Research Paper

Phytochemical Characterization and Evaluation of Crude Extract from *Ruellia patula* Leaves for Antimicrobial, Antioxidant and *In Vitro* Anti-Inflammatory Activities

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Ruellia patula **belongs to the family Acanthaceae. The leaves of this plant possess tremendous medicinal values like treating wounds, eyesores, gonorrhea, syphilis and renal infections. Phytochemicals extracted using four different solvents namely hexane, acetone, methanol and water by maceration technique were qualitatively analysed by chemical tests. Total phenolic content was estimated as 142.94±1.01, 104.41±7.06, 14.37 mg Gallic Acid Equivalent/g of extract in acetone, methanol, and aqueous extracts respectively. Total flavonoid content was estimated as 37.49±1.83, 26.73±10.65 mg Quercetin Equivalent/g of extract in acetone and methanol extracts respectively. The antimicrobial activity of plant extracts was determined by agar well diffusion assay and minimum inhibitory concentration test, acetone extract showed better results for both assays. The minimum inhibitory concentration of acetone extract against five different organisms were determined as,** ***Bacillus subtilis*****-202 mg/ml,** ***Escherichia coli*****-208 mg/ml,** ***Staphylococcus aureus*****-208 mg/ml,** ***Pseudomonas aeruginosa*****-203 mg/ml and for** ***Proteus mirabilis*****-200 mg/ml. Antioxidant activity of extracts was evaluated by 2,2-diphenyl-1-picryl-hydrazyl-hydrate assay, and IC_{50} values of methanol and acetone extracts were estimated as 146.45 and 153.81 µg/ml respectively. Anti-inflammatory activity of extracts estimated using protein denaturation inhibition assay resulted in better anti-inflammatory property associated with acetone extract. Based on the results, the acetone and methanol extracts showed better activities at a lower concentration, further gas chromatography-mass spectrometry analysis of these extracts was carried out and biological activity of components were determined from literature survey.**

Keywords: ***Ruellia patula*****, phytochemical compounds, gas chromatography-mass spectrometry, antimicrobial and anti-inflammatory activity, 2,5-diphenyl-2H-tetrazolium bromide assay**

Phytochemicals are produced by plants for their growth, reproduction and protection against microorganisms or predators like insects and animals[1]. Phytochemicals show drug-likeness (antimicrobial, antioxidant, anticancer, etc.), biological friendliness and as alternatives to eradicate antibiotic-resistant pathogens[2-4]. Initially it is essential to screen and study the plants which are used as folklore medicines[5] to commercialize natural compounds as drug. The present study focuses on *Dipteracanthus patulus* (Jacq.) Nees. Synonym *Ruellia patula* (Vernacular name: Vedichedi, Kiranthinayagam) belongs to the family Acanthaceae[6]. When 5-6 leaves of this plant are chewed, the phytochemicals of these leaves act as an antidote to snakebite. Leaves are also used to treat cuts, wounds, an eyesore, gonorrhea, syphilis and renal infections[7]. *Ruellia patula* is an erect, pubescent, taproot, 50 cm tall, much-branched shrublet. Leaves are elliptic ovate. The fruit capsule is glabrous, 1.4-1.8 cm, 8-10 seeded. Fruits have flat and orbicular seeds. The plant is widely distributed in Arabia, India, Pakistan, Africa and Sri Lanka. In India, these plants are found in Western Ghats, Tamil Nadu, Rajasthan, Andhra Pradesh, and Haryana[8,9].

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As *Ruellia patula* is traditionally used more frequently as a wound healer, wound healing properties of the extracts from leaves are studied. Wounds are either acute or chronic types. Acute wounds heal within the expected time, chronic wound types, take more time to heal and difficult to predict the healing time[10]. Acute wounds undergo four phases in the following order hemostasis, inflammation, proliferation, and remodelling[11]. Chronic wounds on the other hand show an extended inflammatory phase, proliferative phase, or remodelling phase leading to wound ulcers[12]. Hypoxic condition is created in the wound region due to two main reasons such as vascular disruption which leads to diminished oxygen delivery[13] and the exposure of subcutaneous tissue (due to loss of skin integrity in the wound region) to microorganisms causes existing oxygen to be utilized by the aerobic microorganisms. This hypoxic condition becomes an appropriate region for the anaerobic microorganism survival[10]. Hence the wound is not only occupied by aerobic microorganisms like *Staphylococcus aureus*, *Pseudomonas aeruginosa*, etc but also by the anaerobic microorganisms during chronic stage. So, there is a need for antimicrobial activity of the compound against those microorganisms to decrease the healing time.

Further the healing time also delayed by production of excess Reactive Oxygen Species (ROS) by phagocytes. ROS being oxidizing molecules when present at a low-level help in getting rid of the infection and stimulate wound healing by producing cell survival signals[14,15], but when present in excess causes oxidative stress, cell damage and pro-inflammatory condition[16]. Antioxidant molecules on the other hand help to overcome oxidative stress by donating electron or hydrogen ions to ROS and prevent ROS from taking away electrons from macromolecules such as DNA or protein[17], thus preventing host cell damage.

The present study focuses on the phytochemical analysis, evaluation of antimicrobial, antioxidant, and anti-inflammatory properties[18] of four different solvent extracts of *Ruellia patula* leaves and determining the more effective extract among the four. Identification of phytochemicals present in effective extract using gas chromatography-mass spectrometry analysis.

MATERIALS AND METHODS

Collection of plant materials:

Ruellia patula plant was collected from different regions of Virudhunagar district in Tamil Nadu, India. Plant materials were taxonomically identified and authenticated by the Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamil Nadu, India.

Extraction:

Leaves of collected plants were washed, shade dried and powdered using an electric blender. One nonpolar solvent hexane, one mid polar solvent acetone, and two polar solvents methanol and water were chosen. The maceration technique was carried out to avoid the loss of thermolabile compounds and to ensure a longer exposure time of leaf powder to the solvent[19]. The leaf powder was added to solvents in the ratio of 1:20 (W/V). Maceration was carried out for 24 h. Then the contents were filtered using Whatman filter paper No.1 obtained filtrate was air-dried[20].

