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DETECTION AND ESTIMATION OF FLAVONOIDS AND PHENOLIC ACID IN *INULA RACEMOSA* AND *SAUSSUREA COSTUS* EXTRACTS BY HPTLC TECHNIQUE

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ABSTRACT

The main purpose of the study is to observe the flavonoids and phenolic acids in two herbal raw materials *inula racemosa* and *saussurea costus* from Asteraceae family respectively. Traditional siddha practitioners instead of *inula racemosa* using *saussurea costus* as substitute in herbal formulation. Our aim of interest to detect and estimate antioxidant markers in *inula racemosa* and *saussurea costus.* Results of the study clearly revealed that *inula racemosa* exhibits quercetin, rutin and gallic acid and *saussurea costus* exhibit quercetin, gallic acid, caffeic acid. Quercetin was found to be 0.383%, 0.122% in *inula racemosa*, and 0.042% and 0.1301% in *Saussurea costus* and rutin was found in 0.007% in *inula racemosa* and gallic acid was found in 0.02 in *inula racemosa* and 1.36% and 0.0323% in *saussurea costus* and caffeic acid was found to be 0.2096% and 0.5811%. In conclusion quercetin and gallic acid was present in both plant; rutin was present only in *inula racemosa*, and caffeic acid was present only in *saussurea costus* and vitixen was not present in both plant. Quercetin content is more in *inula racemosa* when compare to *saussurea costus*, But Gallic acid is comparatively more in *saussurea costus than inula racemosa.*

KEYWORD: Caffeic acid, Gallic acid, HPTLC, Quercetin, Rutin.

INTRODUCTION

Traditional herbs are used to treat various number of illness, disease and disorder. But due to shortage of herbs used to prepare medicinal preparation we use the substituent of the herbs

used for medicinal preparation. Due to cost effective and better performance using HPTLC is convenient for the comparative studies of the genuine plant and substituent and also used to evaluate, detect, qualify marker compound.[1] *inula racemosa* The fresh root is brownish externally and white internally on drying it become greyish root is aromatic and irregularly wrinkled. Root is used for various herbal formulation preparation. *Saussurea costus* Root are stout up to 60cm long having a penetrating characteristic odour, it is brown with longitudinal ridges and rough reticulated surface it is brittle, fractured surface having resinous appearance. Both plants generally used as an aphrodisiac, anodyne, carminative, diaphoretic, febrifuge, foul ulcer, asthma, amenorrhoea, dysmenorrhea.[2] Quercetin, a polyphenol derived from plants, has a wide range of biological actions including anti-carcinogenic, anti-inflammatory and antiviral activities; as well as attenuating lipid peroxidation, platelet aggregation and capillary permeability.[3] Rutin (3,3′,4′,5,7-pentahydroxyflavone-3-rhamnoglucoside) is oflavone, abundantly found in plants, Citrus leaves contain rutin at concentrations of 11and 7 g/kg in orange and lime trees respectively.[4] Gallic acid is main ingredient and responsible for pharmacological mechanisms in the pathophysiological process of the oxidative damage diseases, such as cancer, cardiovascular, degenerative and metabolic diseases.[5] Caffeic acid is a phenolic compound synthesized by all plant species and is present in foods such as coffee, tea and popular medicines such as propolis. This phenolic acid and its derivatives have antioxidant, anti-inflammatory and anticarcinogenic activity.[6] Vitexin (apigenin-8-C-glucoside) has recently received increased attention due to its wide range of pharmacological effects, including but not limited to anti-oxidant, anti-cancer, anti-inflammatory and neuroprotective effects.[7] High performance thin layer chromatography (HPTLC) is a preferred analytical tool for fingerprints and quantification of marker compounds in herbal drugs due to its simplicity, high sensitivity, accuracy and less expensive.[8] There is no simultaneous HPTLC method is reported in single mobile phase in the literatures for identification of five standard markers for the selected herbal raw materials *Inula racemosa* and *Saussurea costus*. From the above findings we are plan to carry out chromatogram using HPTLC technique using standard antioxidants marker by simultaneous technique.

