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PHYSICOCHEMILCAL AND PHYTOCHEMICAL INVESTIGATIONS ON THE PLANT *PLUCHEA LANCEOLATA*

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ABSTRACT

Medicinal plants are in the richest bioresource of drugs of traditional system of medicine in many countries. The phyto-physicochemical evaluation of the plant material is an important parameter in detecting adulteration or improper handling of drugs. *Pluchea lanceolata* is a small shrub found in hotter parts of India including Punjab, Rajasthan, Upper West Bengal, Uttar Pradesh and neighboring Asian countries. It is locally known as Rasana. It is one of the important medicinal plant having many therapeutic uses. The present study deals with the physicochemical and preliminary phytochemical investigations of *Pluchea lanceolata.* The study on plant was conducted following the reported methods in literature and also from World Health Organization (WHO) guidelines.The physicochemical analysis showed convincing results. The phytochemical screening of the whole plant revealed the presence of flavonoids, alkaloids, terpenoids, steroids, glycosides, tannins, carbohydrates, proteins, amino acids, oils and fats. Thin layer chromatography exhibited a number of compounds in different extracts and the fluorescence study showed promising results.The present study revealed that *Pluchea lanceolata* is an important source of many therapeutically and pharmacologically active compounds. The work further provides information for the correct identification and standardization of the plant.

Keywords: *Pluchea lanceolata,* Whole plant, Physicochemical, Phytochemical, Standardization.

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INTRODUCTION

Medicinal plants have been identified and used throughout human history. It has been a major source of treatment for human diseases since time memorial. The study of traditional human uses of plants is recognized as an effective way to discover future medicines and an effective number of modern drugs have been isolated from natural sources. Nature is a source of medicinal plants for thousands of years. The herbal medicines are known to be oldest health care products that have been used for mankind all over the world in the form of folklore medicines or traditional medicines or ethnic medicines. The World Health Organization (WHO) estimates that 80 percent of the population of some Asian and African countries presently use herbal medicine for some aspect of primary health care [1-2]. Plant derived medicines are promising choice over modern synthetic drugs and has recently become a great interest owing to their versatile applications. They show minimum/no side effects and are considered to be safe. Medicinal plants are the richest bioresource of drugs of traditional system of medicines, modern medicines, food supplements, folk medicines, pharmaceutical intermeciates and chemical entities for synthetic drugs [3]. Generally herbal formulations involve the use of fresh or dried plant parts. Correct knowledge of such crude drugs is very important aspect of preparation, safety and efficacy of the herbal

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product. The curative properties of medicinal plants are mainly due to the presence of various complex substances of different compositions which occur as secondary metabolites [4]. The most important of these bioactive constituents of plants are terpenoids, alkaloids, steroids, flavonoids, tannins, acids, coumarins, anthraquinones and many phenolic compounds. It is therefore necessary to know the phytochemical composition of the plant material before testing their efficacy for medicinal purpose. The quality control standards of various medicinal plants used in Indigenous system of medicine are becoming more relevant today in view of commercialization of formulations based on medicinal plants. Due to natural heterogenity, the quality of the herbal starting materials obtained from different geographical locations together with different vernacular names shows more and more fluctuations and adulterations. Therefore, reproducible standards of each plant are necessary for effective quality control [2]. For this reason, World Health Organization (WHO) has issued guidelines for quality control methods for medicinal plant materials in 1992 with a clear objective to provide general test methods for correct botanical evaluation and identification of medicinal plants widely used in traditional and home remedies [1]. As per World Health Organization (WHO) guidelines, it is thus necessary to study the pharmacognostic (macroscopic and microscopic) characters, physicochemical constants, extractive values with various solvents, fluorescence analysis, its reaction after treatment with chemical reagents under visible and UV light and preliminary phytochemical screening of extracts. The results help to establish the standardization for the drug and to ensure that every packet of medicine that is being sold has the correct amount and induce its therapeutic effect.

