International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.10 No.7, pp 446-454, 2017

Qualitative Analysis of Alkaloids Exist in the Hydroalcoholic Extract of *Ipomoea aquatica* for SSK. in Tamil Nadu

Sasikala M* and Sundaraganapathy R

Faculty of Pharmacy, Karpagam University, Karpagam Academy of Higher Education, Coimbatore – 641 021, Tamil Nadu, India

Abstract : Introduction: Qualitative analysis will help in the detection of phytoconstituents present in the herbal source accurately. The preliminary phytochemical screening and the thin layer chromatographic analysis are found more simple and sensitive and selective techniques in this way. **Aim and Objective:** The research was aimed to reveal the secondary metabolites like alkaloids by using chemical tests and TLC methods. **Methods:** The tests for detecting alkaloids were using acetate: Dragendorff's, Hager's and Mayer's, Wagner's and Tannic acid reagents. The TLC parameters were set silica gel G as adsorbent, Ethly acetate : Methanol: Water in the ratio of 10.0:1.35:1.0 v/v/v as mobile phase, UV light of longer wavelength at 365nm as detection wavelength and R_f value as qualitative respect. **Results and Discussion:** The phytochemical tests were shown positive results for alkaloids. The TLC analysis stated that the presence of nearly nine different fluorescence spots with R_f values of 0.07, 0.13, 0.22, 0.35, 0.48, 0.55, 0.78, 0.88 and 0.94 subsequently. **Conclusion:** Hence, revealing new class of components will assist to develop herbal drug products in the global market. This study could be used in research laboratories for recognizing similar type of compounds using TLC analysis. Definitely this will give the good opportunity for isolating out many therapeutically acting compounds.

Keywords : Detection, Alkaloids, R_f value, Dragendorff, TLC.

Introduction and Experimental

Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms. The name derives from the word alkaline and was used to describe any nitrogen - containing base. Alkaloids are produced by a large variety of organisms, including bacteria, fungi, plants, and animals and are part of the group of natural products (also called secondary metabolites). Many alkaloids can be purified from crude extracts by acid - base extraction. Many alkaloids are toxic to other organisms. They often have pharmacological effects and are used as medications, as recreational drugs, or in entheogenic rituals. Examples are the local anesthetic and stimulant cocaine, the stimulant caffeine, nicotine, the analgesic morphine, or the antimalarial drug quinine. Some alkaloids have a bitter taste[1,2,3].

Extraction methods are widely used to get phytoconstituents from the plant. This is the basic method to detect the compounds present in each and every plant. The application of this process for an important medicinal plant *Ipomoea aquatica* helps in revealing its phytochemicals. These are formed in the primary and secondary metabolism of the herb. The metabolic products are extracted into suitable solvents in which those are soluble. The solvents for extraction are available with various polarities. Mostly therapeutically active

secondary metabolites of the plant will come into polar solvents like alcohol, water, etc. Thereby extraction is found as the most important basic method to recognize the phytochemical potential of a natural source[4,5,6].

Phytochemical Analysis is devoted to the publication of original articles concerning the development, improvement, validation and/or extension of application of analytical methodology in the plant sciences. The spectrum of coverage is broad, encompassing methods and techniques relevant to the detection (including bio-screening), extraction, separation, purification, identification and quantification of compounds in plant biochemistry, plant cellular and molecular biology, plant biotechnology, the food sciences, agriculture and horticulture[7,8,9,10].

Thin layer chromatography, or TLC, is a method for analyzing mixtures by separating the compounds in the mixture. TLC can be used to help determine the number of components in a mixture, the identity of compounds, and the purity of a compound. By observing the appearance of a product or the disappearance of a reactant, it can also be used to monitor the progress of a reaction. TLC is a sensitive technique - microgram (0.000001 g) quantities can be analyzed by TLC - and it takes little time for an analysis (about 5-10 minutes). TLC consists of three steps - spotting, development, and visualization. Photographs of each step are shown on the course website. First the sample to be analyzed is dissolved in a volatile (easily evaporated) solvent to produce a very dilute (about 1%) solution. Spotting consists of using a micro pipette to transfer a small amount of this dilute solution to one end of a TLC plate, in this case a thin layer of powdered silica gel that has been coated onto a plastic sheet. The spotting solvent quickly evaporates and leaves behind a small spot of the material[11,12,13,14,15].

