Journal of Agriculture, Pure and Applied Science and Technology (JAPAST) ISSN 2073-8749
Printed by Moi University Press

Evaluation of biocidal properties of *Terminalia spinosa* and their role in heartwood durability

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JAPAST, **1**, 61-68 (2009); received January 10/February 20, 2009

Heartwood of *Terminalia spinosa* was subjected to sohxlet extraction and analysed for anti-fungal and anti-termite assays. It was also analysed for causes of its exceptional durability. Two white rot fungi, *Coriolus versicolor* and *Antrodia species*, two brown rot fungi *Poria placenta* and *Gloeophyllum trabeum* and the termite *Macrotermes jeanneli w*ere used to determine durability based on growth inhibition, weight loss and termite mortality. ^{1}HNMR and ^{13}C NMR spectra of heartwood extractives were recorded in methanol on a Bruker AM 400 spectrometer. All extracts caused significant growth inhibition to the white rot and brown rot fungi. *T. spinosa* heartwood was very resistant termites. When eaten by the termites, the extracts caused significant percent loss in body weight accompanied by deaths of about 95% of the worker and soldier termites. They were also fungitoxic rather than fungistatic. ^{1}HNMR and ^{13}C NMR and FTIR spectra revealed presence of steareic acid and palmitic acid.

Key words: *Terminalia spinosa*, isolates, fungitoxic, fungistatic, durability, steareic acid and palmitic acid.

Introduction

Green plants act as a reservoir of inexhaustible source of innocuous fungicides and pesticides, which are not toxic to mammals and easily biodegradable than synthetic chemicals (Onuorah, 2006). Efforts have been made by many workers to use these plant products with the amendment of toxic metals and tested for durability against termites or fungi (Jain and virendra, 1991; Jain *et al*., 1989; Purushotham and Tewari, 1961; Indrader and Nautiyal, 2004).The leaves of *Ipomea carnea* (saxena et al., 2002) and Neem tree (Swathi et al., 2004) posses a number of toxic constituents exhibiting high toxicity against wood-destroying microbes. *Terminalia spinosa*, also known under the Swahili name (mwangati) is a tree which is distributed in dry savanna forests and coastal bush lands throughout tropical Africa. It belongs to the family of *Combretaceae*. Its wood is hard, heavy and particularly useful for construction works such as bridges, sleepers, fence and housing. The heartwood is dark brown or reddish brown and described to be very resistant to fungi and termites (Mburu *et al*., 2007; Mburu *et al*., 2008). Extracts from leaves and bark of *Terminalia spinosa* have important medicinal properties (Kokwaro, 1976; Mwambo, 2007). Natural resistance to fungi and termites is primarily attributed to the content of secondary metabolites present in heartwood, since these compounds frequently exhibit antifungal (Gomez-Garibuy et al, 1990; scheffer and Cowling, 1966) and antitermitic properties (McDaniel, 1992; Scheffrahn, 1991; Reyes Chilpa *et al.*, 1995) Knowledge about natural durability comes from practical experiences of end- users, from field tests or from standardized laboratory tests. For

instance, *Terminalia spinosa* has been used by the Pokot community in construction of their dwellings lasting for over 100 years. The use of durable wood species is becoming increasingly important by helping to conserve forests, discourage the use of preservatives which are not environmentally friendly and costly as well as provide economic and social benefits. Due to the negative influence on the environment by preservatives justifies the development of new wood preservation systems from naturally occurring compounds.

Materials and Methods

Collection of plant parts

Terminalia spinosa heartwood was collected from Wei Wei Valley in West Pokot District, which is about 600 Km North West of Nairobi, Kenya. The plant was identified in the Department of Biological sciences, Moi University where a Voucher specimen was deposited.

Quantitative analysis of extractives

Heartwood of was ground into fine sawdust powder, passed through a 115 microns mesh sieve and dried at 60°C before extraction with different solvents in a soxhlet extractor. The temperature of 60°C was used instead of the normal 103°C to avoid possible degradation of some of the extracts that have been observed to occur at 103^0C. About 10 g of wood powder was extracted with 180 mL of *n*-hexane, dichloromethane, methanol, acetone, toluene/ ethanol and water for 15 hr at the rate of 8-12 cycles per hr. After extraction, the solvent was evaporated under reduced pressure and the residue dried over P_2O_5 in a dessicator before weighing. The experiments were done in three replicates. Mean % extract content was determined by indirect (IM) and direct methods (DM) shown below. Similarly series extraction was carried out starting with the least polar *n*-hexane solvent to the most polar water solvent on same wood dust.

