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## Phytochemistry and Antibacterial Assessment of Ethyl acetate Leaf Extract of *Newbouldia laevis*

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### Abstract

A current trend in drug research is the desire to obtain novel antimicrobials with plant origins as an alternative therapy and a means to curb antimicrobial resistance. This study evaluated the phytochemistry and antibacterial activity of ethyl acetate leaf extract of *Newbouldia laevis*. The leaf of *Newbouldia laevis* was cold extracted with Ethyl acetate. The extract was evaluated for its phytochemical constituents using standard methods and antibacterial effect against some clinical bacterial isolates using well diffusion technique. The study revealed the presence of some phytochemicals, alkaloids, saponins, flavonoids, phenol, anthraquinones and steroids in the plant extract, which were believed to be a contributing factor to the observed antibacterial effects. The test isolates' susceptibility to the crude extract was measured at 25 mg/mL and 50 mg/mL, 75mg/mL and 100mg/mL. The growth inhibition of *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* ranges from 9.66±1.54 to 13.00±0.00, 10.00±1.00 to 11.00±1.00, 9.33±0.57 to 11.00±1.00, 14.00±0.00 to 17.33±1.52 and 9.33±0.57 to 10.66± 1.52 mm respectively. The Minimum Inhibitory Concentration was observed at 25mg/mL, 12.5mg/mL, 25mg/mL, 12.5mg/mL and 25mg/mL against *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* respectively while the Minimum Bactericidal Concentrations was at 50mg/mL, 25mg/mL, 50mg/mL, 25mg/mL and 50mg/mL respectively. The observed antibacterial effects were believed to be due to the presence of alkaloids, saponins, flavonoids, phenol, anthraquinones and steroids detected in the extract. The results apparently justified the traditional use of this plant in treating bacterial infections whose causative agents are the organisms used in this study.

**Keywords:** Antibacterial, Clinical isolates, Cold maceration, *Newbouldia laevis*, Phytochemicals.

### INTRODUCTION

The use of medicinal plants dates back to antiquity and has been improved upon over time. (Salmerón-Manzano *et al.*, 2020). Natural medicinal resources are among the first contributions of nature to human well-being, as the relationship between medicinal plants and human health has been inextricably intertwined for generations (Theodoridis *et al.*, 2023).

Evidence-based studies have verified with evidence the effectiveness of medicinal plants globally. This evidence has also provided insights into the application of synthesized plant-based compounds in therapeutics (Dhama *et al.*, 2014; Ugboko *et al.*, 2020). Medicinal plants used in traditional medicine generally contain various phytochemicals, some of which have biological functions. World health organization (WHO) defined traditional medicine as “the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the

maintenance of health, as well as in the prevention, diagnosis, improvement or treatment of physical and mental illnesses” (WHO, 2023; Salmerón-Manzano *et al.*, 2020). WHO reports documented that about 80% of the world population relies on traditional medicine for their primary health care, especially in developing countries (WHO, 2022).

Antimicrobial resistance (AMR) is a serious public health problem, and may be made worse in developing nations by egregious overuse of antibiotics, availability of antimicrobials over the counter, without a prescription, and non-compliance to antimicrobial policies (Ayukekbong *et al.*, 2017). However, Nigeria as a developing country presents a long history of use of herbal medicine to minimize antimicrobial resistance and cases of mortality associated with AMR. The increase in the use of these herbal products is due to their cultural acceptability, availability, affordability, efficacy, and safety claims (Msomi and Simelane, 2019). Several traditional medicinal

plants, including *Newbouldia laevis*, has been a candidate for research because of its perceived medicinal properties in the management of diseases and conditions like skin infection, tooth, and stomach aches, pains, diabetes, hypertension, tumour, malaria, sickle cell anaemia (Amujoyegbe *et al.*, 2016; Ukwubile *et al.*, 2020; Okagu *et al.*, 2021). The herb is well-known for treating inflammation, edema, septic wounds, eye issues, and sexually transmitted infections in southeast and Midwest Nigeria (Umeyor *et al.*, 2011; Anaduaka *et al.*, 2014; Umeyor *et al.*, 2016a; Umeyor *et al.*, 2016b). To the best of our knowledge, studies on the plant *Newbouldia laevis* in Bida local government of Niger State, Nigeria are sparse. Also, there is little or no records on the minimum inhibitory and minimum bactericidal concentration of ethyl acetate leaf extract of *Newbouldia laevis* in the available literatures. Given this, the present study is designed to enrich the pool of available scientific data on the phytochemistry and the antibacterial efficacy of ethyl acetate leaf extract of *Newbouldia laevis*.

