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CHEMOSYSTEMATICS OF TEN *IPOMOEA* JACQ. SPECIES.

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ABSTRACT

The interest for pharmaceutical products in plants is channelled into the discovery of new biologically-active molecules by the pharmaceutical industry and into the adoption of crude extracts of plants for medication by the general public. Ipomoea Jacq. of family Convolvulaceae is medicinally important and used as a mild purgative,carminative, cure inflammations, fevers, headache and bronchitis.

Phytochemical studies were carried out on ten wild species of genus Ipomoea Jacq. Extracted phytochemicals were analyzed using T.L.C. and HPTLC using 0.2mg/ml 'Ergometrin' standard. The Jaccard's Similarity Coefficient amongst ten species of Ipomoea was calculated using UPGMA followed by constructing a dendrogram. On the basis of similarity index, maximum similarity was observed between I. carnea of sub genus Eriospermum, section Jalapae and I. hederifolia of sub genus Quamoclit, section Mina while I. aquatica and I. violacea, as also I. triloba and I. violacea have no similarity with respect to their alkaloids component. In the dendrogram I. nil of the most primitive sub genus Pharbitis was found to be out grouped, indicating its separate existence within the genus. The variations could be due to minor chemical changes that have occurred during the course of evolution leading to formation of different but related alkaloids.

Key words: *Ipomoea*, Phytochemical analysis, alkaloids, phylogeny.

Introduction

While traditional systematics generally focuses on morphological and anatomical characters of plants, in some cases chemotaxonomic aspects with regard to low molecular weight secondary metabolites are also considered (Erdtman, 1963). Chemical compounds have been used extensively in Plant systematic from analysis of intraspecific variation (Harborne and Turner, 1984) to determine phylogenetic relationships of families and other high level taxonomic groups. There are enormous numbers of flora from all over the world for which details of their morphologic-anatomical characters of the different taxa as well as their phytogeographic distribution are well documented. As a rare exception, the comprehensive monograph "Genera Solanacearum" (Hunziker, 2001), is based on morphological and anatomical characters along with detailed information on chromosome numbers, and aspects of the secondary metabolism from the phytochemical point of view. However, this information is more genus-orientated, i.e., not species-specific.

Inter species relationship can be traced to their respective chemical constituents (say alkaloids) profile. This type of profile can be obtained by relative TLC / HPTLC fingerprinting. The fingerprinting lends to the detection of similar chemicals in each of the species under review. A comparative analysis as the absence or presence of constituent in the leaves of several species of a genus, would therefore identify the extent of chemical similarity or dis-similarity among the species.

The interest for pharmaceutical products in plants leads to the discovery of new biologically-active molecules by the pharmaceutical industry which can be adapted for medication by the general public. Chemotaxonomy of Convolvulaceae has been reported by Eckart Eich (2005), of *Ipomoea* taxon subgenus *Quamoclit*, section *Mina* by Jenett-Siems (2005) , of Some Ipomoea by Rao and Leela (1992), of some infraspecific taxa of Ipomoea by Daniel (1988) and by Nair *et al*. (1988). The phytochemicals Calystegines, Indole Alkaloids, Polyhydroxyalkaloids, Ergot Alkaloid, Eriodictyol and Flavonoid Glycoside and Anthocyanin (Eich, 2008) have been reported in the Genus *Ipomoea* of family Convolvulaceae. Several species of *Ipomoea* are medicinally important and used as a mild purgative, carminative, to cure inflammations, fevers, headache and bronchitis (Pullaiah, 2006). *I. aquatica, I. bahiensis* and *I. cairica* possess anticancer activity against certain cell lines (Prasad *et al.,* 2005). There is a search for an alternative source when a particular drug plant is not available. Invariably the alternative source is a closely related taxon – another species of the same genus or another genus of the same family.

With the above objectives in mind, phytochemical studies were carried out on ten wild species of *Ipomoea* Jacq. growing in and around Mumbai, India, for determining the phylogenetic relationship based on Alkaloids amongst these species.

Materials and Methods:

Ten species of *Ipomoea* were collected from wild habitat, in and around Mumbai, India, identified and authenticated in consultation with Blatter Herbarium, St. Xavier's College, Mumbai, India. One copy of the herbarium was deposited in the Blatter Herbarium and the other copy is preserved in The Late Yashwantrao Adbal Herbarium, of the Department as voucher specimen (Table 1).

Table 1:- The ten species of *Ipomoea* studied as per the classification of Austin and Simao-Bianchini (1998) and deposited in The Late Yashwantrao Adbal Herbarium, Department of Botany, R. Ruia College, Mumbai, India.

