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be found in resiniferous channels, xylem rays and at cellular level
([Cambie et al., 1984](#)). They are known to participate in plant defence
mechanism by their repellent or attractive properties, protection
against biotic and abiotic stresses, and maintenance of structural
integrity of plants ([Becerra et al., 2002](#); [Silva et al., 2007](#)). Hydric
stress, radiation, temperature changes, luminosity, environmental
pollution and infections by microbial phytopathogens can elevate
the concentration of reactive species of oxygen or other free radicals
that cause oxidative damage to plant membrane lipids, nucleic
acids, and proteins. It has been suggested that wood extractives can
protect heartwood by free radical scavenger (antioxidant) mechanism
and glutathione, ascorbic acid, tocopherol, carotenoids,
flavonoids, mannitol as well as hydroquinones correspond to plant
antioxidative defence system ([Larson, 1988](#); [Schultz and Nicholas,
2002](#); [Sampath-Wiley et al., 2008](#)).

Degradation of non-durablewoods by fungi is related to action of
fungal enzymes on plant cell wall including extensive oxidative
depolymerization of polymeric polysaccharides ([Green and Highley,
1997](#); [Silva et al., 2007](#)). *Fusarium* species are able to attack woods
when the humidity is favourable. [Becerra et al. \(2002\)](#) correlated
concentration of phenolic diterpenes with wood resistance to *Fusarium*
fujikuroi and *Fusarium ciliatum* suggesting in situ *Fusarium*
inhibition. Study conducted to identify the relationships between
climatic conditions, wood species and distribution of biodeteriorating
agents on *Celtis mildbraedii*, *Ceiba pentandra* and *Pterygota macrocarpa*
woods indicated *F. solani* as the most abundant and common
fungi isolated from wood samples. *F. oxysporum*, *Fusarium sacchari*,
and *F. decemcellulare* occurred only sporadically. The authors suggested
that prophylactic treatment should be done against these
organisms aiming wood preservation ([Apetorgbor et al., 2004](#)). Plant
extract with antifungal activity against *F. oxysporum* was also suggested
as alternative to inhibition of this phytopathogenic fungi aiming
to reduce yield losses ([Khalil and Dababneh, 2007](#)).

Extractives from species of Podocarpaceae family showed antifungal
activity on *Aspergillus* sp., *F. fujikuroi*, *F. ciliatum*, *Mucor*
miehei, *Nematospora coryli*, *Paecilomyces variotii* and *Penicillium*
notatum and the authors suggested that the wood resistance to
fungus attack was due to the high concentration of phenolic
diterpenes ([Becerra et al., 2002](#)). It has been suggested that tannin–
protein interaction explains insecticide and antimicrobial activities
of tannins ([Aerts et al., 1999](#)).

Termites are able to feed on woods because of the presence of
symbiotic cellulose decomposing microorganisms in their gut
([Breznak, 1982](#); [Hojo et al., 2005](#)). *Nasutitermes* species are broadly
distributed in the tropics worldwide and cause damage in buildings,

preferentially attacking roofs, linings, and structural spans (Edwards and Mill, 1986; Scheffrahn et al., 2002). *N. corniger* is one of the most dominant and broadly distributed species of the genus and is able to invade the urban environment attacking wood employed in structures of buildings (Scheffrahn et al., 2005; Vasconcellos et al., 2005; Paes et al., 2007). It has been suggested that natural resistance of *Caesalpinia echinata* heartwood to termite *Cryptotermes brevis* is due to presence of wood toxic extractives (Silva et al., 2007) and termiticidal activity on termite *Cryptotermes formosanus* was already detected in vegetal oil from heartwood from coniferous tree (Cheng et al., 2007). The treatment of woods, i.e., the incorporation of substances that promote improvement in resistance, has been used worldwide for reduction of wood loss, preventing expenses with replacement of spoiled parts and, consequently, reducing the impact on remaining forests. In Brazil, the annual production of treated wood in 2005 was about 685,000 m³ and only 3% was used for building industry. This amount is very small in comparison to United States estimate of treated wood annual production of 15 million m³ with more than 70% used in building industry (Silva, 2006; Paes et al., 2007). This work reports the phytochemical analysis of methanolic and saline extracts from *M. urundeuva* heartwood and the investigation of their antioxidant, antifungal and termiticidal activities. The tissue was chosen since it is considered to be resistant to phytopathogen attack. The study of secondary metabolites present in *M. urundeuva* heartwood may contribute to understand the causes of its natural durability and additionally, may indicate a new source of extractives for treatment of less resistant woods.

