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In Vitro Antioxidant and Antimicrobial Properties of *Murraya Paniculata* L. Extracts as well as Identification of Their Active Secondary Metabolites by HPLC-ESI-MS

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ABSTRACT

The aim of this study was to determine the total phenolic content, antioxidant and antimicrobial properties of the methanolic extract of Murraya paniculata leaves and its derived fractions as well as characterization the active chemical composition of these extracts by High-Performance Liquid Chromatography/Electrospray Ionization Tandem Mass Spectrometry (HPLC-ESI-MS). The antioxidant activity was evaluated by using two methods; DPPH free radical scavenging activity and total antioxidant capacity. The identification of certain chemical constituents of M. paniculata methanolic extract, ethyl acetate and butanolic fractions using HPLC-ESI-MS were carried out. The obtained results revealed that the ethyl acetate fraction contains highest contents of phenolic content (607.88 ± 6.87 mg gallic acid eq./g ext.) and flavonoids content (41.53 ± 0.63 mg rutin eq./g ext.). Also, ethyl acetate fraction exhibited the most potent antioxidant and antimicrobial activities.

Keywords: Antimicrobial, Antioxidants, Phenolic compounds, HPLC-ESI-MS, *Murraya paniculata* L.

INTRODUCTION

Infectious diseases are the world's leading cause of premature deaths and led to killing many people every day. Morbidity and Mortality due to diarrhea are continuous to be a major problem in many developing countries, especially amongst children due to infections with a variety of bacterial agents [1]. Although the antibiotics have been proved to be an excellent antimicrobial agent the resistant to these antibiotics is an increasing public health problem [2]. In addition, in developing countries, the synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often with adulterations and side effects [1]. Therefore, there is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases [2]. Medicinal plants have played an important role in the healthcare system since the dawn of human civilization and remain one of the major sources of drugs in modern and in traditional medicine because it contains different bioactive compounds [3]. These bioactive compounds such as terpenoids (essential oils and carotenoids), vitamins, and phenolic compounds have a medical important such as antimicrobial and antioxidants [4].

The *Murraya* (family rutaceae) is genus made up of 14 species. *Murraya paniculata* L. Jack is a small tropical evergreen shrub, native to the tropical and subtropical parts of the world [5]. For many years, *M. paniculata* has been used as a medicinal plant and used in traditional medicine [6,7]. Previous reports have shown that the extracts from bark and leaf of this plant had antioxidant, anti-inflammatory, antidiabetic, antimalarial, antidiarrheal, antifungal and antibacterial activities [8-10]. *M. paniculata* has been investigated for its bioactive ingredients such as coumarins, indole, alkaloids, phenolic acids, flavonoids, and terpenoids. These compounds serve as the scientific evidence for the traditional usage of *M. paniculata* [11,12].

The aims of this study were to evaluate *in vitro* the antioxidant and antimicrobial properties of the methanolic of *M. paniculata* and certain fractions derived from it as well as identify their chemical composition using High-Performance Liquid Chromatography/Electrospray Ionization Tandem Mass Spectrometry (HPLC-ESI-MS).

MATERIAL AND METHODS

Reagents and standards

DPPH (1, 1-diphenyl-2-picrylhydrazyl) and Folin- Ciocalteu's reagent were purchased from Sigma -Aldrich (Steinheim, Germany). Ammonium molybdate, sodium carbonate and aluminum chloride were purchased from Merck (Darmstadt, Germany). Gallic acid, rutin and ascorbic acid

were purchased from Sigma-Aldrich (St. Louis, USA). All solvents used for HPLC analysis and biological experiments are analytical grade.

Plant materials

The leaves of *M. paniculata* were collected from Giza Governorate, Egypt. The plant under study was identified by Prof. Dr. Rim Samir Hamdy Professor of Plant Taxonomy, Faculty of Science, Cairo University. The voucher specimen was stored in Medicinal Chemistry laboratory, Theodor Bilharz Research Institute, Giza, Egypt. The leaves of the plant were washed with distilled water and air dried at room temperature. The dried leaves were ground using an electric mill and the dry powers of the leaves were stored in dried vials.

Preparation of plant extract

The dried leaves powder of *M. paniculata* (700 g) was extracted several times with 80% methanol. The methanolic (MeOH) extract was filtered and concentrated by the rotatory evaporator (BUCHI, Switzerland) until drying then kept in a desiccator. The methanolic was defatted using petroleum ether. The defatted methanol extract of leaves of the plant was suspended in water and successively fractioned with different solvents chloroform ($CHCl_3$), ethyl acetate (EtOAc) and n-butanol (BuOH) respectively. Each fraction was concentrated under vacuum using rotatory evaporator till dryness and kept in dry conditions.

