

Research Article



Pharmacognostic Profile and Phytochemical Investigation of *Pluchea lanceolata* Oliver & Hiern. *In vivo* and *In vitro*

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ABSTRACT

India has a rich treasure of medicinal plants. Medicinal plants have played a significant role in ancient traditional systems of medicine. *Pluchea lanceolata* is one of the important medicinal plants having many therapeutic uses. The present study deals with pharmacognostic profile and preliminary phytochemical investigations in *in vivo* (leaf, stem and root) and *in vitro* (callus) plant parts of *Pluchea lanceolata*. Various parameters like ash value, fluorescence analysis of powdered as well as its extractives and phytochemical screening of different extractives were studied in all *in vivo* (leaf, stem and root) and *in vitro* (callus) plant parts. Ash value was found to be maximum in stem (17.24%) and minimum in leaf (7.5%) while extractive value was found to be maximum in leaf (41.40%) in methanol and minimum in stem (3.25%) in ethyl alcohol. Phytochemical screening of all plant parts revealed the presence of alkaloids, tannins, phenolics, flavonoids, steroids, glycosides, triterpenoids, proteins and carbohydrates. Therefore, the present study revealed that *Pluchea lanceolata* is an important source of many therapeutically and pharmacologically active constituents. The plant has been widely studied for its pharmacological activities and finds its position as a versatile plant having a wide spectrum of medicinal activities.

Keywords: Flavonoids, *Pluchea lanceolata*, Pharmacognosy, Phytochemical analysis, Steroids.

INTRODUCTION

Nature is a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Plant-derived substances has recently become a great interest owing to their versatile applications. Medicinal plants are the richest bioresource of drugs of traditional systems of medicine, modern medicines, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs.¹ Medicinal plants form a large group of economically important plants that provide the basic raw materials for indigenous pharmaceuticals.² Plant products still remain the principal source of pharmaceutical agents used in traditional medicine.³ According to the WHO the first step for identification and purification of herbal drugs is the pharmacognostic (macroscopic and microscopic) studies which are essential for any phyto pharmaceutical products used for standard formulation.⁴ Preliminary phytochemical studies are helpful in finding out chemical constituents in the plant material that may well lead to their quantitative estimation.^{5,6} Recently much attention has directed towards extracts and biologically active compounds isolated from popular plant species. In the present era of drug development and discovery of newer drug molecules, many plant products are evaluated on the basis of their traditional uses. The curative properties of medicinal plants are mainly due to the presence of various complex chemical substances of different compositions which occur as secondary metabolites.⁷ The most important of these bioactive constituents of plants are alkaloids, steroids, tannins, flavonoids and phenolic compounds. Hence, it is desirable to know the

phytochemical composition of the plant material before testing its efficacy for medicinal purpose.

Pluchea lanceolata is an important xerophytic herb belonging to family Asteraceae and commonly known as Rasna. All parts of the plant are extensively used in indigenous system of medicine. It has anti-inflammatory and analgesic activity and is greatly used in rheumatoid arthritis, neurological diseases, sciatica, edema, bronchitis, dyspepsia, cough, psoriasis and piles.⁸⁻¹⁰ The plant contains different secondary metabolites viz. flavonoids (quercetin, isorhamnetin, daidzein), triterpenes, sitosterols, taraxosterols, pluchine etc. which gives it anti-inflammatory and analgesic properties.¹⁰⁻¹³ The main aim of the present investigation was to study the pharmacognostic profile and phytochemical constituents in *in vivo* (leaf, stem, root) and *in vitro* (callus) plant parts of *Pluchea lanceolata* Oliver & Hiern.

