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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITIES OF METHANOLIC EXTRACT OF *NEWBOULDIA LAEVIS* ROOTS

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ABSTRACT

The plant *Newbouldia laevis* belongs to the Bignoniaceae family and is commonly called Seem or boundary tree in English. Different parts of *N. laevis* are used by herbal practitioners to treat several diseases such as chest pain, sore feet, earache, epilepsy and children's convulsion, malaria and others. Phytochemical screening of extract *N. laevis* root obtained from Bori metropolis was carried out. Antimicrobial activities of the plant extract were evaluated using agar well diffusion method. The phytochemical screening of *N. laevis* roots extract revealed presence of secondary metabolites such as steroids, alkaloids, flavonoids, triterpenoids, tannins, anthraquinone, glycosides, reducing sugars and saponins. Quantitation of phytochemicals by gas chromatography coupled with flame ionization detector (GC-FID) showed that the plant roots contained more of alkaloids like quinine, sparteine, ribalidine and lunamirine. Quinine (36.5481 µg/g)) and lunamarine (36.0202 µg/g)) were highest. The effective MIC of the plant extract was observed at 10 mg/mL against *Proteus mirabilis, MRSA, E. coli, S. aureus, C. albicans and C. stellatoidea.* The MIC 5 mg/mL was effective against *A. nigre, Coniophora puteana, Estrophoria raclentii, Fusarium prokforatum, Scloratium rolfsii, Rhizopus sp.* and *Serpula lacrymans*. The MBC and MFC were further carried out to ascertain whether certain concentrations of the plant extract would kill the test pathogens. The extract showed effective (MBC/MFC) (20 mg/mL) against all the test pathogens that were sensitive to the extract such as *E. coli, MRSA, S. aureus, Candida albicans,* and others. The presence of the detected secondary metabolites in the plant confirms the usefulness of the plant. The activity *N. laevis* against pathogenic strains makes

the plant a potential drug development candidate for treatment of diseases caused by these pathogens.

KEYWORDS: *Newbouldia laevis*, phytochemical, antimicrobial, GC-FID, pathogen.

INTRODUCTION

The plant *Newbouldia laevis* belongs to the Bignoniaceous family and is commonly called Seem or boundary tree in English. It is locally referred to as *Akoko* in Yoruba, *Aduruku* in Hausa and *Ogirisi* in Igbo language. It grows up to about 7-15 m high,[1,2] *N. laevis* originated in the African tropics and extends from Guinea Savannahs to the dense Forests zones where grows on moist and well-drained soils.[1] In Nigeria, different parts of *N. laevis* are used as remedy for chest pain, sore feet, earache, epilepsy and children's convulsion. The leaf, stem and fruits are used for wound dressing and stomach ache.[1] The plant is also used in Nigeria to treat malaria, diarrhea, epilepsy, dysentery, bacterial and fungal infections, mental illness, asthma, diabetes, worm infestation, pains and ulcers[3], [2] demonstrated that the leaf of *N. laevis* has good antibacterial potential.[3] reported the hepatoprotective activity of extracts of root and leaf of *N. laevis.* A study carried out by[4] showed that, ethanol extract of *N. laevis* stem bark has antinociceptive effects. Both stem bark powder and methanol extract of *N. laevis* showed insecticidal activity against *Sitophilus oryzae* and *Sitophilus zeamais* that infest rice and maize grains.[5] The ethanol extracts of *N. laevis* leaves and stem possessed antioxidant activity. This was evident from increased activities of superoxide dismutase and catalase, and glutathione levels of the diabetic rats after treatment. High levels of alkaline phosphatase (ALP), and alanine amino transaminase (ALT), which are associated with oxidative stress condition, were differentially reduced after treatment with the ethanol extracts of *N. laevis* leaves and stem in a dose dependent manner.[6] Preliminary phytochemical analysis of the flower extract of *Newbouldia leavis* revealed the presence of flavonoids, cardiac and steroidal glycosides, tannins while other phytochemicals such as alkaloids, saponins was not detected.[1] Leaves of *Newbouldia leavis* contain several secondary metabolites such as flavonoids, terpenoids, tannin, saponins, steroids, cardiac glycosides, alkaloids, and phenols.[7,8,9] [10] reported the isolation of a naphthoquinone anthraquinone, Newbouldiaquinone A from the leaves of *N. laevis*. Other compounds isolated were apigenin, newbouldiamide, newbouldiaquinone, lapachol, 2-methylanthraquinone, 2-acetylfuro-1,4-naphthoquinone, 2,3-dimethoxy-1,4-benzoquinone, 5,7-dihydroxydehydroiso-α-lapachone, chrysoeriol, oleanolic acid, canthic acid, 2-(4-hydroxyphenyl) ethyl

