.

2.7.7 Lipid peroxidation measures

At day 9, 6 mice from each group were euthanized by decapitation, and both hippocampi were rapidly removed, weighed and kept in liquid nitrogen until assayed. After thawing, one hippocampus per mice was homogenized in cold methanol (1/10 w/v), centrifuged at 1,000 g for 5 min and the supernatant placed in Eppendorf tube. The reaction volume of each homogenate was added to $FeSO_4$ 1 mM, H_2SO_4 0.25 M, xylenol

orange 1 mM and incubated for 30 min at room temperature. After reading the absorbance at 580 nm ($A_{580}1$), 10 µL of cumene hydroperoxide (CHP) 1 mM was added to the sample and incubated for 30 min at room temperature, to determine the maximal oxidation level. The absorbance was measured at 580 nm ($A_{580}2$). The level of lipid peroxidation was determined as CHP equivalents according to CHPE = $A_{580}1/A_{580}2$ x [CHP (nmol)] and expressed as CHP equivalents per mg of tissue and as a percentage of control group data (V-treated Sc.Aβ-administered mice).

2.7.8 Statistical analyses

All values, except passive avoidance latencies, were expressed as mean ± S.E.M. Statistic analyses were performed on the different conditions using one-way ANOVA (*F* value), followed by the Dunnett's post-hoc multiple comparison tests. Passive avoidance latencies do not follow a Gaussian distribution since upper cut-off times were set. Therefore, they were analyzed using a Kruskal-Wallis non-parametric ANOVA (*H* value), followed by a Dunn's multiple comparison tests. $p < 0.05$ was considered as statistically significant.

86. Results and Discussion

3.1 Antioxidant activity

To determine the antioxidant potential of our extract, we evaluated the reducing power by the widely used DPPH test, and the ability to delay the oxidation of fluorescein by the ORAC assay.

$DPPH^{\cdot}$ is a stable radical, nitrogen-centered and colored due to the delocalization of electron with a visible absorption maximum at 517 nm in an alcoholic solution. The reduction of the DPPH radical by the antioxidant compound is manifested by a decrease in the absorbance of the DPPH radical and by its discoloration (from violet to yellow) [34]. The ORAC assay is currently considered the most relevant chemical test for measuring antioxidant activity since it is a dynamic test based on stress induced by peroxyl radicals mimicking the cellular mechanisms induced by them [35, 36]. The DPPH scavenging effect of AT000 extract increased with increasing concentration of extract (Fig. 1), which demonstrated strong antioxidant ability at 2 mg/mL and 10 mg/mL (82.08 and 93.39%) respectively. Table 2 shows the antioxidative potency of AT000 extract compared with foods known to have substantial antioxidant activity (USDA Database for ORAC): Nutrient Data Laboratory, Agriculture Research Service, United States Department of Agriculture). AT000 extract exhibited a powerful antioxidant activity with a value of 301774 Trolox equivalents compared to the reference compounds.

This strong antioxidant activity is due to the richness of the plants constituting AT000 extract in phenolic compounds having the capacity to trap free radicals. Eugenol, the main component isolated from *Eugenia caryophyllata* possesses potent antioxidant properties [37, 38]. The ethanolic extract of *Liquidambar orientalis* leaves also showed a high antioxidant activity [39]. Ethyl acetate extract of *Aquilaria agallocha* have been reported to have free radical scavenging activity [40], and antioxidant properties *in vitro* at different concentrations by inhibition of nitrite-induced oxidation of hemoglobin in human blood hemolysate [41].

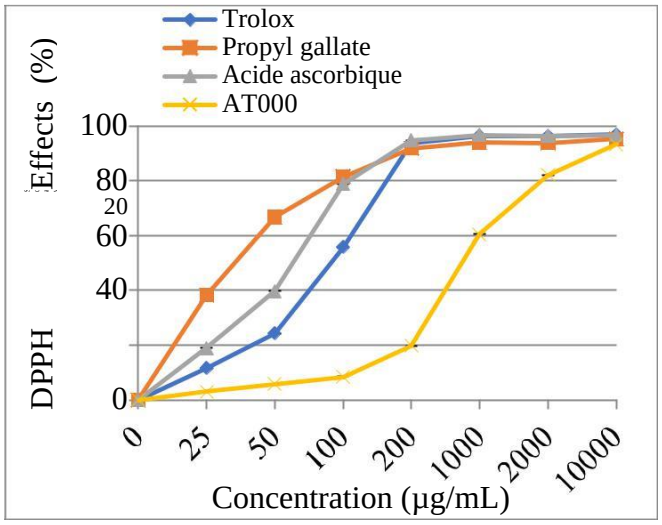

Figure 1. Free radical scavenging activity of methanol extract of AT000, trolox, propyl gallate, and ascorbic acid by 2,2-diphenyl-1-picrylhydrazyl radicals.

Table 2. ORAC values of AT000 and foods expressed as µmol Trolox Eq. (µmol/100g)

Sample	**Trolox Eq. (µmol/100 g)**
AT000	301774
Ground cloves spice	290283
Indian gooseberry, dried	261500
Oregano spice, dried	175295
Rosemary spice, dried	165280
Cinnamon spice, ground	131420

Coumarins, tannins, and flavonoids isolated from *Cyperus rotundus* tuber extracts exhibited antibacterial, antioxidant, antimutagenic, cytotoxic and apoptotic properties [42, 43]. Cyanidin-3-glucoside, the primary pigment identified in *Santalum album* berries extract possessed antioxidant potentials and high scavenging activity [44]. This combination of molecules acts synergistically to boost the overall antioxidant activity of the extract.

3.2 Anti-inflammatory and cytotoxicity assays

Anti-inflammatory activity and cytotoxicity of AT000 extract were tested *in vitro* and compared to dexamethasone as a positive control. The results are summarized in Table 3. AT000 showed the release of NO with IC_{50} value < 20 µM on macrophages RAW 264.7 and exhibited cytotoxicity with IC_{50} value > 50 µM.

Aging is characterized by inflammation involved in the pathogenesis of all age-related diseases such as AD along with increased expression of inflammatory mediators [45]. this chronic inflammation is stimulated by Aβ plaques and tangles [46]. The hallmark of the inflammatory state is the increase of serum levels of inflammatory mediators such as cytokines [47, 48], free radicals as reactive oxygen species and nitric oxide [49, 50]. AT000 extract showed an effective anti-inflammatory activity compared to dexamethasone, by inhibiting NO release from LPS-stimulated murine mouse macrophages RAW 264.7 cells and reduced LPS-induced cytotoxicity (Table 3). The plants constituting AT000 extract are rich in molecules with high anti-inflammatory properties; Eugenol has shown an anti-inflammatory activity by suppressing the expression of cyclooxygenase-2 in mouse macrophage cells stimulated with lipopolysaccharide (LPS) [51]. *Aquilaria agallocha* oil demonstrated anti-inflammatory activity, thus significantly reducing edema in carrageenan-induced paw edema model [52]. Triterpene acids isolated from *Boswellia carteri* resin demonstrated potent anti-inflammatory activity in the mouse ear edema assay [53]. Delivery of Boswellia resin extract resulted in a dose-dependent inhibition of TH1 cytokines

coupled with a dose-dependent potentiation of TH2 cytokines indicating that purified mixture of BAs from *Boswellia carteri* plant resin exhibits carrier-dependent immunomodulatory properties *in vitro*

