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Identification and Charaterization of Fatty Acids, Phytohemical Properties and Antibacterial Effect of the Ethyl Acetate Extract of Piliostigma Reticulatum

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ABSTRACT

The ethyl acetate extract of Piliostigma reticulatum was investigated for its antibacterial activity, phytochemical contents and the fatty acid composition using known methods. The plant was active against three of the five test organisms Shigella dysenteriae, Staphylococus aureus and Streptococcus pyogenes with zones of inhibition of 20mm, 16mm and 18mm respectively. Whereas Salmonella typhi and Pseudmonas aeruginosa were resistant to the plant extract. The comparative antibiotic test showed Salmonella typhi to be largely resistant to the five antibiotics used while other bacteria were sensitive to only one antibiotic ranging from mildly sensitive to sensitive according to the classification of the CLSI. The Multiple antibiotic resistance index (MARi) of the test bacteria showed high level of antibiotic resistance in the bacteria strains used. All bacteria had MARi of 5 except Pseudomonas aeruginosa which had MARi of 2.5.Phytochemicals in P. reticulatum include Alkaloids, glycosides, steroids, phenols, tanins and saponins. Nutritional elements include Na ,K, Ca, Mg, Zn, Fe, Pb and Cu. Fatty acids composition include decanoic acids, octdecanoic acids, hectadecanoic acids, 6- Octadecanoic, 1-(+)-Ascorbic acid (2.87%), 2, 6-dihexadecanoate ;Dipalmitate, Octadecanoic acid, 2-hydroxyl-1, 3, propanediylester, 15-Hydroxypentadecanoic acid, Hexadecanoic acid, 1-(hydroxymethyl)-1, 2-ethanediylester. Docosanoic anhydride, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethylester, Hexadecanoic acid, 2, 3- dihydroxypropyl ester, Hexadecanoic acid, 1-(hydroxymetyl)-1, 2-ethanediyl ester and Pentadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester. The fatty acid profile of the plant could be responsible for the antibacterial activity of the plant. The result of this study underlines the chemotherapeutic potential of this plant.

Keywords: *Fatty acid profile. Antibacterial, phytochemicals, Multiple antibiotic resistance index (MARi), Piliostigma reticulatum, Ethyl acetate, nutritional elements.*

INTRODUCTION

Recently, due to the increase in the rate of resistance to conventional antibiotics, serious attention has shifted to investigating medicinal plants to substantiate the claims of cure made by traditional healers and thus provide scientific basis for their efficacy (Akinsinde and Olukoya, 1995).Medicinal plants and marine organisms are rich in phytochemicals and are natural sources of many antimicrobial compounds (Hughes and Fenical, 2010; Smith,*et al*., 2010;Shannon, and Abu-Ghannam, 2016; Hayashi, 2013). Plant componentswith antimicrobial activity include alkaloids, sulfur-containingcompounds, diterpenes/terpenoids, (Khameneh,*et al*, 2019) fatty acids (Yoon*et al*., 2018; McGaw *et al*., 2002; Bergsson, 2010), some carbohydrates (Pasdaran and Hamedi, 2017), steroidalglycosides, and phenolic compounds (Hemaiswarya,*et al*., 2008).

Piliostigma reticulatum(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 upto 30ft in height with an evergreen, dense spreading crown.*Piliostigma reticulatum* is widely used in Africa as a traditional medicine for the treatment of a wide range of diseases including epilepsy, anxiety, and agitation. The leaf extract was found to have antimicrobial activity. In Sudan (Nuba mountains in particular), it is widely used to dress new wounds and as well puerperal sepsis. Moreover

its fruit is eaten and used to prepare juice (Kafi *et al.*, 2018). The present study seeks to evaluate the antimicrobial properties and the fatty acid components of the ethyl acetate extract of the stem bark of *Piliostigma reticulatum.*

Essential oils and their components are catching attention as natural antimicrobial agents that can be used in treating many diseases, in food preservation, complementary medicine and natural therapeutics and also for other cosmetic purposes (Mohadjerani *et al*., 2016). Fatty acids are important constituents of plants and are commonly known to possess antimicrobial activities. They can act as anionic surfactants and are known to have antibacterial and antifungal properties at low pH (Hayes and Berkovitz, 1979).

MATERIALS AND METHODS

Sampling and Preparation of Plant Sample

The test bacteria and fungi were obtained from the Department of Microbiology, Obafemi Awolowo University, Ile-ife, Osun State. The organisms were collected and maintained by regular subculturing on nutrient and potato dextrose agar slants respectively. The test organisms were subjected to biochemical test to confirm the authenticity of the organisms.

Extraction Procedure

Ethyl acetate extract was prepared by dissolving 250gm of powdered plant sample in 500mls of the solvent. The suspension was allowed to stand for 5 days (120hr) at room temperature of 28^{o}C +1^{o}C with constant agitation using the magnetic stirrer.The suspension was then filtered using a sterile muslin cloth and a Whatmann No 1 filter paper as described by Fabricant and Fansworth (2001). The collected filtrate was evaporated to dryness using the rotary evaporator after which the extracts were kept in sterile bottles until ready for use. The crude extract was reconstituted using 25% DMSO for antimicrobial test.

Collection and Maintenance of Microorganisms

The test bacteria and fungi were obtained from the Department of Microbiology, Obafemi Awolowo University, Ile-ife, Osun State. The organisms were collected and maintained by regular sub-culturing on nutrient and potato dextrose agar slants respectively. The test organisms were subjected to biochemical test to confirm the authenticity of the organisms.

