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Research Article

Pharmacognostic standardization and quality control of stem bark of *Cinnamomum cassia* (L.) J.Presl

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ABSTRACT

Cinnamomum cassia (L.) J.Presl stem bark is one of the most popular spice used for cooking as well as in traditional and modern medicines. *C.cassia* is a tree of tropical region of Lauraceae family, commonly known as Chinese cinnamon. Bark has been used as anti-inflammatory, analgesic, antioxidant, antimicrobial, anti-obesity, mosquito larvicidal, anti-diabetic, antitumour, anti-tyrosinase activity and cardiovascular protective. Traditionally, it is used to cure dental problems, oral microbiota, and bad breath. In Indian traditional medicine, especially in Unani and Ayurveda, the bark is used medicinally to treat various of illnesses, including diarrhoea, kidney pain, cough & cold, cobra bite, headaches, acne, dysuria, burning micturition, indigestion, weakness of liver, amenorrhea, fungal infection and for the management of sexual dysfunction. Pharmacognostic studies help to validate crude drugs and set standardization parameters that can assist in the prevention of adulteration. The present study will help to set the specific macro-microscopic and physico-chemical parameters to identity *C. cassia* stem bark which certainly assurance the quality of crude drug and will definitely help in standardization of various traditional herbal formulations containing *C.cassia* stem bark as ingredient.

Keywords: Pharmacognosy, HPTLC, Quality control, *Cinnamomum cassia*, spice.

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INTRODUCTION

Worldwide, Cinnamon is an important and popular spice used for cooking as well as traditional and modern medicines.[1]All over the world, about 250 species of the *Cinnamomum* genus have been identified. [2,3] *Cinnamomum cassia* (L.) J. Presl is an aromatic and evergreen tree of Lauraceae family native to southern China. It is widely cultivated there and elsewhere in South and Southeast Asia countries such as Indonesia, India, Malaysia, Laos, Vietnamand Thailand.[4,5] Cinnamon is collected from the bark of young branches and has historic medicinal uses in addition to being used in food and as preservative for bakery products, beverages, sweets, cosmetics, medical products, and perfumes.[6,7] About 1–2% of volatile oil, knowns as cassia oil, is its primary chemical constituents, of which cinnamaldehyde constituent 65–80%, where as eugenol is in very less quantity.[8] It also contains procyanidins, catechins, linalool, methoxycinnamic acid, camphene, cinncassiol, geranyl acetate, ethyl cinnamate, as well as mucilage, starch and tannins.[8,9,10] It has various pharmacological effects including anti-inflammatory, antioxidant, analgesic, antitumour, anti-obesity, anti-diabetic, antimicrobial, neuroprotective, cardiovascular protective, anti-tyrosinase activity. [4,11] Traditionally, it is used to dental problems, oral microbiota, and bad breath.[11] Traditional medicine continues to be the most preferred primary health care system due to their low toxicity and potent healing abilities. [12,13,14)

AYUSH (Ayurveda, Yoga & Naturopathy, Unani, Siddha, Sowa-Rigpa, and Homeopathy) is recognized by the Government of India as alternative systems of medicine is

predominately based on medicinal plants for therapeutic purposes. [15,16] Cinnamon has been extensively used in traditional system of medicine for centuries. In Unani system of medicines, bark of *C. cassia* is used in diarrhoea, cough & cold, headaches, kidney pain, acne, cobra bite, dysuria, burning micturition, indigestion, weakness of liver, amenorrhea, and fungal infection. [17] While in Ayurveda, it is used herbs for the management of sexual dysfunction [18,19].

All medicines, whether plant derived or synthetic, should meet the fundamental standards for efficacy and safety [20]. The stability and quality of herbal remedies are largely dependent on the quality of the raw ingredients used. Pharmacognostic studies validate plant identity and set standardization parameters that can assist in the prevention of adulteration. A number of modern tools and techniques are employed for standardization of herbal drugs. The present study was done with the aim to lay down the pharmacopoeial standards for bark of *C.cassia* by using paparamters viz. macro-morphological and micro-anatomical study, powder analysis, physico-chemical parameters assessments such as foreign matters, total ash value, acid insoluble ash, pH values, alcohol-soluble extractives, water-soluble extractives.To evaluate the quality of the crude drug, quality control measures such pesticide, heavy metal, aflatoxins, and microbiological load estimation are carried out.

