Emir. J. Food Agric. 2013. 25 (7): 524-530
doi: 10.9755/ejfa.v25i7.15987
http://www.ejfa.info/

PLANT SCIENCE

Dissuasive effect of an aqueous extract from *Enterolobium cyclocarpum* (Jacq) Griseb on the drywood termite *Incisitermes marginipennis* (Isoptera:Kalotermitidae) (Latreille)

David Raya-González, Rosa E. Martínez-Muñoz, Oscar A. Ron-Echeverría, Alberto Flores-García, Lourdes I. Macias-Rodríguez and Mauro M. Martínez-Pacheco*

Instituto de Investigaciones Quimico Biologicas, Universidad Michoacana de San Nicolas de Hidalgo, Edificio B-3, Cuidad Universitaria, Francisco J. Mujica, Morelia, Michoacán, C. P. 58060, México

Abstract

Heartwood aqueous extracts from *Enterolobium cyclocarpum* (Jacq.) Griseb. exhibited chronic toxic lethal effect against *Incisitermes marginipennis* (Latreille) with more than 60% mortality at a concentration of 56.63 mg. Feeding rate correlated positively with the mortality of termites. The termite hindgut cellulolytic activity, the symbiotic microorganism population in termite hindgut and fungal cellulase activity were not inhibited by the aqueous extract. Two major chemical groups were detected in aqueous extract by gas chromatography; they were monoterpenes (46.5%) and phenols (18.7%). Fifty seven constituents, including, D-limonene, terpineol and eugenol were identified; these terpenes have repellent activity against insects. These results suggest that essential oils from *E. cyclocarpum* contain a slow acting toxin with a dissuasive effect on termites.

Key words: *Enterolobium cyclocarpum*, Hindgut, Antitermite activity

Introduction

One drywood boring insect is *Incisitermes marginipennis,* this termite lives in small colonies into drywood almost all its life. Each year these insects emerge from wood to infest other drywood and establish a new colony. A small number of termites can cause a big damage to low density and durability of drywood from *Pinus* spp or *Abies* spp when they have not been treated with conventional preservatives, such as boron, chrome, copper and arsenic salts or phenolic derivatives and organophosphates. These preservatives produce environmental damage, e.g. they have forced a great selective pressure on insects and others organisms that have generated resistance to the pesticides.

Research has been conducted to find a new, environmentally friendly alternative to control the drywood termites. The secondary metabolites of plants with natural resistance to pests and pathogens are a source of potential insecticides for wood preservation exhibiting new mechanisms of biological action capable of replacing the present ones. The understanding of the natural resistance to biodegradation that some heartwood possess and the secondary metabolites extracted from them, begins to be applied to the treatment of wood showing low resistance to biodegradation and in strategies for the rational utilization of pesticides in the pest management. It has been reported that extracts of heartwood from *Thuja plicata* and *Chamaescyparis nootkatensis* applied to softwood of the same tree species avoided degradation by termites and fungi (Taylor et al., 2006).

The tree *E. cyclocarpum* (native name parota) is a Central American native species that grows in central Mexico, from the Pacific Ocean coasts and the Gulf of Mexico to the North of Brazil and Colombia (Martínez Pacheco et al., 2012). In this region, it is used to obtain aromatic rubber freed from the bark (latex) which is used for pulmonary and bronchial affections treatment, also in cabinetmaking and production of other furniture, as well as a specie capable of restoring the environment and as fodder (Carranza Montaño et al., 2003). It was observed that antique furniture, household articles and the housing construction industry of heartwood derivatives from *E.*

Received 19 December 2012; Revised 13 February 2013; Accepted 21 February 2013

*Corresponding Author

Mauro M. Martínez-Pacheco
Instituto de Investigaciones Quimico Biologicas, Universidad Michoacana de San Nicolas de Hidalgo, Edificio B-3, Cuidad Universitaria, Francisco J. Mujica, Morelia, Michoacán, C. P. 58060, México

Email: pacheco@zeus.umich.mx

cyclocarpum in use nowadays, show a high durability, indicating that wood possesses a natural resistance to the attack of drywood boring insects and to degradation by fungi.