% extract (IM) = $((s_1 - s_e)/s_1) \times 100$,

where S_i is the initial weight of dried un-extracted saw dust and S_e is the weight of extracted saw dust.

% extract (DM) = $(s_{we}/s_1) \times 100$,

where s_{we} is the weight of the dried extracts.

Decay experiments on heartwood

Rot causing fungi

Two white rot, *Coriolus versicolor* (CV) (Strain FPRL 28) and *Antrodia species*, and two brown rots *Poria placenta* (PP) (Strain FPRL 280) and *Gloeophyllum trabeum* (GT) (Strain BAM Ebw 109) fungi were used. The fungi species were obtained from a culture maintained in Wood science laboratory of Moi University, Kenya. The termite species used was *Macrotermes jeanneli.*

Growth inhibition

Potato dextrose/agar medium were prepared by mixing 30 g potato dextrose and 40 g agar per litre of distilled water. The solution was warmed and stirred thoroughly stirred. The P^H of the solution was made to 4.8 by addition of the necessary quantity of 0.1M HCL and sterilized in the autoclave for 25 minutes at 120^0C.

In a sterile chamber the1 litre potato dextrose/agar medium was partitioned into ten portions each 100 ml in empty sterile conical flasks. Different *T. spinosa* heartwood extractives in 5 ml of methanol were introduced to each flask to prepare 50 ppm, 100 ppm and 500 ppm of solutions. Each conical flask was properly shaken and 15 ml of each solution poured into 9 cm petri dishes, allowed to solidify and inoculated in the center by a 10 mm diameter plug of fully-grown test fungi, cut from the edge of a colony growing on potato dextrose-agar medium. Inoculated petri dishes were kept in a growth chamber at 25^0C and relative humidity of 85% until mycelium of control cultures (without extractives) reached the edge of dish. 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 surface area available for growth. All experiments were done in triplicates.

The rate (T) of inhibition was determined for each concentration as:

$$T = 100 \times \left[1 - \frac{\text{Surface area covered by fungi at each concentration}}{\text{Surface area covered by the control}}\right]$$

Decay tests on wood blocks

The test assumed correctness of theoretical dry mass of wood because for natural durability test, samples should not be dried at 103^0C to avoid degradation of the wood components. Test blocks of *Terminalia spinosa* (*2*5 × 25 × 5 mm in longitudinal, radial and tangential directions) were conditioned at room temperature, 20^0C to 22^0C and a relative humidity of 60-70% until they achieved a constant weight (Mμ). Theoretical dry mass (M_{to}) of test blocks was determined by calculating the average percent (%) moisture of similar samples dried at 103^0C to get μ (average % humidity after conditioning) hence:

$$M_{to} = \frac{100M\mu}{100 + \mu}$$

Influence of extractives on durability was evaluated after extraction with toluene/ethanol mixture (2/1, v/v), methanol or acetone in a soxhlet extractor for 15 hr. Two UV sterilized solvent extracted or not test blocks and weight (m_1) were introduced to Petri dishes with healthy fungal colony on potato dextrose/agar medium and incubated for 16 weeks at 25^0C and relative humidity of 85%. The experiment was in triplicates. After the period, fungal mycelium was removed and all blocks dried at 80°C until stabilization of their mass and weighed (m_2) were attained. Weight loss (W_L) was expressed as a percentage of the initial oven dried weight of the sample according to the formula:

$$W_L\% = ((m_1 - m_2)/m_1) \times 100$$

where m_1 is the dried initial weight of the block and m_2, the dried weight after exposure to test fungi. *Pinus patula* was used as control and treated in a similar manner.

Resistance termites

Wood blocks (30 × 10 × 20 mm in longitudinal, radial and tangential directions) were used for termite resistance tests using *Macrotermes jeanneli*. Pine blocks were used as controls. The tests were done in chambers free of organic material in glass jars of diameter 80 mm and 100 m in height. One hundred and fifty (150) grams of screened, washed and sterilized sand was added to each container. 30 ml of distilled water was added to the sand in each jar and allowed to stand for 2 hr prior to introducing test blocks and termites.Four hundred termites from a single colony were added to each of the previously prepared jars at a ratio of 360:40 (worker: soldier) respectively.

One weighed test block (W_1) measuring (30x10x20 mm) extracted by each solvent was placed on the surface of the sand with two corners of the block against the side of the glass jar. Termites were placed on opposite side of the test sample. The setup was replicated five times. Five other jars were assembled with sand, water and test block but without termites were used as control. The glass jars were weighed individually at the beginning of the experiment and re-weighed on a weekly basis. Distilled water was added to all the glass jars if the moisture content of the sand dropped two percent below the original moisture content. All jars were placed in an incubator and maintained at 25^0C for 28 days. After the duration, all the glass jars were dismantled, test samples brushed and oven dried to a constant weight (W_2). % weight loss (W_L) was evaluated as below:

$\%W_L = \{(W_1 - W_2)/ W_1\}$ X100.