2 Extract of rhizomes of *Cyperus rotundus* increased HO-1 expression in a concentration-dependent manner, which was correlated with significant inhibition of iNOS/NO production in LPS-activated RAW 264.7 cells [55]. α-Cyperone, the primary compound isolated from the rhizomes of *Cyperus rotundus*, inhibits LPS-induced COX-2 expression and PGE2 production through the negative regulation of NFκB signalling in RAW 264.7 cells [56]. The (9R,10E)-9-hydroxy- α - santalal, a sesquiterpene isolated from *Santalum album* exhibited tumor-selective cytotoxicity against HL-60 cells [57]. Cyclosaplin, A new cyclic octapeptide purified from somatic seedlings of *Santalum album L.* exhibited cytotoxic activity against cultured human breast cancer (MDA-MB-231) cell line, by inducing apoptotic cell death by caspase 3 activation [58]. *Saussurea lappa* exhibited inhibitory effects on IL-8 Induction in Lipopolysaccharide-Activated Rat Macrophages

80. Ethanolic extract of *Saussurea lappa* affects acute and chronic inflammation induced in mice and rats by inhibiting carrageenan-induced paw edema and preventing accumulation of inflammatory cells in carrageenan-induced peritonitis *in vivo* [60]. 'Dehydrocostus lactone', a sesquiterpene lactone from *Saussurea lappa* suppressed LPS-induced nitric oxide production [61] and inhibited release of TNF-α [62]. Another sesquiterpene lactone 'cynaropicrin' from *Saussurea lappa* inhibited TNF-α murine macrophage cell line and dose-dependently suppressed the proliferation of lymphocytes stimulated by Con-A [63]. Santamarin, a sesquiterpene lactone isolated from *Saussurea lappa*, represses LPS-induced inflammatory responses via expression of heme oxygenase-1 in murine macrophage cells [64]. Borneol, the main component isolated from *Dryobalanops aromatica* inhibit nicotinic receptor-mediated catecholamine secretion in bovine adrenal chromaffin cells [65, 66]. All these properties add up to demonstrate the anti-inflammatory activity of AT000 extract.

3.3 AT000 extract recovers cognitive functions in Aβ25-35-treated mice

To determine the effective dose and the optimal injection time of the extract, the neuroprotective effect of AT000 was examined on the attenuation of the Aβ25-35-induced learning deficits according to two administration procedures (7-days and 21-days injection).

3.3.1 Spontaneous alternation performances

The spatial working memory was first examined by evaluating spontaneous alternation in the Y-maze test. As shown in Fig. 2a and 2c, the Aβ25-35 treatment induced highly significant spontaneous alternation deficits as compared to Sc.Aβ/V-injected mice. The 7-days AT000 treatment tended to attenuate the Aβ25-35-induced deficits, but no significant effect vs. Aβ25-35-treated animals was noted (Fig. 2a). No effect was noted on locomotion (Fig. 2b). On the other hand, the 21-days AT000 treatment prevented the Aβ25-35-induced deficits at the highest dose tested (250 mg/Kg/day) with a highly significant effect vs. the Aβ25-35 treated group (Fig. 2c). A mild effect was noted on locomotion since the ANOVA was significant, but no group effect was observed (Fig. 2d). Compared to 21-days pre-treatment, the daily injection of AT000 extract starting on the same day as Aβ25-35 injection and lasting 7 days after, reduced Aβ25-35-induced deficits at 250 mg/kg/day but without having a significant effect on spontaneous alteration in the Y-maze (Fig. 2a, b). This result

indicates that the extract acts according to a preventive way and not the curative way. In the case of a preventive treatment, the optimal effect was observed with increased treatment duration. The hypothesis is that the extract administered as a prolonged treatment induces a modification in the neurons, which makes the nervous system more resistant and less sensitive to aggression by $A\beta_{25-35}$-induced toxicity. Treatment for 21 days produces a more significant effect than 7 days treatment because it has more time to induce these modifications which are still poorly understood.

Table 3. Anti-inflammatory, cytotoxic activity and the anti-inflammatory ratio of AT000 and Dexamethasone.

	NO release IC_{50} (µM)	Toxicity IC_{50} (µM)	Anti-inflammatory ratio
AT000 (10 mg/mL)	1.06	> 50.00	> 47.00
Dexamethasone	4.31 ± 1.45 µM	163.22 ± 74.96 µM	37.87

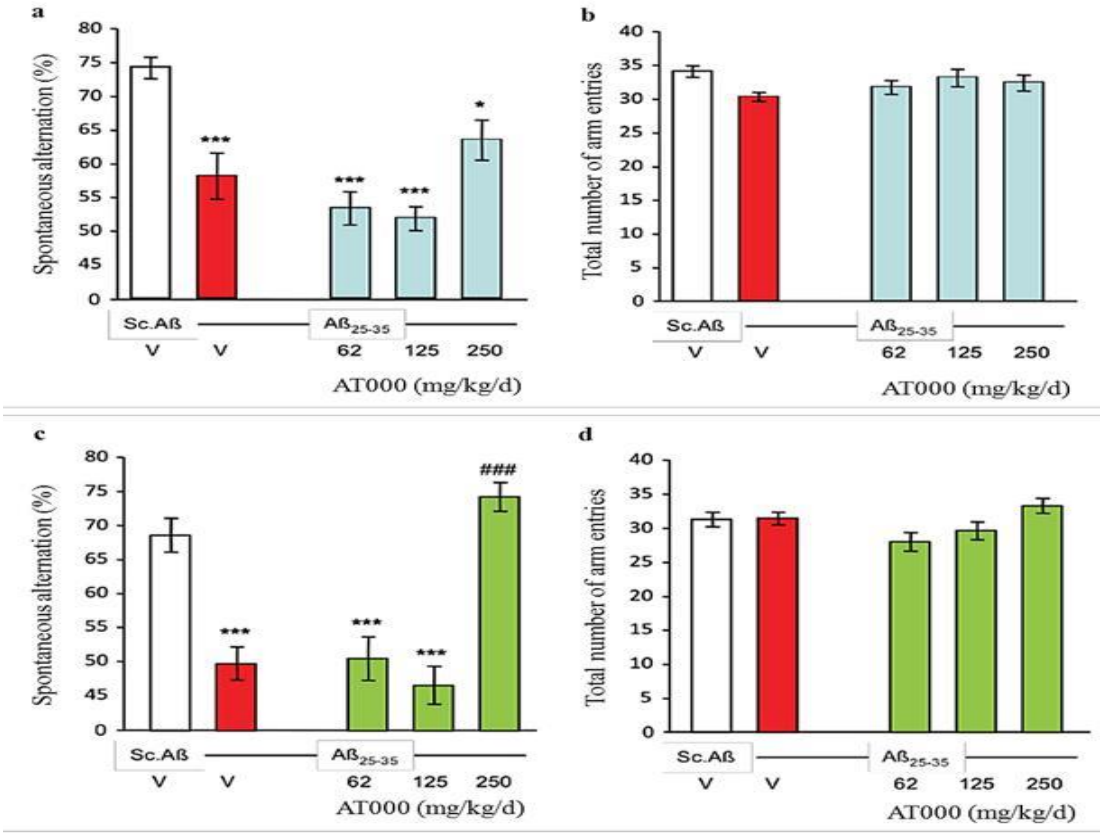

Figure 2. Effects of AT000 p.o. administration during 7 days (a, b) and 21 days (c, d) on $A\beta_{25-35}$-induced spontaneous alternation deficits in mice. V, vehicle solution. *** $p < 0.001$, * $p < 0.05$ vs. the V-treated Sc.Aβ group; ### $p < 0.001$ vs. the $A\beta_{25-35}$ treated group; Dunnett's test.

3.3.2 Step-through passive avoidance test

The contextual long-term memory was evaluated using the step-through passive avoidance test. The $A\beta_{25-35}$ treatment led to highly significant passive avoidance deficits as compared to Sc.Aβ/V-injected mice, both regarding step-through latency (Fig. 3a, c) and escape latency (Fig. 3b, d) during the retention session. The 7-days AT000 treatment alleviated the $A\beta_{25-35}$-induced deficits, with important prevention at the highest dose tested (250 mg/Kg/day) on the step-through latency parameter (Fig. 3a). However, the 21-days AT000 treatment dose-dependently alleviated the $A\beta_{25-35}$-induced deficits, with a significant prevention at the highest dose tested (250 mg/Kg/day) on both the step-through latency (Fig. 3c) and escape latency parameter (Fig. 3d). Note that the treatments did not affect the step-through latency or shock sensitivity during the training session.

