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Antimicrobial, Phytochemical Screening and Radical Scavenging of *Piliostigma Reticulatum* and *Anogiessus Liocarpus* Against Two Enteric Organisms

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Abstract

The Ethanol, Methanol and Ethylacetate extracts of *Piliostigma reticulatum* and *Anogeissus liocarpus* were screened for their antimicrobial activities against *Salmonella typhi,* and *E.coli.* The results obtained indicate that all solvent extracts were active against the test organisms at different concentrations with zones of inhibition ranging from 10mm to 30mm. The positive and negative control using standard antibiotics showed that some organisms like *Salmonella* that are resistant to antibiotics are sensitive to some of the plants' extracts. The phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, steroids, glycosides. The Oxidative ability of the plant extracts revealed the ethanol and methanol extracts to have high scavenging ability.

Key Words: Antimicrobial activity, *Piliostigmareticulatum, Anogeissusleiocarpus*, Phytochemicals, Oxidative ability, Radical scavenging.

INTRODUCTION

The use of and search for drugs and dietary supplements derived from plants have accelerated in recent years. Ethno-pharmacologists, botanists, microbiologists, and natural-products chemists are combing the Earth for phytochemicals and "leads" which could be developed for treatment of infectious diseases. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties.

(Cowan, 2013). There had been, for half a century, a trickle of drugs of modest efficacy and awesome toxicity, however, there has been a rapid discovery of several excellent medications (Shamim, 2010).

The use of medicinal plants all over the world predates the introduction of antibiotics and other modern drugs into the African continent. Herbal medicine has been widely used and formed an integral part of primary health care in China, Ethiopia, Argentina and Papau New Guinea (Akinyemi*et al.*, 2005).

Due to the rate of resistance of microorganisms to antibiotics, the emergence of new mutated strains of microorganisms and adverse effects of some antibiotics, there is a need for alternative prevention and treatment options that are safe, effective and economical. While several agents are commercially available, these chemicals can alter microbiota and have undesirable side-effects such as vomiting, diarrhea and rashes (Palombo, 2011). Hence, the search for alternative products continues and natural phytochemicals isolated from plants used as traditional medicines are considered as good alternatives. So many plants have been studied for activities against pathogens with the aim of developing active drugs against them. The most problematic bacteria include but not limited to extended broad spectrum β- lactamase-producing *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa*, methycillin resistant *Staphylococcus aureus* (Giamarellou, 2010, Magiorakos*et al.,*2012, Talbot *et al*., 2006).

Plants produce natural chemicals used as protection against predators. Most of these biochemical constituents are potent bioactive compounds found in medicinal plants which are precursors for the synthesis of useful drugs (Sofowora,1993). The use of medicinal plants as curative agents spans over long historical records as they have been subjected to very intense pharmacological studies in the last few decades (Matu and Van Staden, 2003). In view of these, many Nigerian medicinal plants are being investigated and their natural products isolated and assayed for biological activities (Almeida *et al.,* 2005). For many of the medicinal plants of interest currently, a primary focus of research has been in the areas of phytochemistry, pharmacognosy and horticulture.

The beneficial medicinal effects of plant materials typically result from the combination of secondary products present in the plants (Briskin, 2000). These metabolites known as phytochemicals are present in plants that are physically active and which exhibit the curative properties of the plants (Ogundare and Onifade, 2009). Some of the biologically active metabolites include but not limited to alkaloids, flavonoids, glycosides, tannins, terpenoids, saponins. Many phytochemicals exert their beneficial effects through the additive of synergistic actions of several chemical compounds acting at a single or multiple target sites associated with physiological process (Tyler, 2006).

