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DETECTION OF ADULTERATION IN COMMERCIAL SAMPLES OF CINNAMOMUM VERUM J. S. PRESL FROM KERALA

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ABSTRACT

Background: Dried inner bark of *Cinnamomum verum* J. S. Presl (Syn. *C.zeylanicum*), commonly known as Cinnamon (True cinnamon/ Ceylon cinnamon) has been used as a raw drug in many Ayurvedic therapeutic formulations. Moreover, it is one of the most commonly used spices worldwide. But commercial samples of *C. verum* are often adulterated with its inferior species *C.cassia*, *C.malabatrum, C.burmanni* and *C.loureiroi* which often leads to serious adverse effects such as hepatotoxicity and carcinogenesis due to the presence of high amount of coumarin. This study aims to detect adulteration in commercial samples of *C.verum* from Kerala. **Methods:** Commercial samples of *C.verum* were collected and compared with genuine and API standards by macroscopy, microscopy and quantification of coumarin using a validated HPTLC (High Performance Thin Layer Chromatography) method. Moreover, heavy metal analysis was done by Atomic Absorption spectroscopy. **Results:** The study revealed that majority of *C.verum* barks available in Kerala were adulterated with other Cinnamomum species which contained high amount of coumarin. **Conclusion:** Authentication of raw drug materials is necessary in Ayurvedic drug industry to ensure safety and efficacy of therapeutic formulations.

KEYWORDS: *Cinnamomum verum,* adulteration, microscopy, HPTLC, coumarin.

1. INTRODUCTION

Herbs and herb-derived medicines have played a crucial role in health and disease management for many centuries. The World Health Organization (WHO) estimates that 80 percent of the population of some Asian and African countries presently uses herbal medicine for some aspect of primary health care.[1] Owing to the medicinal properties attributed to a crude drug used for manufacturing therapeutic formulations, it is necessary to maintain its quality and purity in commercial market. It is, however, observed that the commercial drugs are frequently adulterated and do not fulfill the standards prescribed for authentic drug.[2] A recent study done on authentication of raw drugs used in Ayurvedic system of medicine in Tamil Nadu using DNA barcoding found that approximately one fifth of the market samples were adulterated.[3]

Cinnamomum verum J.S.Presl (Syn. *Cinnamomum zeylanicum* Nees.), commonly known as „Cinnamon" or „True/Ceylon Cinnamon" (*Karuva patta* in *Malayalam*) belonging to the family Lauraceae and native to Srilanka is a versatile medicinal plant in Ayurvedic system of medicine and is used in single or in combination with other drugs for treating a variety of ailments like respiratory disorders, diarrheal diseases, anorexia and urinary disorders.[4] Its dried inner bark known as „*Tvak'* is the key ingredient in many Ayurvedic therapeutic formulations such as *Twageladi choornam*, *Thaleesapatradi choornam*, *Sithopaladi choornam*, *Eladi rasayana* and many *Arishtasavas*. As per Ayurvedic literature, it is *sukrala* (promotes spermatogenesis), *balya* (strengthening), *varnya* (increases body complexion) and *grahi* (water absorbent).[4] The major compounds reported in it are trans-cinnamic aldehyde, eugenol, cinnamyl acetate, cinnamyl alcohol and cinnamic acid.[5] Pharmacological and biological activities of Cinnamon are anti-oxidant, hypoglycaemic, hypolipidemic, hepatoprotective, wound healing, spermicidal, anti-bacterial, anti-fungal, anthelmintic, insecticidal, leech repellant and mutagenic properties.[6] Besides these, Cinnamon bark has multifaceted utilities in spice industry, perfumery, flavoring, beverages and culinary fields.

