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Phytochemical screening and antimicrobial activities of *Flueggea virosa* crude extracts

Ushie O A[1*], Kendeson A C[2], Longbab B D[1], Aasegh T J[1], Ama S O[1], Iortim A[1]
[1] Department of Chemical Science, Federal University Wukari, Taraba State, Nigeria
[2] Department of Chemical Sciences, Federal University of Kashere, Gombe State, Nigeria

Abstract
Flueggea virosa belongs to the family Phyllanthaceae, commonly called white berry-bush and commonly found in Southern part of Nigeria, and also in southern part of China. The leaves of *F. virosa* were extracted using soxhlet extractor with solvents of increasing polarity - hexane, ethyl acetate, acetone and methanol. The phytochemical analysis of the leave of *Flueggea virosa* were carried out using standard procedures and the results obtained reavealed that it contains phytochemical constituents such as alkaloids, flavonoids, anthraquinones, glycosides, tannins, terpenoids, saponins, steroids, and phenols. The quantitative phytochemical analysis showed highest concentration of alkaloids, which indicates that the leaves are rich in alkaloids. The antimicrobial screening of the crude extracts were carried out using the following microorganisms: Methicilin resistant *Staphylococcus aureus (MRSA),* Vancomycin resistant *Enterococci (VRE), Staphylococcus aureus*, *Escherichia coli*, *Helicobacter pylori*, *Campylobacter jejuni*, *Salmonella typhi*, *Candida albicans*, *Candida krusei* and *Candida tropicalis.* It was observed that *MRSA*, *VRE*, *C. jejuni*, and *C. krusei* were completely resistant to the plant's crude extracts, while, *S. aureus*, *E. coli*, *H. pylori*, *S. typhi*, *C. albicans*, and *C. tropicalis* were susceptible to the crude extracts. The activities of the extracts against the microorganisms was concentration dependent, at a higher concentration of 10.00 mg/mL, the extracts were more bactericidal which implies that the plant possess antimicrobial properties as reported by the locals and can be a source of antimicrobial candidate.

Keywords: alkaloid, *flueggea virosa*, leaves, crude extracts, polarity

Introduction
The importance of medicinal plants is due to the secondary metabolites (phytochemicals) which are present in various parts of the plants such as in leaves, fruits, roots, and stems. These secondary metabolites are present in legumes, fruits, nuts, whole grains, spices, seeds, and vegetables; they are biologically active and used for the treatment of various human and animal ailments (Folorunso *et al*., 2019) [1]. Traditionally, plants have been used as a source of medication for treatment of almost all kinds of ailment ranging from unknown to the known, chronic to infectious (Ushie *et al* 2019) [2], but scientific validations for most of these medications are still not available (Akintelu *et al*., 2019) [1, 3]. A large number of plants can be investigated on the basis of their traditional use by the local people as aboriginal people to attain the knowledge of plants and the types of ailments used for from their ancestors.

Flueggea virosa belongs to the family Phyllanthaceae, commonly called white berry-bush and commonly found in Southern part of Nigeria (Abba *et al*., 2009) [4], and also in southern part of China (Wang *et al*., 2018) [5]. It is known 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) [5], reducing cholesterol, fever, respiratory illness, malaria, snake bite, liver, heart and kidney infection (Manandhar *et al*., 2019) [6].

It has been reported that *F. virosa* possess antimicrobial (Mwitari, *et al*., 2013) [7], antiprotozoan, insecticide, larvicide, selective cytotoxicity to tumoral cells, anxiolytic, anti-stress, anti-ulceric (Yuan *et al*., 2016) [8], antidiabetic (Manikkuwadura *et al*., 2019) [10], wound healing, anti-icteric, hepatoprotective, hypoglycemic, antioxidant (Tai & Ayesha, 2014; Manandhar *et al*., 2019) [6], and anti-HIV (Zhang *et al*., 2015a & b). The plant is being reported by the locals of Wukari in Taraba State, Nigeria to possess anti cancer, as well as antimicrobial properties. This research is targeted at scientifically evaluating these claims, by screening the plant for its phytochemical properties both quantitative and qualitative; and antimicrobial properties activity which could help in improving the prevention and treatment of cancer and other diseases using alternate medicine therapy in the high risk population who at the same time have very little or limited access to modern hospitals and treatments.

Materials and Methods
Sample collection and Preparation
The leaves of *F. virosa* were collected in the fields in Wukari; they were identified and authenticated by a Chemist in Department of Chemistry, Faculty of Science, Federal University Wukari, Taraba State, Nigeria. The samples were dried under room temperature and grinded into powder form and stored in clean containers for future use.