MATERIALS AND METHODS

Plant material and culture establishments

The plant parts of *Pluchea lanceolata* were collected during the month of July from the forest regions of Jaipur and adjacent areas. The plant material was authenticated by herbarium of the Department of Botany, University of Rajasthan, Jaipur. A voucher specimen was deposited in the herbarium of the Department. The plant materials (leaves, root and stem) were air dried at room temperature and under shade, and then powdered to a fine grade by using a laboratory scale mill. These shade dried parts of the plant were powdered and kept in air tight plastic bag until use. Un organized callus cultures (eighteen months old) were grown on MS medium consisting of basal salts and vitamins with 3% (w/v)



sucrose and 0.8% agar with NAA (1.0 mg/l) and BAP (0.5 mg/l) using leaf explants.^{14,15} These cultures were allowed to grow up to their maximum growth age (6 weeks).¹⁶ The developed undifferentiated homogenous cell mass was repeatedly subcultured to maintain cell growth. The collected cell mass was allowed to dry at room temperature and then used for further investigation.

About 10 g of all the powdered samples (leaf, stem, root and callus) was refluxed with petroleum ether, methanol, ethanol and ethyl acetate in the ratio of 1:10 (w/v). The crude extracts were collected in amber colored sample bottles and stored. All chemicals and reagents used including the solvents were of analytical grade.

Pharmacognostic Profile

Ash value

Total ash, acid insoluble ash and water soluble ash were determined in all *in vivo* (leaf, stem and root) and *in vitro* (callus) plant samples.¹⁷

Extractive values

Extracts of the all *in vivo* (leaf, stem and root) and *in vitro* (callus) plant samples were prepared with different solvents for the study of extractive values.

Fluorescence analysis

The dried powder (1g) of each plant samples (leaf, stem and root) was extracted with desired quantity (50 ml) of different organic solvents and after 24 hours, fluorescence of each extraction was observed and recorded in both day light and UV light.¹⁸

Phytochemical analysis

Phytochemical analysis was carried out in the petroleum ether, ethyl acetate, ethanol and methanol extracts in all *in vivo* (leaf, stem and root) and *in vitro* (callus) plant samples of *Pluchea lanceolata* using standard procedures.^{19,20}

Test for Alkaloids

- Dragendroff's Test: To 5 ml of the extract few drops of Dragendroff's reagent was added for the formation of orange coloured precipitate.
- Mayer's Test: To 5 ml of the extract few drops of Mayer's reagent was added for the formation of cream coloured precipitate.

Test for Flavonoids

- Alkaline Reagent Test: Extracts were treated with few drops of NaOH solution. Formation of an intense yellow colour which becomes colourless on addition of few drops of dilute acid indicates the presence of flavonoids.
- Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow coloured precipitate indicates the presence of flavonoids.

Test for Tannins

- Gelatin Test: To the extract 1% (w/v) gelatin solution containing 10% (w/v) sodium chloride is added and observed for the formation of white precipitate. The method is based on the ability of tannins to precipitate proteins

Test for Sterols

- Liebermann-Burchard Test: To a small amount of the extract few drops of chloroform, acetic anhydride and H₂SO₄ was added along the sides of the test tube to observe the formation of dark red or pink colour.

Test for Glycosides

- Baljet's Test: To 5 mL of the extract few drops of sodium picrate was added to observe yellow to orange colour.
- Keller Killiani Test: 0.5 g of the extract was treated with 2 ml of glacial acetic acid and a drop of 5% (w/v) FeCl₃ was added to it. Glacial acetic acid containing 1% (w/v) FeCl₃ gives a brown ring in the presence of 2-deoxysugar in the glycone portion of the phytochemical.

Test for Saponins

- Foam Test: To a small amount of the extract few drops of distilled water was added and shaken vigorously until persistent foam was observed.

Test for Terpenoid

- Chloroform Test: To 5 ml of the extract few drops of chloroform and concentrate H₂SO₄ was added carefully along the sides of the test tube to form a layer and observed for the presence of reddish brown colour.

Test for Proteins

- Biuret Test: To 3 ml of the extract few drops of 10% sodium chloride and 1% copper sulphate was added for the formation of violet or purple color. On addition of alkali, it becomes dark violet.
- Millon's Test: To 3 ml of the extract few drops of Millon's reagent was added for the formation of red colour.