Sub genus	**Section**	**Series**	**Species**
I. *Ipomoea*	*Ipomoea*	*Heterophyllae*	*I. nil*
II. *Quamoclit*	*Mina*		*I. hederifolia* *I. quamoclit*
	Batatas		*I. triloba*
	Calonyction		*I. turbinate*
	Pedatisectae		*I. cairica*
III. *Eriospermum*	*Eriospermum*	*Jalapae*	*I. carnea*
	Eripomoea		*I. aquatica* *I. pes-caprae* *I. violacea*

Preparation of Plant Powder: The leaves of the ten *Ipomoea* species under study were collected during flowering season washed in running water, rinsed in deionized water and air dried for 10 days. They were powdered and sieved through BSS mesh No. 85. Loss on drying was determined. The powders were stored in a cool dry place.

Preparation of Plant Extract: 10g of the dry leaf powders of the ten species were weighed and extracted in 100ml Methanol for 3hr. in Soxhlet Extractor at 70^{o}C. The extract was

filtered using Whatman filter paper no. 41 under vacuum. Solvent extract was concentrated on Rotavapor (under vacuum).

Chromatographic Separation: Initially, the methanolic extracts of leaves, followed by separated Alkaloid fraction (Harbone, 2007) from powdered leaves was used for HPTLC screening. Based on the observations of Hofmann (1968), Genest (1966), Niwaguchi and Inoue (1969) and Taber *et al*. (1963) who have identified ergoline compounds in the leaves and seeds of several *Ipomoea* species, 0.2mg/ml 'Ergometrin' (Sigma Aldrich) was used as standard alkaloid for HPTLC analysis of alkaloid fraction of leaf extracts. Silica gel 60 F254, pre-coated silica plates were used as stationary phase and methanol: chloroform 9:1 (v/v) was used as the mobile phase for HPTLC.

Ascending mode of chromatographic development was selected. The densitometric evaluation of separated bands on the plate was performed from 190 to 400nm in ultraviolet and fluorescence mode using D2 lamp with a CAMAG scanner 3 in conjunction with winCATS software. The plates of methanolic extracts separation were initially scanned at 254nm, followed by complete UV spectrum (190 to 400nm) scanning to obtain individual methanolic extract profile of all the *Ipomoea* species. The HPTLC plates of alkaloids fraction separation were scanned initially at 254nm, followed by scanning at 366nmto obtain individual alkaloids profile of all the ten *Ipomoea* species.

Statistical Analysis: The data was subjected to statistical analysis using *MVSP-3.2* version software. The genetic similarity matrix for alkaloids profile of all *Ipomoea* species was calculated using Jaccard's co-efficient and the dendrogram was based on Jaccard's similarity Index which was obtained using UPGMA.

Results:

Similarity in phytoconstituents/ chemical constituents of the ten species of *Ipomoea* species leaves was studied using methanolic extract of leaves through HPTLC separation and scanning. The plates were scanned initially under visible light (Fig. 1b) and at 254nm (Fig. 1c).Since all the bands were not detected at 254nm, a complete UV- spectrum scanning was carried out (190nm – 400nm). Some bands were distinctly seen as common to all plants at 366 nm scanning (Fig. 1d) and similarly sharp peaks were observed when complete spectrum scanning was carried out (Fig. 2a to j). Based on this and the chemical tests; alkaloids fraction of the leaf extracts was further analysed. The HPTLC plates of alkaloids fraction were scanned at 254nm and at 366nm (Fig. 1- e and f). Ergometrin separated at *Rf*= 0.15 (Fig. 1e -f), with % AUC as 38.63 % at 254nm (Fig. 3f) and 37.42 % at 366nm (Fig. 4f). Bands

matching to this *Rf* values were observed in *I.hederifolia, I. cairica* at 254nm and so also in *I. nil, I. hederifolia* and *I. violacea*at 366nm.

Among the ten species studied, methanolic extract of leaves and the alkaloid fraction of the same showed some of the common bands. *I. pes-caprae* showed the presence of uncommon bands at Rf= 0.1, 0.16, 0.19 and 0.23.

When the Alkaloid extracts were separated by HPTLC and scanned at two different wavelengths of light (254 nm and 366 nm), it was found that some of the bands were detected at both the wave lengths (Table 2). As the similarities were observed between the results of 254nm and 366nm scanning of alkaloids HPTLC, the data was combined to determine the similarities and dissimilarities among the ten *Ipomoea* species under study. The similarity amongst the ten species of *Ipomoea* was calculated using UPGMA for Jaccard's Similarity Coefficient as given in Table 4.

