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Simultaneous Estimation of Naturally Occurring Flavonoids by RP-HPLC Method in Dasmoola Churna

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ABSTRACT:

Flavonoids are pivotal in plant biochemistry and physiology, contributing significantly to the biological activities of plants. Flavonoids are polyphenolic compounds recognized for their antioxidant properties and numerous health benefits. This study aimed to evaluate and quantify the flavonoids catechin, mangiferin, scopoletin, rutin, ellagic acid, baicalein, quercetin, naringenin, kaempferol, chrysin in dasmoola root. High-Performance Liquid Chromatography (HPLC) was used for the simultaneous estimation of flavonoids utilizing a reverse-phase column Optimix C8/C18 with a mobile phase A consisting of Buffer Solution (10 ml of Orthophosphoric into 1000 ml water): Methanol: Acetonitrile in 80: 10: 10 and mobile phase B consisting of 50 :20: 30 ratios at a flow rate of 1 mL/min, with detection at 323 nm. The standard retention times for catechin, mangiferin, scopoletin, rutin, ellagic acid, baicalein, quercetin, naringenin, kaempferol, chrysin were 7.09, 9.52, 21.0, 21.89, 22.64, 25.03, 28.24, 31.43, 34.64, and 37.14 respectively. In the root extract catechin, mangiferin, scopoletin, rutin, ellagic acid, baicalein, quercetin, naringenin, kaempferol, chrysin were detected. These findings confirm the presence of significant flavonoids with potential health benefits in Dasmoola Churna.

Keywords: Dasmoola Churna, flavonoids, RP-HPLC, simultaneous estimation

INTRODUCTION:

The role of flavonoids in enhancing the bioavailability of Dasmoola, a traditional Ayurvedic formulation composed of ten medicinal roots such as Bilva, Gambhari, Patala, Shalaparni, Gokhru, Agnimantha, Shyonaka, Brihati, Kantakari, and Prishnaparni. Flavonoids are naturally occurring compounds found in various plants, and their ability to modulate cytochrome P450 (CYP450) enzymes, particularly CYP3A4, plays a crucial role in influencing drug metabolism, absorption, and overall efficacy, thereby highlighting their importance in therapeutic contexts. Dasmoola may enhance the bioavailability of its active compounds by improving their solubility, stability, and absorption in the body, thereby potentially maximizing therapeutic outcomes [1]. The modulation of CYP450 activity by flavonoids is particularly notable as it can lead to increased plasma concentrations of co-administered drugs, which may improve their clinical effectiveness [2].

Dasamkla, commonly referred to as Dashamoola, is a significant Ayurvedic formulation composed of ten medicinal roots, each contributing unique therapeutic properties to the blend. This classical formulation is recognized for its efficacy in treating a variety of health concerns, particularly those associated with the nervous system, musculoskeletal system, and digestion [3]. The term "Dashamoola" derives from the Sanskrit words "Dasha," meaning ten, and "moola," meaning root [4].

The formulation Dashamoola consists of ten specific roots, categorized into two groups: Brihat Panchamoola (five large roots) Bilva (*Aegle marmelos*), Arani (*Premna serratifolia*), Gambhari (*Gmelina arborea*), Shyonaka (*Oroxylum indicum*), Patala (*Stereospermum suaveolens*), and Laghu Panchamoola (five small roots) Brihati (*Solanum indicum*), Shalaparni (*Desmodium gangeticum*), Kantakari (*Solanum xanthocarpum*), Gokhru (*Tribulus terrestris*), and Prishnaparni (*Uraria picta*) [5]. These roots work synergistically to promote overall health and well-being, addressing various ailments, particularly those related to Vata dosha, such as inflammation, pain, and respiratory issues [6].

