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Phytochemical Study and Antimicrobial Activity of *Murraya paniculata* (L.) Extracts

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Abstract

The present study focused on the phytochemistry and antimicrobial potential of the stems of *M. paniculata*, an ornamental and medicinal plant used in Benin for oral care and hygiene. The objective of this work was to determine the chemical composition and antimicrobial activity of the aqueous and ethanolic extracts, as well as the decoction of this plant. For this purpose, the total phenolic compounds and flavonoid content were determined by the Folin-Ciocalteu and aluminum trichloride methods, respectively. The antioxidant activity was assessed using the DPPH and FRAP methods. The antimicrobial tests were conducted using antibiograms and the determination of MIC and MBC. The identification of bioactive molecules was performed by HPLC. The results reveal that the three extracts are rich in phenolic compounds. The ethanolic extract exhibits the highest content of total phenolic compounds with a value of 109.678 ± 0.757 µg GAE/mg DE, while the decoction has the highest flavonoid content, measuring 150.538 ± 2.020 µg QE/g DE. The antioxidant activity demonstrated that the decoction possesses the best activity according to the DPPH method, with an IC_{50} of 0.181 ± 0.015 mg/ml, while the aqueous extract proved to be the most effective by the FRAP method, with a reducing power of 74.022 ± 1.152 mg E AG/g DE and 153.009 ± 3.127 mg EC/g DE. Only the decoction displayed activity against the tested microorganisms, showing an inhibition diameter of 23.333 ± 11.666 mm on *Staphylococcus aureus*, whereas vancomycin, the reference compound, had an inhibition diameter of 17.666 ± 0.577 mm. The MICs of the decoction on the various strains range from 3.125 to 25.000 mg/ml. Tannic acid is the major bioactive compound identified in

the three extracts, with the highest content found in the decoction. The traditional use of this plant could be well justified by the results obtained.

Keywords

Murraya paniculata, Phenolic Compound, Oral Care, Antimicrobial

1. Introduction

Despite the evolution of modern medicine and the discovery of new drugs, traditional medicine remains an inexhaustible source of solutions for the treatment and prevention of several diseases of bacterial, fungal, and even viral origin. In this perspective, it is appropriate to establish the chemical profile and elucidate the potential of medicinal plants to effectively combat microorganisms responsible for these diseases in order to better promote them. Oral diseases constitute a major public health problem due to the dangers to which they expose victims and also because of the high cost of their treatments [1] [2]. Several plants are used in developing countries as chewing sticks to maintain healthy oral hygiene and treat oral diseases [2]. The stem of *Murraya paniculata*, an ornamental plant of the Rutaceae family originating from Asia and transported to several African countries, including Benin, is one of these plants [3]. The genus Murraya has been commonly used to treat many diseases owing to its antibacterial properties due to the presence of highly valuable phytochemicals [4] [5]. The stems of *Murraya paniculata* are used in India as chewing sticks for oral hygiene and for the treatment of toothaches [6]. In Bangladesh, the leaves are boiled in water, and the extract is used for gargling three to four times daily for three days [7]. The plant is also used to treat many other diseases, like headaches, bruises, gastralgia, stomachaches, rheumatism, skin irritation, swelling, menstrual problems, and snake bites [8]. Several phytochemical studies have been carried out on the leaves, roots, and bark of *Murraya paniculata* to highlight their antimicrobial and antioxidant activity, as well as their chemical composition [8] [9]. Meanwhile, no studies have been conducted specifically in the Republic of Benin on the stems of this plant with the aim of finding scientific justification for its traditional use in oral hygiene and care, in order to discover new medical remedies. This is why the present study aims to determine the chemical composition of the stems of *Murraya paniculata* in relation to its antimicrobial and antioxidant activity.

2. Materials and Methods

2.1. Plant Material

The twigs of *Murraya paniculata* were collected in the southern part of the Benin Republic and kept in the laboratory until dry. After that, they were ground to a fine powder and used for further experiments.