The plants used in this study are traditional medicinal plants used basically in the treatment of neonatal skin infection (Personal contact). However, part of the objectives of this work is to investigate the possibility of the plant to be used in the treatment of other infections particularly enteric infections. *Anogeissusleiocarpus* is a deciduous tree species that can grow up to 15–18 m of height and measure up to 1m diameter (El Ghazali *et al*., 2003, Ouedraogo*et al*., 2013). Many traditional uses have been reported for the plant. In Sudanese traditional medicine the decoction of the barks is used against cough (El Ghazali *et al*., 2003). Rural populations of Nigeria use sticks for orodental hygiene, the end of the sticks are chewed into fibrous brush which is rubbed against teeth and gum (Rotimi,1988). Ivory Coast traditional practitioners use the plant for parasitic disease such as Malaria, Trypanosomiasis, Helminthasis and dysenteric syndrome. In Togolese traditional medicine, it is used against fungal infections such as dermatitis and Mycosis; also, the decoction of leaves is used against stomach infections. The plant is also used for the treatment of diabetic ulcers, general body pain, blood clots, asthma, coughing and tuberculosis (Victor, 2013). *Anogeissusleiocarpus* is a tree that is considered to be sacred. It is widely used to dye textiles and leather as well as in the traditional treatment of many diseases. The decoction and maceration of the stem bark are used against anorexia, constipation, malaria, jaundice, itching, wounds, eczema, psoriasis, carbuncles, boils and

various forms of ulcers (Kerharo*et al*., 1974, Nacoulma, 1996). The decoction of the leaves is used in the treatment of jaundice, various forms of hepatitis and amoebic dysentery.

Anogeissusleiocarpus Tree

Piliostigmareticulatum (DL.) Hochst. (common name; Yoruba: 'abafin', Hausa: 'kalgo', Igbo: okpoatu') belongs to the family Leguminosae - Caesalpiniaceae and is found in the savannah region of Nigeria. It is a tree, occurring up to 30ft in height with an evergreen, dense spreading crown (Keay, 1989).It is used traditionally in the treatment of diarrhea. Tea from the leaves is used to treat colds, bark is astringent and used against diarrhea and dysentery; leaves and bark have haemostatic and antiseptic properties, cures also ulcers, boils, wounds and syphilitic cancer. Other medical uses are against coughs, bronchitis, malaria, hepato-biliary ailments, hydropsy, sterility, rachitis and kwashiorkor.

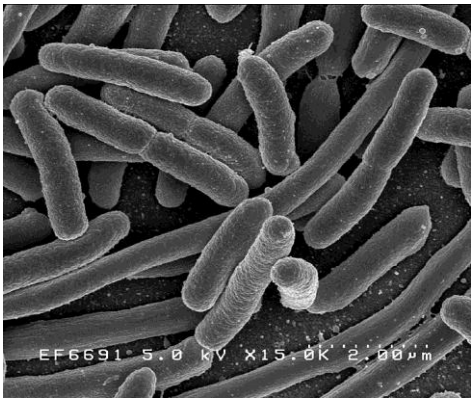
Piliostigmareticulatum

Enteric organisms are bacteria of the intestines and belong to the family enterobactericeae. They are gram negative rods with facultative anaerobic metabolism that live in the intestinal tract of animals in health and diseases. (Todar, 2015).

*Escherichia coli*is a bacterium that is commonly found in the gut of humans and other warm-blooded animals. While most strains are harmless, some can cause severe foodborne disease. *E. coli* infection is usually transmitted through consumption of contaminated water or food, such as undercooked meat products and raw milk (Kappeli*et al*., 2009).

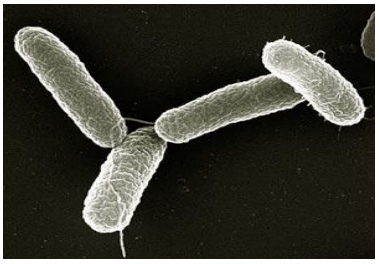
Escherichia coli is one of the most frequent causes of many common bacterial infections, including cholecystitis, bacteremia, cholangitis, urinary tract infection (UTI), and traveler's diarrhea, and other clinical infections such as neonatal meningitis and pneumonia (Buchholz *etal.,* 2011). *Escherichia* organisms are gram-negative bacilli that exist singly or in pairs. *E coli*isfacultatively anaerobic with a

type of metabolism that is both fermentative and respiratory. They are either non-motile or motile by peritrichous flagella. *E coli*is a major facultative inhabitant of the large intestine.