It is believed that, in part, this is due to secondary metabolites present in the tree (Carter et al., 1975; Chudnoff, 1984; Grace and Yamamoto, 1994). All this evidence is a motivation to study the potential anti-termite property from aqueous extract of *E. cyclocarpum* heartwood.

Materials and Methods

Volatile constituents extraction

One tree sample from each sampling location was collected. *E. cyclocarpum* heartwood logs from four mature trees more than 15 years old which had recently fallen were used to obtain the aqueous extracts. The extracts were elaborated with 100 g of heartwood sawdust in 500 ml of hot deionized water during 20 min at a temperature of 45-50 ºC (Raya-González et al., 2008). A reddish brown color extract was obtained, filtered and concentrated by liophylization (14.157 g) and stored under vacuum cooling to 0ºC until its use.

Biological material

Drywood termites of the species *I. marginipennis* from blocks of infested dry pine wood were cultivated in a container (40x50x150 cm), and maintained in the laboratory in darkness at 25ºC for a month for acclimatization prior to the test of toxicity (Raya González, 2013).

Oral toxicity assay

Whatman number 3 filter paper disks (diameter at 8.5 cm), were previously impregnated with the aqueous extract (0, 0.57, 5.66, 28.3 and 56.63 mg) and then dried to a constant weight. The treated papers were placed in a Petri-dish with 25 termites and covered to protect them from light. It was verified that the termites fed with cellulose impregnated with the aqueous extract by visual inspection of the pigmentation of its abdomen and they were contrasted with the control termites.

Every week, for eight weeks both the live and the dead termites were counted. Other toxic effects leading to death, such as those that suffered a reduction on the dimensions of the abdomen were not taken into account. To determine the percentage of feeding, each week the weight of the control filter paper and the impregnated paper were registered. The controls were termites fed with filter paper without aqueous extract (Martínez Muñoz et al., 2009).

Aqueous extract effect on micro flora and cellulytic enzymes of hindgut termite. Termites were starved for 48 h, then, several groups of fifteen termites each, were fed for 24 h with the following diets: 1) filter paper impregnated with (56.63 mg) *E. cyclocarpum* heartwood aqueous extract and, 2) a plain filter paper (control). After a period of 24 h post treatment, each termite was extracted by suctioning the hindgut to measure cellulolitic activity, presence and viable count of bacteria, protozoa and spirochaetes.

Cellulases preparation (crude enzymatic extract) from termite was done pool the hindgut of 15 termites and macerating them with liquid nitrogen on saline solution. Crude enzymatic extracts were used to measure the cellulolytic activity. Also, cellulases obtained from the phytopathogen fungi *Colletotrichum lindemuthianum,* the causal agent of *Phaseolus* (common bean) anthracnose, which was obtained by fungus growing on cellulose and salt medium were used.

The extracellular enzymes were precipitated with 40% NH_4SO_4, then desalted by dialysis and concentrated by liophylization and refrigerated until their use.

The enzymatic activity determinations of the β-glycosidase and endo cellulase were made using the method reported by Habu et al. The assay mixture contained 10 U (enzyme activity units) of enzymes in 20 to 80 µg protein (enzymatic extract), the substrates were *p*-nitrophenyl glycoside (PNPG) and carboxymethyl cellulose (CMC) for the determination of β-glycosidase and endo cellulase activities, respectively. The specific activity is reported as *p*mol·min^{-1}·mg^{-1} protein.

Phytochemical analysis

Test of total phenolics was done by the method reported by Swain and Hillis, 1995, using the Folin-Ciocalteu´s Phenol reagent and cinnamylic alcohol as standard. Test of total flavonoids was done by the method reported by Nieva Moreno et al. (2000), using aluminum nitrate and quercitin as standard. Test of reducing sugars was done as reported by Miller, using dinitrosalicylic acid (DNS) and glucose which was used as standard and was reported as glucose equivalents (Miller, 1959).

