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Research Article

ESTIMATION OF ANTI-OXIDANT MARKERS IN TRIPHALA TABLET AND TRIPHALA CHOORNAM BY HPTLC TECHNIQUE

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ABSTRACT:

The study aims to analyse flavonoids and phenolic acids in two indigenous herbal formulations: marketed formulation tablet, marketed formulation choornam, these formulations, commonly used in daily domestic needs, were examined to confirm the presence of antioxidant secondary metabolites in marketed formulations. Results revealed that both marketed formulation choornamontain flavonoids and phenolic acids. The developed simultaneous HPTLC method can be employed for routine investigations. The formulations were procured from a drug store and analysed for Quercetin, Rutin, Gallic acid, Tannic acid, Ellagic acid, Catechin, and Vitexin. In marketed formulation tablet, Quercetin (0.1617%), Rutin (0.41%), Gallic acid (1.60%), Tannic acid (0.51%), Ellagic acid (2.27%), Catechin (0.26%), and Vitexin (0.66%) were detected. In marketed formulation choornam, Quercetin (0.0008%), Rutin (0.85%), Gallic acid (1.0%), Tannic acid (1.06%), Ellagic acid (4.73%), and Catechin (0.56%) were found, but Vitexin was absent. In conclusion, key antioxidant markers—Tannic acid, Gallic acid, and Catechin—were present in both formulations. Marketed formulation tablet contained all tested compounds, whereas marketed formulation choornam lacked Vitexin. These findings confirm the presence of essential antioxidant constituents in both marketed formulations, reinforcing their therapeutic potential.

Keywords: Marketed formulation tablet, marketed formulation choornam, gallic acid, tannic acid, catechin, HPTLC.

INTRODUCTION

In ayurveda, Triphala is a well-known poly herbal formulation. In Indian system of medicine (ISM) it is a rasayana drug. Triphala is one among the ayurvedic medicinal herbal formulation mostly preferred by medical practicioners[1]. Triphala (Sanskrit; tri = three and phala = fruits) is a well-recognized and revered polyherbal medicine consisting of dried fruits of the three plant species Emblica officinalis (Euphorbiaceae), Terminalia bellerica (Combretaceae), and Terminalia chebula (Combretaceae) that are native to the Indian subcontinent. It is classified as a tridoshic rasayana in Ayurvedic medicine as it promotes longevity and rejuvenation in patients of all constitutions and ages. The formula consists of the fruits Amalaki or the Indian Gooseberry, Bibhitaki, and Haritaki of the three plants generally in equal proportions and has been used in traditional medicine in India for over 1000 years according to the writings of the great physician Charak in a foundational text of Ayurveda called the Charaka Samhita as well as in another key text called the Sushruta Samhita [2]. Triphala is an antioxidant-rich herbal formulation, frequently used for immune system stimulation, improvement of digestion, relief of constipation, gastrointestinal tract cleansing, relief of gas, treatment of diabetes, eye disease, anemia, jaundice, asthma, fever, chronic ulcers etc [3]. Amla fruits are globose, fleshy, pale yellow with six obscure vertical furrows enclosing six trigonous seeds in two seeded three crustaceous cocci. Is highly nutritious and is one of the richest sources of vitamin C, amino acids and minerals. It contains several chemical constituents like tannins, alkaloids and phenols. Among all hydrolysable tannins, Emblica nin A and B; Gallic acid, Ellagic acid

