PHYTOCHEMICAL INVESTIGATION, ANTIBACTERIAL AND ANTI-INFLAMMATORY ACTIVITIES OF MUSSAENDA ELEGANS AND SMEATHMANNIA LAEVIGATA: CONTRIBUTION TO THE VALORIZATION OF GUINEAN MEDICINAL PLANTS.

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Abstract

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Plants in general and medicinal plants in particular, play an important role in human health. This work aims to study the phytochemical properties and evaluate the antibacterial and anti-inflammatory activity of *Mussaenda elegans* and of*Smeathmannia laevigata*, cultivated in the Republic of Guinea. Tannins, flavonoids, mucilage, and reducing compounds were identified in the leaves of both plants. *Mussaenda elegans* showed the highest content of phenolic compounds, with total polyphenols (22.006 ± 0.648 mg GAE/g), flavonoids (2.360 ± 0.110 mg QE/g), and tannins (4.087 ± 0.312 mg EC/g).For antibacterial activity, three strains were found to be sensitive to the hydroethanolic extract of *Mussaenda elegans*, namely, *Pseudomonas aeruginosa* and *Staphylococcus aureus* with aMIC of 1.25 mg/mL and *Candida albicans* (MIC 2.5 mg/mL) Hydroethanolic extract of *Smeathmannia laevigata* showed similar sensitivity against *Escherichia* coli, *Pseudomonas aeruginosa* and *Candida albicans* with MIC of 2.5 mg/mL. *The anti-inflammatory activity observed for these two extracts was moderate. However, the Mussaenda elegans* extract showed 18.29% inhibition, which is more promising than *Smeathmannia laevigata extract's* activity. *These findings position Smeathmannia laevigata* and *Mussaenda elegans* extracts as promising natural candidates for developing alternatives to conventional non-steroidal anti-inflammatory drugs, although their efficacy remains below the reference standard and requires further investigation to confirm their therapeutic potential and safety profile.

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Introduction:-

The increasing ineffectiveness of antibacterial treatments (Klobucar and Brown , 2022; Prasad et al., 2021) is largely attributed to the misuse and inappropriate use of antibiotics, which promotes the emergence and spread of multidrug-resistant bacterial strains. Faced with this threat, the discovery and development of new molecules with effective antibacterial biological activity against these pathogenic strains are essential, both to improve therapeutic management and to limit the health and socioeconomic impact of bacterial resistance.

Inflammation (Nathan and Ding, 2010; Mack, 2018) is a complex and highly regulated biological response of the body, involving the coordinated activation of immune cells and the release of pro-inflammatory mediators such as cytokines, chemokines, prostaglandins, and reactive oxygen species. It is triggered in response to various pathogenic stimuli, including microbial infections, tissue damage, and chemical insults, and plays a crucial role in tissue defense and repair mechanisms. However, when this response becomes excessive or chronic, it contributes to the development and progression of many inflammatory and degenerative diseases.

From a therapeutic standpoint, the management of inflammation relies primarily on the use of non-steroidal anti-inflammatory drugs (NSAIDs), which act notably by inhibiting cyclooxygenases (COX-1 and COX-2), and steroidal anti-inflammatory drugs (corticosteroids), which modulate the gene expression of multiple inflammatory mediators. Despite their clinical efficacy, these synthetic molecules are frequently associated with significant adverse effects,

including gastrointestinal, renal, cardiovascular, and immunosuppressive disorders, thus limiting their long-term use (Blain et al., 2000).

The Republic of Guinea is characterized by remarkable plant biodiversity, constituting an important source of medicinal plants widely used in traditional medicine. Numerous studies have reported the use of these plant resources in the management of various pathologies, including infertility (Bah et al., 2025) , dermatoses (Goumou et al., 2018), malaria (Haba et al., 2022), hemorrhoids (Camara et al., 2024), oral and dental diseases (Camara et al., 2023), and others. In this context, the present study aims to contribute to the scientific valorization of traditional Guinean medicine through a phytochemical investigation, coupled with the evaluation of the biological activities (antibacterial and anti-inflammatory activities) of two plant species: *Mussaenda elegans* and *Smeathmannia laevigata* , commonly used in Upper Guinea in the treatment of dermatoses.