3.3.3 Lipid peroxidation measure

Lipid peroxidation, one of the essential biochemical parameters of amyloid toxicity, was also analyzed in the hippocampus to validate the neuroprotective activity of the extract. According to the 7-days AT000 injection procedure, $A\beta_{25-35}$ treatment induced highly significant increase (+87%) in lipid peroxidation in the hippocampus, as compared to Sc.Aβ/V-injected mice (Fig. 4a). The 7-days AT000 treatment dose-dependently attenuated the $A\beta_{25-35}$-induced increase, with a significant effect at the two highest doses tested (125 and 250 mg/Kg/day) (Fig. 4a). At 250 mg/kg/day, the lipid peroxidation level remained significantly higher than Sc.Aβ/V-injected mice (+29%). According to the 21-days AT000 injection procedure, $A\beta_{25-35}$ treatment induced highly significant increase (+33%) in the level of peroxidized lipids in the hippocampus, as compared to Sc.Aβ/V-injected mice (Fig. 4b). The 21-days AT000 treatment dose-dependently attenuated the $A\beta_{25-35}$-induced increase, with a significant and complete blockage at the highest doses tested (Fig. 4b).

The pre-treatment by oral administration of AT000 extract for 21 days, alleviated the pathology induced intracerebroventricularly in mice and showed significant activity on the attenuation of $A\beta_{25-35}$-induced learning deficits, with a highly significant effect at 250 mg/kg/day (Fig. 2, 3, 4). This neurotoxic peptide leads to cognitive and behavioral disorders including spatial working memory and contextual long-term memory, as well as biochemical modifications such as lipid peroxidation. The effect inducing concentration of AT000 extract was significantly higher than the previously reported concentration for Amino-tetrahydrofuran derivative ANAVEX1-41 [10 μg/kg; [32]. However, ANAVEX1-41 was administered intraperitoneally suggesting that the mode of administration plays a role in determining the effective dose. Koo *et al.* and Jeon *et al.* studied the effect of SuHeXiang essential oil on attenuation of amyloid-beta-induced alteration of memory. However, their studies did not report an effective dose [24, 25]. On the other hand, oral administration in the present study showed an advantage over inhalation for the determination of an active dose of this extract *in vivo* for the first time.

3.4 Experiment 3 (Evaluation of synergy)

In this experiment, we evaluated the impact of *Dryobalanops aromatica and Saussurea lappa* in the composition to demonstrate synergy between the different plants constituting the AT000 extract. For this, we prepared 4 extracts by eliminating these two plants. We evaluated the neuroprotective effect of these 4 extracts on the attenuation of $A\beta_{25-35}$-induced learning deficits first with spatial working memory: spontaneous alternation in the Y-

maze and contextual long-term memory: passive avoidance test. The impact of these extracts on lipid peroxidation in the hippocampus was also evaluated. This last experiment was performed at the active dose of the 21-days injection procedure.

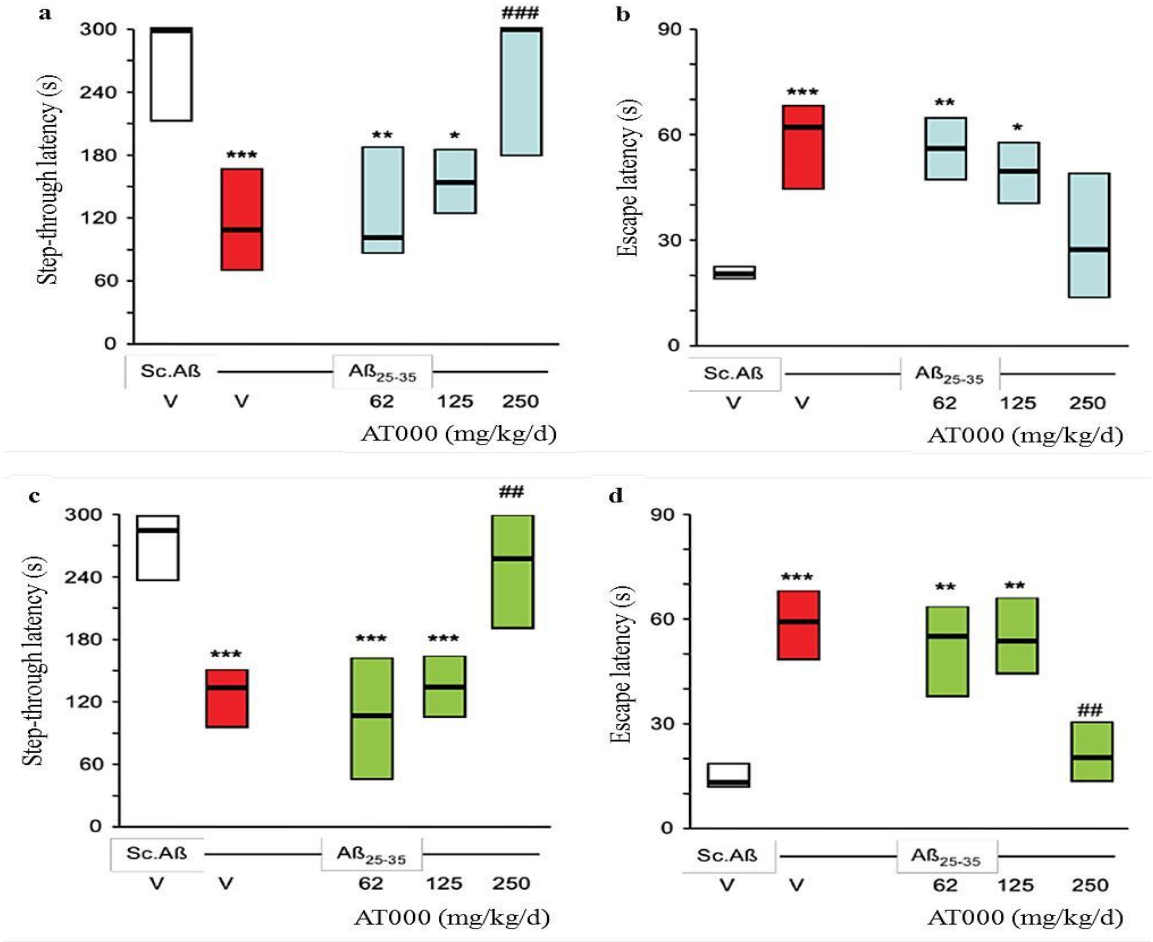

Figure 3. Effects of AT000 administration during 7 days (a, b) and 21 days (c, d) on Aβ25-35-induced passive avoidance deficits in mice: (a, c) step-through latency and (b, d) escape latency measured during the retention session. V, vehicle solution. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. the V-treated Sc.Aβ group; ## $p < 0.01$, ### $p < 0.001$ vs. the Aβ25-35-treated group; Dunn's test.

