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DETECTION AND ESTIMATION OF FLAVONOIDS, PHENOLIC ACIDS AND XANTHONE IN *CLERODENDRUM SERRATUM* ROOT AND *PREMNA HERBACEAE* ROOT EXTRACTS BY HPTLC TECHNIQUE

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ABSTRACT

The prime aim of the study is to observe the flavonoids, phenolic acids and xanthone in two herbal raw materials *clerodendrum serratum* root and *Premna herbaceae* root from verbanaceae family. Traditional siddha practitioners instead of *clerodendrum serratum* root using *Premna herbaceae* root as substitute in herbal formulations because both are belongs to Verbenaceae and used for similar symptoms of disease. Our aim of interest to detect and estimate antioxidatant markers in *clerodendrum serratum* root and *Premna herbaceae* root. Results of the study clearly revealed that *clerodendrum serratum* root reveals caffeic acid and rutin but *premna herbaceae* root exhibits only caffeic acid. Rutin and caffeic acid was found to be 0.04 and & 0.27% respectively in *clerodendrumserratum* root. Caffeic acid was found to be 5.536% in *Premnaherbaceae* root. In Conclusion the antioxidant markercaffeic acid were present in both extracts but *Premna herbaceae* root extracts only caffeic acid. The *clerodendrum serratum* literature reveals anti-inflammatory, antioxidant, antibacterial, hepatoprotective and anticancer and *Premna herbacea* possess antipyretic, anti-inflammatory and anti nociseptic activity may be due to presence of antioxidant markers like caffeic acid and rutin present in the extracts.

KEYWORDS: Caffeic acid, Gallic acid, HPTLC, Mangiferin, Rutin, Quercetin.

INTRODUCTION

Clerodendrum serratum is a shrub belong to Verbenaceae.[1] The leaves are ternate opposite which is usually 5 to 7 cm. The flowers are numerous. Rootof *clerodendrum serratum* has a pungent, bitter, acrid taste. *C.serratum* is used as stomachic, anthelmintic and useful in bronchitis, asthma, ozoena, fevers, diseases of the blood, tumours, burning sensation, hiccough, epilepsy, tuberculosis glands, wounds. The leaves of *clerodendrumserratum* used in fevers and tridosha(Ayurveda). The root increases appetite, it also less the expectoration and used in febrile, catarrhal affections. The Ratnagiri peoples consider it is very effective in malarial fevers. Ointment was made by using the *C. serratum* leaves boiled with oil and butter which is used in cephalgia and ophthalmia. The seeds bruised and boiled in butter milk used as aperient and in dropsy. *C. Serratum* is also used in dyspepsia. The leaves are used as one of the snake remedies (sushruta, Bapat), and the root issued for scorpion-sting (susruta) but they are no antidote to either snake venom (Mhaskar and Caius) or scorpion venom (Caius and mhaskar).[2] The aqueous extract obtained from *clerodendrum serratum* roots have an immunostimulatory activity in mice[3]; The *clerodendrum serratum* leaves and root extract was used in the treatment of rheumatism and asthma.[4] *C.serratum* having a pharmacological activity such as anti-inflammatory, antioxidant, antibacterial, hepatoprotective and anticancer[5] *clerodendrum serratum* having antiviral activities.[6] *Premna herbaceae* it is a small undershrub, belongs to verbanaceae The leaves are sessile, obtuse, obovate, mature, pubesant on the nerves, microscopically dotted above, minutely deciduously pubescent beneath, nerves 5 pairs. It distribute over the subtropical Himalayas, from kumaon to Bhutan, N. circara, W. Ghats of the madras presidency. *Premna herbacea* possess antipyretic, anti-inflammatory and anti nociseptic activity.[7] it is used to treat several diseases like bronchitis asthma, blood pressure, tumour, cough, epilepsy and helminthiasis.[8] Both the plants are used by traditional practises for symptoms of inflammation, lung diseases, respiratory infections, liver diseaseas and tumors at the same time *clerodendrum serratum*literature exhibits anti-inflammatory, antioxidant, antibacterial, hepatoprotective and anticancer.[9] *Premna herbacea* possess antipyretic, anti-inflammatory and anti nociseptic activity.[10] From the above findings, Our aim of interest to identify the similar antioxidatant markers in *clerodendrumserratum* root and *Premnaherbaceae*root. High performance thin layer chromatography (HPTLC) is a preferred analytical tool for fingerprints and quantification of marker compounds in herbal drugs dueto its simplicity, high sensitivity, accuracy and less expensive.[11] 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.[12] Rutin(3,3′,4′,5,7-pentahydroxyflavone-3-rhamnoglucoside) is a flavonol, abundantly found in plants, Citrus leaves contain rutin at concentrations of 11 and 7 g/kg in orange and lime trees respectively.[13] 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.[14] 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.[15] Mangiferin is a C-glycosyl compound consisting of 1,3,6,7-tetrahydroxyxanthen-9-one having a beta-D-glucosyl residue at the 6-position. Mangiferin and its derived lead molecule have proven its effectiveness as an antioxidant, analgesic, antidiabetic, antiproliferative, chemopreventive, radioprotective, cardiotonic, immunomodulatory and diuretic.[16] There is no simultaneous HPTLC method is reported in single mobile phase in the literatures for identification of five srtandard markers for the selected herbal raw materials *clerodendrumserratum* root and *Premnaherbaceae* root. From the above literature review 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 Varuni Exports – Medicinal Plants Cultivators and Exporters in Tirunelveli district of Tamilnadu, India and plant was authenticated by Dr.V.Chelladurai, Professor, Department of Botany Medicinal plant Survey for Siddha, Government of India.

