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EVALUATION OF ANTIBACTERIAL AND PHYTOCHEMICAL PROPERTIES OF METHANOLIC LEAF AND ROOT BARK EXTRACT S OF *Newbouldia leavis* Seeman ex Bureau (Bignoniaceae)

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ABSTRACT

Methanol fraction of Newbouldia leavis root bark and leaf extract were analyzed in-vitro for their antibacterial and phytochemical activities. Spot test and gas chromatography-mass spectrometry (GC-MS) were used to determine the phytoconstituents of the extracts. The plant extracts were tested against three multi-resistant bacteria isolated from infected wound. The antibacterial activity of the plant extracts was evaluated against Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli using agar well diffusion method. Broth dilution method was used to determine the minimum inhibitory concentration (MIC) and minimum bacteriocidal concentration (MBC). The extracts showed the presence of alkaloid, tannins, flavonoids, saponins, cardio-active glycosides, terpenoids, phenols and steroids. The GC-MS analysis showed the presence of 17 compounds in the leaf extract and 16 compounds in root bark extract. The two extracts have 7 compounds in common. The results of the zone of inhibition test showed that the root extract at the concentration of 50 and 100 mg/mL inhibited the growth of E. coli, P. aeruginosa and S. aureus while the leaf extract inhibited the growth of E. coli and S. aureus at the concentration of 25, 50 and 100 mg/mL and P. aeruginosa at 50 and 100 mg/mL. The MIC of the root bark extract against all the test bacteria was at 50 mg/mL while the MIC of leaf extract against E. coli and S. aureus was at 25 mg/mL and P. aeruginosa at 50 mg/mL. MBC of both extracts against the three test bacteria was at 100 mg/mL except root bark extract that has no MBC against P. aeruginosa. The study shows that methanol extract of the leaf and root-bark of Newbouldia leavis possess antibacterial activity and justified the traditional use of this plant in the treatment of wound and other bacterial infection.

Keywords: Antibacterial, phytochemical, *Newbouldia leavis,* broth dilution, Gas Chromatography-Mass Spectrometry.

INTRODUCTION

The traditional medicinal practice is employed for the treatment of various ailments in many societies especially the African society. This practice continues to exist in the developing nations including Nigeria. It is on this basis that researchers keep on working on medicinal plants in order to produce the best medicines for therapeutic uses (Yadav and Munin, 2011).

Nowadays, the use of phytochemical constituents for pharmaceutical purposes has gradually increased in many countries. The World Health Organization (WHO, 2001) estimate shows that 80 % of the world population depends on traditional medicine, predominantly originated from plants for their primary healthcare (WHO, 1996).

The medicinal values of plant lie in the presence of some endogenous substances that produce definite physiological or pharmacological actions on the human body (Ullah *et al*. 2012). Phytochemicals, being natural and bioactive compounds are produced by plants as protective agents against external stress and pathogenic attack hence are source for plant defense and survival (Ullah *et al.,* 2012).

Wounds have a potential for serious bacterial infections, including gas gangrene and tetanus and these in turn may lead to long term disabilities, chronic wound or bone infection, and death (Kotz *et al*., 2009). Wound infection is particularly of concern when multi-resistance organisms are involved in the infection. In recent times, there has been increase in bacterial resistant strains of clinical importance which have resulted in the emergence of new multi-drug resistant bacterial strains (WHO, 2001). The non-availability and high cost of synthetic drugs with limited efficacy has led to increased morbidity and mortality (Williams, 2000). This has led to the search for new, safe and effective antibacterial agent of plant origin with the aim of discovering potentially useful active ingredient that can serve as source and template for the synthesis of new anti-bacterial drugs which can be used to treat bacterial wound infection and other infectious diseases (Mamah *et al.,* 2014; Pretorious *et al.,* 2003).

