INTERNATIONAL RESEARCH JOURNAL OF PHARMACY

www.irjponline.com ISSN 2230 – 8407

Research Article

PHYTOCHEMICALS ANALYSIS AND TLC FINGERPRINTING OF METHANOLIC EXTRACTS OF THREE MEDICINAL PLANTS

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Article Received on: 11/03/13 Revised on: 06/04/13 Approved for publication: 01/05/13

DOI: 10.7897/2230-8407.04627

IRJP is an official publication of Moksha Publishing House. Website: www.mokshaph.com

ABSTRACT

The present work is done on three medicinal plants (*Enhydra fluctuans, Lecuas aspera* and *Dillinia indica*) in order to investigate the presence of the various types of Phytoconstituents. The leaves of all three plants were extracted using methanol as solvents. For the purpose of phytochemical investigation, Preliminary qualitative chemical test and TLC were mainly used. Thin layer chromatography (TLC) has been carried out on all the three plants in two different solvent systems, which showed different Rf value. TLC profiling of all these three extracts confirm about the presence of various phytochemicals. Different Rf (Retention factor) value of various phytochemicals provide valuable clue regarding their polarity and selection of solvents for separation of phytochemicals.

Keywords: Phytochemical screening, TLC, *Enhydra fluctuans, Lecuas aspera, Dillinia indica*

INTRODUCTION

India has one of the oldest, richest and most diverse cultural traditions associated with the use of medicinal plants. This knowledge is accessible from thousands of medical texts and manuscripts. The substances having medicinal value have been extensively used for treating various disease conditions. Herbs being easily available to human beings have been explored to the maximum for their medicinal properties. Products of primary metabolism such as amino acids, carbohydrates and proteins are vital for the maintenance of life processes, while others like alkaloids, phenolics, steroids, terpenoids are products of secondary metabolism and have toxicological, pharmacological and ecological importance[1]. Flavonoids consist of a central three-ring structure. Proanthocyanidins are oligomers of flavonoids. All compounds of flavonoids contain phenol-groups involved in an effect as general antioxidant. Other actions are diverse-several structures reduce inflammation or carcinogenicity. The group isoflavones are primarily known as phytoestrogens. Flavonoids and proanthocyanidins are all pigments occurring in a long range of plant families. Tannins are used as astringents in cases of diarrhea, skin bleedings and transudates. Tannins are very widely distributed in the plant kingdom. The terpenoids are synthesized via the five-carbon building block isoprene. They show property like antineoplastic, antibacterial, antiviral effects as well as gastrointestinal stimulation. The alkaloids are heterocyclic, nitrogen containing compounds, usually with potent activity and bitter taste. They are of limited distribution in the plant kingdom. The various groups have diverse clinical properties. Most saponins- "soap forming compound"- occur as glycosides[2]. The importance of antioxidant activities of phenolic compounds and their possible usage in processed foods as a natural antioxidant have reached a new height in recent years. Plant phenolics, originally hypothesized to inhibit carcinogenesis by virtue of antioxidant or electrophile trapping mechanisms, can also act as potent modulators of arachidonic metabolism cascade pathways[3]. Plant products have been part of phytomedicines since time immemorial. This can be derived from barks, leaves, flowers, roots, Fruits, seeds. Knowledge of the chemical constituents of plants is desirable because such information will be value for synthesis of complex chemical substances[4]. Various parts of the plants like roots, leaves, bark, exudates etc. are used as per medicinal properties.[5] *Leucas aspera* (Willd.) Linn. (Family: Lamiaceae) is distributed throughout India from the Himalayas down to Ceylon. The plant is used traditionally as an antipyretic and insecticide. Flowers are valued as stimulant, expectorant, aperients, diaphoretic and insecticide. Leaves are considered useful in chronic rheumatism, psoriasis and other chronic skin eruptions. Bruised leaves are applied locally in snake bites[6]. *Leucas aspera is* also used for treatment of respiratory tract disorders, edema, gastrointestinal disorders, pain, and as an antidote to poison[7]. *Dillenia indica* L. (Family – Dillineacea) commonly known as elephant apple is distributed in the sub-Himalayan tract of India. *Dillenia indica* is an ethno-medicinally important plant used for the treatment of severe diseases like cancer and diarrhea. The fruit extract has shown significant anti-leukemic activity in human leukemic cell lines. The fruit possesses tonic and laxative properties and is used for abdominal pains[8]. A total of four compounds namely, lupeol, betulinaldehyde, betulinic acid and stigmasterol were isolated from the stem extract of *Dillenia indica* Linn. The structures of the isolated compounds were established by extensive spectroscopic studies[9]. *Enhydra fluctuans Lour* (Family: Asteraceae) is commonly called Water Cress. Its leaves are used in the treatment of skin diseases, nervous affection and also useful to cure inflammation, leucoderma, bronchitis and biliousness.[10] In the present work phytochemical analysis and TLC profiling were carried out in three selected medicinal plants *Lecuas aspera, Dilenia indica* and *Enhydra fluctuans* which are commonly found in the Northeastern region of India The selection of the above said plants were mainly based on their wide ethno pharmacological use and their easy availability in local market and nearby areas of Gauhati University.

