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Research Article

DETECTION AND ESTIMATION OF FLAVONOIDS PHENOLIC ACIDS AND XANTHONE IN HERBAL RAW MATERIALS BELONGS FAMILY OF CAESALPINACEAE, ACANTHACEAE, MENISPERMACEAE BY HPTLC TECHNIQUE

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ABSTRACT:

The prime aim of the study is to observe the flavonoids phenolic acid and xanthone in six commercial herbal raw materials namely Cassia lanceolata (Caesalpinaceae), Cassia auriculata flower (Caesalpinaceae), Cassia auriculata bark (Caesalpinaceae), Andrographis paniculata (Acanthaceae), Adhatoda vasica (Acanthaceae), Pachygone buxifolia (Menispermaceae). family respectively used in daily domestic needs to confirm the presence of common antioxidant secondary metabolites in herbal raw materials. Results of the study clearly revealed that this raw materials from the above family contains flavonoids and phenolic acids. The developed simultaneous HPTLC method can be employed for the routine investigations of flavonoids and phenolic acids in herbal raw materials and sample. The above-mentioned raw materials are procured from authenticated country shop.In Cassia lanceolata both quercetin & magniferin is absent. Cassia auriculata flower extract contains quercetin-0.49%, magniferin-0.04%. Cassia auriculata bark extract contains quercetin-1.38%, magniferin-0.06%. Andrographis paniculata extract contains quercetin-0.48%, magniferin-0.19%. Adhatoda vasica extract contain quercetin-0.57%, magniferin-0.08%. Pachygone buxifolia contain only quercetin-0.36%. In conclusion, both quercetin & magniferin present in Cassia auriculata flower, Cassia auriculata bark, Andrographis paniculata, Adhatoda vasica.In Cassia lanceolata both quercetin & magniferin is absent. Pachygone buxifolia contain only quercetin. Quercetin is present in maximum number of herbal extracts hence it possesses anti-oxidant property.

Key words: Cassia lanceolata, Cassia auriculata flower & bark. Andrographis paniculata, Adhatoda vasica, pachygone buxifolia, HPTLC.

INTRODUCTION

The World Health Organization (WHO) estimates that about 80% of people living in developing countries rely exclusively on traditional medicines for their primary health care need. India is virtually a herbarium of the world, using plants and herbs as the basic source of medicine. Herbals which form a part of our nutrition and provide us an additional therapeutic effect are in demand and Cassia species is one of such plant. Cassia species (Caesalpinaceae) are well known medicinal plant commonly found in India and other tropical countries. The extract of Cassia species leaves has been found to possess significant hepatoprotective activity and anti-inflammatory activity [1]. Cassia auriculata (Caesalpinaceae), Linn commonly known as Tanners Senna, is also known as Avaram tree. The Cassia flower is bright yellow, bisexual, with irregular petals, 10 anthers (three sterile), a superior unilocular ovary, and marginal ovules [2]. Chemical constituents such as protein, carbohydrate, alkaloids, flavonoids and tannin [3]. Bark of C. auriculata (Caesalpinaceae), the chemical investigation of the stem

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bark of the plant yielded two new triterpenoid glycosides [5]. C. auriculata is a shrub that is used as 'Avarai Panchaga Choornam' (mixture of five parts of the shrub i.e. roots, leaves, flowers, bark and unripe fruits) which establishes good control on sugar levels [4]. Andrographis paniculata Acanthaceae, is a plant that has been effectively used in traditional Asian medicines for centuries. Its perceived "blood purifying" property results in its use in diseases where blood "abnormalities" are considered causes of disease, such as skin eruptions, boils, scabies, and chronic undeter mined fevers, A. paniculata contains diterpenes, lactones, and flavonoids. Flavonoids mainly exist in the root, but have also been isolated from the leaves [6]. Adhatoda vasica Nees belonging to family Acanthaceae, commonly known as Adosa, is a small, evergreen shrub found many regions of India and throughout the world, with a multitude of uses in traditional Ayurveda. The vast variety of pharmacological uses of Adhatoda is believed to be the result of its rich concentration of alkaloids. The prominent alkaloid found in Adhatoda leaves is the quinazoline alkaloid known as vasicine [8]. Mangiferin and its derived lead molecule have proven its effectiveness as an antioxidant, analgesic, antidiabetic, antiproliferative, chemopreventive, radioprotective, cardiotonic, immunomodulatory and diuretic.[9]

MATERIALS AND METHODS:

Collection of herbal raw materials for HPTLC screening:

Raw materials of Cassia lanceolata (Caesalpinaceae), Cassia auriculata flower (Caesalpinaceae), Cassia auriculata bark (Caesalpinaceae), Andrographis paniculata (Acanthaceae), Adhatoda vasica (Acanthaceae), Pachygone buxifolia (Menispermaceae) procured from authenticated country shop.

