

Antioxidant, Antimicrobial Studies and Investigation of Secondary Metabolites from Stem Bark of *Barringtonia acutangula* (L.)

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ABSTRACT

In the present study, the bioactive components of *Barringtonia acutangula* stem barks have been evaluated using Gas Chromatography - Mass Spectrum (GC-MS). Seven compounds in ethanolic extract were identified. 9-Octadecenoic acid, (E) (21.64%) was the prevailing compound in ethanolic extract, which is suggested to be an anticancer compound. The ethanol extract of stem bark was found to exhibit *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella typhi*, *Klebsiella* and *Escherichia coli*. The extract and fractionates of fresh stem bark of *Barringtonia acutangula* showed a significant and remarkable activity against *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella typhi*, *Klebsiella* and *Escherichia coli* when compared to standard. It indicates the presence of these biologically active chemical in *Barringtonia acutangula* may justify their wide usage in traditional medicine. Based on the antioxidant analysis obtained, it was showed that the extract of *Barringtonia acutangula* stem bark exhibits the greatest antioxidant activity through the DPPH radicals scavenging activity. The present study concludes that the ethanol extract of stem bark of *Barringtonia acutangula* contains broad spectrum of bioactive compounds and also exhibit antimicrobial activity against all the tested microorganisms. It indicates the presence of these biologically active chemical in *Barringtonia acutangula* may justify their wide usage in traditional medicine.

Keywords: *Barringtonia acutangula* Linn, *Salmonella typhi*, Antioxidant, Antimicrobial activity, GC – MS.

INTRODUCTION

Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years – such extensive dependence of human being on “Mother Nature” has invoked tremendous interest in the scientific world, which ultimately led to the isolation of a vast number of chemical agents with potentials for multipurpose uses. Plants have economic and environmental uses, depending on the natural characteristics. Some are consumed in human diet, while other species have medicinal values and still other species are good resource of minerals and vitamins.

Barringtonia acutangula is a species of *Barringtonia* native to coastal wetlands in southern Asia and northern Australasia, from Afghanistan east to the Philippines and Queensland. The plant has Antitumor, Antibiotic, Antifungal and Inhibit growth of *Helicobacter pylori* activities.

The word “phyto” is the Greek word for plant. Phytochemicals which not only that they are non nutritive plants chemicals they have protective or disease preventive properties but also protect human from a host of disease. Phytochemical studies have shown that plants with antimicrobial activity contain bioactive constituents such as tannins, flavonoids, alkaloids and saponins. Alkaloids and flavonoids have been used as antiviral,

antibacterial, antimicrobial and anticancer agents. Phenolic and polyphenolic are the other group of secondary metabolites. The uses of plant – derived products as disease control agents have been studied, since they tend to have low Mammalian toxicity, less environmental effects and wide public acceptance.

Antioxidants are substances that are used to fight against the free radicals which has involved in food and chemical material degradation and oxidize nucleic acids, proteins and lipids which will cause oxidative stress and initiating degenerative diseases such as cancer, Alzheimer’s disease, Parkinson’s diseases and some cardiovascular diseases Over the years, prevalence of the diseases result from oxidative stress has been increasing over the year.

Hence the present paper reports the antimicrobial, antioxidant properties and Gas Chromatography - Mass Spectrometry (GC – MS) analysis on the isolated essential oil of *Barringtonia acutangula* Linn stem and barks.