Newbouldia leavis commonly called 'Aduruku' in Hausa, 'Ogirisi' in Igbo and 'Akoko' in Yoruba languages (Hutchinson and Dalziel, 1963) used for this research is a medium sized angiosperm which belongs to the Bignoniaceae family. It grows to a height of about 7-8 (up to 15) metres, more usually a shrub of 2-3 meters, many-stemmed forming clumps of gnarled branches. *Newbouldia leavis* is native to tropical Africa and grows from Guinea Savannahs to dense forests (Arbonnier, 2004). It is one of the plants with magical effect (Idu *et al.,* 2003). Scientifically it has been reported to have medicinal value ranging from anti-inflammatory, antimalarial, antioxidant, anti-bacterial, antifungal, analgesic and wound healing properties (Akerele *et al.,* 2011; Omokpo *et al.,* 2012). Specifically, the leaves have been used in the South-Eastern and Western part of Mid-Western Nigeria, for the treatment of septic wounds and eye problems (Akerele *et al.,* 2011). While Usman and Osuji, (2007) reported the remarkable antibacterial potentials of the methanol leaf extract. Chukwujekwu *et al.* (2005) investigated the anti-inflammatory antibacterial and the antimalarial activities of the methanol root bark extracts.

Recent phytochemical studies on the methanol leaf and root bark of this plant revealed the presence of alkaloid, tannins, flavonoids, saponins, terpenoids, cardio-active glycosides and steroids (Aladesanmi *et al.,* 1998; Germann *et al.,* 2006). Based on the ethnomedicinal information on the use of the plant to treat wound infection, this work is designed to investigate the antibacterial and phytochemical properties of *N. leavis* against some bacteria commonly found responsible for wound infection.

MATERIALS AND METHODS

Sample collection and identification

Newbouldia leavis (leaf and root-bark) samples were collected on the 17th day of December, 2018, from the premises of Humid Forest Research Outstation, Umuahia, Abia State, Nigeria. The plant leaf and root bark sample were identified and authenticated by Prof. M.C. Dike of the college of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike, Nigeria. The voucher sample of root and leaf of *N. leavis* were deposited in the herbarium of the Department of Veterinary Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike, Nigeria, with the voucher no: MOUAU/VPP/18/016 for reference. The bacteria (*Escherichia coli*, *Pseudomonas aeruginosa and Staphylococcus aureus*) were obtained from the Microbiology Laboratory of the Federal Medical Center, Umuahia, Abia State, Nigeria. They were sub cultured and re-identified.

Preparation and extraction of the plant sample

The leaf and root-bark of *N. leavis* were washed with tap water and air dried for 2 weeks with intermittent turning to prevent fungal growth (Bonjar, 2004). They were pulverized into fine powder using a milling machine. The powdered form of the plant sample was stored in air tight sterile container at room temperature. Eighty grams each of the pulverized leaf and root bark powder were extracted with 400 mL methanol with the aid of soxhlet

apparatus. The extract was concentrated in a hot air oven at 40 ℃ and the concentrated extracts gave dark green paste which was stored aseptically in the refrigerator till needed (Bonjar, 2004).

Preparation of stock solution of the extracts

The plant leaf and root bark crude extract were reconstituted, 1.0 g each with 10 mL of methanol to give a concentration of 100 mg/ mL (NCCLS, 2000).

Phytochemical and Gas Chromatography-Mass Spectroscopy (GC-MS) screening of the plant sample

The preliminary phytochemical test was done on the fine powdered form of the plant samples using standard procedure as described by Sofowora (1993), Trease and Evans (2002). Gas chromatography-mass spectroscopy (GC-MS) were also used on the crude extract (N.I.S.T, 2009).

Antimicrobial Bioassay

Bacteria growth of 24 hours were prepared and adjusted to 0.5 McFarland standard solution which corresponded to approximately $1.5x10^8$ CFU/ml (NCCLS 2000). Agar well diffusion method as described by El-Mahmood (2009) was used to determine the antibacterial activities of the extracts against the test organisms – *Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus.*

Determination of the Zone of Inhibition Diameter of the Leaf and Root Bark Extracts of *N. leavis*