Salmonella Typhi

S. typhi causes typhoid fever. It is an obligate multi-organ parasite that inhabits the lymphatic tissues of the small intestine, liver, spleen and bloodstream of infected host. It is a gram negative enteric bacillus belonging to the *enterobacteriaceae.* It is a motile, facultative anaerobe that is susceptible to various antibiotics (Pollack, 2003). Its toxicity is due to the outer membrane made up of lipopolysaccharide which protects the bacterium from the environment (Surette *and* Bonnie.1998).



MATERIALS AND METHODS

Plant Collection and Preparation

Stem bark and roots of plants were sampled from traditional healers at Ibode in Ibadan, Oyo State. The plant materials were washed with distilled water, air dried at room temperature, milled into powder and kept in sterile containers till use.The plants were identified by Mr. Olatunde of the Department of Biological Sciences of Achievers University, Owo, Ondo State. Voucher specimens were deposited in the herbarium of the University.

Extraction Of Crude Extracts

The ethanol, methanol and ethyl acetate extracts were prepared by dissolving100gm amount of powered plant samples in 500mls of each solvent. The solutions were allowed to stand for 5 days (120hr) after which each solution was filtered using a sterile muslin cloth and a whatmann no 1 filter paper. The collected filtrate was evaporated to dryness under sterile condition at room temperature. The extracts were kept in sterile bottles until ready for use.

***Crude Extract Yield*:** The percentage yield of the extracts were determined by weighing the crude plant extractsand before extraction and after concentration and then calculated using the formula.

$$\text{Percentage (\%) yield} = \frac{\text{Weight (g) of the concentrated extracts}}{\text{Weight (g) of the ground plant extracts}} \times 100.$$

***Antimicrobial Test*:** The crude extracts were reconstituted in DMSO. The agar well diffusion method of Perez, 1990 was adopted for the antimicrobial assay. Test organisms were suspended in Nutrient broth and incubated for 4hours to obtain a concentration corresponding to MacFarlands constant.

The sterile petri-plates were inoculated by pour plate method. About 1ml amount of the test organisms were poured aseptically into the plates and about 20 ml of sterilized Nutrient Agar was poured into sterile petri-plate. The agar was allowed to set in the plates. Wells were punched over the agar plates using sterile gel puncher (Cork borer) and about 1ml of each plant extracts were added to the wells. The plates were incubated for 24 h at 37 ^{O}C. After incubation the diameter of inhibitory zones formed around each wells were measured in mm and recorded. Experiments were carried out in triplicates.

Table 1: Plants names, parts used and traditional uses

	Anogeissusleiocarpus	*Piliostigmareticulatum*
Plants (English name)	African birch	Piliostigma, Kargo.
Local names	Ayen	Abafi
Family	combretaceae	ceasalpiniaceae
Part used.	Stem/leaves	Leaves, pods, stem twigs, roots
Part used in this work	Stem bark	Stem bark
Traditional uses	Diabetes, ulcer, bloodclot, asthma and TB	Fever, ulcer, rheumatism, cough, tooth ache, hookworms, diuretic, diabetes.

Phytochemical Screening.

Active plants were screening quantitatively and qualitatively for phytochemicals using the methods of Trease and Evans (2002) and Odebiyi and Sofowora (1993).

Test For Alkaloids; About 0.2g of plant extract was acidified with 1% hydrochloric acid (HCL) for 2 min and was then treated with a few drops of Dragendorff's reagent in a test tube. The formation of white precipitate indicates the presence of alkaloids. (Odebiyi and Sofowora, 1978, Banso and Ngbede,2006).

Test For Saponins; Sterile distilled water was used to dissolve 0.2g of plant extract. A 2ml amount of the solution was placed in different test tubes and were shaken vigorously for a few minutes. Frothing which persists on warming was taken as an evidence of the presence of saponnin (Odebiyi and Sofowora, 1978).

***Test For Tanins; Gelatin Test*.** To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

Test For Flavonoids (Shinoda's Tests); Plant extract was dissolved in 2ml of dilute NaOH. A yellow solution that turns faint or colorless on addition of a few drops of hydrochloric acid and a change in colour while standing indicates the presence of flavoniods.