Table 2-- Common Bands of Alkaloids Fraction at 254nm and 366nm

***Ipomoea* species**	**Rf**	**%AUC at**		***Ipomoea* species**	**Rf**	**%AUC at**	
		254 nm	**366 nm**			**254 nm**	**366 nm**
I. nil	0.02	8.33	10.72	***I. triloba***	0.07	1.40	2.10
	0.77	54.82	31.50		0.72	46.25	33.77
I. aquatica	0.02	7.91	6.09		0.80	4.63	3.78
	0.16	0.69	0.67	***I. cairica***	0.08	3.23	3.34
	0.29	15.58	11.69		0.23	8.25	8.73
	0.46	1.05	1.96		0.28	2.95	4.37
	0.75	62.87	58.47		0.35	6.67	15.06
I. pes-caprae	0.02	8.36	12.67		0.48	4.77	1.83
	0.29	20.81	41.29		0.57	6.77	15.21
I. carnea	0.01	3.82	3.19		0.69	8.40	13.00
	0.19	0.54	0.38		0.73	1.16	23.42
	0.25	4.21	4.00	***I. turbinata***	0.01	1.18	1.42
	0.31	14.22	18.05		0.10	0.90	0.93
	0.41	13.10	7.41	***I. quamoclit***	0.08	0.75	0.32
	0.75	45.81	32.58		0.18	3.07	12.72
I. hederifolia	0.02	1.19	1.01		0.22	0.99	1.63

	0.12	1.46	1.82		0.26	1.89	4.32
	0.15	0.25	0.40		0.29	3.02	9.08
	0.25	1.07	1.37		0.31	3.53	9.00
	0.27	1.43	1.55		038	4.78	7.85
	0.31	8.29	13.83	***I. violacea***	0.26	4.90	2.27
	0.49	4.08	1.19				
	0.74	57.99	54.78				

Based on UPGMA Jaccard's Coefficient of Similarity, a Dendrogram (Fig. 5) was made using MVSP2 software, which showed four major clusters connected to each other at different nodes with variation in similarity index.

	I. nil	*I. aquatica*	*I. pes-caprae*	*I. carnea*	*I. hederifolia*	*I. triloba*	*I. cairica*	*I. turbinata*	*I. quamoclit*	*I. violacea*
I. nil	1									
I.aquatica	0.16	1								
I. pes-caprae	0.037	0.19	1							
I. carnea	0.107	0.037	0.125	1						
I.hederifolia	0.111	0.038	0.238	**0.318**	1					
I. triloba	0.071	0.174	0.04	0.074	0.12	1				
I. cairica	0.172	0.033	0.111	0.222	0.143	0.103	1			
I.turbinata	0.032	0.16	0.167	0.148	0.154	0.154	0.133	1		
I.quamoclit	0.103	0.074	0.167	0.148	0.154	0.111	0.133	0.231	1	
I.violacea	0.032	**0**	0.12	0.148	0.111	**0**	**0.259**	0.231	0.231	1

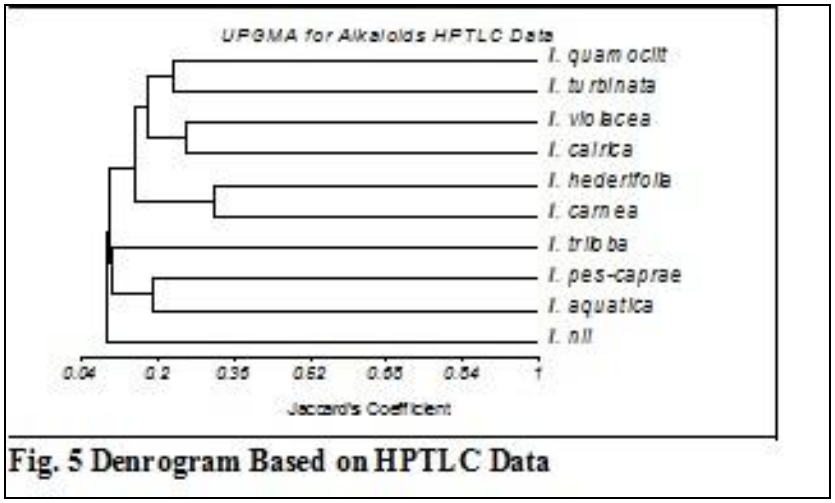

Fig. 5 Denrogram Based on HPTLC Data

Table 3: Jaccard's Similarity Matrix of HPTLC Data

Discussion:

As per the classification based on morphological characters, Austin (1979) has divided the genus *Ipomoea* into three subgenera – *Ipomoea, Quamoclit* and *Eriospermum* and each of these is further divided into sections and series by Austin and Simao-Bianchini (1998). The ten species studied here fall in the sections and series as shown in Table 1.

Based on the HPTLC analysis data of Alkaloids - not a single band was monomorphic, indicating that each of the ten species analysed had a unique set of alkaloids.

On the basis of similarity index (Table 3), maximum similarity was observed between *I. carnea* of sub genus *Eriospermum*, section *Jalapae* and *I. hederifolia* of sub genus *Quamoclit*, section *Mina* at 31.8%, followed by *I. cairica* of Sub genus *Quamoclit*, Section *Pedatisectae* and *I. violacea* of Sub genus *Eriospermum*, Section *Eripomoea* at 25.9%.On the other hand it was noticed that, *I. aquatica* and *I. violacea*, as also *I. triloba* and *I. violacea* have no similarity with respect to their alkaloids component.