MATERIALS AND METHODS

Plant material Collection

The Plants were collected from local market and nearby areas of Guwahati University Campus. Plants are authenticated from department of Botany (Gauhati University) using standard reference. The collected aerials parts of plant were

made thoroughly free from any foreign organic matter, Dried under shade and powdered.

Chemicals and reagents
All the solvents used for extraction, phytochemicals screening and TLC profiling of plant were of analytical grade, respectively. Silica gel GF-254 (Merck) was used for preparation of TLC plate.

Preparation of plant extract
Sample (30gm) of the leave of all the three plants (*Lecuas aspera, Dilinia indica, Enhydra fluctuans*) were extracted separately in a soxhlet apparatus with 200ml methanol for 72 hours until extract was obtained. The solvent extracts were concentrated separately under reduced pressure in a rotator evaporator. After complete solvent evaporation, each of these solvent extracts were weighed and subjected to phytochemical Screening and TLC fingerprinting.

Phytochemical screening
The Phytochemical screening of the three plants showed the presence of different primary and secondary bioactive molecules like Carbohyrate, proteins, fixed oils, alkaloids, flavonoids, terpenoids, tannins, and saponin. The results and observations were summarized in Table 1.
The methanolic extract of *Leucas aspera, Dilinia indica* and *Enhydra fluctuans* were tested for the presence or absence of different primary and secondary compounds like carbohydrates, proteins, fixed oils, flavonoids, alkaloids, saponins, terpenoids, tannins and phenolic compounds by using following standard methods.[11, 12]

- **Test for proteins**

Millon's test
Crude extract when mixed with 2ml of Millon's reagent, white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein. Ninhydrin test Crude extract when boiled with 2ml of 0.2% solution of Ninhydrin, violet colour appeared suggesting the presence of amino acids and proteins.

- **Test for carbohydrates**

Fehling's test
Equal volume of Fehling A and Fehling B reagents were mixed together and 2ml of it was added to crude extract and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars.

Benedict's test
Crude extract when mixed with 2ml of Benedict's reagent and boiled, a reddish brown precipitate formed which indicated the presence of the carbohydrates.

Molisch's test
Crude extract was mixed with 2ml of Molisch's reagent and the mixture was shaken properly. After that, 2ml of concentrated H2SO4 was poured carefully along the side of the test tube. Appearance of a violet ring at the inter phase indicated the presence of carbohydrate.

Iodine test
Crude extract was mixed with 2ml of iodine solution. A dark blue or purple coloration indicated the presence of the carbohydrate.

- **Test for phenols and tannins**

Crude extract was mixed with 2ml of 2% solution of FeCl3. A blue-green or black coloration indicated the presence of phenols and tannins.

- **Test for flavonoids**

Shinoda test
Crude extract was mixed with few fragments of magnesium ribbon and concentrated HCl was added drop wise. Pink scarlet colour appeared after few minutes which indicated the presence of flavonoids.

Alkaline reagent test
Crude extract was mixed with 2ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids.

- **Test for saponins**

Crude extract was mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

- **Test for terpenoids**

Crude extract was dissolved in 2ml of chloroform and evaporated to dryness. To this, 2ml of concentrated H2SO4 was added and heated for about 2 minutes. A grayish colour indicated the presence of terpenoids.

- **Test for alkaloids**

Crude extract was mixed with 2ml of 1% HCl and heated gently. Mayer's And Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids

Thin Layer Chromatography
The thin layer chromatography result confirmed the presence of different bioactive compounds (Figure 1 and 2). The results and observations were summarized in Table 2**.** TLC plates were prepared by using Silica Gel-GF 254 as adsorbent. 15gm silica gel-G was mixed with 30ml of distilled water (1:2) to make slurry. The slurry was immediately poured into the plates. Plates were then allowed to air dry for one hour and layer was fixed by drying at 110 0C for one and half hours. Using a micropipette, about 10µml of extracts were loaded gradually over the plate and air dried. The plates were 1st developed in chloroform: Methanol in 5:1 ratios. The plates were again loaded with sample and developed in Toluene: Chloroform: Acetone (5:3.1:4.3). Both the solvent showed different Rf value for the same plant extract. The chromatograms were observed under visible light and were photographed. The Rf value was obtained by using the following formula.

