Science Journal of Chemistry
2025, Vol. 13, No. 2, pp. 33-40
https://doi.org/10.11648/j.sjc.20251302.11

Research Article

Phytochemical Screening and Antimicrobial Properties of Leaf Extracts from *Newbouldia laevis* (P. Beauv) and *Flueggea virosa* (Roxb. ex Willd.)

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Abstract

Using plants to treat human diseases is very old and has evolved throughout human history. *Flueggea virosa* and *Newbouldia laevis* are two plant species acclimatized in Benin and used in Africa to treat several diseases such as malaria, liver diseases; fever, migraine, diarrhea, dysentery, intestinal worms, diabetes, sexually transmitted diseases, etc. In this work, we studied methanolic and hydroethanolic leaf extracts from those species. Phytochemical screening was determined by the Hounton and Raman methods, and the antimicrobial activity of the leaf extracts was evaluated by the microdilution method. The antimicrobial properties of the leaf extracts were examined on five bacteria strains. The phytochemical analysis revealed the presence of alkaloids, polyphenols, tannins, flavonoids, anthocyanins, coumarins, mucilages, reducing compounds, and bound anthracene derivatives (O-heterosides and C-heterosides). Quinonine derivatives were absent in the leaves of *Flueggea virosa*, but present in the leaves of *Newbouldia laevis*. In addition, the leaves of *Flueggea virosa* contained saponosides, triterpenoids compared to those of *Newbouldia laevis*. Moreover, *Flueggea virosa* leaves enclosed no cardenolides, cyanogenic derivatives as well as, free leuco-anthocyanins and anthracene derivatives. Evaluation of antimicrobial activity in the five strains included in this study showed better results (minimum inhibitory concentrations between 31.2 and 1000 µg/mL), specifically with the methanolic extract from *Flueggea virosa* leaves that showed a Minimum Inhibitory Concentration (MIC) equal to 31.2 µg/mL against the reference strain of *Staphylococcus aureus* ATCC 12600. Among the two plants, *Flueggea virosa* extracts showed more interesting antimicrobial activity then those of *Newbouldia laevis* on strains.

Keywords

Flueggea virosa, *Newbouldia laevis*, Phytochemical Screening, Antimicrobial Activity

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Received: 3 March 2025; **Accepted:** 18 March 2025; **Published:** 2 April 2025

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1. Introduction

Plants have proved to be one of the major sources of primary health care in developing countries [1]. In Benin, several ethnobotanical surveys reveal the effectiveness of plants in treating some illnesses. Among those plant species, *Flueggea virosa* (Roxb. ex Willd.) Euphorbiaceae and *Newbouldia laevis* (P. Beauv) Bignoniaceae play great roles. Thus, they are used in traditional medicine for the treatment of diverse diseases using several methods for preparation. Leaf and root decoctions of *Flueggea virosa* are applied against liver diseases [2], cough [3]. *Flueggea virosa* leaf infusion is used for the treatment of diarrhea stomachaches [4], as well as in the treatment of sexual impotence and erection dysfunction. Leaves and roots crushed or decocted [5] treat infertility problems in women [6]. Leafy stem decoction is used in the treatment of diabetes in pregnant women [7]. The decocted leaves of *Newbouldia laevis* are also used against different types of ulcers, hemorrhoids and constipation [8]. These plants are also useful in the treatment of ear and chest pains, epilepsy and convulsions affecting children. Several studies have shown the application of plants in traditional and modern medicine systems. They are particularly the richest source of medicine utilized by local populations in Africa, Asia and Latin America [9]. Some previous reports showed that *N. laevis* possesses antibacterial [10], antiplasmodial, anthelmintic *in vitro* and antiradical [11] activities. *Flueggea virosa* presented also in previous studies antiplasmodial activities [12]. *F. virosa* is also an important medicinal plant in tropical Africa, used alone or in combination with other plants, for a variety of purposes, including liver, kidney, urinary and venereal diseases, bile deficiency, testicular inflammation, frigidity, sterility, heavy menstruation, rheumatism and arthritis. The plant has been around for a long time [13-15]. According to the WHO in 2022, 7.7 million people die each year from bacterial infections [16]. Therefore, the present investigation reported here focused on the phytochemical screening and antimicrobial activity of the methanolic and hydroethanolic extracts of these two medicinal plant species.

