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Antimicrobial and Free Radical Scavenging Potentials of *N*-Hexane and Ethyl Acetate Fractions of *Phyllanthus Fraternus*

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ABSTRACT

The genus *Phyllanthus* (Phyllantaceae) is widely used in the african system of traditional medicine and is reported to have various biological activities. In this study, antimicrobial and antioxidant activities of *n*-hexane and ethyl acetate fractions of *Phyllanthus fraternus* leaves were investigated. The antimicrobial screening was carried out against *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Salmonella typhi and Klebsiella pneumoniae,* using Agar-well diffusion method. The antioxidant activity was carried out using DPPH free radical scavenging capacity. The results show that fractions of *Phyllanthus fraternus* leaves have DPPH radical scavenging activities with IC_{50} value of 263.53 mg/mL and 143.56 mg/mL for *n*-hexane and ethyl acetate fractions respectively. For n-hexane fraction, the MICs of the extract were; 80 mg/mL against *K. pneumoniae* and *S. aureus*,120 mg/mL against *P. aeruginosa* and *S. typhi,* and 160 mg/mL against *E. Coli*. However, ethyl acetate fraction had MICs of 80 mg/mL against all test organisms except *S. aureus* (40 mg/mL). The n-hexane and ethyl acetate fractions of *Phyllanthus fraternus* leaves exhibited considerable antioxidant and antimicrobial properties, with ethyl acetate fraction been the most potent. This plant extract can be regarded as promising resource for antimicrobial and antioxidant drugs.

Keywords: Antioxidant; Antimicrobial; *Phyllanthus fraternus;* n-hexane, ethyl acetate fractions.

INTRODUCTION

Africa is endowed with large amounts of medicinal plants used for therapeutic intervention (Bashir *et al.,* 2015; Lawal *et al.,* 2015; Lawal *et al.,* 2016a). The importance of plants in medicine remains of greater relevance with the current global shift to obtain drugs from plants sources, as a result of which attention has been given to the medicinal value of herbal remedies for safety, efficacy and economy (Adebayo *et al.,* 2009). Plants constitute an important source of active ingredients which differ widely in terms of structure and therapeutic properties (Lawal *et al.,* 2016b). The continued investigation into the secondary plant metabolites for anti-infective properties has gained importance in recent years because of the alarming increase in resistance of pathogenic microorganisms to existing antibiotics. For instance, the emergence and spread of *Salmonella* resistance to many commonly used antibiotics (Ciprofloxacin, Ampicillin, Chloromphenicol, Amoxicillin) has been a subject of international concern (Tsobou *et al.,* 2015).

The recent growth in knowledge of free radicals and Reactive Oxygen Species (ROS) in biological systems is causing a medical revolution that promises a new age of health (Tsado *et al.,* 2016). Free radicals are highly reactive molecules generated during oxidation reactions which in turn initiate chain reactions resulting in to cellular damage (Lawal *et al.,* 2015b). There is substantial evidence implicating free radicals especially reactive oxygen species (ROS) in the etiology of more than one hundred degenerative disorders in humans including, arthritis, atherosclerosis, ischemia and reperfusion injury of many tissues, gastritis, diabetics, central nervous system injury, acquired immunodeficiency syndrome (AIDS) and cancer (Lawal *et al.,* 2016a)

Reports abound on the antioxidant activities of phytochemical constituents of medicinal plants (e.g. polyphenols, carotenoids, flavonoids, phenolics, vitamins C and E). These phytochemicals act as antioxidants by preventing damages to cell membrane due to cellular oxidative processes that may result in diseases (Soni *et al.,* 2015). For instance, natural polyphenols from plants have been found to exert their beneficial effect by removing free radicals, chelating metal catalyst, activating antioxidant enzymes, etc (Lawal *et al.,* 2016a).

Phyllanthus fraternus G.L.Webster (Phyllantaceae) is widely distributed in most tropical and subtropical countries, and have long been extensively used in folk medicine in Africa and most other countries for thousands of years in the treatment of a broad spectrum of diseases, such as disturbances of the kidney and urinary bladder, intestinal infections, diabetes, and the hepatitis B virus (Manjulatha *et al.,* 2008). The present study sought to evaluate antimicrobial and antioxidant activities of *n*-hexane and ethyl acetate fractions of *phyllanthus fraternus.*

MATERIALS AND METHODS

Chemicals

DPPH (2,2-diphenyl-1-picrylhydrazyl) and solvents use were obtained from Sigma-Aldrich (Steinhein-Germany), All solvents used for extraction were of analytical grade.

Plant Collection

Freshly harvested *Phyllanthus fraternus* leaves were procured from Bosso, area of Minna, Niger State, Nigeria. The plant was authenticated by a botanist at National Institute of Pharmaceutical Research and Development, Abuja, Nigeria.

Sources of Microorganisms

Pure isolates of *K. pneumoniae, S. aureus*, *P. aeruginosa, E.coli* and *S. typhi* were procured from Microbiology Unit, Faculty of Life Sciences Federal University of Technology, Minna, Nigeria. Biochemical test and Gram staining test were used to confirm the identity of the organism.

