

DETECTION AND ESTIMATION OF RUTIN, QUERCETIN, GALLIC ACID AND MANGIFERIN IN MELIACEAE, PIPERACEAE, AND ROSACEAE FAMILY BY HPTLC TECHNIQUE

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Article Received on
01 December 2023,

Revised on 22 Dec. 2023,
Accepted on 11 Jan. 2024

DOI: 10.20959/wjpr20242-31071



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ABSTRACT

The prime aim of the study is to detect and estimate the flavonoids, and phenolic acids in commercial herbal raw materials namely *Azadiracta indica* flower, *Azadiracta indica* leaf, *Ocimum santum* leaf, *Melia composita* leaf, *Piper cubeba* bud, *Piper nigrum* bud, *Rosa damacena*, obtained from *Meliaceae*, *Piperaceae*, and *Rosaceae* species. 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 *Meliaceae*, *Piperaceae*, and *Rosaceae* species contains flavonoids and phenolic acids. The developed HPTLC method shows the presence of rutin in *Azadiracta indica* flower, *Azadiracta indica* leaf, *Melia composita* leaf, *Piper nigrum* bud and it was estimated as 0.56%, 0.37%, 0.18%, and 0.02%. Gallic acid was estimated in *Azadiracta indica* leaf, *Piper nigrum* bud, and *Rosa damacena*, as 0.02%, 0.03% and 0.86% respectively. Quercetin and Mangiferin was predictable in all the raw materials and estimated less than 1% respectively.

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

INTRODUCTION

Global health and medical practice seek to merge alternative medicine with evidence-based medicine for a better understanding of the metabolic process and its effects in the human body. *Azadirachta indica* (Neem) – *meliaceae* family, called as “The village pharmacy” or “Divine tree” because of its many health properties. In recent times, Neem-derived extracts have been shown to work from anywhere from insect repellent, to supplements to lower inflammation, diabetic control, and even to combat cancer.^[1] The drugs of this family majorly

used as cytotoxic activity and antimicrobial activity, insect anti-feedant, anti-malarial activity.^[2] Many species of Meliaceae family has been investigated for their phytochemical and pharmacological activities. Leaves of *Azadirachta* are used for Leprosy, Scrofula, diuretic, antilithic. Seeds are used for rheumatism. Leaf extracts showed the anti-insecticidal property. Oil extracted from this species has antiseptic properties. Oil is also used for fever and leprosy treatment.^[3] Several species of piperaceaea have great economic and cultural importance and are used as foods, medicines, stimulants, antiseptics and antioxidants.^[4] *Piper nigrum* is the most important cultivated species due to its economic value. Geographically, it is confined to Western-Ghats of South India. Piperine is an active component in *Piper nigrum* and contributes to its pungency. *Piper nigrum* is reputed in the local system of medicine of India, Latin America and West-Indies for its multidimensional medicinal properties. Secondary metabolites from *Piper nigrum* play defensive role against infections by microbes, insects and animals. Another important component of pepper volatile oil is pipene, which is a famous odorants. *Piper nigrum* is anti-microbial, anti-mutagenic, a free-radical scavenger, immuno-modulator, anti-tumor, anti-depressant, anti-apoptotic, anti-metastatic, anti-thyroid, hepatoprotective, immune stimulator, anti-diarrheal and anti-spasmodic.^[5] Rosaceae is one of the important families possessing a variety of diversified plant species. It includes many economically valuable crops that provide nutritional and health benefits for the human. Whole genome sequences of valuable crop plants were released in recent years. The primary aim of the study is to detect the flavonoids, phenolic acids and xanthones in seven effortlessly available commercial herbal raw materials. These herbal materials are in daily domestic needs as antioxidants, antidiabetic, antipyretic, ulcer healing, diuretic, anti-fertility and anti-inflammatory agent and dysentery, diarrhea, relieves morning sickness, nausea, eliminates bacteria, heals wounds, burns and reduces stress. The marker selected for detection of flavonoids and phenolic acids were quercetin, rutin, gallic acid and a xanthone Mangiferin. 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] 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.^[7] 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.^[8] 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.^[9] High performance thin layer chromatography (HPTLC) is a preferred analytical tool for fingerprints and quantification of marker compounds in herbal raw materials owing to its simplicity, high sensitivity, accuracy and less expensive.^[10, 11]

