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JULIFLORA

Author(s): P Sirmah, F Mburu, K Iaych, S Dumargay and P Gérardin

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# POTENTIAL ANTIOXIDANT COMPOUNDS FROM DIFFERENT PARTS OF *PROSOPIS JULIFLORA*

P Sirmah<sup>1, 2</sup>, F Mburu<sup>2</sup>, K Iaych<sup>1</sup>, S Dumarçay<sup>1</sup>, \* & P Gérardin<sup>1</sup>

<sup>1</sup>Laboratoire d'Etudes et de Recherche sur le Matériau Bois, EA 4370, Equipe Chimie du Bois et Procédés de Préservation, Nancy Université, BP 70239, 54506 Vandoeuvre les Nancy Cédex, France

<sup>2</sup>Department of Forestry and Wood Science, Moi University, PO Box 1125 Eldoret, Kenya

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**SIRMAH P, MBURU F, IAYCH K, DUMARÇAY S & GÉRARDIN P. 2011. Potential antioxidant compounds from different parts of *Prosopis juliflora*.** Research was carried out to explore antioxidant properties of *Prosopis juliflora* extractives and their additional utilisation value. Results showed that *P. juliflora* contained important amounts of flavanols, constituting of 4'-O-methyl-gallocatechin, (+)-catechins and (-)-mesquitol. Their amounts are different and highly dependent on the wood sample location in the tree. Reasons for these differences are not yet fully understood and need further investigation. Heartwood contained (-)-mesquitol as the main secondary metabolite responsible for the strong antioxidant properties of crude extracts obtained with solvents of different polarities. (-)-Mesquitol was also the main component of gums exuding from the stem. Crude bark extractives showed lower antioxidant properties which were attributed to the presence of 4'-O-methyl-gallocatechin. The lower antioxidant activity may be attributed to the presence of some inactive components in bark extractives which reduced overall activity of the extract. Extractives of *P. juliflora* could therefore be of interest as source of natural antioxidants for applications in food, cosmetic or pharmaceutical industries.

Keywords: Flavanols, catechin, (-)-mesquitol extractives, metabolite, bark extractives

**SIRMAH P, MBURU F, IAYCH K, DUMARÇAY S & GÉRARDIN P. 2011. Sebatian antioksidan yang berpotensi daripada bahagian *Prosopis juliflora* yang berbeza.** Kajian dijalankan untuk meninjau ciri antioksidan ekstrakatif *Prosopis juliflora* serta kegunaannya yang lain. Keputusan menunjukkan bahawa *P. juliflora* mengandungi jumlah flavanol yang penting yang terdiri daripada 4'-O-metil-galokatekin, (+)-katekin and (-)-mesquitol. Jumlah ketiga-tiga komponen adalah berbeza dan bergantung pada lokasi sampel di pokok. Namun sebab perbezaan ini berlaku belum difahami sepenuhnya dan perlu dikaji lebih lanjut. Kayu teras mengandungi (-)-mesquitol sebagai metabolit sekunder utama yang bertanggungjawab untuk ciri antioksidan yang kuat pada ekstrak mentah yang diperolehi daripada pelarut berlainan polariti. (-)-Mesquitol juga merupakan komponen utama damar daripada batang pokok. Ekstratif kulit kayu mentah menunjukkan ciri antioksidan yang lebih rendah yang dikaitkan dengan kehadiran 4'-O-metil-galokatekin. Aktiviti antioksidan yang lebih rendah mungkin disebabkan oleh kehadiran komponen tak aktif dalam ekstrakatif kulit kayu yang menurunkan aktiviti keseluruhan ekstrak. Oleh itu, ekstrakatif *P. juliflora* mungkin dapat dijadikan sumber antioksidan semula jadi bagi penggunaan dalam industri makanan, kosmetik dan farmaseutikal.

## INTRODUCTION

Flavane derivatives are versatile natural products found in fruits, vegetables, flowers, barks and wood. They possess protective effects against oxidation reactions involving free radicals and reactive oxygen species (ROS) generated in numerous oxidative processes (Whiteside et al. 2004, Raza & John 2007). Flavonoids also protect plants from ultraviolet induced injury and contribute to diversity in wood colorations (Tanaka et al. 2008). In addition, flavonoids also possess anti-inflammatory, antiallergic, antiviral

and anticarcinogenic properties. Flavonoids in wood have an important effect on its durability (Wang et al. 2004). It has been hypothesised that flavonoids protect heartwood against fungal colonisation by a dual mechanism involving fungicidal and antioxidant activities (Schultz & Nicholas 2000, Pongtip et al. 2007). The radical scavenging activity is particularly important because both white-rot and brown-rot fungi are believed to use radicals to disrupt cell walls (Pietarinen et al. 2006). In woody tree species,

\*Author for correspondence. E-mail: [stephane.dumarcay@lermab.uhp-nancy.fr](mailto:stephane.dumarcay@lermab.uhp-nancy.fr)

flavonoids accumulate in bark, leaves and heartwood, while lesser amounts are found in sapwood and seeds. Galocatechin, catechin and many condensed tannins present in extractives of bark and heartwood of many wood species have strong biological activities such as enzyme inhibition, antioxidant and antifungal activities (Mihara et al. 2005, Wu et al. 2005, Khairullina et al. 2006).