2. Materials and methods

2.1. Plant material

M. urundeuva (Engl.) Fr. Allemão belongs to the Division Magnoliophyta, Class Magnoliopsida, Subclass Rosidae, Order Sapindales, Family Anacardiaceae and its popular names are “aroeira do sertão” in Portuguese and “urundel” in Spanish (Leite, 2002). A sample of *M. urundeuva* central heartwood from a tree of 6 m of height and 20 cm of diameter was collected in the State of Maranhão, northeastern Brazil and a voucher specimen, identified by Mr. Gonalo Mendes da Conceio, is archived under number 054 at the Herbarium Aluisio Bittencourt, Centro de Estudos Superiores de Caxias (CESC), Universidade Estadual do Maranho, Brazil.

2.2. *M. urundeuva* heartwood extracts

The heartwood was air dried and powdered (40 mesh). Saline extract (SE) was obtained by soaking the powdered heartwood (10 g) in 0.15 mol kg⁻¹ NaCl (100 ml) and magnetic stirring for 16 h at 4 °C. After filtration, the homogenate was filtered through gauze, centrifuged (3000g, 15 min, 4 °C), and the supernatant was dialyzed (against distilled water for 4 h at 4 °C) and dried by lyophilisation to yield SE. Methanolic extract (ME) was obtained by soaking the powdered material (3 g) in methanol (10 ml) at 25 °C for 3 h. After extraction, the mixture was filtered and evaporated to dryness on

a rotary evaporator to yield ME. The extracts were diluted in methanol or water and used in phytochemical analysis, hemagglutinating activity assay, antioxidant determination as well as biological assays.

Powdered sapwood (40 mesh, 10 g) was extracted with 0.15 mol kg⁻¹ NaCl (100 ml) by 16 h at 4 °C under magnetic stirring. After filtration and centrifugation (3000g, 15 min, 4 °C) of filtrate, the supernatant was dialyzed (against distilled water for 4 h at 4 °C) and dried by lyophilisation to yield sapwood extract.

2.3. Hemagglutinating activity

Hemagglutinating activity assay was carried out in microtiter plates (Kartell S.P.A., Italy) according to [Paiva and Coelho \(1992\)](#) using suspension (2.5% v/v) of rabbit erythrocytes treated with glutaraldehyde ([Bing et al., 1967](#)). Hemagglutinating activity (titer), defined as the reciprocal of the highest dilution of the sample promoting full agglutination of erythrocytes, was reckoned as one hemagglutination unit ([Chumkhunthod et al., 2006](#)). Specific hemagglutinating activity was defined as the ratio between the titer and protein concentration (unit mg⁻¹).

2.4. Total phenol content

Total phenol content of SE and ME was determined by the Folin-Ciocalteu method based on the reduction of phosphomolybdic-phosphotungstic acid reagent by phenols in alkaline solution ([Morais et al., 1999](#)). Folin-Ciocalteu reagent (1:10 solution in distilled water; 2.5 ml) and sodium carbonate (75 g l⁻¹ solution in distilled water; 2 ml) were added to SE or ME (0.5 mg ml⁻¹ solution R.A. Sa' et al. / International Biodeterioration & Biodegradation 63 (2009) 470–477 471

in distilled water; 0.5 ml) as well as tannic acid (standard calibration curve: 9.6–48 mg ml⁻¹ solutions in distilled water; 0.5 ml), and the mixtures were kept at 50 °C for 5 min. After cooling for 30 min, the absorbance was measured at 760 nm.