Materials and Methods

Plant material:

The leaves of *Mussaenda elegans* and *Smeathmannia laevigata* were harvested in the commune of Kankan (Republic of Guinea) and then dried in the laboratory at Julius Nyerere University of Kankan. The dried leaves were then ground into a powder and stored for analysis, which was carried out at the University of Abomey-Calavi in Benin.

Microorganisms

Microorganism used in this study were constituted of *Enterococcus faecalis* , *Staphylococcus aureus* ; *Pseudomonas aeruginosa* , *Escherichia coli* ; *Salmonella typhi*and *Candida albicans* .They were provided by the Bacteriology section of the National Laboratory of the Ministry of Health in Benin.

Animal material : The animal material consists of male albino Wistar rats (180-210 g; n = 3 per group), raised under the following conditions: 12 h light/dark; 22 ± 2 °C; humidity 50-60%; water and pellets ad libitum; acclimatization 7 days.

Plant extracts preparation

Hydroethanolic (70%) extract of *M. elegans* and *S. laevigata* were prepared by exhaustive extraction for three successive days in 70% ethanol. Extracts were filtered and concentrated using a rotary evaporator (BUCHI Rotavapor RII). Filtrate were collected and stored at 4°C for further analysis.

Phytochemical screening

Phytochemical screening was carried out using standard methods described by Noudogbessi et *al* ., (2013) and Olayé et al., (2018).

Saponins

The procedure involves preparing a decoction by boiling two (2) grams of dried, ground plant material in 100 mL of distilled water for 30 minutes. After cooling and filtration, 1 mL, 2 mL, 3 mL, ..., 10 mL of the decoction are

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introduced into 10 test tubes (1.3 cm internal diameter). The volume of extract in each tube is then adjusted to 10 mL with distilled water. After this, each tube is shaken.The tube is held horizontally for 15 seconds. The tubes are then left to rest in a vertical position for 15 minutes, after which the height of the remaining moss is measured in centimeters. If it is close to 1 cm in the tenth tube, then the moss index is calculated using the following formula: I = Height of moss (in cm) in the tenth tube × 5/0.0X or (1000/N), where N is the number of the tube where the moss height is equal to 1 cm. The plant is considered to contain saponins when the index is greater than 100.

Tannins

Tannins are identified by preparing an aqueous infusion. Mix 5g of plant powder with 100mL of boiling distilled water. This infusion is left to steep for 15 minutes, then filtered and rinsed with a little hot water to adjust the filtrate volume to 100mL. A 5ml aliquot of this filtrate (5%) is then added to 1mL of 1% ferric chloride. The presence of tannins is indicated by the observation of a greenish or bluish-black coloration.

Mucilage

Five milliliters of absolute ethanol are added to 1 milliliter of a 10% aqueous decoction prepared for 15 minutes. The formation of a flocculent precipitate within the mixture is an indicator of the presence of mucilage.

Anthraquinones

In a test tube containing 2 mL of the extract, 5 mL of 10% ammonium hydroxide (NH4OH) is added. The presence of anthraquinones is characterized by the appearance of a yellow color.

Flavonoids

The identification of flavonoids was carried out by the cyanidin reaction consisting of adding 5 mL of hydrochloric alcohol, a few magnesium shavings and 1 mL of isoamyl alcohol to a test tube containing 5 mL of filtrate of a 5% infusion. The observation of a pink-orange, pink-purple or red color collected in the supernatant layer of isoamyl alcohol reveals the presence of flavonoids respectively flavones, flavanones or flavonols and flavanonols.

Alkaloids

These are precipitation reactions. In acidic solutions at pH 1 or 2, alkaloid salts react with iodine compounds of heavy metals to form characteristic colored precipitates. A sulfuric acid extract is prepared from 10 g of powdered leaves, bark, or roots and 50 mL of concentrated $_{H_2SO_4\ diluted}$ to 10% in a 250 mL Erlenmeyer flask. After maceration for 24 hours at room temperature, the macerate is filtered through filter paper and washed with distilled water to obtain 50 mL of filtrate. One (1) mL of filtrate is introduced into a test tube, to which approximately five drops of Bouchardat's reagent are added. In the presence of alkaloids, a brown precipitate is obtained with Bouchardat's reagent.