Gas chromatography-mass spectrometry analysis (GC-MS)

The GC-MS analysis was performed using an Agilent 6850 Series II with a mass selective 5973 MS detector gas chromatographer. The run conditions were: HP-5 MS column (30 m x 0.25 mm x 0.25 µm thick film), Helium was used as carrier gas at 0.935 Kp·cm^{-2}, column flow rate of 1 ml·min^{-1}. One microliter of sample extract was injected at a split ratio 1:10.

Table 1. Natural variability of extractable, phenolics and flavoniods from an aqueous extract of *E. cyclocarpum* heartwood.

Sampling locations	Extractables (g/g dw)	Phenolics (mg/ml)	Flavonoids (mg/ml)
Apatizingan, Mich (19º16' N and 102º22' W)	1.20	0.36 ± 0.009^{c}	11.62 ± 0.003^{b}
Tacambaro, Mich (19º14' N and 101º28' W)	1.84	0.77 ± 0.024^{a}	12.4 ± 0.007^{b}
Tuzantla, Mich (19º12' N and 100º34' W)	1.42	0.31 ± 0.017^{d}	19.4 ± 0.008^{a}
Veracruz, Ver (17º09' N and 98º39' W)	1.33	0.54 ± 0.025^{b}	8.25 ± 0.026^{c}

The oven temperature was held at 150ºC for 3 min with increments of 5ºC·min^{-1} to 278ºC. Other chromatography conditions were; injector temperature, 270ºC; transference line temperature, 300ºC; scanning range, 50 to 600 a.m.u.; voltage ion source 70 eV, scanning speed 1.9 scan·s^{-1}; source pressure at 50 mTorr.

Constituents identification of aqueous extract was done by comparing the mass spectral data with those reported in NIST Rev. D.04:00 2002 mass spectral database.

Data analysis

Five independent experiments were done using three independent replicates. Feeding and survival rates were measured each time. The percentages of

The biological variability is a natural factor that depends on the genome and life history of the organism, e.g. Lewis et al. (2004) observed twice the amount of extractables in *Arabidopsis* extracts from individual plants grown under controlled environmental conditions compared to a pool of combined material extracts from all 24 plants in a tray. To reduce the variation of extractables quality and amount from individual tree in the extract preparation procedure, the sawdust from all trees samples were mixed to obtain a single aqueous extracts from the heartwood of *E. cyclocarpum*.

The concentration dependent toxic effect of an aqueous extract from *E. cyclocarpum* on the survival and feeding rates of *I. marginipenis* (termite) is summarized in Table 2. Termites were susceptible to 56.63 mg of extract with survival rate of 38 % after five weeks, the termites were fed with paper impregnated containing aqueous plant extract in a dependent concentration way.

Table 2. Survival and feeding rates of *I. marginipennis* exposed to aqueous extract of *E. cyclocarpum* after five weeks of exposure.

Heartwood extract (mg)	Survival rate (%)	Feeding rate (%)
0.00	97	100
0.57	96	92
5.66	77	63
28.30	44	29
56.63	38	26

mortality were calculated according to the Abbot equation (Abbot, 1925). The percentage of feeding was calculated on the basis of the consumption of the paper in the control test as 100% of feeding.

All the cases reflect the differences of values among the treatments and the control according to one way ANOVA, the statistical analysis of correlation was done with the Statistic 7.0 software.

Results and Discussion

The heartwood aqueous extracts from four trees with different harvest origin and development stage had a natural biological variability in the amounts of extractables, phenolics and flavonoids, therefore aqueous extract induced different feeding and mortality rates on termites, see Table 1.