2.2. Microorganisms

The microorganisms used in this study consisted of *Enterococcus faecalis* ATCC 10240, *Staphylococcus aureus* ATCC 29223, *Proteus mirabilis* ATCC 24974, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, and *Candida albicans* IP 4872. They were provided by the Bacteriology section of the National Laboratory of the Ministry of Health in Benin.

2.3. Plant Extracts Preparation

Hydroethanolic (50%) extract (eth) and aqueous extract (aq) of the twigs of *M. paniculata* were prepared by exhaustive extraction for three successive days in 50% ethanol and water, respectively. The decoction extract (de) was prepared by boiling the plant powder for 30 minutes in distilled water. All extracts were filtered and concentrated using a rotary evaporator (BUCHI Rotavapor RII). The filtrate was collected and stored at 4˚C for further analysis. The diagrams in Figure 1 and Figure 2 summarize the extraction methods used in this work.

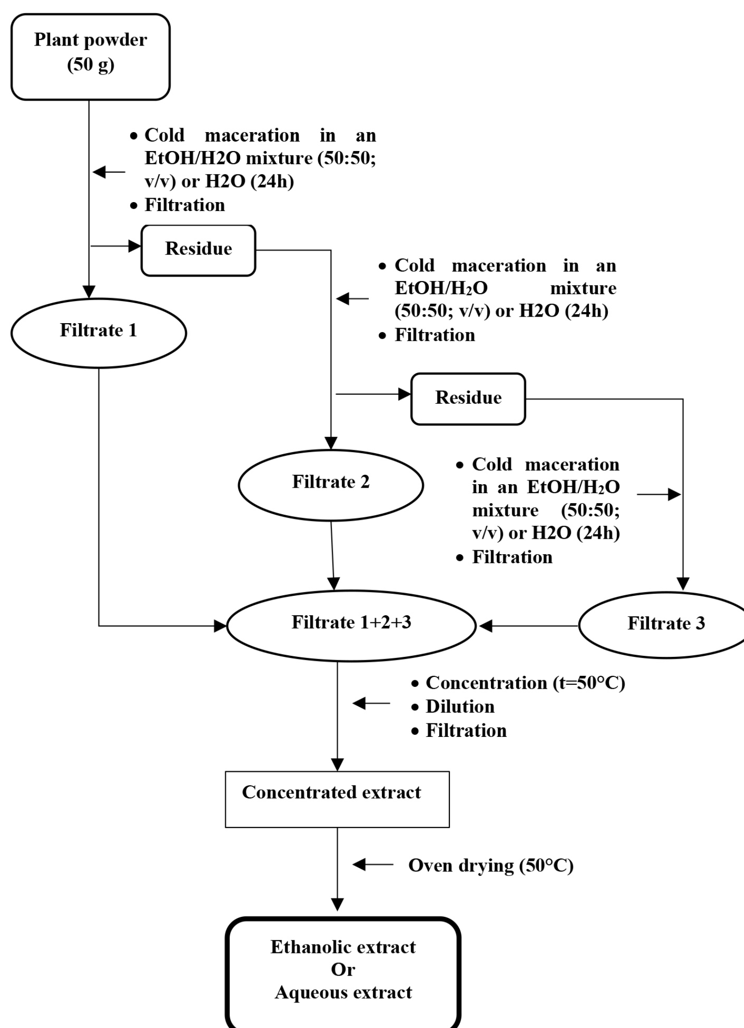

Figure 1. Extraction diagram of ethanolic and aqueous extract.