Determination of phenolic content of the extracts

Total phenolic content

Total phenolic content were evaluated using Folin–Ciocalteu technique (Koudoro et al., 2021). Briefly, 50 µL of plant extract previously prepared or reference compound (gallic acid) were mixing with 200 µL of distillated water, 125 µL of Folin–Ciocalteu reagent (1 N), and 625 µL of sodium carbonate (20%, w/v). The mixture were kept in dark for 30 minutes and then ultraviolet absorbance were measured at 760 nm using UV-visible spectrophotometer.

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TPC was expressed as microgram (µg) of gallic acid equivalents per **milligram (mg) of dry extract (mg GAE**/g) using a calibration curve plotted with pure gallic acid in a series of different concentrations.

Total flavonoid content

Aluminum chloride colorimetric method was used to determine the total flavonoid contents of plant extracts (Koudoro et al., 2021). A volume of 50 µL of extracts were added to a solution containing 30 µL of 10% $NaNO_2$, 60 µL of 20% ($AlCl_3$, $6H_2O$), 200 µL of 1 N NaOH, and 660 µL of distillated water. After mixing, the absorbance at 510 nm of each sample was determined with a UV–visible spectrophotometer. Standard calibration curve was plotted with quercetin and total flavonoid content was expressed as microgram of quercetin equivalents **per milligram of dry extract (mg QE/g)**.

Tannins content

The tannin content was determined according to the method described by Koudoro et al. (2021), which involves mixing 50 µl of the extract with 3 mL of 4% vanilla in ethanol and 1.5 mL of fuming hydrochloric acid. Absorbance was measured spectrophotometrically after 10 minutes of mixing in the dark at 510 nm against a blank without extracts, which served as a reference. The results are expressed as mg catechin equivalent per g of extract (Olayé et al., 2018).

Determination of antimicrobial activity

The antimicrobial test was performed according to the method described by Atindehou et al. (2013). Bacteria were cultured aerobically at 37°C in Mueller-Hinton broth (MHB) for 18 hours. The hydroethanolic extract of each sample was suspended in a DMSO /water mixture (10:90, v:v) and diluted to concentrations of 10, 5, 2.5, 1.25, 0.625, etc., mg/mL in MHB in 100 µL 96-well microplates. 100 µL of bacterial culture in logarithmic phase with a concentration of 10^6 CFU/mL at 620 nm was added. Each test was performed in triplicate. A positive DMSO/water control and a negative control consisting of an MHB mixture without bacteria were performed. After 18 hours of incubation at 37°C with shaking, the minimum inhibitory concentration (MIC) was determined by adding 40 µL of iodonitrotetrazolium chloride at 0.2 mg/mL to each well. Bacterial growth was determined by a reddish-pink color in the well after 1 hour of incubation of the plate at 37°C.

Determination of Anti-inflammatory Activity

The 1% (v/v) formalin-induced edema model is a classic paradigm of acute local biphasic inflammation described by Sen et al. (1991). Rats were divided into four (4) groups of three (3) rats per group. The rats were fasted for 12 h prior to the experiment. The initial thickness (T0) of the left hind leg of each rat/group was measured. Subsequently, a volume of 0.1 ml of 1% formalin was injected into the plantar fascia of the rats' feet. Leg thickness measurements were taken hourly from 1 to 4 h after formalin injection using a digital caliper (0.01 mm accuracy). Two control groups were prepared for the experiment. These were:

- Lot 1: negative control that received formalin without being treated;
- Lot 2: positive control which was treated with the reference product, here acetylsalicylic acid.

Thirty (30) minutes before the formalin injection, the rats to be tested received orally 300 mg/kg of hydroethanolic extracts of the leaves of *Smeathmannia laevigata* and *Mussaenda elegans* respectively. Aspirin (acetylsalicylic acid)

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was used as the reference product at a dose of 100 mg/kg. The negative control group received 0.9% isotonic saline NaCl (10 ml/kg).

