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PHYTOCHEMICAL SCREENING AND TLC PROFILE OF ETHYL ACETATE EXTRACT OF *LEUCAS BIFLORA* LEAVES

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ABSTRACT

The present study was aimed to carry out preliminary phytochemical screening and thin layer chromatography of Ethyl acetate extract of *Leucas biflora* leaves. The plant has been reported to be used traditionally for the treatment of conjunctivitis, nosebleed and white discharge in women. The plant material was collected, leaves were shade dried and pulverized into coarse powder. It was then extracted with Ethyl acetate solvent by soxhlation. The extract was subjected to preliminary phytochemical screening to detect the presence of primary and secondary metabolites. It showed the presence of Carbohydrate, Lipids, Tannins, Flavonoids and Steroids. A number of different solvent system were tried for developing TLC profile of Ethyl acetate extract of *L.biflora* leaves and the better separation of the components was observed in Methanol: n-Butanol: Formic acid (8:1:1) solvent system. 5 components were found to be separated with corresponding Rf values 0.109, 0.581, 0.727, 0.927 and 1.00. In future, these phyto-constituents can be isolated by column chromatography and can be characterized by Mass spectroscopy, Infrared spectroscopy, Nuclear magnetic resonance spectroscopy etc. Their pharmacological potential can be evaluated and can be formulated into a suitable dosage form.

KEYWORDS: *Leucas biflora*, Ethyl acetate, TLC, Phytochemical screening.

INTRODUCTION

Plants and other natural substances have been used for the treatment of diseases and disorders in virtually all cultures from the early ages. The widespread use of medicinal plants has been

found to be described in various ancient texts all over the world. All different systems of medicine use plants as a source of medicines. It is estimated that more than 80 % of world population uses medicinal plants and plant derived products for primary healthcare.

Leucas biflora is a perennial herb belonging to family Lamiaceae is commonly found throughout India and propagate at river bank and frequent on rocky wet soil.Traditionally mature leaf decoction of *Leucas biflora* is used as eye drop twice a day in case of conjunctivitis. The mature leaves ground with leaves of *Centella asiatica* in a ratio of 2:1 and juice extracted from this mixture is applied directly to stop instance of bleeding from nose (nose bleed) and 4 to 5 leaves are also prescribed to chew with a leaf of *Piper betel* for the women suffering from white discharge. No scientific work has been found to be reported regarding the phyto-constituents of leaves.

MATERIALS AND METHODS

Collection and authentication of plant

Leaves of *Leucas biflora* were collected from campus of Smt. Kishoritai Bhoyar College of Pharmacy, Kamptee and authenticated from Botany Department of RTMNU, Nagpur University, Nagpur **(Specimen Voucher no. 9998).**

Method of Extraction of leaves of *Leucas biflora*

The collected leaves were dried in shade, pulverised into coarse powder. 100 gm extracted of leaves powder was extracted with Ethyl acetate solvent by continuous hot percolation method using soxhlet apparatus. The extract so obtained was then filtered and concentrated using rotatory vacuum evaporator. % yield of extract was calculated and was evaluated for its organoleptic characteristics.

Preliminary phytochemical screening

Ethyl acetate extract of *L. biflora* leaves was subjected to preliminary phyto-chemical screening to detect presence of various primary and secondary metabolites like proteins, amino acids, fats and oils, carbohydrates, alkaloids, glycosides, flavonoids, tannins, steroids, saponins etc.

1) Tests for Carbohydrates

Molish's test

To 2 ml of test solution, two drops of alcoholic solution of α-Naphthol was added. The

mixture was shaken well and few drops of concentrated sulphuric acid were added slowly along the sides of test tube. A violet ring at the junction of two lights indicates the presence of carbohydrates.

Benedict's test

To 2 ml of test solution, 2 ml of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 minutes. A characteristic coloured precipitate indicates the presence of sugar.

Fehling's Test

1ml of Fehling's A solution and 1ml of Fehling's B solution were mixed, boiled for 1 minute, equal amount of test solution was added to it. Reaction mixture was heated in boiling water bath for 5-10 minutes. First yellow, then red precipitate of cuprous oxide indicates the presence of sugar.