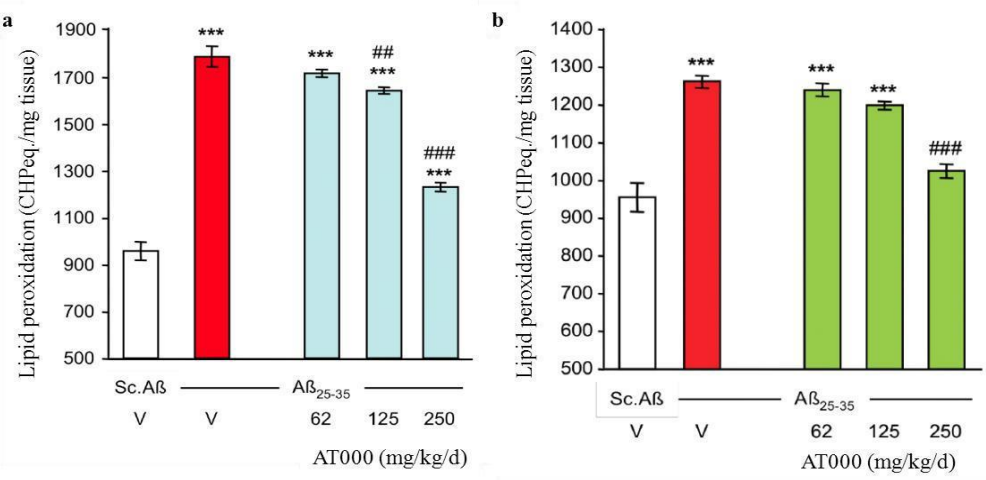

Figure 4. Effects of AT000 administration during 7 days (a) and 21 days (b) on Aβ25-35-induced increase in lipid peroxidation in the mouse hippocampus. V, vehicle solution. *** $p < 0.001$ vs. the V-treated Sc.Aβ group; ## $p < 0.01$, ### $p < 0.001$ vs. the Aβ25-35-treated group; Dunn's test.

3.4.1 Spontaneous alternation performances

Spontaneous alternation in the Y-maze was evaluated first, as shown in Fig. 5a and 5b. Aβ25-35 treatment induced highly significant spontaneous alternation deficits as compared to Sc.Aβ/V-injected mice. The AT000 treatment blocked the Aβ25-35-induced deficit, when solubilized in Vehicle 1 solution (Fig. 5a) as well as in-vehicle 2 solution (Fig. 5b). Neither the 1 or 2 plants formulations (AT001 or AT002) or the remaining 7 or 8 plants formulations (AT007, AT008) affected the Aβ25-35-induced alternation deficit (Fig. 5b). No effect was noted on locomotion.

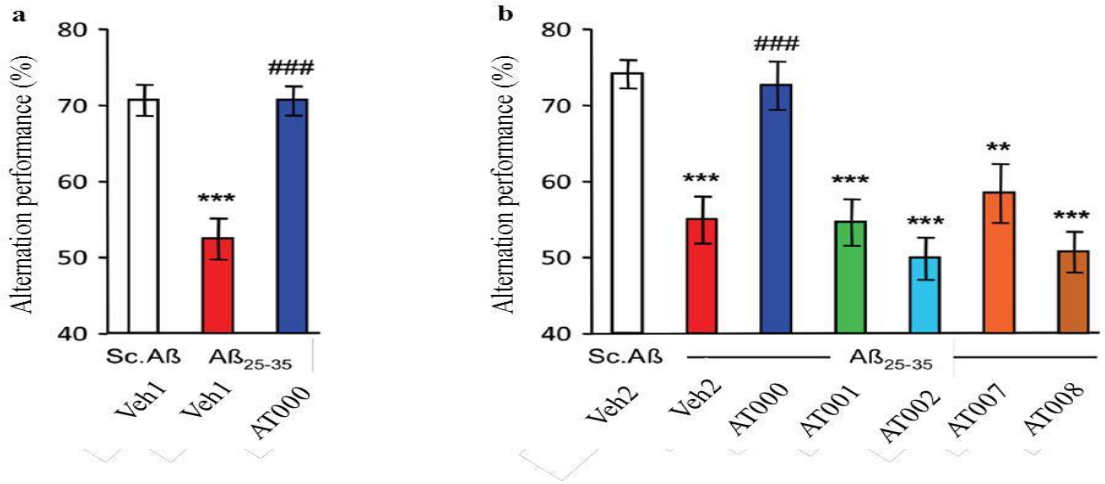

Figure 5. Comparative effects of *Dryobalanops aromatica, Saussurea lappa* and AT formulations p.o. administration during 21 days on Aβ25-35-induced spontaneous alternation deficits in mice. Veh1, vehicle 1 solution (DMSO 5% in water); Veh2, vehicle 2 solution (DMSO 10% in grapeseed oil). *** $p < 0.001$, ** $p < 0.01$ vs. the V-treated Sc.Aβ group; ### $p < 0.001$ vs. the V-treated Aβ25-35 group; Dunnett's test.

3.4.2 Step-through passive avoidance test

The contextual long-term memory was evaluated using the step-through passive avoidance test. Aβ25-35 treatment induced highly significant passive avoidance deficits as compared to Sc.Aβ/V-injected mice, both regarding step-through latency (Fig. 6a, c) or escape latency (Fig. 6b, d). AT000 treatment blocked the Aβ25-35-induced deficits when solubilized in Vehicle 1 solution (Fig. 6a, c) as well as in-vehicle 2 solution (Fig. 6b, d). Neither the 1 or 2 plants formulations (AT001 or AT002) or the remaining 7 or 8 plants formulations (AT007, AT008) affected the Aβ25-35-induced passive avoidance deficits, both in terms of step-through latency (Fig. 6a, c) or escape latency (Fig. 6b, d).