The samples (100 g) were extracted for eight (8) hours by Soxhlet extractor using hexane, ethyl acetate, acetone, and methanol as solvents in increasing polarity. The crude extracts were concentrated using rotary evaporator at 40 ^{o}C and stored in labeled sample bottles for further use.

Phytochemical Analyses
The quantitative and qualitative phytochemical analyses were done following standard procedures as reported by Ushie, (2019 & 2022) [2, 15] and Perez *et al*., (2016) [17].

Antimicrobial Screening

The antimicrobial activities of the crude extracts were determined following standard procedure described by Ushie, (2021) [19] using pathogenic microbes, obtained from the Department of Medical Microbiology Ahmedu Bello University teaching hospital Zaria, Kaduna State, Nigeria.

About 0.1 g each of the crude extracts were weighed and dissolved in 10 mL of DMSO to obtain a concentration of 10 mg/mL. This was the initial concentration of the extract used to determine its antimicrobial activities.

Results and Discussions

The hexane, ethyl acetate, acetone and methanol of the *F. virosa* leaves were screened for the presence of some phytochemicals constituents such as alkaloids, flavonoids, anthraquinones, glycosides, tannins, terpenoids, saponins, steroids, and phenols. The antimicrobial screening of the crude extracts were carried out using the following microorganisms: Methicilin resistant *Staphylococcus aureus (MRSA)*, Vancomycin resistant *Enterococci (VRE)*, *Staphylococcus aureus*, *Escherichia coli*, *Helicobacter pylori*, *Campylobacter jejuni*, *Salmonella typhi*, *Candida albicans*, *Candida krusei* and *Candida tropicalis.* The results are shown below:

Table 1: Qualitative Phytochemical Analysis

S/N	Phytochemical	Crude Extracts			
		Hexane	Ethyl acetate	Acetone	Methanol
1	Alkaloids (a) Mayer	-	-	-	-
	(b) Wagner	+	+	+	+
2	Flavonoids	+	+	+	-
3	Anthraquinones	-	-	-	-
4	Steroids	-	-	-	+
5	Glycosides	+	+	+	-
6	Tannins	+	+	+	+
7	Terpenoids	-	-	-	+
8	Saponins (Froth test)	-	-	-	+
9	Phenols	-	-	-	+

Key: - implies absence of constituents; + implies presence of constituents

Table 2: Quantitative Phytochemical Analysis

S/N	Phytochemical	Concentration (mg/g)
1	Alkaloids	1.816 ± 0.02*
2	Flavonoids	1.208 ± 0.15*
3	Saponins	0.854 ± 0.21
4	Tannins	1.326 ± 0.21**
5	Phenols	0.194 ± 0.03*

