ejpmr, 2015,2(6), 253-255

SJIF Impact Factor 2.026

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 3294-3211

EJPMR

BIOCHEMICAL ANALYSIS OF VARIOUS PLANTS USING H.P.T.L.C. TECHNIQUES FROM SHIVALIK RANGE (SAHARANPUR DIST)

Swati Singh[1], Krishan Pal[2] and Shrikant Sharma*[3]

[1]Research Scholar, OPJS University, Rajgarh, India.
[2]Associate Professor, OPJS University, Rajgarh, India.
[3]Kisan Sewa aivam Paryavaran Sanstha, Saharanpur, India.

***Correspondence for Author: Shrikant Sharma**
Kisan Sewa aivam Paryavaran Sanstha, Saharanpur, India.

Article Received on 11/09/2015 **Article Revised on 04/10/2015** **Article Accepted on 27/10/2015**

ABSTRACT

The present study was carried out to predict the presence of bioactive molecules like, Anthraquinone Alkaloids, Bioflavonoid and steroids etc, which are very helpful in health industry sector in plant parts of our interest using HPTLC. HPTLC is suitable for qualitative, quantitative and micro preparative chromatography. This technique was deployed for identification of bioactive molecules from plant parts collected from different areas of Shivalik Hill in the area of Saharanpur Dist. These test confirm that selected plant have very important chemical compound which has great importance in Pharma industry.

KEYWORDS: HPTLC, Pharma Industry, Shivalik Hill, Medicinal Plants.

1. INTRODUCTION

The value of the medicinal plants is very much essential for commercial exploitation as well the medicinal value of the raw drugs. Even authenticated plant material may not be of desired quality and strength and not conforming to the physio-Chemical parameters or the concentration of the active phytochemicals or marker compounds as per the pharmacopoeia standard or the consumer/industry requirements because there are many photochemical clones and variation in concentration of photochemical may be ecologically induced.

Chemotaxonomic studies help the science in several ways and these are used in the clarity and understanding of taxa at any level. Whenever there is a conflict between two species than chemotaxonomic studies evaluated along with classical taxonomic feature may solve the status of taxa (Upadhyaya and Singh, 1989).

Chemotaxonomic Studies also help pharmaceutical industries. Phytochemicals studies with the help of spot tests indicate presence of many biologically active molecules like Bioflavonoid, Alkaloids, Anthraquinone and steroids etc. besides this a chemically similar species may be used as substitute for the other. (Upadhyaya et al., 1977).

Out of the total of 944 drugs described, 657 are of plant origin, with descriptions of the outward appearance, locality, mode of collection, formulation of the medicinal preparations, and their therapeutic effect. Discords' mostly appreciated domestic plants like: willow, camomile, garlic, onion, marsh mallow, ivy, nettle, sage, common centaury, coriander, parsley, sea onion, and false hellebore). It is worth underscoring that Dioscorides pointed to the possibility of forgery of drugs, both the domestic ones such as opium forged by a yellow poppy (*Glaucium flavum*) milk sap and poppy, and the more expensive oriental drugs, transported by the Arab merchants from the Far East, such as iris, calamus, caradmomum, incense, etc.

Marco Polo's journeys (1254-1324) in tropical Asia, China, and Persia, the discovery of America (1492) and Vasco De Gama's journeys to India (1498) resulted in many medicinal plants being brought into Europe. Botanical gardens emerged all over Europe, and attempts were made for do mastic cultivation of regional medicinal plants and of the ones imported from the old and the new world. With the discovery of America, materia medica was enriched with a large number of new medicinal plants: *Cinchona, Ipecacuanha, Cacao, Ratanhia, Lobelia, Jalapa, Podophylum, Senega, Vanilla, Mate*, tobacco, red pepper, etc. In 17th century, *Cortex Chinae*, yielded from quinine bark *Cinchona succirubra* Pavon, under the name countess' powder, since the Countess of Chinchon was the first one who used it, was introduced to European medicine. Quinine bark rapidly overwhelmed England, France, and Germany despite the fact that there were many opponent to its use among distinguished physicians—members of a range of academies.