In another study we described isolation and characterisation of (-)-mesquitol as the major secondary metabolite of acetic heartwood extractive of *Prosopis juliflora* (Sirmah et al. 2009). This flavonoid which was present in high yield and purity exhibited strong antioxidant activity similar to those of (+)-catechin and butylated hydroxytoluene (BHT) chosen as reference antioxidants. Due to the growing interest in the development and use of chemicals from natural origin as antioxidants for food, cosmetic and pharmaceutical industries, we investigated antioxidant properties of *P. juliflora* extractives obtained from different parts of the tree using a wide range of solvents in order to explore their additional utilisation value. The chemical composition of these extractives were then characterised and molecules responsible for antioxidant properties, identified.

## MATERIALS AND METHODS

### Plant material preparation and solvent extraction

Heartwood and bark of *P. juliflora* was collected from Baringo Forest (0° 20' N, 35° 57' E), Kenya. The voucher specimen was kept at the department of Forestry and Wood Science, Moi University. Air-dried samples were separately ground to fine powder, passed through a 115-mesh sieve and dried at 60 °C to constant weight. Individual extraction was performed with dichloromethane, acetone, toluene/ethanol (2/1, v/v) or water using an accelerated solvent extractor (Dionex ASE 200) in 33-ml cell size on 8 g of sawdust at 100 °C under a pressure of 100 bars (3 static cycles of 5 min each). After extraction the solvent was evaporated under reduced pressure and the crude extract dried under vacuum in a desiccator over P<sub>2</sub>O<sub>5</sub>. The percentage of extractives was evaluated according to the formula:

$$\text{Extract \%} = \frac{m_e}{m_s} \times 100$$

where  $m_e$  is the weight of extracts after solvent evaporation and  $m_s$  is the dry mass of the sawdust before extraction.

### Antioxidant activity by 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  $9 \times 10^{-3}$  M solution of 2,2'-azobis[2-methylpropionitrile] (AIBN) in butan-1-ol 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 1 ml of a  $10^{-4}$  M, solution in butan-1-ol of *P. juliflora* extractives, or not for the control. The volumes of the liquid and gas phases were 4 and 100 ml respectively.

### <sup>1</sup>H-NMR analysis

<sup>1</sup>H-NMR spectra of the crude extracts were recorded in methanol-D<sub>4</sub> on a Brüker DRX 400 MHz spectrometer. Chemical shifts were expressed in ppm and calculated relative to TMS.

### GC-MS analysis

*Prosopis juliflora* extracts were analysed as trimethylsilyl derivatives using the following procedure. In a screw-capped vial, an individual sample of approximately 1 mg of dry extract, (+)-catechin or (-)-epicatechin was dissolved in 0.5 ml of anhydrous acetonitrile and 0.4 ml of *N,O*-bis-(trimethylsilyl) trifluoroacetamide containing 1% trimethylchlorosilane (BSTFA/1% TMCS) 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 coupled to a Clarus® 500 MS quadrupole mass spectrometer. Gas chromatography was carried out on a 5% diphenyl/95% dimethyl polysiloxane fused-silica capillary column (Elite-5ms, 30 m ×

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  $\mu$ l) was performed at 250 °C in the split mode (split flow of 20 ml min<sup>-1</sup>). Helium was used as carrier gas, with a constant flow of 1.2 ml min<sup>-1</sup>. The oven temperature program was as follows: 200 °C constant for 4 min, 200 to 330 °C at a rate of 5 °C min<sup>-1</sup> and then constant at 330 °C for 10 min. Ionisation was achieved under the electron impact mode (ionisation energy of 70 eV). The source and transfer line temperatures were 250 and 330 °C respectively. Detection was carried out in scan mode: m/z (i.e. molecular mass/charge) 35 to 700 amu. The detector was switched off in the initial 2 min (solvent delay).