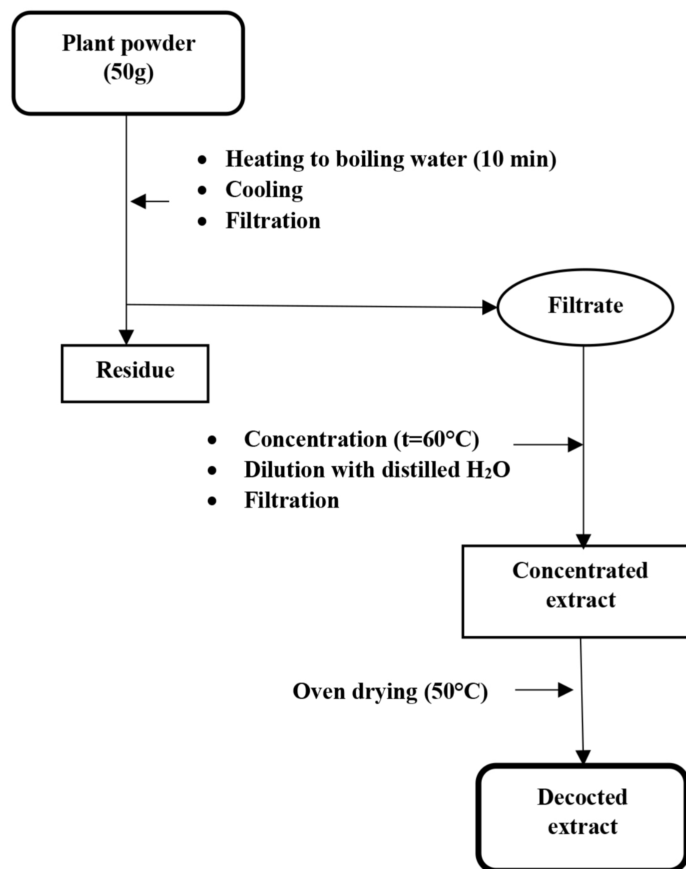

Figure 2. Extraction diagram of the decoction.

2.4. Determination of Phenolic Content of the Extracts

2.4.1. Total Phenolic Content (TPC)

Total phenolic content was evaluated using the Folin-Ciocalteu technique [9]. Briefly, 50 μL of plant extract previously prepared or reference compound (gallic acid) were mixed with 200 μL of distilled water, 125 μL of Folin-Ciocalteu reagent (1 N), and 625 μL of sodium carbonate (20%, w/v). The mixture was kept in the dark for 30 minutes, and then ultraviolet absorbance was measured at 760 nm using a UV-visible spectrophotometer. TPC was expressed as micrograms (μg) of gallic acid equivalents per milligram (mg) of dry extract (μg GAE/mg DE) using a calibration curve plotted with pure gallic acid in a series of different concentrations.

2.4.2. Total Flavonoid Content (TFC)

The aluminum chloride colorimetric method was used to determine the total flavonoid contents of plant extracts [9]. A volume of 50 μL of extracts was 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 distilled water. After mixing, the absorbance at 510 nm of each sample was determined with a UV-visible spectrophotometer. The standard calibration curve was plotted with quercetin, and total flavonoid content was expressed as micrograms of quercetin equivalents per milligram of dry extract (μg

QE/mg DE).

2.5. Antioxidant Activity by DPPH Method

The DPPH assay has been realized according to the method described by the authors [10]. The first step was to find the right dilution series, which was 0.1 mg/mL, 0.2 mg/mL, 0.3 mg/mL, 0.4 mg/mL, 0.5 mg/mL, and 0.6 mg/mL for the extracts. Gallic acid and catechin used as references were prepared in a series of dilutions as follows: 0.005 mg/mL, 0.01 mg/mL, 0.02 mg/mL, 0.04 mg/mL, 0.06 mg/mL, 0.08 mg/mL, 0.1 mg/mL, 0.12 mg/mL, 0.14 mg/mL, and 0.16 mg/mL. Then, 50 µl of each diluted extract or reference was added to 1950 µl of DPPH (130 Mm) and kept in the dark for 45 min. After that, the absorbance of each sample was measured at 516 nm against the blank solution realized with 50 µl of solvent (distillated water or ethanol/distillated water (50:50) and 1950 µl of DPPH (130 Mm).

