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Research Article
Comparative Study of Identification of Bioactive Compounds from *Barringtonia acutangula* Leaves and Bark Extracts and its Biological Activity

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Abstract

Background and Objective: There has been not much information available on phytochemical components and biological activity in the bark and leaves extract of *Barringtonia acutangula* L. The objective of this study was to evaluate *in vitro* antioxidant activity, antimicrobial activity and characterize the chemical constituents of leaves and bark extract of *Barringtonia acutangula* L. by using gas chromatography-mass spectrometry (GC-MS). **Materials and Methods:** This investigation was carried out to determine the possible chemical components from *Barringtonia acutangula* L. by GC-MS.-QP 2010 PLUS SHIMADZU JAPAN. The antioxidant activity of plant material was assayed by reducing power assay. **Results:** This analysis revealed that the ethanol extract of *Barringtonia acutangula* L. (Bark) contained 32 phyto components, mainly 1,2-Benzenedicarboxylic acid diethyl ester, Dibutyl phthalate, 1,2- Benzenedicarboxylic acid, etc. The leaves extract contained 37 phyto components, among them 2,6,10-Trimethyl, 14-ethylene-14-pentadecene, 4,4a,6b,8a,11,11,12b,14a-Octamethyl-eicosahydro-picen-3-one, Dibutyl phthalate, 1,2- Benzenedicarboxylic acid, Friedelan-3-one were the major components. The reducing power of the plant extract was compared with the standard ascorbic acid and found to be dose-dependent. **Conclusion:** From the results, it is evident that *Barringtonia acutangula* L. contains various bioactive compounds and is recommended as a plant of phytopharmaceutical importance.

Key words: GC-MS analysis, phytocomponents, *Barringtonia acutangula* L., *in vitro* antioxidant, TPC, TFC, UTI

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Bioactive compounds commonly found in medicinal plants and vegetables exhibit possible health benefits, such as antioxidative, anticancer, antidiabetic and angiogenesis inhibitory activities[1]. Phenolic antioxidants in herbs mainly consist of phenolic acids flavonoids and catechin[2-4]. The antioxidant activities of phenolic compounds are mainly due to their redox properties, which play an important role in the abilities of these compounds to adsorb and neutralize free radicals, quench singlet and triplet oxygen and decompose peroxides[5]. *Barringtonia acutangula* (L.) Gaertn. (Family: Lecythidaceae) known as Kadambu in Tamil; *Samudraphal* in Hindi, is a medium-sized tree found throughout India. Barringtonia acutangula is used as a folk medicine for curing various diseases like pain in joints, eye diseases, stomach disorders, diarrhea, cough, leprosy and spleen disorders[6]. An aqueous extract of the bark is found hypoglycemic and is reported to be used in pneumonia, asthma and leaf juice is given for diarrhoea[7]. From the previous study, 7 compounds have been identified from ethanolic extract of the stem bark of *Barringtonia acutangula* by Gas GC-MS analysis[8]. Kathirvel and Sujatha[9] investigated the antioxidant potential of *Barringtonia acutangula* leaves in various extracts. So, the objectives of the present study were (1) To identify and compare the bioactive compounds in the bark and leaves extract of *Barringtonia acutangula* by GCMS analysis, (2) To compare total phenolic and flavonoids in the bark and leaves extract of *Barringtonia acutangula*, (3) To determine antioxidant activity based on their reducing power and (4) To compare antimicrobial activity of the bark and leaves extract of *Barringtonia acutangula*.

MATERIALS AND METHODS

Chemicals: All the chemicals and solvents were of analytical grade and procured from E. Merck (India) Ltd., Mumbai.

Plant material: The bark and leaves of *Barringtonia acutangula* were collected from Madurai, Tamil Nadu, India. Nearly 1.0 kg of shade-dried coarse powders of the leaves and bark material were extracted with 80% v/v aqueous ethanol by maceration at room temperature for 72 h. After the completion of each extraction, the extracts were filtered, concentrated to dryness in a rotavapor under reduced pressure and controlled temperature (40-50°C). The residues were stored in a vacuum desiccator for further use.

