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Valorisation du *Prosopis juliflora* comme alternative à la diminution des ressources forestières au Kenya

Towards valorisation of *Prosopis juliflora* as an alternative to the declining wood resource in Kenya

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Sommaire

INTRODUCTION GENERALE

Ce travail trouve son origine dans les problèmes environnementaux occasionnés par le développement incontrôlé du *Prosopis juliflora* DC (Swartz) dans les régions arides et semi arides du Kenya. Suite aux inquiétudes des populations locales devant le caractère invasif de cette essence et à plusieurs problèmes de toxicité vis-à-vis du bétail mettant en cause cette espèce, il a semblé opportun de mener une étude sur les propriétés et les possibilités de valorisation de cette essence.

Le *P. juliflora* est une essence exogène native d'Amérique du Sud et d'Amérique centrale, qui a été introduite au Kenya au début des années 1970 pour améliorer la production de biomasse et lutter contre la désertification. Au cours des dernières années, il s'est rapidement acclimaté colonisant rapidement les terres agricoles et les pâturages. Sa capacité à faire des rejets après la taille le rend difficile à contrôler. Aujourd'hui, il a commencé à envahir tout le réseau routier, détourne le lit de cours d'eau, forme des bosquets impénétrables et est à l'origine de différents cas de mortalité de bétail ayant consommé son écorce, ses feuilles ou les cosses de ses graines. Il apparaît toutefois envisageable, par analogie avec d'autres régions arides comme en Inde où cette essence est présente, de transformer le caractère invasif de cette essence plutôt négatif en un atout pour les populations locales en valorisant la production de biomasse générée par cette essence. L'exploitation et la valorisation du *Prosopis juliflora* pourraient donc constituer une opportunité pour remédier aux problèmes de pénurie de bois existant actuellement au Kenya et remédier aux problèmes environnementaux causés par le développement rapide de cette essence.

. Sur demande du gouvernement kenyan, le ministère de la Forêt et de l'Environnement a mandaté l'Université Moi de mettre en place une étude sur les propriétés technologiques de cette essence. La recherche a été réalisée en collaboration entre le LERMAB (Nancy Université, France) et le département de Foresterie et des Sciences du Bois (Moi University, Kenya) dans le cadre d'une bourse d'étude accordée par le gouvernement français par le biais de l'ambassade de France à Nairobi.

La première partie de l'étude concerne l'évaluation des propriétés technologiques du bois de *Prosopis juliflora* poussant dans les zones arides et semi arides du Kenya. Pour cela nous sommes intéressé à la quantification des substances extractibles présentes dans le bois et à

leurs effets sur la durabilité du bois, mais aussi à la caractérisation des propriétés mécaniques, de l'anatomie, de l'imprégniabilité et du pouvoir calorifique de cette essence. Parallèlement à la caractérisation des propriétés technologiques du bois nécessaires pour valoriser cette essence comme possible alternative aux problèmes de pénurie de bois au Kenya, nous nous sommes intéressé à la nature des substances extractibles présentes dans l'écorce, les feuilles et gousses des graines pour essayer de comprendre les problèmes de toxicité liés à cette essence.

La seconde partie de l'étude concerne l'identification des composés chimiques présents dans les substances extractibles et l'évaluation des propriétés antioxydantes et antibactériennes de ces dernières. Les caractérisations chimiques ont été réalisées par différentes méthodes spectroscopiques (RMN ^{1}H et ^{13}C, FTIR) et différentes méthodes chromatographiques (GC-MS, HPLC). L'évaluation des propriétés antioxydantes a été réalisée en utilisant la méthode basée sur l'inhibition de l'oxydation du linoléate de méthyle et la méthode au DPPH. Différentes possibilité de valorisation des extractibles comme antioxydant ou antibactérien peuvent être envisageables suite à cette étude.

1.0. GENERAL INTRODUCTION

This study was motivated by the magnitude of the *Prosopis juliflora* (Swartz) DC invasion in Kenya, the level of public and government concern about the invasion, environmental, human and animal toxicity associated to this tree species in the arid and semi arid land and wood shortage resource across Kenya.

P. juliflora, an exogenous wood species native from South and Central America was introduced in Kenya in the early 1970's in order to remedy environmental problems, improve biomass cover and rehabilitate disused quarries. Over the years it spread rapidly and colonized agricultural lands and pasture. Its suckering ability after cutting made it difficult to control. Today it has overgrown road networks, diverted the flow of water, formed impenetrable thickets and caused death to livestock feeding on its bark, pods and leaves. Given experience where local inhabitants of arid and semi-arid areas such as India benefit from sale of *P. juliflora* products, the research began with a presumption that the invasion in Kenya can be turned into a significant resource for the local population. Efficient utilization of *P. juliflora* may therefore provide a solution to wood shortage resource and remedy the negative effects associated to this timber species in Kenya.

The Kenyan government, through the Ministry of Forestry and Environment requested Moi University to provide technological information on this wood species. The research was conducted in collaboration between LERMAB at Nancy University (France) and the Department of Forest and Wood Science of Moi University (Kenya). Financial assistance was provided by the French government through the French Embassy in Nairobi, under staff development training programme in Moi University, Kenya.

The first part of my study was to evaluate technological wood properties of *P. Juliflora*, growing in the semi-arid and arid land of Kenya. This involved working on the quantity of extractives from wood, leaves, pods and bark as well as understanding heartwood natural durability, contribution of wood extractives to wood natural durability, wood physical, mechanical, anatomical, energy and treatability characteristics. Technological wood properties will contribute to valorization of this species as an alternative construction material hence alleviating the reported wood shortage resource in Kenya.

The second part of the study was to identify and characterise the chemical nature, antioxidant and antibacterial properties of *P. juliflora* extractives. Chemical characterisation of extractives was based on ^{13}C $^{1}HNMR$, IR, GC-MS and HPLC analysis. Antioxidant properties were estimated using methyl linoleate oxidation inhibition and DPPH radical scavenging actions. Nature and characteristics of extractives from pods, leaves and bark is important to explain the reported toxicity of this species to animals, human and the environment. Valorisation of identified biomolecules as antioxidant and antibacterial agents is discussed.

2.0. LITERATURE REVIEW

2.1. What is known about *P. juliflora*?

Prosopis juliflora belongs to the family *Leguminosae* and is a fast growing and drought resistant plant originating from South and Central America. It grows in all kinds of soil conditions, including wastelands at altitudes ranging from 0 to 1,500 m above sea level, under mean annual temperature of 14 to 34°C and under annual rainfall of 50 to 1,200 mm (Pasiecnik *et al.*, 2001). Mature trees grow up to 17 m in height. The species is characterised by a green-brown, sinuous and twisted stem, with axial thorns situated on both sides of the nodes and branches. It is reported to dry out the soil and compete with grasses, particularly in dry areas and is therefore considered in some areas as a weed (Choge *et al.,* 2007). Indeed, it has been declared a noxious plant with legal disputes over compensation for its spread and subsequent loss of livelihoods in dry lands of Kenya.

Figure 1 reports distribution of *P. Juliflora* in Kenya. It is widely distributed in semi arid and arid regions such as Tana River, Baringo and Mandera. Kenya is facing wood resource deficit and challenges over reliance on natural resources (Mburu *et al.,* 2007; Mburu *et al.,* 2005). It is therefore important for national development that *P. Juliflora* is conserved, protected and sustainably used as an alternative material.

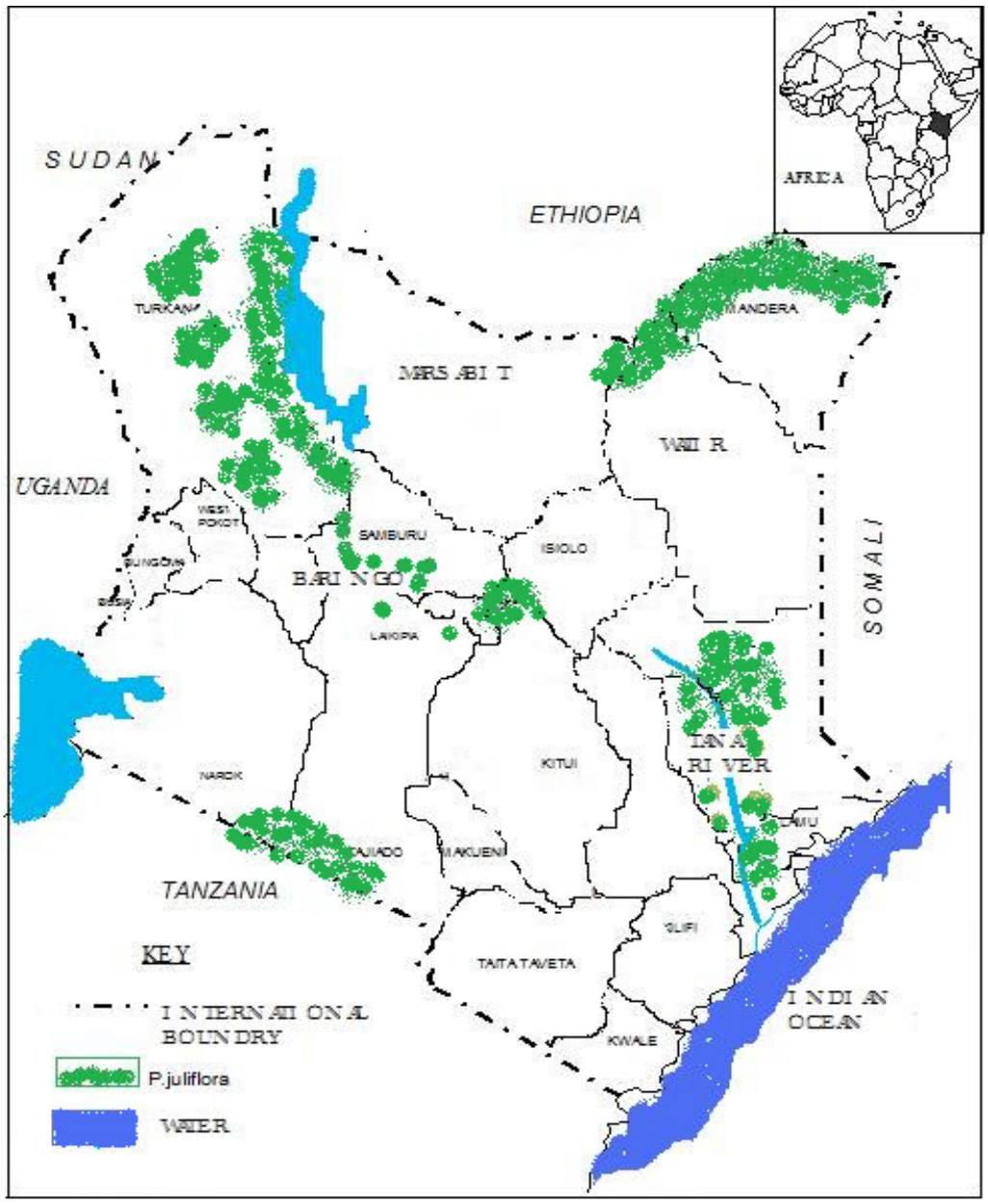


Figure 1. Distribution of *P. juliflora* in Kenya

P. Juliflora is valued for its ability to prevent soil erosion and to grow where nothing else can, but it is noted to colonize agricultural land and overgrow some road network in arid lands of Kenya (figure 2).

A **B**

C **D**

Figure 2. *P.juliflora* in Kenya
A. Over growing road network. **B**. Colonizing agricultural land
C. Growing were nothing can. **D**. Thorns that cause injury

Despite the negative effects, valorisation of *P. juliflora* pod flour, beans and gums as food supplement and medicine in animals and humans has been described (Barba *et al.,* 2006; Choge *et al.,* 2007). Chemical characterization of gums by Lopez *et al*. (2006) indicated an

arabinogalactan protein with potential uses for beverages and pharmaceutical products. Fatty acids and free sugar such as glucose, sucrose, rhamnose, fructose, glucose, galactose and galactouronic acid in the seeds and pods enhances its use as a food supplement (Fatma *et al.,* 1991; Sawal *et al.*, 2004; Silva *et al.,* 2002). Saturated and unsaturated fatty acids including caproic, lauric, myristic, palmitic, stearic, oleic, linoleic and linolenic acids and amino acids such as valine, leucine, tyrosine, and phenylalanine have been isolated from the pods of *P. Juliflora* (Marangoni *et al.,* 1988; Fatma *et al.,* 1991; Liu *et al.,* 2008). Mixtures have been reported to posses some antifungal and antibacterial properties.

Malhotra and Misra (1981) isolated from the fresh pods of *P. juliflora* ellagic acid glycosides such as rhamnosylgentiobiosyl, while Wassel *et al.* (1972) identified the flavonoïd patulitrin (figure 3). Galactomannan present in the seed gum of *P. juliflora* has excellent thickening properties useful for textile, pharmaceutical and food industries (Chaires-martinez *et al.,* 2008). Galactomannans have the fundamental structure consisting of a main chain of β-(1-4)-D-mannopyranose units substituted by a single α- (1- 6) galactopyranose units, although there are few deviations (Vieira *et al,* 2007). Choge *et al*. (2007) analysed the food quality and safety of flour produced from *P.juliflora* pods in Kenya and found to be rich in sugar, carbohydrates and proteins therefore can be useful as a supplement in human and animal feeds.

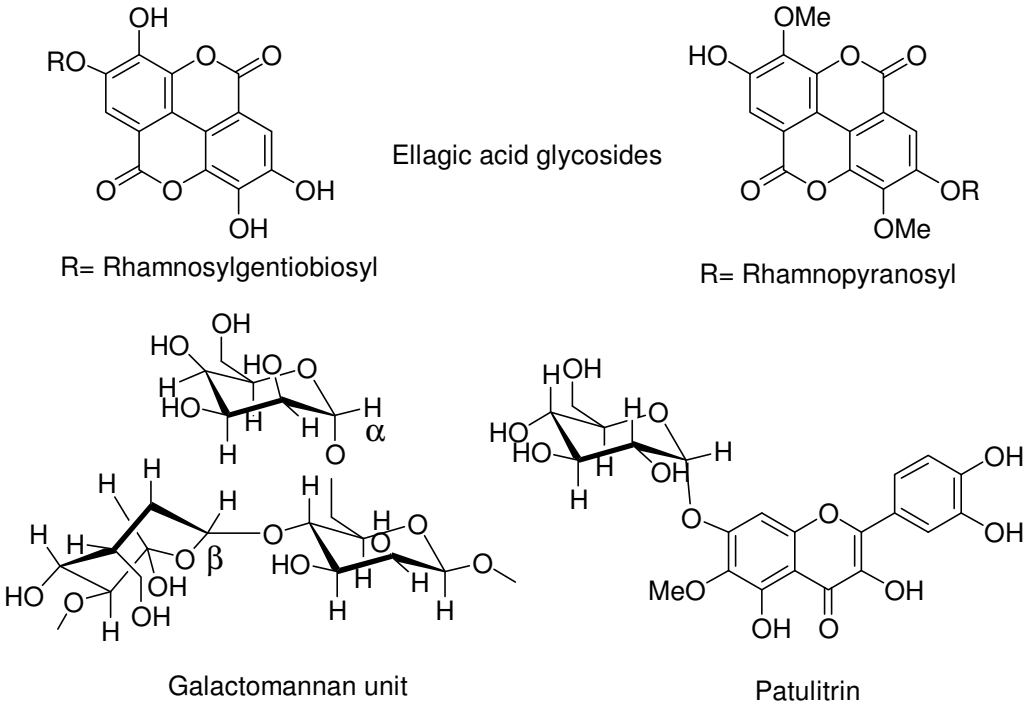


Figure 3. Some components contained in pods extractives of *P. juliflora*

Antifungal, plant growth inhibiting and DNA binding alkaloids such tryptamine, piperidine, phenethylamine and juliprosopine (figure 4) and their isomers have been isolated from the leaves of *P. juliflora* (Tapia *et al.,* 2000; Ahmed *et al.,* 1989).

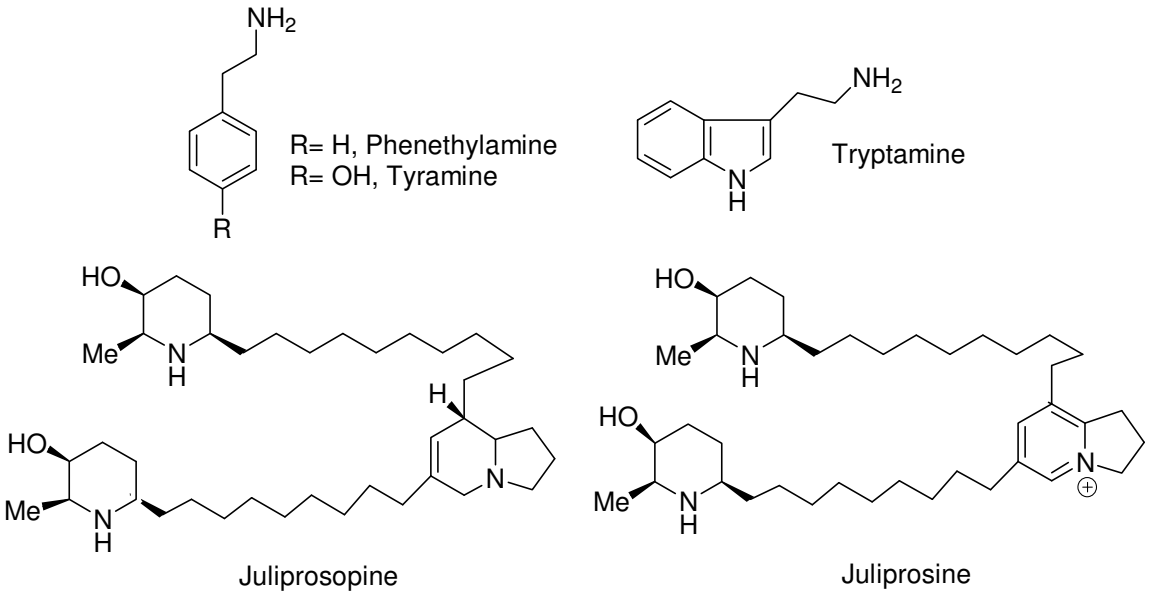
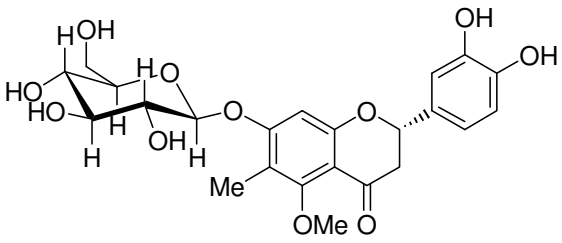


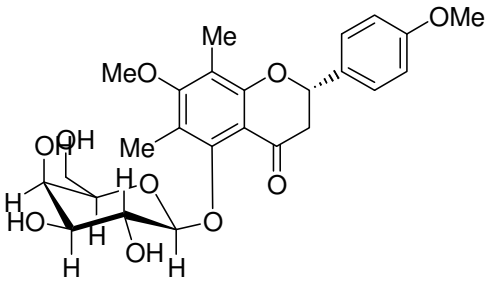
Figure 4. Some components contained in leaf extractives of *P. juliflora*

P. juliflora leaf alkaloids and their different isomers have the ability to inhibit growth of surrounding plants (Nakano *et al*., 2004; Kishore and Pande, 2005; Pasiecnik *et al.*, 2001). Fractionated alkaloids isolated from leaves have been shown to induce cytotoxicity leading to neuronal damages, neuromuscular alterations and gliosis in animals (Silva *et al.,* 2007). The leaves are reported to contain crude protein levels of 14-22%, crude fibre 21-23%, nitrogen free extract 43-50%, calcium 1.5% and phosphorus 0.2% while mineral content is directly related to the levels of minerals in the soil (Pasiecznik *et al.,* 2001).

Flavanones constituted of 3′,4′-dihydroxy-5-methoxy-6-methylflavanone,7-O-β-D-glucopyranoside and 7,4′-dimethoxy-6,8-dimethylflavanone5-0-β-D-galactopyranoside shown in figure 5 have been isolated from its roots (Malhotra and Misra, 1983). Catechins, shown to display a powerful protective effect against lipid peroxidation of cell membranes has been isolated at (73%) from exudates of *P. flexuosa* as the main constituent (Tapia *et al.,* 2000).



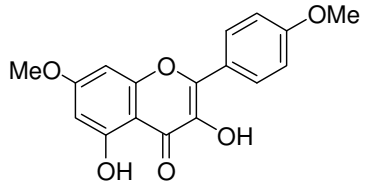
3',4'-dihydroxy 5-methoxy-6-methylflavanone 7-O-β-D-glucopyranoside



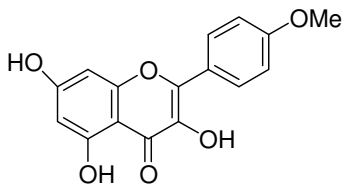
7,4'-dimethoxy-6,8-dimethylflavanone 5-O-β-D-galactopyranoside .

Figure 5. Main components contained in root extractives of *P. juliflora*

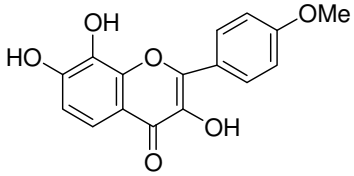
Some components which have been isolated from *P. juliflora* bark are shown in figure 6. Bark extractives exhibit antifungal properties (Caceres *et al.,* 1995). Two leucoanthocyanin flavonoïds (leucodelphinidin-3-O-α-L-rhamnopyranoside and flavonoïd leucodelphinidin-3-O-β-D-glucopyranosyl-(1-4)-O-α-rhamnopyranoside were isolated by Shukla *et al.* (1980). Ranjana and Misra (1981b and 1981b) later isolated several flavonoïd glycosides derived from kaempferol or retusin along with ombuin and a triterpenoid glycoside.



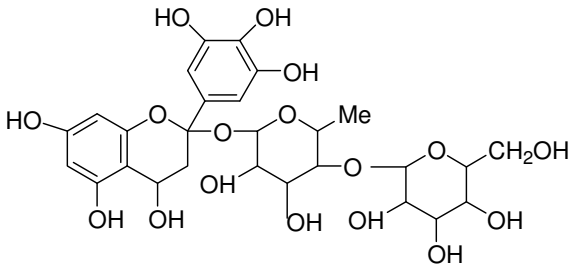
Quercetin 4',7-dimethylether



Kaempferol 4'-O-methyether



Retusin



L- Manopyranoside

Figure 6. Some components contained in bark extractives of *P. juliflora*

P. juliflora wood has been described as a source of lumber, firewood, activated carbon and barbecue charcoal (Kailappan *et al.*, 2000; Goel and Behl, 2001). There is considerable potential for *P. juliflora* as a source of fibre for the paper, paperboard and hardboard industries. The heartwood of different *Prosopis* species contains significant amounts of wood extractives and polyphenol compounds (Goldstein *et al.,* 1972). A reddish-amber gum with properties similar to the gum arabica produced by *Acacia Senegal*, often exudes from the stem and older branches. Little information about the chemical nature of *P. juliflora* wood extractives is available.

2.2. Plant extractives

Extractives are low molecular weight compounds present in plants in more or less important quantity, alongside cellulose, lignin and hemicelluloses (Chang *et al.,* 2001). They are obtained by extracting grounded plant material using neutral organic solvents of different polarities such as hexane, dichloromethane, acetone, toluene/ethanol mixture or water through cold soaking, steam distillation, Soxhlet or dionex extraction. Some are obtained as exudates from wounded trees (Toshiaki, 2001).

Many extractives have specific biological activities such as tannin able to bind to protein through complexation. They are therefore useful as sources of crude human and animal drugs. Biological and pharmacological studies strongly suggest that plant extractives used as additives in foods and beverages give various health benefits (Harborne and Williams, 2000). Extractives such as taxane diterpene (taxol), shown to have strong antitumor activity has been used as sources of crude drugs and medicines for centuries (Toshiaki, 2001). Similarly other group of extractives such as aryltetralin lignin (podophyllotoxin) alkaloid have both toxic (gastrointestinal toxicity) as well as benefic effects (antitumor) on human health (Harborne and Williams, 2000; Toshiaki, 2001).

A great diversity in extractive composition and distribution is found throughout wood species. Individual extractive compounds are often found in specific tissues of individual trees. The amount of extractives in the wood from the temperate zone ranges from 5 to 10% (Toshiaki, 2001) but higher amounts of up to 17.5% have been reported in *Prosopis africana*

and other tropical wood species (Gérardin *et al*., 2004). The amount however can vary from season to season even in the same tissue or are restricted in certain wood species (Taylor *et al.,* 2006).

Many phenolic compounds are accumulated in heartwood, whereas they are found only in trace amounts in the corresponding sapwood (Toshiaki, 2001). Extractives compounds in wood, bark, and leave range from simple products such as vanillin to polymeric condensed tannins (Taylor *et al.,* 2006). Such features provide the basis of chemotaxonomy of woody plants (Toshiaki, 2001).

High amount of extractives are found in the heartwood tissue of plant species in comparison with the seeds, leaves and bark. Recent progress of the biosynthesis of secondary metabolites of woody plants in the seeds, leaves, bark and heartwood has suggested the possibility of biotechnological control of their biosynthesis. This may lead to biotechnological production of biologically active extractives hence explaining molecular mechanisms of heartwood formation and the different physiological properties in woody plants (Toshiaki, 2001).

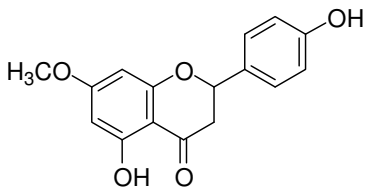
Wood extractives such as tannins, flavanoids, lignans, stilbenes, terpenes and terpenoids have crucial effects on the natural durability of wood explaining the resistance of some wood species to bio-degraders (Toshiaki, 2001; Windeisen *et al.,* 2002). Similarly extractives may influence color, fragrance, pulping, drying, adhesion, hygroscopicity and acoustic properties of wood (Tanaka *et al.,* 2008). Wood extractives have been shown, for example, to cause technical and economic pitch problems in the pulp and paper industry (Toshiaki, 2001).

The nature of extractives in bark and wood of same species are generally similar. The content can vary according to numerous factors, including environmental, genetic, age and seasonal variations (Toshiaki, 2001; Haupt *et al.,* 2003). Recent work has demonstrated that changes in the tree's environment during growth can alter heartwood extractives content in some species. If such variations in heartwood extractives affect wood properties, then silvicultural treatments and changes in how forest resources are managed may impact on the valorization of future wood products (Taylor *et al.,* 2006).

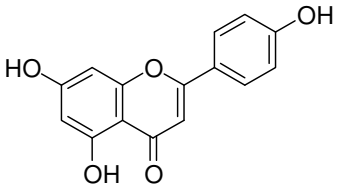
Variability in the amount of extractives exists between hardwood and softwoods with hardwoods having higher tannin contents than softwoods (Pizzi *et al.,* 1986). Some compounds present in plant extractives and their contributions to wood properties are presented thereafter.

2.2.1. Flavonoïds

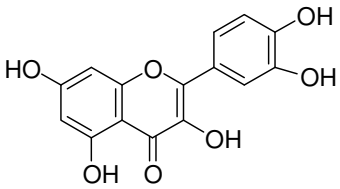
Flavonoïds are diphenyl propane (C_6-C_3-C_6) compounds. They are classified into flavanones, flavones, chalcones, dihydroflavonols (flavanonols), flavonols, aurones, flavan-3-ols (catechins), flavan-3,4-diols (leucoanthocyanidins), anthocyanidins, isoflavonoïds, and neoflavonoïds. Flavonoïds occur in bark, heartwood, flower, fruit, seed and root of many plants (Toshiaki, 2001). Some examples of each class of flavonoïds are described in figure 7.



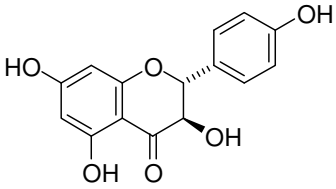
Sakuranetin-(flavanone)



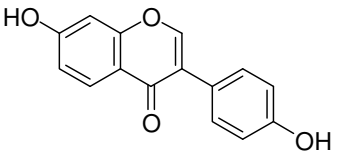
Apigenin-(flavone)



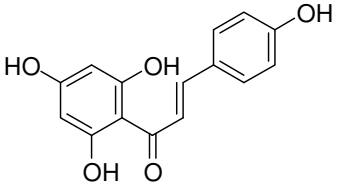
Quercetin-(flavonol)



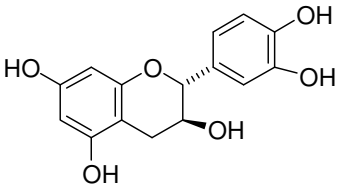
Dihydro-kaempferol-(flavanonol)



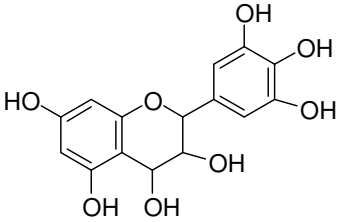
Daidzein-(Isoflavonoid)



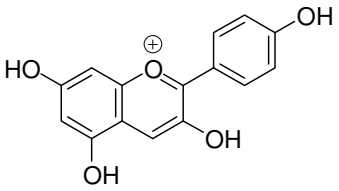
Chalconaringenin-(chalcone)



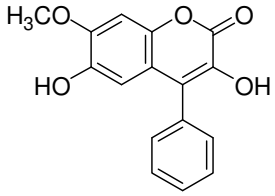
Catechin-(flavan-3-ol)



Leucodelphinidin-(flavan-3,4-diol)



Pelargonidin-(Anthocyanidin)



Dalbergin-(Neoflavonoid)

Figure 7. Some examples of each class of flavonoïds.

Flavonoïds protect plants from ultraviolet-induced injury and contribute to diversity in wood colorations (Tanaka *et al.,* 2008). They are biosynthesized in response to external stresses (ultraviolet light, microbial attack, and physical injury) (Tanaka *et al.,* 2008). Flavonoïd biosynthesis is a metabolic event which helps scientists to understand stress-gene expression relation in plants (Toshiaki, 2001).

More than 4000 different types of flavonoïds have been isolated from different plants (Neacsu *et al.,* 2007). The flavonoïds constitute a large potential resource of natural antioxidants and radical scavengers for the food and pharmaceutical industries and for use as technical antioxidants (Neacsu *et al*., 2007). Some flavonoïds such as 3,4,7,8-tetrahydroxyflavanone and 4,7,8-trihydroxyflavanone have important antifungal, antibacterial and antitermitic properties with great potential for further development as wood biocides (Khairullina *et al*., 2006; Mihara *et al.,* 2005).

Different flavonoïds such as naringenin and tamoxifen are reported to enhance the antibacterial, antiviral, or anti-cancer activities (Arima *et al.,* 2002). Combination with some drugs lead to synergistic effects. Some flavonoïds such as taxifolin and quercetin show antifeedant activity against subterranean termites (Ohmura *et al.,* 2000). *Melicoccus bijugatus* fruits rich in catechins and other flavonoïds are consumed for dietary and medicinal purposes, treatment of diabetes, cardiovascular and gastrointestinal disorders (Bystrom *et al,* 2008). Tannins from Mimosa are useful as additives in glue for plywood bonding (Stefani *et al,* 2007).

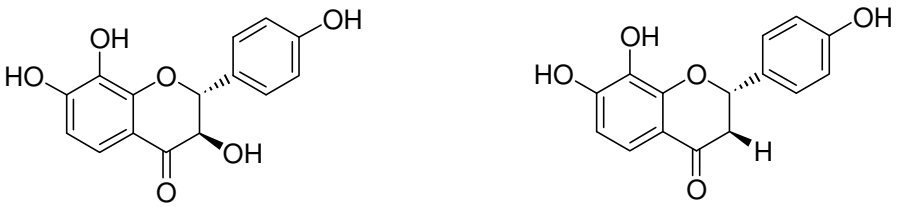
Harborne and Williams, (2000) suggest that flavonoïds contribute to colour in plants, protection against UV-B, microbial and animal invasion. Glycoflavones in the sap of many plants and glycosides in leaves are deterrent to insect attack. Occurrence of flavonoïds in pollens may facilitate insect pollination by stimulating the insects to visit the flowers. Flavonoïds with medicinal properties stop lipid peroxidation, inhibit oxidase enzymes and low density proteins (Harborne and Williams, 2000; Nijveldt *et al.,* 2001).

Flavonoïds in wood have an important effect on the durability (Chang *et al.,* 2001; Wang *et al*., 2004). It has been hypothesized that flavonoïds protect heartwood against fungal colonization by a dual function: fungicidal activity and being excellent free radical scavengers (antioxidants) (Schultz and Nicholas, 2000; Pongtip *et al.,* 2007). Pietarinen (2006) showed

that the radical scavenging activity is particularly important because both white-rot and brown-rot fungi are believed to use radicals to disrupt cell walls.

Heartwood formation is one of the metabolic events specific to woody plants, but little is known about its biochemical mechanisms. It is accompanied by deposition of significant amounts of secondary metabolites (heartwood extractives) such as flavonoïd, stilbene, lignan, norlignan, etc in to the heartwood cells (Toshiaki, 2001).

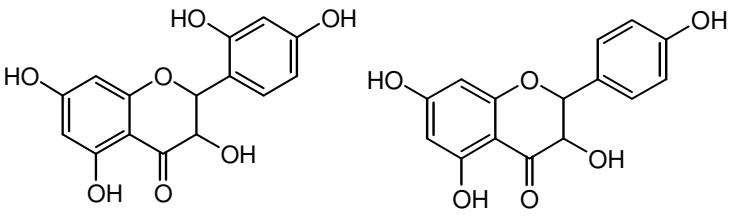
Flavonoïds such as 3,4,7,8-tetrahydroxyflavanone and 4,7,8-trihydroxyfavanone represented in figure 8, are associated to heartwood durability of *Acacia mangium* and *Acacia auriculiformis*. They also have properties that afford plant tissues protection from the external environment including antimicrobial, antifeedant and antioxidant properties (Mihara *et al.,* 2005).



3,4,7,8-tetrahydroxyflavanone 4,7,8-trihydroxyfavanone

Figure 8. Flavonoïds present in acacia heartwood

In woody tree species, flavonoïds accumulate in bark, leaves, and heartwood, while lesser amounts are found in sapwood and seeds. Extractives from bark and heartwood of many woody tree species have strong biological activities, such as enzyme inhibition, antioxidant and antifungal activities (Mihara *et al.,* 2005). Schultz *et al.* (1995) attributed the durability of acacia to the presence of dihydromorine and dihydrokaempferol (figure 9). Dihydromorine was also reported to contribute to the durability of *Morus mesozygia* (Déon *et al.*, 1980).



