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Phytochemical and antimicrobial study of *Polyalthia Longifolia*

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Abstract
Medicinal and aromatic plants represent a consistent part of the natural biodiversity endowment of many states in India, as well as the world at large. Medicinal and aromatic plant species are widely distributed due to a variety of climatic factors and altitudinal variations coupled with varied ecological habitats. These plant species are basic ingredient of the ethno-botanical and traditional health care system. The premillinary phytochemical analysis and antimicrobial activity of different extracts (petroleum ether, chloroform, ethyl acetate, ethanol and aqueous) of leaves of *Polyalthia longifolia* was studied against six different bacteria by disc diffusion method. The various metabolites present in all the extracts. Among various solvent extracts studied, chloroform extract showed higher degree of inhibition followed by ethylacetate, ethanol, petroleum ether and aqueous.

Keywords: *polyalthia longifolia*, phytochemical analysis, antimicrobial activity, disc diffusion method

1. Introduction
Plants are the essential and integral part in Complementary and Alternative medicine and due to this they develop the ability for the formation of secondary metabolite substances which are in turn used to restore health and heal many diseases. Plants and plants products are being used as a source of medicine long. According to the world health organization more than 80% of the world population mostly in poor and less developed countries depends on traditional plant based medicine for their primary health care needs. Herbs are natural form of whole plants or their parts such as flower, root, oil, stem rich in bioactive chemical compounds so called "Herbiceuticals". The main difference between pharmaceutic drug and herbal principles is their isolation method and purification level. The pharmaceutical drugs are available with high purity as artificial chemical(s) while herbs are in rich natural complex chemicals (Sharma, 2010) [1]. Herbal medicine is a major component in all indigenous people's traditional medicine and a common element in Ayurvedic, Homeopathic, Naturopathic, Traditional, Oriental and Indian medicine. Many drugs commonly used today are of herbal origin because of their safety, quality and efficacy. *Polyalthia longifolia* is native to the drier region of India and it is locally known as "Nakali Ashoka". The plant grows throughout the tropical and subtropical parts of India up to an altitude of 1500 m. A tall, evergreen, handsome, pyramid like, columnar tree, undivided, growing up to 12m or more. Branches, Short, about 1-2 m long, glabrous and pendulous. Leaves are alternate, estipulate, distichous, mildly aromatic. Shining, glabrious, narrowly lanceolate. Polyalthia is a large genus of shrubs and trees distributed in tropic and subtropic regions (Krishnamurthy) [2]. It belongs to the family Annonaceae. Various parts of *P. longifolia* are used to treat fever, gonorrhea, uterus ailment, leucorrhoea and menorrhagia (Kritikar and Basu, 1998 and Rosakutty, *et al.* 2000) [3-4]. Decoction made from bark is used as cure for mouth ulcers (Garg and Jain, 1999) [5]. *P. longifolia* mainly contains diterpenoids, alkaloids, tannins, and mucilage.

Bacterial resistance to antimicrobial drugs is a worldwide problem that has emerged even among the common poultry pathogens. Nowadays, the use of antibiotics to control diseases is producing adverse toxicity to the host organs, tissues and cells. The toxicity produced by the antimicrobial agents can be cured or prevented or antagonized using herbs. Herbal medicines are in great demand in both developed and developing countries as a source of primary healthcare owing to their attributes having wide biological and medicinal activities, high safety margins and lesser costs. Herbal molecules are safe and will overcome the resistance produced by the pathogens as they exist in a combined form or in a pooled form of more than one molecule in the protoplasm of the plant cell. Some herbs were known to prevent cancer. Some herbs have antibacterial and antifungal properties that are useful for clinical use. Some of the in vitro studies have been conducted, in which herbal extracts were given to clinical drug resistant strains and different serotype strains of infection (Amin and Kapadnis, 2005) [6]. Hence the present study reports on the preliminary phytochemical analysis and antibacterial activity of *Polyalthia longifolia*.

2. Material and Methods
Plant collection
Healthy disease free, mature fresh plant leaf sample were collected in Namakkal Dt., Tamilnadu, India. The plants were identified in Botanical Survey of India (Central circle), Coimbatore. A voucher specimen of the plant has been deposited at the Department herbarium. Fresh leaves were washed thoroughly 2-3 times with running tap water and once with sterile water, shade-dried without any contamination. The dried leaves were then powdered using an electric mill.