Antioxidant activity

Reducing power assay: Different concentration of extract was mixed with 2.5 mL of 1% potassium ferricyanide and 2.5 mL of phosphate buffer (pH 6.6). The mixture was incubated at 50°C for 20 min. Trichloroacetic acid (10%) was added to it and centrifuged at 3000 rpm for 10 min. Then the supernatant was taken, 2.5 mL of water and $FeCl_3$ (0.1%) is added to it. The absorbance was measured at 700 nm.

Determination of the antioxidant components

Total phenols determination: The amounts of total phenolic contents of plant were determined by the spectrophotometric method of Kim *et al.*[10] with slight modification. A diluted plant sample (1 mL) or standard phenolic compound (Gallic acid) was added to a 25 mL volumetric flask, containing 9 mL of distilled water. To this mixture 1 mL of Folin-Ciocalteu's phenol reagent was added and shaken. After 5 min, 10 mL of 7% Na_2CO_3 solution was mixed in to the test sample solution was diluted to 25 mL distilled water and mixed thoroughly. The mixture was kept in the dark for 90 min at 23°C after which the absorbance was read at 750 nm. Total phenol content was determined from extrapolation of calibration curve which was made by preparing gallic acid solution (20-100 μg mL^{-1}). The estimation of the phenolic compounds was carried out in triplicate. The total phenolic content was expressed as milligrams of gallic acid (GAE) equivalents per gram dried sample.

Total flavonoids determination: The total flavonoids assay was conducted according to Damodar *et al.*[11]. Total flavonoids content was determined by using Aluminium chloride colorimetric method. Plant sample (0.5 mL) was mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. It remained at room temperature for 30 min. The absorbance of the reaction mixture was measured at 510 nm using UV-Visible spectrophotometer. The calibration curve was prepared by preparing quercetin solutions at concentrations 20-100 μg mL^{-1} in methanol. The total flavonoids content was expressed as milligrams of quercetin equivalents per gram of dried sample.

Assay of antimicrobial activity: About 25 g of plant powder was weighed and macerated in methanol, individually in the ratio of 1:4 (w/v). They were kept at the room temperature for 24 h. Each mixture was stirred every hours using sterile glass rod. Then it was filtered through the Whatmann No. 1 filter

paper. Bacteria causing UTI were used in the present study. They were both Gram-positive and Gram-negative. Such as *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus* and *Enterococcus faecalis* were used. They were collected from the microbial type culture collection (MTCC) at Chandigarh, India. Pure cultures of bacterial pathogens in nutrient agar slant was transferred to tryptone broth and incubated at 37°C for 24 h. The 6 mm (diameter) discs were prepared from Whatmann No. 1 filter paper. The discs were sterilized by autoclave at 121°C. After the sterilization the moisture discs were dried on hot air oven at 50°C. Then discs were mixed with chemical compounds separately and control discs were prepared. Antibacterial activity test was carried out following the modification of the method originally described by Bauer *et al.*[12]. Muller Hinton agar was prepared and autoclaved at 15 lbs pressure for 20 min and cooled to 45°C. The cooled media was poured on to sterile Petri plates and allowed for solidification. The plates with media were seeded with the respective microbial suspension using sterile swab. The ethanol extract soaked discs were placed on the each Petri plates and also placed control and standard (Nitrofurantoin) discs. The plates were incubated at 37°C for 24 h. After incubation period, the diameter of the zone formed around the paper disc were measured and expressed in mm.

RESULTS

In-vitro antioxidant activity

Assay of reducing power: The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, the activities of antioxidants have been attributed to various mechanisms such as prevention of chain initiation, decomposition of peroxides, reducing capacity and radical scavenging. The reducing power of the leaves and bark extracts were shown in Fig. 1.

Phenolic and flavonoid contents: The total phenolic content of the leaf and bark extract, calculated from the calibration curve (R^2 = 0.997, y = 0.014x-0.090) were 74.68±4.14 and 42.20±2.2 gallic acid equivalents/g and the total flavonoid content (R^2 = 0.997: y = 0.021x+0.242) were 98.4±2.24 and 70.8±4.6 quercetin equivalents/g (Table 1).

Chemical composition by GC-MS analysis: Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST). The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. In the GC-MS analysis, 37 bio active phytochemical compounds were identified in the ethanol extract of leaves (Fig. 2, Table 2) and 32 bio active compounds were identified in the ethanol extract of bark (Fig. 3, Table 3). Biological activity of some identified components listed in Table 4.