GC/MS spectrum of the penta-TMS derivative of (-)-mesquitol: retention time = 26.05 min, m/z (%): 650 (M<sup>+</sup>, 1.6), 383 (5.8), 370 (6.1), 369 (12.9), 368 (39.7), 357 (1.3), 356 (2.8), 355 (8.7), 281 (1.3), 280 (2.2), 268 (4.5), 267 (19.5), 249 (1.9), 179 (4.7), 147 (3.8), 133 (2.2), 75 (5.3), 74 (7.4), 45 (6.7), 73 (100).

GC/MS spectrum of the penta-TMS derivative of 4'-OMe-galocatechin: retention time = 26.89 min, m/z (%): 680 (M<sup>+</sup>, 4.65), 665 (3.1), 592 (3.1), 591 (4.0), 590 (9.7), 400 (17.5), 399 (35.9), 398 (97.1), 385 (2.3), 384 (3.5), 383 (16.1), 370 (2.9), 369 (5.7), 368 (29.3), 355 (26.5), 297 (16.2), 267 (7.9), 147 (5.2), 133 (2.6), 73 (100).

GC/MS spectrum of the penta-TMS derivative of (+)-catechin: retention time = 26.86 min, m/z (%): 650 (M<sup>+</sup>, 1.6), 383 (2.0), 370 (6.2), 369 (15.3), 368 (47.8), 357 (2.6), 356 (5.5), 355 (17.8), 281 (2.0), 280 (3.3), 268 (1.4), 267 (6.1),

249 (2.1), 179 (9.1), 147 (4.2), 133 (1.6), 75 (5.1), 74 (7.5), 73 (100), 45 (6.1).

### Acetylation of extractives

To investigate the structure of the flavanols present in bark, the dark brown crude bark acetone extractives were acetylated in acetic anhydride/anhydrous pyridine (1/2v/v), under inert air (N<sub>2</sub>) and at 0 °C for 6 hours, followed by ethyl acetate extraction in an ice packed separating funnel. The organic phase was washed with 10 to 20 ml of 2 N H<sub>2</sub>SO<sub>4</sub> followed by a saturated solution of NaHCO<sub>3</sub> and finally water. The resulting organic phase was dried in anhydrous MgSO<sub>4</sub>, filtered and the filtrate evaporated under reduced pressure to yield a light brown solid. Column chromatography of this product over silica gel using EtOAc/hexane (2/1 v/v) as eluent led to a compound labelled as CX<sub>1</sub> (1.5%, R<sub>f</sub> 0.55, silica gel, EtOAc). <sup>1</sup>H-NMR and GC-MS analysis was carried out on compound CX<sub>1</sub> as well as other crude extractives.

## RESULTS AND DISCUSSION

### Quantity of extractives

The quantities of extractives contained in bark and heartwood of *P. juliflora* are reported in Figure 1. Contrary to what was generally observed, quantities of extractives present in bark of *P. juliflora* for a given solvent were slightly lower than those of heartwood. Quantity of extractives increased with increasing solvent polarity to reach quite high values of 9% of initial dry mass for the bark and 11% for the heartwood.

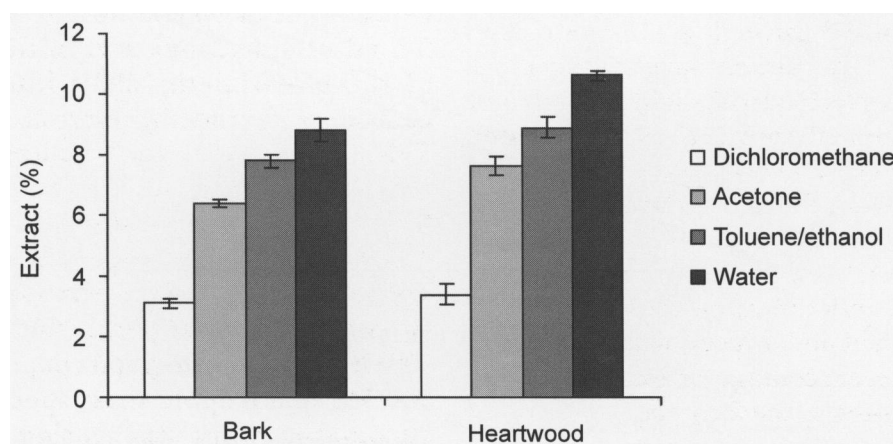


Figure 1 Quantities of *Prosopis juliflora* extractives obtained from different solvents

