ANTIOXIDANT ACTIVITIES OF *flueggea virosa* CRUDE EXTRACTS

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Abstract

F. virosa is of the family Phyllanthaceae, also called white berry-bush found in the Southern parts of Nigeria. The leaves of *F. virosa* were collected from the environments in Wukari, dried under room temperature and crushed into powdered form using mortar and pestle. The samples were macerated for 72 hours using each of hexane, ethyl acetate, acetone, and methanol as solvents in increasing polarity. The crude extracts were concentrated using rotary evaporator at 40 ^{o}C; it was observed that methanol extract had the highest yield (4.42 %); followed by acetone (1.05 %); hexane (1.10 %); and ethyl acetate had the least yield (0.99 %), this could be attributed to the nature of the constituents as well as the polarity of the solvents. The antioxidant activity of the crude extracts were then determined using DPPH (1,1-diphenyl-2-picryl hydrazyl) free radical scavenging assay at various concentrations of 0.0313, 0.0625, 0.1250, 0.2500, and 0.500mg/mL, with vitamin C as standard. The IC_{50} values were calculated using the linear regression equations and observed that the standard had the least IC_{50} value of 22.52 mg/mL indicating highest antioxidant activity, comparing to that of the extracts, methanol extract (37.58 mg/mL) was closely comparable to that of the standard, closely followed by that of acetone extract (40.26 mg/mL), then, hexane (43.67 mg/mL), and lastly ethyl acetate (56.89 mg/mL). This has shown that the plant has antioxidant properties and it can be a good source of antioxidant validating its uses by the locals. This research work is in continuation of our previous works - phytochemical and antimicrobial activities.

Keywords: Antioxidant, *flueggea virosa*, Phyllanthaceae, Southern parts of Nigeria, phytochemical, antimicrobial

Introduction

Traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs rely mostly on medicinal plants which are regarded as the richest bio-resources (Ncube et al., 2008).These bio-resources are the phytochemical which are responsible for their reported activities such as antimicrobial, anti-inflammatory, anti-stress, anti-ulcer, anti-HIV, antioxidant etc. Antioxidants have been reported to improve the quality of life through protection against free radicals which are often obtained from intake of dietary antioxidants, thereby, preventing degenerative diseases (Alam et al., 2013; Kumar et al., 2013; Kendeson et al., 2021). These antioxidants are gaining more attention due to their scavengers potential especially those of the plant based origin such as *Flueggea virosa* (Agbo et al., 2015).

F. virosa is of the family Phyllanthaceae, also called white berry-bush found in the Southern parts of Nigeria and China (Abba et al., 2009; Wang et al., 2018). It has been reported to possess various health benefits which include preventing and treating infection, treating of eczema, allergic dermatitis, scald and rheumatoid arthritis hemorrhoid (Wang, et al., 2018), reducing cholesterol, fever, respiratory illness, malaria, snake bite, liver, heart and kidney infection (Manandhar et al., 2019). Also, *F. virosa* has been reported to possess antimicrobial (Mwitari, et al., 2013), antiprotozoan, insecticide, larvicide, selective cytotoxicity to tumoral cells, anxiolytic, anti-stress, anti-ulceric (Yuan et al., 2016), antidiabetic (Manikkuwadura et al., 2019), wound healing, anti-icteric, hepatoprotective, hypoglycemic, antioxidant (Tai & Ayesha, 2014; Manandhar et al., 2019), and anti-HIV (Zhang et al., 2015a & b).

This research work is in continuation of our previous works - phytochemical and antimicrobial activities - done on the plant to validate its uses by the locals. Presently, the aim is to determine the antioxidant activity of the crude extracts, thereby establishing more facts to the claim for its medicinal use.

Materials and Methods

Sample collection and Preparation

The leaves of *F. virosa* were collected from the environments in Wukari; they were identified and authenticated in the Department of Biological Science, Faculty of Science, Federal University Wukari, Taraba State, Nigeria. The samples were dried under room temperature and crushed into powder form using mortar and pestle, and stored in clean containers for future use.