Preparation and Standardization of Inocula

Test organisms were suspended in Nutrient broth and incubated for 4 hours to obtain a concentration corresponding to McFarlands constant (0.5 X 10^{8}cfu/ml). The inocula were standardized with the prepared barium sulphate according to Lonsway and Kevin, (2016). One percent (1%) of solution of sulphuric acid was prepared and mixed properly. Also, 1% solution of Barium chloride was prepared by dissolving 0.5g of dehydrated barium chloride ($BaCl_2.H_2O$) in 50ml of distilled water. A 0.5ml aliquot of barium chloride solution was added to 99.5ml of the sulphuric acid solution and it was the mixed together. The solution was transferred into a capped tube of the same type used for both the control and the test inocula. The solution was kept at room temperature of +4^{O}C (Cheesebrough, 2000).

Determination of Antibacterial Activities of Extract

Antibacterial Assay of the plant extract was carried out using the agar well diffusion methodof Perez *et al.*, (1990),Sterile Petri dishes were inoculated by the pour plate method. One ml (1ml) of the test inoculum was pipetted aseptically into each Petri dish and about 20 ml of sterilized nutrient agar was poured into the inoculated Petri dish.

The agar plates were allowed to set. Wells of 6mm diameter were made over the agar plates equidistant from each other using sterile cork borer and 0.5ml of each plant extracts of different concentrations as prepared by the serial dilution were added to the wells using a micropipette. The extracts were allowed to diffuse into the agar for about 20 minutes after which the plates were incubated for 24 h at 37^{o}C. Thereafter, the diameter of inhibition zones formed around each well was measured in mm and recorded. The experiments were carried out in triplicates and the average values recorded.

Determination of Activity of Standard Antibiotics on Test Organisms

Multiple Antibiotic Resistant Index (Mari) of test Organisms

The MAR index for the resistant bacterial isolates was determined according to the procedure described by Krumperman (1983). The indices were determined by dividing the number of antibiotics to which the organism were resistant to (a) by the number of the antibiotics tested (b), MARi= a/b Resistance to

three or more antibiotics is taken as MAR and MAR greater that 0.2 indicates a high risk source of contamination where antibiotics are often used.

Determination of Minimum Inhibitory Concentration (MIC) of Plant Extracts Against test Organisms

A modified method of Weigand *et al.* (2008) was adopted in the determination of MIC. The MIC of the extracts was determined by diluting the various concentrations with nutrient broth. A 1ml aliquot of a serial dilution of 100mg/ml, 60mg/ml, 40mg/ml, 20mg/ml and 10mg/ml of the extracts was separately added to test tubes containing specifically 0.1ml of standardized inoculum of 1 to 2 X 107cfu/m. The tubes were incubated aerobically at 37^{o}C for 18-24hrs. Two control tubes were prepared for each test batch. This is as follows: tube containing extracts and the growth medium without inoculums (antibiotic control) and the tube containing the growth medium and the inoculums (organism control). The inocula were then plated and inoculated at 37^{o}C for 24 hrs. The MIC was determined as the lowest concentration of the extracts exhibiting no visible growth (no turbidity) when compared with the control tubes.

Determination of Antifungal Properties

The radial well diffusion method of Duru *et al.* (2003) was employed in determining the antifungal effect of plant extracts. Prepared plates of potato Dextrose agar already mixed with concentrations extract was allowed to gel. A 3mm mycelia plug of a 7 day old culture of each fungus was placed in inverted position at the center of the plate for the organism to be in direct contact with the extracts. The plates were incubated at 28^{o}C ± 1^{o}C for 5 days. The experiment was carried out in triplicate and the average mycellial growths were recorded. The percentage radial inhibition was calculated and recorded. Control experiment was also carried out using plates without extracts.

Phytochemical Screening of Plants

Phytochemical screening were performed using standard procedures Qualitative phytochemical screening of medicinal plant parts and their recipes were carried out by means of some specific methods. Alkaloids, flavonoids, tannins and saponins were detected by the Tyler (2019) and Harborne [1973] method. Quantitative phytochemical analyses were carried by using the Harborne [1973] and Obadoni 2002] methods for the determination of alkaloids, the Boham [1994] method for flavonoids and the Obadoni (2002] method for saponins.

Elemental Evaluation of Plant

Elemental content of plant were determined using known methods (Brima, 2017; Hseu, 2004; Zafar *et al.*, 2010). Samples in powder form were used for Atomic Absorption Spectrophotometer (AAS). Each plant material (0.25 g) were taken in 50 ml flask and add 6.5 ml of mixed acid solution that is, Nitric acid (HNO3), Sulfuric acid (H2SO4) and Perchloric acid ($HClO_4$) (5:1:0.5) The sample boiled in acid solution in fume hood on hot plate (model VWR VELP scientifica, Germany) till the digestion has been completed which was indicated by white fumes coming out from the flask. Thereafter, few drops of distilled water were added and allowed to cool. Then these digested samples were transferred in 50 ml volumetric flasks and the volume was made up to 50 ml by adding distilled water in them. Then filter the extract with filter paper (Whatmann No. 42) and filtrate were collected in labeled plastic bottles. The solutions were analyzed for the elements of interest utilizing Atomic Absorption Spectrometer Shimadzu AA-670 with suitable hollow cathode lamps. The percentages of different elements in these samples were determined by the corresponding standard calibration curves obtained by using standard AR grade solutions of the elements i.e. K+, Mg+2, Ca+2, Na+, Fe+2, Co+3, Mn+2, Cu+3, Cr+3, Zn+2, Ni+3, Li+1, Pb+4 and Cd+2.

Purification of Crude Extracts

Crude extract was subjected to purification by column chromatography using the method of Cosa *et al.*, (2006). Fraction collected were spotted on chromatographic plates and subjected Thin Layer chromatography to ascertain purity of fractions.