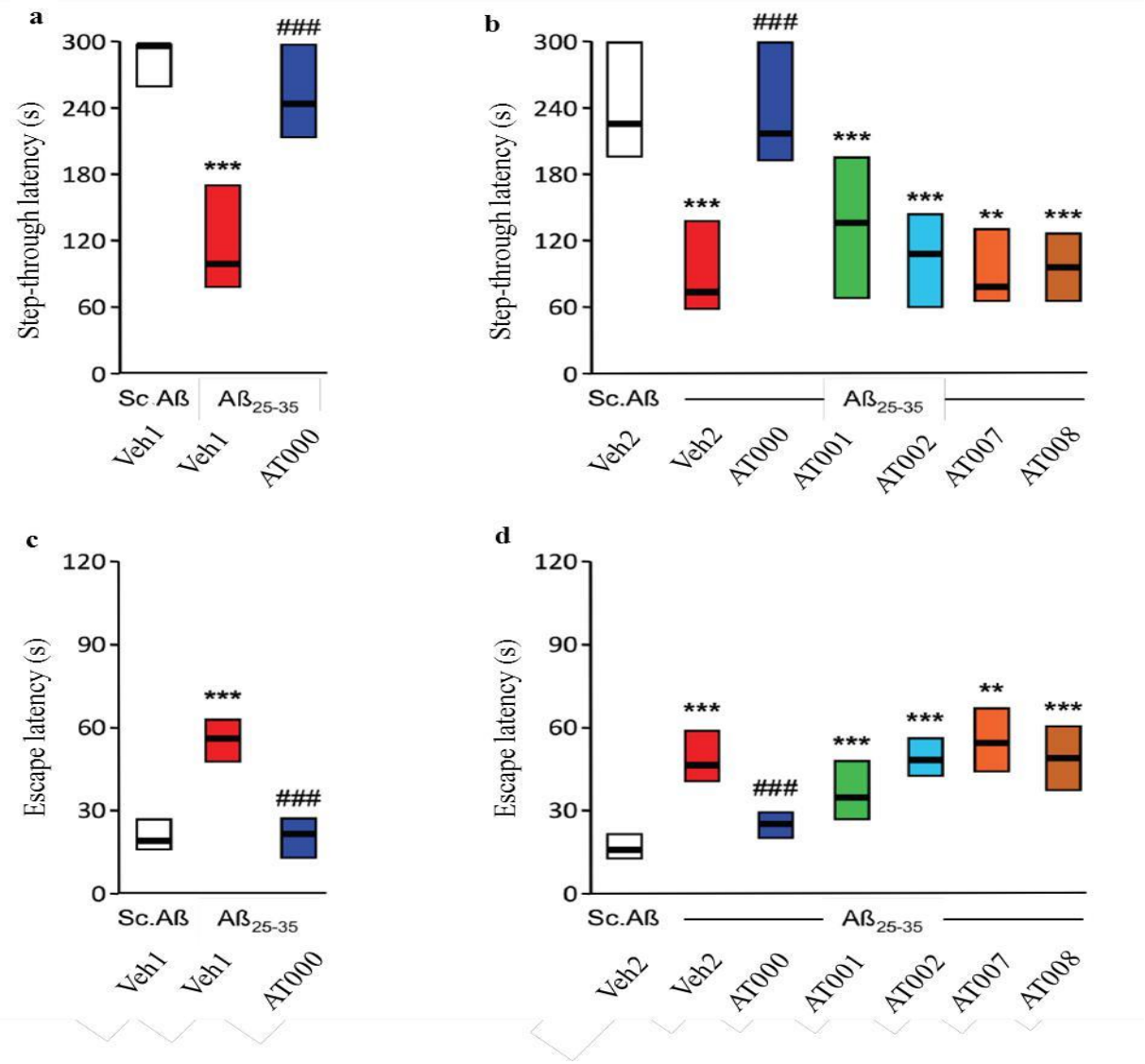

Figure 6. Comparative effects of *Dryobalanops aromatica*, *Saussurea lappa* and AT formulations on Aβ25-35-induced passive avoidance deficits in mice: (a) step-through latency and (b) escape latency measured during the retention session. Veh1, vehicle 1 solution (DMSO 5% in water); Veh2, vehicle 2 solution (DMSO 10% in grapeseed oil). ** $p < 0.01$, *** $p < 0.001$ vs. the V-treated Sc.Aβ group; ### $p < 0.001$ vs. the Aβ25-35-treated group; Dunn's test.

3.4.3 Lipid peroxidation measure

Finally, we evaluated lipid peroxidation, an index of oxidative stress. The Aβ25-35 treatment induced highly significant increase (+44%) in lipid peroxidation in the hippocampus, as compared to Sc.Aβ/V-injected mice in Veh1 conditions (Fig. 7a) and a highly significant increase (+63%) in lipid peroxidation in the hippocampus, as compared to Sc.Aβ/V-injected mice in Veh2 conditions (Fig. 7a). AT000 treatment blocked the Aβ25-35-induced increase highly significantly as compared to Aβ25-35/V-treated mice in both vehicle conditions (Fig. 7a, b). Neither the 1 nor 2 plants formulations (AT001 or AT002) or the remaining 7 or 8 plants formulations (AT007, AT008) affected the Aβ25-35-induced increase in lipid peroxidation (Fig. 7b).

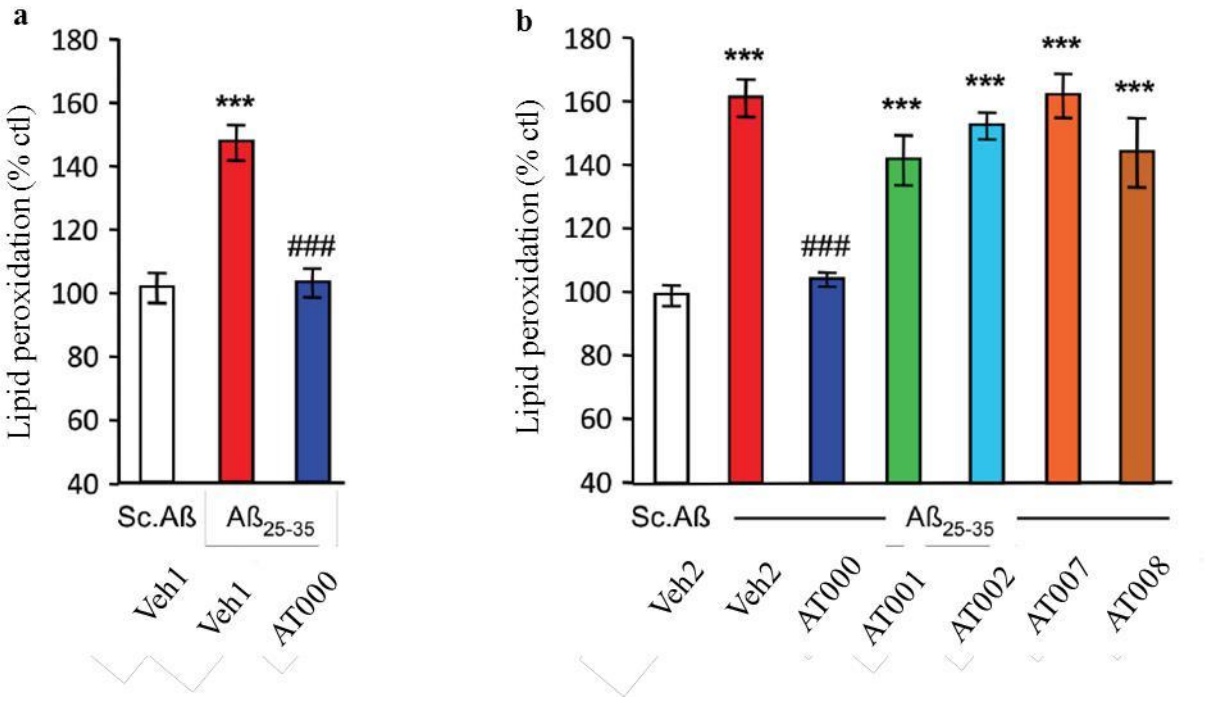

Figure 7. Comparative effects of *Dryobalanops aromatica*, *Saussurea lappa,* and AT formulations p.o. administration during 21 days on Aβ25-35-induced increase in lipid peroxidation in the mouse hippocampus. Veh1, vehicle 1 solution (DMSO 5% in water); Veh2, vehicle 2 solution (DMSO 10% in grapeseed oil). *** $p < 0.001$; ### $p < 0.001$ vs. the V-treated Aβ25-35 group; Dunnett's test.

The synergistic effect of *Dryobalanops aromatica* and *Saussurea lappa* was evaluated. For this purpose, 4 different extracts were prepared to study the impact on the overall activity of the extract. The 4 extracts were tested on spatial working memory, contextual long-term memory and lipid peroxidation at the active dose (250 mg/kg/day) and 21-days treatment. The results obtained showed that the elimination of these plants from the extract led to the total disappearance of the neuroprotective activity (Fig. 5, 6, 7). The results also showed that *Dryobalanops aromatica* and *Saussurea lappa* alone do not show any neuroprotective activity. The activity is due to the combination of these 9 plants which, by extracting them together, will interact according to the potentiation and the effect will be boosted. Additionally, the two controversial plants were

found to be essential for the neuroprotective activity of the extract.

The mechanisms of action of the plants constituting AT000 extract have been previously studied. Essential oil of *Eugenia caryophyllata* possesses anticonvulsant activity against maximal electroshock (MES)-induced tonic seizures in male mice [67]. *Liquidambar orientalis* has also been reported to have anticonvulsant and sedative properties by substantially delaying the appearance of PTZ-induced convulsion [68]. Aqueous extract of *Aquilaria agallocha* stems showed inhibitory effects on histamine release from rat peritoneal mast cells leading to inhibition of immediate hypersensitivity reactions [69]. Benzylacetone, α-gurjunene and calarene, principles volatile obtained from Agarwood oil showed sedative activity in mice using a system of spontaneous vapor administration [70]. Alcoholic extract of *Aquilaria agallocha* (AEAA) has anticonvulsant activity using PTZ (Pentylenetetrazole) to induced convulsion. At a higher dose, an AEAA showed a significant anticonvulsant effect by increasing latency of clonus, an onset of tonic seizures and declined mortality of mice [71]. *Cyperus rotundus* tubers extract attenuated significantly learning and memory disturbance in passive avoidance paradigm and spatial cognitive deficit in Morris water maze produced by lesioning of the NBM in rats [72]. *Cyperus rotundus* tubers extract treatment showed therapeutic potential in aging and age-related neurodegenerative disorders by preventing the cognitive impairments significantly following NBM lesion [72]. Pre-treatment of neurons with *Cyperus rotundus* extract ameliorates the mitochondrial and plasma membrane damage induced by SIN-1, restores the cellular morphology and improves the antioxidant status by regulating the oxidative stress biomarkers [73]. Total oligomeric flavonoids (TOFs) extracted from *Cyperus rotundus* demonstrated neuroprotective effect against the ischemic–reperfusion, induced neurodegeneration in the rat model by reducing oxidative stress, excitotoxicity, neurological and behavioral alterations [74]. Pre-treatment with an alcoholic extract of *Saussurea lappa* roots increased latency and also reduced mortality rate which indicates anticonvulsant property against Pentylenetetrazole and picrotoxin-induced convulsions in mice [75].

