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DETECTION AND ESTIMATION OF RUTIN, QUERCETIN, GALLIC ACID, CATECHIN, ELLAGIC ACID AND FERULIC ACID IN HERBAL RAW MATERIALS AVAILABLE IN MARKET TO TREAT VIRAL PATHOGENS SARS-COV-2 BELONGS TO *PHYLLANTHUS* & *FICUS* SPECIES BY HPTLC TECHNIQUE

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ABSTRACT

The prime aim of the study is to detect and estimate the flavonoids, and phenolic acids in five commercial herbal raw materials namely *Phyllanthus Emblica* dry fruit, *Phyllanthus pinnatus* entire herb, *Ficus bengalensis* bark, *Ficus religiosa* bark, *Ficus racemosa* bark obtained from *Phyllanthus* and *Ficus* species used in treatment for SARS-CoV-2 infection. We plan to confirm the presence of mentioned common secondary metabolites in herbal raw materials. Results of the study clearly revealed that these raw materials from *Phyllanthus* and *Ficus* species contains flavonoids and phenolic acids. The developed HPTLC method can be employed for the routine investigations of flavonoids and phenolic acids in herbal raw materials. *Phyllanthus Emblica* dry fruit, *Phyllanthus pinnatus* entire herb and *Ficus racemosa* bark was ascertain the presence of Ellagic acid and be evidence for the 0.80%, 0.68% and 0.02% respectively. Gallic acid was estimated in *Phyllanthus Emblica* dry fruit, *bengalensis* bark, *Ficus religiosa* bark as 0.54%, 0.03% and 0.09% respectively. Quercetin was predictable in all five herbal extracts *Phyllanthus Emblica*, *Phyllanthus pinnatus*, *Ficus bengalensis*, *Ficus religiosa*, *Ficus racemosa* 0.03%, 0.05%, and 0.12%, 0.12% and 0.27% respectively.

KEYWORDS: Ellagic acid, Quercetin, Gallic acid, Rutin HPTLC.

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is the causative agent of Coronavirus Disease 2019 or COVID-19, triggered a pandemic affecting over 215 countries and territories around the world.[1,2] As of August 2020, there are more than 23 million cases worldwide with over 800,000 deaths, indicating that the virus is highly infectious with its pathogenicity being a global health threat.[3–5] 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.[6] Catechin is a flavan-3-ol, a type of natural phenol and antioxidant.[7] Catechins have been shown to demonstrate a variety of antimicrobial properties. Consumption of green tea has been shown to distribute catechin compounds and/or their metabolites throughout the body, which allows for not only the possibility of treatment of infections but also the prevention of infections.[8] 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.[9] 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.[10] Ferulic acid is most often found in anti-aging skin creams, where is it believed to neutralize free radicals that damage and age cells.[11,12] Ellagic acid has been marketed as a dietary supplement with a range of claimed benefits against cancer, heart disease, and other medical problems. Ellagic acid has been identified by the U.S.Food and drug Aministration as a "fake cancer 'cure'.[13] *Ficus religiosa, Sesbania grandiflora, Moringa oleifera, Avicennia marina, Terminalia bellirica, Phyllanthus amarus, Hippophae rhamnoides*, are few of these plants having antiviral activity, however, their therapeutic applications in COVID-19 are yet to be investigated.[14] Flavonoids and polyphenolic compounds like luteolin, quercetin and tetra-O-galloyl- β-D-glucose have been shown to significantly block the entry of SARS-CoV into the cells.[15] Flavonoids including flavones and flavonoids were investigated for having antiviral potential, and many of them showed significant antiviral responses in both in vitro and in vivo studies. Naringenin and hesperetin (flavanon), hesperidin (flavanonone glycoside), baicalin and neohesperidin (flavone glycoside), nobiletin (O-methylation), scutellarin (flavone), nicotinamin (nonproteinogenic amino acids), and glycyrinodin (methylated-eminin-1,3,8-trihydroxyanthraquinone)are amongst natural ACE2 inhibitors.[16,17,18] *Phyllanthus emblica* is antioxidative and anti-inflammatory, and its extract Phyllaemblicin G7 has the potential to