Assay of antimicrobial activity: The antimicrobial activity of *Barringtonia acutangula* was tested against UTI causing bacteria *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus* and *Enterococcus faecalis* compared with the standard, the diameters of inhibition zones increased for all the test pathogens (Table 5, Fig. 4).

DISCUSSION

As shown in Fig. 1, the reducing power of the plant extracts were compared with the standard ascorbic acid and found to be dose-dependent, reducing power activity increases gradually with the increase of concentration of extracts. Reducing power of any extract will be given by the amount of reductones present in them. The ability of the hydroxyl groups present in the flavonoids/phenolics to reduce the free radicals by donating their electrons will determine

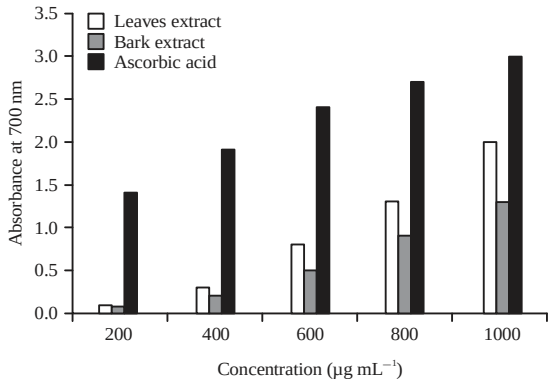

Fig. 1: Assay of reducing power of *Barringtonia acutangula* leaves and bark extract

Table 1: Quantitative analysis of total phenols and flavonoids content of leaves and seeds

Name of samples	Flavonoids (Expressed as milligrams of quercetin equivalents per gram of dried sample)	Phenol (Expressed as milligrams of gallic acid (GAE) equivalents per gram dried sample)
Leaf	98.4±2.24	74.68±4.14
Bark	70.8±4.60	42.20±2.20