MATERIALS AND METHODS

Drug procurement and authentication

The bark samples of *C. cassia* were purchased from local raw drug dealers of New Delhi and Ghaziabad. The botanist of DSRI, Ghaziabad identified by using pharmacognostic methods. [21,22,223] The drug was ground to a coarse powder using a griding paste and stored in airtight containers for powder microscopy. The pharmacopeia standards of drug were developed as per WHO (2011) guidelines).[24]

Physicochemical analysis

The physical-chemical characteristics viz.foreign matter, moisture content, ash values,ethanol extractive values and water extractive values, pH values (1% & 10% aq. solution) were carried out as per standard methods. [25,26]

HPTLC Profile

The extract was prepared by sonicating 2g of drug samples for 20 minutes with 30 ml of Chloroform.The extract was filtered, concentrated to a volume of 20 ml, and used as test solutions. About 10µl of chloroform extract was applied to a TLC plate as a 10mm band using an automatic HPTLC sample applicator.The plate was developed in the solvent system Toluene: Ethyl acetate: Formic acid (9: 1: 0.5) up to a distance of 9 cm. The plate was air dried and then subjected to UV light at 254 and 366 nm. Further, the plate was immersed in 1% vanillin-sulphuric acid reagent, heated to 105^0C until colored bands appeared, and the plate was examined in visible light. [27,28,29]

Quality control analysis

The drug was examined for potential contamination by using various quality control measures, such as pesticide residues, heavy metals, aflatoxins, and microbial load. Heavy metals and aflatoxins analysis were carried out as per standard method by using Atomic Absorption Spectrophotometer HPLC (Thermo Fisher) and Atomic Spectro photometer (LABINDIA) respectively. [30,31] Estimation of microbial load and pesticide residues were carried out as per standard methods. [30,32]

RESULT AND DISCISSION:

Macro-microscopic study

Macro-microscopy evaluation of crude drug materials is a key point for detecting source materials.[12,33,34] World Health Organization (WHO) state that morphological and anatomical assessment of a medicinal plant are imperative diagnostic tool for determining the identity and the degree of purity of crude drug material.[35,36,37] The drug in present study subjected to both macro-microscopic studies.

Macroscopic characters

The macroscopic study revealed dried bark earthly brown in colour and smooth but greyish cork persists and have channelled or single quill of bark (Figure 1A). Drug pieces possess an average width 1-2 cm and 0.3-0.5 cm thickness of various length from 5 cm to 40 cm in size or single quill of bark which is extremely hard, fracture uneven and short.The outer layer is relatively rough and brown in colour, while the inner layer is oily and reddish brown with a brown-yellowishline between two layers.

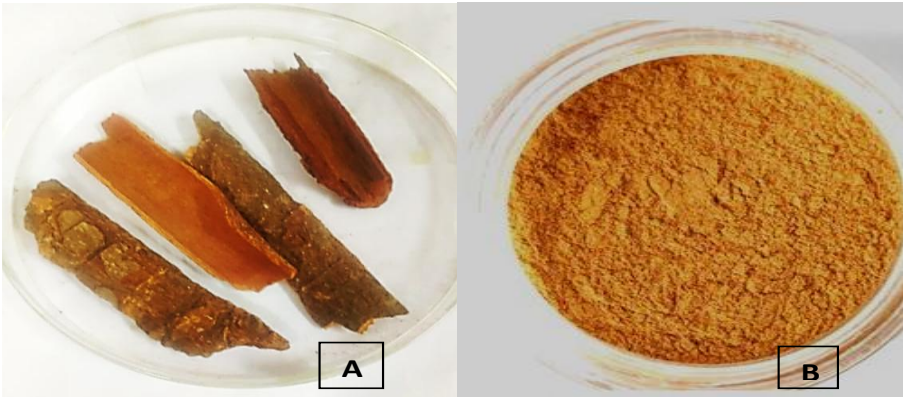

Figure1: (A) Stem bark of *C. cassia*; (B) Powder of stem bark

Organoleptic characters

A comprehension of the organoleptic properties of crude drug is necessary for their identification and authenticity. The evaluation of organoleptic characters of plant material is based on colour, odor, taste, shape, texture, etc. [38] The bark of *C. cassia* has a strong and spicy taste. Odour and taste resemble as like Dalchini (*Cinnamomum zeylanicum*) but harder and more mucilaginous and astringent in taste. The bark powder is yellowish brown in colour, oily in nature with typical sweet smell of orange (Figure 1B).

