African Journal of
Biochemistry and Molecular Biology Research

ISSN : 0000-0000

Index: Harvard, Boston, Sydney University, Dimensions, Lens, ResearchGet Scilit, Semantic, Google Scholar, Base etc

https://doi.org/10.58578/AJBMBR.v1i1.3311

ANTIMYCOBACTERIAL ACTIVITY OF NEWBOULDIA LAEVIS ROOT AND STEM BARK EXTRACTS: IN VITRO EVALUATION AND PHYTOCHEMICAL COMPOSITION

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Article Info:

Submitted:	Revised:	Accepted:	Published:
Jun 6, 2024	Jun 30, 2024	Jul 3, 2024	Jul 6, 2024

Abstract

This present study aimed to determine the phytochemical screening, antimicrobial and anti-tuberculosis activity of the root and stem bark of methanol extracts of the N. laevis plant from Gashaka Gumti national park of Taraba state. Phytochemical screening was carried out using standard methods. For the two extracts obtained from the root and stem bark which revealed the presence of all except cardiac glycosides and quinones in the root, while all were the stem bark extracts. The Anti-bacterial assay was carried out using Agar-well diffusion technique. The highest zone of inhibition for the root crude extract of E. coli was (18mm) and its lowest zone of inhibition was (2mm), the highest zone of inhibition of Stap. A was (22mm) and the lowest was (8mm), the highest zone of inhibition of Bacillus cereus (22mm) and its lowest was (10mm). Salmonella typhii had the highest zone of inhibition for the root extract at (24mm) and its lowest at (4mm). The highest zone of inhibition for stem bark crude extract of E. coli was (12mm) and its lowest zone of inhibition was (5mm), the highest zone of inhibition of Stap. A was

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(13mm) and the lowest was (6mm), the highest zone of inhibition of Salmonella typhii was (18mm) and its lowest was (18mm),the highest zone of inhibition of Bacillus cereus (12mm) and its lowest was (4mm). The anti-tuberculosis assay was carried out using Broth micro dilution method. The result concerning the mycobacterium TB (anti-tuberculosis) of the root and stem bark crude extract of N. laevis plants inhibit the growth of bacterium with inhibitory zone within the range 6-16mm. This study reveals that the methanolic crude extract of N. laevis root and stembark can be utilized as a potential anti-tuberculosis (TB) drug for novel discovery.

Keywords; Newbouldia laevis, Anti-tuberculosis, Anti-bacterial, phytochemical

INTRODUCTION

In many parts of the world, traditional herbal medicine has been used for centuries to treat various ailments, including infectious diseases.(11). Tuberculosis treatment by use of traditional medicine may offer a new hope as source of bioactive molecules for developing alternative medicines for the mycobacterial diseases (8). The production of commercial medication formulations is largely dependent on plants and natural sources, which are used extensively in modern medicine. In the world, over 25% of prescribed medications come from plants. In spite of this, medicinal herbs are often employed instead of pharmaceutical medications (1). The application of plant extracts as infusions, decoctions, and mixtures to treat a variety of illnesses has gained a great deal of attention. Health issues such as malaria, epilepsy, convulsions, diarrhea, bacterial and fungal infections, mental illness, asthma, hypertension, worm infestation, ulcers, and many more conditions that have proven resistant to conventional medicine have reportedly been successfully treated locally with these remedies (2). One of the most common infectious diseases and a major cause of illness and death globally is tuberculosis (TB). TB ranked higher than HIV/AI DS as the primary cause of death from a single infectious agent prior to the coronavirus (COVID-19) pandemic. (9) It is estimated that TB infections affect about 25% of the world's population (*Newbouldia laevis* (commonly known as the African Border Tree or Tree of Life) *'Aduruku'* in Hausa, *'Ogirisi'* in Igbo and *'Akoko'* in Yoruba languages (10). It grows to a height of about 7 – 8 (up to 15) metres, more usually a shrub of 2 – 3 metres, many–stemmed forming clumps of gnarled branches. It belongs to the family Bignoniaceae and is known as a medicinal plant indigenous to Africa due to its various therapeutic properties. (10).

The leaves, root, and stem bark of *Newbouldia laevis* have been traditionally used by local communities to treat a wide range of health conditions, including respiratory problems, fever, and infectious diseases. These traditional uses have piqued the interest of researchers and pharmacologists, leading to investigations into the pharmacological activities of the plant.(10).