Phytochemical analysis:

For phytochemical analysis, crude extracts were dissolved in their respective solvents. Phlobatannins were detected by the formation of a red precipitate when a few drops of extract boiled in 1 % HCl. Terpenoids or sterols were detected by the formation of a reddish-brown ring when 1 ml of the extract was mixed with 0.5 ml of chloroform followed by the addition of a few drops of concentrated sulphuric acid. Tannins or phenolics were detected by brownish-green colour formation when 2 ml of the extract was added with a few drops of 10 % ferric chloride[21]. Alkaloids were detected by adding a few ml of extract and a few ml of diluted HCl, the mixture was then filtered, and filtrate obtained was added with drops of saturated picric acid (Hager's reagent), and the formation of a yellow precipitate indicates the presence of alkaloids. Flavonoids were detected by an alkaline reagent test where 2 ml of the extract was added with a few drops of 1 N NaOH, the formation of a yellow solution indicates the presence of flavonoids. Glycosides detection was done by following the Keller-Killiani test, in which 2 ml of extract was added with 2 ml of water, 0.5 ml of lead acetate and shaken well, mixture obtained was then filtered, filtrate then added with an equal volume of chloroform, evaporated, the

obtained residue dissolved in glacial acetic acid and few drops of ferric chloride was added, the obtained mixture was then transferred to test tube containing 2 ml of concentrated sulphuric acid[19]. The formation of a reddish-brown layer at the interface which on standing turns to bluish green indicates the presence of glycosides.

Total phenolic content:

Assay carried out based on Ainsworth *et al.*[22] with slight modifications, 0.25 ml of each gallic acid standard aliquots of following concentrations, 10, 20, 40, 60, 80, 100 µg/ml prepared in 10 % (v/v) Dimethylsulfoxide (DMSO) was added with 1 ml of 10 % (v/v) Folin-Ciocalteu reagent and 2 ml of 7.5 % (v/v) sodium carbonate. Shaken well and kept in dark condition for 30 min. Spectrophotometric reading of absorbance taken at 765 nm. Blank was prepared by replacing gallic acid aliquot with 10 % (v/v) DMSO. Whereas extracts at two different concentrations were prepared as 0.1 and 1 mg/ml in 10 % (v/v) DMSO, 0.25 ml of extracts were added in place of gallic acid aliquot. The standard curve of gallic acid concentration versus absorbance plotted was used to determine the total phenolic content in extract and expressed as mg of Gallic Acid Equivalent (GAE) per g of extract.

Total flavonoid content:

The assay was carried out based on Correa *et al.*[23] with slight modifications. 0.25 ml of each Quercetin standard aliquots of concentrations, 20, 40, 60, 80, 100 µg/ml prepared in 80 % ethanol was added with 0.65 ml of 80 % ethanol, 0.05 ml of 1 M sodium acetate, and 0.05 ml of aluminium chloride (10 %) and placed in dark condition for 40 min. Absorbance was read in the spectrophotometer at 415 nm. Blank was prepared by mixing 0.95 ml of 80 % ethanol and 0.05 ml of 1 M sodium acetate. Extracts of 0.5 mg/ml (in 10 % DMSO) were prepared and 0.25 ml of extract was used in place of Quercetin. Negative control samples were prepared by replacing 0.05 ml of aluminium chloride with 0.05 ml of distilled water. The standard curve of quercetin concentration *vs.* absorbance plotted was used to determine the total flavonoid content in the extracts and expressed results as mg of Quercetin Equivalent (QE) per g of extract.

Antimicrobial assay:

Preparation of Inoculum: The antimicrobial activity of the extracts was tested against bacteria such as *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Proteus mirabilis*. The bacterial strains were procured from Microbial Type Culture Collection, India. Microorganisms were cultured overnight in nutrient broth (Peptone 5 g/l, NaCl 5 g/l, Meat Extract 1.5 g/l, Yeast Extract 1.5 g/l) at 37° in a rotary shaker. Later, each strain was adjusted to a concentration of 10^8 cells/ml using 0.5 McFarland standard using a spectrophotometer at a wavelength of 600 nm[24].

Agar well diffusion assay: The antimicrobial activity of extracts was tested by Agar well diffusion method. The surface of the agar plate was inoculated by spreading a 100 µl volume of the microbial culture over the entire surface of the agar plate. A well was punched aseptically using a sterile cork well borer, and 100 µl of plant extract was introduced into the well. Then the agar plates were incubated at 37° in an incubator. For the diffusion of the extracts into agar, the plates were refrigerated for 30 min[25]. After 24 h of incubation, the zone of inhibition (diameter) was measured in mm. This assay was repeated twice to minimize the errors.

Minimum Inhibitory concentration (MIC): MIC was identified by the microtiter broth dilution method. The procedure involved preparing different concentrations of plant extracts in nutrient broth in a 96 well microtitration plate. Different serial dilutions were made for different organisms for each extract. Then each well was inoculated with a microbial culture which was adjusted to a 0.5 McFarland scale. After mixing, the microtiter plates were incubated at 37° for 24 h[25]. The concentration of extract at which no visible growth was observed was taken as MIC value. This experiment was repeated twice to minimize errors and to confirm the activity.

Antioxidant activity:

2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) free radical scavenging assay: Different concentrations of 20, 40, 60, 80 and 100 µg/ml of ascorbic acid and extracts were prepared in absolute ethanol and 10 % DMSO respectively. 0.25 ml of each standard aliquots and samples was added with 1 ml of 0.1 mM DPPH reagent freshly prepared in absolute ethanol. The reaction mixture was incubated at room temperature in dark conditions for 30 min. Spectrophotometric reading of absorbance at 517 nm for all standard and sample aliquots was determined[26]. Percentage of inhibition estimated using the formula as follows:

$$\%Inhibition=((A_{control}-A_{sample})/A_{control})\times 100$$

Where, $A_{control}$=Absorbance of control and

A_{sample}=Absorbance of sample/standard

Control is 0.25 ml of ethanol or 10 % DMSO and 1 ml of 0.1 mM DPPH for standards or samples respectively. Blank is absolute ethanol or mixture of 0.25 ml of 10 % DMSO and 1 ml of ethanol for standards or samples respectively.

