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DETECTION AND ESTIMATION OF FLAVONOIDS AND PHENOLIC ACIDS IN HERBAL RAW MATERIALS AVAILABLE IN MARKET BELONGS TO FAMILY OF ANACARTIACEAE, APOCYNACEAE, ASCLEPIADACEAE, CAPPARACEAE, FABACEAE AND LAMIACEAE BY HPTLC TECHNIQUE USING ANTIOXIDANT MARKERS

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ABSTRACT

The aim of the study to analyse flavonoids and phenolic acids in seven indigenous herbal raw materials. *Glycyrrhiza glabra, Rauwolfia serpentina, Ocimum kilimandscharicum, Crateva nurvala, Gymnema sylvestre, Acacia nilotica ssp indica, Mangifera indica*. Results reveals that seven herbal raw materials contain flavonoids and phenolic acids. The developed simultaneous HPTLC method can be employed for routine investigations. The seven herbal raw materials were procured from a drug store and analysed for the presence of Quercetin, Rutin, Gallic acid and mangiferin. Quercetin was found to be in *Glycyrrhiza glabra* 0.26%, *Rauwolfia serpentina* 0.08, *ocimumkilimandscharium* 0.18%, *crateva nurvala* 0.18%, *gymnema sylvestre* 0.06%, *acacia nilotica ssp indica* 0.16%, *mangifera indica* 0.25%. Rutin was present only in four herbals such as *glycyrrhiza glabra* 0.69%, *ocimumkilimandscharium* 0.07%, *gymnema sylvestra* 0.02, and *acacia nilotica ssp indica* 0.02. Gallic acid was found to be only in *glycyrrhiza glabra* 0.03%, *acacia nilotica ssp indica* 0.51%, mangifera indica 0.26%. Mangiferin shows very minimum percentage in *Glycyrrhiza glabra* 0.01%, *Crateva nurvala*-0.005%, *Acacia nilotica ssp indica* 0.02%. Quercetin was present in all seven herbal raw materials hence all seven herbal raw materials possesses antioxidant property.

KEYWORDS: Quercetin, Gallic acid, Mangiferin, *Asclepiadaceae, Fabaceae, Lamiaceae.*

INTRODUCTION

Glycyrrhiza glabra is a widely used medicinal plant known for its numerous therapeutic properties, including antioxidant, anti-inflammatory, antimicrobial, antiviral, anticancer, anti-obesity, anti-tussive, diuretic, immune-boosting, hepatoprotective, muscle-relaxing, and gastroprotective effects. It plays a significant role in benefiting digestion, respiratory health, and metabolic disorders. *Glycyrrhiza glabra* root is commonly used in herbal teas, cough syrups, and digestive remedies, making it a staple in traditional medicine.[1] *Rauvolfia serpentina*, known as Sarpagandha, is highly valued for its role in treating hypertension, insomnia, schizophrenia, and other nervous disorders. It contains potent alkaloids such as reserpine and ajmaline, which have neuroleptic, sedative, and antihypertensive effects. Stigmasterol, a phytosterol present in many plant species, contributes to cell elongation, division, and vegetative growth. It has also been identified as an effective compound in neutralizing the lethal effects of rattlesnake venom.[2] *Ocimum kilimandscharicum* is a perennial herb that propagates through seeds and cuttings. It thrives in various soil types, requiring well-drained conditions with an annual rainfall of approximately 1250 mm. The plant grows at altitudes up to 900 mm and can be harvested three times a year for more than three years. Due to its aromatic properties, it is often used in herbal remedies for respiratory infections, fever, and asthma. Its essential oils have strong medicinal properties and are widely used in natural treatments.[3] *Crateva nurvala*, commonly known as Varuna, is an evergreen tree native to India. It is primarily used for treating urinary disorders, prostate enlargement, bladder sensitivity, and kidney stones. The plant exhibits anti-arthritic, hepatoprotective, and cardio-protective properties. Lupeol, a bioactive compound found in its bark, has shown promising anti-inflammatory, antimicrobial, anti-protozoal, anti-proliferative, and cholesterol-lowering properties. Notably, lupeol selectively targets diseased cells while sparing healthy ones, making it a potential therapeutic agent in modern medicine.[4] *Gymnema sylvestre* is a key medicinal plant in Ayurvedic, Siddha, and Homeopathy medicine, widely recognized for its anti-diabetic properties. It restores pancreatic function, increases insulin levels, and helps regulate blood glucose levels. Gymnema-based supplements and herbal formulations are commercially available worldwide and commonly used for diabetes management and weight control.[5] *Acacia nilotica* is a powerful medicinal plant rich in phenolic compounds like quercetin. It possesses strong antioxidant, anti-inflammatory, and antibacterial properties, making it useful in treating infections, wounds, and digestive ailments. Researchers are currently developing a rapid HPTLC quantification method to analyze herbal raw materials more efficiently.[6] *Mango*

