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Comparative analysis of Phytochemicals present in *Polyalthia longifolia* and *Justicia adhatoda*

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Abstract

The aim of this study was to evaluate the presence of phytochemicals in the leaves of *Polyalthia longifolia* and *Justicia adhatoda* .*P. longifolia* has been used in the traditional system of medicine for the treatment of fever, skin diseases, diabetics and hypertension. *J. adhatoda* is a well-known drug in Ayurvedic and Unani medicine and has been used for the treatment of various diseases such as Asthma, bleeding, bronchitis, cough, diabetes, diarrhea, dysentery, epilepsy, fever, flu, hysteria, insainty, neuralgia, rheumatic pain, skin disorders, swelling, urinary disorders, vomiting. The phytochemical screening of the extracts was analyzed for the presence and absence of Alkaloids, Carbohydrates, Tannins, Flavanoids, Phytosterols, Volatile oils and Saponins. The leaves extract of the plants were prepared using different solvents like Petroleum ether, Benzene, Chloroform, Acetone, Alcohol, Methanol, and Water. Alkaloids, Flavanoids and volatile oils were the major compounds found in all solvent fractions of *J. adhatoda* except in petroleum ether fraction in which flavonoids were absent whereas in *P. longifolia* also Alkaloids, Flavanoids and volatile oils are present in most of the fractions where Alkaloids are absent in benzene and chloroform fractions, Flavanoids are absent in petroleum ether and benzene fractions and volatile oils are absent in petroleum ether and chloroform fractions. The percent extractive value of methanolic fraction was found to be maximum around 22% in *J. adhatoda* whereas in *P. longifolia* maximum percent extractive value was found to be around 13% in its alcoholic fraction. The present study provides evidence that solvent extracts of *P. longifolia* and *J. adhatoda* contain medicinally important bioactive compounds and this justifies the use of plant species as traditional medicine for treatment of various diseases.

Key words: *Polyalthia longifolia, Justicia adhatoda,* phytochemical screening, percent extractive value

Introduction

Plants are the main source of food and are rich in compounds which have pain relieving and healing abilities. The wide spread use of herbal remedies and health care preparations obtained from commonly used traditional herbs and medicinal plants have been raised due to the occurrence of natural products with medicinal properties.

Many of today's modern drugs have their origin in traditional plant medicine (Blanks *et al*., 1998). The therapeutic efficacies of many indigenous plants for several disorders have been described by practitioners of traditional herbal medicines (Natarajan *et al*., 2003). Natural products are a significant source of synthetic and traditional herbal medicine and are still the primary health care system (Singh and Singh, 2001). Being sources of many life sustaining metabolites, the research is still on for plants to be used in healing.

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This in part is due to the growing problem of worldwide antibacterial resistance (Amadou, 1998). Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has also increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents (Gislene, 2000). The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted to prove such efficiency. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, the phenolic compounds which are part of the essential oils, as well as in tannin (Saxena *et al*., 1994).

The Indian shrub *Justicia adhatoda* (Malabar nut) belongs to family Acanthaceae and is commonly known as ''Arusa or Vasaka''. It is a perennial shrub

1–2.5 m high and has been used in the indigenous system of medicine in India for over 2,000 years. The importance of *J. adhatoda* plant in the treatment of respiratory disorders (Dymock *et al.*, 1893). It has simple, acute, shiny, lance-shaped, oppositely arranged, smooth-edged leaves borne on short petioles. Flowers are white in capsule shape. They are dull brownish-green colour when dry and are bitter in taste. When a leaf is cleared with chloral hydrate and examined microscopically the oval stomata can be seen. They are surrounded by two crescent-shaped cells at right angles to the ostiole. The epidermis bears simple one to three-celled warty hairs, and small glandular hairs. Cystoliths occur beneath the epidermis of the underside of the blade.

Quinazoline alkaloid i.e. vasicine are the most important chemical components in *J. adhatoda* and are present in the leaves, roots and flowers. Apart from vasicine, the leaves contain several alkaloids (vasicinone, vasicinol, adhatodine, adhatonine, adhavasinone, anisotine and peganine), betaine, vitamins (vitamin C, β-carotene), steroids (vasakin), glycosides, phenolic components, sterols, essential oils, and alkanes a mixture of fatty acids (Lahiri and Prahdan 1964; Bhat *et al*., 1978; Atal, 1980; Chowdhury and Bhattacharyya, 1987). These constituents have been identified as potential contributor to the observed medicinal effects of the plant. Bromohexine and ambroxol, the semi-synthetic derivatives of vasicine are useful in anti-tuberculosis therapy (Grange and Snell, 1996).

