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Chromatographic Fingerprint Analysis of *Ixora coccinea* Methanolic Flower Extract

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ABSTRACT
Ixora coccinea (R.) belonging to the Rubiaceae family is an ornamental plant claimed for the management of oral cancer and one of ingredients in curing different ailments. The present investigation was carried out to determine the chemical composition of *Ixora coccinea* methanolic flower extract using HPLC, HPTLC and Gas Chromatography–Mass Spectrometry technique. HPLC analysis of *Ixora cocccinea* methanolic flower extract revealed the presence of Biochin A, Myricetin, Quercetin, Rutin, Diadzein and formononetin, HPTLC fingerprint revealed the presence of ursolic acid and GC-MS with 24 phytochemicals among which some are of biological importance. The result of this study offers a platform for using *Ixora coccinea* pharmacologically.

Keywords: *Ixora cocccinea,* HPLC, HPTLC, GC-MS techniques, Quercetin, Ursolic acid.

INTRODUCTION

Herbal medicinal preparations and their proprietary products are being used more and more widely throughout the world, for treating various ailments. Hence evaluating and ensuring their quality becomes increasingly urgent. Last few decades have seen rapid worldwide growth in demand for herbal formulation as medicines and their proprietary products in the pharmaceutical industry and medicinal markets, especially in India, China, Japan and countries in Europe and North America. Population of developing countries relay on the herbal medicine for treating primary health care problems due to their less adverse complications, ease self administration, affordability and because of limitations of current conventional methods. As demand grows, demand for mass production and quality assurance that each batch of medicine meets certain standards both at the time of production and over its shelf life. Quality control for herbal preparations or proprietary products, however, is much more difficult than for synthetic drugs because of the chemical complexity of the ingredients any loss in a particular chemical may result in loss of pharmacological action of that herb. As herbal preparations comprise hundreds of mostly unique or species-specific compounds, it is difficult to completely characterize all of these compounds. It is also equally difficult to know precisely which one is responsible for the herbs or herbal preparation's therapeutic action because these compounds often work synergistically in delivering therapeutic effects. Thus, maintaining quality in herbal preparations, both from batch to batch is as problematical as it is necessary and has drawn serious attention recently as a challenging analytical task. In recent years, significant efforts have been made for the quality control of herbal materials as well as herbal preparations by utilizing quantitative methods and/or qualitative fingerprinting technologies. [1]

Ixora coccinea (Family: Rubiaceae), a small to medium sized hardy shrub is cultivated for ornamental purpose and also it finds place in traditional Indian medicine. IC is reported for diverse pharmacological properties including anti-inflammatory and antimitotic activities. [2] Leaves and flower extracts of IC were reported to possess antimicrobial activitties. [3] Flower extract contains triterpenoid and ursolic acid. [4] The flowers afforded two new cycloartenol esters, lupeol fatty ester, lupeol, oleanolic acid and sitosterol. Flower extract of IC showed protective effect against cyclophosphamide and cisplastin induced systemic toxicity. [5-6] It has been also reported to possess cytotoxic and anti-tumour activities in mice injected with Dalton's lymphoma ascetic (DLA) cells. [7] The present communication was the first of its kind to summarize the bioactive information on IC methanolic flower extract.

MATERIALS AND METHODS

Plant material

Flowers of *Ixora coccinea* were collected from a local commercial source in Chennai and authenticated by Prof. P. Jayaraman, Director, Plant Anatomy Research Centre,

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Chennai. Herbarium specimen of the flower was prepared and preserved for future references. Fresh flowers were washed thoroughly with water to remove the earthy matters and freed from debris. They were shade dried, powered (80% coarse: 20% fine) and subjected to successive (petroleum ether, ethyl acetate and methanol) extraction by hot continuous method using Soxhlet's apparatus. Extracts was concentrated under vacuum, in rotary evaporator, dried and stored in vacuum desiccators for future analysis.