Worker termites were susceptible to the plant aqueous extract; inducing a 62% termite mortality rate after five weeks, following oral feeding rate at 26%; these values did not change during the next three weeks. A strong positive correlation was observed between termite feeding and mortality rates. The range of termite feeding values was small and termite mortality matching the sample was positive by linear regression analysis, see Figure 1.

The regression line slope ($r = 0.967$, d.f. = 1, $F = 48$, s.e = 2.2) represents the efficiency of termites for eating filter paper impregnated with aqueous extract. *E. cyclocarpum* heartwood aqueous extract exhibited a toxic effect against *I. marginipennis* which was dependent on the time and the amount of extract consumed. The survival rate of termites in starvation was similar to the subterranean termite *Coptotermes formosanus* Shiraki and different from the termites of the genus *Reticulitermes* (Boue and Raina, 2003).

Nevertheless, the toxic effect was less effective than the one caused by tannins and modified tannins against *C. formosanus* (Yamaguchi et al., 2002).

Also, it was observed that in termites fed with paper impregnated with the extract, the size of the abdomen diminished visibly and finally led to their death. The termite population with these symptoms was roughly 20% after four weeks.

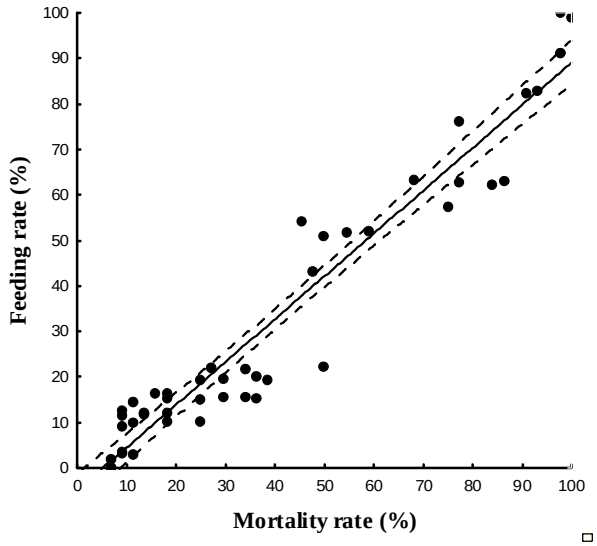

Figure 1. Consumption correlation of cellulose impregnated with aqueous extract of *E. cyclocarpum* and the mortality rate. The data of mortality and feeding rates were used, the line is a linear regression with r^2 = 0.94 and $\alpha = 0.05$ as a consequence the correlation is significant.

The no-selection food assays showed that concentrations over 28.3 mg of the plant aqueous extract caused a decrease in the consumption of cellulose due to a slow acting toxin with a lethal toxic effect. This reduced the number of termites, and no increase in the consumption of the filter paper was detected, similarly to the chlordane and chlorpyrifos effect on formasan subterranean termite or the imidinohydrazone effect on American cockroaches (*Periplaneta americana* Lin.) and German cockroaches (*Blotella germanica* Lin.) (Su et al., 1987; Lovell, 1979). From these results it was concluded that death from termites correlated with the consumption of increasing concentrations of the aqueous extract.

E. cyclocarpum aqueous extract presented a lethal toxic effect against *I. marginipennis* for a long therm. The understanding of the factors involved in the toxicity of aqueous extract on termites is difficult because of the presence of complex mixtures of secondary metabolites in the aqueous heartwood extract, however, it is possible that some of these affected the symbiotic relationship between the gut microbiota and the termite, in analogy to the sulfuramide effect on termite cellulases (Zhou et al., 2008).

It is thought that some constituents of the aqueous extract exhibit inhibitory activity on termite hindgut cellulases (β-glycosidase and endocellulases) or have a microbiocide activity that affects the digestive function of termites. A change on activity in termite hindgut was taken as an indicator of the kind of toxic effect caused by hardwood aqueous extract on termites. The β-glycosidase and endocellulase enzymes were taken as representatives of intestinal cellulolytic activity on termites. Administration of cellulose impregnated with heartwood aqueous extract did not alter the intestinal cellulolytic activity compared to that observed in termites fed with only cellulose (Table 3).

