Dynamic Multidisciplinary Journal of Nigeria: Delta State University Library, Abraka, Delta State, Nigeria
Email: Dmjon2080@Gmail.Com, ISSN: 2955-0564, Articles Are Indexed in Google Scholar
Volume 6, Number 3, May, 2024

INVESTIGATION OF ANTIMICROBIAL PROPERTIES AND PHYTOCHEMICAL SCREENING OF THE AQUEOUS AND METHANOLIC EXTRACTS OF NEWBOULDIA LAEVIS (P. BEAUV) ON SELECTED PATHOGENIC MICROORGANISMS

By

Ifelolu Adeseye REMI-ESAN
Environmental Biology Unit, Department of Science Laboratory Technology,
The Federal Polytechnic Ilaro, Ogun State, Nigeria.
Email: ifelolu.remiesan@federalpolyilaro.edu.ng

and

Azeez Adewale ADENIJI
Environmental Biology Unit, Department of Science Laboratory Technology,
The Federal Polytechnic Ilaro, Ogun State, Nigeria.

Abstract

Aqueous and methanolic extracts of *Newbouldia laevis* were evaluated for their phytochemical and antimicrobial properties against *Escherichia coli, Candida albicans,* and *Staphylococcus aureus*. The phytochemical composition of this medicinal plant was estimated using standard procedures, and the findings indicated that both forms of extract included oxalate, alkaloids, phenols, flavonoids, tannins, terpenoids, anthraquinones, and glycosides. For *S. aureus, E. coli,* and *Candida albicans*, the antimicrobial activity inhibition zones were 9–23 mm, 8–25 mm, and 7–24 mm, respectively. The extracts' minimum inhibitory concentrations (MIC) against *S. aureus, E. coli,* and *Candida albicans* varied from 6.25 mg/ml to 25 mg/ml. The isolates of *S. aureus, E. coli,* and *Candida albicans* had MBCs ranging from 50 to 100 mg/ml. These findings suggest that *N. laevis* methanolic leaf extracts can naturally limit the growth of the species under investigation (*S. aureus, E. coli, and C. albicans*).

Keywords: *Escherichia coli*, Methanolic, *Candida albican, Newbouldia laevis, Staphylococcus aureus.*

1.0 Introduction

In the past, plant herbal mixtures have greatly enhanced human health and welfare and guaranteed the development of novel medicinal substances. In order to battle various infectious diseases, scientific research nowadays often seeks for plant components that have antibacterial capabilities (Idu *et al*., 2009). This is because plants are widely used as therapies for a variety of viral ailments, one of these botanical plants with antibacterial qualities is *Newbouldia laevis* (P. Beauv) which belongs to the Bignoniaceae family and the Newbouldia genus. It has characteristic features such as being glossy, with dark-green leaves that are frequently planted as ornamentals, big, spectacular terminal purple blooms, and is simple to produce from cuttings. It is essentially a holy or symbolic tree that is placed properly as a barrier and frequently allowed to develop into a stockade when fully matured (Shibata *et al*., 2005).

Numerous secondary metabolites, including tannins, terpenoids, alkaloids, and flavonoids, have been found to have antimicrobial properties in vitro and are abundant in plants. Scientific studies on the phytochemical components of plants have identified the majority of these secondary metabolites in numerous writings (Betoni *et al*, 2006). African folk medicine has documented the usage of *N. laevis*, which is common in the rural areas of the countries for the treatment of malaria fever, stomach aches, coughs, toothaches, breast cancer, constipation, as well as other diseases like *Diabetes mellitus.* This is because the plant parts are said to contain a variety of phytochemical constituents that have been pharmacologically proven to be active and have been used in the management and treatment of many animal and human diseases (Arbonnier, 2004).

According to scientific research, *N. laevis* provides medical benefits including analgesic, anti-inflammatory, antioxidant, antibacterial, and anti-fungal qualities. Particularly, the stem bark when used in combination with red pepper has been said to be useful in treating many illnesses such as bone lesions as well as pneumonia, fever, colds, and coughs (Arbonnier, 2004). The current work aims to determine the phytochemical composition and also investigate the antibacterial activity of the methanol and aqueous extracts of *Newbouldia laevis* on several pathogenic microorganisms.

2.0 Materials and Methods

2.1 Materials

Newbouldia laevis, Nutrient agar, Potato dextrose agar, cork borer, sterile swab, syringe, cotton wool, aluminum foil, petri dish, distilled water, conical flask, blender, vacuum, evaporator, beaker, Whatman's No. I, centrifuge, weighing scale, incubator, autoclave, transparent ruler, spirit lamp, and paper tape.

