



## **Phytochemical Screening and Anthelmintic Activity Study of Different Stem Bark Extract of *Barringtonia Acutangula*.**

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### **ABSTRACT**

Medicinal plants have much creative property due to the presence of many complex chemical substances with different chemical composition which are found as secondary plant metabolites in many parts of these plants. The aim of the study is to investigate Phytochemical screening of Petroleum ether and methanol of the stem bark extract of *Barringtonia Acutangula* powdered and the presence of different secondary metabolites responsible for the therapeutic values of the drug. Qualitative phytochemical studies were performed on petroleum ether; Ethyl acetate and methanolic extract of *Barringtonia Acutangula* stem bark. The study was performed to identify its Alkaloid, Carbohydrate, Glycoside, Protein, Amino acid and flavonoids by using suitable chemicals and reagents of the different extract. The anthelmintic study shows all the two *in vitro* test species (*Pheretima posthuma* and *Ascaridia galli*) responded towards our plant extracts by showing the sign of paralysis and death finally. and observed that the Ethyl acetate extract was most potent which is well comparable with both standard drugs as Albendazole and Piperazine citrate, followed by Methanolic extract at higher doses. Petroleum Ether extracts of all the plants were endowed with minute anthelmintic property, The Preliminary Phytochemical studies of stem was performed on its different extracts to identify its Alkaloid, Carbohydrate and Glycoside, Saponin, Protein & Amino acid, and many mores. The anthelmintic activity of the extract of ethyl acetate showing most potent as it contains mainly flavones and flavonoids, triterpenoids and phenolic compounds which may be responsible for this activity.

**Keyword:** *Barringtonia Acutangula*, Phytochemical evaluation, Anthelmintic activity. piperazine citrate, *Pheretima. posthuma*, , *Ascaridia galli*.

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## INTRODUCTION

Historically, the most important uses of herbs were medicinal. For most of his existence, man had various but limited resources for treating injuries and disease. Plant remedies represent the most continuous and universal form of treatment. Ancient wisdom has been the basis of modern medicine and will remain as one important source of future medicine and therapeutics. The future of natural product drug discovery will be more holistic, personalized and involve wise use of ancient and modern therapeutic skills in a complimentary manner so that maximum benefits can be accrued to the patients and the community [1]. There are approximately 1,250 Indian medicinal plants that are used in formulating therapeutic preparation according to Ayurvedic & other traditions [2]. India has about 45,000 plant species; medicinal properties have been assigned to several thousands. The government of India has format structure to regulate quality, safety, efficacy & practice of herbal medicine. A number of companies, including some multinational are entering into the area of herbal medicines. The medicines are available for each & every disorder ranging from diabetes to rejuvenators. Demand of medicinal plants is increasing in both developing and developed countries due to growing recognition of natural products [3]

### **Impact of ethno pharmacology in Modern Medicine**

Traditional medicine is a general, powerful source of biological activity. Ethnopharmacology is not just a science of the past using an out molded approach. It still constitutes a scientific backbone in the development of active therapeutics based upon traditional medicine. The ultimate aim of ethnopharmacology is the validation of these traditional preparations. Natural products & especially those derived from higher plants have historically played a pivotal role in the discovery o new pharmaceuticals [4]

## MATERIALS AND METHOD

The different Mayer's, Hager's, Barfoed's, Benedict's and millon's reagent, Wagner's, Dragendorff's, Fehling's A & B,  $\alpha$ -naphthol, Ferric chloride, Conc. Sulphuric acid, Pyridine, Sodium nitropruside, Acetic anhydride, were purchased from S.D. Fine Chemical, Mumbai. The solvents petroleum ether, Chloroform, and Ethanol were purchased from Hi Media Laboratories Pvt. Ltd., Mumbai. All others chemicals, solvents and reagents were of analytical grade and procured from authorized dealer. Other chemicals were prepared in the laboratory as, 10 % Lead acetate, 10 % Ammonium hydroxide solution, 10 % Ammonia. And to carry out the anthelmintic study of different extracts, we have taken following chemicals as Saline water (Claris

Lifesciences Ltd.,Ahmedabad). Albendazole (Alkem Ltd.) and Piperazine citrate (Glaxo Smithkline Ltd.) were used as reference standards.