Key:* show significant at 5% level while ** show significant at 1% level

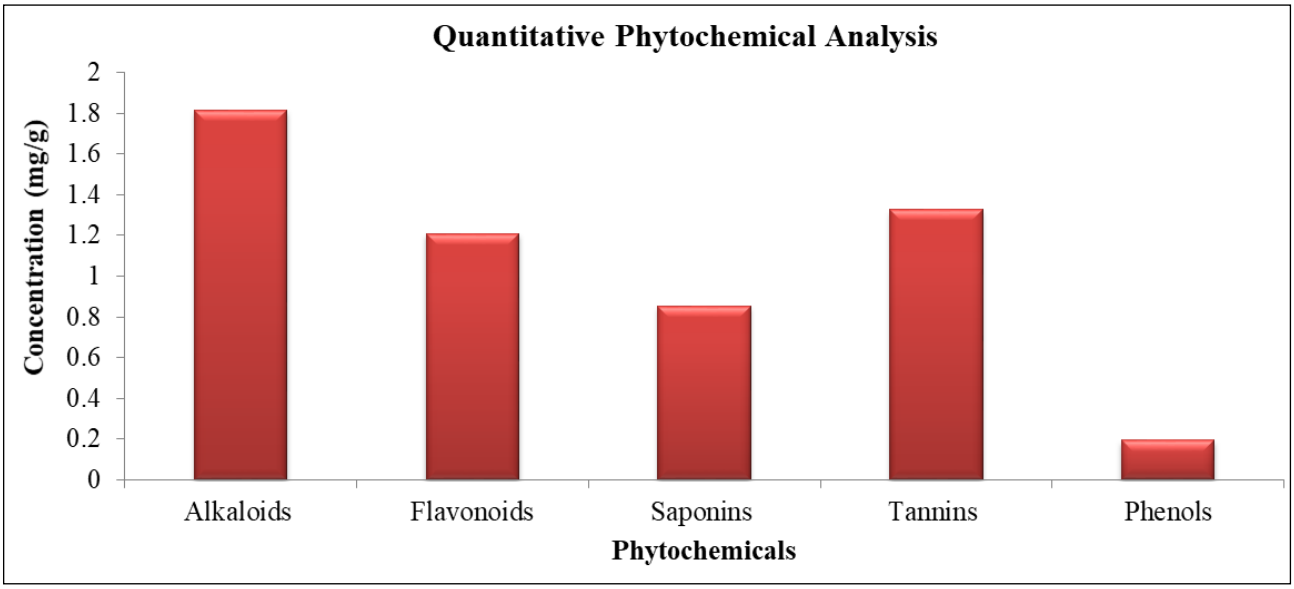

Fig 1: Quantitative Phytochemical screening of *F. virosa* Leaves

The phytochemical results of the crude extracts of *F. virosa* leaf as shown in Table 1a &b and Fig. 1 indicates that the plant is rich in phytoconstituents especially in the methanol crude extract. The presence of these bio-active constituents attests to the use of *F. virosa* leaves for medicinal purposes by the locals. Usually, water being a universal solvent is mostly used for extraction by locals who consume herbs for healing; this may suggest that due to its high polarity, water will probably contain similar phytoconstituents as methanol. This result agrees with the reports of authors like Alali *et al*., (1999) [20], Wang *et al*., (2008) [21] and, Zhao *et al*., (2011) [22] who were able to identify and isolate some.

The result has shown (Fig. 1) that alkaloid is the major constituent present in the plant's leaves, this also, agrees with most of the findings reported. Securinega alkaloids a class of natural products discovered only from plants of the genera Securinega and Flueggea of the Euphorbiaceae family are of norsecurinine-derived oligomers reported to possess medicinal properties (Wehlauch and Gademann, 2017) [23] example include flueggether A and virosinine A isolated from *F. virosa* plant (Zhang *et al*., 2015a) [12], flueggenines E and F were reported to be isolated as novel hydrid structures, flueggenines G–I (Zhang *et al*., 2015b) [13]. Likewise, Fluvirosaones A and B, together with virosecurinine (3), were isolated from *F. virosa* plant (Luo *et al*., 2018) [24], Wang *et al*., 2008 [21], also isolated alkaloids from the leaves; this supports the high alkaloid content in this present studies.

Table 3: Antimicrobial Activities of *F. virosa* Crude Extracts

Test organisms	Crude Extracts			
	Hexane	**Ethyl acetate**	**Acetone**	**Methanol**
Methicilin resistant *Staphylococcus aureus (MRSA)*	R	R	R	R
Vancomycin resistant *enterococci (VRE)*	R	R	R	R
Staphylococcus aureus	S	S	S	S
Escherichia coli	S	S	S	S
Helicobacter pylori	S	S	S	S
Campylobacter jejuni	R	R	R	R
Salmonella typhi	S	S	S	S
Candida albicans	S	S	S	S
Candida krusei	R	R	R	R
Candida tropicalis	S	S	S	R

Key: R = Resistance and S = Susceptible

Table 4: Mean Zone of Inhibition of the Extract against the Test Micro-organism

Test organisms	Zone of Inhibition/Crude Extracts Concns			
	Hexane	**Ethyl acetate**	**Acetone**	**Methanol**
Methicilin resistant *S. aureus (MRSA)*	0	0	0	0
Vancomycin resistant *enterococci (VRE)*	0	0	0	0
S. aureus	18	25	20	21
E.coli	16	22	18	20
H. pylori	20	24	21	23
C.jejuni	0	0	0	0
S.typhi	18	23	20	20
C. albicans	17	21	19	20
C. krusei	0	0	0	0
C. tropicalis	0	21	20	19

Table 5: Minimum Inhibitory Concentration (MIC) of *F. virosa* Leaf Crude Extracts