Pluchea lanceolata Oliver &Hiern (*Asteraceae*) is a small shrub found in hotter parts of India including Punjab, Rajasthan, Upper West Bengal, Uttar Pradesh and neighboring Asian countries. It is locally known as Rasna. It groups mainly in sandy and saline soil. In Indigenous system of medicine its use has been described as an antipyretic, analgesic, bitter, laxative, nervine tonic and recommended for dyspepsia, rheumatoid arthritis and bronchitis [5-6]. Rasna is reported for treatment of rheumatism. The plant is greatly used in neurological diseases, sciatica, cough, psoriasis and piles. It is used for treating pain and swelling of the body joints. Methanol extract of the plant has been reported to possess anti-inflammatory and anti-arthritic activities [7-11]. Neolupinol isolated from the plant possess anti-inflammatory activity. A number of secondary metabolites eg. flavonoids, triterpenes, sterols and alkaloids have been isolated from *Pluchea lanceolata*[7, 12-16]. The plant possesses valuable medicinal properties but most of advantages are still confined to tribal areas because of raw knowledge and absence of proper scientific standardization. Only a few systematic report on quality control parameters of the plant parts (leaf, stem, root) of *Pluchea lanceolata* was available [11, 17-18]. No such report on the whole plant is available. The present investigation has therefore undertaken to more detail set standards on the whole plant of *Pluchea lanecolata* and to characterize by various physicochemical parameters and phytochemical techniques.

MATERIALS AND METHODS

Chemical/reagents: BDH grade chemicals/reagents were used in present study.

Plant material

Whole plants of *Pluchea lanceolata* were collected in the month of october around Varanasi district. The plant was botanically indentified by Prof. N. K. Dube, Department of Botany, Banaras Hindu University, Varanasi and voucher specimen is kept in the Department of Rasa Shastra, Faculty of Ayurveda, IMS, BHU.

The collected whole plant materials were cleaned and air dried at room temperature under shade for three weeks. Then they were powered to a fine grade by using a laboratory scale mill and kept in air tight plastic bags until use.

Organoleptic evaluation.

Organolepitc evaluation refers to evaluation of the whole plant poweder of *Pluchea lanceolata* by colour, odour, taste, touch etc. The organoleptic characters of the sample were evaluated based on the method described earlier [19].

Physicochemical investigations

Loss on drying (Moisture content)

About 5.0 g of whole plant powder of *Pluchea lanceolata* was accurately weighed in a dried and tared flat weighing bottle and dried at 105^{o}C for 5h in an oven, cooled in desicator for 30 min and weighed without delay. The percentage in loss of weight was calculated with reference to initial weight [20].

Determination of total ash

5 g of the whole plant powder of *Pluchea lanecolata* was placed in a previously ignited (300°C for 1 h) and tarred crucible accurately weighed. The powder was spread into an even layer in the crucible and the powder ignited to gradually increasing the heat, not exceeding, 450°C until free from carbon, cooled in a desicator, weighed and percentage ash was calculated by taking in account the difference of empty weight of crucible and that of crucible with total ash [21].

Determination of acid insoluble ash

The ash obtained as above was boiled for 5 min with 25 ml of dilute hydrochloric acid. The insoluble matter was collected on an ashless fitter paper by filtration, washed with hot water and ignited to constant

weight. The percentage of acid-insoluble ash with reference to the air dried drug was calculated [19-20].

Determination of water soluble ash

The ash was boiled for 5 min with 25 ml of water, collected insoluble matter in an ashless filter paper by filtration, washed with hot water and ignited for 15 min at temperature of 450°C. Subtract the weight of the insoluble matter with water from the weight of the ash; the difference in weight represents the water soluble ash. The percentage of water soluble ash with reference to the air dried drug was calculated [20-21].