The formulation of Dasmoola Churna is not only rooted in traditional practices but is also backed by modern studies highlighting the presence of significant flavonoids and other beneficial compounds. Flavonoids contribute to the antioxidant properties of Dasmoola, potentially lowering the risk of chronic diseases and enhancing overall health [7][8]. This combination has been effectively used for treating respiratory ailments, joint pain, fevers, and digestive disorders, showcasing its holistic approach to wellness in Ayurveda [9][10]. The preparation method involves washing, drying, and grinding the roots into a fine powder, which ensures that the active ingredients remain intact and effective for medicinal use [11]. This careful process of formulation is crucial in preserving the therapeutic potential of each root, contributing to the overall efficacy of Dasmoola Churna in promoting health and vitality [12]. Health Benefits of Flavonoids Flavonoids, a diverse group of phytochemicals found in various fruits, vegetables, and plants, are well

recognized for their numerous health benefits. Research has demonstrated that these compounds possess antioxidant, anti-inflammatory, and anti-cancer properties, making them crucial in reducing the risk of chronic diseases such as cardiovascular ailments and certain cancers [13].

METHODOLOGY

Collection and Authentication of Plant Material:

Ten plants part of Dasmoola roots were collected during February 2024 and was authenticated by Dr. Nita Raval, M.D., Dravya Guna from the State Model Institute of Ayurveda Sciences Kolavada, Gandhinagar, Gujarat, India. Roots of dasmoola were further identified its Microscopical studies. The voucher specimen KB/24/0001 to 0010, and they were deposited in K. B. Institute of Pharmaceutical Education and Research, Gandhinagar, Gujarat, India.

Preparation of Dasmoola churna (In-house)

As per the procedure outlined in the Ayurvedic formulary of India, in house preparation of churna was done. All the ingredients viz., Bilva - Aegle marmelos, Arani - *Premna serratifolia*,

Gambhari - *Gmelina arborea*, Shyonaka - *Oroxylum indicum*, Patala - *Stereospermum suaveolens*, Brihati - *Solanum indicum*, Shalaparni - *Desmodium gangeticum*, Kantakari - *Solanum xanthocarpum*, Gokhru - *Tribulus terrestris*, Prishnaparni - *Uraria* were individually grinded, sieved through 80# screen and then blended in proportions as specified in Ayurvedic formulary of India.

Preparation of Extract:

The selected plant parts of Dasmoola roots were separated and dried under sunlight. Dried powdered passed through a sieve of 60 mesh (#) size and stored in airtight containers and then used for present work. The shade-dried powder of dasmoola roots were extracted with alcohol (methanol), water and Hydroalcoholic (50:50) (methanol: water). The extraction was carried out by Soxhlet assembly for 6-8 h. Then the solvent was filtered and repeat the process for three times in the same manner. The extracts were concentrated and dried under a controlled temp of 60 ºC on a water bath and reported the % yield. Dried extract of the dasmoola roots were used for further investigation.

For microscopical studies free hand section of the dasmoola roots were taken, and performed as per Schulz maceration method [14],[15],[16]. Photomicrographs were shot for histological observation (Labomed) of ten roots of dasmoola were carried out by using standard methods [17]. The freshly prepared root extracts of dasmoola were quantitatively and qualitatively tested for the presence of chemical constituents and these constituents were identified by characteristic color changes as described by the standardized procedures [18], [19], [20].

Estimation of Flavonoids:

Total flavonoid content was determined as per Aluminium chloride method by using Rutin as a standard [21], [22].

Chemicals and reagents

Rutin was purchased from Yucca enterprises (Mumbai, India). Sodium nitrite (5%), Aluminium chloride (10%), Sodium hydroxide (1M) were prepared with material procured from S.D. Fine Chemicals Pvt. Ltd., Mumbai.

Preparation of standard solution

A solution of Rutin (100 µg/mL) was made by dissolving 10 mg of rutin in a 100 mL volumetric flask using alcohol as the solvent. From the standard stock solution, aliquots of 2 -10 mL were pippeted out in volumetric flask (10 mL) and the final volume was made with alcohol to get concentration of 20-100µg/mL.

Preparation of test sample

1 gm powder of all exploratory samples were extracted using each of 100 mL of distilled water, alcohol and hydro alcohol. The solutions were filtered and diluted up to 100 mL with distilled water, alcohol and hydro alcohol (100mg/mL) respectively. In 1mL of standard Rutin solution having concentration 20-100 µg/mL and different extracts of test solutions were diluted in volumetric flask (10 mL) and 4 mL of water was added. Additionally, to this 5 % sodium nitrite (0.3 mL), 10% aluminium chloride (3 mL) were added and set aside for 5 minutes. 2mL of sodium hydroxide (1 M) was added to each sample. Instantly the final volume was adjusted 10 mL with distilled water and the absorbance of the resultant red reaction mixtures was measured against blank at 510 nm on a UV-Vis spectrophotometer. The concentration of total flavonoids content (TFC) in the samples was estimated from the regression equation expressed as rutin equivalent (mg) /g of dried plant material. All experiments were performed in triplicate and values were represented in mean ± SEM.