triacontanoate, β-sitosterol, and β-sitosterol glucopyranoside.[11] reported the isolation of apigenin from methanol extract of the plant leaves.

MATERIALS AND METHODS

Plant collection and Preparation

The roots of *Newbouldia laevis* were collected from Bori metropolis in July, 2019. The fresh roots were dried under shed for three weeks. The dried roots were then pulverized using pestle and mortar.

Extraction Procedure

The powdered sample (100 g) of *Newbouldia laevis* root was extracted with methanol 200 cm^3 by cold maceration for 72 hours with occasional shaking. Mixture was filtered to obtain crude methanol crude extracts.[12]

Preliminary Phytochemical Screening

Preliminary phytochemical screening *N. laevis* roots was carried out based on the procedures described by.[13,14]

Quantitation of Phytochemicals by GC-FID

BUCK M910 Gas chromatography (BUCK Scientific, USA) was used for quantification of the phytochemicals in the extract. The gas chromatography was with a flame ionization detector, a RESTEK 15 m MKT-1 column (15 m× 20 m×0.15 um). The injection temperature was set at 280 °C and the injection velocity 30cm/s was used with 2 µL splitless injection of sample. Helium (5.0pa) was used as carrier gas with a flow rate of 40 mL/min. The initial oven temperature was 200 °C, the oven was then heated at the rate of 3 °C/min until a temperature of 330 °C was achieved, while the detector was operated at 320 °C. Phytochemicals were determined by the ratio between the area and mass of internal standard and the area of identified phytochemicals. The concentrations of individual phytochemicals were expressed in µg/mL.[12]

Antimicrobial Analysis

The antimicrobial activity of plant extract was determined using some pathogenic microbes. The microbes were obtained from Department of Medical Microbiology Ahmadu Bello University Teaching Hospital, Zaria.

Test organisms used for antimicrobial activities were; *methicillin resistant staphylococcus aureus* (MRSA), *vancomycin resistant enterococci, Staphylococcus aureus, Streptococcus pyogenes, Streptococcus feacalis, Bacillus subtilis, Escherichia coli, Klebscilla pneumonia, Salmonella typhi, Shigella dysenteriae, Proteus mirabilis, Pseudomonas eruginosa, Candida albicans, Candida krusei, Candida tropicalis* and. All bioassays were carried out in triplicates and the mean was taken and recorded as results.

Cultivation and standardization of test organisms

A loop full of test organism was taken from agar slant and sub-cultured into test tubes containing 20 mL sterile nutrient agar for bacterial and Sabourand dextrose medium for fungi. The test tubes were incubated for 48 hours at 37 ^{0}C. The broth culture was standardized using sterile normal saline to obtain a density of 106 cfu/mL for bacteria. A sporulated test fungal spore was harvested with 0.05% tween 80 in sterile normal saline and standardized to106 spores/mL.[15]