J. adhatoda is a well-known drug in Ayurvedic and Unani medicine and has been used for the treatment of various diseases such as Asthma, bleeding, bronchitis, cough, diabetes, diarrhea, dysentery, epilepsy, fever, flu, hysteria, insainty, neuralgia, rheumatic pain, skin disorders, swelling, urinary disorders, vomiting (Atal, 1980). The antimicrobial activity of *J. adhatoda* leaf extracts against *Staphylococcus aureus, S. epidermidis, Bacillus subtilis, Proteus vulgaris* and *Candida albicans* had been established (Karthikeyan *et al*., 2009). The bronchodilatory activity of *J. adhatoda* compounds have been well studied by Dorch and Wagner (1991). Grange and Snell (1996) have studied the antimycobacterial activity of semi-synthetic derivatives of compounds of *J. adhatoda* against *M. tuberculosis* by conventional method. . It is a potent expectorant (Atal 1980), antiseptic (Patel and Bhatt 1984), antihelmintic (Mathew *et al*., 1998), bronchodilatory (Lahiri and Pradhan, 1964; Gupta *et al*., 1977) and uterotonic. The leaves as well as flowers, fruits, and roots extracts are extensively used for treating cold, whooping-cough, asthma and intestinal worm infection (anthelmintic). The leaf juice is stated to cure diarrhoea, dysentery and glandular tumors.

Polyalthia longifolia is a member of order Magnoliales and family Annonaceae. *Polyalthia longifolia* is a tree, which is widely distributed in Bangladesh, Srilanka and throughout the hotter parts of India (Hooker and Clarke, 1875). It is a lofty evergreen plant, commonly planted due to its effectiveness in alleviating noise pollution. It is known by various common names like False Ashoka, the Buddha Tree and Indian Fir tree, Ashoka or Devadaru in Sanskrit, Debdaru in Bengali and Hindi, Asopalav (Gujarati), Glodogan tiang (Indonesian), devdar in Marathi and Nettilinkam in Tamil. It exhibits symmetrical pyramidal growth with willowy weeping pendulous branches and long narrow lanceolate leaves with undulate margins. The tree is known to grow over 30 ft in height (Wu *et al*., 1990).

A number of biologically active compounds have been isolated from this plant. *P. longifolia* mainly contains diterpenoids, alkaloids, tannins, and mucilage. It is proved to show Antibacterial activity, Antioxidant activity, Anti-inflammatory activity, Anti-cancer activity, Hepato-protective activity, Anti-hyperglycemic activity. In India, the seeds and bark of this plant were used as febrifuge (Raghunathan and Mitra, 1985; Kirtikar and Basu, 1993). Literature survey revealed that most of the plants of annonaceae family contain antitumor and anticancer principles (Chakrabarti and Mukherjee, 1968). The extract of stem bark and the alkaloids isolated from this were found to demonstrate a good antibacterial and antifungal activities (Hasan *et al*., 1988). In traditional medicines various herbal preparations are being used for treating duodenal ulcers. The plant has been used in traditional system of medicine for the treatment of fever, skin diseases, diabetics, hypertension and helmiinthiasis.

The present work aims to study the comparative analysis of phytochemicals present in *J. adhatoda* and *P. longifolia* and calculate their percent extractive value. The percent extractive value determines the amount of active constituents extracted with solvents from a given amount of plant material (Kamalesh Upreti *et al.*, 2013).

Material and Methods

Collection of plant material

The healthy leaves of *P. longifolia* and *J. adhatoda* were collected from the Botanical garden of University College of Science, Mohanlal Sukhadia University, Udaipur, Rajasthan, India. The herbarium specimen plants were identified from Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India and the voucher number for *P. longifolia* is RUBL211518

and for *J. adhatoda* is RUBL211516. The leaves were shade dried at room temperature and was grounded in an electrical grinder. The ground material was passed through sieve of mesh size 60mm to obtain a fine powder which was used to prepare the extract.

Extract preparation

Different partially purified organic constituents were successively separated from dried leaves of *P. longifolia* and *J. adhatoda* by reflux method of solvent extraction (Harborne, 1984). The solvents are arranged in a series from non-polar to polar nature in a manner mentioned below:

Petroleum ether → Benzene → Chloroform → Acetone → Alcohol →Methanol →Water

40 gm leaf powder was kept in Soxhlet extraction unit and extracted with 280 ml petroleum ether in the round bottom flask till all petroleum ether soluble fractions were extracted. The leaves residue remained was dried in an oven below 50°C and used for extraction with next solvent in series. Fractions obtained at every step including aqueous fraction were vacuum dried in a rotary evaporator and the dried residue was used as extract.

Calculation of percent extractive value

The dried extract was used for calculation of percent extractive value which was calculated by using formula mentioned below:

$$\text{Percent extractive value} = \frac{\text{Weight of dried extract}}{\text{Weight of dried plant material}} \times 100$$

Phytochemical tests

The phytochemical tests were carried out in the extracts using the standard procedures as described by Sofowara(1993), Trease and Evans (1989) and Harbone (1973) and is mentioned in Table No. 1.

Results and Discussion

The comparative analysis of percent extractive values of different solvent extracts of *Justicia adhatoda* and *Polyalthia longifolia* was done and is mentioned in Table 2. It was observed that the chloroform extract has minimum percent extractive value in both the plants. The maximum percent extractive value was around 22.75% in methanol fraction of *J. adhatoda* whereas the alcohol fraction of *P. longifolia* showed maximum percent extractive value of around 13.375%.

