Phytochemical Profiling and Evaluation of Antioxidant and Anti-inflammatory Activities *in vitro* of *Newbouldia laevis* and *Flueggea virosa* Leaf Extracts

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ABSTRACT

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| Traditional medicine use has surged over the past 20 years, with many people using it to treat various health issues. Ethnobotanical and pharmaceutical surveys revealed their application in many areas.  Ethnobotanical and pharmaceutical surveys have revealed their application in many areas. Analyzing the antioxidant and anti-inflammatory properties and quantifying the polyphenol, flavonoid, and tannin contents of the methanolic and hydroethanolic extracts from the leaves of *Flueggea virosa* and *Newbouldia laevis*.is the goal of this study. All extracts exhibited notable antioxidant activity. Among them, we noted that the hydroethanolic extract of F. virosa stood out by displaying the highest antioxidant performance, with values of 3.30 ± 0.05; 7.81 ± 0.54 and 18.72 ± 0.05 mMol AAE/mg according to the ABTS, APM, and DPPH methods, respectively. The quantitative analysis of phenolic compounds showed that extracts from *F. virosa* leaves were so rich in polyphenols (14328 and 22512 μg EAG/mg extract). From the study of anti-inflammatory activity, it was found that a good activity was obtained for all extracts in particular the hydroethanolic extract of *N. laevis* (IC50 = 0.87 ± 0.02 mg/mL). From these results, it is deduced that the content of phenolic compounds is very high for *F. virosa* and *N. laevis* in contrast to flavonoids and tannins that give them these anti-inflammatory and antioxidant powers.  The study supports the use of these plants in traditional medicine. |

*Keywords: antioxidant, anti-inflammatory, leaves, Newbouldia laevis, Flueggea virosa, extracts.*

1. INTRODUCTION

Plants have been used in traditional medicine for several centuries. This, because of their therapeutic properties, attributed to the presence of bioactive compounds. One of the most important bioactive compounds is the antioxidants which play a significant role by preventing various diseases related to oxidative stress, such as cardiovascular diseases, diabetes, and hypertension (Wang and Kang, 2020). Similarly, in developing countries such as Benin, there are many other diseases that are now scourges that cause a large number of deaths every (Washington, 2024). Plants with their effectiveness and their wide variety of phytochemical constituents, have an important potential in the treatment of several human diseases (Da et al., 2016; Nguemo Dongock et al., 2018).

*Newbouldia laevis* as tree of life, is a purple-flowering plant that is widely distributed in many parts of Africa and *Flueggea virosa* is a plant with whitish flowers. They offer a promising source of active drug in the treatment of diseases. Many parts of these plants such as leaves, flowers, roots, bark, seeds, etc., can generate phytochemicals of therapeutic interest with varying levels.

These biologically active compounds can be isolated by biological techniques, physico-chemical and also traditional processes namely maceration, decoction, infusion, etc (Sasidharan et al., 2011). *Newbouldia laevis* and *Flueggea virosa* are two plant species widely used in African pharmacopeia to treat various ailments. *Newbouldia laevis*, belongs to the family Bignoniaceae. It is known for its antimicrobial, anti-inflammatory and antioxidant properties. *Flueggea virosa*, is used in traditional medicine for relieving pain and reducing inflammation, particularly in cases of muscle pain, gout, and arthritis. It’s from the family of Euphorbiaceae and subfamily of Phyllanthaceae. Its extracts are applied to reduce swelling and manage chronic inflammatory disorders (Noudamadjo et al., 2025a). The pharmacological properties as antioxidant and anti-inflammatory of these plants are based on the types of compounds and their proportion in the plant species. Thus, this study aims to analyze the antioxidant and anti-inflammatory properties of the leaf extracts from *Newbouldia laevis* and *Flueggea virosa,* but also the dosage of certain chemical families of these plants by different methods to better understand their therapeutic potential and use in herbal medicine.

2. material and methods

**2.1 Material**

After collecting the plant material, the different parts were separated from each plant (leaf, stem, fruit, etc.). These plant organs were dried. The leaves, stem and flowers of *N. laevis* and *F. virosa* are presented on Fig. 1. These 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.