MATERIALS AND METHODS

Collection of herbal raw materials for HPTLC screening

Two herbal raw material were procured from the traditional siddha practitioner (Balavidayambigai siddha hospital, Bommidi small town in Dharmapuri district of Tamilnadu, India) who is using this material for various herbal formulations. The Two herbal

raw materials were *Inula racemosa* root is obtained from asteracea family, *saussurea costus* root is obtained from asteracea family. The traditional medical practitioners use this raw material for various formulations like aphrodisiac, anodyne, carminative, diaphoretic, febrifuge, foul ulcer, asthma, amenorrhoea, dysmenorrhea.

EQUIPMENT

A CAMAG HPTLC system comprising of a Linomat-5 applicator and CAMAG TLC Scanner-3 and single pan balance of Shimadzu model was used, for weighing the samples.

CHEMICALS AND SOLVENT

Quercetin, Rutin, Gallic acid, Caffeic acid, Vitexin were procured from Sigma Chemical Company Inc., USA. Solvents for extraction were purchased from Qualigens fine chemical (P) limited Mumbai. HPTLC was carried out using Merck aluminum sheet coated with silica gel GF254 (0.2 mm).

PREPARATION OF STANDARD AND EXTRACT FROM THE HERBAL RAW MATERIAL

One gram of each dried powdered material was taken and sonicated with 10 ml of methanol. Filtered and the filtrate solution was used for HPTLC analysis. Standard marker compounds were prepared using methanol to get a concentration 1 mg/1ml.

APPLICATION OF SAMPLE

The sample solutions were spotted in the form of bands of width 6 mm with a Hamilton 100µl syringe on precoated plate 60 F254 (10 cm × 10 cm with 0.2 mm m thickness, E. Merck) using a Camag Linomat V applicator. The slit dimension was kept 5 mm × 0.45 mm. Eight µl of each sample and five µl of standard solutions were applied on to the plate. The migration distance was 80 mm. TLC plates were dried with air dryer. Densitometric scanning was performed using Camag TLC Scanner-3 at 254 nm and 366 nm operated by a wincat software.

DEVELOPMENT

The chromatogram was developed in CAMAG glass twin-through chamber (10-10cm) previously saturated with the mobile phase toluene: ethyl acetate: formicacid:methanol [3:6:1.6:0.4] for 10 min (temperature 25 °C, relative humidity 40%). The development was done for 8 cm from bottom.

DETECTION

The plate was scanned at UV 254 and 366 nm using CAMAG TLC Scanner-3 and LINOMAT-V. Rf value of each compound which were separated on plate and data of peak area of each band was recorded.

RESULTS AND DISCUSSION

The following different solvent compositions were tried for monitor the elution of components in herbal extracts[9,10] Ethyl acetate: glacial acetic acid formic acid: water (100:3:3:28), Ethyl Acetate: Methanol: Water Toluene (100:13:10:13), Chloroform: ethyl acetate: methanol (6:4:0.3), Toluene: ethyl acetate: formic acid: methanol (3:6:1.6:0.4), Toluene: ethyl acetate (93:7).Among the 5 mobile phases attempted, Toluene: ethyl acetate: formic acid: methanol in the ratio of 3:6:1.6:0.4 gave better elution for all the extracts tested and hence it was used as mobile for detection of constituents in herbal extracts. The optimized chamber saturation time for mobile phase was 10 min at room temperature (25 ± 1°C). The densitometric analysis was performed at 254 nm in reflectance mode. The Rf values of the marker compounds were in the range of 0.08 to 0.91. The detection and quantity of marker in herbal raw material extracts were given in Table 1. The identity of components in herbal extracts was ascertained by chromatogram. Results of the study clearly revealed that *Inula racemosa* exhibits quercetin, rutin, gallic acid and *Saussurea costus* exhibits quercetin, gallic acid, caffeic acid. Quercetin was found to be 0.076%, 0.122%, 0.042%, 0.130% in *Inula racemosa* root and *Saussurea costus* root respectively. Gallic acid was found to be 0.021%, 1.36% and 0.032% in *Inula racemosa* root and *Saussurea costus* root respectively. Rutin was to be 0.007% in *Inula racemosa* root. Caffeic acid was found to be 0.209%, 0.581% in *Saussurea costus* root. In Conclusion the antioxidant markers Quercetin and Gallic acid were present in both extracts but Rutin were present in *Inula racemosa* root extracts. Caffeic acid were present in *Saussurea costus* root extracts. From the above findings that *Inula racemosa* and *Saussurea costus* exerts its characteristic activity due to presence of antioxidant marker present in the extracts.