Wood blocks were further classified from 0 to 10 according to the significance of attack as summarized in Tab I.

Table I. Classification of natural durability of wood towards termites according to EN 117 standard

Block aspect after test	Classification
Failure	0
Attack (50-75%) cross section	4
Attack (30-50%) cross section	6
Attack (10-30%) cross section	7
Slight(3-10%) cross section	8
Slight(3%) cross section	9
Sound	10

Chemical analysis

Utilization of ^{1}HNMR, ^{13}C NMR and FTIR data and comparison standard compounds in MSD ChemStation led to elucidation of palmitic acid (1) and stearic acid (2).

Results and Discussions

The direct method and indirect method used to quantify extractives based on the weight of the extracts after evaporation of the solvent on the mass loss of sawdust respectively, gave comparable results indicating that no products were lost during vacuum evaporation. Quantities of extracts increased with the solvent polarity in agreement with the strong contents of extractives often observed in some tropical wood (Mburu *et al*., 2007; Gerardin *et al*., 2004; Hakkou *et al*., 2006)

Table II. % yield of extractives from direct and indirect methods of extraction

Solvent	**Yield of Extractive (%)**	
	DM	IM
Hexane	0.98	1.12
Dichloromethane	0.57	0.72
Methanol	3.40	3.51
Acetone	4.13	4.50
Toluene/ethanol	4.00	4.20
Water	4.85	5.00

Table 4. Efficacy of *Terminalia spinosa* against *Macrotermes jeanneli*

Extracting solvents	*Visual Rating Degree of attack*	*% Weight Loss*	*%Workers survivors*	*Soldier survivors*
Hexane	*9*	*8*	*3.5*	*4.5*
Dichlomethane	*9*	*10*	*3*	*3.5*
Methanol	*7*	*16*	*4*	*4*
Acetone	*7*	*15*	*3*	*3.5*
Toluene/ ethanol	*9*	*8*	*2*	*2*
Water	*9*	*19*	*5*	*6*
Un-extracted	*10*	*1*	*0*	*0*
Control (Pinus patula)	*4*	*32*	*60*	*65*

At 50 ppm to 100 ppm concentration of extracts, significant anti-fungal effects were observed on the growth of the mycelium of *Coriolus versicolor, Antrodia species, Poria placenta* and *Gloeophyllum trabeum*. The toluene/ethanol and *n*-hexane extracts displayed comparable results against the four fungi used with the extracts of the heartwood being fungitoxic rather than fungistatic. The white rot and brown rot fungi responded to the extract to the same degree. Respective ant-fungal activities of dichloromethane, methanol and acetone extracts are summarized in Fig.1, Fig. 2 and Fig. 3.

The results suggested that *Terminalia spinosa* heartwood is very durable toward termites. *T. spinosa heartwood* was very resistant termites. When eaten by the termites, the extracts caused significant percent loss in body weight accompanied by deaths of about 95% of the worker and soldier termites. They were also fungitoxic rather than fungistatic. Hydrophobic compounds contained in dichloromethane extracts play an important role in the dimensional stability of *Terminalia spinosa* heartwood, which was demonstrated by the high values of swelling coefficient (3.3%) and anti swelling efficiency measured (Table 4).