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are reported to possess biological activity. T. Chebula contains 32% of tannin [4]. T. Chebula the fruit are yellowish brown in colour on ripe when unripe the fruit is green, 20 to 25 mm long and 15 to 25 mm wide in size. The fruit is Ovate and wrinkled longitudinally in shape, they contain 14 components of hydrolysable tannins (Gallic acid, chebulic acid, punicalagin, chebulanin, corilagin, neochebulinic, Ellagic acid, chebulegic acid, chebulinic acid, 1,2,3,4,6- penta-Ogalloyl-ß-D-glucose, 1,6, -diogalloyl-D-glucose, casuarinin, 3,4,6- tri-O-galloyl-D-glucose and terchebulin). The tannin content varies with the geological variation. Flavonol glycosides, triterpenoids, coumarin conjugated with Gallic acid called chebulin, as well as phenolic compounds were also isolated. In addition, ethyl gallate and luteolin were isolated from the fruit of T. Chebula.[5] Terminalia Bellerica is light yellow in colour. It is drupe, globose or ovoid, densely velutinous or sericeous, 2-4 x 1.8-2.2 cm. It is slightly 5 ridged, 3cm across. It is one seeded and covered with minute pale pubescence. It contains flavone, steroids, lignans, tannins, glycoside, terpenoids, saponin, cardenolide, fatty acids [6]. Methanolic extract of Chebula shows best anit- bacterial activity against E. coli [7]. Quercetin has been reported to inhibit the allergic and inflammatory responses of the immune system [8] by modulating several aspects of cell function relevant to inflammatory arthritis. At the molecular level, Quercetin is known to inhibit nuclear factor kappa B (NF-kb), a central transcription factor in-flammatory and proliferative diseases [9]. Quercetin inhibits inflammatory aspects of synovial cell function, neutrophil activation and hence Quercetin could be an effective anti-arthritic agent. Caffeic acid and Quercetin, the well-known phenolic compounds widely present in the plant kingdom, were investigated for their possible protective effects against paracetamol and ccl4-induced hepatic damage. Paracetamol at the oral dose of 1 g/kg produced 100% mortality in mice while pretreatment of separate groups of animals with Caffeic acid (6 mg/kg) and Quercetin (10 mg/kg) reduced the death rate to 20% and 30%, respectively [10]. Cowan, 1999 [11] explained that, tannin is a general descriptive name for a group of Phyto constituents of polymeric phenolic substances. Many physiological activities such as stimulation of phagocytic cells, host mediated tumour activity and wide range of anti – infective actions have been assigned to tannins. This mode of antimicrobial action may be related to their ability to inactivate microbial enzymes and transport proteins. Tannic acid is commercial form of tannins, a type of poly phenol. It has been described as having antimutagenic, anticarcinogenic and antioxidant activities. It may also be found on fruits of many plants [12]. Gallic acid is phenyl propanoid, chemically it is 3, 4, 5, - Tri hydroxybenzoic acid, and possess astringent activity [13]. It is commonly used in pharmaceutical industry. It seems to have antifungal, antiviral and antioxidant activities. Yoshimura et al., 2005, [14] has stated that, Ellagic acid is a natural phenol found in numerous fruits. It is a di lactone of hexahydroxydiphanic acid. It has been found to have anticarcinogenic, anti-fibrosis and antioxidant activities. It has a high affinity for copper at the active site of tyrosinase and inhibits its activity. Kaempferol is a common type of dietary flavonoid with anti-oxidative and anti-inflammatory properties. Studies also indicated that KP decreased lipopolysaccharide (LPS)-induced tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1) expression by increasing the number of activated macrophages; suppres1sion of TNF-α mediates the translocation of NF-κb p65 to the nucleus [15].No HPTLC method is reported in the literature for detection and estimation of well-known free radical scavengers Rutin, Quercetin and Gallic acid in market herbal anti-inflammatory, antarthritic formulations and hence this paper describes the same.

MATERIALS AND METHODS:

Collection of marketed formulations for HPTLC screening:

The tablet and choornam marketed formulations were procured from authenticated drug store. Marketed formulation choornam - each 5gm contain 1.66gm Emblica officinalis, Terminalia chebula, Terminalia belirica. Marketed formulation tablet contains extracts of triphala 250mg.

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 MNC tablet formulations.