(Mangifera indica), originating from India, has over 1,000 cultivars and is valued for its exceptional taste, high nutritional content, and diverse medicinal uses. Apart from its edible fruit, mango bark and leaves have been utilized in traditional medicine for their anti-diabetic, digestive, and immune-boosting properties.[7] There is no simultaneous HPTLC method is reported in single mobile phase in the literatures for detection of antioxidant markers in seven herbal raw materials from Anacartiaceae, Apocynaceae, Asclepiadaceae, Capparaceae, Fabaceae and Lamiaceae and hence this paper describes detection of flavonoids, phenolic acids and xanthones in raw materials by HPTLC method.

MATERIALS AND METHODS

Collection of seven herbal raw materials for HPTLC screening

The *Glycyrrhiza glabra, Rauwolfia serpentina, Ocimum kilimandscharicum, Crateva nurvala, Gymnema sylvestre, Acacia nilotica ssp indica, Mangifera indica* of seven herbal raw materials were procured from authenticated drug store.

Instruments

A CAMAG HPTLC system comprising of a Linomat-V applicator and CAMAG TLC Scanner-3 and single pan balance of Shimadzu model were used, for weighing the herbal raw materials for preparation of extracts.

Chemicals and solvents

Quercetin, Rutin, Gallic acid, Rutin, 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 plant's formulations

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 concentration 1 mg/1 ml.

Application of sample

The herbal formulations solutions were spotted in the form of bands of width 4 mm with a Hamilton 100µl syringe on recoated 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 6mm × 0.45

mm. Eight µl of each herbal raw material extract 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 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.

Table 1: Rf value of 1-*Glycyrrhiza Glabra,* 2-*Rawolfia Serpentina,* 3-*Ocimumkilimandscharium,* 4-*Cretavea Nurvala,* 5-*Gymnema Sylvestra,* 6-*Acacia Nilotica Ssp Indica,* 7- *Mangifera Indica, 8*- Quercetin, Ruttin, Gallic Acid, 9- Mangiferin.

Track no	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/ 8µl Of Extracts/ 5µl Of Standards	%Of Marker In Extracts
T-1	*Glycyrrhiza Glabra*	0.05,0.16,0.28, 0.44,0.52,0.79, 0.87,0.90	0.87	Quercetin	5854.5	2.10	0.26
			0.79	Gallic Acid	618.4	0.24	0.03
			0.16	Rutin	24334.5	5.59	0.69
			0.79	Mangiferin	618.4	0.24	0.03
T-2	*Rauwolfia Serpentina*	0.09,0.19,0.24, 0.31,0.37,0.44, 0.52,0.60,0.65, 0.72,0.74,0.87, 0.91	0.87	Quercetin	1794.3	0.64	0.08
T-3	*Ocimumki limandscha rium*	0.16,0.23,0.39, 0.86	0.86	Quercetin	4195.6	1.51	0.18
			0.16	Rutin	2478.3	0.57	0.07
T-4	*Crateva Nurvala*	0.05,0.09,0.18, 0.28,0.31,0.54, 0.60,0.67,0.87	0.87	Quercetin	1406.6	0.50	0.06
T-5	*ymnema Sylvestre*	0.02,0.06,0.13, 0.16,0.22,0.38, 0.46,0.53,0.87,	0.87	Quercetin	3534.1	1.27	0.15
			0.13	Rutin	9015.6	0.20	0.02