Identification of Fatty Acid Components Using GCMS

Ethyl acetate extracts of Stem bark of *P. reticulatum*were analyzed with the help of GC-MS analyzer (Perkin Elmer Gas Chromatography- Mass Spectrum). On Elite-1 column the date was generated. The carrier gas helium (99.999%) was used at flow rate of 1 ml

per min in split mode (10:1). 8µ of sample was injected to column at 250°C injector temperature. Temperature of oven starts at 60°C and hold for 6min and then it was raised at rate of 10°C per min to 300°C without holding. Holding was allowed for 6 min at program rate of 5°C per min. temperature of ion sources was maintained at 240°C. The injector temperature was set at 250°C and detector temperature was set at 260°C. The mass Spectrum of compounds present in samples was obtained by electron ionization at 70eV and detector operates in scan mode 50 to 600Da atomic units. A 0.5 seconds of scan interval and fragments from 50 to 600Da was maintained. Total running was 40 minutes.

RESULTS AND DISCUSSION

Antibacterial Effect of Plant Extract

The extract of *P. reticulatum* at 100mg/ml was active against S, *dysenteriae, S. aureus* and S. *pyogenes* with zones of inhibition of 20mm, 16mm and 18mm respectively while it had no effect of *S. typhi.* Comparative antibiotic sensitivity test showed the test organisms to be resistant to the five antibiotics used in this student except for *S. aureus* that was susceptible to chloramphenicol and *S. pyogenes* that was sensitive to cotrimoxazole with zones of inhibition of 18 and 14 mm respectively (Table 1).

Table1. *Antibacterial activity of plant extract*

	Zone of inhibition in mm					
	P. reticulatum extract	Amoxyllin	Chloramphenicol	Cotrimoxazole	Gentamycin	Ciprofloxacin
Shigella dysenteriae	20	-		-	-	-
Staphylococcus aureus	16	-	18	-	-	-
Streptococcus pyogenes	18	-	-	14	-	-
Salmonella typhi	-	-	-	-	-	-
Pseudomonas aeruginosa	-	-	-	-	12	12

Legend - Means no Activity

According to theCLSI document M100-S23 (M02-A11) Disc diffusion supplemental tables, zone of inhibition ≥18 for chloramphenicol is said to be sensitive, so *S. aureus* can be said to be sensitive to chloramphenicol. Other organisms are resistant according to the table.

Mari of Test Organisms

The multiple antibiotic resistance index calculated is shown in table 2. From the standard rule, an organism that is resistant to two or more antibiotics is said to be a high risk organism (Rotchdell and Paul, 2016). All test organisms used were resistant to more than one antibiotics. *S,dysenteriae* was resistant to all the antibiotics used with MARi of 5. Similarly, *S. aureus*, *S. typhi*and*S. pyogenes* had MARi of 5 while P. aeruginosa had MARi of 2.5. The two parameters used to measure the level of resistance (i.e MARi and number of antibiotics resistant to) show all the test organisms to be highly resistant to standard antibiotics.

Table2. *Multiple antibiotic resistance index of test Organisms*

Organisms	MARi
`Shigella dysenteriae	5
Staphylococcus aureus	5
Streptococcus pyogenes	5
Pseudomonas aeruginosa	2.5
Salmonella typhi	5

MIC of Plant Extract

The MIC values of the plant extracts against test organisms are represented in fig 1. The MIC of the extract for *S. dysenteriae* and *S. pyogenes* was 20mg/ml while MIC for *S. aureus* was 40mg/ml while the extract did not show any inhibition for *S. typhi* even at 100mg/ml.

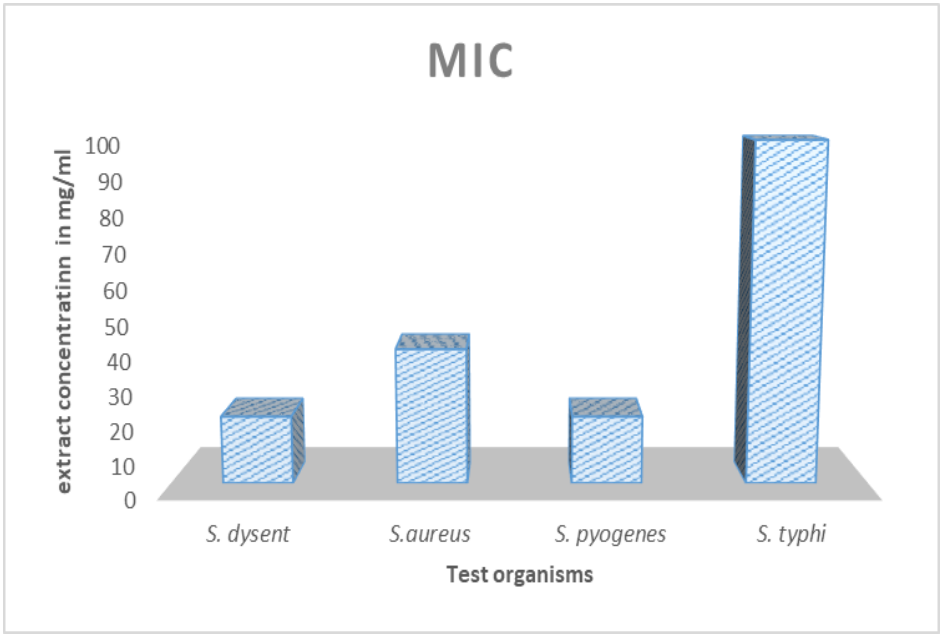


Fig1. *The Minimum inhibitory concentration (MIC) of plant extract against test organisms*

Antifungal Activity of Plant Extract

P. reticulatum did not show any antifungal activities against *Aspergillus flavus, Cuninghemella elegans, Aspergillus fumigatus and Syncephalastrum racemosum* used in this study.

Phytochemical Content of Plant

This study revealed the presence of alkaloids, glycosides, steroids, phenols, tanin, and saponin in *P. reticulatum*while Anthraquinone was not discovered *(*Table 4).