Values are expressed as Mean±SD for triplicates

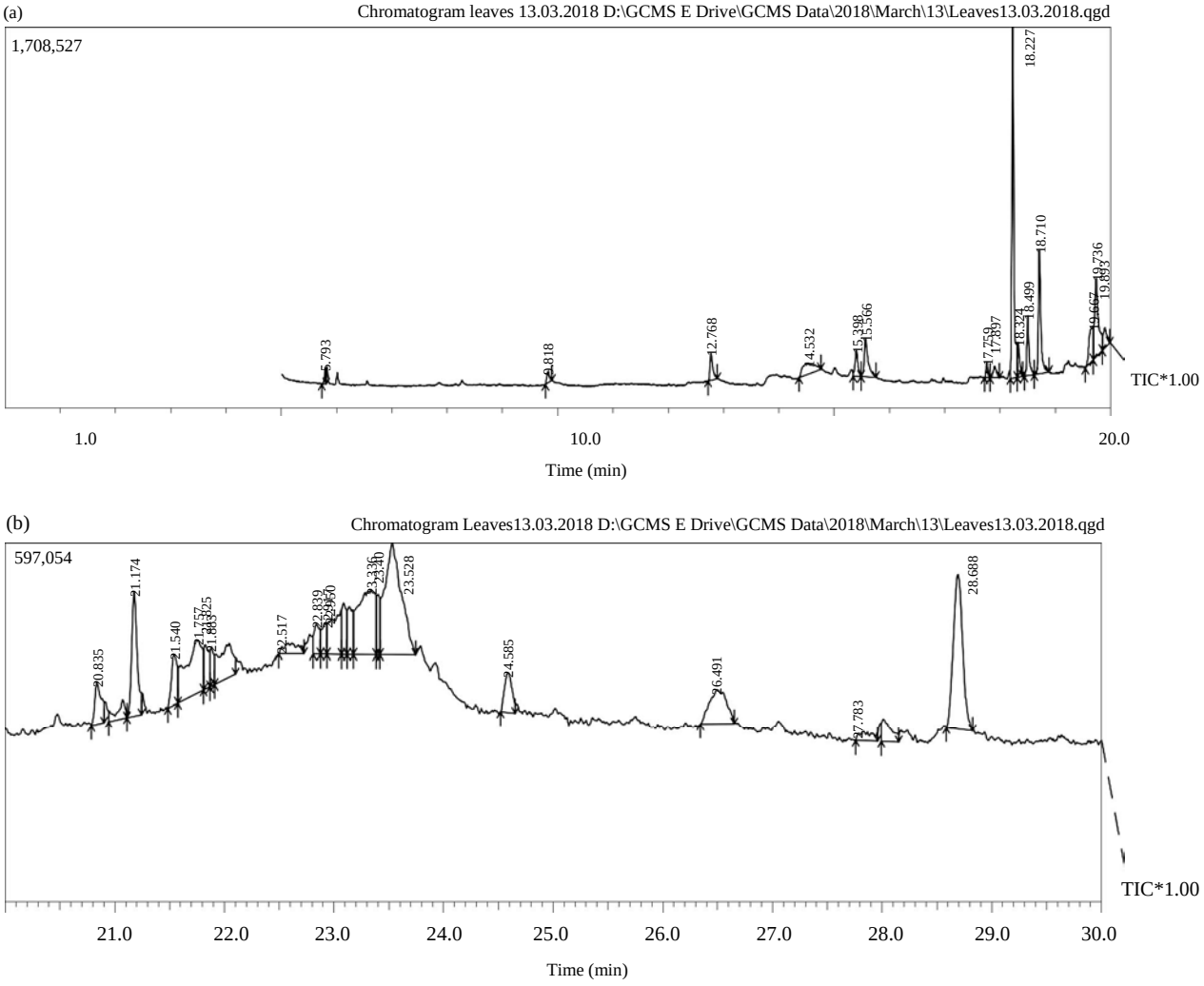

Fig. 2(a-b): GCMS chromatogram of *Barringtonia acutangula* leaves extract

Table 2: Compounds identified in the leaves extract of *Barringtonia acutangula*

Peak#	R. Time	Area (%)	Height (%)	Name
1	5.793	0.69	1.26	Butane, 1,1-diethoxy-3-methyl-
2	9.818	0.68	0.89	Tridecane
3	12.768	1.81	2.14	Heptadecane
4	14.532	2.95	0.81	beta-D-Glucopyranose, 1,6-anhydro-
5	15.398	1.69	2.07	Dodecane, 2,7,10-trimethyl
6	15.566	3.43	3.01	1,Benzenedicarboxylic acid, diethyl ester
7	17.759	0.69	1.18	Dodecane, 2,6,10-trimethyl-
8	17.897	0.93	0.84	2(4H)-Benzofuranone, 5,6,7,7A-tetrahydro-6-hydroxy-4,4,7A-trimethyl-, (6S)
9	18.227	17.06	27.77	2, 6, 10-trimethyl 14-ethylene-14-pentadecane
10	18.324	1.79	2.71	2-Pentadecanone
11	18.499	3.11	4.66	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
12	18.710	7.07	9.74	Cyclopropanenonanoic acid, 2-[(2-butylcyclopropyl)methyl]-, methyl ester
13	19.667	3.41	2.69	n-Hexadecanoic acid
14	19.736	7.20	6.26	Dibutyl phthalate
15	19.893	1.78	1.63	Hexadecane, 2,6,10,14-tetramethyl-
16	20.835	1.22	1.25	n-Pentadecanol
17	21.070	0.67	0.56	11-Oxa-dispiro[4.0.4.1]undecan-1-ol
18	21.174	3.01	3.67	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-