Table 1: Experimental groups (random allocation):

Batch	Band	Treatment	Dose	Way
Lot 1	Negative test	saline solution	0.9% NaCl (10 ml/kg)	Oral
Lot 2	Positive test	Acetylsalicylic acid	100 mg/kg	Oral
Lot 3	*Mussaenda elegans*	Tested extract/compound	300mg/kg	Oral
Lot 4	*Smeathmannia laevigata*	Tested extract/compound	300 mg/kg	Oral

The percentage increase (P) and inhibition (I) of edema were calculated using the following formulas:

$$P = \frac{Vt - Vo}{Vo} \times 100$$

With

P: percentage increase in edema

Vo = volume of the right hind leg at time 0 (before treatment)

Vt = volume of the right hind leg at time t

- **(%) Inhibition** :

$$I = \frac{(PAO)\ groupe\ contrôle - (PAO)\ groupe\ traité}{(PAO)\ groupe\ contrôle} \times 100$$

I: Percentage of edema inhibition

Statistical analysis

The data were entered into an Excel spreadsheet. Mean values ± SEM and one-way ANOVA followed by Tukey's test ($p < 0.05$) were calculated *using* software (e.g., GraphPad Prism v.8).

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Results and discussion

Phytochemical study

Table 1 presents the results of the phytochemical analysis of the hydroethanolic extract of plant material from *Mussaenda elegans* and *Smeathmannia laevigata* .

Table 1: Phytochemical screening of the two plants

Secondary metabolites	Plants	
	Mussaenda elegans	*Smeathmannia laevigata*
Anthraquinone	-	-
Mucilage	+	-
Tannins	+	+
Flavonoids	+	+
Saponins	+	-
Alkaloids	-	-
Reducing compounds	+	+

- : Absent; + : Present

Phytochemical analysis of plant material from *Mussaenda elegans* and *Smeathmannia laevigata* reveals the presence of several secondary metabolites, including phenols, tannins, and flavonoids. Nwafor et al. (2022) identified alkaloids, tannins, flavonoids, saponins, and reducing compounds in *M. elegans leaves* harvested in Nigeria. Their results are nearly identical to those of our work, with the difference that these authors demonstrated the presence of alkaloids in this plant, unlike our findings.

The total estimated content of phenolic compounds, flavonoids and tannins is presented in Table 2.

Table 2: Phenolic compound content

Extract	**Total phenolics (mg AG/ g)**	**Flavonoid (mg EQ/ g)**	**Tannins (mg EC/g)**
M. elegans	22.006 ± 0.648	2.360 ± 0.110	4.087 ± 0.312
S. laevigata	11.239 ± 0.422	0.940 ± 0.052	9.945 ± 0.622

Mussaenda elegans plant material has a significantly higher phenol content (22.006 ± 0.648 mg GA/g) than the hydroethanolic extract of *Smeathmannia laevigata plant material* (11.239 ± 0.422 mg GA/mg). The same observation was made for flavonoid content (2.360 ± 0.110 mg QE/g for *Mussaenda elegans* versus 0.940 ± 0.052 mg QE/g for *Smeathmannia laevigata*, and the opposite was found for tannin content for which content was 4.087 ± 0.312 mg CE/gfor *Mussaenda elegans* versus 9.945 ± 0.622 mg EC/g for *Smeathmannia laevigata*. These observations suggest that the hydroethanolic extract of *Mussaenda elegans* plant material has superior biological potential, particularly in terms of antibacterial and anti-inflammatory activities, compared to the hydroethanolic extract of *Smeathmannia laevigata* plant material.

Nwafor et al. (2022) found respective total phenol, flavonoid, and tannin levels of 659.75, 34.13, and 304.11 mg/100 g, or approximately 6.59, 0.34, and 3.04 mg/g. Compared to the results of our work, the tannin contents are similar, while the total phenol and flavonoid contents found by these authors are lower than those of our work. Hassler (2025) showed that *Rosa laevigata* is synonymous with *Smeathmannia laevigata*. According to Quan et al. (2022) **,** approximately 148 chemical components have been isolated from this plant, including flavonoids, lignans, polyphenols, steroids, triterpenoids, tannins, and other components.