### PLANT PROFILE:

#### Morphology [5, 6, 7, 8]

A medium sized glabrous tree 10-15 m in height with pale grey slender young branches and rough dark brown bark; leaves simple, alternate, obovate-oblong or elliptic-cuneate, the margins minutely denticulate or crenulated, main nerves 10-13 pairs; flowers fragrant, dark scarlet, in pendulous many flowered racemes; fruits bluntly quadrangular, narrowed towards the ends, crowned by a small persistent calyx (1,2). The tender leaves are edible. It has a brownish grey bark with glabrous young parts. The leaves are simple, alternate and stipulate, and are 7.5 -12.5 cm long. Leaves are oval shaped, bright green in colour with tapering bases and contain a reticulate venation pattern. Their petioles are 0.6-1.2 cm long. Flowers are regular, bisexual and about 2.5 cm in diameter. Petals are cream coloured and stamens are dark bright crimson. Ovaries are inferior to the flowers in long pendulous racemes, fragrant, with bright red stamens. Fruits are bluntly quadrangular and 2.5- 4.0 cm long.



Figure 1 Leaf of *B. acutangula*



Figure2 Bark of *B. acutangula*

#### Taxonomy [9, 10]

<b>Kingdom</b>	:	Plantae
<b>Division</b>	:	Magnoliophyta
<b>Class</b>	:	magnoliopsida
<b>Order</b>	:	ericales
<b>Family</b>	:	Leythidaceae
<b>Genus</b>	:	<i>Barringtonia</i>
<b>Species</b>	:	<i>acutangula</i>

**Synonyms:** *Barringtonia spicate*, *Eugenia accutangula*

#### Other Names

English	:	Indian oak, Small Indian oak
Hindi	:	Samudraphal
Kannada	:	Holekavau
Malayalam	:	Nirpelu, Attupelu, Samudraphalam
Sanskrit	:	Samudraphalah , Abdhiphala
Tamil	:	Samuttirappalam , Aram Kadambu
Telugu	:	Kanapa, Kanigi
Assamese	:	Hendol, Hinyon, Pani Amra.
Bengali	:	Hijjal
Marathi	:	Tiwar, Newar, Sathaphala.
Oriya	:	Nijhira, Kadappaia, kinjol

**Habitat:**

It's found throughout India, in deciduous and evergreen forests, mostly along the banks of rivers, streams and tanks.

**CHEMICAL CONSTITUENTS:**

The plant *Barringtonia Acutangula* contains triterpene dicarboxylic acid, barringtonic acid. Tanginol, barringtogenol E and barrinic acid [8-11]. Leaves contain acutangulic acid, barringtogenic acid, tangulic, oleanolic acids,  $\beta$ -sitosterol and stigmasterol. stigmasterol -3- $\beta$ -O-D-glucoside,  $\beta$ -amyrin, acutangulic acid have been isolated from the leaves [8-11]. Fruit contain barringtogenol D, C and B [8-11]. Bark and leaves contain small amount of a sapogenin [8-11]. Bark contains tannin 16% [8-11].

**TRADITIONAL USES:**

The different parts are used as roots, leaves, axillaries bud, fruit, bark and seeds for different traditional uses [5, 6, 7, 8,]. It is very effective in skin related ailments. It improves the skin complexion. It prevents indigestion and is effective in diarrhoea. It prevents dehydration in the body helping proper absorption of water. It purifies blood and prevents any infection happening in body. It helps in expelling out the mucus from the body and hence it keeps the respiratory tract clean. It regularizes the menstrual cycle and also manages urinary tract. It is helpful in relieving from fever. It is a general health tonic and hence is very effective in avoiding general health weakness. The roots are bitter, cooling, aperients, antipyretic, stimulant and emetic. They are useful in catarrh, intermittent fevers. Splenomegaly and constipation. It is similar to 'cinchona' in action when administered in malarial fever. The leaves are bitter, constipating and tonic, and are useful in diarrhoea and dysentery. The fruits are acrid, bitter, cooling, anthelmintic,

