

Original Research Article

Phytochemical Screening, Mineral Content Analysis and Assessment of Antibacterial Activity of Methanol Extract of *Newbouldia laevis* Leaf

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Abstract: This study dealt with the assessment of the phytochemicals, antibacterial and mineral analysis of methanol extract of leaf *Newbouldia laevis* and its fractions (hexane, chloroform and ethyl acetate). Quantitative phytochemical screenings were done by various standard laboratory methods. Quantitative phytochemical screening was carried out using spectrophotometry method while mineral analysis was determined by atomic absorption spectrophotometric method. Antibacterial activity was investigated using agar well diffusion method. The result obtained from the phytochemical screening revealed the presence of alkaloids, phenols, oxalate, tannin, saponin among others. The antibacterial activity of *Newbouldia laevis* leaf extracts was tested on *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia*, and *Streptococcus pneumonia*. Antibacterial effect of *Newbouldia laevis* leaf showed that only n-hexane fraction was capable of inhibiting all microorganisms used except *Staphylococcus aureus* in concentration dependent manner. Chloroform fraction was found to inhibit only *E.coli* at higher concentration (100-150 mg/ml) as indicated in the zone of inhibition, while it has no effect on other bacteria. Methanol extract showed inhibition activity only on *S. aureus* at 100-150 mg/ml. All the microorganisms showed resistance to the aqueous fraction of *Newbouldia laevis* leaf. Mineral analysis revealed the presence of Zn, Co, Cd, Cu, Ca, Fe and Mn. These results could suggest the promising chemopreventive use of *Newbouldia laevis* and some of its active principles in the treatment of infections.

Keywords: *Newbouldia laevis*; phytochemical; mineral analysis; bacterial activity.

INTRODUCTION

Newbouldia laevis (L) is a medium sized angiosperm which belongs to the Bignoniaceae family [1]. In Nigeria vanacular, it is called *Aduruku* in Hausa, 'Ogirisi' in Igbo and 'Akoko' in Yoruba. The plant has been found to be effective in the treatment of elephantiasis, dysentery, rheumatic swellings, syphilis, constipation, pile and as a vermifuge to round worms. It has also been found useful for earache, sore feet, chest pain, epilepsy and children's convulsion [2].

Considering the ability of gastrointestinal pathogens to give rise to resistant strain to antibiotics, there is a need for alternative therapy. Since *Newbouldia laevis* leaf is an antimicrobial agent there will need to validate its potency against these resistant strains of selected pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia* and *Streptococcus pneumonia* [3]. Elemental minerals play important roles in human as cofactors in metabolic pathways, blood clotting; maintenance of membrane integrity, connective tissues and so on. In view of this,

the present study is designed to analyze the mineral content and to study the antibacterial activity of methanol extract, n-hexane fraction and chloroform fraction of *Newbouldia laevis* leaf.

MATERIALS AND METHODS

Experimental Design

Collection and identification of plants

The leaf of *Newbouldia laevis* was collected from Anyigba, Kogi State and was authenticated by a taxonomist in the Herbarium Unit, Salem University Lokoja, Kogi State with Voucher Specimen Number SU11012.

Extraction

The leaves of *Newbouldia laevis* was dried at room temperature (28 – 30°C) for four (4) weeks (30 days) and pulverized to a fine powder using a blender. The extract was obtained by cold extraction method with methanol in 1:10 (w/v) ratio. After thoroughly mixed, it was allowed to stand for 72 hours and filtered using sterile Whatman No 1 filter paper.

The green colour filtrate (extract) was concentrated using rotary evaporator. The resulting crude extract was evaporated to dryness using water bath. The crude methanol extract was partitioned successively between n-hexane, chloroform, ethyl acetate and distilled water using vacuum liquid chromatography (VLC).