Chemicals and solvents:

Quercetin, Rutin, Gallic acid, caffeic acid, Tannic acid, Ellagic acid, Catechin, Kaempferol, vitexin, 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 marketed 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 marketed formulations solutions were spotted in the form of bands of width 6 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 5mm × 0.45 mm. Three µl of each MNC tablet formulation and three 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 standard markers in extracts of marketed formulation 1& marketed formulation 2.

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	Marketed formulation tablet	0.01,0.08,**0.18**,0.22, 0.29,**0.34**,0.41,**0.52**, 0.60,0.69,**0.77**,**0.81**, **0.86**.	0.86	Quercetin	2527.2	0.8087	0.1617%
			0.18	Rutin	18656.0	2.0521	0.4104%
			0.77	Gallic acid	30791.9	8.0058	1.6011%
			0.34	Tannic acid	28499.3	2.5649	0.5129%
			0.81	Catechin	5347.6	1.3369	0.2673%
			0.34	Ellagic acid	28499.3	11.3997	2.2799%
			0.52	Vitexin	7899.4	3.3177	0.6635%
T-2	Marketed formulation choornam	0.01,0.07,**0.16**,0.21, 0.30,**0.37**,0.60,0.68, **0.76**,**0.81**,**0.86**.	0.86	Quercetin	1253.2	0.4041	0.0008%
			0.16	Rutin	38793.3	4.2672	0.8534%
			0.76	Gallic acid	19321.0	5.0234	1.0046%
			0.37	Tannic acid	59170.8	5.3253	1.0650%
			0.81	Catechin	8330.5	2.0826	0.5605%
			0.37	Ellagic acid	59170.8	23.6683	4.7336%
T-3	Quercetin	0.85			15528.0	5µl	100%
	Rutin	0.18			43717.9	5µl	100%
	Gallic acid	0.76			19036.1	5µl	100%
T-4	Caffeic acid					5µl	100%
T-5	Tannic acid	0.35			54279.3	5µl	100%
T-6	Ellagic acid	0.37			12316.7	5µl	100%
T-7	Catechin	0.83			19300.4	5µl	100%
T-8	Kaempferol	0.89			7742.6	5µl	100%
T-9	vitexin	0.51			11841.8	5µl	100%

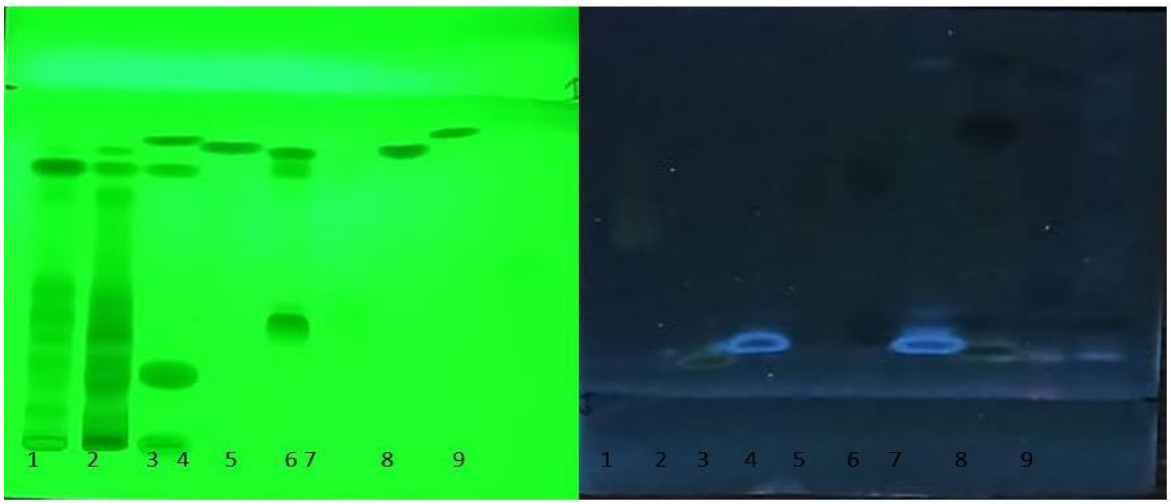

Figure 1: 1- Marketed formulation tablet, 2- Marketed formulation choornam, 3-QRG, 4-Caffic Acid, 5- Tannic Acid, 6- Ellagic Acid, 7- Catechin, 8- Kaempferol, 9-Vitexin