2.2 Collection Of Sample And Identification

The leaf of the *Newbouldia laevis* plant was collected in Ilaro, Ogun State, and a botanist from the Federal Polytechnic Ilaro, department of Science Laboratory Technology identified the plant.

2.3 Sample Preparation

To get rid of dirt and other foreign objects, distilled water was used to wash the freshly acquired leaf of *N. laevis*. The leaf was then blended into a fine powder after being allowed to dry completely at room temperature.

2.4 Phytochemical Analysis

This was achieved using standard methods as described by; (Sofowora, 1993; Ogundare, 2007; Trease and Evan, 2002).

2.4.1 Alkaloids

After filtering, 5 ml of 1% aqueous HCL was added to mix it (0.5 g/ml for the extract). Three sections will be separated out of three milliliters of the filtrate. A few drops of recently made dragendoff reagent were transferred to the first milliliter. Meyer's reagent was added in one drop to the second. After adding Wagner's reagent to the third, it was checked for a comparable alteration.

2.4.2 Flavonoids (Ferric chloride test)

The solution remains dark green or blue in color. A drop of ferric chloride is added to this solution and results are noted.

2.4.3 Saponins

0.5 g of the extract in a test tube was added with water and kept watch over twelve minutes.

2.4.4 Tannins (Lead test)

The extract was mixed with distilled water. After which, about 3 – 5 drops of lead acetate solution was added and changes in the mixture were monitored.

2.4.5 Phenols (Ferric chloride test)

A drop of the extract, aqueous and stir with distilled water. Results: A drop of ferric chloride was added to the solution and recorded.

2.4.6 Glycosides (Legal's test)

Sodium nitroprusside dissolved in pyridine and sodium hydroxide was applied to a tiny percentage of the extracts, and the outcomes were examined.

2.4.7 Ferric chloride test

A small amount of the extract was mixed and dissolved in glass-distilled water, then a few drops were added to a solution of ferric chloride. Results were later observed.

2.5 Test Organism

Escherichia coli, Staphylococcus aureus, and Candida albicans were obtained from the Federal Institute of Industrial Research (FIIRO) as test organisms for antimicrobial testing. The bacterial and fungal isolates were further preserved at 4°C on both potato dextrose agar slants and nutritional agar slants.

2.6 Extract Preparation

2.6.1 Aqueous Extract

100 ml of distilled water was used to dissolve 10 grams of powdered *N. laevis* leaves over the course of 24 hours. The mixture was concentrated by drying in a vacuum at 37°C using a rotary evaporator, filtered via Whatman's No. 1 filter paper, and kept at 4°C.

2.6.2 Methanolic Extract

Ten grams of powdered *N. laevis* leaves were dissolved in 100 mL of 95% methanol and left to stand for 24 hours. The mixture was then filtered through Whatman filter paper No. 1, and the solvent was removed with the aid of an evaporator. The resulting extract was stored at 4°C.

2.7 Extract Dilution

In order to acquire concentrations of 3.13, 6.25, 12.5, 50, 100, 150 and 200 mg/ml, respectively, both aqueous and methanolic extracts were reconstituted using sterile distilled water after the extract was prepared as stated.

2.8 Mode Of Action Of The Extracts

The fact that there was no observable growth on any of the nutrient agar (NA) plates suggested that the concentration of the extract used had a bactericidal effect. Plates with minimal growth demonstrated the extract concentration's bacteriostatic properties. Extract concentrations that exhibited heavy and moderate growth were assumed to have no inhibitory effects on the test organisms observed.

2.9 Antimicrobial Assay

Using the Agar-well diffusion Technique, the antibacterial potency of plant leaf extracts was evaluated against test isolates. By streaking the isolates with a sterile swab stick, test organisms for bacteria (*Escherichia coli* and *Staphylococcus aureus*) and fungus (*Candida albicans*) were inoculated on freshly gelled sterile nutrient agar and potato dextrose agar plates, respectively. On each agar plate, wells were aseptically drilled and labeled using a sterile cork borer (6mm). Following that, fixed amounts (0.1 ml) of the extracts at varied concentrations (aqueous and, correspondingly, methanol) were added to the plates. The last

two wells served as positive and negative controls, respectively. The positive control wells contained Gentamicin, whereas the negative control wells contained sterile water. The plates underwent a 24-hour incubation period at 37 degrees after being put on a bench for 40 minutes to allow for pre-diffusion of the extract. The diameter of the inhibitory zone was consequently measured with a calibrated transparent ruler in millimeters. The data was analyzed to show the zones of inhibition for that particular bacterial strain at that dosage. (NCCLS, 2000).