Test for Carbohydrates

- Molisch's Test: To a small amount of the extract few drops of Molisch's reagent was added followed by the addition of concentrate H₂SO₄ along the sides of the test tube. The mixture was then allowed to stand for 2 min and then diluted with 5 ml of distilled water. Formation of red or dull violet colour at the inter phase of two layers indicates the presence of carbohydrates.
- Fehling's Test: The extract was treated with 5 ml of Fehling's solution (A and B) and kept in boiling water

bath. The formation of yellow or red color precipitate indicates the presence of reducing sugar.

Test for phenols

- a) Ferric chloride Test: A fraction of the extract was treated with 5% ferric chloride solution and observed for the formation of deep blue or black colour.

RESULTS AND DISCUSSION

Pharmacognostic Profile

Ash Value

Results of total ash value, acid insoluble ash and water soluble ash in all *in vivo* (leaf, root and stem) and *in vitro* (callus) plant samples are shown in figure-1. Total ash value was found to be maximum in stem (17.24 %) and minimum in leaf (7.5 %).

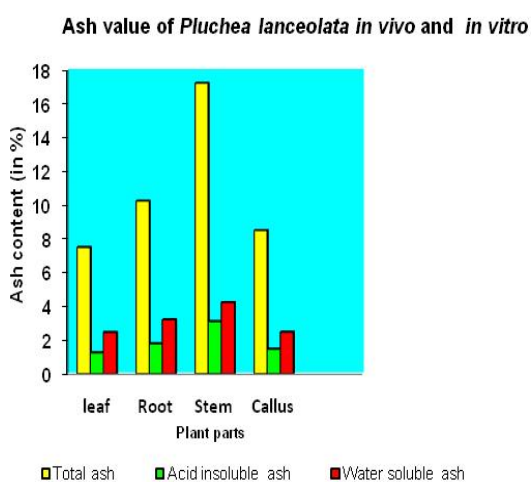


Figure 1: Ash value in *Pluchea lanceolata* in vivo and in vitro

Extractive value

Results of extractive value in different organic solvents, in all *in vivo* (leaf, root and stem) and *in vitro* (callus) plant samples are shown in figure-2. Among all the organic solvents which were used in the present experiment, maximum extractive value was found to be in leaf in methanol (41.40 %) and minimum in stem in ethyl alcohol (3.25 %).

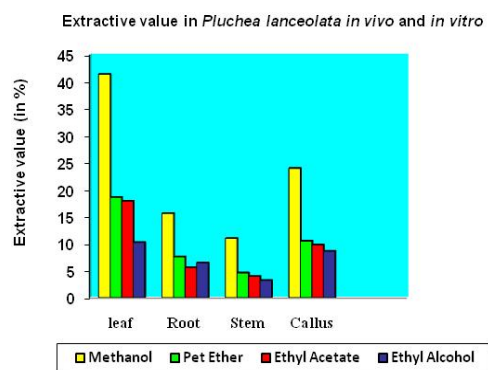


Figure 2: Extractive value in *Pluchea lanceolata* in vivo and in vitro

Fluorescence analysis

The analytical results of fluorescence analysis in all *in vivo* and *in vitro* plant samples extracts are tabulated in table-1.

Phytochemical analysis

All *in vivo* (leaf, stem and root) and *in vitro* (callus) plant samples of *Pluchea lanceolata* were subjected to various qualitative tests for the identification of phytochemical constituents are tabulated in table-2.