Dihydromorine Dihydrokaempferol

Figure 9. Structure of flavonoïds responsible for the durability of acacias

There are up to five components which are responsible for the durability of *plalymiscium yucatanum* against fungi among which 4, 2',5'-trihydroxychalcone (figure 10) was identified as particularly active against brown and white rots (Reyes chilpa *et al.* 1998).

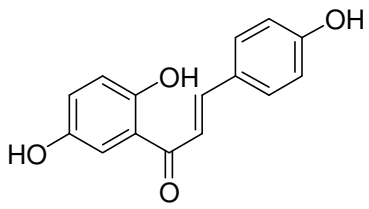
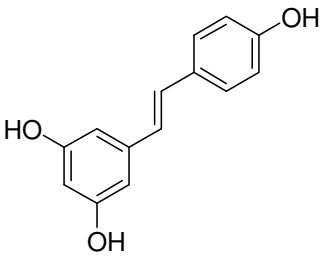


Figure 10. Structure of 4, 2', 5'-trihydroxychalcone

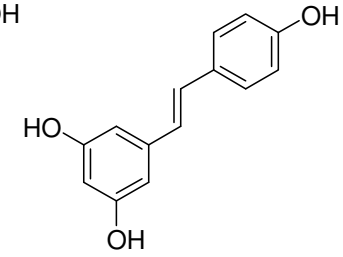
Bark of many plants contain substances like gallocatechin, catechin and many condensed tannins. The role of flavanoids in biodegradation resistance is variable and dependent on the test fungi. Exceptional resistance of *Robinia pseudoacacia* to biodegradation is attributed to the concentration of robinetine and dihydrobine (Rudman, 1963).

2.2.2. Stilbenes

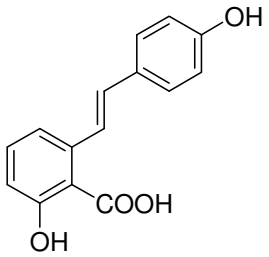
These are compounds possessing the 1,2-diphenylethene structure, as well as bibenzyls and phenanthrenes, which are composed of C_6-C_2-C_6 skeleton (Toshiaki, 2001). Typical examples of this class are shown in figure 11.



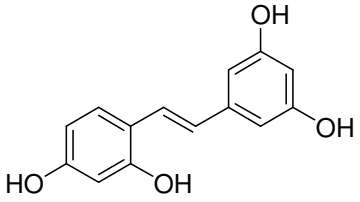
Resveratrol



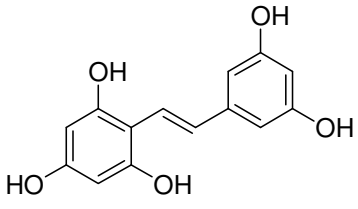
Pinosylvin



Hydrangeric acid



2,4,3',5'-tetrahydroxystilbene



3,4,5,3',5'-pentahydroxystilbene

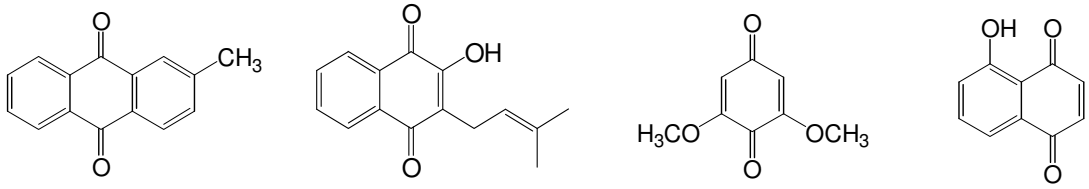
Figure 11. Structure of some stilbenes isolated from wood

Pinosylvin present in the heartwood of *Pinus* spp is formed as a response to external stress such as fungal infections or UV light. The role of stilbene in decay resistance and induction of its synthesis has therefore attracted much attention (Toshiaki, 2001). Pinosylvin, resveratrol and other stilbenes have three defense functions: as radical scavengers, as biocides and metal chelators (Pietarinen *et al.,* 2006). The radical scavenging activity is important because of the numerous enzymatic mechanisms involving radicals developed by both white-rot and brown-rot fungi to disrupt cell walls polymers (Pietarinen *et al.,* 2006).

Stilbenes are responsible for resistance against fungi even in vegetables (Celimene *et al.*, 1999). The 2,4,3',5'-tetra and 3,4,5,3',5'-pentahydroxystilbenes are responsible for wood resistance against brown rot fungi (Schultz *et al.*, 1995). Stilbene phytoalexins can be modified *in vivo* by partial methylation to enhance bioactivity (Schultz *et al.*, 1997). Indeed, generally all stilbenes (pinosylvin, pinosylvin monoethylether and pinosylvin dimethylether) are known to control the activity of brown and white rot fungi (Schultz *et al.,* 1997).

2.2.3. Quinones

Various types of quinones (benzoquinones, naphthoquinones, or anthraquinones) occur in many plant families (Toshiaki, 2001). Some examples are shown in figure 12. Quinones are pigments and have various biological activities e.g. Juglone in black walnut *(Juglans nigra),* is skin-irritating and well known allelochemical (Toshiaki, 2001). Tectoquinone has strong antitermitic activity and is assumed to be at the origin of the resistance of teak wood to termites (Levya *et al.*, 1998). *Tectona grandis* wood contain about 0.3% of 2-methylanthraquinone (tectoquinone) as well as naphtoquinone (Thévenon *et al.*, 2001).

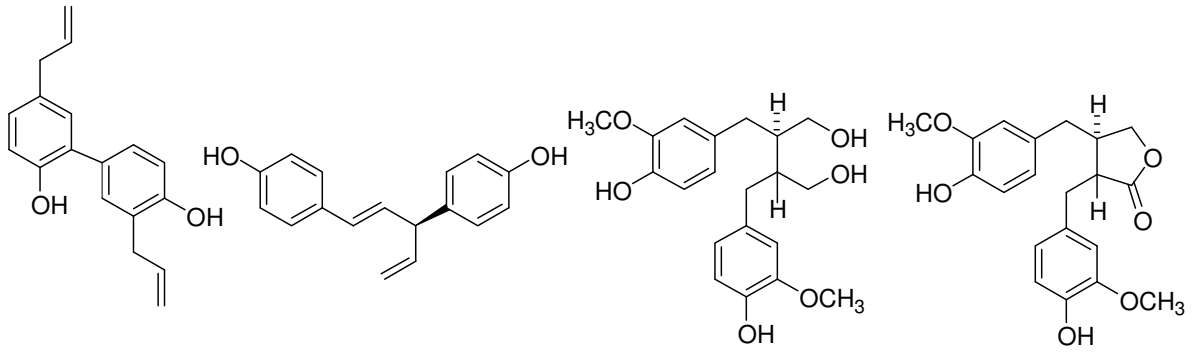


Tectoquinone Naphtoquinone 2, 6-dimethoxy-p-benzoquinone Juglone

Figure 12. Structure of some quinones

2.2.4. Lignans, Neolignans and related compounds

Lignans and neolignans are phenylpropanoids that occur in many woody plants including softwoods, hardwoods, and medicinal plants. Neolignan describe compounds containing two phenylpropane units (Toshiaki, 2001). Lignans are a group of phenylpropanoid dimmers (dibenzylbutanes, dibenzylbutyrolactones, furans, furofurans, aryltetralins, arylnaphthalenes, and dibenzocyclooctadienes), where the phenylpropane units are linked by different bonds (Toshiaki, 2001). Some examples of this class are shown in figure 13.



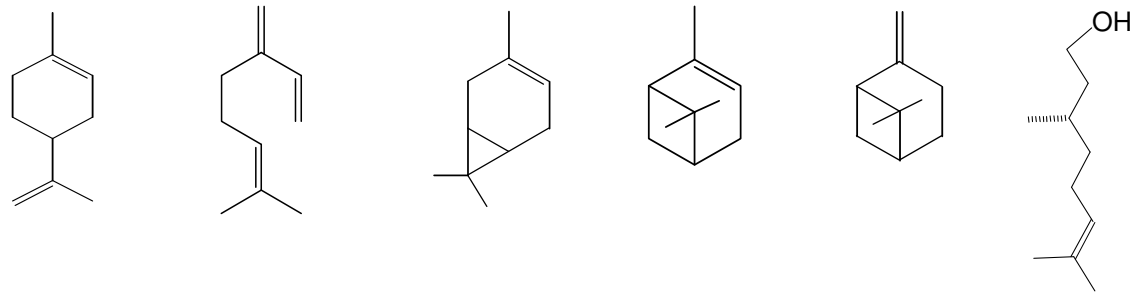
Honokiol Hinokiresinol (-)-Secoiso-lariciresinol (-)-Matairesinol

Figure 13. Structures of some lignans found in woody plants

Lignans are intermediates in the biosynthesis of lignin derived from oxidative coupling of coniferylic or coumarylic alcohols. Some of them have been identified as toxic to biodegraders and difficult to decompose (Pandey and Pitman, 2004). Different structures, identified in the bark of *Obvata species* suppress the activity of fungi. Among these, eudesmol, is particular important in controlling activities of basidiomycetes (Mori *et al.*, 1997). Furthermore, lignans have such biological activities as antitumor (Matairesinol, podophyllotoxin and steganacin), antimitotic (podophyllotoxin), antioxidant (nordihydroguaiaretic acid and sesaminol), antiviral (podophyllotoxin) against cytomegalovirus and (-)-arctigenin against herpes simplex l-virus, (Toshiaki, 2001).

2.2.5. Terpenes

Specific fragrances of different woods are usually due to their high monoterpenes and volatile sesquiterpenes concentration (Toshiaki, 2001). Some examples of this compounds are shown in figure 14.



Limonene Myrcene Δ^3-carene α-pinene β-pinene (-)citronellol

Figure 14. Structure of different monoterpenes

Terpenes can be easily separated from wood by steam distillation to yield essential oil. Turpentine and essential oil from *Pinus* spp. is obtained by steam distillation of exudates from pine trees (oleoresin). The residue of steam distillation is gum rosin that is composed mainly of diterpene acids (rosin acids) such as abietic acid (Toshiaki, 2001). Turpentines obtained from pine wood and those recovered from kraft pulp waste liquor are called wood turpentine and sulfate turpentine, respectively. Rosins are used for sizing of papers (Toshiaki, 2001). Monoterpenes like α and β-pinene are toxic to *Heterobasidon annosus*. Mixtures of monoterpenes like limonene, myrcene, 3-carene, α and β-pinene, from oleoresin of *Pinus ponderosa*, inhibit growth of fungi and bacteria (Flodin and Fries, 1978).

2.2.6. Tannins

Tannins are water-soluble phenolic compounds with the ability to precipitate alkaloids, gelatin, and other proteins. This class of compounds has high astringency and gives blue or green coloration with ferric chloride. Tannins are distributed widely in wood, bark, and leaves of many plants. They are classified into hydrolysable and condensed tannins (Toshiaki, 2001). Hydrolysable tannins are esters of an aliphatic polyo1 and phenolic acids and can be hydrolyzed into gallic or ellagic acid (Toshiaki, 2001). Condensed tannins are oligomers and polymers of polyhydroxyflavan-3-o1 units. In spite of their difference in the basic structures, hydrolysable and condensed tannins have a similarity in that they have many phenolic units and therefore are often called plant polyphenols. Tannins have antioxidant, radical-scavenging, biological and pharmacological activities (Toshiaki, 2001). Condensed tannins, especially wattle (or mimosa) tannins, have been used to produce adhesives for wood-based materials such as particle board and plywood. Wood preservative formulations based on tannin/copper,

tannin/zinc, tannin/boron complexation have been proposed (Thévenon *et al.*, 2001). Wood preservatives based on tannin/copper are very effective against fungi and insects (Toussaint, 1997). Some examples of this class are shown in figure 15.

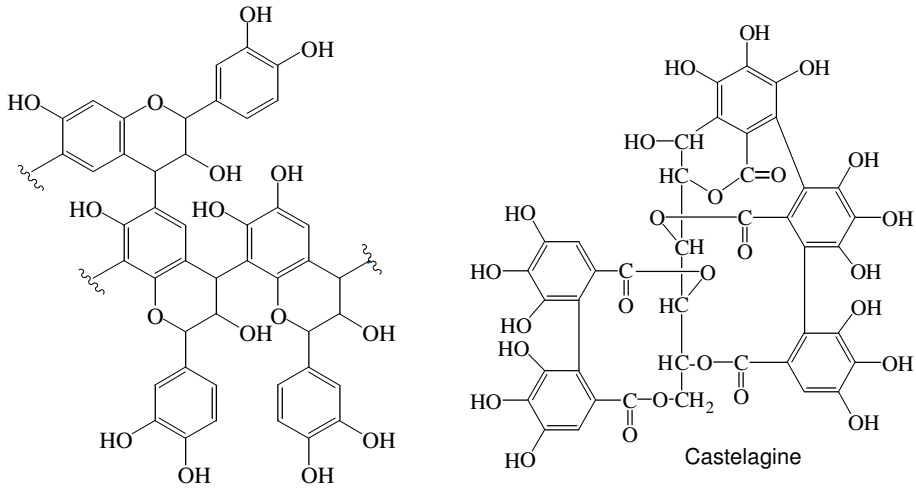
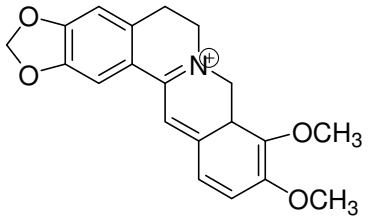


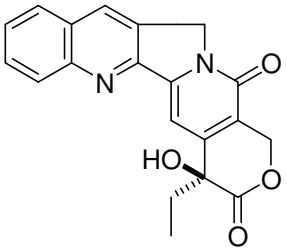
Figure 15. Structure of different plant tannins

2.2.7. Other extractives

Besides the compounds mentioned above, sugars, triglycerides and waxes, monomeric aromatic compounds, simple phenols like vaniline, syringaldehyde, sinapaldehyde, ferulic acid, guaiacol or eugenol, and alkaloids occur as extractives in woody plants (Toshiaki, 2001). D-Glucose and D-fructose are found, along with sucrose, in the seeds, sapwood and leaves of woody plants, while L-arabinose is found in heartwood. Waxes are complex mixtures of aliphatic compounds. To a lesser extend, some plant alkaloids such as berberine, quinine, emetine and reserpine (figure 16) have been reported to possess important medicinal properties (Toshiaki, 2001). These last compounds do not represent an important category of wood extractives, but are more frequently reported in leaves.



Berberine



Camptothecin

Figure 16. Structures of some alkaloids

2.3. Biodegradation of wood

2.3.1. Contribution of extractives to wood natural durability

The amount of extractives in the heartwood of a number of wood species such as *Thuja plicata* has been correlated with the observed termite and fungal resistance (Taylor *et al*., 2006). Termite resistance of wood is a function of heartwood extractive variability while individual extractives inhibit fungal growth (Neya *at al.,* 2004; Arango *et al.,* 2006).

Schultz and Nicholas (2000) suggested that extractives protect wood by combination of fungicidal and antioxidant properties. Phenolic extractives form complexes with metals as metal chelators to provide an additional means of wood protection (Hillis and Sumimoto, 1989; Slabbert, 1992). Wood degradation by fungi involves various metals, either in free form or as key components of enzymes (Ericksson *et al.*, 1990; Green *et al.,* 1997).

Biological deterioration of wood is of concern to the timber industry due to the economic losses caused to wood in service or in storage. Fungi, insects, termites, marine borers and bacteria are the principal wood biodegraders. They attack different components of wood at different rates giving rise to a particular pattern of damage (Silva *et al.,* 2007b). Degradation is influenced by environmental conditions of the wood; whether in storage or in use. The degraded wood material is returned into the soil to enhance its fertility (Silva *et al.,* 2007b).

2.3.2. Wood degrading fungi

Wood degrading fungi begin life as spores (round or oval bodies, which are not visible individually without the aid of a microscope). Under conditions of appropriate moisture, temperature, acidic PH, readily utilizable nutrients especially sugars at the wood surface and a certain amount of carbon dioxide, a spore germinates and produces a fine thread, or hypha. The thread like hyphae penetrates into the wood cells and obtains nutrients for growth by means of chemical reactions aided by enzymes. In damp conditions, hyphae interweave to form a mat, or mycelium. On maturity, fruiting bodies, or carpophores form and produce spores in quite remarkable numbers (Silva *et al.,* 2007b). *Ganoderma applanatum* is a large, highly virulent bracket fungus that produce large number of spores that are carried great distances by air current, water and animals (Beets *et al.,* 2008).

Wood decay fungi mainly gain access to wood using the water in the cell lumen as transport vessels to colonize the wood and begin to break down the structural components of wood. Most wood decay fungi initially attack wood by non-enzymatic methods, which allow them to gain access to the cell wall so that the larger enzymes can enter. These non-enzymatic pathways produce hydroxyl radicals that contribute to the breakdown of the structural components (cellulose and hemicelluloses) and lignin. Extracellular enzymes catalyze the dissolution of the cell wall components and therefore cause progressive changes of wood properties (Silva *et al.,* 2007b).

Decrease in toughness, a mechanical property most sensitive to wood deterioration is noted even before weight loss is detected. Other mechanical properties affected as degradation progresses include bending strength, compression, hardness and elasticity (Yen *et al.,* 2008; Schirp and Wolcott, 2005). As decay progresses the wood becomes discolored, loses strength, weight, and density. Decay and discoloration caused by fungi are major sources of loss in both timber production and wood use, with losses of 15 to 25% marketable wood volume in standing timber and 10 to 15% in wood products during storage and conversion. Brown rots rapidly and drastically reduce wood strength early in the decay process, while white rots causes a slower progressive decrease in wood strength. Brown-rot fungi can reduce wood strength by as much as 75% at less than 5% weight loss of the wood. For this reason it is important to develop methods which can detect incipient wood decay prior to the occurrence of significant strength loss (Zabel and Morrell, 1992).

Wood decay fungi are divided into: white rot, brown rot and soft rot fungi (Anke *et al.,* 2006). Brown-rot fungi preferentially attack and rapidly de-polymerize the structural carbohydrates (cellulose and hemicellulose) in the cell wall leaving the modified lignin behind. White-rot fungi can progressively utilize all major cell wall components, including both the carbohydrates and the lignin. As the primary biotic decomposers of wood (basidiomycetes) fungi can attack and degrade both wood in the forest and wood in service. They colonize and degrade wood using enzymatic and non-enzymatic processes. All groups of fungi have the ability to detoxify toxic chemicals such as wood preservatives and extractives (Schultz and Nicholas, 2000). This means that they can cause extensive damage to both treated and untreated wood. They hydrolyze and assimilate as food, wood components by injecting enzymes into the wood cells (Erickson *et al.*, 1990).

Fungi and bacteria attack both living and dead trees in different ways. The organisms have different demands in terms of conditions that sustain life. These conditions are specific to the species of organism and substrate they feed on (Beets *et al.,* 2008; Morton and Eggins, 1976). However, all living organisms need water and nutrition. At very high moisture content, wood degrading fungi are not able to grow, while bacteria survive. Fungi are members of the plant kingdom but differ from other plants by not processing the green colouring matter, chlorophyll. Despite the great diversity in form of fungal fruit bodies, the life cycle pattern in the majority of filamentous fungi involves the sexual and asexual parts.

The asexual part of the life cycle involves production of large number of spores, which are disseminated into the environment. Spores germinate when the environmental conditions (temperature, pH and moisture levels) are suitable to support mycelia colony. Sexual reproduction involves the fusion of two differentiated cells followed by meiosis, which result in segregation of genetic characters in progeny. Sexual reproduction is initiated by the onset of adverse climatic conditions. Organic and inorganic compounds as a source of energy and carbon are derived from cellulose, hemicelluloses, lignin, soluble sugars and minerals from wood (Presnell and Nicholas, 1990). The different types of wood rot fungi are discussed thereafter.

2.3.2.1. Brown rot

Brown rot is caused by *Basidomycota,* a group of fungi common on wood used above ground with characteristic fruit bodies and asexual reproduction system (Deacon, 1997). Brown rot fungi are equipped with cellulase that break down the linear structure of wood and leaves the brown cubical remnants of lignin (Niemenmaa *et al*., 2008). The optimal moisture content (MC) in wood for brown rot attack is 30-70% (Viitanen and Ritschkoff, 1991b).

Oxalic acid ($C_2O_4H_2$), the strongest of the organic acids (PKa_1=1.23, PKa_2=4.26) is secreted by both brown and white rot decay fungi and has been connected to various aspects of the Fenton reaction in brown rot decay (Micales, 1994). Oxalic acid secreted into the wood cell lumen quickly dissociates into hydrogen ions, decreases the pH of the medium and forms complexes with various cations. The reported tolerance of brown rot fungi to copper appears to be due to the complexing ability of oxalate with copper (Micales, 1994).

Brown rot decay is characterized by wood color changes, softening, brashness and brittleness. Fungus mycelium develops on wood surface and in latter stages of decay the wood shrink and cracks along and across the grain. Cellulose and hemicelluloses is degraded in the cell wall, leaving the wood to turn brown due to the presence of oxidised lignin (Green *et al.*, 1997). A typical cubical cracking is observed in wood with brown rot. However, in contrast to soft rot, brown rot degrades almost the whole of the S_2-layer of the cell wall (Anke *et al*., 2006). There is no localization of decay around the hyphae of the brown rot fungus. Instead, the fungus decays the cell walls by means of a generalized thinning (Carlile and Watkinson, 1994). In the later stages, the cell wall collapse and small splits very close to the S_2 microfibrillar orientation become visible (Anke *et al.*, 2006).

2.3.2.2. White rot

White rot is caused by both *Ascomycota* and *Basidomycota* (Anke *et al*., 2006). *Ascomycota* is distinguished by sexual reproduction in so-called *ascus*. White rot fungi produce laccases and peroxidases that cleave the ring structures of lignin and celluloses hence break down the linear cellulose molecules. White rots degrade all components of the wood structure leaving behind bleached strands of cellulose.

Indeed white rot do not only degrade the S_2-layer (as does soft rot), but also erodes the S_1 and S_3-layer sequentially. In the early stages of an attack, each hypha is surrounded by an erosion zone or a bore-hole (Carlile and Watkinson, 1994). In the advanced stages of white rot there is little or no shrinkage or collapse of wood cells. Severely attacked wood appears white and fibrous due to residual cellulose and has reduced mechanical properties (Beets *et al.,* 2008). Certain white rot fungi have mutualistic associations with bacteria and are effective in degrading various classes of hydrophobic extractives found in Scots pine (Dorado *et al.*, 2001).

2.3.2.3. Soft rot

Soft rot is caused by *Deuteromycota* and *Ascomycota.* *Deuteromycota*, propagate through asexual spores, *conidia*. Soft rot fungi decay wet wood in ground contact, since it requires nitrogen. The nitrogen is taken up from the surrounding soil (Anke *et al*., 2006). Soft rot is reported to cause serious economic loss through lose in pulp yield from wood chips in ground contact by hydrolyzing cellulose during storage (Mouzouras, 1989).

Soft rot prefer to degrade cellulose in the S_2 and S_3-layers of the cell wall. In the initial colonization, it grows in the cell lumen, and from this position a lateral branch penetrates the thin lignified S_3-layer. As the hyphae enter the S_2-layer, it branches into two hyphae and grows up and down the cell wall in the direction of the microfibrils (Carlile and Watkinson, 1994). Degradation occurs slowly in softwood when compared to white or brown rot. Wood subjected to a soft rot attack looks similar to that attacked by brown rot, but has a somewhat softer surface and reduced mechanical properties (Erickson *et al.*, 1990).

2.3.2.4. Moulds

Moulds are members of ascomycetes and fungi imperfecti that cause molds and stains on wood surface. Moulds colonize freshly felled wood without causing significant damage to cell walls. The presence of water (≥20%) in wood is an essential requirement for fungal colonization and decay to occur (Eaton and Hale, 1993). Moulds cause little damage to the structure of wood, provided their action does not reach a more aggressive stage where it would be considered soft rot (Schultz *et al*., 1995).

Moulds are always present in our surroundings as airborne spores. They do not have any of their own enzymes for degrading wood, and only live on the surface of the wood (Fengel and Wegener, 1984). They are more dependent on wood surface air humidity than on the moisture content in the substrate (Assersson and Bergman, 1971). The lowest relative humidity (RH) that can support mould growth on pine and spruce has been shown to be 80% (Viitanen and Ritschkoff, 1991a). The more hygroscopic a material is, the lower the relative humidity required for supporting mould growth (Block, 1953).

Moulds demonstrate a large range of temperature tolerances. Some mould fungi generally grow in temperatures around 0-50˚C, while other species propagate at temperatures down to negative 6˚C. Mould on a wooden surface can have both an antagonistic and stimulating effect on other fungi (Fengel and Wegener, 1984). Their ability to bind water, cause higher moisture content near the wood surface, and consequently make the wood more susceptible to other fungi. Mould attack on wood is an indication of impending rot attacks.

2.3.2.5. Sapstain

"Sapstain" refer to different microfungi that cause wood discolouration, often dispersed by insects. They survive in wood having moisture content (MC) ≥25%, and temperatures between 0-50˚C depending on species (Käärik, 1980). Fungal hyphae contain the pigment (melanin) of a blue, green or black colour, responsible for wood discoloration (Fengel and Wegener, 1984; Brisson *et al.*, 1996). The pigment is not produced at lower temperatures therefore a seemingly unaffected wooden surface can be extensively discoloured as the temperature increases. In contrast to moulds, sapstain hyphae grow into the wood through the rays, bordered pits and cell lumen (Fengel and Wegener, 1984). Although it grows in the cells, it cannot feed on cellulose and therefore it does not affect the strength properties of the wood (Blanchette *et al.*, 1992).

2.3.3. Bacteria

Bacteria of the class *Actinomycetes* are normally the first colonizers of wood. They create conditions necessary for fungal attack by altering the nutrient status of the wood and production of synergistic secondary metabolites (Holt *et al.,* 1979). Bacterial attacks need free

water in the wood cell to propagate and therefore are slower than attacks from most fungi (Fengel and Wegener, 1984).

Bacterial degradation of the cell wall occurs by tunnelling the pit membranes in the sapwood and erosion of ray parenchyma cells (Clausen, 1996; Fengel and Wegener, 1984). Some bacteria are capable of fixing atmospheric nitrogen for subsequent fungal colonization of the wood (Dickinson and Levy, 1979). They also have the ability to detoxify and degrade wood preservative elements including heavy metals such as Cu, Cr and As rendering them harmless. Bacteria are more tolerant to high lignin and extractive contents in wood, high preservative loadings and low levels of oxygen than fungi. They may be the sole agents of decay in situations where other decay organisms are excluded. The three different forms of bacterial decay are recognized as: erosion, tunnelling and cavitations (Singh and Kim, 1997).

2.3.4. Other wood degraders

2.3.4.1.Termites

Termites are social insects living in well-defined colonies under crowded conditions. The size of colonies varies from a few thousand individuals to millions. They require high moisture and carbon dioxide levels and shun the light (Cartwright and Findlay, 1958; Kollman and Cote, 1984; Hickin, 1971). Termites exist worldwide and may be found in latitudes 50^{o} North and 50^{o} South. They cause heavy destruction by hydrolyzing wood and other cellulosic components using enzymes in a short period of time (Theodore, 1973). Of about 2000 termite species, a few subterranean termites (Rhinotermitidae, Coptotermes, heterotermes and shedorhinotermes) in the tropics and reticulitermes found in subtropical and temperate regions are of economic importance (Hickin, 1975).

Termites feed almost exclusively on vegetable materials although extensive damage is often done to other materials in their efforts to find cellulose (Hickin, 1975). They are able to digest cellulose with the aid of protozoa secreting cellulase, which break down cellulose into simpler materials capable of being digested (Nunes and Dickson, 1996). The higher termites, particularly macrotermes in Africa and odontermes in Indomalaya, construct nests in which they bring in fungi to break down wood components into simpler digestible substances (Lee and Wood, 1971). A study carried out by Peralta *et al.* (2004) on wood consumption rates of

different forest species by termites under field conditions, did not find a strong correlation between wood density and termite resistance. The authors did, however, acknowledge the importance of wood hardness as a deterrent to termite damage, concluding that wood density alone cannot be considered the single most important factor in determining termite resistance.

2.3.4.2. Insects

Wood destroying insects are from the order coleoptera, isoptera and hymenoptera in decreasing order of importance. The families which are most destructive to wood in storage include anobiidae, lyctidae and bostrichidae collectively referred to as "powder post beetles" and platypodidae and scolytidae, referred to as "a*mbrosia beetles*" or pinhole borers. Powder post beetles constitute the most economically important group of wood destroying insects and are only overshadowed by termites. Larval stage of these beetles can last up to 5 years hence causes extensive damage (Hickin, 1975).

Lyctus beetles are often associated with sapwood and they derive their nourishment primarily from the starch reserve stored in the parenchyma ray cells. The larvae bore small tunnels approximately 1.5 mm in diameter and repeated attacks by the insects reduce wood into fine flour-like powder. They invade wood with moisture content as high as 30% (Schultz *et al.,* 2005). *Ambrosia beetles* are associated with dead and dying wood. They bore into the wood and feed on wood-inhabiting ectosymbiotic fungi "ambrosia fungi" introduced into the gallery system by the beetles. "Ambrosia fungi" are able to breakdown cellulose and lignin into organic molecules that can be assimilated by the beetles. They can also synthesize chemicals essential for beetle development (Silva *et al.,* 2007b; Collins and Weber, 1987).

2.3.4.3.Marine borers

These are the most important degraders of wood in marine environments. They are categorized into two families: molluscans and crustaceans. Molluscans include teredo (shipworm), pholads and bankia. Teredos are filter feeders and they go into the wood for food and shelter. They can be identified by their worm-like bodies, which sometimes extend up to 2m and calcified tunnels, where they live in. They have two siphons, one used for sucking water, which contain food nutrients and the other for pumping out water from the body. They cause extensive damage

to the wood by deep tunneling, leaving behind a small diameter hole on the surface that may not show the extent of damage inside the wood (Johnson *et al.*, 1973). The crustaceans include limnoria, sphaeroma and chelura. These are crab-like creatures, which have mandibles and have the ability of moving hence are not restricted to attacking the same piece of wood. They are 3-4 mm long and attack wood by boring shallowly about 1cm below the surface. Repeated attacks on the same wood cause an hourglass appearance on pilings, which develops a weak point in the intertidal zone (Lori *et al*., 1999). Marine borers cause economic losses on marine pilings and other structures along the coastal waters.

3.0. MATERIALS AND METHODS

3.1. Determination of extractive content

3.1.1. Plant material preparation

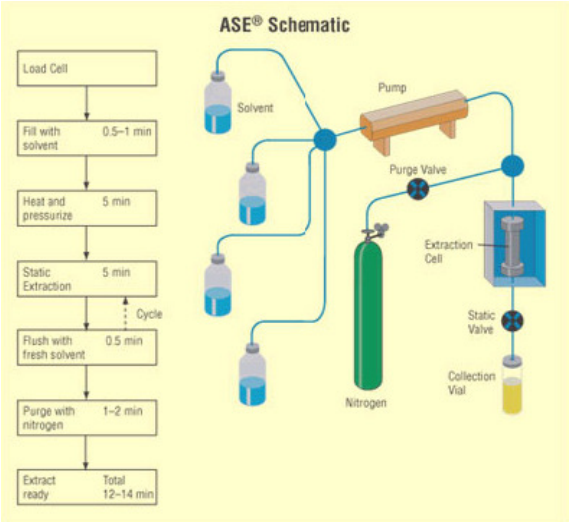
27 year old *Prosopis juliflora* growing at different sites were selected randomly from Baringo forest (latitude 0°, 20'N, longitude 35°, 57'E), Kenya. The trees were marked for identification purposes, felled and debarked. Bark, wood, leaves, pods and stem bark exudates from each tree were picked separately and transported while fresh to Moi University Wood Science laboratories.

In the laboratory samples were conditioned for two months, reduced to appropriate sizes for packaging and transported to LERMAB laboratory, Nancy University (France). In the laboratory samples were reduced further to appropriate sizes as indicated for different tests.

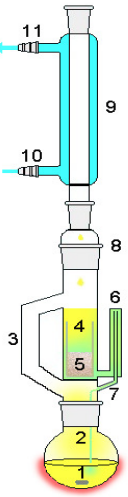
3.1.2. Solvent extraction

Heartwood, sapwood, bark, leaves and pods were separately ground to fine powder using a vibrating hammer mill, passed through a 115-mesh sieve and dried at 60°C to constant weights before extraction.

Two methods of extraction namely Soxhlet and accelerated solvent extractor (Dionex ASE 200) were used throughout this study (figure 17). Different solvents including hexane, dichloromethane, acetone, toluene/ethanol (2/1 v/v) mixture and water were selectively used.



A. Dionex extractor



1: Stirrer
2: Still pot
3: Distillation path
4: Thimble
5: Solid
6: Siphon top
7: Siphon exit
8: Expansion adapter
9: Condenser
10: Cooling water in
11: Cooling water out

B. Soxhlet extractor

Figure 17: Extraction equipments used

3.1.3. Extraction procedure

Soxhlet extraction was done using hexane, dichloromethane, acetone, toluene/ethanol (2/1 v/v) and or water. 10gm of sample powder were extracted with 180ml of the solvent for 15 hours at the rate of 10 to 12 cycles per hour.

Dionex ASE 200 (Voisins Le Bretonneux, France) extraction was done using dichloromethane, acetone, toluene/ethanol (2/1 v/v) and or water. Extraction was performed in 33 mL cell size on 10gm of sample powder at 100°C under a pressure of 100 bars (3 static cycles of 5 minutes each). To quantify the total amount of extractives series extraction was done successively on same sample using hexane, dichloromethane, acetone, toluene/ethanol (2/1 v/v) and or water in that order. Three replicate extractions were done for each sample and test.

3.1.4. Quantification of extractives

After each extraction, the solvent was evaporated under reduced pressure in a Büchi rotavapor and the residue dried over P_2O_5 under vacuum before weighing.

Two methods based either on direct determination of extractives after solvent evaporation (direct methods, DM) or on the difference between dry weight of sawdust before and after extraction (indirect method, IM) were used to evaluate extractive contents.

The percentage of extractives was evaluated according to the formula:

$$\%\,\mathrm{DM} = \frac{m_e}{m_s} \times 100$$

$$\%\,\mathrm{IM} = \frac{(m_s - m_d)}{m_s} \times 100$$

where m_e is the weighed mass of extracts after solvent evaporation m_s is the dry mass of the sawdust before extraction, and (m_d) is the dry mass of extracted sawdust.