Extraction of plant Materials

Fresh leaves of *Phyllanthus fraternus* were grounded using a grinder mill. Exactly 200 g of the powdered plant was extracted with 600ml of methanol. The resulting extract was concentrated using rotary evaporator. The methanol extract was partitioned between n-hexane and water. The aqueous layer was further fractionated using different solvents in increasing order of polarity: *n*-hexane, chloroform and ethyl acetate. The fractions were collected and concentrated using rotary evaporator (Resona, Germany). The concentrated fractions were investigated for antimicrobial and antioxidant activities

Assay for antibacterial activity

Stock cultures were maintained at 4^{o}C on nutrient agar (HiMedia) slants. Active cultures for experiments were prepared by transferring a loopful of culture to 10 mL of nutrient broth (HiMedia) and incubated at 37 ^{o}C for 24 hours for bacterial proliferation (Jayaraman *et al.,* 2008). Antibacterial activity of n-hexane fraction of *Phyllanthus fraternus* leaves was carried out using agar-well diffusion method as described by Jayaraman *et al.,* (2008), using Ciprofloxacin (40 µg/mL) as standard drug. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by tube dilution method for each of the test organism in triplicates.

Estimation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity

The free radical scavenging activity of the *n*-hexane fraction was assayed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical was determined (Szabo *et al.,* 2007).

Statistical Analysis

All the experiments were carried out in triplicate and data obtained from the study were subjected to analysis of variance using statistical package for Social Science (SPSS) version 21 and presented as means ± SE of the mean.

RESULTS AND DISCUSSION

Figure 1 shows the results of scavenging radical ability of *n*-hexane and ethyl acetate fractions of *Phyllanthus fraternus* at various concentrations in comparison with same doses of ascorbic acid. The extract was found to exert antioxidants effect in DPPH radical scavenging assay with IC_{50} value of 263.53 mg/ml and 143.56mg/ml for *n*-hexane and ethyl acetate fractions respectively (Figure 2). The decrease in absorbance of DPPH caused by *n*-hexane fraction of *Phyllanthus fraternus* was due to the reaction between antioxidant molecules and radicals, which results in the scavenging of the radical by hydrogen donation.

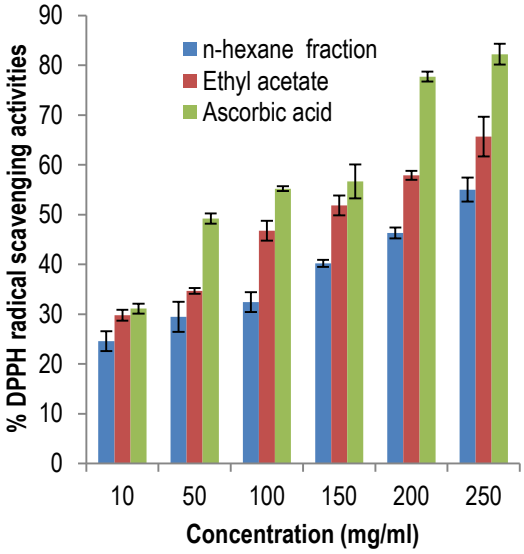

Figure 1: DPPH radical scavenging activities of *n*-hexane and ethyl acetate fractions of *Phyllanthus fraternus* leaves.

Many antioxidants compounds are present in natural products. Flavonoids are phenolic compounds with important functions in scavenging free radicals and thus play vital roles in preventing oxidative stress associated disorder (Nahak and Sahu, 2010). However, the IC_{50} value recorded in this study were higher than IC_{50} values of 41.05, 17.52 and 32.66 µg/mL reported for crude methanol fruit extracts of *Phyllanthus acidus*, *Phyllanthus emblica* and *Phyllanthus fraternus,* respectively (Manjulatha *et al.,* 2014). The quality and quantity of bioactive antioxidative agents in plants vary with the plant species, part of the plant used as well as the solvents used in the extraction process (Lawal *et al.,* 2014). Thus the higher IC_{50} value observed for fractions of *Phyllanthus fraternus* leaves could be attributed to species differences and part of the plant used.

The antimicrobial effects of plant extracts have been the subject of many studies during the last three decades (Tsobou *et al.,* 2015). Recenlty, many antimicrobial screening evaluation studies have been published based on traditional Chinese, African and Asian use of extractives that are plant-based (Suffredim *et al.,* 2004). In the present study, the results of antibacterial property of *n*-hexane and ethyl acetate fractions of *Phyllanthus fraternus* leaves (Tables 2 and 3 respectively) against tested organisms varied depending on bacteria tested and concentration (Ravikumar *et al.,* 2007; Rajasekharan and George, 2010).

