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Phytochemical Analysis and Antimicrobial Activity of *Newbouldia Laevis* Leaves Extracts

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Abstract
The aim of this research is to determine the qualitative and quantitative phytochemical analysis and antimicrobial activity of *Newbouldia laevis* leaves extract. Extraction of the crude was carried out using cold maceration method with absolute ethanol solvent. Phytochemical screening was carried out using standard method. Qualitative phytochemical screening result shows the presence of phenol, tannins, steroid, terpenes and glycoside, while flavonoid and saponins were not detected. The quantitative phytochemical analysis shows a significant amount of secondary metabolite with high values of 0.00618 and 0.00616 in 1 g of sample for steroid and glycosides where as low value of 0.00241 in 1 g of sample for tannins. The antimicrobial activity was determined using agar well diffusion method with different concentrations against the following test microorganisms, Staphylococcus aureus, Escherichia coli, Salmonella typhi, Shigella sp, Streptococcus pneumoniae, and Pseudomonas aeroginosa with the highest zone of inhibition of 26 mm and 23 mm at 400 mg/ml concentration against Escherichia coli and Staphylococcus aureus. The phytochemical analysis and antimicrobial activity justify the claim of the traditional use of the plant for medicinal purpose.

Keywords: phytochemical analysis, antimicrobial activity, *Newbouldia laevis* leaves extract

1. Introduction

Medicinal plants remain a major source of drug discovery and play an important role in the management of diseases such as infections. It is also worth noting that over 85% of the population in Africa, Asia, and the Middle East use herbal medicine as their first line of treatment [Adeleye *et al.,* 2022].

Medicinal plants are essential natural resources for the treatment of more persistent diseases, various medicinal plants can be used to treat similar diseases, depending on the country in which the disease occurs [Miranda, 2021]. Medicinal plants are used as traditional herbal remedies for some illnesses and disorders such as diabetes, sweating, bleeding, and reduction of extensive haemorrhage regulation of the menstrual cycle, stomach pain, inflammation, and toothache [Chen *et al.,* 2021]. Most of the potent medicinal plants have relatively no toxic or adverse effects when used by humans [Okeye *et al.,* 2014].

Many African plants are used in traditional medicine as antimicrobial agents but only a few are documented. Long before mankind discovered the existence of microbes, the idea that certain plants had healing potential, that they contained what would currently be characterized as antimicrobial was well accepted. Man has used plants to treat common infectious diseases and some of them possess antimicrobial activity [Rios and Recio 2005]. Nowadays an

increasing number of infectious agents are becoming more resistant to commercial antimicrobial compounds, therefore there is a need to develop new drugs from medicinal plants [Oliveira *et al*.2013].

Different phytochemicals are present in plants, these phytochemicals contribute to plant aroma, colour, flavor and protect plants from predators and infections plant-derived components conduct certain biological functions that enhance therapeutic activities such as anti-carcinogenic, anti-mutagenic, anti-inflammatory, and antioxidant properties [Tezere *et al*., 2020].

2. Materials And Method

2.1 Materials/ Equipment

The equipment that were used for this experiment include UV-Visible spectrophotometer, incubator, oven, measuring cylinders, beakers, spatula, filter paper, stirrer, distilled water, test-tubes, round bottom flask, weighing balance, and other laboratory Apparatus.

2.2 Sample Collection and preparation

Fresh leaves of *Newbouldia laevis* leaves were collected from Azare LGA of Bauchi State, Nigeria. The sample was identified by Dr Hayatu Usman Dukku of Biological Science Abubakar Tafawa Balewa University Bauchi. The Sample was washed with distilled water and dried on a clean surface at room temperature.

2.2.1 Grinding and Extraction

The Sample was ground into powder using laboratory mortar and pestle to obtain a homogenous sample. The powdered sample was extracted sequentially by Cold maceration extraction using absolute ethanol to extract much of the most active components from the sample. One hundred grams (100 g) of the powder sample was mixed with 500 mL of absolute ethanol in a conical flask, the mixture was stirred thoroughly with a glass rod, and the conical flask was kept with intermittent shaking for 72 hours. The mixture was filtered using muslin cloth and through whatman No.1 filter paper. The extract was dried in the oven at 40˚c. The resultant residue was kept in a refrigerator at 4˚c till further use [Sankeshwari *et al.,* 2018].

2.2.2 Percentage Recovery

Percentage recovery of the extracts were calculated as shown in the equation below:

$$\%\ \text{extract recovery} = \text{weight of extract} / \text{weight of dried powder sample} \times 100$$

2.3 Qualitative Phytochemical Screening

This is a qualitative analysis which involves performing some simple chemical test in order to detect the kind of secondary metabolites present in the plant extract. The phytochemical analysis of the plant extracts will be performed as described by [Santhi and Sangottuvel, 2016] to identify the presence of the classes of secondary metabolite.