4. Conclusions

Our results demonstrated that AT000 extract has a significant neuroprotective effect on the attenuation of the $A\beta_{25-35}$-induced learning deficits and lipid peroxidation in the hippocampus. The extract also showed an effective antioxidant and anti-inflammatory activities, which indicates that AT000 extract has potential as a potent therapeutic agent for neuroprotection and prevention of AD. We are continuing studies on the AT000 extract to better understand the synergy mechanisms between the different plants in the mixture as well as chemical composition of AT000 extract. For that the analytical study part including the chemical composition of the plants is crucial.

Conflict of interest

The authors declare that they have no conflicts of interest to disclose.

Acknowledgments

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References

[1] V. Lahmy, R. Long, D. Morin, V. Villard, T. Maurice, Mitochondrial protection by the mixed muscarinic/σ1 ligand ANAVEX2-73, a tetrahydrofuran derivative, in Aβ25–35 peptide-injected mice, a nontransgenic Alzheimer's disease model, *Frontiers in Cellular Neuroscience* **8** (2015) 1-11.

[2] H. Thonberg, H.-H. Chiang, L. Lilius, C. Forsell, A.-K. Lindström, C. Johansson, J. Björkström, S. Thordardottir, K. Sleegers, C. Van Broeckhoven, A. Rönnbäck, C. Graff, Identification and description of three families with familial Alzheimer disease that segregate variants in the SORL1 gene, *Acta Neuropathologica Communications* **5**(1) (2017) 43-55.

[3] A. E. Budson, B. H. Price, Memory dysfunction, *The New England Journal of Medicine* **352**(7) (2005) 692-699.

[4] I. Chumakov, S. Nabirotchkin, N. Cholet, A. Milet, A. Boucard, D. Toulorge, Y. Pereira, E. Graudens, S. Traoré, J. Foucquier, M. Guedj, E. Vial, N. Callizot, R. Steinschneider, T. Maurice, V. Bertrand, C. Scart-Grès, R. Hajj, D. Cohen, Combining two repurposed drugs as a promising approach for Alzheimer's disease therapy, *Scientific Reports* **5** (2015) 7608-7620.

[5] R. Katzman, Alzheimer's disease, *The New England Journal of Medicine* **314**(15) (1986) 964-973.

[6] D. J. Selkoe, Cell biology of protein misfolding: the examples of Alzheimer's and Parkinson's diseases, *Nature Cell Biology* **6**(11) (2004) 1054-1061.

[7] J. Hardy, D. J. Selkoe, The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics, *Science (New York, N.Y.)* **297**(5580) (2002) 353-356.

[8] S. Barghorn, V. Nimmrich, A. Striebinger, C. Krantz, P. Keller, B. Janson, M. Bahr, M. Schmidt, R. S. Bitner, J. Harlan, E. Barlow, U. Ebert, H. Hillen, Globular amyloid beta-peptide oligomer - a homogenous and stable neuropathological protein in Alzheimer's disease, *Journal of Neurochemistry* **95**(3) (2005) 834-847.

[9] M. Townsend, G. M. Shankar, T. Mehta, D. M. Walsh, D. J. Selkoe, Effects of secreted oligomers of amyloid β-protein on hippocampal synaptic plasticity: a potent role for trimers, *The Journal of Physiology* **572**(Pt 2) (2006) 477-492.

[10] D. J. Selkoe, D. Schenk, Alzheimer's disease: molecular understanding predicts amyloid-based therapeutics, *Annual Review of Pharmacology and Toxicology* **43** (2003) 545-584.

[11] D. J. Selkoe, Molecular pathology of amyloidogenic proteins and the role of vascular amyloidosis in Alzheimer's disease, *Neurobiology of Aging* **10**(5) (1989) 387-395.

[12] M. A. Gruden, T. B. Davidova, M. Malisauskas, R. D. E. Sewell, N. I. Voskresenskaya, K. Wilhelm, E. I. Elistratova, V. V. Sherstnev, L. A. Morozova-Roche, Differential neuroimmune markers to the onset of Alzheimer's disease neurodegeneration and dementia: autoantibodies to Abeta((25-35)) oligomers, S100b and neurotransmitters, *Journal of Neuroimmunology* **186**(1-2) (2007) 181-192.

[13] T. Kubo, S. Nishimura, Y. Kumagae, I. Kaneko, In vivo conversion of racemized beta-amyloid ([D-Ser 26]A beta 1-40) to truncated and toxic fragments ([D-Ser 26]A beta 25-35/40) and fragment presence in the brains of Alzheimer's patients, *Journal of Neuroscience Research* **70**(3) (2002) 474-483.

[14] A. Y. Abramov, L. Canevari, M. R. Duchen, Beta-amyloid peptides induce mitochondrial dysfunction and oxidative stress in astrocytes and death of neurons through activation of NADPH oxidase, *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* **24**(2) (2004) 565-575.

[15] R. C. Frederickson, Astroglia in Alzheimer's disease, *Neurobiology of Aging* **13**(2) (1992) 239-253.

[16] D. A. Butterfield, D. Boyd-Kimball, Amyloid beta-peptide(1-42) contributes to the oxidative stress and neurodegeneration found in Alzheimer disease brain, *Brain Pathology (Zurich, Switzerland)* **14**(4) (2004) 426-432.

[17] H. A. Jung, T. Yokozawa, B.-W. Kim, J. H. Jung, J. S. Choi, Selective inhibition of prenylatedflavonoidsfromSophora flavescens against BACE1 and cholinesterases, *The American Journal of Chinese Medicine* **38**(2) (2010) 415-429.

[18] J. Misik, J. Korabecny, E. Nepovimova, P. Cabelova, J. Kassa, The effects of novel 7-MEOTA-donepezil like hybrids and N-alkylated tacrine analogues in the treatment of quinuclidinyl benzilate-induced behavioural deficits in rats performing the multiple T-maze test, *Biomedical Papers* **159**(4) (2015) 547-553.

[19] R. Cacabelos, Donepezil in Alzheimer's disease: From conventional trials to pharmacogenetics, *Neuropsychiatric Disease and Treatment* **3**(3) (2007) 303-333.

[20] M.-J. R. Howes, P. J. Houghton, Plants used in Chinese and Indian traditional medicine for improvement of memory and cognitive function, *Pharmacology Biochemistry and Behavior* **75**(3) (2003) 513-527.

[21] M. Adams, F. Gmünder, M. Hamburger, Plants traditionally used in age related brain disorders--a survey of ethnobotanical literature, *Journal of Ethnopharmacology* **113**(3) (2007) 363-381.

[22] M. Ayaz, A. Sadiq, M. Junaid, F. Ullah, F. Subhan, J. Ahmed, Neuroprotective and anti-aging potentials of essential oils from aromatic and medicinal plants, *Frontiers in Aging Neuroscience* **9** (2017) 1-16.