**B**



**A**

**Fig.1**. **Leaves, stems and flowers of *Newbouldia laevis* (A) and *Flueggea virosa* (B).**

**2.2 Methods**

**2.2.1 Method of extract preparation**

Extracts were prepared according to the method of and Goueti et al., (2023) and Noudamadjo et al. (2025b). Methanolic extracts were obtained by maceration of 2.5 kg and 1.5 kg of dry leaf powder from *Flueggea virosa* and *Newbouldia laevis*, respectively, in 18 L and 10 L of methanolic solvent, for 72 hours. After filtration using filter paper, the extracts collected were concentrated at 55°C using a rotary evaporator. The crude extracts were then oven-dried at 48°C. The mass of each extract was accurately measured. The hydroethanolic extract was prepared by macerating 50 g of leaf powder in 500 mL of a 70/30 (V/V) ethanol-water mixture for 72 hours. The extract obtained was concentrated using a rotary evaporator and then dried at 50°C before being accurately weighed. Methanol is used in this work because of the micro-extractions carried out at the beginning of our work. Thin-layer chromatography with these different extracts showed that several compounds migrated from the methanol extract to the hydroethanol extract. Methanol is also a volatile solvent and is completely eliminated from the extraction after evaporation and drying. Hence the choice of methanolic solvent.

**2.2.2 Assessment of the antioxidant activity of leaf extracts from *Newbouldia laevis* and *Flueggea virosa***

The antioxidant activity of leaf extracts from *N. laevis* and *F. virosa* was determined *in vitro* by three methods as described below:

**2.2.2.1 DPPH trapping capacity (2.2’-diphenyl-1-picrylhydrazyl)**

The protocol used for trapping DPPH of our extracts is that used by Vamanu and Nita (2013) with a few modifications in the presence of a radical H•. For this, an amount of 1 mL of DPPH solution at a concentration of 0.25 mM was mixed with 0.2 mL of the diluted extract and 0.9 mL of absolute ethanol. The package is inserted into all tubes and has been stirred and allowed to stand for 30 min in the dark at room temperature. Optical densities were measured at 517 nm using a Helios Ω spectrophotometer. The tests were repeated in triple. Free radical trapping capability was achieved using GraphPad Prism 8.0.2 (263) software. The following equation was used to calculate the DPPH radical trapping activity (%):

(1)

Where: AAs is the absorbance in the presence of extract and AAc is the absorbance in the absence of extract.

**2.2.2.2 Inhibition test ABTS (2. 2'-azinobis-[3-ethylbenzothiazolin-6-sulfonic] acid)**

The protocol of Vamanu and Nita (2013) with some modifications was used to carry out the ABTS anti-radical activity. To generate ABTS radical cations, we react ABTS solution (7mM) with potassium persulfate (2.45 mM) in a 1:0.5 ratio. To get the resulting solution, a series of activity have been done. The mixture of extract plants is incubated at room temperature in the dark for 12 hours. The resulting solution is further diluted with phosphate-buffered saline (PBS; pH = 4) to obtain an absorbance between 0.70 and 1.00 at 734 nm. A volume of 0.5 mL of diluted extracts is then mixed with 0.3 mL of the ABTS solution and 0.2 mL of ethanol, making a final volume of 1 mL. Absorbance was recorded after 15 minutes in the dark at 734 nm with the Helios spectrophotometer λ.

The percentage of inhibition was calculated with the following equation:

(2)

**2.2.2.3 Phosphorus and molybdenum test**

We also evaluated the phosphorus and molybdenum assay using the protocol used by Kedir et al. (2023). We prepared an aliquot of 0.1 mL of the sample extract dilution in a triple test tube. Each tube was treated with 1 mL of reactive solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated at 95° C in a water bath for 90 min. The samples were cooled to room temperature and their absorbance was recorded at 765 nm. Ascorbic acid was used as a positive control to generate a standard curve (y = 1.4831x - 0.1568; R2 = 0.9957). The equivalent antioxidant activity (AAE) was derived from the standard cure and expressed using the following equation:

(3)

With: X = trapping activity (mM/mL); Df = dilution factor; Cm = concentration of the initial extract solution (mg/mL).

**2.2.3 Determination of the secondary metabolite contents**

***2.2.3.1 Content of total polyphenolic compounds***

The method described by Vermerris and Nicholson (2006) using the Folin-Ciocalteu reagent has determined the content of phenolic compounds in the various extracts. The reference for the calibration curve was gallic acid. The total phenol in the extract is expressed as μg gallic acid equivalent (GAE, μg gallic acid/mg of extract) (Kim et al., 2003). For this, 125 µL of each extract prepared at 1 mg/mL was mixed with 625 µL of Folin-Ciocalteu reagent (at a concentration of 10%). After mixing, the mixture was incubated for 5 minutes. Then, they added 500 µL of sodium carbonate (75 mg/mL) and 4.75 mL of distilled water (H2O). The mixture was vortexed and incubated at room temperature in the dark for 1 hour, after which the absorbance is measured at 760 nm using a spectrometer, with a blank as reference.