Phytochemical screening of plants

Flavonoids were detected with ethanolic KOH/ethyl acetate and alkaloids were discovered using Mayer's, Wagner's, Dragendorff reagents and picric acid. Also, saponins were identified using froth and haemolytic tests and cardiac glycosides were identified using chloroform/H₂SO₄. Tannins and phenols were identified using ferric chloride reagent, whereas ethanolic NaOH was used to determine the presence of anthraquinones [4, 5].

These phytochemicals were quantitatively measured using spectrophotometric method. Alkaloid was quantified using ethanol/H₂SO₄ treatment with formaldehyde and absorbance taken at 565nm [6]. Flavonoid was determined by HCl hydrolysis and subsequent treatment with ethyl acetate and ammonium solution detectable at 420nm- 520nm [6]. Saponin was detected by spectrophotometer at wavelength 490nm [7]. The quantity of tannins is determined by using spectrophotometric method measured at 395nm [6]. Total phenolic content was determined by standard method of Makkar *et al.* [8].

Method for Mineral Analysis

Atomic absorption spectrophotometry method was used in the analysis of the elemental mineral, Zn, Co, Cd, Cu, Ca, Fe and Mn according to by Rowe, 1973 and Vishal and Vishwa [9,10]. In this technique, the sample was digested by organic matter constituent oxidation and aspiration into characteristic flame. Perkin-Elmer Atomic Spectroscopy Instrument- L-301 was used.

Sources and Maintenance of Organisms

Gram-positive organisms (*Staphylococcus aureus*, *Staphylococcus pneumonia*), Gram-negative organisms (*Klebsiella pneumonia*, *Escherichia coli*) were obtained from State Specialist Hospital, Lokoja, Kogi State and confirmed at the Research Laboratory of the Department of Microbiology, College of Natural and Applied Science, Salem University, Lokoja, Kogi State, Nigeria.

Standard Drug

Streptomycin, the standard antibiotic used was purchased from Sigma Chemicals Co, London.

Antimicrobial Screening Method

Antimicrobial activities of methanol crude extract and its hexane, chloroform, aqueous fractions

were carried out using the agar well diffusion method [11]. 0.2ml of an overnight broth culture of test micro-organisms was added to 20ml of cooled molten agar. It was well mixed and then poured into a sterile petri-dish and allowed to set. The stock was maintained on nutrient agar slant and sub-cultured in nutrient broth for incubation at 37°C prior to each antimicrobial testing.

Inoculation of the test organisms on nutrient agar-prepared plates was achieved by flaming a wire loop on a spirit lamp, cooling the wire loop (air cooling) and fetching the test organisms. The discs were prepared using a Grade No. 1 Whatman filter paper. 100 discs were obtained by punching and putting in vials and sterilizing in an oven at 150°C for 15 min. Thereafter the cups (9mm diameter) were aseptically bored into the solid nutrient agar using a sterile cork borer.

The test solutions of extracts at various concentrations of 50mg/ml, 100mg/ml and 150mg/ml were then introduced into each of the designated cups on each plate ensuring that no spillage occurred. The same amount of the standard antimicrobial agent and solvents (DMSO, dimethyl sulphoxide) were introduced into the remaining cups on each plate to act as positive and negative controls respectively. The plates were left at room temperature for 1 hour, allowed to diffuse into the medium, turned upside-down and thereafter incubated at 37°C for 24 hrs in an incubator. Clear zones of inhibition were observed. Activity or inactivity of each extract was tested in triplicate and the diameters of zones of inhibition were measured in millimeter.

RESULTS

Table 1 shows the antibacterial activity that only n-hexane fraction was capable inhibiting all organisms in concentration dependent manner. However, *Staphylococcus aureus* was not affected.

Table 2 shows the antibacterial activity profile of chloroform fraction of *Newbouldia laevis* leaf. Except for *K. pneumonia*, other bacteria were not affected.

Table 3 shows antibacterial activity profile of methanol extract of *Newbouldia laevis*. Except for *S. aureus*, other bacterial organisms were not affected.