RESULT:

Estimation of total flavonoids (TFC)

As a standard, Rutin was used and calibration curve shown in Figure. Linearity was observed from 20-100 µg/mL of Rutin concentration. Total phenols and flavonoids of different sample were represented in table.

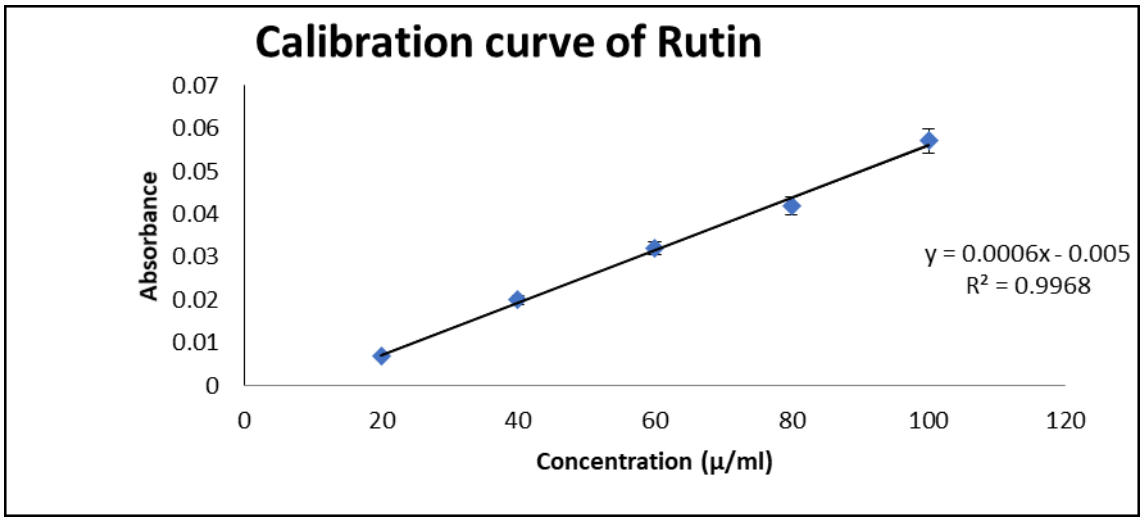

Figure 1: Calibration curve of Rutin

Table 1: % of Flavonoids present in different extracts of Dasmoola

Plant Name	**% Flavonoid (%w/w)**		
	Aqueous extract	**Alcoholic extract**	**Hydroalcoholic extract**
Bilva	6.92 ± 0.09	8.04 ± 0.09	7.72 ± 0.21
Gambhari	6.71 ± 0.17	7.89 ± 0.06	7.45 ± 0.08
Patala -	2.45 ± 0.06	3.12 ± 0.11	2.81 ± 0.17
Shalaparni	7.67 ± 0.88	8.78 ± 0.12	8.44 ± 0.11
Gokhru	3.82 ± 0.18	4.73 ± 0.09	4.24 ± 0.84
Agnimantha	5.92 ± 0.08	6.01 ± 0.16	7.02 ± 0.21
Shyonaka	8.71 ± 0.09	7.95 ± 0.08	8.04 ± 0.11
Brihati	3.45 ± 0.07	2.72 ± 0.19	4.14 ± 0.07
Kantakari	7.70 ± 0.24	8.08 ± 0.12	8.11 ± 0.10
Prishnaparni	2.82 ± 0.18	3.70 ± 0.11	4.24 ± 0.84

* Mean ± SEM of three values.

Simultaneous Estimation of flavonoids in-house Dasmoola churna by RP-HPLC method

Instruments

High-Performance Liquid Chromatography (HPLC), Agilent's RP-HPLC (Infinity 1200) apparatus was used.