However, commercially available *C.verum* is often adulterated with its inferior species such as *C.cassia* (Chinese cinnamon), *C.malabatrum*, *C.burmanni* (Java

Cinnamon/Indonesian Cinnamon) and *C.loureiroi* (Saigon Cinnamon from Vietnam) jungle Cinnamon and Cinnamon chips (untrimmed barks).[7,8,2] One important difference between *C.cassia* and *C.verum* is their coumarin (1, 2-benzopyrone) content.[9] Coumarins are secondary phyto-chemicals with strong anticoagulant, carcinogenic and hepato-toxic properties.[10] Evidence of the hepatotoxic effects of this compound in animal models led the U.S. Food and Drug Administration to ban coumarin as a food flavoring agent.[11] In a study conducted at United States, it was found that coumarin content was high in all locally bought Cinnamon, cinnamon-flavored foods, and cinnamon food supplements and most of Cinnamon samples were Indonesian cassia, *C. burmanni*, which has replaced the more expensive true or Ceylon cinnamon (*C. verum*) in Europe, the United States, and Canada. The experimental results indicated that *C. verum* bark contained only traces of coumarin, whereas barks from all three cassia species, especially *C. loureiroi* and *C. burmanni*, contained substantial amounts of coumarin.[12] FSSAI (Food Safety and Standards authority of India) advocates maximum permissible limit of coumarin content to be not more than 0.3% by weight. *C.Cassia* contains coumarin content in the range of 0.8-10.63%. Cinnamon varieties have coumarin content around 0.2%.[7]

Sophisticated analytical methods such as UPLC-UV/MS (Ultra performance liquid chromatography),[12] gene sequencing,[13,14] ambient ionization (DART-Direct analysis in Real Time), and single quadrupole mass spectrometry[15] were developed to ddetermine coumarin and related compounds in Cinnamon and detect the adulteration in Cinnamoum species. But these expensive methods cannot be done at quality control labs associated with Drug manufacturing units. So simple, cost-effective, but precise methods should be developed to ensure the quality and purity of raw drugs. Hence the present study aimed to detect the adulteration of *Cinnamomum verum* bark commercially available in Kerala using macroscopy, microscopy and Coumarin content estimation by a validated HPTLC method.

2. MATERIALS AND METHODS

2.1. Cinnamon genuine sample collection

The genuine sample of *Cinnamomum verum* fresh bark was procured from Botanical garden of Govt. Ayurveda College, Thiruvananthapuram (8.4916^0 N, 76.9787^0 E) and was authenticated by botanist of Pharmacognosy unit, Govt. Ayurveda College, Thiruvananthapuram. Voucher specimens were deposited at drug museum of Drug Standardisation unit, Govt. Ayurveda College, Thiruvananthapuram (No.108/DSU/AVC) for future reference. The bark was thoroughly cleaned; outer cork was scrapped and dried in shade. It was powdered in a mini pulveriser, sieved through mesh size 80 and kept in labeled air tight container.

2.2. Cinnamon commercial sample collection

Commercial samples of Cinnamon were purchased randomly from spice markets, hypermarkets and raw drug markets of various parts of Kerala. All the samples were separately packed in labeled zip locked polythene bags in crude form and in labeled air tight containers in powder form.

2.3. Methods

The methods used to detect adulteration in this study are macroscopic and microscopic evaluation, High Performance Thin Layer Chromatography and Heavy metal analysis and the results were compared with genuine sample and Ayurveda Pharmacopoeial standards. The investigations were carried out at Instrumental and Botany lab of Drug Standardization unit, Govt. Ayurveda College, Thiruvananthapuram.

2.3.1. Macroscopical evaluation: Different macroscopic parameters like appearance, color, surface characters, odor, fracture and thickness of all samples were observed and analyzed.

2.3.2. Microscopical evaluation: Fine hand sections of bark of all Cinnamon samples were taken using standard procedures and were stained with Aqueous Safranin 1% and mounted in glycerin. Microphotographs of sections were made by using Olympus Microscope (Model CX 41; Tokyo, Japan) with CCD camera 2 mega pixel.

