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Pharmacognostic Studies of Selected Species of *Curcuma* L. a Medicinally Important Member of the Family Zingiberaceae

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A B S T R A C T

Present investigation deals with the pharmacognostic studies of some medicinally important species of *Curcuma* L. namely *Curcuma amada* Roxb., *Curcuma aromatica* Salisb., *Curcuma caesia* Roxb., *Curcuma longa* L. and *Curcuma zedoaria* (Christm.) Roscoe. Distinct variation was observed during foliar epidermal micromorphology study. Epidermal cell shape was found distinctly variable though cell wall outline was found to be straight in all the selected species. Leaves of the investigated taxa were found to be amphistomatic and the stomata are of tetracytic type. Stomatal index ranges from 13.54 (*Curcuma longa*) to 3.25 (*Curcuma zedoaria*). Trichomes were found to be present on both the epidermal surfaces of all the investigated taxa except *Curcuma caesia* where trichomes were found to be restricted to the upper epidermal surface. Petiole outline is almost concavo- convex in T.S. and the number of vascular bundle were ranges from 57 (*Curcuma longa*) *to* 39 (*Curcuma caesia*). Colour of the powdered crude drug varies from colourless to bright yellow. Textures of the fresh powders were smooth and fibrous. Moisture content ranged from 8.02 % (*Curcuma longa*) to 14.53 (*Curcuma amada*). Total ash value ranged from 3.91% to 5.49%. Microchemical colour reaction test of the ethanolic plant extracts showed the presence of important phytochemical groups like- alkaloids, flavonoids, proteins, saponins reducing sugars etc.

Introduction

Medicinal plants are the fundamental components of any indigenous medicine system. The use of different plant species for the treatment of different kinds of health hazards is the oldest form of health care system acknowledged in human civilization all over the globe (Fransworth, 1994), and presently more than 80% of the world populations, mostly of the third world countries depend on herbal medication for their primary health care (WHO, 1993). The genus *Curcuma* L. belonging to the family Zingiberaceae, originated in the Indo – Malayan region (Purseglove, 1974) has a wide spread occurrence in the tropics of Asia to Africa and Australia.

The genus globally consists of about 80 species of which 40 are reported from India (Sashikumar, 2005). The word Curcuma derived from the Arabic word "Kurkum" which means yellow colour, it is mainly due to the yellow colour of the underground rhizome. *Curcuma* is known as the golden spies of India.

The rhizome is vegetative in nature as well as the propagating part which is traditionally used in medicine and food since ancient times (Srimal, 1997). The use of different *Curcuma* species has been given in the vedic culture of India, nearly 4000 years back.

It reached China before the 7th century, East Africa in the 8th century and West Africa in the 13th century. This multipurpose ancient and sacred spice of India, also known as '*Indian Saffron*', finds a place in offerings on religious and ceremonial occasions (Khan *et al.,* 2014).

India is the largest producer and exporter of turmeric in the world accounting for more than 50% of the world's trade; fulfilling 90% of world's demand (APEDA, 2018; Olojede *et al.,* 2009).

Turmeric occupies about 6% of the total area under spices and condiments in India, and has considerable importance in Indian economy (Choudhury, 2018). Turmeric has already gained importance all over the world for therapeutic uses owing to its anti-inflammatory, anti-diabetic, anti-carcinogenic, anti-hepatotoxic, anti-viral, choleretic , antibiotic, anti-venomous and anti-rheumatic properties (Singh and Aggarwal, 2003; Wilken *et al.,* 2011; Lin *et al.,* 2008; Kaypee *et al.,* 2015) and is frequently used in ayurvedic medicine of India as '*haridra*'. Pharmacognostic, phytochemical and antimicrobial activity studies of medicinal plants have been carried out by many workers earlier for proper identification and standardization of natural products obtained from respective medicinal plants (Choudhury *et al.,* 2012; Choudhury *et al.,* 2013; Pal and Rahaman 2014; Ghosh and Rahaman 2015).

Though a good number of quality research has been undertaken on the therapeutic values and bioprospecting of *Curcuma* species mostly concentrated on the species *Curcuma longa* L. Research work relating to pharmacognostic, phytochemical studies of different species of the genus *Curcuma* L. are limited in number.