Chemicals

The flavonoids were bought from Yucca enterprises, Mumbai. All the reagents used were of HPLC grade. Orthophosphoric acid was procured from Spectrochem, Acetonitrile from Merck and Methanol obtained from Fisher chemical ltd. All sample solutions were prepared in HPLC grade reagents.

HPLC Conditions

High-Performance Liquid Chromatography (HPLC), Agilent's RP-HPLC (Infinity 1200) apparatus was used for the simultaneous estimation of flavonoids utilizing a reverse-phase column Optimix C8/C18 with a mobile phase A consisting of Buffer Solution (10 ml of Orthophosphoric into 1000 ml water): Methanol: Acetonitrile in 80: 10: 10 and mobile phase B consisting of 50 :20: 30 ratios at a flow rate of 1 mL/min, with detection at 323 nm. The standard retention times for catechin, mangiferin, scopoletin, rutin, ellagic acid, baicalein, quercetin, naringenin, kaempferol, chrysin were 7.09, 9.52, 21.0, 21.89, 22.64, 25.03, 28.24, 31.43, 34.64, and 37.14 respectively.

Table 2: HPLC parameters

Parametes:	**Description**
Column:	Optimix C8/C18 (250 mm x 4.6 mm), 5 µm
Wavelength:	323 nm
Flow rate:	1.0 ml/min
Injection volume:	10 µl
Column Oven Temp.:	25℃
Sample Cooler Temp.:	25℃
Run Time:	50 mins.
Diluent:	Methanol
Buffer Soln.:	10 ml of Orthophosphoric into 1000 ml water
MP A:	Buffer Soln.: Methanol: Acetonitrile (80: 10: 10)
MP B:	Buffer Soln.: Methanol: Acetonitrile (50 :20: 30)

Table 3: Gradient programme:

Time (mins)	**Mobile phase A MP A (%)**	**Mobile phase B MP B (%)**
0.0	90	10
15.0	90	10
20.0	10	90
40.0	10	90
41.0	90	10
50.0	90	10

Preparation of standard stock solution:

Alcoholic solution of flavonoids was prepared to obtain 1000 µg/mL. They were further diluted to obtain 10 µg/ mL and 50 µg/ mL. A 10 mL volumetric flask was used to mix 1 ml of flavonoids (10ppm) to make a reference solution, which was diluted with methanol.

Sample Preparation:

Initially weighed and transferred 27.0 mg of samples into 20 ml volumetric flask. Then add about 10 ml of diluent, Sonicate for 20 minutes, below 25^0 C with intermediate shaking, allowed the flask to attain a room temperature and make-up volume up to mark with dilutant and mix well. Then after, filtered the solution through 0.45 µm MDI make PVDF+ prefilter by discarding not less then 5 ml of filtrate [23].

Note: Remaining sample (i.e. sample-2 to sample-13) were prepared same as above procedure

RESULTS:

High-Performance Liquid Chromatography (HPLC) was used for the simultaneous estimation of flavonoids. The standard retention times for catechin, mangiferin, scopoletin, rutin, ellagic acid, baicalein, quercetin, naringenin, kaempferol, chrysin were 7.09, 9.52, 21.0, 21.89, 22.64, 25.03, 28.24, 31.43, 34.64, and 37.14 respectively. In the root extract catechin, mangiferin, scopoletin, rutin, ellagic acid, baicalein, quercetin, naringenin, kaempferol, chrysin were detected. These findings confirm the presence of significant flavonoids with potential health benefits in Dasmoola Churna.