**Antioxidant properties of extractives**

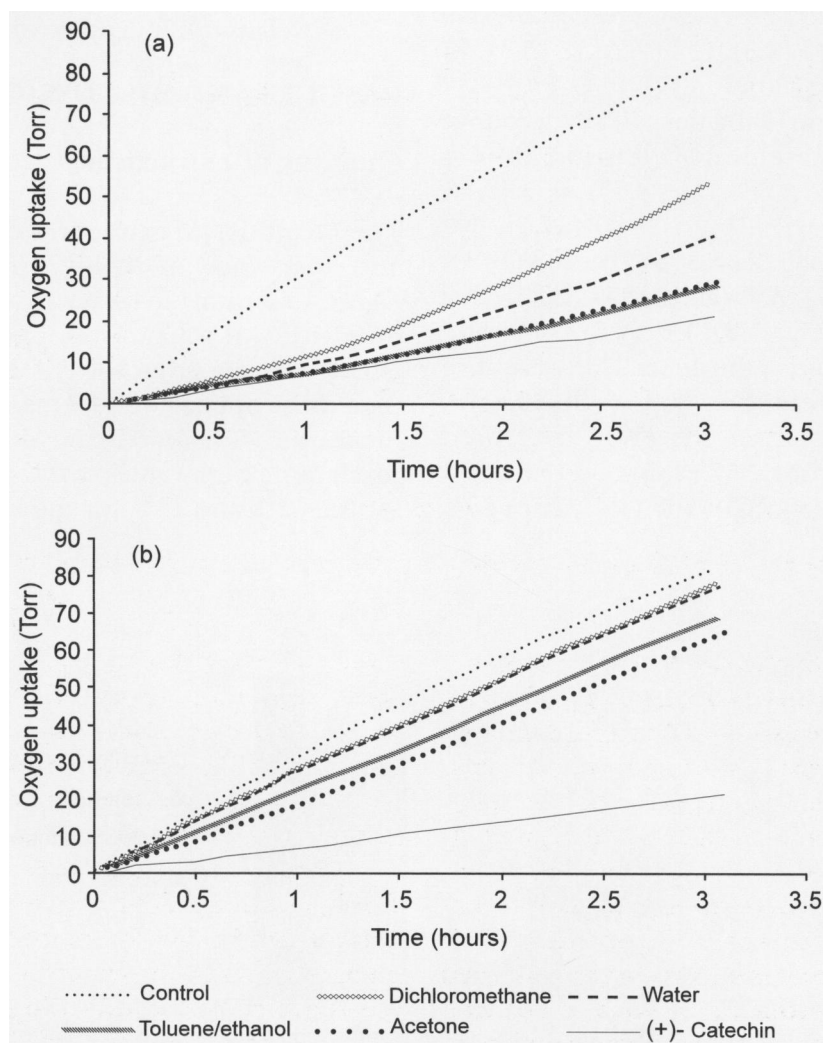
Antioxidant properties of the different *P. juliflora* extractives, estimated using methyl linoleate oxidation inhibition, are shown in Figure 2. Heartwood extractives (Figure 2a) had higher antioxidant properties compared with bark extractives (Figure 2b). Antioxidant properties were strongly correlated with the nature of extracting solvent. Extractives obtained with dichloromethane gave lower antioxidant properties, while those obtained with toluene/ethanol mixture or acetone were more effective, suggesting a quite similar chemical composition. Hydrophilic extractives showed intermediate behaviour. This could be attributed to the presence of different families of extracts which may or may not have antioxidant properties, therefore, limiting overall antioxidant properties. Regardless of the solvent used, all heartwood

extractives were able to slow down the oxidation of methyl linoleate. Spectroscopic studies showed that (-)-mesquitol was the main component of acetone heartwood extractives (Sirmah et al. 2009). Antioxidant properties, estimated using methyl linoleate oxidation inhibition test, showed that (-)-mesquitol presented strong antioxidant properties similar to those of (+)-catechin and BHT.