Table 4 shows antibacterial activity of aqueous fraction of *Newbouldia laevis*. Aqueous fraction was devoid of any antibacterial activity.

Table 5 showed the result of mineral composition of the *Newbouldia laevis* leaf. The leaf extract was found to contain magnesium (Mg), zinc (Zn), copper (Cu), calcium (Ca), iron (Fe), manganese (Mn), while cadmium (Cd) and cobalt was below detectable level (BDL).

Table 6 showed the qualitative phytochemical screening of methanol extract of *Newbouldia laevis* leaf and its fractions. It was observed to be composed of alkaloid, carbohydrate, cardiac glycoside, oxalate, tannin, terpenoids, phenols and saponins are present in the methanol extract and its fractions of *Newbouldia laevis* (NL) leaf.

Table 7 revealed the quantitative phytochemical analysis of NL leaf methanol extract and its chloroform fractions. The result indicated that 0.47% alkaloids, 1.13% saponins, 0.58% phenol, 0.88% oxalate and 9.2% tannins were found in the crude methanol extract. Whereas, 0.3% alkaloid, 0.72% saponins, 0.38% phenol, 0.96% oxalate and 7.6% tannin were present in the chloroform.

Table 1: Antibacterial activity of n-hexane fraction of *Newbouldia laevis* leaf

Concentration of extracts (mg/ml)	Organisms/Zone of inhibition (mm)			
	<i>S. pneumonia</i>	<i>E.coli</i>	<i>K. pneumonia</i>	<i>S.aureus</i>
150	10±0.2	16± 0.4	9± 0.7	-
100	6 ±0.4	11± 0.2	-	-
50	-	10± 0.2	-	-
Control +	17 ± 0.2	17± 0.4	19±0.2	15±0.7
Control -	-	-	-	-

Values are of means of two replication ± SD

Control + : Streptomycin Control - : Water

Table 2: Antibacterial activity of chloroform fraction of *Newbouldia laevis*

Concentration of extracts (mg/ml)	Organisms/Zone of inhibition (mm)			
	<i>S. pneumonia</i>	<i>E.coli</i>	<i>K. pneumonia</i>	<i>S.aureus</i>
150	-	-	10±06	-
100	-	-	8±0.2	-
50	-	10± 0.2	-	-
Control +	13± 0.2	16±0.4	16 ± 0.7	11±0.2
Control -	-	-	-	-

Values are of means of two replication ± SD

Control + : Streptomycin Control - : Water

Table 3: Antibacterial activity of methanol extract of *Newbouldia laevis* leaf

Concentration of extracts (mg/ml)	Organisms/Zone of inhibition (mm)			
	<i>S. pneumonia</i>	<i>E.coli</i>	<i>K. pneumonia</i>	<i>S.aureus</i>
150	-	-	-	11±0.3
100	-	-	-	-
50	-	-	-	-
Control +	15± 0. 6	18±0.2	17± 0.4	15±0.6
Control -	-	-	-	-

Values are of means of two replication ± SD

Control + : Streptomycin Control - : Water

Table 4: Antibacterial activity of aqueous fraction of *Newbouldia laevis* leaf

Concentration of extracts (mg/ml)	Organisms/Zone of inhibition (mm)			
	<i>S. pneumonia</i>	<i>E.coli</i>	<i>K. pneumonia</i>	<i>S.aureus</i>
150	-	-	-	-
100	-	-	-	-
50	-	-	-	-
Control +	16 ±0.2	14± 0.4	11 ± 0.7	12± 0.6
Control -	-	-	-	-

Values are of means of two replication ± SD

Control + : Streptomycin Control - : Water

Table 5: Mineral compositions of *Newbouldia laevis*

Elemental analysis	Amount of minerals (mg /g)
Mg	0.496
Zn	0.066
Co	BDL
Cd	BDL
Ca	0.015
Fe	7.166
Mn	0.060