galactagogue, alexeteric, vulnerary, depurative, emetic, purgative, expectorant, diuretic, emmenagogue and antipyretic. They are useful in colic, intestinal worms, agalactia, wounds, ulcers, skin diseases, leprosy, splenomegaly, cough, bronchitis, strangury, dysmenorrhoea, intermittent fevers, ophthalmitis, cephalalgia, lumbago, syphilis, nasal catarrh and hallucinations [9, 10] Phytochemically, the bark and the root of *B. acutangula* contain saponins, tannins and triterpenes, and phenolic compounds. The juice of the leaves is used for diarrhoea. The bark provides a scorpion venom antidote and is also used on wounds. The powdered seeds are inhaled as snuff for relief in headache and externally applied for insect stings. The water extracts of roots and barks are employed as a febrifuge for malaria as these supposedly possess similar properties to the cinchona bark [5, 6].

#### EXPERIMENTAL WORK

##### **Collection and Authentication:**

The fresh bark *Barringtonia Acutangula* was collected from local area in the month september 2017 of Barpali, (Dist-Bargarh, and Odissa). The plant was authenticated by Prof. (Dr.) Santosh Kumar Dash, Retired Professor and H.O.D., P.G Dept. of Biosciences, C.P.S, Mohuda, Berhampur, Ganjam, Odisha. The plant was washed properly with water to remove the mud or dust, and then it was dried in sun light for one hour and the stem bark was dried under shade and was powdered by the help of mechanical process. The coarse powder have stored in air tight container for further studies.

##### **MATERIALS AND METHOD:**

The different Mayer's, Hager's, Barfoed's, Benedict's and millon's reagent, 70 % alcohol, extracts.  $\alpha$ -naphthol, Conc. Sulphuric acid, 10 % Ammonia, Pyridine, Sodium nitropruside, Acetic anhydride, Ferric chloride, 10 % Lead acetate, 10 % Ammonium hydroxide solution were purchased from S.D. Fine Chemical, Mumbai. The solvents petroleum ether, Chloroform, and Ethanol were purchased from Hi Media Laboratories Pvt. Ltd., Mumbai. All others chemicals, solvents and reagents were of analytical grade and procured from authorized dealer.

##### **EXTRACTION:**

Extraction involves the separation of bioactive portion of the plant tissues from inactive components by using selective solvents with different extraction technique [12]

##### **Hot successive extraction (By soxhlet apparatus):**

The shade dried coarse powder of stem bark *Barringtonia Acutangula* (100 g) was subjected to continuous hot extraction with solvent methanol and Pet. ether successively. The extracts were

filtered and dried, weighed and percentage of yield was calculated in terms of air-dried crude powdered materials

**Table 1 Extractive values of *Barringtonia acutangula***

Sl. No	Extract	Stem
1	Petroleum ether	3.50% w/w
2	Methanol	6% w/w
3	ethyl acetate	4.5%w/w

## RESULTS AND DISCUSSION

The results of extractive value were showed the methanolic extract has 6%w/w in stem bark (Table-1). The soxhlet extraction was done using the Pet. Ether, Methanol and ethyl acetate respectively and the finding showed methanol has a higher percentage of extract 6% w/w in stem bark. From the finding of extractive value of the extracts has been selected for further studies.

### **Qualitative phytochemical screening:**

The plant may be considered as a biosynthetic laboratory, not only for the chemical compounds such as Carbohydrates, Protein and Lipids that are utilized as food by human being but also for a multitude of compounds like Glycosides, Alkaloids, Volatile oils, Tannins etc., that exerts a physiological effect. The compounds that are responsible for therapeutic effect are usually the secondary metabolites. A systemic study of a crude drug embraces through consideration of both primary and secondary metabolites derived as a result of plant metabolism. The plant material may be subjected to preliminary phytochemical screening for the detection of various plant constituents. The different qualitative chemical tests can be performed for establishing profile of given extract for its chemical composition. The following tests may be performed on extracts to detect various phytoconstituents present in them [13-15].