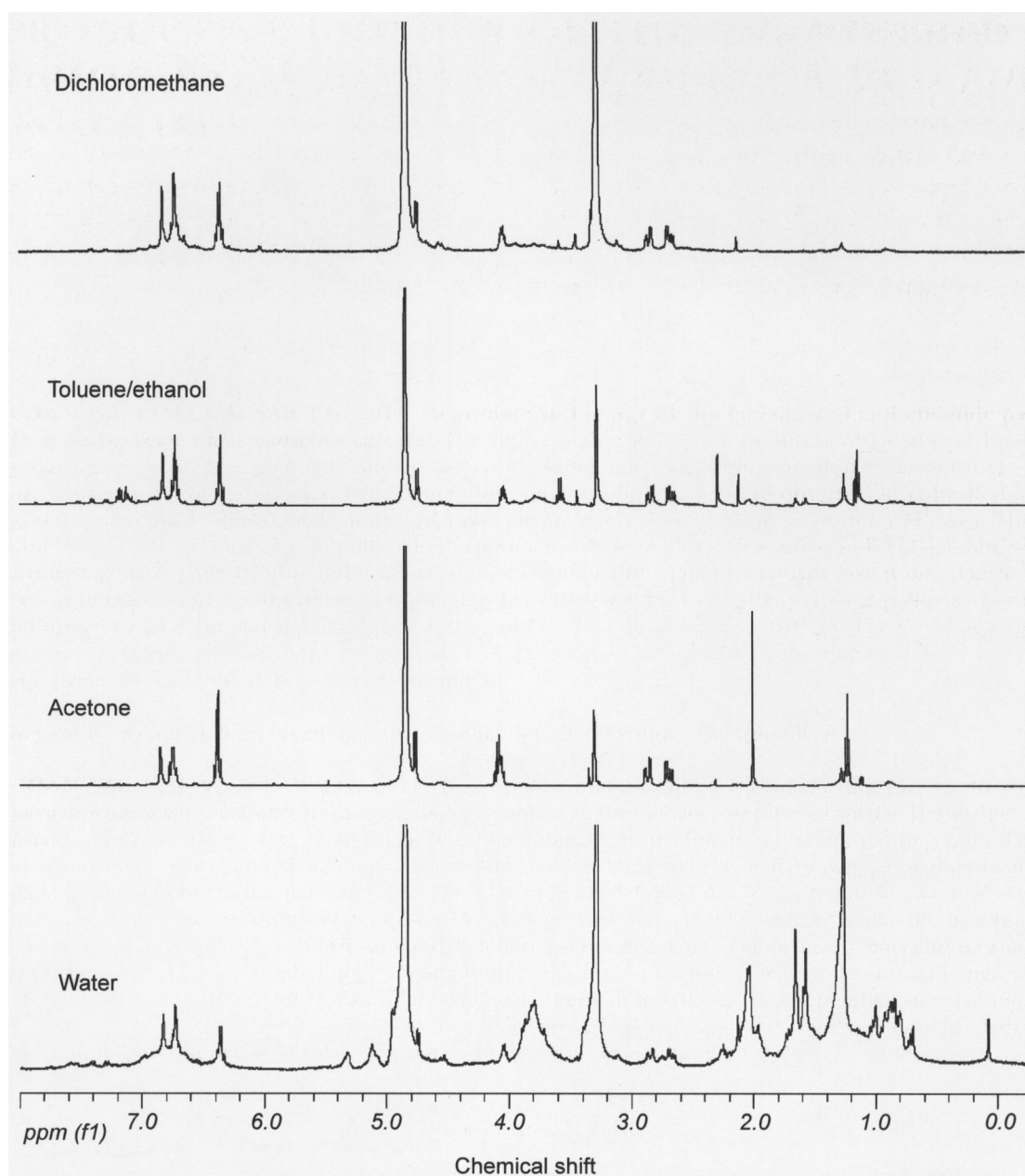
**Spectroscopic analysis of *P. juliflora* extractives**

To check the presence of (-)-mesquitol in different heartwood extractives and in the bark extractives, <sup>1</sup>H-NMR spectra of the crude extracts were recorded (Figures 3 and 4).

Results indicated quite similar signals ascribable to (-)-mesquitol as the main components. The signal at 2.8 ppm was characteristic of the ABX