Anti-inflammatory activity:

The anti-inflammatory activity of *Ruellia patula* extracts was studied according to the protocol of Padmanabhan *et al.*[27], Gunathilake *et al.*[28]. The activity of the extracts was evaluated using the "Albumin Denaturation Inhibition Assay". The reaction mixture was prepared by mixing 50 µl of egg albumin (from fresh hen's egg), 700 µl of phosphate-buffered saline of pH 7.4, and 0.5 ml of plant extract (0.6 to 1.6 mg/ml). Positive controls were prepared using 0.5 ml of diclofenac sodium (0.6 to 1.6 mg/ml) in the place of plant extract. Negative control was prepared by mixing 50 µl of Albumin and 750 µl of phosphate-buffered saline. Controls and test samples were incubated at 37° for 15 min and again incubated at 75° for 10 min. Then the absorbance was read at 660 nm (To nullify the absorbance due to extracts at different concentrations, mixture of 0.5 ml of extract (0.6 to1.6 mg/ml) and 750 µl of 10 % DMSO was prepared and spectrophotometric reading of absorbance taken at 660 nm). The percentage of inhibition was calculated using the formula

$$\% \text{ Inhibition} = ((A_{control} - A_{sample})/A_{control}) \times 100$$

Where $A_{control}$=Absorbance of negative control and A_{sample}=Absorbance of sample (extract)/Absorbance of positive control

Gas Chromatography-Mass Spectroscopy (GC-MS) analysis of *Ruellia patula* leaf extracts:

Plant extracts were subjected to GC-MS. The analysis was performed using Agilent GC 7890A/MS 5975C, an inert mass spectrometer fused with the capillary column of 30 m×0.25 mm with a film thickness of 0.25 µm. Pure nitrogen gas was used as the carrier gas at a flow rate of 1 ml/min and the pressure was maintained at 7.6522 psi. The oven temperature was increased from 50° to 300° at a rate of 50°/min. A 1 µl aliquot of the crude extract of acetone and methanol leaf extracts was injected in split mode. The runtime for analysis was 23.833 min. The peak retention time, peak area (%), and mass spectral fragmentation patterns to that of the known compounds described by the National Institute of Standards and Technology (NIST) library[29].

RESULTS AND DISCUSSION

According to some investigators, it is best to carry out initially the phytochemical analysis before evaluating the biological activity of plant extracts. Determining the phytoconstituents helps to forecast the pharmacological function of the plant extracts[30,31]. The presence of phytochemicals such as flavonoids, tannins or phenols, alkaloids, glycosides, phlobatannins, terpenoids, or sterols of each extract was detected by chemical tests. Flavonoids, glycosides, and phlobatannins were detected in acetone and methanol extracts, tannins or phenols detected in acetone, methanol and aqueous extracts, terpenoids detected in hexane and methanol extracts (Table 1).

Under basic conditions (sodium carbonate) phenolic compounds lose their proton and form phenolate anions which can reduce Folin-Ciocalteu's Reagent (FCR) which results in blue color formation due to the reduction of molybdate to molybdenum oxide in the FCR. Formed molybdenum oxide shows maximum absorbance at 765 nm. Hence the intensity of blue coloration is directly proportional to the concentration of phenolics[32]. The standard curve for total phenolic content was obtained (fig. 1), and the total phenolic content of extracts was interpolated from the standard curve. Total phenolic content in extracts decreases as follows, acetone extract>methanol extract>aqueous extract, and no phenolic content was detected in hexane extract (Table 2).

TABLE 1: PHYTOCHEMICAL ANALYSIS OF THE EXTRACTS BY CHEMICAL TESTS

Solvent	Hexane	Acetone	Methanol	Aqueous
Flavonoids	-	+	+	-
Tannins or phenolic	-	+	+	+
Alkaloids	-	-	-	-
Glycoside	-	+	+	-
Phlobatannins	-	+	+	-
Terpenoids or sterols	+	-	+	-

Note: Presence (+) and Absence (-)

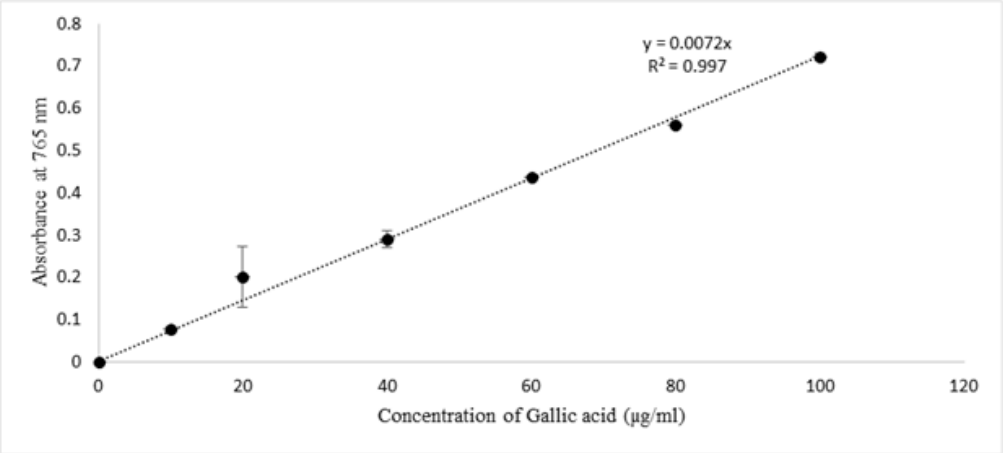

Fig. 1: Total phenolic content-Gallic acid standard plot

TABLE 2: ESTIMATION OF TOTAL PHENOLIC CONTENT OF FOUR EXTRACTS

Solvent	Total phenolic content (mg GAE/g of extract)
Acetone	142.94±1.01
Methanol	104.41±7.06
Aqueous	14.37

Note: Values obtained are mean values of two observations (mean±standard deviation). As phenolic compounds were absent in hexane extract, the assay showed positive outcomes for acetone, methanol and aqueous extracts

Flavonoids are estimated by the aluminium chloride colorimetric method where aluminium chloride forms the complex with either C-5 or C-3 hydroxyl, the C-4 group of flavonols, and flavones. Those mentioned complexes are said to be acid stable complexes. Additionally, aluminium chloride forms ortho-dihydroxyl groups in the B- or A- ring of flavonoids which are acid labile complexes[33]. Standard plot for total flavonoid content obtained, it was estimated that only the acetone and methanol extracts possess the flavonoid content which is correlating to the phytochemical analysis data of four different extracts. Among acetone and methanol extracts, acetone extract contains a higher content of flavonoids than methanol extract (fig. 2 and Table 3).

Antibiotic resistance is a challenge that continues to affect the healthcare industry in both developing and developed countries all over the world. The rise and spread of multidrug-resistant organisms have put conventional antibiotic therapy in jeopardy. This has forced a search for new antimicrobial substances, such as plants, which produce a wide range of bioactive chemicals with recognized medicinal characteristics. In this study, the antimicrobial property of extracts was studied against five microorganisms *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Proteus mirabilis*.