Microscope characters (Anatomical study)

Transverse section of bark shows the presence of lenticels. Cork is made up of multiple layers of cells with lignified outer walls and a thick innermost layer. Cortex 12 to 15 layered, parenchymatous with abundant simple starch grains, secretory cells, scattered stone cells with more lignified and pitted tangential and lateral walls; pericycle fibres in tangential rows embedded among stone cells group. Sclerenchyma cells lignified, pitted inner and radial wall thick. Phloem in isolated groups along with isolated or short tangential rows of thick,lignified and striated fibres with narrow lumen; secondary phloem parenchymatous with starch grains,oil cells and acicular crystal. Oil cellsthin, large, oval, associated with thin-walled parenchyma and medullary rays. Medullary rays narrow on inner side and wider towards peripherywith starch granules and acicular calcium oxalate crystals (Figure 2).

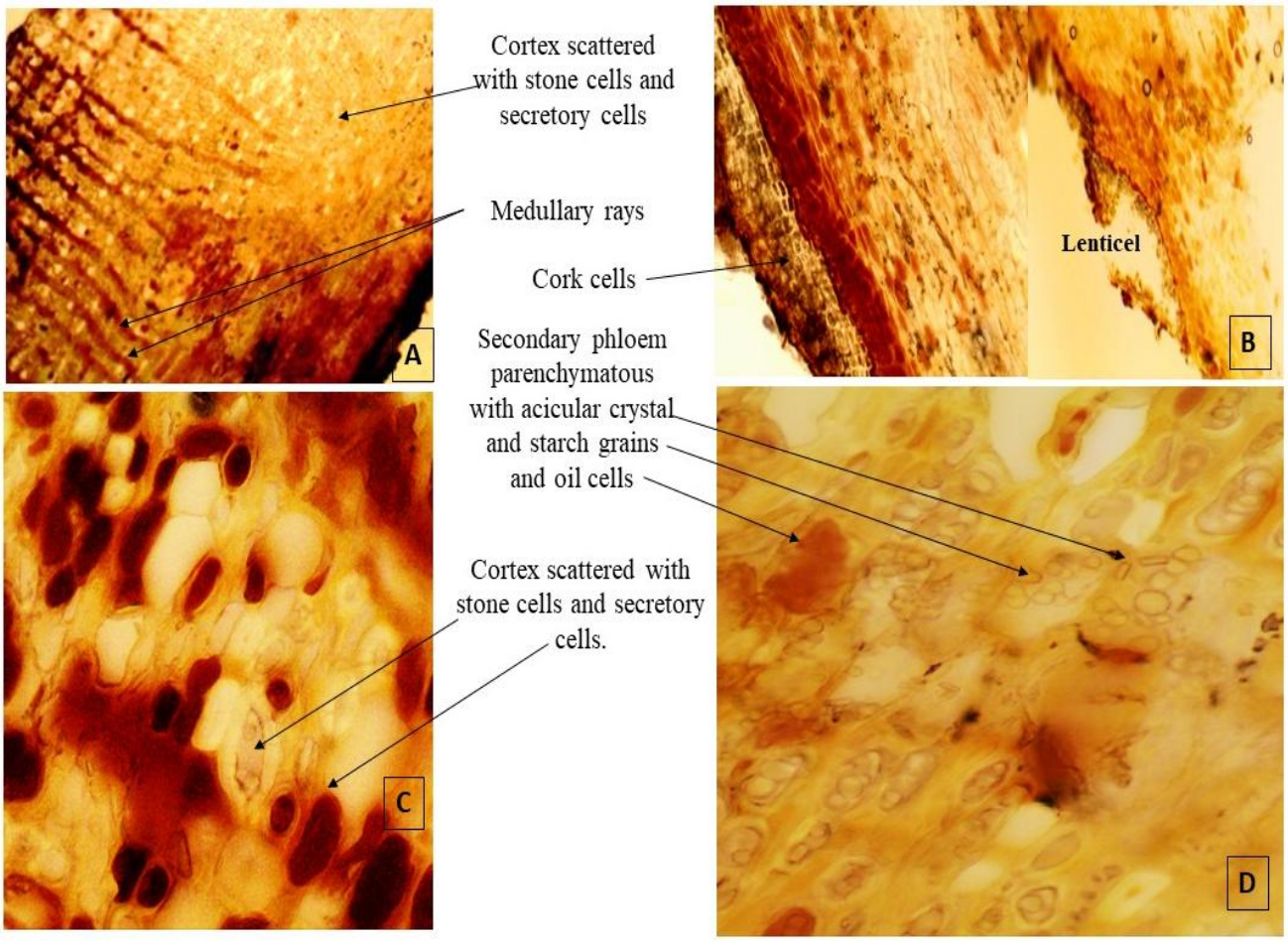

Figure 2: T.S. of stem bark of *C. cassia* - A) 10x; B) 20x; C&D) 40x

Powder microscopy