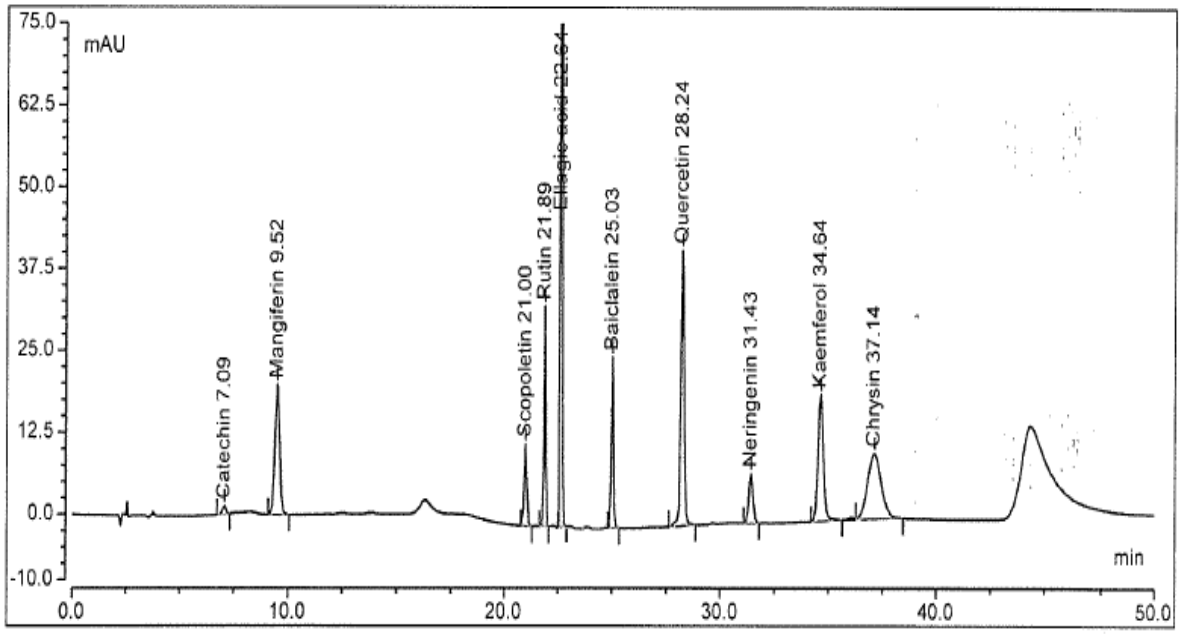

Figure 2: Chromatograph of Standard Mixture (10 PPM)