The scavenging percentage P was calculated, and the inhibitory concentration necessary for trapping 50% of free radicals of DPPH (IC_{50}) was graphically determined. Catechin and gallic acid were used as positive controls.

$$P = [(Ab - As)/Ab] \times 100 \quad (1)$$

where P: percentage of trapping; Ab: absorbance of the blank; As: absorbance of the sample.

2.6. Ferric Reducing Antioxidant Power (FRAP) Method

The FRAP assay was determined according to the method described by the authors [10]. A volume of 2 ml of extract was mixed with 2 ml of phosphate buffer (0.2 M, pH 6.6) and 2 ml of the aqueous solution (1%) of potassium hexacyanoferrate [$K_3Fe(CN)_6$]. The mixture was incubated for 20 min at 50˚C, and then 2 ml of trichloroacetic acid (10%) was added. After that, the mixture was centrifuged at 3000 rpm for 10 min, and 2 ml of the supernatant was mixed with the same volume of water and 20 µl of $FeCl_3$ (0.1%). Absorbances were measured at 700 nm against a calibration curve obtained from gallic acid and catechin. The calibration curves were realized with a series of dilutions. The different concentrations of gallic acid were: 10 µg/mL, 20 µg/mL, 30 µg/mL, 40 µg/mL, 50 µg/mL, 60 µg/mL, 70 µg/mL and 80 µg/mL. Catechin was prepared at the following concentrations: 20 µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL, 100 µg/mL, 120 µg/mL, 140 µg/mL, 160 µg/mL, 180 µg/mL, and 200 µg/mL. Then, 2 mL of each dilution was reacted in the same conditions with the extracts as previously described.

The reducing power was expressed as a function of milligrams of gallic acid equivalent per gram of crude extract (mg eq AG/g CE) and also as a function of milligrams of catechin equivalent per gram of crude extract (mg eq EC/g CE).

2.7. Determination of the Antimicrobial Activity

2.7.1. Agar Well Diffusion Method

The preliminary screening of the antimicrobial activity was carried out by the

diffusion method of the extracts in wells dug in Mueller Hinton agar plates described by authors [10]. Briefly, inoculums of bacteria and fungi were prepared, and a swab of each inoculum was cultured onto Mueller-Hinton II agar plates. Then, 50 mL of the plant extracts previously prepared at a concentration of 100 mg/ml in DMSO and filtered using 0.4 µm mullipore membranes were transferred in each of 16 wells of about 6 mm dug in the agar plates. DMSO solution was used as the negative control. The positive control was conventional vancomycin (30 µg) antibiotic discs for Gram-positive cocci, imipenem (10 µg), and colistin (10 µg) discs for Gram-negative bacilli. The different Petri dishes were left at room temperature for 1 h for pre-diffusion and then incubated at 37˚C for 18 h. Each test was conducted three times for quality control purposes. The zones of inhibition were measured and compared to the positive control antibiotics.

2.7.2. Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC)

The determination of the MIC was performed according to the microwell methodology described by the authors [10]. Different successive dilutions of 180 µl of the extract at initial concentrations of 50 mg/mL prepared in Mueller Hinton broth were distributed in the wells. Then, 20 µl of a 10% dilution of a suspension of 0.5 McFarland strains in Mueller Hinton broth were distributed in all wells. On each plate, bacterial suspension + Mueller Hinton broth served as a positive control, and the negative control was DMSO + Mueller Hinton broth. The plates were then stirred for 5 min and placed in an oven at 37˚C for 18 h. After that, 40 µl of a solution of 0.2% piodonitrotetrazolium (INT) prepared in distilled water was added to each well. After 20 min in the dark, the presence of a red color in a well indicates the presence of viable bacteria. The MIC is the first concentration for which viable bacteria are present. Wells that did not show a red color are seeded on Mueller Hinton agar. CMB is the first concentration for which there is a presence of surviving bacteria.