The pharmacological properties of *Newbouldia laevis* are attributed to its diverse array of bioactive compounds, such as alkaloids, flavonoids, tannins, terpenoids, and phenolic compounds. These compounds have been known to possess antimicrobial, anti-inflammatory, antioxidant, and immune modulatory activities, among others. Given the long history of traditional use and the presence of potentially bioactive compounds, there is a scientific rationale to explore the pharmacological potential of *Newbouldia laevis* against *Mycobacterium tuberculosis*.(7) If proven effective, the plant could serve as a valuable source for the development of new anti-TB drugs or adjunct therapies to enhance the current treatment regimens.

MATERIAL AND METHODS

Sampling and sample preparation

The Root and stem bark of *Newbouldia laevis* research was collected from Gashaka gumti National Park in Gashaka, Taraba State, Nigeria. The plant was authenticated by Dr Yekini at Department of Forestry, Moddibo Adama University, Yola, Adamawa State. The sample was allowed to air dry under shade in Chemistry Laboratory at MAU, Yola. The dried plant materials were pulverized using a pestle and mortar and the pulverized sample was kept dry in a container for further use.

Sample Extraction

100 g of the sample's powdered *N. laevis* root and stem bark were contained in a timble attached to a soxhlet extraction apparatus seperately. The soxhlet containing around 300 milliliters of methanol was allowed to be extracted for an hour at 60^{o}C. The extract(root and stem bark) was dried using a rotary extractor after being reduced to a tenth of its original volume in a water bath heated to 60^{o}C (3).

Qualitative phytochemical analysis

The same procedure was repeated for the root and stem bark extracts resspectively

Test for alkaloids

About 3 ml of the extract and precisely 1 ml of 1% HCl were combined in a test tube. The mixture was heated, cooled, and filtered after 20 minutes. One milliliter of the filtrate was mixed with around two drops of Mayer's reagent. Alkaloids are present when the substance is creamy (4)

Test for Saponins

Precisely five drops of olive oil were added to three milliliters of extract in a test tube and the mixture was violently agitated. The presence of saponin is indicated by the lack of foaming and a stable emulsion (6)

Test for tannins

In a test tube with 20 ml of water, precisely 0.5 g of the dry powder sample was boiled before being filtered. A few drops of 0.1% ferric chloride were added and the coloration was checked for brownish green or blue-black (5)

Test for phenolic compound

To one gram of dried plant material, exactly 10 ml of ethanol was added, then ultra-sonication at 30 ºC was carried out for 15 minutes. Two milliliters of the filtrate were added to five milliliters of distilled water after the combination had been filtered. For treatment, a few drops of 5% FeCl3 were added to the filtrate. The dark green hue indicated phenolic compounds (6)

Test for flavonoids

A quantity of 1 ml of 10% NaOH was added to 3 ml of the extract. The absence of flavonoids shows yellow coloration (4)

Test for Quinones

Ten milliliters of ethanol were precisely mixed with one gram of the dried material, and the combination was ultrasonically sonicated for fifteen minutes at thirty degrees Celsius. The mixture underwent filtration. H_2SO_4 and filtrate were combined in one milliliter. Quinones could be identified by their red hue (4)

Test for terpenoids

About Five (5) ml of the extract was mixed in 2 ml of chloroforms and 3 ml H_2SO_4 concentrated was carefully added to form a layer. A reddish brown coloration of the interface formed shows the presence of terpenoids (4).

Test for Cardiac glycoside

To one mill (1m) of the extract, 2ml of 3.5% ferric chloride solution was added and allowed to stand for one minute. H_2SO_4 was carefully poured down the test tube so as to form a lower layer. A reddish-brown ring, the interface indicates the presence of cardiac glycoside.

Antimicrobial Study

Collection of Test Organisms

The Micro Organisms, *Staphylococcus Aureus, Escherichia Coli, Salmonella Typhi* and *Bacillus Cereus* were used for the analysis. They were obtained from the Microbiology department, Modibbo Adama University, Yola. Cultured and were brought to the laboratory and were resuscitated in peptone water and thereafter sub cultured into nutrient agar medium and incubated at 37^{o}C for 24 hrs.

