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CHEMICAL COMPOSITION, ANTIMICROBIAL AND ANTIOXIDANT PROPERTIES OF *BARRINGTONIA ACUTANGULA* BARK EXTRACT

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ABSTRACT: *Barringtonia acutangula* (L.) Gaertn. (Family: Lecythidaceae), is a medium-sized tree found throughout India. It has been used in folk medicine to treat tuberculosis, skin disease, inflammation, hemorrhoids and diarrhea. The present study deals with *in-vitro* antioxidant, antimicrobial and GC-MS study on the bark extract of *B. acutangula*. In order to investigate the antioxidant activity, reducing power activity was used. Antibacterial activity was evaluated by disc diffusion method. Identification of extract bioactive compounds by GC-MS revealed that alpha-Pinene oxide, 1,2-benzoldicarbonsaeure di-(hex-1-en-5-yl-ester), 3-methyl-4-heptanone, (Z)-3-hexadecene are the major components. The antioxidant activity of the bark extract increased with increasing concentration; the values are comparable with the standard antioxidant ascorbic acid. From the results of antibacterial activity, *B. acutangula* bark extract could inhibit gram-negative and gram-positive pathogenic bacteria. Thus, *B. acutangula* could be considered as an excellent broad-spectrum antibacterial agent.

INTRODUCTION: Herbal plants play a key role in the treatment of complex diseases in humans due to their complex mixture of secondary metabolites [1]. *Barringtonia acutangula Linn.* is a medium size glabrous tree found throughout India in deciduous and evergreen forests, mostly along the bank of rivers and streams [2]. *B. acutangula* is one of the useful traditional medicinal plant in India. It is used in various Ayurvedic formulations for the treatment of various diseases [3]. *B. acutangula is used in managing diabetes, obesity, hypertension, and cardiovascular diseases an*d in various polyherbal formulations [4, 5].

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The roots, leaves, fruits, and seeds are used for therapeutic purposes. Traditionally, parts of B. acutangula, like root, seed, bark, and leaf, are used as carminative, expectorant, bitter tonic, emetic, and also in treating diarrhea and gonorrhea. Seeds and leaves are useful in treating colic, intestinal worms, wounds, ulcers, skin diseases, and hallucinations; the bark is an astringent in diarrhea and a febrifuge in malaria [6-12]. Natural antioxidants present in plants scavenge harmful free radicals from our bodies.

Free radicals are any species capable of independent existence that contains one or more unpaired electrons which react with other molecules by taking or giving electrons and are involved in many pathological conditions [13]. These free radicals are highly unstable, and when the amount of these free radicals exceeds in the body, it can damage the cells and tissues and may involve in several diseases. Thus, antioxidants of natural

origin need because they can protect the human body from diseases caused by free radicals [14, 15]. An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Currently available synthetic antioxidants like BHT, butylated hydroxyl anisole (BHA), and tertiary butylated hydroquinones have been suspected of causing or prompting negative health effects [16, 17]. Hence, strong restrictions have been placed on their application, and there is a trend to substitute them with naturally occurring antioxidants [18]. Recently, medicinal plant, herb, and spice extracts are emerging as alternatives to traditional natural preservatives, as they are generally healthy for humans and environmentally friendly [19-21]. This study aims to evaluate *in-vitro* antioxidant and antimicrobial activity and characterize the chemical constituents of *Barringtonia acutangula* L. bark extract using gas chromatography-mass spectrometry (GC–MS).

MATERIALS AND METHODS: The plant material (bark) was collected from Madurai, Tamil Nadu. The bark was collected in bulk, shade dried for 2 days and then dried in the hot air oven at 50 °C for 12 h. Dried bark were pulverized by a mechanical grinder and the coarse powder obtained was taken for ethanol (95%) extraction using Soxhlet assembly for 48 h each. The collected specimens are authenticated by Dr. S. John Brito, The Director, The Rapinat Herbarium and center for Molecular systematics, St. Joseph's college, Trichy, Tamil Nadu, India.

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.