Therefore, the present investigation has been undertaken to study the pharmacognostic potential of these medicinal plant which will be very helpful in proper identification and standardization of the natural products obtained the plant.

Materials and Methods

Five medicinally important species of the family Zingeberaceae namely *Curcuma amada* Roxb., *Curcuma aromatica* Salisb., *Curcuma caesia* Roxb., *Curcuma longa* L. and *Curcuma zedoaria* (Christm.) Roscoe. have been selected for this study.

Plant specimens of those five selected taxa have been collected from the Department of Botany, University of North Bengal, West Bengal and have been identified by Prof. A. P. Das of the Department of Botany, North Bengal University.

Those plant specimens have been grown and maintained in the medicinal plant garden of Department of Botany, Visva-Bharati, Santiniketan, and the voucher specimens have been kept in Visva-Bharati Herbarium, Department of Botany, Visva- Bharati, Santiniketan, India for future reference.

Scientific name, Local name and Medicinal uses of the selected plant species have been presented in Table 1.

Epidermal micromorphology

Leaf samples were cleared following the Bokhari's method (1970). The cleared leaf samples then mounted on the slide with a drop of 10% glycerine & 1% aqueous safranin and observed under the compound light microscope.

Vegetative anatomy

Vegetative anatomy method was carried out following the standard methods of Johansen (1940).

Preliminary microchemical screening

Detection of different phytochemical groups was carried out by following different standard methods (Evans, 1996; Harbrone, 2002).

Physical evaluation

Physical constant

The physical evaluation of the powder was done following different methods (Peach and Tracy, 1955, Evans 2008) which includes determination of ash value and moisture content.

Histochemical study

Few drops of different reagents (Mayre's reagent, Wagnor's reagent, Lugols reagent, Phloroglucinol, lead acetate) were added to the thin sections of the rhizome of the investigated species and then those sections were observed under light microscope. These tests were done to detect different phytochemicals localized in different tissue zones of the rhizome (Trease and Evans, 1983).

Results and Discussion

General description and measurement of epidermal cells, stomata, microchemical colour reaction tests, physical parameters, histochemical localization tests of the investigated plant species were given below (Tables 2, 3, 4, 5 and Fig. 1–13).

Foliar micromorphology

Epidermal cell shape was found to be irregular in *Curcuma amada*, hexagonal in case of *Curcuma aromatica* and *Curcuma longa* though the shape was found to be polygonal in case of *Curcuma caesia* and *Curcuma zedoaria.* Cell wall outline was found to be straight in both the epidermal surfaces in all the selected species. Epidermal cell length was ranging from 82.61 µm (upper surface of *Curcuma longa*) to 41.46 µm (upper surface of *Curcuma amada*). Epidermal cell width was highest on upper surface of *Curcuma aromatica* (40.34 µm) and lowest in case of upper surface of *Curcuma amada* (24.67 µm). Epidermal cell frequency was found to be highest in upper surface of *Curcuma amada* (977.69/mm^2) and lowest in lower surface of *Curcuma zedoaria* (352.85/mm^2). Pallisade ratio was highest in *Curcuma amada* (14.21) and lowest in *Curcuma caesia* (6.2) (Table 2).

Stomatal features were also studied and all the investigated taxa showed amphistomatic type of stomata which are of tetracytic type. Stomatal length ranged from 38.13 µm (upper surface of *Curcuma zedoaria*) to 19.32 µm (lower surface of *Curcuma amada*) and the width of the stomatal ranges from 21.64 µm (*Curcuma aromatica* upper surface) to 11.76µm (lower surface of *Curcuma amada*). Stomatal index was ranging from 13.54%

(lower surface of *Curcuma longa*) to 3.25% (upper surface of *Curcuma zedoaria*). Highest stomatal frequency was found in the lower surface of *Curcuma zedoaria* (163.52/mm^2) and lowest at both upper surface of *Curcuma aromatica* (28.20 /mm^2) (Table 3).