Preparation of Extracts (Sani, *et al.* 2009) [7]
For water extract: Leave powder (20g) was subjected to boil in 200 ml doubled distilled water in a 500 ml flask till

the total volume becomes one fourth. The water extract was filtered through a 420 µm stainless steel filter, cooled and transferred to screw capped glass vials. For organic solvent extract: 10g of plant leave material was powdered and extracted with solvents of different polarities (petroleum ether, chloroform, ethyl acetate and ethanol) by cold maceration for 24 h. The extracts were filtered through Whatman No. 1 filter paper into screw capped vials. The entire extract was concentrated to dryness using rotary flash evaporator under reduced pressure.

Phytochemical Screening

Phytochemical screening was done for analyzing secondary metabolites that are responsible for curing ailments. The Phytochemical screening of the plant extract was carried out (Trease and Evans, 1978) [8] in all the extracts.

Collection and maintenance of Microbial culture

The strains were collected from the Department of Biotechnology and Microbiology, A.P.S. University, Rewa (M.P.) and freeze preserved in nutrient agar slants. The bacterial strain such as (*Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Salmonella typhi Pseudomonas aeruginosa* and *Proteus vulgaris*) were inoculated in a nutrient broth at 37°C for 24 hour in incubator.

The 36g of Muller Hinton agar (Himedia) was mixed with distilled water and then stabilized in autoclave at 15lbs pressure for 15 min. The sterilized media was poured into Petri dishes; the solidified plates were bored with 5mm diameter cork bearer. The plates with wells were used for the antimicrobial studies.

The various extracts were tested against the Escherichia coli, Bacillus subtilis, Staphylococcus aureus, and *Salmonella typhi* for antimicrobial activity. Wells of equal size were cut and the antibiotic was added into it for positive control; respective solvents acting as a negative control. The plates were incubated at 37°C, overnight.

Antibacterial sensitivity (Bauer *et al.* 1966) [9]

The antibacterial activity of crude plant extracts of *Polyalthia longifolia* were determined by well diffusion method. Plates were prepared by pouring sterile Muller Hinton agar (Himedia) into sterile petri dishes that were previously autoclaved. Sterilized cotton swabs were dipped in the bacterial culture in nutrient broth and then swabbed on the agar plates. Wells of equal size were cut with proper gaps in the medium and the plant extracts were added into it. Then the plates were incubated at 37°C and observed for zones of growth inhibition after 24 hours.

3. Results and discussion

Phytochemical analysis

Table 1 shows the result of the preliminary phytochemical analysis. The results showed the Carbohydrates, Oils and fats, Terpenoids, Steroids and sterols and amino acids showed the positive result in all the extracts. Saponins were absent in all the extracts. Cardio glycosides, tannins, phenolic compounds and flavanoids were absent in aqueous extract but other extracts exhibited positive result. Alkaloids were present only in chloroform, ethanol and aqueous extracts, petroleum and ethyl acetate extracts showed negative result.

Table 1: Preliminary phytochemical analysis of *Polyalthia longifolia* in different plant extracts

S. No.	Compound	Extracts				
		PE	CH	EA	EH	AQ
1.	Carbohydrates	+	+	+	+	+
2.	Cardio glycosides	+	+	+	+	-
3.	Saponins	-	-	-	-	-
4.	Oils and Fats	+	+	+	+	+
5.	Terpenoids	+	+	+	+	+
6.	Alkaloids	-	+	-	+	+
7.	Steroids and Sterols	+	+	+	+	+
8.	Flavanoids	+	+	+	+	-
9.	Tannins and Phenolic compounds	+	+	+	+	-
10.	Amino acids	+	+	+	+	+

\+ - Positive
\- - Negative
PE - Petroleum ether
CH - Chloroform
EA - Ethyl Acetate
EH - Ethanol
AQ - Aqueous

Table 2: Antimicrobial activity of *Polyalthia longifolia* in different plant extracts

