The phytochemical and antimicrobial activities of *Terminalia laxiflora* Engl. & Diels root bark extract

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Abstract: The root bark of *Terminalia laxiflora* Engl. & Diels is used traditionally as gastric stimulant to prevent and cure diarrhea in infants and children, aids digestion and relieves constipation in adults. As quality antibiotics are rarely possessed, human pathogens are fast developing resistance to synthetic drugs yet medicinal plants are scantily validated. This has necessitated the investigation of *T. laxiflora* root bark for its phytochemical and antimicrobial values. Petroleum ether, aqueous and ethanoic extracts of *Terminalia laxiflora* root bark were tested using agar well diffusion technique on the following selected microorganisms: *Shigella sonnei, Salmonella typhi, Klebsiella aerogenes, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Penicillium chrysogenum, Aspergillus flavus, Lasidiophobia discorea* and *Saccharomyces cerevisae*. *Terminalia laxiflora* showed the presence of alkaloids, flavonoids, tannins, saponins and cardiac glycosides as its non nutritive metabolites. Ethanoic and aqueous extracts of the plant root were found to be potent on all the bacteria used at minimum inhibitory concentration of 58mg/ml and 54mg/ml respectively. Besides, ethanoic extract also reacted with *Penicillium sp* and *Aspergillus sp* while the aqueous and petroleum ether extracts were non reactive on all of the fungi used. The pharmaceutical use of this extract may be found to be bactericidal and weakly fungistatic in properties. This finding will help a long way to complement the worldwide efforts of providing safe, potent and cheap antibacterial drug of natural origin for all and sundry.
[Taiye R. Fasola, M.E. Oluwole, I. F. Olaniyi and I.E. Adeboye **The phytochemical and antimicrobial activities of *Terminalia laxiflora* Engl. & Diels root bark extract.** *Nat Sci* 2013;11 (8):122-127]. (ISSN: 1545-0740). http://www.sciencepub.net. 18

Keywords: *Terminalia laxiflora,* antibacterial, secondary metabolites, plant root bark

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Introduction

The dependence of man on nature for survival has led aboriginal people from time immemorial to live in harmony with nature in order to study a unique system of plant wealth by trial and error methods. This treasure of knowledge has been orally passed from generation to generation and is being retained by diverse indigenous groups around the world. These have variously enriched the knowledge of the people in their inherited wealth such as culture, food habit, religious rites, and phytochemicals such as herbal medicine until these ages of documentation (Samy *et al,* 2011). For the fact that Nair *et al*, 2005 among other scientists reported lower incidence of adverse reaction in plant preparations compared to modern conventional pharmaceuticals have made medicinal plants more relevant in both developed and developing countries. Basha and Sudarshanam (2011) reported that herbs provide many unique qualities that are very limited in conventional medicine, such as anticancer, antiviral and immunoregulatory properties and that herbs are excellent alternative to antibiotics in the treatment of infectious diseases, with wider antibacterial effects as well as various antifungal and antiviral activities. *Terminalia laxiflora* belongs to the family Combretaceae and is widely distributed in Africa, Middle and South East Asia. The Africa's distribution cuts across the savannah region, from the eastern Sudan to the western Senegal, and the northern Tunisia to the central Congo. It is a common indigenous tree in the woodland and semi - arid savannah of Sudan with high multipurpose potentials (Adam and Kerharo, 1974). In West Africa, there are 14 species spread from Senegal, across Nigeria to Cameroon. The germination of this species varies from 0-70% under the same condition. The tree is of 12m in height and nearly 1m width with the usual crooked bole, dark grey, deeply fissured and scaly bark (Adam and Kerharo, 1964). The wood is fire resistant because of its thick corky bark (Savill and Fox 1967). The plant has a variety of medicinal applications across the areas of occurrence. These include the stem as chewing stick in Nigeria, and as gastric stimulant to prevent and cure diarrhoea in infants and children. It also aids digestion and relieves constipation in adults in Senegal. The leaves and the bark of the root are used as anti-dysentery while the stem bark for the treatment of tuberculin cough and the yellow pulp of

root and the black leaves are used as dye. The scented heart wood is used as perfume called "amu" and the root bark is used to treat wound and strains. The macerated stem bark serves as antiseptic to wash mouth in order to resist gingivitis and thrush and general body pains in Congo. The plant also serves as wound dressing, diuretic management, pile and yaws treatment (Ivory coast), anti-skin inflammation, sores and ulcers treatment (Sierra Leone), eye lotion (Gambia), hair perfume, severe jaundice and chewing stick (Cameroon) across other African countries (Savil and Fox 1967; Adam and Kerharo, 1964, 1974; Abbow, 1990)