2.3.1 Test for phenols

The extract was dissolved in 10 cm^3 of distilled water. To 2 cm^3 of extract 3-4 drops of ferric chloride solution was added. formation of dark green indicates the presence of phenols.

2.3.2 Test for Alkaloids

To 1 cm^3 of the extract few drops of concentrated hydrochloric acid and Dragendorf´s reagents was added. Formation of white precipitate indicates the presence of Alkaloids.

2.3.3 Test for Saponins

A 2 cm^3 of the extract was shaken in test tube for 30 seconds. Formation of foam indicated the presence of saponins.

2.3.4 Test for flavonoids

To 1 cm^3 of extract, few drops of 10 % lead acetate solution was added sodium hydroxide solution was added. Yellow colour formation indicates the presence of flavonoids.

2.3.5 Test for Tannins

To 2 cm^3 of extract, 1.0 cm^3 of distilled water and 3 drops of 10 % ferric chloride solution was added. Formation of blue or green colour indicated the presence of tannins.

2.3.6 Test for steroid

To 1 cm ³ of the extract 2 cm³ of chloroform and a few drops of concentrated sulphuric acid was added sidewise. Production of red colour in the lower chloroform layer indicated the presence of steroid.

2.3.7 Test for glycoside

To 5.0 cm³ of the extract, 2.0 cm³ of glacial acetic acid containing one drop of ferric chloride solution was added. The resulting solution was underlayed with 1.0 cm³ of concentrated sulphuric acid. Formation of a brown ring of the interface indicated the presence glycosides.

2.3.8 Test for Terpenes

To 1.0 cm³ of extract, 3 cm³ of chloroform was added. The resultant solution was mixed with 2.0 cm³ of concentrated sulphuric acid. Reddish-brown colour at the interface indicated the presence of terpenoids.

2.4 Quantitative Phytochemical Determination

2.4.1 Determination of Tannins Content

The sample (1.0 g) was weighed into a plastic bottle and 50 ml of distilled water was added and shaken for 3 hours in a vibrator. The sample was filtered into a 50 ml volumetric flask and make up to mark. A 5.0 mL portion of the filtrate was dispensed into a test tube and mixed with 1.0 mL of 0.1M FeCl3 in 0.1M HCl and 0.008M potassium ferrocyanide, the absorbance was measured at 720 nm for 10 mins. [Eze *et al.*,2020]. The tannin concentration was determined using the following equation.

Concentration of tannin = Abs ×D.F /100×weight of sample used

where, Abs= value of absorbance read, D.F = dilution factor

2.4.2 Determination of Steroids Content

One gram 1.0 g of sample was dispersed in 100 mL of distilled water into a conical flask, the mixture was shaken for 3 hours and allowed to stand overnight. Then it was filtered, the filtrate was eluted with 100 mL normal ammonium hydroxide solution, 2 mL of the elute was put into a test tube and mixed with 2 mL of chloroform and also 3 mL of acetic hydride was added to the mixture, followed by 2 mL of concentrated H2SO4 drop wisely. The absorbance was measured in a spectrophotometer at 240 nm [Eze *et al.*, 2020] The steroid concentration was determined using the following equation.

Concentration of steroid = Abs ×D.F /100×weight of sample used

2.4.3 Determination of Terpenoid Content

One gram of sample 1.0 g was weighed out separately, macerated with 20 mL of ethanol and filtered through whatman No. 1 filter paper. The filtrates (1ml) was pipette out and 1 mL of 0.5% phophorylbdic acid solution was added and shaken. Gradually 1mL of concentrated H2SO4 was added to each. The mixtures were left to stand for 30 minutes, 2 mL of Ethanol was added and absorbance was measured at 700 nm [Eze *et al.*,2020].

Concentration of terpenoid = Abs ×D.F /100×weight of sample used

2.4.4 Determination of Glycoside Content

One gram 1.0 g of sample was weighed out separately, mecerated with 20 mL of distilled water and 2.5 mL of 15% lead acetate was added and filtered. Chloroform 2.5 mL was added to the filtrate, shaked vigorously and the lower layer was collected and evaporated to dryness. Glacial acetic acid 3 mL was added together with 1 mL of 1% ferric chloride and 1 mL of concentrated H2SO4. The mixture was shaken and put in the dark for 2 hours. Absorbance was measured at 530nm [Eze *et al.*,2020].