		0.91					
T-6	*Acacia Nilotica SSP Indica*	0.01,0.09,0.17, 0.25,0.31,0.38, 0.50,0.70,0.77, 0.83,0.87,0.92	0.87	Quercetin	3633.9	1.30	0.16
			0.77	Gallic Acid	10386.9	4.15	0.51
			0.17	Rutin	967.6	0.22	0.02
			0.77	Mangiferin	10386.9	4.15	0.51
T-7	*Mangifera Indica*	0.08,0.19,0.24, 0.34,0.43,0.53, 0.61,0.77,0.85, 0.88,0.91	0.85	Quercetin	5670.1	2.04	0.25
			0.77	Gallic Acid	5300.2	2.12	0.26
			0.77	Mangiferin	5300.2	2.12	0.26
T-8	Quercetin	0.86				5	100
	Gallic Acid	0.79				5	100
	Rutin	0.15				5	100
T-9	Mangiferin	0.27				5	100

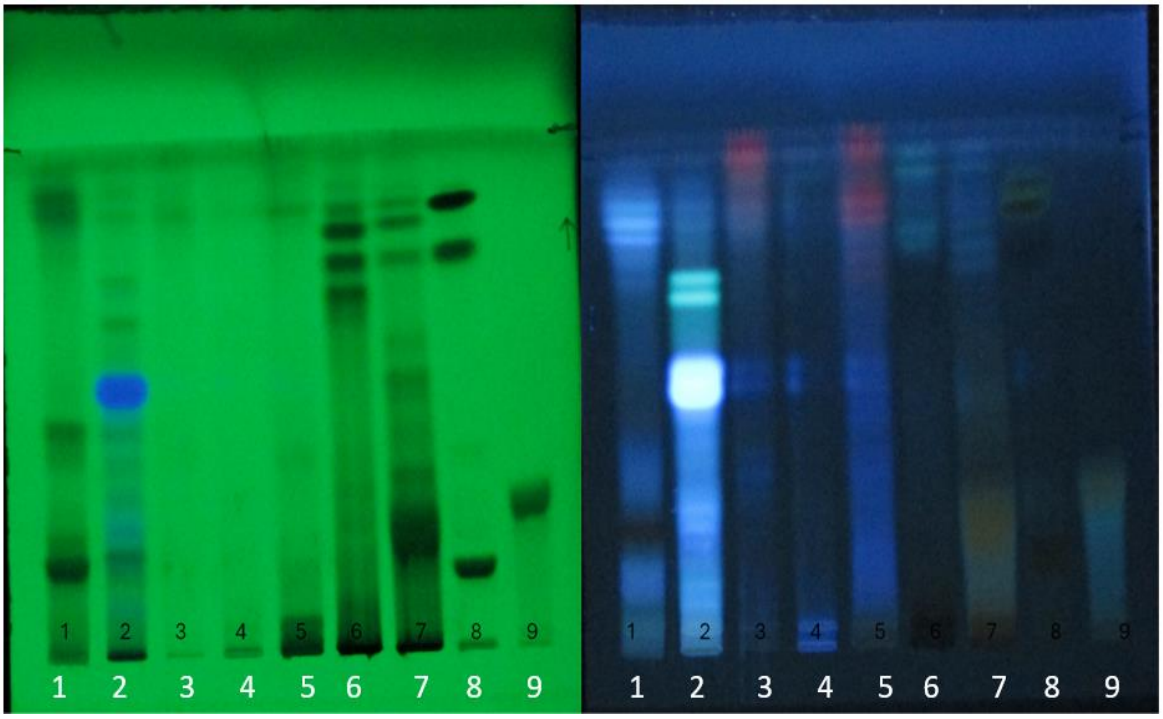

Figure-1: ***1- Glycyrrhiza Glabra, 2-Rawolfia Serpentina, 3-Ocimumkilimandscharium, 4-Cretavea Nurvala, 5-Gymnema Sylvestra, 6-Acacia Nilotica SSP Indica, 7- Mangifera Indica, 8-*** **Quercetin, Ruttin, Gallic Acid, 9- Mangiferin.**