Other than the aqueous extract of leaves, all other extracts showed a significant zone of inhibition. Zone of inhibition decreased as positive control>acetone extract>methanol extract>hexane extract. Ampicillin was used as positive control against *Escherichia coli*, *Bacillus subtilis* and *Proteus mirabilis*. Levofloxacin and penicillin were used as positive control against *Staphylococcus aureus* and *Pseudomonas aeruginosa* respectively. Ampicillin was used in three different concentrations against *Escherichia coli* and *Bacillus subtilis* (fig. 3 and fig. 4). Disc containing 2 µg/disc of ampicillin developed zone of inhibition of 18.55 mm against *Proteus mirabilis*. Disc containing 2 units/disc of penicillin developed zone of inhibition of 15.2 mm against *Pseudomonas aeruginosa* and levofloxacin disc (5 µg/disc) developed zone of inhibition of 22.2 mm against *Staphylococcus aureus*. Acetone showed a better zone of inhibition from 100-300 mg/ml. Zone of inhibition of acetone extract at 300 mg/ml against microbes such as *Escherichia coli*-18.75 mm, *Bacillus subtilis*-20.5 mm, *Staphylococcus aureus*-20.5 mm, *Pseudomonas aeruginosa*-22.3 mm and *Proteus mirabilis*-18.5 mm (fig. 3- fig. 7).

MIC is defined as the lowest concentration of the antimicrobial agent that prevents visible microbial growth after 24 h of incubation. Extracts exhibited antimicrobial activity against all the 5 microorganisms such as *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas*

aeruginosa, and *Proteus mirabilis*. MIC of acetone extract was lower than other extracts, *Escherichia coli*-208 mg/ml, *Bacillus subtilis*-202 mg/ml, *Staphylococcus aureus*-208 mg/ml, *Pseudomonas aeruginosa*-204 mg/ml, *Proteus mirabilis*-204 mg/ml (Table 4). The aqueous extract didn't inhibit the growth of microorganisms.

DPPH assay works on the principle of free radical scavenging. The highly sensitive and simple nature of the assay made the assay popular nowadays. DPPH free radical is purple which turns to yellow color DPPH when antioxidant provides hydrogen atom to the free radical. As the concentration of antioxidants increases, there occurs a decrease in the intensity of the purple color. DPPH free radical shows maximum absorbance at 517 nm[34]. Absorbance at 517 nm *vs.* concentration of extract (µg/ml) was plotted (fig. 5) and analysed that percentage of inhibition was directly proportional to the concentration of extract. The IC_{50} value of methanol extract in DPPH free radical scavenging was interpolated as 146.4483 µg/ml which is less than the IC_{50} value of acetone extract of 153.8131 µg/ml (Table 5). While other extracts did not show radical scavenging activity within a concentration range of 10-200 (µg/ml).

The egg albumin (protein) denaturation inhibition assay provides a cheap alternative method for testing the anti-inflammatory activity of herbal extracts. As the concentration of extract had increased, absorbance decreased, indicating that denaturation of egg albumin by the applied heat was inhibited by leaf extract. Percentage of inhibition by the extracts at 1.6 mg/ml decreased as diclofenac (90 %)>acetone extract (89 %)>aqueous extract (86 %)>methanol (79 %)>hexane (45 %) (fig. 6). The result was compared with Ruellia tuberose. Hexane extract of *Ruellia tuberosa* was not showed antimicrobial activity on *Escherichia coli*, *Pseudomonas aeruginosa* and *Protease* sp at any concentration[78]. Anti-inflammatory activity of *Ruellia tuberose* was studied by *in vivo* experiment and it showed the activity at maximum concentration 300 mg/kg[79].

As acetone and methanol extracts showed better activities than other two extracts, acetone and methanol extracts were further analysed by GC-MS method. In the case of acetone extract, compounds with greater percentage area are phytol (14.99) and diethyl phthalate (10.05) (fig. 8 to fig. 11). Phytol possesses both anti-inflammatory and antimicrobial activities. Diethyl phthalate possesses antimicrobial activity (Table 6)[35-77]. In the case of methanol extract, compounds with greater percentage area are beta-sitosterol (17.01), dimethyl sulfone (7.53), beta-amyrin (6.51) and alpha-amyrin (6.51). Antimicrobial activity associated with dimethyl sulfone, antibacterial and antioxidant activities associated with beta-sitosterol, anti-inflammatory activity associated with beta-sitosterol, dimethyl sulfone, beta- amyrin, alpha-amyrin (Table 7)[78,79].

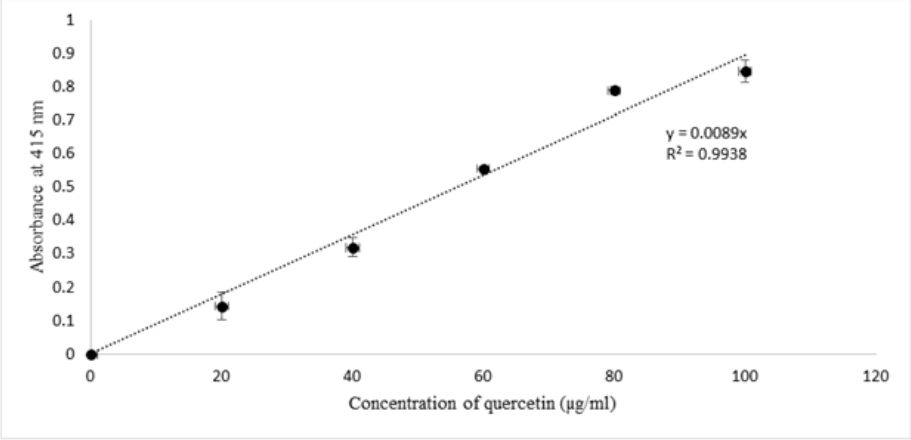

Fig. 2: Total flavonoid content-Quercetin standard plot

TABLE 3: ESTIMATION OF TOTAL FLAVONOID CONTENT OF FOUR EXTRACTS

Solvent	Total flavonoid content (mg QE/g of extract)
Acetone	37.49±1.83
Methanol	26.73±10.65

Note: Values obtained are mean values of two observations (mean±standard deviation). As flavonoid present only in acetone and methanol extracts, aluminium flavonoid complex concentration was estimated only in those extracts from the assay