The samples (100 g) were macerated for 72 hours (3 days) using 250 ml each of hexane, ethyl acetate, acetone, and methanol as solvents in increasing polarity (Ushie et al., 201). The crude extracts were concentrated using rotary evaporator at 40 ^{o}C and stored in labeled sample bottles for further use. The percentage yields were calculated and noted.

Antioxidant Activity

The antioxidant activity of the *F. virosa* extracts was determined using DPPH (1,1-diphenyl-2-picryl hydrazyl) free radical scavenging assay described by Mahdi-Pour et al., (2012) and Kendeson et al., (2021) with some slight

modifications. The extracts/standard (1.5 mL each) at various concentrations of 0.0313, 0.0625, 0.1250, 0.2500, and 0.500mg/mL were mixed with 1.5 mL of DPPH solution and incubated at 37°C for 30min. The absorbance of each mixture was measured at 517 nm using a spectrophotometer (V-730 UV-Vis Spectrophotometer, Jasco, USA). The DPPH scavenging activity was calculated as follows:
Percentage (%) inhibition = $A_0 – A_1 / A_0 \times 100$; Where A_0 = the Absorbance of control and A_1 = the Absorbance of standard/sample. All measurements of free radical scavenging activity were performed in duplicate. The (%) inhibition values were plotted against the sample concentration and the concentrations of standard/sample resulting in 50% inhibition on DPPH (IC_{50} value) were calculated using the linear regression equation (Do et al., 2014).

Results and Discussion

F. virosa leaves were extracted and the percentage yields were calculated and presented as shown on Table 1; Figure 1. It was observed that methanol extract had the highest yield (4.42) and ethyl acetate had the least yield (0.99). This could be attributed to the nature of the constituents as well as the polarity of the solvents. The high yield of methanol extract could be an indication that polarity facilitated the extraction of the active constituents which may be attributed to higher solubility of proteins and carbohydrates in methanol than in acetone and ethyl acetate (Do et al., 2014). The high yield observed in hexane, being a non-polar solvent could be mostly non polar constituents.