Preparation of Nutrient Agar

This was done in accordance with (3). 28 grams of dehydrated powdered nutrient agar was weighed and then dissolved in a 1.5-liter conical flask with one liter of distilled water. The mixture was then left to dissolve on the bench for five to ten minutes before being boiled and having non-absorbent cotton wool inserted to seal the flask's mouth. After that, the flask was wrapped in aluminum foil and autoclaved for 15 minutes at 121 degrees Celsius to disinfect it. After allowing the media to cool to between 40 and 45 degrees Celsius, an aliquot of 20 milliliters each plate was added, and it was stored in a 40 degree refrigerator for internal use. Ciprofloxacin was used as a novel control medication in this investigation to determine the Minimum Bacteria Inhibitory Concentration utilizing the Gel-Diffusion method on the stem, leaf, and root extract of the *Newbouldia laevis* plant extract.

Antimycobacterial activity susceptibility test

The susceptibility test was conducted using the broth micro dilution method (BMM) as described by (12) Extracts were first dissolved in DMSO and then diluted in Middle brook 7H9 broth, to give a stock concentration of 500 ug/ml. The same procedure was repeated for the control (Streptomycin) with the initial concentration of 30 ug/ml with the subsequent dilution to the final testing concentrations of 100, 200, 300 and 400 ug/mL. Appropriate DMSO, growth and sterile controls were carried out with Streptomycin as positive control. Streptomycin control and negative control sets were triplicated for all the sets on each plate. The plates were inoculated with the diluted culture for well 1-12.The plates were then incubated for 5-7days at 37^{O}C, after this Incubation, any well before those that turned cloudy were recorded as the highest dilution of test compound that prevented bacterial growth (MIC). The minimum inhibitory concentration (MIC) was defined as the lowest extract concentration at which no mycobacterial growth was observed

RESULTS AND DISCUSSION

Table 1: Qualitative analysis of the methanolic crude extracts(root and stembark) of *N. laevis*

Phytochemicals	Root Extract	Stembark Extracts
Alkaloids	+	+
Flavonoids	+	+
Phenols	+	+
Terpenoid	+	+
Tannin	+	+
Saponins	+	+
Quinones	_	+
Cardiac Glycosides	_	+

Keys: + = Detected, - = Not detected

Phytochemicals are secondary plants metabolites responsible for many observed bioactivity of plant extracts. They are known to possess antioxidant, anti-inflammatory, antibacterial, immunomodulatory and anti-sickling activities.

Phytochemicals are secondary plants metabolites responsible for many observed bioactivity of plant extracts. They are known to possess antioxidant, anti-inflammatory, antibacterial, immunomodulatory and anti-sickling activities. The documented medicinal

plant species used by the people of Gashaka community in Gashaka local government area of Taraba State showed a potential source of a new class of anti-tuberculosis drugs. Plant phytochemical screening differs from location to location, which could be caused by local soil composition, climate, and geographic variance. Therefore, it is feasible for the same plant being studied in different fields to have a diverse chemical composition. (16).

The most effective class of secondary chemical elements found in plants and the well-researched in terms of their antibacterial, anthelmintic, and antidiarrheal properties are alkaloids. Alkaloids have extremely bitter solutions. Due to their strong biological properties, these heterocyclic nitrogenous compounds are frequently used as stimulants, narcotics, medicines, and poisons. They also help plants defend themselves against diseases and herbivores. They regulate growth in living systems and have certain metabolic functions. In addition, they serve a protective role for animals and are employed in medicine, particularly steroidal alkaloids. Because of their analgesic and antispasmodic qualities, pure separated alkaloids and their synthetic derivatives are utilized as the primary therapeutic agent (14). As a result, phytochemical analysis is required to determine any potential health risks associated with using plant extracts for medicinal purposes. Crude extracts are complex mixtures of biologically active compounds, some of which may exhibit genotoxic and antigenotoxic effects (15).

Table 2: Antimicrobial Activity of Methanolic Extract of Root of *N. laevis* plant (Zone of inhibition in mm).

Organisms	Concentrations					Positive Control Ciprofloxacin 30mg/ml
	100 mg/ml	200 mg/ml	300 mg/ml	400 mg/ml	500 mg/ml	
E.coli	2	5	7	14	18	28
Salmonella thypii	4	7	13	22	24	33
S. Aureous	8	12	17	21	22	32
B.cereus	__	__	10	21	13	26

KEY-- = No zone of inhibition

The highest zone of inhibition for the root crude extract of E. coli was (18mm) and its lowest zone of inhibition was (2mm), the highest zone of inhibition of *Stap. A* was (22mm) and the lowest was (8mm),) the highest zone of inhibition of *Bacillus cereus*

(22mm) and its lowest was (10mm). *Salmonella typhii* had the highest zone of inhibition for the root extract at (24mm) and its lowest at (4mm).