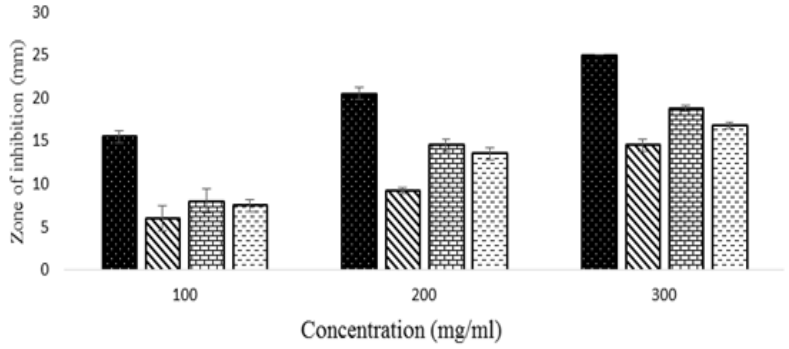

Fig. 3: Antimicrobial activity-zone of inhibition developed by antibiotic (Ampicillin) and extracts in the concentration range of 100-300 mg/ml against *Escherichia coli*
Note: (■): Ampicillin; (▨): Hexane; (▤): Acetone and (□): Methanol

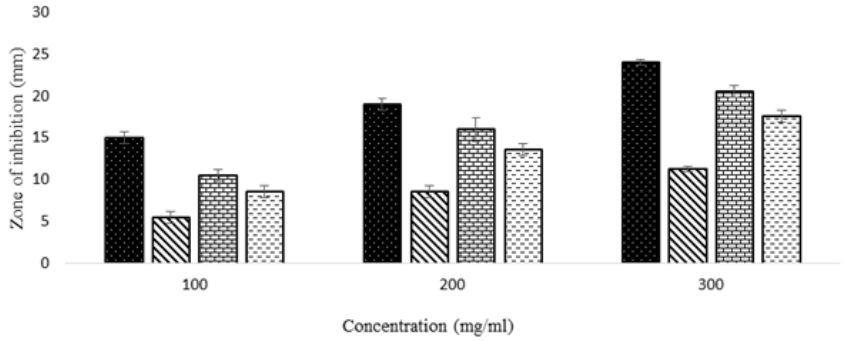

Fig. 4: Antimicrobial activity-zone of inhibition developed by antibiotic (Ampicillin) and extracts in the concentration range of 100-300 mg/ml against *Bacillus subtilis*
Note: (■): Ampicillin; (▨): Hexane; (▤): Acetone and (□): Methanol

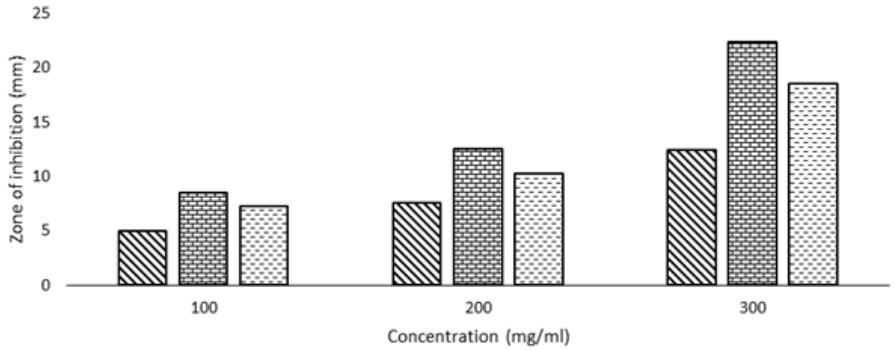

Fig. 5: Antimicrobial activity-zone of inhibition (mm) developed by extracts in the concentration range 100-300 mg/ml against *Pseudomonas aeruginosa*
Note: (▨): Hexane; (▤): Acetone and (□): Methanol

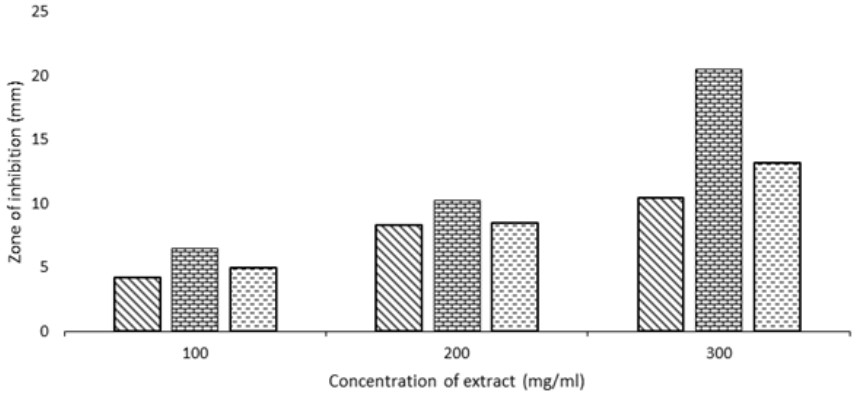

Fig. 6: Antimicrobial activity-zone of inhibition (mm) developed by extracts in the concentration range of 100-300 mg/ml against *Staphylococcus aureus*
Note: (▨): Hexane; (▤): Acetone and (□): Methanol

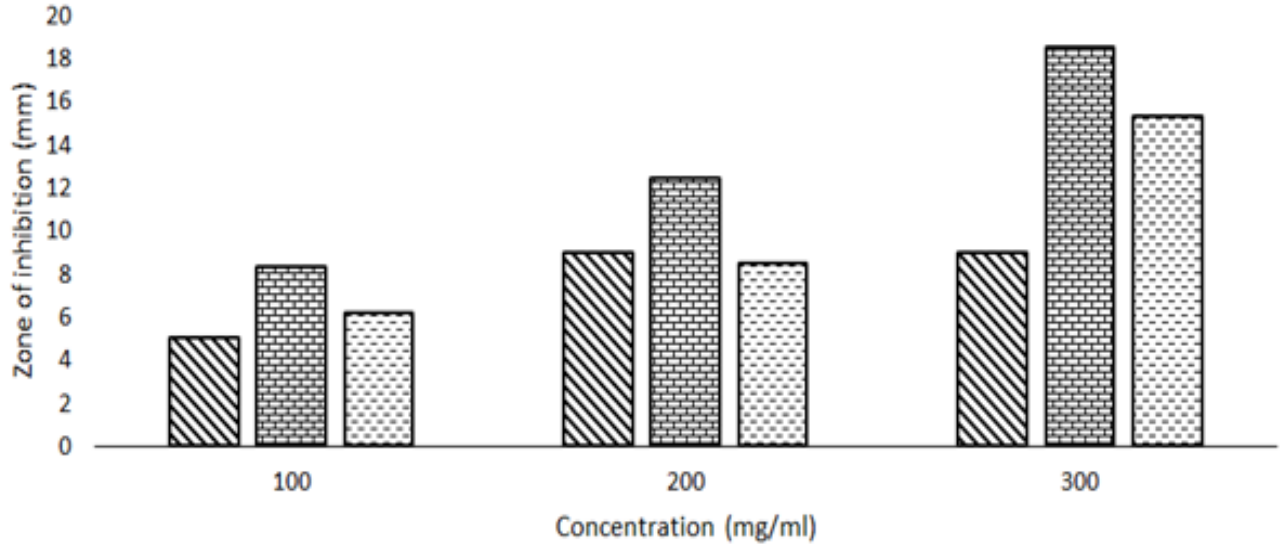

Fig. 7: Antimicrobial activity-zone of inhibition (mm) developed by extracts in the concentration range of 100-300 mg/ml against *Proteus mirabilis*
Note: ( ): Hexane; ( ): Acetone and ( ): Methanol