BDL: Below detectable level

Table 6: Qualitative phytochemical analysis of *Newbouldia laevis* leaf extracts

Phytochemical	Methanol extract	Hexane fraction	Chloroform fraction	Aqueous fraction
Alkaloid	+	-	+	-
Carbohydrate	+	-	+	+
Cardiac glycoside	+	-	+	+
Phenol	+	-	+	+
Saponin	+	-	+	+
Tannins	+	-	+	+
Terpenoids	+	-	+	+
Quinone	+	-	+	+
Oxalate	+	-	+	+

Table 7: Quantitative phytochemical analysis of *Newbouldia laevis* leaf extracts

Phytochemical	Methanol extract %	Chloroform fraction %	Aqueous fraction %
Alkaloid	0.47	0.30	-
Phenol	0.58	0.38	0.15
Saponin	1.13	0.72	0.62
Tannins	9.20	7.20	2.71
Oxalate	0.88	0.96	-

DISCUSSION

The results in Table 1 support the antibacterial activities of *Newbouldia laevis* as a broad spectrum antibacterial agent since hexane fraction was able to inhibit the growth of gram- positive (*S. pneumonia*) and gram negative bacteria (*E. coli* and *K. pneumonia*). This is consistent with the previous report showing the efficacy of methanol extract of the stem bark of *N.laevis* against some gram negative and gram positive bacteria [12]. Chloroform fraction (Table 2) was capable of preventing the growth of gram negative *K. pneumonia*. It was also discovered that methanol extract (Table 3) was able to inhibit only *S.aureus* and this may be attributed to the modulation of the active components in the crude extract by substances that could alter their activity [12]. Aqueous fraction (Table 4) is incapable of causing any growth inhibition in all the tested bacteria, probably because the active components are in low concentrations in the fraction.

The fact that the n-hexane and other fractions (except aqueous fraction) of *Newbouldia laevis* showed activity against most of the test organisms is a major breakthrough in appreciating the medicinal potential of the plant especially in the management of clinic community acquired infections (nosocomial associated infections).

Comparison of the hexane fraction with the commercial antibiotic showed that the fraction shows high level of inhibition towards the tested organisms as the commercial antibiotics. This shows that the n-hexane fraction of *Newbouldia laevis* can be used as a broad spectrum antibiotic since it exhibited a profound effect on most of the organisms tested than other fractions. The ability of this plant to be capable of inhibiting the activity of various bacteria may be associated with the presence of the bioactive principle or the secondary metabolites including tannins which have cytotoxic potentials hence are effective against some bacterial activities. Also, hexane fraction inhibitory activity on the bacteria may be adduced to the presence of oxalate in high concentration which might have overwhelmed the oxalate-degrading capability of the microorganisms, thus, resulting in oxalate toxicity and eventually in bacterial inhibition.

Also, the presence of quinone might have provided a source of stable radicals which can irreversibly bind with nucleophile amino acids in proteins thereby causing inactivation and loss of protein function in bacteria [13, 14]. Saponins too possess detergent-like properties which may increase the permeability of bacterial cell membranes thereby facilitating antibiotic influx through the bacterial cell wall [15, 16, 17].

Mineral analysis (table 5) of the leaf powder revealed the presence of Mg, Zn, Co, Cd, Cu, Ca, Fe, and Mn. Reports showed that Zn, Co, Cd and Cu have been implicated in the regulation of acid-base balance as well as condition of nerve impulse [18]. Mg and Ca functions as enzymes activator (ATPase, lipase) and enzyme cofactor respectively. They are also vital constitute of bones and teeth. In this regard therefore, *Newbouldia laevis* leaves could be a good supplement in animal feeds.

CONCLUSION

From this study, it may be concluded that the presence of some secondary metabolites and mineral components in *Newbouldia laevis* leaf may be responsible for the inhibition of the bacteria observed in this present study and could also justify its local use in the treatment of sore feet. Therefore, it could be useful chemotherapeutic agent in prevention, inhibition or retardation of pathogenesis of some infectious diseases.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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