Trichomes were found to be present on both the epidermal surfaces of all the investigated taxa except the lower surface of *Curcuma caesia*. Trichomes are of unicellular, non glandular type with swollen base and pointed tip in all the investigated taxa. Trichome length ranged from 218.2 µm (upper surface of *Curcuma aromatica*) to 94.93 µm (lower surface of *Curcuma zedoaria*).

Trichome frequency was highest on the lower surface of *Curcuma longa* (13.45/mm^2) and lowest on the upper surface of *Curcuma amada* (4.29/mm^2). Highest trichome index was found at upper surface of *Curcuma amada* (12.32%) and lowest was at lower surface of *Curcuma zedoaria* (3.48%) (Table 4).

Petiole anatomy

T.S. of the petiole revealed more or less similar type of histological features in petiole shape, outline and orientation of vascular bundle. Shape of the petiole is more or less concavo-convex in T.S. Petiole is surrounded by unicellular epidermal layer followed by a massive parenchymatous ground tissue, composed of thin walled cells.

Various numbers of air space were noted in the ground tissue. But, a distinct variation was recorded in the number of vascular bundle in the T.S of the petiole.

Highest number of vascular bundle was observed in *Curcuma longa* (57 Nos.) and lowest number of vascular bundles were observed in *Curcuma caesia* (39 Nos).

Organoleptic study of powdered plant parts

Crude drugs obtained from the powdered rhizome pieces were evaluated with different sensory organs are tested for the colour, odour, taste and texture which are listed below (Table 5).

Physical parameters

Moisture contents were also studied among the investigated taxa and they were found to be very distinct. Lowest value of moisture content was found in case of *Curcuma longa* (8.02%) and the highest value was observed in case of *Curcuma amada* (14.53 %). Total ash-value ranged from 3.91% to 5.49% and the value was very diverse among the investigated species. Lowest ash value was found in case of *Curcuma zedoaria* (3.91%) and the highest value was observed in *Curcuma aromatica* (5.49%) (Table 6).

Microchemical colour reaction test

Through microchemical colour reaction tests of the ehanolic plant extracts, important phytochemical groups like alkaloids, flavonoids, proteins, saponins etc have been detected which indicated the medicinal properties of the selected plant species (Table 7).

Histochemical study

Histochemical study has been carried out to detect various phytochemical groups localized in different tissue zones of the rhizomes. Different phytochemical groups like alkaloids, proteins, flavonoids, lignin, saponins etc. were found in different tissue zones of the rhizome. It has been found that the connective tissues and cortical zones are main active sites for synthesis of high content of different phytochemical groups (Table 8).

Table.1 Materials and their medicinal importance incorporated in the present investigation (Anonymous 2005)

Sl No.	Scientific name	Local name	Medicinal importance of different plant parts
1	*Curcuma amada* Roxb.	Aam aada	Rhizome: Used to treat pruigo, sprains, itch, skin disease, bronchitis, asthama, hic cough; crushed in water and juice taken in empty stomach as carminative and to improve appetite. Root: Used to treat diarrhoea, gleet, lumbago, scabies and ulcer on penis.
2	*Curcuma aromatica* Salisb.	Ban halud, Gyan churamani	Rhizome: Used to treat scabies, eruption of small pox, and headache, paste applied to affected parts as antidotes to snake bite. Root: Paste with Kujri oil as massage to cool down high fever. Leave: Decoction with honey given to treat dropsy.
3	*Curcuma caesia* Roxb.	Kalo halud, Kala haldi	Rhizome: Purifies blood; used as tonic to brain and heart; used to treat leucoderma, piles, bronchitis, asthama, tumours, tuberculous glands on the neck, enlargement of the spleen, to check leucorrhal and gonorrhoeal discharges. Root: Fine paste of rhizomes and roots applied on the affected parts on the body as an antidote to snake bite.
4	*Curcuma longa* L.	Halud, Haldi	Rhizome: Used to treat disease of the blood, leucoderma, scabies, urinary discharges, inflammation , ozonea, biliousness, dyspepsia, elephantiasis, small pox, swellings, boils, catarrh, purulent opthalmia, diarrhoea, interminnet fever, dropsy, bronchitis , paste with mustard oil applied on skin disease. Leave: Juice mixed with water given twice daily to treat dysentery.
5	*Curcuma zedoaria* (Christm.) Roscoe	Sathi, Palo	Rhizome: Used to treat asthama, bronchitis, piles, leucoderma, tumours, tuberculous glands on the neck, enlarged spleen, gripping of children, pains inflammation, tooth aches, bruises, sprains ; to cure weakness after childbirth; to purify blood; given to treat intestinal worms of children. Root: Decoction taken to treat diarrhoea and piles. Leave: Used to treat dropsy.