2.5. Phytochemical evaluation by thin layer chromatography (TLC)

A sample (15 ml) of SE and ME (1.0 mg ml⁻¹ in methanol) was submitted to phytochemical evaluation using silica gel TLC sheets (60F254 aluminium backed, Merck Germany). Different systems of development and adequate visualization techniques were used: 1% (w/v) iron alum for hydrolysable tannins ([Stiasny, 1912](#)), Neus reagent for gallic acid ([Neu, 1956](#)) and flavonoids ([Markhan, 1982](#)), vanillin–hydrochloric acid for condensed tannins and leucoanthocyanidins ([Roberts et al., 1956](#)), Lieberman-Burchard reagent for steroids ([Harborne, 1991](#)), Dragendorffs reagent for alkaloids, vanillin–sulphuric acid for terpenes and iridoids, anisaldehyde for saponins and UV light for coumarins [all last four techniques described by [Wagner and Bladt \(1996\)](#)].

2.6. DPPH free radical scavenging activity

Qualitative assay of free radical scavenger capacity (antioxidant activity) from SE and ME (1.0 mg ml⁻¹ in methanol; 2 ml) as well as ascorbic acid, catechin, epigallocatechin and epigallocatechin gallate standards (1.0 mg ml⁻¹ in methanol; 2 ml) was performed

according to [Soler-Rivas et al. \(2000\)](#) using dot-blot on thin layer chromatography (TLC) stained with a 0.4 mM 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) solution. The dilutions of applied samples were 1:2, 1:4 and 1:8. Positive results were observed by yellow color on silica sheet.

Quantitative assay for antioxidant activity from solutions (1.0 mg ml⁻¹ in methanol) of SE, ME as well as ascorbic acid and catechin standards was performed according to [Brand-Williams et al. \(1995\)](#) and [Soler-Rivas et al. \(2000\)](#) with slight modification. An aliquot (20 ml) of each solution was mixed with 980 ml of DPPH methanolic solution (90 mM) to a final volume of 1 ml. Absorbance at 515 nm was read using a spectrophotometer and the disappearance of DPPH was monitored by decrease in absorbance, which was recorded after 0, 1, 2, 3, 4, and 5 min, and subsequently every 5 min up to 30 min. The concentration of DPPH in the reaction mixture was calculated using a calibration curve according to the linear regression $A_{515nm} = 0.00853 [DPPH] + 0.00929$ ($R^2 = 0.99695$), being [DPPH] expressed in mg ml⁻¹. The percentage of remaining DPPH (%DPPHREM) was calculated according to [Brand-Williams et al. \(1995\)](#), as follows: $\%DPPHREM = \frac{[DPPH]_T}{[DPPH]_{T_0}} \times 100$ where T is the time when absorbance was determined and T₀ is the time zero.

The determination of inhibition percentage (IP) was made adding SE and ME (50 ml) to 90 mM methanolic solution of DPPH (2 ml) and the absorbance (515 nm) at the steady state was determined. IP was calculated according the expression: $IP = 100 \left(\frac{AT_0 - AT_S}{AT_0} \right)$ where AT₀ is the absorbance at time zero and AT_S is the absorbance in steady state. The IP corresponds to the amount of total DPPH that was inhibited, i.e., the DPPH that reacted with antioxidant at steady state. Results were expressed as % of inhibition of the oxidant agent (reduction of DPPH).

2.7. Antifungal activity

Fusarium species were obtained from Culture Collections of University Recife Mycologia (URM, Departamento de Micologia, Universidade Federal de Pernambuco, Brazil). *F. solani* (URM-2490), *F. oxysporum* (URM-2489), *F. moniliforme* (URM-3226), *F. decemcellulare* (URM-3006) and *F. lateritium* (URM-2491) were identified by Dr. De' bora Maria Massa Lima (Departamento de Micologia, Universidade Federal de Pernambuco, Brazil).

Antifungal activity was performed according to [Cunico et al. \(2004\)](#). The method has been modified by application of samples in solid potato-dextrose agar (PDA) medium rather than liquid medium used by [Cunico et al. \(2004\)](#). SE and ME (1.0 mg ml⁻¹ in distilled water) were filtered using a 0.45 mm sterile syringe filter (Minisart[®]). Next, the samples (50 ml) were spread on solidified PDA medium in Petri plates (100 × 15 mm) and a fungal mycelium disk (0.625 cm in diameter) was disposed in the center of Petri plate. Distilled water was used as negative control and all assays were carried out in triplicate. The plates were incubated at 28 °C for 72 h. Antifungal activity was indicated by a reduction of the fungal growth

zone (diameter) in the plates in comparison to negative controls.