The qualitative study of phytochemicals present in the different solvent extract of *P. longifolia* and *J. adhatoda* are mentioned in Table 3 and 4 respectively. In *P. longifolia* alkaloids are absent in benzene and chloroform fractions whereas in *J. adhatoda* alkaloids are major phytochemical and is present in all fractions. Carbohydrates are present in alcohol and methanol fractions of *P. longifolia* whereas in *J. adhatoda* it is present in petroleum ether and benzene fractions. In *P. longifolia* tannins are present only in alcohol fraction whereas in *J. adhatoda* tannins are present in chloroform fraction. Flavanoids are present in all fractions of both the plants except petroleum ether fraction of *J. adhatoda* and petroleum ether as well as benzene fraction of *P. longifolia*. Phytosterols are completely absent in *J. adhatoda* fractions but in *P. longifolia* it is present only in petroleum ether and benzene fraction. In *P. longifolia* volatile oils are absent in petroleum ether and chloroform fractions whereas these are major phytochemical present in all fractions of *J. adhatoda*. Alcohol, methanol and water fractions of both *P. longifolia* and *J. adhatoda* contain saponins.

Conclusion

In the present study different solvent fractions of *J. adhatoda* and *P. longifolia* leaves were analysed for the presence and absence of phytochemicals in them. It was observed that both the plants are rich in Alkaloids, Flavanoids and Volatile oils. The percent extractive value of methanol fraction was maximum in *J. adhatoda* and was found to be around 22.75% whereas in case of *P. longifolia* the alcohol fraction showed maximum percent extractive value which is around 13.375%. The biomolecules present in these plants can be evaluated for large number of applications in the field of development of biomedicine, antibiotics etc. Some herbs have antibacterial and antifungal properties that are useful for clinical use. This will go a long way in the scientific exploration of medicinal plants for the benefit of man and is likely to decrease the dependence on drugs.

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Table 1. Procedure of Phytochemical screening

Tests	Results
For Alkaloids	
1. Extract + 3-4 drops of Wagner's Reagent	Reddish brown ppt. or colouration
2. Extract + 1.5ml HCl + Mayer's Reagent + Wagner's Reagent	Yellow white ppt.
For Carbohydrate	
1. Benedict's test: Extract + Benedict Reagent + heat	Orange red ppt.
2. Fehling Test: Extract + dil HCl + dil NaOH + heat + Fehling A + Fehling B	Red ppt.
For Tannins	
1. Gelatin Test Extract + 1% gelatin solution containing NaCl	White ppt.
2. Braymmer's Test Extract + 10% Alcoholic $FeCl_3$	Blue or greenish colour
For Flavanoids	
1. Alkaline reagent test: Extract + 20% NaOH → Yellow color + dil HCl	Colourless
2. Lead Acetate test Extract + 10% Lead Acetate	Yellow ppt.
For Phytosterol	
1. Salkowski's Test: Extract + Chloroform → Filtered. Filterate + conc. H_2SO_4 + Shake	Golden yellow colour
2. Extract + Choroform + conc. H_2SO_4 + acetic acid	Green colour
For Volatile Oil	
1. Extract + alcoholic Sudan III	Red Colour
For Saponin	
1. Extract + Distilled water → shake for 15 minutes	Layer of foam at the surface

Table 2. Percent extractive value of *J. adhatoda* and *P. longifolia*

S. No.	Name of Solvent	Percent extractive value of *J. adhatoda*	Percent extractive value of *P. longifolia*
1.	Petroleum Ether	2.09 %	8.95%
2.	Benzene	1.675 %	5.05%
3.	Chloroform	0.775 %	1.325%
4.	Acetone	1.875 %	3.125%
5.	Alcohol	18.8 %	13.375%
6.	Methanol	22.75 %	4.575%
7.	Water	17.275 %	7.225%

Table 3. Phytochemical screening of *P. longifolia*

S. No.	Phytochemicals	Solvents						
		Petroleum Ether	Benzene	Chloroform	Acetone	Alcohol	Methanol	Water
1.	Alkaloids	+	-	-	+	+	+	+
2.	Carbohydrates	-	-	-	-	+	+	-
3.	Tannins	-	-	-	-	+	-	-
4.	Flavanoids	-	-	+	+	+	+	+
5.	Phytosterols	+	+	-	-	-	-	-
6.	Volatile oils	-	+	-	+	+	+	+
7.	Saponins	-	-	-	-	+	+	+

Table 4. Phytochemical screening of *J. adhatoda*

S. No.	**Phytochemicals**	**Solvents**						
		Petroleum Ether	**Benzene**	**Chloroform**	**Acetone**	**Alcohol**	**Methanol**	**Water**
1.	Alkaloids	+	+	+	+	+	+	+
2.	Carbohydrates	+	+	-	-	-	-	-
3.	Tannins	-	-	+	-	-	-	-
4.	Flavanoids	-	+	+	+	+	+	+
5.	Phytosterols	-	-	-	-	-	-	-
6.	Volatile oils	+	+	+	+	+	+	+
7.	Saponins	-	-	-	-	+	+	+

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