Increase in the concentration of *n*-hexane and ethyl acetate fractions of *Phyllanthus fraternus* resulted in corresponding increase in the zones of inhibition. This linear relationship between the concentrations of extracts and zones of inhibition could be that the higher concentration of extracts causes a higher diffusion of the substances in the nutrient agar (Tsado *et al.,* 2016) The extracts were more active with greater zone of inhibition observed at concentrations of 120 and 160 mg/mL suggesting a dose dependendent growth inhibition (Tsado *et al.,* 2016). Antimicrobial activities of most medicinal plants are attributted to the presence of bioactive phytochemicals (Rice-Evans *et al.,* 1995). The methanol extract of *Phyllanthus fraternus* leaves have been reported to contain tannins, saponins, alkaloids, anthraquinones and resins. These phytochemicals reported to offer great pharmacological activites both in traditional and orthodox medicine could be responsible for the enhanced activity of the fractions of *Phyllanthus fraternus* leaves as shown in Tables 1 and 2. For n-hexane fraction, the MICs of the extract were 80 mg/mL against *K. pneumoniae* and *S. aureus*, 120 mg/mL against *P. aeruginosa* and *S. typhi,* and 160 mg/mL against *E. Coli*. The ethyl acetate fraction had MIC of 80 mg/mL

against all test organisms except for *S. aureus* where the MIC was 40 mg/mL (see Table 3). However, despite the higher zones of inhibition demonstrated by fractions of *Phyllanthus fraternus* leaves, the zones of inhibitions were lower on all test organism compare to zone of inhibitions demonstrated by standard antibiotics drugs (ciprofloxacin).

Table 1: Zones of inhibition of *n*-hexane fraction of *Phyllanthus fraternus* leaves against some pathogenic organism

Concen. (mg/mL)	*E. coli*	*K. pneumoniae*	*S. aureus*	*P. aeruginosa*	*S. typhi*
			Zone of inhibition (mm)		
40	-	-	-	-	-
80	-	12.00±0.50	14.00±0.10	-	-
120	-	16.00±0.50	21.00±0.10	12.00±1.00	16.00±0.50
160	18.00±0.00	-	11.00±0.50	15.00±0.50	16.00±0.10
180	20.00±0.50		11.00±0.05	23.00±0.60	20.00±0.05
Control (40 µg//mL)	18.00±0.00	28.00±0.00	32.00±0.56	28.50±0.40	19.00±0.55

Data represent means ± SEM of triplicate determination.

Table 2: Zones of inhibition of ethyl acetate fraction of *Phyllanthus fraternus* leaves against some pathogenic organism

Concen. (mg/mL)	*E. coli*	*K. pneumoniae*	*S. aureus*	*P. aeruginosa*	*S. typhi*
			Zone of inhibition (mm)		
40	-	-	5.03±0.70	-	-
80	5.89±0.59	12.45±0.46	9.47±0.38	8.90±0.89	9.08±0.90
120	7.45±0.90	11.45±0.21	9.92±0.36	12.35±0.79	12.30±0.52
160	11.80±0.46	19.90±0.05	13.90±0.55	13.79±0.29	17.08±0.79
180	13.89±0.97	24.79±0.55	16.05±050	19.56±0.89	22.47±0.92
Control (40 mg/mL)	18.00±0.00	28.00±0.00	32.00±0.56	28.50±0.40	19.00±0.55

Data represent means ± SEM of triplicate determination.

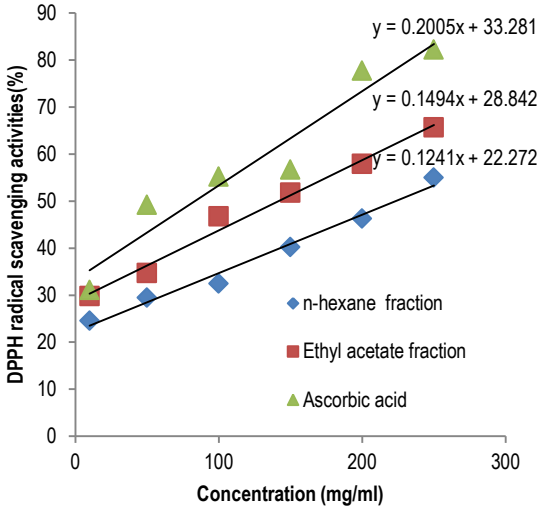

Figure 2: DPPH radical scavenging assay for determination of IC_{50} of *n*-hexane and ethyl acetate fractions of *Phyllanthus fraternus* leaves.

Table 3: Minimal inhibitory concentrations (MIC) of n-hexane and ethyl acetate fractions of Phyllanthus fraternus leaves against some pathogenic organisms

Test organisms	MIC (mg/mL)	
	N hexane	Ethyl acetate
E. coli	160	80
K. pneumoniae	80	80
S. aureus	80	40
P. aeruginosa	120	80
S. typhi	120	80

CONCLUSION

The n-hexane and ethyl acetate fractions of *Phyllanthus fraternus* leaves exhibited antioxidant and antimicrobial properties with ethyl acetate fraction been the most potent. The observed activities sports the ethno medicinal use of this plant. The plant extracts could be regarded as a promising source for antimicrobial and antioxidant agents.

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