2.3.3.High Performance Thin Layer Chromatography: Alcoholic extracts of all Cinnamon samples were prepared and HPTLC was done (CAMAG, Switzerland) using $60F_{254}$ TLC plate, keeping in TLC twin trough developing chamber (after saturation with solvent vapor) with respective mobile phase Toluene: Methanol: Water: Ammonia in the ratio of 35:7:2.5:1to 70mm. The developed plate was dried and kept in Photo-documentation chamber (CAMAG REPROSTAR 3). The plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at 254 and 360 nm. The software used was Win CATS 1.3.4 version. The content of coumarin in all the Cinnamon samples was estimated using linear regression equation. The developed method was validated for specificity and linearity. All the solvents used were of HPLC/ Chromatographic grade and were purchased from Merck and Qualigens Fine Chemicals, India.

Preparation of Standard Solution: The standard compound coumarin was purchased from Sigma-Aldrich (Bengluru, Inda). Standard stock solution was prepared at a concentration of 2.0 mg/ mL in methanol. One ml of this solution is diluted with 10ml of methanol and a working solution of 0.2mg/ml concentration was prepared and used for HPTLC analysis. Calibration curves were prepared using five different concentration levels.

2.3.4. Heavy Metal Analysis: Atomic absorption Spectroscopy was used to determine the heavy metal elements and some nonmetal elements in atomic state. Determination of Lead, Copper, Zinc and Nickel in ppm levels in all Cinnamon samples were carried out using Atomic absorption Spectrophotometer (Software Solar AA, 11.01 version, Thermo-scientific iCE 3500 series, United States).

3. RESULTS

3.1. Macroscopical evaluation: As per API (Ayurveda Pharmacopoeia of India, a legally valid drug document in Ayurveda), the macroscopic features of *C.verum* are single, double or compound quills, 0.5mm thick, dull yellowish brown outer surface, splintery fracture and free from cork portion.[16] The samples D, E, G, J and K comply with these features. The results are shown in Table No.1 and Fig. No.1

Table 1: Macroscopical evaluation of all Cinnamon samples.

Samples	Macroscopical features				
	Appearance & Color	**Surface characters**	**Thickness**	**Fracture**	**Odour**
A	Thick Bark; Outer: greenish red with white patches, Inner: reddish yellow.	Rough, numerous warts	3mm	Tough, short	Intense aromatic
B	Thick Bark; Outer: Reddish green, Inner: Reddish yellow colored.	Rough, numerous warts	3mm	Short, Tough	Intense aromatic
C	Single Quills; rolls from both sides; Outer: dull yellow colored, inner dark yellow.	Smooth, longitudinal striations	3mm	Short, Tough	Intense aromatic
D	Compound Quills, rolls from one side, Dull yellowish red color; occasional scars & holes	Smooth, longitudinal striations	0.5-1mm	Brittle, Short & splintery	fragrant
E	Compound Quills; outer: smooth dull yellowish red color, fibrous inner.	Rough, numerous warts	1mm	Brittle, Fibrous	Fragrant
F	Thick bark; outer: greenish red, inner : dull yellowish red colored with longitudinal fibres	Rough, numerous warts, inner: smooth	4mm	Short, Tough	Intense aromatic
G	Compound Quills rolling from one side mixed with untrimmed bark; dull yellow colored.	Smooth, longitudinal striations on inner side	0.5mm	Brittle, Short & splintery	Fragrant
H	Thin bark; Outer: greenish red, Inner: reddish yellow	Rough	1mm	short	Fragrant
I	Thin bark; Outer: greenish red, Inner: reddish yellow	Rough	1.25mm	short	Fragrant
J	Compound quills; rolls from one side, outer: dull yellowish red, Inner: Dark red.	Smooth	0.5mm	Brittle, splintery	Fragrant
K	Compound quills; rolls from one side, occasional scars & holes, dull yellowish red colored	Smooth	0.5mm	Brittle, splintery	Fragrant
L	Thin bark; outer: greenish red, Inner: red.	Rough , numerous warts	0.7mm	Short, Tough	Strong Aromatic
M	Trimmed bark; Outer: dull yellowish red, Inner: dark yellowish red	smooth	o.5mm	Brittle, splintery	Fragrant