Concentration of glycoside = Abs ×D.F /100×weight of sample used

2.4.5 Determination of Phenolic compounds

The sample 1.0 g was defatted with 100 mL of diethyl ether using a soxhlet apparatus for two hours. The defatted sample was boiled with 50 mL of ether for 15 minutes, then 5 mL of the extract was pipetted into a 50 mL flask and 10 mL of distilled water was added. 2 mL of ammonium hydroxide and 5 mL of ethanol was added. The absorbance was measured at 505 nm [Iwu *et al.*, 2018].

2. 5 Antibacterial Assay

2.5.1 Preparation of the Bacterial Inoculum

Broth cultures of desired organism (*Escherichia coli, Staphylococus aureus Salmonella typhi, Shigella species, Streptococcus pneumonia* and *Pseudomonas aeroginosa*) was prepared by suspending two colonies of each organism in nutrient broth and incubated aerobically at 37 ˚C for 12 hours. After incubation the cultures was used for experiments [Akerele *et al.,*2011].

2.5.2 Screening for Antibacterial Activity

Antibacterial activities of ethanol extract was carried out using the agar well difusion method as describe by Obum-Nnadi *et al.* 2020. The isolate was inoculated on the surface of freshly gelled nutrient agar plates by streaking using sterile swab stick. Wells were bored on each agar plate using a sterile cork borer and wells were properly labelled. A mass of 0.4g of the extract was dissolved in distilled water to make different concentrations of 400 g/ml, 200 g/ml, 100 g/ml and 50 g/ml. The extract was introduced into the wells in the plates respectively, the plates was allowed for 40 minutes for pre-diffusion of the extract to occur and incubated at 37 ˚C for 24 hours. The zone diameter of inhibition was measured using a transparent ruler calibrated in millimetres. The readings were taken to be the zone diameter of inhibition, chloramphenicol (250 mg/ml) was used as a positive control and water as negative control

2.5.3 Minimum inhibitory concentration

Minimum inhibitory concentration was carried out by broth dilution method. The extracts was serially diluted to give a concentration of 400, 200, 100 and 50 mg/ml in test tubes. Dilution of the extract is in ratio 1:1 with nutrient broth and incubated for 18-24 hours. After incubation the tubes were examine for microbial growth by observing turbidity. The test tube with least concentration which showed no turbidity indicates the MIC. [Obum-Nnadi *et al.,* 2020].

2.5.4 Minimum bactericidal concentration

Minimum bacterial concentration was determined from broth dilution test resulting from MIC tubes as described previously by inoculating the content of each test tube on a nutrient agar plate. The plates was incubated at 37°C for 24 hours. The lowest concentration of the extract that showed no growth will be noted and recorded as the MBC. [Obum-Nnadi *et al.,* 2020].

3. Results And Discussion

3.1 Percentage of Extraction

Table 1: Extraction Percentage Recovery

Crude Extract	Mass Recovery (g)	Percentage Recovery (%)
Newbouldia laevis leaves	7.60	7.60

3.2 Phytochemicals

3.2.1 Qualitative Phytochemical Screening Result

Table 2: Qualitative Phytochemicals of *Newbouldia laevis* Leaves Results

Secondary Metabolite	Inference	Reported Literature (Nwachukwu *et al*., 2017)
Saponins	-	-
Alkaloids	-	-
Flavonoids	-	+
Phenols	+	+
Tannins	+	-
Steroids	++	+
Terpenes	+	+
Glycosides	++	+

Key: + means presence of phytochemical - means absence of phytochemical

Table 2 shows the qualitative phytochemical screening of ethanolic extract of *Newbouldia laevis* leaves. The result revealed the presence of phenols, tannins, steroid, terpenes, glycoside but alkaloid, saponin and flavonoids were found to be absent. This partially agrees with the one reported by Nwachukwu *et al*. (2017). The disparity may be because of the sample difference, polarity of the solvent used for extraction of the bioactive compounds or geographical location of the sample plants. This justify the medicinal properties of the plant as these phytochemicals has been reported for their pharmacological activities such as antifungal, antibacterial activities and anti-inflammatory [Akinpelu *et al*., 2009].

3.2.2 Quantitative phytochemical Analysis Results

Table 3: Quantitative Analysis of *Newbouldia laevis* Leaves

Secondary Metabolite	Quantity (in 1.00 g)
Phenols	0.00482 g
Steroids	0.00618 g
Tannins	0.00241 g
Terpenoids	0.00318 g
Glycoside	0.00616 g

Quantitative phytochemical composition reveals that *Newbouldia laevis* leaves cointain a reasonable amount of secondary metabolite that were screened in this research. High values of 0.00618 and 0.00616 were obtained for steroids and glycosides where as low value of 0.00241 was obtained for tannins.