Antimicrobial Assay: The *in-vitro* screening for antimicrobial was carried out using selected pathogens which includes two gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two gram negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae)* and two fungal species (*Aspergillus niger* and *Aspergillus flavus*).

Disc Preparation: The 6mm (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 in hot air oven at 50°C. Then various solvent extract discs and control discs were prepared.

Collection of Test Microorganism: The Bacterial strains of *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae* and *Staphylococcus aureus* were obtained from Microbial Type culture Collection Centre (MTCC), Chandigarh. The fungal strains of *Aspergillus niger* and *Aspergillus flavus* were obtained from the Microbial Type culture Collection Centre (MTCC), Chandigarh.

Assay of Antibacterial Activity: Antibacterial activity test was carried out following the modification of the method originally described by Bauer *et al.,* (1966) [22]. Muller Hinton agar was prepared and autoclaved at 15 lbs pressure for 20 minutes and cooled to 45ºC. The cooled media was poured onto sterile petriplates and allowed for solidification. The plates with media were seeded with the respective microbial suspension using sterile swab. The various solvents extract-prepared discs individually were placed on each petriplates and also placed control and standard (Nitrofurantoin (300 µg) for Bacteria) discs. The plates were incubated at 37ºC for 24 hrs. After incubation, the diameter of the zone formed around the paper disc was measured and expressed in mm.

Assay of Antifungal Activity: Antifungal activity test was carried out following the modification of the method originally described by Bauer *et al.* (1966) [22]. Potato Dextrose Agar (PDA) was prepared and autoclaved at 15 lbs pressure for 20 minutes and cooled to 45ºC. The cooled media was added 10ml/L tartaric acid (10%) to act as antibacterial agents, poured onto sterile petriplates, and allowed for solidification. The plates with media were seeded with the respective microbial suspension using sterile swab. The various solvents extract-prepared discs were individually placed on each petriplates and control and standard (Amphotericin-B (10 µg)) discs. The plates were incubated at 28ºC for 72 hrs. After incubation, the diameter of the zone formed around the paper disc was measured and expressed in mm.

Reducing Power Activity: The reducing power of the bark extract was determined according to the method of Oyaizu, (1986) [23]. The sample extract of varying concentrations (20–100 µg/ml) were taken in 2 mL of 0.2M phosphate buffer in a test tube and 5 mL of 0.2 M phosphate buffer (pH 6.6). To this 5 ml of 1% potassium ferricyanide was added. After incubation at 50 °C for 20 minutes, the mixture was mixed with 2.5 ml of 10% trichloro acidic acid and then centrifuged at 3000 RPM for 10 minutes. The supernatant (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride.

Then the absorbance of the reaction mixture was measured at 700 nm by the spectrometer. The increased absorbance of the reaction mixture indicated increasing reducing power.

RESULTS AND DISCUSSION:

Chemical Composition by GC-MS Analysis: Interpretation on mass spectrum GC-MS was conducted using the National Institute Standard and Technology (NIST) database. 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, 20 bioactive phytochemical compounds were identified in bark's ethanol extract (**Fig. 1** and **Table 1.**