3.2. Natural wood durability

3.2.1. Biological organisms

Macrotermes natalensis (MN) termites common in Kenya and brown rot fungi *Poria placenta (PP) s*train FPRL 280*, Gloephyleum trabeum (GT), Coniophora puteana (CP)* and white

rot fungi *Coriolus versicolor (CV)* strain CTB 863A, *Pycnoporus sanquineus (PS) and Antrodia species* strain CIRAD/2304/1 and coloration fungi *Aureobasidium pullulans (AP)* were used throughout this study. All fungal strains were obtained from CIRAD Forêt (Montpellier, France).

3.2.2. Decay resistance of *P. juliflora* wood against fungi

Heartwood samples 35mm × 25mm × 5mm (longitudinal, radial, tangential) Soxhlet extracted or not extracted were conditioned to a constant weight (m_u) at temperature of 20° to 22°C and relative humidity of 60 to70%.

Theoretical dry mass (m_t) of test samples was determined from the averaged percentage moisture (μ) obtained from similar samples dried at 103°C according to the formula:

$$m_t = \frac{m_u}{100 + \mu} \times 100$$

Petri dishes (9cm diameter) were filled with sterile culture of malt-agar medium (30 grams malt, 40 grams agar in 1L of distilled water) allowed to solidify, inoculated with fungal culture and incubated at 22°C and 85% RH until full colonization by mycelium.

In a sterile chamber, two UV sterilized samples were introduced to cultures of the brown rot fungi *Poria placenta, Gloephyleum trabeum* and white rot fungi *Coriolus versicolor* and *Antrodia species*. Each experiment was repeated three times. Beech wood (*Fagus sylvatica*) was used as control for white rot fungi and *Pinus sylvestris* sapwood for brown rot. Incubation was carried out for four months at 22°C and RH of 70%. Assessment of decay was done by determination of mass loss using the following formula:

$$\text{mass loss } (\%) = \frac{(m_t - m_f)}{m_t} \times 100$$

where m_f is the final weight of dry samples after attack

3.2.3. Inhibition of fungal growth by extractives

To understand contributions of extractives towards wood decay resistance fungal mycelium was grown in 9 cm Petri dishes filled with 20 ml of malt-agar medium (30 grams malt, 40 grams agar in 1L) treated to 50, 100, 500 and 1000 ppm of extracts. Control dishes were not treated with the extracts.

Introduction of the extracts was carried out after medium sterilization (20 min, 120°C, 1 bar) by addition of the necessary quantity of extract solubilized in 5ml of ethanol. Dishes were inoculated in their centre with a 10 mm portion of healthy fungal colony. Incubation was carried out at 22°C and 70% RH.

Growth was evaluated every 2 or 3 days by measuring the diameter of the colony estimated from the mean of two perpendicular diameters and expressed as a percentage of the room available for growth. Growth inhibition was calculated when the diameter of the control culture reached 9 cm according to the formula:

$$\text{growth inhibition } (\%) = 100 \times (1 - d_1/d_0)$$

where d_0 is the diameter of the control culture and d_1 the diameter of the culture in the presence of extracts. All experiments were repeated two times.

3.2.4. Resistance of *P. juliflora* wood against termite attack

Field test: A standard field evaluation method to determine resistance to subterranean termites AWPA: E7-1993 was adopted. Eighteen heartwood test samples measuring 50mm × 15mm × 15mm were Soxhlet extracted separately using hexane, dichloromethane, acetone, toluene/ethanol (2/1 v/v) and or water and conditioned to constant weight (t_1). Eighteen unextracted test samples of *P. juliflora* and *Pinus sylvestris* were used as positive controls.

Three termite nests were randomly selected at Kerio Valley, Kenya. The test site was cleared of vegetation and any wood debris before exposing the samples. Test samples were

exposed at a distance of 0.3m from the termite nest with a third of their lengths above the ground and spaced 30cm apart in a randomized design (Figure 18).

Figure 18. Termite field layout at KerioValley, Kenya

The soils around each test sample were compacted after installation. The positions of the installed test samples were mapped in the field to facilitate inspection and record keeping. Every month for six months three test samples extracted or not by each solvent and controls were pulled out of the ground carefully with minimal soil disturbance, cleaned by gently brushing off any soil on its surface and oven dried to a constant weight (t_2). % weight loss (w_t) was then evaluated as below:

$$w_t\,(\%) = ((t_1 - t_2)/\, t_1) \times 100$$

Laboratory test: Resistance to *M. natalensis* in the laboratory was evaluated on E1-97 standard method. Six test blocks 30mm x 10mm x 20mm were Soxhlet extracted using fresh hexane, dichloromethane, acetone, toluene/ethanol (2/1 v/v) and or water, conditioned to constant weight (m_1) and labeled. Two test samples were placed in sterile glass jars with two corners against the side of the container. The jars measuring 80mm diameter and 100mm high contained 150 grams of sterile sand and 30 ml distilled water (figure 19).

Figure 19. Termite test in the laboratory

Four hundred termites were added to each jar at a ratio of 360:40 workers to soldiers respectively and incubated at 25^0 C for 28 days. This was replicated three times. *Pinus sylvestris* and unextracted *P. juliflora* samples were used as the control.

Both percentage of live termites and weight loss (w_l) of the test samples were determined after the experiment as follows:

$$w_l\,(\%) = ((m_1-m_2)/\,m_1)x\ 100$$

where m_1 is the dried initial weight of the block and m_2 the dried weight after exposition to the termites.

Classification of wood durability against termites was done as described in table 1.

Table 1. Classification of wood durability against termites

Block aspect after test	Classification
Sound, no attack	10
Light attack	9
Moderate attack	7
Heavy attack	4
Failure	0

3.2.5. Anti-termitic test of *P.juliflora* heartwood extractives

No- choice bioassay method was used to evaluate the anti-termitic activity of heartwood extractives. Whatman n°3, 9.0cm diameter filter paper was treated with solution of 10, 50, 100, and 500mg/g of extractives. This was done by soaking the filter paper overnight in acetone containing the different amounts of extractives and drying at 60°C to remove the solvent. A piece of filter paper treated with the solvent only was used as the control.

Fifty (50) active termites (45 workers, 5 soldiers) were placed onto each filter paper impregnated with the test materials in a Petri-dish 9cm diameter x 1.5cm height (figure 20). The test dishes with perforated covers, sterilised sand and filter paper were then placed into an incubator maintained at 26.5^0 C and R.H of 80% for 21 days.

Figure 20. Antitermitic test of extractives in the laboratory

A few drops of water were periodically added to the bottom edge of each Petri dish. Three replicates were prepared for each test sample and the mortality of the termites evaluated daily

3.3. Other wood properties

3.3.1. Heartwood anatomy

Microscopic observations were performed with an environmental scanning electron microscope (ESEM Quanta 200) for heartwood samples Soxhlet extracted using different solvents or not extracted. The transverse surface of test samples was microtomed and analyzed without further preparation. Photomicrographs were taken at different magnifications.

3.3.2. Physical and fuel wood characteristics

Moisture content was determined using the oven dry method. Basic density was determined from freshly cut wood samples using water displacement method hence:

$$\text{wood density} = w_o / v_o$$

where v_o is the volume of displaced water and w_o is the oven dried weight of the sample.

Ash content was determined as per ASTM D 3174-89. 1 g of wood sample in a tared porcelain capsule was subjected to a temperature of 815^{o}C in a muffle furnace and the amount of ash generated measured. Calorific value was determined as per ASTM D 2015-93. 1 g of ground wood was pressed into a pellet and charged into a calorimetric bomb standardised by benzoic acid with a heat value of 26.453 MJ/kg. Each experiment was repeated three times.

3.3.3. Dimensional stability

Twenty four samples of *P. juliflora* were cut into 20mm × 20mm × 20mm and dried at 103± 2°C. Dimensions were measured to the nearest 0.01 mm using Veneer callipers and their dry volume determined (v_1). The samples were then put in a desiccator containing a saturated copper sulphate solution.

Weight of these blocks was measured every two days until stabilization to constant mass indicating that the wood blocks have attained maximum moisture level. Dimensions were measured again and wet volume determined (v_2). Swelling coefficient (S) was determined according to the formula:

$$S\,(\%) = \frac{v_2 - v_1}{v_1} \times 100$$

3.3.4. Mechanical strength

Specimens from freshly cut mature *P. juliflora* were extracted from billets cut at breast height. Clear-wood specimens were sampled and tested according to British Standard, BS 373:1957 with specimens obtained along and across the grain. Specimens were dried to approximately 12% moisture content.

Bending strengths (compression and shear parallel to the grain) and hardness was tested. Modulus of rupture and elasticity and Janka hardness was evaluated. A Calibrated Universal Strength Testing Machine (USTM) was used.

3.3.5. Wood treatability

Industrial-quality CCA was used at concentration of 6% (Tanalith-C, Arch Timber). Air dried round wood samples of 140-150mm diameter and 2m lengths were vacuum-pressure treated with an initial vacuum of 500 mm Hg for 15 min, pressure of 150 KN/m^2 for 4 hours and a final vacuum of 500 mm Hg. Retention and penetration were determined using the following formula:

$$\text{Retention} = (w_2 - w_1) \times c/v$$

where w_1 is the weight of the air dried untreated wood (kg), w_2 is the weight of the pressure treated wood (kg), c is the solution concentration (%) and v is the sample volume.

Penetration was determined by measuring the depth (mm) impregnated by chemical from the periphery in the radial direction at the middle length.

$$\text{Penetration} = [1-(r-r_1)/r] \times 100$$

where r_1 is the depth of preservative penetration (cm) and r is the sample radius. Each experiment was repeated three times.

3.4. Identification and characterisation of *P. juliflora* extractives

3.4.1. General information

Melting points were measured on a Buchi Melting Point B-540 apparatus. Micro-analysis was carried out on a Thermofinnigan Flash EA 1112 apparatus. Specific rotations were determined on a Perkin–Elmer 141 polarimeter (10 cm cell) at room temperature. Analytical thin-layer chromatography was performed on Merck 60 F254 pre-coated silica gel plates. Compounds were visualised with UV light. All compounds and reagents used unless stated were purchased from Fluka-Sigma-Aldrich Chimie SARL (St Quentin Fallavier, France).

3.4.2. ^{1}H NMR, ^{13}C NMR and FTIR analysis

^{1}H and ^{13}C NMR spectra were recorded in chloroform-D, methanol-D_4, acetone-D_6 or dimethylsulfoxide-D_6 as required on a Brüker DRX 400 spectrometer. Chemical shifts were expressed in ppm and calculated relative to tetramethylsilane (TMS). FTIR spectra were recorded as KBr disks on a Perkin Elmer FTIR spectrometer SPECTRUM 2000 between wave number ranges of 4000-500 cm^{-1}. Finely divided samples were dispersed in a matrix of KBr and pressed to form disks.

3.4.3. GC-MS analysis

Test samples were analyzed as trimethyl derivatives using the following procedure. In a screw-capped vial, a sample of approximately 1 mg of dry sample was dissolved in 0.5 ml of anhydrous acetonitrile (Acros Organics) and 0.4 ml of *N,O*-bis-trimethylsilyl (trifluoroacetamide) containing 1% of trimethylchlorosilane (BSTFA / 1%TMCS) (Acros Organics) was added. The solution was sonicated for about 1 min and heated at 60°C for 60

min. After evaporation of the solvent in a stream of dry nitrogen, the residue was diluted in 1 ml of anhydrous acetonitrile.

GC-MS analysis was performed on a Clarus® 500 GC gas chromatograph (Perkin Elmer Inc., USA) coupled to a Clarus® 500 MS quadrupole mass spectrometer (Perkin Elmer Inc., USA). Gas chromatography was carried out on a 5 % diphenyl / 95 % dimethyl polysiloxane fused-silica capillary column (Elite-5ms, 60 m x 0.25 mm, 0.25 mm film thickness, Perkin Elmer Inc, USA). The gas chromatograph was equipped with an electronically controlled split / splitless injection port. The injection (injection volume of 1 µl) was performed at 250°C in the split mode (split flow of 20 ml/min). Helium was used as carrier gas, with a constant flow of 1.2 ml/min.

The oven temperature program was as follows: 200°C constant for 4 min, 200°C to 330°C at a rate of 5°C/min and then constant for 330°C. Ionization was achieved under the electron impact mode (ionization energy of 70 eV). The source and transfer line temperatures were 250°C and 330°C, respectively. Detection was carried out in scan mode: m/z 35 to m/z 700 a.m.u. The detector was switched off in the initial 10 min (solvent delay).

3.4.4. HPLC analysis

HPLC analysis was performed using a Supercosil ^{TM} LC-18 column (250 mm x 4.6 mm i.d,) at 35°C on a Waters liquid chromatograph (Waters SAS, Saint Quentin-en Yvelines, France) equipped with a system controller 600E, a manual injector system with a 20µl loop and a Waters 2996 photo diode array (PDA) detector. The data were recorded on a 210 nm to 400 nm range with the Empower software.

Solvents used for elution were solvent A (water containing 0.05% of trifluroacetic acid) and solvent B (methanol (HPLC grade) containing 0.05% of TFA) at a flow rate of 1ml/min. Elution was achieved with a binary gradient starting at 95% A and 5% B for 1 min followed by a linear ramp to 50% A and 50% B at 10 min to finish at 100% B after 20 min.

3.4.5. Antioxidant tests

Two methods were used to evaluate antioxidant properties:

3.4.5.1. Methyl linoleate oxidation inhibition

Oxidation of methyl linoleate (2 ml of a 0.4 M solution in 1-butanol) was performed in a closed borosilicate glass reactor containing 1 ml of a 9.10^{-3} M solution of 2,2'-azobis[2-methylpropionitrile (AIBN) in 1-butanol as initiator. The double shell reactor was thermostated at 60°C by an external heating bath. Oxygen (150 Torr) was bubbled by a gas-tight oscillating pump. A small condenser was inserted on the reactor in the gas circulation to ensure condensation of the solvent. Oxygen uptake was monitored continuously with a pressure transducer (Viatron model 104) in the presence of 1ml of a 10^{-4} M, 10^{-5} M, and 6.10^{-6} M solution in butan-1-ol of the test samples to evaluate antioxidant properties. The volumes of the liquid and the gas phases were respectively of 4 and 100ml.

3.4.5.2. Radical-scavenging activity

Scavenging actions of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical by different extracts were also measured. Heartwood, sapwood and bark extracts and reference controls like (+)-catechin and BHT (Butylated hydroxytoluene) were prepared to concentrations of 10^{-4} M, 4.10^{-5} M and 2.10^{-5} M. The reaction mixture contained 100µM of 0.09g/l DPPH solution and 10, 20, and 50μg/ml of test samples in butanol. DPPH free radical and the different extractive concentrations were rapidly mixed in the kinetic accessory SFA- II (Hi-Tech Scientific, Salisbury, United Kingdom). Injection was carried out in UV Perkin Elmer spectrophotometer (Lambda 16). Reduction of the DPPH free radical was measured by reading the absorbance at 520nm exactly 20 min after adding each of the extracts and evaluating IC_{50} value which corresponds to the antioxidant concentration scavenging 50% of DPPH.

DPPH inhibition ratio was expressed as a percentage after being calculated from the following equation:

$$\% \text{ inhibition} = 100 \times (a_c - a_e / a_c)$$

where a_c is the absorbance of control and a_e the absorbance of test extracts. All experiments were repeated two times.

3.4.6. Antibacterial test

3.4.6.1. Minimal inhibitory concentration

The minimal inhibitory concentration (MIC) was determined by the critical dilution method in 96- well microtitre plates. The target bacterial strains: *Escherichia coli (strain CIP53126), Salmonella anterica (strain CIP81.32), Staphylococcus aureus (strain CIP4.83), Listeria monocytogenes (strain CIP82110)* were cultured in Trypcase Soja broth enriched with yeast extract (TSB-YE broth) and *Escherichia faecalis (strain CIP76117)* cultured in Elliker broth to final optical density (OD_{620nm}) ≈ 0.01 by two fold dilution.

P. juliflora bark and heartwood acetone extractives were separately prepared to stock solutions of 10mg/ml and immediately frozen at -20°C. Under sterile conditions, test medium was prepared by two fold dilution of the 10mg/ml stock solution to the desired working solutions range (5mg/ml to 2.44×10^{-3}mg/ml) in the well plates. 100 µl of distilled water was introduced into each well of the 96-well microtitre plate followed by 20 µl bacterial culture (OD) ≈ 0.01. Positive controls were not exposed to the extract. The plates were shacked (Titramax 100, Bioblock Fischer Scientific, Illkirch, France) for one minute before incubating.

Enriched suspensions in plated wells were incubated at 37°C for 24 hours. The growth of bacteria was followed by measuring OD_{620nm} of bacterial suspension using Titertek multiscan MCC/340P, version 2.20 (Huntsville, Al) densitometer before and after 24 hours of incubation. Each test was triplicated. Inhibition of extractives on bacterial growth was evaluated as below:

$$\% \text{ Inhibition} = (OD_c - OD_t) / OD_c \times 100$$

where OD_c is optical density control, OD_t is optical density in presence of extractives after 24 hours. MIC value (mgL^{-1}) is the inverse of the highest dilution where no growth is detected.

3.4.6.2. Enzyme oxidation

Direct oxidation of bark and heartwood extractives by laccase enzyme was carried out as follows: In a test tube, 5µl of 10mg/ml extractive, 485 µl of Phosphate buffer (pH 6.5, concentration 100Mm) and 10µl laccase enzyme at concentration of (5040µI/ml) were tested for UV absorbance (UV-spectrophotometer Cary 50 scan). Oxidation of extractives was followed by reading UV absorbance every 2 minutes for 2 hours at wavelength range of 250nm to 650nm.

4.0. RESULTS AND DISCUSSION

4.1. Technological wood properties

4.1.1. Extractive content of *P. juliflora*

The quantities of extractives in wood, bark, pods and leaves of *P. juliflora* by Dionex extraction are reported in table 2. The amounts of extractives in the bark are slightly higher than that of wood (sapwood and heartwood) taken together and are in agreement with observations reported by Toshiaki (2001). The quantity of extractives in the pods and leaves are lower than those for heartwood. For all cases the yield of extractives increases with increasing solvent polarity.

Table 2. Percentage extractives from different parts of *P. juliflora* by Dionex (n = 3)

Solvent	Dichloromethane		Acetone		Toluene/ethanol		Water	
	IM	DM	IM	DM	IM	DM	IM	DM
Bark	3.3	3.1	7.1	6.4	8.3	7.8	9.2	8.8
SD±	0.25	0.18	0.26	0.12	0.17	0.22	0.36	0.39
Leaves	3.2	3.1	5.1	4.9	6.1	5.6	8.5	7.8
SD±	0.45	0.47	0.13	0.12	0.14	0.33	0.16	0.12
Pods	2.6	2.1	3.8	3.2	4.3	4.1	6.3	6.2
SD±	0.16	0.28	0.24	0.41	0.21	0.27	0.09	0.13
Heartwood	3.7	3.4	7.7	7.6	9.6	8.9	10.8	10.6
SD±	0.19	0.33	0.29	0.31	0.35	0.34	0.19	0.18
Sapwood	1.3	1.2	2.6	2.2	4.8	4.6	5.4	5.2
SD±	0.09	0.04	0.15	0.08	0.14	0.14	0.16	0.19

DM: Direct method, IM: Indirect method, SD: Standard deviation

The two methods of extract quantification based either on the weight of extracts (DM) after evaporation of the solvent or on the mass loss of extracted sawdust (IM) gave close values indicating that practically no products were lost during vacuum evaporation.

The quantities of extractive contained in the wood of *P. juliflora* by Soxhlet extraction are reported in table 3. The amounts of extractives are similar to those obtained by Dionex extraction and in all cases increase with increasing solvent polarity. In the heartwood, the lowest percentage of 2.4% extract was recorded with hexane, followed by dichloromethane

with about 3.4%. The highest values, of 10.8% and 8.9% respectively, were recorded with water and a toluene/ethanol mixture. The high extractive content reported is in agreement with extractive content reported generally for other tropical wood species (Neya *et al.,* 2004).

Table 3. Percentage extractives from heartwood and sapwood by Soxhlet extraction (n = 3)

Solvent	**Heartwood**		**Sapwood**		**Series**
	IM	DM	IM	DM	DM
Hexane	2.7	2.4	1.9	1.8	2.3
Dichloromethane	3.7	3.4	1.9	1.9	0.8
Acetone	7.7	7.6	2.1	2.0	7.1
Toluene/Ethanol	9.6	8.9	6.4	6.0	2.4
Water	10.8	10.6	6.6	6.2	2.6
				Total	15.2

DM: Direct method, IM: Indirect method

Heartwood extractives content was obtained in a yield of 15.2% by extractions realized successively (series) on the same batch of sawdust with different solvents of increasing polarity. Highest yield of 7.1% was by acetone (table 3).

To confirm the quantity of extractives and generalize their occurrence in *P. juliflora* heartwood, Dionex extraction of six different trees selected randomly gave amounts similar to those obtained previously (table 4).

Table 4. Percentage heartwood extractives from different *P. juliflora* trees (n = 3)

Solvent	Dichloromethane		Acetone		Toluene/ethanol		Water	
Tree N°	IM	DM	IM	DM	IM	DM	IM	DM
1	3.5	2.9	7.5	7.5	9.1	9.1	10.9	10.6
2	3.7	3.4	8.2	7.9	9.5	9.1	11.1	10.7
3	3.8	3.5	7.4	7.7	9.2	9.1	11.2	10.9
4	3.9	3.8	7.6	7.8	9.6	9.4	10.9	10.6
5	3.8	3.5	7.7	7.1	8.8	8.5	10.5	10.4
6	3.5	3.3	7.4	7.3	8.8	8.6	10.7	10.6
MEAN	3.7	3.4	7.7	7.6	9.6	8.9	10.8	10.6
SD±	0.17	0.3	0.3	0.31	0.34	0.35	0.26	0.16

DM: Direct method, IM: Indirect method, SD: Standard deviation

High quantities of extractives recorded on *P. juliflora* are similar to values described in the literature on other *prosopis* wood species. These high amounts may play an important effect on wood durability and offer more generally different possibilities of chemical valorization (Neya *et al.,* 2004).

4.1.2. Heartwood anatomical characteristics

In order to understand reasons for the high extractive content in heartwood, we investigated its microscopic characteristics. *P. juliflora* shows a marked difference between its yellowish coloured sapwood and dark brown heartwood. The wood has a slight lustre, straight to wavy grain and medium to coarse texture. It has a fragrant odour when freshly cut.

Figure 21. Sapwood and heartwood delimitation

The annual growth rings are distinct marked by marginal parenchyma bands. The vessels are either solitary or in clusters of 2 to 3 with a diffuse porous pattern in arrangement. Early wood and latewood vessels had a mean diameter range comprised between 110 to 130µm. Mean vessel density was evaluated at 6/mm² (Figure 22). The diameter and density of vessels in *P. juliflora* can be described as normal for this species. Villalba (1985) reports vessel diameters in *P. flexuosa* of 130 µm in earlywood and 40µm in latewood. Other authors do not separate between earlywood and latewood vessels and give medium diameters ranging from 40 µm for *P. argentina* (Villagra and Roig, 1997) up to 140µm for *P .pallida* (Lopez *et al*., 2005).

Density of vessels per square millimetre were evaluated to 10 in *P. laevigata*, 12 in *P. kunzei* and 5 in *P. palida* (Lopez *et al.,* 2005). Vessels and parenchyma cells are filled with a light brown compound. Vessel diameter and density influence wood density and mechanical

properties (Leal *et al*., 2006). If the vessel proportion is high and diameters are large then wood density and strength properties are lowered.

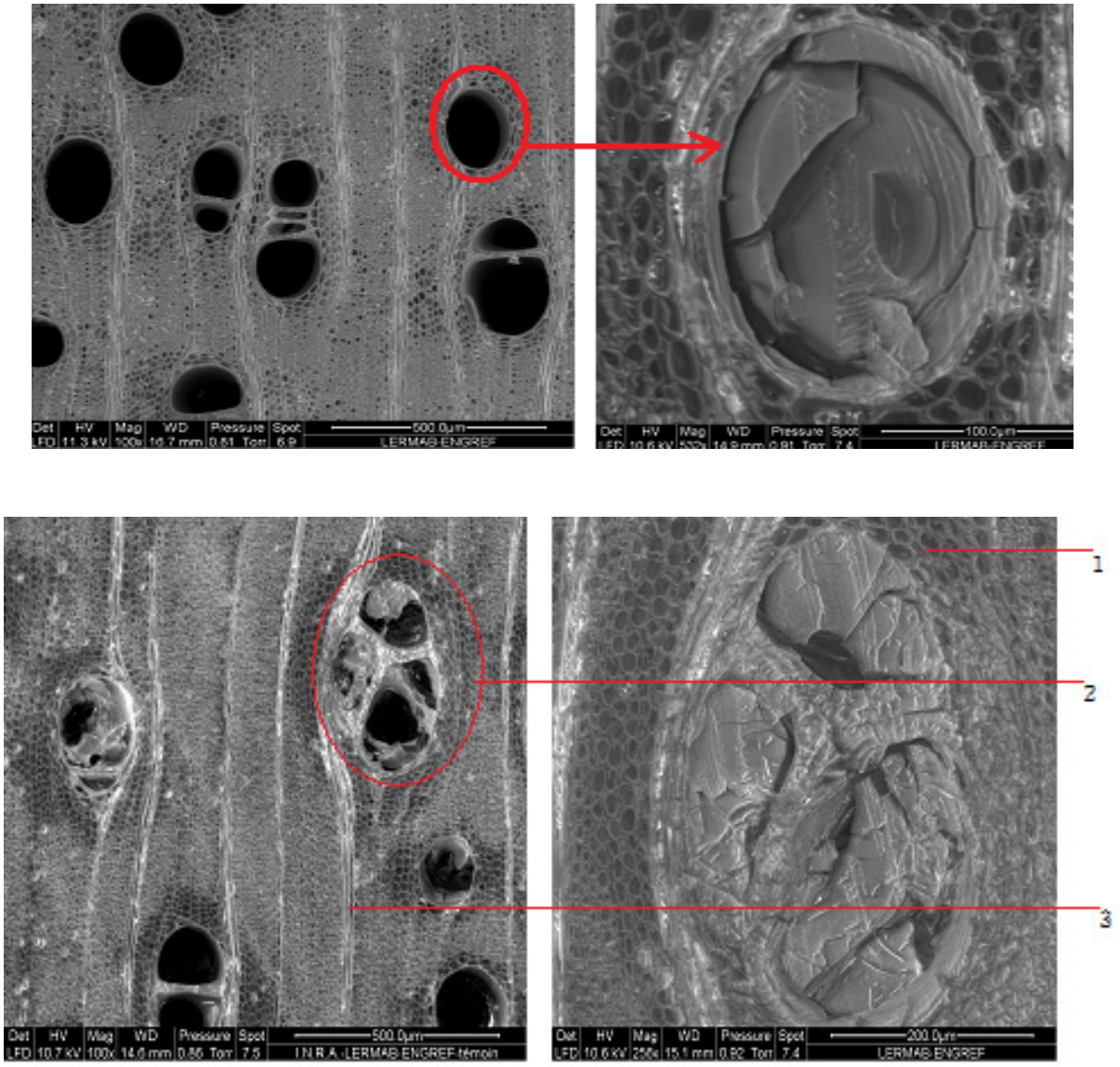


Figure 22. Photomicrographs of *P. Juliflora* heartwood (1) Parenchyma (2) Vessel (3) Ray cells distribution and density

Figure 23 presents cross-sectional views of *P. juliflora* heartwood vessel before and after successive extraction with acetone followed by water. Un-extracted wood vessel (C) is filled with a light brown solid which soften and partially removed after solvent extraction with acetone (A). Further extraction with water, a solvent of higher polarity removes more of this product (B) suggesting presence of gums in *P. Juliflora* heartwood.

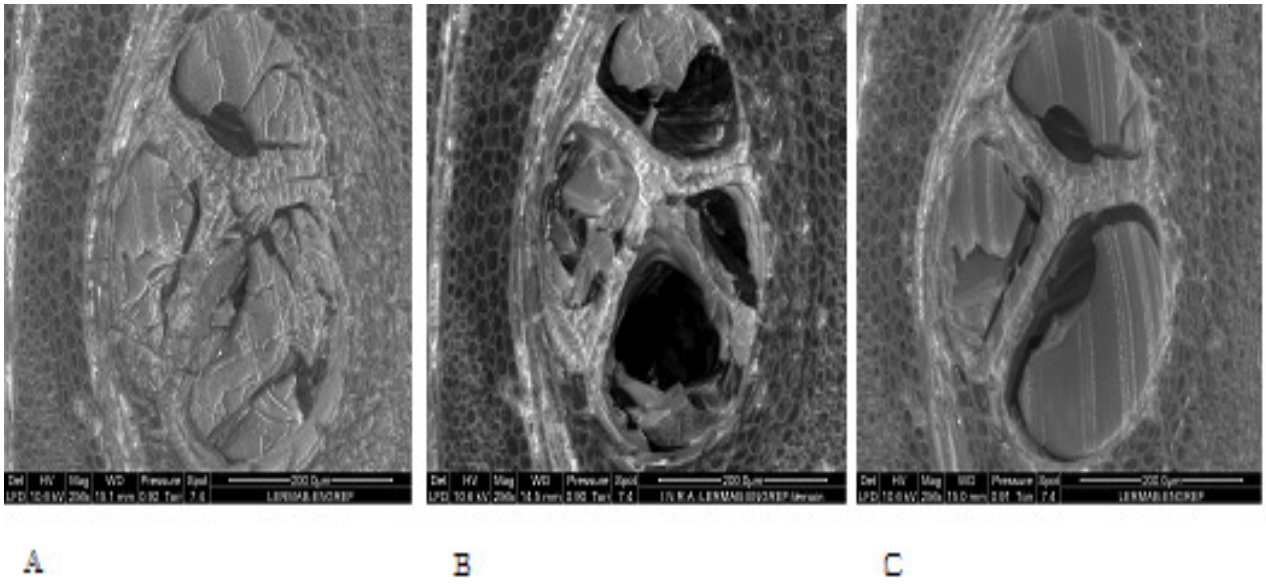


Figure 23. Photomicrographs of *P. Juliflora* heartwood showing vessels: A. Acetone extracted. B. Water extracted. C. Unextracted.

Literature studies indicate that gums in wood are present even after acetone extraction (Gérardin *et al*., 2004) and may be extracted by solvents of higher polarity such as water. Gums soften during Soxhlet extraction and can completely fill the wood lumens. Presence of gums in the wood cells of *Prosopis africana* enhanced its wood dimensional stability and natural durability (Gérardin *et al*., 2004).

4.1.3. Heartwood natural durability

4.1.3.1. Natural durability against fungi

Non-extracted, non-dried heartwood samples were exposed to the action of fungi for 4 months under sterile conditions to evaluate natural durability. The results showed high natural durability against all tested fungi. In all cases, no significant weight losses were observed (table 5), while beech (*F. sylvatica)* and pine (*P. sylvestris)* controls were strongly degraded.

Table 5. Percentage weight loss on wood samples after four months exposure to fungi

	Fungal species			
Wood species	*C. versicolor*	*P. placenta*	*Antrodia*	*P. sanquineus*
P. juliflora	1.5	2.3	2.5	3.0
P. sylvestris	-	20.5	-	-
F. sylvatica	30.1	-	24.5	12.6

The natural durability was determined based on theoretical weight to avoid modification of extractives during the drying process. This test reflects the natural situation in the field. It has been observed that oven dried samples are attacked slightly more as compared to samples which are not dried. Drying the wood at 103°C modifies slightly extractives composition reducing their effect against fungal attack (Gérardin *et al*., 2004).

After four months of exposure, beech (*F. sylvatica)* and pine (*P. sylvestris)* control samples were highly colonized by fungi while *P. juliflora* were observed to have minimal surface attack as shown in figure 24.

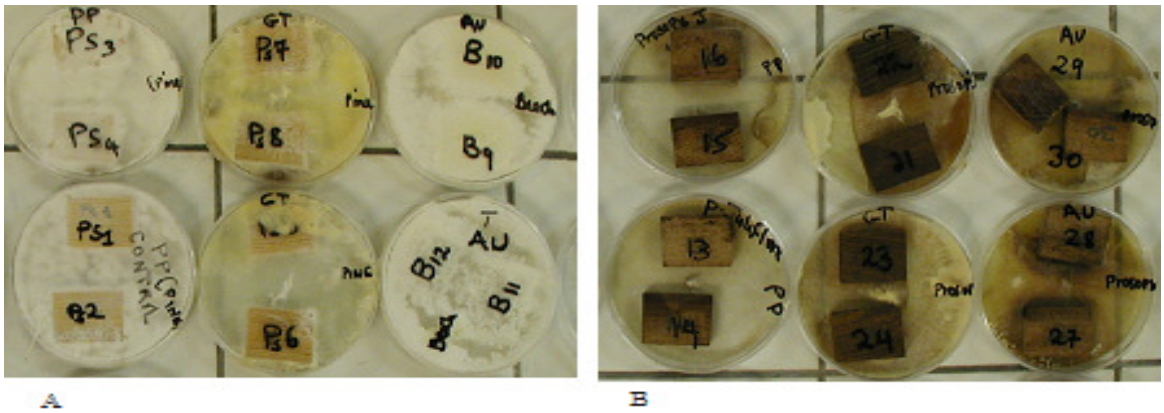


Figure 24. *P. juliflora* wood samples (**B)** and control samples (**A)** after 4 months exposure to fungi

4.1.3.2. Contributions of extractives to natural durability against fungi

The contribution of extractives to the natural durability of *P. juliflora* heartwood was investigated on resistance of solvent extracted wood blocks, exposed to fungi in a sterile chamber for four months. The results are reported in figure 25.