MATERIALS AND METHODS

Collection of herbal raw materials for HPTLC screening

Seven 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 seven herbal raw materials *Azadiracta indica* flower, *Azadiracta indica* leaf, *Ocimum santum* leaf, *Melia composita* leaf, *Piper cubeba* bud, *Piper nigrum* bud, *Rosa damacena*. 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, Quercetin, Gallic acid and 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 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 6 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 X 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. R_f 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.

- 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.

The optimized chamber saturation time for mobile phase was 10 min at room temperature ($25 \pm 1^\circ\text{C}$). The densitometric analysis was performed at 254 nm in reflectance mode. The Rf values of the marker compounds were in the range of 0.10 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).

Table 1: Rf values of standard markers in extracts of *Azadiracta indica* flower, *Azadiracta indica* leaf, *Ocimum santum* leaf, *Melia composita* leaf, *Piper cubeba* bud, *Piper nigrum* bud, *Rosa damacena*.

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 $\mu\text{g}/8\mu\text{l}$ of extracts/ 5 μl of standards	% of marker in Extracts
T-1	<i>Azadiracta indica</i> flower extract / 8 μl	0.02, 0.08, 0.17, 0.24 , 0.31, 0.36, 0.41, 0.50, 0.58, 0.69, 0.87	0.17	Rutin	10016.2	1.2520	0.56%
			0.24	Mangiferin	8917.2	1.1146	0.44%
			0.87	Quercetin	7869.5	0.9836	0.55%
T-2	<i>Azadiracta indica</i> leaf extract / 8 μl	0.18, 0.24 , 0.37, 0.42, 0.51, 0.58, 0.74, 0.84 , 0.86	0.18	Rutin	8116.6	1.0145	0.37%
			0.24	Mangiferin	3855.5	4.8193	0.82%
			0.74	Gallic acid	1357.8	0.1697	0.02%
			0.86	Quercetin	4408.7	0.5510	0.17%
T-3	extract <i>Ocimum santum</i> / 8 μl	0.01, 0.13, 0.23 , 0.44, 0.80, 0.86	0.23	Mangiferin	999.1	0.1248	0.006%
			0.86	Quercetin	3104.4	0.3880	0.09%
T-4	<i>Melia composita</i> extract / 8 μl	0.01, 0.07, 0.14, 0.22 , 0.30, 0.36, 0.38, 0.41, 0.51, 0.60, 0.86	0.14	Rutin	5717.2	0.7146	0.18%
			0.22	Mangiferin	6319.9	0.789	0.22%
			0.86	Quercetin	12776.1	1.5970	1.46%
T-5	<i>Piper cubeb</i> extract / 8 μl	0.05, 0.08, 0.22 , 0.80, 0.86 , 0.88, 0.92	0.22	Mangiferin	2019.2	0.2524	0.002%
			0.86	Quercetin	1475.7	0.1844	0.02%
T-6	<i>Piper nigrum</i> extract / 8 μl	0.05, 0.16 , 0.23 , 0.31, 0.41, 0.49, 0.73 , 0.80, 0.83, 0.87	0.16	Rutin	2148.6	0.2698	0.026%
			0.23	Mangiferin	2614.3	0.3267	0.038%
			0.73	Gallic acid	654.9	0.0818	0.0038%
			0.87	Quercetin	10837.0	1.353	1.05%
T-7	<i>Rosa damascena</i>	0.04, 0.25 , 0.35, 0.39,	0.25	Mangiferin	36945.5	4.618	1.01%
			0.76	Gallic acid	9818.8	1.2272	0.86%

	extract / 8µl	0.50, 0.57, 0.68, 0.76 , 0.82, 0.85	0.85	Quercetin	2196.2	0.2745	0.043%
T-8	Rutin/ 5µl	0.16			22061.8	5.0	100%
	Gallic acid/ 5µl	0.76			14005.9	5.0	100%
	Quercetin / 5µl	0.85			13951.8	5.0	100%
T-9	Mangiferin / 5µl	0.26			22415.6	5.0	100%