***Test For Cardiac Glycosides* (Liberman's Test);** The Liberman's test was used to test for the presence of cardiac glycosides. A 5g amount of plant was dissolved in 20ml of acetic anhydride and cooled with ice. Concentrated H_2SO_4 was then carefully added. A color change from violet to blue and then to green indicated the presence of a steroidal nucleus (a glycone portion of the cardiac glycoside).

***Test For Steroids* (Salkowski Test);** About 0. 5g amount of plant extract was dissolved in 2ml of chloroform. 0.2ml of concentrated H_2SO_4 was carefully added to form a layer. A reddish –brown colour ring at the interface between the layers indicated the deoxy- sugar characteristic of cadenolides which indicated the presence of steroids.

Positive And Negative Control

Standard antibiotics were tested against test organisms to detect the susceptibility or resistance of microorganisms to the antibiotic disc to justify the need for new antimicrobial. Commercial antibiotic discs were used in this work and the agar disc diffusion method of Kirby- Bauer (1966) was employed. Standard antibiotic discs used include; Amoxylin, Ofloxacin, Streptomycin, Chloramphenicol, Ceftriazone, Gentamycin, Pefloxacin, Cotrimoxazole, Ciprofloxacin and Erythromycin as the negative control and Augmentin, Ceftriazone, Nitrofurantoin, Gentamycin, Cotrimoxazole, Amoxylin, Ciprofloxacin, Tetracyline, PefloxacinWere used for the negative control.

Scavenging Activity against 1,1-Diphenyl-2-Picryl Hydrazyl Radical (DPPH)

The assay was carried out as described *by* Ayoola *et al.,* 2006. DPPH(1,1--diphenyl-2-picrylhydrazyl) was prepared by dissolving 0.03g of DPPH into 200 ml of distilled water.1ml of DPPH was pipetted into 1 ml of different concentration of each solvent extract. The solution was incubated in the dark for 30min.The DPPH solution in methanol was prepared daily before the absorbance measurements. DPPH is a purple-colored, stable free radical. When reduced it gives the yellow colored Diphenylpicrylhydrazine. All experiments were performed thrice and the results were averaged. The absorbance was read off the spectrophotometer at 570nm.Radical scavenging ability was calculated using the formula:

$$\%Ihn=\frac{1-(\text{Abs DPPH} + \text{Sample} - \text{Sample+distilled water})}{\text{Abs DPPH}} \times 100$$

RESULTS AND DISCUSSION

The percentage yield extracts of the plants' extract as depicted in table 1 showed *Anogeissusleiocarpu*s to have the highest percentage yield with the ethanol extract having 2.76%, methanol, 2.61% and the ethyl acetate having 1.75%. Factors like age of the plant and the polarity of the solvent used may affect the yield. Ethanol seems to be a good solvent for this plant which supports the use of water and alcohol as traditional solvent.

The antibacterial screening of the two plants revealed that *P. reticulatum*was was active against all the test organisms used in this work. The ethanol extract showed activity with the 100mg/ml having a zone of inhibition of 18mm against Salmonella typhi while the same concentration had 14mm against *Shigella*. The same concentration of extract had 16mm against *E.coli*. The ethyl acetate showed highest activity against Salmonella with the 100mg/ml having a zone of inhibition of 30mm while the same concentration of extract had 22mm as diameter zone of inhibition against *Shigella*. However, the ethyl acetate extract was not active against *E. coli.*

The ethanol extract of *Anogeissusleiocarpus* inhibition against Salmonella with inhibition zone ranging from 10mm to 30mm while the ethanol and ethyl acetate extract were active against *E. coli*. (Table 2) The positive and negative control using standard antibiotics revealed the plants to have activities against organisms that are resistant to some of the antibiotics (Table 3). *Salmonella* was resistant to all the antibiotics used but sensitive to the plants extracts used.