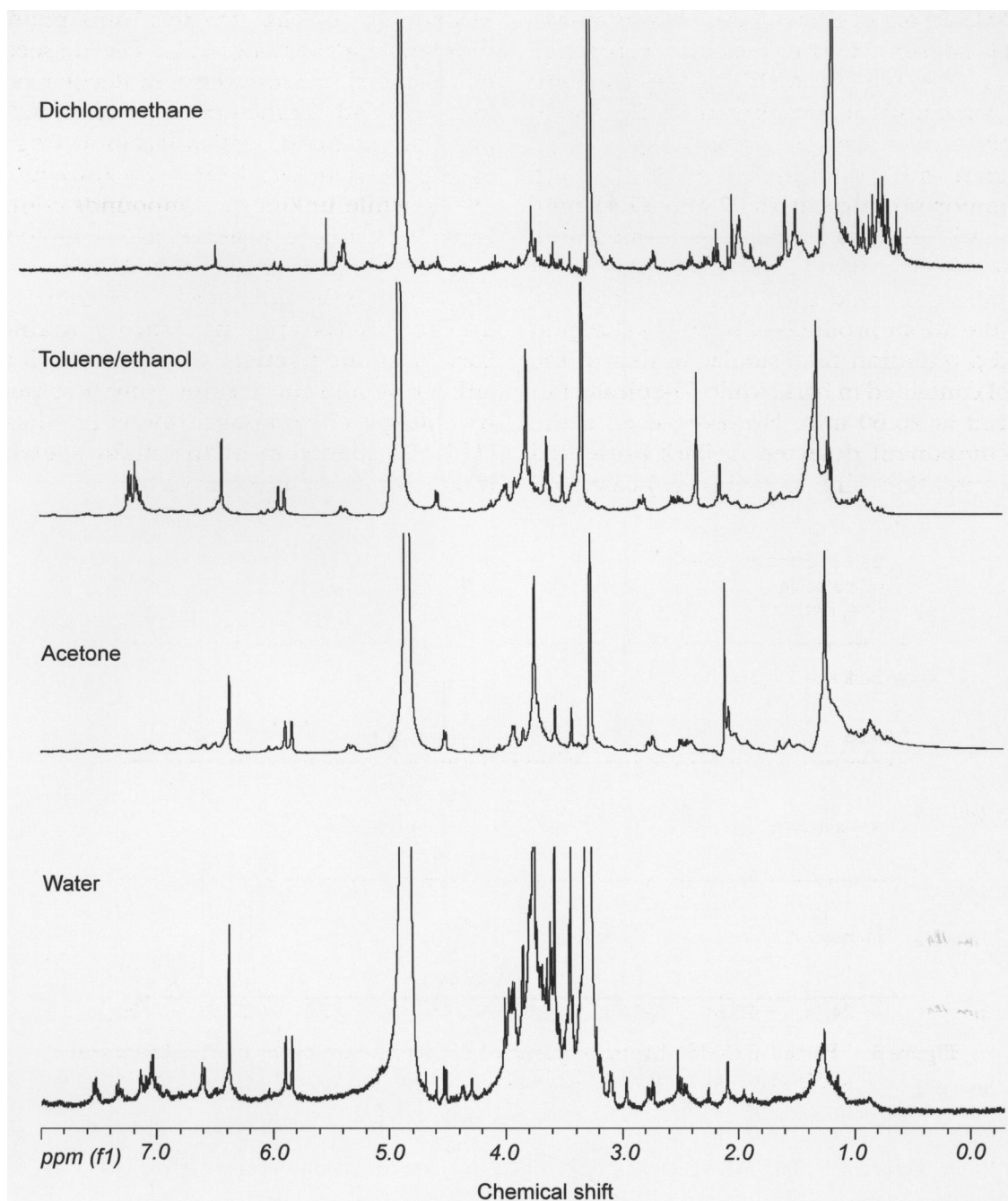
**Figure 2** Antioxidant properties of *P. juliflora* (a) heartwood and (b) bark extractives estimated using methyl linoleate oxidation inhibition



**Figure 3**  $^1\text{H-NMR}$  spectra of different *P. juliflora* heartwood crude extracts

system corresponding to two hydrogen atoms at the  $\text{C}_4$  position of the (C) ring of the flavanol structure, while the two signals at approximately 4.0 and 4.8 ppm were characteristic of hydrogen at  $\text{C}_3$  and  $\text{C}_2$  positions respectively. The large coupling constant observed between  $\text{H}_2$  and  $\text{H}_3$  ( $J=6.75$  Hz) indicated that the 3', 4'-dihydroxyphenyl group at the  $\text{C}_2$  position was in *trans* position of the hydroxyl group at the  $\text{C}_3$  position. This value concurs with values that were observed for (+)-catechin. Aromatic protons of (B) ring appeared as a singlet at approximately 6.80 ppm and two doublets with a typical benzenic ortho

coupling constant ( $J = 8.0$  Hz) at 6.70 and 6.67 ppm, while those of (A) ring appeared as two doublets, also with a typical benzenic ortho coupling constant ( $J = 8.2$  Hz) at 6.38 and 6.42 ppm. Spectrum of acetone extracts indicated the presence of (-)-mesquitol as the sole compound without any noticeable impurities. This product was also present as the main component in dichloromethane and toluene/ethanol extracts. Spectrum of water extracts was more complex indicating the presence of several components in addition to mesquitol. These can be associated with the presence of



**Figure 4**  $^1\text{H-NMR}$  spectra of different *P. juliflora* bark crude extractives

fats or more probably to terpenic compounds resulting from hydrolysis of glycosidic bonds of saponins during hot water extraction. The presence of these compounds without any antioxidant properties explained the lower activity of heartwood water extractives.