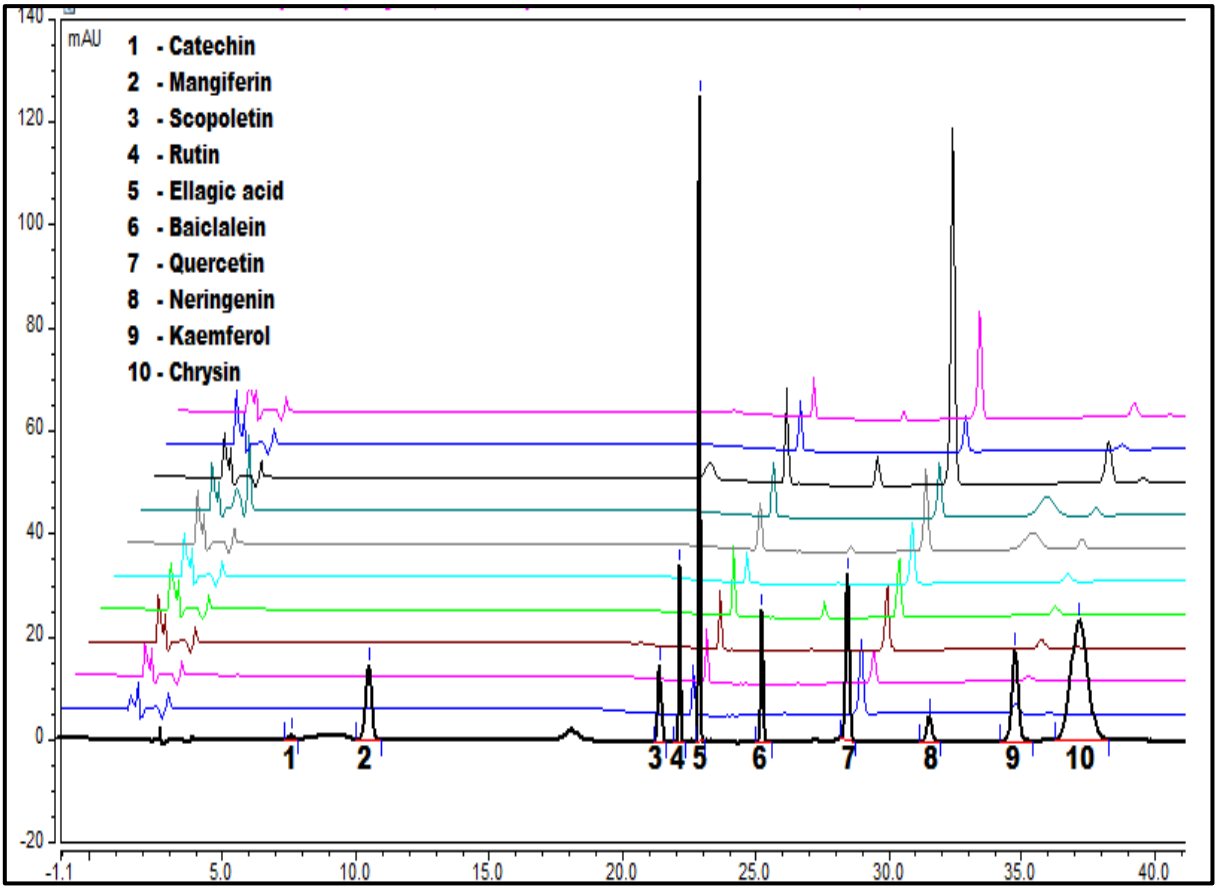

Figure 3: Chromatograph of Overlaid of 10 different roots extracts of Dasmoola churna