The microscopic study of herbal powder is a simple and reliable method for the identification of the drug.[39] It helps in evaluating the purity of the prepared herbal drug.The microscopic analysis of bark powder revealed several anatomical features that are peculiar and useful for diagnostic purposes.The powder microscopic study indicated the presence of polygonal cork cells containing reddish brown contents. Fibres are singly scattered. Parenchymatous tissue having some part of oil gland, oil droplets, starch grains; minute needle crystals of calcium oxalate, solid yellowish bodies; oil cells sub-rounded or oblong. Sclereids cells are associated with parenchymatous cells and are in sub square or sub-rounded, or elongated shapes (Figure 3 & 4).

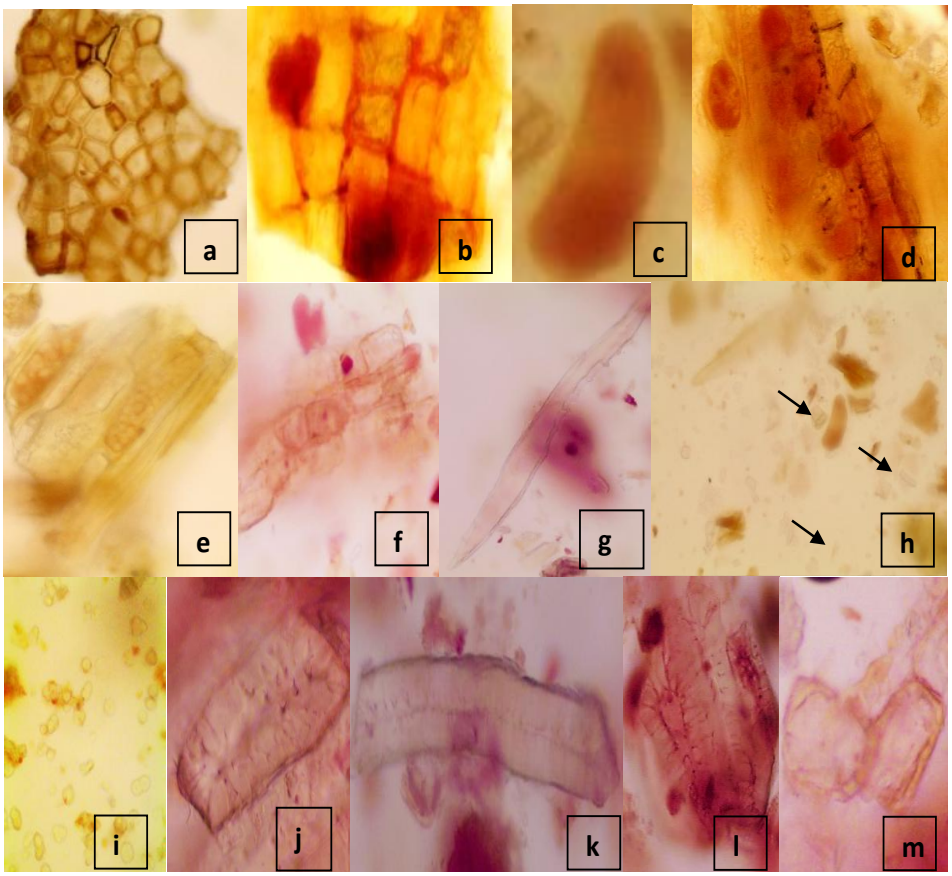

Figure 3: Powder microscopic characters of *C. cassia*stem bark:(a) cork cells in surface view (20x); **b)**part of the cork in sectional view with brownish content (20x); **c)** oil cell (40x); **d)** parenchymatous tissue having oil droplets, starch grains and yellowish bodies (20x); **e)** secondary phloem parenchymatous with starch grains (40x); **f)**sclereids associated with parenchymatous cells (20x); **g)** fibre (20x); **h)** acicular crystals (40x); **i)** starch grains (40x);**j,k,l,m)** different types of stone cells (40x)