TABLE 4: MINIMUM INHIBITION CONCENTRATION OF EXTRACTS AGAINST FIVE MICROORGANISMS

Solvent	*Escherichia coli*	*Bacillus subtilis*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Proteus mirabilis*
Hexane (mg/ml)	288	264	289	313	315
Acetone (mg/ml)	208	202	208	204	204
Methanol (mg/ml)	215	210	210	208	207

Note: Aqueous extract did not show microbial growth inhibition, thus minimum inhibitory concentration was estimated only for hexane, acetone and methanol extracts

TABLE 5: DETERMINATION OF IC_{50} VALUE OF FOUR EXTRACTS IN SCAVENGING DPPH FREE RADICALS

Solvent	IC_{50} (µg/ml)
Methanol	146.4483
Acetone	153.8131

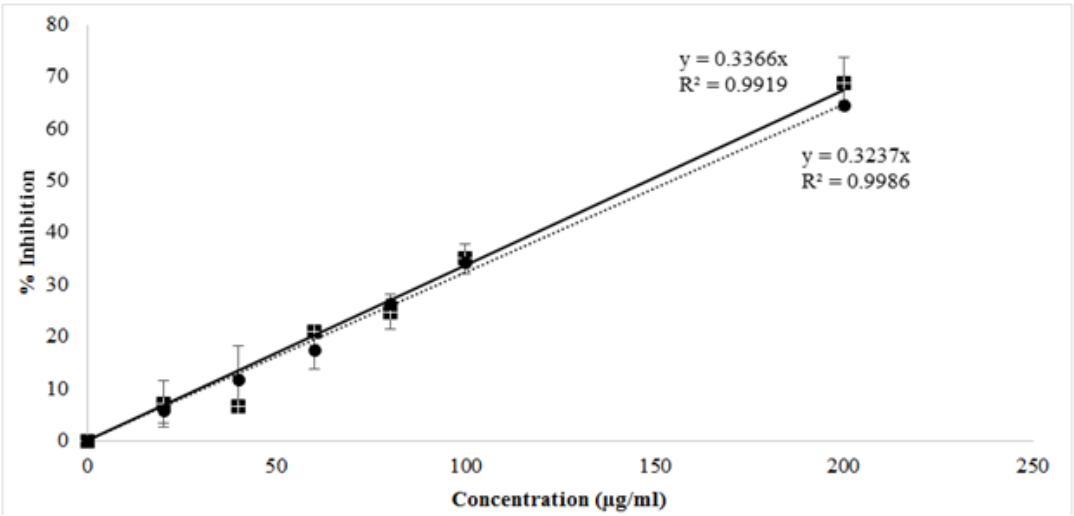

Fig. 8: Antioxidant assay-DPPH free radical scavenging assay
Note: ( ): Methanol; ( ): Acetone; ( ): Linear (Methanol) and ( ): Linear (Acetone)

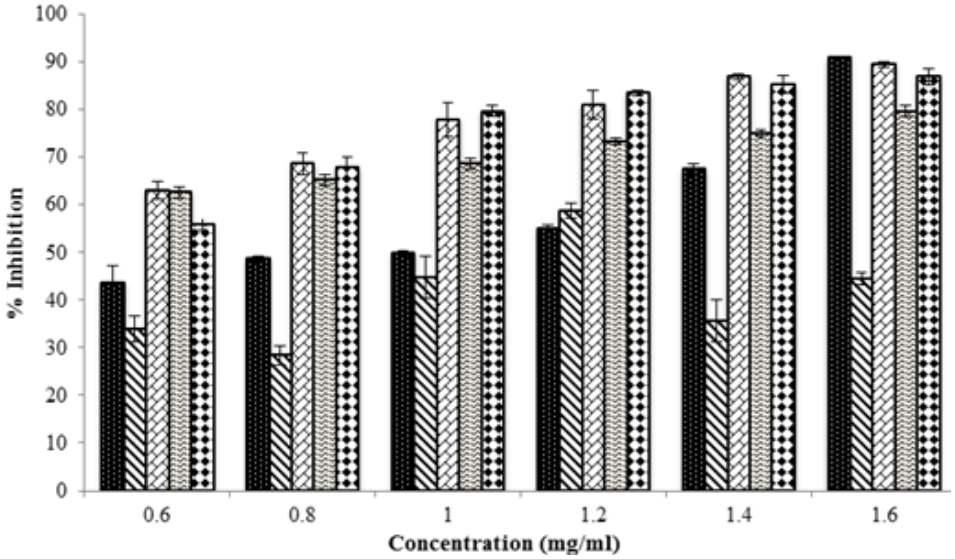

Fig. 9: Anti-inflammatory assay-protein denaturation inhibition assay
Note: (■): Diclofenac; (▨): Hexane; (▨): Acetone; (▨): Methanol and (▨): Aqueous