## MATERIALS AND METHODS

### Identification of Plant Materials

Fresh samples of leaves of *Newbouldia laevis* were collected separately from Bida town, 9°05'N 6°01'E / 9.083° N 6.017°E of Niger State (Tiptopglobe.com, 2018). The plant material was identified by a local herbalist (Mallam Hashimu Muhammad) and authenticated by a Botanist (Mr. Lateef Akeem, Herbarium and Ethno-botany Department, National Institute for Pharmaceutical Research Development, Idu, Abuja) and voucher specimen NIPRD/H/6909 was kept in the herbarium of the Institute.

### Preparation of Extracts

The extraction technique employed by Ali *et al.*, (2011) was employed. The plant's leaves were thoroughly dried by air-drying them at room temperature. The sample was dried and ground into a fine powder. Each 400g ground sample was cold-macerated for 72 hours with occasional stirring in 1200 mL of ethyl acetate in a screw-cap bottle. A clean piece of muslin cloth and Whatman No. 1 filter paper were used to filter the extract. The extract was concentrated at 45°C in a water bath, transferred to a sample vial, and refrigerated for additional analysis.

### Phytochemical Screening

According to the methods of Ushie and Adamu (2013), Kendeson *et al.*, (2019), and Ushie *et al.*, (2019), the phytochemical screening of the extract was done to determine whether alkaloids, total phenol, cardiac glycosides, reducing sugars, anthraquinones, flavonoids, phlobatannins, saponins, steroids, and tannins were present or absent.

### Test Organisms

From the Microbiology Laboratory of the General Hospital Minna, Niger state, clinical strains of *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* have been isolated. By using a conventional bacteriological approach, the isolates were verified (Omar-Zahid, 2013). The organisms were kept in slants and stored in the refrigerator for further analysis

### Standardisation of bacteria

The standardization of the bacterial cultures was done using the technique described by Akerele *et al.*, (2011). Each organism's 18-hour culture was suspended in 0.2 mL of sterile universal bottles holding 20 mL of nutrient broth, and the bottles were incubated at 37°C for 5 hours. Using 0.5 McFarland Standard as a visual reference, the suspension was adjusted to a turbidity equal to roughly  $1.5 \times 10^8$ cfu/mL. The antibacterial assay utilized the standardized cultures.

### Antibacterial Assay of Extracts

The study employed an in vitro test utilizing the agar well diffusion method as published by Idu and Igeleke (2012). A sterile 6 mm cork borer was used aseptically to hole four wells on Mueller Hinton agar media surfaces that were roughly equally spaced apart from one another. On the surfaces of the agar plates, 0.2 mL of the standardized ( $1.5 \times 10^8$ cfu/mL) cultures of the examined organisms were seeded equally. Different amounts of the extract at concentrations of 25, 50, 75, and 100 mg/mL reconstituted with dimethylsulfoxide were added to each well. The plates were allowed to sit for 30 minutes to allow for pre-diffusion of the extract before being incubated for 37°C for 16-18 hours. A conventional antibiotic (Ampiclox at a concentration of 30 g/mL) and dimethylsulfoxide were employed as positive and negative controls respectively. The nearest millimeter was used to measure the zones of inhibition. Results in triplicate were averaged, and standard deviations were calculated.

### Determination of Minimal Inhibitory Concentration (MIC)

The Broth dilution method, as reported by Ubulom *et al.*, (2013), was used to determine the minimum inhibitory concentration (MIC). In order to achieve a concentration of 100 mg/mL, 1 mL of the reconstituted extract solution at a concentration of 200 mg/mL was added to another test tube containing 1 mL of sterile broth. Up until the sixth test tube, one mL of this dilution was transferred to another test tube. The seventh test tube did not contain extract but instead contained a pure solvent (DMSO) solution as a negative control. Each tube was then filled with 1 mL of an 18-hour culture of the bacterium that had been

previously adjusted to 10<sup>6</sup> cfu/mL and carefully vortexed. For every test organism, this was repeated. The tubes were kept at 37°C for 24 hours while growth in the form of turbidity was monitored. The least extract concentration in test tubes that did not exhibit turbidity upon visual inspection was noted as the minimum inhibitory concentration.

**Minimal Bactericidal Concentration (MBC)**

The bacterial suspension from the MIC tubes that showed no growth were subcultured onto Mueller Hinton agar plates and incubating at 37°C for 24 h, and the MBC values were ascertained. The tube with the lowest extract concentration that did not exhibit any apparent

growth after incubation was identified as having the lowest minimal bactericidal concentration (Ubulom *et al.*, 2013).

