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Assessment of Antibacterial Properties of Methanolic Leaf Extracts and Their Fractions from Beninese Medicinal Plants

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Abstract

The use of plants in disease treatment is an ancient practice that laid the groundwork for modern pharmacology. This study assessed the antibacterial activities of crude extracts and solvent fractions from *Flueggea virosa* and *Newbouldia laevis*, two species extensively used in African traditional medicine. Methanolic leaf extracts were fractionated with hexane, dichloromethane, ethyl acetate, and water. Antibacterial screening was performed via agar well diffusion and broth microdilution methods to determine inhibition diameters (ID), minimum inhibitory concentrations (MIC), and minimum bactericidal concentrations (MBC). Toxicity was assessed using *Artemia salina* larvae. Phytochemical screening revealed the presence of diverse secondary metabolites. Extracts and fractions from *F. virosa* exhibited stronger antibacterial effects, particularly against *Staphylococcus aureus* and *Enterococcus faecalis*, with IDs of 7–15 and 7–13.5 mm, respectively. The methanolic extract (FvM) showed MIC values of 2.5 and 5 mg/mL, while fractions Fv1 and Fv2 recorded MICs of 10 and 2.5 mg/mL, respectively, on the two bacteria. MBC values ranged from 7.5 to 10 mg/mL, indicating good bactericidal potency (P = 1–4). Conversely, *N. laevis* displayed weak or no inhibition, except for fraction Nl2, which significantly inhibited *S. aureus* (ID = 13 mm, MIC = 2.5 mg/mL, MBC = 10 mg/mL, P = 4). Neither plant showed antibacterial activity against *Escherichia coli*. All tested extracts and fractions were non-toxic to *A. salina* (LC_{50} > 0.30 mg/mL). The findings presented the significant antibacterial potential of plant, likely linked to synergistic phytocompounds, providing scientific support for its ethnomedicinal application. Further bio-guided fractionation and compound isolation are recommended to identify their active antibacterial constituents.

Keywords: Medicinal plants; Extracts and fractions; Antibacterial activity; Micro-organisms

1. Introduction

Medicines are essential for treating various diseases. But they are often used incorrectly [**1-3**]. Irrational use of medicines can cause many problems. These include increased mortality and morbidity. They can also result in poor treatment outcomes. In addition, it can lead to wastage of healthcare resources [**1**, **4**]. Antibiotics are pharmaceuticals employed in the treatment of bacterial infections, including, but not limited to, pneumonia, bronchitis, ear infections, meningitis, urinary tract infections, septicemia and sexually transmitted diseases. This is one of medicine's most significant discoveries, saving millions of lives [**5-8**]. Antibiotics kill bacteria or stop them from reproducing, allowing the body's natural **defenses** to eliminate them. However, they are ineffective against viruses and most other types of