Total phenolic content

The phenolic content of the defatted methanolic extract of the leaves of *M. paniculata* and its fractions were determined using spectrophotometric method [13]. The reaction mixture was prepared by mixing 0.5 ml of sample soluble in MeOH (250 mg/ml), 2.5 ml of Folin-Ciocalteus reagent (10 %) dissolved in water and 2.5 ml $NaHCO_3$ (7.5%). Blank was prepared containing 0.5 ml methanol, 2.5 ml of Folin-Ciocalteus reagent (10 %) and 2.5 ml $NaHCO_3$ (7.5%). The mixture was shaken and incubated for 45 min at 45°C. The absorbance was measured at 760 nm against the blank sample and gallic acid used as a standard. All determinations were carried out in triplicate. The total content of phenolics was expressed in terms of gallic acid equivalent per gram dry weight extract (mg GAE/g of extract).

Total flavonoid content

The content of flavonoids of the defatted methanolic extract of the leaves of *M. paniculata* and the fractions were determined using a colorimetric assay [14]. 0.5 ml of extract was mixed with 2 ml distilled water and 150 µl of $NaNO_2$ (5%) for 6 min, then 150 µl of $AlCl_3$ (10%) was added and allow to stand 5 min then 2 ml of NaOH (4%) was added and adjusted to 5 ml with 200 µl distilled water. The mixture was incubated at room temperature for 30 min. The absorbance was measured at 510 nm against blank sample and rutin used as a standard. Estimation of flavonoid content was carried out in triplicate. The total flavonoid content was estimated as mg rutin equivalents per gram extract (mg RE/g extract).

***In vitro* antioxidant assays**

DPPH radical scavenging activity assay

DPPH (2, 2'-diphenyl-1-picrylhydrazyl) free radical scavenging method was carried out [15]. 2 ml of extract concentration solution was mixed with 2 ml of DPPH in methanol (0.1 m mol/l). The control takes in 2 ml of MeOH and 2 ml DPPH solution without the extract. The mixtures were shaken well and kept in dark for 30 min at 37°C. The absorbance was measured at 517 nm against the blank. Ascorbic acid was used as standards and the experiment was carried out in triplicate. The DPPH scavenging activity expressed as SC_{50} (Concentration of sample required to scavenge 50% of DPPH radicals compared with that of ascorbic acid). The lower SC_{50} value is an indication of higher scavenging activity. The antioxidant activity of plant extracts was calculated from the following equation:

$$\text{Scavenging activity \%} = [(\text{control absorbance} - \text{sample absorbance})/(\text{control absorbance})] \times 100$$

Total antioxidant capacity (Phosphomolybdate assay)

The total antioxidant capacity assay was carried out [16]. An aliquot of 0.5 ml of sample (200 µg/ml) solution get mixed with 5 ml of reagent solution (0.6 M sulphuric acid, 28 mM disodium hydrogen phosphate and 4 mM ammonium molybdate). Blank was prepared to contain 5 ml of the reagent solution and the appropriate volume of the same solvent used for the samples. The tubes were coverd and incubated at 95°C for 90 min. After the samples had cooled down to room temperature, the absorbance of the mixture was measured against the blank at 695 nm. The experiment was repeated for three times. The antioxidant activity of the extracts was expressed as the number of equivalents of mg ascorbic acid per gram extract (mg AAE/g extract).

Determination of antimicrobial activity

Test microorganisms

The pathogenic microbes used in this study were gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and gram positive bacteria (*Bacillis subtilis* and *streptococcus pneumonia*). In addition to four fungal strains which are *Aspergillus fumigates*, *Syncephalastrum*, *Candida albicans* and *Geotrichum candida*. They were obtained from the regional center for Mycology and Biotechnology Unit, Al-Azahar University, Cairo, Egypt.

Disc diffusion assay

The antimicrobial activity assay is based on the clear zone formed around the disk. Partial inhibition was indicated by a semi-clear zone and complete inhibition by a clear zone. The sterilized media was poured onto sterilized Petri dishes (20 ml, each Petri dish) and be allowed to solubility. Wells of 6 mm diameter were made in the solidified media with the help of sterile borer. A sterile swab was used to evenly distribute microbial suspension over the surface of solidified media and solutions of the tested samples were added to each well with the help of micropipette. The plates were incubated at 37°C for 24 h in case of antibacterial activity and 48 h at 25°C for antifungal activity. Ampicillin and gentamycin were used as antibacterial standards and ampholericin was used as an antifungal standard. This experiment carried out in triplicate and zones of inhibition measured in mm scale. This test was carried out at Antimicrobial activity unit Faculty of Science, Al-Azhar University, Cairo, Egypt.