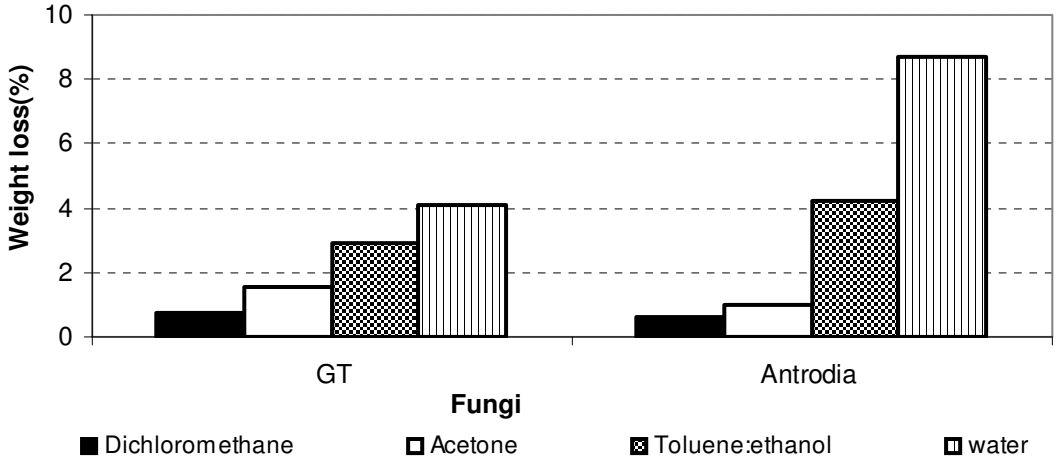


Figure 25. Percentage weight loss on solvent extracted *P. juliflora* wood samples after 4-month exposure to fungi

Independent of the type of fungi tested, weight losses observed on solvent extracted or not extracted *P. juliflora* test samples are low but increase with increasing solvent polarity. Beech and pine test samples used as the control were strongly degraded. Similar observations were made for all fungi. Indeed these results suggest that durability of *P. juliflora* heartwood is not only due to the presence of extractives but depends also on other factors. Vessel materials that cannot be extracted by solvents such as gums have been reported to limit fungal mycelium penetration into the wood hence improving durability (Gérardin *et al*., 2004; Adikwu *et al.*, 2000; Adikwu *et al.*, 2001).

Solvent extracted wood samples were fully colonized by fungi, while the un-extracted samples showed a brown coloration on the malt/agar medium (figure 26), suggesting diffusion of extractives followed by a detoxification of the medium by fungal enzymes, allowing further development of the fungus.

It should also be noted that extractions performed on solid wood are probably not able to remove all extractives present in wood blocks. Superficial removal of these latter ones could explain the slight weight losses observed after fungal exposure, while wood blocks were not attacked in their middle.

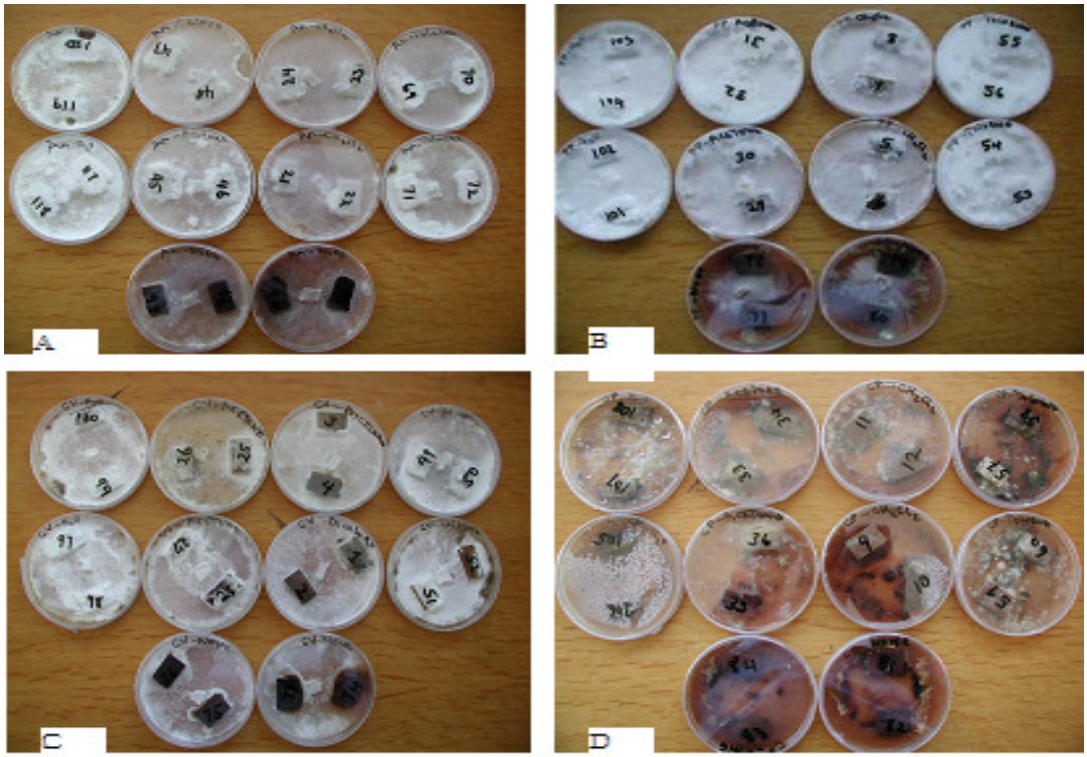


Figure 26. *P. juliflora* heartwood samples extracted or not extracted after 4 months exposure to: **A**. *Antrodia* species **B**. *Poria placenta* **C**. *Coriolus versicolor* **D**. *Coniophora puteana*

4.1.3.3. Effect of extractives on fungal growth

Effects of heartwood extractives on the growth of fungi were evaluated by malt agar inoculation in order to assess their effects on wood durability. The results showed that in all cases, the extracts were more or less efficient as fungal growth inhibitors at the tested concentrations (figure 27).

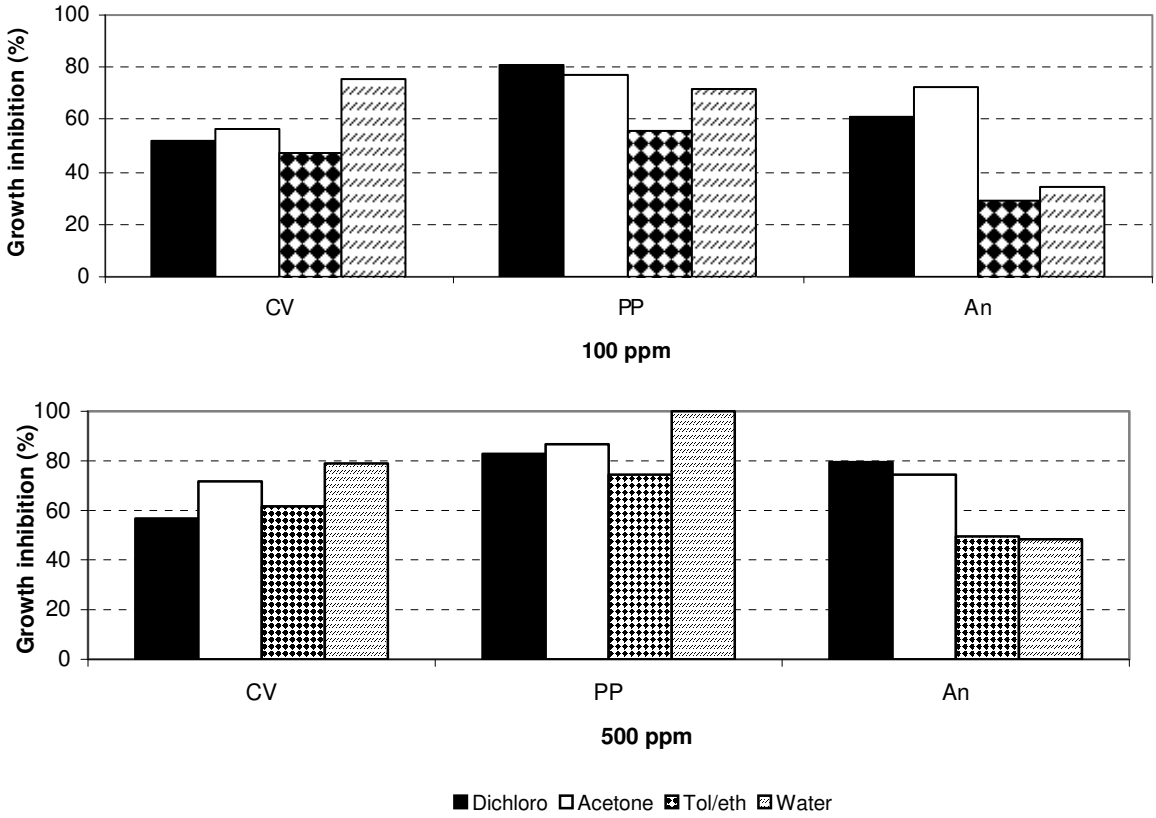


Figure 27. Effect of extractives tested on fungal growth

However, their action seems to stem from a fungistatic effect rather than a fungicidal effect. Indeed, independently of the tested extracts and according to tested concentration, development of the mycelium on the treated medium started after a more or less lengthy inhibition period for all fungi and extractives. Figures 28 and 29 showed the effect of acetonic heartwood extractives tested at different concentrations on the growth of the different fungi and the effect of the different types of extractives tested at different concentrations on growth of *Antrodia* species, respectively.

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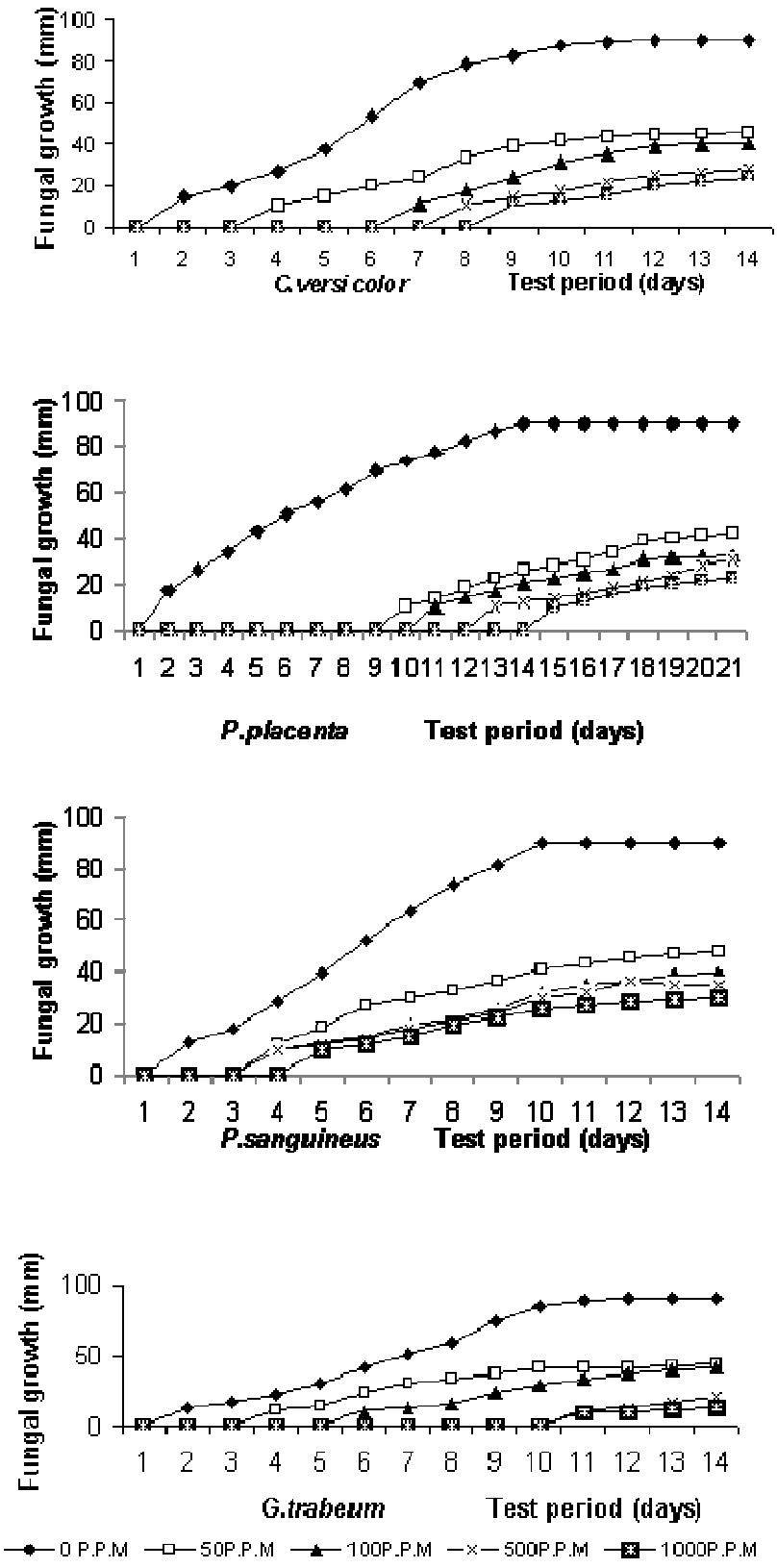


Figure 28. Fungistatic effects of acetone heartwood extractives on growth of different fungi.

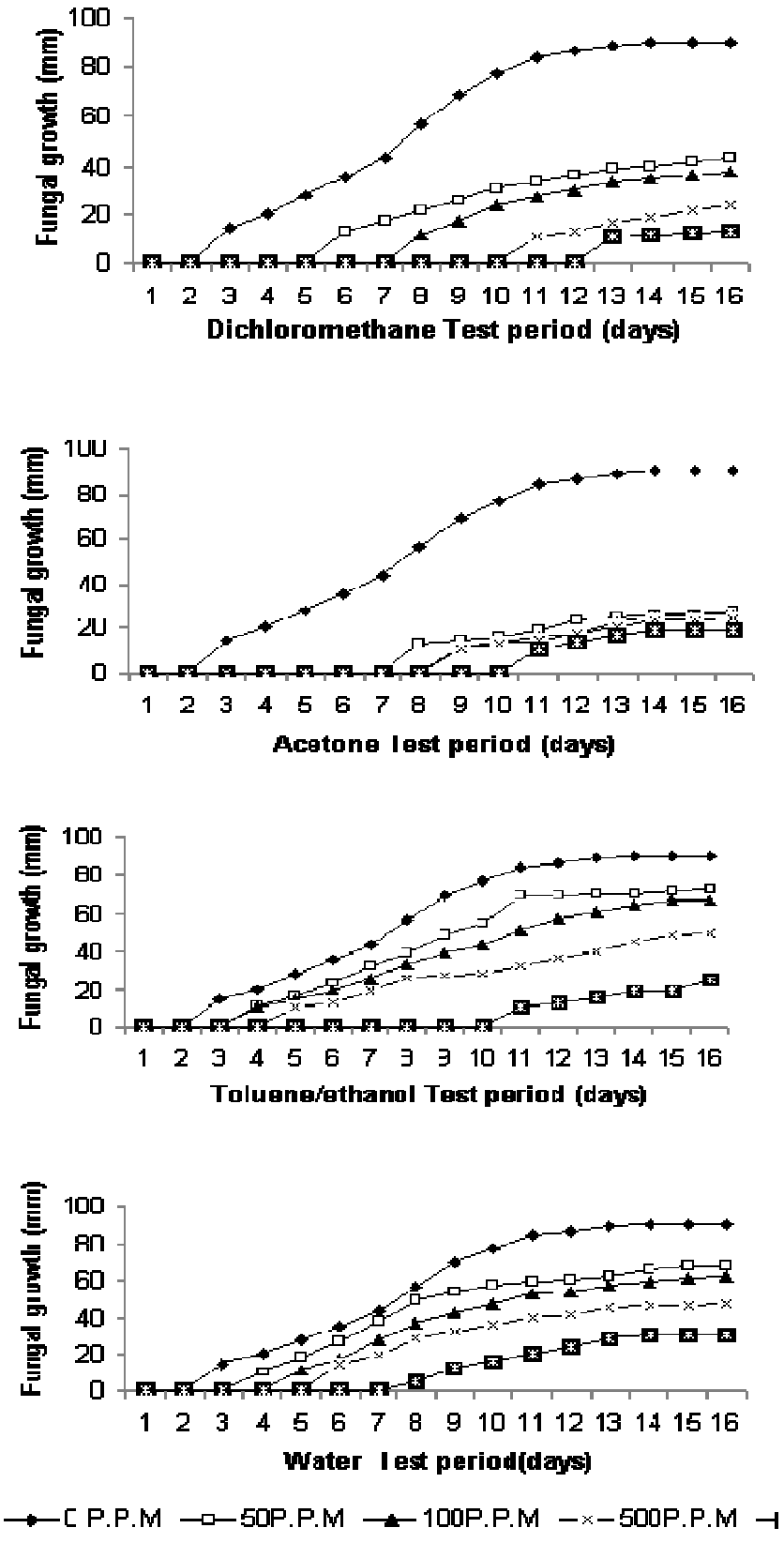


Figure 29. Fungistatic effects of different heartwood extractives on growth of *Antrodia* species.

During this period, fungal activity was detected by the formation of a colored area around the fungal inoculate (figure 30). This behavior is probably associated with detoxification of the medium by fungal enzymes, allowing further development of the fungus. Increasing the extract concentration increased the inhibition period.

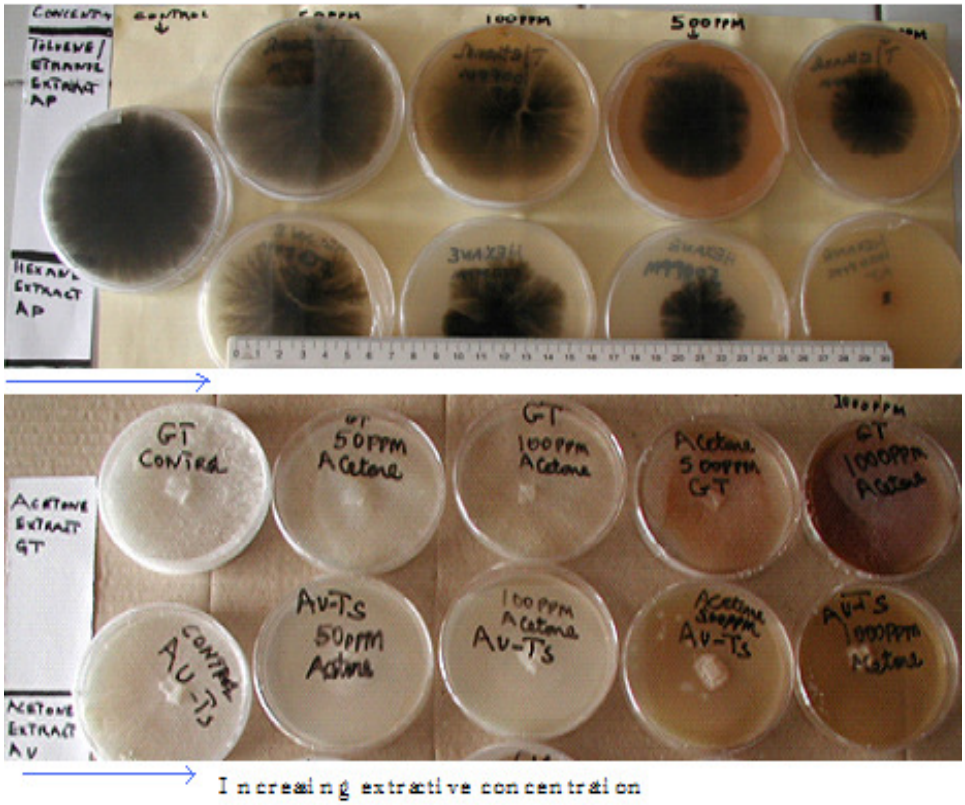


Figure 30. Growth of fungal mycelium on malt/agar treated to various concentrations of extracts

According to these observations, it seems that heartwood extracts possess strong fungistatic properties even at low concentrations which increase with increasing extract concentration. Similar observation was made for all the extractives and fungi.

4.1.3.4. Natural durability against termites

To assess resistance to termites and check the influence of extractives, test samples (extracted or not) were subjected to termite action in the field for 6 months and in the laboratory for 28 days. The results are reported in figure 31 and table 6 respectively.

Table 6. Effect of extract removal or not on resistance of *P. juliflora* heartwood to termites in the laboratory

Solvent	Results (%)			Classification
	Workers surviving	Soldiers surviving	Weight loss (WL)	
Hexane	0	0	0.3	sound
Dichloromethane	8.5	4.5	2.5	light
Acetone	9	10	8.4	light
Toluene/ethanol	6	4	4.7	light
Water	8	7	12.8	moderate
Un-extracted	0	0	0.15	sound
Control	62	53	29.8	heavy

Independently of the nature of the extraction and the test method, weight losses in the test samples were low for the un-extracted test samples and rose with increasing extracting solvent polarity, while the pine blocks were heavily degraded.

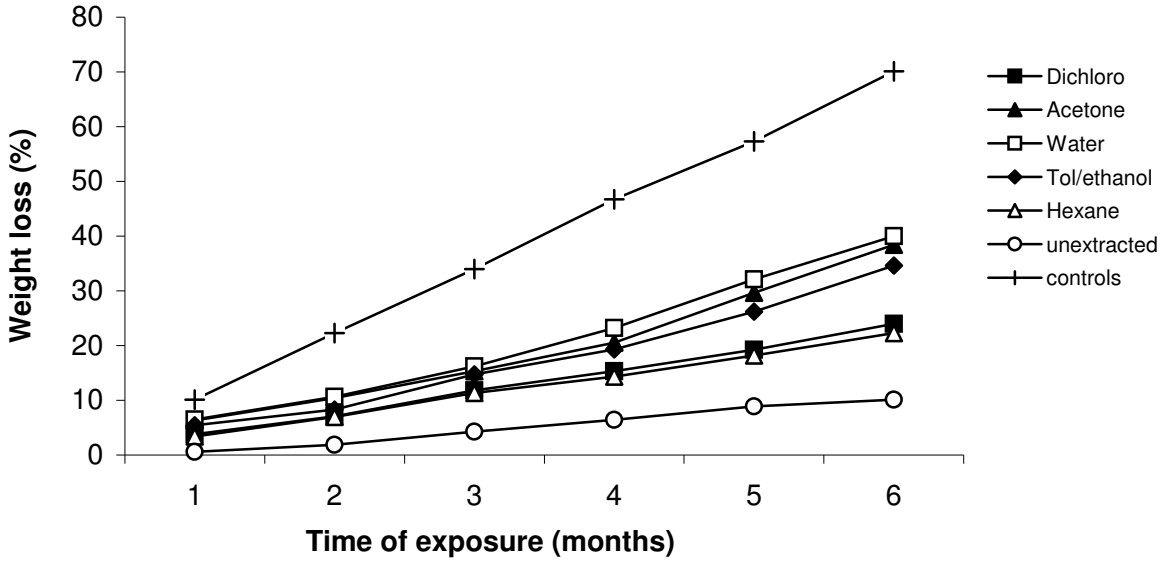


Figure 31. Effect of extractive removal or not on resistance of *P. juliflora* heartwood to termites

These results suggest that extractives play an important part in natural wood durability.

4.1.3.5. Effect of extractives on termites

No- choice bioassay method was used to evaluate the anti-termitic activity of heartwood extractives. The results are reported in figure 32 and 33.

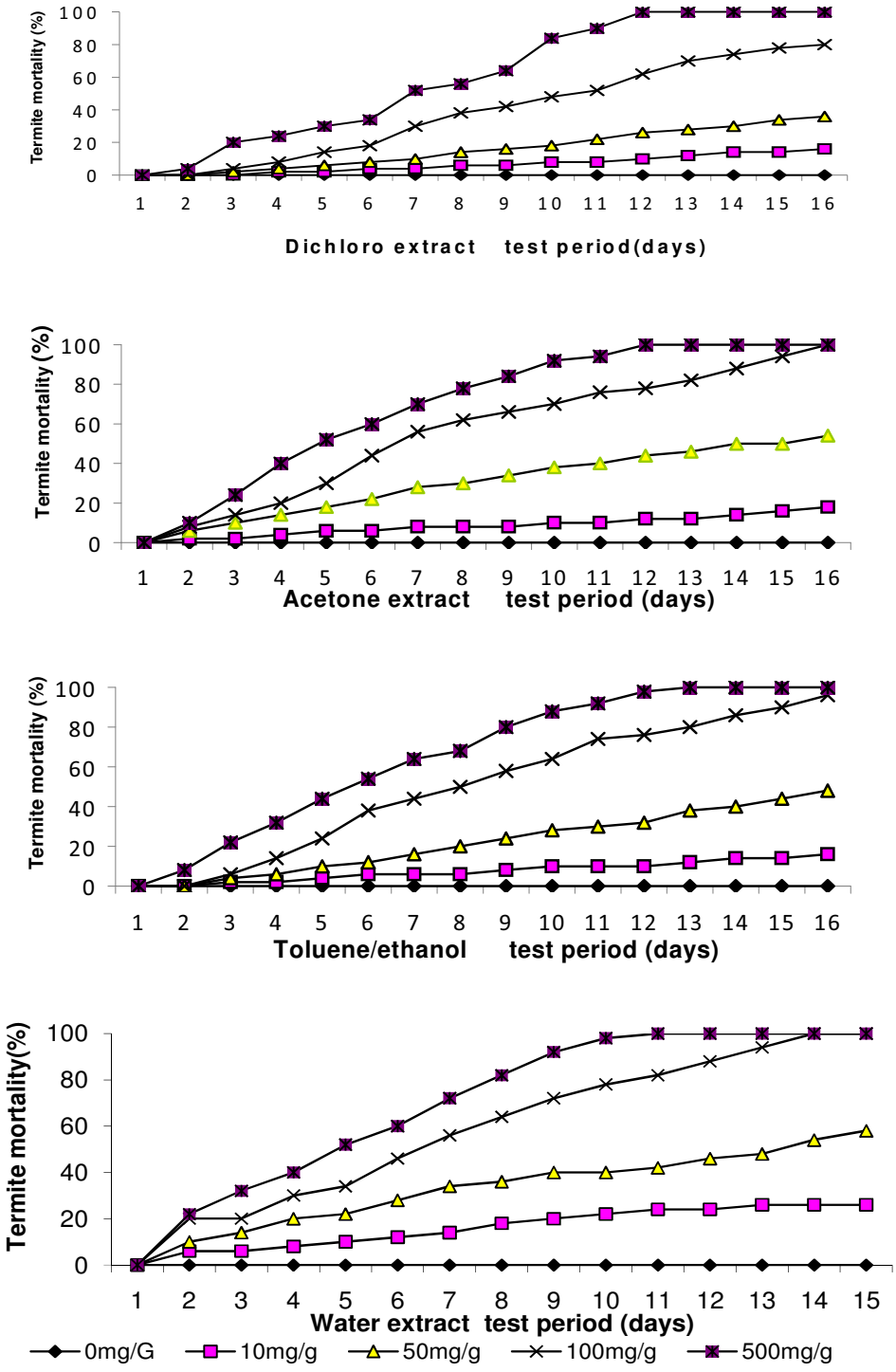


Figure 32. Termite mortality on different extractives and concentrations (N = 3) using 50 termites per replicate

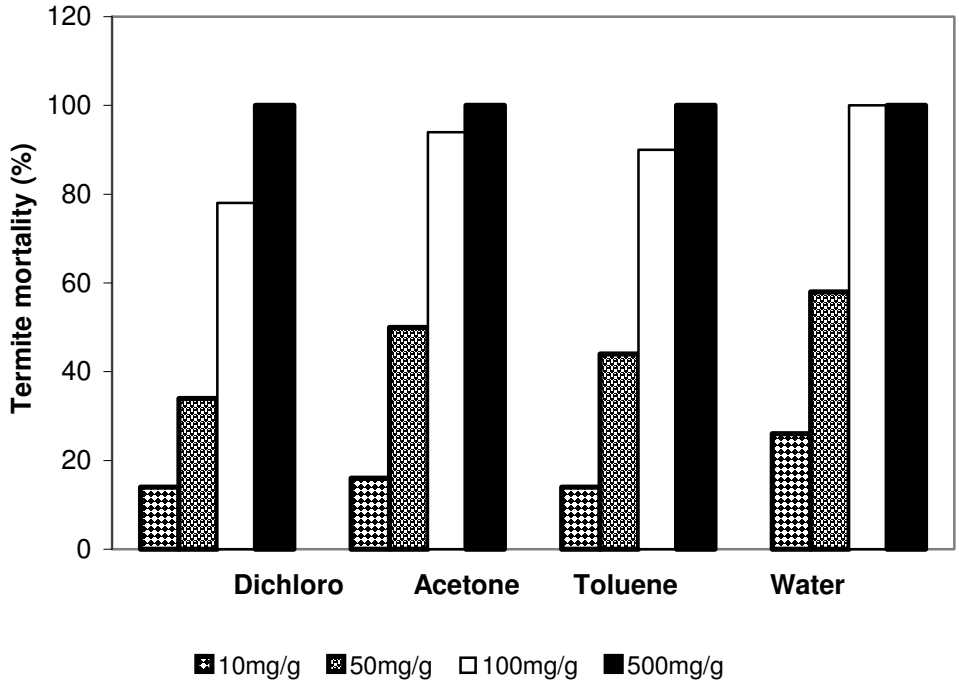


Figure 33. Termite activity of different heartwood extractives at different dosage levels

Termite mortality was observed at all types and concentration levels of extractives tested. Increasing the extractive concentration increases termite mortality, confirming contribution of extractives to termite resistance of this wood species.

Literature studies indicate that wood extractives may act as anti-feedant or feeding deterrent to termites. Indeed, in their studies (Ohmura *et al.,* 2000) found that taxifolin, quercetin and related flavonoïds from Japanese larch wood showed anti-feedant activities against the subterranean termites *Coptotermes formosanus*. Other class of wood extractives such as alkaloids may have potent toxicity, irritant, unpalatable and repellent properties to termites hence protecting wood (Neya *et al.,* 2004; Gérardin *et al*., 2004). In another study, Cheng *et al*. (2004) observed that T-muurolol, a wood extractive was able to cause 100 % termite mortality at 5mg/g after 14 days exposure to termites presenting properties as a potential termiticide for the future.

For all the types of heartwood extractives tested in our work, 100% mortality was recorded at a dosage of 500mg/g extractive concentration after 14 days (figure 33). These results suggest that *P. juliflora* extractives confer heartwood resistance to *Macrotermes natalensis* a subterranean termite commonly found in Kenya.

4.1.4. Mechanical and fuel wood characteristics

Moisture content, swelling coefficient, ash content, density and calorific values of *P.Juliflora* and *Pinus patula* a widely used Kenyan species in construction are shown in table 7.

Table 7. Physical and fuel wood characteristics values

Species	Physical and fuel wood characteristics				
	Moisture content (%)	Density (g/m^3)	Dimensional stability (%)	Ash content (%)	Calorific values (kJ/g)
P. juliflora *P. patula*	32.62 46.30	0.828 0.696	2.3 14.5	2.84 0.4	21.50 20.11

The results indicate that the species is dimensionally stable with calorific values consistent with those of other prosopis wood species currently used as fuel wood. Ash content is relatively high but in good agreement to contents generally found in tropical wood species (Goel and Behl, 1996). Bending strengths values of *P. juliflora* and *P. patula* are shown in (table 8).

Table 8. Mean mechanical property values

Species	Compression (N/mm^2)	MOR (Nmm^{-2})	MOE ($kNmm^{-2}$)	Shear (Nmm^{-2})	Hardness (kN)
P. juliflora[1] *P. juliflora*[2]	73.32 62.05	124.1 113.7	15.2 14.2	20.1 15.03	7.3 13.0
P. patula	56.93	82.1	4.9	13.1	2.0

[1]Found, [2]Reported (http:www2.fpl.fs.fed.us/Techsheets/hardwoodNA/pdf-files/prosoeng.pdf)

The found values are higher than reported with exception of hardness however are consistent with those of prosopis species. The results suggest that *P. juliflora* can be a substitute for parquetry, carving, fuel wood or charcoal production mainly using the traditional

earth kilns. In this latter case traditional earth kilns could be an interesting alternative to produce charcoal for local population.

4.1.5. Wood treatability properties

The sapwood penetration for *P. juliflora* (46.4%) is only half that of *P. patula* (100%) but higher than those of other tropical hardwood species in literature while retention ranged from 36 kg/m^3 in *P. juliflora* to 49 kg/m^3 in *P. patula*. Generally sapwood is more vulnerable to biological degradation than heartwood for most wood species (Grace, 1996). Both heartwood and sapwood are susceptible to fungi, termite and powder-post-beetle infestation depending on the durability (Kamweti, 1992). According to Food and Agriculture Organization (FAO, 1986), the recommended retention of CCA for interior timber not in ground contact such as trusses, rafters is 6 kg/m^3 and exterior timber not in ground contact for example doors and windows is 8 kg/m^3. Timber in ground contact such as fence posts, railway sleepers and bridges need a retention of 12 kg/m^3, timber permanently immersed in fresh waters require 16 kg/m^3 while in seawater e.g. jetties, boat building 24 kg/m^3 respectively (FAO, 1986). The relatively high retention and higher sapwood treatability indicate that this species could be a potential alternative source of material for railway sleepers and fencing posts. However based on the strength parameters, this species is unsuitable for heavy construction works.

4.2. Identification and characterisation of *Prosopis juliflora* extractives

4.2.1. Heartwood extractives

^{1}HNMR spectra of the heartwood crude extractives by different solvents in methanol-D4 are showed in figure 34.