MATERIALS AND METHODS

Plant Collection: The fully mature *Barringtonia acutangula* stem barks were collected in July 2014 from Karaikudi (Village), Paramakudi (Taluk), Ramnad District, Tamil Nadu, India from a single tree. The stem barks were washed to remove soil particles and impurities

Table 1: Bioactive components detected in the solvent extracts of the stem and barks of *Barringtonia acutangula*

S.No	RT	Name of the Compound	Molecular Formula	Molecular Weight	Peak area %
1	8.72	1-methoxy-2-(methoxymethyl) benzene	C ₉ H ₁₂ O ₂	152	9.02
2	15.12	2[(5,6-dimethyl-1H-benzimidazol-2-yl) sulfanyl] ethanol	C ₁₁ H ₁₄ N ₂ OS	222	13.40
3	15.33	5-amino-2-hydroxy-3-propylbenzoic acid	C ₁₀ H ₁₂ NO ₃	195	9.02
4	17.25	Pentadecenoic acid, 13-methyl-methyl ester	C ₁₇ H ₃₄ O ₂	270	10.82
5	18.13	5-methyl-7-phenyl-1,3-diazaadamantan-6-one Hydrazone	C ₁₅ H ₂₀ N ₄	256	15.20
6	19.03	3-Octadecenoic acid, methyl ester	C ₁₉ H ₃₆ O ₂	296	20.87
7	19.88	9-Octadecenoic acid, (E)	C ₁₈ H ₃₄ O ₂	282	21.64

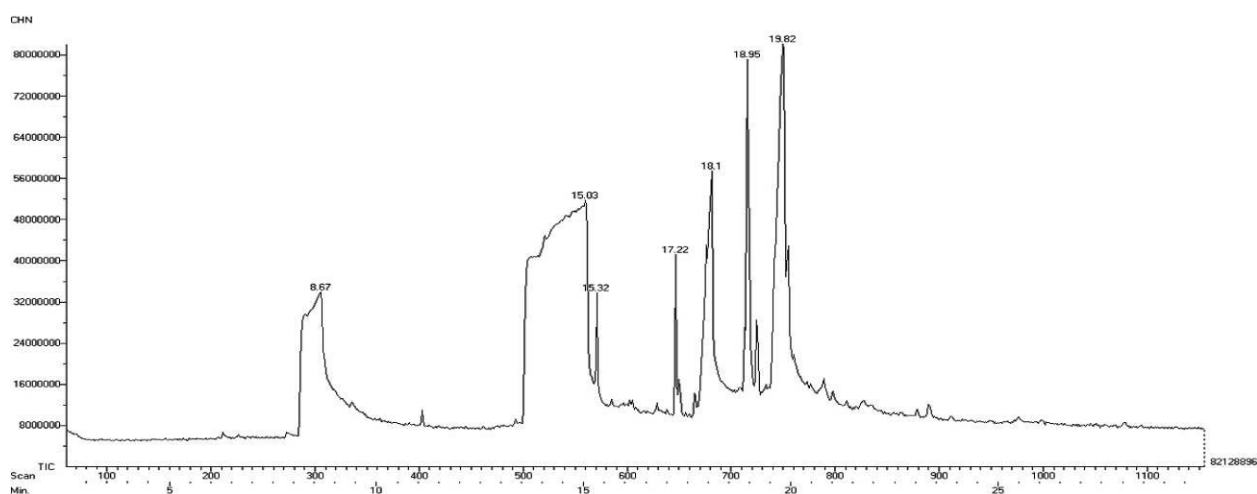
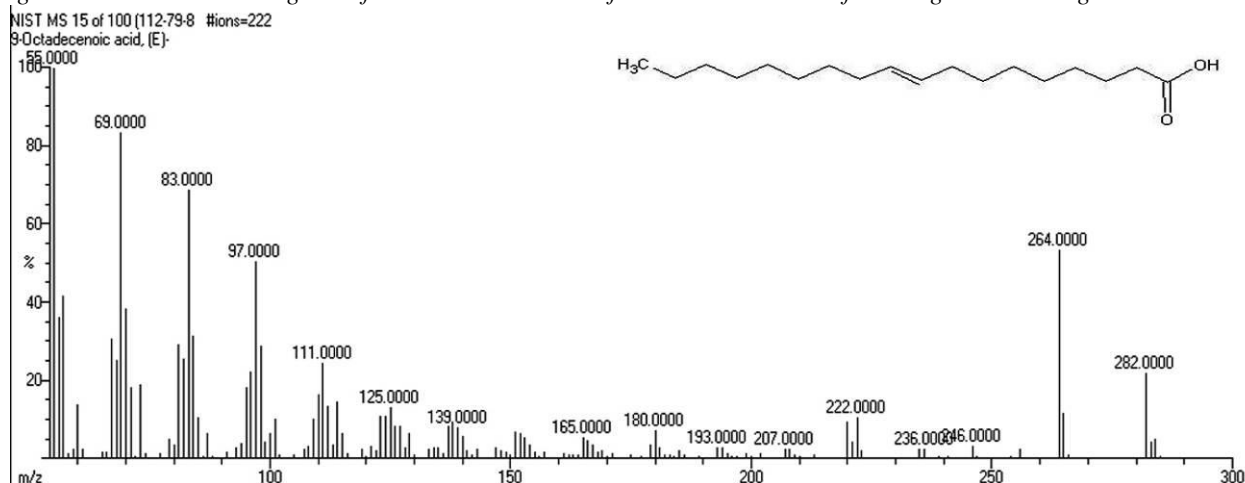
Fig. 1: GC – MS Chromatogram of the solvent extracts of the stem and barks of *Barringtonia acutangula*