HPLC-ESI-MS analysis

HPLC-ESI-MS analysis was performed using HPLC instrument (Waters Alliance 2695) and mass spectrometry (Waters 3100). The mobile

phase was filtered carefully by using 0.45 μm filter membrane disc and degassed by sonication before analysis. 20 µl of samples volume of MeOH extract and EtOAc fraction of *M. paniculata* were injected into the HPLC instrument contain reversed phase C-18 column (Phenomenex 250 mm, 5 µm particle size). Mobile phase elution was made with the flow rate of 0.4 ml/min using gradient linear mobile phase containing two solvents: solvent A is H_2O acidified with 0.1% formic acid and eluent B is CH_3CN/MeOH (1: 1, v/v) acidified with 0.1% formic acid. The mobile phase elution was carried out by the following gradient: 5% B, 0-5 min; 5%-10% B, 5-10 min; 10%-50% B, 10-55 min; 50%-95% B, 55-65 min; 5% B, 65-70 min. The parameters for analysis were performed by using negative ion mode as follows: source temperature 150°C, capillary voltage 3 kV, cone voltage 50 eV, cone gas flow 50 L/h, desolvation temperature 350°C, and desolvation gas flow 600 L/h. Mass spectra were detected in the ESI negative ion mode between *m/z* 50-1000. The peaks and spectra were processed using the Maslynx 4.1 software and tentatively characterized by comparing its mass spectrum and retention time (t_R) with literature.

Statistical analysis

The data analysis was carried out using SPSS (13) software for evaluation of the IC_{50}. The data were presented as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Total phenolic and flavonoid contents

It is well-known that plant phenolic compounds are highly effective free radical scavengers and have high antioxidant potential. In this study, the total phenolic and flavonoid contents of the methanolic extract of *M. paniculata* and certain fractions derived from this extract were evaluated as shown in Table 1. EtOAc fraction exhibited the highest total phenolic and flavonoid contents (607.88 ± 6.87 mg GAE/g ext. and 41.53 ± 0.63 mg RE/g ext., respectively), followed by BuOH fraction (405.86 ± 4.17 mg GAE/g ext. and 39.9 ± 0.12 mg RE/g ext., respectively), the defatted MeOH extract (370.37 ± 8.34.17 mg GAE /g ext. and 35.92 ± 0.11 mg RE/g ext., respectively), $CHCl_3$ fraction (344.89 ± 8.78 mg GAE /g ext. and 30.56 ± 0.50 mg RE/g ext., respectively). It was documented that the secondary metabolites isolated from medicinal plants are very reactive in the neutralization of free radicals by donating the odd electron or hydrogen atom due to the presence of hydroxyl groups on their chemical structures [16].

Antioxidant properties

DPPH scavenging activity

The model of scavenging the stable DPPH radical is a widely used method to evaluate antioxidant activities in a relatively short time compared with other methods [17]. The effect of antioxidants on DPPH radical scavenging was thought to result from their hydrogen donating ability. DPPH is a stable free radical which accepts an electron or hydrogen radical to become a stable molecule. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm induced by antioxidants. It is visually noticeable as a discoloration from the purple color to yellow color. The results in Table 1 showed that the EtOAc fraction had the highest antioxidant activity (SC_{50}=87.58 ± 1.06 µg/ml) > BuOH fraction (SC_{50}=162.53 ± 1.27 µg/ml) > the defatted MeOH extract (SC_{50}=206.05 ± 1.09 µg/ml) > $CHCl_3$ fraction (SC_{50}=259.12 ± 3.00 µg/ml), respectively.

Total antioxidant capacity

The Total antioxidant capacity or phosphomolybdenum assay is a routinely applied method to evaluate the total antioxidant capacity of plant extracts. It is based on the reduction of Mo (IV) to Mo (V) generating the green phosphate/Mo (V) by the antioxidant molecules of the extract. The subsequent reaction is the formation of a green phosphate/Mo (V) complex, at acidic pH, which absorbs at 695 nm. The total antioxidant capacity of the tested extract was expressed as ascorbic acid equivalents. The obtained results in Table 1 showed that the EtOAc fraction showed the more potent antioxidant activity (149.42 ± 3.33 mg ascorbic acid eq./g ext.). The other extracts arranged in the following order BuOH fraction > the defatted MeOH extract > $CHCl_3$ fraction. These results indicated that the total antioxidant activity of the extract is increased by increasing of the phenolic contents and there is a positive correlation between them.