Similarly, *in vitro* assays of cellulases partially purified from fungus *C. lindemuthianum* were not affected by any chemical component of heartwood aqueous extract from *E. cyclocarpum*, see Table 3. Also, it was observed that termite symbiotic hindgut microorganisms were not severely affected by aqueous extract. The protozoa and spirochetes abundance did not show any change, and bacteria were weakly affected. The termite hindgut cellulolytic activity (cellulases secreted by protozoa, fungi, bacteria and probably by the insect) was not affected by any component of heartwood aqueous extract nor inhibited by *in vitro* activity of fungal cellulases.

Chromatographic analysis of the heartwood aqueous extract allowed detecting some groups of chemical, which were clustered in three groups; major abundance, monoterpenes (46.5%) and phenols (18.7%); intermediate abundance, alkanes (6.8%), alcohols (4.17%), aromatic alkenes (3.36%), aromatics (4.26%), ketones (6.55%), sesquiterpenes (5.82%) and minor constituents, carboxylic acids (0.39%) and quinones (0.74%).

Table 3. Effect of *E. cyclocarpum* heartwood aqueous extract on cellulolytic activity present in *I. marginipennis* hindgut.

Enzymatic source	β-Glycosidase (*p*mol·min^{-1}·mg^{-1} prot.)	Endocellulase (*p*mol·min^{-1}·mg^{-1} prot.)
Hindgut of termite fed without *Ec*	93 ± 12	2.23 ± 0.07
Hindgut of termite fed with *Ec* (56.63 mg)	102 ± 13	2.98 ± 0.11
Cellulase-*Cl* (10 U)	152 ± 10	7.02 ± 0.75

Cl, C. lindemuthianum; Ec, E. cyclocarpum aqueous extract

Table 4. Relative abundance of volatile constituents identified from *E. cyclocarpum* heartwood.

Constituents	Retention time (min)	Relative abundance (%)
α-pinene	3.119	0.15
D-limonene	6.538	17.83
p-cymene	8.720	10.93
exo-brevicomin	10.349	0.18
Methylheptenone	12.242	0.87
Tetradecane	13.533	0.50
2-butoxy ethanol	15.362	0.40
α,*p*-dimethylstyrene	15.690	2.14
Farnesane	16.602	0.21
Octen-3-ol	17.061	0.15
trans linalool oxide	17.679	0.22
Acetic acid	17.908	0.37
Pentadecane	18.062	1.89
Octyl alcohol	18.592	3.41
Linalool	20.859	0.44
α-bergamotene	21.081	1.69
Caryophyllene	21.213	1.08
(+)-fenchol	21.911	0.98
Aristolene	22.000	1.01
Hexadecane	22.328	2.75
4-terpeneol	22.583	0.99
2,5-dimethylhydroqinone	23.421	0.70
β-damascone	23.621	1.09
β-terpineol	23.845	0.51
Menthol	24.109	0.22
Acetophenone	24.494	0.53
γ-valerolactona	25.299	3.38
Pristane	25.549	0.23
2,6,10,14-tetramethyl hexadecane	25.601	0.20
trans-*p*-2,8-menthadien-1-ol	25.927	0.43
(-)-α-terpineol	26.310	5.60
β-bisabolene	26.756	1.55
Napthalene	27.364	0.62
Epizonarene	27.608	0.30
Veratrole	27.718	1.90
Carveol	28.279	0.28
Acetophenone	28.935	0.68
methoxy-phenyl oxime	29.594	0.52
Octadecane	30.022	0.65
Methyl veratrole	30.579	0.51
methyl napthalene	31.296	0.54
p-cymen-8-ol	31.892	4.84
Guaiacol (natural)	32.332	2.77
Paeonal	33.045	0.36
Butylated hydroxytoluene	33.848	7.01
Phenylethyl alcohol	33.998	0.40
Benzothiazole	35.063	0.55
2-methoxy-4-methylphenol	35.522	2.72
1,2,3-trimethoxy benzene	35.856	0.30