2. Material and Methods

2.1. Chemicals Used

The ethanol (95%) and methanol used for extraction were produced by ADER-Cameroon. Distilled water was obtained from POBEL DETA (made in Spain) and used as a hydroalcoholic extraction solution. The resulting filtrate from the extraction was concentrated using the Heidolph rotavapor (water bath). Microbial strains were cultured on agar to confirm their identity and kept in tilted Msouculler Hinton agar in a test tube. Strains were then reactivated for 24 hours at 37 ℃ used each test.

2.1.1. Plant Material

The plant material consisted of leaves from *Flueggea virosa* (Roxb. ex Willd.) Euphorbiaceae and *Newbouldia laevis* (P. Beauv) Bignoniaceae (Figures 1 and 2) harvested respectively in Parana and Djadjo, located in the municipality of Abomey-Calavi (Atlantic Department) in the Southern Benin Republic on 20 December 2022 and 09 January 2023 and was dried at room temperature in the Laboratory of Physics Organic Chemistry and Synthesis (LaCOPS) of the University of Abomey-Calavi (UAC). Both species were identified, certified and authenticated by the National Herbarium with specimens N ° YH1060/HNB and N ° YH1061/HNB at the UAC National Source Herbarium.

Figure 1. *Flueggea virosa leaves, flowers and stems.*

Figure 2. *Newbouldia laevis leaves, flowers and stems.*

2.1.2. Process for Obtaining the Plant Leaf Powder

The dried leaves were ground to a fine powder using a blender, and the resulting powder was used later for the preparation of extracts.

2.1.3. Preparation of the Leaf Extracts

The methanolic extracts were prepared by maceration of 2.5 kg and 1.5 kg of dried leaf powder of *F. virosa* and *N. laevis*, respectively in 18 L and 10 L of methanolic solvent and left for extraction during 72 hours. After filtration using filter paper, the extract obtained was concentrated using a rotary evaporator at 55 ℃. The raw extracts were then dried in the oven at 48 ℃. The extract's weight was measured with accuracy. The hydroethanolic extract was obtained by mac-

erating 50 g of the leaf powder from plants for 72 hours in 500 mL of a 70/30 (V/V) ethanol-water mixture. The resulting extract, which was concentrated with a rotary evaporator, was dried at 50 ℃ and weighted with precision.

2.2. Phytochemical Screening of Leaf Powder from *N. laevis* and *F. virosa*

Phytochemical screening is one of the techniques used for identifying the different major chemical groups contained in a plant organ. This technique is based on coloration and precipitation reactions that allow the detection of chemical families in the powders using the Houghton and Raman method adapted to our laboratory condition [17].

2.3. Strains Used

Five bacterial strains were used. These are the bacterial strains of reference: (Gram-positive): *Enterococcus faecalis* ATCC 51299, *Staphylococcus aureus* ATCC 12600, and (Gram-negative): *Klebsiella pneumoniae* NR 4188, *Escherichia Coli* ATCC 25922 and finally a clinical strain: *Salmonella typhimirim* STM CPC.

2.3.1. Preparation of Stock Solutions

The extract stock solutions were prepared at 10 mg/mL by dissolving 10 mg of extract powder in 1 mL of DMSO 10%. The stock solutions of ciprofloxacin (Gibco, China) used as a positive control for strain testing were prepared under the same conditions at 1 mg/mL by dissolving 1 mg of powder from each plant organ in 1 mL of acidified distilled water [18].

The assessment of antibacterial activity was carried out using liquid microdilution methods. The antimicrobial activity study was evaluated by testing the sensitivity of extracts on bacterial strains. Activity indicators were determined, namely the Minimum Inhibitory Concentrations (MIC) [19] as shown below.

2.3.2. Methods for the Evaluation of Antimicrobial Activities

(i). Preparation of Bacterial Suspension

For the bacterial suspension preparation, two decimal dilutions were made. Indeed, in 900 µL of sterilized distilled water, 100 µL of the bacterial strain suspension enriched in nutrient broth was added. The mixture is vortex-stirred for a seconds. This was the first decimal dilution. From this first dilution, 100 µL was taken and supplemented with 900 µL of sterilized distilled water. The whole was stirred in a vortex for a few seconds. This was the second decimal dilution. The latter was used for the flood of Petri dishes.