Table 1: Total phenolic content, flavonoid content, DPPH scavenging activity, and total antioxidant capacity of various *Murraya paniculata* extracts

Extract	Total phenols (mg gallic acid eq./g ext.)	Total flavonoids (mg rutin eq. /g ext.)	DPPH scavenging activity SC_{50} (µg/ml)	Total antioxidant capacity (mg ascorbic acid eq. / g ext.)
MeOH extract	370.37 ± 8.34	35.92 ± 0.11	206.05 ± 1.09	100.32 ± 0.61
$CHCl_3$ fraction	344.89 ± 8.78	30.56 ± 0.5	259.12 ± 3.00	43.26 ± 2.03
EtOAc fraction	607.88 ± 6.87	41.53 ± 0.63	87.58 ± 1.06	149.42 ± 3.33
BuOH fraction	405.86 ± 4.17	39.9 ± 0.12	162.53 ± 1.27	113.24 ± 2.15

Values are expressed as mean of triplicate determinations ± standard deviation

Antimicrobial activity

The antimicrobial activities of the tested plant extracts were measured by disk assay procedure against indicator microorganisms such as gram positive and negative bacteria as well as four fungal strains. The antimicrobial activities were expressed as inhibition diameter zones in millimeters (mm). The obtained results in Table 2 showed that, EtOAc fraction of *M. paniculata* exhibited the highest inhibitory effects against *S. pneumoniae* (20.6 ± 0.44 mm), *E. coli* (18.6 ± 0.46 mm), *B. subtilis* (23.4 ± 0.67 mm), as well as against all fungi strains; *A. fumigatus* (20.3 ± 0.46 mm), *S. racemosum* (21.6 ± 0.34 mm), *G. candidum* (23.4 ± 0.58 mm). It was reported that *M. paniculata* leaves extracts had antimicrobial and antioxidant activities [12]. The antioxidant and antimicrobial results exhibited that, the methanolic extract and ethyl acetate fraction of *M. paniculata* were the most active.

Table 2: Diameter of the inhibition zones of bacterial and fungal strains growth of *Murraya paniculata* extracts

Sample or antibiotic	Microorganism	
	Bacterial	*Fungal*

	S. pneumonia	*E. coli*	*B. subtilis*	*P. aeruginosa*	*A. fumigatus*	*Syncephal- astrum*	*G. candida*	*C. albicans*
MeOH extract	20.6 ± 0.63	16.9 ± 0.58	22.4 ± 0.44	NA	17.8 ± 0.63	18.9 ± 0.44	20.3 ± 0.23	NA
$CHCl_3$ fraction	14.6 ± 0.43	13.7 ± 0.25	16.2 ± 0.53	NA	12.6 ± 0.58	13.6 ± 0.25	15.2 ± 0.38	NA
EtOAc fraction	20.6 ± 0.44	18.6 ± 0.46	23.4 ± 0.67	NA	20.3 ± 0.25	21.6 ± 0.34	23.4 ± 0.58	NA
BuOH fraction	16.9 ± 0.44	14.9 ± 0.44	19.3 ± 0.25	NA	15.6 ± 0.44	16.2 ± 0.58	17.9 ± 0.37	NA
Ampicillin	23.8 ± 0.3	NA	32.4 ± 0.3	NA	NA	NA	NA	NA
Gentamycin	NA	19.9 ± 0.30	NA	NA	NA	NA	NA	NA
Ampholericin	NA	NA	NA	NA	23.7 ± 0.10	NA	NA	NA

NA: not active

HPLC-ESI-MS analysis of *M. paniculata* extracts

HPLC–ESI-MS have been becoming a very powerful approach for the rapid identification of constituents in botanical extracts. In the present study, the HPLC-ESI-MS analysis of *M. paniculata* methanolic extract and its derived fractions (EtOAc and BuOH) in negative ion mode lead to a different chemical composition as shown in Tables 3-5 and Figures 1 and 2.