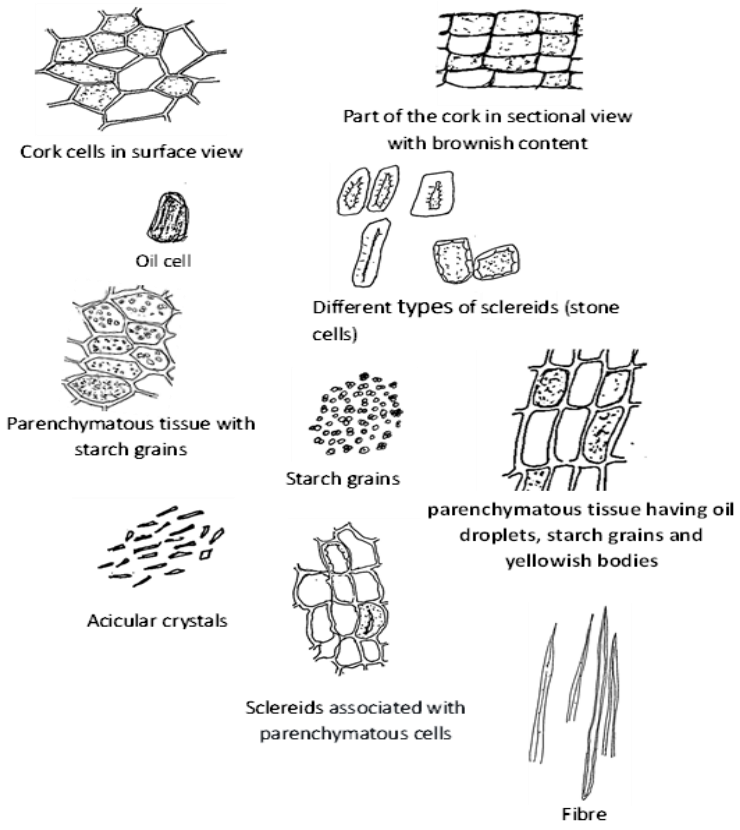

Figure :4 Digramatic representation of different type of cells found in powder microscopy of *C. cassia*.

Physico-chemical parameters

Physicochemical parameters are essential for the standardization and quality control of herbal drugs.The results of various physico-chemical parameters analysis of bark of *C. cassia* are presented in Table 1.The analysis of foreign matter in powdered drugs is crucial to detect any contamination. In order to prevent the growth of microbes such as bacteria, yeast, or fungi during storage, drug should have a very low moisture content.[40] Loss on drying is method for determination of moisture content in a powdered sample.Quantitative analysis of the drug revealed that the moisture content percentage is less than 10% which shows an ideal range for minimum bacteria as well as for fungal growth during herbal drug storage.[41] The quality and purity of crude drugs are evaluated using by ash values.Total ash content of the drug was ranges between 6.18-6.29% and acid insoluble ash is lower than 1% which is within the limit. Significantly water extractive values (13.61-13.83 %) higher than ethanol soluble extractive (11.45-11.67%) and the hexane soluble extractive values (1.80-1.92 %) came out to be on lowest side; which indicated the presence of distinctly polar constituents.The pH of drug ranges between 6.46–4.92% range, indicating the drug slightly acidity in nature.

Table 1: Physicochemical parameters of Stem bark

S. No.	Parameters	Values
1.	Foreign Matter (%)	1.55
2.	Loss in weight on drying at 105^0 C (%)	6.20-6.31
3.	Total Ash (%)	6.18-6.29
4.	Acid insoluble ash (%)	0.40-0.55
5.	Ethanol Soluble Extractive (%)	11.45-11.67
6.	Water Soluble Extractive (%)	13.61-13.83
7.	Hexane Soluble Extractive (%)	1.80-1.92
8.	pH 1% Soln.	6.46
	pH 10% Soln.	4.92

HPTLC fingerprinting

High-performance thin-layer chromatography (HPTLC) fingerprint analysis is now the most effective method for identification, authentication and to measure quality control of herbal medicine.[42] The HPTLC finger printing of chloroform extracts of stem bark of *C. cassia* (three samples) were observed under UV 254nm, UV 366 nm and under white light after derivatization. All three samples illustrate similar colourful bands with similar Rf values (Figure 5). It shows the reliability of the results.