2.8. HPLC Assay of Phenolic Compounds

Quantitative and qualitative analysis of phenolic compounds in plant extract was achieved through the CECIL Wagtech HPLC EN 91-500 equipped with a C18 120 Å column (4.6 mm × 100 mm, 5 µ), Acclaim™ for chromatographic analysis. The extracts were solubilized in methanol at 1 mg/ml and filtered using a 0.22 µm Millipore filter. Detection was performed with a variable wavelength detector (200, 254, 272, and 365 nm), UV-Visible Adept CE 4201. A binary system made up of methanol (1% phosphoric acid) was used as follows: 0 - 20 min, 20% - 50% B; 20 - 25 min, 50% - 70% B; 25 - 30 min, 70% - 80% B; 30 to 35 min, 80% - 20% B; 35 - 50 min, 20% B. The flow rate was 1 ml/min, and the injection volume was 20 mL. The content and qualitative analysis of phenolic compounds in fractions were achieved by comparing their retention times and UV-Vis spectra with those of standard phenolic compounds [9].

3. Results and Discussion

3.1. Phenolic Content of the Extracts

The total phenolic and total flavonoid contents of the extracts are shown in Table 1. Analysis of the results shows that all three extracts have high contents of total phenolic and flavonoid compounds. The ethanolic extract has the highest content of total phenolic compounds with a value of 109.678 ± 0.757 µg GAE/mg DE, while the decoction has the lowest content with a value of 62.178 ± 1.767 µg GAE/mg DE. As for the flavonoid content, it is rather the decoction that presents the best content with a value of 150.538 ± 2.020 µg QE/mg DE. The low content of total phenolic compounds in the decoction compared to other extracts could be explained by the destructive effect of heat on some phenolic compounds [11]. The authors [12] confirmed a high content of total phenolic compounds and flavonoids in the ethanolic extract of the stem bark of *M. paniculata*, *i.e.* 70.81 ± 0.31 mg GAE/g extract and 101.94 ± 0.73 mg QE/g extract, respectively.

Table 1. Total phenolic compound and total flavonoid content of the extracts.

Contents	Extracts		
	Aqueous	Decocted	Ethanolic
Total Phenols (µg GAE/mg DE)	83.428 ± 1.010	62.178 ± 1.767	109.678 ± 0.757
Flavonoids (µg QE/mg DE)	83.490 ± 1.243	150.538 ± 2.020	96.900 ± 99.098

3.2. Antioxidant Activity

Antiradical activity of the extracts of *M. Paniculata,* as well as that of the standards by the DPPH method, is presented in Table 2. Only the aqueous extracts and the decoction were able to inhibit more than 50% of the free radicals of DPPH with respective IC_{50} of 0.216 ± 0.022 and 0.181 ± 0.015 mg/mL. These results show that the two extracts present an interesting antioxidant activity but remain significantly lower than that of catechin and gallic acid used standards with respective IC_{50} of 0.071 ± 0.012 and 0.028 ± 0.001 mg/mL. The authors [13] also revealed in their study an interesting antioxidant activity of the stem barks of the same plant with an IC_{50} = 1.36 mg/mL. The difference in IC_{50} values would be due to the difference in concentration of DPPH and reaction time used in the protocols.

Table 2. Antiradical activity of *M. paniculata.*

Extracts			Catechin	Gallic Acid
Aqueous	Decocted	Ethanolic		
0.216 ± 0.022	0.181 ± 0.015	NA	0.071 ± 0.012	0.028 ± 0.001

NA: Not Active.