treat COVID-19.[19] Quercetin along with vitamin C has been proposed to have the synergistic effect in treating COVID-19 patients.[20] Medicinal plants inhibit protease enzymes of the SARS-CoV-2. Many medicinal plants are believed to target the viral 3-chymotrypsin-like cysteine protease (3CLpro) enzyme, which is essential for replication of coronavirus.[21] Isoflavone extracted from *Psorothamnus arborescens*, (2S)-Eriodictyol 7-O-(6”-O-galloyl)-beta-d-glucopyranoside from *Phyllanthus emblica*, 3,5,7,30,40,50 - hexahydroxy flavanone-3-O-beta-d-glucopyranoside from *Phaseolus vulgaris*, methyl rosmarinate from *Hyptis atrorubens*, myricitrin from *Myrica cerifera*, myricetin 3-O-beta-d-glucopyranoside from *Camellia sinensis*, amaranthin from *Amaranthus tricolor* and licoleafol from *Glycyrrhiza uralensis* are some of the potent phytochemicals against SARS-CoV-2.[22] Norsesquiterpenoids isolated from the roots of *Phyllanthus emblica* showed, again, some activity against Coxsackie B3 virus.[23] Vitamin C regulates immunity by enhancing differentiation and proliferation of B- and T-cells, and it is beneficial in preventing and treating respiratory and systemic infections.[24,25,26] Vitamin C potentially protects against infection caused by CoVs due to its benefits on immune function.[27] Quercetrin, Rutin, Cinanserin (1 and 2 dpi) isolated from *H. cordata* reported showed significant against CoV murine at 15.63 –500 µg/m.[28] Note that the SARS-CoV spike protein (S) uses ACE2 as a responsive binding site to invade host cells.[29] A recent research showed that rutin was a powerful inhibitor, which could bind to the active site of the SARS-CoV-2 protease (PDB: 6Y84).[30] From the above background our analysis focuses on the outlook of estimating flavonoids and phenolic acid in *Phyllanthus* & *Ficus* Species by Hptlc Technique for potential treatment for SARS-CoV-2 infection.

MATERIALS AND METHODS

Collection of herbal raw materials for HPTLC screening

Five herbal raw material were procured from the traditional siddha practitioner (Bala vidayambigai siddha hospital, Bommidi small town in Dharmapuri district of Tamil nadu, India) who is using this material for various herbal formulations. The five herbal raw materials were *Phyllanthus Emblica* dry fruit, *Phyllanthus pinnatus* entire herb, *Ficus bengalensis* bark, *Ficus religiosa* bark, *Ficus racemosa* bark obtained from *Phyllanthus* and *Ficus* species. The traditional medical practitioners use this raw material for various formulations like skin care chooranam, Expectorant Chooranam, anti-histamine chooranam, antidiabetic chooranam, anti inflammatory Chooranam, Blood purifier chooranam, Blood pressure reducing chooranam and blood sugar reducing chooranam.

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 solvents

Rutin, Gallic acid, Quercetin, Catechin, Ellagic acid and Ferulic acid 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 GF_{254} (0.2 mm).

Preparation of standards and extracts from the herbal raw materials

One gram of the 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/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 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-trough 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[31] in herbal extracts

- 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)
- Ethyl Acetate: Methanol :Water Toluene (100:15.5:13.5:2)
- Ethyl acetate: methanol: water (100:15.5:13.5)
- Chloroform: ethyl acetate :formic acid (6:4:0.3)
- Toluene: ethyl acetate :formic acid :methanol (3:6:1.6:0.4)
- Ethyl acetate: methanol: water (100:13.5:10)
- Toluene: ethyl acetate (93:7)

Among the 9 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.

Table 1: Rf values of standard markers in extracts of 1. *Phyllanthus Emblica* dry fruit Methanol ext.2. *Phyllanthus pinnatus* entire herb methanol ext 3. *Ficus bengalensis* bark Methanol ext 4. *Ficus religiosa* bark Methanol ext 5. *Ficus racemosa* bark Methanol ext.

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/ 8 µl of extracts/ 5 µl of standards	% of marker in Extracts
T-1	*Phyllanthus Emblica* dry fruit Methanol extract / 8 µl	0.07,0.24,0.59 ,**0.74**,**0.79**,0.85, and **0.88**	0.74	Ellagic acid	21329.7	6.352	0.80%
			0.79	Gallic acid	12589.7	4.319	0.54%
			0.88	Quercetin	612.9	0.204	0.03%
T-2	*Phyllanthus pinnatus* entire herb methanol extract /8 µl	0.13,0.15,0.35 ,**0.74**,0.84, and **0.88**	0.74	Ellagic acid	18188.7	5.41	0.68%
			0.88	Quercetin	1189.3	0.396	0.05%
T-3	*Ficus bengalensis* bark Methanol extract /8 µl	0.09,**0.17**,0.27 ,0.43,0.61, 0.65,069,**0.76**, 0.85 and **0.87**.	0.17	Rutin	4357.0	0.827	0.11%
			0.76	Gallic acid	676.9	0.232	0.03%
			0.87	Quercetin	2806.8	0.935	0.12%
T-4	*Ficus religiosa*	0.23,0.42,0.53	0.76	Gallic acid	1867.6	0.640	0.09%