Table 1: Percentage yield of Crude Extracts

Crude Extracts	% yield
Hexane	1.10
Ethyl acetate	0.99
Acetone	1.05
Methanol	4.42

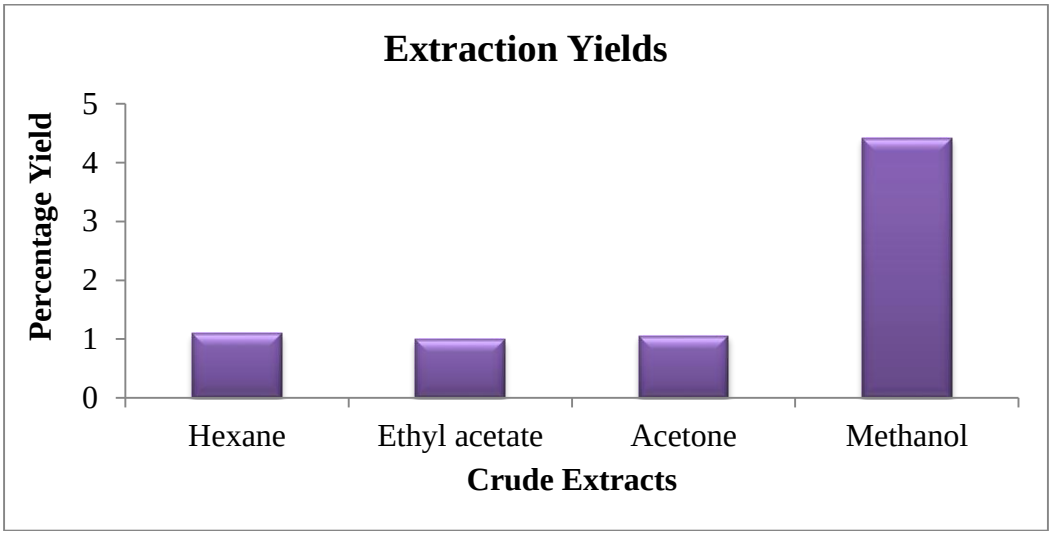

Figure 1: Chart showing the % Yield of the Extracts

DPPH assay is a very common method that is frequently used for *in vitro* radical scavenging activity of a sample and it is based on hydrogen or electron reduction (Johari and Khong, 2019; Kendeson et al., 2021).
The antioxidant activity of the crude extracts and the standard (Vitamin C) are as shown on Tables 2 & 3 and Figures 2 & 3a, b, c. Comparing the antioxidants activities of the crude extracts to that of the standard at the various concentrations of 0.0313, 0.0625, 0.125, 0.250, 0.500 mg/mL, the samples showed lower activity than that of the standard. From the absorbance readings Table 2; Figure 2, it was observed that the absorbancies were decreasing as the concentration increased from 0.313 to 0.500 mg/mL; while the percentage inhibition increased as the concentration increased (Table 3; figure 3a). The maximal inhibitory concentration (IC_{50}) was obtained from the linear regression analysis for the extract and standard (Table 2; Figure 3b); it was observed the standard had the least IC_{50} value of 22.52 mg/mL indicating highest antioxidant activity, comparing to that of the extracts, methanol extract (37.58 mg/mL) was closely comparable to that of the standard, closely followed by that of acetone extract (40.26 mg/mL), then, hexane (43.67 mg/mL), and lastly ethyl acetate (56.89 mg/mL). This result clearly indicates that the leaves of *F. virosa* possess antioxidant activities, as documented by Chauke et al., (2012) and Narain et al., (2018), in their various researches in South Africa.

Table 2: Antioxidant Activity of the Crude Extracts (Absorbance @ 517 nm)

Concentration (mg/mL)	Absorbance				
	Methanol	Acetone	Ethyl acetate	Hexane	Vitamin C
0.0313	0.154	0.156	0.160	0.144	0.147
0.0625	0.146	0.145	0.156	0.137	0.134
0.125	0.130	0.129	0.145	0.129	0.127
0.250	0.117	0.120	0.131	0.115	0.081
0.500	0.082	0.088	0.109	0.095	0.025

Table 3: Percentage Inhibition and the IC_{50} of the Crude Extracts

Concentration (mg/mL)	% Inhibition				
	Methanol	Acetone	Ethyl acetate	Hexane	Vitamin C
0.0313	22.61	21.56	19.60	27.71	26.13
0.0625	26.63	27.14	21.56	31.16	32.66
0.125	34.64	35.18	27.14	35.13	36.18
0.250	40.20	39.70	34.21	42.21	59.30
0.500	58.79	55.78	45.23	52.23	87.44
IC_{50}	**37.58**		**40.26**	**56.89**	**43.67**	**22.52**