2.8. Termiticidal activity

Colonies of *N. corniger*, identified by Dr. Luiz Roberto Fontes (Superintendência de Controle de Endemias, SUCEN, Brazil) were maintained in the vegetation house at the Departamento de Agronomia of Universidade Federal Rural de Pernambuco. Termiticidal activity was evaluated by a no-choice bioassay based on the method described by Kang et al. (1990). Each experimental unit consisted of a Petri plate (90 × 15 mm) with the lower plate covered by filter paper. A filter paper disk (4 cm of diameter) impregnated with 200 µl of SE or ME (0.5, 1.0, 2.0 and 3.0 mg ml⁻¹ in distilled water) was put in each plate. A total of 20 active termites (workers and soldiers, in the proportion of 4:1, respectively) was transferred to each plate and the plates were maintained at 28 °C in darkness. Evaluation of insect survival was made daily until the death of all insects. Distilled water was used in negative control. Bioassay was achieved in quintuplicate and survival rates (%) were obtained for each treatment.

2.9. Repellence activity

Assay based on Su et al. (1982) was used for evaluation of repellent activity. Petri plates (100 × 15 mm) were filled up with 2% agar solution until there was no space between the superior surface of agar and plate covers. After solidification, wells are made in agar by the removal of a central cylinder with 25 mm diameter and 10 peripheral cylinders (6 mm diameter). In each peripheral well was put a filter paper soaked with 15 µl of SE and ME (0.5, 1.0, 2.0 and 3.0 mg ml⁻¹ in distilled water) or distilled water (negative control) solutions. Each treatment solution was present in double in each plate. Termites (16 workers and 4 soldiers) were then transferred to central well and the plates were maintained at 28 °C in darkness. Assay was made in triplicate and the parameters' absence or presence of termites in peripheral wells, construction standards of tunnels in agar and closing by insects of constructed galleries were observed during 15 days.

2.10. Statistical analysis

Regression equations in quantitative assay for antioxidant activity were established using Origin 6.0 program (Microcal, Northampton, MA, USA). Statistical analysis of antifungal activity was performed using GraphPad Prism version 4.0 for Windows (GraphPad Software, San Diego, California, USA) and data were expressed as a mean of three assays ± SD. The data from termiticidal assay were expressed as a mean of five experiments ± SD and analyzed by Student's t-test (significance at $p < 0.05$) using Origin 6.0 program. The lethal concentration required to kill 50% (LC₅₀) of workers or soldiers in 4 days was determined by probit analysis (472 R.A. Sá et al. / International Biodeterioration & Biodegradation 63 (2009) 470–477

with a reliability interval of 95% using the computer software StatPlus_2006 (AnalystSoft, Canada).

3. Results

3.1. Evaluation of hemagglutinating activity and secondary metabolites

Methanol extracted highest phenol content and the values obtained for total phenol of SE and ME were 10 and 27 mg per g of dry heartwood, respectively. Hemagglutinating activity (titer of 32,768) was detected in SE and was absent in ME and sapwood extract. TLC chromatograms showed the presence in both extracts of cinamic derivatives, flavonoids, gallic acid, luteolin, proanthocyanidins (condensed tannins), hydrolysable tannins, and leucoanthocyanidins but with less intensity in SE. No alkaloids, coumarins, iridoids, phenylpropanoid glycosides, saponins, steroids, and terpenes were present in the extracts.

3.2. Antioxidant activity

The ability of SE and ME to act as a free radical scavenger was revealed by DPPH reduction on TLC sheet. Antioxidant activity was detected after 30 min and the extracts showed high (DPPH) radical scavenging activity, similar to ascorbic acid, catechin, epigallocatechin and epigallocatechin gallate (positive controls).

The capacity of SE and ME for scavenging free radicals was also quantified by determination of IP values. The steady state was reached in less than 10 min and the amount of DPPH radical that reacted with SE and ME was 84.7% and 91.1%, respectively.