2.10 Minimum Inhibitory Concentration

MIC was determined to be the lowest dose at which test tube turbidity could not be seen. The concentrations were established somewhat differently from how Vollekovà *et al*. (2001) had previously described them. The microorganisms with appropriate sensitivity to the test extracts had their MICs calculated. The microorganisms for this test were created using the broth dilution method. Before being inoculated with a 0.25 ml culture of the test organisms, the appropriate concentrations were obtained using a two-fold serial dilution procedure using nutrient broth that varied from 0.20 mg/ml to 50 mg/ml. A stock extract concentration of 100 mg/ml was produced as a result. The tubes were examined for turbidity after being incubated for 24 hours at 37°C. The lowest amounts without turbidity were identified and recorded.

2.11 Minimum Bactericidal Concentration

The broth dilution test yielded the minimum bactericidal concentration (MBC). This entailed plating the contents of each test tube on nutritional agar plates in order to examine them. The minimum bactericidal concentration of the extract was found to be the lowest concentration that did not result in any bacterial growth after the plates were incubated at 37°C for 24 hours.

2.12 Control

As positive and negative controls, gentamicin and distilled water, respectively, were used.

2.13 Statistical Analysis

The Statistical Package for Social Sciences, version 20.0, was used to analyze the data (SPSS). ANOVA, was used to see if the results differed significantly from one another. P-values were used to determine statistical significance, with a 0.05 cut-off point. When the p-value was less than this cut-off, the null hypothesis was rejected.

3.0 Result and Discussion

Table 1: The qualitative results of the phytochemical screening of *Newbouldia leavis*

Phytochemical	A1	A2
Alkaloids	+	+
Saponins	+	+
Tannis	+	+
Phlobatannin	-	-
Anthaquinones	-	-
Flavonoids	+	+
Caroliaglycoside	-	+
Carbohydrate	+	+
Oxalate	+	+

Key: A1= methanol extract + = present
A2= aqueous extract - = absent

Table 2: shows the quantitative phytochemical evaluation of extracts from *Newbouldia laevis*

Phytochemical	Methanol extract %	Aqueous fraction %
Alkaloid	0.43	-
Phenol	0.55	0.13
Saponin	1.15	0.68
Tannins	9.30	2.79
Oxalate	0.81	-

Table 3: *Newbouldia laevis* leaf extract's methanolic and aqueous antibacterial effects against *S. aureus, E. coli, and C. albican*

	ZONE OF INHIBITION (mm)						
Isolates	200	150	100	50	C+	C-	Extracts
S. aureus	17	16	12	9	19	0	AQ
S. aureus	23	21	18	12	19	0	MET
E. coli	21	18	12	8	16	0	AQ
E. coli	25	24	20	19	16	0	MET
C. albican	18	14	10	7	18	0	AQ
C. albican	24	20	16	16	18	0	MET

Key: AQ = Aqueous Extracts MET = Methanolic Extracts

C+ = Gentamicin (+ve control) C- = Sterile water (-ve control)

Table 4: *S. aureus, E. coli*, and *C. albican Newbouldia laevis* minimum inhibitory concentration

	Conc of Extracts (mg/ml)									
Isolates	3.13	6.25	12.5	25	50	100	150	200	Extracts	MIC
E. coli	+	+	+	-	-	-	-	-	AQ	25.00
E. coli	+	-	-	-	-	+	-	-	MET	6.25
C. albican	+	+	+	-	-	-	-	-	AQ	25.00
C. albican	+	-	-	-	-	+	-	-	MET	6.25
S. aureus	+	+	+	-	-	+	-	-	AQ	25.00
S. aureus	+	-	-	-	-	-	-	-	MET	6.25

KEY: AQ = Aqueous Extracts MET = Methanolic Extracts

+= Present - = Absent

Table 5: Minimum bactericidal concentration of *Newbouldia laevis* leaves on *S. aureus*, *E. coli* and *C. albican*

	Extract concentration (mg/ml)									
Isolates	3.13	6.25	12.5	25	50	100	150	200	Extracts	MBC
S. aureus	+	+	+	+	+	-	-	-	AQ	100
S. aureus	+	+	-	+	-	-	-	-	MET	50
E. coli	+	+	+	+	+	-	-	-	AQ	100
E. coli	+	+	+	+	-	+	-	-	MET	50
C. albican	+	+	+	+	+	-	-	-	AQ	100
C. albican	+	+	+	+	-	-	-	-	MET	50

Key: AQ = Aqueous Extracts MET = Methanolic Extracts += Present - = Absent

3.1 Discussion

The phytochemical examination found the availability of terpenoids, glycosides, steroids, flavonoids, tannins, alkaloids and saponins. The leaf extract's inclusion of steroids, tannins, flavonoids, cardiac glycosides and terpenoids was consistent with Usman and Osuji's (2007) results.