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infection [**9**]. The overuse of antibiotics has led to an increase in bacterial resistance to antimicrobial agents [**10**]. This resistance can arise either endogenously, through mutation and selection within the pathogen itself, or exogenously, through horizontal gene transmission (HGT) from environmental organisms (e.g. antibiotic producers, commensals and non-human pathogens) to human pathogens [**11-14**]. Infections caused by resistant bacteria can lead to serious consequences, including longer illnesses and hospitalisations, increased mortality, and reduced patient protection during surgery and other medical procedures [**10**, **15**]. Antimicrobial resistance (AMR) is one of the top global public health and development threats. It is estimated that bacterial AMR was directly responsible for 1.27 million global deaths in 2019 and contributed to 4.95 million deaths [**16**]. The world faces an antibiotics pipeline and access crisis. There is an inadequate research and development pipeline in the face of rising levels of resistance, and urgent need for additional measures to ensure equitable access to new and existing vaccines, diagnostics and medicines [**17**]. In addition to death and disability, antimicrobial resistance (AMR) has significant economic costs. The World Bank estimates that AMR could result in US$ 1 trillion additional healthcare costs by 2050, and US$ 1 trillion to US$ 3.4 trillion gross domestic product (GDP) losses per year by 2030 [**18**]. Faced with increasing antibiotic resistance and a shortage of new antibiotics, it is essential to use antibiotics rationally [**10**, **19**, **20**]. A wide range of human infections are caused by the following pathogens : *Staphylococcus aureus*, *Enterococcus faecalis* and *Escherichia coli*. They are responsible for a number of problems that affect people's lives and mobility. Furthermore, the development of resistance to existing treatments, the high cost of antibiotics, and their inaccessibility to most of the population make it essential to find more accessible solutions. Thus, urgent actions need to be taken to effectively control human, animal and plant AMR pathogens. Plants are currently of paramount importance in all civilisations that use them, whether wild or cultivated, for food, defense and clothing [**21**]. The therapeutic use of plants to treat human diseases is very old [**22**] and has evolved alongside humanity throughout history [**23**]. *Staphylococcus aureus*, *Enterococcus faecalis* and *Escherichia coli* are important pathogens that cause a wide range of human infections. Traditional healers use medicinal plants to treat various diseases, including malaria, ulcers, diabetes and cancer…. Unlike modern medicine, this form of medicine is accessible to all and less expensive [**24-26**]. In previous work, we explored the phytochemical screening and biological activities of extracts from plants *Flueggea virosa* and *Newbouldia laevis*. This paper studies the antibacterial activities of these two plants on three strains *Staphylococcus aureus*, *Enterococcus faecalis* and *Escherichia coli*, as well as their larval toxicity.

2. Material and methods

2.1. Material

2.1.1. Plant material

The plant material consists solely of leaves (Figure 1). After harvesting, they were identified, certified and authenticated by the National Herbarium of the University of Abomey-Calavi under the numbers YH1060/HNB and YH1061/HNB.

Figure 1 Left «*Flueggea virosa*» (leaves and fruits) ; Right «*Newbouldia laevis*» (leaves and stems)

2.1.2. Chemical material

The various solvents were purchased directly from the manufacturers and are used without further purification. These are n-hexane (Merck), dichloromethane (ReAgent), and ethyl acetate (Sigma-Aldrich), iodo-nitrotetrazolium chloride (Sigma-Aldrich).

2.1.3. Microbiological material

All biological material is acquired and used under the conditions of the Laboratory of Biology and Molecular Typing in Microbiology.

2.2. Methods

Our previous work [**27, 28**] described the harvesting of plant leaves ; the preparation of methanol extracts and their phytochemical analysis. This paper described the procedure for obtaining fractions from these extracts and some bacterial tests. Two methods were used to fractionate the extracts, depending on the quantity of crude extracts available.

2.2.1. Fractionation method for F. virosa crude extract

A total of 211 g of crude extract obtained by macerating *F. virosa* powder in methanol, was treated with a methanol-water mixture (20:80, v/v) for solvent fractionation (Figure 2). The solvents used were of increasing polarity: hexane, dichloromethane (DCM), ethyl acetate. Each solvent was added to the extract three times in succession: 500 mL of hexane, ethyl acetate and dichloromethane. Each of these fractions was concentrated using a rotary evaporator and then dried. Four fractions were obtained: hexane (Fv1 = 26 g), dichloromethane (Fv2 = 14 g), ethyl acetate (Fv3 = 49 g), and the aqueous residue (Fv4 = 47 g).