Table.2 Epidermal cell characters of the investigated plant species

Investigated taxa	Leaf surface	Cell shape	Cell length (µm)	Cell width (µm)	Cell frequency (No./mm^2)	Cell wall out line	Palisade ratio
C. amada	Upper	Irregular	41.46	24.67	977.69	Straight	14.21
	Lower	Irregular	43.65	29.72	770.84	Straight	
C. aromatica	Upper	Hexagonal	61.12	40.34	405.58	Straight	12.5
	Lower	Hexagonal	57.83	37.91	456.13	Straight	
C. caesia	Upper	Polygonal	46.27	29.28	738.12	Straight	6.2
	Lower	Polygonal	49.18	33.27	611.16	Straight	
C. longa	Upper	Hexagonal	52.41	30.1	633.89	Straight	11.73
	Lower	Hexagonal	48.73	34.37	597.06	Straight	
C. zedoaria	Upper	Polygonal	82.61	31.07	389.60	Straight	7.2
	Lower	Polygonal	79.81	35.51	352.85	Straight	

Table.3 Stomatal features of the investigated species

Investigated taxa	Leaf surface	Stomatal type	Stomatal length (µm)	Stomatal width (µm)	Stomatal index (%)	Stomatal frequency (No./mm^2)
C. amada	Upper	Tetracytic	27.44	12.34	8.10	61.01
	Lower	Tetracytic	19.32	11.76	13.40	76.04
C. aromatica	Upper	Tetracytic	36.62	21.64	6.08	28.20
	Lower	Tetracytic	31.33	20.07	7.44	35.67
C. caesia	Upper	Tetracytic	30.06	15.64	9.55	60.60
	Lower	Tetracytic	28.63	13.72	12.26	66.75
C. longa	Upper	Tetracytic	36.20	20.31	11.11	51.36
	Lower	Tetracytic	34.69	19.77	13.54	63.23
C. zedoaria	Upper	Tetracytic	38.13	14.88	3.25	146.39
	Lower	Tetracytic	34.76	19.72	7.16	163.52

Table.4 Trichome features of the investigated taxa

Investigated taxa	Leaf surface	Trichome type	Trichome size (µm)	Trichome frequency (No./mm^2)	Trichome Index (%)
C. amada	Upper	Nonglandular, unicellular with swollen base and pointed tip	157	4.29	6.38
	Lower		164.8	12.31	12.32
C. aromatica	Upper	Nonglandular, unicellular with swollen base and pointed tip	218.2	6.42	4.86
	Lower		157.3	10.14	8.54
C. caesia	Upper	Nonglandular, unicellular with swollen base and pointed tip	131.73	11.79	5.2
	Lower	Absent	-------	--------	--------
C. longa	Upper	Nonglandular, unicellular with swollen base and pointed tip	164.37	9.34	7.64
	Lower		132.82	13.45	6.51
C. zedoaria	Upper	Nonglandular, unicellular with swollen base and pointed tip	104.89	5.89	4.97
	Lower		94.93	4.72	3.48

Table.5 Findings of the organoleptic study among the investigated taxa

Investigated taxa	Plant parts	Colour	Odour	Taste	Texture
C. amada	Rhizome	Pale yellow	Fresh, slightly rosy with a fruity top note of raw mangoes	Sweet, mildly spicy	Smooth
C. aromatica	Rhizome	Greenish brown	Pungent, warm, woody-rooty with a camphory top note	Bitter, flat earthy and mildly spicy	Fibrous
C. caesia	Rhizome	Colour less, become blue on exposure to air	Warm, mildly spicy, camphoraceous with woody top note	Slightly bitter, warm with a spicy after taste	Fibrous
C. longa	Rhizome	Bright yellow	Spicy, mildly earthy, smooth warm with a rooty top note	Pleasantly warm with a spicy after taste	Smooth
C. zedoaria	Rhizome	Light yellow	Warm, penetrating, pleasant, camphoraceous with a flowery top note	Slightly bitter with a spicy after taste	Smooth

