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Quantifications of phytochemicals and biocide actions of *Lawsonia inermis* linn. Extracts against wood termites and fungi

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ABSTRACT

In this study, *Lawsonia inermis* stem bark and leaf extracts compounds were quantitatively determined using Spectrophoto-metric and Forlin-Ciocalteu methods. The wood protection abilities of the leaf extract (LILE) against termites and fungi were investigated on *Triplochiton scleroxylon* and *Vitex doniana* woods. Sample woods were treated with four different formulations (5%–20%) of LILE in 70% ethanol for three days. The treated wood block samples including control as reference were exposed to termites under field conditions and two fungi (white rot, *Ganoderma lucidum* and brown rot, *Sclerotium rolfsii*) under laboratory (RH = 65%, Temperature = 27 °C) conditions for six months. The leaf extract yield was 5.7%. The stem bark of *L. inermis* contained 273.16 ± 0.25 mg/g total alkaloids, 6.81 ± 0.10 mg/g total flavonoids, 51.39 ± 0.28 mg/g total phenol, 47.98 ± 0.27 mg/g total saponins and 54.22 ± 0.30 mg/g total tannins while the leaf contained 236.60 ± 1.32 mg/g total alkaloids, 4.43 ± 0.05 mg/g total flavonoids, 30.96 ± 1.15 mg/g total phenol, 21.41 ± 0.44 mg/g total saponins and 37.44 ± 0.24 mg/g total tannins. The results showed that the extractives compounds from *L. inermis* had significant biocide actions against both termite attack and fungi decay, and conclusively rendering the study findings promising for the future use as protective agent for wood.

1. Introduction

Exploring the antidegradation potentials of plant tissues' extracts has hitherto long been one of the cardinal goals of foresters and wood protection scientists, and more recently has found crucial implications in researches into global functional biosecurity and wood protection technology. Selection preferences of plant tissues have been majorly based on the medicinal potency understanding of indigenous knowledge for specific plants in varied cultural uses. Natural products derived from plant extracts have widely been tested in a discovery programme for effective, environmental-friendly termite control agents (Meepagala et al., 2006), which could satisfactorily replace conventional chemicals for controlling wood pests (European Commission, 2004; Tiilikkala et al., 2010). Among the varying antidegradation or antimicrobial potential extract producing plant species in Nigeria, many like *Lawsonia inermis* which is known to contain array of natural compounds exhibiting antioxidant, insecticidal and antimicrobial as well as fixative properties have not been explored for wood protection purposes. For instance, *L. inermis* leaf grown in African soils (Egypt, Morocco, Ethiopia) has been reported to contain p-Coumaric acid, lawsone, apigenenin, luteolin, 2-methoxy-3-methyl-1,4-naphthoquinone, cosmosiin and apiin with good antioxidant activity (Mikhaeil et al., 2004), polyphenols, tannins, flavonoids, anthocynanins with interesting effects against contact dermatitis to severe angioneurotic oedema and haemolysis (Babili et al., 2013), and eugenol, hexadecanoic acid, phytol, α-terpineol and ether-phenylvinyl, and bisabolene (Kidanemariam et al., 2013). Lawsone (2-hydroxy-1,4-naphthoquinone) has been identified as the chief colouring compound responsible for good colour fixation and fastness on wool, silk and tenaciously on human skin and keratin in nails (Mondal et al., 2009; Tommasi, 1920 in Babili et al., 2013; Yusuf et al., 2011).

In Nigeria, *Lawsonia inermis* locally known as Lali in Yoruba language has a long traditional history of cosmetic and curative/preventive medicinal benefits. The plant is renowned for its leaf

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colouring fixative values and its extract that transform into blackish brown coloured paste as cosmetic product is bio-available for use throughout the year. The colourants and fixatives that occur naturally in *L. inermis* leaf have been the source of the natural cosmetics of raw, as well as processed product for fingernails and body tattooing. While *L. inermis* is considered the most famous fixative cosmetic flora, its leaf extracts have greatly been investigated for manifold insecticidal effects against human (*Pediculus humanus*) and small ruminants (*Bovicola ovis*) louse species (Marimuthu et al., 2012), stored maize seeds weevils (*Sitophilus zeamais*) (Suleiman et al., 2012), mosquito larvae (*Anopheles stephensi*) (Bakhshi et al., 2014), (*Culex quinquefasciatus*) (Dass and Mariappan, 2014), stored cowpea seeds weevils (*Callosobruchus maculatus*) (Jose and Adesina, 2014), stored ground nut seeds beetles (*Tribolium castaneum*) (Onoja, 2015).