Strangely the species with some amount of similarity belonged to different Sub genera of Austin (1997). This could be because during the course of evolution minor chemical changes occurred leading to the formation of different but related alkaloids.

The first major cluster from top of dendrogram is formed of *I. quamoclit* and *I. turbinata*both of sub genus *Quamoclit*but belonging to different sections *Mima* and *Calonyction* at Node – 3 with 23.1% similarity. Second cluster is connecting two plants as *I.*

cairica and *I. violacea* of different sub- genera *Quamoclit* and *Eriospermum* respectively at Node - 2 with 25.9% similarity. Plants at Node – 2 (*I. quamoclit*+ *I. turbinata*) and Node – 3 (*I. cairica* + *I. violacea*) are in turn connected to each other at Node – 5.

Third major cluster is formed of *I. carnea* and *I. hederifolia* of different sub- genera – *Erospermum* and *Quamoclit* respectively at Node -1, with maximum similarity of 31.8%. This cluster is connected to plants at Node -5 [(*I. quamoclit*+ *I. turbinata*)+ (*I. cairica* + *I. violacea*)] forming Node – 6.

Fourth cluster initially has *I. aquatica* and *I. pes-caprae* both of sub-genus *Eriospermum,* section *Eripomoea*are connected to each other at Node – 4 with the similarity of 19% followed by addition of *I. triloba* at Node – 7 with 10.7% similarity. These plants at Node – 7 [(*I. aquatica*+ *I. pes-caprae*) + *I. triloba*] are connected to all the plants at Node -6 {[(*I. quamoclit*+ *I. turbinata*)+ (*I. cairica* + *I. violacea*)][*I. carnea* + *I. hederifolia*] at Node -8 with similarity of 10.2%.

Finally, *I. nil* of sub-genus *Ipomoea* section *Pharbitis,* the most primitive species (Austin and Huaman, 1996) amongst those studied is added to the dendrogram at Node – 8 with least similarity of 9.2%; thus outgrouping *I. nil t*he most primitive species of *Ipomoea* amongst those studied and showing that several biochemical changes have occurred in the genus during the course of evolution.

Conclusion:

One can conclude that based upon the phytochemical analysis the dendrogram (Fig. 5), derived from HPTLC profile, revealed that each of the ten species analysed had a unique set of alkaloids since not a single band was monomorphic. Maximum similarity was observed between *I. carnea*and *I. hederifolia* followed by *I. cairica* and *I. violacea*. On the other hand it was noticed that, *I. aquatica* and *I. violacea,* as also *I. triloba*and *I. violacea* have no similarity with respect to their alkaloids component. Strangely the species with some amount of similarity belonged to different Sub genera of Austin. This could be because during the course of evolution minor chemical changes occurred leading to the formation of different but related alkaloids.

References:

- Austin D.F. 1979. An infrageneric classification for *Ipomoea*.(Concolvulaceae). Taxon **28:** 359-361.
- Austin D.F. andHuaman Z. 1996. A synopsis of *lpomoea*(Convolvulaceae) in the Americas. Taxon 45: 3-38.
- Austin, D.F., Bianchini, R.S. (1998) Additions and corrections in American Ipomoea (Convolvulaceae).*Taxon* **47:** 833-8.
- Daniel, M., Nair, G. G., Sabnis, S.D. (1988).A Chemotaxonomic aseesment of the status of some infraspecific taxa of *Ipomoea*Linn.*Current Science***. 57 (10):** 527-529.
- Eich E.,Schimming, T., Jenett-Siems, K., Siems, K., Witte, L., (2005) N1,N10-ditifloylspermidine, a novel alkaloid from the seeds of *Ipomoea nilPharmazie*.**60(12):** 958-9
- Eich, E. (2008) *Solanaceae and Convolvulaceae: Secondary Metabolites Biosynthesis, Chemotaxonomy, Biological and Economic Significanc*e(A Handbook) Springer-Verlag Berlin Heidelberg. pp-644.
- Erdtman, H. (1963). *Chemical Plant Taxonomy* (edited by T. Swain), Academic Press, London, New York.p.89—126, 543pp.
- Genest, K. (1966) Changes in ergoline alkaloids in seeds during ontogeny of *Ipomoeaviolacea*.*J. Pharm. Sci*. **55:** 1284.
- Harbone J.B., (2007) *Phytochemical Methods – A Guide to Modern Techniques ofPlant Analysis*, Champan& Hall London UK, 4th edition, pp. 302.
- Harborne, J.B. and Turner, B.L. (1984) *Plant chemosystematics.*Academic Press, San Diego, California, USA.
- Hofmann, A. (1968) *Psychotomimetic agents*.InBurgar, A., editor; Drugs Affecting the Central Nervous System.Vol. 2. Marcel Dekker, New York., pp. 184-185.
- Hunziker, A. T. (2001) *Genera Solanacearum. The genera of Solanaceae illustrated, arranged according to a new system* .Liechtenstein :GantnerVerlag .
- Jenett-Siems, K., Ott, S.C., (2005) Ipangulines and minalobines, chemotaxonomic markers of the infrageneric Ipomoea taxon subgenus Quamoclit, section Mina. *Phytochemistry* (Amsterdam) **66:** 223-31.
- Nair G G , Daniel M, Sabnis S D.(1988). Chemosystematics of Convolvulaceae (B. *et* H) *Geobios***15**:241-244.
- Niwaguchi, T. and T. Inoue.(1969) Chromatographic separation of lysergic acid amide and isolysergic acid amide in morning glory seeds.*J. Chromatog*.**43**: 510.