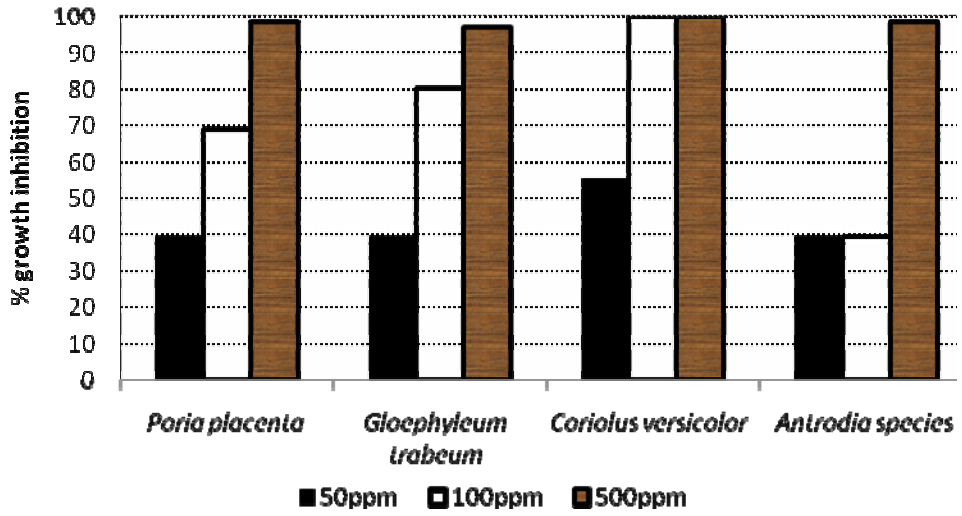


Fig. 1. Effect of dichloromethane extracts on growth of wood rotting fungi

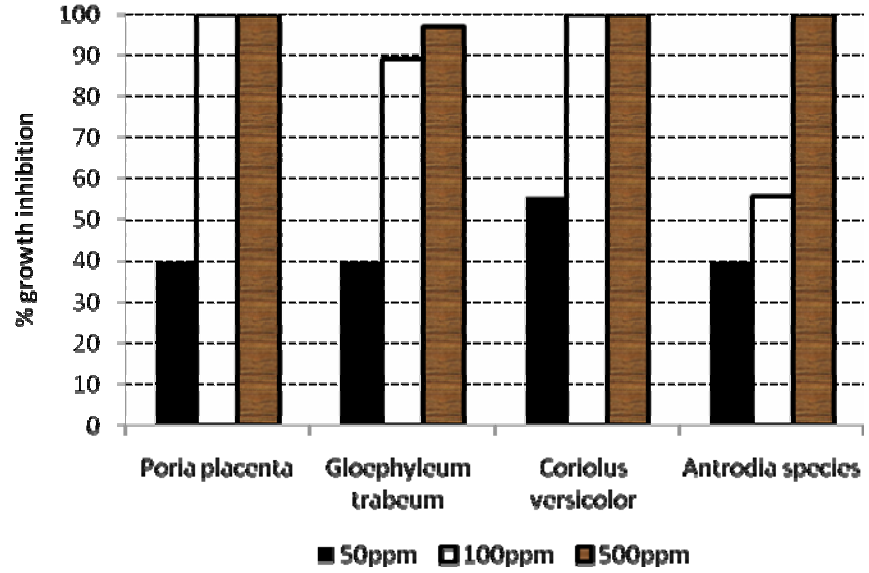


Fig. 2. Effect of methanol extracts on growth of wood rotting fungi

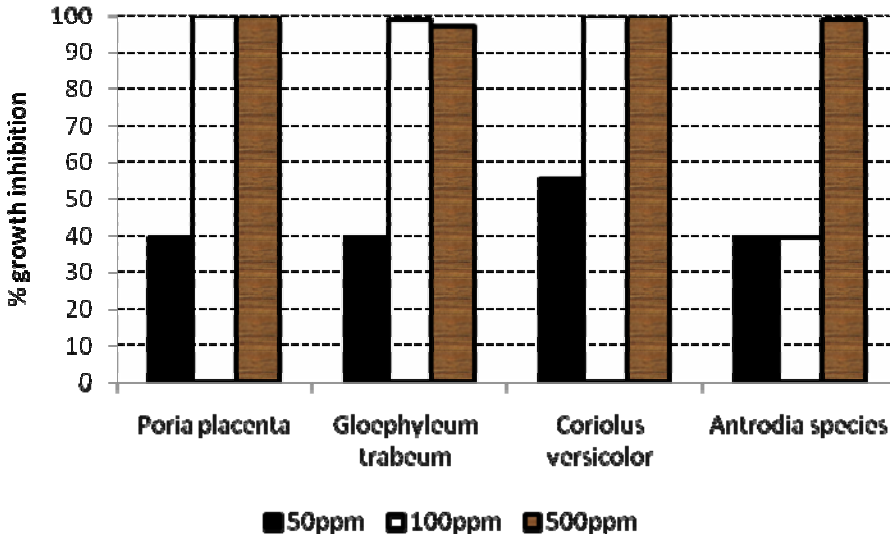
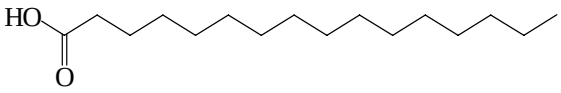
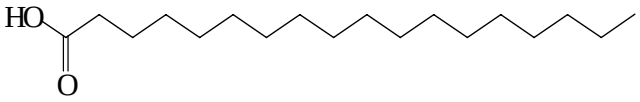


Fig. 3. Effect of acetone extracts on growth of wood rotting fungi

Structure of compounds



Palmitic acid



Stearic acid

Acknowledgement

The authors convey their appreciation to the Department of Biological Sciences and Moi University for availing facilities and technical support.

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