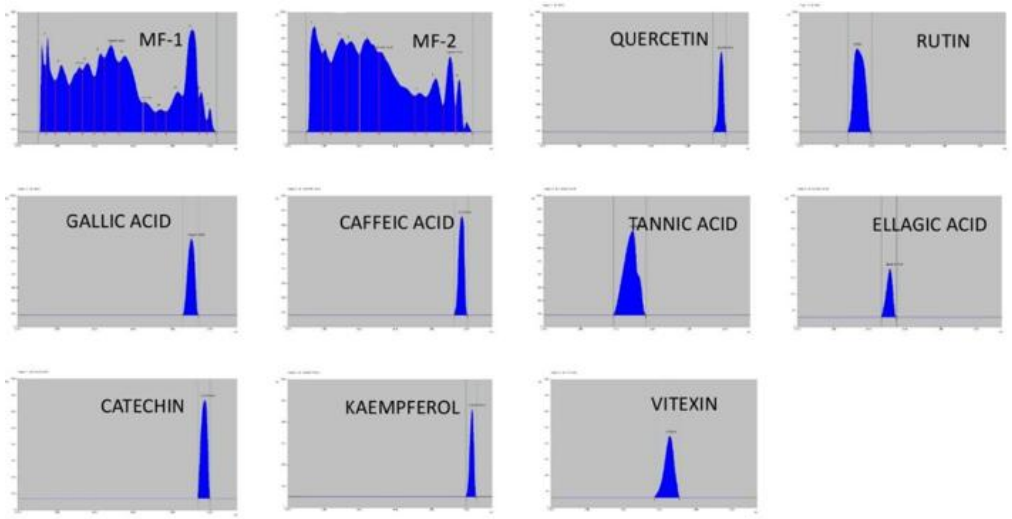

Figure 2: Chromatogram of 1- Marketed formulation tablet, 2- Marketed formulation choornam, 3-QRG, 4-Caffic Acid, 5- Tannic Acid, 6- Ellagic Acid, 7- Catechin, 8- Kaempferol, 9-Vitexin

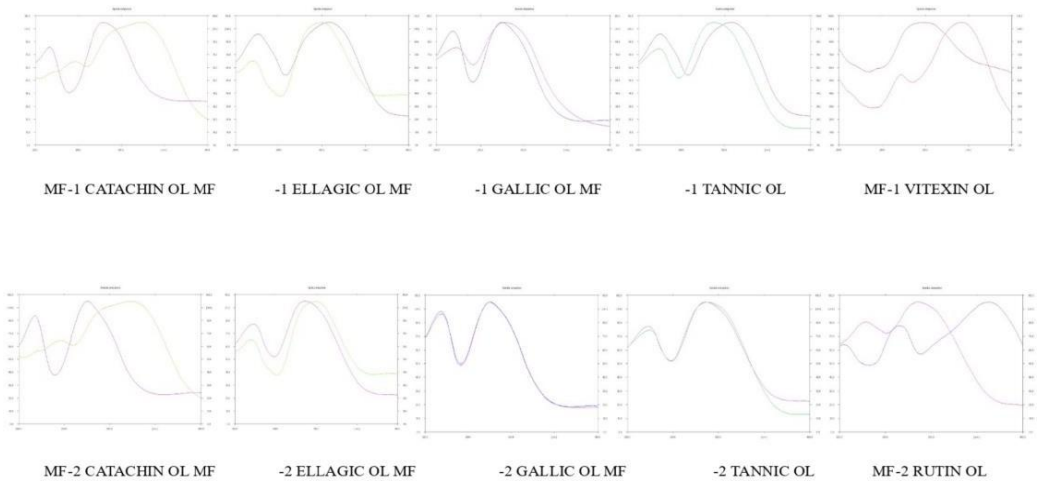

Figure 3: Overlay of 1- Marketed formulation tablet, 2- Marketed formulation choornam, 3-QRG, 4-Caffic Acid, 5- Tannic Acid, 6- Ellagic Acid, 7- Catechin, 8- Kaempferol, 9-Vitexin