Instruments:

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, catechin, magniferin, 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 standards and extracts from the herbal raw materials:

One gram of each dried herbal 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 raw material herbal sample solutions were spotted in the form of bands of width 6 mm with a Hamilton 100 µl 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 5mm × 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 withthe 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.

Table 1: Rf values of standard markers in extracts of Cassia lanceolata leaf extract , Cassia auriculata flower extract,Cassia auriculata bark extract,Andrographis paniculata extract,Adhatoda vasica extract, Pachygone buxifolia extract

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	Cassia lanceolata leaf ext	0.07, 0.09, 0.16, 0.22, 0.27, 0.35, 0.44.0.54,0.87.0.89	-				
T – 2	Cassia auriculata Flower Ext	0.10, 0.19, 0.33, 0.39,0.69, 0.85	0.84	Quercetin	10429.0	3.9630	0.4953%
T – 3	*Cassia auriculata* Bark Ext	0.07, 0.10, 0.19, 0.26, 0.32, 0.37, 0.46,0.53,0.84	0.84	Quercetin	29071.6	11.0472	1.3809%
T-4	Andrographis paniculata	0.04, 0.07, 0.09, 0.16, 0.24, 0.34, 0.42, 0.50,0.56,0.67,0.73,0.80,0.83	0.84	Quercetin	10272.1	3.9033	0.4879%
			0.32	Mangiferin	10657.7	1.5347	0.1918
T – 5	Adhatoda vasica	0.08, 0.10, 0.19, 0.25, 0.30,0.36,0.46,0.59,0.63, 0.65, 0.71, 0.84,	0.32	Mangiferin	4451.6	0.6410	0.0801
T – 6	Pachygone buxifolia	0.20,0.27,0.37,0.44,0.69, 0.84,	0.84	Quercetin	7675.6	2.9167	0.3645%
T – 7	Reference marker Quercetin Rutin Gallic Acid	0.20,0.75,0.84	0.84	Quercetin	12928.1	5µg	100%
			0.75	Gallic acid	14320.7	5µg	100%
			0.20	Rutin	26135.1	5µg	100%
T-8	Reference marker catechin	0.74	0.74	Catechin	22341.0	5µg	100%
T-9	Reference marker Mangiferin	0.32	0.32	mangiferin	34525.5	5µg	100%

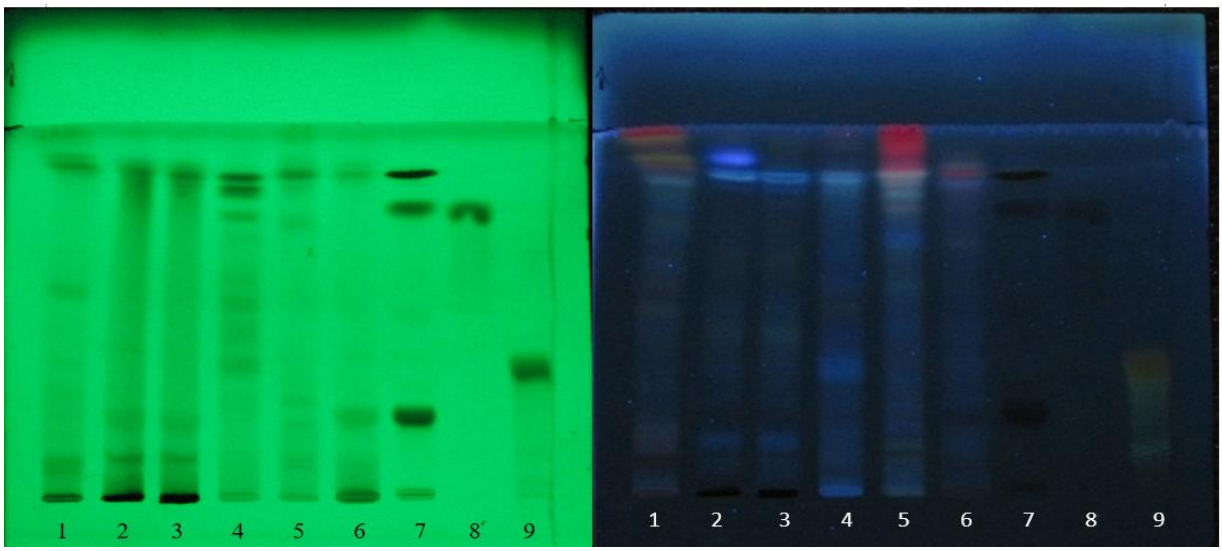

Figure 1: 1. Cassia lanceolata leaf extract 2. Cassia auriculata flower extract 3. Cassia auriculata bark extract 4. Andrographis paniculata 5. Adhatoda vasica 6. Pachygone buxifolia 7. Quercetin rutin gallic acid 8. Catechin 9. Mangiferin