Fig. 2: Mass Spectrum of 9-Octadecenoic acid, (E)

by using distilled water. The stem bark was dried for 20 days under low sun intensity. The dried materials were powdered mechanically and they were stored in polythene bag to be used as samples for the extraction. Preparation of Extracts: 100 g of the *Barringtonia acutangula* stem bark was extracted with 250 ml of Ethanol in a round bottom flask using reflux condenser apparatus. The reaction was carried out for 24 hours and the extract was collected the excess ethanol was removed by using a distillation process. The extract was stored in refrigerator until used. The extract contained both polar

and non-polar phyto components of the plant material used. (R. Sasi Kumar, 2014).

GC – MS Analysis: GC-MS analysis of the methanol extract of *Barringtonia acutangula* was performed in a Perkin–Elmer GC Clarus 500 system comprising an AOC-20i auto-sampler and a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with a Elite-5MS (5% diphenyl/95% dimethyl poly siloxane) fused a capillary column (30 × 0.25 μm ID × 0.25 μm df). For GC-MS detection, an electron ionization system was operated in electron impact mode with

Table 2: Antimicrobial activity of *Barringtonia acutangula* L. ethanol extract against bacteria pathogens with reference to Amikacin

Compound	Weight of the Compound (µg/ml)	Zone of inhibition (in mm)				
		<i>Salmonella Typhi</i>	<i>Staphylococcus aureus</i>	<i>Bacillus cereus</i>	<i>Klebsiella</i>	<i>Escherichia coli</i>
<i>Barringtonia acutangula</i>	30	14	12	9	12	14
<i>Barringtonia acutangula</i>	50	21	18	15	20	21
Amikacin (Standard)	30	17	18	18	17	17