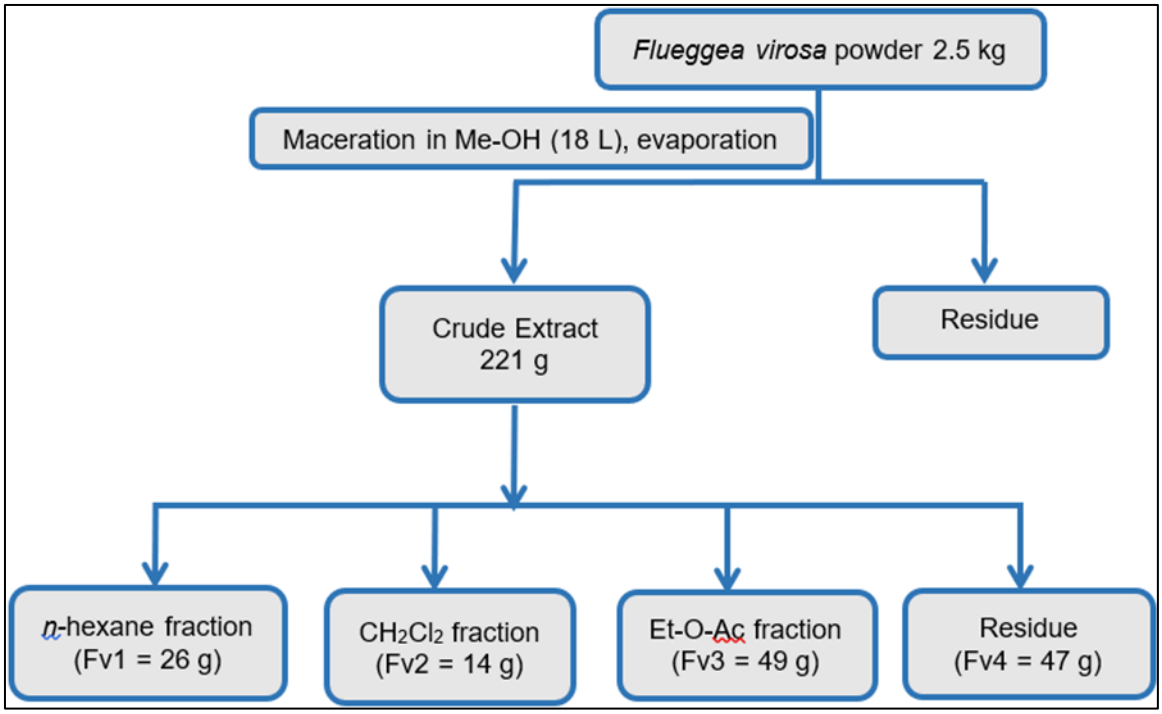

Figure 2 Procedure for fractionating of *F. virosa* crude extract

2.2.2. Fractionation method for N. laevis crude extract

70 g of the crude extract of *N. laevis* by maceration were subjected to liquid-liquid partitioning using solvents of increasing polarity (Figure 3). Using the same procedure as described above, four fractions were obtained such as fraction of hexane (Nl1 = 30 g), dichloromethane (Nl2 = 9 g), ethyl acetate (Nl3 = 6 g) and the aqueous residue (Nl4 = 20 g).