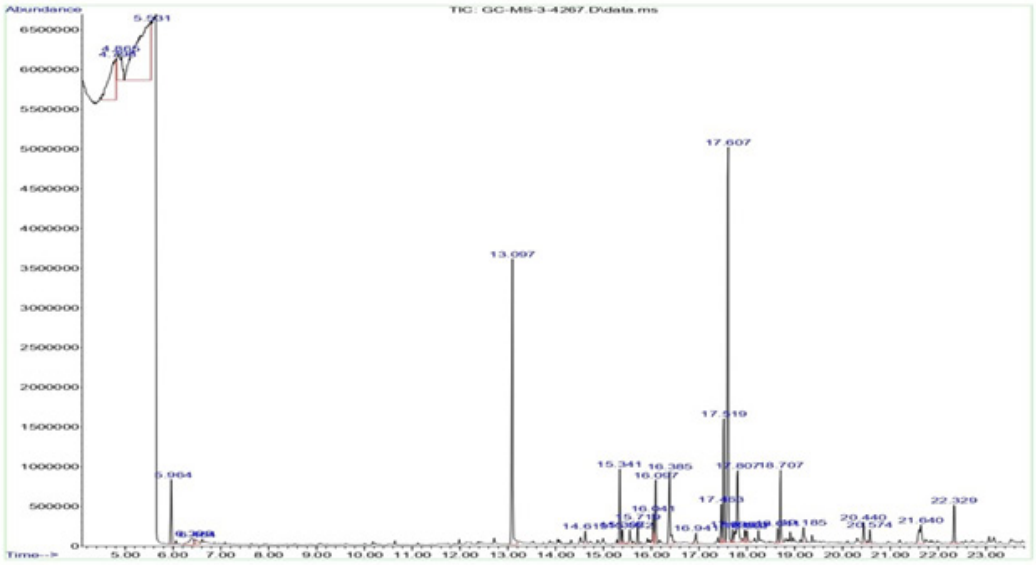

Fig. 10: GC-MS analysis of acetone extract

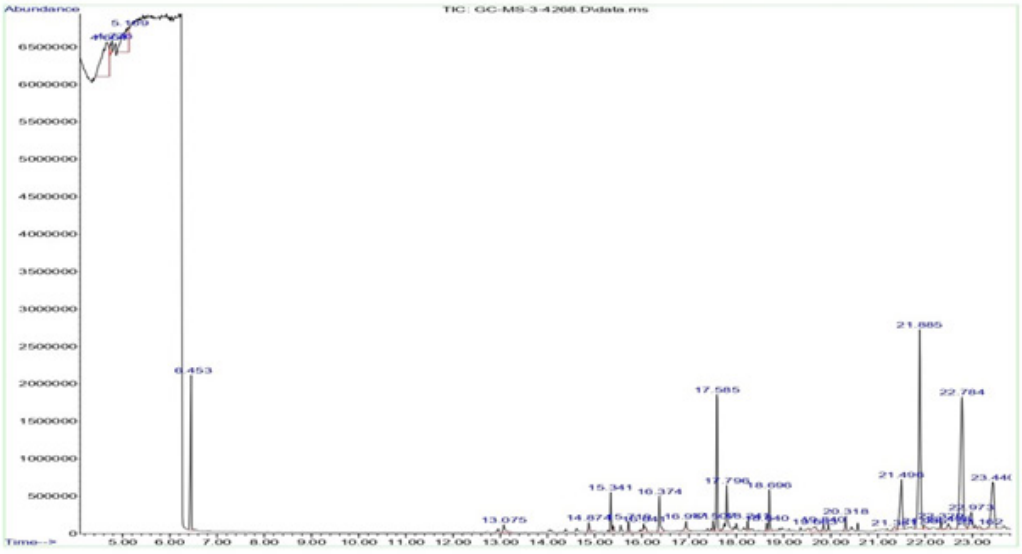

Fig. 11: GC-MS analysis of methanol extract

TABLE 6: COMPOUNDS IDENTIFIED FROM GC-MS ANALYSIS OF ACETONE EXTRACT

Name	RT	% Area	MW	Molecular formula	Biological activity
Dimethyl sulfone	5.964	2.1	94.14	$C_2H_6O_2S$	Antimicrobial and anti-inflammatory[35]
Diethyl Phthalate	13.1	10.05	222.24	$C_{12}H_{14}O_4$	Antimicrobial[36]
Tetradecanoic acid	14.62	0.51	228.37	$C_{14}H_{28}O_2$	Antimicrobial[37]
Tridecanoic acid	14.62	0.51	214.34	$C_{13}H_{26}O_2$	Antimicrobial, anti-inflammatory[38]
2-Hexadecene, 3,7,11,15-tetramethyl	15.4	0.55	280.5	C20H40	Antimicrobial and antioxidant[39]
5-Nonadecen-1-ol	15.55	0.59	282.5	$C_{19}H_{38}O$	Antimicrobial and anti-inflammatory[40]
1,4-Eicosadiene	15.55	0.59	278.5	$C_{20}H_{38}$	Antimicrobial[41]
Cyclohexanol, 1-ethynyl	15.72	0.69	124.18	$C_8H_{12}O$	Anticancer and antioxidant[42]
Dodeca-1,6-dien-12-ol, 6,10-dimethyl	15.72	0.69	210.36	$C_{14}H_{26}O$	Antimicrobial, antioxidant, and anti-inflammatory[43]
Squalene	16.04	1.08	410.7	$C_{30}H_{50}$	Antioxidant, antibacterial[44]
Hexadecanoic acid, methyl ester	16.1	2.23	270.451	$C_{17}H_{34}O_2$	Antibacterial and antifungal[45]
Pentadecanoic acid, 14-methyl-, methyl ester	16.1	2.23	270.5	$C_{17}H_{34}O_2$	Antimicrobial, antifungal[46]
n-Hexadecanoic acid	16.39	3.3	256.42	$C_{16}H_{32}O_2$	Antioxidant[47]
Nerolidol 1	16.94	0.55	222.37	$C_{15}H_{26}O$	Antimicrobial, anti-biofilm, antioxidant, anti-inflammatory[48]
Methyl 10-trans,12-cis-octadecadienoate	17.64	1.18	294	$C_{19}H_{34}O_2$	Antioxidant and antimicrobial[47]
9,12,15-Octadecatrienal	17.52	4.13	262.43	$C_{18}H_{30}O$	Antimicrobial[49]
Phytol	17.61	14.99	296.5	$C_{20}H_{40}O$	Anti-inflammatory and antimicrobial[50]
Octadecanoic acid, methyl ester	17.7	0.44	294.47	$C_{19}H_{34}O_2$	Antiviral[51]
Octadecanoic acid	17.95	0.59	284.48	$C_{18}H_{36}O_2$	Anti-inflammatory and antioxidant[47]
Tetradecanoic acid	17.95	0.59	228.37	$C_{14}H_{28}O_2$	Antimicrobial[52]
3-Hydroxymyristic acid	18.64	0.61	244.37	$C_{14}H_{28}O_3$	Antifungal and antimicrobial[53]
7-Tetradecyne	19.19	0.78	194.36	$C_{14}H_{26}$	Antimicrobial[54]
1,2-Benzenedicarboxylic acid, mono(2-Ethylhexyl) ester	20.57	0.5	278.34	$C_{16}H_{22}O_4$	Antimicrobial[46]
Di-n-octyl phthalate	20.57	0.5	390.6	$C_{24}H_{38}O_4$	Antimicrobial[55]
Phthalic acid, isohexylisoporpyl ester	20.57	0.5	292.4	$C_{17}H_{24}O_4$	Antimicrobial[56]
Nonanoic acid, 9-(3-hexenylidenecyclopropylidene)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	21.64	1.79	52	$C_{21}H_36O_4$	Antioxidant, antimicrobial[57]
i-Propyl 9,12,15-octadecatrienoate	21.64	1.79	320.5	$C_{21}H_{36}O_2$	Anti-inflammatory[58]
2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl	22.33	1.5	410.72	$C_{30}H_{50}$	Antioxidant[59]