In 1938, Izmailow and Schraiber pioneered the thinlayer chromatography (TLC) method for the analysis of plant material containing alkaloids. TLC is particularly well suited for checking the processes of synthesis as well as for establishing the progress of reactions and testing of products in pharmaceutical preparations. The importance of alkaloids is also fundamental in toxicological analysis; many are used as narcotics and hallucinogenic drugs, as doping substances and as poisons. The presence of alkaloids in drugs of abuse and their metabolites in biological fluids such as urine and blood has also been tested by means of TLC[16,17,18,19,20].

Thin layer chromatography is a simple, cost-effective, and easy-to-operate planar chromatographic technique which has been used in general chemistry laboratories for several decades to routinely separate chemical and biochemical compounds. Traditionally, chemical and optical methods are employed to visualize the analyte spots on the TLC plate. Also it has a wide application in identifying impurities in a compound. Study highlights the review on TLC and its application of qualitative and quantitative estimation of bio-active compounds from medicinal plants[21,22,23,24,25].

Materials

The proposed plant contents are given in the table 01. The instruments, chemicals/reagents and glass wares/apparatus used for the research are denoted in the table 02, table 03 and table 04 respectively.

Table 01: Plant details

S. No.	Parameters	Subject
1.	Plant Name	Water Spinach
2.	Botanical Name	*Ipomoea aquatica* FORSSK.
3.	Family	Convolvulaceae
4.	Location	Parambikulam – Aliyar Riverine, Pollachi
5.	Part of the plant	Whole plant
6.	Authentication No.	BSI/SRC/5/23/2017/Tech./3269
7.	Place of Authentication	BSI, Coimbatore-641003, Tamil Nadu

Table 02: Instruments used

S. No.	Name of the Instrument	Model Name
1.	Precision Balance	Wensar
2.	Hot plate	Cintex
3.	Electrical Water bath	Technico
4.	UV cabinet	CAMAG and Deep Vision

Table 03: Chemicals/Reagents used

S.No.	Name of the Reagent	Company	Location
1.	Petroleum Benzine boiling range 60.0^{0}C-80.0^{0}C GR (Petroleum ether)	Merck Specialities Private Limited	Mumbai – 400 018
2.	Ethanol AR 99.9%	Jiangsu Huaxi International Trade Co., Ltd.	China
3.	Distilled water		
4.	Methanol LR	S d Fine Chemicals Limited	Mumbai – 400 030
5.	Ethyl acetate LR		
6.	Silica gel G for TCL	Loba Chemie Private Limited	Mumbai – 400 005

Table 04: Glass wares/Apparatus used

S. No.	Name of the Glassware	Capacity	Brand Name
1.	Round bottomed flask	1000.0ml	Riviera
2.	Funnel	Medium Size	Sh Borosilicate Glass
3.	Beaker	1000.0ml	Borosilicate Glass
4.	Measuring cylinder	10.0ml	Riviera
5.	Measuring cylinder	50.0ml	Sh Borosilicate Glass
6.	China dish	Big & Small size	Chinese Porcelain
7.	Stirrer	Small size	Sh Borosilicate Glass
8.	Conical flask	250.0ml	Borosilicate Glass
9.	Test tubes	10.0ml	Borosilicate Glass
10.	Pipettes	5.0ml	Borosilicate Glass
11.	Mobile phase chamber (Twin trough)	20×10cm	CAMAG
12.	Glass plate	20.0×10.0cm	Borosilicate Glass
13.	Glass slide	8.0×3.0cm	Borosilicate Glass
14.	Beaker	250.0ml	Borosilicate Glass
15.	Petridish lid	Medium size	Borosil S - Line

Miscellaneous

Aluminium foil, Muslin cloth, Filter paper, Tripod stand, Test tube holders and test tube stands and butter paper.