**RESULTS**

**Phytochemical Constituents of Ethyl Acetate Leaf Extract of *Newbouldia laevis***

The phytochemicals obtained in the leaf extract of *Newbouldia laevis* are presented in Table 1. The result revealed the presence of alkaloids, flavonoids, total phenol, saponins, steroids and anthraquinones in varying concentrations. Tannins, cardiac glycosides, reducing sugars and phlobatannins were shown to be absent.

**Table 1: Phytochemical constituents of ethyl acetate leaf extract of *Newbouldia laevis***

Phytochemicals	Leaf Extract
Alkaloids	+
Tannins	-
Flavonoids	+
Total phenols	+
Saponins	+
Cardiac glycosides	-
Phlobatannins	-
Steroids	+
Reducing sugars	-
Anthraquinones	+

Key: -- = absent + = present

**Antibacterial Susceptibility Inference**

The result of antibacterial screening of the different concentrations of the extract on the test organisms is shown in Table 2. The results show that there is no significant difference in the zones of inhibition with increased concentrations of the extract. The zones of inhibition ranges from 9.33mm to 10.66mm for *Pseudomonas aeruginosa*, 9.33mm to 11.00mm for *Klebsiella pneumoniae*, 10.00mm to

11.00mm for *Salmonella typhi*, 14.00mm to 17.33mm for *Staphylococcus aureus* and 9.66mm to 13.00mm for *Escherichia coli* as compared to the standard control Ampiclox (20.00mm to 35.00mm). The highest zone of growth inhibition of 17.33mm was exhibited at 100mg/mL against *Staphylococcus aureus*. The lowest zone of inhibition was observed as 9.33mm at 25mg/mL against *Pseudomonas aeruginosa* and *Salmonella typhi*

**Table 2: Antibacterial activities of ethyl acetate leaf extract of *Newbouldia laevis* and control antibiotic on test bacteria**

Concentration (Mg/mL)	<i>Pseudomonas aeruginosa</i>	<i>Klebsiella pneumoniae</i>	<i>Salmonella typhi</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>
25	9.33± 0.57 <sup>b</sup>	9.33±0.57 <sup>b</sup>	10.00± 1.00 <sup>b</sup>	14.00± 0.00 <sup>d</sup>	9.66± 1.54 <sup>d</sup>
50	10.00±1.00 <sup>b</sup>	10.67± 0.57 <sup>b</sup>	10.67± 1.52 <sup>b</sup>	15.00± 1.00 <sup>cd</sup>	11.00± 0.00 <sup>c</sup>
75	10.66±1.52 <sup>b</sup>	10.67± 0.59 <sup>b</sup>	11.67± 1.52 <sup>b</sup>	17.00± 2.00 <sup>cb</sup>	11.67± 0.58 <sup>c</sup>
100	10.66±1.52 <sup>b</sup>	11.00± 1.00 <sup>b</sup>	11.00± 1.00 <sup>b</sup>	17.33± 1.52 <sup>b</sup>	13.00± 0.00 <sup>b</sup>
30µg/mL(Amplx)	20.00± 0.00 <sup>a</sup>	32.00± 0.00 <sup>a</sup>	35.00± 0.00 <sup>a</sup>	35.00± 0.00 <sup>a</sup>	26.00± 0.00 <sup>a</sup>
DMSO (50%)	0.00±0.00 <sup>e</sup>	0.00±0.00 <sup>e</sup>	0.00±0.00 <sup>e</sup>	0.00±0.00 <sup>e</sup>	0.00±0.00 <sup>e</sup>

Key: Amplx= Ampiclox DMSO= Dimethyl sulfoxide, Values are means ± standard deviation. Values ≥ 10mm are considered active (Usman *et al.*, 2007). According to New Duncan Multiple Range Test, Means with the same superscript across the column are not significantly different (P> 0.05).

**Minimum Inhibitory Concentration**

The minimum inhibitory concentration (MIC) of the extract on the isolates is shown in Table 3.

The result showed minimum inhibitory concentration at 50mg/mL, 25mg/mL, 12.5mg/mL, 12mg/mL and 50mg/mL

**Table 3: Minimum inhibitory concentration (MIC) of ethyl acetate leaf extract (mg/mL) of *Newbouldia laevis***

Organisms	100	50	25	12.5	6.25	3.125
<i>P. aeruginosa</i>	-	-	-	+	+	+
<i>K. pneumoniae</i>	-	-	-	+	+	+
<i>S. typhi</i>	-	-	-	-	+	+
<i>S. aureus</i>	-	-	-	-	+	+
<i>E. coli</i>	-	-	-	+	+	+

Keys: + = Turbid, - = Not turbid. The MIC ranged between 12.5mg/mL - 50mg/mL, at any concentration below the least concentration will render the extract ineffective.