HPLC Chromatographic condition

Chromatographic analysis was performed on a 250 mm × 4.6 mm i.d., C18 (ODS), LACHROM L-7000 with 0.5% aqueous solution of Methanol (HPLC Grade) as mobile phase at a flow rate of 1 ml /min. HPLC equipment comprised of Hewlett-Packard (HP) 1050 ChemStation Software, an HP model 35900 interface units, an HP 9000 Series 300 computer, and an HP DeskJet 500 Printer. A Waters 486 tuneable absorbance detector was operated at 254 nm; detector sensitivity was 0.05 AUFS and the column oven temperature was 30°C.

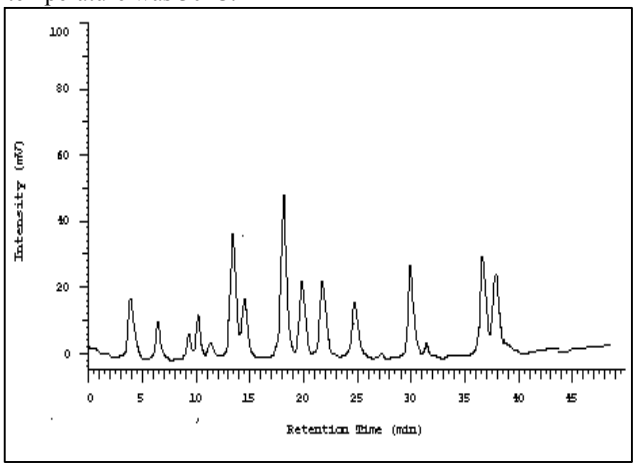

Fig. 1: HPLC separation of IC methanolic flower extract

HPTLC finger print

The IC methanolic flower extract was standardized by high performance thin layer chromatography (HPTLC) using Camag Linomat applicator V and TLC Scanner-III. IC extract (10mg/ml) and Ursolic acid (1mg/ml) were dissolved in ethyl acetate and 10µl was used for spot application. IC and ursolic acid were applied to pre-coated silica gel plates (Merck 60F254), and developed using the solvent system Chloroform:Methanol (95:5) up to 8 cm. Developed plates were dried and scanned at 535 nm. Ursolic acid was used as standard and its content in IC was computed from the peak areas.

Gas Chromatography-Mass Spectrum Analysis (GC-MS)

GC-MS technique was used in this study to identify the phytocomponents present in IC methanolic flower extract and the technique was carried out at Sargam laboratory, Chennai, Tamil Nadu. GC-MS analysis of this extract was performed using GC SHIMADZU QP2010 system and gas chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with Elite-1 fused silica capillary column (Length : 30.0m, Diameter : 0.25 mm, Film thickness : 0.25 µm Composed of 100% Dimethyl poly siloxane). For GC-MS detection, an electron ionization energy system with ionization energy of 70eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1.51ml/min and an injection volume of 2µl was employed. Injector temperature was set atn200°C and the ion-source temperature was at 200°C. The oven temperature was programmed from 70°C (isothermal for 2 min.), with an increase of 300°C for 10 min. Mass spectra were taken at 70eV with scan interval of 0.5 seconds with scan range of 40 – 1000 m/z. Total GC running time was 35 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was GC MS solution ver. 2.53.

Table 1: Percentage content of components in the extract

Component name	R.T.	Area	Content in %
Biochin A	10.22	118913	0.675
Myricetin	11.67	96765	0.212
Quercetin	13.45	706765	0.123
Rutin	14.89	226751	0.067
Daidzein	37.93	332334	0.8966
Formononetin	39.34	954523	0.00564