T. laxiflora called Idi-pupa in Yoruba was also codenamed Ewe Osanyin (deity leaves) as reported by Verger (1967). It was so called for its perceived ability to safely prevent still-birth. Historically, it was assumed among the Yorubas to posses the power to close ("Idi" means to block and "pupa" means yellow) the path of evil and death. It is also known that this plant synthesizes derivatives useful for the maintenance of health in human and animals (Srivastaraj *et al,* 1996; Tapsell, 2006).

Records have shown that a few reports are available on the antimicrobial potentials of this African plant of rich heritage. This study is therefore conducted to explore its phytochemical and antimicrobial qualities in order to complement efforts to develop and produce quality drugs that would improve human wellbeing.

Materials and Method

Plant material

Terminalia laxiflora root was purchased at Bode herbal market, Ibadan, Oyo State; Oja-Oba market, Ilorin, Kwara State and Isanlu-Mopo market, Yagba East Local Government Area, Kogi State, all in Nigeria. The bark was cut into chips, dried and powdered for *in vitro* analysis.

Collection of microorganisms

Except for *Saccharomyces cerevisae, Lasidiophobia discorea* and *Pseudomonas aeruginosa* that were collected from Cocoa Research Institute of Nigeria, Ibadan, most microorganisms used for this work were obtained from the Microbiology Department, Lead City University, Ibadan, Nigeria.

Plant Extract Preparation

About 30g of milled extract powder was separately measured and dissolved into 400ml (75 mg/ml) of each of these solvents (Aqueous, Ethanol, and Petroleum Ether). They were allowed to soak for 24 hours after which the supernatant was filtered into a conical flask using muslin cloth reinforced with Whatman No 1 filter paper. They were used immediately and the remaining extracts stored in the refridgerator for further study (Harrigan and McCane, 1986). Also, different concentrations were prepared from above filtrate by mixing 20ml of undiluted extracts with 2ml solvent (68mg/ml), 20ml with 4ml solvent (63mg/ml), 20ml with 6ml solvent (58mg/ml) and 20ml with 8ml solvent (54mg/ml).

Phytochemical analysis

The powdered root bark was extracted with water, ethanol and petroleum ether and subjected to the phytochemical analysis according to Kumar *et al* (2011).

***In vitro* antimicrobial analysis**

The Disc-diffusion method was used for the evaluation of the antimicrobial activity of the extracts according to Aubreville (1950); Andrew (1990) and Tajkarimi *et al.,* (2010). The 8mm diameter size cork borer used for the well cavity was re-sterilized and equally used to cut across the fungi colony. This was then planted into the PDA well at the centre while the marked well surrounding the centre well contains the extract in different concentrations. In the same vein, the Nutrient Agar was thoroughly streaked with the bacterial isolate before the wells were bored, marked and the extracts in different concentrations dispensed into them.

The plates thus prepared were incubated at 30^{o}C and 37^{o}C for fungal and bacterial growth respectively. The zone of clearance around each colony was determined (Guptee, 2001and Nester *et al*., 2004)

Results and Discussion

Table.1: Phytochemical analysis of *Terminalia laxiflora* root bark

Cardiac glycosides	Tannins	Alkaloids	Saponins	Flavonoids	Phenols	Steroids
+	+	+	+	+	-	-

+ means present
- means absent

Among the seven phytochemical screened for, only five were present in the root bark of *Terminalia laxiflora* while phenols and steroids were absent

.**Table 2: Zone of inhibition (mm) of *Terminalia laxiflora root bark* extracts on some microorganisms using different solvent concentrations (mg/ml).**