Determination of sulphated ash

1 g of the whole plant powder of *Pluchea lanceolata* was placed in a previously ignited and weighed crucible, ignited gently at first, heated strongly until the substance was thoroughly white. Cooled and added 1 ml of dilute sulphuric acid, heated gently until the sample is charred. After cooling, added 1 ml of sulphuric acid, heated gently until white fumes no longer evolved, then ignited at high temperature around 750°C till the residue is completely incinerated. Cooled the crucible and weighed accurately. Calculated the percentage of sulphated ash.

Determination of ethanol and aqueous extracts

Dried and powdered whole plant powder was extracted with ethanol and water using hot extraction techniques separately. A 20 g of powdered whole plant and 100 ml of ethanol was taken in a round bottom flask fitted with condenser and refluxed for 2 h. The residue was filtered and the ethanol soluble filtrate was concentrated in a Rotary Vacuum Evaporator to yield solid of ethanol extract. Same procedure was followed using water instead of ethanol to prepare the hot aqueous extract. The percentage of extracts were calculated with reference to the air dried drug [21].

Phytochemical screening

Determination of successive solvent extractive values

100 g Air dried whole plant powder of *Pluchea lanceolata* was filled in a Soxhlet extractor apparatus and extracted by successive extraction method with various solvents of increasing polarity such as petroleum ether (60-80°), hexane, benzene, chloroform, ethylacetate, methanol and water. The extracts were concentrated by evaporation, dried and percentage yield was determined [21].

Screening of phytochemicals

Phytochemical analysis was carried out in the petroleum ether (60-80°), hexane, benzene, chloroform, ethylacetate, methanol and water extracts of the whole plant powder of *Pluchea lanceolata* using standard procedures to identify phytoconstituents, as described earlier [22-24]. The procedures for identification of constituents are given below.

Test for flavonoids

To 3 ml of each extract in a test tube, few magnesium pieces are added and conc. HCl was added in it and heated gently. Observed for the formation of magenta (brick red) color indicating the presence of flavonoids.

Test for alkaloids

1 ml of each extract solution was taken in a test tube, acidified with 2-3 drops of dil. HCl and treated with 4-5 drops of Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellowish cream precipitate indicates the presence of alkaloids.

Test of terpenoids

To 1 ml of solution in chloroform of each extract, few drops of acetic anhydride was added in a test tube and then few drops of conc. H_2SO_4 was added by the side of the tube. Terpenoids were indicated by pink colored ring at the junction of the two layers which changes to brown on standing.

Test for sterols

To a small amount of each extract, few drops of chloroform and acetic anhydride was added in a test tube. Further, added few drops of conc. H_2SO_4 by the side of the test tube. Dark red or pink colored ring was observed at the junction of the two layers.

Test for steroids

To 1 ml of solution in chloroform in a test tube of each extract, few drops of acetic anhydride was added and then few drops of conc. H_2SO_4 was added by the side of the test tube. Steroids were indicated by reddish brown colored ring at the junction of two layers which changes to green on standing.

Test for coumarins

The 1 ml of portions of 1% solution of each extract in a test tube was treated with 3-4 drops of 1% KOH to absolute ethanol. Coumarins formed a yellow color.

Test for quinones

To each test samples, sodium hydroxide is added. Formation of blue, green or red color indicates the presence of quinones.

Test for anthraquinones

To each test samples, added KOH solution. Blood red color indicated the presence of anthraquinones.

Test for tannins

Few drops of $FeCl_3$ solution was added to the test solution. Blackish precipitate indicates the presence of tannins.

Test for lignins

To 1 ml of each extract in a test tube, 2% furfuraldehyde was added. Red colour indicates the presence of lignins.

Test for glycosides

To 1 ml of test solution, 1 ml of glacial acetic acid was added, dissolved by heating and then cooled. After cooling, 2-3 drops of ferric chloride was added. Then carefully 2 ml of conc. H_2SO_4 was added along the walls of test tube. Reddish brown ring at the junction of two layers indicates the presence of glycosides

Test for saponins

To a small amount of the extract is a test tube, few drops of distilled water was added and shaken vigorously until persisted foam was observed.