- Prasad.K.N., Raghu, A.G., Shivamurthy G.R., Vijayan, P. and Aradhya, S.M. (2005).In vitro cytotoxic properties of *Ipomoea aquatica. Ind. J. Pharmacol*.**37:** 397-398.
- Pullaiah, T. (2006) *Encyclopedia of World Medicinal Plants,* Vol. 1, Regency Publication, 1149-1160.
- Rao, S.R.S. and Leela, M. (1992) Chemotaxonomy of some Ipomoea L. Convolvulaceae*FeddesRepertorium***103** : 351-355
- Taber, W. A., L. C. Vining, and R. A. Heacock (1963) Clavine and lysergic acid alkaloids in varieties of morning glory. *Phytochem*.**2**: 65.

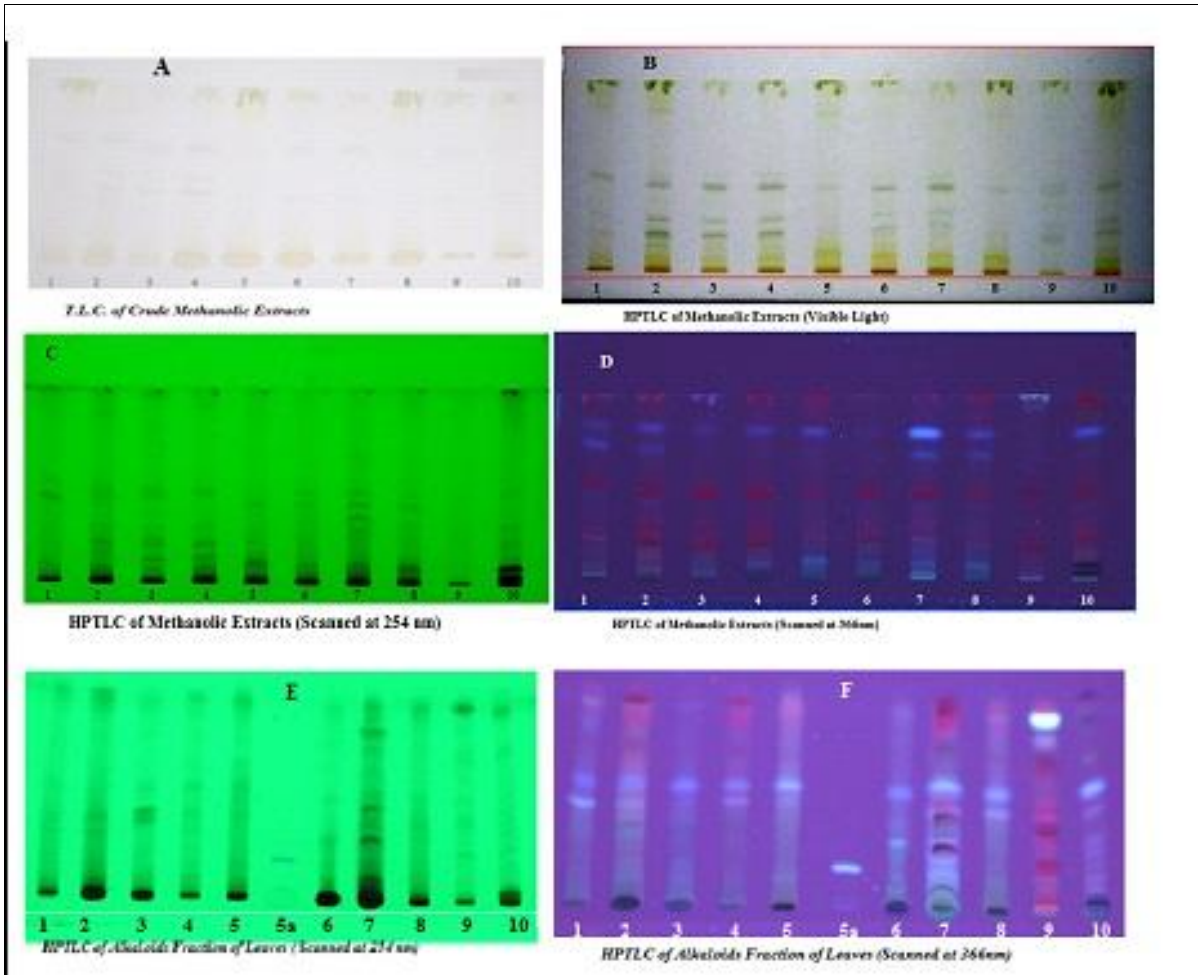

Fig. 1
A. T.L.C. of Crude Methanolic Extracts, B. HPTLC of Methanolic Extracts (Visible Light) C. HPTLC of Methanolic Extracts (Scanned at 254nm), D. HPTLC of Methanolic Extracts (Scanned at 366nm), E. HPTLC Alkaloids Fraction of Leaves (Scanned at 254nm), F. HPTLC Alkaloids Fraction of Leaves (Scanned at 366nm)

1. *I. nil*, 2. *I. aquatica*, 3.*I. pes-caprae*, 4.*I carnea*, 5.*I. hederifolia*, 6.*I. triloba*, 7.*I. cairca*, 8.*I. turbinata*, 9.*I. quamoclit*, 10.*I. violacea*, 5a.*Ergometrin*