Test organism	10.00 mg/ml	10.00 mg/ml	10.00 mg/ml	10.00 mg/ml
	5.00 mg/ml	5.00 mg/ml	5.00 mg/ml	5.00 mg/ml
	2.50 mg/ml	2.50 mg/ml	2.50 mg/ml	2.50 mg/ml
	1.25 mg/ml	1.25 mg/ml	1.25 mg/ml	1.25 mg/ml
	0.63 mg/ml	0.63 mg/ml	0.63 mg/ml	0.63 mg/ml
	0.31 mg/ml	0.31 mg/ml	0.31 mg/ml	0.31 mg/ml
	(Hexane)	(Ethyl acetate)	(Acetone)	(Methanol)
MRSA				
VRE				
S. aureus	- - 0* + ++ +++	- - 0* + ++ +++	- - 0* + ++ +++	- 0* + ++ ++ +++
E. coli	- 0* + ++ ++ +++	- - 0* + ++ +++	- - 0* + ++ +++	- 0* + ++ ++ +++
H. pylori	- - 0* + ++ +++	- - 0* + ++ +++	- - 0* + ++ +++	- - 0* + ++ +++
C. jejuni				
S. typhi	- - 0* + ++ +++	- - 0* + ++ +++	- - 0* + ++ +++	- 0* + ++ ++ +++
C. albicans	- 0* + ++ ++ +++	- - 0* + ++ +++	- - 0* + ++ +++	- 0* + ++ ++ +++
C. krusei				
C. tropicalis	- - 0* + ++ +++	- - 0* + ++ +++	- 0* + ++ ++ +++	

Key: - = No turbidity (No growth); 0* = MIC; + = Turbidity (light growth); ++ = Moderate turbidity; +++ = High Turbidity.

Table 6: Minimum Bactericidal Concentration (MBC)/Minimum Fungicidal Concentration (MFC) of *F. virosa* Leaf Crude Extracts

Test organism	10.00 mg/ml	10.00 mg/ml	10.00 mg/ml	10.00 mg/ml
	5.00 mg/ml	5.00 mg/ml	5.00 mg/ml	5.00 mg/ml
	2.50 mg/ml	2.50 mg/ml	2.50 mg/ml	2.50 mg/ml
	1.25 mg/ml	1.25 mg/ml	1.25 mg/ml	1.25 mg/ml
	0.63 mg/ml	0.63 mg/ml	0.63 mg/ml	0.63 mg/ml
	0.31 mg/ml	0.31 mg/ml	0.31 mg/ml	0.31 mg/ml
	(Hexane)	(Ethyl acetate)	(Acetone)	(Methanol)
MRSA				
VRE				
S. aureus	- - 0* + ++ +++	- - 0* + ++ +++	- - 0* + ++ +++	- 0* + ++ ++ +++
E. coli	- 0* + ++ ++ +++	- - 0* + ++ +++	- - 0* + ++ +++	- 0* + ++ ++ +++
H. pylori	- - 0* + ++ +++	- - 0* + ++ +++	- - 0* + ++ +++	- - 0* + ++ +++
C. jejuni				

S. typhi	- - 0* + ++ +++	- - 0* + ++ +++	- - 0* + ++ +++	- 0* + ++ ++ +++
C. albicans	- 0* + ++ ++ +++	- - 0* + ++ +++	- - 0* + ++ +++	- 0* + ++ ++ +++
C. krusei				
C. tropicalis	- - 0* + ++ +++	- - 0* + ++ +++	- 0* + ++ ++ +++	

Key: - = No colony growth; 0* = MBC; + = Scanty colonies growth; ++ = Moderate colonies growth; +++ = Heavy colonies growth.

The results (Table 3 - 5), clearly shows that some of the microorganisms used (Methicilin resistant *S. aureus (MRSA)*, Vancomycin resistant *Enterococci (VRE)*, *C. jejuni*, and *C. krusei*) were completely resistant to the plant's crude extracts. *S. aureus*, *E. coli*, *H. pylori*, *S. typhi*, *C. albicans*, and *C. tropicalis* were susceptible to the crude extracts. From the result in Tables 3, the ethyl acetate extract showed more activity with the highest zones of inhibition, this was followed by the methanol, and acetone extracts, hexane extract was the least. This could be as a result of the polarity of the solvent (hexane). In Tables 4 and 5, the activities of the extracts against the microorganisms was concentration dependent, at a higher concentration of 10.00 mg/mL, the extracts were more bactericidal. This implies that the plant possess antimicrobial properties as reported by the locals and can be a source of antimicrobial candidate. This result agrees with that of Mwitari, *et al*., (2013) [7], Manandhar *et al*., (2019) [6] who also reported the antimicrobial activities of the plant.

Conclusion

The analyses conducted on the leaves of *F. Virosa* have revealed that the plant's leaves contains phytochemical constituents, especially alkaloids, which are known to work in synergy thereby giving plants their medicinal properties. Hence, it can be inferred that the phytochemicals contribute to the antimicrobial activities exhibited by this plant, hence its use for medicinal purposes.

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