Table.6 Comparison of ash value and moisture content of the investigated taxa

Investigated taxa	Ash value			Moisture content
	Total ash (%)	Water soluble ash (%)	Acid insoluble ash (%)	Loss on drying (%)
C. amada	5.19	1.28	1.06	14.53
C. aromatica	5.49	1.37	2.07	9.37
C. caesia	3.99	0.96	1.83	12.43
C. longa	4.08	1.58	2.02	8.02
C. zedoaria	3.91	1.42	1.07	9.19

Table.7 Microchemical tests of the investigated species

Test/ Reagent	Test for	Nature of change	*C. amada*	*C. aromatica*	*C. caesia*	*C. longa*	*C. zedoaria*
Dragendroff's reagent	Alkaloids	Orange brown ppt	+	++	++	++	++
Wagner's reagent	Alkaloids	Orange brown ppt	++	+	+	+	++
Mayer's reagent	Alkaloids	White/cream ppt	+	++	++	++	++
Shinoda test	Flavonoids	Magenta colour	+	--	+	+	--
10% NaOH	Flavonoids	Yellow colour	+	+	+	++	_
Salkowski test	Steroids and Triterpenoids	Reddish blue and green fluorescence	++	++	--	++	+
Benedict's reagent	Reducing sugars	Brick red ppt	_ _	+	+	++	++

Fehling's reagent	Reducing sugars	Brick red ppt	_ _	+	+	++	+
Molish's test	Gums	Red violet ring	_	+	-	+++	+
10% K_2Cr_2 O_7 solution	Tannins	Yellow brown ppt	+	++	++	++	++
5% $FeCl_3$ solution	Tannins	Greenish black ppt	_	_ _	++	+	+
1% lead acetate solution	Saponins	White ppt	+	+++	--	+	+
Bentrager's test	Anthraquinones	Pink colour	_	_ _	--	_	++
Ninhydrine test	Amino acids	Purple colour	_	+++	+	_	--
Phloroglucinol test	Lignin	Red colour	+	++	+	+	++
Millon's reagent	Proteins	White ppt.	++	++	++	++	++
Lugols	Proteins	Faint yellow colour	++	++	++	++	++

Table.8 Findings of the histochemical localization of the investigated taxa

Investigated taxa	Alkaloid	Tannin	Protein	Lignin	Flavonoid	Reducing sugar
C. amada	Outer cortex, few cells of inner cortex, vascular bundle	Outer cortex, xylem	Few cortical cells and phloem tissue	Endodermis	Pith and phloem	Xylem tissue and inner cortical cells
C. aromatica	Vascular bundle	Few cells of cortex	Epidermis, few cells of hypodermis and cortex	Not detected	Phloem tissue	Not detected
C. caesia	Few cells of xylem and pith	Pith and cortex cells	Xylem cells	Vascular bundles	Few cells of xylem	Pith zone
C. longa	Few cells of pith and cortex	Few cells of pith and cortex	Few cells of pith and cortex	Few cells of pith and cortex	Few cells of pith and cortex	Few cells of xylem tissue
C. zedoaria	Outer cortex and xylem tissue	Few cells of xylem, pith and cortex	Vascular bundle	Endodermis and xylem tissue	Phloem zone	Few cells of cortex and endodermis