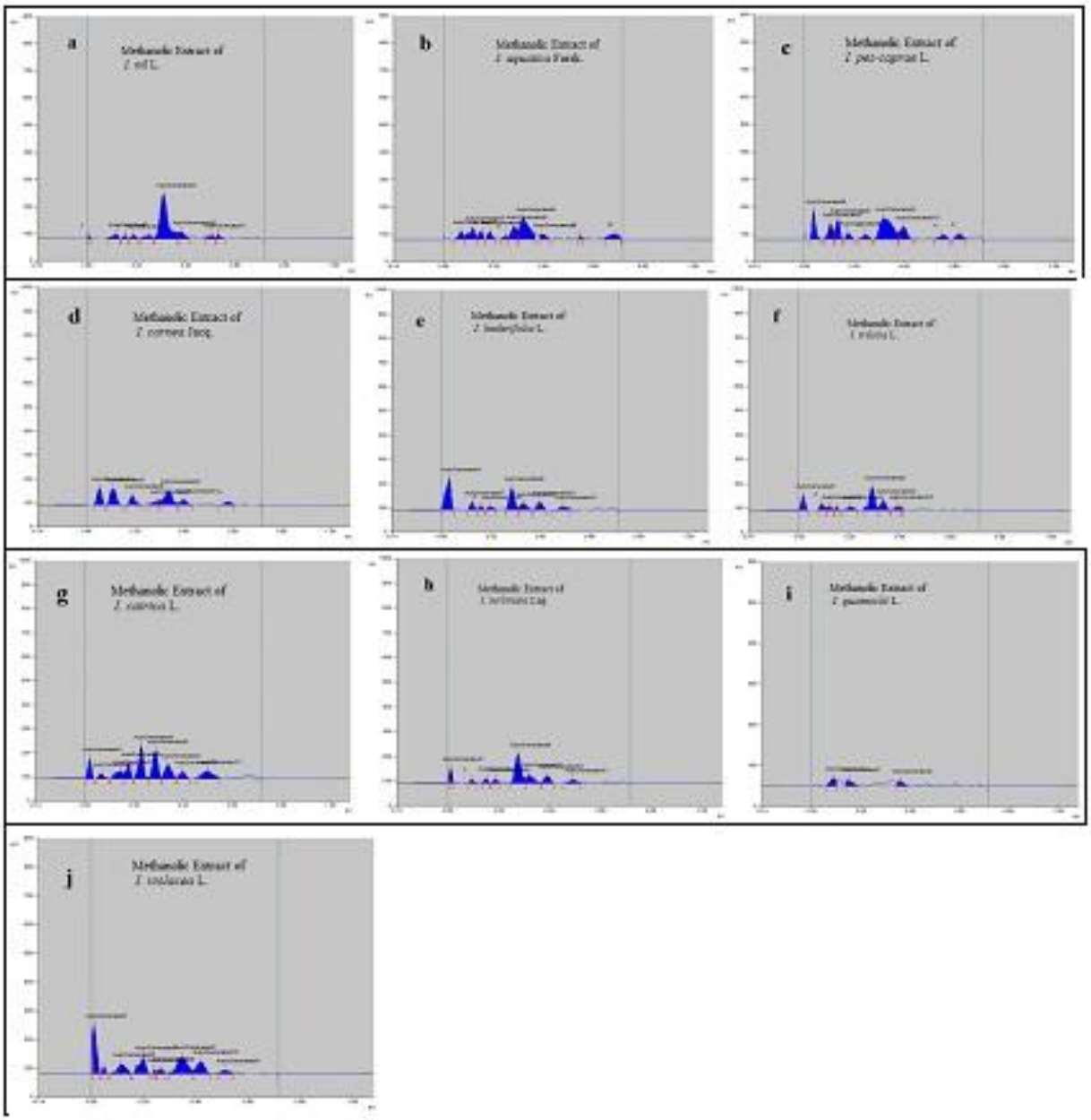

Fig. 2
HPTLC 254nm Profile of Methanolic Extracts of a. *I. nil*, **b.** *I. aquatica*, **c.***I.pes-caprae*, **d.** *I. carnea*, **e.** *I. hederifolia*, **f.** *I. triloba*, **g.** *I cairca*, **h.** *I. turbinata*, **i** *I. quamoclit*, **j.** *I. violacea*