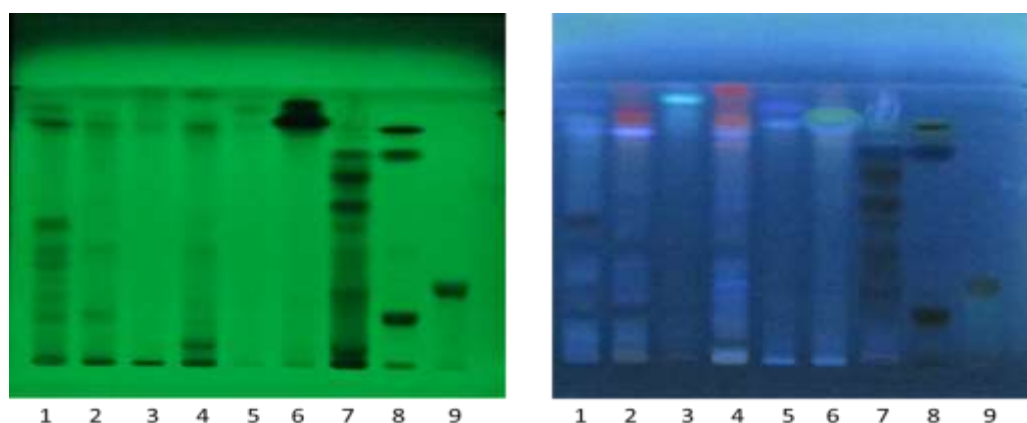


Figure 1: T-1 *Azadiracta indica* flower, T-2 *Azadiracta indica* leaf, T-3 *Ocimum santum* leaf, T-4 *Melia composita* leaf, T-5 *Piper cubeba* bud, T-6 *Piper nigrum* bud, T-7 *Rosa damacena*, T-8 Rutin, Gallic acid and Quercetin Markers, T-9 Mangiferin Marker.