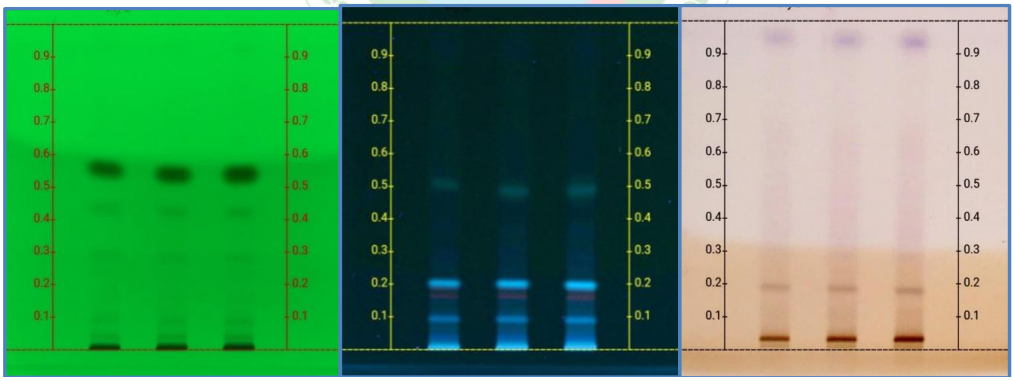

Figure 5: HPTLC of Chloroform extracts of stem bark of *C. cassia.*

Quality control parameters

Plant based herbal drugs and remedies are considered to be pure and safe as it is obtained from natural resources.The herbal materials are being used worldwide as effective remedies since they exhibit less adverse effects. The main objective of developing pharmacopoeial standards for herbal medicines is to ensure quality assurance.[43] Thus, WHO has emphasized to analysis the quality control of herbal drugs by using modern techniques and appropriate practices to assure herbal drug quality.[44] Analysing any contamination in herbal drug is crucial in order to ensure their safe consumption by humans. The result of various quality control parameters such as microbial load, aflatoxins & heavy metals analysis, pesticide residue for bark of *C. cassia* is respectively shown in Table 2, 3, 4 & 5.

Heavy metal analysis

The accumulation of heavy metals by plants and their uptake along the food chain can potentially harm animal and human health.[45] Herbal medicines contaminated with heavy metals pose a global threat to humans, particularly at levels beyond established threshold concentrations.[46] Even at extremely low

quantities, heavy metals can still be harmful to human health, according to the World Health Organization.[47] Quantitative analysis of the content of heavy metal in bark of *C. cassia* was not detected which indicated that the drug was free from heavy metal contamination. The results for heavy metal analysis are shown in Table 2.

Table 2: Heavy Metals Estimation**.**

S.N.	Heavy metals		WHO Limits
1	Arsenic	Not Detected	10 ppm
2	Cadmium	Not Detected	0.3 ppm
3	Lead	Not Detected	3.0 ppm
4	Mercury	Not Detected	1.0 ppm

Aflatoxins

Molds especially *Aspergillus parasiticus, A. flavus.* and *A. nomius* produce a potent hepatic carcinogen secondary metabolites knows as aflatoxins, which can cause a serious threat for both animals and humans . The results reveal that there are no aflatoxin contents (B1, B2, G1, and G2) in drug samples. The analysis of aflatoxins in the drug has been presented in Table 3.

Table 3: Aflatoxins analysis.

S.N.	*Aflatoxins*	Results
1.	$B_{1:}$	Not Detected
2.	$B_{2:}$	Not Detected
3.	G_1	Not Detected
4.	G2	Not Detected

Microbial load

Medicinal pants often carry a number of microbiota (bacteria, molds), which are often soil origin. Moreover, unscientific harvesting methods, inadequate cleaning and drying, and inappropriate storage may also cause contamination.[46] Microbial load analysis help in determining if the spoilage causing microorganisms are within acceptable range. In present study, the total aerobic microbial count and fungal count as well as the total Enterobacteriaceae count were analysed. The analysis for the presenceof bacterial account especially for *Escherichia coli*, *Salmonella spp.*, *Shigella*, and *Pseudomonas aeruginosa* and *Staphylococcus aureus* were also performed. The analysis resulted that the drug is free of any microbial growth and safe for human consumption as shown in Table 4.