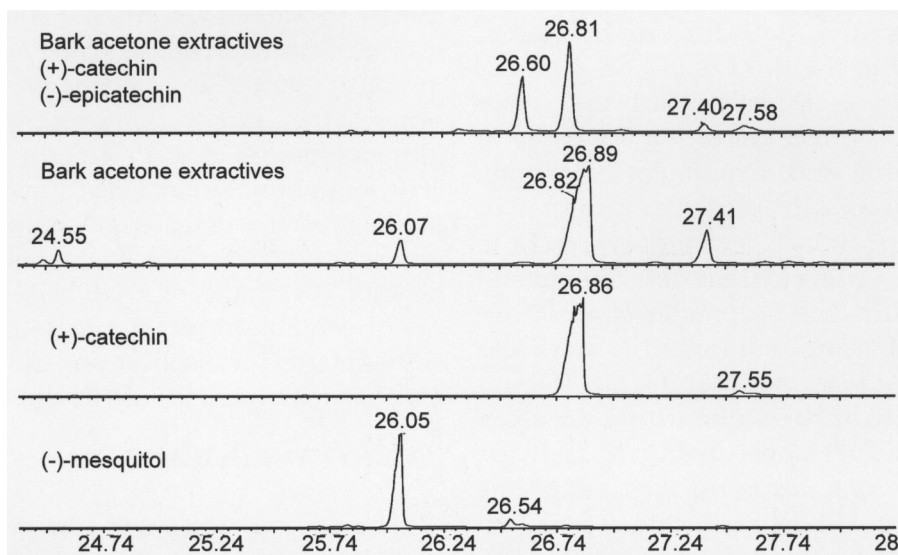
Contrary to heartwood,  $^1\text{H-NMR}$  analysis of the crude bark extractives indicated the presence of several products belonging to different families. Dichloromethane, acetone and toluene/ethanol extractives present signals ascribable to flavonoids, fats and/or terpenes. Surprisingly, chemical

shifts of signals attributed to proton of flavonoids are quite different from those observed for (-)-mesquitol. Indeed, signals that appeared at 2.40 and 2.75 ppm corresponded to the two hydrogen atoms at the  $\text{C}_4$  position of the (C) ring of the flavanol, while signals at 3.9 and 4.5 ppm were characteristic of hydrogen at  $\text{C}_3$  and  $\text{C}_2$  positions respectively. Aromatic protons of (A) and (B) rings appeared as three singlets at 5.8, 5.9 and 6.4 ppm. Spectrum of water extractives indicated the presence of important amounts of sugar units mixed with small amount of flavonoids.

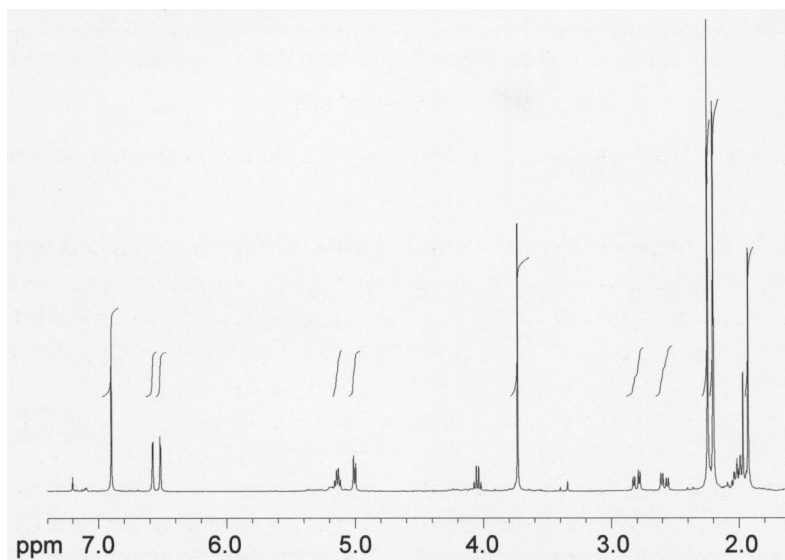


GC-MS chromatograms of the TMS derivatives of bark acetone extractives and reference flavanol such as (+)-catechin, (-)-epicatechin and (-)-mesquitol are shown in Figure 5. Bark extractives presented a main product at 26.89 min with a shoulder at 26.82 min and some minor products at 26.07 and 27.41 min. Comparative to bark extractives, (-)-mesquitol identified in heartwood extractives appeared at 26.05 min and could therefore correspond to one of the minor products of bark. (+)-Catechin showed a retention time similar to that of the flavanol contained in bark, while (-)-epicatechin appeared at 26.60 min. However, even if the main component detected in bark presented similar retention time to that of (+)-catechin,

MS spectra of the two products produced different characteristic peaks. The MS spectrum of (+)-catechin indicated a molecular peak at  $m/z$  650 and characteristic peaks at  $m/z$  355 and 368 similar to typical flavanols fragments as reported in the literature (Soleas et al. 1997), while unknown compounds contained in bark produced peaks at  $m/z$  680 and 398. This difference of 30 units of mass can be attributed to the presence of a methoxy group. To identify flavanol structure contained in bark, acetic fraction was reacted with acetic anhydride and the resulting mixture purified by column chromatography over silica gel.  $^1\text{H-NMR}$  spectrum of the main acetylation product is shown in Figure 6.