This plant appears to be effective and can be used in the treatment of enteric infections.The phytochemical screening of the crude extracts showed the plants to be rich in active compounds as shown in table 4; alkaloids, flavonoids, tannins, and saponins. They were known to show medicinal activity as well as exhibiting physiological activity (Edeoga*et al*., 2005) and exhibit anti-inflammatory, anti-oxidant and membrane stabilizing property (Perez *et al.,* 1995) These phytochemicals also have some strong antimicrobial significance against some potential enteric pathogens (El-Mahmood, 2009) and this may justify their use in traditional medicine(Shinde and Mulay,2015). The presence of Alkaloids in significant quantities may be used as antimalarial, analgesics and stimulants (Mantle *et al.,* 2000). The other phytochemicals present in the plants are known to inhibit tumor growth, treatment of intestinal disorder like diarrhea and dysentery, tannin are used in treating wounds, sprains, bruises and arresting bleeding (Nwaeze and Abarikwu,2006; Akinpelu and Onakoya, 2006). Studies carried out by Ijeh and Agbo (2006)indicated the possibility that the use of plant extract in high doses could lead to toxic injury to kidney which may interfere with renal tubular functioning and could induce acute renal failure. Steroids have been reported to have antibacterial properties, the correlation between membrane lipids and sensitivity for steroidal compound indicates the mechanism in which steroids specifically associate with membrane lipid and exerts its action by causing leakages

from liposomes (Raquel *et al.,* 2007). Tannins bind toproline rich proteins and interfere with the protein synthesis (Shimada *et al.,* 2006).

Table 2: Percentage yield of all extract of plants.

Plant	Part	Percentage Yield of Extract (%)		
		Ethanol	Methanol	Ethyl Acetate
Anogeissusleiocarpus(Ayin)	Stem bark	2.76	2.61	1.75
Piliostigmareticulatum	Stem bark	1.68	1.54	1.02

Table 3.Antibacterial activities of solvent extracts of plants.

	S A L M O					E . C O L I				
Plants/concentration of extracts	100	60	40	20	10	100	60	40	20	10
Piliostigmatareticulatum in ethanol	18	16	-	-	-	16	12	10	16	14
Piliostigmatareticulatum in ethyl acetate	30	26	24	20	18	22	20	18	-	-
Piliostigmatareticulatum in methanol	18	14	-	-	-	-	-	-	-	-
Anogeissusleicarpus in ethanol	30	22	18	16	14	14	12	10	-	-
Anogeissusleicarpus in Ethyl acetate	-	-	-	-	-	24	20	18	14	10
Anogeissusleicarpus in methanol	-	-	-	-	-	-	-	-	-	-

Table 4: Positive and negative control using standard antibiotics.

A B	P o s i t i v e C o n t r o l			N e g a t i
	S a l m o n e l l a .	E.coli.	Shigella	
E R Y	-	20	-	A U
A M X	-	14	-	C R
O F L	-	24	-	N I
S T R	-	12	-	G E
C H L	-	24	**24**	C O
C R O	-	-	-	O F
G E N	-	2 . 0	**20**	A M
P F X	-	-	-	C P
C O T	-	-	-	T E
C P X	-	-	-	P F

Table 5; Qualitative Analyses of the Phytochemical Screening of Medicinal Plant

s a m p l e	Alkaloids	Glycosides.	Steroids	Anthraquinone	Phenol	Tanin	Saponin	Flavonoids
Piliostigmareticulatum	+ V E	+ V E	+ V E	N D	+ V E	+ V E	+ V E	+ v e
Anogeissusleicarpus	+ V E	+ V E	- V E	+ V E	+ V E	+ V E	+ V E	+ v e

Table 5; Quantitative Analyses of Minerals present in Plants (mg/ml)

SAMPLE	N a	K	C a	M g	Z n	F e	P b	c u	M n	p
Piliostigmareticulatum	14.65	15.24	19.78	20.10	20.32	21.14	1.20	1.24	6.21	18.99
Anogeissusleicarpus	120.93	23.15	29.37	24.72	17.38	20.31	2.78	3.09	4.96	27.21