Iodine Test

1 ml of Iodine solution was mixed with 1ml of test solution. Formation of deep blue colour indicates the presence of starch.

2) **Tests for Proteins**

Millon's test

To 3 ml of test solution, 5 ml of Millon's reagent was added. A white precipitate, which on warming turns brick red indicates the presence of proteins.

Biuret test

To 2ml of test solution, 2ml of Biuret reagent was added, violet colour indicates presence of proteins.

3) Tests for Amino acids

Ninhydrin Test

To 2ml of test solution, 2 ml of ninhydrin solution was added. Appearance of purple colour indicates the presence of amino acids.

4) Tests for fixed oils and fats

Spot test

A small quantity of powder was pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oils.

5) **Tests for Alkaloids**

Mayer's test

To 2-3 ml of test solution, 2 ml of Mayer's reagent were added. Appearance of white creamy precipitate indicates the presence of alkaloids.

Wagner's test

To 2-3 ml of test solution, 2 ml of Wagner's reagent were added. Appearance of cream colour precipitate indicates the presence of alkaloids.

Dragendorff's Test

To 2-3 ml test solution, 2 ml of Dragendorff's reagent were added. Formation of reddish brown precipitate indicates the presence of alkaloids.

Hager's Test

To 2-3 ml test solution, 2 ml of Hager's reagent were added. Formation of yellow orange precipitate indicates the presence of alkaloids.

6) Tests for Glycosides

A. For Anthraquinone glycoside

Borntrager's Test

To 2 ml of test solution, few ml of dil. Hydrochloric acid was added and boiled for few minutes, filtered and cooled to it, 3 ml of chloroform was added and shaken, chloroform layer was separated and 10% ammonia solution was added to it. Appearance of pink or red colour indicates presence of glycosides.

B. For Cardiac glycosides

Keller-Killiani test (for deoxy-sugars)

To the alcoholic extract of sample add 5 ml of water and 0.5 ml of strong solution of lead acetate. Filter and treat the clear filtrate with equal volume of chloroform and evaporated to yield the dry residue. Add glacial acetic acid, 0.5 ml of ferric chloride solution and 2 ml of

concentrate sulphuric acid. Formation of red brown layer changes to blue green indicates presence of Cardiac glycosides.

Legal's test

1 ml of test solution was treated with 2 ml of pyridine and 1 ml of alkaline sodium nitroprusside solution, blood red colour indicates presence of cardiac glycoside.

Baljet's test

The 2ml of test solution was treated with sodium picrate solution, formation of orange colour indicates presence of cardiac glycoside.

C. For Saponin glycosides

Froth formation test

2 ml test solution was shaken well with water in a test tube; formation of stable froth (foam) indicates the presence of saponin glycoside.

Haemolysis test

To 2ml test solution, 1 drop of blood was added and allowed to stand for 15 minutes, settling down of RBCs indicates the presence of saponin.

7) Tests for Tannins and phenolic compounds:

Ferric chloride test

2-3 ml of test solution was treated with ferric chloride solution, appearance of blue colour indicates presence of hydrolysable tannins and appearance of green colour indicates the presence of condensed tannins.

Gelatin test

To the test solution, 1% Gelatin solution containing 10% sodium chloride was add, appearance of precipitate indicates the presence of tannin.

Lead acetate Test

To the 2-3 ml of aqueous test solution, few drops of Lead acetate solution was added, formation of precipitate indicates the presence of tannin.

8) Tests for Steroids

Liebermann-Burchard test

To 2 ml of the test solution, 1-2 ml of acetic anhydride were added, boiled and cooled. Then concentrated sulphuric acid was added from the side of the test tube, brown ring was formed at the junction two layers and upper layer turns green which shows presence of steroids and formation of deep red colour indicates presence of triterpenoids.

Salkowski test

To 2 ml of the test solution, 2 ml of chloroform was added and 2 ml of concentrated sulphuric acid was added from the side of test tube, the chloroform layer shows red to blue colour and acid layer shows greenish yellow florescence.