TABLE 1: CHEMICAL COMPONENTS IDENTIFIED IN THE BARK EXTRACT OF *B. ACUTANGULA* BY GC-MS

Peak#	R. Time	Area	Area%	Height	Height%	A/H	Name
1	5.067	267085	13.06	122103	21.65	2.19	alpha.-Pinene oxide
2	5.420	69815	3.41	22439	3.98	3.11	Cyclohexane, octyl- (CAS) n-Octylcyclohexane
3	7.217	72206	3.53	34042	6.04	2.12	1-Heptanol, 6-methyl- (CAS) 6-Methyl-1-heptanol
4	9.445	32483	1.59	8328	1.48	3.90	1,2-Propadiene (CAS) Allene
5	9.742	176912	8.65	54438	9.65	3.25	3-HEXADECENE, (Z)-
6	9.823	157143	7.68	46423	8.23	3.39	4-Heptanone, 3-methyl- (CAS) 3-Methyl-4-heptanone
7	9.995	504152	24.65	60574	10.74	8.32	1,2-BENZOLDICARBONSAEURE, DI-(HEX-1-EN-5-YL-ESTER)
8	10.108	42845	2.09	12205	2.16	3.51	1-Propyne (CAS) Propyne
9	11.817	43526	2.13	9597	1.70	4.54	Heptanoic acid (CAS) Heptoic acid
10	12.090	72377	3.54	35750	6.34	2.02	1-Heptanol, 6-methyl- (CAS) 6-Methyl-1-heptanol
11	12.152	26512	1.30	12781	2.27	2.07	Docosane (CAS) n-Docosane
12	13.000	45461	2.22	7269	1.29	6.25	2-[3'-(1"-HYDROXY-1"-METHYLETHYL)-2',2'-DIMETHYLCYCLOBUTYL] ETHANAL
13	13.126	30210	1.48	12135	2.15	2.49	Pentane, 3-methyl- (CAS) 3-Methylpentane
14	14.000	86810	4.24	26177	4.64	3.32	Hexadecanoic acid (CAS) Palmitic acid
15	14.137	74556	3.64	17687	3.14	4.22	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester (CAS) Isobutyl phthalate
16	14.236	88659	4.33	29310	5.20	3.02	1-Undecene, 9-methyl-
17	14.292	28444	1.39	14262	2.53	1.99	Heptane (CAS) n-Heptane
18	15.400	41511	2.03	7250	1.29	5.73	DIDECYL 1,4-DIHYDRO-2,6-DIMETHYL-3,5-PYRIDINEDICARBOXYLATE
19	20.001	50604	2.47	7020	1.24	7.21	[1,1':4',1"-terphenyl]-2',5'-diamine, N,N'-dicyclohexyl-
20	22.925	134345	6.57	24137	4.28	5.57	1-OCTANOL, 2-METHYL-
		2045656	100.00	563927	100.00		

Biological activity of some identified components is listed here. Alpha-pinene oxide (RT 5.067) is an epoxide of alpha-pinene. It has a role as a fragrance, a bacterial xenobiotic metabolite and a human metabolite. This enzyme participates in limonene and pinene degradation. Allenes (RT 9.445) are classified as cumulated dienes. Compounds with an allene-type structure but more than three carbon atoms are members of a larger class of compounds called cumulenes. These compounds are not just interesting intermediates but synthetically valuable targets themselves; for

example, over 150 natural products are known with an allene or cumulene fragment [24]. 3-methylpentane (RT 13.126) is an alkane that is pentane which a methyl group substitutes at position 3.

It is used as a solvent in organic synthesis, lubricant, and raw material for producing carbon black. Compounds n- octylcyclohexane (RT 5.420), heptanoic acid (RT 11.817), and n-docosane (RT 12.152) act as plant metabolites.

Palmitic acid (RT 14.000) induced significantly elevated levels of biologically active neutrophil chemoattractant. Cells can use it as fuel to generate energy and palmitic acid is a key component of cell membranes, which regulate cellular activities. Cells can use palmitic acid as fuel to generate the energy necessary to perform their biological functions [25].

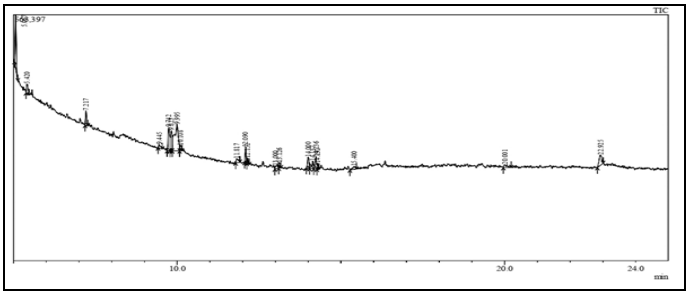

FIG. 1: GC-MS CHROMATOGRAM OF *B. ACUTANGULA* BARK EXTRACT

Assay of Antimicrobial Activity: The antimicrobial activity of *Barringtonia acutangula* was tested against disease-causing bacteria *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Bacillus subtilis*, *Aspergillus niger* and *Aspergillus flavus* compared with the standard, the diameters of inhibition zones increased for all the test pathogens **Table 2** & **Fig. 2.**