(ii). Evaluation of Antibacterial Activity

The antibacterial activities of the leaf extracts were evaluated by determining the minimum inhibitory concentrations (MIC) using the liquid micro-dilution method. The antimicrobial activity study was estimated by testing the sensitivity of extracts on bacterial strains. Activity indicators were determined, namely the Minimum Inhibitory Concentrations (MIC).

(iii). Determination of the Minimum Inhibitory Concentrations [19]

Minimum inhibitory concentrations of the selected samples were determined using 24-hour young colony technique. In this method, 50 µL sterile MH broth was distributed in the 96 wells of the microplate except for the first line. A volume of 50 µL of the extract stock solution were then added to the first two columns of the microplate. Two from the first well on the second line to the last well of the line were diluted; 50 µL inoculum was distributed into all wells. The microplate was then incubated for 24 hours at 37 ℃. Afterward, 20 µL of 0.01% INT was in the 96 wells and then; re-incubated for 30 minutes at 37 ℃. The test was read. Wells that turn pink indicate bacterial growth. MIC in the first well was not pink-coloured.

3. Results

3.1. Yield of Extract

The extraction yield (r) was calculated using the mass of raw extract obtained (m_{Ex}) for a given mass of the powder (m_p) as related in the equation below:

$$r = \frac{m_{EX}}{m_p} \times 100$$

The results obtained on each plant extract and according to the solvent used as well as some physical characteristics are presented in Table 1 below.

Table 1. *Yield and appearance of methanol and hydroethanol extracts from the leaves of F. virosa and N. laevis.*

Plants	Extracts	Yields (%)	Color	Physical aspect
Flueggea virosa	Methanolic	8.84	Brown	Paste
	Ethanol/Water (70/30)	10.85	Green	Powder

Plants	Extracts	Yields (%)	Color	Physical aspect
Newbouldia laevis	Methanolic	5.2	Black	Powder
	Ethanol/Water (70/30)	8.5	Green	Powder

3.2. Chemical Composition of *Flueggea virosa* and *Newbouldia laevis*

Table 2. *Phytochemical screening of leaves powders from Flueggea virosa and Newbouldia laevis.*

Active Ingredient Class	*Flueggea virosa* leaves	*Newbouldia laevis* leaves
Alkaloids	+	+
Polyphenols	+	+
Tannins	+	+
catechin tannins	+	+
gallic tannins	+	+
flavonoids (flavones)	+	+
anthocyanins	+	+
leuco anthocyanins	-	-
quinonic derivatives	-	+
saponins	+	-
triterpenes	+	-
cardenolides	-	-
cyanogenic derivatives	-	-
mucilages	+	+
coumarins	+	+
reducing compounds	+	+
free anthracene derivatives	-	-
anthracenic O-heterosides	+	+
anthracenic C-heterosides	+	+

+: present in the leaf –: absent in the leaf

Table 2 presents the phytochemical screening of *Flueggea virosa* and *Newbouldia laevis*. This analysis revealed severals metabolites in the extracts.

3.3. Antibacterial Activities of *F. virosa* and *N. laevis*

The biological activity obtained from the extracts of these plants are presented in Table 3.

Table 3 showed that, apart from the hydroethanolic extract which was not active on the strain *E. faecalis* ATCC 51299, all other extracts of *Flueggea virosa* were active on all five strains.

Table 3. *Minimum inhibitory concentrations (MIC in µg /mL) of F. virosa and N. laevis in five strains, including 04 reference strains and one clinical strain.*

Name of the plant	Sample code	*E. coli* ATCC 25922	*K. pneumoniae* NR 4188	*S. typhimirim* STM CPC	*S. aureus* ATCC 12600	*E. faecalis* ATCC 51299
Positive control	Ciprofloxacin	0.34	0.66	0.33	0.32	0.62
F. virosa	FVH	500	500	1000	250	NA
	FVM	250	1000	500	31.2	500
N. laevis	NLH	1000	NA	NA	1000	NA
	NLM	500	NA	NA	250	NA

FVM: Metanolic extract of *Flueggea virosa*, FVH: Hydroethanolic extract of *Flueggea virosa*, NLM: Methanolic extract of *Newbouldia laevis*, NLH: Hydroethanolic extract of *Newbouldia laevis.*

According to the classification criteria of Kuete and Efferth [20], extracts and fractions presented: high activity when MIC is between 0 and 100 µg/mL; moderate (100 < MIC < 625 µg/mL) and low or no (MIC > 625 µg/mL).