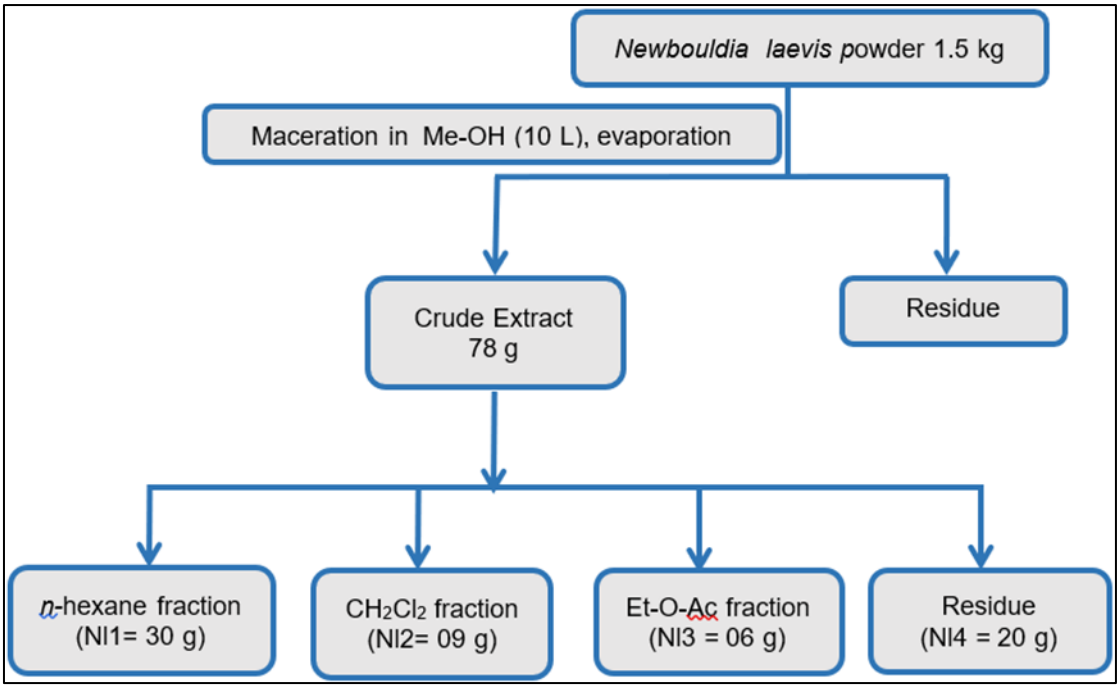

Figure 3 Procedure for fractionating of *N. laevis* crude extract

2.3. Antibacterial test

The following microorganisms were used in the study: two Gram-positive (*Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212) and one Gram-negative (*Escherichia coli* ATCC 25922). Antimicrobial activity was assessed by sensitivity testing of extracts and fractions on bacterial strains. Indicators of activity will be minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs).

The tests were performed using a standard methodology that has been adapted for use in our laboratory.

2.3.1. Sensitivity test

The Müller-Hinton (MH) solid medium diffusion method, as described by Anani et al. [**29**], will be used to test the sensitivity of microbial strains to extracts. The previous day's bacterial pre-culture (one colony in one millilitre of liquid Mueller-Hinton) will be diluted to achieve a turbidity of 0.5 on the McFarland scale (i.e. 10^8 CFU/mL), then reduced to 10^6 CFU/ml in sterile distilled water. This bacterial suspension (1000 µL) will be used to flood a Petri dish containing Mueller-Hinton agar medium (Bio-Rad, France) [**30-32**]. Using a perforator, 6 mm diameter paper disks will be made. The sterile disks will be deposited under aseptic conditions onto plates that have previously been flooded with bacterial culture. 30 µL of the extract to be tested will be inoculated onto the disks under aseptic conditions. For each extract, the experiment will be duplicated and a negative control will be performed using the solvent instead of the extract. The plates are then left for 15–30 minutes at room temperature before being incubated at 37 °C in an oven for 24 and 48 hours [**33**]. After the incubation times of 24 and 48 hours, inhibition diameters are measured using a graduated ruler [**34**].

2.3.2. Determination of the minimum inhibitory concentration (MIC)

In addition to testing bacterial sensitivity to various mixtures, the MICs were determined. The MIC of an extract for a strain is the lowest concentration at which no visible growth occurs within 24 hours. In this study, they were determined using the micro-dilution method and iodo-nitrotetrazolium (INT) as a bacterial viability indicator [**35**]. The method involved preparing the bacterial inoculum with 24-hour-old young colonies, distributing 50 µL of sterile MH broth into 96 wells of a microplate (except the first row), adding 50 µL of the stock extract solution to the first two columns of the microplate, creating a twofold dilution from the first well of the second row to the last well, dispensing 50 µL of inoculum into each well, incubating the microplates for 24 hours at 37 °C, adding 20 µL of 0.01% INT solution to each well; Re-incubate for 30 minutes at 37°C ; Take the reading. Wells turning pink indicate bacterial growth.

The MIC corresponds to the first well in which no pink coloration is observed due to the presence of INT.

2.3.3. Determination of the minimum bactericidal concentration (MBC)

The MBC will be determined on the basis of the MIC results. After identifying the MIC, all the other tubes from the MIC to the high concentrations will be inoculated onto Petri dishes containing MH agar medium using a loop. The plates will be examined after an incubation period of 24 hours at 37°C. The MBC is the extract concentration at which no bacterial growth is observed [**36**].