Table 4: Microbial load.

Total aerobic bacterial Count (TABC):	< 10 CFU/gm
Total yeast and molds count (TYMC):	< 10 CFU/gm
Enterobacteriaceae members	
Escherichia coli	ND
Salmonella sp.	ND
Shigella sp.	ND
Klebsiella sp.	
Specific objectionable pathogens	
Pseudomonas aeruginosa	ND
Staphylococcus aureus	ND
Candida albicans	ND
Aflatoxin producing fungi	
Aspergillus flavus	ND
Aspergillus parasiticus	ND

Pesticide residue

According to WHO, pesticide residues in any herbal drugs must be in permissible limits. The analysis of pesticide residue in bark of *C. cassia* is confirmed the below the quantification limit. The pesticide residues results are given in Table 5.

Table 5: Pesticide Residue Analysis: (Analyzed by Thermo Fisher TSQ 9000 Triple Quadrupole GC-MS/MS system).

S.No	Pesticide	Result(mg/Kg)	Permissible limit(mg/Kg)
1.	Alachlor	BLQ	0.02
2.	Aldrin (Aldrin and dieldrin combined expressed as dieldrin)	BLQ	0.05
3.	Azinophos-methyl	BLQ	1.0
4.	Bromopropylate	BLQ	3.0
5.	Chlordane (cis, tans and oxychlordane)	BLQ	0.05
6.	Chlorfenvinphos	BLQ	0.5
7.	Chlorpyrifos	BLQ	0.2
8.	Chlorpyrifos-methyl	BLQ	0.1
9.	Cypermethrin (and isomers)	BLQ	1.0
10.	DDT (all isomers, sum of p,p'-TDE (DDD) expressed as DDT)	BLQ	1.0
11.	Deltamethrin	BLQ	0.5
12.	Diazion	BLQ	0.5
13.	Dichlorvos	BLQ	1.0
14.	Dithiocarbamates (as CS2)	BLQ	2.0
15.	Endosulphan (sum of isomers &Endosulphan sulphate)	BLQ	3.0
16.	Endrin	BLQ	0.05
17.	Ethion	BLQ	2.0
18.	Fenitrothion	BLQ	0.5
19.	Fenvalerate	BLQ	1.5
20.	Fonofos	BLQ	0.05
21.	Heptachlor (sum of Heptachlor & Heptachlor epoxide)	BLQ	0.05
22.	Hexachlorobenzene	BLQ	0.1
23.	Hexachlorocyclohexane isomer (other than γ)	BLQ	0.3
24.	Lindane (γ – Hexachlorocyclohexane	BLQ	0.6
25.	Malathion	BLQ	1.0
26.	Methidathion	BLQ	0.2
27.	Parathion	BLQ	0.5
28.	Parathion methyl	BLQ	02
29.	Permethrin	BLQ	1.0
30.	Phosalone	BLQ	0.1
31.	Piperonyl butoxide	BLQ	3.0
32.	Pirimiphos methyl	BLQ	4.0
33.	Pyrethrins (sum of isomers)	BLQ	3.0
34.	Quintozen (sum of Quintozene, pentachloroaniline and methyl pentachlorophenylsulphide)	BLQ	1.0

***BLQ** – Below limit of quantification

CONCLUSION

Standardizing drugs means confirming their identity and determining their quality and purity. The herbal materials are being used world wide as effective remedies since they exhibit less adverse effects.The present study can be concluded that the macro-microscopic findings along with physical chemical parameters together will help future investigators in proper identification of the bark of *C. cassia*. The study sets the specific macro-morphology, micro-morphology, organoleptic tests, physicochemical evaluations and HPTLC parameters to identity *C. cassia* stem bark which will certainly assurance the quality of crude drug. Subsequently, it will help in standardization of various traditional herbal formulations of Unani, Siddha and Ayurveda containing *C.cassia* bark as an ingredient.

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