S/No	Microbial Isolates used	Solvent Extracts	Undiluted Extracts (75mg/ml)	20ml + 2ml (68.2mg/ml)	20ml + 4ml (62.5mg/ml)	20ml + 6ml (57.7mg/ml)	20ml + 8ml (53.6mg/ml)	Control
1	*Klebsiella aerogenes*	Petroleum Ether	-	-	-	-	-	-
		Aqueous	10	7	7	-	-	-
		Ethanol	9	8	7	7	5	5
2	*Escherichia coli*	Petroleum Ether	-	-	-	-	-	-
		Aqueous	10	10	9	9	8	-
		Ethanol	28	12	10	10	10	5
3	*Proteus mirabilis*	Petroleum Ether	-	-	-	-	-	-
		Aqueous	13	-	-	-	-	-
		Ethanol	11	7	-	-	-	5
4	*Salmonella typhii*	Petroleum Ether	8	6	-	-	-	6
		Aqueous	18	16	16	-	-	-
		Ethanol	26	22	10	10	-	5
5	*Pseudomonas aeruginosa*	Petroleum Ether	-	-	-	-	-	-
		Aqueous	13	12	8	5	5	-
		Ethanol	19	17	7	7	-	5
6	*Shigella sonnei*	Petroleum Ether	-	-	-	-	-	-
		Aqueous	14	10	7	4	-	-
		Ethanol	28	22	12	11	4	4
7	*Penicillium chrysogenum*	Petroleum Ether	20	-	-	-	-	12
		Aqueous	-	-	-	-	-	-
		Ethanol	36	35	29			20
8	*Aspergillus flavus*	Petroleum Ether	14	-	-	-	-	8
		Aqueous	-	-	-	-	-	-
		Ethanol	-	-	-	-	-	-
9	*Saccharomyces cerevisae*	Petroleum Ether	-	-	-	-	-	-
		Aqueous	-	-	-	-	-	-
		Ethanol	-	-	-	-	-	-
10	*Lasidiophobia discorea*	Petroleum Ether	-	-	-	-	-	-
		Aqueous	-	-	-	-	-	-
		Ethanol	38	29	-	-	-	20

Table 3: Minimum Inhibitory Concentration (mg/ml) of *Termilalia laxiflora root bark extracts* on some microbes

S/No	Microbial Isolates used	Solvent Extracts	Undiluted Extracts (75mg/ml)	20ml + 2ml (68.2mg/ml)	20ml + 4ml (62.5mg/ml)	20ml + 6ml (57.7mg/ml)	20ml + 8ml (53.6mg/ml)
1	*Klebsiella aerogenes*	Petroleum Ether	-	-	-	-	-
		Aqueous	10	7	7	-	-
		Ethanol	4	3	2	2	-
2	*Escherichia coli*	Petroleum Ether	-	-	-	-	-
		Aqueous	10	10	9	9	8
		Ethanol	23	7	5	5	5
3	*Proteus mirabilis*	Petroleum Ether	-	-	-	-	-
		Aqueous	13	-	-	-	-
		Ethanol	6	2	-	-	-

4	*Salmonella typhii*	Petroleum Ether	2	0	-	-	-
		Aqueous	18	16	16	-	-
		Ethanol	21	17	5	5	-
5	*Pseudomonas aeruginosa*	Petroleum Ether	-	-	-	-	-
		Aqueous	13	12	8	5	5
		Ethanol	14	12	2	2	-
6	*Shigella sonnei*	Petroleum Ether	-	-	-	-	-
		Aqueous	14	10	7	4	-
		Ethanol	24	18	8	7	0
7	*Penicillium chrysogenum*	Petroleum Ether	8	-	-	-	-
		Aqueous	-	-	-	-	-
		Ethanol	16	15	9	-	-
8	*Aspergillus flavus*	Petroleum Ether	-	-	-	-	-
		Aqueous	-	-	-	-	-
		Ethanol	-	-	-	-	-
9	*Saccharomyces cerevisae*	Petroleum Ether	-	-	-	-	-
		Aqueous	-	-	-	-	-
		Ethanol	-	-	-	-	-
10	*Lasidiophobia discorea*	Petroleum Ether	-	-	-	-	-
		Aqueous	-	-	-	-	-
		Ethanol	18	9	-	-	-

Phytochemical analysis of *Terminalia laxiflora* root bark extract revealed the presence of tannins, alkaloids, saponins, flavonoids and Cardiac glycosides (Table 1). Records have shown that some of these phytochemicals have antimicrobial - related composition such as tannins (an aromatic substance), alkaloids (serves as defense against microbial predations), spices (used to season food) and Flavonoids having antimicrobial medicinal properties (Lai, 2004).