Antimicrobial profile sensitivity test

Antimicrobial screening was carried out using agar diffusion method as described by[16] with slight modification. Mueller Hinton and Sabourand dextrose agar were the medium used for growth of bacteria and fungi, respectively. The entire medium was prepared according to manufacturer's instructions, sterilized at 121 ^{0}C for 15 minutes and poured into sterile petri dishes. The plates were allowed to cool and solidify. Diffusion method was used for screening of extracts. Sterilized Mueller Hinton agar was seeded with 0.1 mL of standard inoculum of the test bacteria and Sabourand dextrose was seeded with 0.1 mL of standard inoculum of the test fungi. The inoculums were evenly spread over the surface of the media using a sterile swap. A sterile standard cork borer of 6 mm in diameter was used to cut a well at the center of each inoculated medium. About 0.1 mL of solution of extract of concentration of 5 mg/mL was then introduced into the well in the medium. Incubation for bacteria was at 37 ^{0}C for 24 hours and at 27 ^{0}C for one week for fungi. Each plate was then observed for zone of inhibition of growth, which was measured with a transparent ruler and the result recorded in millimeters.

Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration was carried out on extracts that showed growth inhibitory activity on test organisms. It was carried out using broth dilution method as described by[17] and modified by.[2] Mueller Hinton and sabourand dextrose broth were prepared according to

manufacturer's instruction. About 10 mL of broth was dispensed into test tubes, separated and were sterilized at 121 ^{0}C for 15 minutes and allowed to cool. Mc-farland's turbidity standard scale number 0.5 was prepared to give a turbid solution. Normal saline was prepared and was used to make a turbid suspension of the microbes. About 10 mL was dispensed into test tubes and the test microbes were inoculated and incubated for 6 hours at 37 ^{0}C. Dilution of micro-organism in normal saline was continuously done until the turbidity (1.5x 10^6 cfu/mL) matched that of the Mc-farland scale by visually comparing them. Two fold serial dilution of extract in sterile broth was done to obtain concentration of 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL, 0.625 mg/mL, and 0.3125 mg/mL. Having obtained different concentrations of extracts in the broth, 0.1 mL of standard inoculum of the microbes was inoculated into the different concentrations. Incubation for bacteria was at 37 ^{0}C for 24 hours and at 30 ^{0}C for one week for fungi. The test tubes were then observed for turbidity. The lowest concentration of an extract in the broth which showed no turbidity was recorded as the minimum inhibition concentration (MIC).[15]

RESULTS AND DISCUSSION

Preliminary phytochemical screening of crude extract of roots of *Newbouldia laevis* showed the presence of phytochemical compounds such as steroids, alkaloids, flavonoids, triterpenoids, tannins, anthraquinone, glycosides, reducing sugars and saponns (Table 1). These phytochemicals are known to have important biological activities. Flavonoids for example, have antioxidant, hepatoprotective, antibacterial, antiviral, anticarcinogenic and anti-inflammatory activities.[18] Some of these components were further quantified by Gas Chromatography Flame Ionization detector (GC-FID) (Table 2). The current produced in FID is proportional to the information which depends on the composition of the separated sample. Flame ionization detectors are extremely sensitive and have a wide range of linearity.[19]

Significant quantities of alkaloids such as quinine (36.5481 µg/g), lunamarine (36.0202 µg/g) and sparteine (19.8053 µg/g) were recorded by GC-FID from roots of *N. laevis*. Quinine is antimalarial drug that was very efficient but has been discontinued due to its side effects. The leaves and roots of this plant are used as remedy for malaria in different parts of Nigeria[20] which could be due to presence of quinine. Lunamarine has been reported to have radical scavenging and anti-amoebic activities.[20,21] Another alkaloid detected was ribalidine (4.5924 µg/g). Flavonoids also rich in the plant extract, such as epicatechin (17.8974 µg/g), anthocyanin (27.4182 µg/g), resveratol (15.8736 µg/g), flavonones (8.2861 µg/g) rutin