2.3.4. Determination of the antimicrobial potency of extracts and fractions

Antibacterial potency (AP) is defined as the ratio of the minimum bactericidal concentration (MBC) to the minimum inhibitory concentration (MIC). This provides information on the bactericidal and bacteriostatic properties of a product [**37**].

$$AP = \frac{MBC}{MIC}$$

If the antibacterial power (AP) is less than or equal to four (AP ≤ 4), the product tested is bactericidal. If AP is greater than four, the product tested is bacteriostatic [**38**].

A bacteriostatic effect means that bacteria stop multiplying without necessarily being destroyed. It involves the reversible inhibition of certain biological functions necessary for microbial metabolism, growth and multiplication without affecting all vital functions [**39**]. The bactericidal effect results in the definitive destruction of the microbe over a period of time. This destruction is linked to an irreversible lesion that renders further proliferation impossible [**40**].

2.4. Toxicity screening

Larval toxicity was assessed using the method outlined by Dougnon et al. [**41**]. *Artemia salina* Leach eggs were incubated in seawater for 48 hours until the young larvae hatched. A series of second-order dilutions of the extract were made to obtain a range of concentrations. Sixteen larvae were placed in 1 mL of seawater and then transferred to each dilution of the extract. All dilutions, as well as the control solution containing no extract, were left under agitation for 24 hours. The number of dead larvae in each solution was counted under a microscope to assess the larval cytotoxicity of the extract. Dose-response data were expressed as logarithms to base 10 and the mean lethal concentration (LC_{50}) was determined by linear regression. Finally, larval toxicity was assessed using the Mousseux scale [**42**]. An LC_{50} value greater than or equal to 0.1 mg/mL indicates that the extract is non-toxic, while a value between 0.1 mg/mL and 0.05 mg/mL indicates low toxicity. An LC_{50} value between 0.05 mg/mL and 0.01 mg/mL indicates moderate toxicity, while an LC_{50} value below 0.01 mg/mL indicates high toxicity.

3. Results and discussion

3.1. Phytochemical screening

In our previous work, it was demonstrated that extracts from plant leaf powder were rich in various secondary metabolites. Their extracts were screened for the presence of alkaloids, carbohydrates, phenols, gums and mucilage, flavonoids, steroids, proteins, tannins, and saponins using standard qualitative methods (table 1). Several phytochemicals responsible for pharmacological activities, such as antimicrobial, antiparasitic, analgesic, and anti-inflammatory properties, have been identified.

These include phytochemicals with antimicrobial and anti-inflammatory properties, and several recognised activities of these plants are reported in the literature [**27**, **28**, **43-46**]. In last works, MIC of methanolic extract = 31.2 and 250 µg/mL against *S. aureus* strain ATCC 12600 were obtained from *F. viroa* and *N. laevis* respectively ; hyodextranolic extract moderately impacts cell growth. This explains why methanolic extract was chosen to obtain the different fractions for this work.

Table 1 Secondary metabolites from phytochemical screening

Secondary metabolites	***F. virosa* leaves**	***N. laevis* leaves**	**Secondary metabolites**	***F. virosa* leaves**	***N. laevis* leaves**
Alkaloids	+	+	Triterpenes	+	-
Polyphenols	+	+	Cardenolides	-	-
Tannins	+	+	Cyanogenic derivatives	-	-
Catechin tannins	+	+	Mucilages	+	+
Gallic tannins	+	+	Coumarins	+	+
Flavonoids (flavones)	+	+	Reducing compounds	+	+
Anthocyanins	+	+	Free anthracene derivatives	-	-
Leuco anthocyanins	-	-	Anthracenic O-heterosides	+	+
Quinonic derivatives	-	+	Anthracenic C-heterosides	+	+
Saponins	+	-			

3.2. Antibacterial Activity

The results of the sensitivity test were shown in the following table. The influence of the extracts and fractions on the bacterial strains tested made it possible to measure the diameters of the inhibition zones of the samples recorded in the table above.