3.3 Antibacterial Activity Result

Table 4: Zone of Inhibition (mm) for the antibacterial activity of *Newbouldia laevis* Leaves

Conc.(mg/ml)	SA	EC	ST	Ssp	SP	PA
50	10	14	11	03	00	00
100	15	17	12	06	05	04
200	18	22	15	10	10	06
400	23	26	20	13	12	08
Control	25	28	22	20	20	20

Key: Control = chloramphenicol SA= *Staphylococus aureus*, EC= *Escheria coli*, ST= *Salmonella typhi*, Ssp = *Shigella sp*, SP= *Sterptococus pneumonia*,PA= *Pseudomonas aeroginosa*.

Table 4 shows the antibacterial activity of Newbouldia laevis leaves ethanol extract. Extract has antibacterial activity with zone of inhibition range of 0-26 mm for all test organism with highest value observed at 26 mm of 400 mg/ml concentration against *E. coli* and lowest values of 08 mm at 400 mg/ml concentration against *P. aeroginosa.* Though both values indicate that the extract were active except for *P. aeroginosa, S. pneumonia* and *Shigella sp* with values of 08, 12 and 13 mm at 400 mg/ml which is partially active. In addition, extract was considered relatively active at 200 mg/ml concentration for *S. aureus, S. typhi* and *E. coli* with inhibition range of 22, 18 and 15 mm, while *P. aeruginosa, S. pneumonia* and *Shigella sp* was considered non active from 200, 100 and 50 mg/ml because of lower values of inhibition of 00-10 mm. The activity against S. *aureus, S. typhi* and *E. coli* started to decrease significantly at 100 mg/ml and 50 mg/ml and was considered partially active because of the lower values of inhibition zone which range from 10-17 mm. The activity was found to be concentration-dependent, as the concentration increases the activity also increases.

Table 5: Minimum Inhibitory Concentration (MIC) Result

Conc.(mg/ml)	SA	EC	ST	Ssp	SP	PA
50	+	+	+	+	+	+
100	-	-	+	+	+	+
200	-	-	-	-	-	+
400	-	-	-	-	-	-
MIC	100	100	200	200	200	400

Key: + = Inhibition observed, - = No inhibition observed

Table 5 shows the minimum inhibitory concentration of the extract. *S. aureus* and *E. coli* showed no inhibition at 100 mg/ml, *S. typhi, Shigella sp* and *S. pneumoniae* showed no inhibition at 200 mg/m while *P. aeroginosa* showed no inhibition at 400 mg/ml. This showed that all test organism are sensitive to the extract. Result showed lower value of MIC for *S. aureus* and E.*coli* while *S. typhi, Shigella sp* and *S. pneumoniae* showed a moderate value of MIC, except *P. aeroginosa* which showed a high value of 400 mg/ml which is less sensitive to the extract.This result correspond with the one reported by Nwachukwu *et al*.(2017) in phytochemical and antibacterial activity of Ethanolic *Newbouldia laevis* leaves thus concentration of the plant and some test organisms used differs.The activitities of this extract against tested organism could be connected to the presence of phytochemical cointained in plant. The ability of the extract to inhibit the growth of the organism indicates that, the plant can be use for the treatment of diseases associated with these bacteria.This justifies the medicinal use of the plant.

Table 6: Minimum Bactericidal Concentration (MBC) Result

Conc.(mg/ml)	SA	EC	ST	Ssp	SP	PA
50	+	+	+	+	+	+
100	+	+	+	+	+	+
200	-	-	+	+	+	+
400	-	-	-	-	-	+
MBC	200	200	400	200	400	-

Key: + = Growth, - = No Growth

Table 6 shows the minimum bactericidal concentration of the extract. *S. aureus* and *E. coli* showed no growth at 200 mg/ml, *S. typhi, Shigella sp* and *S. pneumoniae* showed no growth at 200 mg/m while *P. aeroginosa* showed growth even at 400 mg/ml that means no MBC indicating growth at 400 mg/ml. This showed that all test organism were sensitive to the extract except for *P. aeroginosa*. Result showed lower value of MBC for *S. aureus* and *E. coli* while *S. typhi, Shigella* sp and *S. pneumoniae* showed a higher value of MBC, except *P. aeroginosa* which showed growth at a high value of 400 mg/ml.

Conclusion

Base on the result obtained in this research, it may be concuded that the plant *Newbouldia laevis* leaves has cointain some amount of phytochemicals. The antibacterial result indicate that the plant possesses sufficient medicinal properties thus justifying the use of the plant for treatment of some diseases by traditional medicinal practitioners.

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