Table4. *Phytochemical content of P. reticulutum*

Plant	Alkaloids	Glycosides	Steroids	Anthraquinones	Phenol	Tanin	Saponin
P. reticulatum	+ve	+ve	+ve	ND	+ve	+ve	+ve

Legend;

+ve----- Present

ND-----Not discovered

Elemental Composition of Plant

P. reticulatum was found to contain Na (14.62%),, K (15.24%), Ca (19.78%), Mg (20.10%), Zn (20.32%), Fe (21.14%), Pb (1.20%) and Cu (1.24%) (Table 5).

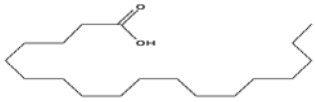
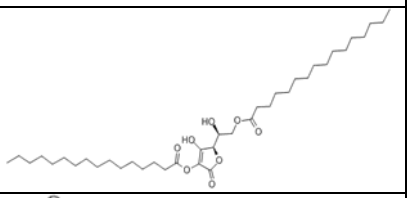
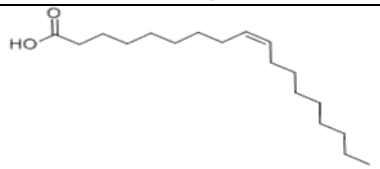
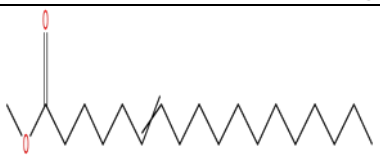
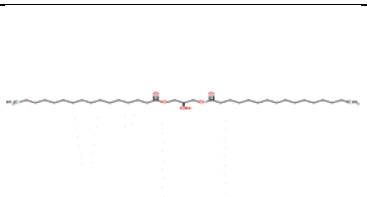
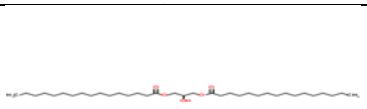
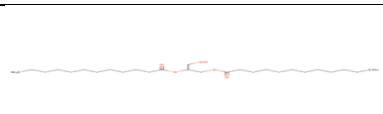
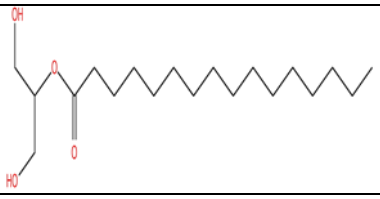
Table5. *Elemental content of P. reticulatum*

	Elemental content in mg/g							
Plants	Na	K	Ca	Mg	Zn	Fe	Pb	Cu
P. reticulatum	14.62	15.24	19.78	20.10	20.32	21.14	1.20	1.24

Fatty Acid Content of P. Reticulatum

The GCMS analysis of the ethyl acetate extract of *P. reticulatum* revealed the presence of fatty acid likeOleic acid (50.80%) which is the most abundant fatty acid in *P. reticulatum*followed by the octadecanoic acids (10.78%) The next most abundant is, hexadecanoic acids (3.15%),and finally the octadecanoic acids (2.87%), the others are stereoisomers of each class of fatty acids which are; 6- Octadecanoic, 1-(+)-Ascorbic acid (2.87%), 2,6-dihexadecanoate; Dipalmitate, Octadecanoic acid, 2-hydroxyl-1, 3, propanediylester, 15-Hydroxypentadecanoic acid, Hexadecanoic acid,1-(hydroxymethyl)-1, 2-ethanediylester. Docosanoic anhydride, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethylester, Hexadecanoic acid, 2, 3- dihydroxypropyl ester, Hexadecanoic acid, 1-(hydroxymetyl)-1, 2-ethanediyl ester, Pentadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester. The structures, chemical formula, molecular weight and quantity of the identified fatty acids are presented in table 6.

Table6. *Fatty acid components of ethyl acetate fraction of P. reticulatum*

Peaks	R. Time	Compound / mol formula	Mol weight	area	structure
49	22.383	N, hexadecanoic acid(Palmitic acid) $C_{16}H_{32}O_2$	256	10.78%	
		Octadecanoic acid $C_{18}H_{36}O_2$; Stearic acid Hydrofol	284	10.78%	
		1-(+)-Ascorbic acid,2,6-dihexadecanoate;Dipalmitate $C_{38}H_{68}O_8$	652	10.78%	
75	24.525	Oleic acid $C1_8H_{34}O_2$ Octadeer	282	50.80%	
		6- Octadecanoic $C_{18}H_{34}O_2$	282	50.80%	
55	27.183	Octadecanoic acid,2-hydroxyl-1,3, propanediylester. $C_{39}H_{76}O_5$	624	2.87%	
		Hexadecanoic acid,2-hydroxy-1,3-propanediylester. $C_{35}H_{68}O_5$	568	2.87%	
		15-Hydroxypentadecanoic acid $C_{15}H_{30}O_3$	258	2.87	
		Hexadecanoic acid,1-(hydroxymethyl)-1,2-ethanediylester. Palmityl. $C_{53}H_{68}O_5$	568	2.87	
		Docosanoic anhydride $C_{44}H_{86}O_3$	662	2.87	
56	27.433	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethylester $C_{19}H_{38}O_4$	330	3.15	

		Hexadecanoic acid, 2,3-dihydroxypropyl ester $C_{19}H_{38}O_4$	330	3.15	
		Hexadecanoic acid,1-(hydroxymetyl)-1,2-ethanediyl ester $C_{35}H_{68}O_5$	568	3.15	
		Pentadecanoic acid,2-hydroxy-1-(hydroxymethyl)ethyl ester $C_{18}H_{36}O_4$	316	3.15	

DISCUSSION

More than 60% of the word population depend on medicinal plants and herbal medicine in the treatment of various ailments and diseases (Akinyemi *et al.,* 2000) such as diarrhea (Yelemou *et al.,* 2007; Dosso and N'Guessan, 2012). This could be attributed to affordability, accessibility, in the economic sense and socially, an uneven distribution of health personnel between rural and urban areas.