Table 2: Continue

Peak#	R. Time	Area (%)	Height (%)	Name
19	21.540	1.29	1.49	Palmitaldehyde, diallyl acetal
20	21.757	3.94	1.55	Silane, dimethyl(dimethyl(dec-4-enyloxy)silyloxy)(dec-4-enyloxy)-
21	21.825	0.96	1.31	Pyridine, 2,4-dihydroxy-3,5-dibromo-
22	21.883	0.60	1.14	Pyrimidine, 4,6-dichloro-2-(methylthio)-
23	22.043	2.13	0.98	Hexadecane, 2,6,10,14-tetramethyl-
24	22.517	0.71	0.16	2-(2-Methyl-5-oxo-tetrahydrofuran-3-ylthio] benzoic acid, methyl ester
25	22.839	0.73	0.89	Dodecanoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester
26	22.917	0.68	0.91	Benz[e]azulene-3,8-dione, 5-[(acetyloxy)methyl]-3a,4,6a,7,9,10,10a,10b-octahydro-3a,10a-dihydroxy
27	22.950	2.01	0.97	1-Propen, 3-(2-Cyclopentenyl)-2-methyl-1,1-diphenyl-
28	23.086	0.99	1.50	Carbonic acid, methyl 3-(trifluoromethyl)phenyl ester
29	23.142	1.09	1.39	2,7-Methanonaphthalen-3-amine,
30	23.336	4.89	1.90	Friedelan-3-one
31	23.400	0.81	1.78	4-Amino-N'-[4-Pyridinylmethylidene]-1,2,5-oxadiazole-3-carbohydrazide
32	23.528	8.06	3.27	4,4a,6b,8a,11,11,12b,14a-Octamethyl-eicosahydro-picen-3-one
33	24.585	1.33	1.18	4,8,12,16-Tetramethylheptadecan-4-olide
34	26.491	2.61	1.02	Squalene
35	27.783	0.58	0.22	Propanenitrile, 3-(5-diethylamino-1-methyl-3-pentynyloxy)-
36	28.008	0.94	0.68	Naphthalene, 1-[1-(3-cyclohexylpropyl)undecyl]decahydro-
37	28.688	6.45	4.56	1,2- Benzenedicarboxylic acid
		100.00	100.00	