The majority of the reported bioactivity of plant extracts is caused by phytochemicals, which are secondary plant metabolites. According to Egba *et al*. (2012), they are known to have anti-oxidant, anti-inflammatory, antibacterial, immunomodulatory, and anti-sickling properties. There is no question that the existence of such metabolites indicates the potential therapeutic value of the plant extracts. It has been demonstrated that saponin has enormous relevance as an anti-hypercholesterolemia, and cardiac arrhythmias. The presence of tannins indicated by the results, points to this plant's potential to be a significant antidiarrheal and antihaemorrhagic agent.

According to Alan and Miller (1996), flavonoids also demonstrated to have antiviral antineoplastic, antibacterial, anti-allergic, and anti-inflammatory action. According to Nakayama and Lindsey (2013), many of these purported benefits are related to their well-known roles as potent antioxidants, free radical scavengers, and metal chelators. Terpenoids have been demonstrated in animal tests to lower blood sugar levels, making them important in medicines due to their link with substances used as sex hormones (Okwu, 2012). Glycosides may play a key role in transmitting intracellular signals carried by neurotransmitter, hormone, and neuromodulator receptors that are triggered by hydrolysis, which separates the sugar part of several biological enzymes. These molecules have a variety of intracellular targets (glycoside-linked signal transduction proteins) where they can function when activated.

The existence of various component groups that may be working synergistically with one another may be the cause of the bacteria' vulnerability to the extracts. Plant extracts can be divided into two immiscible solvents to help separate the active components of the plant, with the ultimate goal of determining where such components may be located (Martin, 2005).

The inhibitory effects grew stronger for the aqueous extract in this study, indicating that the contents of the extract were responsible for the reported antibacterial property. The secondary metabolites including tannins, alkaloids, and flavonoids that were discovered to be present in the plant material may be the cause of the extracts' generally observed antibacterial properties (Vlietinck, 2010).

The study's findings demonstrate that the methanolic extracts of *N. laevis* provide a larger zone of inhibition than the positive control drug gentamicin does at all doses used. This suggests that *N. laevis* may be more effective than the majority of currently available conventional antibiotics in treating infections brought on by *S. aureus*, *E. coli*, and *C. albican*. Similar findings were made in the study of Vlietinck (2010), who found that the extract had significant antibacterial properties against both Gram-negative (*E. coli*) and Gram-positive (*S. aureus*).

Due to the existence of a thick layer that tends to block the entry of inhibitors, Gram-negative bacterial species are typically thought to be more difficult to eliminate (Vlietinck, 2010). The fact that Staphylococci, which have a history of acquiring resistance to antibiotics quickly and effectively, were the ones most vulnerable to the methanol leaf extract is unanticipated. The MIC and MBC of the *E. coli, S. aureus, and C. albican* species that had previously shown resistance to the extracts were found to be between 6.25mg/ml and 25mg/ml; 50mg/ml to 100mg/ml. These showed that doses previously utilized were entirely ineffective for controlling the organisms' development. Additionally, based on this increased MIC and MBC, it can be claimed that Gram negatives are the most resistant bacteria.

4.0 Conclusion

The results of the study indicate that the leaf extract of *Newbouldia laevis* has the natural ability to prevent the growth of *S. aureus, E. coli,* and *Candida albicans*. It's possible that the plant's secondary metabolites are what give it its antimicrobial properties. Furthermore, the study found that *N. laevis* methanolic extracts produced a larger zone of inhibition at all tested doses when compared to gentamicin, the positive control drug. These findings suggest that *N. laevis* leaf extracts might be effective as an antimicrobial agent against the pathogens being studied.

4.2 Recommendation

Based on the findings of this research, it is recommended that *N. laevis* plants should serve more as medicinal plant due to its wide availability and accessibility in the environment. The presence of notable secondary metabolites in the plant avails its antimicrobial capacity and hence should be a plant of choice in combating bacterial infections. The plant shows high anti-inflammatory and analgesic capacity and hence should be used to relieve pain and inflammation, especially for conditions like arthritis, rheumatism, and other joint-related issues. The usage of this plant's leaves and bark for treating related digestive issues such as constipation, dysentery, and abdominal pain is highly recommended.

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