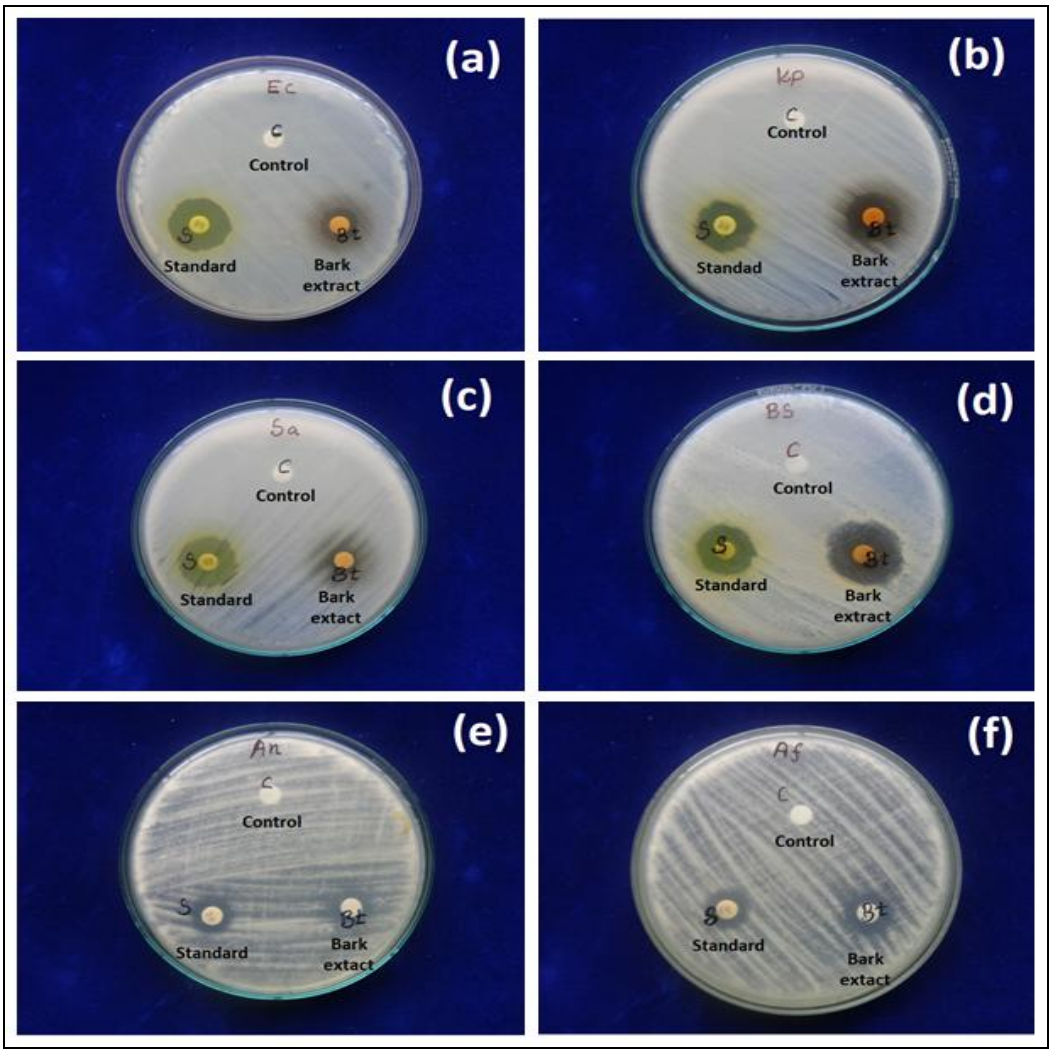

FIG. 2: ANTIBACTERIAL ACTIVITY OF *BARRINGTONIA ACUTANGULA* BARK EXTRACT AGAINST PATHOGENS (A) *ESCHERICHIA COLI* (B) *KLEBSIELLA PNEUMONIA* (C) *STAPHYLOCOCCUS AUREUS* (D) *BACILLUS SUBTILIS (E) ASPERGILLUS NIGER AND (F) ASPERGILLUS FLAVUS*