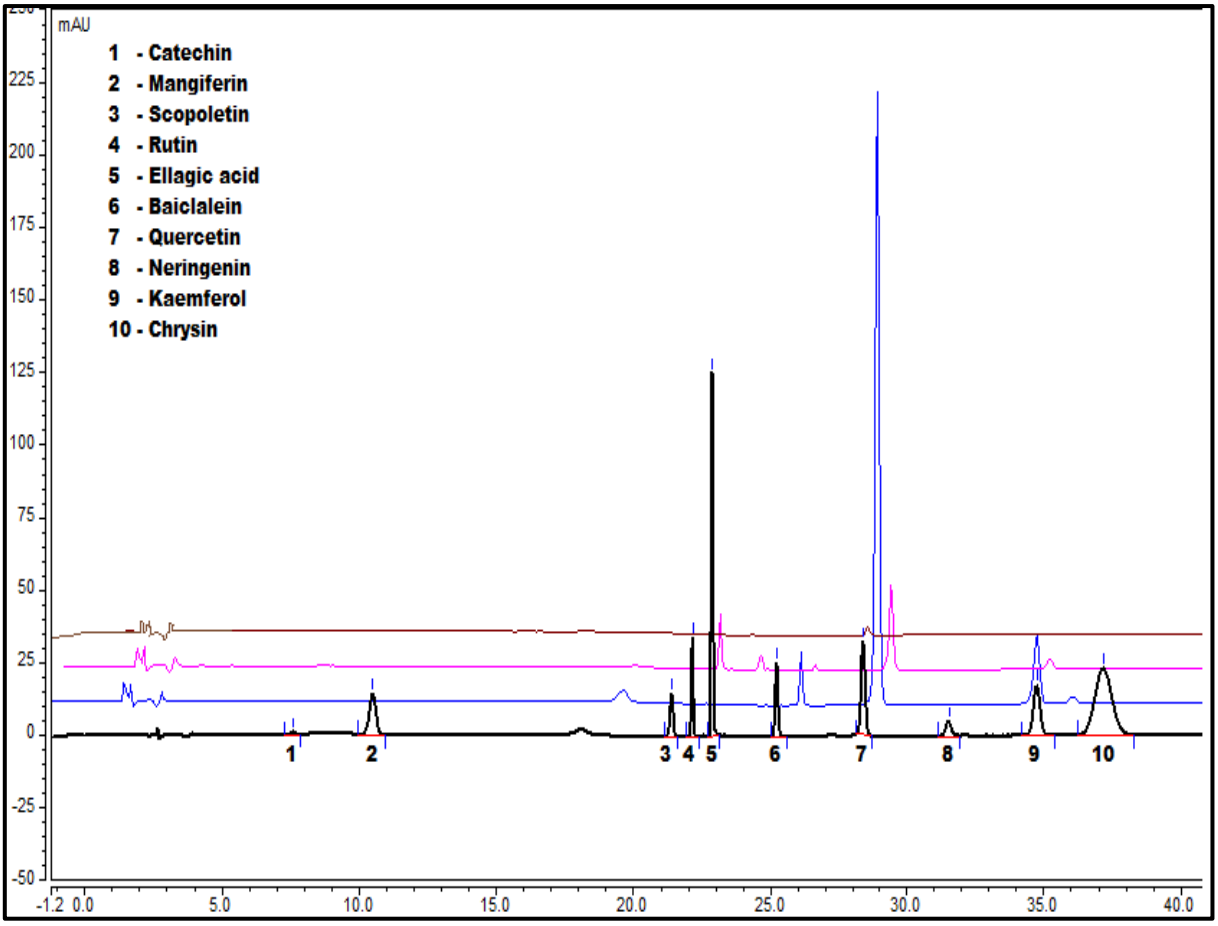

Figure 4: Chromatograph of Overlaid of 3 different roots extracts of Dasmoola churna

Moreover, current investigations emphasize the necessity for further studies to explore the precise mechanisms of action of these flavonoids and their bioavailability, as well as their interactions with cellular receptors in different pathological contexts [24]. Overall, the scientific exploration of Dashamoola Churna and its flavonoids highlights their potential role in integrating traditional Ayurvedic practices with modern medicinal applications [25].

DISCUSSION

Flavonoids have been extensively studied for their ability to interact with cytochrome P450 (CYP450) enzymes, which play a crucial role in the metabolism of various therapeutic drugs. These compounds can modulate the activity of CYP450 enzymes through mechanisms such as induction or inhibition, impacting the bioavailability and efficacy of drugs that are substrates of these enzymes [26][27].

The interactions between flavonoids and CYP450 enzymes primarily involve the modulation of the enzymes' biosynthesis and catalytic activities. For instance, flavonoids have been shown to inhibit the activity of CYP3A4, one of the most significant CYP450 enzymes involved in drug metabolism [28], [29]. This inhibition can lead to increased bioavailability of co-administered drugs, as less of the drug is metabolized and eliminated from the body [30]. Conversely, flavonoids may also induce specific CYP isozymes, leading to enhanced metabolism of certain drugs [31], [32].

Dashmoola has demon strated a multifaceted approach to managing inflammation and associated disorders, such as arthritis, asthma, and pain conditions. By potentially influencing CYP450 enzyme activity, Dashmoola may enhance the pharmacokinetics of co-administered medications, allowing for lower doses and minimizing the risk of side effects associated with high drug concentrations [33][34]. This property could be particularly beneficial in the treatment of chronic conditions requiring long-term medication administration, where the risks of drug toxicity are elevated due to cumulative exposure [35]. In the context of Dasmoola, a traditional Ayurvedic formulation, the incorporation of flavonoids can facilitate better absorption of active components through several mechanisms [36].

CONCLUSIONS:

Recent studies have concluded the presence of significant levels of flavonoids and polyphenols in Dasmoola Churna, compounds that are associated with various health benefits, including reduced oxidative stress and inflammation [37]. These findings align with traditional Ayurvedic beliefs that emphasize the therapeutic efficacy of herbal formulations in promoting health and treating diseases [38]. Overall, Dasmoola Churna represents a bridge between ancient wisdom and mod ern science, embodying the rich tradition of Ayurvedic medicine while addressing contemporary health challenges. Its growing

popularity and recognition in the field of herbal medicine underscore the importance of further research and sustainable practices to ensure its continued availability and efficacy [39].

This finding showed that Dasmoola and its bio-markers have very less inhibitory effect on CYP2D6 and CYP3A4 iso-enzymes [40]. The effect exhibited against the CYP450 enzymes was in a dose dependent manner. Certain major factors such as interaction between co-administered drugs, mechanism-based inhibition, unacceptable interactions with proteins, enzyme etc. need to be addressed further for better knowledge on herb–drug interaction [41], [42]. The developed RP-HPLC method can be used for the simultaneous quantitative estimation of Flavonoids present in the natural plant extracts.

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