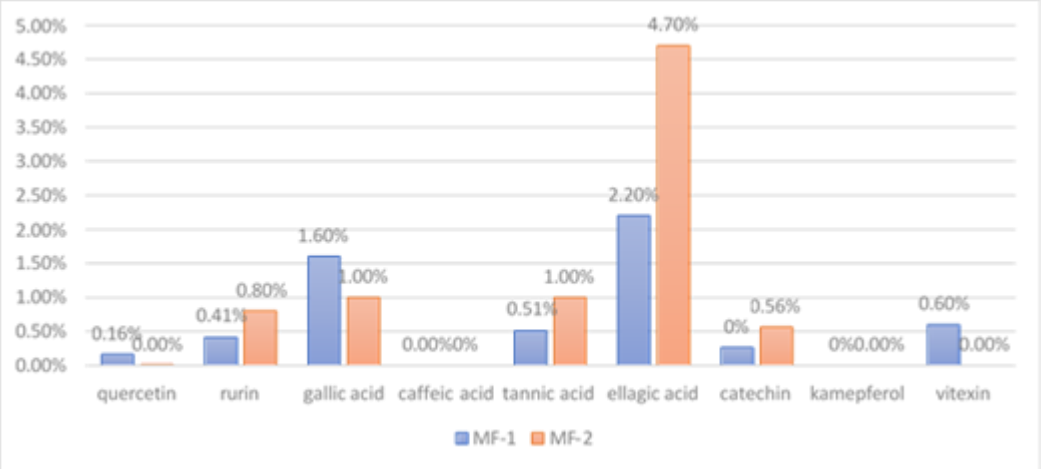

Figure 4: percentage of quercetin, rutin, gallic acid, caffeic acid, tannic acid, ellagic acid, catechin, kaempferol, vitexin in present in MF-1, MF-2

Result:

In marketed formulation tablet quercetin was found to be 0.1617%, In marketed formulation choornam Quercetin was found to be 0.0008%. In marketed formulation tablet rutin was found to be 0.41%,%,In marketed formulation choornam rutin was found to be 0.85%. In marketed formulation tablet gallic acid was found to be 1.60% In marketed formulation choornam gallic acid was found to be 1.0%. In marketed formulation tablet tannic acid was found to be 0.51%, In marketed formulation choornam tannic acid was found to be 1.06%. In marketed formulation tablet ellagic acid was found to be 2.27%, In marketed formulation choornam ellagic acid was found to be 4.73%. In marketed formulation tablet catechin was found to be 0.26%, In marketed formulation choornam catechin was found to be 0.56%, Vitexin was found only in Marketed formulation tablet 0.66%. In conclusion the antioxidant marker Tannic acid, Gallic acid, Catechin was found in the both marketed formulations, Marketed formulation tablet & Marketed formulation choornam. In marketed formulation tablet Quercetin, Rutin, gallic acid, tannic acid, ellagic acid, catechin, vitexin are all found. In marketed formulation choornam except vitexin all the other compounds are found.

Conclusion:

In conclusion the antioxidant marker Tannic acid, Gallic acid, Catechin was found in the both marketed formulations, Marketed formulation tablet & Marketed formulation choornam. In marketed formulation tablet Quercetin, Rutin, gallic acid, tannic acid, ellagic acid, catechin, vitexin are all found. In marketed formulation choornam except vitexin all the other compounds are found.

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