TABLE 2: ZONE OF INHIBITION VALUES OF BARK EXTRACT AGAINST DIFFERENT PATHOGENS

S. no.	Microorganism	Zone of Inhibition (mm in diameter)		
		Control	Standard*	Sample
1	*Escherichia coli*	-	21	15
2	*Klebsiella pneumoniae*	-	18	18
3	*Staphylococcus aureus*	-	20	14
4	*Bacillus subtilis*	-	20	24
5	*Aspergillus niger*	-	13	13
6	*Aspergillus flavus*	-	14	15

*NIT-Nitrofurantoin (300µg); AP-Amphotericin-B

The results of antibacterial activity showed that *B. acutangula* could inhibit gram-negative and gram-positive pathogenic bacteria. Thus, *B. acutangula* could be considered an excellent broad-spectrum antibacterial agent. Bioactive phytocompounds extracted from the plant can serve as an efficient drug in controlling microorganism development. The phytochemicals in the bark can be responsible for the observed antimicrobial property.

Antioxidant Activity: The reducing property is generally associated with the presence of reductants. The antioxidant action of reductants is based on breaking the free radical chain by donating a hydrogen atom. Reductants also react with certain precursors of peroxide, thus preventing peroxide formation. The presence of phytochemicals may act as reductants by donating the electrons and reacting with free radicals to convert them to more stable products and terminate radical chain reactions. **Fig. 3** showed the reducing power (as indicated by absorbance at 700 nm) of extract, which increased concentration to a certain extent and then levelled off with a further increase in concentrations. A strong reducing power was noted at 100 µl and the values are comparable with the standard antioxidant ascorbic acid.

A similar relationship between the chemical constituents and reducing power activity has been reported for several plants extracts [26, 27]. The results elucidate that phytochemicals of the extract appear to function as good electron and hydrogen donors and, therefore, should be able to terminate radical chain reactions by converting free radicals to more stable products.

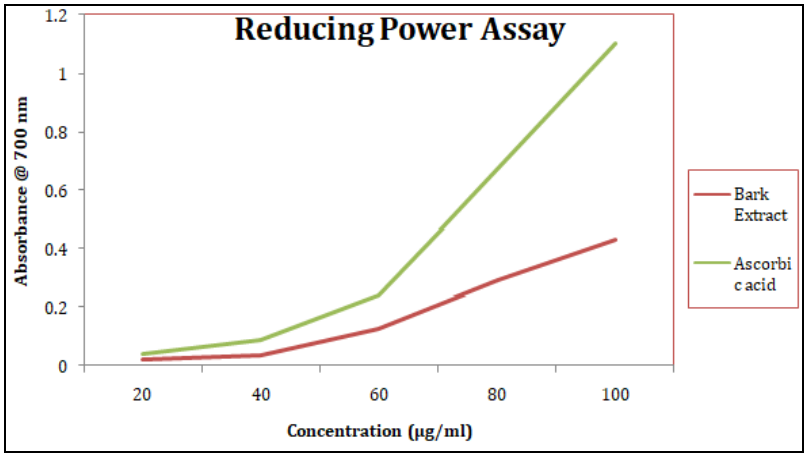

FIG. 3: REDUCING POWER ACTIVITY OF *B. ACUTANGULA* BARK EXTRACT

CONCLUSION: Herbal medicines occupy a distinct position from the primitive period to the present day. Therapeutically, important drugs have been developed from plant sources used in traditional medicine systems. It may be concluded that the ethanol extract of *B. acutangula*, demonstrated antioxidant and antimicrobial activity. This finding substantiates its traditional uses in treating various disorders and increases the interest and potential use of this sample as a nutraceutical and pharmacological agent. Further isolation and purification of compounds from this extract and study of their biological effects may provide further information on their medicinal value.

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CONFLICTS OF INTEREST: The author declares that no conflicts of interest exist.

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