P. reticulum is used in the treatment of ailments such as epilepsy, anxiety, and agitation (Kafi *etal.,* 2018). The result obtained in this study confirms the traditional uses of this plant in the treatment of bacterial infections. *S. dysenteriae* which is often implicated in bloody or hemorrhagic diarrhea/ Shigellosis (Al-Dahmoshi *et al.,* 2020; Chang *et al.,* 2016) was sensitive to the extract of *P. reticulatum.*This report is supported by the report of several authors.In their work, Awe and Omojasola (2009) demonstrated that ethanolic leaf extract of *P. reticulatum* had appreciable antibacterial properties. Similarly, N'Guessan *et al* (2015) also reported the antimicrobial activity of the dichloromethane fraction of the stem bark of *P. reticulatum* against resistant strains of bacteria used except *S. dysenteriaea* and *S typhimurium.*

Interestingly, the plant extract was not active against *Pseudomonas aeruginosa*, an organism known to infect wounds (Augustine, 2016) whereas, *P. reticulatum* is known to be used in the treatment of wounds (Kafi *et al.,* 2018). Several factors could be responsible for this. The compound extracted by the solvent used may not have effect on the organisms. Zerbo et *al.*.2010) recorded high activity of the methanol and aqueous extract against all the test organisms used.

Although the extract used in this study showed no antifungal activities against the test fungi, in a similar study by the same author, the ethanol and methanol extracts of the same plant showed appreciable fungistatic activity against *A, flavus* and *S. racemosum* respectively (Daniels, 2018).. On the Other hand Osuntokun and Thonda (2016) recorded antifungal activity of the leaf extract of *P. reticulatum* against*Aspergillus flavus* and *Candida albicans* This inconsistencies may also be due to the extraction solvent as well as the part of the plant used in each study.

There was a high level of resistance of the test organisms to standard antibiotic. All the test organisms were resistant to Amoxyllin, while *S, aureus* was sensitive to chloramphenicol (18mm), *S. pyogenes*was mildly sensitive to cotrimoxazole (14mm) and *P. aeruginosa* was also mildly sensitive to Gentamycin (12mm) and ciprofloxacin (12mm).

Multiple antibiotic resistance (MAR) has beenattributed to the presence of plasmids whichcontain one or more resistance genes, eachencoding a single antibioticresistance phenotype(Daini *et al.,* 2005).Antibiotic resistance increasingly compromises theoutcome of many infections that were, hitherto treatable (Okeke *et al.,* 2005).Multiple antibiotic resistance (MAR) indexing hasbeen shown to be a cost effective and valid methodof bacteria source tracking. The high prevalence of antibiotic resistance observed in this study is a cause for alarm. *P. aeruginosa* is notorious for the high level of antibiotic resistance. The values of MARi for *P. aeruginosa* is higher than as observed by Olayinka *et al.* (2009) and Davis and Brown(2016). According to Deyno *et al.,* (2017) the multidrug resistant status of *S. aureus*

used in their study was 100% which agrees with the result of this study.*Shigella dysenteriae* showed absolute resistance to all the antibiotics used. This result is contrary to the opinion ofPourakbari*et al.*,(2010) who found *S. dysenteriae*to have 100% sensitivity to the antibiotics used in their study. The difference in opinions of this and other authors could be due to the type of antibiotics used. Some authors reported the resistance of *S. dysenteriae* toampicillin, co-trimoxazole (Jain *et al*., 2005) and chloramphenicol (Ozmert *et al.,* 2005) but *S. dysenteriae* is known to be sensitive to second generation cephalosporin (Pourakbari*et al.*, 2010). Rahman, (2015) reported the resistance of *Salmonella typhi* toceftriaxone (Mushtaq, 2006) cefixime (Capoor, 2006). This also supports the result of this study. Multiple drug resistance against 6 to 8drugs has been reported in several Salmonellaserotypes of animal and human origin in India byseveral workers (Ashwini *et al*., 2013; Singh *et al*.,2012).

The antimicrobial activity of this plant may not be unconnected with the phytochemicals present. In this work, the phytochemicals identified include; Alkaloids, Glycosides, Steroid, Phenol, Tannin and Saponin. These chemical compounds are known to play important roles in the medicinal values of plants, these embedded phytochemicals produce definite physiological actions on human body (David *et al.*, 2014). Tanin is useful to human physiological activities such as phagocytic cells, host mediated activity and a wide range of anti-effective action (Lamai, 2009). Alkaloids, tannins and other biomolecules are known for their antioxidant, antifungal, anticancer, antiviral, anti-inflammatory and antiophidic activities (Osuntokun and Thonda, 2016).

The mineral content of the plant shows the high content of iron, zinc, magnesium calcium, potassium and sodium. These minerals help in maintaining the salt and ionic balance in the human body and also helps the nerves, cells and tissues to function properly. This result suggests that the stem bark may be of great physiological significance especially in part of the world were muscle weakness, increased nervous system irritability and spontaneous action potential generation in neurons are relatively rampant (Ighodarro *et al.*, 2012). Calcium helps to keep teeth and bones strong, iron is important for cell growth, development, and normal body functions. It also helps the body make some hormones and connective tissue and also helps to transport blood from the lungs to the tissues. Magnesium helps the body regulate muscle and nerve function, blood sugar levels, and blood pressure. It also helps the body make protein, bone, and DNA (Zhang *et al.*,2020). Potassium is a mineral that cells, nerves, and muscles need to function properly. It helps the body regulate blood pressure, heart rhythm and the water content in cells. It also helps with digestion ( ). Zinc is found in cells throughout the body. It helps the immune system fight off invading bacteria and viruses. The body also needs zinc to make proteins and DNA, the genetic material in all cells, it also helps in wound healing (Romito and Rhonda,2019).