Table 2 Inhibition diameters of extracts and fractions used in the study

	Inhibition diameters (mm)		
Extracts and fractions	***S. aureus ATCC 29213***	***E. foecalis ATCC 29212***	***E. coli ATCC 25922***
FvM	8±0	7±0	-
Fv1	15±0	13.5±1,5	10±3
Fv2	7±0	7±0	-
Fv3	-	-	-
Fv4	-	-	-
NlM	-	-	-
Nl1	-	10±0	-
Nl2	13±0	-	-
Nl3	-	-	-
Nl4	-	-	-

FvM : methanolic extract of *F. virosa* ; Fv1 : hexane fraction ; Fv2 : DCM fraction ; Fv3 : AcOEt fraction and Fv4 : aqueous residue.
NlM : methanolic extract of *N. laevis* ; Nl1 : hexane fraction ; Nl2 : DCM fraction ; Nl3 : AcOEt fraction and Nl4 : aqueous residue

Results showed that the strains are more sensitive to the methanolic extract and fractions from *F. virosa* than to those from *N. laevis*, which showed no reduction in sensitivity. The methanol extract FvM and the dichloromethane fraction Fv2 showed affinity for the Gram-positive bacteria *S. aureus* and *E. faecalis* with inhibition diameters of 07 mm and 08 mm, respectively. Meanwhile, the hexane extract Fv1 showed particular sensitivity to the three strains, with inhibition diameters of 15, 13.5, and 10 mm for *S. aureus*, *E. faecalis*, and *E. coli*, respectively. Furthermore, the fraction Fv3 and the residue Fv4 showed no effect on the strains studied. Regarding *Newbouldia laevis*, only the hexane (Nl1) and

dichloromethane (Nl2) fractions had an effect on the *E. faecalis* and *S. aureus* strains, respectively. Therefore, the methanolic extract and the other fractions showed no results. No effect was observed on the strains, either for the ethyl acetate fractions or for the aqueous residues from the plants. Our results are consistent with those of Amenu et al. [**47**], which vary depending on the microorganisms studied. In their research, it was demonstrated that significant activity against S. aureus and *E. coli* was exhibited by the ethanol extract from *S. virosa* roots, with variable inhibitory activity being shown against other organisms. The lowest inhibition was observed against *Micrococcus luteus*. The inhibition zone ranged from 6.33 to 17.67 mm.

During this study, we found that neither the extract nor the fractions of the two plants exhibited any antibacterial activity against *E. coli*. This strain was resistant to all extracts. Studies have been continued on the two Gram-positive bacterial strains. The minimum inhibitory concentrations (MIC), and minimum bactericidal concentrations (MBC), and then the antibiotic power (AP) were determined for the extracts and fractions of each plant. Results were described in the following table.

Table 3 MICs (mg/mL), MBCs(mg/mL) and antibiotic potency (AP) of *F. virosa*

Extracts and fractions	***S. aureus ATCC 29213***				***E. Foecalis ATCC25922***			
	ID	**MIC**	**MBC**	**AP**	**ID**	**MIC**	**MBC**	**AP**
FvM	8±0	2,5±0	10	4	7±0	5±0	7,5	1,5
Fv1	15±0	10±0	10	1	13,5±1,5	10±0	10	1
Fv2	7±0	2,5±0	7,5	3	7±0	2,5±0	10	4
Fv3	NA	NA	NA	ND	NA	NA	NA	ND
Fv4	NA	NA	NA	ND	NA	NA	NA	ND