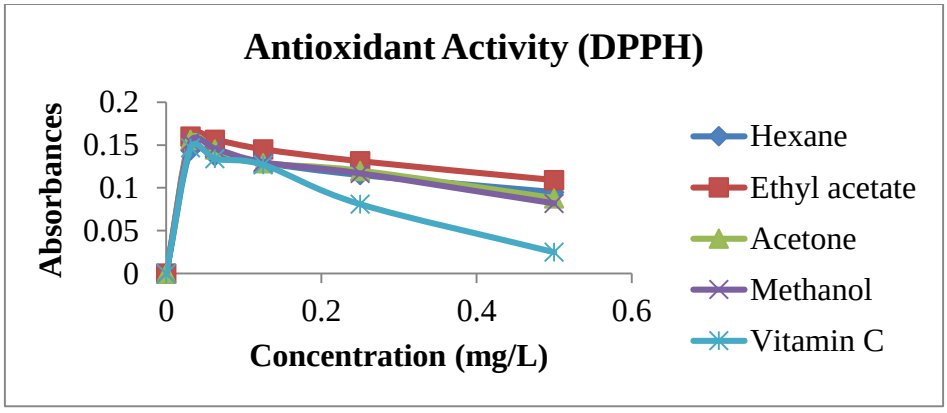

Figure 2: Chat showing the Absorbance of the Extracts and Standard

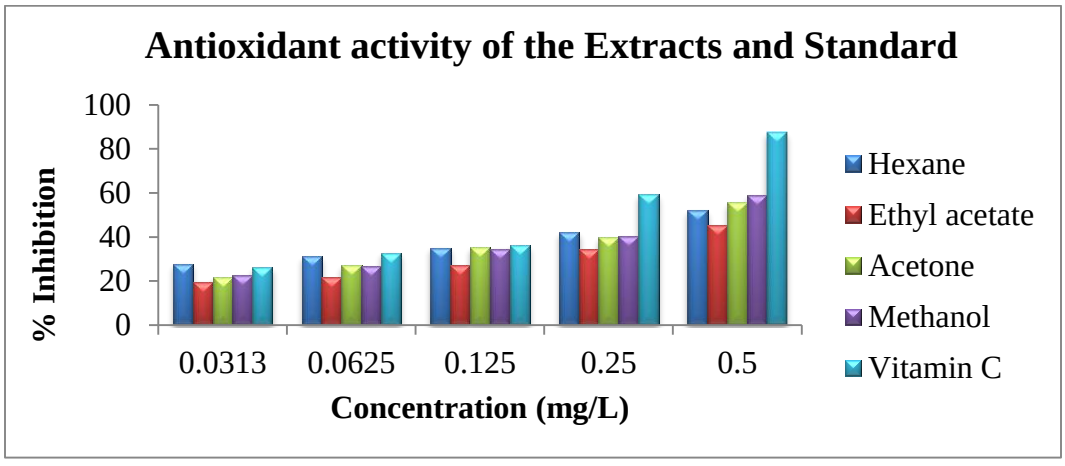

Figure 3a: Chat showing the % Inhibition of the Extracts and Standard

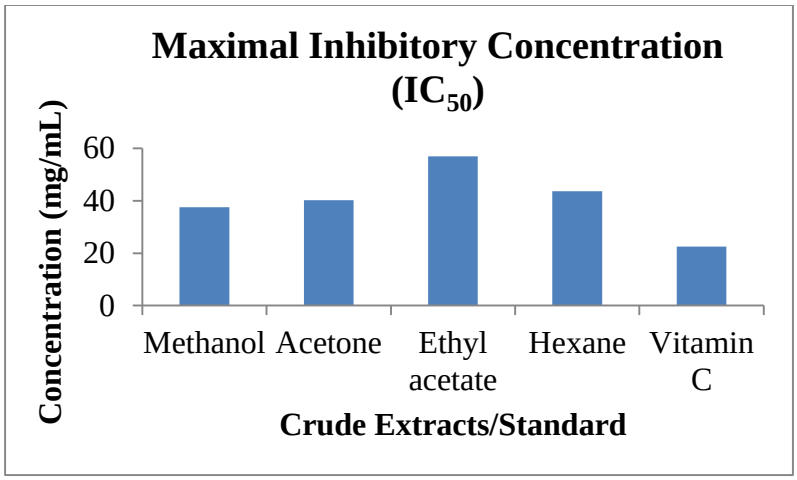

Figure 3b: Chat showing the IC_{50} of the Extracts and Standard

Conclusion

The antioxidant study of the leaves of *F. Virosa* crude extracts was done using DPPH method and the results shows that the plant had antioxidant properties as indicated by the results and it can be a good source of antioxidant.

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