The reducing power of the different plant extracts expressed in gallic acid equivalence and catechin equivalence is recorded in Table 3. Analysis of the results shows

that the aqueous extract is the most active with reducing powers of 74.022 ± 1.152 mg E AG/g of dry extract and 153.009 ± 3.127 mg EC/g dry extract. The decoction, which had presented the best antiradical activity with respect to DPPH, presents a weak power of reduction of Fe(III) ions in Fe(II) with reducing powers of 18.392 ± 2.199 mg E AG/g DE and 5.629 ± 0.853 mg EC/g DE. This latter observation could be explained by the fact that the effectiveness of flavonoids, largely responsible for antioxidant activities in plants, strongly depends on their structures and the extraction solvent [14]. Another reason is that DPPH• presenting steric hindrances due to its structure could negatively influence the access to the radical location by large molecules such as flavonoids, while iron(III) ions, being of reasonable size, can easily react with these phenolic compounds [15]. The good antioxidant observed is of great importance because studies have shown the responsibility of free radicals and Reactive Oxygen Species (ROS) in the inflammatory response. In addition, imbalances in the levels of free radicals, ERO, and antioxidants in saliva could play an important role in the appearance and development of dental caries [16].

Table 3. Reducing power of different plant extracts.

Extracts	Reducing Power	
	(mg E AG/g DE)	(mg EC/g DE)
Aqueous	74.022 ± 1.152	153.009 ± 3.127
Decocted	18.392 ± 2.199	5.629 ± 0.853
Ethanolic	44.392 ± 1.152	74.594 ± 3.127

3.3. Antimicrobial Activity

Table 4 presents the inhibition diameters of the microbial strains tested against the plant extracts and the reference antibiotics. Analysis of the table reveals that none of the strains were sensitive to the aqueous extract and the ethanolic extract of *M. paniculata*. The decoction was rather sensitive to all the microbial strains and sometimes more effective than the reference antibiotics, mostly against *Staphylococcus aureus,* with an inhibition diameter of 23.333 ± 11.666 mm, while vancomycin, which is the reference compound, has an inhibition diameter of 17.666 ± 0.577 mm. The decoction also showed an inhibition power close to that of vancomycin on *Enterococcus faecalis* with inhibition diameters of 15.666 ± 1.154 mm for the plant extract and 17.666 ± 0.577 mm for vancomycin. The authors [5] reported that the methanolic extract of *M. paniculata* stems inhibited multi-resistant strains over a diameter of 22 mm *Acinetobacter baumannii*, involved in infections.

Table 4. Inhibition diameters in mm of strains by extracts and antibiotics.

Mic	Plant extracts			Reference Antibiotics		
	Aq	De	Eth	Vancomycin	Imipenene	Colestine
Sa	0	23.333 ± 1.666	0	17.666 ± 0.577	-	-
Ec	0	11.666 ± 0.577	0	-	26.666 ± 1.154	19.333 ± 0.577
Ef	0	15.666 ± 1.154	0	17.666 ± 0.577	-	-

Continued

Pm	0	13.333 ± 0.577	0	-	26.666 ± 1.154	-
Pa	0	11.000 ± 1.000	0	-	26.666 ± 1.154	19.333 ± 0.577
Ca	0	15.333 ± 0.577	0	-	-	-

Mic: Microorganism; Aq: Aqueous; Eth: Ethanolic; De: Decoction; Sa: *Staphylococcus aureus*; Ec: *Escherichia coli; Ef: Enterococcus fecal*; Pm: *Proteus mirabilis*; Pa: *Pseudomonas aeruginosa*; Ca: *Candida albicans*.