Scientific evidences have also proved that leaf extract has several antimicrobial bioactive uses such as antifungal (Saadabi, 2007; Satish et al., 2010; Yusuf et al., 2012a; Gozubuyuk et al., 2014; Sherifa et al., 2015), antimold (El Bergadi et al., 2015), antibacterial (Habbal et al., 2005, 2011; Abulyazid et al., 2013; Kawo and Kwa, 2011; Yusuf et al., 2012a; Gull et al., 2013; Rahmoun et al., 2013; Maqtari and Maqtari, 2014), antioxidant (Al-Damegh, 2014), including colour/dye fastness on flora and fauna tissues (Chukwu et al., 2011; Jan et al., 2011; Yusuf et al., 2011, 2012b; Kannanmarikani et al., 2015), Ultraviolet (UV) protection (Dweck, 2002; Alebeid et al., 2015) and corrosion inhibitor of metals (Al-Sehaibani, 2000; El-Etre et al., 2005; Nik et al., 2012). The multiple ability of *L. inemis* leaf for protecting materials from peculiar wood degradation agents, fixing in the keratin in nails, and exhibiting good colour fastness on wool/cotton could find wider application in wood protection industry as attractive alternative. Natural dyes including *L. inemis* leaf extract (or dye) are widely use in particular, cosmetic and cloth industries as fixative, UV protective and antimicrobial agent around the world but its use as wood protective agent remain unexplored. In this study, the phytochemicals (total alkaloids, flavonoids, phenol, saponins, and tannins) of extracts from *L. inermis* stem bark and leaf were quantitatively determined using Spectrophoto-metric and Forlin-Ciocalteu methods and the leaf extract biocide actions against termites and fungi on two tropical non-durable white wood were investigated.

2. Material and methods

2.1. Wood test blocks preparation

Wood bolts were obtained from a 22 years *Triplochiton scleroxylon* and 28 years *Vitex doniana* trees and processed into 2 cm × 2 cm x 6 cm samples (that is, 2 cm in thickness by 2 cm in width by 6 cm in length). Woods' samples were oven dried at 103 ± 2 °C for 22 h (until constant weight was researched) according to the ASTM D-1413 (2007) norm, conditioned, and determined the initial oven-dry weight of each test block as ($\mathbf{W_0}$). The sample woods were then kept under air-tight Ziploc nylons prior to treatments and tests. For each wood species, 36 and 72 samples containing mixtures of sapwood and heartwood were selected for biocides' treatments for termites and fungi tests respectively.

2.2. Extract preparation and yield estimation

Fresh leaves of *Lawsonia inermis* were obtained from standing living plants at Imeko Nigeria between February and June 2012, cleaned and air-dried for three months. The dried samples were ground into smaller particles (chips) using hammer milling machine so as to increase the surface area. Five hundred grammes (500 g) of the powdered *L. inermis* leaf were weighed into flask and soaked with 2.5 L of 70% ethanol for 5 days with constant shaking. The ethanol extract was then filtered and concentrated using a rotary evaporator at 40 °C and yield determined. Yield was measured as the dry weight of the extract to the original dry weight of the sample material in percentage following the formula (Eqn. (1)) thus:

$$\text{Yield estimation} = \frac{\text{Dry weight of concentrated extracts} \times 100}{\text{Weight of the ground sample}} \quad (1)$$

2.3. Organoleptic characterisation of the extract

Aliquots (samples) of the extract were aseptically taken with aid of sterilised spoons and organeloptically assessed for colour, odour and texture under damp and dry conditions by visual, olfactory, and tactile methods respectively.

2.4. Determination of phytochemicals of the extract and test wood species

The extract and test wood blocks species dust samples were quantitatively assessed for total content of phytochemicals compounds such as alkaloids, flavonoids, phenol, saponins and tannins using the standard procedures reported by Adedeji et al. (2013).

2.4.1. Procedures

Methanolic extract of the samples was prepared following the method of Chan et al. (2006), by adding 25 mL of methanol to 0.5 g of sample contained in a covered 50 mL centrifuge tube, and shaking continuously for 1 h at room temperature. The mixture was centrifuged at 3000 rpm for 10 min, and then the supernatant was collected and store at −20 °C until analysis was done.

2.4.1.1. Quantification of total alkaloids content (TAC). The total alkaloid contents in the samples were measured using 1, 10-phenanthroline method described by Singh et al. (2004) with slight modifications. 100 mg sample powder was extracted in 10 ml 80% ethanol. This was centrifuged at 5000 rpm for 10 min. Supernatant obtained was used for further estimation of total alkaloids. The reaction mixture contained 1 mL plant extract, 1 mL of 0.025 M $FeCl_3$ in 0.5 M HCl and 1 mL of 0.05 M of 1, 10-phenanthroline in ethanol. The mixture was incubated for 30 min in hot water bath with maintained temperature of 70±2 °C. The absorbance of red coloured complex was measured at 510 nm against reagent blank. Alkaloid contents were estimated and it was calculated with the help of standard curve of qui-nine (0.1 mg/mL, 10 mg dissolved in 10 mL ethanol and diluted to 100 mL with distilled water). The values were expressed as g.100 g-1of dry weight.

2.4.1.2. Quantification of total flavonoids content (TFC). TFC was determined by Aluminium chloride method as reported by Kale et al. (2010). Sample (0.5 mL) of extract was dispensed into test tube, followed by 1.5 mL of methanol, 0.1 mL of aluminium chloride (10%), 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. The reaction mixture was mixed, allowed to stand at room temperature for 30 min, before absorbance was read at 514 nm. TFC was expressed as quercetin equivalent (QE) in mg/g material.

2.4.1.3. Quantification of total phenolic content (TPC). The total phenolic content of samples extracts was determined according to