2,6-dimethyl naphthalene	36.010	0.21
p-mentha-1(7),8(10)-dien-9-ol	36.726	0.47
2,4-dimethylphenethyl alcohol	37.468	0.35
4-ethyl-2-methoxy phenol	37.892	0.17
1,6,7-trimethyl naphthalene	39.727	0.44
4-methyl phenol	39.949	4.51
2,6-dimethoxy phenol	40.097	0.06
Eugenol	42.145	0.20

The GC-MS analysis allowed to identify fifty seven constituents (Table 4), some of them like D-limonene have a repellency activity against insects including mealybugs, whiteflies, ticks, louses, acaruses and fleas (Hollingsworth, 2005). Another example is terpineol used with eugenol and cinnamic acid produce a neuro-insecticide mixture used against carpenter ants *Camponotus pennsylvanicus* De Geer and German cockroaches *B. germanica* (Enan, 2001).

It is possible that the major constituents detected in the essential oil from *E. cyclocarpum* heartwood, terpineol, eugenol, cinnamic acid and D-limonene act on *I. marginipennis* as repellent or as neuro insecticide as has been described.

Also, a small group of components that are not plant natural products and the tree intakes from the

environment by the following two mechanisms were detected: a) pollutants acquired by bioconcentration such as 2-butoxy ethanol, 1,2,3-trimethoxy benzene (used in paint industry), 2-ethyl-1-hexanol (a product of the biodegradation of the phthalate plasticizers and oil additives) and 2,5-dimethylhydroquinone (urease inhibitor) or, b) components acquired by microbial interaction such as octen-3-ol, 4-ethyl-2-methoxy phenol and 4-methyl phenol, all of them are fungal metabolites.

Finally, it is considered that dissuasive agents such as the anti-feedants and the repellents are a class of environmentally friendly drywood preservatives. These metabolites do not cause death in target and others organisms therefore it is better to use an agent with a dissuasive effect on drywood termite *I. marginipennis* than one with a lethal effect. The results described herein suggest that the essential oil from heartwood of *E. cyclocarpum* induced a dissuasive effect on termites.

Acknowledgments

We deeply thank Dr. Rosa Santillan (IPN-CINVESTAV Mexico) for proof reading and discussion. Financial support was provided by UMSNH (CIC-2.1-MMP-2006) and Fondo Mixto CONACYT-Government of the State of Michoacan (2005-C01- 009). DRG and AFG (postgraduate fellowships CONACYT). REMM and OARE hold a medical fellowship.

References

Abbot, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18:265-267.

Boue, S. M. and A. K. Raina. 2003. Effects of plant flavonoids on fecundity, survival, and feeding of the formasan subterranean termite. J. Chem. Ecol. 29:2575-2582.

Carranza Montaño, M. A., L. R. Sánchez Vázquez, M. R. Pineda López and R. Cuevas Guzmán. 2003. Calidad y potencial forrajero de especies del bosque tropical caducifolio de la Sierra de Manatlan, México. Agrociencia 37:203-210.

Carter, L. F., R. H. Beal and J. D. Bultman. 1975. Extraction of antitermitic substances from 23 tropical heartwoods. Wood Sci. 8:406-410.

Chudnoff, M. 1984. Tropical timbers of the world. U.S. Department of Agriculture Eds, Washington, D.C. pp.466.

Enan, E. 2001. Insecticidal activity of essential oil: octopaminergic sites of action. Comp. Biochem. Physiol. 130:325-337.

Grace, J. K. and R. T. Yamamoto. 1994. Natural resistance of Alaska-cedar, redwood, and teak to formasan subterranean termites. Forest Prod. J. 44:41-45.

