ejpmr, 2015,2(6), 250-252

SJIF Impact Factor 2.026

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 3294-3211

EJPMR

CHEMICAL ANALYSIS OF MEDICINAL PLANTS USING SPOT TEST TECHNIQUES FOR IDENTIFICATION OF BIOACTIVE COMPOUNDS

Swati Singh[1], Krishan Pal[2], Shrikant Sharma*[3] and Mohd. Javed[4]

[1]Research Scholar, School of Sciences, OPJS University, Rajgarh, (R.J.)
[2]Associate Professor, School of Sciences, OPJS University, Rajgarh, (R.J.)
[3]Kisan Sewa Aivam Paryavaran Sanstha, Saharanpur (U.P.)
[4]Botany Dept. M S College Saharanpur (U.P.)

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

Article Received on 10/09/2015 **Article Revised on 03/10/2015** **Article Accepted on 26/10/2015**

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. The spot test techniques was deployed for identification of bioactive molecules from plant parts collected from different areas of Shivalik Hill from Saharanpur Dist. These test confirm that selected plant have very important chemical compound which has great importance in Pharma industry.

KEYWORDS: Spot Test, Pharma Industry, Shivalik Hill, Medicinal Plants.

INTRODUCTION

In Indian culture has a centuries old history use of medicinal plants and approximately two million traditional health practitioners called "Vaids" still depends on medicinal plants for curing various diseases (Venketa et al, 2008). These practitioners, sizable great number look upon the mountains and called them store house of varieties of herbal medicines. The vegetation wealth of the Uttaranchal as well as Uttar Pradesh which are located in the Himalayas has received significant attention throughout the ages for cure of various chronic human health problems (Gaur, 1999). Today, many plants of medicinal use threats due to pollution and human population. except this, may modern scientist declines these traditional knowledge on the use of some important medicinal plant and techniques of making many herbal formulations have declined over the few decades due to lack of awareness and spread of allopathic medicines (Kala, 1998a). It is well known fact that Chemotaxonomic analysis of plants is very helpful in designing of new drugs by pharmaceutical industries. Besides this, phyto-chemical test indicate presence of many bioactive molecules like, Anthraquinone Alkaloids, Bioflavonoid and steroids etc, which are very helpful in health industry (Upadhyaya et al., 1977).

MATERIAL AND METHOD

Raw material was collected was from Shivalik region of Dist Saharanpur (Shakambhari Devi area and Mohand). Plant material has collected on the basis of botanic field notes during three different seasons for chemical analysis using spot testing techniques. Different spot test was used for identification are as follows:

Raphides Test: Material of young leaves treated with a saturated aqueous solution of cupric acetate.

Syring in Test: Hand cut sections of fresh plant material (young shoots) were mounted in 50-55% aqueous sulphuric acid.

Hot Water Test: In this test a fresh mature leaf was dipped half way into hot water at about 85^0C for about 8-15 seconds.

HCN Test for Cynogens: Picrate papers prepared by dipping rectangular pieces of filter paper in saturated (0.5M) aqueous picric acid previously neutralized with sodium bicarbonate were stored in a dessicator. Some dried leaves were taken in a glass stoppered test tube, 1 cc water and 2 cc toulence were added to the tube and the material was crushed by a glass rod. The test tube was then closed firmly by a glass stopper with a piece of picric acid paper fastened on its underside with melted paraffin wax. The test tube was incubated at 40^oC for 2 hours.

Formic Acid Test

Dried leaves were extracted in ethyl alcohol in a 250 cc round bottom flask, using a reflex condenser. The extract was evaporated over a water bath and washed successively by petroleum ether and benzene. The residue left behind was dissolved in water. Two cc of this

stock solution was taken in a test tube and 1 cc of 2 N hydrochloric acid and magnesium dust was added to it until no gas evolved. Then 3 cc of 72% sulphuric acid and a little chromatotropic acid was added and the tube was heated at 60^{o}C for 10 minutes.