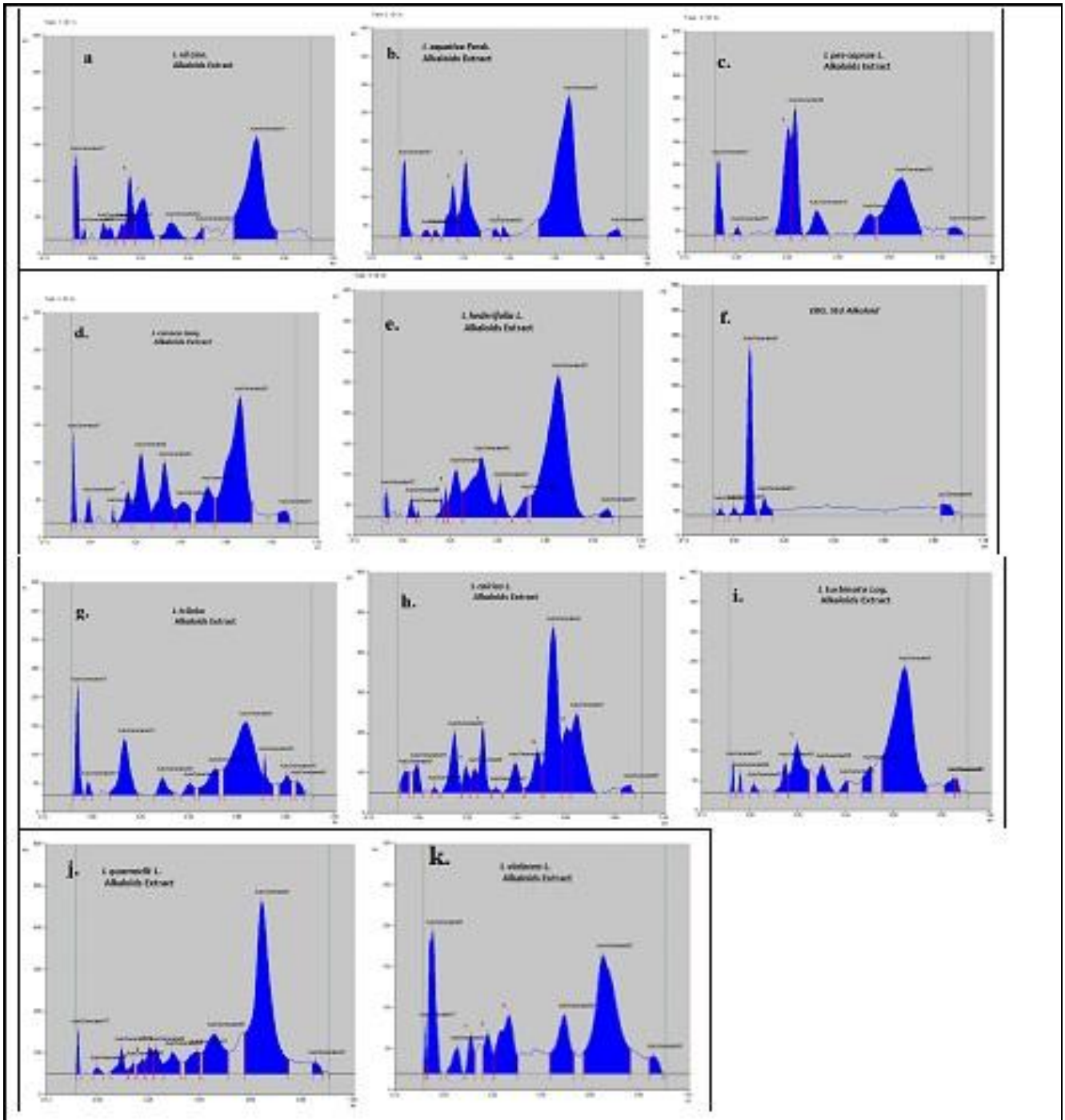

Fig. 3
HPTLC 254nm Profile of Alkaloids Extracts of a. *I nil*, **b.** *I. aquatica*, **c.***I.pes-caprae*, **d.** *I. carnea*, **e.** *I. hederifolia*, **f.** *Ergometring.I. triloba*, **h.** *I. cairca*, **i.** *I. turbinata*, **j.** *I. quamoclit*, **k.** *I. violacea*.