Methods

Plant Collection, drying and powdering

The plant was collected from Parambikulam – Aliyar Riverine in Pollachi, Tamil Nadu. The collected portions of the plant were washed with distilled water three times. They were allowed to dry under shade kept

over the news paper. Then the half dried portions were cut into small pieces using stainless steel knife and kept under shade only for drying completely. It took 22 days for complete drying. The dried material was pulverized into coarse powder by means of manual blender. The powdered plant material was stored in air tight containers at 4.0^0C for further use. 350.0g of coarse powder of drug was weighed and was taken in a 5000.0ml Round bottomed flask. Petroleum ether was added to remove the fatty matters associated with the powder. The solvent retained was evaporated at room temperature after rinsing for few minutes. Then the dried defatted powder was immersed in 2000.0ml of solvents which comprises 1000.0ml of distilled water and 1000.0ml of ethanol (50:50 v/v). After 7 days, the content of extraction was strained through a muslin cloth. The marc was separated from the menstrum. The extract was kept at 40.0^0C for concentration and evaporation at the same temperature. Then the completely dried extract was cooled to room temperature and weighed[26,27,28,29,30,31,32,33,34,35].

Preliminary phytochemicals Evaluation

Tests for Alkaloids

Dragendorff's test

To 2.0ml filtrate of plant drug extract, 2.0ml of reagent was mixed. Formation of reddish brown precipitate indicated the presence of alkaloids.

Hager's test

To 2.0ml filtrate of plant drug extract, 2.0ml of reagent was mixed. Formation of yellow colour indicated the presence of alkaloids.

Mayer's test

To 2.0ml filtrate of plant drug extract, 2.0ml of reagent was mixed. Formation of reddish brown precipitate indicated the presence of alkaloids.

Wagner's test

To 2.0ml filtrate of plant drug extract, 2.0ml of reagent was mixed. Formation of reddish brown precipitate indicated the presence of alkaloids.

Tannic acid test

To 2.0ml filtrate of plant drug extract, 2.0ml of tannic acid solution was mixed. Formation of buff colour precipitate indicated the presence of alkaloids[36,37,38,39,40,41,].

Thin Layer Chromatographic Analysis

The existing constituents were separated by using proper mobile phase which was selected based on trial and error method [42,43,44,45,46,47].

Chromatographic parameters

Stationary phase selection

Principle: Adsorption
Support material: Glass plate
Dimension of the plate: 20.0×10.0cm
Adsorbent: Silica gel G
Method of thin layer preparation: Pouring method
Layer thickness: 1.0mm
Plate activation temperature: at 105.0^0C for one hour

Mobile phase selection

Chamber: Twin trough mobile phase chamber

Chamber dimension: 20.0×10.0cm
Mobile phase selected for separation:
Ethyl acetate: Methanol: Water (10.0:1.35:1.0 v/v/v).
Chamber saturation time: 45.0 minutes
Fluorescence detection: at 365.0nm (Longer wavelength)
Calculation of R_f value:

$$R_f = \frac{Distance travelled by the solute from the sample application position}{Distance travelled by the solvent from the sample application position}$$

Ideal $R_f$value: 0.1-0.9

Results and Discussion

From the hydroalcoholic extract, the phytoconstituents detection was focused on the main group of compounds such as alkaloids. So, the tests carried out were shown positive results for the same. The details are represented in the Figure 01 and in the table 05. The mobile phase for the separation of alkaloids was found through the trial and error method and that is mentioned in the figure 02. The fixed solvents with the ratio were used to detect the constituents of interest using R_f value qualitatively. The separated spots were found with their their property of showing particular fluorescent colour at 365nm under UV light. The details are illustrated in the figure 02 and in the table 06.

Figure 01: Preliminary Phytochemical Tests for Alkaloids

Figure 02: TLC for mobile phase selection (Trial and error method)

Table 05: Report for Preliminary Phytochemical Tests for Alkaloids

S. No.	Phyto-constituents	Chemical test	Observation	Inference
1.	Alkaloids	Dragendorff's test	Reddish brown precipitate	+
2.		Hager's test	Yellow colour	+
3.		Mayer's test	Reddish brown precipitate	+
4.		Wagner's test	Reddish brown precipitate	+
5.		Tannic acid test	Buff coloured precipitate	+

Table 06: Thin Layer Chromatographic Analysis for alkaloids in Hydroalcoholic extract of *Ipomoea aquatica*