***2.2.3.2 Content of flavonoids***

The total flavonoid content of extracts from *Flueggea virosa* and *Newbouldia laevis* was estimated by the aluminium trichloride (AlCl3) method. Quercetin is used as a reference compound to produce the calibration curve (Kim et al., 2003). To 500 µL of AlCl3 (2%) was added 500 µL of the extract. To the mixture, 3 mL of distilled water was added, and after 10 min incubation, the reading was taken against a blank at 415 nm (Assogba, 2016).

***2.2.3.3 Determination of condensed tannins***

Different methods were described to determine the concentration of condensed and hydrolysable tannins in the different extracts of these plant leaves. The reagent used to determine the content of condensed tannins is sulphuric vanillin according to the method of Assogba (2016). To 500 µL of each extract, 1.5 mL of vanillin solution (4%) was added. 750 µL of concentrated HCl was then added to the mixture, incubated for 20 min and read on the spectrometer at 500 nm.

**2.2.4 Evaluation of anti-inflammatory activity in vitro**

To assess the in vitro anti-inflammatory activity, we used the method of heat-induced denaturation of egg albumin, as described by Chandra et al. (2012). The reaction took into account a set of mixing: 0.2 mL of freshly prepared egg albumin, 2.8 mL of phosphate-buffered saline (PBS, pH= 6.4), and 2 mL of extract at varying concentrations (0.625 to 5 mg/mL). The mixture was incubated at 37 ± 2°C for 15 minutes, then heated to 70°C in a water bath for 5 minutes.

The absorbances were read at 660 nm with a spectrophotometer after cooling to room temperature. Diclofenac sodium with concentrations between 1.25 and 10 g/mL was used as the reference molecule. The test was performed in duplicate for each sample and the percentage of denaturation inhibition was calculated using the following formula:

(4)

With : Vt represents the volume of the solution tested and Vc the volume of the control solution.

3. results and discussion

**3.1 Antioxidant activities**

The antioxidant power of our extracts was determined by three methods: DPPH, ABTS and APM. For each plant, all three methods were explored. The graphs obtained are shown below.



**NLH** : hydroethanolic extract of *Newbouldia laevis ;* **NLM** : methanolic extract of *Newbouldia laevis*

**Fig. 2. Summarizes the antioxidant capacity of *N. laevis* extracts**

The methanolic extract had the highest inhibition while the lowest was exerted by the hydroethanolic extract with respectively corresponding values of 3.32 ± 0.01 mMol AAE/mg and 2.95 ± 0.07 mMol AAE/mg. For the APM method, the reducing power of the methanolic extract was higher than that of the hydroethanolic extract which is the lowest with a capacity of 2.17 ± 0.66 and 2.47 ± 0.01 mMol AAE/ mg for the methanolic extract. The DPPH trapping capacity of samples ranged from 5.55 ± 0.83 to 17.83 ± 0.18 mMol AAE/mg. As far as the methanolic extract is concerned, it exhibited the highest activity, with a value of 17.83 ± 0.18 mMol AAE/mg. For the hydroethanolic extract, it showed the lowest activity, with a value of 5.55 ± 0.83 mMol AAE/mg. The DPPH method proved to be the most effective, straightforward, and commonly used for preliminary testing of free radical scavenging activity in plant extracts or compounds. It should be noted that the methanolic extract of *N. laevis* leaves showed good antioxidant activity in vitro using all three methods (Fig. 2).

These results are in accordance with previous study conducted by Okagu et al. (2021), who revealed the presence of good antioxidant scavenging activities in the methanol extract of *N. laevis* stem bark with superoxide anion, hydrogen peroxide and DPPH radical scavenging tests. Our results corroborate the work of Bothon et al. (2014), who found in their study that the aqueous extract of *Newbouldia laevis* leaves with the DPPH method presented the best activity. The above results showed that the different parts of *N. laevis* possess antioxidant and radical scavenging properties and those activities vary from part to part, with leaves consistently showing higher activity than stem bark and other parts of the plant. Our results are similar to those obtained by Ngozi Okafor et al. (2020), indicating that the extract of the leaves macerated with aqueous ethanol (50:50) may possess antioxidant activities to treat the rat diabetics. Solomon et al. (2019) showed that the methanolic extract of the leaves from these plants presented a good antioxidant activity with the method of DPPH.