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Antimicrobial activity

Table 3 presents the antibacterial activity of the plant extracts studied. Analysis of this table shows that the hydroethanolic extracts of *S. laevigata* and *M. elegans* both exhibited inhibitory activity against the tested microbial strains, with MICs ranging from 1.25 to 10 mg/mL. The hydroethanolic extract of *M. elegans* proved particularly effective against *Pseudomonas aeruginosa* and *Staphylococcus aureus* with an MIC of 1.25 mg/mL. The hydroethanolic extract of *S. laevigata* was more effective against *Escherichia coli*, with an MIC of 2.5 mg/mL. Pharmacological studies have already confirmed that the leaves of *Rosa laevigata* , synonym of *S. laevigata* , have antioxidant, anti-inflammatory, antiviral and antitumor activities (Quan et al., 2022) **.**

Table 3: Minimum Inhibitory Concentration (MIC)

Microbial strains	MIC (mg/mL)	
	S. laevigata	*M. elegans*
Salmonella typhi	5	5
Escherichia coli	2.5	5
Pseudomonas aeruginosa	2.5	1.25
Staphylococcus aureus	10	1.25
Candida albicans	2.5	2.5
Enterococcus faecalis	5	5

Anti-inflammatory activity

Figure 1 shows the anti-inflammatory activity of extracts from the two plants studied, as well as aspirin taken as a reference compound

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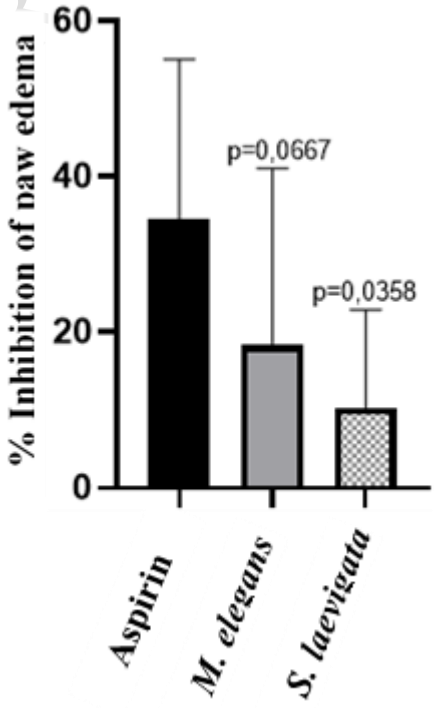

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Figure 1: Edema-inhibiting power of the tested extracts and reference compound

In a 1% formalin-induced rat leg edema model reproducing the acute and late phases of inflammation, extracts of *Smeathmannia laevigata* and *Mussaenda elegans* showed moderate anti-inflammatory activity, although lower than that of the pharmacological standard. Post-hoc statistical analysis using Tukey's test, performed after ANOVA, revealed a significant superiority of the aspirin-treated positive control, with a mean inhibition of 34.49%, compared to the *Smeathmannia laevigata extract* (10.25% inhibition; $p = 0.0358$). In contrast, the difference observed between aspirin and the *Mussaenda elegans extract* (18.29% inhibition) did not reach statistical significance ($p = 0.0667$). Furthermore, direct comparison between extracts of *Smeathmannia laevigata* and *Mussaenda elegans* revealed no statistically significant difference (mean difference: 8.04%; $p = 0.3752$), although *Mussaenda elegans* showed a tendency towards greater efficacy.

The anti-inflammatory activity observed for these two extracts could be linked to a partial inhibition of prostaglandin synthesis via the cyclooxygenase-2 (COX-2) pathway, associated with modulation of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, possibly under the control of the NF-κB signaling pathway. These results position the extracts of *Smeathmannia laevigata* and *Mussaenda elegans* are considered promising natural candidates for developing alternatives to conventional non-steroidal anti-inflammatory drugs (NSAIDs), although their efficacy remains below the reference standard and requires further investigation to confirm their therapeutic potential and safety profile. The phytochemicals primarily responsible for the anti-inflammatory activities belong to the families of polyphenols, terpenoids, flavonoids, saponins, and tannins (Azab et al., 2016; Bunte et al., 2019; N'guessan et al., 2021). The fact that the phenolic compound content of the two plants is comparable to their anti-inflammatory activity could mean that the anti-inflammatory activity observed in both plants could be largely due to these phenolic compounds.

Conclusion

In conclusion, this study suggests that the leaves of Smeathmannia laevigata and Mussaenda elegans are two potential sources of bioactive molecules. This observation is supported by the phenolic compound content of these two plants, which correlates with their antimicrobial and anti-inflammatory activities. Further investigations are

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warranted on these two plants to identify the secondary metabolites responsible for their biological activity and to assess their potential toxicity.

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