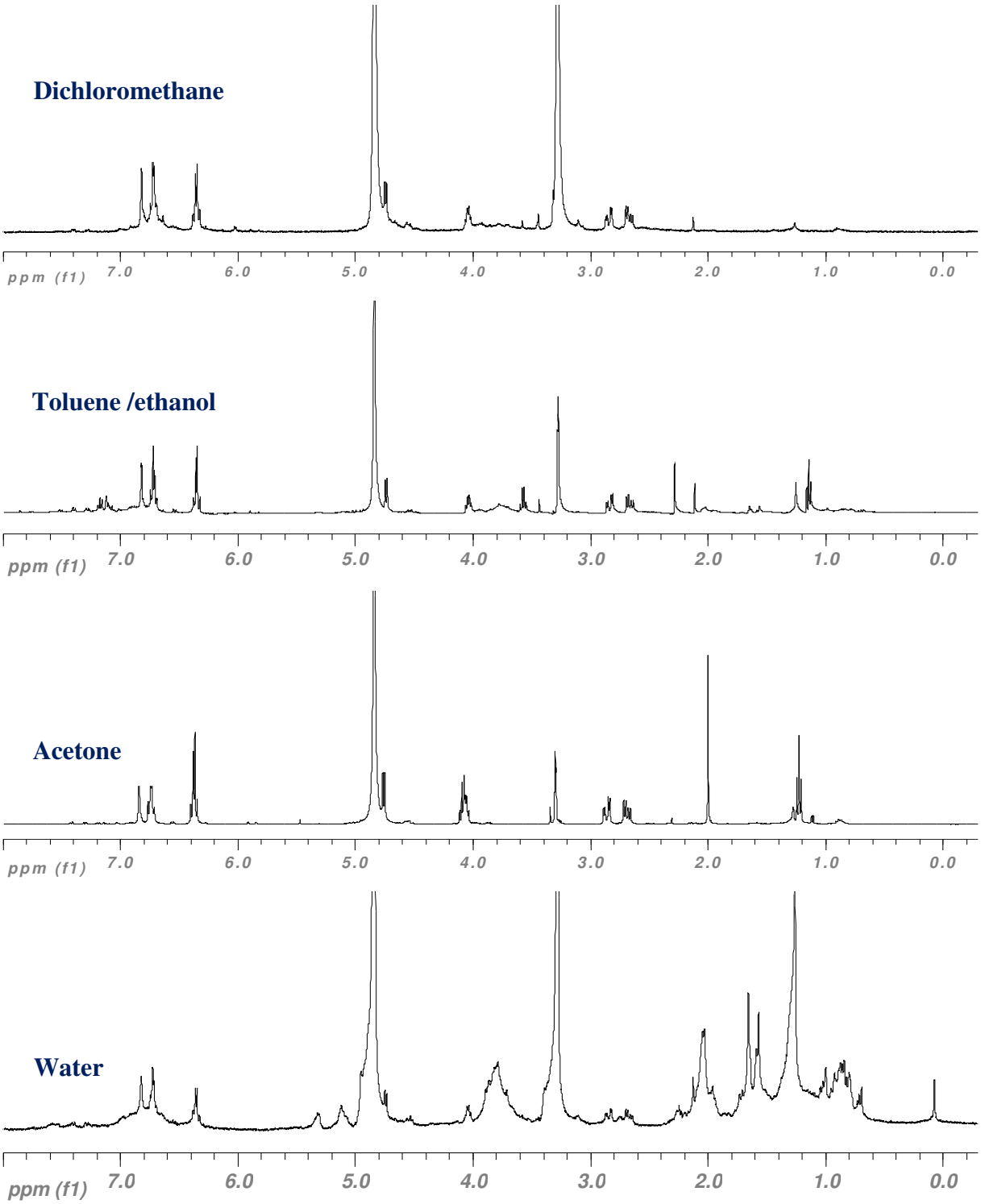


Figure 34. ^{1}HNMR spectra of different heartwood crude extractives

All spectra indicated more or less similar signals excepted for the spectrum of water soluble extractives. The different signals indicated the presence of a main component showing typical flavanol signals (figure 35).

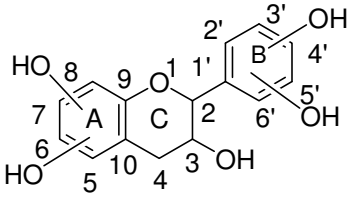


Figure 35. General structure of flavanols

Indeed, ^{1}HNMR analysis of the different types of extractives indicate a characteristic ABX system corresponding to two hydrogen atoms at the C_4 position of the (C) ring of a flavanol structure at approximately 2.8 ppm and two additional signals at approximately 4.0 and 4.5 ppm, characteristic of hydrogen at C_3 and C_2 positions respectively. Indeed signals at 2.71 (dd), 2.89 (dd) and 4.09 (m) ppm are characteristic of an ABX system corresponding to the two hydrogen atoms at the C_4 position and to the hydrogen at the C_3 position of the (C) ring of a flavanol structure. This attribution is corroborated by the presence of a doublet at 4.79 ppm corresponding to the hydrogen atom at the C_2 position. Moreover, additional signals appearing in the NMR spectra of the crude extractives concern aromatic signals, which could be attributed to (A) and (B) rings.

Comparison with spectral data of reference flavanols like (+)-catechin and (-)-epicatechin, reported in the literature to be present in several wood species (Pietarinen *et al.,* 2006; Mayer *et al*., 2006; Mammela, 2001) indicates that the flavanol present in *P. juliflora* heartwood has a different structure. The crude acetone extract was subjected to purification to confirm purity and identification purposes. Column chromatography over silica gel using EtOAc/hexane (3/1v/v) mixture lead to a pale yellow solid in 60% yield (Rf 0.45, silica gel, EtOAc) melting at 81-83 °C.

^{1}HNMR and FTIR analysis of the purified product presented quite similar signals to those of the crude acetone extract discussed above. For identification purposes, ^{1}H-^{13}C HMQC and ^{1}H-^{13}C HMBC NMR coupling analysis was carried out. According to the large coupling constant observed between H_2 and H_3 (J = 6.75 Hz), the 3′, 4′,-dihydroxyphenyl group at the C_2

position is in position *trans* of the hydroxyl group at the C_3 position. This value is in good agreement with that observed for (+)-catechin. The main difference between spectra of reference flavanols (figure 36) and that of purified extract of *P. juliflora* concerns the signals of the hydrogen atoms present on the (A) aromatic cycle. These two protons appear as 2 singlets just under 6 ppm in reference compounds, while they appear as 2 doublets with a typical benzenic ortho coupling constant (J = 8.2 Hz) at 6.38 and 6.42 ppm in the isolated product from the heartwood of *P. juliflora*.

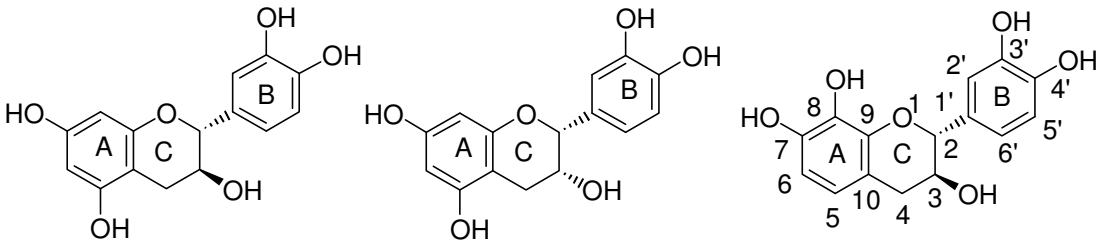


Figure 36. Structure of the different flavanols

Further detailed analysis of ^{1}H-^{13}C HMQC and ^{1}H-^{13}C HMBC NMR data (table 9) allowed unambiguous assignments of the structure of the main compound present in *P. juliflora* extractives as 2, 3-*trans*-3′, 4′, 7, 8-tetrahydroxyflavan-3-ol (figure 35).

Table 9. NMR data for (-)-mesquitol

Carbon No	δ_C (ppm), multiplicity	^{1}H - ^{13}C HMQC	^{1}H - ^{13}C HMBC
2	83.4, CH	4.79 (d, J = 6.75 Hz, 2H)	2H-4, H-2', H-6'
3	69.2, CH	4.09 (m, 1H)	2H-4, H-2
4	33.3, CH_2	2.71 (dd, J = 15.7, 7.5 Hz, 1H)	H-2, H-5
		2.89 (dd, J = 15.8, 5.0 Hz, 1H)	H-2, H-5
5	120.7, CH	6.42 (d, J = 8.2 Hz, 1H)	2H-4
6	109.8, CH	6.38 (d, J = 8.2 Hz, 1H)	H-5
7	145.5, qC	-	H-6, H-5
8	134.3, qC	-	H-6, H-5
9	151.1, qC	-	-
10	113.7, qC	-	2H-4, H-5
1'	129.2, qC	-	H-2, H-6', H-5'
2'	115.4, CH	6.87 (d, J = 1.6 Hz, 1H)	H-2
3'	146.7, qC	-	H-2', H-6', H-5'
4'	146.7, qC	-	H-2', H-6', H-5'
5'	120.2, CH	6.78 (d, J = 8.0 Hz, 1H)	H-2, H-2'
6'	116.5, CH	6.75 (dd, J = 8.0, 1.6 Hz, 1H)	H-2

Such a compound has been previously isolated from the bark of *Dichrostachys cinerea* and described as (-)-mesquitol (Mammela, 2001; Madhusudana *et al*., 2004). The specific rotation of the isolated product is similar to that of (-)-mesquitol reported in the literature (Madhusudana *et al.,* 2003; Madhusudana *et al.,* 2004) allowing assignment of product configuration (compare $[\alpha]_{D\ =}$ –39 (c 1.0, CH_3OH) to a value of –36 in the literature). Found microanalysis is in good agreement with calculated one for $C_{15}H_{14}O_6$ (compare C, 60.92; H, 5.29; O, 33.79 to C, 62.07; H 4.86, O, 33.07 respectively).

The FTIR spectrum of heartwood acetonic extractives is presented in figure 37.

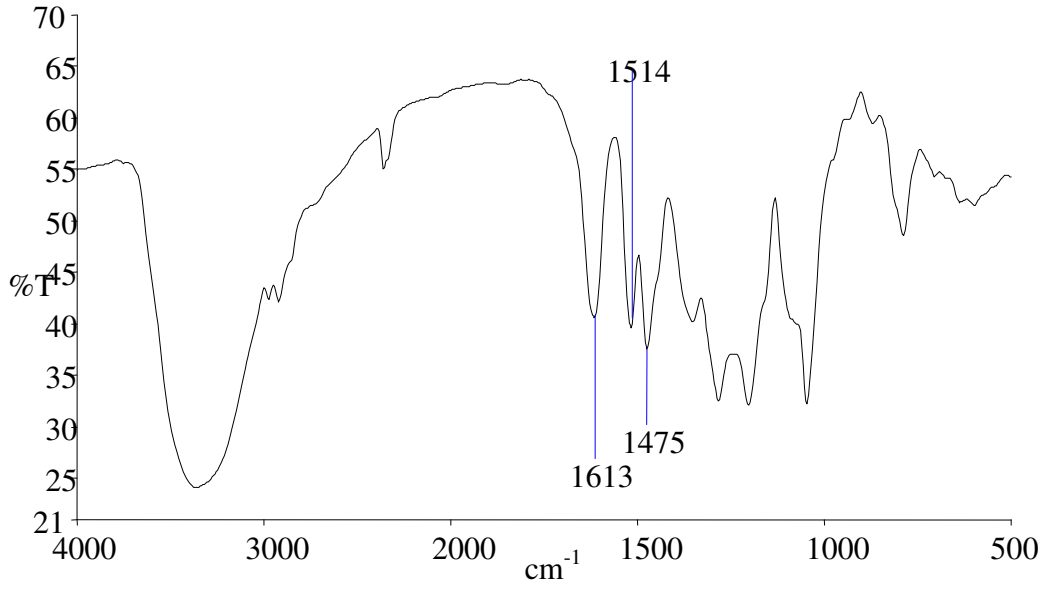


Figure 37. FTIR spectrum of acetone extractives of *P. juliflora* heartwood

The spectrum indicated hydroxyl group absorption at 3350 cm^{-1} and aromatic C=C skeletal vibrations at 1613, 1514 and 1475 cm^{-1}typical characteristic of the flavanol structure corroborating the structure attribution made by NMR.

To check purity and analyse compounds present in the different extracts, GC-MS analysis has been performed on the crude extracts (figures 38, 39 and 40). Analyses were performed after derivatization of the crude extracts as tetramethylsilyl derivatives.

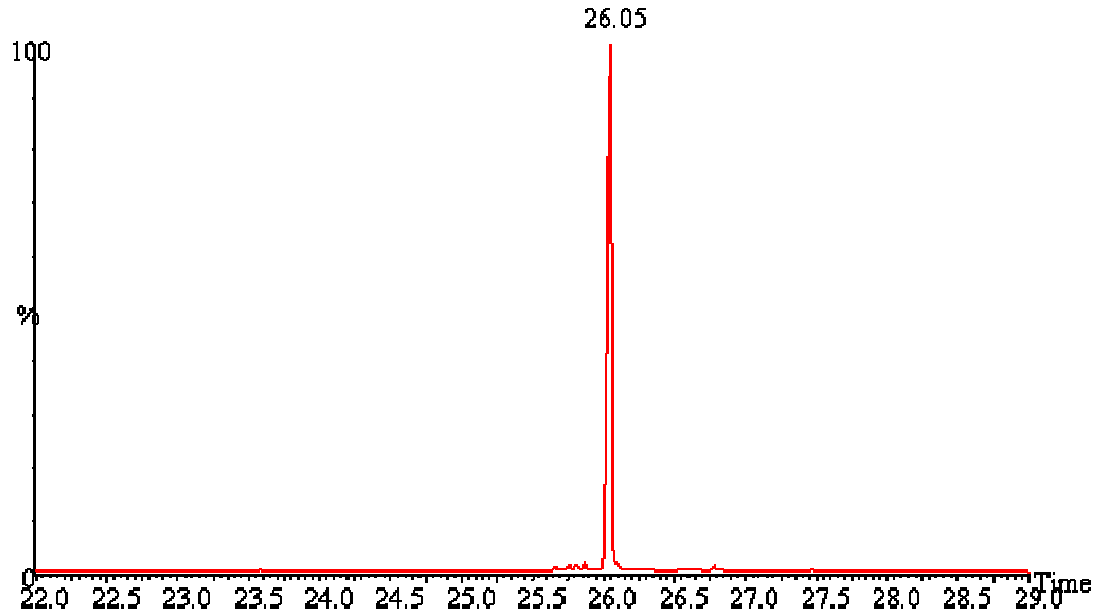


Figure 38. GC-MS chromatogram of toluene/ethanol heartwood extractive

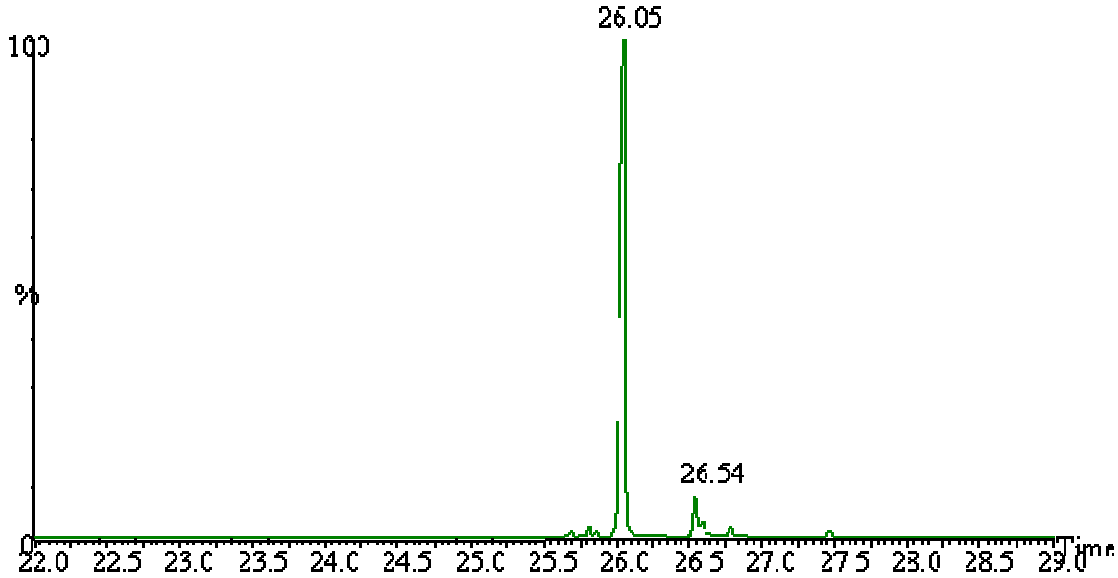


Figure 39. GC-MS chromatogram of acetone heartwood extractive

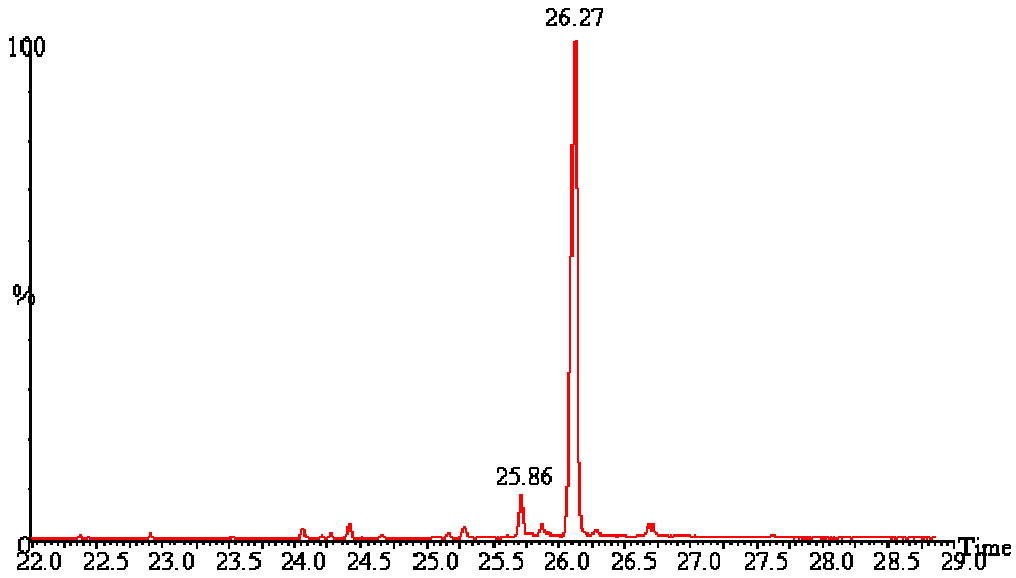


Figure 40. GC-MS chromatogram of dichloromethane heartwood extractive

In all cases, chromatograms indicated the presence of a main component with a retention time of approximately 26 minutes. GC-MS analysis of the TMS derivatives of the purified product and of reference flavanols (catechin and epicatechin) in a mix ratio (ratio 1:1:1) confirm the existence of three isomers appearing at distinct retention times (figure 41).

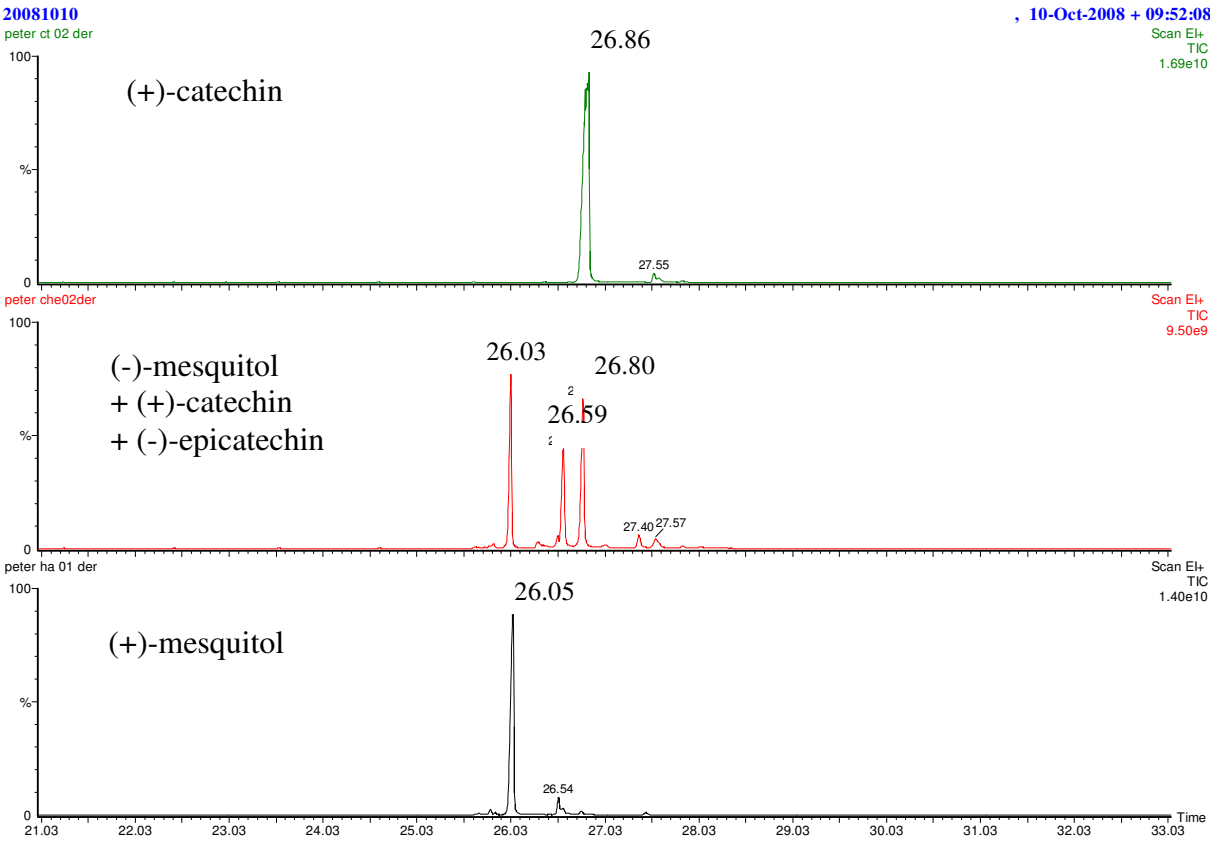
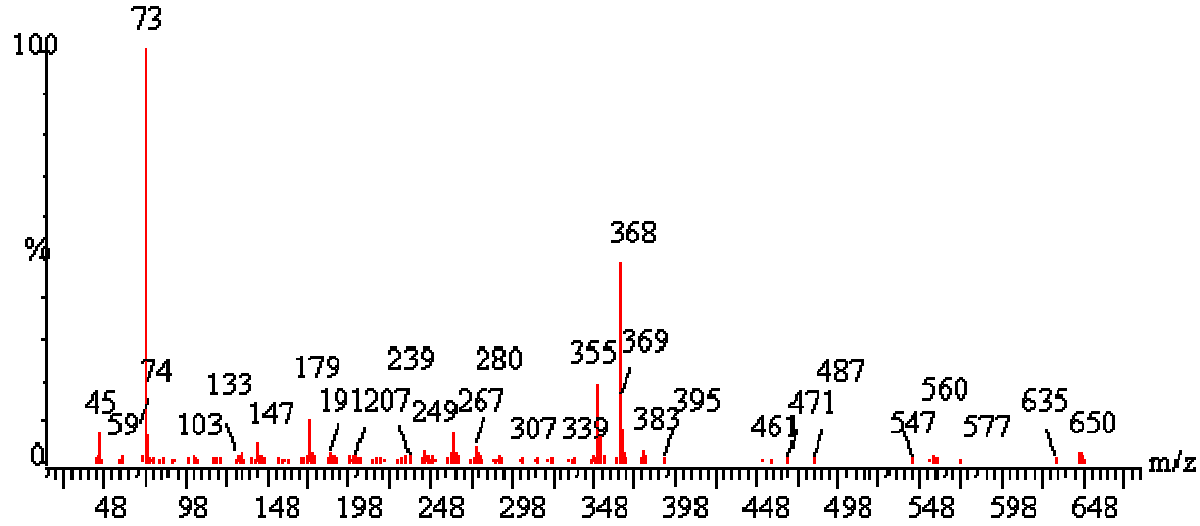


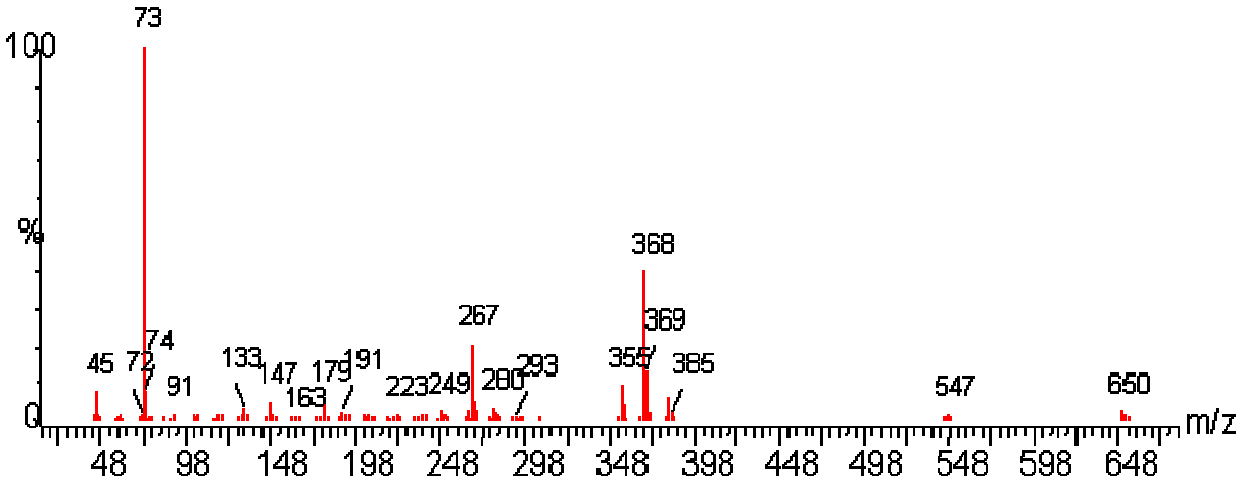
Figure 41. GC-MS chromatogram of (-)-mesquitol and reference controls

MS spectra of the different products present quite similar fragmentations with molecular ion peak for the penta-TMS derivative at m/z 650 and characteristic peaks at 355 and 368 (figures 41, 42 and 43) similar to those reported in the literature (Soleas *et al.,* 1997).



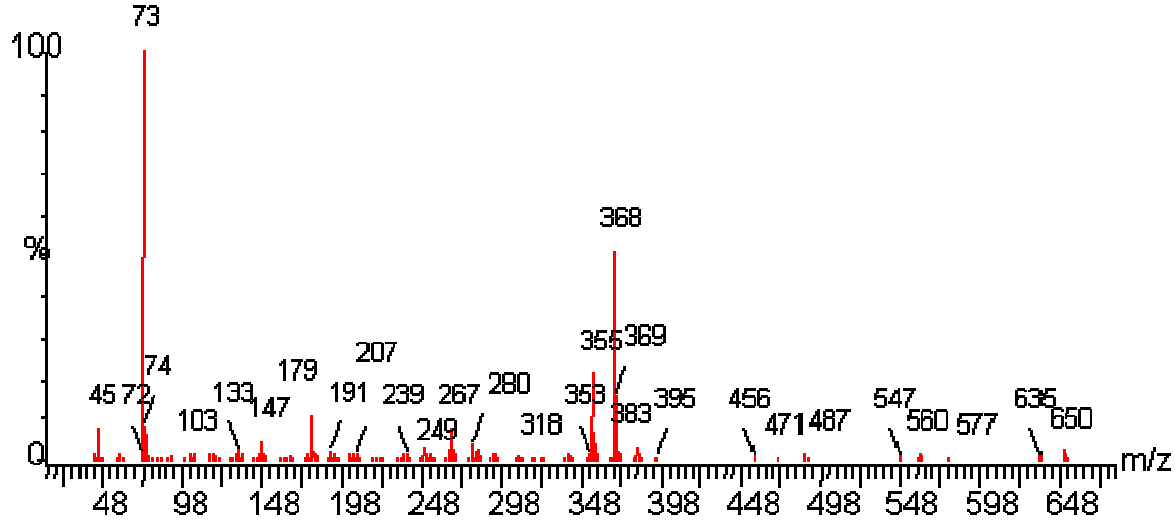
rt = 26.03. m/z (%): 650 (M^+, *1.6*), 73 (100), 368 (*39.7*), 267 (*19.5*), 369 (*12.9*), 355 (*8.7*), 74 (*7.4*), 45 (*6.7*), 370 (*6.1*), 383 (*5.8*), 75 (*5.3*), 179 (*4.7*), 268 (*4.5*), 147 (*3.8*), 356 (*2.8*), 280 (*2.2*), 133 (*2.2*), 249 (*1.9*), 281 (*1.3*), 357 (*1.3*).

Figure 41. MS spectrum for (-)-mesquitol



rt = 26.59 min. m/z (%): 650 (M^+, *1.7*), 73 (100), 368 (*47.5*), 355 (*20.1*), 369 (*14.3*), 179 (*9.5*), 74 (*7.3*), 267 (*7.2*), 45 (*7.1*), 370 (*6.9*), 356 (*6.3*), 75 (*5.7*), 147 (*4.1*), 280 (*3.1*), 357 (*2.9*), 383 (*2.5*), 249 (*2.0*), 281 (*1.9*), 133 (*1.7*), 268 (*1.6*).

Figure 42. MS spectrum for (-)-epicatechin



Rt = 26.80. m/z (%): 650 (M^+, *1.6*), 73 (100), 368 (*47.8*), 355 (*17.8*), 369 (*15.3*), 179 (*9.1*), 74 (*7.5*), 370 (*6.2*), 45 (*6.1*), 267 (*6.1*), 356 (*5.5*), 75 (*5.1*), 147 (*4.2*), 280 (*3.3*), 357 (*2.6*), 249 (*2.1*), 383 (*2.0*), 281 (*2.0*), 133 (*1.6*), 268 (*1.4*).

Figure 43. MS spectrum for (+)-catechin

HPLC analysis of (-)-mesquitol from *P. juliflora* heartwood and of reference flavanols (catechin and epicatechin) in a mix ratio (ratio 1:1:1) corroborate preceding results allowing identification of the three different flavanols (figure 44).

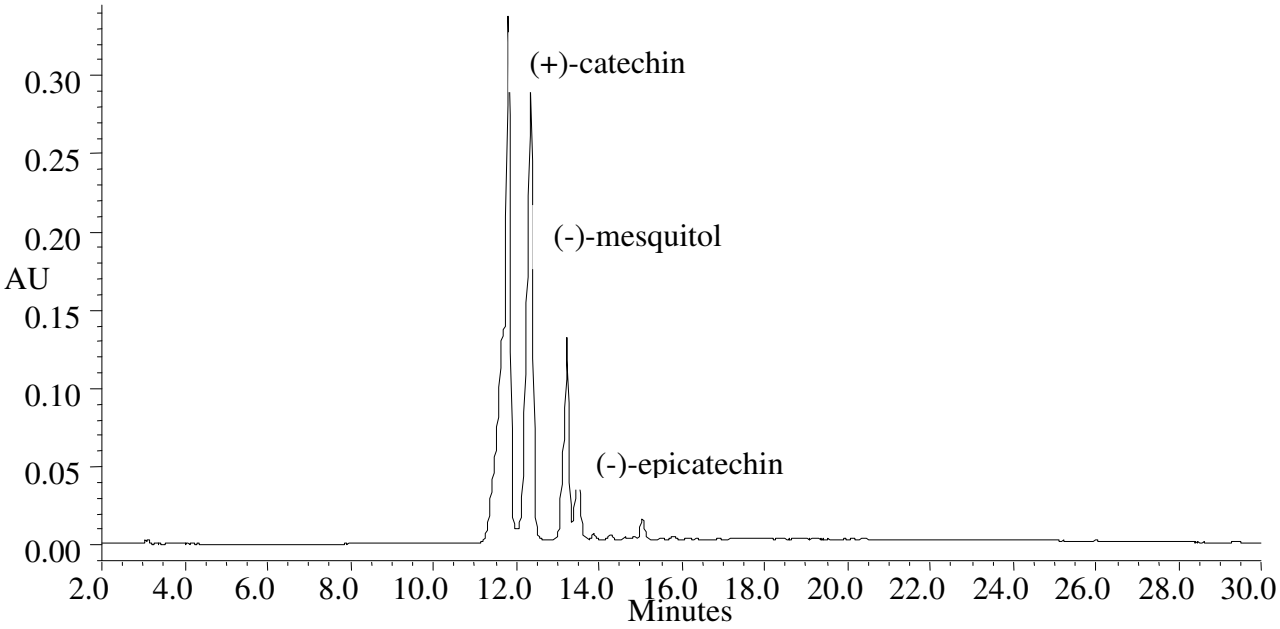


Figure 44. HPLC chromatograms of the mixture of reference flavanols and (-)-mesquitol

All the flavanols present quite similar UV absorptions but appear at distinct retention times. UV spectrum of mesquitol, showed in figure 45, presents characteristic $\pi \rightarrow \pi^*$ absorption of the conjugated doubles bonds of the aromatic ring.

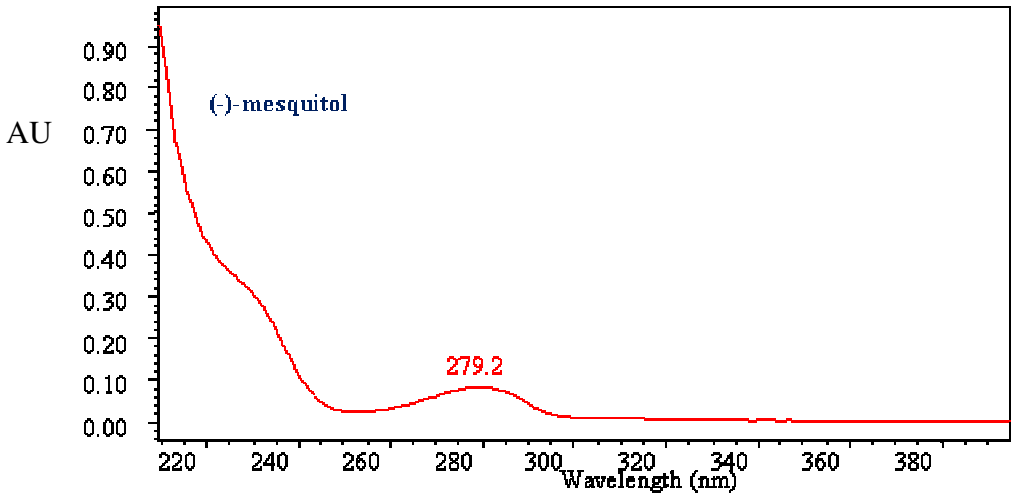


Figure 45. UV absorption spectrum of mesquitol

According to NMR and to the different liquid and gas chromatographic analyses, it appears that mesquitol is the main component of acetonic extracts. To confirm and generalize the presence of (-)-mesquitol in the heartwood of *P. juliflora*, analysis of extractives was investigated on six different trees selected randomly. Amount of extractives are similar to those obtained previously (7.5% ± 0.3). ^{1}H NMR and HPLC analyses clearly demonstrated the presence of (-)-mesquitol as the sole compound without any noticeable impurities.

4.2.2. Bark extractives

^{1}H NMR analyses of bark crude extractives by different solvents in methanol-D4 are presented in figure 46. In a general manner, spectra showed a higher complexity indicating the presence of different families of products among which: fats, sugar and flavanol structures.