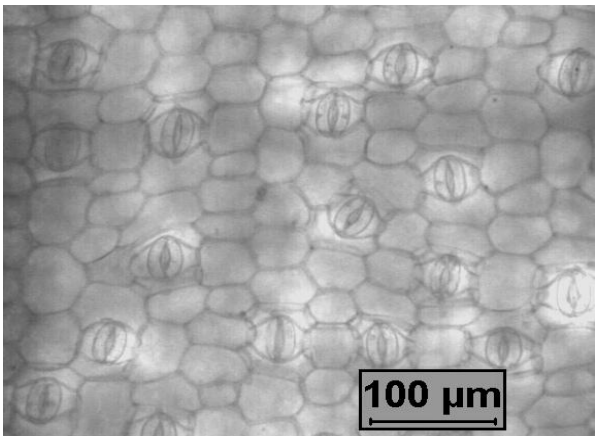

Fig.1 Foliar epidermal micromorphology of *Curcuma aromatica*

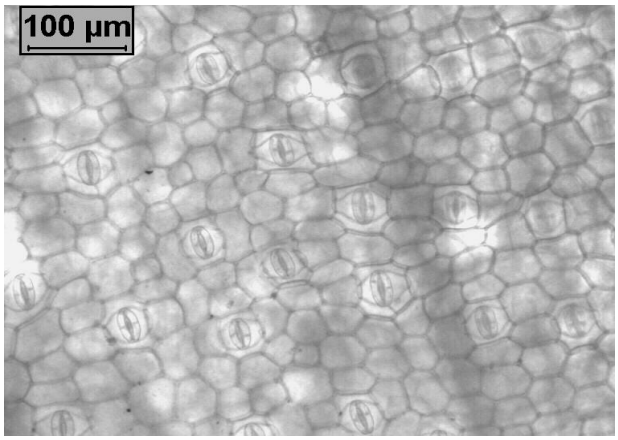

Fig.2 Foliar epidermal micromorphology of *Curcuma caesia*

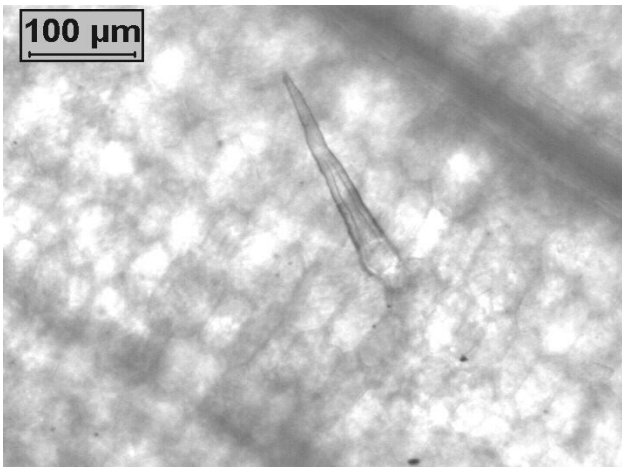

Fig.3 Trichomes of *Curcuma zedoaria*

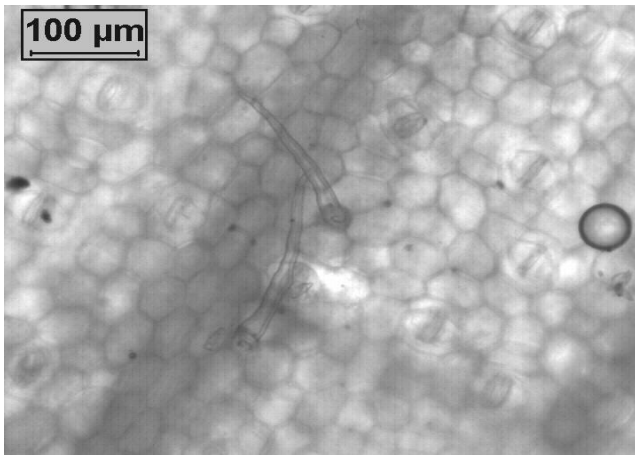

Fig.4 Trichomes of *Curcuma aromatica*