Essential oils are the main essence of medicinal plants. The GCMS profile of ethyl acetate extract of *P. reticulatum* showed the presence of fourteen (14) fatty acids. Fatty acids have on occasions been discovered to be responsible in part for the antimicrobial activities of plants (Cerdeiras *et al.* 2000, Dilika *et al.* 2000, McGaw *et al*. 2002, Yff*et al*. 2002). The plant oils are a heterogeneous mixture of fatty acids where some existed in trace amounts. The most abundant of these fatty acids is Oleic acid (50.80%). Oleic acid have been recorded to exhibit the inhibition of bacterial enoyl-acyl carrier protein reductase (FabI) (Zheng*et al.*, 2005).Fatty acids are also reported to have also been reported to have antifungal and antibacterial properties (McGaw, (2002; Seidel and Taylor, 2004). The antibacterial properties of this plant can be attributed to the high amount of oleic acid, however, the synergistic effect of other fatty acids and other components cannot be underestimated as many authors have authenticated the synergy of phytochemicals in medicinal plants. Dr.Duke's Phytochemical and Ethnobotanical databases [online] claimed octadecanoic acid acts as antiandrogenic, antiarthritic and anticoronary. . Another author also reported octadecanoic acid act as 5-alpha-reductase-inhibitor, cosmetic, flavor, hypocholesterolemic, lubricant, perfumery, propecic and suppository (Hema *et al.*, 2011). Hexadecanoic acid acts as anticancer, antioxidant, antimicrobial (Graikou *et al*., 2011) hypocholesterolemic, nematicide, pesticide, anti-androgenihemolytic and 5- Alpha reductase inhibitor (Praveenkumar*et al.*, 2010).

CONCLUSION

Although no research has been carried out on the fatty acid content of *Piliostima reticulatum.*

In the present investigation, the result justified the use of the plant in traditional medicine. The fatty acid composition and reference documents on the biological activities of fatty acid presupposes that the fatty acid content could be responsible for the antibacterial effect. The ethyl acetate extract of *Piliostigma reticulatum* had a wide range of antibacterial activity comparable to standard antibiotics as well as contain many biologically active components.

REFERENCES

[1] Akinyemi, K. O, Coker, A. O; Bayagbon C, Oyefolu, A.O.B, Akinside, K. A. and Omonigbehin, E. O. (2000). Antibacterial screening of five Nigerian medicinal plants against *S. typhi* and *S. paratyphi*. *Journal of the Nigerian Infection Control Association* **3**(1).

[2] Akinside, K. A. and Olukoya, D. K. (1995). Vibriocidal activities of some local herbs. *Journal of Diarrhoea Disease Research.***13**: 127-129.

[3] Ashwini C. Gopalakrishnan, , Nambi,R P, V Ramasubramanian, V Ghafur, K, and Thirunarayan, M A (2013), Antimicrobial susceptibility of Salmonella enterica serovars in a tertiary care hospital in southern India. *Indian Journal of Medical Research*. **137**. (4). 800-802

[4] Awe, S, and Omojasola, P.F. (2009). A Comparative Study of the Antibacterial Activity of *Piliostigma reticulatum* Bark Extract with Some Antibiotics. *Ethnobotanical Leaflets* **13**: 1197-1204.

[5] Bergsson, G; Hilmarsson, H; Thormar, H. (2010). Antibacterial, antiviral and antifungal activities of lipids. In Lipids and Essential Oils as Antimicrobial Agents; Thormar, H., Ed.; Wiley: Chichester, UK, pp. 47–80

[6] Boham B., Kocipai-Abuyazan R. (1994). Flavonoids and condensed tannins from the leaves of *Vaccinum raticulatum* and *Vaccinum calycinium. Pacific. Science*.; **48**: 458–463

[7] Brima, E. I. (2017). Toxic elements in different medicinal plants and the impact on human health," *International Journal of Environmental Research and Public Health*, vol. **14**(10) 1209, 2017.

[8] Capoor MR, Nair D, Hasan AS, Aggarwal P and Gupta, B. (2006). Narrowing therapeutic options in typhoid fever, India. *Southeast Asian Journal of Tropical Medicine and Public Health*. ; **37**: 1170-1174.

[9] Cerdeiras MP, Fernández J, Soubes M, Vero S, Ferreira F, Moyna P, Olano I, Vázquez A (2000) A new antibacterial compound from *Ibicella lutea*. *Journal of Ethnopharmacology* **73**: 521–525

[10] Zheng.C.J, Yoo, J ., Lee, T.G, Cho, H.Y. Kim, Y.H. and Kim, W.G.(2005). Fatty acid synthesis is a target for antibacterial activity of unsaturated fatty acids. *FEBS Lett.*; **579**(23): 5157-62.

[11] Daini, O.A., Ogbolu, O.D. and Ogunledun A. (2006). Quinolone resistance and R-plasmids of some Gram negative enteric bacilli. African Journal of Clinical Experiments in Miccrobiology, 2005: 6 (1): 14-20.

[12] Daniels, A.O. (2018). : The antimicrobial effect, phytochemical properties and toxicological studies of *Piliostigmareticulatum* (Benths) and *Cleistopholis patens* (Hoesch). (PhD thesis).

[13] David A. A, Emmanuel O. A, Olayinka A. A, Olusegun F. A, and Anthony I. O (2014). Evaluation of Antibacterial and Antifungal Properties of *Alchornea laxiflora*(Benth.) Pax and Hoffman Evidence-*Based Complementary and Alternative Medicine*: 1-6 http://dx.doi.org/10. 1155/2015/684839

[14] Davis, R and Brown, P.D. (2016). Multiple antibiotic resistance index, fitness and virulence potential in respiratory Pseudomonas aeruginosa from Jamaica. Journal of Medical Microbiology**; 65**(4): 261-271.