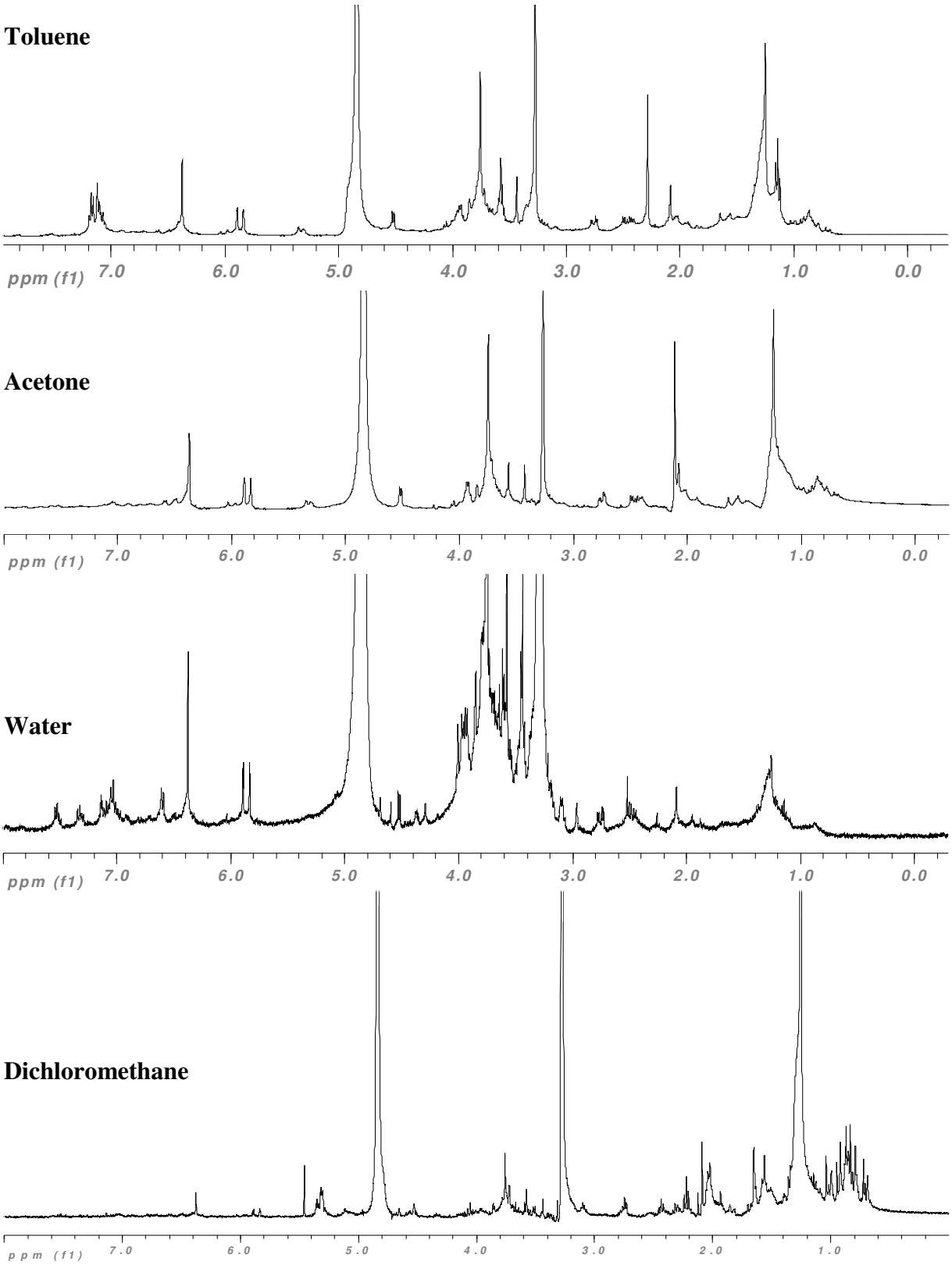


Figure 46. ^{1}HNMR spectra of different bark crude extractives

NMR signals between 0.8 and 2.1 ppm are typical for aliphatic structures, while signals between 3 and 4 ppm are typical of sugar or glycerol units. Quantity of sugar units increases with the polarity of the extraction solvent, water giving the higher amounts.

Analysis of ^{1}H NMR spectra of acetone extractives indicates two doublet of doublet at 2.4 and 2.7 ppm and two additional signals at approximately 3.9 and 4.6 ppm that are characteristic of hydrogen atoms at C4, C3 and C2 positions of the (C) ring of flavanol structure. Signals of the hydrogen atoms present on the (A) aromatic cycle appear as 2 singlets just under 6 ppm. These signals can not be attributed as previously demonstrated in the case of heartwood extractives to the presence of mesquitol. Chemical shifts and coupling constants of aromatic hydrogen atoms of (A) ring are more consistent with the presence of a 6, 8-dihydroxylated (A) ring similar to that of catechin.

FTIR analysis of bark acetone extractives was carried out (figure 46).

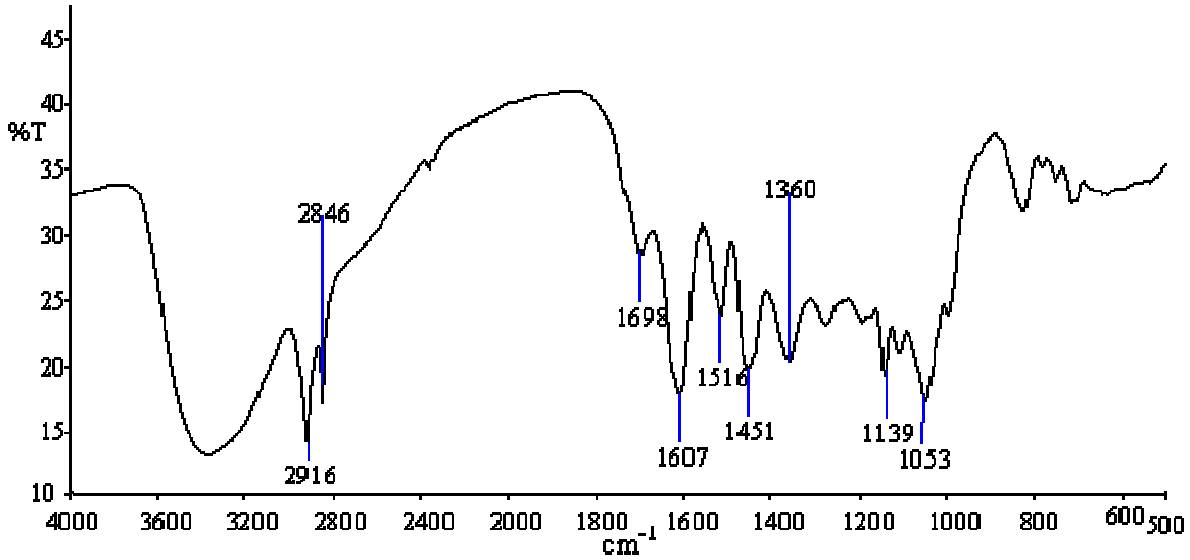


Figure 46. FTIR spectrum of acetone extractives of *P. juliflora* bark

FTIR spectrum indicates characteristic hydroxyl group absorption at 3350 cm^{-1}. Fatty acids present in fats are identified by the presence of C-H vibrations between 2850 and 2920 and carbonyl bands at 1698 cm^{-1}. Aromatic C=C skeletal vibrations at 1605, 1514 and 1454 cm^{-1} are typical of aromatic structure in flavanols. A strong absorption at 1050 cm^{-1} is characteristic of C-O vibrations in sugar units.

In order to investigate the structure of the flavanols present in bark, the crude bark acetone extractives were acetylated at 0°C in a mixture of acetic anhydride / anhydrous pyridine (1/2, v/v), under inert atmosphere (N_2) for 6 hours, followed by ethyl acetate extraction. The organic phase was washed with 10 to 20ml of 2N H_2SO_4 followed by a saturated solution of $NaHCO_3$ and finally water. The resulting organic phase was dried with anhydrous $MgSO_4$, filtered and the filtrate evaporated under reduced pressure to yield a light brown solid. Column chromatography of the mixture over silica gel using EtOAc/hexane (2/1, v/v) as eluent led to a compound labelled as CX_1 (Rf = 0.55, silica gel, EtOAc). ^1H and ^{13}C NMR, HPLC and GC-MS analysis was carried out on CX_1 as well as other crude extractives. ^1H NMR analysis of CX_1 is reported in figure 47.

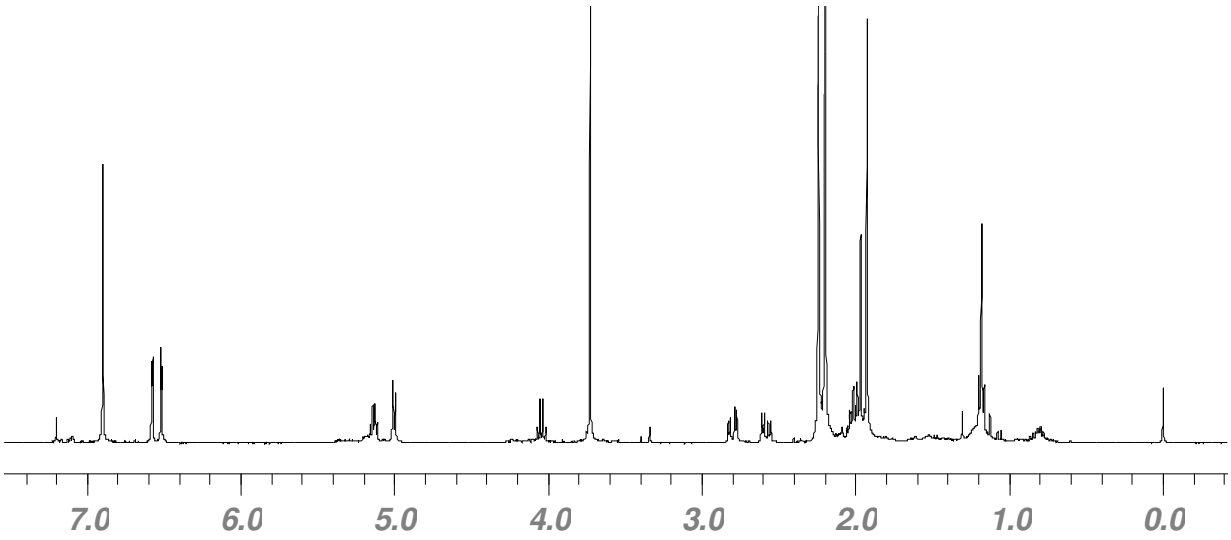
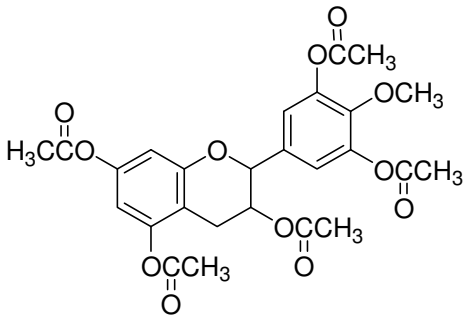


Figure 47. ^{1}HNMR spectra and possible structure of compound CX_1

Analysis of CX_1 indicates the presence of a typical flavanol structure: two doublets of doublets at 2.6 and 2.8 ppm characteristic of the two hydrogen atoms at C4, while signals at 5.0 and 5.15 ppm are characteristic of hydrogen atoms at C2 and C3 position respectively. The large coupling constant observed between H_2 and H_3 (J = 6.75 Hz) indicated a *trans* relation between the two hydrogen atoms. Signals between 1.8 and 2.3 ppm are attributed to CH_3 groups of acetyl groups. Integration of this signals (not reported on the figure) indicated the presence of five acetyl groups. Signal at 3.7 ppm is ascribable to CH_3 of methoxy group. Aromatic signals indicated the presence of two singlets at 6.7 and 6.8 ppm similar to signals observed for catechin (B ring) and of another singlet at 6.9 ppm integrating for two hydrogens.

According to these observations, it seems that the isolated CX1 product correspond to 3, 5, 7, 3′, 5′-penta-O-acetyl-4′-O-methylgallocatechin (figure 48).



3,5,7,3',5'-penta-O-acetyl-4'-O-methylgallocatechin

Figure 48. Proposed structure for compound CX_1

Further detailed ^{1}H-^{13}C HMQC and ^{1}H-^{13}C HMBC NMR analysis confirmed the structure attributed to CX_1. GC-MS chromatograms of the TMS derivatives of heartwood and bark acetone extractives and reference flavanols confirm the existence of the different flavanols and isomers appearing at distinct retention times (figure 49).

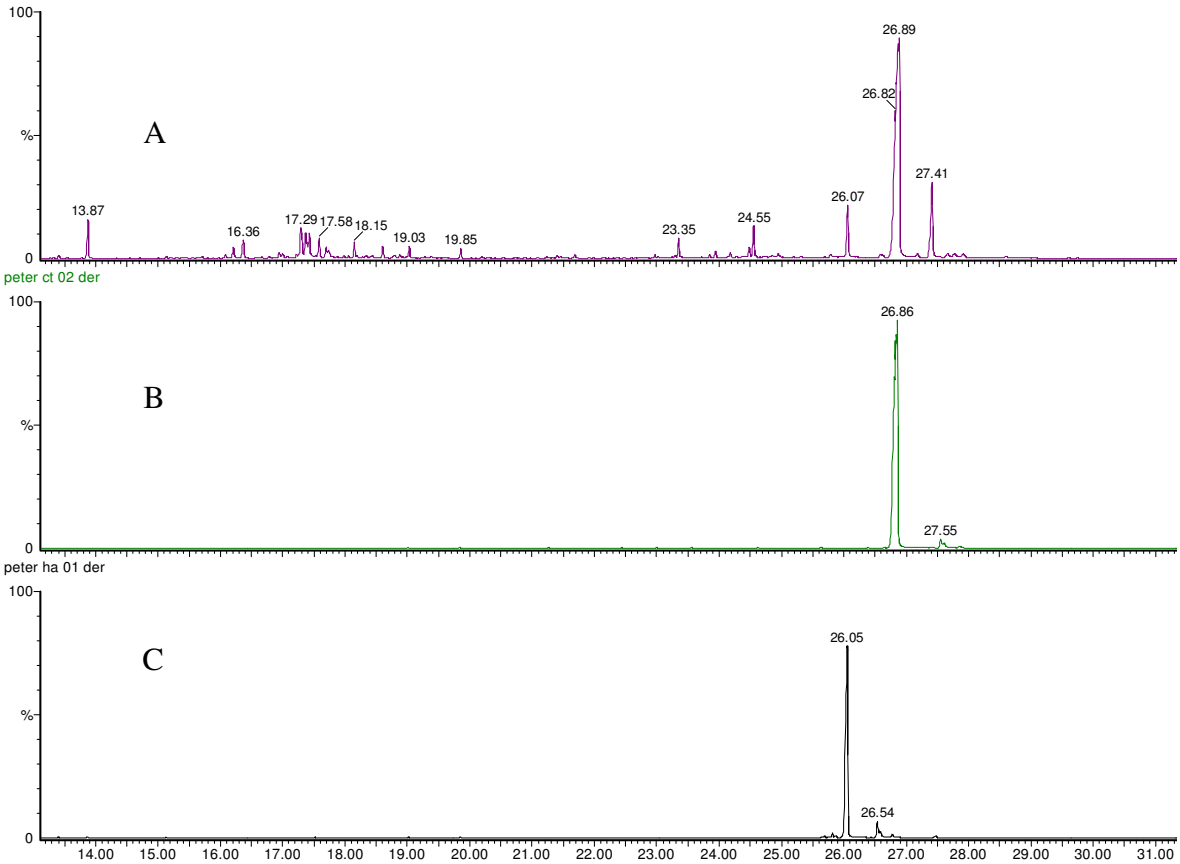


Figure 49. GC-MS chromatograms of bark acetone extractives (A), (+)-catechin (B) and heartwood acetone extractives (C)

Magnification of the GC chromatograms in the area corresponding to flavanols is shown in in figure 50.

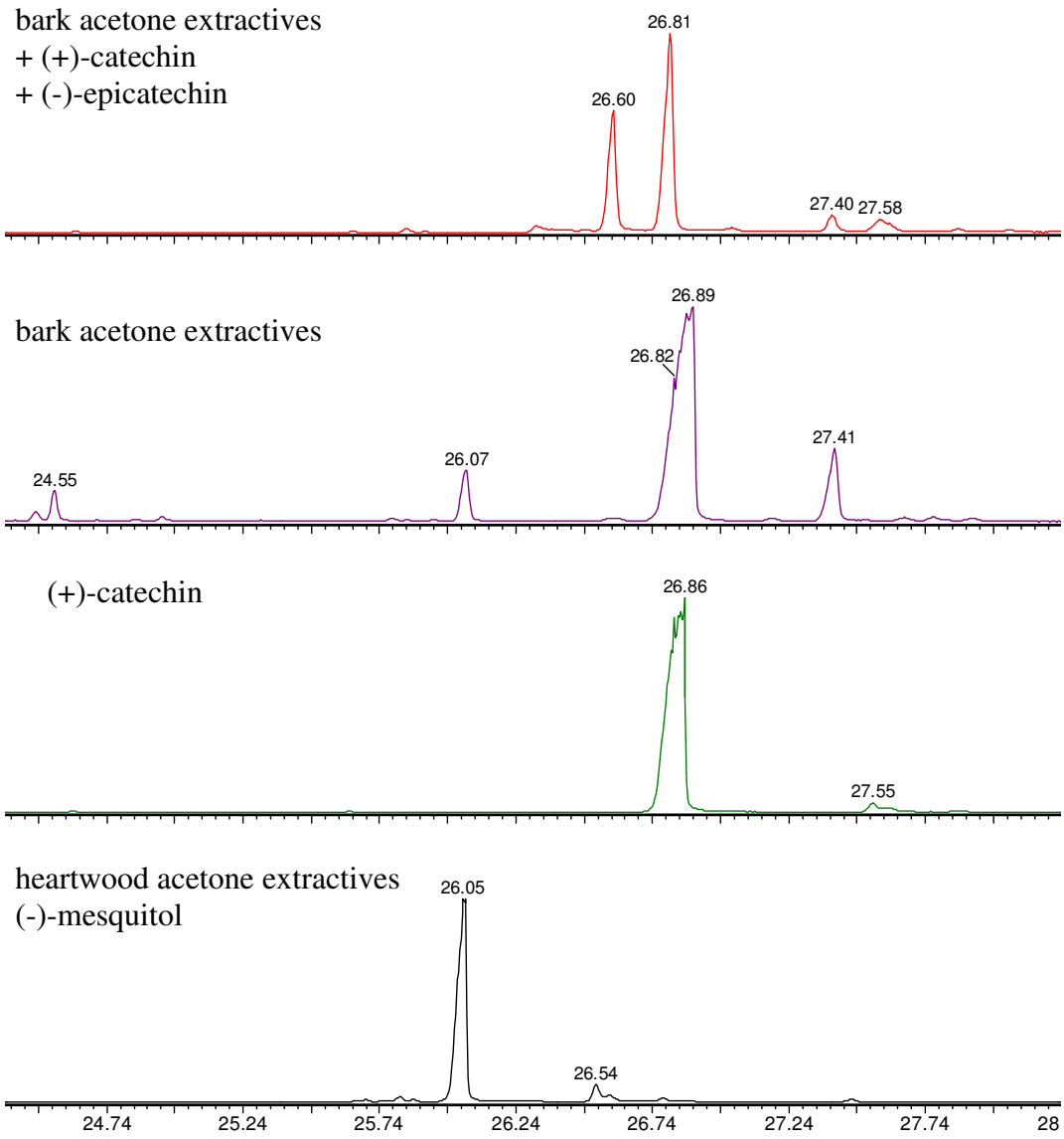
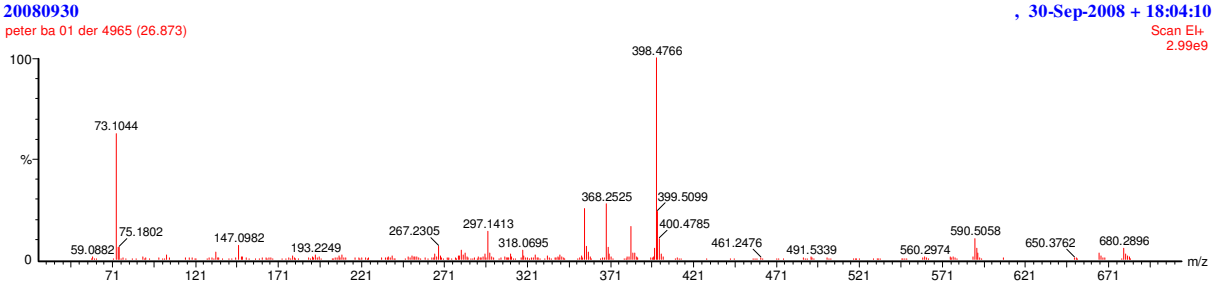


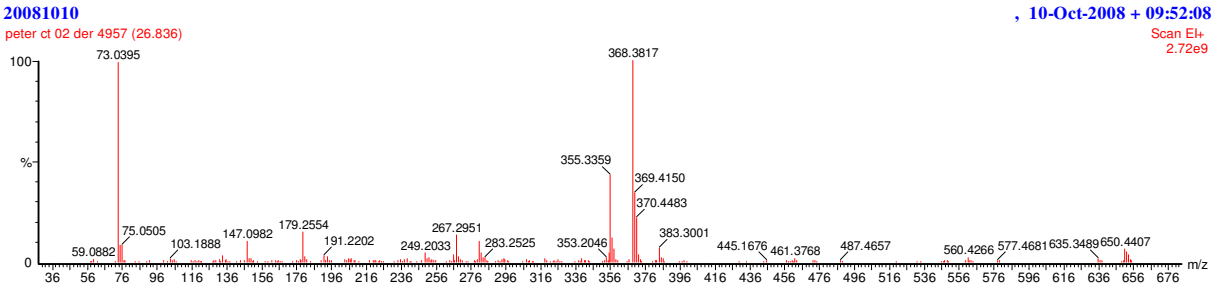
Figure 50. Magnification of GC-MS chromatograms of bark acetone extractives and reference flavanols

Bark extractives present a main product at 26.89 min. with a shoulder at 26.82 min. and some minor products at 26.07 and 27.41 min. Comparatively to bark extractives, mesquitol identified in heartwood extractives appears at 26.05 min. and could therefore corresponds to one of the minor products of bark. Catechin presents a retention time similar to that of the main products of bark.

According to the aromatic signals observed in the crude ^{1}H NMR spectrum of bark extractives, it was supposed in a first time that the main product contained could correspond to catechin. However, ^{1}H NMR spectrum of the purified acetylated product indicated the presence of another favanol structure corresponding more probably to 4′-O-methylgallocatechin. This result is confirmed by the analysis of the MS spectra (figure 51).



bark main component, rt = 26.87 min.



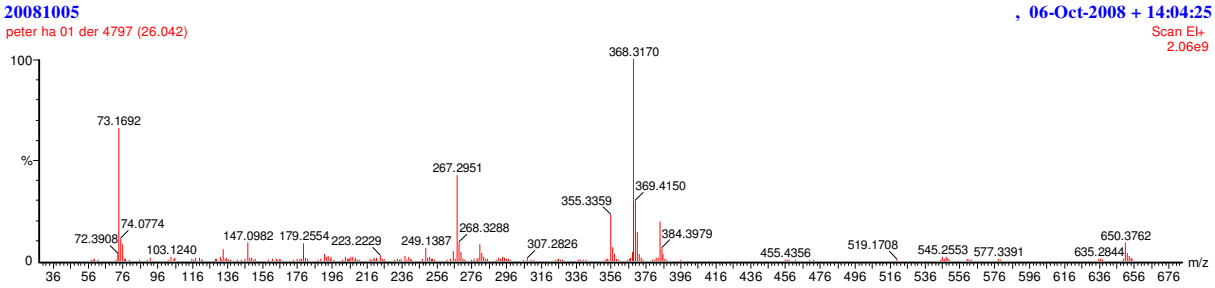
(+)-catechin, rt = 26.83 min

Figure 51. Comparison of MS spectra of acetone bark main component and (+)-catechin

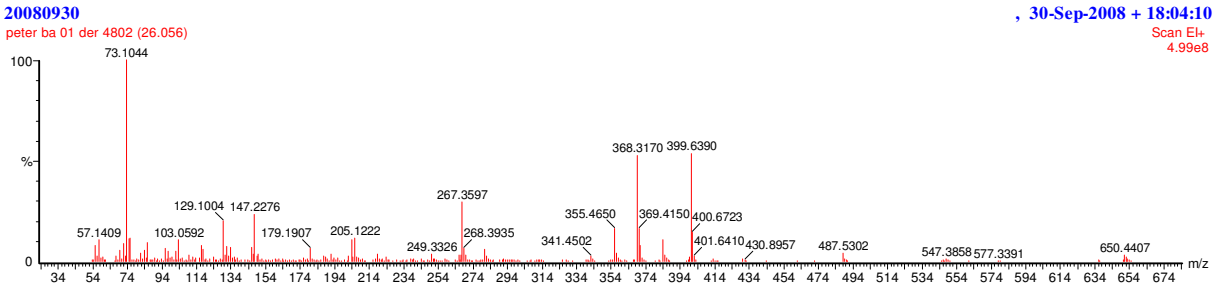
Even if main component detected in bark and (+)-catechin present quite similar retention times, MS spectra of the two products present different characteristic peaks. The MS spectrum of catechin indicates molecular peak at m/z 650 and characteristic peaks at 355, 368 as reported in the literature (Soleas *et al.,* 1997). Molecular peak and peak at 368 observed for catechin are shifted at 680 and 398 in the case of bark main component. This difference of 30 units of mass can be attributed to the presence of a methoxyl group confirming the attribution made by NMR.

Similar observations have been performed between (-)-mesquitol (rt = 26.05 min.) present in heartwood and product detected at 26.07 min in bark (figure 52). The two MS

spectra indicated the same difference of 30 units of mass corresponding probably to the presence of another methoxylated isomer of gallocatechin.



(-)-mesquitol, rt = 20.04 min



bark component, rt = 20.05 min.

Figure 52. Comparison of MS spectra of (-)-mesquitol and unknown product of bark

A possible structure for this isomer is proposed thereafter.

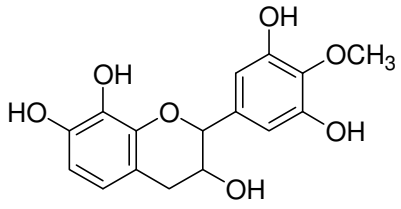


Figure 53. Possible structure for bark component, rt = 20.05 min.

GC-MS analysis of toluene/ethanol bark extractives indicated numerous products among which 4′-O-methylgallocatechin as the main component (figure 53).

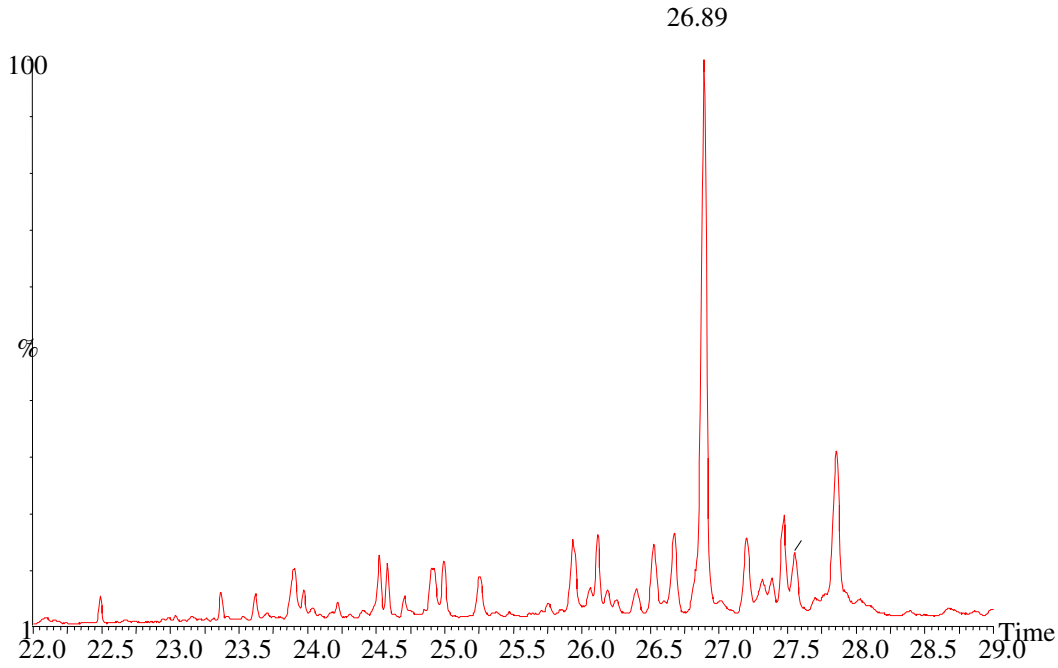


Figure 53. GC- MS chromatograms of toluene/ethanol bark extractives.

Further identifications of different compounds in bark extractives using NIST library and literature informations indicate that this latter one contain epicatechins, catechins, methylgallocatechins, gallocatechins, fatty acids and sugars.

Comparatively to literature data, where flavones like kaempferol, quercetin and retusin have been identified in different studies concerning chemical composition of *P. juliflora* bark extractives (Caceres *et al.,* 1995; Ranjana and Misra, 1981b; Shukla *et al.,* 1980; Malhorta and Misra, 1983), our results indicated rather the presence of flavanol among which different isomers of methylgallocatechins, which have been identified for the first time in *P. juliflora* bark extractives.

4.2.3. Leaves extractives

^{1}H NMR analysis of leaves crude extractives by different solvents is reported in figure 54.

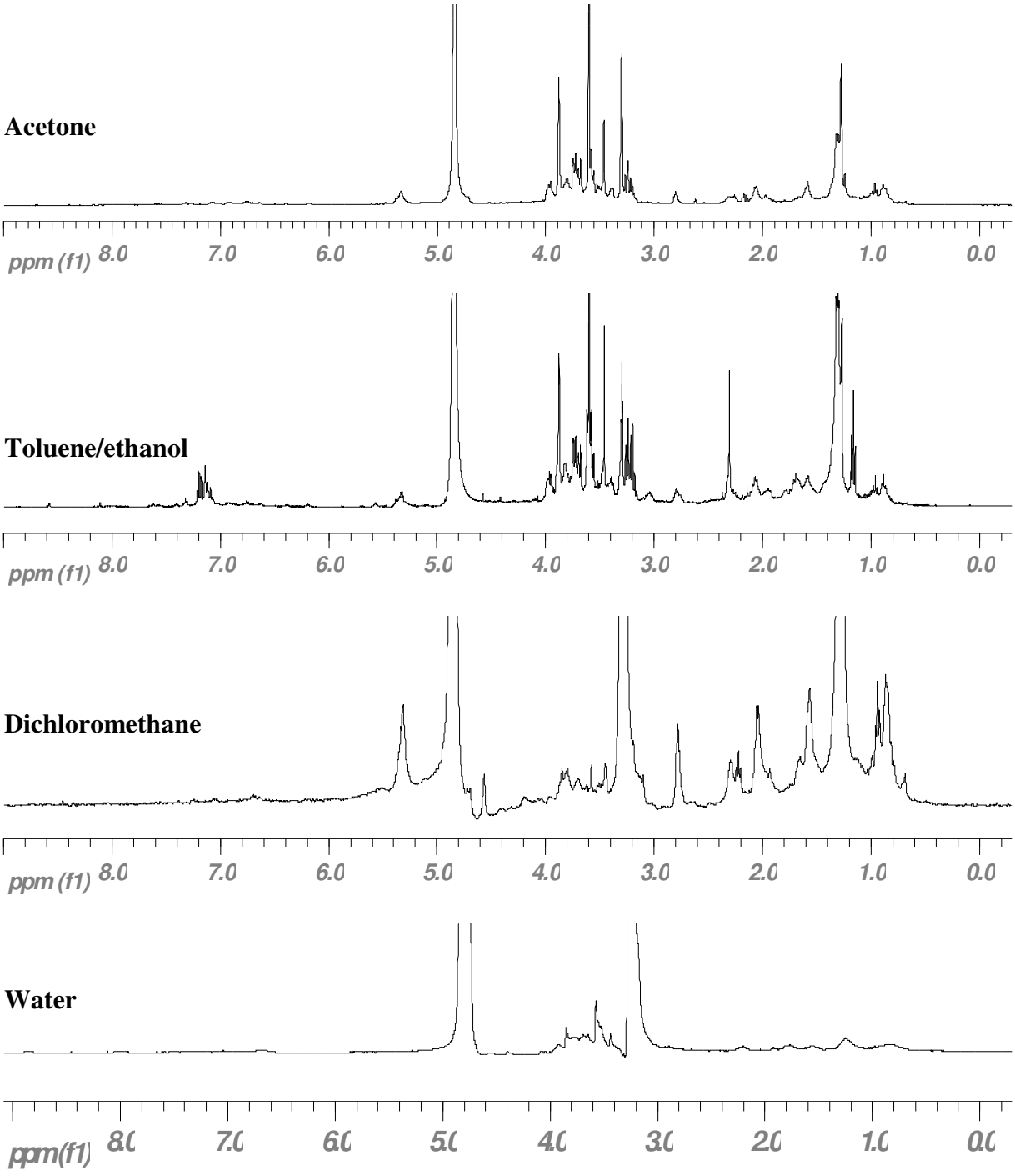


Figure 54. ^{1}H NMR analysis for leaves extractives by different solvents

^{1}H NMR analysis of leaves crude extractives by different organic solvents indicates mainly the presence of signals between 0.8 and 2.0 ppm typical of fatty alkyl chains of fatty acids, fats or waxes and signals between 3.0 and 4.0 ppm ascribable to sugars or glycerol moiety of fats. Presence of allylic protons between 2.0 and 2.5 ppm and vinyl protons at 5.4 ppm is characteristic of unsaturated fatty alkyl chains. In their study on the leaf extracts of *P. juliflora,* Ahmed *et al.* (1998) showed the presence of alkaloids. These latter ones are characterized by different signals at 0.8, 1.3, 3.1, 3.4 and 3.6 ppm. According to our NMR analysis, such products can not be totally excluded, even if they do not represent the main components. FTIR spectra of leaves extracts are shown in figure 55.