Sterile Mueller Hilton agar plates were seeded with 0.1 mL of overnight standardized broth culture of each bacterial isolate. The seeded Petri dishes were uniformly distributed and allowed to set. Standard cork-borer of 6 mm in diameter was used to cut uniform wells on the surface of the agar. Serial dilution of the extract stock solution was prepared in five-fold (6.25, 12.5, 25, 50 and 100 mg/ mL). Each plate inoculated with each bacterial isolate has three holes. Each of the holes contained a concentration of the extract, positive and negative control respectively. The well was filled with 0.5 mL of each solution with the aid of sterile Pasteur pipettes and allowed to stand for 45 minutes at room temperature for proper diffusion. Methanol used as diluent was used as negative control while Gentamicin was used as positive control. The holes were 22 mm from each other and 14 mm from the edge of the plate. They were all incubated at 37 ℃ for 24 hours. The antimicrobial activity was determined by measuring the diameter of zones of inhibition produced after incubation using a transparent meter rule in millimeter (mm) (Okwu, 2007). The tests were carried out in replicates.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The Minimum Inhibitory Concentration (MIC) of the extract was determined using broth dilution method (El-Mahmood, 2009). Synthetic drug (Gentamicin) was used as positive control while the diluent (methanol) was used as negative control. All tubes were plugged with cotton wool, incubated for 24 hours at 37 ^oC and observed visually for growth which was indicated by turbidity. The lowest concentration that inhibits the growth of the test organisms (absence of turbidity) were recorded as the Lowest Inhibitory Concentration of the extract (Bonjar, 2004).

The minimal bactericidal concentration test was determined from broth dilution test resulting from the MIC tubes and the concentration of the extract before and after the MIC test tube were used as described by (Usman *et al.*, 2005). A loop full of the content of each test tube were sub cultured on Mueller Hinton agar plates and incubated at 37 ^oC for 24 hours and observed for growth. The lowest concentration of the extract that showed no growth was noted and recorded as the minimum bactericidal concentration (MBC).

RESULTS

Phytochemical screening

The presence of flavonoids, alkaloid, steroids, cardiac glycosides, terpenoids and tannins were identified in both leaf and root bark of *N. leavis*. Saponin was present only in the root bark while phenol was present only in the leaf extract (Table 1). GC-MS analysis of the methanol extract of the leaf and root bark of *N. laevis* reveals the presence of 16 compounds (Table 2) in the root bark extract while the leaf extract contains 17 compounds (Table 3). The two extracts have some compounds (MDMA methylene homolog, amionooxy acetic acid, N-methyl-3,4-methylenedioxyphenyl propan-3-amine,

beta-d-Lyxofuranoside, methyl, isobutylamine, benzene acetic acid and L-aspartic acid) in common. The results of the zone of inhibition test (Table 4) showed that the root extract (50 and 100 mg/mL) inhibited the growth of *E. coli*, *P. aeruginosa and S. aureus* while the leaf extract inhibited the growth of *E. coli* and *P. aeruginosa*, but did not inhibit the growth of *S. aureus.* The result of the MIC is presented in Table 5. The MIC of the leaf extract against *E. coli* and *S. aureus* was 25 mg/mL while the MIC against the *P. aeruginosa* was 50 mg/mL. The MIC of the root extract against *P. aeruginosa* and *E. coli* was 50 mg/mL while the MIC against the S.aureus was 25 mg/mL. The MBC of the leaf extract against *E. coli*, *P. aeruginosa and S. aureus* was 100 mg/mL while the MBC of the root extract against *E. coli and S. aureus* was 100 mg/mL (Table 6).

Table 1: Preliminary Phytochemical tests of the methanolic leaf and root bark extract of *Newbouldia laevis*.

Phytochemical Constituents	Leaf	Root bark
Saponins	-	+
Alkaloids	+	+
Tannins	+	+
Flavonoids	+	+
Terpenoids	+	+
Cardiac glycosides	+	+
Steroids	+	+
Phenol	+	-