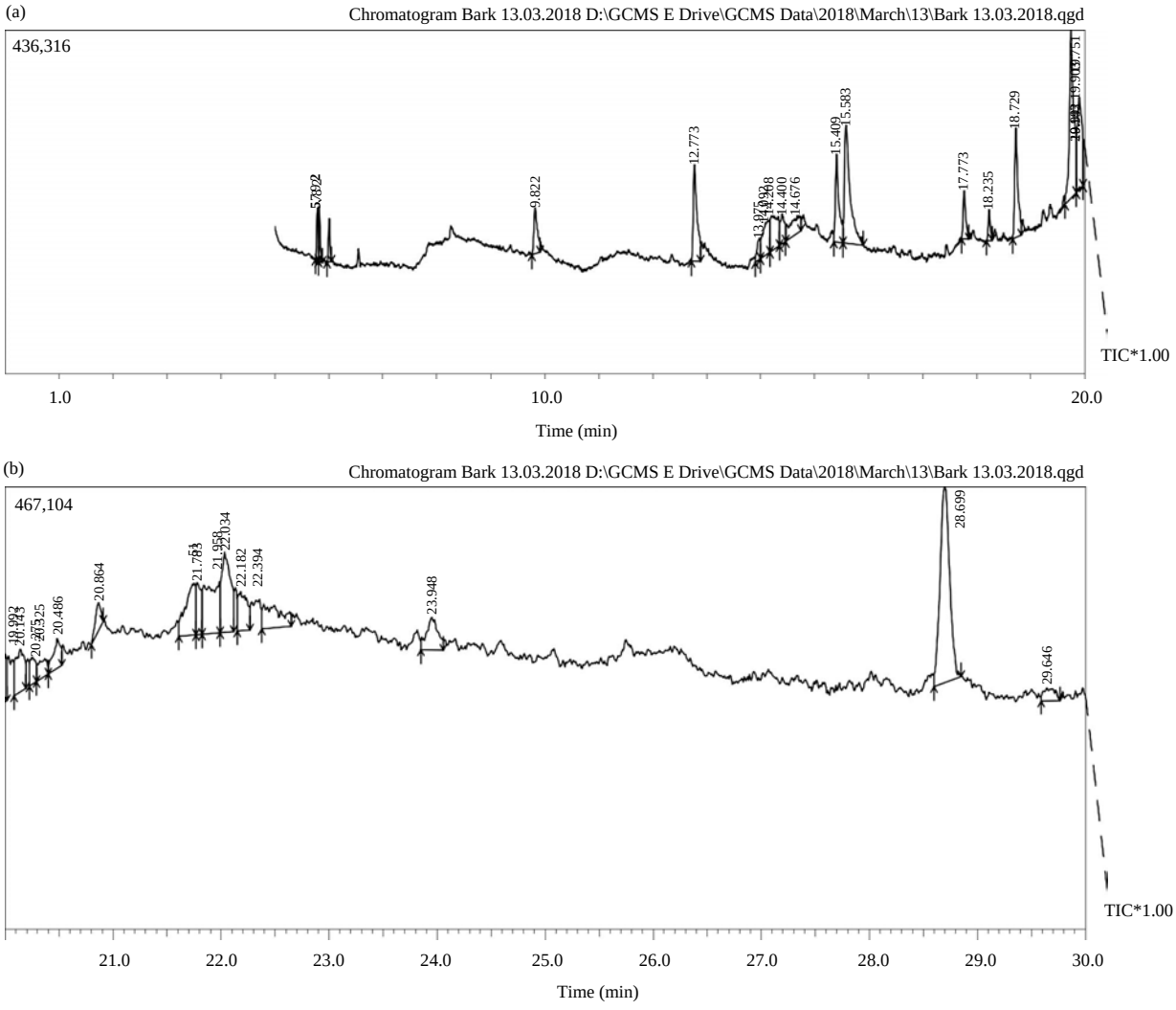

Fig. 3(a-b): GCMS chromatogram of *Barringtonia acutangula* bark extract

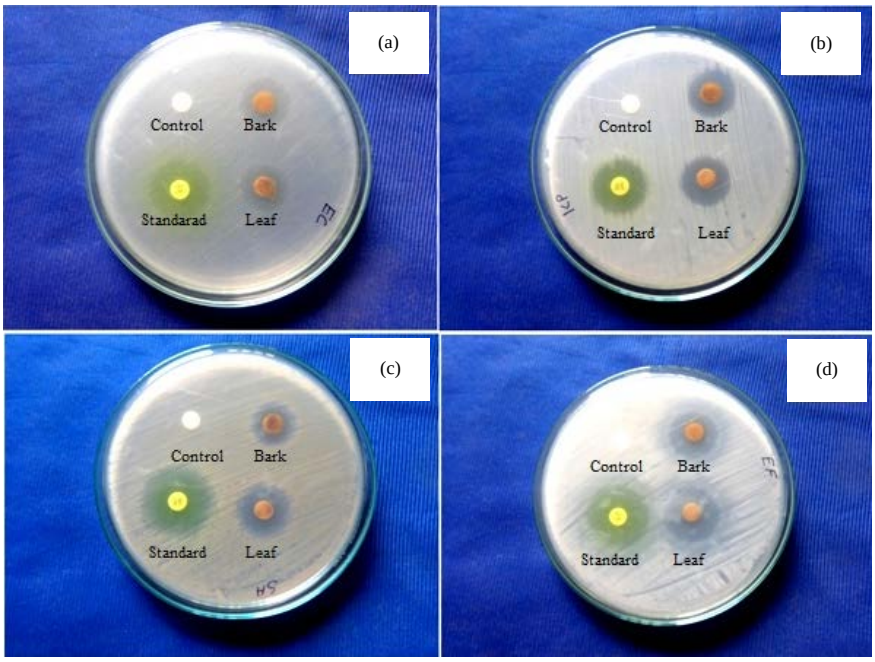

Fig. 4(a-d): Antibacterial activity of *Barringtonia acutangula* bark and leaves extract against UTI causing pathogens (a) *Escherichia coli*, (b) *Klebsiella pneumonia*, (c) *Staphylococcus aureus* and (d) *Enterococcus faecalis*