[23] Z. Lin, J. Gu, J. Xiu, T. Mi, J. Dong, J. K. Tiwari, Traditional Chinese Medicine for Senile Dementia, *Evidence-Based Complementary and Alternative Medicine* **2012** (2012) 1-13.

[24] B.-S. Koo, S.-I. Lee, J.-H. Ha, D.-U. Lee, Inhibitory effects of the essential oil from SuHeXiang Wan on the central nervous system after inhalation, *Biological & Pharmaceutical Bulletin* **27**(4) (2004) 515-519.

[25] S. Jeon, J. Hur, H. J. Jeong, B.-S. Koo, S. C. Pak, SuHeXiang Wan essential oil alleviates amyloid beta induced memory impairment through inhibition of tau protein phosphorylation in mice, *The American Journal of Chinese Medicine* **39**(5) (2011) 917-932.

[26] W. Brand-Williams, M. E. Cuvelier, C. Berset, Use of a free radical method to evaluate antioxidant activity, *LWT - Food Science and Technology* **28**(1) (1995) 25-30.

[27] B. Ou, M. Hampsch-Woodill, R. L. Prior, Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe, *Journal of Agricultural and Food Chemistry* **49**(10) (2001) 4619-4626.

[28] E. Garayev, G. Herbette, C. Di Giorgio, P. Chiffolleau, D. Roux, H. Sallanon, E. Ollivier, R. Elias, B. Baghdikian, New sesquiterpene

acid and inositol derivatives from Inula montana L, *Fitoterapia* **120** (2017) 79-84.

[29] T. Maurice, B. P. Lockhart, A. Privat, Amnesia induced in mice by centrally administered β-amyloid peptides involves cholinergic dysfunction, *Brain Research* **706**(2) (1996) 181-193.

[30] T. Maurice, T. P. Su, A. Privat, Sigma1 (sigma 1) receptor agonists and neurosteroids attenuate B25-35-amyloid peptide-induced amnesia in mice through a common mechanism, *Neuroscience* **83**(2) (1998) 413-428.

[31] J. Meunier, J. Ieni, T. Maurice, Antiamnesic and neuroprotective effects of donepezil against learning impairments induced in mice by exposure to carbon monoxide gas, *The Journal of Pharmacology and Experimental Therapeutics* **317**(3) (2006) 1307-1319.

[32] V. Villard, J. Espallergues, E. Keller, T. Alkam, A. Nitta, K. Yamada, T. Nabeshima, A. Vamvakides, T. Maurice, Antiamnesic and neuroprotective effects of the aminotetrahydrofuran derivative ANAVEX1-41 against amyloid beta(25-35)-induced toxicity in mice, *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology* **34**(6) (2009) 1552-1566.

[33] V. Villard, J. Espallergues, E. Keller, A. Vamvakides, T. Maurice, Anti-amnesic and neuroprotective potentials of the mixed muscarinic receptor/sigma 1 (σ1) ligand ANAVEX2-73, a novel aminotetrahydrofuran derivative, *Journal of Psychopharmacology (Oxford, England)* **25**(8) (2011) 1101-1117.

[34] M. N. Alam, N. J. Bristi, M. Rafiquzzaman, Review on in vivo and in vitro methods evaluation of antioxidant activity, *Saudi Pharmaceutical Journal* **21**(2) (2013) 143-152.

[35] R. L. Prior, X. Wu, K. Schaich, Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements, *Journal of Agricultural and Food Chemistry* **53**(10) (2005) 4290-4302.

[36] N. P. Seeram, M. Aviram, Y. Zhang, S. M. Henning, L. Feng, M. Dreher, D. Heber, Comparison of antioxidant potency of commonly consumed polyphenol-rich beverages in the United States, *Journal of Agricultural and Food Chemistry* **56**(4) (2008) 1415-1422.

[37] K. Chaieb, H. Hajlaoui, T. Zmantar, A. B. Kahla-Nakbi, M. Rouabhia, K. Mahdouani, A. Bakhrouf, The chemical composition and biological activity of clove essential oil, Eugenia caryophyllata (Syzigium aromaticum L. Myrtaceae): a short review, *Phytotherapy Research* **21**(6) (2007) 501-506.

[38] İ. Gülçin, İ. Güngör Şat, Ş. Beydemir, M. Elmastaş, Ö. İrfan Küfrevioğlu, Comparison of antioxidant activity of clove (Eugenia caryophylata Thunb) buds and lavender (Lavandula stoechas L.), *Food Chemistry* **87**(3) (2004) 393-400.

[39] N. Saraç, B. Şen, Antioxidant, mutagenic, antimutagenic activities, and phenolic compounds of Liquidambar orientalis Mill. var. orientalis, *Industrial Crops and Products* **53** (2014) 60-64.

[40] O. P. Sharma, T. K. Bhat, DPPH antioxidant assay revisited, *Food Chemistry* **113**(4) (2009) 1202-1205.

[41] P. B. Miniyar, T. S. Chitre, H. J. Deuskar, S. S. Karve, K. S. Jain, Antioxidant activity of ethyl acetate extract of Aquilaria agallocha on nitrite-induced methaemoglobin formation,

International Journal of Green Pharmacy **2**(2) (2008) 116-117.

[42] S. Kilani, R. Ben Ammar, I. Bouhlel, A. Abdelwahed, N. Hayder, A. Mahmoud, K. Ghedira, L. Chekir-Ghedira, Investigation of extracts from (Tunisian) Cyperus rotundus as antimutagens and radical scavengers, *Environmental Toxicology and Pharmacology* **20**(3) (2005) 478-484.

[43] S. Kilani, M. Ben Sghaier, I. Limem, I. Bouhlel, J. Boubaker, W. Bhouri, I. Skandrani, A. Neffatti, R. Ben Ammar, M. G. Dijoux-Franca, K. Ghedira, L. Chekir-Ghedira, In vitro evaluation of antibacterial, antioxidant, cytotoxic and apoptotic activities of the tubers infusion and extracts of Cyperus rotundus, *Bioresource Technology* **99**(18) (2008) 9004-9008.

[44] P. S. C. Sri Harsha, M. I. Khan, P. Prabhakar, P. Giridhar, Cyanidin-3-glucoside, nutritionally important constituents and in vitro antioxidant activities of Santalum album L. berries, *Food Research International* **50**(1) (2013) 275-281.

[45] G. Candore, G. Colonna-Romano, C. R. Balistreri, D. D. Carlo, M. P. Grimaldi, F. Listì, D. Nuzzo, S. Vasto, D. Lio, C. Caruso, Biology of Longevity: Role of the Innate Immune System, *Rejuvenation Research* **9**(1) (2006) 143-148.

[46] T. Town, V. Nikolic, J. Tan, The microglial "activation" continuum: from innate to adaptive responses, *Journal of Neuroinflammation* **2** (2005) 24-34.

[47] K. S. Krabbe, M. Pedersen, H. Bruunsgaard, Inflammatory mediators in the elderly, *Experimental Gerontology* **39**(5) (2004) 687-699.

[48] H. Bruunsgaard, The clinical impact of systemic low-level inflammation in elderly populations. With special reference to cardiovascular disease, dementia and mortality, *Danish Medical Bulletin* **53**(3) (2006) 285-309.

[49] C. E. Finch, T. E. Morgan, Systemic inflammation, infection, ApoE alleles, and Alzheimer disease: a position paper, *Current Alzheimer Research* **4**(2) (2007) 185-189.

[50] J. M. Rubio-Perez, J. M. Morillas-Ruiz, A review: inflammatory process in alzheimer's disease, role of cytokines, *The Scientific World Journal* **2012** (2012) 1-15.

[51] S. S. Kim, O. J. Oh, H.-Y. Min, E.-J. Park, Y. Kim, H. J. Park, Y. Nam Han, S. K. Lee, Eugenol suppresses cyclooxygenase-2 expression in lipopolysaccharide-stimulated mouse macrophage RAW264.7 cells, *Life Sciences* **73**(3) (2003) 337-348.