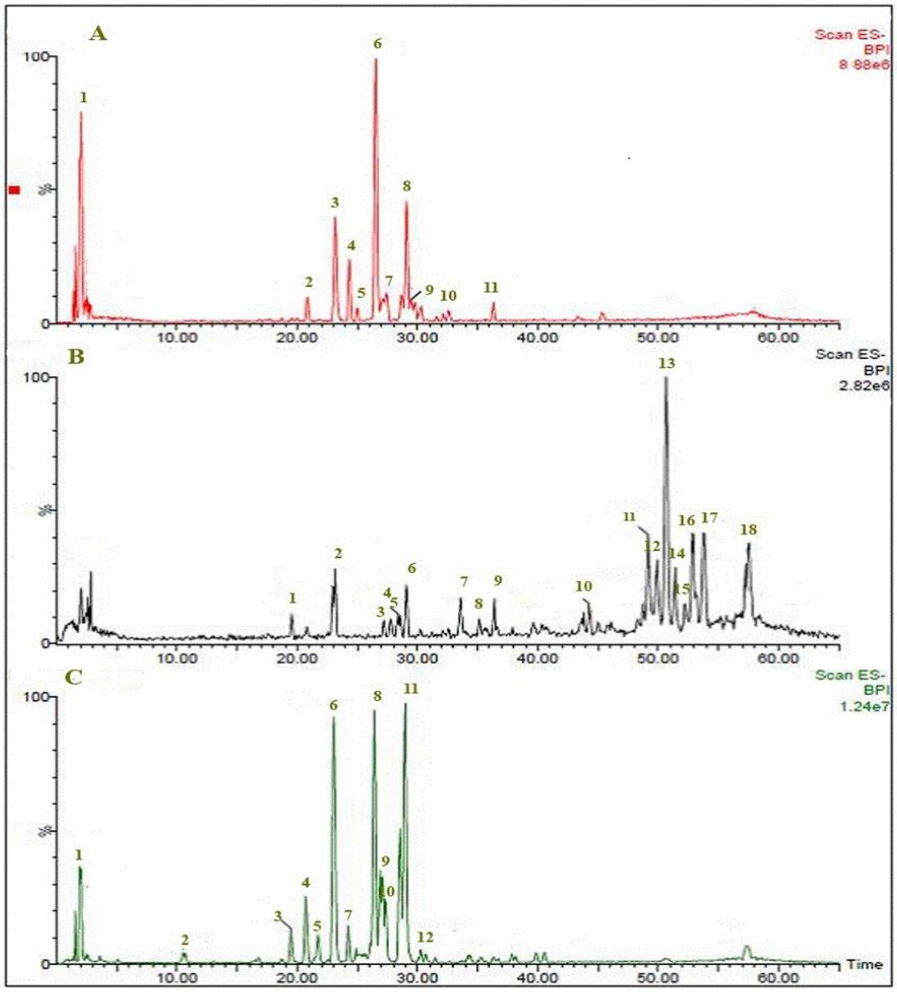

Figure 1: HPLC-ESI-MS total ion current chromatogram of *Murraya paniculata* defatted MeOH extract (A), EtOAc fraction (B), and BuOH fraction (C) at the negative ion mode

Flavonoids

Peak 4 in MeOH extract and peak 7 in BuOH fraction (t_R=24.29 min) exhibited molecular ion peak at *m/z* 609 $[M-H]^-$ and main two fragments at *m/z* 489 $[M-H-120]^-$ revealed to liberation of C-glucoside unit and another signal at *m/z* 369 $[M-H-120]^-$ revealed the presence of two C-glucoside units. Therefore, this compound was tentatively identified as luteolin-6, 8- di-C-glucoside (Lucenin-2). Peaks 5 and 6 in MeOH extract (t_R=25.05 and 26.55 min) and peak 8 in BuOH fraction (t_R=26.38 min) showed precursor ion peak at *m/z* 593 $[M-H]^-$ and fragment peaks at *m/z* 473 $[M-H-120]^-$ and *m/z* 353 $[M-H-120]^-$ revealed to the liberation of two C-glucoside units. Therefore, this compound was tentatively identified as apigenin 6, 8-di-*C*-glucoside (Vicenin-2) [18,19]. Peak 7 in MeOH extract (t_R=27.38 min) exhibited deprotonated molecule at *m/z* 623 $[M-H]^-$ and two fragments at *m/z* 503 $[M-H-120]^-$ refers to the loss of C-glucoside unit and *m/z* 383 $[M-H-120]^-$ revealed to the loss of two C-glucoside units. Therefore, this compound was characterized as diosmetin-6, 8- di-C-glucoside [18,20]. Peak 9 in MeOH extract (t_R=30.22 min) gave molecular ion peak at *m/z* 609 $[M-H]^-$ and other fragments at *m/z* 447 $[M-H- 162]^-$, 357 and 327 (100%). So, this compound was tentatively identified as orientin-*O*- glucoside. Peak 10 in MeOH extract (t_R=32.64 min) showed deprotonated molecule at *m/z* 737 $[M-H]^-$ and other