S. No.	Sample of interest	Mobile phase	Develop-ment time (min)	Fluorescence spot colour at 365nm	Distance travelled by the solute (cm)	Solvent Front (cm)	R_f value
1.	Alkaloids	Ethyl acetate: Methanol: Water (10.0: 1.35: 1.0 v/v/v)	33	Green Blue Green Blue Pink Blue Dark Blue Green Pink	0.9 1.8 3.0 4.7 6.5 7.5 10.6 11.9 12.8	13.6	0.07 0.13 0.22 0.35 0.48 0.55 0.78 0.88 0.94

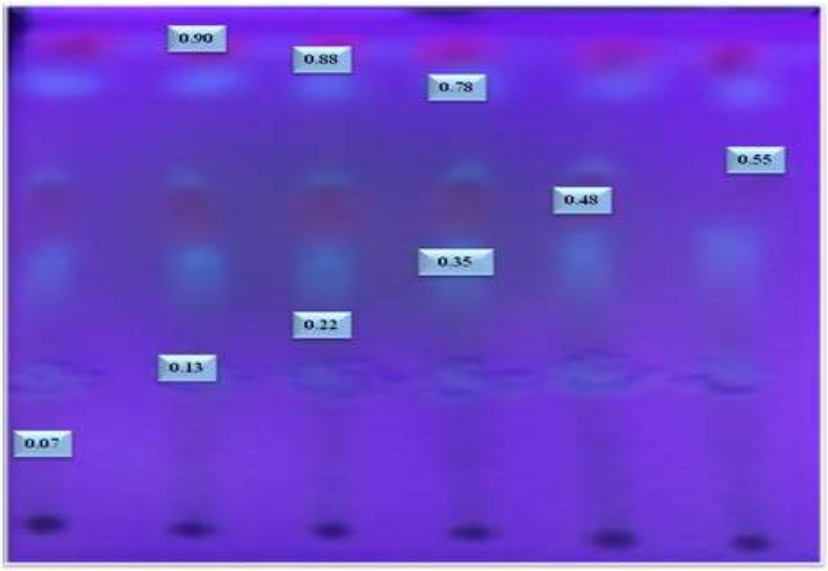

Figure 03: TLC Profile for alkaloids in Hydroalcoholic extract of *Ipomoea aquatica*

Conclusion

Generally alkaloids play major role in treating many life threatening diseases. Hence, revealing new class of components will assist to improve herbal drug products in the global market. Nearly nine numbers of different compounds were found by choosing the fixed mobile phase. This study could be used in research laboratories for detecting similar type of compounds using TLC analysis. The presence of alkaloids in the chosen extract was confirmed by means of phyto compounds tests specific for the mentioned group of compounds. Definitely this will give the good for opportunity for isolating out many therapeutically acting compounds.

Acknowledgement:

This research was supported by the Management and Principal of Karpagam Educational Institutions, Coimbatore. We thank our colleagues from Karpagam College of Pharmacy and Faculty of Pharmacy, Karpagam University, Karpagam Academy of Higher Education, Coimbatore who contributedperception and expertise that enormouslyexpedited the investigation.

References:

1. Wagner H. In Plant Drug Analysis: A Thin Layer Chromatography Atlas. Ist Edn. Springer, New Delhi, 2004,195.
2. Gangwal A., A review on extraction, estimation and thin layer chromatography of alkaloids, Inventi Rapid: Planta Activa, 2013, Article IDInventi: ppa/252/13, 2013.
3. Schugerl K., Extraction of primary and secondary metabolites, Adv Biochem Eng Biotechnol, 2005, 92, 1-48.
4. Harborne JB., In Phytochemical Methods: A guide to modern technique of plant Analysis, 3rd Edn., Chapman & Hall, London, 1998, 11.
5. Makkar HPS, Siddhuraju P and Becker K., In Plant secondary metabolites, Ist Edn., Humana Press, New Jersey, 2007, 93.
6. Sarkar SD, Latif Z and Gray AI., In Natural product isolation, 2nd Edn., Springer, New Delhi, 2005, 323-351.
7. Mukherjee PK., In Quantity Control of Herbal drug: An approach to evaluation of Botanicals, 3rd Edn., Business Horizons, New Delhi, 2010, 379-422.
8. Haughton PJ and Raman A., In Laboratory Handbook for the fractionation of natural Extracts, Ist Edn., Springer, New Delhi, 2011, 39- 43.
9. Beckett AH and Stenlake JB, Practical pharmaceutical chemistry,Thin layer chromatography, CBS publishers, 4th edition, 2005, 115-128.
10. Kasture AV, Mahadik KR, Wadodkar SG and More HN, A textbook of pharmaceutical analysis, instrumental methods,9th edition,Nirali Prakashan, 2005, 18-30.
11. Fried B and SharmaJ, Thin-layer chromatography,Fourth edition,Revised and expanded, Marcel Dekker inc., New York – Basel, 1999, 499.
12. Sharma B.K., Instrumental methods of chemical analysis, 5th edition, Goel publishing house, Meerut, 2007, 241-264.
13. SkoogD.A., Holler F.J.and Nieman T.A.,Principles of instrumental analysis,5th edition,Saunders college publishing, 2006, 761-766.
14. Dreassi E, Ceramelli Gand Corti P, Thin-layer chromatography in pharmaceutical analysis. In practical thin-layer chromatography—a multidisciplinary approach. Fried b., Sharma J. (eds.) Crc press, Boca Raton, fl, 1996, 231.
15. SzepesiG and Gazdag M, Steroids,In handbook of thin layer chromatography, Sharma G, Fried B (eds.) Marcel Dekker, New York, 1996, 971.
16. Justus G and Kirchner, Thin-layer chromatographic quantitative analysis, Journal of Chromatography a-1, 1973, 82(1), 101- 115.
17. Chatwal G.R. and Anand S.K, Instrumental methods of chemical analysis, 5th edition, Himalaya publishing house, 2008, 2.599-2.616.