Table 3: Compounds identified in the bark extract of *Barringtonia acutangula*

Peak#	R.Time	Area (%)	Height (%)	Name
1	5.792	1.27	3.11	Butane, 1,1-diethoxy-3-methyl-
2	5.822	1.09	3.29	Pentane, 1,1-diethoxy-
3	6.007	0.99	2.56	3,3-Diethoxy-2-butanone
4	9.822	2.26	2.73	Tetradecane
5	12.773	5.33	5.76	Dodecane, 2,6,10-trimethyl-
6	13.975	1.05	1.27	Methyl-.alpha.-d-ribofuranoside
7	14.092	3.31	1.93	Oxalic acid, ethyl neopentyl ester
8	14.208	3.58	2.09	2,2,4-Trimethyl-3-pentanol
9	14.400	1.79	1.77	1,4-Methanocycloocta[d]pyridazine, 1,4,4a,5,6,9,10,10a-octahydro-11,11-dimethyl-, (1.alpha.,4.alph)
10	14.676	2.71	1.05	Chloroethyl 2-hexyl ether
11	15.409	4.51	5.22	Heptadecane
12	15.583	9.40	7.01	1,Benzenedicarboxylic acid, diethyl ester
13	17.773	1.82	2.90	Sulfurous acid, 2-ethylhexyl nonyl ester
14	18.235	0.90	1.85	2,6,10-Trimethyl,14-ethylene-14-pentadecane
15	18.729	4.95	6.50	1,2- Benzenedicarboxylic acid, bis(2-methylpropyl) ester
16	19.751	10.35	9.90	Dibutyl phthalate
17	19.903	5.94	5.44	Pentadecane, 2,6,10,14-tetramethyl-
18	19.992	1.10	2.67	2,4(1H,3H)-Pyrimidinedione, 6-amino-1,3-di-2-propenyl-
19	20.143	2.14	2.10	Silane, [(11-fluoroundecyl)oxy]trimethyl-
20	20.275	0.87	1.12	Fumaric acid, heptyl non-5-yn-3-yl ester
21	20.325	0.93	0.91	O,O-Dimethyl N-ethyl N-methylphosphoramidate
22	20.486	1.33	1.45	2-Ethylhexyl methyl isophthalate
23	20.864	0.95	1.37	1-Pentadecanol
24	21.751	3.09	2.52	2,4-Di-tert-butylthiophenol
25	21.783	1.50	2.49	N-Acetoacetyl valine, methyl ester
26	21.958	4.10	2.21	2-Thiatricyclo[3.3.1.1(3,7)]decane
27	22.034	4.07	3.91	Pentacontanoic acid, ethyl ester
28	22.182	2.01	1.89	Iron tritropolanate
29	22.394	2.47	1.19	p-Fluorobenzamidoxime
30	23.948	2.20	1.61	Tridecanal
31	28.699	11.12	9.57	1,2- Benzenedicarboxylic acid
32	29.646	0.86	0.63	Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate
		100.00	100.00	

Table 4: Biological activity of some identified compounds

Some identified compounds	Biological activity
1,6-Anhydro-beta-D-glucopyranose	Antihuman immunodeficiency virus and blood anticoagulant activities[17]
Butane, 1,1-diethoxy-3-methyl-	Food additives, flavouring Agents[21]
n-Hexadecanoic acid	Antioxidant[22]
Squalene	Antibacterial, antioxidant, antitumour and cancer preventive, immunostimulant, chemo preventive, lipoxygenase inhibitor, pesticide and diuretic[23]
Tridecane	Antimicrobial activity[24]
Friedelan-3-one	Antimicrobial activity[25]
Heptadecane	Antiinflammatory, antioxidant[26]
2, 6, 10-trimethyl 14-ethylene-14-pentadecane	Antiproliferative [27]
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Antimicrobial, anti-inflammatory, antidiuretic, anticancerous[28]
1-Pentadecanol	Antioxidant, antimicrobial[29]
1,2-benzene dicarboxylic acid (Chemical Name : Phthalic acd)	Antioxidant, antimicrobial, antifouling[29]
Hexadecane, 2,6,10,14-tetramethyl- (Chemical Name : Phytane)	Biomarkers in petroleum studies[29]
Pentadecane, 2,6,10,14-tetramethyl- (Chemical Name : Pristane)	Pathogenesis of rheumatoid arthritis and lupus[29]
Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate	Antimicrobial, anti-inflammatory, antiarthritic, antiuretic, antiasthmatic[29]
Tetradecane	Antifungal, antibacterial and nematicidal activity[29]
1,2-benzene dicarboxylic acid, diethyl ester	Cosmetics, insecticides, plasticizer[29]
Dibutyl phthalate	Antifungal, antimicrobial, antimalarial, plasticizer[29]
2-hexadecen-1-ol 3 7 11 15-tetramethyl-, [R-[R*,R*-(E)]] (Chemical Name : Phytol)	Anti-inflammatory activity, antimicrobial, anticancer, diuretic[18,30]

Table 5: Size of the inhibition zone for bark and leaf extract against UTI causing pathogens

	Zone of Inhibition (mm in diameter)			
Bacteria	Control	Standard*	Leaf (20 mg)	Bark (20 mg)
Escherichia coli	-	22	18	20
Klebsiella pneumonia	-	20	20	20
Staphylococcus aureus	-	26	20	18
Enterococcus faecalis	-	24	23	24

*Nitrofurantoin (100 µg)

their activity. Plant phenolics contribute a major group of compounds that act as primary antioxidants[13] which can react with hydroxyl radical [•OH], superoxide anion radicals [O^{2-}] and lipid peroxy radicals[9]. Hence, such a strong reducing activity is given by the leaves and bark extract of *Barringtonia acutangula*.