+ Present; – Absent

Table 2: GC-MS Analysis of the Methanolic Fraction of *N. leavis* Root Extract

S/N	RT	Area	Compound	MF	MW (g/mol)
1	68.152	1.21	Carbonyl sulfide	COS	60.07
2	82.000	9.94	MDMA methylene homolog	$C_{12}H_{17}NO_2$	207.273
3	83.502	32.83	Acetic acid(aminooxy)	$C_2H_5NO_3$	91.066
4	85.077	3.03	Ethanol,1-(methylenecyclopropyl)	$C_6H_{10}O$	98.145
5	85.516	12.19	N-methyl-3,4-methylenedioxyphenyl propan-3-amine	$C_{11}H_{15}NO_2$	193.246
6	87.055	7.17	Trimethylsilyl-di(timethylsiloxy)silane	$C_9H_{27}O_2Si_4$	279.653
7	88.008	7.52	Beta-d-Lyxofuranoside, methyl	$C_6H_{12}O_5$	164.156
8	89.436	2.69	1,3-Bis-(2-cyclopropyl,2-methylcyclopropyl) -but-2-en-1-one	$C_{18}H_{26}O$	258.405
9	89.656	2.41	Isobutylamine	$C_4H_{11}N$	73.139
10	91.195	3.28	Benzene acetic acid	$C_{14}H_{15}NO_4$	261.277
11	91.488	2.15	L-Aspartic acid	$C_9H_{17}N_3O_5$	247.251
12	92.257	1.36	Silane	SiH_4	32.117
13	92.697	3.41	Malic acid	$C_4H_6O_5$	134.087
14	94.126	5.19	Propanamide	C_3H_7NO	73.095
15	94.639	1.22	Benzotriazol-1-carboxylic acid,3-oxide,ethyl ester	$C_9H_9N_3$	207.186
16	95.261	4.41	Thiirane	$C_9H_{14}OS$	170.27

Key: RT: retention time, MF: molecular formula, MW: molecular weight

Table 3: GC-MS analysis of the methanol fraction of *N. leavis* leaf extract

S/No.	RT	Area	Compound	MF	MW(g/mol)
1	79.032	1.19	Carbamodithioic acid	$C_5H_{12}N_2S_2$	164.285
2	79.948	38.74	Acetic acid, (aminooxy)	$C_2H_5NO_3$	91.066
3	80.095	1.21	5-Chlorovaleric acid	$C_5H_9ClO_2$	136.575
4	82.476	4.05	Isobutylamine	$C_4H_{11}N$	73.139
5	83.502	1.22	1-Methylverbenol	$C_{12}H_{20}O$	180.291
6	83.722	11.33	MDMA-Methylene homolog	$C_{12}H_{17}NO_2$	207.273
7	84.784	1.23	N-Desmethyl- Tapentadol	$C_{13}H_{21}NO$	207.317
8	85.480	1.30	1-Methylverbenol ether	$C_{11}H_{18}O$	166.264
9	86.286	5.99	L-Aspartic acid	$C_9H_{17}N_3O_5$	247.251
10	87.971	5.92	Beta-d-Lyxofuranoside, methyl	$C_6H_{12}O_5$	164.156
11	88.154	4.37	1-(2-Adamantylidene) semicarbazide	CH_5N_3O	75.071
12	88.447	12.97	N-methyl-3,4-methylenediox-yphenylpropan-3-amine	$C_{11}H_{15}NO_2$	193.246
13	89.400	1.81	Arachidonic acid	$C_{20}H_{32}O_2$	304.474
14	89.840	2.19	1,3-Bis-t-butylperoxy-Phthalan	C16H24O5	296.363
15	89.986	3.46	Benzene acetic acid	$C_{14}H_{15}NO_4$	261.277
16	96.104	1.62	2-Cyclopentene-1-thione, 2,3,4,4-tetramethyl	$C_{11}H_{16}S$	180.310
17	96.947	2.30	Benzonitrile,3,5-dinitro-	$C_7H_3N_3O_S$	193.116

Key: RT: retention time, MF: molecular formula, MW: molecular weight

Table 4: The Mean Zone of Inhibition Diameter of The Leaf and Root Bark Extracts of *N. leavis*