	bark Methanol extract /8 µl	,**0.76**,0.84 and **0.87**	0.87	Quercetin	2813.3	0.938	0.12%
T-5	*Ficus racemosa* bark Methanol extract /8 µl	0.11,0.34,0.46 ,**0.74**, and **0.84**	0.74	Ellagic acid	493.1	0.146	0.02%
			0.84	Quercetin	6382.0	2.128	0.27%
T-6	Rutin/ 5 µl	0.19			26310.4	5.0	100%
	Gallic acid/ 5 µl	0.78			14574.7	5.0	100%
	Quercetin / 5 µl	0.87			14994.7	5.0	100%
T-7	Catechin / 5 µl	0.76			17130.3	5.0	100%
T-8	Ellagic acid/ 5 µl	0.76			16789.5	5.0	100%
T-9	Ferulic acid / 5 µl	0.42			12397.3	5.0	100%

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 0.88. (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 (Figure 1).

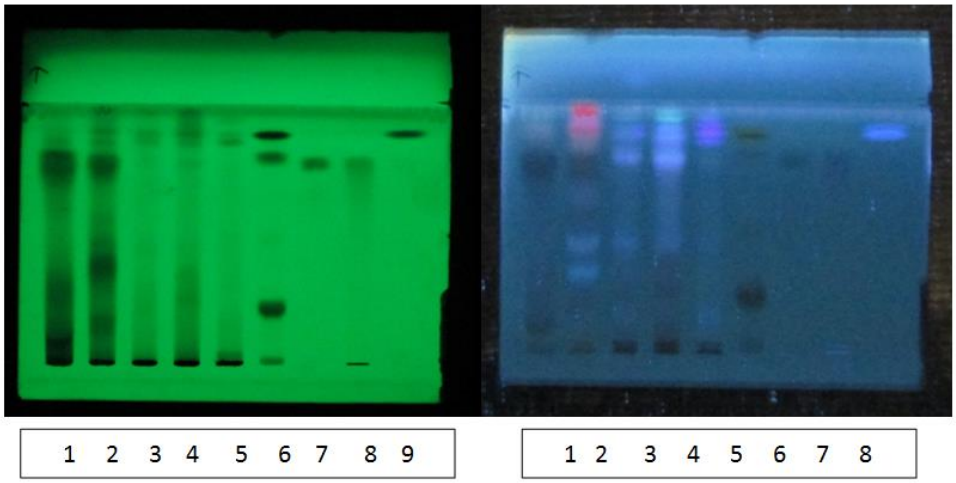


Figure 1: HPTLC Profile Of Methanol Extracts Of Herbal Raw Extracts And Markers After Development In Mobile Phase And Visualised At 254 Nm & 366 Nm.

1. *Phyllanthus Emblica* dry fruit Methanol ext
2. *Phyllanthus pinnatus* entire herb methanol ext
3. *Ficus bengalensis* bark Methanol ext
4. *Ficus religiosa* bark Methanol ext
5. *Ficus racemosa* bark Methanol ext
6. Rutin, Gallic acid, Quercetin markers
7. Catechin marker
8. Ellagic acid marker
9. Ferulic acid marker.