The minimum inhibitory and bactericidal concentrations of the decoction on the microbial strains studied are recorded in **Table 5**. It appears from the analysis of this table that the extract was effective against all strains, in particular on *S. aureus*, *E. faecalis*, *P. mirablis* and *C. albicans* with respective MICs of 3.125 mg/mL, 6.250 mg/ml, 12.500 mg/ml, 6.250 mg/ml and respective MBCs of 6.250 mg/ml, 12.500 mg/ml, 25.000 mg/ml and 12.500 mg/mL. These results could justify the use of this plant for oral care and hygiene. Studies conducted by authors [5] on extracts of the flowers, roots, and stems of *M. paniculata* revealed good antioxidant, antimicrobial, anticancer, and antidiabetic activity of these extracts. The antimicrobial activity of extracts of stem barks of *M. paniculata* carried out on 5 microbial strains, including *S. aureus*, *E. coli*, and *P. aeruginosa,* revealed the potential to inhibit these strains. *In vivo* tests performed on animal models showed that *M. paniculata* stem extracts could be considered as a main candidate drug against infections, including pulmonary infection [5].

Table 5. MIC and MBC of *M. paniculata* decoction.

C (mg/mL)	Microorganisms					
	S. aureus	*E. coli.*	*E. faecalis*	*P. mirablis*	*C. albicans*	*P. aeruginosa*
CMI	3.125	25.000	6.250	12.500	6.250	25.000
CMB	6.250	25.000	12.500	25.000	12.500	25.000

3.4. HPLC Analyse

Many phenolic compounds, including tannic acid, chlorogenic acid, ferulic acid, ellagic acid, and luteolin, were identified in all the extracts of *M. paniculata* through the HPLC assay (**Table 6**). Tannic acid is the major compound identified with contents of 34.289 µg/mg, 39.953 µg/mg, and 111.024 µg/mg, respectively, for the ethanolic extract, the aqueous extract, and the decoction. The tannic acid content of the decoction is much higher than that of the other two extracts. This could explain the antioxidant and antimicrobial activity observed with this extract. Indeed, all the hydroxyl groups in the tannic acid molecule (**Figure 3**) are potential donors of either electrons or hydrogen, which could stabilize free radicals. Many studies have demonstrated that an increase in the number of hydroxyl (-OH) groups significantly increased the antioxidant activity of phenolic acids and their esters [17]-[19].

Table 6. HPLC profile of crude extracts of *M. paniculata.*

Extract	Retention Time	Compound	Percentage	Content (µg/mg)
Ethanol (50%)	2.08	Nd	30.64	Nd
	3.35	Gallic Acid	3.88	0.047
	6.79	Chlorogenic Acid	0.68	0.023
	8.16	Syringic Acid	0.29	0.003
	10.19	Tannic Acid	7.72	34.289
	12.70	Ferulic Acid	0.15	0.004
	13.77	Nd	19.66	Nd
	16.67	Hyperoside	0.07	0.004
	18.71	Ellagic Acid	1.55	0.012
	24.45	Luteonine	4.34	0.029
	26.58	Isorhamnetin	0.98	0.079
	32.35	Nd	11.11	Nd
Aqueous	2.08	Nd	24.23	Nd
	4.35	Nd	5.30	Nd
	6.81	Chlorogenic Acid	3.89	0.359
	10.35	Tannic Acid	3.32	39.953
	12.62	Ferulic Acid	2.33	0.186
	13.87	Nd	5.11	Nd
	18.62	Ellagic Acid	2.21	0.048
	25.21	Nd	22.83	Nd
	27.21	Isorhamnetin	0.08	0.018
	27.42	Nd	10.00	Nd
	27.91	Chrysine	0.25	0.005
Decoction	2.09	Nd	21.58	Nd
	3.46	Gallic Acid	0.01	0.001
	6.13	Nd	10.20	Nd
	7.42	Chlorogenic Acid	2.82	0.405
	9.70	Nd	8.07	Nd
	10.59	Tannic Acid	5.93	111.024
	12.12	Ferulic Acid	7.36	0.915
	14.09	Nd	6.74	Nd
	16.54	Hyperoside	0.01	0.002
	19.08	Ellagic Acid	6.65	0.225
	24.60	Luteolin	11.75	0.335
	27.84	Chrysin	0.44	0.013

Nd: Not determined.