From the Table 1, the results showed remarkably higher total phenolic and flavonoid content in the leaves than bark extract. Phenolic compounds have redox properties, which allow them to act as antioxidants[14]. Plant flavonoids have antioxidant activity *in vitro* and also act as antioxidants *in vivo*[15,16].

From the GC-MS analysis, 37 phytocompounds were identified in the leaves and 32 phytocompounds were identified in the bark extract. Particularly 1,1-diethoxy-3-methylbutane identified both in leaves and bark extract. The presence of 1,1-diethoxy-3-methylbutane (DMB), never detected before in *Barringtonia acutangula*. The compound 1, 2-benzenedicarboxylic acid found in leaves and bark extract, known as Phthalic acid, a colourless, crystalline organic compound ordinarily produced and sold in the form of its anhydride. Smaller quantities were consumed in the manufacture of anthraquinone (a dye intermediate), phenolphthalein (a laxative and acid-base indicator) and phthalocyanine pigments. 1,6-Anhydro-beta-D-glucopyranose found in leaves extract commonly known as Levoglucosan, an important starting material for the synthesis of stereoregular polysaccharides possessing biological activities, such as the antihuman immunodeficiency virus and blood anticoagulant activities[17]. A compound 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]- found in leaves extract (RT 21.174), its chemical name is Phytol, an acyclic diterpene alcohol found in the essential oils of some aromatic plants. Various therapeutic activities of phytol have been reported in previous studies, including its activity against myco bacteria and anticonvulsant, antispasmodic and anticancer activities[18]. Pentadecane, 2,6,10,14-tetramethyl-(RT 19.903) found in leaves extract, commonly known as Pristane, a natural saturated terpenoid alkane obtained primarily from shark liver oil. Pristane is known to induce autoimmune diseases in rodents. It is used in research to understand the pathogenesis of rheumatoid arthritis and lupus[19,20].

The antimicrobial activity of *Barringtonia acutangula* was tested against UTI causing bacteria shown in Fig. 4. From the results *Barringtonia acutangula* could inhibit different typical pathogenic bacteria. Thus, *Barringtonia acutangula* could be considered as excellent broad-spectrum antibacterial agents. Phytocompounds extracted from the plant source can serve as a prototype to develop less lethal and efficient drug in controlling the development of micro-organism. These antimicrobial drugs have enormous potentially effective therapeutic value which involve in the treatment of infectious disease caused by microbes. In the present study, bark and leaf extracts of the *Barringtonia acutangula* were tested against Gram-negative and Gram-positive organisms. The presence of bioactive compounds in the plant which can be responsible for the observed antimicrobial property.

CONCLUSION

The investigation presents the first report on comparative analysis of GCMS, antioxidant and antimicrobial potential of leaf and bark extract of *Barringtonia acutangula.* The present study indicated that *Barringtonia acutangula* contains considerable amount of total phenols and flavonoids and exhibited good antioxidant and antimicrobial activity. The antioxidant and biological activities might be due to the synergistic actions of bioactive compounds present in them.

SIGNIFICANT STATEMENT

Plant extracts containing many active constituents which lead to the production of new drugs from various parts of plants. The objective of present study was to investigate the GC-MS analysis, antioxidant and antibacterial activity of *Barringtonia acutangula* L. leaves and bark extracts. The present study helped in identifying phytoconstituents present in the extracts, which are responsible for various biological and antibacterial activities. The significant activity of the leaves and bark extract shall be attributed particularly to the phenolic and flavonoid compounds, which forms the base of antioxidant properties. Based on the biological activity the *Barringtonia acutangula* leaf and extract, which can be used as a safe nutritional supplement without any adverse side effects.

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