1.1. Location of Study Area

This district is situated in the west of U.P in the catchment area of Ganga and Yamua rivers. It is surrounded by Dehradun Haridwar, Yamunanagar Shamli and Muzaffarnagar districts. The Ganga, Yamuna, Dhamola, and Paon Dhoi are the important rivers of the district (Negi, 2008).

In another zone it is having sandy, sandy-loamy or clay loam broadly classified into Ganga Alluvium soil group. It is found near the river Ganga. Saharanpur located between the Ganga and Yamuna River and their tributaries. The zone is highly productive with light coloured loam soil. The average annual rainfall is 795 mm. Relative humidity ranges from 32 to 85% and the temperature ranges from 1.5^0C to 4.3^0C. Rice wheat sugarcane based cropping system is prevalent in the zone. Inside the region of Shivalik range in Saharanpur region, people do not live but live in small villages adjacent to the area of forest. They graze the cattle in the forest and for the better grassy fodder they cause forest fire, as after the fire grasses reappear with delicate leaves and are much liked by their cattle. These villagers also take fire wood and some medicinal plant to cure the ailment of their cattle also for themselves (Negi, 2008).

2. MATERIAL AND METHODS

2.1. Material: To conduct this study, we have collected the samples of medicinal plants our interest. Different plant parts like Root, Shoot, Stem, Leaves, and flowers etc has been collected from our study area for HPTCL Studies.

2.2. Methodology

2.2.1. High performance thin layer chromatography analysis

High performance thin layer chromatography (HPTLC) is a planer chromatography where separation of the sample components is achieved on high performance layer with detection and data acquisition using an advanced work station. These high performance layer are pre-coated plates, coated with a sorbent (mostly silcagel G-60 PF254) of particle size, 5-7 microns and a layer thickness of 150-200 microns. The reduction in the thickness of the layer and the particle size results in increasing the plate efficiency as well as nature of separation. Separation on high performance thin layer plates gives sharper and more compact bands with shorter distances of migration. HPTLC is suitable for qualitative, quantitative and micro preparative chromatography (Touch stone, 1988).

The main steps for the isolation and identifications of organic molecules are extraction of powdered plant material followed by fractionation with suitable solvents of increasing polarity and monitored by HPTLC (Seth, 2013, tendon, 2010). The identification of separated compound is usually based on their reaction with suitable location reagents and their position in the chromatogram. The fundamental measurement in chromatography is that of Rf. The Rf value is constant for a given compound under standard condition and formula as given bellow:

$$Rf = \frac{distance\ substance\ has\ traveled\ from\ the\ origin}{distance\ solvent\ front\ has\ traveled\ from\ the\ origin}$$

$$Result\ (\%) = \frac{Area\ of\ sample\ x\ wt.\ of\ std.x\ vol.\ of\ sample\ x\ potency\ of\ std.distance\ substance\ has\ traveled\ from\ the\ origin}{Are\ of\ std.x\ vol.\ of\ std.x\ wt.\ of\ sample}$$

The structures of the isolated pure compounds on silica plates are elucidated on the basis of physio-chemical and spectral studies taking advantage of U.V. scanner.

2.2.2. Sample preparation

Take 2 gm dried pant material (leaves) in a 100 ml volumetric flask, add 20 ml methanol (CH_3OH); sonicate it for 5-10 minutes, filter through whatman No. 41 with anhydrous sodium sulphate. Evaporate the filterate to dryness. Reconstitute the residue with 10 ml methanol.

2.2.3. Chromatographic Condition

We use standard condition for H.P.T.L.C. for stationary phase; we take HPTLC Plate 10x10 cm silica gel 60f 254.0 f 0.2 mm thickness whereas for mobile phase; we take Toluene: Ethylacetate: Formic acid (5:4.5:05) ml.