18. QuachH.T., SteeperR.L. and Griffin G.W., Separation of plant pigments by thin layer chromatography, Journal of Chemical Education, 81, 2004, 385-7.
19. Ali M and AgrawalV, Thin-layer chromatography of aromatic amines, Separation Science and Technology, 37, 2002, 363-377.
20. Scott R.M., Clinical analysis by thin-layer chromatography techniques, Ann Arbor science publishers, Ann Arbor, mi, 1969.
21. SinghalS, SinghalN and AgarwalS, Pharmaceutical analysis-II, Thin Layer Chromatography, First edition, Pragati Prakashan, 2009, 98-111.
22. Vidya Sagar. Instrumental methods of drug analysis. First edition. Pharma Med Press. 2009:263.
23. KhadabadiS.S., DeoreS.L. and BaviskarB. A., Experimental Phytopharmacognosy – A Comprehensive guide,Second edition, Nirali Prakashan, Pune, Maharashtra,2013, 3.1-3.13, A 1.1-2.8.
24. Ranjith Kumar D, Mohan S., Vanathi P., Prakash G., Sasikala M. and Karpakavalli M,Development and Validation of UV Spectroscopic Method for Estimation of Milrinone in Formulation, World Journal of Pharmaceutical Research,2016,5(4), 1501-1508.
25. Sasikala M., Karpakavalli M., Ranjithkumar D., Prakash G., Vanathi P. and Mohan S., In-vitro free radical scavenging activity studies of extracts and isolated compounds of Eugenia jambolana lam. Seeds,World Journal of Pharmaceutical Research,2016, 5(6), 2021-2030.
26. Sasikala M., Karpakavalli M., Vanathi P., Ranjithkumar D., Prakash G. and Mohan S., Validated UV spectrophotometric method development for evaluation and stability analysis of Tolvaptan in bulk drug and its formulation, World Journal of Pharmacy and Pharmaceutical Sciences,2016, 5(6), 2048-2063.
27. Karpakavalli M, Sangilimuthu AY, Sasikala M, Komala M, Ranjithkumar D and Mohan S, A Predominant Evaluation of Viable Templates for Asthma Therapy Exerting an In silico Analog Method, Virtual Library Screening, Journal of Chemical and Pharmaceutical Research, 2017, 9(1), 43-53.
28. Sasikala M, Sundara Ganapathy R, Karpakavalli M and Mohan S, Chemotaxonomical and pharmacological review on medicinal plants in temperate region, International Journal of Pharmacy & Technology, 2017, 9(1), 29165-29172.
29. Wallis T.E., Text Book of Pharmacognosy, 3rd edn., CBS Publishers and Distributors, Delhi, 1985.
30. Evans WC,Trease and Evan's Pharmacognosy, 14th Edition, W. B. Saunders Company Limited, London, 1998, 119.
31. Sambamurthy K, Pharmaceutical Engineering, Reprint, New Age International (P.) Ltd., New Delhi, 2002, 173-194.
32. Khandelwal KR, Practical Pharmacognosy – Techniques and Experiments. 9th Edn., Nirali Parkashan, Pune, 2002, 82.
33. Quality control methods for medicinal plant material by WHO; 1998**.**
34. Anonymous. Quality control methods for medicial plant materials. WHO, Geneva. 1998.
35. Anonymous, the Ayurvedic Pharmacopoeia of India, Vol-I, the Controller of Publication, New Delhi, 2001, 321-322.
36. Anonymous, The Wealth of India: A Dictionary of Indian Raw Materials and Industrial Products, Vol-2 (B), CSIR, New Delhi, 1998, 49-52.
37. WHO. WHO traditional medicine strategy, [cited May 9]; Available from:http:// www.who.int/ medicines/ publications/ traditional/trm_strategy14_23/en2014. 2014-23p.
38. WHO. Quality control methods for medicinal plant materials, [cited May 9]; Available from:http:// apps.who.int/ medicinedocs/en/d/Jh1791e2014.
39. WHO. General guidelines for methodologies on research and evaluation of traditional medicine, [cited May 9]; Available from: http://www.who.int/medicines/areas/traditional/definitions/en2014.
40. Kokate CK, Purohit AP and Gokhale SB, Pharmacognosy, 51st edition, Nirali Prakashan, Pune, Maharashtra, 2015, A.23-A.27.
41. Junli Hu, Fuyong Wu, Shengchun Wu, Cheung Lung Lam, Xiangui Lin and Ming Hung Wong, Biochar and Glomus caledonium Influence Cd Accumulation of Upland Kangkong (Ipomoea aquatica Forsk.) Intercropped with Alfred Stonecrop (Sedum alfredii Hance), Scientific Reports, 2014, 4, 4671. DOI: 10.1038/srep04671.
42. Dhore Mukund Manohar and Lachure Paresh Shyaam, Survey of Aquatic Macrophyte diversity in Yavatmal District, Maharashtra, India, Int. J. of Life Sciences, 2014, 2(3), 273-275.

43. Parimala K and Sukumar D, Phytochemical Studies on the Flowers of *Ipomoea Aquatica*, International Journal of Scientific Research, 2013, 2(6), 53-55.
44. Elija Khatiwora, Vaishali B. Adsul, Manik Kulkarni, Deshpande NR and Kashalkar RV, Antibacterial activity of Dibutyl Phthalate: A secondary metabolite isolated from Ipomoea carnea stem, Journal of Pharmacy Research, 2012, 5(1), 150-152.
45. Shirin Akhtar, Chandan Karak, Priyanka Biswas, Arup Chattopadhyay and Pranab Hazra, Indigenous Leafy Vegetables: A Potential Source of β-Carotene and Ascorbic Acid, International Journal of Vegetable Science, 2012, 18, 370–375.
46. Elija Khatiwora, Vaishali B. Adsula , Anjali D. Ruikar, Tushar Gadkari, N.R. Deshpande and R.V Kashalkar. Isolation and Characterization of Dibutyl phthalate from Ipomoea carnea stem and its Qantification by HPTLC. Journal of Pharmacy Research. 2011, 4(10), 3264-3265.
47. Hongfei Fu, Bijun Xie, Shaojun Ma, Xinrong Zhu, Gang Fan and Siyi Pan., Evaluation of antioxidant activities of principal carotenoids available in water spinach (*Ipomoea aquatica*), Journal of Food Composition and Analysis, 2011, 24, 288–297.

*****