ID : inhibitory diameters (mm) ; NA : not active ; ND not determined

It should be noted that the two bacteria used for the antimicrobial test are highly sensitive to extracts and fractions derived from *Flueggea virosa* leaf powder, with MICs ranging from 2.5 to 10 mg/mL and MBCs from 7.5 to 10 mg/mL. In particular, the antibiotic potency (or power) (AP ≤ 4) varies between the methanol extract (FvM) and the hexane (Fv1) and dichloromethane (Fv2) fractions, which display AP values : 1, 1.5, 3, and 4. *S. aureus* and *E.* faecalis showed high sensitivity to the hexane partition, with an inhibitory diameter of 15 mm and 13.5 mm, respectively. The methanol extract and dichloromethane fraction exhibited the greatest antibiotic potency (1.5, 3 and 4). The two strains were resistant to the ethyl acetate fraction (Fv3) and aqueous residue (Fv4). These findings support those in the literature in which *S. virosa*'s most active extract was the chloroform one, showing activity against 13 test organisms with MIC values ranging from 15.6 µg/mL to over 1000 µg/mL. The petroleum spirit, chloroform and ethanol extracts of *S. virosa* combined with standard antibiotics showed effects against antibiotic-resistant strains of *S. aureus*. Moreover, previous reports have documented the antibacterial properties of securin and viroallosecurnin, which are alkaloids derived from the leaves of *F. virosa* [48, 49]. In 2020, Anarado et al. in their study showed that *S. aureus* was highly sensitive to the ethyl acetate extract of *Securinega virosa* but resistant to the n-hexane extract [50]. Other research has demonstrated that a methanol extract of *S. virosa* root bark (6.25 to 25 mg/kg body mass, by intraperitoneal injection) considerably suppressed ($P < 0.05$) acetic acid-induced stomach contractions and reduced formalin-induced neurogenic pain (phase 2). It also significantly slowed the reaction time ($P < 0.01$) of mice to thermal pain induced by a heating plate. At the doses tested, the extract reduced paw swelling by 12%, 52%, and 52% by the third hour [51]. Recently, an ethanolic extract derived from the aerial parts of *F. virosa* demonstrated antisickle cell activity by normalising the shape of abnormal circulating erythrocytes, a property known as antifalcemic or antisickling activity [52]. Previously, the same type of activity was demonstrated with an aqueous methanolic leaf extract, which inhibited sodium metabisulphite-induced sickling of HbSS red blood cells in a concentration-dependent manner [53].

The following results were obtained from the methanol extract and fractions of the *N. laevis* plant (table 4).

Table 4 MICs (mg/mL), MBCs(mg/mL) and antibiotic potency (AP) of *N. laevis*

Extracts and fractions	*S. aureus* ATCC 29213				*E. Foecalis* ATCC25922			
	ID	MIC	MCB	AP	ID	MIC	MCB	AP
NlM	NA	NA	NA	NA	NA	NA	NA	ND
Nl1	NA	NA	NA	NA	10±0	NA	NA	ND
Nl2	13±0	2,5±0	10	4	NA	NA	NA	ND
Nl3	NA	NA	NA	NA	NA	NA	NA	ND
Nl4	NA	NA	NA	NA	NA	NA	NA	ND

ID : inhibitory diameters ; NA : not active ; ND not determined

Overall, all fractions except the dichloromethane fraction remained inactive against the bacteria used. The *E. faecalis* strain showed highly resistant to all extracts and fractions. Only Nl2 inhibited bacterial activity on *S. aureus* (MIC = 2.5; MBC = 10 mg/mL). This extract exhibited a bactericidal effect with antibiotic potency equivalent to 4. Our work yielded different results to those obtained by Suleiman et al. in 2024 in the ethyl acetate leaf extract of *N. laevis*. They demonstrated that the extract from the aerial parts exhibited antibacterial activity against the pathogenic strains *K. pneumoniae*, *P. aeruginosa*, *S. aureus*, *E. coli* and *S. typhi* at varying concentrations depending on the bacterial species [**54**]. Other researcher concluded that microbes could not grow at varying doses of plant extracts and that no significant differences ($P > 0.05$) were observed in the inhibition zones as the extract concentrations increased [**55**]. In contrast, Okeke et al. found that higher concentrations resulted in significant growth inhibition. The results suggest that the broad spectrum of antibacterial activity exhibited by *F. virosa* can be attributed to the presence of diverse active secondary metabolites in the extract and its various partitions or fractions [**56**]. The inhibitory effect could be a result of tannins and flavonoids, which have been employed as antimicrobials for a long time. On the *N. laevis* side, where the bacteria showed strong resistance to the extracts and fractions, perhaps the solvents used did not sufficiently extract the metabolites responsible for the activities. The next step in the work will be to carry out a phytochemical analysis of each extract and fraction of each plant. This could help us to better understand and explain the different results obtained.