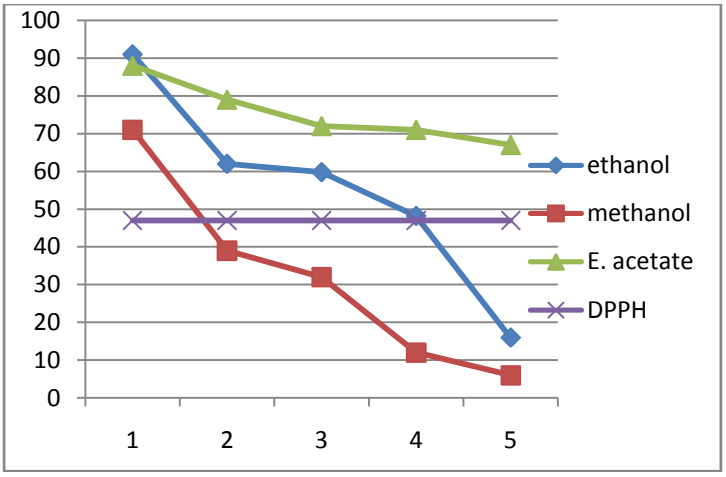


Fig 1: Graph Showing The Radical Scavenging Ability Of*a. Leiocarpus* % Inh

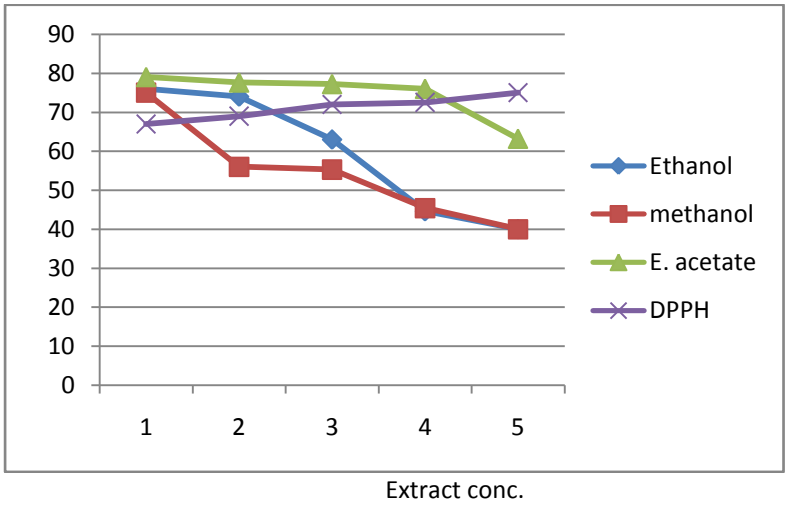


Fig 2. Graph Showing the Radical Scavenging Ability Of*p. Reticulatum %Inh.*

The scavenging ability of the two plants (Figs.1 and 2) .reflect he oxidative ability of the plants. The methanol extract of *Piliostigmareticulatum* showed the greatest scavenging ability while the ethanol and methanol extracts of *Anogeissusleiocarpus* showed similar scavenging ability.

Antioxidant agents are compounds that have the potentials to scavenge reactive oxygen species of free radicals. These free radicals play important roles in energy production, synthesis of some biomolecules, phagocytosis and cell growth (Adeyanju *et al.,* 2013). The results of the scavenging test showed that ethanol extract was the best scavenger of the solvents and was able to reduce the oxygen attached to the free radical by about 60%. Alcohol preparation is more useful than the aqueous one in medical approach, particularly in case when high activity of preparation is desired during anti-cancer therapy or other degenerative diseases.

CONCLUSION

In *vitro* evaluation of plants for antimicrobial property is the first step towards achieving the goal for developing eco-friendly management of infectious diseases of humans by search for new bio-molecules of plant origin. The optimal effectiveness of a medicinal plant may not be due to the one main active onstituent, but may be due to the combined action of different compounds originally in the

plant. The effectiveness of plant extract against a particular pathogen is affected by various intrinsic and extrinsic factors. The results of this study revealed that the stem bark of *P. reticulatum* and *A. leiocarpus* have antimicrobial activities and high radical scavenging ability.These properties lends support to the traditional use of these plants. These plants can thus be included in the phytomedical database due to their broad-spectrum and radical scavenging ability. However, further studies could be carried out to ascertain their level of toxicity and the mode of action in the human body

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