**FVH :** hydroethanolic extract of *Flueggea virosa* ; **FVM :**  methanolic extract of *Flueggea virosa*

**Fig. 3. Antioxidant capacity of *Flueggea virosa* extracts.**

It has been noted that the highest inhibition was observed with the hydroethanolic extract of *Flueggea virosa* (FVH) (3.3 ± 0.05 mMol AAE/mg). As far as the methanolic extract of *F. virosa* (FVM) is concerned,it showed a lower inhibition, with a value of 3.11 ± 0.09 mMol AAE/mg. ABTS inhibition ranged from 3.11 ± 0.09 to 3.3 ± 0.05 mMol AAE/ mg dry extract. The highest inhibition was recorded with the hydroethanolic extract FVH (3.3 ± 0.05 mMol AAE/ mg) while a low inhibition was observed in the methanolic extract FVM with a value of 3.11 ± 0.09 mMol AAE/mg. We noted a reduction capacity between 6.19 ± 0.74 mMol AAE/mg and 7.81 ± 0.54 mMol AAE/mg for the antioxydant activity measured by the APM reduction method. The DPPH scavenging capacity of the samples ranged from 18.65 ± 0.05 mMol AAE/mg to 18.72 ± 0.06 mMol AAE/mg. The methanolic extract showed the lowest activity with a value of 18.65 ± 0.05 mMol AAE/mg while the hydroethanolic extract displayed the highest activity with a value of 18.72 ± 0.06 mMol AAE /mg (Fig. 3).

Some studies showed that the plant is more active with ethanolic leaf extracts, while hexane extracts were less active (Uzama Danlami et al., 2013). Methanolic leaf extract showed higher antioxidant effects than methanolic stem bark extract (Bailly, 2024).The leaf extract was as effective as the reference product ascorbic acid in a DPPH test (Chauke et al., 2012). The results obtained from the various phytochemical and antioxidant activities could partly justify the traditional use of this plant in the treatment of patients suffering from certain diseases. According to Thiombiano et al. (2022), the best antioxidant activity was observed at the ABTS method with the best results obtained for the methanolic extracts, among the three methods used. The ethanolic extract of *F. virosa* was found to be more antioxidant activity according to Agbodan et al. (2017).

**3.2 Determination of total polyphenols**

The total polyphenol contents of the *Flueggea virosa* and *Newbouldia laevis* extracts are shown in Figure 4.

**Fig. 4. Total polyphenol content of *F. virosa* and *N. laevis* extracts**

The results showed that the methanolic extract of *Flueggea virosa* (FVM) is richer in polyphenolic compound with 14328 µg EAG/mg extract than that of *Newbouldia laevis* with a value of 11928 µg EAG/mg extract (Fig. 4). This difference in content could be due to extraction solvents or to the genus or family of the species.

Researchers working on *F. virosa* in South Africa found by (Chauke et al., 2012) as the present in the total phenolic content of acetonic extracts from *F. virosa* roots. Compared with our work, which gave 22512 µg EAG/mg of extracts as total phenolic content, the results of Chauke et al. (2012) are lower than ours. This difference in our results could be justified by the type of solvent and the part of the plant used. Indeed, the composition of secondary metabolites in the two types of extract is different, as acetone is a less polar organic solvent than methanol. The aqueous and hydroethanolic extracts of *N. laevis* were evaluated for their total polyphenol content. Thus, the high polyphenol content of the various extracts of the plant leaves studied would justify their use in traditional medicine in the care of sickle-cell patients (Sibri et al., 2023).

**Fig. 5. Flavonoid content of *Flueggea virosa* and *Newbouldia laevis* extracts**

The hydroethanolic extracts (NLH and FVH) of *N. laevis* and *F. virosa* showed the highest contents, respectively 4.996 and 62.9827µg EqQCT/mg extract, while the methanol extract contained the lowest total flavonoid contents (Fig. 5).

According to Thiombiano et al. (2022), the methanolic leaf extract of *F. virosa* gave the highest content of total flavonoids (3.30 ±0.32 EQ/100mg extract) (Thiombiano et al., 2022). Similarly, from the work of Ajaib et al. (2021), the wide range of flavonoid contents was provided by methanolic leaf extract of *F. virosa* (Ajaib et al., 2021). It is also interesting to know that the antioxidant potentials of *N. laevis* ethanolic extracts of the plant were shown to be proportional flavonoid contents of the extracts (Salemcity et al., 2020).