fragments at *m/z* 593 [M-H- 144]$^-$ due to the loss of rhamnose unit, *m/z* 431 [M-H-144-162]$^-$ correspond to losing of another glucose unit, and *m/z* 311(100%). So, this compound was tentatively identified as vitexin-*O*- rhamnosyl-glucoside [19-21]. Peak 11 in MeOH extract (t_R=36.40 min) showed deprotonated molecule at *m/z* 507 [M-H]$^-$ and other fragments at *m/z* 344 (100%) [M-H- 163]$^-$ refers to loss of glucoside unit and another fragment at *m/z* 301, therefore, this compound was tentatively identified as syringetin-3-*O*- glucoside [22,23].

Table 3: Tentative identification of phenolic compounds of *Murraya paniculata* defatted MeOH extract by HPLC-ESI-MS

Peak No.	$t_{R\ (Min)}$	MW	[M-H]$^{-m/z}$	Mass fragments	Identified compounds
1	2.08	192	191	173, 127, 85	Quinic acid
2	20.87	368	367	193 (100%), 134	3- Feruloylquinic acid I
3	23.13	368	367	193 (100%), 134	3- Feruloylquinic acid II
4	24.29	610	609	489, 369	Luteolin-6,8-di-C-glucoside (Lucenin-2)
5	25.05	594	593	473, 383, 353	Apigenin-6,8-C-di-glucoside (Vicenin-2) I
6	26.55	594	593	473, 383, 353	Apigenin-6,8-C-di-glucoside (Vicenin-2) II
7	27.38	624	623	503, 383	Diosmetin-6, 8- di-C-glucoside
8	29.05	368	367	193, 173 (100%), 134, 93	4- Feruloylquinic acid
9	30.22	610	609	447, 357, 327 (100%), 295, 147	Orientin-*O*-glucoside
10	32.64	738	737	593, 431, 311 (100%), 283, 147	Vitexin-*O*-rhamnosyl-glucoside
11	36.4	508	507	344 (100%), 301	Syringetin-3-*O*-glucoside

(t_R): retention time; (MW): Molecular weight

Phenolic acids

Peak 1 in MeOH extract and peak 1 in BuOH fraction (t_R=2.08 min) exhibited precursor ion at *m/z* 191 [M-H]$^-$ and other fragments *m/z* 173, 127 and 85 characteristics for quinic acid [24]. Peaks 2 and 3 in MeOH extract (t_R=20.87 and 23.13 min), peak 8 in EtOAc fraction (t_R=35.23 min) and peaks 4, 5 and 6 in BuOH fraction (t_R=20.70, 21.71 and 23.04 min, respectively) showed deprotonated molecule at *m/z* 367 [M-H]$^-$, base peak *m/z* 193 (100%) [M-H-174]$^-$ correspond to lose of quinic acid unit and other fragment *m/z* 134. Therefore, these compounds were tentatively identified as 3-feruloylquinic acid [25,26]. Peak 8 in MeOH extract (t_R=29.05 min), peak 3 and 6 in EtOAc fraction (t_R=27.13 and 29.14 min), peaks 9, 10 and 11 in BuOH fraction (t_R=27.13, 28.55 and 28.97 min, respectively) showed deprotonated molecule at *m/z* 367 [M-H]$^-$, m/z 193 [M-H-quinic acid]$^-$, base peak *m/z* 173 (100%) other fragment *m/z* 134 and 93. S, these compounds were characterized as 4-feruloylquinic acid [25-27]. Peak 2 in EtOAc fraction (t_R=23.96 min) and peak 12 in BuOH fraction (t_R=30.31 min) exhibited a precursor ion at *m/z* 389 [M-H]$^-$ and other fragments *m/z* 193[M-H-196]$^-$ due to losing of sinapoyl unit, and 134 (100%), therefore this compound was characterized as sinapoyl-ferulic acid. Peak 7 in EtOAc fraction (t_R=33.56 min) exhibited precursor ion at *m/z* 431 [M-H]$^-$ and other fragments *m/z* 311, 175 (100%) and 147 characteristics for cinnamic acid derivatives [28]. Peak 11 in EtOAc fraction (t_R=49.18 min) had deprotonated molecule at *m/z* 369 [M-H]$^-$ and other fragments *m/z* 193 (100%) [M-H-176]$^-$ reflect to lose of glucuronic acid unit. Therefore, this compound was identified as Ferulic acid-3-*O*- glucuronide [29]. Peaks 12 and 15 in EtOAc fraction (t_R=49.93 and 52.27 min, respectively) showed deprotonated molecule at *m/z* 349 [M-H]$^-$, m/z 193 and base peak *m/z* 175 (100%). So, these compounds were characterized as 3-Feruloyl-1, 5-quinic acid lactone. Peaks 13, 14, 16 and 17 in EtOAc fraction (t_R=50.68, 51.43, 52.85 and 53.85 min, respectively) showed deprotonated molecule at *m/z* 367 [M-H]$^-$, *m/z* 349 [M-H-H_2O] and base peak *m/z* 193 (100%). So, these compounds were tentatively identified as 1-Feruloyl-quinic acid [29,27].