Test for carbohydrates

Test solution was mixed with few drops of Benedict's solution (alkaline solution containing cupric citrate complex) and boiled on water both, observed the formation of reddish brown precipitate indicates the presence of carbohydrates.

Test for phenolic compounds

2 to 3 Drops of 1% $FeCl_3$ solution was added to 2 ml portion of each extract. Phenolic compounds produce a deep violet color with ferric ions.

Test for proteins

The extracts were taken in test tube, treated with few drops of conc. HNO_3. Formation of yellow color indicates the presence of proteins.

Test for amino acids

Test solution, when boiled with 2% ninhydrin solution, would result in the formation of purple color suggesting the presence of amino acids.

Test for vitamin-C

Test solution was treated with dinitrophenylhydrazine (DNPH) dissolved in conc. H_2SO_4. The formation of yellow precipitate would suggest the presence of vitamin-C.

Test for starch

The test sample dissolved in water, was treated with reagent of starch (Iodine). The blue color developed, indicates the presence of starch.

Test for fixed oils and fats

Small quantity of extract was taken between the two Whatman No. 1 papers, the stain on the filter paper indicates the presence of fixed oil and fats.

Test for resins

To 1 ml of the test solution, 2-3 ml of copper sulphate solution was added, the contents were mixed well for 2 minutes, and then the solution was allowed to separate. Resins indicated green colored precipitate.

Thin layer chromatography (TLC) of different successive extracts

TLC is used for the separation of mixture of compounds. It is performed on thin layer coated silica gel (F_{254}, Merck) on aluminium foil. All the extracts were spotted on silica gel plates as single spot separately with capillary tube (Mendham et al., 2002). The plate is run with suitable solvent system in TLC chamber upto the top of the plate. The plate is then taken out from the chamber, dried at room temperature and the spots were visualized by developing with suitable reagent eg. Iodine vapour/Lieberman-Burchard reagent. Rf values of each spot was calculated by distance travelled by the solute divided by distance travelled by the solvent.

Fluorescence study of whole plant powder

Fluorescence study is an essential parameter of first line standardization of crude drug [25]. Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material. Some show fluorescence in the visible range in day light. The ultraviolet light produces fluorescence in many natural products which do not visibly fluorescence in day light. Some of the substances may be often converted into fluorescent derivatives by using different chemical reagents though they are not fluorescent. A small amount (0.5 g) of whole plant powder was taken into clean and dried text tubes. To each tube, 5 ml of the different reagents/solvents like distilled water, 10% aqueous NaOH, 10% alcoholic NaOH, 10% aqueous KOH, 10% alcoholic KOH, 5% HCl, 5% H_2SO_4, 5% HNO_3, 5% picric acid, 5% CH_3COOH, 5% $FeCl_3$, 5% I_2, NH_3 solution, petroleum ether ($60\text{-}80^0$), hexane, benzene, chloroform, ethylacetate, acetone, ethanol, methanol, dimethylether and methylene dichloride were added separately. All the tubes were shaken and they were allowed to stand for about 1 h. The solutions obtained were observed under the visible light and U.V. light (245 nm) for their characteristic color reaction and colors were recorded [26-27].

Statistical analysis

The statistical analysis of the results obtained was carried out by the use of the statistical software MS Excel 2007 and the mean values along with standard deviation were recorded.

RESULTS

The results of organoleptic evaluation, physicochemical analysis, successive solvent extractive values, phytochemical screening, TLC and fluorescence analysis are shown respectively in Tables: 1–6. Results shown in Table 1, 2, 4, 5, 6 are obtained from experiments repeated three times. In table 3 all the extractives were obtained at a time.

Table 1. Organoleptic properties of whole plant powder of *Pluchea lanceolata*.