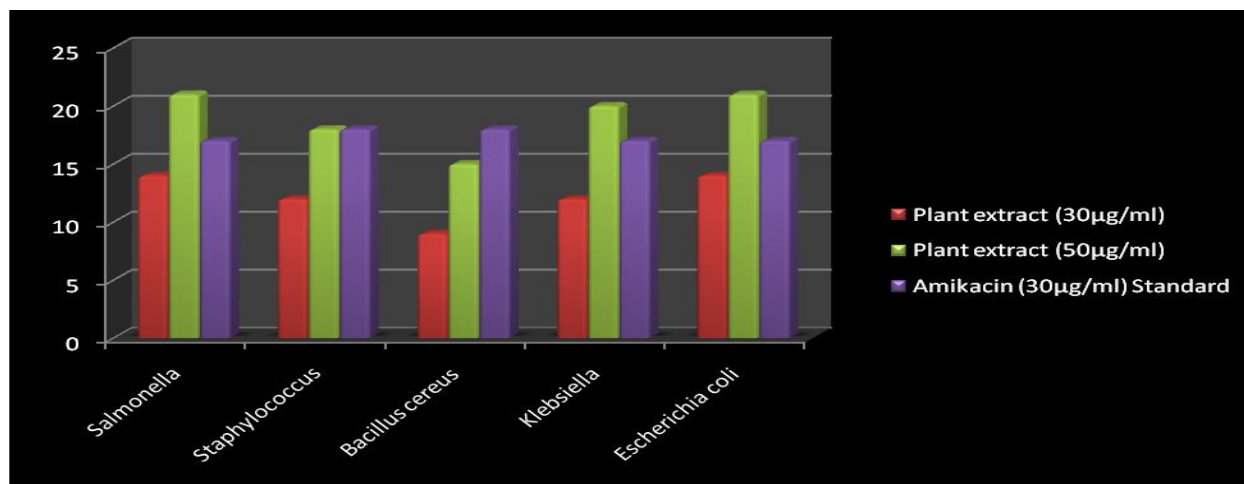
Fig. 3: Comparison of Antimicrobial activity of *Barringtonia acutangula* L. stem and barks and Standard Amikacin

Table 3: DPPH radical scavenging activity of plant extract and standard (Ascorbic acid)

Concentrations (µg/ml)	Plant extract (% of inhibition)	Ascorbic acid (% of inhibition)
20	21.34±1.49	41.00 ± 9.4
40	43.37±3.03	68.10 ± 8.6
60	61.45±4.30	84.64 ± 7.9
80	89.54±6.26	98.23 ± 5.4
IC ₅₀ (µg/ml)	45.78	25.57

Values are expressed as Mean ± Standard deviation for triplicates

ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 2µl was employed (a split ratio of 10:1). The injector temperature was maintained at 250°C, the ion-source temperature was 200°C, the oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min to 200°C, then 5 °C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450 Da. The solvent delay was 0 to 2 min, and the total GC-MS running time was 36 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The mass-detector used in this analysis was Turbo-Mass Gold-Perkin-Elmer, and the software adopted to handle mass spectra and chromatograms was a Turbo-Mass ver-5.2.

Identification of Bioactive compounds: Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST)

having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

Antimicrobial Activity: The anti bacterial activity for the stem bark of *Barringtonia acutangula* was determined by Disc-Diffusion method. The antibacterial activity studies in nutrient agar medium for the following organisms *Salmonella typhi*, *Escherichia coli*, *Klebsiella pneumonia*, *Bacillus cereus*, and *Staphylococcus aureus* were carried out.

Preparation of Nutrient Agar Medium: Exactly 1g of peptone 0.5g of Beef extract and 0.5 g of sodium chloride were weighed and transferred into conical flask and dissolved in 100ml of distilled water after the pH range was checked for 7.0 – 7.2 finally added 1.5 g of agar into the conical flask. It was closely packed with cotton plug and placed in an autoclave for 15 minutes for sterilization. The antimicrobial assay was carried out