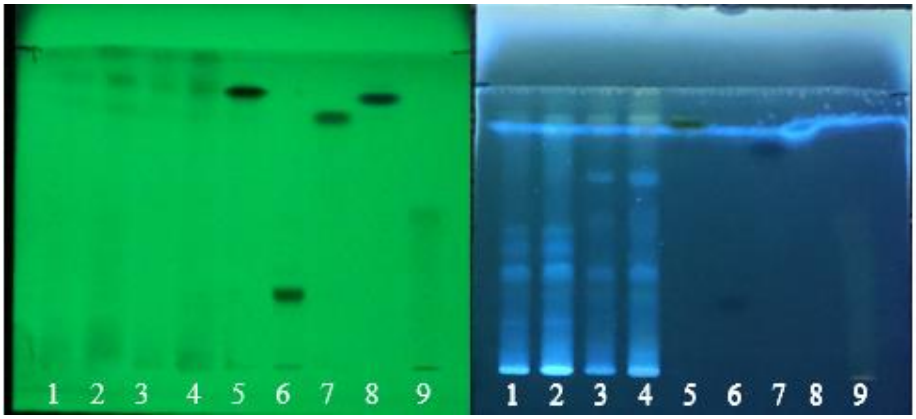

Figure 1: 1- *Inula racemosa* (5µl), 2- *Inula racemosa* (10µl), 3- *Saussurea costus* (5µl),4- *Saussurea costus* (10µl), 5- Quercetin, 6- Rutin, 7- Gallic acid, 8-Caffeic acid, 9-Vitexin.

Table 1: Rf values of standard markers in extracts of *Inula racemosa* and *Saussurea costus*.

Track Number	Name / Amount of Sample in µl	Rf values of compounds in extracts/Standards	Rf value of the marker in extracts	Name of marker in extracts	Area of Standard Marker in sample	Amount of marker present in µg/ 5 µl and 10 µl ofextracts/ 5 µl of standards	% of marker in Extracts
T – 1	*Inula racemosa* whole plant extract /5µl	0.08, 0.11, 0.19, 0.23, 0.33, 0.37, 0.56,0.58, **0.87**	0.87	Quercetin	1137.6	0.383	0.076%
T – 2	*Inula racemosa* whole plant extract/10µl	0.09, 0.19, **0.25**, 0.38, 0.43, 0.55, 0.70, **0.81**, **0.86**	0.86	Quercetin	1813.1	1.223	0.122%
			0.25	Rutin	159.0	0.075	0.007%
			0.81	Gallic acid	253.6	0.211	0.021%
T – 3	*Saussurea costus* whole plant extract / 5 µl	0.09, 0.11, 0.19, 0.32, 0.50, 0.56, 0.70, **0.80**, **0.86**, 0.88	0.86	Quercetin	624.7	0.210	0.042%
			0.80	Gallic acid	164.2	0.068	0.0136%
			0.88	Caffeic acid	696.0	1.048	0.209%
T – 4	*Saussurea costus* whole plant extract/ 10 µl	0.08, 0.19, 0.31, 0.38, 0.47, 0.56, 0.70, **0.81**, **0.89**	0.89	Quercetin	1928.0	1.301	0.130%
			0.81	Gallic acid	389.0	0.323	0.032%
			0.89	Caffeic acid	1928.0	5.811	0.5811%
T – 5	Quercetin / 5µl	0.88			14816.7	5.0	100%
T – 6	Rutin / 5µl	0.26			21031.6	5.0	100%
T – 7	Gallic acid / 5µl	0.81			12014.4	5.0	100%
T – 8	Caffeic acid / 5µl	0.90			3317.8	5.0	100%
T – 9	Vitexin / 5µl	0.48			1448.9	5.0	100%