Equipment

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

Chemicals and solvents

Quercetin, Rutin, Gallic acid, Caffeic acid, mangiferin 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 aluminium sheet coated with silica gel GF254 (0.2 mm).

Preparation of standards and extracts from the herbal raw materials

One gram of clerodendrumserratum root sonicated with 10 ml of methanol and named as Methanolic Extract of clerodendrumserratum (MECS) and Methanolic Extract of and *Premnaherbaceae* root (MEPH). Filtered and the filtrate solution was used for HPTLC analysis. Standard marker compounds Quercetin, Rutin, Gallic acid, Caffeic acid, Mangiferinwere prepared using methanol to get at 1 mg/1 ml.

Application of sample

The sample solutions were spotted in the form of bands of width 6 mm with a Hamilton 100 µl syringe on precoated plate 60 F254 (10 cm × 10 cm with 0.2 mm m thickness, E. Merck) using a CamagLinomat 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 (10-10 cm) previously saturated with the mobile phase toluene: ethyl acetate: formic acid: 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.[17,18] 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 0.09 to 0.89.(Table 1) 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. *Clerodendrum serratum* root reveals caffeic acid and rutin but *premnaherbaceae* root exhibits only caffeic acid. Rutin and caffeic acid was found to be 0.0228 & 0.0425 and 0.223 & 0.2274% respectively in *clerodendrumserratum* root. caffeic acid was originated to 4.284 & 5.536% in *Premnaherbaceae* root. In Conclusion the antioxidant marker caffeic acid were present in both extracts but *Premna herbaceae* root extracts only caffeic acid 4.284 & 5.536%. The results confirm the presence of antioxidant marker in both the extracts *Clerodendrum serratum* root and *premna herbaceae* roots and it reveals that they are given by traditional siddha practitioners for similar symptoms of inflammation, lung diseases, respiratory infections, liver diseases and tumours, since the therapeutic action of both the plants are may be due the presence of caffeic acid in both the plants.