The best activity of the methanolic extract was achieved at 31.2 µg /mL with a minimum inhibitory concentration (MIC) against *S. aureus* strain ATCC 12600. The hydroethanolic extract had moderate activity on the growth of bacterial strains.

4. Discussion

Based on the results of the yield obtained in Table 1, the extraction yield for *Flueggea virosa* and *Newbouldia laevis* is considerable. The efficiency of hydroethanol extraction (10.85%) and (8.5%) is better than that of methanolic extraction (8.84%) and (5.2%), respectively. The solvent ethanol/water (70:30, V/V) is more polar than methanol. This variation in yield is explained by the influence of the polarity of the extraction solvent on those different leaves. Similar results were obtained by Oghenemaro *et al.* (2021), who reported 10.3% for the yield of the ethanolic extract of the leaves of *F. virosa* [21]. Different results were obtained with another report [22], from the soxhlet-based methanolic extraction of *F. virosa* leaves which led to a yield of 26.34%, on the one hand, and with those of Bothon et *al* [10], during the aqueous and hydroethanol extraction of *Newbouldia laevis* that permitted the obtention of 6.03% and 5.23% respectively. The difference observed when compared with the results presented here could be explained by the time, the provenance of the plant materials, as well as the difference in solvent applied and the method used during extractions.

In our study, it was noted that different extracts presented alkaloids, tannins, flavonoids, anthocyanins, coumarins, mucilages, reducing compounds, anthracene derivatives (O-heterosides and C-heterosides), and a total absence of cardenolides, cyanogenic derivatives, leuco anthocyanins and anthracene free derivatives. It should be noted that this test revealed the presence of terpenoids and saponosides in the leaves of *Flueggea virosa.* They are not detected in the leaves of *Newbouldia laevis*. No quinonic derivatives were observed either in the leaves of *Flueggea virosa* or those of *Newbouldia laevis.* Our results are different from those obtained by Bothon *et al.*, (2014) in the phytochemical screening of *N. laevis* leaves [10]. These authors reported the presence of alkaloids, saponins, anthocyanins and tannins in this plant. Our results are similar to those reported by Udeozo and colleagues [23] who showed the presence of flavonoids, tannins, terpenes, steroids and alkaloids. The results of Usman and Osuji [24] for the *N. laevis* screening are different from those concerning saponins and anthocyanins. Our results justified partly those obtained by Tinn ẻo *et al.* [25], during the phytochemical analysis of aqueous and hydroethanolic extracts of *F. virosa* leaves. There was the absence of alkaloids, reducing compounds, cardiotonic glycosides, cyanogenic derivatives, and C-heterosides in the extracts as previously proved by Traor é *et al.* [26].

The differences in these results when compared to earlier findings could be explained using the location (where the plant materials have been collected), period of collection, or the sensitivity of the screening method. Our results might justify the frequent use of these plants as a health remedy in traditional medicine. They could find applications in the manufacture of antibiotics after their toxicological studies.

In pharmacological screening, the hydroethanolic extract presented had moderate activity efficiency on the bacterial growth of strains [27, 28]. Extracts from *N. laevis* did not show any activity on *K. pneumoniae* NR 4188, *E. faecalis* ATCC 51299 and *S. typhimirim* STM CPC, but presented low activities, with hydroethanolic extract and moderated with methanolic extract on *E. coli* and *S. aureus* strains with MIC equal to 1000, 500 and 250 µg/mL of the respective extracts. The methanolic extract of *N. laevis* leaves was most active on the strains. Various research has been conducted on the anti-

microbial activity of this plant species, revealing its ability to control a wide range of bacterial, fungal and sometimes parasitic strains.