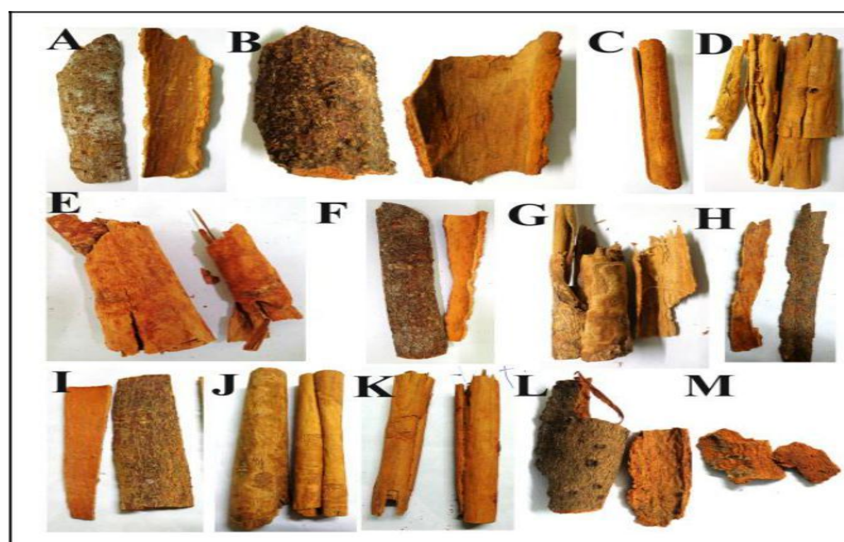

Fig. 1: Commercial (A-L) and Genuine (M) samples of Cinnamon.

3.2.Microscopical evaluation: Microscopical evaluation was done based on the presence of cork and cortex, size of stone cells, occurrence of starch grains, number of secretory cells, presence of crystals and medullary rays. The results of microscopical evaluation are shown in Table No.2 and Fig.No.2.

Table 2: Microscopical evaluation of all samples of Cinnamon.

Sample	Microscopical features					
	Cork &Cortex	**Stone cells**	**Starch grains**	**Secretory cavities**	**Crystals**	**Medullary rays**
A	Present	Bigger	Abundant & thickly packed in cells	A few	Present	Present
B	Present	Bigger	Abundant & thickly packed	A few	Not seen	Not seen
C	Present	Bigger & Longer	Not abundant	A few	Not seen	2-3 layered
D	Absent	Medium sized	Abundant & thickly packed	many	Present	Biseriate
E	Absent	Medium sized	Numerous	many	Acicular crystals present	Biseriate
F	Present	Bigger & Wider	Abundant & thickly packed	many	Not seen	Multilayered
G	Traces of primary cortex seen	Big	Fewer	many	Not seen	Biseriate
H	Present	Big	Abundant	Many, Oil globules present	Present	2-3 layered
I	Present	Medium sized	Abundant & thickly packed	many	Not seen	2-3 layered
J	Absent	Elongated	Fewer	Many, Oil globules present	Not seen	Biseriate
K	Absent	Medium sized	Numerous	Many, Oil globules present	Not seen	3-4 layered
L	Present	Bigger & thicker	not numerous	Fewer	Present	Biseriate
M (Genuine)	Absent	Medium sized	Present, not numerous	Numerous, Oil Globules present	Acicular crystals present in parenchyma& medullary rays	Biseriate