Habu, N., K. Igarashi, M. Samejima, B. Pattersson and L. K. L. Ericsson. 1997. Enhanced production of cellobiose dehydrogenase in cultured of *Phanerochaete chrysosporium* supplemented with bovine calf serum. Biotech. Appl. Biochem. 20:97-102.

Hollingsworth, R. G. 2005. Limonene, a citrus extract, for control of mealybugs and scale insects. J. Econ. Entomol. 98:772-779.

Lewis, J., J. M. Baker, M. H. Beale and J. L. Ward. 2004. Genomics for biosafety in plant

biotechnology. J. P. H. Nap, A. Atanossov and W. J. Stiekema (Eds.), IOS Press.

Lovell, J. B. 1979. 1,5-Bis(α,α,α-trifluoro-p-tolyl)-1,4-pentadien-3-one (1,4,5,6-tetrahydro-2-pyrimidinyl) hydrozones. United States Patent 4163102.

Martínez Muñoz, R. E., D. Raya González, M. Cajero Juárez, M. Calderón Raya, R. E. del Rio and M. M. Martínez Pacheco. 2009. Innocuous use of aqueous extract obtained from the heartwood of *Enterolobium cyclocarpum* (Jacq.) Griseb. Pharmacologyonline 2:1091-1096.

Martínez Pacheco, M. M., R. E. N. del Rio, A. Flores García, R. E. Martínez Muñoz, O. A. Ron Echeverría and D. Raya González. 2012. *Enterolobium cyclocarpum* (Jacq.) Griseb.: The biotechnological profile of a tropical tree. BLACPMA 11:385-399.

Miller, G. L. 1959. Use of dinitrosalisylic acid reagent for determination of reducing sugar. Anal. Chem. 31:426-429.

Nieva Moreno, M. I., M. I. Isla, A. R. Sampietro and M. A. Vattuone. 2000. Comparison of the free radical scavenging activity of propolis from several region of Argentina. J. Ethnopharmacol. 71:109-114.

Raya González, D., Flores García, A., Morales López, M.E. and Martínez Pacheco, M.M. 2013. Pinewood preserved with aqueous extract from *Enterolobium cyclocarpum* (Jacq) Griseb. heartwood. Afr. J. Biotech. In press.

Raya-González, D., T. Pamatz-Bolaños, R. E. del Rio-Torres, R. E. Martínez-Muñoz, O. Ron-Echeverría and M. M. Martínez-Pacheco. 2008. D-(+)-Pinitol, a component of the heartwood of *Enterolobium cyclocarpum* (Jacq.) Griseb. Zeitschrift fur Naturforsch. 63c:922-924.

Su, N. Y., M. Tamashiro and M. I. Harverty. 1987. Characterization of slow-acting insecticides for the remedial control of the formasan subterranenan termite (Isoptera: Rhinotermitidae). J. Econ. Entomol. 80:1-4.

Swain, T. and W. E. Hillis. 1995. The phenolic constituents of *Prunus domestica* I. The quantitative analysis of phenolic constituents. J. Sci. Food Agric. 10:6368-6376.

Taylor, A. M., B. L. Gartner, J. J. Morrell and K. Tsunoda. 2006. Effect of heartwood extractives fraction of *Thuja plicata* and *Chamaecyparis nootkatensis* on wood degradation by termites or fungi. J. Wood Sci. 52:147-153.

Yamaguchi, H., H. Yoshino and A. Kido. 2002. Termite resistance and wood-penetrability of chemically modified tannin and tannin-copper complexes as wood preservatives. J. Wood Sci. 48:331-337.

Zhou, X., M. M. Wheeler, F. M. Oi and M. E. Scharf. 2008. Inhibition of termite cellulose by carbohydrate-based cellulose inhibitors: Evidence from *in vitro* biochemistry and *in vivo* feeding studies. Pest. Biochem. Physiol. 90:31-41.