Note: RT: Retention Time and MW: Molecular Weight

TABLE 7: COMPOUNDS ANALYSED FROM GC-MS ANALYSIS OF METHANOL EXTRACT

Name	RT	% Area	MW	Molecular formula	Biological activity
Dimethyl sulfone	6.453	7.53	94.13	$C_2H_6O_2S$	Anti-inflammatory and antimicrobial[35]
Diethyl Phthalate	13.075	0.79	222.24	$C_{12}H_{14}O_4$	Antimicrobial[36]
2-Furanethanol, .beta.-methoxy-(S)	14.874	0.58	142.15	$C_7H_{10}O_3$	Antimicrobial[40]
1-Methoxy-3-(2-hydroxyethyl) nonane	15.341	1.59	202.33	$C_{12}H_{26}O_2$	Antimicrobial, antioxidant activity[60]
9-Nonadecyne	15.719	0.51	264.5	$C_{19}H_{36}$	Antifungal, antioxidant and antimicrobial[40]
Squalene	16.041	0.85	410.7	$C_{30}H_{50}$	Antioxidant[44]
n-Hexadecanoic acid	16.374	2.28	256.42	$C_{16}H_{32}O_2$	Antioxidant[47]
5,9-Undecadien-2-one, 6,10-dimethyl	16.941	1.06	194.31	$C_{13}H_{22}O$	Antimicrobial[61]
9,12,15-Octadecatrienoic acid, methyl ester	17.507	0.49	292.5	$C_{19}H_{32}O$	ALA–omega 3 fatty acid Reduce blood clots[47]
7,10,13-Hexadecatrienoic acid, methyl ester	17.507	0.49	264.4	$C_{17}H_{28}O_2$	Fatty acids that reduce blood clots[62]
Phytol	17.585	0.87	296.539	$C_{20}H_{40}O$	Anti-inflammatory and antimicrobial[50]
Isophytol	17.585	0.87	296.5	$C_{20}H_{40}O$	Antimicrobial and antifungal[63]
9,12,15-Octadecatrienoic acid	17.796	3.85	278.4	$C_{18}H_{30}O_2$	ALA – omega 3 fatty acid Reduce blood clots[47]
2,6-Octadien-1-ol, 3,7-dimethyl	18.241	0.46	308.5	$C_{20}H_{36}O_2$	Antimicrobial[64]
Tetradecanoic acid, 2-hydroxy-	18.64	0.46	244.37	$C_{14}H_{28}O_3$	Antioxidant[65]
17-Pentatriacontene	18.696	1.97	490.9	$C_{35}H_{70}$	Antibacterial[66]
Vitamin E	19.663	0.93	430.71	$C_{29}H_{50}O_2$	Antioxidant[67]
Phenol, 2,4-bis(1-phenylethyl)-	19.84	0.49	302.4	$C_{24}H_{26}O$	Anti-inflammatory[68]
Xanthen-9-one, 1-hydroxy-3,5,8-trimethoxy-	19.84	0.49	302.28	$C_{16}H_{14}O_6$	Antimicrobial, Antioxidant, Anti-inflammatory[69]
Methanone, [1,4-dimethyl-7-(1-methylethyl)-2-azulenyl]phenyl-	19.84	0.49	302.42	$C_{22}H_{22}O$	Antimicrobial[70]
Phenol, 2,4-bis(1-phenylethyl)-	20.318	0.8	302.4	$C_{22}H_{22}O$	Antioxidant[71]
Xanthen-9-one, 1-hydroxy-3,5,8-trimethoxy	20.318	0.8	302.28	$C_{16}H_{14}O_6$	Antimicrobial, Antioxidant, Anti-inflammatory[69]
Ergosta-5,24-dien-3-ol, (3.beta.)-	21.351	0.58	398.7	$C_{28}H_{46}O$	Moderate cytotoxicity against the human foreskin fibroblast cell line (Hs27 cells)[72]
Ergost-5,8(14)-dien-3-ol	21.351	0.58	398.7	$C_{28}H_{46}O$	Immunosuppressive, anti-tumor[73]

Stigmasta-5,24(28)-dien-3-ol, (3.beta.,24Z)	21.351	0.58	412.7	$C_{29}H_{48}O$	Antioxidant, anti-inflammatory[74]
Pregnenolone	21.985	0.5	316.47	$C_{21}H_{32}O_2$	Anti-tumor[75]
2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	22.329	0.57	410.71	$C_{30}H_{50}$	Antioxidant[59]
gamma.-Sitosterol	22.784	17.01	432.7	$C_{29}H_{52}O_2$	Anti-cancer[76]
					Antibacterial[44]
beta.-Sitosterol	22.784	17.01	414.71	$C_{29}H_{50}O$	
26,26-Dimethyl-5,24(28)-ergostadien-3.beta.-ol	22.973	1.94	426.7	$C_{30}H_{50}O$	maintain cell membrane integrity[77]
beta.-Amyrin	23.44	6.51	426.7	$C_{30}H_{50}O$	Antimicrobial[44]
Alpha.-Amyrin	23.44	6.51	426.7	$C_{30}H_{50}O$	Antimicrobial[44]

Note: RT: Retention Time and MW: Molecular Weight

In conclusion, the results of this study show the presence of phytochemicals such as flavonoids, tannins/phenolics, glycosides, and phlobatannins, higher phenolics and flavonoids content in both acetone and methanol extracts than in the other extracts. Higher antimicrobial, antioxidant and anti-inflammatory properties are associated with acetone and methanol extracts than the other extracts due to the presence of bioactive compounds illustrated in GC-MS analysis. Purification of most essential compound from the *Ruellia patula* leaves extract, investigating the wound healing properties by performing *in vivo* studies can be explored to bring natural product as a wound healing drug.

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Conflict of interests:

The authors declare that they have no competing interest.

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