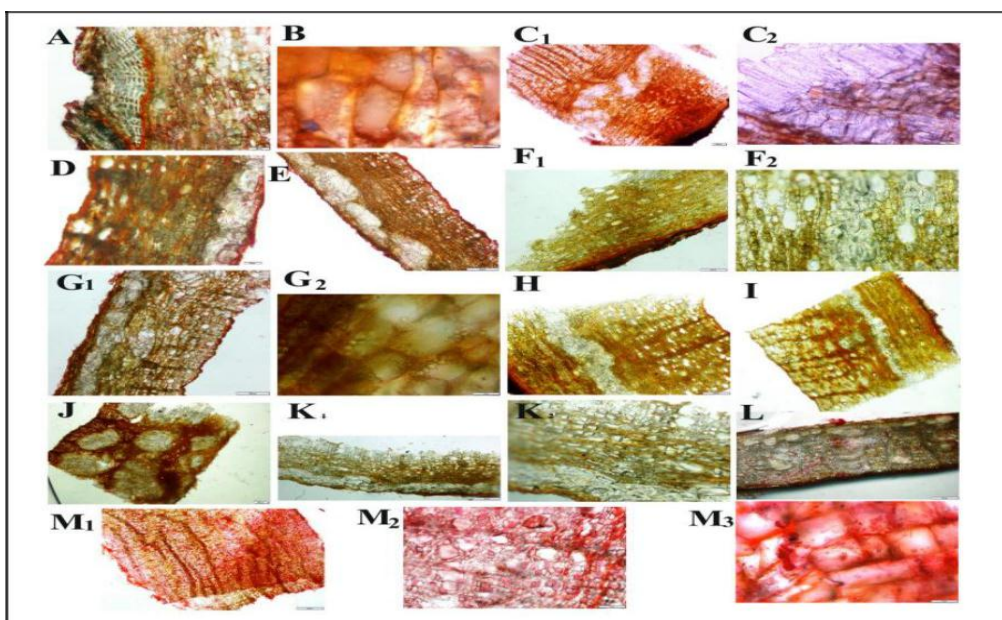

Fig. 2: Transverse sections of all samples of Cinnamon. (A) Cork and cortex were present with bigger stone cells thickly packed with starch grains, 10x. (B) Bigger stone cells with many starch grains, 40x. (C_1)Thick bark with Cork & Cortex, 2x. (C_2) Bigger and longer stone cells, 10x. (D)Thin bark with medium sized stone cells, 10x. (E)Thin bark with medium sized stone cells and starch grains, 4x. (F_1)Thick bark with cork & cortex, 4x.(F_2) Bigger & wider stone cells thickly packed with starch grains,10x. (G_1)Thin bark, 4x. (G_2)a few starch grains, 40x. (H)Thin bark with many secretory cavities & oil globules, 4x. (I)Thick bark with medium sized stone cells, 2x. (J)Elongated stone cells with a few starch grains and many oil globules, 2x. (K_1)Thin bark, 4x. (K_2)Medium sized stone cells with many oil globules, 10x. (L)Thick bark with bigger and thicker stone cells, 4x. (M_1)Thin bark, 4x. (M_2)Many secretory cavities, 40x. (M_3)A few starch grains & Acicular Calcium oxalate crystals, 40x.

3.3. High Performance Thin Layer Chromatography

In HPTLC analysis, five different concentrations of coumarin standard was spotted in tracks 1-5 (S1-S5) and Cinnamon samples were spotted in tracks 6-18(A-M). The spots were visualized under UV 254 and 360 nm. The data of analysis is depicted in Fig.No.3, 4 and Table No.3 and the peak display of all the samples are shown in Fig.No.4.