[52] H. Rahman, K. Vakati, M. C. Eswaraiah, In-Vivo and In-Vitro Anti-Inflammatory Activity of Aquilaria agallocha Oil, *International Journal of Basic Medical Sciences and Pharmacy* **2**(1) (2012) 7-10.

[53] N. Banno, T. Akihisa, K. Yasukawa, H. Tokuda, K. Tabata, Y. Nakamura, R. Nishimura, Y. Kimura, T. Suzuki, Anti-inflammatory activities of the triterpene acids from the resin of Boswellia carteri, *Journal of Ethnopharmacology* **107**(2) (2006) 249-253.

[54] M. R. Chevrier, A. E. Ryan, D. Y. W. Lee, M. Zhongze, Z. Wu-Yan, C. S. Via, Boswellia carterii extract inhibits TH1 cytokines and promotes TH2 cytokines in vitro, *Clinical and Diagnostic Laboratory Immunology* **12**(5) (2005) 575-580.

[55] K. Tsoyi, H. J. Jang, Y. S. Lee, Y. M. Kim, H. J. Kim, H. G. Seo, J. H. Lee, J. H. Kwak, D.-U. Lee, K. C. Chang, (+)-Nootkatone and (+)-valencene from rhizomes of Cyperus rotundus increase survival rates in septic mice due to

heme oxygenase-1 induction, *Journal of Ethnopharmacology* **137**(3) (2011) 1311-1317.

[56] S.-H. Jung, S. J. Kim, B.-G. Jun, K.-T. Lee, S.-P. Hong, M. S. Oh, D. S. Jang, J.-H. Choi, α-Cyperone, isolated from the rhizomes of Cyperus rotundus, inhibits LPS-induced COX-2 expression and PGE2 production through the negative regulation of NFκB signalling in RAW 264.7 cells, *Journal of Ethnopharmacology* **147**(1) (2013) 208-214.

[57] Y. Matsuo, Y. Mimaki, α-Santalol derivatives from Santalum album and their cytotoxic activities, *Phytochemistry* **77** (2012) 304-311.

[58] A. Mishra, S. S. Gauri, S. K. Mukhopadhyay, S. Chatterjee, S. S. Das, S. M. Mandal, S. Dey, Identification and structural characterization of a new pro-apoptotic cyclic octapeptide cyclosaplin from somatic seedlings of Santalum album L, *Peptides* **54** (2014) 148-158.

[59] G. I. Lee, J. Y. Ha, K. R. Min, H. Nakagawa, S. Tsurufuji, I. M. Chang, Y. Kim, Inhibitory effects of Oriental herbal medicines on IL-8 induction in lipopolysaccharide-activated rat macrophages, *Planta Medica* **61**(1) (1995) 26-30.

[60] A. B. Gokhale, A. S. Damre, K. R. Kulkami, M. N. Saraf, Preliminary evaluation of anti-inflammatory and anti-arthritic activity of S. lappa, A. speciosa and A. aspera, *Phytomedicine: International Journal of Phytotherapy and Phytopharmacology* **9**(5) (2002) 433-437.

[61] M. Jin, H. J. Lee, J. H. Ryu, K. S. Chung, Inhibition of LPS-induced NO production and NF-kappaB activation by a sesquiterpene from Saussurea lappa, *Archives of Pharmacal Research* **23**(1) (2000) 54-58.

[62] F. Zhao, H. Xu, E.-Q. He, Y.-T. Jiang, K. Liu, Inhibitory effects of sesquiterpenes from Saussurea lappa on the overproduction of nitric oxide and TNF-alpha release in LPS-activated macrophages, *Journal of Asian Natural Products Research* **10**(11-12) (2008) 1045-1053.

[63] J. Y. Cho, K. U. Baik, J. H. Jung, M. H. Park, In vitro anti-inflammatory effects of cynaropicrin, a sesquiterpene lactone, from Saussurea lappa, *European Journal of Pharmacology* **398**(3) (2000) 399-407.

[64] H.-G. Choi, D.-S. Lee, B. Li, Y. H. Choi, S.-H. Lee, Y.-C. Kim, Santamarin, a sesquiterpene lactone isolated from Saussurea lappa, represses LPS-induced inflammatory responses via expression of heme oxygenase-1 in murine macrophage cells, *International Immunopharmacology* **13**(3) (2012) 271-279.

[65] K.-S. Oh, T.-J. Park, B.-H. Choi, D.-K. Lee, T.-K. Lee, K.-T. Kim, Inhibition of nicotinic receptor-mediated catecholamine secretion by dryobalanops aromatica in bovine adrenal chromaffin cells, *Pharmacological Research* **42**(6) (2000) 559-564.

[66] T.-J. Park, Y.-S. Park, T.-G. Lee, H. Ha, K.-T. Kim, Inhibition of acetylcholine-mediated effects by borneol, *Biochemical Pharmacology* **65**(1) (2003) 83-90.

[67] M. H. Pourgholami, M. Kamalinejad, M. Javadi, S. Majzoob, M. Sayyah, Evaluation of the anticonvulsant activity of the essential oil of Eugenia caryophyllata in male mice, *Journal of Ethnopharmacology* **64**(2) (1999) 167-171.

[68] J. Guo, J.-A. Duan, Y. Tang, Y. Li, Sedative and anticonvulsant activities of styrax after oral and intranasal administration in mice, *Pharmaceutical Biology* **49**(10) (2011) 1034-1038.

[69] Y. C. Kim, E. H. Lee, Y. M. Lee, H. K. Kim, B. K. Song, E. J. Lee, H. M. Kim, Effect of the aqueous extract of Aquilaria agallocha stems on the immediate hypersensitivity reactions, *Journal of Ethnopharmacology* **58**(1) (1997) 31-38.

[70] H. Takemoto, M. Ito, T. Shiraki, T. Yagura, G. Honda, Sedative effects of vapor inhalation of agarwood oil and spikenard extract and identification of their active components, *Journal of Natural Medicines* **62**(1) (2008) 41-46.

[71] T. Alla, M. Handral, K. Nandakumar, N. Venkatrao, Shalam, Satyanaryana, S. M. Shantakumar, Anxiolytic and anticonvulsant activity of alcoholic extract of heartwood of Aquilaria agallocha roxb, (Thymelaeceae) in mice, *Pharmacologyonline* **1** (2007) 564-572.

[72] Z. Rabiei, M. Hojjati, M. Rafieian-Kopaeia, Z. Alibabaei, Effect of Cyperus rotundus tubers ethanolic extract on learning and memory in animal model of Alzheimer, *Biomedicine & Aging Pathology* **3**(4) (2013) 185-191.

[73] K. Hemanth Kumar, A. Tamatam, A. Pal, F. Khanum, Neuroprotective effects of Cyperus rotundus on SIN-1 induced nitric oxide generation and protein nitration: Ameliorative effect against apoptosis mediated neuronal cell damage, *NeuroToxicology* **34**(Supplement C) (2013) 150-159.

[74] A. G. Sunil, K. S. Kesavanarayanan, P. Kalaivani, S. Sathiya, V. Ranju, R. J. Priya, B. Pramila, F. D. S. Paul, J. Venkhatesh, C. S. Babu, Total oligomeric flavonoids of Cyperus rotundus ameliorates neurological deficits, excitotoxicity and behavioral alterations induced by cerebral ischemic–reperfusion injury in rats, *Brain Research Bulletin* **84**(6) (2011) 394-405.

[75] S. D. Ambavade, N. A. Mhetre, A. P. Muthal, S. L. Bodhankar, Pharmacological evaluation of anticonvulsant activity of root extract of Saussurea lappa in mice, *European Journal of Integrative Medicine* **1**(3) (2009) 131-137.

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