Table 3: Antimicrobial Activity of Methanolic Extract of Stem bark of *N. laevis* plant (Zone of inhibition in mm).

Organisms	Concentrations					Positive Control Ciprofloxac in 30mg/ml
	100mg/ml	200mg/ml	300mg/ml	400mg/ml	500mg/ml	
E.coli	--	6	12	5	12	28
S. Aureous	--	6	12	13	10	32
Salmonella thypii	12	13	14	18	18	34
B.cereus	6	4	10	11	13	26

KEY **-- =** No zone of inhibition

The highest zone of inhibition for stem bark crude extract of E. coli was (12mm) and its lowest zone of inhibition was (5mm), the highest zone of inhibition of *Stap. A* was (13mm) and the lowest was (6mm), the highest zone of inhibition of *Salmonella typhii* was (18mm) and its lowest was (18mm),the highest zone of inhibition of *Bacillus cereus* (12mm) and its lowest was (4mm).

Table 4: Antimicrobial Activity of Methanolic Extract of Stem bark of *N. laevis* plant (Zone of inhibition in mm).

Organisms	Concentrations					Positive Control Ciprofloxac in 30mg/ml
	100mg/ml	200mg/ml	300mg/ml	400mg/ml	500mg/ml	
E.coli	--	6	12	5	12	28
S. Aureous	--	6	12	13	10	32
Salmonella thypii	12	13	14	18	18	34
B.cereus	6	4	10	11	13	26

KEY -- = No zone of inhibition

The highest zone of inhibition for stem bark crude extract of E. coli was (12mm) and its lowest zone of inhibition was (5mm), the highest zone of inhibition of *Stap. A* was (13mm) and the lowest was (6mm), the highest zone of inhibition of *Salmonella typhii* was (18mm) and its lowest was (18mm),the highest zone of inhibition of *Bacillus cereus* (12mm) and its lowest was (4mm).

Table 5: Anti-tuberculosis activity of the Methanolic Crude Extracts of the Leaves, Root and Stem bark of *N laevis* plant in (Zone of inhibition in mm).

Samples extract	**Concentrations**				**Positive Control**
	400 mg/ml	300 mg/ml	200 mg/ml	100mg/ml	Streptomycin 30mg/ml
Stem	16	12	9	8	23
Stem	14	10	8	7	21
Stem	13	13	8	7	18
Root	15	10	8	6	13
Root	10	9	7	6	10
Root	10	8	7	6	22

The results of anti-tuberculosis activity of the root and stem bark crude extract of *N. laevis* plant were shown in (Table 5). The results of anti-tuberculosis are presented at different concentrations. The stembark extract had the highest zone of inhibition than that of the roots of the plants as can be seen in Table 5. The result concerning the *mycobacterium* TB (anti-tuberculosis) of the root and stem bark crude extract of *N. laevis* plants inhibit the growth of bacterium with inhibitory zone within the range 6-16mm. The zone of inhibition (sensitivity) of the plant extract considers from 7mm and above while 6mm is considered to be resistance and antimicrobial method used is Agar diffusion method. The plant extract used shows effectiveness against the test organism at the higher concentrations.

It was observed that the higher the concentration of the crude extract, the stronger the activity. The root extract showed that at 400ug/ml extract concentration an ZOI of 16mm mm was observed. Streptomycin, the positive control in this study had

higher activity (23mm) against the test organisms when compared to the methanol crude extracts. This may be as a result that the antibiotics (streptomycin) are in their pure form unlike the crude extract which are still in the unpurified form, which still needed to be purified to remove all the inhibitory substances to its activity.

CONCLUSION

This study demonstrated that the methanolic extracts of the root and stem bark of Newbouldia laevis contain a diverse range of phytochemicals, including alkaloids, flavonoids, phenols, terpenoids, tannins, saponins, and quinones. Both extracts exhibited significant antimicrobial activity, with the root extract showing superior effectiveness compared to the stem bark extract against various bacterial pathogens. Additionally, both extracts displayed notable anti-tuberculosis activity, with the root extract being slightly more effective. These results validate the traditional medicinal use of N. laevis for treating infections and suggest that the plant's extracts have potential as novel anti-tuberculosis agents. Future research should focus on isolating and characterizing the specific bioactive compounds responsible for these activities and assessing their efficacy and safety in clinical trials. Further studies could explore the mechanisms of action of these compounds and their potential integration into existing tuberculosis treatment regimens.

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