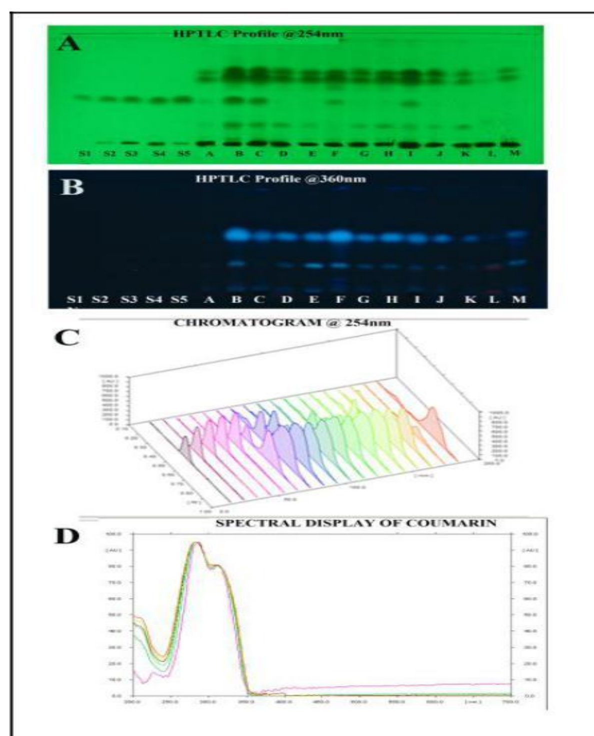

Fig. 3(A) HPTLC profile of all Cinnamon samples @ 254nm; S1, S2, S3, S4& S5 were Coumarin standards; A-M are commercial samples of Cinnamon. (B)HPTLC profile @360nm. (C)Colour display of chromatogram @254nm. (D) Spectral display of Coumarin.