OBSERVATIONS

- The crystals of calcium oxalate dissolved and oxalic acid so formed yellow crystals of cupric oxalate appeared (Johansen, 1940) [**Raphides Test**].
- Development of blue colour was taken as positive indication of the presence of syring in and rose red colour for absence of syring in (Gibbs, 1974) [**Syring in Test**].
- Instant appearance of brown or black band at the juncture of dipped and undipped area indicated a strongly positive reaction (Gibbs, 1974) [**Hot Water Test]**.
- Appearance of yellow to reddish brown colour on picrate paper indicated the presence of cynogens (Harborne, 1973) [**HCN Test for Cynogens**].
- Apperance of a violet pink colour indicated the presence of formic acid (Feigl, 1947) [**Formic Acid Test**].

RESULTS AND DISCUSSION

Spot tests and chromatography spot patterns have been used directly in chemical evaluation of plants. They have been successfully used for infrageneric classification in several taxa e.g Ficus (Saini and Mukherjee, 1969), Avena (Iiyama and Grant, 1972), Blumea (Singh and Dakshini, 1977) and Aquilegia (Taylar and Compbell, 1969). Several workers have cautioned against such studies (crawford and Dorn, 1974). They are of the opinion that spot tests and spot patterns of Chromatography have their limitations.

1. Differences or similarities between spots based on crude Rf values and colour reagents may be apparents.
2. Artifacts may given a spot on chromatogram or colour in spot tests. These artifacts may develop during handling, normal extraction and concentration, acidic extraction, or chromatographic solvents may cause isomerization of phenolic acid resulting is appearances of numerous blue, green and yellow spots on chromatographic paper.
3. While o-glycosides breakdown, c-glycoside under isomerization in several ways thereby spuriously increasing the apparent but not actual number of spots on a chromatogram (Ribereau-Gayon, 1972; Chopin and Bouillant, 1975).
4. If even a blank sheet of chromatographic paper is developed in as solvent, it will have a few spots of its own.
5. Personal error in observation.
6. Synergistic effect of different chemicals in a spot test.
7. Vast deviation in colour due to slight change in structure of a molecule or PH etc. of the test.
8. Different colours by same class of compounds eg. Luteolin gives orange colour and kaempferol red in shinoda test, while both are flavonoids.
9. Some different class of compounds may respond similarly to these test. For example, leucoanthocyanine, catechol tannins, biflavonoids, and flavonoids respond alike for shinoda test.
10. Over estimation of spot numbers due to slight change in Rf values and colour reaction, which is possible due to change in concentration of chemicals.

Evaluation of chemical data in species under studies

The aim of photochemical approach in taxonomy is refinement an illumination of phyletic problems which remains unsolved by other means. Retention of a primitive chemical character by an advanced taxonor an advanced chemical character by a primitive taxon,arising from a different pathways also needed a different retention. For example,is xanthone present in *Mangifera* as well as *Cassia*. In the former it is derived from anthraquinones by fusion in the central ring (Gatenbeck and Malmstrom, 1969) and hence an advanced character while in the latter it is not derived from anthraquinones and hence a primitive character. Handling and analysis of data also need some comment. Just numerical evaluation of characters by giving equal or different weightage may not be useful. Handling of data for such a limited study of almost sympatric species, representing all major natural groups, be done with character to character discussion. Morphological, Cytological and Geographical data should also be taken into consideration to test the results for congruency derived from analysis of independent chemical data.

RECOMMENDATIONS

Present studies are limited. Tall claim cannot be made out of such small studies. However it is evident that-

1. Spot tests are indication of phytochemicals in a plant.This information may be used by phytochemists and pharmaceutical industry.
2. These tests will also guide solvent selection for our further studies Spot patterns on chromatogram may further be evaluated numerically
3. Data base for phytochemicals in form of finger prints may serve as quick identification tool.

It has been found that some chemicals are specific for one plant as it not present in any other plant analyzed in this study.

REFERENCES

1. Gaur, R.D., 1999. Flora of the District Garhwal: North West Himalaya (with Ethnobotanical Notes). Transmedia, Srinagar (Garhwal).
2. Kala C.P. (1998) Ecology and conservation of alpine meadows in the VAlly of Flowers National Park Garhwal Himalayas (PhD Thesis submitted TO FRI, Dehradun).

3. 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.
4. Venkata Krishnbana P., Venkata Ratnam, K. and Venkata Raju. R.R., 2008: Preliminary phytochemical Evolution of certain anticaneer crude drugs used by advosis of Rayaloscenma region. Andhra Pradesh, India. Ethnobotanical leaflets, 2018; 12: 693-697.