The authors [20] showed that tannic acid exhibited interesting antimicrobial activity on various microorganisms, namely, *Staphyloccocus aureus*, *Salmonella enterica* Typhimurium, *Klebsiella oxytoca*, *Klebsiella aerogenes*, *Enterobacter cloacae*, *E. coli*, *Streptococcus pneumonia*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* with MICs between 53.1 and 425 µg/ml. The authors [21] showed in a literature review that tannins are potential

substitutes for antibiotics in view of the various interesting biological activities they present. The author [22] also reported through a literature review an antiviral activity of tannic acid on the Influenza A virus, the Human Immunodeficiency Virus (HIV) and many others, as well as an antimicrobial activity against microorganisms, such as *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pyogenes*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Yersinia enterocolitica*, and *Listeria innocua*. *Streptococcus pyogenes* is recognized as a pathogen of oral diseases [2]. This further justifies the traditional use of *M. paniculata* for oral care and hygiene. The authors [5] identified mostly compounds such as Murrangatin, sinensetin, isosinensetin, ferulic acid, 5,7-Dimethoxy-8-[(Z)-30-methylbutan-10,30-dienyl]coumarin, agmatine feruloyl, and chlorogenic acid in the stems of *M. paniculata*.

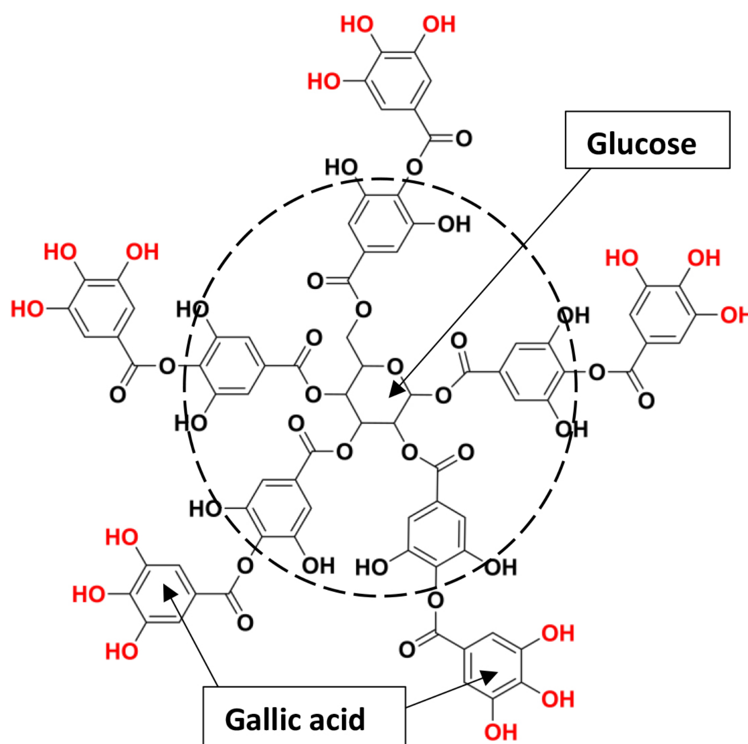

Figure 3. Chemical formula of tannic acid, the main compound identified from *M. paniculata* extracts. Tannic acid is a decapalloylglucose characterized by a central glucose moiety and two gallic acid moieties, exhibits the esterification of all five hydroxyl groups present on the glucose molecule. The shaded circle underscores the fundamental architecture of the pentagalloylglucose and tannic acid [23].

4. Conclusion

This work has highlighted the chemical profile, the antiradical activity and the antimicrobial activity of *Murraya paniculata* stem extracts. *Murraya paniculata* extracts have been found to be rich in phenolic compounds. The antioxidant and antimicrobial activity of extracts from this plant, particularly that of the decoction, was revealed to be interesting against several microbial strains involved in the development of oral infections. The results obtained justify the use of this plant for oral

care and hygiene.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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