Parameters	Raw	Ethanol extract	Aqueous extract
Appearance	Powder	Liquid	Liquid
Touch	Coarse	Smooth	Smooth
Colour	Yellowish light green	Light brown	Dark brown
Taste	Light biter	Bitter	Bitter
Odour	Characteristic	Characteristic	Characteristic

Table 2. Physicochemical parameters of the whole plant powder of *Pluchea lanceolata*

Parameters	Whole plant powder (%w/w)
Loss on drying (moisture content)	8.32± 0.44
Total ash value	23.20±0.41
Acid insoluble ash	4.23±0.32
Water soluble ash	3.50±0.22
Sulphated ash value	3.96±0.62
Ethanol soluble extractive value	26.53±5.2
Water soluble extractive value	38.45±0.36

Table 3. Successive solvent extractive values of the whole plant powder of *Pluchea lanceolata*

Solvents	Extractive values (% w/w)
Petroleum ether (60-80°)	4.50
Hexane	5.35
Benzene	5.55
Chloroform	8.26
Ethyl acetate	10.73
Methanol	11.85
Water	13.28

Table 4. Phytochemical screening of different successive extracts of whole plant of *Pluchea lanceolata*

Components	Pet ether ($60\text{-}80^0$) extract	Hexane extract	Benzene extract	Chloroform Extract	Ethylacetate extract	Methanol extract	Aqueous extract
Flavonoids	-	-	+	+	+	+	-
Alkaloids	-	-	+	+	+	+	+
Terpenoids	+	+	+	+	-	-	-
Sterols	+	+	+	+	-	-	-
Steroids	-	-	+	+	+	+	-
Coumarins	-	-	-	-	-	-	-
Quinones	-	-	-	-	-	-	-
Anthraquinones	-	-	-	-	-	-	-
Tannins	-	-	+	+	+	+	+
Lignins	-	-	-	-	-	-	-
Glycosides	-	-	+	+	+	+	+
Saponins	-	-	-	+	+	+	+
Carbohydrates	-	-	-	+	+	+	+
Phenolic compounds	-	-	+	+	+	+	+
Proteins	-	+	+	+	+	-	+
Amino acids	-	+	+	+	+	-	-

Vitamin-C	-	-	+	+	-	-	-
Starch	-	-	-	-	-	-	-
Fixed oils and fats	+	+	+	+	-	-	-
Resins	-	-	-	+	+	+	+

Table 5. Thin layer chromatographic pattern of various successive extracts of whole plant of *Pluchea lanceolata*

Extracts	Adsorbent	Solvent System	Viewing medium	No. of spots on TLC sheet	Rf values
Petroleum ether (60-80^0)	Silica gel	C_6H_6-$CHCl_3$ (2:1)	Iodine vapour	5	0.21, 0.28, 0.45, 0.68, 0.78
Henxane	Silica gel	C_6H_6-$CHCl_3$ (1:2)	Iodine vapour	6	0.33, 0.43, 0.55, 0.68, 0.76, 0.85
Benzene	Silica gel	C_6H_6-$CHCl_3$ (1:8)	Iodine vapour	5	0.25, 0.35, 0.48, 0.61, 0.79
Chloroform	Silica gel	$CHCl_3$-EtOAc (1:2)	Iodine vapour	7	0.28, 0.36, 0.44, 0.51, 0.68, 0.81, 0.92
Ethylacetate	Silica gel	$CHCl_3$-MeOH (8:1)	Iodine vapour	6	0.35, 0.46, 0.55, 0.65, 0.78, 0.88
Methanol	Silica gel	$CHCl_3$– MeOH (1:8)	Iodine vapour	5	0.37, 0.49, 0.52, 0.78, 0.85
Water	Silica gel	$CHCl_3$–MeOH-H_20 (65:35:10), Organic phase	LeibermanBurchard reagent	6	0.34, 0.49, 0.58, 0.63, 0.79, 0.80