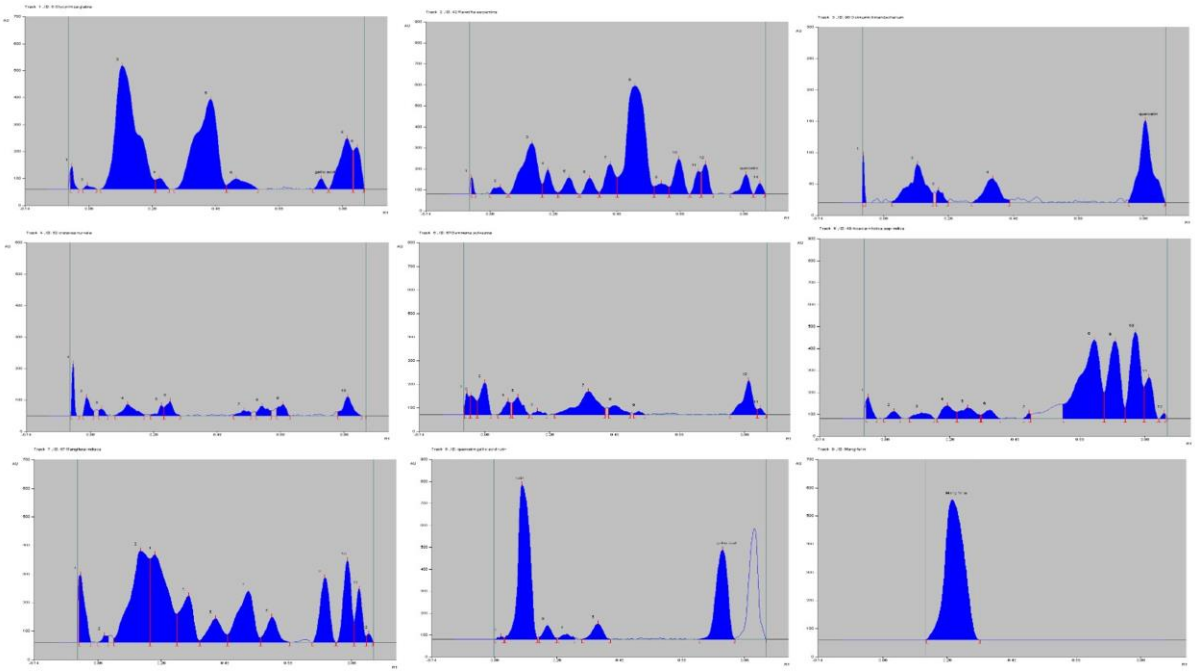

Figure-2: chromatogram of *1- Glycyrrhiza Glabra, 2-Rawolfia Serpentina, 3-Ocimumkilimandscharium, 4-Cretavea Nurvala, 5-Gymnema Sylvestra, 6-Acacia Nilotica Ssp Indica, 7- Mangifera Indica, 8-* Quercetin, Ruttin, Gallic Acid, 9- Mangiferin.