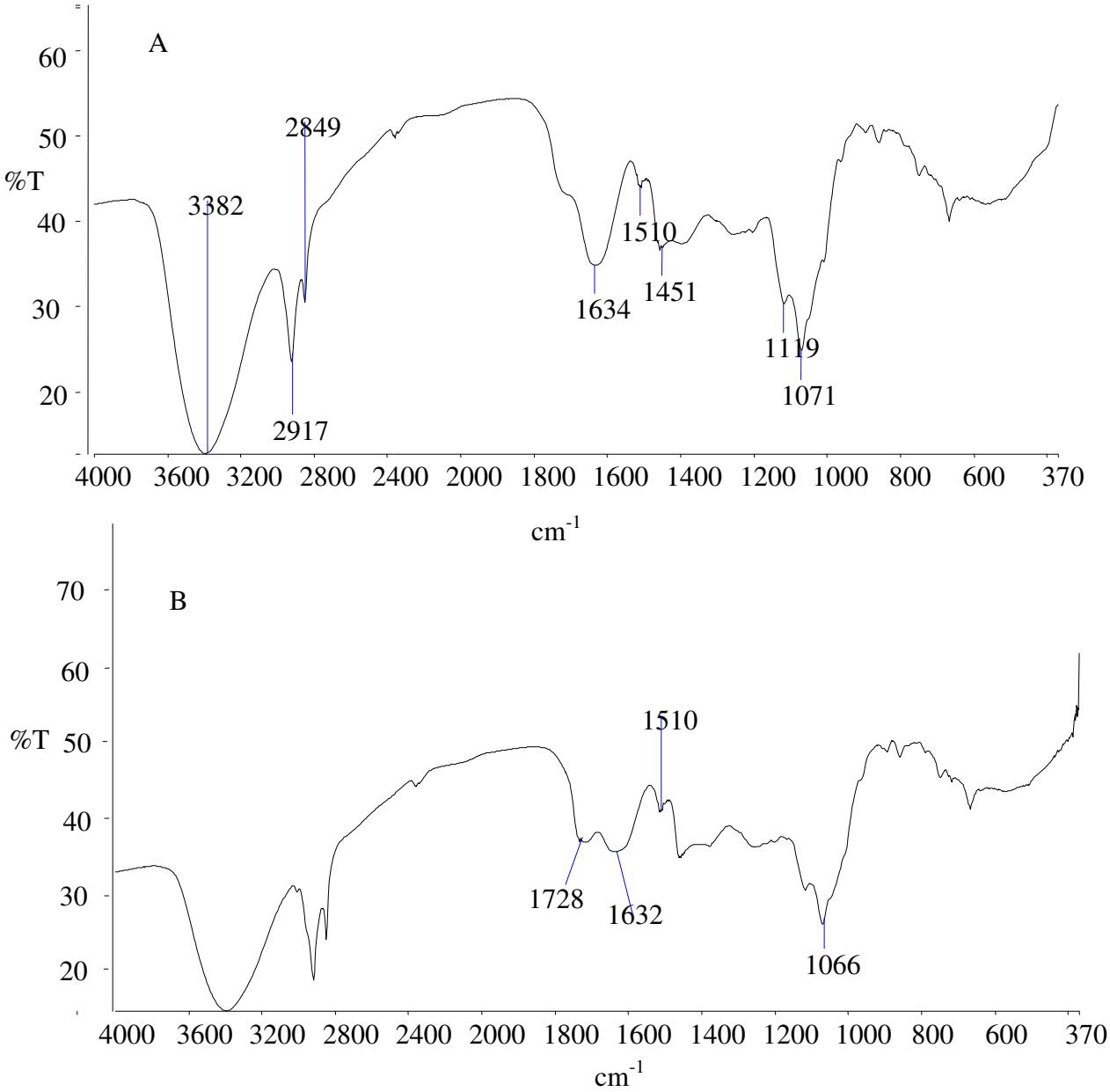


Figure 55. FTIR spectrum of (A) toluene/ethanol and (B) acetone extractives of *P. juliflora* leaves

FTIR analyses indicated absorption bands at 3400 cm^{-1} characteristic of OH group and bands at 1119 and 1071 cm^{-1}, which could be ascribable to sugars detected by NMR. Strong absorption bands at 2917 and 2849 cm^{-1} characteristic of C-H vibrations and 1728 cm^{-1} characteristic of C=O vibrations can be attributed to the presence of fatty acids in their free or esterified form, while bands at 1510 and 1450 cm^{-1} are ascribable to aromatic structures.

Identification of products based on GC-MS chromatograms, NIST library and other literature information indicate that leaves are constituted of fatty acids such hexadecanoic acid or linolenic acid and different sugar units.

4.2.4. Whole pods extractives

^{1}H NMR analysis recorded in methanol-D4 of whole pods crude extracts by different solvents is presented in figure 56.

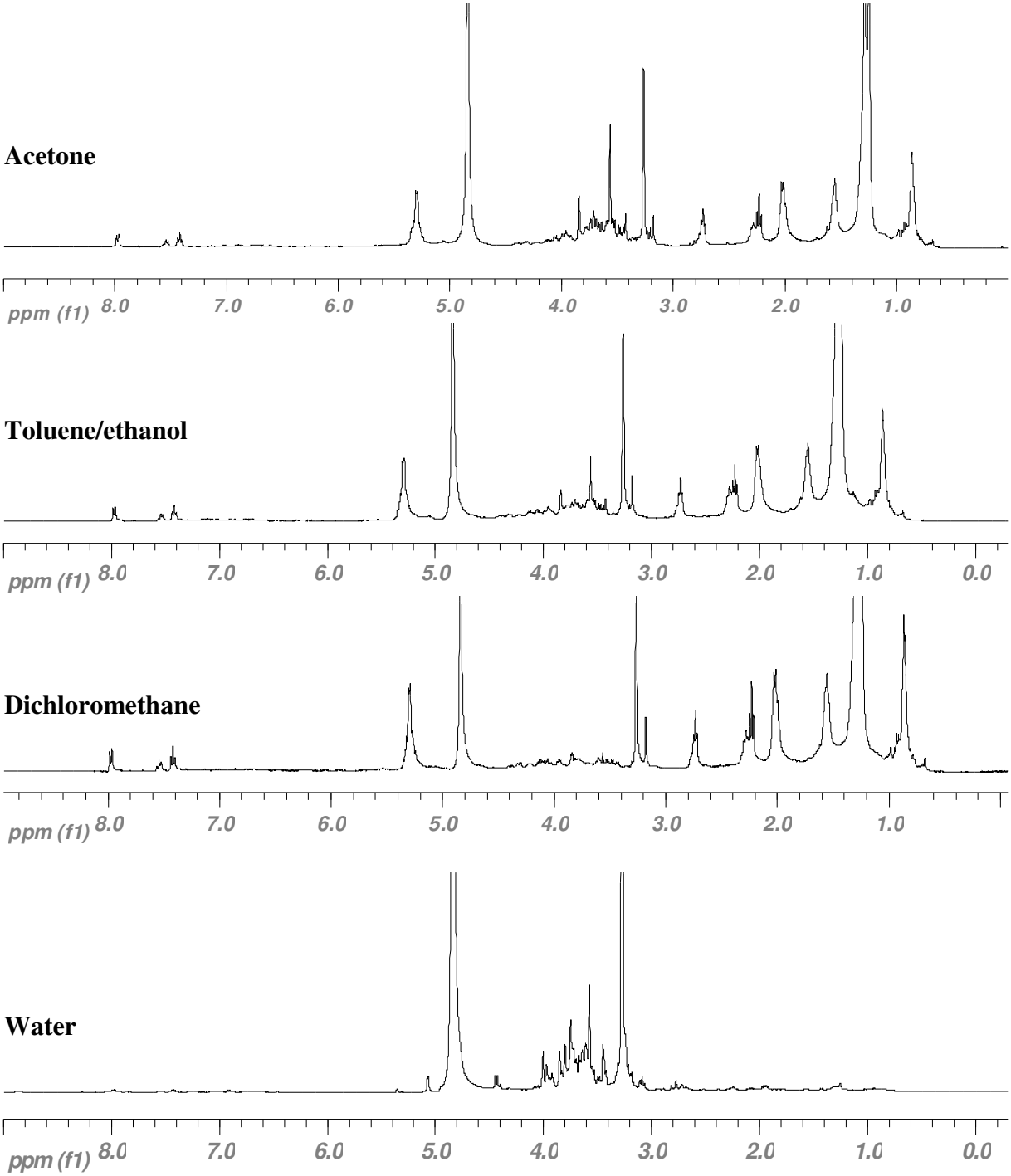


Figure 56. ^{1}H NMR analysis for whole pods extractives by different solvents

Dichloromethane, acetone and toluene/ethanol extractives present quite similar NMR spectra, while water extractives present completely different signals.

Spectrum of water extractives indicates mainly the presence of signals comprised between 3.2 and 4.0 ppm ascribable to sugar units of mono or polysaccharides. Such chemical shifts have been reported from *P. juliflora* pods (Vierira *et al.,* 2007; Gallao *et al.,* 2007). Indeed, *P. juliflora* pods are reported to contain galactomannan: mannan ratio of 1.0: 1.1 (Vierira *et al.,* 2007). Spectra of extractives obtained with other solvents indicate the presence of fats, waxes or fatty acids. Signal at 0.8 ppm is typocal of terminal CH_3 group, signals between 1.2 and 1.6 ppm characteristic of methylene groups (CH_2) of the fatty alkyl chain, signals between 1.8 and 2.8 ppm typical of methylene groups in α position of the carbonyl group or allylic position. Vinylic hydrogen atoms appear around 5.2 ppm. Signals comprised between 3 and 4 ppm can be ascribable to hydrogen atoms of glycerol unit of fats or to the presence of some sugar units. These latter ones are more important in acetone and toluene / ethanol extracts comparatively to dichloromethane extractives. Aromatic signals present different chemical shifts at 7.4, 7.5 and 8.0 ppm, which can be attributed to alkaloids like juliprosine.

FTIR spectrum is reported in figure 57.

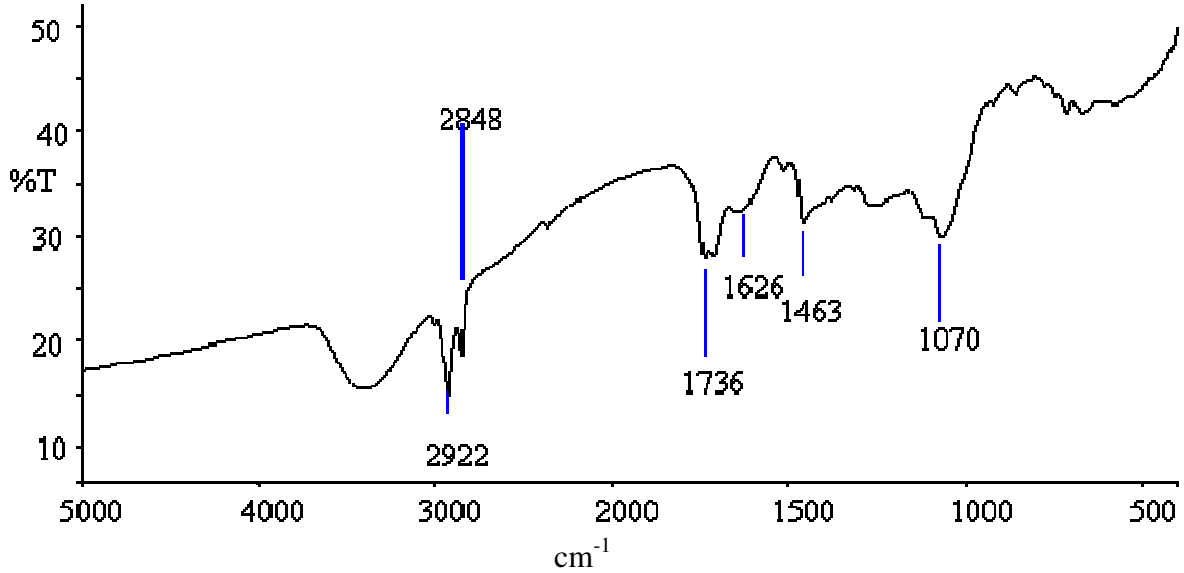
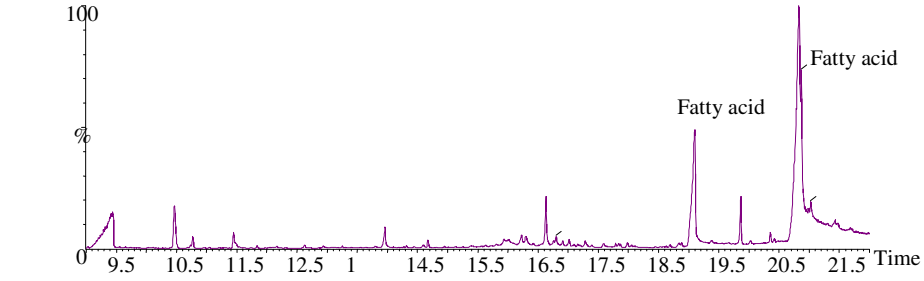


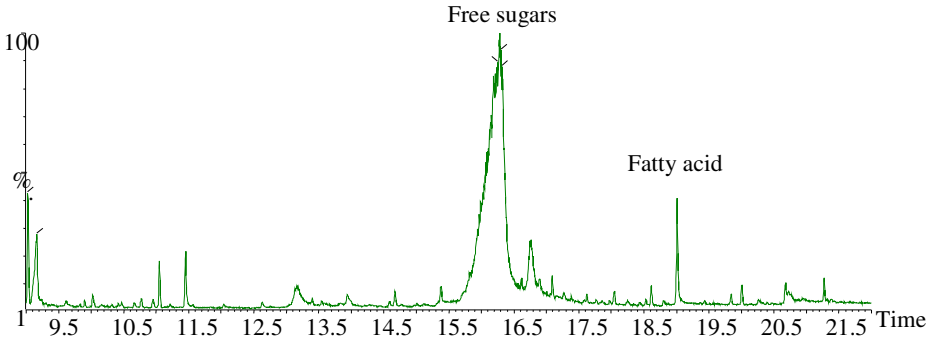
Figure 57. FTIR spectrum for pods dichloromethane extractives

FTIR analysis indicated characteristic hydroxyl group absorption of a sugar unit at 3350 cm^{-1} and C-H vibrations present in aliphatic structure of fatty acids between 2849 and 2922. The C-O absorption of polysaccharides compounds is evident at 1070 and C=O absorption at 1736 confirms a saturated fatty acid. Aromatic C=C skeletal vibrations are weak and difficult to exploit.

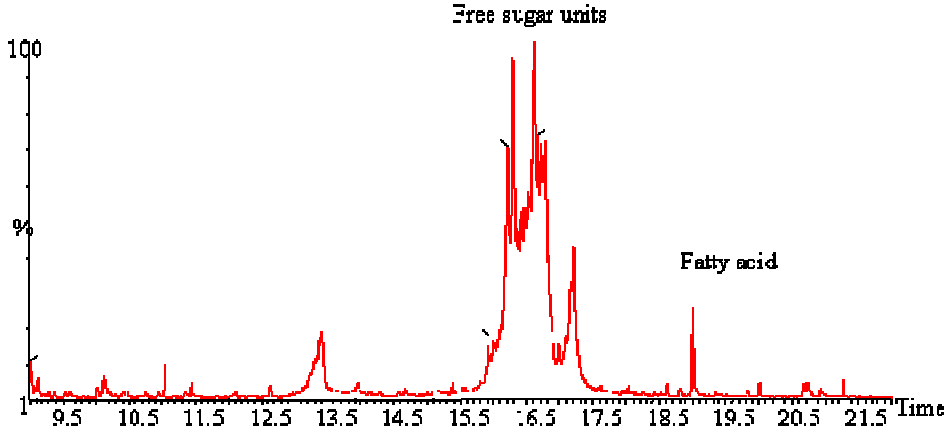
GC-MS analyses of the TMS derivatives of different extractives from pods are presented in figure 58.



Dichloromethane extractives



Acetone extractives



Toluene/ethanol extractives

Figure 58. GC-MS chromatograms of the different compounds in pods extractives

GC chromatograms are difficult to exploit due to the presence of numerous products. Toluene / ethanol and acetone extractives are very similar showing sugars and fatty acids. Chromatogram of dichloromethane indicates mainly the presence of fatty acids.

Based on GC-MS, FTIR, ^{1}H NMR, NIST library and literature information it can be concluded that the pods contain fatty acids such as hexadecanoic acid, octadecanoic acid, palmitic acid, dehydroabietic acid, oxyhydroabietic acid, free sugars such as sucrose and glucose, mannose, galactomanans and traces of aromatic compounds. Presence of such products in the pods of *P. juliflora* has been previously reported corroborating our results (Liu *et al.,* 2008; Lopez *et al*., 2006; Silva *et al.,* 2002; Sawal *et al.,* 2004; Choge *et al.,* 2007).

4.2.5. Stem bark exudates

Mature, standing *P. juliflora* produce thick brown exudates from knots and stem bark, that solidify on the stem surface to form gum like structures. It was therefore of interest to analyse this product using the procedures described in 3.0. FTIR spectrum is shown in figure 59.

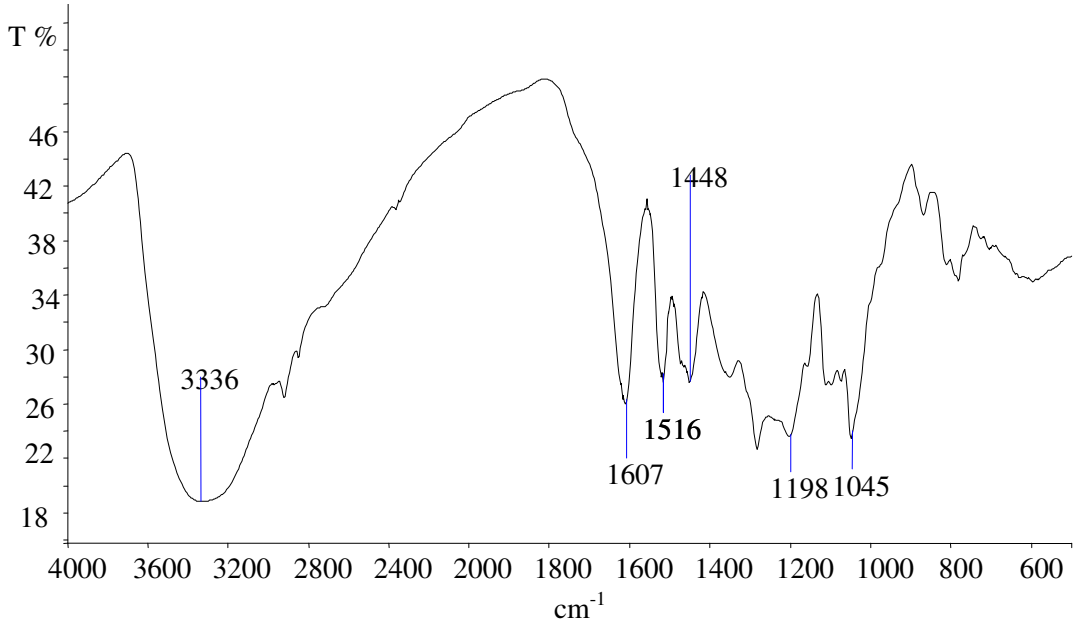


Figure 59. FTIR spectrum for stem bark exudates

The FTIR spectrum indicated characteristic hydroxyl group absorption at 3336 cm^{-1} and aromatic C=C skeletal vibrations at 1607, 1516 and 1448 cm^{-1}. The spectrum presents important similarities with that of mesquitol (figure 36). GC-MS spectrum is shown in figure 60.

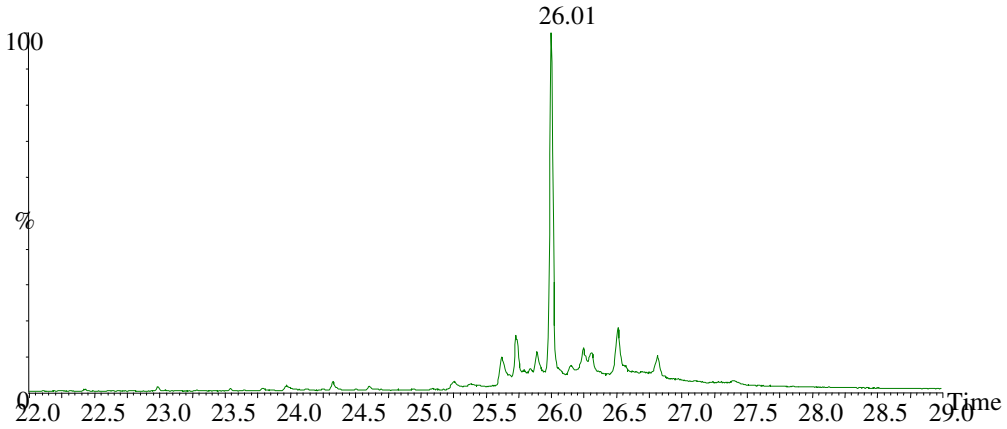


Figure 60. GC-MS chromatogram of stem bark exudates

The chromatogram indicates the presence of different compounds with a main products appearing at a retention time of 26.01 minutes. MS spectrum of this product is similar to that of (-)-mesquitol obtained previously from the heartwood presenting molecular peak for the penta-TMS derivative at m/z 650 and characteristic peaks at 355 and 368.

^{1}H NMR analysis is described in figure 61.

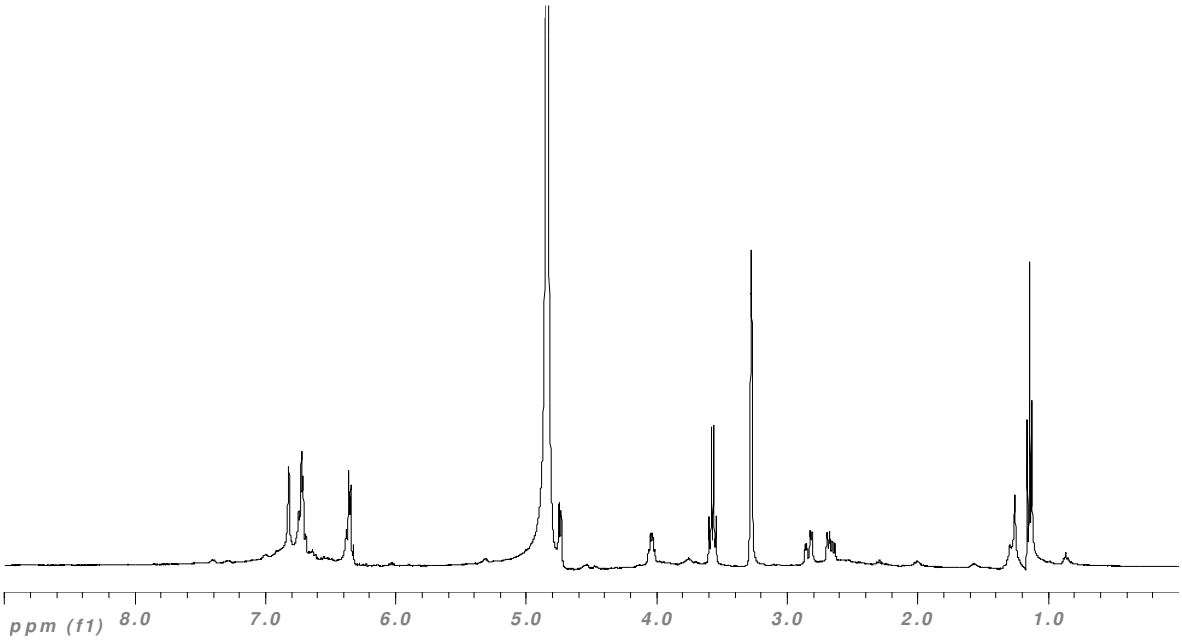


Figure 61. ^{1}H NMR spectra of stem bark exudates

Similarly with the results obtained for heartwood, the ^{1}H NMR of stem bark exudates indicates the presence of main component showing characteristic aromatic hydrogen atoms and ABX system corresponding to (A) ring of (-)-mesquitol. According to the NMR spectrum, it seems that (-)-mesquitol present a high degree of purity allowing to envisage further valorisation.

These results suggest that accumulation of (-)-mesquitol in the heartwood could be a mean for the tree to respond to external injuries caused by mechanical damage, herbivores, or infections inducing secretion of phytoalexin, mainly constituted by mesquitol, as exudates.

4.2.6. Antioxidant properties of extractives

4.2.6.1. According to methyl linoleate oxidation inhibition

The inhibition of oxygen uptake with methyl linoleate (LH) as the substrate was used. L° free radicals are generated by the initiator AIBN (2,2'-azobis(2-methylpropionitrile)), and oxidation of methyl linoleate is a chain reaction propagated by the processes:

$$L^{\circ} + O_2 \leftrightarrows LOO^{\circ}$$
$$LOO^{\circ} + LH \rightarrow LOOH + L^{\circ}$$

In the presence of an inhibitor having a labile hydrogen and called AH, chaincarriers easily abstract an H atom from AH (called a chain-breaking antioxidant),giving an unreactive free radical A°:

$$LOO^{\circ} + AH \rightarrow LOOH + A^{\circ}$$

and so inhibiting chain propagation. Oxidation was monitored by measuring the oxygen pressure for the reaction. Antioxidant properties of the different *P. juliflora* extractives, estimated using methyl linoleate oxidation inhibition induced by AIBN, are presented in figures 62, 63 and 64.

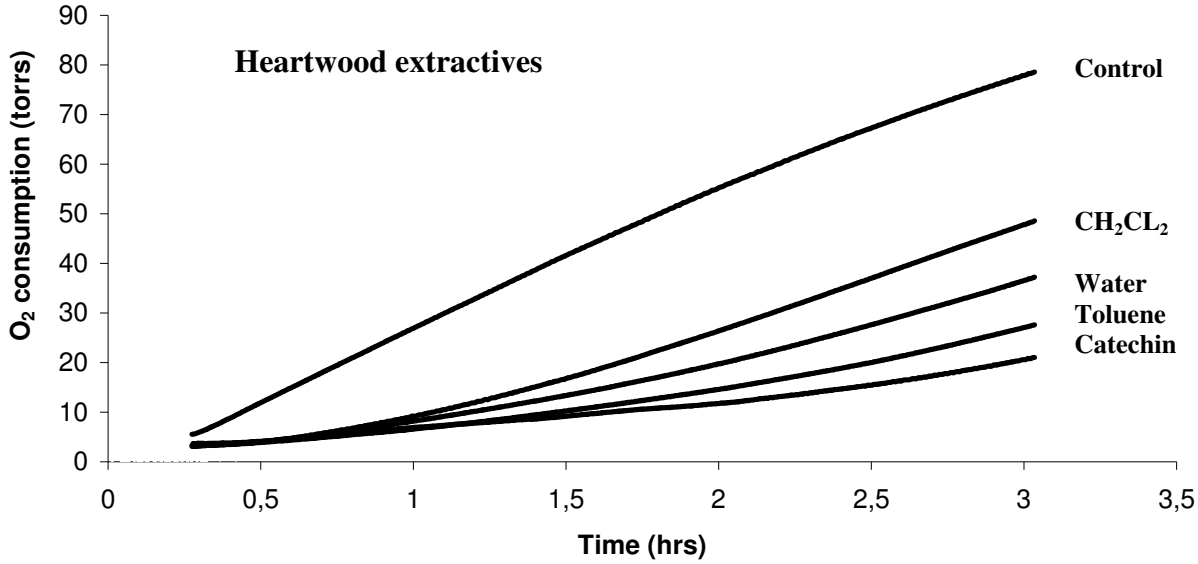


Figure 62. Antioxidant properties of heartwood *P. juliflora* extractives at 0.09g/l estimated using methyl linoleate oxidation inhibition

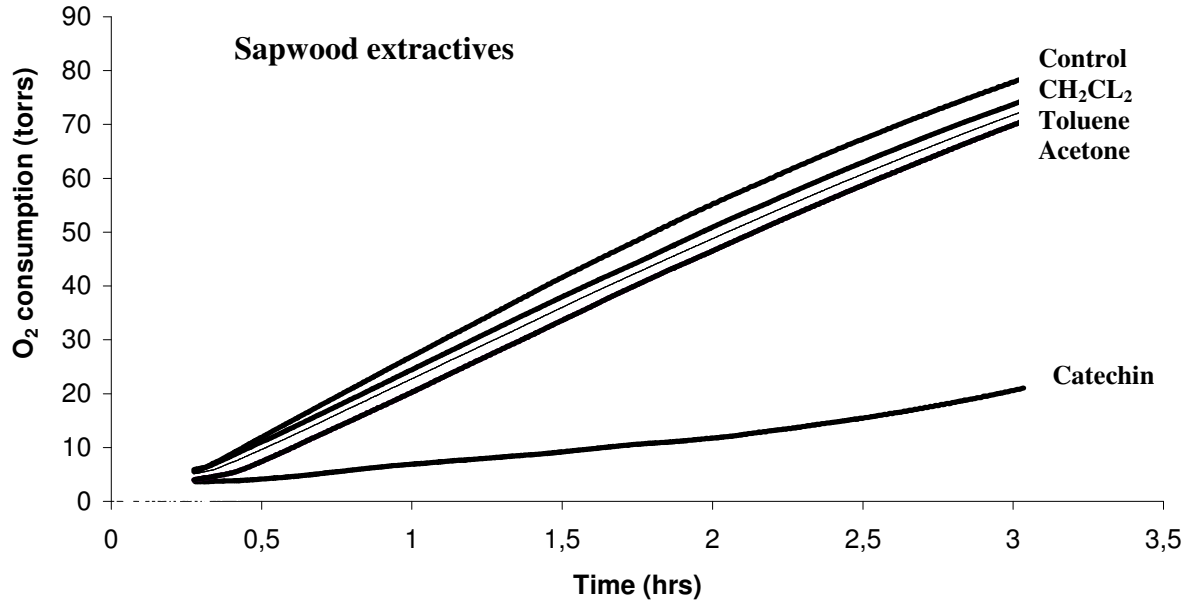


Figure 63. Antioxidant properties of sapwood *P. juliflora* extractives at 0.09g/l estimated using methyl linoleate oxidation inhibition

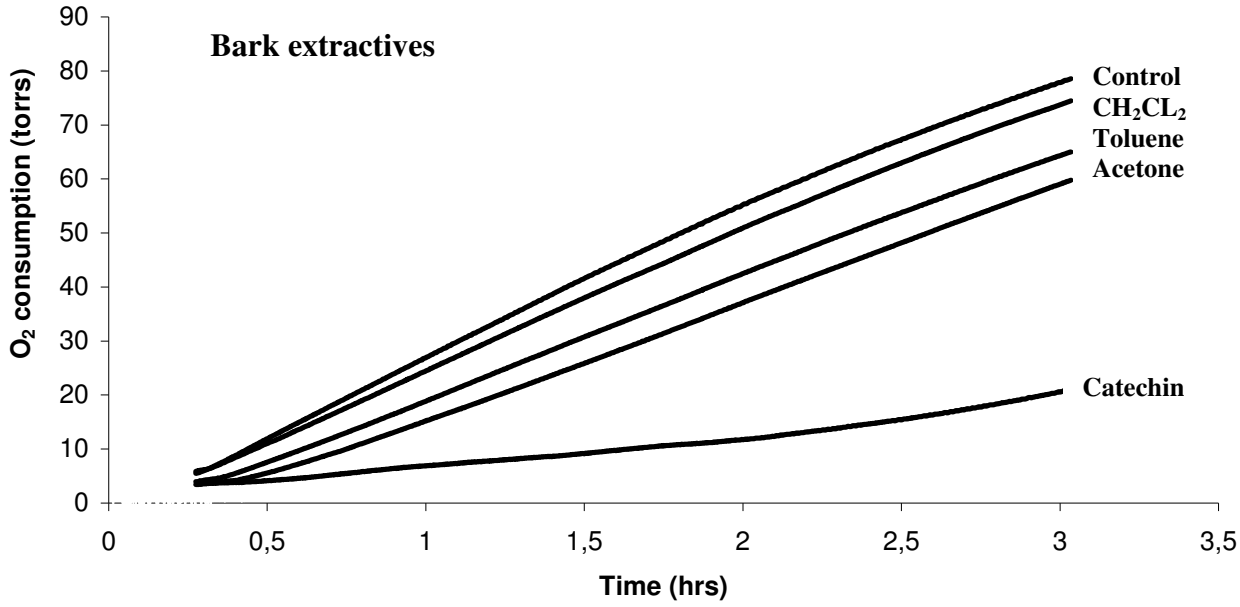


Figure 64. Antioxidant properties of bark *P. juliflora* extractives at 0.09g/l estimated using methyl linoleate oxidation inhibition

Generally heartwood extractives presented higher antioxidant properties in comparison with bark and sapwood extractives. Hydrophilic extractives from the more polar solvents such as acetone and toluene/ethanol mixture gave higher antioxidant properties than lipophilic extractives present in dichloromethane extractives, suggesting that flavanols such as (-)-mesquitol present in these extracts are responsible for the observation. Indeed correlation of phenolic contents in plants to their antioxidant activities has been reported (Wang *et al*., 2004; Haupt *et al*., 2003). Antioxidant properties of bark extractives can be explained by the presence of methylgallocatechins described in literature as having important antioxidant properties (Valcic *et al*., 2000; Sang *et al*., 2003; Bors, 1990).

To evaluate potential of mesquitol present in heartwood acetonic extractives as antioxidant, additional experiments have been performed with BHT a widely used synthetic antioxidant and catechin (figure 65).

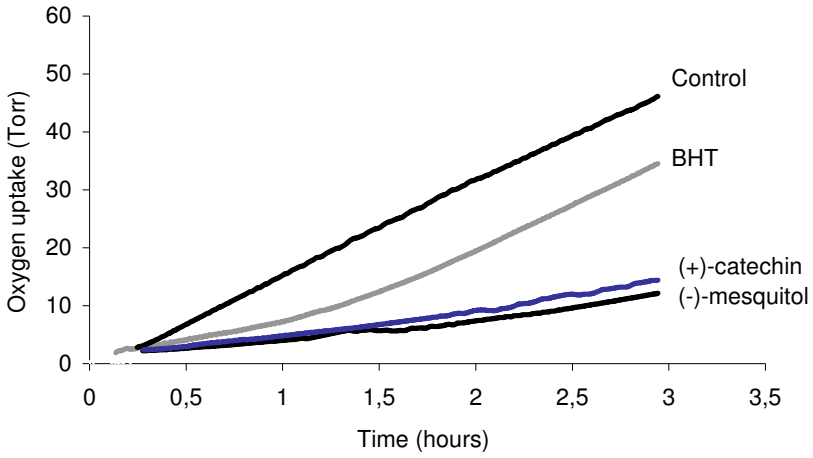


Figure 65. Antioxidant properties of heartwood flavanols (0.09g/l) estimated using methyl linoleate oxidation inhibition

Acetonic heartwood extractives presented similar antioxidant properties than (+)-catechin. In both cases, the two flavanols present higher antioxidant properties compared to BHT chosen as reference antioxidant. Antioxidant properties of (-)-mesquitol (M) and (+)-catechin (C) at different concentrations are presented in figure 66.