Table 6. Fluorescence study of whole plant powder of *Pluchea lanceolata*

Powder+reagents/solvents	Visible light	U.V. light (245 nm)
Powder as such	Yellowish light green	Yellowish green
Powder + distilled H_2O	Yellowish brown	Green
Powder + 10% aqu. NaOH	Light brown	Greenish yellow
Powder + 10% alc. NaOH	Light green	Green
Powder +10% aqu. KOH	Yellowish brown	Brown
Powder + 10% alc. KOH	Brown	Light green
Powder + 5% HCl	Greenish brown	Black
Powder + 5% H_2SO_4	Brown	Light violet
Powder +5% HNO_3	Light green	Yellowish orange
Powder + 5% picric acid	Yellowish green	Yellow
Powder + 5% CH_3COOH	Light green	Green
Powder + 5% $FeCl_3$	Greenish yellow	Greenish yellow
Powder + 5% I_2	Light brown	Light orange
Powder + NH_3 solution	Yellowish brown	Green
Powder + pet ether (60-80^0)	Greenish yellow	Light green
Powder + hexane	Greenish yellow	Greenish black
Powder + benzene	Greenish black	Dark black
Powder + chloroform	Dark green	Yellowish green
Powder + ethyl acetate	Greenish black	Light green
Powder + acetone	Greenish black	Brownish green
Powder + ethanol	Yellowish brown	Yellow

Powder + methanol	Dark brown	Greenish yellow
Powder + diethyl ether	Light green	Green
Powder + methylene dichloride	Light green	Yellowish brown

DISCUSSION

The whole plant of *Pluchea lanceolata* was subjected to systematic organoleplic evaluation, physicochemical and phytochemical screening which are helpful in determining the quality and purity of a crude drug.

As represented in Table-1, both the ethanol and aqueous extracts of the whole plant of *Pluchea lanceolata* has similar organoleptic properties, except in color of both extracts. As exhibited from Table-2, the percent weight of moisture content was found to be 8.32 ± 0.44. The total ash value was found to be 23.20 ± 0.41% whereas acid insoluble ash, water soluble ash and sulphated ash values were found to be low. High content ethanol soluble and water soluble values were found to be 26.53 ± 0.52 % and 38.45 ± 0.36% respectively. Ash values are used to find out quality, authenticity and purity of drug, hence these values are important quantitative standards [28]. Table-3 showed the successive extractive values of different solvent system which assist in evaluation of definite constituents soluble in a particular solvent.

As seen in Table-4, the phytochemical screening of different extracts indicated the presence of flavonoids, alkaloids, terpenoids, sterols, steroids, tannins, glycosides, saponins, proteins, carbohydrates, amino acids, phenolic compounds and fixed oils and fats. The extracts does not indicate the presence of coumarins, quinones, anthraquinones, lignins and starch. The presence of the above constituents in the plant may possibly be responsible for biological activities of *Pluchea lanceolata*.

As seen in Table-5, TLC analysis of whole plant successive extracts of *Pluchea lanceolata* resulted in identification of 5 spots in petroleum ether, benzene and methanol extracts each, 6 spots in hexane, ethylacetale and aqueous extracts each and 7 spots in chloroform extract. This shows the presence of phytochemicals in varying quantities with the respective extracts.

The results of fluorescence analysis of whole plant powder (Table-6) of *Pluchea lanceolata* showed characteristic coloration in treatment with various reagents/solvents, which plays an important role in determination of quality and purity of drug.

CONCLUSION

In the present study, whole plant of *Pluchea lanceolata* was thoroughly investigated for their organoleptic characters, physicochemical properties and phytochemicals, to analyze their quality, safety and standardization for its safe use. Phytochemical analysis of *Pluchea lanceolata* showed the presence of various bioactive compounds in all the extracts in varying qualities. TLC analysis revealed the presence of various types of phytochemicals based on the number of spots. The phytochemicals of different extracts of *Pluchea lanceolata* would be helpful is treating many diseases. The fluorescent analysis of powdered plant plays an important role in the determination of quality and purity of the drug. The generated information of the present study will provide data which is helpful in the correct identification and authentication of *Pluchea lanceolata* and may help in preventing its adulteration.

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CONFLICT OF INTEREST

No interest

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