Fig. 4: Zone of inhibition – bacteria *Salmonella typhi*



Fig. 5: Zone of inhibition – bacteria *Staphylococcus aureus*



Fig. 6: Zone of inhibition – bacteria *Bacillus cereus*

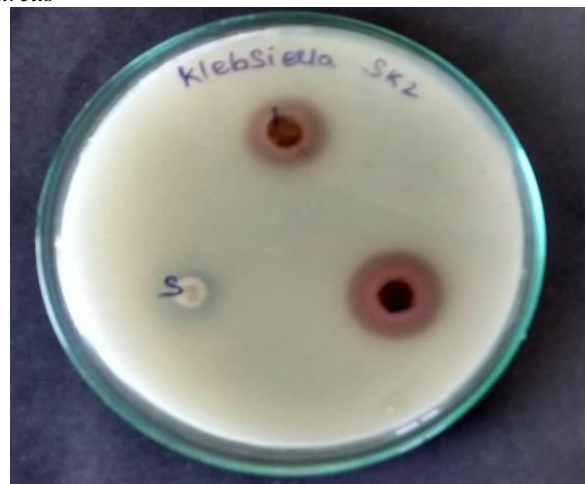


Fig. 7: Zone of inhibition – bacteria *Klebsiella*

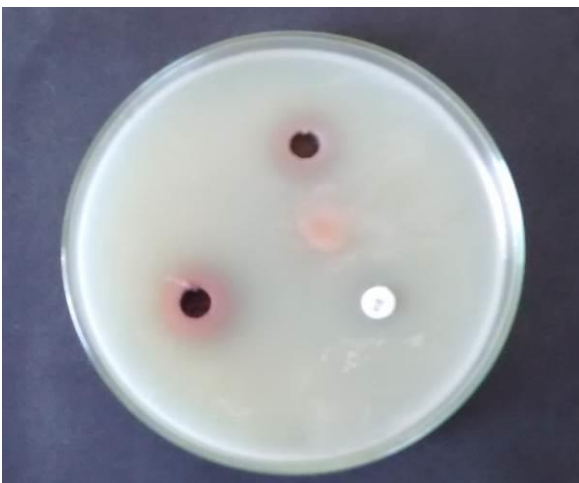


Fig. 8: Zone of inhibition – bacteria *Escherichia coli*

using Agar well diffusion method. Amikacin is used as reference drug and corresponding solvent (Ethanol) is used as positive controls. About 20 ml of nutrient agar medium for bacteria poured sterilized Petri dishes and allow solidifying. The agar medium was spread was 24 hrs cultured 10⁸ CFU/ml microbial sterilized rod. Wells of 6 mm diameter were made in the culture medium using

sterile cork borers. About 30 µg and 50 µg of the plant extracts (1 µg/ml) was added the wells. Plates were than incubated at 37 °C for 24 h. Antimicrobial activity was evaluated by measuring the inhibition zone diameters in mm formed around the well. The assay was carried out in triplicates and the results thus obtained are taken as the mean of the three readings for each concentration and no statistical tools were used calculate the standard deviation. The Antimicrobial activity of *Barringtonia acutangula* L. stem barks of ethanol extract against bacteria pathogens with reference to Amikacin is reported in table 3 and also represented as Figure 3.

Screening for Antioxidant assay

Preparation of sample: The different concentrations of plant extract (20, 40, 60 and 80 µg/ml) were chosen for *in vitro* antioxidant activity. L-Ascorbic acid was used as the standard.

DPPH radical-scavenging activity: DPPH radical-scavenging activity was determined by the method of Shimada, *et al.*, (1992) [14]. To added 2 ml aliquot of DPPH solution (25µg/ml) to 0.5 ml sample solution at different concentrations. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. Then the absorbance was measured at 517nm in a spectrophotometer. Lower absorbance of