Qualitative phytochemical screening of *in vivo* and *in vitro* plant parts of *Pluchea lanceolata* in different organic solvents showed the excessive medicinal value of the plants. These plant samples contain majority of metabolites except saponins. For the purpose of screening methanolic and ethanolic extracts of plant were found better suited for maximum metabolites viz. alkaloids, tannins, glycosides, flavonoids, steroids, terpenoids, phenols, carbohydrates and proteins. Leaves part was found to be richer in metabolites as compare to other *in vivo* (stem and root) and *in vitro* (callus) plant parts. These phytochemicals are known to be of therapeutic importance since they have biological roles. For example, alkaloid extracts obtained from medicinal plant species have multiplicity of host-mediated biological activities, including antimalarial, antimicrobial, antihyperglycemic, anti-inflammatory and pharmacological effects.²¹ Flavonoids possess potential pharmacological activities such as antioxidant activity, vitamin C sparing activity, protein kinase C, tyrosine kinase, genetic toxicity etc. Flavonoids have anti-allergic, anti-inflammatory, anti-microbial and anticancer activity.²² Phenols are shown to have antioxidant activity.²³ Tannins are shown to have antiviral, anti-tumour, wound healing and anti-parasitic effects.²⁴ Steroids compounds are of importance and of interest in pharmacy due to their relationship with sex hormones.²⁵ Flavonoids, steroids and terpenoids are found to be rich in most of the parts of the medicinal plant. Hence In the present study, the preliminary phytochemical screening of the various extracts of *in vivo* and *in vitro* plant parts of *Pluchea lanceolata* revealed the presence of major bioactive compounds which may retain a wide range of pharmaceutical and therapeutical actions.

CONCLUSION

Medicinal plants form a large group of economically important plants that provide the basic raw materials for indigenous pharmaceuticals. Results of phytochemical evaluation revealed the presence of alkaloids, flavonoids, proteins, carbohydrates, tannins, phenols, glycosides, and terpenoids. The pharmacognostic profile and phytochemical screening of the present study showed favorable effects for the standardization parameters of plant parts. Hence, based on the phytochemical of interest, it is necessary to use the appropriate solvent for extraction and isolation. This could be the crucial step in further studies on the phytochemical, biological

structure-function relationship of the study plant which are already reported to be of therapeutic importance. This established a significant scope to develop a broad

spectrum use of *Pluchea lanceolata* in herbal medicine and as a base for the development of novel potent drugs and phytomedicine.

Table 1: Fluorescence analysis of intact parts of *Pluchea lanceolata*

Drug powder in organic Solvents	Plant parts					
	Leaf		Stem		Root	
	Day light	UV light	Day light	UV light	Day light	UV light
50 % H ₂ SO ₄	Greenish black	Greenish black	Black	Black	Black	Black
1N HCl	Black	Black	Black	Black	Black	Black
Aqueous 1N NaOH	Light brown	Pale green	Light brown	Light green	Yellowish brown	Green
Alkaline 1N NaOH	Light green	Green	Yellowish brown	Green	Brown	Light green
H ₂ O	Green	Green	Yellowish brown	Light green	Brownish red	Greenish yellow
Chloroform	Dark green	Dark green	Light green	Yellowish green	Dark brown	Dark brown
Benzene	Dark greenish black	Dark greenish black	Light green	Light green	Brown	Light green
Hexane	Greenish black	Greenish black	Light green	Light green	Brown	Light green
Acetone	Greenish black	Brownish green	Brownish green	Yellowish green	Light Brown	Light green
Methanol	Greenish dark brown	Yellow	Yellowish brown	Green	Brown	Light green

Table 2: Phytochemical screening of Plant parts of *Pluchea lanceolata*

Phytoconstituents	Plant parts															
	Leaf				Stem				Root				Callus			
	ME	EE	EAE	PEE	ME	EE	EAE	PEE	ME	EE	EAE	PEE	ME	EE	EAE	PEE
Alkaloids	+	-	-	+	-	-	-	-	+	-	-	+	+	-	-	+
Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
Tannins	+	+	+	-	-	-	-	-	+	+	-	-	+	-	-	-
Steroids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glycosides	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
Saponins	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Terpenoids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Proteins	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+	-
Carbohydrates	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phenols	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+	-

ME: methanol extract; EE: ethanol extract; EAE: ethyl acetate extract; PEE: petroleum ether extract; '+': presence of phytochemical; '-': absence of phytochemical.

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