Table 4: Tentative identification phenolic compounds of *Murraya paniculata* EtOAc fraction by HPLC-ESI-MS

Peak No.	t_R (Min)	MW	[M-H]$^{-m/z}$	Mass fragments	Identified compounds
1	19.53	400	399	191, 176(100%)	Scopoletin-*O*-glucoside (Format adduct)
2	23.96	390	389	193, 134 (100%), 62	Sinapoyl-ferulic acid
3	27.13	368	367	193, 173 (100%), 134, 93	4- Feruloylquinic acid I
4	27.8	206	205	161, 133 (100%), 62	Scoparone
5	28.47	390	389	276, 191, 176, 119 (100%), 62	Unidentified
6	29.14	368	367	193, 173 (100%), 134, 93	4- Feruloylquinic acid II
7	33.56	432	431	311, 263, 175 (100%), 147	Cinnamic acid derivatives
8	35.23	368	367	193 (100%), 134	3- Feruloylquinic acid
9	36.4	418	417	344, 193	Unidentified
10	44.33	594	593	344, 243, 205	Unidentified
11	49.18	370	369	193 (100%), 93	Ferulic acid-3-*O*- glucuronide
12	49.93	350	349	193, 175 (100%)	3-Feruloyl-1,5-quinic acid lactone I
13	50.68	368	367	193(100%), 173	1-Feruloylquinic acid I
14	51.43	368	367	193(100%), 173	1-Feruloylquinic acid II
15	52.27	350	349	193, 175 (100%)	3-Feruloyl-1, 5-quinic acid lacton II
16	52.85	368	367	193(100%), 173	1-Feruloylquinic acid III
17	53.85	368	367	193(100%), 173	1-Feruloylquinic acid IV
18	57.28	759	758	555(100%), 469	Unidentified

(t_R): retention time; (MW): Molecular weight

Coumarins

Peak 1 in EtOAc fraction (t_R=19.53 min) and peak 3 in BuOH fraction (t_R=19.45 min) exhibited precursor ion at *m/z* 399 [M+HCOOH-H]$^-$, fragment ion at *m/z* 191 suffered a loss of format- ion adduct and hexoside unit and base peak at *m/z* 176 (100%). Therefore, this compound was characterized as scopoletin-*O*-hexoside [30,31]. Peak 4 in EtOAc fraction (t_R=27.80 min) showed molecular ion peak at *m/z* 205 [M-H]$^-$, and

other fragments at *m/z* 161 and 133 (100%) characteristic for fragmentation of scoparone [32].

Table 5: Tentative identification of phenolic compounds of *Murraya paniculata* BuOH fraction by HPLC-ESI-MS

Peak No.	t_R (Min)	MW	$[M-H]^{-}$ m/z	Mass fragments	Identified compounds
1	2.08	192	191	173, 127, 85	Quinic acid
2	10.6	484	483	344, 153	Unidentified
3	19.45	400	399	191, 176(100%)	Scopoletin-*O*-hexoside (Format adduct)
4	20.7	368	367	193 (100%), 134	3- Feruloylquinic acid I
5	21.71	368	367	193 (100%), 134	3- Feruloylquinic acid II
6	23.04	368	367	193 (100%), 134	3- Feruloylquinic acid III
7	24.29	610	609	489, 369	Luteolin-6, 8- di-C-glucoside (Lucenin-2)
8	26.38	594	593	473, 383, 353	Vicenin-2
9	27.13	368	367	193, 173 (100%), 134, 93	4- Feruloylquinic acid I
10	28.55	368	367	193, 173 (100%), 134, 93	4- Feruloylquinic acid I
11	28.97	368	367	193, 173 (100%), 134, 93	4- Feruloylquinic acid I
12	30.31	390	389	193, 134 (100%), 62	Sinapoylferulic acid