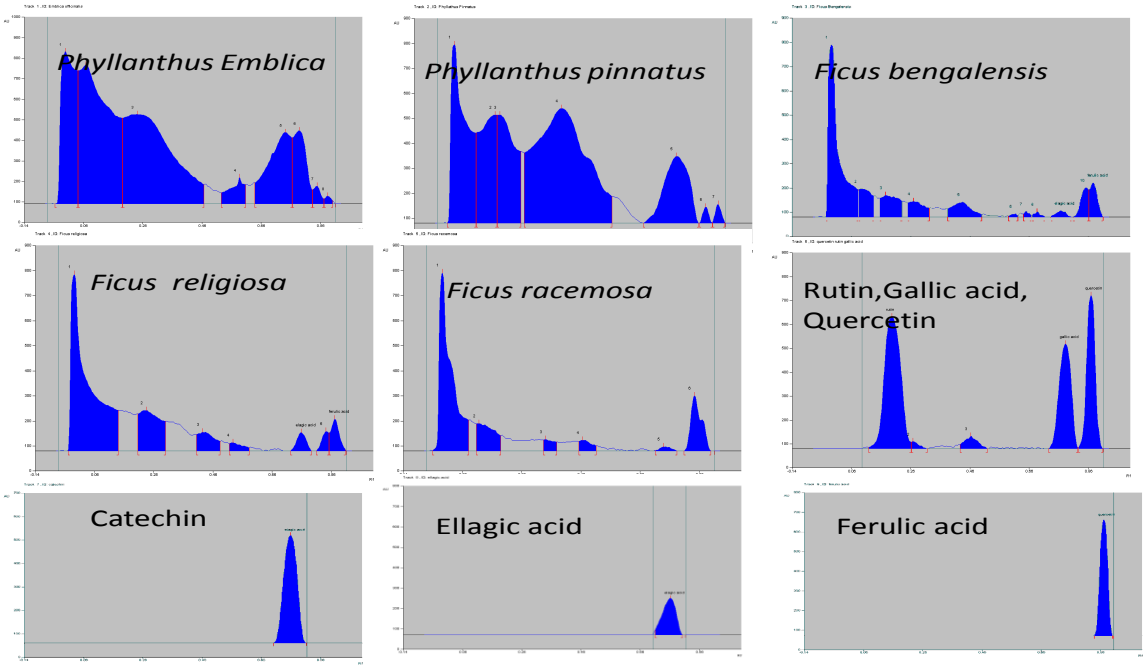
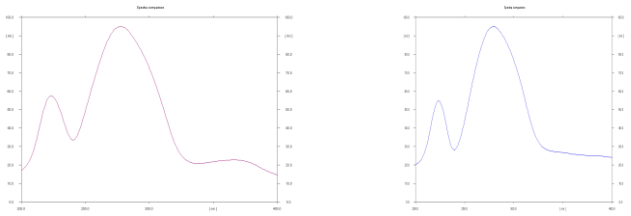
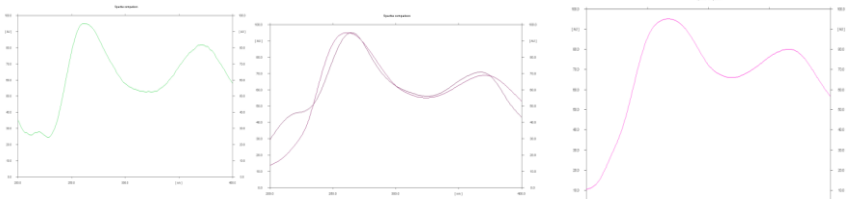


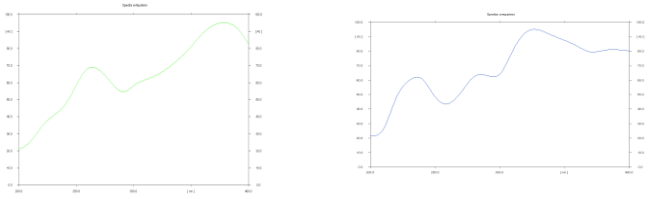
Figure .2 Chromatogram of raw material extracts and Standard markers



Gallic acid marker Track-1 Gallic acid in Phyllanthus emblica



Ellagic Acid in marker Track -1Ellagic acid in P. emblica Track-2 P. pinnatus



Quercetin in marker Track -4 Quercetin in Ficus religiosa

Figure .3 Resemblance of marker lambda max and compounds in extracts

Phyllanthus Emblica dry fruit, *Phyllanthus pinnatus* entire herb and *Ficus racemosa* bark showed same Rf values as that of Ellagic acid 0.74. Gallic acid was detected by its Rf value 0.79 in *Phyllanthus Emblica* dry fruit, *Ficus bengalensis* bark, *Ficus religiosa* bark.

Quercetin detected by its Rf value 0.88 in all the five extracts. Rutin is detected only in *Ficus bengalensis* bark with Rf value of 0.17. Ellagic acid and gallic acid content was comparatively higher in *Phyllanthus Emblica* dry fruit extract. Quercetin showed increasd percnetage in *Ficus racemosa* bark. By estimation of flavonoids and phenolic acid from above mention marketed raw materials we confirm the percentage of the constituents in the same, which is responsible for the treatment of SARS-CoV-2 infection.[20,32]

CONCLUSION

The estimation can be concluded that flavonoids and phenolic acids were present in the five herbal extracts. Presence of quercetin was confirmed in all five herbal extracts. Gallic acid was attendance in *Phyllanthus Emblica* dry fruit, *Ficus bengalensis* bark, *Ficus religiosa* bark. Rutin is detected only in *Ficus bengalensis* bark.

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