**Figure 5** Partial GC-MS chromatograms of different extractives of *P. juliflora* and reference flavanols in a mix ratio



**Figure 6**  $^1\text{H-NMR}$  spectrum of the main acetylation product (CX1) of bark acetone extractives from *P. juliflora*



Analysis of acetylation product CX<sub>1</sub> (Figure 6) indicated the presence of a typical ABX flavanol structure, i.e. 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 were characteristic of hydrogen atoms at C2 and C3 position respectively. The large coupling constant observed between H<sub>2</sub> and H<sub>3</sub> ( $J = 6.75$  Hz) indicated a *trans* relation between the two hydrogen atoms. Signals between 1.8 and 2.3 ppm were attributed to CH<sub>3</sub> groups of acetyl groups. Integration of these signals indicated the presence of five acetyl groups. Signal at 3.7 ppm was ascribable to CH<sub>3</sub> of methoxy group. Examination of aromatic signals indicated the presence of three different singlets at 5.7, 5.8 and 6.9 ppm. The singlets at 5.7 and 5.8 ppm, integrating both for one hydrogen, were attributed to the two C6 and C8 aromatic protons of (A) ring by analogy with the signals observed for (+)-catcechin which appeared at the same chemical shift. The singlet at 6.9 ppm, integrating for two hydrogens, was attributed to the two symmetrical hydrogen atoms present in the (B) ring. According to these observations and the bibliographic data (De Mello et al. 1996), the unknown flavanol contained in bark was identified as 4'-O-methylgallocatechin (Figure 7). Lower purity of bark, due to the presence of several products presenting not always antioxidant properties extractives, explains the weaker antioxidant properties recorded. Additional analyses (P Sirmah, personal observation) performed on gums exuding from stem indicated that these stem gums were also mainly constituted of (-)-mesquitol. This result suggests that (-)-mesquitol accumulated

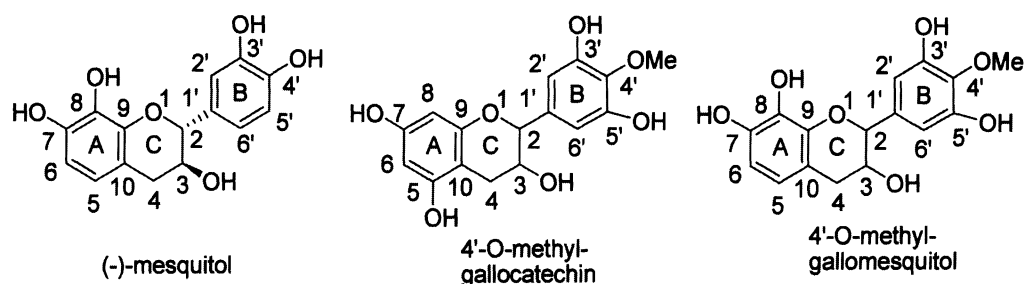
in heartwood act as phytoalexin in response to exterior aggression.

## CONCLUSIONS

*Prosopis juliflora* contained important amounts of flavanols, which differed in structure according to their location in the tree. Reasons for these differences are not yet fully understood and need further investigations. Heartwood contained important amounts of (-)-mesquitol responsible for the strong antioxidant properties of crude extracts obtained with solvents of different polarities such as dichloromethane, acetone, toluene/ethanol or water. (-)-Mesquitol was the main secondary metabolite of the extractives obtained with all organic solvents. However, its purity in water extractives was less important. Contrary to heartwood, crude bark extractives presented lower antioxidant properties, which were attributed to the presence of another flavanol identified as 4'-O-methylgallocatechin. The lower antioxidant activity may be attributed to the presence of different components in bark extractives which reduced overall activity of the extract. Extractives of *P. juliflora* could therefore be of valuable interest as a source of natural antioxidants for applications in food, cosmetic or pharmaceutical industries.

## ACKNOWLEDGEMENTS

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**Figure 7** Structure of the different flavanols isolated in *P. juliflora* extractives

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