(t_R): retention time; (MW): Molecular weight

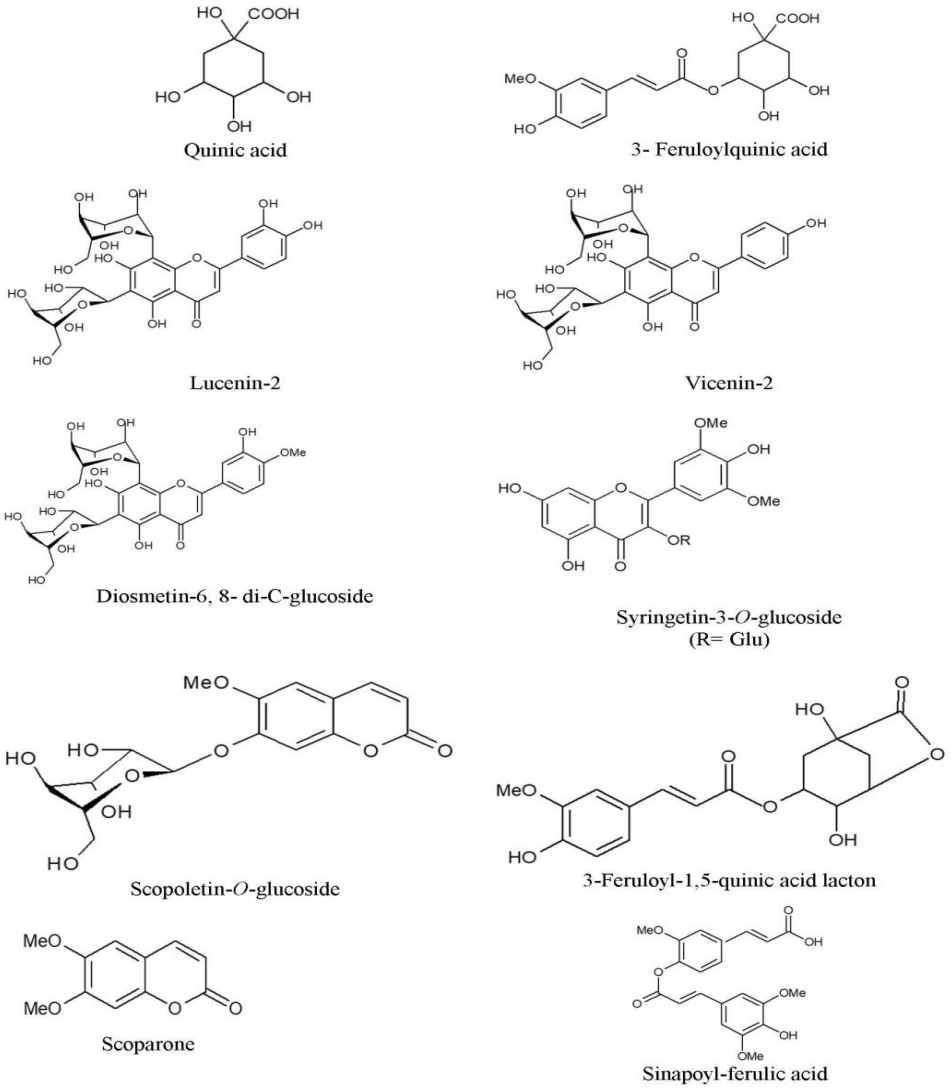

Figure 2: Chemical structures of some of the identified compounds of *Murraya paniculata* found in the defatted MeOH extract, EtOAc and BuOH fractions

CONCLUSION

As a conclusion, EtOAc fraction derived from the defatted MeOH extract of *M. paniculata* exhibited significant the highest content of antioxidants compared to the tested extracts. The antimicrobial test showed that the ethyl acetate and methanol extract had similar results against S. pneumonia. On the other hand, the results for other bacteria and fungi showed that the ethyl acetate extract has the lead compared to other extracts. HPLC-ESI-MS analysis of MeOH extract and its derived fractions (EtOAc and BuOH) demonstrated the presence of three major bioactive compounds, *viz*. flavonoids, phenolic acids, and coumarins. These bioactive secondary metabolites are responsible for the different bioactivities of *M. paniculata* extracts. Therefore, it would suggest that *M. paniculata* extracts may be used for the treatment of microbial infections.

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