This study also investigated the toxicity of the extracts and fractions that reacted with bacteria (table 5). To achieve this, *Artemia salina* L. larvae were used to evaluate the toxicity of each extract. We used Camptothecin as a positive control, a well-justified choice. Camptothecin and its derivatives are used in chemotherapy because they are cytotoxic to mammalian cells. It targets topoisomerase I, a highly conserved enzyme, all of whose residues in contact with the drug in a crystalline structure of topotecan/human topoisomerase I [**57**, **58**] are conserved in insects. Several studies have been conducted on this molecule, with varying results [**59-61**]. It is increasingly used in preliminary toxicity studies of plant extracts and fractions, as well as isolated or synthesized compounds. The lethal half-concentrations (LC_{50}) of each solution were determined (see the table below). This test is a preliminary *in vitro* toxicity method that is not entirely comparable to a test on human cells in culture. Although there is a correlation between the two types of cells, Carballo et al. suggested performing both tests before drawing conclusions, which would give a reliability of 75% compared to 50% for the test on shrimp larvae alone [**62**].

Table 5 Toxicity of extracts and partition of plants

F. virosa			*N. laevis*		
Extracts and fractions	**LC_{50} (mg/mL)**	**Toxicity**	**Extracts and fractions**	**LC_{50} (mg/mL)**	**Toxicity**
FvM	4.00	no toxic	NlM	3.01	no toxic
Fv1	1.11	no toxic	Nl1	2.99	no toxic
Fv2	1.02	no toxic	Nl2	0.30	no toxic
Control	LC_{50} = 0.13 mg/mL		non toxic		

In our study, and according to the toxicity scale used, camptothecin has an LC_{50} value of 0.13 mg/mL, which is also higher than the limit of 0.1 mg/mL [**42**] that indicates whether a substance under study is toxic or not. It was found that

all our extracts and fractions had an LC_{50} > 0.30 mg/mL. This result is consistent with others that have been carried out on other parts of the plant. In 2022, an acute toxicity study showed that the *F. virosa* root extracts tested did not cause any mortality in mice up to 5000 mg/kg body weight [**63**]. This preliminary toxicity test is performed to gain an understanding of the potential harmful effects of using these extracts. It also proves that the bactericidal effects (PA) of these extracts on the strains are not caused by the solvents, which were thoroughly evaporated using appropriate methods. However, given the correlation between cytotoxicity in shrimp larvae and in 9PS and 9KB cells (human nasopharyngeal carcinoma) on the one hand [**64**] and in A-549 lung carcinoma cells and HT-29 colon cells on the other [**62**], it can be concluded, pending further investigation, that the tested extracts do not exhibit cytotoxic activity and can therefore be used without risking toxicity in the short and medium term.

4. Conclusion

This study demonstrated that, of the methanolic leaf extracts and their fractions tested, *Flueggea virosa* exhibited significantly higher antibacterial activity against Gram-positive and Gram-negative bacterial pathogens than *Newbouldia laevis*. The superior efficacy of *F. virosa*, particularly evident in several of its fractions, suggests the presence of potent bioactive compounds with broad-spectrum potential. In contrast, *N. laevis* showed little or no activity against the bacterial strains tested. None of the plant extracts or fractions exhibited larval toxicity. These results confirm the traditional use of plants by the general public for treating infectious diseases. Our work also corroborates the findings of other pharmacological studies, which support its value as a source of new antibacterial agents.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest in our manuscript

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