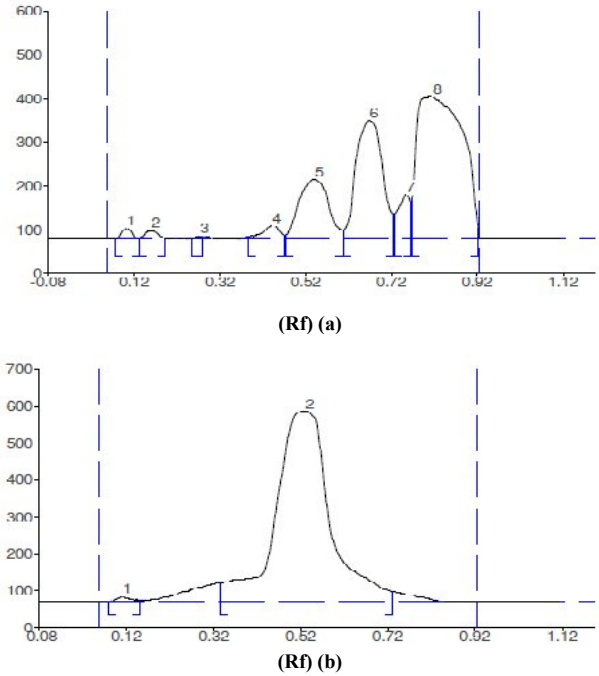

Fig. 2: (a) HPTLC chromatogram of methanol extract of IC flowers. (b) HPTLC chromatogram of Petroleum ether extract.

Table 2: Ursolic acid content of both ME of IC flowers

Drug	Max Rf	Area	Content %
IC ME	0.54	6831.9	1.45
Ursolic acid	0.53	47980.4	89.2

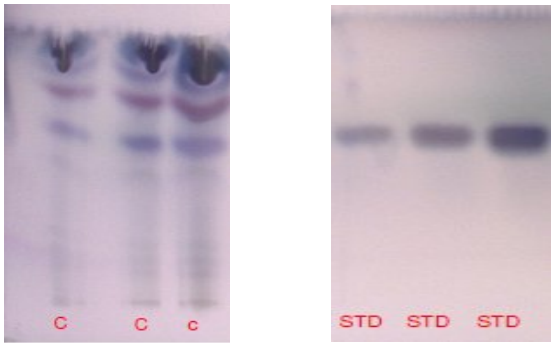

Fig. 3: Represents the HPTLC chromatogram of IC methanolic flower extract. C and STD represents the methanolic extract and Ursolic acid