3.3. Antifungal activity

The extracts from *M. urundeuva* heartwood showed antifungal activity (Fig. 1) but differences were detected in relation to the effect on growth of tested fungi. SE was efficient against *F. decemcellulare* (inhibition of 46.7% $\pm$ 6.7), *F. moniliforme* (50.4% $\pm$ 2.6), *F.*

oxysporum (50% $\pm$ 0.0) and *F. solani* (68.5% $\pm$ 3.1) but it had little effect against *F. lateritium* (14% $\pm$ 2.8). ME had almost no effect on *F. decemcellulare* with minimal growth inhibition (9.5% $\pm$ 4.1) and was more active than SE on *F. lateritium* (growth inhibition of 25% $\pm$ 0.0).

The effect of SE and ME on *Fusarium* was compared with fungus growth in water (negative control) and in presence of antifungal cercobin (positive control). SE was more efficient than cercobin in inhibiting the growth of *F. oxysporum* and *F. lateritium* and promoted effect similar to the antifungal on *F. decemcellulare* and *F.*

Fig. 1. Growth in PDA medium of *F. decemcellulare* (A), *F. lateritium* (B), *F. moniliforme* (C), *F. oxysporum* (D) and *F. solani* (E) at presence of methanolic (6) and saline (:) extracts,

distilled water (,, negative control) and 10 ppm cercobin (B, positive control). Each point represents the mean $\pm$ SD of three experiments.

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solani (Fig. 1A, B, D, and E). ME was more efficient than cercobin in inhibiting the growth of *F. lateritium*, promoted effect similar on *F. oxysporum* and had no effect on *F. decemcellulare* (Fig. 1A, B, and D). The extracts showed lower antifungal activity than cercobin against *F. moniliforme* (Fig. 1C).

3.4. Termiticidal and repellent activities

The effect of SE and ME on *N. corniger* was performed using filter

paper soaked with the extracts and the insect mortality was evaluated daily during 22 days. The survival rate (%) for soldiers and workers in contact with ME was similar to that determined for insects in contact with water (Fig. 2A). Thus, the extract had no termiticidal activity since the detected mortality in all ME concentrations was not statistically different to the control. SE showed termiticidal activity with LC50 values of 1.81 and 2.59mgml⁻¹ for soldiers and workers after 4 days, respectively and the assay revealed that soldiers (Fig. 2C) were more sensible than workers (Fig. 2D) to SE. Statistical analysis using Student's t-test showed that data of all SE concentrations tested on soldiers were significantly different ($p < 0.05$) while on workers the 0.5 and 1.0 mgml⁻¹ concentrations were not significantly different to control. The building of tunnels and closing of galleries by insects was investigated by assay in agar (Fig. 3) aiming to determine if extracts promote repellent action on termites. Tunneling termites can react to the presence of possibly toxic substances by closing galleries to avoid contact with these substances (Su et al., 1982). SE was not repellent since no gallery was closed and termites were observed exploring peripheral wells treated with the extract. ME had repellent property since termites closed the gallery constructed next to the peripheral well containing ME. After 6 days termites were opening a gallery to the well of highest (3 mg ml⁻¹) ME concentration (Fig. 3B); the same gallery was partially closed after 11 days and totally after 13 days (Fig. 3C and D). The gallery to 1mgml⁻¹ well was opened and partially closed after 11 (Fig. 3C) and 13 days (Fig. 3D), respectively. After 11 days of test termites were observed near the negative control wells (Fig. 3C) and at the end of the observation period, termites were mainly found in the center of plate (Fig. 3D).

4. Discussion

The resistance of *M. urundeuva* heartwood to biological agents stimulated studies that aimed the identification of constituents that may be involved in the defence mechanism. It has been suggested that the structure of *M. urundeuva* lignin and phenolic compounds could account for the resistance but this seems not sufficient to explain the wood durability (Morais et al., 1999). In fact, recently, a lectin was also suggested as bioactive molecule in the defence mechanism of *M. urundeuva* heartwood (Sa' et al., 2008, 2009a, 2009b). The present work is another step in the study of chemical resistance of *M. urundeuva* heartwood and we report antioxidant, antifungal and termite repellent activities of extracts with and without hemagglutinating activity.