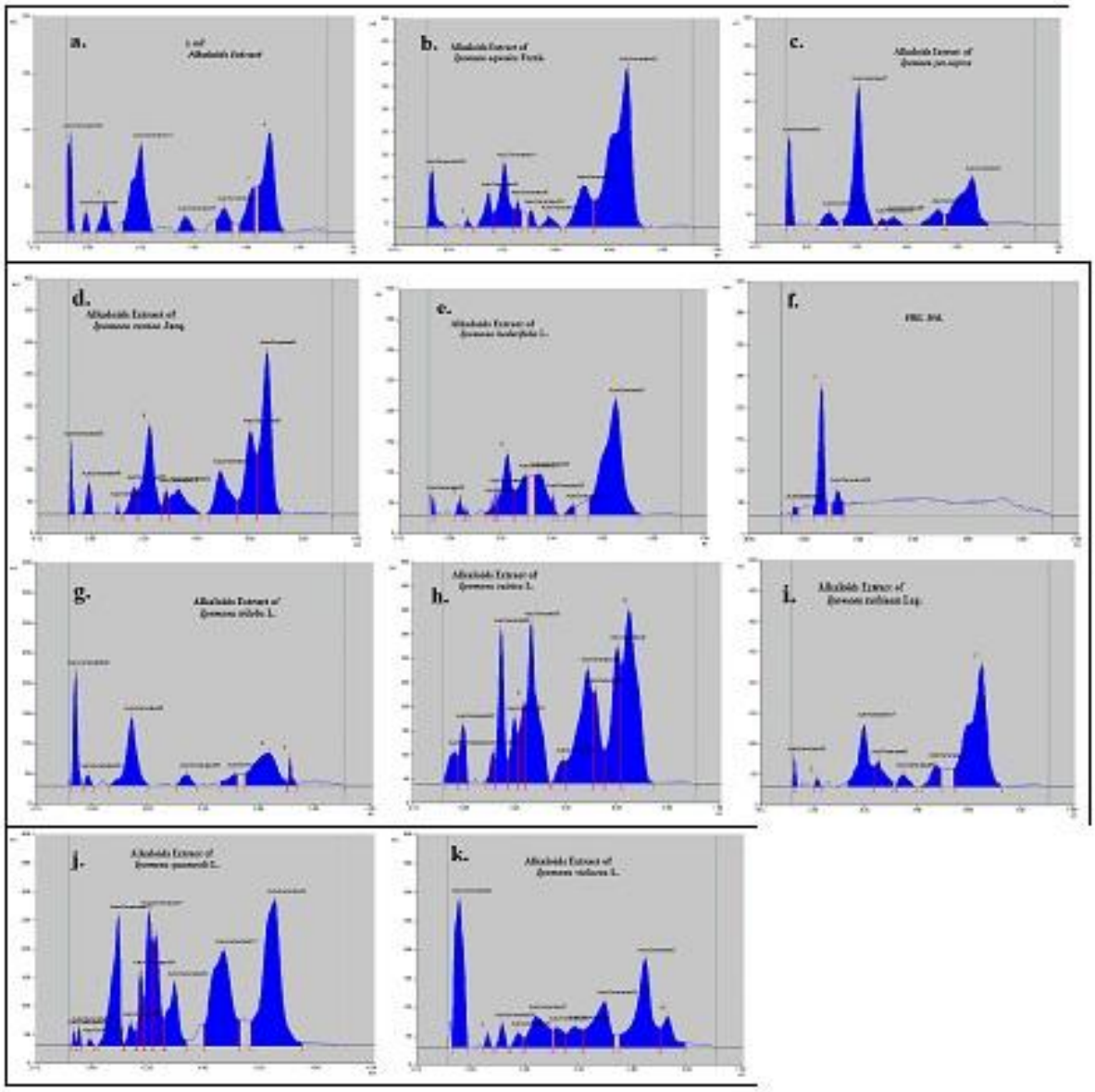

Fig. 4
HPTLC 366nm Profile of Alkaloids Extracts of a. *I nil*, **b.** *I. aquatica*, **c.***I.pes-caprae*, **d.** *I. carnea*, **e.** *I. hederifolia*, **f.** *Ergometring.I. triloba*, **h.** *I. cairca*, **i.** *I. turbinata*, **j.** *I. quamoclit*, **k.** *I. violacea*