**Minimum Bactericidal Concentration**

The minimum Bactericidal concentration (MBC) of the extract on the isolates is shown in Table 4. The result showed minimum bactericidal

concentration at 50mg/mL for *K. pneumoniae* and *S. aureus* and at 100mg/mL for *P. aeruginosa*, *S. typhi* and *E. coli*.

**Table 4: Minimum bactericidal concentration (MBC) of ethyl acetate leaf extract (mg/mL) of *Newbouldia laevis***

Organisms	100	50	25	12.5	6.25	3.125
<i>P. aeruginosa</i>	-	-	+	+	+	+
<i>K. pneumoniae</i>	-	-	+	+	+	+
<i>S. typhi</i>	-	-	-	+	+	+
<i>S. aureus</i>	-	-	-	+	+	+
<i>E. coli</i>	-	-	+	+	+	+

Keys: + = Turbid, = Not turbid. The MBC ranged between 25mg/mL - 100mg/mL, at any concentration below the least concentration will render the extract bacteriostatic.

**DISCUSSION**

Phytochemicals of plant origin with therapeutic purposes can now be identified and extracted through research. In this investigation, the phytochemical components saponins, anthraquinones, total phenol, flavonoids, steroids, and alkaloids were identified. In a similar study by Ushie *et al.*, (2021), using serial exhaustive extraction method, similar phytoconstituents were identified in ethyl acetate leaf extract of *Newbouldia laevis* with the exception of saponins, steroids and anthraquinones which could be due to the prior extraction with hexane and chloroform which could have led to insufficient quantity of the said compounds to be detected in ethyl acetate. Phlobatannins, Cardiac glycosides, reducing sugars and tannins were not detected in this study, this could be attributed to their absence in the plant part or poor solubility in ethyl acetate solvent. According to Falahati *et al.*, (2005), not all phytochemicals are found in therapeutic plants, and those that are, vary depending on the extraction solvents. Obum-Nnadi *et al.*, (2020) revealed the presence of alkaloids, phenols, flavonoids, anthraquinones and saponins in methanolic and aqueous extracts of *Newbouldia laevis* which are similar to those detected in this study.

The antibacterial activity of *Newbouldia laevis* was evaluated in this study and the results showed that the extract of the plant part has exhibited antibacterial activity against pathogenic strains of *E. coli*, *S. typhi*, *K.pneumoniae*, *S. aureus* and *P. aeruginosa* at varying concentrations depending on the bacterial species, this is in response to the finding of El-Mahmood and Ameh (2007), that different microbes could not thrive at varying doses of plant extracts. However, despite an increase in extract concentrations, there was no significant difference (P> 0.05) in the inhibitory zones. This finding is at variance with the report of Okeke *et al.*, (2023), who observed that higher concentrations showed appreciable and significant growth inhibition. One of the issues that frequently arises while using medicinal plants is the quantity of extract needed to improve successful treatment; in most circumstances, the quantity of extract consumed is unclear. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) predict therapeutic results are one of the criteria used to gauge how effective an antimicrobial agent is. When compared to the typical MIC values of 0.01 - 10 g/mL for conventional antibiotics, the MIC values found

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for the complete test microorganisms were high. The minimum bactericidal concentration of the crude extracts produced higher MBC values, ranging from 25 to 50 mg/mL, suggesting that very high concentrations of the extracts are necessary to have a bacteriocidal effect on the organisms. This corresponds with the findings of Kuta *et al.*, (2015) and Abalaka *et al.* (2011).

## CONCLUSION

Findings from this study revealed that ethylacetate leaf extract of *Newbouldia laevis* contains Alkaloids, Steroids, Phenols, Anthraquinones, Flavonoids and Saponins. However, Tannins, Phlobatannins, Cardiac glycosides and Reducing sugar were absent in the medicinal plant leaf extract. The medicinal plant extract exhibited antibacterial activity on the test organisms with zones of inhibition equal or above ten millilitres (value  $\geq 10$ mm). The minimum inhibitory concentration of the extract varied from 12.5mg/ mL, 25mg/ mL, to

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(12.5 to 25mg/mL). 50mg/ mL while the minimum bactericidal concentration varied from 50mg/ mL to 100mg/mL. The extract's efficacy, probably due to the effects of the secondary metabolites, confirms its uses as an antibacterial agent in traditional medicine. Thus, the extract if well refined could be an effective alternative to some antibiotics to which the test organisms display resistance. However more studies need to be carried in order to discover, refine and validate the active component responsible for antibacterial activity of the plant extracts.

## Conflict of Interest

All authors declare no conflict of interest

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