The antimicrobial properties of the plant extracts showed a broad spectrum against the bacterial isolates. Though in Table 2, there was no inhibition observed with aqueous control but ethanol control was observed to have the highest inhibitory zone on the tested bacterial and a few of the fungi; even as high as 20mm diameter on *Penicillium chrysogenum*. This was not surprising as it is generally known that alcohols are useful chemical agents employed against bacteria and fungi, but they have no effect on bacterial spores (http://www.cliffsnotes.com/study_guide/Chemical-Methods-of Control). Phytochemical and antimicrobial results of the extracts of this plant (Tables 1 and 2) are of pharmaceutical importance as it is broad - based bactericidal though weakly fungistatic in properties. This can help a long way to complement worldwide efforts of providing safe, potent and cheap antibacterial drugs for all and sundry.

In Table 3, aqueous extract was tested to have the Minimum Inhibitory Concentration (M.I.C) of 54mg/ml with 8mm and 5mm diameter of inhibition on *Escherichia coli* and *Pseudomonas aeruginosa* respectively. At the same concentration it was closely followed by ethanoic extract with 5mg/ml on *E. coli*. In the undiluted extracts of 75mg/ml, ethanoic extract was the most potent of the solvent used with 23mm and 24mm clearing zones on *E. coli* and *Shigella sonnei* respectively. Aqueous extract was also found reactive but Petroleum ether extract was observed to be non reactive on both the bacteria and fungi isolates used in this study. As Table 3 revealed the efficacy of different concentrations of *Terminalia laxiflora* root extracts, ethanoic and aqueous extracts were found to be potent on bacteria at M.I.C of 58mg/ml and 54mg/ml respectively. However, ethanoic extract reacted weakly on the fungal isolates (fungistatic) while aqueous petroleum ether extracts were non reactive on all the fungi used.

Though it has been proven that different parameters in this test (like the depth of the gel) can affect the result (diameter of inhibition), it has guided the exact precision of 4mm gel dept level used according to Brandi *et al, (*2006), Burt *et al. (*2007) and Kil *et al. (*2009). The method allows the

diffusion of various extract concentrations from the cavity well to diffuse into the solidified Agar gel in the Petri dishes such that the plated microorganisms are inhibited from growing towards the well containing the various extract concentration thereby forming clear circular zone around the well. The diameter of such clear zone is directly proportional to the efficacy of the concentration of the solvent extract (Guptee, 2001).

In modern medicine, medicinal plants are used as basic knowledge for studying substances the plant or its organs contain and how they can be used for therapeutic purposes directly or indirectly as described by Razaq *et al*., (2003). These diverse substances are of various range of bioactive molecule that makes them a rich source of production of different types of medicine useful for the maintenance of human health. Most of the bioactive substances are secondary metabolites that are always found in small quantity and constitute over 50% of all modern clinical drugs of natural origin. Primary metabolites like fats, sugars, alcohols, acids, aldehydes and a few antibiotics and secondary metabolites like tannins, saponins, anthraquinones, alkaloids, phenols, Flavonoids, terpenoids, steroids, sterols, cardiac glycosides and phlobatannins are often used as sources of food, spices, and drug production while others can be toxic (to deter predators), pheromones (to attract insects for pollination), phytoalexins (to protect them against bacterial and fungal attacks and phyto alleto (to inhibit rival plants from competing for mineral resources and light) (Nair *et al*. 2005, Edeoga *et al*. 2005). This study has proved the worth of this plant in ethnomedicine. Fabricant and Farnworth (2001) discussed the tremendous value of plants in traditional medicine and drug discovery.

Conclusion

Extracts from *Terminalia laxiflora*, if developed into drug, could be safely administered across ages (i.e young, old, infant, male, female, pregnant or aged). Properties displayed both by aqueous and ethanoic extracts from this study would effectively encourage such development. Local use of the stem as chewing stick will help to dislodge and disinfect the microbes in the gingival creeks and gum of the tooth and prevent weakness of the gum. Also, the development and production of antibiotics from this plant as herbal drug for all and sundry will be significant efforts towards controlling diarrhoea among the developing nations.

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