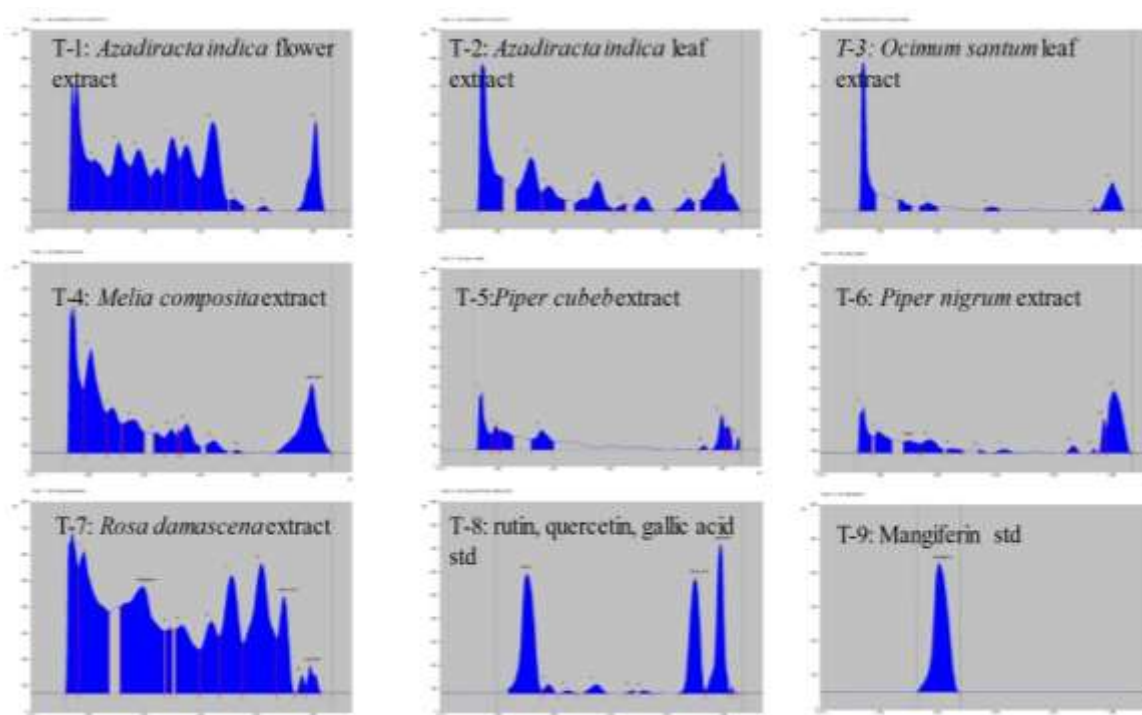


Figure 2: chromatogram of herbal raw materials and standard marker compounds.

CONCLUSION

From our findings we concluded that presence of rutin and quercetin was confirmed comparatively more in *Azadiracta indica* flower among the seven herbal raw materials. Mangiferin was maximum in *Azadiracta indica* leaf among the seven raw materials. Gallic acid was present only in *Piper nigrum* bud, *Rosa damacena*, and *Azadiracta indica* leaf extract. From the literature cited, the marketed herbal raw materials. *Azadiracta indica* flower, *Azadiracta indica* leaf, *Ocimum santum* leaf, *Melia composita* leaf, *Piper cubeba* bud, *Piper nigrum* bud, *Rosa damacena*, were used for the treatment of various conditions, and in our findings the action recognized may be due to presence of flavonoids, phenolic acids and xanthones.

REFERENCE

1. Jose Francisco Islas, Zuca G-Buentello, An overview of Neem (*Azadirachta indica*) and its potential impact on health, journal of functional foods, November 2020; 74.
2. Rashmi Yadav, Akshada Pednekar, Amruta Avalaskar, Madhuri Rathi, Yogesh Rewachandani, A comprehensive review on Meliaceae family, World Journal of Pharmaceutical Sciences, 3(8): 1572-1577.
3. Muzafer Hussain Dar¹, S.D. Singh, Taxonomy and Medicinal uses of Meliaceae Family at District Bhopal, Research & Reviews: A Journal of Life Sciences, 2019; 9(3).
4. A.K. Meena ¹, M.M. Rao, V. Nageswara Rao, Komalpreet, M.M. Padhi and Ramesh Babu, Comparative Study of Various Plants of Piperaceae Family Commonly Used in Ayurvedic Formulations, Research Journal of Pharmacognosy and Phytochemistry. 2(5): Sept.-Oct. 2010, 407-410.
5. Hussein, G., Miyashiro, H., Nakamura, N., Hattori, M., Kakiuchi, N., Shimotohno, K., Inhibitory effects of Sudanese medicinal plant extracts on hepatitis C virus (HCV) protease. Phytotherapy research, 2000; 14: 510-516.
6. Pietta, PG. Flavonoids as antioxidants. Journal of natural products, 2000; 63(7): 1035– 42.
7. Jiayu Gao, Jiangxia Hu, Dongyi Hu, and Xiao Yang. A Role of Gallic Acid in Oxidative Damage Diseases: A Comprehensive Review Natural Product Communications, 2019; 1– 9. 4. Yao Li et al Quercetin, Inflammation and Immunity Nutrients, 2016; 8: 167.
8. Jyotshna Puja Khare and Karuna Shanker Mangiferin: A review of sources and interventions for biological activities, 2016; 42(5): 504–514.

9. Harish Chandra Andola, Vijay Kant Purohit. High Performance Thin Layer Chromatography (HPTLC): A Modern Analytical tool for Biological Analysis. *Nature and Science*, 2010; 8(10): 58-61.
10. Meier B, Spriano D. Modern HPTLC--a perfect tool for quality control of herbals and their preparations. *J AOAC Int*, 2010; 93(5): 1399-409.
11. Arivukkarasu R, and Rajasekaran A. Detection of flavonoids, phenolic acids and xanthonones in commercial herbal formulations by HPTLC technique. *Research Journal of Pharmacognosy and phytochemistry*, 2015; 7(1): 13-27.