TLC chromatograms revealed the presence of secondary metabolites in SE and ME. The composition of a methanolic extract from *M. urundeuva* wood has been already analyzed by ¹³C-NMR spectrum (proanthocyanidins) and high performance liquid chromatography detected flavonoids, ellagic acid, hydrolysable tannins, and gallic acid (Morais et al., 1999). The choice of the concentration of NaCl in SE used here aimed to minimize eventual decreasing in

phenol solubility since it has been demonstrated that phenol solubility decreased when NaCl concentration increased from 0.5 to 3.5 mol kg⁻¹ by salting-out effect (Noubigh et al., 2007).

Fig. 2. Survival of workers (A and C) and soldiers (B and D) of *N. corniger* at presence of methanolic (A and B) and saline (C and D) extracts at concentrations of 0.25 (:), 0.5 (>), 1.0

(A), 2.0 (-) and 3.0 (6) mgml⁻¹. Distilled water (.) was used as negative control. Each point represents the mean $\pm$ SD of five experiments. The concentration required to kill 50%

of insects in each treatment (LC50) was determined using probit analysis with a reliability interval of 95%.

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Additionally, previous study revealed that 0.15 mol kg⁻¹ NaCl was able to solubilize the *M. urundeuva* heartwood lectin (Sa' et al., 2008, 2009a,b). Thus, SE would be a preparation richer in bioactive molecules than ME whose extraction process would abolish the hemagglutinating activity. In fact, hemagglutinating activity was detected only in SE.

IP values are a good measure of the antioxidant efficiency of pure compounds or extracts and the IP value obtained for SE and ME revealed high antioxidant activity. Typical IP values of plant tissues with high antioxidant activity were 93% and 75% for *Cassia fistula* stem bark and leaves, respectively (Siddhuraju et al., 2002). A methanolic extract from stem bark of *M. urundeuva* also showed strong antioxidant activity in vitro (Desmarchelier et al., 1999). The presence of phenols, proanthocyanidins and flavonoids in SE and ME would certainly contribute to the antioxidant activity since these compounds are known to be good antioxidants (Erasto et al., 2004; Podsędek, 2007).

Studies have demonstrated that the use of the synthetic antioxidant butylated hydroxytoluene (BHT) enhanced the activity and decreased the depletion of organic biocides when it was co-added in a solution for wood treatment (Schultz et al., 2004; Schultz et al., 2005; Schultz et al., 2006). Extracts of *Castanea sativa* shell and *Eucalyptus globulus* bark with antioxidant activity have been indicated as additives to increase the stability of food and pharmaceutical compositions (Va'zquez et al., 2008).

Purified *M. urundeuva* heartwood lectin has already been shown to be an antifungal agent against *Fusarium* (Sa' et al., 2009a) with growth inhibition of 60.8% (*F. oxysporum*), 51% (*F. decemcellulare*), 31.8% (*F. moniliforme*), 36% (*F. solani*) and 38.6% (*F. lateritium*) and thus the antifungal activity of SE can be due to lectin presence in this active hemagglutinin preparation. The antifungal effect of ME, a preparation without hemagglutinating activity, indicates that extractive components with antioxidant activity may contribute to *M. urundeuva* heartwood resistance to fungus attack. Secondary metabolites from plants and antioxidative compounds have been already reported as antifungal agents. It is interesting to notice that *Linum usitatissimum* transgenic plants with increased flavonoid

content showed an enhanced antioxidant capacity and improved resistance to *Fusarium* (Lorenc-Kuku1a et al., 2007). Essential oils from *Ocimum basilicum* with DPPH free radical scavenging activity inhibited *F. solani* growth (Hussain et al., 2008) and tannins from *Rubus ulmifolius* showed activity against phytopathogenic fungi (Sisti et al., 2008). Flavonoids from *Dianthus caryophyllus* and ethanolic extract of *Varthemia iphionoides* were able to inhibit in 67% and 42.7% the growth of *F. oxysporum*, respectively (Khalil and Dababneh, 2007; Galeotti et al., 2008). Yen et al. (2008) demonstrated that b-thujaplicin and g-thujaplicin isolated from ethanolic extract of *Calocedrus macrolepis* var. *formosana* heartwood were active compounds against tree pathogenic fungus *F. solani* promoting 50% of growth inhibition at 18 and 24 mg ml⁻¹, respectively.