Some of the previous studies have shown the antibacterial activity of these two plant species. In 2008, Usman and Osuji [24] evaluated the antimicrobial activity of ethanol extract from *Newbouldia laevis* leaves in Nigeria. Their work has demonstrated interesting antibacterial activities against *Escherichia coli*, *Klebsiella pneumonia* and *Salmonella typhi* with MIC = 1563; 1563 and 3125 µg/mL respectively. These MICs compared to those from our study are high, so we can assume that our hydroethanolic and methanolic extracts of *N. laevis* are more active than the one from ethanol. The same comment could be made with the results of leaf extract activities of *Flueggea virosa* in our study, compared to those of the work of Osuagwu and Akomas on *E. coli*, *S. typhimirim*, *Shigella flexneri* and *K. pneumonia* [29]. The MIC was high and respectively equaled to: 4000; 3500; 1250 and 1750 µg/mL except for *S. flexneri* which wasinhibited by the same concentration as *Shigella sp* in the present study. These observations could be explained by the fact that aqueous and hydroethanolic extracts are richer in bioactive substances (flavonoids, tannins, saponins, phenols) than the ethanolic extract *F. virosa* was the most active, including chloroform extract derived from root bark, which was found to be active against 13 microorganisms tested but with varying degrees of activity [13].

In vitro study by Okagu *et al.* [30] reported that *N. laevis* hydroethanolic bark extract showed moderate activity against some bacterial species with minimum inhibitory concentrations (MIC) of 25000; 12500; 25000; 25000; 6250 and 25000 µg/mL, respectively on *S. typhi*, *S. aureus*, *S. pneumoniae*, *B. subtilis*, *K. pneumoniae* and *P. aeruginosa*. These MICs are higher than in our study. Overall, in this study, we noticed that the extracts of the leaves of *F. virosa* are more active on the five strains studied than those of *N. laevis*. Gram-negative bacteria showed a more pronounced sensitivity (good activity on *S. aureus* strain ATCC 12600) to the extracts of *F. virosa* while the extracts of *N. laevis* are less active with low or no activities.

From the above, this work focused on the two plant species and showed the significant chemical composition of the extracts studied and great significant antibacterial activities on certain strains, and then this work would partly justify their use by the population.

5. Conclusions

The main objective of this work is to contribute to valorization of traditional medicine through a phytochemical and biological study of *F. virosa* and *N. laevis* leaves, the Beninese medicinal plants. Methanol and hydroethanol extracts from leaves of the plants were prepared to assess their antimicrobial activities. To get an idea of the secondary metabolites present in the extract that could be responsible for the observed biological activities, a phytochemical screening of the leaf powder was performed. According to the results, the extracts from our study focused on methanolic and hydroethanolic extracts of the leaves of *Flueggea virosa* and *Newbouldia laevis*. The work showed that the four extracts studied are rich in phytochemical compounds such as total polyphenols, flavonoids, tannins, alkaloids, saponins, etc. Some metabolites are present or not in a given plant species and absent in the other. Some were absent in both plants. The extracts of the leaves of *F. virosa* are more active on strains studied than those of *N. laevis*. The presence of several common and varied metabolites is responsible for the interesting antimicrobial properties observed and constitutes a therapeutic potential in several areas, especially in traditional medicine. However, more in-depth research such as the study of active fractions as well as isolated compounds, active ingredients, and the determination of larval toxicity tests, acute toxicity, lethal doses, etc. are essential to validate their effectiveness and assurance in their general use.

Abbreviations

NA	Not Active
FVM	Methanolic Extract of *Flueggea virosa*
FVH	Hydroethanolic Extract of *Flueggea virosa*
NLM	Methanolic Extract of *Newbouldia laevis*
NLH	Hydroethanolic Extract of *Newbouldia laevis*

Acknowledgments

The authors express their gratitude the YaBiNaPA project for supporting this work.

Author Contributions

Amandine Noudamadjo: Funding acquisition, Investigation, Methodology, Writing – original draft.

Bénédicta Kpadonou-Kpoviessi: Methodology, Formal Analysis, Writing – original draft.

Bienvenu Glinma: Conceptualization, Formal Analysis, Writing – original draft, Validation, Supervision.

Basile Goueti: Investigation, Methodology.

Blandine Kampa-Kuemkon: Methodology, Visualization

Fernand Ahokanou Gbaguidi: Validation, Resources, Visualization.

Salomé Dossou Sika Kpoviessi: Project administration, Formal Analysis, Investigation.

Conflicts of Interest

The authors declare no conflicts of interest.

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