**Fig. 6. Condensed tannin content of *Flueggea virosa* and *Newbouldia laevis***

Figure 6 shows the results of the tannin assay in our extracts. From the analysis of this figure, our extracts are very rich in condensed tannins. *Flueggea virosa* has the highest content of condensed tannins in methanolic extract (8.609 mg EAG/100 mg). It should also be noted that methanolic extracts have a high content of condensed tannins than hydroethanolic. For example, when comparing the prepared extracts, the methanolic extract is richer in condensed tannins (2.58 ± 1.49 mg EAG/100 mg and 8.61 ± 2.16 mg EAG/ 100 mg) for *N. laevis* and *F. virosa*, respectively, than the hydroethanolic extract. This difference in content may be due to the polarity of the extraction solvents and the plant family.

Our results corroborate those obtained by Olounlade et al. (2012) on *N. laevis*. To our knowledge, there has been no work carried out on the dosage of tannins in the leaves of *Flueggea virosa*.

**3.3. Anti-inflammatory activity: in vitro (PDI)**

* ***Flueggea virosa***



**Fig. 7. Protein denaturation inhibition activity of *Flueggea virosa***

Figure 7 shows the results of anti-inflammatory tests for *Flueggea virosa****.*** Both methanolic and hydroethanolic extracts show better activity, with inhibition percentages of 66.185 ± 0.980% and 66.042 ± 1.332%, respectively. The methanolic extract appeared to be more active than the ethanolic one. The hydroethanolic extract showed the highest IC50 =1.67 ± 0.58 mg/mL attesting to its efficient anti-inflammatory capacity.

Our work corroborates the results obtained by Dénou et al. (2021) and Yerima et al. (2009) in France during the anti-inflammatory test of the methanolic extract of leaves from the same plant in vivo. The results of Ezeonwumelu et al., (2012) suggested that *Flueggea virosa* root aqueous extract produced fairly reasonable amount of extract and contained phytochemicals which possess anti-inflammatory activity. Our result justifies the work by Rutuja et al., (2025) who used the white mice male and female weighing between 18-35 g aqueous extracts from the leaves and stems of *Flueggea virosa* have anti-inflammatory activity.

* ***Newbouldia laevis***



**Fig. 8. Protein denaturation inhibition activity of *Newbouldia laevis***

From the graph analysis (Fig. 8), the hydroethanolic and methanolic extracts of *Newbouldia laevis* showed very good anti-inflammatory activities with inhibition percentages of 89.86 ± 1.63% and 62.73 ± 15.03%, respectively. Thus, the hydroethanolic extract of *Newbouldia laevis* had the best anti-inflammatory activity with the IC50 value of 0.87 ± 0.02 mg/mL

Our results justify those of Udeozo et al. (2014), who showed that the ethanolic extract of *Newbouldia laevis* flowers presented a good anti-inflammatory activity effect in vivo. The findings authenticated the claim that the methanol extract of root of *Newbouldia laevis* is used in treating inflammatory disorder in vivo. This research provides a pharmacological basis for the utilization of *Newbouldia laevis* roots for the treatment of inflammatory disorders and related diseases (Ali et al., 2022). The ethanolic extract of Newbouldia laevis root flower in vivo possesses anti-inflammatory activity according to Usman et al., (2008).

4. Conclusion

The study carried out on leaves extracts of *Newbouldia laevis* and *Flueggea virosa* revealed that they extracts are rich in antioxidants and have very good anti-inflammatory activity. The best activity (3.30 ± 0.05 mMol EAA/ mg) is the methanolic extract of *Flueggea virosa*. Based on these results, these plants have a capacity to reduce free radicals inside the human body. The leaves of these plant species could be potential protective agents against oxidative stress. The evaluation of the anti-inflammatory activity of leaves extracts shows that they have a very interesting inti-inflammatory potential and that the hydroethanolic extract of *Newbouldia* seems to be the most active (IC50 value of 0.87 ± 0.02 mg/mL). These results also confirm their use in traditional medicine for the treatment of inflammatory issues. The determination of phenolic compounds, condensed tannins and flavonoids allowed us to determine the content of bioactive compounds (polyphenols, tannins and flavonoids) in the extracts that could be responsible for the biological activities noticed during this study. These results suggest that extracts of *Newbouldia laevis* and *Flueggea virosa* could be of considerable pharmacological interest, and could be exploited in the development of new drugs that traditionally improve the fight against oxidative stress and inflammatory diseases. However, further studies, especially in vivo, are needed to investigate the mechanism of their action and to confirm the safety of their use.

**Disclaimer (Artificial intelligence)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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