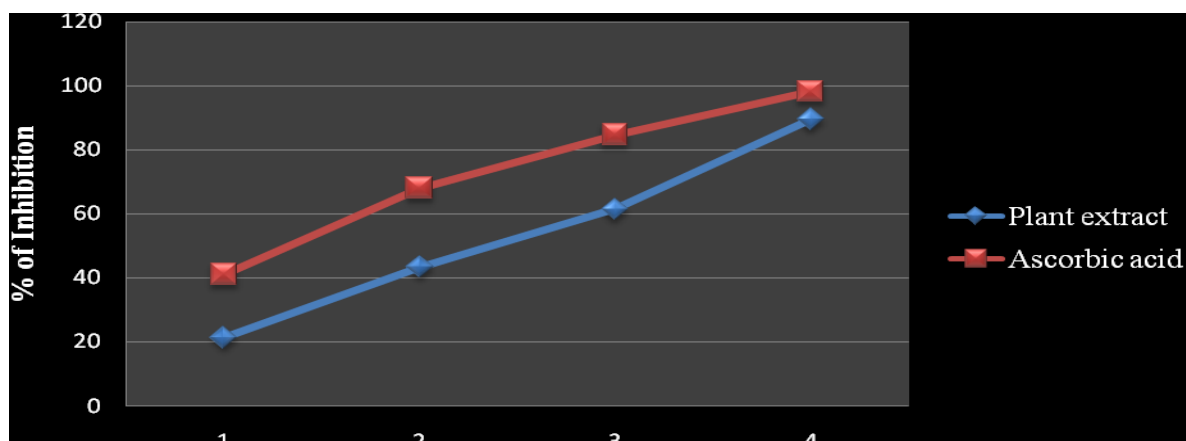


Fig. 9: DPPH radical scavenging activity of plant extract and standard (Ascorbic acid)

the reaction mixture indicated higher free-radical scavenging activity.

Radical scavenging activity (%) =

$$100 - \left(\frac{A_c - A_s}{A_c} \right) \times 100$$

Where A_c = control is the absorbance and A_s = sample is the absorbance of reaction mixture (in the presence of sample).

Statistical analysis: Tests were carried out in triplicate for 3 separate experiments. The scavenging activity of sample was expressed as 50% inhibition concentration (IC_{50}), which represented the concentration of sample having 50% of radical scavenging effect. The amount of extract needed to inhibit free radicals concentration by 50%, IC_{50} , was graphically determined by a linear regression method using Ms - Windows based graph pad

Instant (version 3) software. Results were expressed as graphically / Mean \pm standard deviation.

RESULTS AND DISCUSSION

GC - MS Analysis: The bioactive components present in the ethanolic extracts of *Barringtonia acutangula* were identified by GC-MS analysis (Figure 1). The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) in the ethanolic extracts of *Barringtonia acutangula* are presented in Table 1. Seven compounds were identified in ethanol extract by GC-MS. The major secondary metabolites present in the stem barks of *Barringtonia acutangula* were 9-Octadecenoic acid, (E) (21.64%), 1-methoxy-2-(methoxymethyl) benzene (9.02%), 2[(5,6-dimethyl-1H-benzimidazol-2-yl) sulfanyl] ethanol (13.40%), 5-amino-2-hydroxy-3-propylbenzoic acid (9.02%), Pentadecenoic acid 13-methyl-methyl ester (10.82%), 3-Octadecenoic acid, methyl ester (20.87%) and 5-methyl-7-phenyl-1,3-diazadamontan-6-one Hydrazone (15.20%). Figure 2 shows the mass spectrum and structure of medicinally important bioactive

compound which contribute to the medicinal activity of the ethanolic extracts of *Barringtonia acutangula*. 9-Octadecenoic acid, (E) is the major component found in the whole plant of *Barringtonia acutangula* which is being used for the pharmacological work.

Antimicrobial Activity

DPPH radical scavenging activity of sample: DPPH radical scavenging activity (%) can be calculated by using the formula as mentioned previously. The values obtained are tabulated in Table 3 and it is expressed as mean \pm standard deviation.

CONCLUSION

In the present study, seven compounds have been identified from ethanolic extract of the stem barks of *Barringtonia acutangula* by Gas chromatography – Mass spectrometry (GC-MS) analysis. Secondary metabolites such as alkaloids, flavonoids, terpenoids, sterols and phenolic compounds were detected to be present in the stem bark of *Barringtonia acutangula* plant. The present study portrays that the secondary metabolites in fresh *Barringtonia acutangula* stem barks may contribute in many significant ways for various studies in a truthful manner to the pharmaceutical activity of the plant. The antioxidant results showed that the extract of *Barringtonia acutangula* stem barks exhibits the greatest antioxidant activity through the DPPH radicals scavenging activity. Since this plant had been used in the treatment of different ailment such as malaria, cancer and skin burn etc., the medicinal roles of these plants could be related to such identify bioactive compounds.

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