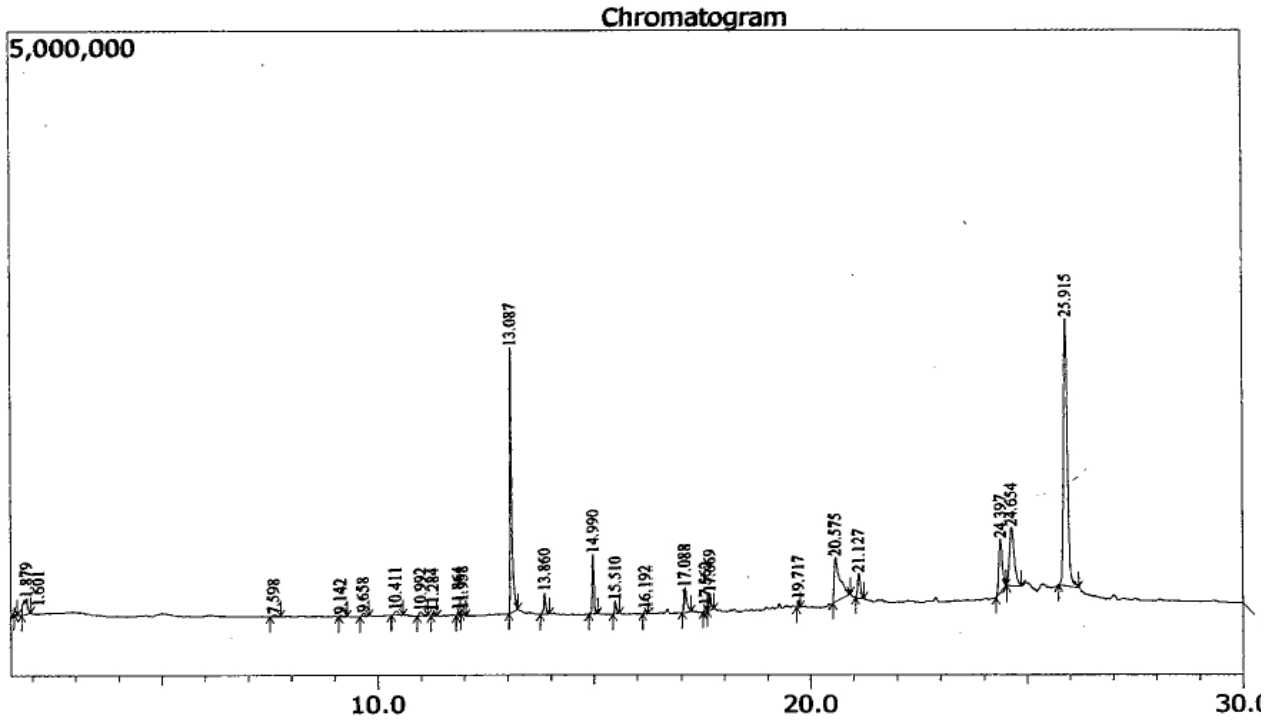

Fig. 4: GC-MS Chromatogram obtained for methanol extract of IC methanolic flower extract

Table 3: Total ionic chromatogram showing the compounds of methanol extract of IC flowers

Peak	R. time	Area	Area %	Name of the compound
1	1.601	127787	0.34	9,12,15-Octadecatrienoic acid (2-phe
2	1.879	907393	2.42	9.12- Octadecadienoic acid (Z,Z)(2-phe
3	7.598	109885	0.29	Propargyl alcohol
4	9.142	85993	0.23	Furfural
5	9.658	149832	0.40	Furfuryl alcohol
6	10.411	251945	0.67	Glycerose
7	10.992	214735	0.57	2-Cyclopenten-1-one, 2hydroxy
8	11.284	105449	0.28	2,4 dihydroxy-2,5 dimethyl furfural
9	11.864	93233	0.25	Ethanimidic acid, ethyl ester
10	11.958	123238	0.33	Dimethyl, tetra butoxysilanol
11	13.087	6602508	17.58	Glycerin
12	13.860	552654	1.47	Cymel
13	14.990	1409196	3.75	4H-Pyran-4-one, 2,3 dihydro
14	15.510	292507	0.78	Benzenecarboxilic acid
15	16.192	103311	0.28	2-acetyl-2-hydroxy-gamma-butyrola
16	17.088	818728	2.18	Hydroxymethylfurfural
17	17.562	123701	0.33	3,4-Dihydroxy-5-methyldihro
18	17.669	503477	1.34	1,3-Propanedial, 2-hydroxymethyl
19	19.717	135467	0.36	Heptadecane
20	20.575	352912	9.40	Xanthosine
21	21.127	928616	2.47	5,5Dimethyl1 oxa-5-silacyclononan
22	24.397	2580751	6.87	(1R,3R,4R,5R) Quinic acid
23	24.654	3818632	10.17	Mome inositol
24	25.915	13993470	37.25	Quinic acid

RESULTS AND DISCUSSION

HPLC analysis of the extract showed several peaks (Fig. 1 and Table 1) one among them is quercetin, a flavonoid which has various biological actions and HPTLC fingerprinting (Fig. 2 & 3, Table 2) had revealed the presence of eight peaks in methanolic extract and ursolic acid used as standard gave a sharp and well-defined single peak with less diffusion and spreading with Rf value of 0.53 and the content was found to be 99.09%. Ursolic acid a terpenoid possess diverse pharmacological activities supported by various investigations. Ursolic acid has shown to have anti angiogenic activity [8], inhibition of DNA replication [9] and activates various caspases. [10] Pharmacological actions reported are antibacterial, hepatoprotective, immunomodulatory, antiproliferative, antitumoral, antiinflammatory, and anti-angiogenic activities. [11] Twenty four compounds were identified in methanol extract by GC-MS analysis. The chromatogram obtained from methanol extract was shown in Fig 4. The active principles, area of the peak, Concentration (%) and Retention Time (RT) are presented in Table 3. The prevailing compounds were Quinic acid, Glycerin, Mome inositol, Xanthosine, (1R, 3R, 4R, 5R) Quinic acid.

The combinative approach of these qualitative and quantitative chromatographic techniques helps in evaluating the quality consistency of herbal preparations. As herbal preparations have chemical complexity it is very difficult to identify all of their constituents. Using these methods their quality and stability can be easily assessed.

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