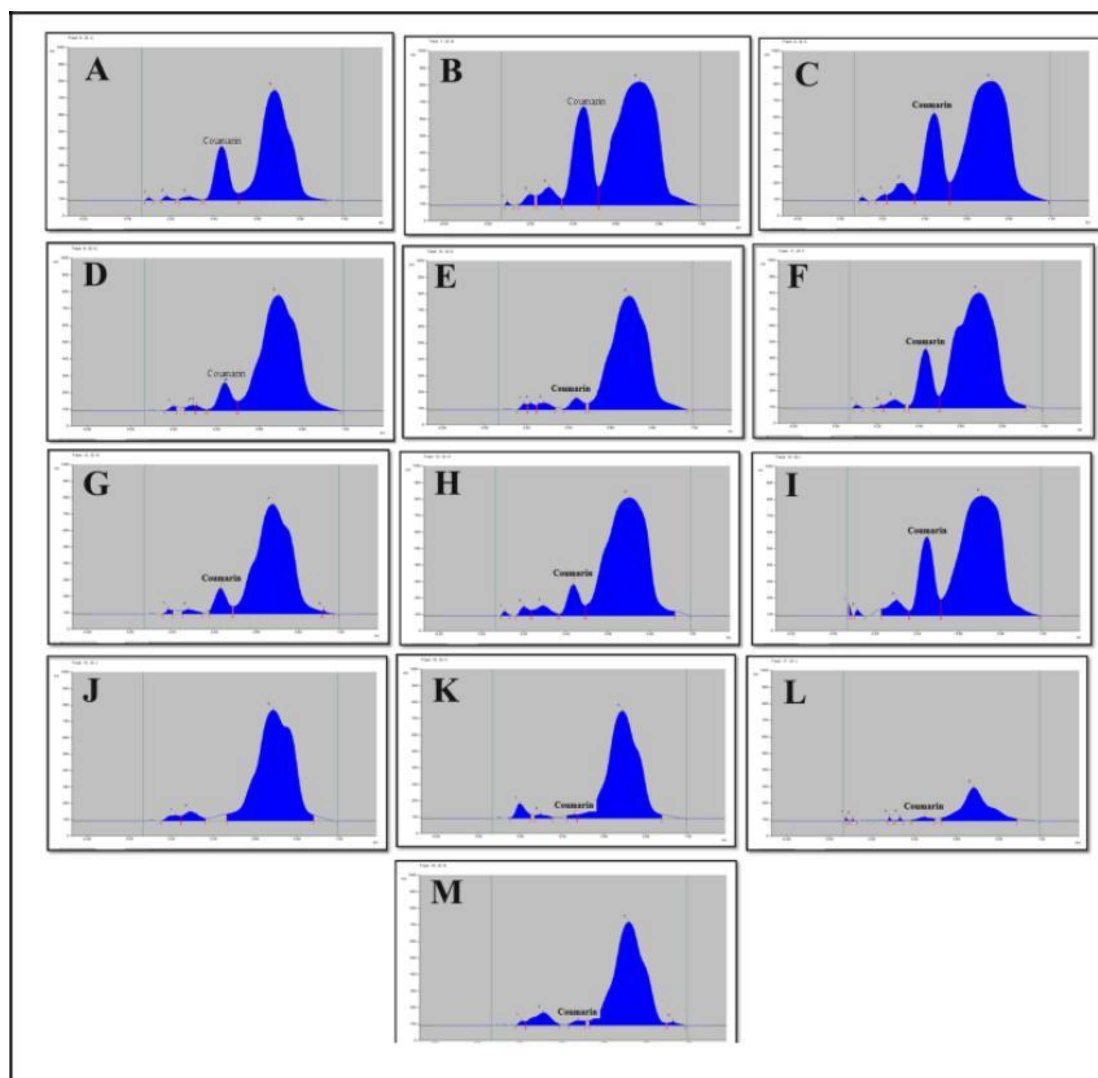

Fig. 4: Showing peak display of all cinnamon samples.

Table 3: HPTLC results showing Rf values, area and concentration of coumarin in the samples.

Track No.	Samples	Rf values	Area of Coumarin (AU)	Estimationof Coumarin (%)
6	Sample A (4µl)	0.10, 0.18, 0.29, 0.44, 0.68	13084.6	4.56
7	Sample B (4µl)	0.09, 0.20, 0.29, 0.45, 0.71	31534.6	11
8	Sample C (4µl)	0.11, 0.21, 0.31, 0.45, 0.72	28249.4	9.85
9	Sample D (4µl)	0.20, 0.29, 0.31,0.44, 0.69	6647.4	2.31
10	Sample E (4µl)	0.19,0.22, 0.29, 0.44, 0.69	2665.3	<2
11	Sample F (4µl)	0.10, 0.22, 0.28, 0.43, 0.69	15633.5	5.45
12	Sample G (4µl)	0.18, 0.28, 0.43, 0.68, 0.92	5837.4	<2
13	Sample H (4µl)	0.11, 0.20, 0.29, 0.44, 0.71	7119.8	2.48
14	Sample I (4µl)	0.08, 0.12, 0.31, 0.45, 0.71	21904.0	7.64
15	Sample J (4µl)	0.22, 0.29, 0.69	Not detected	Not detected
16	Sample K (4µl)	0.20, 0.30, 0.46, 0.69	566.6	<2
17	Sample L (4µl)	0.08, 0.11, 0.29, 0.33, 0.45, 0.68	991.3	<2
18	Sample M (4µl)	0.21, 0.31, 0.48, 0.72, 0.93	1027.8	<2

3.4. Heavy Metal Analysis

The heavy metals lead, Copper, Zink and Nickel were found within permissible limits in all the samples. The results are shown in Table.No.3.

Table 4: Heavy metal levels in samples.

Samples	Lead (ppm)	Copper (ppm)	Zink (ppm)	Nickel (ppm)
A	0.3156	0.2817	4.98	1.91
B	0.4621	0.1679	3.87	1.67
C	0.4006	0.1713	4.06	1.84
D	0.5883	0.2433	3.98	0.30
E	0.5147	0.3248	3.34	0.23
F	0.5284	0.4183	5.21	1.59
G	0.2661	0.1832	5.41	2.11
H	0.1593	0.3233	3.62	0.79
I	2.0168	0.3691	3.02	1.43
J	0.6124	0.2724	5.55	2.50
K	0.2275	0.5409	4.49	1.76
L	0.1848	0.5346	4.58	1.12
M	0.3961	0.2914	3.21	1.51

4. DISCUSSION

In the present era of globalized recognition of Ayurveda, adulteration is a major challenge in Ayurvedic drug industry which affects the quality and efficacy of medicines. Simple, cost effective, but precise methods should be developed which will be useful to pharmaceutical industries for authentication of crude drugs.