SE showed termiticidal activity but no repellent effect. Similarly to SE, *M. urundeuva* heartwood lectin showed termiticidal activity on soldiers and workers (Sa' et al., 2008); thus, the mortality of termites by SE was probably due to ingestion of this active principle, although the possibility of secondary metabolites present in the SE with termiticidal activity cannot be discarded. Comparison of termiticidal effect of SE (Fig. 2D) and heartwood lectin on soldiers revealed that SE only promoted 100% of mortality after 7 days at 3.0 mg ml⁻¹ while lectin promoted 100% of mortality after 7 days of experiment at 0.1, 0.2, 0.4 and 0.8 mg ml⁻¹ concentrations. Fig. 3. Aspects of termite repellence assay at time zero in a superior view (A) and after 6 (B) 11 (C) and 13 (D) days of test in an inferior view. The samples evaluated were distilled

water (1) and methanolic extract at concentrations of 0.5 (2), 1.0 (3), 2.0 (4) and 3.0 (5) mg ml⁻¹. The arrows indicate insects and closed gallery next to the well containing ME at

3.0 mg ml⁻¹.

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The deleterious effect of SE lower than lectin was also detected on workers (Fig. 2C); 100% of mortality was observed after 12, 14, 18 and 19 days with 3.0, 2.0, 1.0 and 0.5 mg ml⁻¹ SE while 0.1 and 0.8 mg ml⁻¹ lectin promoted 100% of mortality after 10 and 8 days of experiment, respectively. The termiticidal efficiency of tested samples was reflected by higher LC50 values of SE (1.81 and 2.59 mg ml⁻¹ for soldiers and workers after 4 days, respectively) higher than isolated lectin (LC50 values of 0.199 and 0.248 mg ml⁻¹ for soldiers and workers after 4 days, respectively). The LC50 value of SE, approximately 10 times higher than isolated lectin, may be justified by the lower amount of lectin in the SE crude preparation. It has been described that sapwood is a *M. urundeuva* tissue lesser resistant to termite attack than heartwood (Paes et al., 2002); the absence of hemagglutinating activity in sapwood extract corroborate with the indication of natural resistance associated to the presence of hemagglutinating activity.

Differently from SE, ME had repellent property but no termiticidal activity. The termiticidal activity of purified lectin from *M.*

urundeuva heartwood reported previously and the detected repellent effect of ME suggest that two different mechanisms may be involved in the resistance of heartwood to *N. corniger*, one by lectin toxicity and another by extractives with repellent action. The main difference between SE and ME was the presence of hemagglutinating and termiticidal activities in SE. Probably if lectin is removed from SE, these activities will be eliminated. It has been suggested that termite repellent properties of plant extractives were due to presence of terpenoids, quinones, flavonoids and fatty acids (Scheffrahn, 1991). Another study has demonstrated that essential oil of *Cryptomeria macrolepis* heartwood obtained by stem distillation was termiticidal on *C. formosanus*, showing 100% mortality after 5 days of test and the anti-termite function was due to its toxicity and its repellent action (Cheng et al., 2007).

5. Conclusions

The study of timbers is important for understanding of natural biochemical processes related to natural resistance. The detection of antioxidant, antifungal and termite repellent activities of extractives from *M. urundeuva* heartwood, although may not reflect their complete in vivo role, is a strong indication of their involvement in heartwood durability. This report, together with other published works, contributes to the understanding of defence mechanisms in *M. urundeuva* heartwood and suggests that the resistance to biodeterioration and biodegradation involves antifungal and termiticidal activities of from *M. urundeuva* heartwood lectin plus antioxidant, antifungal and insect repellent activities of secondary metabolites. The activities detected in *M. urundeuva* heartwood may indicate the use of industrial waste of heartwood manual and industrial management as a source of bioactive molecules for wood treatment.

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