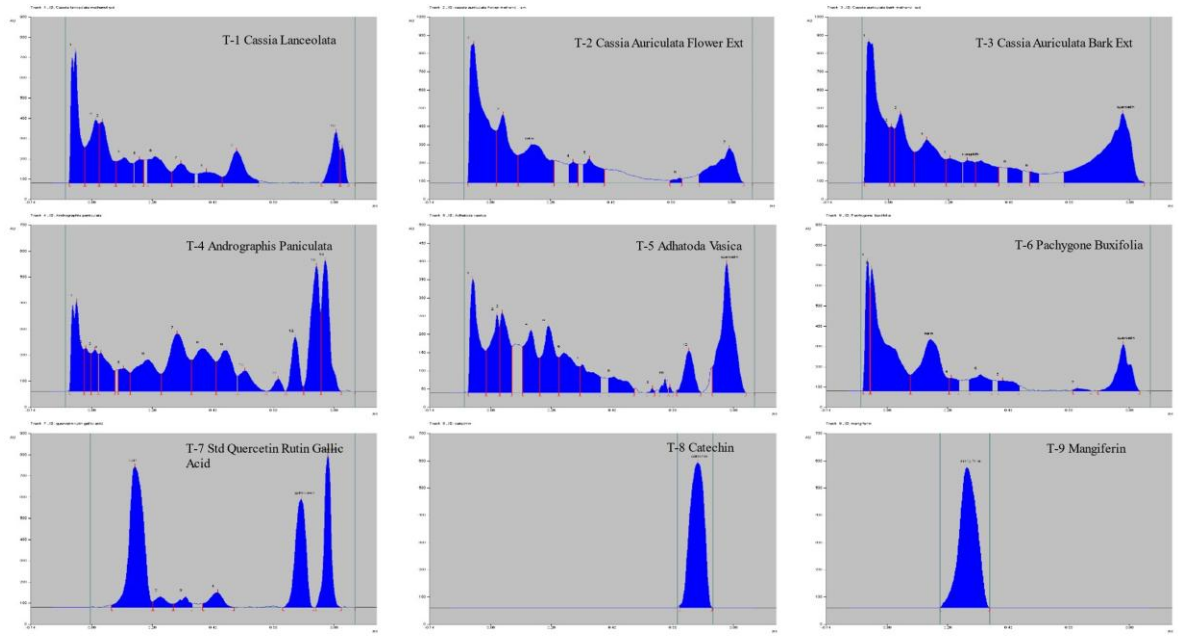

Figure 2: Chromatogram of 1. Cassia lanceolata 2. Cassia auriculata flower extraxt 3. Cassia auriculata bark extract 4. Andrographis paniculata 5. Adhatoda vasica 6. Pachygone buxifolia 7. Std quercetin rutin gallic acid 8. Catechin 9. Mangiferin.

RESULTS AND DISCUSSION:

The following different solvent[10,11] compositions were tried for monitor the evaluation of components in herbal extracts Ethyal acetate: galacial 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 extracts tested and hence it was hence it was used as mobile phase for detection of constituents in herbal extracts. The optimized chamber saturation time for mobile phase was 10 min at a room temperature (25+/- 1°C). The densitometric analysis was performed at 254 nm in reflectance mode. The Rf value of the bio marker compounds range of 0.09 to 0.85. (table 1) the detection and quantity of marker in herbal raw material and sample extracts were given in Table 1. the identity of compounds in herbal extracts was ascertained by chromatogram. From the table 1 results understanding reveal, Cassia lanceolata leaf extract doesn't contain both quercetin and magniferin. Cassia auriculata flower extract contains quercetin-0.49% & magniferin-0.04%. Cassia auriculata bark extract contains quercetin-1.38% & magniferin-0.06%. Andrographis paniculata contains quercetin-0.48% & magniferin-0.19%. Adhatoda vasica contains quercetin-0.57% & magniferin-0.08%. Pachygone buxifolia contains only quercetin-0.36% & not magniferin. In conclusion the two anti-oxidant markers quercetin and magniferin were found in Cassia auriculata flower extract, Cassia auriculata bark extract, Andrographis paniculata, Adhatoda vasica. phagygone buxifolia contains only one anti-oxidant marker quercetin. Cassia lanceolata leaf extract doesn't contain any anti-oxidant markers. Further quercetin a naturally non-toxic flavonoid within the safe dose range with antioxidant, anti-apoptotic and anti-inflammatory properties, plays an important role in the treatment of aging-related diseases. so we confirm the therapeutic activity may due the presence of anti-oxidant marker quercetin in six selected herbal raw materials and samples.

CONCLUSION:

The findings can be concluded that flavonoids a phenolic acids were detected in the six herbal raw material and sample extracts. In conclusion the antioxidant marker quercetin & magniferin were found in Cassia auriculata leaf extract,Cassia auriculata bark extract, Andrographis paniculata, adhatoda vasica. Pachygone buxifolia contains only quercetin. Cassia lanceolata doesn't contain any anti-oxidant marker.The developed HPTLC method may be adopted for routine detection and estimation of flavonoidsand phenolic acids in the six marketed herbal raw materials and sample used in various herbal formulations.

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