### **Determination of biological (anthelmintic) activity:**

The anthelmintic study was done by using two *in-vitro* species i.e. roundworm *Ascaridia galli* Schrank (Nematode) and adult earthworms *Pheretima posthuma* L.Vaill (Annelida). Roundworms were obtained from intestine of freshly slaughtered fowls *Gallus spadiceus* (Phasianidae). Infested intestines of fowls were collected from the local slaughter house and washed with normal saline solution to remove all the faecal matter. These intestines were then dissected and worms were collected and kept in normal saline solution. Earthworms were collected near the swampy water in our locality. The average size of the round worm was 5-7 cm, average size of the earthworm was 8-9 cm. These helminths and earthworms were identified and services of veterinary practioner were utilized to confirm the identity of worms.

The anthelmintic assay was carried out as per the reported method (Subash et al., 2012). The assay was performed *in vitro* using adult earthworm (*Pheretima posthuma*) owing to its anatomical and physiological resemblance with the intestinal roundworm parasites *Ascaris lumbricoids* of human beings for preliminary evaluation of anthelmintic activity (Dash et al., 2002; Shivkumar & Kumar, 2003). Use of *Ascaridia galli* species as a suitable model for screening of anthelmintic drug was advocated earlier (Kaushik et al., 1974; Yadav & Temjenmongla, 2006). Test samples of each extract was prepared at the concentrations of 10, 20 and 40 mg/ml in distilled water and six worms i.e. *Pheretima posthuma* and *Ascaridia galli* of approximately equal size (same type) were placed in each 9 cm Petridish containing 25 ml of above test solution of extracts. Albendazole (10 mg/ml) and Piperazine citrate (10 mg/ml) were used as reference standard and saline water as control (Gbolade & Adeyemi, 2008; Mali & Wadekar, 2008; Lal et al., 1976). This procedure was adopted for all two different types of worms. All the test solutions and standard drug solutions were prepared freshly before starting the experiments. Observations were made for the time taken for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50°C). All the results were shown in (Table. 6.1) and expressed as a mean  $\pm$  SEM of six worms in each group.

The finding of phytochemical detection was shown in Table 2. Qualitative Phytochemical analysis reports for presence of phytoconstituents in *Barringtonia acutangula* are Glycosides, Carbohydrate, Phenolic compounds, Flavonoids and Saponins.

Qualitative phytochemical studies were performed on petroleum ether; methanol and ethyl acetate extract of *Barringtonia Acutangula* stem bark. The study was performed to identify its Alkaloid, Carbohydrate, Glycoside, Saponin, Protein, Amino acid, Phenolic compounds and flavonoids by using suitable chemicals and reagents in the different extract. Alkaloid test showed presence in methanolic extract; however petroleum ether showed least amount of alkaloid but Mayer test showed absence. Carbohydrate and glycosides test showed negative in all tested except the reagent Borntrager's test. Foam test was performed for saponin, it showed positive result but the results were not satisfactory. Results of proteins and amino acids test showed presence of least amount in petroleum ether extract and moderate in ethyl acetate extract. Phenolic compounds & flavonoids test was showed appreciable amount in all tested reagents. Test of glycoside showed presence of least amount of glycoside in methanolic, petroleum ether and ethyl acetate extract.

The finding of anthelmintic activity was shown in Table 3. In this present study, The different extracts exhibited anthelmintic activity in dose dependent manner giving shortest time of paralysis (P) and death (D) with different concentration.all the two *in vitro* test species (*Pheretima posthuma* and *Ascardia galli*) responded towards our plant extracts by showing the sign of paralysis and death finally. It was observed that the Ethyl acetate extract (i.e. EABA) was most potent which is well comparable with both standard drugs Albendazole and Piperazine citrate, followed by Methanolic extract (MEBA) at higher doses. Petroleum Ether extracts (PEBA) of all the plants were endowed with minute anthelmintic property, which were not up to standards.