Organism	Zone of inhibition (mm) ± SD				
	Conc. (mg/mL)	LM	RM	NC	PC
Pseudomonas aeruginosa	6.25	0.00	0.00	0.00	14.00 ± 1.57^{b}
	12.5	0.00	0.00	0.00	14.83 ± 1.45^{b}
	25	0.00	0.00	0.00	18.17 ± 1.30^{a}
	50	15.00 ± 0.58^{a}	11.00 ± 0.00^{b}	0.00	18.33 ± 1.28^{a}
	100	16.67 ± 0.33^{a}	12.33 ± 0.33^{a}	0.00	18.67 ± 1.31^{a}
Escherichia Coli	6.25	0.00	0.00	0.00	16.17 ± 1.64^{b}
	12.5	0.00	0.00	0.00	18.33 ± 1.41^{b}
	25	11.67 ± 0.33^{b}	0.00	0.00	19.67 ± 0.84^{ab}
	50	13.00 ± 0.00^{b}	11.33 ± 0.33^{b}	0.00	21.00 ± 0.45^{a}
	100	17.67 ± 0.67^{a}	14.00 ± 0.00^{a}	0.00	21.00 ± 0.45^{a}
Staphylococcus aureus	6.25	0.00	0.00	0.00	10.50 ± 0.29^{c}
	12.5	0.00	0.00	0.00	12.00 ± 1.35^{bc}
	25	13.00 ± 0.00^{b}	0.00	0.00	14.00 ± 1.41^{ab}
	50	14.00 ± 0.00^{b}	11.00 ± 0.00^{b}	0.00	15.75 ± 1.93^{a}
	100	17.00 ± 0.00^{a}	12.33 ± 0.00^{a}	0.00	16.00 ± 2.04^{a}

Key: LM - leaf methanol, RM - root methanol, NC- negative control, PC - positive control.
^{a,b,c} significantly different vertically at $P < 0.05$.

Table 5: Minimum Inhibitory Concentration of Extracts on Test Organisms

Organisms	Concentrations (mg/mL)			
	LM	**RM**	**NC**	**PC**
Pseudomonas aeruginosa	50.0	50.0	0	6.25
Escherichia coli	25.0	50.0	0	6.25
Staphylococcus aureus	25.0	50.0	0	6.25

Key: LM - leaf methanol, RM - root methanol, NC- negative control, PC - positive control

Table 6: Minimum Bactericidal Concentration of Extracts on Test Organisms

Organisms	Concentrations (mg/mL)			
	LM	**RM**	**NC**	**PC**
Pseudomonas aeruginosa	100.0	0	0	25.0
Escherichia coli	100.0	100.0	0	12.5
Staphylococcus aureus	100.0	100.0	0	25.0

Key*:* LM - leaf methanol, RM - root methanol, NC - negative control, PC - positive control.

DISCUSSION

The phytochemical composition and the antibacterial activities of the methanol extract of the leaf and root-bark of *Newbouldia leavis* against *E. coli*, *P. aeruginosa and S. aureus* were evaluated. The leaf extract exhibited both bacteriostatic and bactericidal activities against all the test bacteria. The root-bark extract elicited bacteriostatic and bactericidal activities against *E. coli and S. aureus* but had only bacteriostatic activity against *P. aeruginosa.* The antibacterial activities of the extracts could be attributed to the high quantity of aminooxy acetic acid (Gloriozova and Dembitsky, 2018; Usman *et al.,* 2005).

The leaf extract of *N. laevis* produced a wider zone of inhibition against Gram negative bacteria, *E. coli* and *P. aeruginosa* than the root extract; thus was more potent than the root extract. This could be due to the differences in the concentration of the phytoconstituents. The leaf extract has higher concentration of aminooxy acetic acid, MDMA-Methylene homolog, Isobutylamine and L-Aspartic acid than the root extract. The leaf and root-bark extract had bacteriostatic activity at lower concentration (25 mg/mL) and bactericidal activity at higher concentration (100 mg/mL). This observation is in agreement with the report of Akerele *et al* (2011). The results of this study suggest that *N. laevis* has antibacterial activities against all the test organisms which implies that the extracts are broad spectrum in activities. This correlates with the observation of the previous works by Ogbe *et al*., (2009) and Muhammad *et al.*, (2012).

CONCLUSION

This study confirms the antibacterial activity of the leaf and root bark extract of *Newbouldia leavis.* This is due to the presence of some indigenous phytocontituents in the extracts. This proves the therapeutic value of using materials of plant origin in the management of bacterial infected wounds. It is further suggested that more studies should be carried out on this important medicinal plant to harness its great utilizable potentials.

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