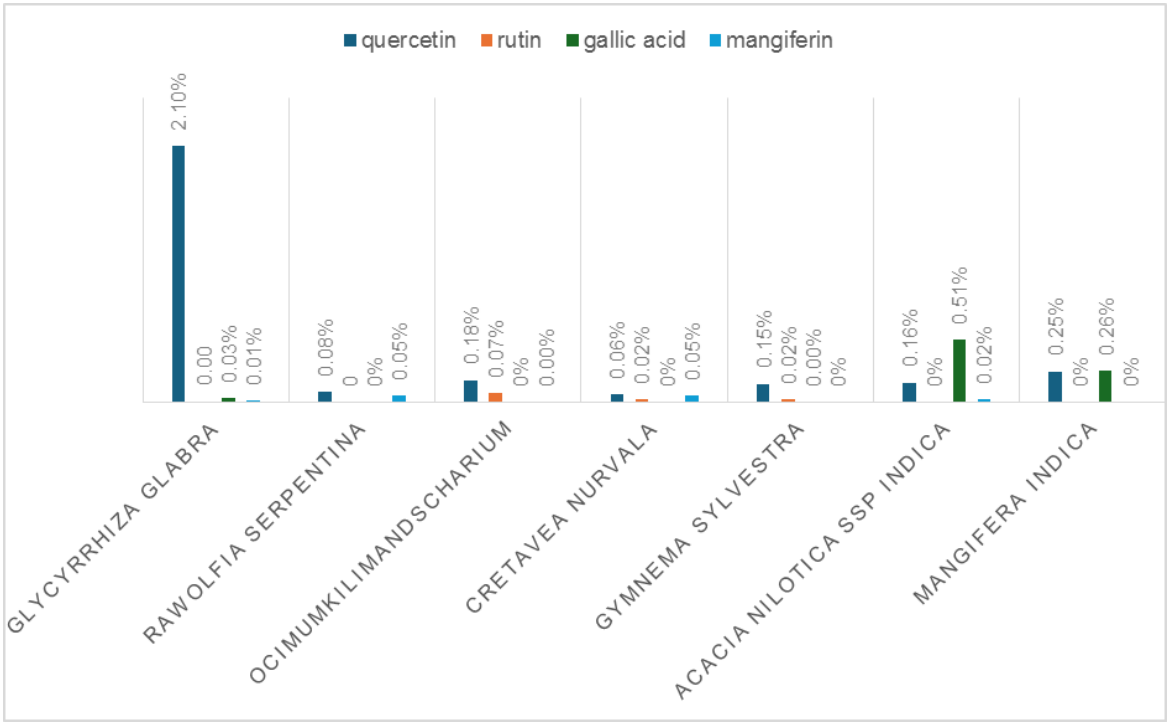

Figure-3: percentage of *1- Glycyrrhiza Glabra, 2-Rawolfia Serpentina, 3-Ocimumkilimandscharium, 4-Cretavea Nurvala, 5-Gymnema Sylvestra, 6-Acacia Nilotica Ssp Indica, 7- Mangifera Indica, 8-* Quercetin, Rutin, Gallic Acid, 9- Mangiferin.

RESULT

Quercetin was found to be in *Glycyrrhiza glabra* 0.26%, -*Rawolfia serpentina*- 0.08, *Ocimumkilimandscharium*- 0.18%, *Cretavea nurvala*-0.18%, *Gymnema sylvestra*-0.06%, -*Acacia nilotica ssp indica*-0.16%, *Mangifera indica*- 0.25%. rutin was found to be *glycyrrhiza glabra*-0.69%, *ocimumkilimandscharium*-0.07%, *gymnema sylvestra*-0.02, *acacia nilotica ssp indica*-0.02. gallic acid was found to be in *glycyrrhiza glabra*-0.03%, *acacia nilotica ssp indica*-0.51%, mangifera indica-0.26%. mangiferin was found to be glycyrrhiza glabra-0.01%, *cretavea nurvala*-0.005%, *acacia nilotica ssp indica*-0.02%. the antioxidant marker quercetin was present in all seven herbal raw materials.

CONCLUSION

In conclusion the quercetin was found in all seven herbal raw materials, rutin was found in *Glycyrrhiza Glabra, Ocimumkilimandscharium, Gymnema Sylvestra, Acacia Nilotica Ssp Indica.* Gallic Acid was found in *Glycyrrhiza Glabra, Acacia Nilotica Ssp Indica, Mangifera Indica.* Mangiferin was found to in *Glycyrrhiza Glabra, Cretavea Nurvala, Acacia Nilotica Ssp Indica*. From the above findings the antioxidant activity may be due to presence of the Quercetin, rutin, gallic acid and mangiferin in herbal raw materials.

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