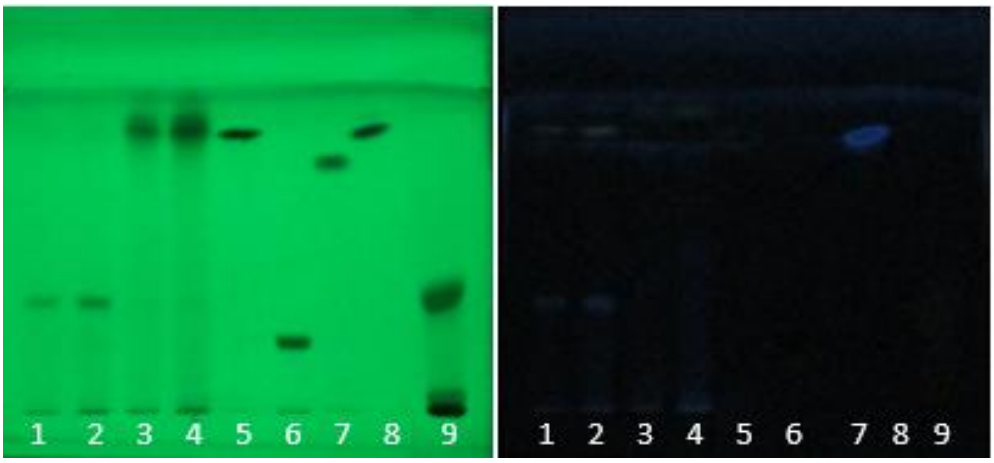

Figure 1: 1. *Clerodendrum serratum* (5µl), 2. *Clerodendrum serratum* (10µl), 3. *Premna herbacea* (5µl), 4. *Premna herbacea* (10µl), 5. Quercetin, 6. Rutin, 7. Gallic acid, 8. Caffeic acid, 9. Mangiferin.

Table 1: Rf values of standard markers in extracts of *Clerodendrum Serratum* and *Premna Herbaceae.*

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	*Clerodendrum Serratum Root* extract / 5µl	0.13, **0.21**, 0.33, 0.5, 0.83, **0.86**	0.86	Caffeic acid	784.5	1.115	0.223%
			0.21	Rutin	420.6	0.114	0.0228%
T – 2	*Clerodendrum Serratum* Root Extract / 10µl	0.07, 0.14, **0.20**, 0.24, 0.33, 0.39, 0.83, **0.86**	0.20	Rutin	777.8	0.425	0.0425%
			0.86	Caffeic acid	965.7	2.747	0.2747%
T – 3	*Premna Herbaceae* Root extract / 5µl	0.29, 0.33, 0.37, 0.48, **0.85**	0.85	Caffeic acid	15065	21.42	4.284%
T – 4	*Premna Herbaceae* Root extract / 10 µl	0.05, 0.33, 0.44, **0.85**	0.85	Caffeic acid	19460.2	55.36	5.536%
T – 5	Quercetin / 5µl	0.85			15361.9	5.0	100%
T – 6	Rutin / 5µl	0.20			18291.3	5.0	100%
T – 7	Gallic acid / 5µl	0.76			10732	5.0	100%
T – 8	Caffeic acid / 5µl	0.86			3515	5.0	100%
T – 9	Mangiferin / 5µl	0.35			36392.5	5.0	100%