S. No.	Microorganisms	Zone of Inhibition (mm)					
		Petroleum ether	Chloroform	Ethy acetate	Ethanol	Aqueous	Control
1.	*Salmonella typhi*	14±0.11	21±0.10	20±0.26	18±0.15	11±0.17	27±0.05
2.	*Escherichia coli*	16±0.13	24±0.27	21±0.22	17±0.23	12±0.24	29±0.03
3.	*Bacillus subtilis*	17±0.19	26±0.25	22±0.20	19±0.24	15±0.17	28±0.04
4.	*Staphylococcus aureus*	15±0.20	25±0.21	21±0.19	18±0.08	14±0.20	25±0.06
5.	*Pseudomonas aeruginosa*	14±0.26	24±0.22	20±0.16	15±0.18	11±0.16	24±0.03
6.	*Proteus vulgaris*	15±0.20	23±0.24	21±0.24	18±0.17	13±0.12	27±0.06

Data given are mean of three replicates ± standard error

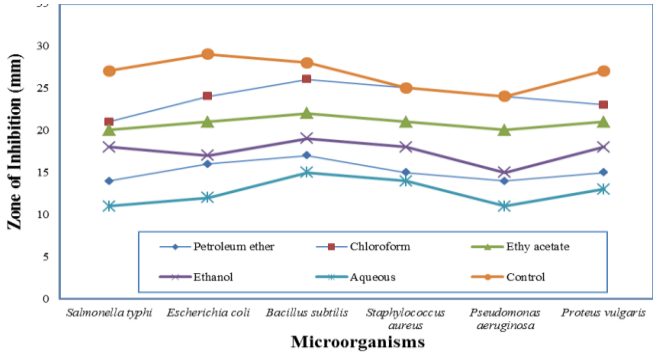

Fig 1: antimicrobial activity of *polyalthia longifolia* in different plant extracts

Antibacterial sensitivity

The results of antibacterial sensitivity of various solvent extracts of *Polyalthia longifolia* leaves by disc diffusion method are in depicted table 2. The results reveal that all extracts are potent antimicrobials against all the pathogenic organisms studied. The antibacterial activity was screened from the zone of inhibition. Among various solvent extracts studied, chloroform extract showed higher degree of inhibition followed by ethylacetate, ethanol and petroleum ether. The aqueous extract show minimum inhibitory effect compare to the all the other extracts. The diameter of inhibition zones for each of the samples were compared with (positive control) standard antibiotic (chloramphenicol 30 mcg/disc). In negative control has not shown any inhibitory effect. Highest antibacterial activity was against *Bacillus subtilis* in chloroform extract, followed by *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris* and *Salmonella typhi*. Similar types of observation were also noticed in all the other extracts.

The antimicrobial activities of various plants have been reported by many Researchers (Dewanjee *et al.* 2008) [10]. The emergence of antibiotic resistance has its roots in the injudicious use of antibiotics and the subsequent transfer of resistance genes and bacteria among animals, animal products and environment. Extra chromosomal genes associated with plasmids were found to be responsible for these antibacterial resistant phenotypes that may impart resistance to an entire antibacterial class (Motamedi *et al.* 2009) [11]. As the plant produce secondary metabolites in order to protect themselves from microorganism, herbivores and insects, thus antimicrobial effect is somehow expected from plants namely flavonoids, alkaloids and triterpenoid and are producing a better opportunity for testing wide range of microorganism. Preliminary phytochemical analysis during the present study also ascertains the presence of some potential group of bioactive substances (Ghosh *et al.* 2008) [12].

4. Conclusion

Plants have an almost limitless ability to synthesize aromatic substances. Most of them are secondary metabolites, of which at least 12,000 have been isolated. In many cases, these substances serve as plant defense mechanisms against predation by microorganisms, insects and herbivores. The potential for developing antimicrobials from higher plants appears rewarding, as it will lead to the development of a phytomedicine to act against microbes. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. The property of active phyotoconstituents responsible for the antibacterial activity cannot be altered. (The nature of active phytochemical responsible for antibacterial activity cannot be ascertained). Further studies of the active principles involved and their mode of action, formulated reparations for enhancing potency and stability are needed to recommend *Polyalthia longifolia* in control of several bacteria associated diseases.

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