9) Tests for Flavonoids

Shinoda test

To 2 ml of test solution, few magnesium turnings and concentrated hydrochloric acid drop wise were added, pink scarlet, crimson red or occasionally green to blue colour appears after few minutes which indicates presence of flavonoids.

Alkaline reagent test

To the test solution, few drops of sodium hydroxide solution was added, there is formation of intense yellow colour which becoming colourless on addition of few drops of dilute acid indicates presence of flavonoids.

Lead acetate Test

To the test solution few drop of lead acetate solution were added. Yellow precipitate indicates presence of flavonoids.

10) Test for terpenoids

1 ml of extract treated with 1% CuSO4 solution, formation of emerald green colour indicates the presence of Diterpene.

Thin layer chromatography (TLC):

The ethyl acetate extract *of L.biflora* leaves was subjected to thin layer chromatography.

Procedure/ method

Silica gel was made into slurry with sufficient quantity of distilled water.The chromatographic plates were thoroughly cleaned with chromic acid mixture followed by washing with distilled water and dried in oven. Silica gel was coated uniformly on the cleaned plates and allowed to dry at room temperature. The plates were then activated in an oven at 120ºC for 20 minutes. The solvent system was poured in the TLC chamber to a depth of 3cm and allowed to saturate. The chamber was lined with the filter paper on the three sides to maintain equilibrium of mobile phase. The test solution of extract was applied on the activated plate with the help of capillary at about 1cm above the origin. The chromatogram was allowed to develop about 80% of solvent run. The plate where observed under UV Lamp for detection of fluorescent spots and sprayed with vanillin- sulphuric acid reagent, and heated in oven at 110ºC for 30minutes. Then Rf value of each developed spots was calculated.

Calculation of Rf value:

The Rf value is calculated as follows.

$$Rf = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

Rf = retardation factor or ratio-to-front value expressed in decimal fraction

RESULTS AND DISCUSSION

Table 1: Organoleptic evaluation of Ethyl acetate extract of *L.biflora* leaves.

Sr.No.	Evaluation	Observations
1.	Colour	Green
2.	Odour	Pungent
3.	Consistency	Sticky, Semisolid
4.	% yield	4.2 %w/w
5.	PH	6.5

Table 2: Preliminary phytochemical screening of Ethyl acetate extract of *L. biflora* leaves.

Sr. No.	Phyto-constituents	Results
1	Carbohydrates	+++
2	Proteins	----
3	Amino acids	----
4	Lipids	+++
5	Alkaloids	----
6	Glycosides	----
7	Tannins and phenolic compounds	+++

8	Flavonoids	+++
9	Terpenoids (sterols)	+++
10	Saponins	----

The preliminary phyto-chemical screening of Ethyl acetate extract of *L.biflora* showed the presence of carbohydrate, lipids, tannins, flavonoids and steroids.

Table 3: TLC profile of Ethyl acetate extract of *L. biflora* leaves.

Solvent system	Proportion (in ml)	Number of spots	Rf value	Colour in UV (254nm)
Methanol: n - Butanol: Formic acid	8:1:1	5	0.109 0.581 0.727 0.927 1.00	Pink

A number of different solvent system were tried but the better separation of the components was observed in Methanol: n- Butanol: Formic acid (8:1:1) solvent system. Five components were found to be separated with corresponding Rf values 0.109, 0.581, 0.727, 0.927, 1.00.

Fig. 1: *L. biflora* leaves.

Fig. 2: *L. biflora* flowers.

CONCLUSION

The results indicate that the Ethyl acetate extract of *L.biflora* leaves contains considerable number of bioactive phyto-constituents and it may be concluded that result obtained from qualitative evaluation of TLC profile could be useful for further studies.

As therapeutic potential of any plant depends on the presence of its chemical composition. The secondary metabolites like steroids, alkaloids, tannins, flavonoids possess a number of pharmacological activities like anti-inflammatory, antimicrobial, antioxidant, analgesic, anti-diabetic, hepatoprotective, etc.

In future these phyto-constituents can be isolated by column chromatography and can be characterized by Mass spectroscopy, Infrared spectroscopy, Nuclear magnetic resonance spectroscopy etc. Their pharmacological potential can be evaluated and can be formulated into a suitable dosage form.

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