(6.2872 µg/g) were detected; others were naringenin, kaempferol and protoanthocyanin. Flavonoids are known prominently for their antioxidant and anti-inflammatory activities.[18] Anthocyanins play an important role in visual acuity, heart disease, treatment of cancer, age-related neurodegenerative disorders and in angiogenesis.[22] Tannins (15.8736 µg/g) were also detected by GC-FID method. Tannins are widely distributed in plants and may be utilized in medicine as antidiarrheal, haemostatic, and antihemorrhoidal compounds. Thay have anti-inflammatory effects and play important role in control of gastritis, esophagitis, enteritis, and irritating bowel disorders. Tannins are able to heal burns, stop bleeding as well as infection while they continue to heal the wound internally.[22] The leaf, stem bark and fruits of the plant are used in wound dressing traditionally.[1] Other phytochemicals detected in *N. laevis* root extract were sapogenin, phenol, oxalate, and steroids (Table 2). The sensitivity test and inhibition zones of plant extract were measured and compared to those of standard drugs such as spirofloxacin/ciprofloxacin and fulcin/ketoconazole for bacteria and fungi, respectively (Table 3). The susceptible bacteria were *Methicillin Resistant Staphylococcus aureus* (MRSA), *Staphylococcus aureus*, *E. coli* and *Proteus mirabilis*. MRSA was the most sensitive with inhibition zones of 25 mm and 30 mm in the extract and spirofloxacin, respectively. It however, appeared resistant to ciprofloxacin. *Psedomonas aeruginosa* and *Salmonella typhi* were resistant to plant extract and spirofloxacin but both sensitive ciprofloxacin. Previous study by[2] showed that *N. laevis* roots have good antibacterial potential. In their study, both *E. coli* and *S. typhi* were sensitive to ethanolic to root extract of *N. laevis*. In the present study however, *S. typhi* was resistant to plant extract.

The susceptible fungi were *Candida albicans*, *Candida stellatoidea*, *Aspergillus nigre*, *Coniophora puteana*, *Estrophoria raclentii*, *Fusarium proliferatum*, *Rhizopus sp*. and *Serpula lacrymans* (Table 3). The most sensitive fungi was *C. albicans* and the least sensitive was *Serpula lacrymans* with inhibition zones of 25 mm and 20 mm, respectively. Coniophora puteana being sensitive to plant extract and fluconazole with common inhibition zone 23 mm was resistant to fulcin. Furthermore, *Estrophoria raclentii* was sensitive to plant extract (23 mm inhibition zone) and fulcin (28 mm inhibition zone) but resistant to fluconazole.

Minimum inhibitory concentration (MIC) is the minimum concentration of a sample that could inhibit growth of microorganisms. Different concentrations of the plant extract were used to evaluate the MIC. The effective MIC of the plant extract was observed at 10 mg/mL against *Proteus mirabilis, MRSA, E. coli, S. aureus, C. albicans and C. stellatoidea.* The

MIC 5 mg/mL was effective against *A. nigre*, *Coniophora puteana*, *Estrophoria raclentii*, *Fusarium prokforatum*, *Scloratium rolfsii*, *Rhizopus sp.* and *Serpula lacrymans*. The MBC and MFC were further carried out to ascertain whether certain concentrations of the plant extract would kill the test pathogens. The extract showed effective (MBC/MFC) (20 mg/mL) against all the test pathogens (Table 4) that were sensitive to the extract such as *E. coli, MRSA, S. aureus, Candida albicans,* and others. The inhibitory effect of extract of *N. laevis* against pathogenic strains makes the plant a potential drug development candidate for treatment of diseases caused by these pathogens. The presence of the detected secondary metabolites in the plant (Table 1) supports the usefulness of the plant. These classes of compounds have various physiological activities.[7,8,9]

Table 1: Qualitative Phytochemical Screening.

Phytochemical Components	Remark
Steroids	+
Flavonoids	+
Alkaloids	+
Tannins	+
Saponins	+
Glycosides	+
Reducing sugars	+
Triterpenoids	+
Anthraquinones	+

Table 2: Quantitative Phytochemical Screening.