2.2.4. Sample Application

Sample has been prepared by taking 15 ml volumes of test solution and applied on thin layer chromatography Plate as 8 mm band length, 6 mm space between two bands, 10 mm from lower edge of plate. The twin chamber of 10CM*10CM with S.S. lid used as development chamber and distance travelled by solvent was 8-9 cm. for Visualization use >260 nm and >380 nm; after trotted with venelline sulphuric acid.

3. OBSERVATION AND RESULTS

Nearly 25 medicinally important plants have been screened through qualitative analysis using HPTLC. These plants contains many photochemical which has been detected at a particular retardation factor (Rf) value (IUPAC, 2006). This study was only based on Rf values and not claiming about the molecular identity in selected plants. Although timid identification may be made as some of them have been reported with molecular details. Although timid identification may be made as some of them have been reported with molecular details by some scientist. Based on number of different Rf values, present study showed qualitative richness.

Many medicinal plants show correlation with one-another such same chemical compounds have been predicted at Rf 0.46 in *M. champaca, A. rohituka E. officinalis* and *D. Montana.* Again at the Rf value of 0.68; *M. azadirachta* and *M. champaca* contained similar chemical compounds. This chemical compound is specific in these plants. Plants *M. champaca S. alata, A. rohituka D. montana* and *E. officinalis* have same compound at Rf 0.64. likewise this; many other plants have same Rf values. Many other genera have been studied but no similar compounds in rest of samples which was analyzed using HPTLC.

At present HPTLC is rottenly used as an option to HPLC for the quantitave analysis of samples retrieved from plants due to its easy handling, precision, low cost and quickness as described by Sethi (1997). According to Mauji et al (2011), the results of H.P.T.L.C. have better pledge and judgment of active compounds with realistic precision petite time period. Chromatographic fingerprinting is the better option for fulfillment of the need of accurate and prevailing value evaluation Indian traditional Medicine.

4. DISCUSSION

As we already described that, this study was only based on high performance thin layer chromatography analysis and has very limited analysis. HPTLC facilitates us for identification of Bioactive compounds present in many herbs or plants which have medicinal properties and may be used in cure of disease. It was found that many compounds were only present in one sample whether some other compounds were detected in two or more. These chemicals may work as important finger prints in identification of plant medicinal values. Some such specific compounds are present at different Rf values, like *Strychnos nux- vomica*, Rf value of 0.05 0.07 &0.39 and *Melia azadirachta* with Rf value of 0.45 & 0.75.

ACKNOWLEDGEMENT

Authors are highly thankful to all researchers whose work was consulted to carry out this research work.

REFERENCES

1. IUPAC, Compendium of Chemical Terminology, 2nd ed. (the "Gold Book") (1997). Online corrected version: (2006–) "Retardation factor, R in column chromatography".
2. Negi, S., S. (2008). Biodiversity and its conservation in India 2nd revised ed. New Delhi, Indus Publishing ISBN 978-81-7387-211-2
3. Sethi P. D. (2013), Sethi's HPTLC, High Performance Thin Layer Chromatography.
4. Tandon, N. (2010) Phytochemical Reference Standards of Selected Indian Medicinal Plants, volume 1, ICMR, New Delhi, ISSN: 0976-6057
5. Touchstone J., C., Instrumentation of Thin Layer Chromatography: a Review. J. Chromatographer, 1988; 26: 645.
6. Upadhyaya, S.K. and Singh, V., 1989: Phytochemical identify of Cassia angustifolia Vahl. And Cassia acutifolia Del. Nat. Acad. Science Letters, 12.
7. Upadhyaya, S.K., Singh, V. and S.P. Srivastava, S.P., 1977.C. spectabilis, a new source of senna drug.J. Res. Indian Med. Yoga Homeo. 12, 1, PP. 129-130.