$$Rf = \frac{\text{Distance travelled by the solute (cm)}}{\text{Distance travelled by the solvent (cm)}}$$

RESULT AND DISCUSSION

Table 1: Result of Phytochemical Screening of Methanolic Extracts of *Enhydra Fluctuans, Lecuas Aspera* and *Dillinia Indica*

Bioactive groups	*Enhydra fluctuans*	*Lecuas aspera*	*Dillinia indica*
Carbohydrate	++	-	++
Protein	-	+	++
Fixed oil	+	+	+++
Tannin and phenolics	++	++	+++
Saponin	+	+	+
Flavanoid	+++	++	+++
Alkaloid	++	+	+
Terpenoids	++	++	+++

- Absent: + Presence, ++ moderate presence, +++ highly presence

Table 2: **Result of TCL Fingerprinting of Methanolic Extract of *Enhydra Fluctuans, Lecuas Aspera* and *Dillinia Indica***

S. No.	Plants	Solvent system used	Rf value obtained
1	*Enhydra fluctuans*	Methanol: chloroform(1:5)	0.25,0.30, 0.60, 0.63
		Toluene : chloroform :Acetone (5:3.1:4.3)	0.25, 0.26, 0.58, 0.63
2	*Lecuas aspera*	Methanol: chloroform(1:5)	0.20,
		Toluene : chloroform :Acetone (5:3.1:4.3)	0.33, 0.58
3	*Dillinia indica*	Methanol: chloroform(1:5)	0.34, 0.41, 0.75, 0.80
		Toluene : chloroform :Acetone (5:3.1:4.3)	0.16, 0.25, 0.66, 0.75

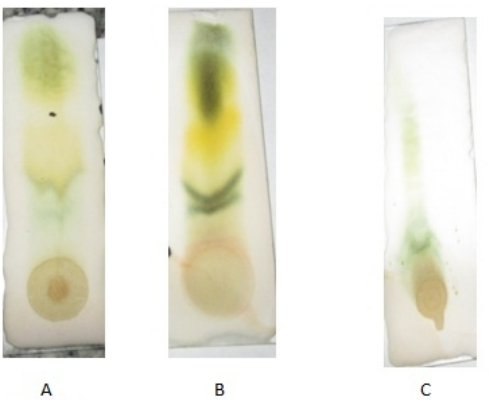

Fig 1 – TLC fingerprint of three plants in Methanol chloroform (1:5)
(A) *Dilinia indica* (B) *Enhydra fluctuans* (C) *Leucas aspera*

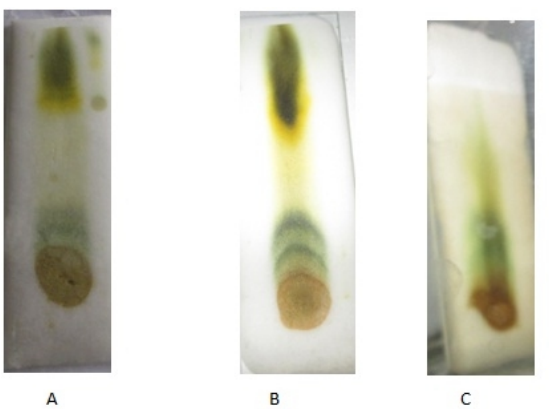

Fig 2 – TLC fingerprint of three plants in Toluene: chloroform: Acetone (5:3.1:4.3)
(A) *Dilinia indica* (B) *Enhydra fluctuans* (C) *Leucas aspera*

For the discovery of any novel drug having pharmacological importance, the essential information's Regarding the chemical constituents are generally provided by the qualitative phytochemical screening of plant extracts, since it gives information about the presence of any particular primary or secondary metabolite in the extracts of the plant which is having a clinical significance. If any such significant bioactive natural product is present, it is necessary to separate that compound from the mixture of compounds by using suitable chromatographic technique. Preliminary phytochemical analysis of methanolic extract of *Enhydra fluctuans, Dillinia indica* and *Leucas aspera* revealed the presence of alkaloid, saponin, Fixed oil, Tannin and phenolics, terpenoids and flavanoid. The TLC profiling of all the three extracts in Chloroform: methanol (5:1) and Toluene: chloroform: Acetone (5:3.1:4.3) solvent system confirms the presence of diverse potent bio molecules in these plants. TLC analysis Provide an idea about the polarity of various chemical constituents, in a way such that compound showing high Rf value in less polar solvent system have low polarity and with less Rf value have high polarity. These potent bio molecules can be further used for development of different drug in future.

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Cite this article as:

Dutta Jayashree. Phytochemicals analysis and TLC fingerprinting of methanolic extracts of three medicinal plants. Int. Res. J. Pharm. 2013; 4(6):123-126

Source of support: Nil, Conflict of interest: None Declared