Phytochemical	Class	Quantity (µg/g)
Protoanthocyanin	Flavonoid	0.2932
Rutin	Flavonoid	6.2872
Ribalidine	Alkaloid	4.5924
Quinine	Alkaloid	36.5481
Anthocyanin	Flavonoid	27.4182
Lunamarine	Alkaloid	36.0202
Sapogenin	Saponin	10.2708
Phenol	Phenol	2.6850
Flavonones	Flavonoid	8.2861
Steroids	Steroids	9.0728
Epicatechin	Flavonoid	17.8974
Kaempferol	Flavonoid	1.9517
Phytate	Phenol	2.5051
Oxalate	-	2.3312
Resveratol	Flavonoid	15.8736
Naringenin	Flavonoid	2.2154
Tannin	Tannins	15.8736
Sparteine	Alkaloid	19.8053

Table 3: Sensitivity and Zones of Inhibition.

Test organisms	Extract	spirofloxacin	Ciprofloxacin	ketoconazole	Fulcin
MRSA	S(25)	S(30)	R		
VRE	R	S(29)	S(30)		
Staphylococcus aureus	S(24)	S(32)	S(26)		
Escherichi coli	S(23)	R	S(37)		
Proteus mirabilis	S(23)	S(32)	S(30)		
Psedomonas aeruginosa	R	R	S(25)		
Salmonella typhi	R	R	S(40)		
Candida albicans	S(25)			S(33)	S(32)
Candida krusei	R			S(34)	R
Candida stellatoidea	S(24)			S(35)	S(30)
Aspergillus fumigatus	R			R	S(28)
Aspergillus nigre	S(24)			R	S(26)
Coniophora puteana	S(23)			S(23)	R
Estrophoria raclentii	S(23)			R	S(28)
Fomitopsis pinicola	R			S(27)	S(30)
Fusarium oxysporum	R			R	S(27)
Fusarium proliferatum	S(21)			S(28)	S(26)
Rhizopus sp.	S(21)			R	S(32)
Scloratium rolfsii	R			S(25)	R
Serpula lacrymans	S(20)			S(26)	S(30)

Key: R= Risistant, S = Sensitive, MRSA= Methicillin resistant staphylococcus aureus, VRE = Vancomycin resistant enterococci.

Table 4: Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) of *N.laevis*.

Test organisms	MIC						MBC and MFC					
	40 mg/mL	20 mg/mL	10 mg/mL	5 mg/mL	2.5 mg/mL	1.25 mg/mL	40 mg/mL	20 mg/mL	10 mg/mL	5 mg/mL	2.5 mg/Ml	1.25 mg/mL
MRSA	-	-	ox	+	++	+++	-	O*	+	++	++	+++
VRE												
Staphylococcus aureus	-	-	ox	+	++	+++	-	O*	+	++	++	+++
Escherichi coli	-	-	ox	+	++	+++	-	O*	+	++	++	+++
Proteus mirabilis	-	-	ox	+	++	+++	-	O*	+	+	++	+++
Psedomonas aeruginosa												
Salmonella typhi												
Candida albicans	-	-	ox	+	++	+++	-	O*	+	+	++	+++
Candida krusei												
Candida stellatoidea	-	-	ox	+	++	+++	-	O*	+	++	++	+++
Aspergillus fumigatus												
Aspergillus nigre	-	-	-	ox	+	++	-	O*	+	++	++	+++
Coniophora puteana	-	-	-	ox	+	++	-	O*	+	++	++	+++

Estrophoria raclentii	-	-	-	ox	+	++	-	O*	+	++	++	+++
Fomitopsis pinicola												
Fusarium oxysporum												
Fusarium prokforatum	-	-	-	ox	+	++	-	O*	+	++	++	+++
Rhizopus sp.	-	-	-	ox	+	++	-	O*	+	++	++	+++
Scloratium rolfsii												
Serpula lacrymans	-	-	-	ox	+	++	-	O*	+	++	++	+++

Key: - = No colony growth; ox = MIC; O* = MBC/MFC; + = scanty colony growth; ++ = moderate colony growth; +++ = heavy colony growth.

4.0 CONCLUSION

The antimicrobial activity exhibited by *Newbouldia laevis* root extract is attributed to several biologically active constituents it contains. The present study is anticipated to add value to the medicinal plants used in Nigeria.

CONFLICT OF INTEREST

None exists.

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