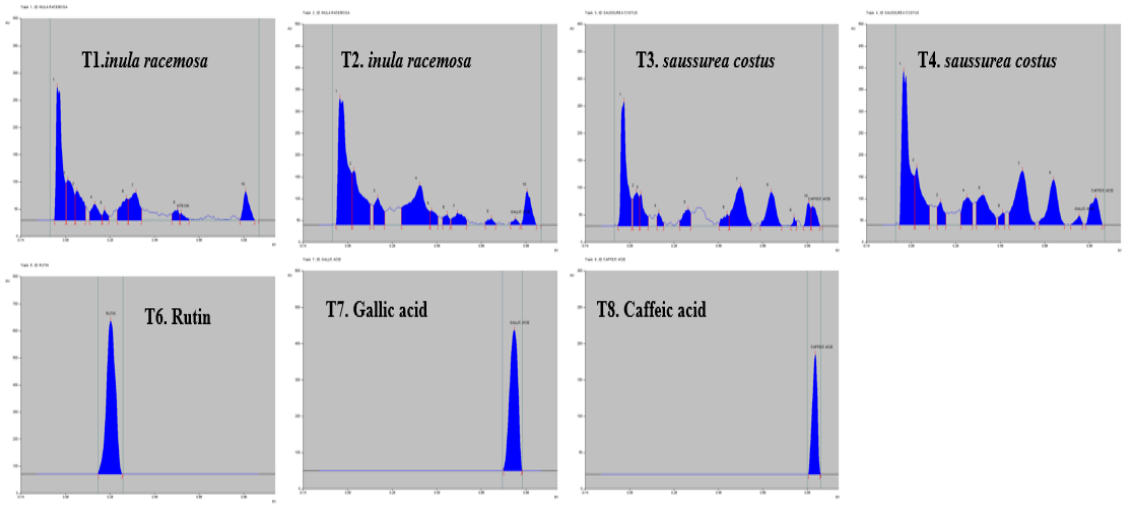

Figure 2: Chromatogram of *Inula racemosa* and *Saussurea costus* extracts and standard markers.

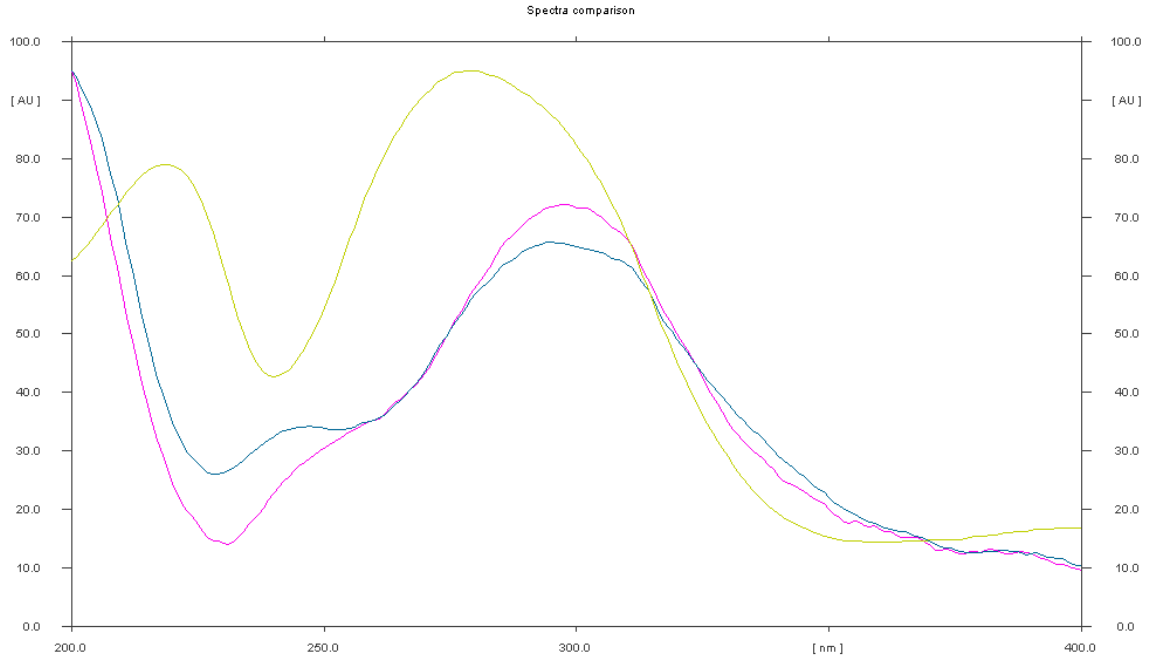

Figure 3: Overlay of Gallic Acid In *Inula Racemosa* and *Saussurea Costus.*

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