The inner bark of *Cinnaomomum verum* is used as a raw drug in many Ayurvedic therapeutic formulations. The present study assessed the genuineness and quality of *Cinnaomomum verum* commercially available in Kerala with the aid of macroscopical and microscopical examinationand coumarin content estimation by HPTLC. Twelve commercial samples of Cinnamon were collected and were compared with genuine Cinnamon and API standards.

According to WHO, the macroscopic and microscopic description of medicinal plant is the first step towards establishing the identity and purity of such materials and should be carried out before any tests are undertaken.[17] Hence macroscopic and microscopic examinations were carried out firstly. The macroscopic and microscopic features of samples D, E, G, J and K appeared to be similar with genuine Cinnamon (Fig. No. 1, 2, Table.No.1, 2), even though sample G contained chips (untrimmed bark) and the features corroborated with API standards. Samples A, B, C, F, H and I appeared similar and may be *Cinnamomum cassia* or *Cinnamomum burmanni*.[2,6] Sample L was entirely different from other samples and was found to be *Cinnamomum malabatrum* with broken stone cell layer, large stone cells and few secretory cells It was clear that all the samples were *Cinnamomum* species only. But the species differentiation can be done accurately with the help of detailed and quantitative microscopy only.[6,18] which was beyond the scope of the study. As per WHO, determination of heavy metals is included in quality control methods for medicinal plant materials.[17] Hence the present study screened heavy metals, Lead, Copper, Zink and Nickel in Cinnamon samples and was found to be within permissible limits.

High performance Thin Layer Chromatography (HPTLC) is a precise method used for separation of bioactive components present in medicinal plants both qualitatively as well as quantitatively. As per FSSAI norms, coumarin content in Cinnamon should not be more than 0.3% by weight and coumarin content can be used as a standard to detect adulteration in commercial Cinnamon samples. Hence coumarin content in all the samples was quantified using HPTLC. The HPTLC densitogram revealed that peak display and Rf values of samples D, E, J and K were found similar with sample M (genuine) and the samples A, B, C, F, H and I were found alike, but sample L showed different peaks and Rf values. Being Cinnamomum species, some peaks were found common in all samples owing to the presence of common chemical constituents.. The results corroborated with macro and microscopic results. Coumarin content was high in samples A, B and C compared with others. Hence it can be concluded that samples D, E, G and J were genuine Cinnamon. Other samples were assumed to be *Cinnamomum cassia* or *Cinnamomum burmanii* and sample L may be *Cinnamomum malabatrum*. However, bark of Cinnamomum verum available in commercial markets of Kerala is adulterated with other species of Cinnamomum having high amount of coumarin which may result in potential adverse effects.

5. CONCLUSIONS

Quality is the sum of all the factors which contribute directly or indirectly to the safety, efficacy and acceptability of the product.[19] Hence medicines prepared with the species other than *Cinnamomum verum* never bring desired pharmacological actions and can cause harmful effects due to the high amount of coumarin The developed active constituent based HPTLC method has the advantage of simple, specific and easy identification of coumarin in Cinnamon samples and could be applied for the routine analysis for Cinnamon species in crude drug or finished products.. The present study reveals the genuineness of commercially available crude drug, bark of *Cinnamomum verum* and is found adulterated with other species of Cinnamon having high amount of coumarin.

Hence manufacturers must ensure proper screening of raw materials for better product development.

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CONFLICT OF INTEREST
The authors have no Conflict of interest.

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