[15] Dilika F, Bremner P.D, and Meyer J.J.M (2000) Antibacterial activity of linoleic and oleic acids isolated from *Helichrysum pedunculatum*: a plant used during circumcision rites. *Fitoterapia* **71:** 450–452.

[16] Dosso K, N'Guessan B.B, Bidie A.P, Gnangoran B.N, Méité S, N'Guessan D, (2012). Antidiarrhoeal activity of an ethanol extract of the stem bark of *Piliostigma reticulatum* (Caesalpiniaceae) in rats. *African Journal of Traditional Complementary and Alternative Medicine*. **9:** 242–9.

[17] Graikou K, Kapeta S, Aligiannis N, (2011). Chemical analysis of Greek pollen – antioxidant, antimicrobial and proteasome activation properties. Chemical Cent Journal**;** **5**(1): 33.

[18] Haley A, Gillis, J and Williams J(2016). *Pseudomonas aeruginosa* wound infections: a critical appraisal of topical antiseptics. Dalhousie Medical Journal, **42**(1). 6434.

[19] Harborne J. (2019). Phytochemical methods: London Chapman and hill Ltd. [(accessed on 23 October 2019); 1973: 49–188. Available online: https://trove.nla.gov.au/version/45450216

[20] Hayashi, M.A.; Bizerra, F.C.; Da Silva, P.I. (2013). Antimicrobial compounds from natural sources. Fronties in Microbioogy.**, 4,** 195.

[21] Hema R, Kumaravel S, Alagusundaram K. (2011). GC-MS Study on the bioactive components and anti-cancer activities of *Solanum surattense*. *Cancer Biology.;***1**(1): 13–17.

[22] Hemaiswarya, S.; Kruthiventi, A.K.; Doble, M. Synergism between natural products and antibiotics against infectious diseases. *Phytomedicine* 2008, 15, 639–652.

[23] Hseu Z.H. (2004) Evoluating heavy metal contents in nine composts using four digestion methods. Bioresource Technology**. 95 (**2004) pp. 53-59.

[24] Hussein O. M. Al-Dahmoshi N. K. -Khafaji, K, WissamH.A. Salman, ,K. and Alabbasi, A.H. (2020). A review on shigellosis: Pathogenesis and antibiotic resistance. *Today Drug Invention Today* **15**(5): 793-798.

[25] Hughes, C.C.; Fenical, W. (2010). Antibacterial from the sea. Chemistry 2010, 16, 12512–12525.[CrossRef]

[26] Ighodaro, O.M Agunbiade, S.O. Omole J.O and. Kuti, O.A (2012). Evaluation of the Chemical, Nutritional, Antimicrobial and Antioxidant-vitamin Profiles of *Piliostigma thonningii* Leaves (Nigerian Species). *Research Journal of Medicinal Plants*, 6: 537-543.

[27] Jain SK, Gupta A, Glanz B, Dick J, Siberry GK. Antimicrobial-Resistant Shigella sonnei: Limited Antimicrobial Treatment Options for Children and Challenges of Interpreting In Vitro Azithromycin Susceptibility. Pediatr Infect Dis J. 2005; 24:494-7.

[28] Khameneh, B.; Iranshahy, M.; Soheili, V.; Fazly Bazzaz, B.S. (2019). Review on plant antimicrobials: A mechanistic viewpoint. *Antimicrobial Resistant Infection Control***. 8**, 118.

[29] Lonsway, D, and Ward, K (2016). Preparation of Routine Media and Reagents Used in Antimicrobial Susceptibility Testing, Clinical Microbiology Procedures Handbook, 4th Edition. *ASM Press*, Washington, DC. p.2.20. 1.1-2.20.1.4

[30] Lamai, R. S. (2009). Preliminary phytochemical and antibacterial screening of the ethanolic stem bark extract of Phyllanthusmuellerianus. Project submitted to the Department of pharmacognosy and drug development. Faculty of pharmaceutical Sciences, Ahmadu Bello University Zaria. **2009;** 34-35.

[31] Mohadjerani, M, Hosseinzadeh, R, and MHosseini, M (2016). Chemical composition and antibacterial properties of essential oil and fatty acids of different parts of *Ligularia persica* Boiss. *Avicenna Journal of Phytomedicine*. **6**(3): 357–365.

[32] McGaw LJ, Jäger AK, Van Staden J (2002) Isolation of antibacterial fatty acids from Schotia brachypetala. *Fitoterapia***73**: 431–433

[33] McGaw, L.J.; Jäger, A.K.; Van Staden, J. (2002). Antibacterial effects of fatty acids and related compounds from plants. South African Journal of Botany. **68,** 417–423.

[34] Mushtaq MA. What after ciprofloxacin and ceftriaxone in treatment of Salmonella typhi. Pakistan Journal of Medical Sciences. **22**: 51-54

[35] Obadoni B., Ochuko P (2000). Phytochemical studies and comparative efficacy of the Crudeextracts of some haemostatic plants in Edo and Delta States of Nigeria. *GlobalJournal of Pure and Applied* Sciences; **8:**203–208. doi:10.4314/gjpas.v8i2.16033. [CrossRef] [Google Scholar]

[36] Olayinka, A.T., Onile, B.A. and Olayinka, B.O. (2009). Antibiotic susceptibility and plasmid pattern of Pseudomonas aeruginosa from the surgical unit of a university teaching hospital in north central Nigeria. *International Journal of Medicine and Medical Sciences*; **1**(3): 079-083.