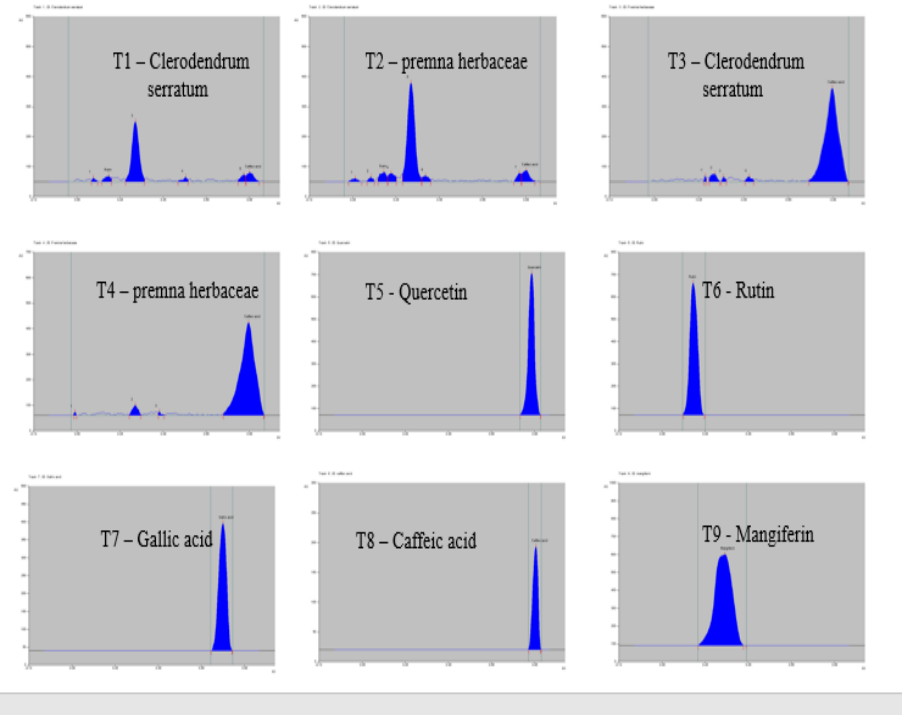

Figure 2: Chromatogram of Raw materials extracts and Standard markers.

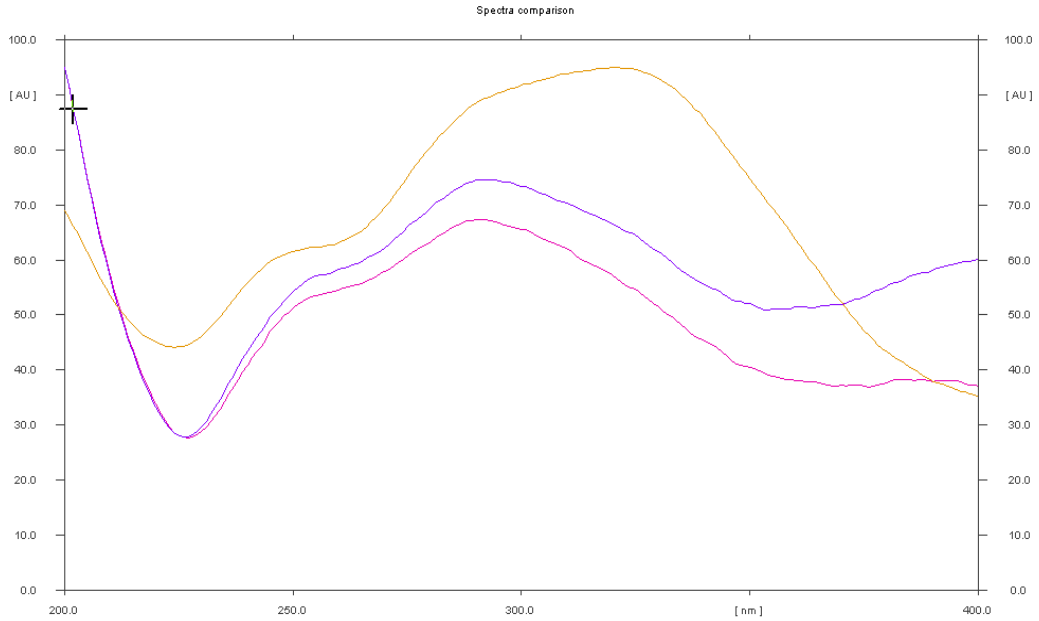

Figure 3: overlay of caffeic acid in *clerodendrumserrratum* and *premna herbaceae.*

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