**Table 2: Qualitative Phytochemical Screening of stem of *Barringtonia acutangula*.**

Phytochemical test	Pet.ether Extract	Methanol Extract	ethyl acetate Extract
<b>Alkaloid test</b>			
Mayer's test	-	-	-
Wagner's test	+	+	+
Hager's test	+	+	+
Dragendorff's test	+	+	+
<b>Carbohydrates &amp; glycosides</b>			
Molish's test	-	-	-
Fehling's test	-	-	-
Barfoid's test	-	-	-
Benidict's test	-	-	-
Borntrager's test	+	+	+
<b>Saponins</b>			
Foam test	+	+	+
<b>Proteins &amp; amino acid</b>			
Millon's test	+	-	+
Biuret's test	-	-	-
Ninhydrin test	-	-	-
<b>Phenolic compounds &amp; flavonoids</b>			
Ferric chloride test	+	-	-
Lead acetate test	+	+	+
Alkaline test	+	+	+

+, present, -, absent

**Table 3: Anthelmintic Activity of selected plant extracts by *in vitro* methods.**

Sample	Conc <sup>n</sup> (mg/ml)	Time taken for paralysis (P) & death (D) of worms in min.			
		<i>Pheretima posthuma</i>		<i>Ascardia galli</i>	
		Paralysis	Death	Paralysis	Death
<b>Control</b>	--	--	--	--	--
<b>PEBA</b>	10	85±0.71	--	78±0.33	90±0.87
	20	79±0.44	95±0.32	65±0.45	82±0.92
	40	65±0.29	88±0.86	55±0.76	71±0.13



<b>EABA</b>	<b>10</b>	<b>45±0.17</b>	<b>87±0.19</b>	<b>45±0.72</b>	<b>67±0.65</b>
	<b>20</b>	<b>40±0.31</b>	<b>67±0.14</b>	<b>35±0.12</b>	<b>59±0.14</b>
	<b>40</b>	<b>33±0.32</b>	<b>59±0.22</b>	<b>26±0.56</b>	<b>50±0.06</b>
<b>MEBA</b>	10	65±0.12	97±0.04	59±0.26	73±0.58
	20	52±0.12	83±0.14	47±0.08	66±0.51
	40	43±0.49	77±0.26	39±0.57	55±0.72
<b>Albendazole</b>	<b>10</b>	<b>32 ±0.28</b>	<b>56± 0.78</b>	<b>26±0.14</b>	<b>53±0.78</b>
<b>Piperazine citrate</b>	<b>10</b>	<b>29±0.68</b>	<b>61±0.28</b>	<b>25±0.18</b>	<b>48±0.84</b>

Each value represents mean ± SEM (n=6)

## CONCLUSION:

The activity revealed concentration dependence nature of the different extracts. Potency of the extracts was found to be inversely proportional to the time taken for paralysis/death of the worms (Table. 6.1). The ethyl acetate extract (showing most potent anthelmintic activity) contains mainly flavones and flavonoids, triterpenoids and phenolic compounds which may be responsible for this activity (Lal *et al.*, 1976; Da Silva *et al.*, 2008; Enwerem *et al.*, 2001; Jabbar *et al.*, 2007). Moderate anthelmintic activity of the methanolic extract may be due to the presence of glycosides, phenolic compounds, saponins and flavonoids (Lal *et al.*, 1976; Da Silva *et al.*, 2008; Enwerem *et al.*, 2001) present in it. Our results from the present study indicate the potential usefulness of *Barringtonia acutangula* in the treatment of helminthiasis. Attempts for the isolation and characterisation of the active constituents responsible for such activities are currently under progress. Further studies are necessary to understand the exact mechanism of action.

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