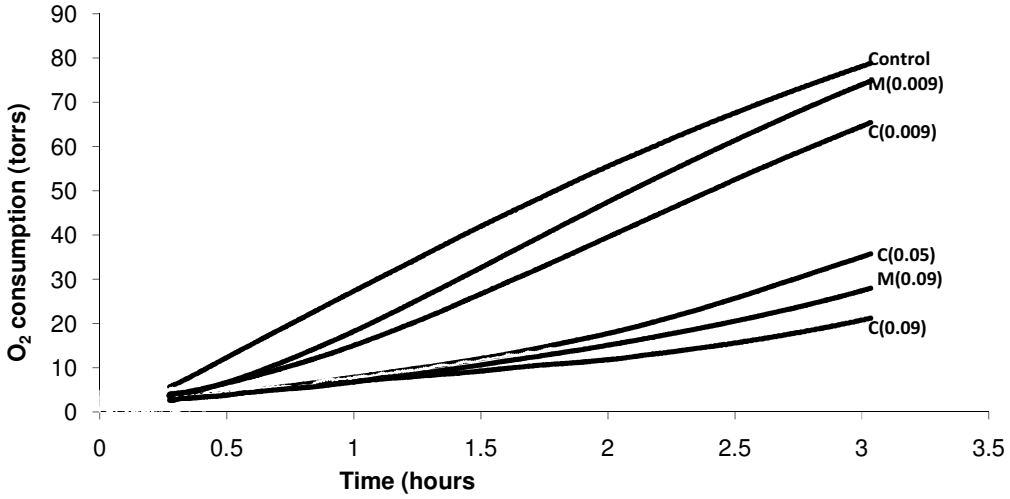
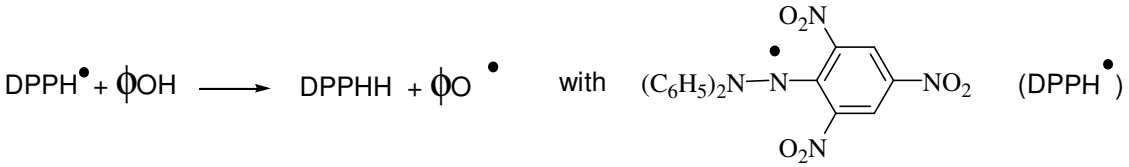


Figure 66. Antioxidant properties of (-)-mesquitol (M) and (+)-catechin (C) at different concentrations(g/l) estimated using methyl linoleate oxidation inhibition

Independent of the concentration level tested (-)-mesquitol and (+)-catechin gave important antioxidant properties which increase with increasing concentration. For a given concentration, (+)-catechin seems to be slightly more effective than (-)-mesquitol.

4.2.6.2. According to DPPH assay

To corroborate the above properties, free radical scavenging activities of *P. juliflora* extractives by different solvents were assessed by DPPH assay. DPPH is a stable radical, dark violet in color. Its color is bleached by its reaction with a hydrogen donor.



Methanolic solutions of 2,2-diphenyl-1-picrylhydrazyl free radical and different extractives were rapidly mixed and the absorbance measured at 517 nm (a wavelength at which only 2,2-diphenyl-1-picrylhydrazyl absorbs). Antioxidant activity of the crude extracts was evaluated according to the remaining DPPH concentration (figure 67).

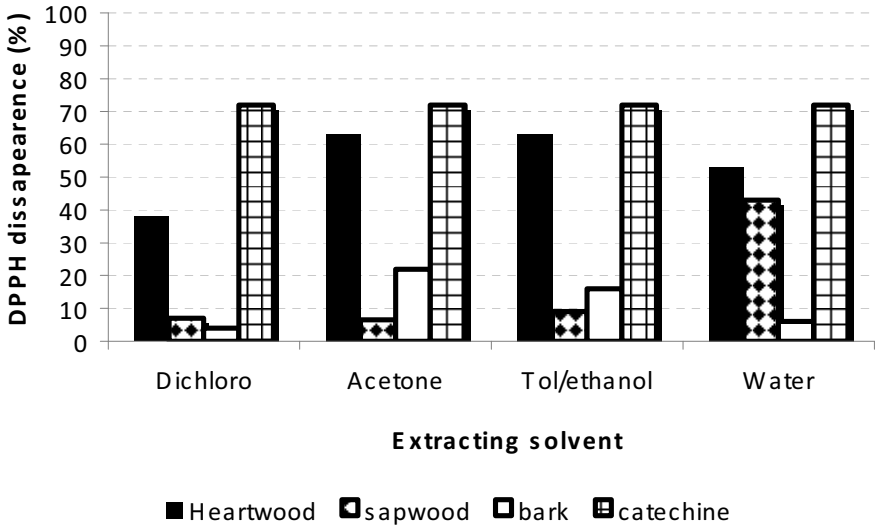
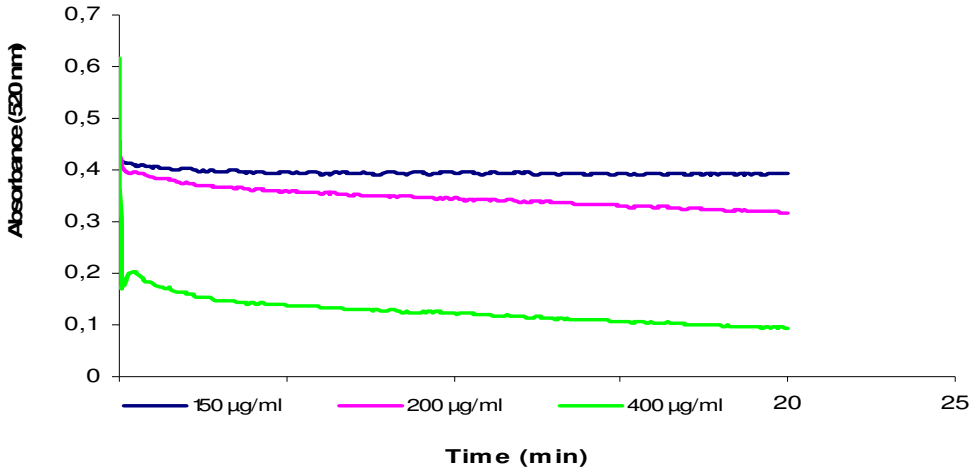


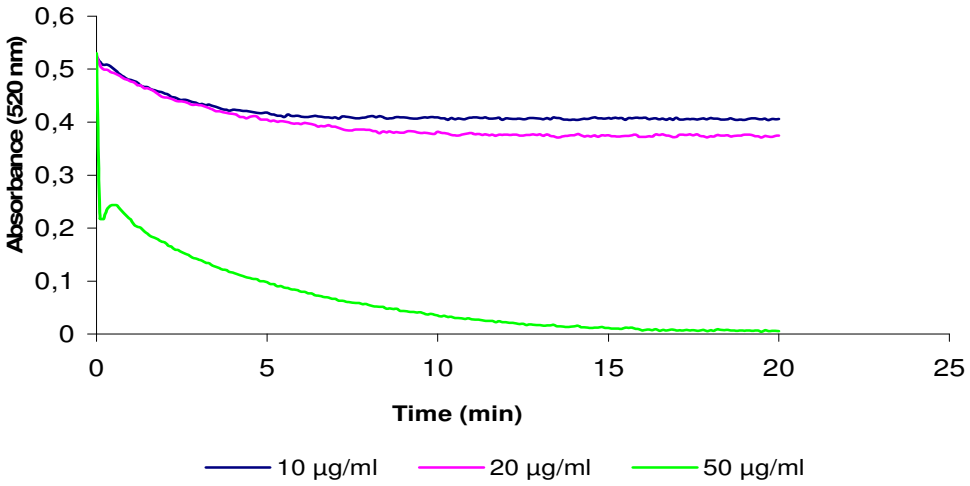
Figure 67. DPPH inhibition by different *P. juliflora* extractives

As shown in figure 67 the heartwood acetone and toluene/ethanol extractives like (+)-catechin exhibited significant inhibitory activity (≥ 60%) against the DPPH radical whereas the bark and sapwood extractives were found to have much less effect (≤ 25%).

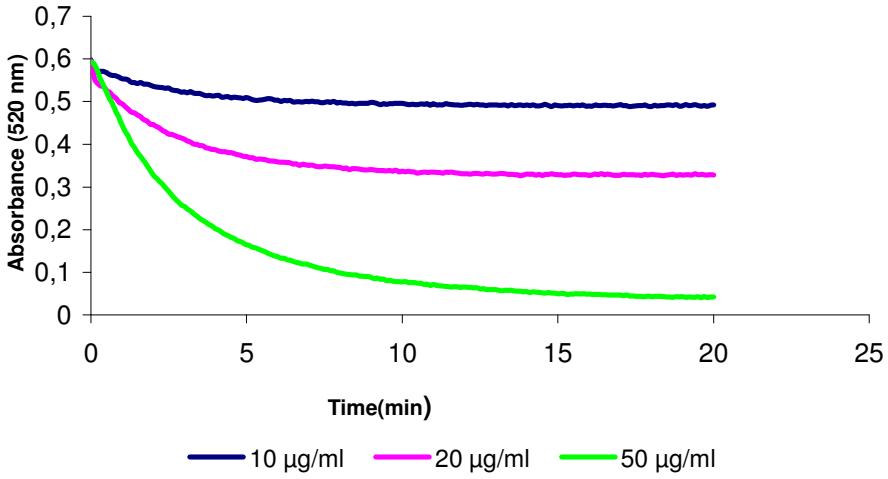
The antioxidant activity of (-)-mesquitol was investigated more precisely by measuring IC_{50} value, which corresponds to the antioxidant concentration scavenging 50% of DPPH. For comparison purposes, IC_{50} values were also determinate for (+)-catechin and BHT. Results are presented in figure 68.



Treated with different concentrations of BHT



Treated with different concentrations of (+)-catechin



Treated with different concentrations of (-)-mesquitol

Figure 68. Absorption of 100µM DPPH treated by different antioxidants

The inhibitory concentration at 50% DPPH (IC_{50}) was determined for (-)-mesquitol, BHT and (+)-catechin by reporting the remaining DPPH concentration as a function of antioxidant concentration. Figure 69 shows the typical curve obtained for (-)-mesquitol allowing determination of IC_{50}.

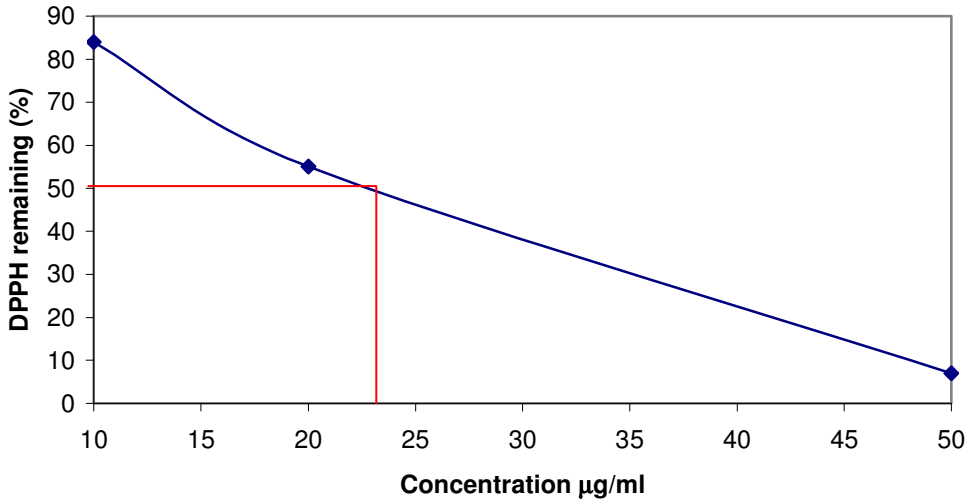


Figure 69. Determination of IC_{50} of (-)-mesquitol

IC_{50} values for (-)-mesquitol from heartwood acetone extractive were observed at approximately 23µg/ml. At 50µg/ml, the extract inhibited more than 90% of the DPPH radical. The IC_{50} value of well-known antioxidant compounds, (+)-catechin and BHT, used as a reference in this study, is approximately 29µg/ml and 268µg/ml.

Free radical scavenging is one of the known mechanisms whereby antioxidants inhibit lipid peroxidation or stop the enzymatic activities of micro-organisms (Pongtip *et al*., 2007). DPPH assay is used extensively for screening antioxidants from natural products such as fruit, vegetable juices and wood extractives (Raza and John, 2007). Our results show that *P. juliflora* extractives have important antioxidant activity to prevent the fungal deterioration of wood. Indeed (-)-mesquitol in comparison with existing antioxidants, such as Probucol and alpha-tocopherol, shows better antioxidant activity, which can be therefore useful in controlling inflammatory diseases such as cancer and diabetes (Madhusudana *et al.,* 2004).

4.2.7. Antibacterial properties and effect on laccases

The minimal inhibitory concentration (MIC) was determined for heartwood and bark acetonic extractives by the critical dilution method in 96- well microtitre plates. Results are presented in table 10.

Table 10. % bacterial growth inhibition by different *P. juliflora* extractives

Extractive (mg/ml)	5.0		2.5		1.3		0.6	
Bacteria	HW	B	HW	B	HW	B	HW	B
E. coli	-	-	-	-	-	-	-	-
S. anterica	-	-	-	-	-	-	-	-
S. aureus	-	-	-	-	-	-	-	-
E. faecalis	≥ 22	-	22	-	-	-	-	-
L. monocytogenes	-	-	-	-	-	-	-	-

HW:Heartwood, B: Bark

The crude acetonic heartwood extractive (HW) was able to inhibit the activities of *E. faecalis* (MIC ≈ 2.5mg/ml) but was less important in other bacterial strains tested. Bark extractives (B) did not show any antibacterial properties.

Even if the results obtained showed poor antibacterial activity for the different *P. juliflora* extractives tested, it is important to notice that concentrations used during this study are relatively low. Indeed, a recent study aimed to evaluate antimicrobial activity of the methanolic extracts and compounds from *Treculia africana* and *Treculia acuminata* (Kuete et al. 2008) indicates MIC values for catechin of 2, 10 and 20 µg/ml for *E. coli*, *S. aureus* and *E. faecalis* respectively. MIC values of crude extracts are generally higher and comprised between 50 and 200 µg/ml according to their chemical composition.

It is therefore necessary to perform additional experiments to evaluate the MIC of mesquitol, which should be probably slightly higher than the maximal concentration tested in our study. If higher concentrations lead to better results, the use of *P. juliflora* extracts could be of valuable interest for the treatment of infections associated to microorganisms. The crude

extracts as well as the isolated compounds found active could be in this case useful for the development of new antimicrobial drug.

4.2.8. Effect of (-)-mesquitol on laccase activity

Degradation of wood by white rot fungi is carried out by several enzymes such as cellulases, peroxidases or laccase, while the initial stages of brown-rot decay is caused by non enzymatic oxidative degradations involving hydroxyl radicals (Cheng *et al.,* 2004). Antioxidant properties of extractives are therefore useful to explain the reasons of natural durability of *P. juliflora.* It seems therefore interesting to investigate the behavior of acetonic extractives on the activity of commercially available laccase to evaluate if (-)-mesquitol is oxidized by the enzyme explaining thus fungistatic properties observed previously. Different assays have been performed with heartwood and bark acetonic extractives and (+)-catechin as reference product. Results are reported in figure 70, 71, 72 and 73.

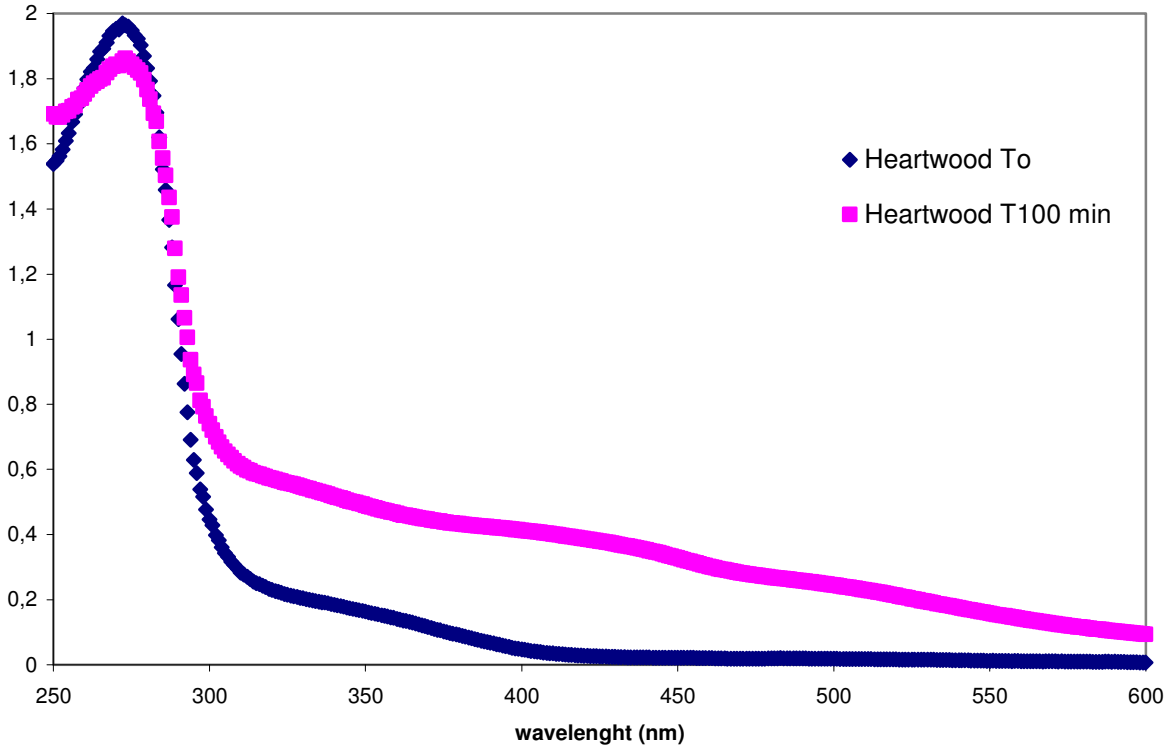


Figure 70. Effect of laccase on heartwood acetone extractives

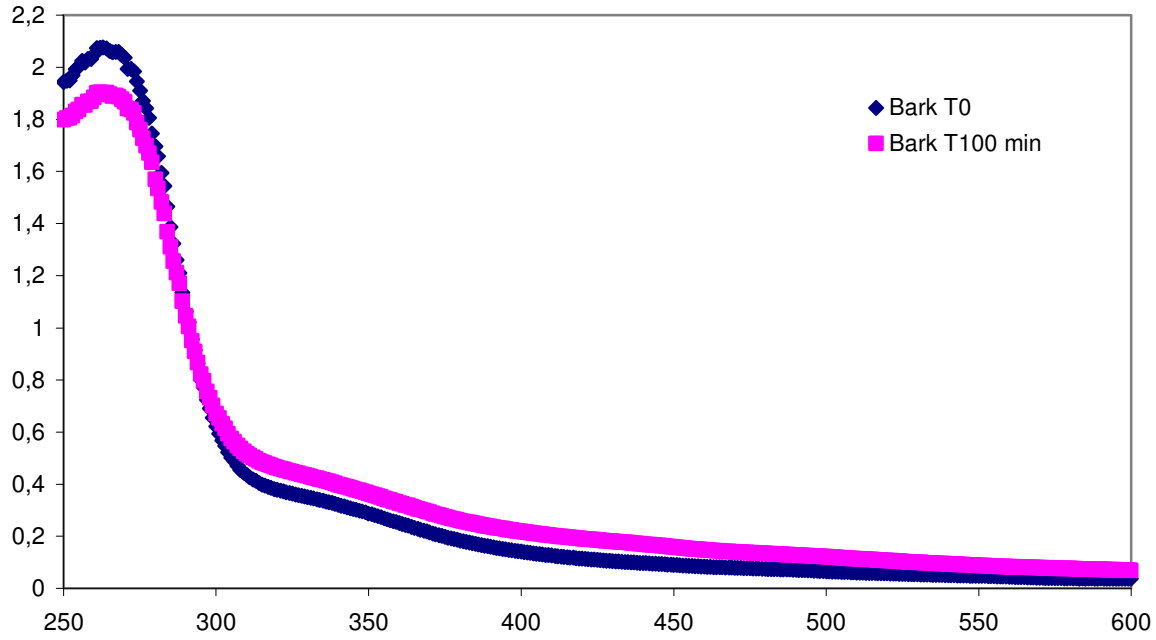


Figure 71. Effect of laccase on bark acetone extractives

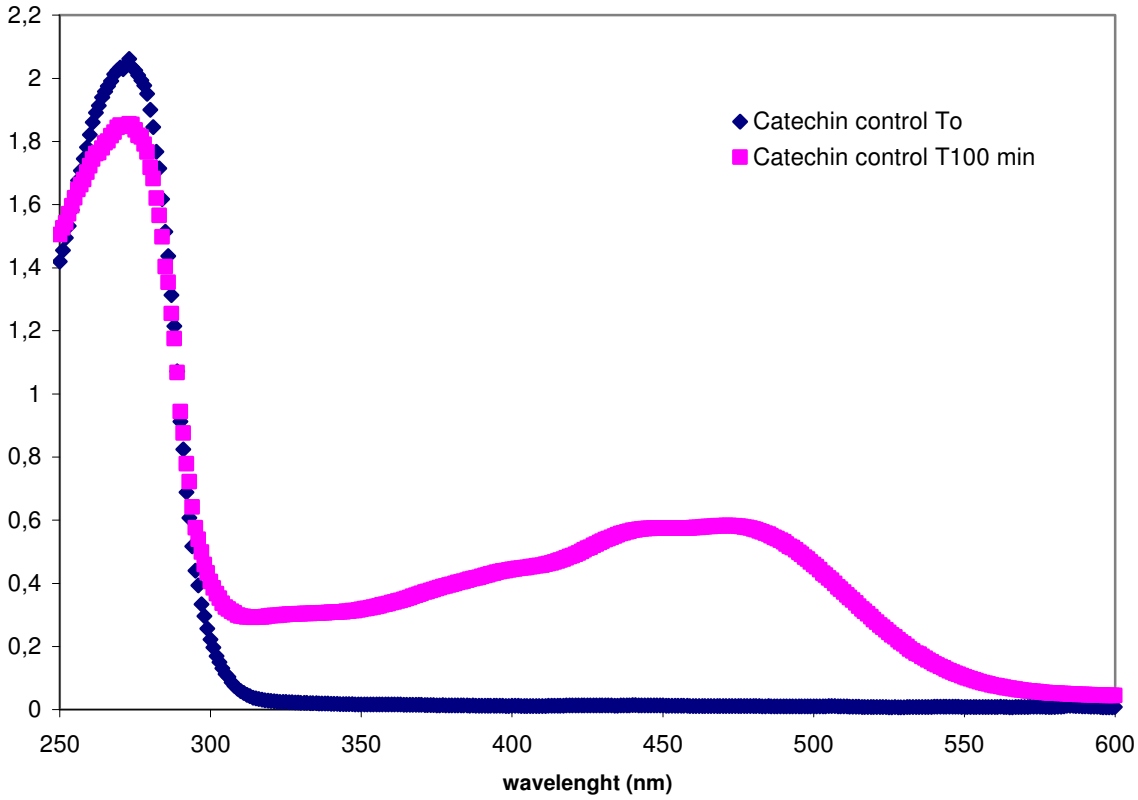


Figure 72. Effect of laccase on (+)-catechin

Evolution of UV absorbance with time depends of the nature of the tested product. After 100 minutes, (+)-catechin is strongly oxidized by laccase as demonstrated by the large increase of the detected absorbance measured between 350 to 600 nm region. In the same time, UV spectra of heartwood acetone extractives exhibit also an important increase of absorbance in the region between 350 to 550 nm. These results demonstrated that (-)-mesquitol present as the main component in acetonic heartwood extractives is also oxidized by laccase. The two flavonoïds tested can be therefore considered as sacrificial antioxidants.

Laccase production has been correlated with wood extractives degradation in particular oxidation of catechin. Wood extractives induce laccase expressions. Initial stages of wood colonization correlate with wood extractives degradation, requiring laccase activity whereas the other wood degrading systems involving peroxidases and polysaccharide hydrolases are still repressed (Lekounougou *et al*, 2007). It is therefore probable to conclude that fungistatic properties observed previously associated with more or less important growth inhibition periods are explained by the ability of laccase enzymes to oxidize different biomolecules in extractives.

Bark extractives are less susceptible to laccase oxidation leading to quite similar UV absorption spectrum after the same incubation period. This behaviour can be attributed the presence of methoxy group slowing down oxidation processes.

5.0. CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

Prosopis juliflora contains important amount of extractives. Heartwood extractive content is comparable to that of the bark but significantly higher than those of sapwood, pods and leaves. High quantities of extractives as described in the literature on other wood species contribute to wood physical, mechanical, colour, durability and toxicological properties.

The heartwood is durable against all tested fungi and resistance to termites is more important. Weight losses on solvent extracted wood exposed to termites and fungi are higher than for the unextracted samples. It seems that the more hydrophilic extractives removed by acetone and water contribute more to heartwood bioresistance than the lipophilic compounds extracted by dichloromethane however does not fully explain heartwood durability.

Heartwood extractives by different solvents were effective in inhibiting the growth of the fungi at all the levels of extractive concentration tested. Development of the fungal mycelium on the treated medium started after a more or less important inhibition period, suggesting that the heartwood extractives possess strong fungistatic properties even at low concentrations. Exposure of filter papers treated with different heartwood extractives to termites caused mortality at different levels of extractive concentrations. Biological resistance of *P. juliflora* wood against termites and fungi is therefore associated to the presence of extractives.

The wood of *P. juliflora* is dimensionally stable with calorific values consistent with those of other *prosopis* wood species currently used as fuel wood. Ash content is relatively high but in good agreement to contents generally found in tropical wood species while anatomical wood characteristics can be described as normal for this species. Bending strengths values are consistent with those reported in literature with exception of hardness. The relatively high retention and higher CCA sapwood treatability indicate that this species could be a potential alternative source of material for railway sleepers and fencing posts however based on the strength parameters is unsuitable for heavy construction works. Put together, these results suggest that *P. juliflora* can be a substitute for parquetry, carving, fuel wood, light construction works and furniture production.

Important amount (≈ 8 %) and high purity of the rare flavonoïd (-)-mesquitol was identified as the major metabolite in heartwood extractives, while (+)-epicatechin, (+)-catechin, gallocatechins, methylgallocatechins, fatty acids and free sugar are present in the bark. *P. juliflora.* Pods contain important quantity of galactommanans, mannose, saturated and unsaturated fatty acids and free sugar described in the literature as food supplement and medicine in animals and humans. Leaves of *P. juliflora* contain alkaloids such as tryptamine, piperidine, phenethylamine and juliprosopine described in literature as having antifungal and plant growth inhibiting properties as well as capable of inducing neuronal damages in animals (Tapia *et al.,* 2000). GC-MS analysis indicates the presence of an important quantity of fatty acids such as hexadecanoic and octadecanoic acids, glucopyranose, hydroquinone, glucopyranosides and galactose sugar in leaves extractives. Screen test suggests presence of flavonoïds in exudates from the stem bark.

Antioxidant properties, estimated using methyl linoleate oxidation inhibition test showed that (-)-mesquitol like (+)-catechin is able to slow down oxidation of methyl linoleate induced by AIBN as well as inhibit the activities of DPPH radical. In both cases, the flavanols presented higher antioxidant properties compared to BHT chosen as reference antioxidant. Heartwood extractives by different solvents presented higher antioxidant properties compared to bark and sapwood extractives. Hydrophilic extractives from the more polar solvents gave better antioxidant properties than lipophilic extractives. Sapwood and bark extractives are weak antioxidants suggesting that extractives protect heartwood by being excellent free radical scavengers and having fungistatic properties. The crude acetonic heartwood extractive was able to inhibit the activities of *E. faecalis* (MIC ≈ 2.5mg/ml), while crude bark extractives were less effective. Extractives of *P. juliflora* could therefore be of valuable interest as potential source of antioxidants and for the development of new antimicrobial drug.

Valorisation of the different *P. juliflora* extractives and biomolecules as additives in products which are required to have mild fungicidal, antioxidant, antitermitic and antibacterial properties such as cosmetics, soaps, termiticides and shampoo could remedy the negative effects associated to this timber species and stop the shortage of wood resources in Kenya. Several studies have reported the possibility of developing new biocides and pharmaceutical products analogous to natural products present in extractive matter (Inamori *et al.*, 2000; Baya *et al.*, 2001). Such products if developed synthetically would be more environmentally

acceptable due to their composition based on analogies with natural extracts present in plants. Indeed the search for potent natural antioxidants from plant sources as nutritional supplements for health foods is gaining a lot of interest worldwide. Antioxidants are also used as a key strategy for inhibiting or reversing carcinogenesis (Wang *et al.,* 2004).

Articles published from the preceding results are as follows:

Review articles:

- Sirmah P., Muisu F., Mburu F., Dumarçay S., Gérardin P. (2008). Evaluation of *Prosopis juliflora* properties as an alternative to wood shortage in Kenya. *Bois et forets des tropiques*, 298, 4, 25-35.

- Sirmah P., Dumarçay S., Masson E., Gérardin P. (2009). Unusual amount of (-)-mesquitol from the heartwood of *Prosopis juliflora*. *Natural Product Research*, 23, (2), 183-189

- Sirmah P., Iaych K., Poaty B., Dumarçay S., Gérardin P. Valuable antioxidant compounds from *Prosopis juliflora*. *Annals of forest science*, to submit.

Conferences and proceedings:

- Sirmah P., Dumarçay S., Masson E., Gérardin P., (2008). Antioxidant and antifungal properties of (-)-mesquitol from the heartwood of *Prosopis juliflora.* Poster presentation, RP2E Doctoral School, Nancy, January, 2008.

- Sirmah P., Muisu F., Mburu F., Dumarçay S., Gérardin P. (2008). Towards valorisation of *Prosopis juliflora* (Mathenge) as an alternative to the declining wood resource in Kenya. 4th Annual International Conference, Moi University, 29th-1st August, 2008.

- Sirmah P., Muisu F., Mburu F., Dumarçay S., Gérardin P. (2008). Contribution of (-)-mesquitol to the natural durability of *Prosopis juliflora* heartwood. 4th Annual International Conference, Moi University, 29th-1st August, 2008.

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5.2. Recommendations

- Additional experiments to evaluate antibacterial properties.

- More studies to understand toxicological properties of the different biomolecules present in *P. juliflora*.

- Chemical valorization of *P. juliflora* heartwood and bark extractives based on their antioxidant properties through establishment of collaboration with industry.

- Further detailed analysis of the leaves and pods extractives in order to understand problems of toxicity caused by this wood species.

- Comprehensive review of various wood technologies for *P. juliflora* wood valorization.

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Towards valorisation of *Prosopis juliflora* as an alternative to the declining wood resource in Kenya

Abstract: The first part of the study was to evaluate technological wood properties of *Prosopis juliflora* growing in the semi-arid and arid land of Kenya**.** Heartwood is durable against all tested fungi and relatively resistant to termites. Heartwood extractives possess fungistatic and antitermitic properties. The wood of *P. Juliflora* is dimensionally stable with normal calorific, anatomical and mechanical characteristics but relatively high ash content and sapwood treatability.
The second part of the study was to identify and characterise the chemical nature, antioxidant and antibacterial properties of extractives. Important amount (≈ 8 %) and high purity of the rare flavonoid (-)-mesquitol was identified in heartwood extractives, while (+)-epicatechin, (+)-catechin, 4'-O-methylgallocatechins, fatty acids and free sugar are present in the bark. (-)-mesquitol like (+)-catechin is able to slow down oxidation of methyl linoleate induced by AIBN as well as inhibit the activities of DPPH radical. Heartwood extractives by different solvents presented higher antioxidant properties compared to bark and sapwood extractives suggesting that antioxidant properties could be at the origin of fungistatic properties. The crude acetonic heartwood extractives were able to inhibit the activities of *E. faecalis* (MIC ≈ 2.5mg/ml). Extractives of *P. juliflora* could therefore be of valuable interest as potential source of flavanols, which have been described as powerful antioxidants. Utilisation of *P. juliflora* extractives as additives in products where antioxidant, mild fungicidal and antibacterial properties are required such as cosmetics, soaps and shampoo could offer another way of valorization in addition to the use of wood which can remedy to the shortage of wood resources in Kenya and the negative effects associated with this species.

Key words: *Prosopis juliflora*, extractives, antioxidant, antibacterial, termite, rot, flavanol, (-)-mesquitol

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Valorisation du *Prosopis juliflora* comme alternative à la diminution des ressources forestières au Kenya

Résumé : La première partie de l'étude concerne l'évaluation des propriétés technologiques du bois de *Prosopis juliflora* poussant dans les régions semi-arides et arides du Kenya. Le duramen est durable vis à vis des champignons et relativement résistant vis à vis des termites. Les extractibles du duramen possèdent des propriétés fongistatiques et antitermites. Le bois de *P. Juliflora* est dimensionnellement stable et présente des caractéristiques calorifiques, anatomiques et mécaniques normales , mais un taux de cendre élevé. L'imprégnabilité de l'aubier est bonne.
La deuxième partie de l'étude concerne la caractérisation chimique des extractibles et l'étude de leurs propriétés antioxydantes et antibactériennes. Le duramen contient une quantité importante (≈ 8 %) d'un flavonoïde rare, le (-)- mesquitol, alors que l'écorce renferme différents produits tels que de la (+)-épicatéchine, de la (+)-catéchine, de la 4-O-methylgallocatechine, des acides gras et des sucres. Le (-)-mesquitol comme la (+)-catéchine sont capables d'inhiber l'oxydation du linoléate de méthyle induite par l'AIBN ainsi que d'inhiber le radical du DPPH. Les extractibles du duramen obtenus avec différents solvants présentent des propriétés antioxydantes plus élevées comparativement à celles des extractibles contenus dans l'écorce ou l'aubier suggérant que les propriétés antioxydantes de ces derniers soient à l'origine des propriétés fongistatiques. Les extraits brutes acétoniques du duramen sont capables d'inhiber l'activité de E. faecalis (MIC≈ 2.5mg/ml). Les extractibles du *P. juliflora* peuvent donc être une source importante de flavanols connus pour leurs propriétés antioxidantes. L'utilisation des extractibles de *P. juliflora* comme additifs dans les produits où des propriétés antioxydantes et des propriétés antifongiques et antibactériennes douces sont requises telles que des produits de beauté, des savons et des shampoings peut constituer une voie de valorisation supplémentaire en plus de la valorisation du bois de cette essence pouvant limiter la pénurie de bois au Kenya et aux effets négatifs associés à cette essence.

Mots clés : *Prosopis Juliflora*, extractibles, antioxydant, antibactérien, termite, pourriture, flavanol, (-)-mesquitol