[37] Osuntokun OludareTemitope1, OA Thonda (2016). Comparative Study of the Antibacterial and antifungal Spectrum, Phytochemical screening, Elemental composition, Proximate analysis, Anti-nutrients, and Antioxidant potentials of Aqueous and Ethanol Extracts of *Alchornea Laxiofolia* and *Piliostigma reticulatum* Leaf on Pathogenic Isolates. *The Pharmaceutical and Chemical Journal*, **3**(2):1-11.

[38] Ozmert EN, Göktürk B, Yurdakök K, Yalçin SS, Gür D. Shigella Antibiotic Resistance in Central Turkey: Comparison of the Years 1987-1994 and 1995-2002. *Pediatric Gastroenterology and Nutrition Journal*. **40**: 359-62.

[39] Pasdaran, A.; Hamedi, A. Natural products as source of new antimicrobial compounds for skin infections. In The Microbiology of Skin, Soft Tissue, *Bone and Joint Infections;* Kon, K., Rai, M., Eds.; *Academic Press:* Cambridge, MA, USA, 2017; pp. 223–253.

[40] Praveenkumar P, Kumaravel S, Lalitha C. (2010). Screening of antioxidant activity, total phenolics and GC-MS study of *Vitex negundo. African Journal of Biochemical Research*; **4**(7): 191–195.

[41] Rahman, M.A. (2015). Antimicrobial Resistance Patterns of SalmonellaTyphi Isolated from Stool Culture. *Chattagram Maa-O- Shishu Hospital Medical College Journal* **14**, (1),

[42] Rotchell D, Paul D. (2016). Multiple Antibiotic Resistance Index. Fitness and Virulence Potential in Respiratory Pseudomonas aeruginosa from Jamaica. *Journal of Medical Microbiology*; **65**:251–271

[43] Romito K. and O'Brien, R (2019). Minerals: Their Functions and Sources. Medical Review

[44] Shamsoun Khamis Kafi Fatima Mekki Abdalla and Mohamedelfateh Salaheldin Eljack (2018). Evaluation of Antimicrobial Activity of a Sudanese Herbal Plant (Piliostigma reticulatum) Sudan Journal of Medical Sciences. 0.18502/sjms.v13i4.3599.

[45] Shannon, E.; Abu-Ghannam, N (2016). Antibacterial derivatives of marine algae: An overview of pharmacological mechanisms and applications. Mar. Drugs, 14, 81.

[46] Seidel, V.; Taylor, P.W. (2004). In vitro activity of extracts andconstituents of Pelagonium against rapidly growing mycobacteria.Int. J. Antimicrob. Agen, 23, 613-619.

[47] Singh, S., Agarwal, R.K., Tiwari, S.C., Singh, H.Antibiotic resistance pattern among the Salmonella isolated from human, animal and meatin India. Trop Anim Health Prod, 2012: 44(3): 665-74.

[48] Smith, V.J.; Desbois, A.P.; Dyrynda, E.A. Conventional and unconventional antimicrobials from fish, marine invertebrates and micro-algae. Mar. Drugs 2010, 8, 1213–1262.

[49] Toma, A., M and Bekele, M. (2017). Antimicrobial resistance profile of Staphylococcus aureus isolates isolated from ear discharges of patients at University of Hawassa comprehensive specialized hospital. BMC Pharmacol Toxicol. 2017; 18:35.

[50] Tyler V.E. (1993) Phytomedicines in Western Europe: Potential impact on herbal medicine inthe United States. [(accessed on 23 October 2019)]; ACS Publ. 1993 Available online: https://pubs.acs.org/doi/10.1021/bk-1993-0534.ch003. [Google Schola]

[51] Yelemou B, Bationo B, Yameogo G, Millogo-Rasolodimby J. Montpellier, France: CODEN; 2007. Traditional use and management of Piliostigma reticulatum on the central plateau of Burkina faso. Woods and Forests of the tropics. No 291(1)

[52] Yff BTS, Lindsey KL, Taylor MB, Erasmus DG, Jäger AK (2002) The pharmacological screening of Pentanisia prunelloides and the isolation of the antibacterial compound palmitic acid. Journal of Ethnopharmacology 79: 101–107

[53] Yoon, B.K.; Jackman, J.A.; Valle-González, E.R.; Cho, N.J. Antibacterial free fatty acids and monoglycerides: Biological activities, experimental testing, and therapeutic applications. Int. J. Mol. Sci. 2018, 19, 1114.

[54] Zafar, M Khan, M. A, Ahmad, M , Jan, G, Sultana, S, Ullah, K, Marwat, S.K, Ahmad, F, Jabeen, Nazir, A., Abbasi, A.M., Rehman, Z and Ullah, Z (2010) Elemental analysis of some medicinal plants used in traditional medicine by atomic absorption spectrophotometer (AAS). Journal of Medicinal Plants Research Vol. 4(19), pp. 1987-1990.

[55] Zhaorui Chang, Jing Zhang, Lu Ran, Junling Sun, Fengfeng Liu, Li Luo, Lingjia Zeng, Liping Wang, Zhongjie Li, Hongjie Yu and Qiaohong Liao (2016). RESEARCH ARTICLE Open Access The changing epidemiology of bacillary dysentery and characteristics ofantimicrobial resistance of Shigellaisolated in China from 2004–2014. BMC Infectious Diseases (2016) 16:685.

[56] Zerbo, A, Koudou, J., Ouédraogo, O. Ouédraogo, R and. GuissouI.P (2010).Antioxidant and antibacterial activities of Piliostigma reticulatum (DC). Hochst extracts. African Journal of Biotechnology Vol. 9(33), pp. 5407-5411.

[57] Zhang, F.F. Barr, S.I. and McNulty, H. Li, D and Blumberg, J.B. (2020). Health effects of vitamin and mineral supplements. BMJ 2020; 369:m2511.

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