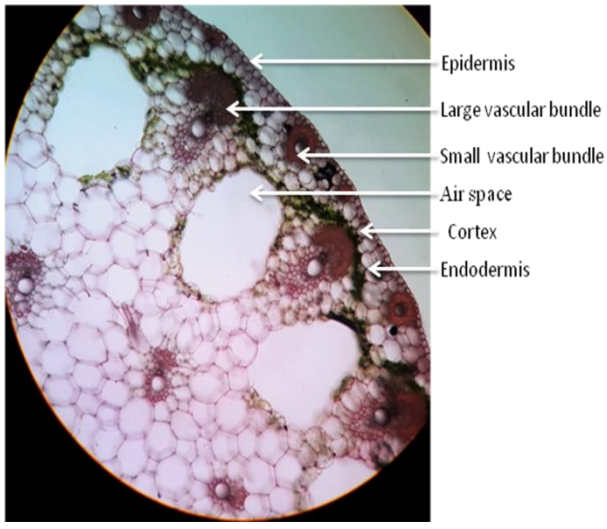

Fig.5 T.S. through the petiole of *Curcuma amada*

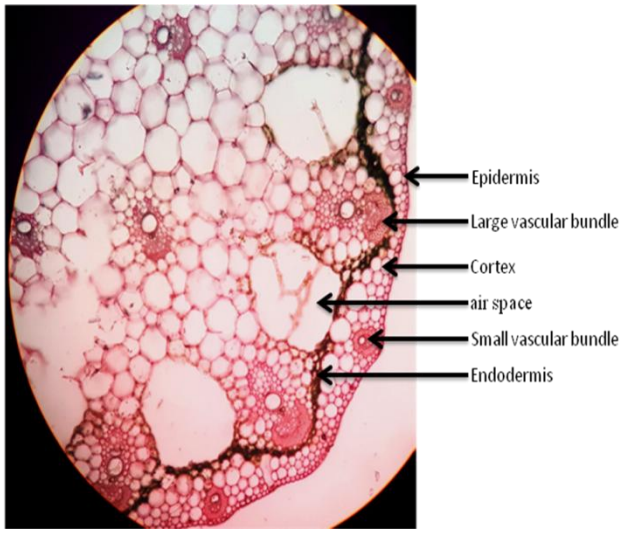

Fig.6 T.S. through the petiole of *Curcuma aromatica*

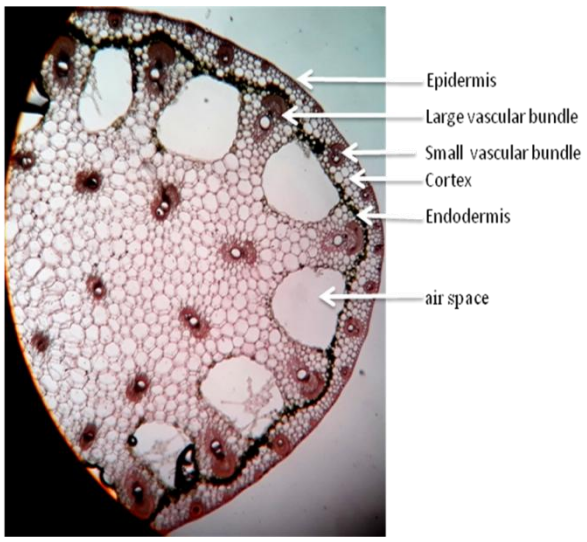

Fig.7 T.S. through the petiole of *Curcuma caesia*

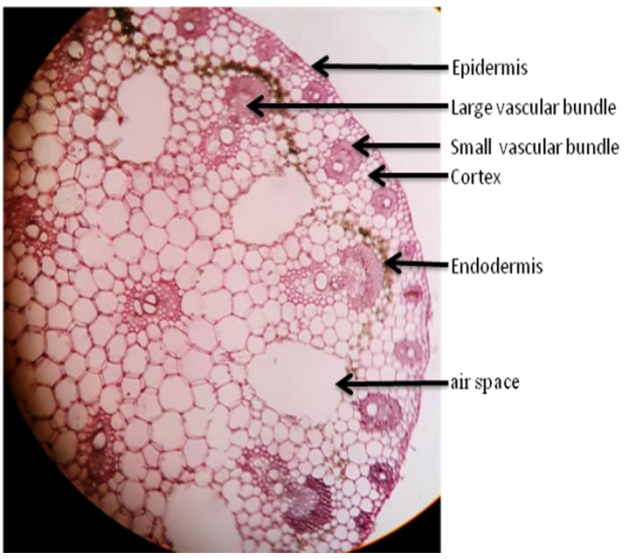

Fig.8 T.S. through the petiole of *Curcuma longa*

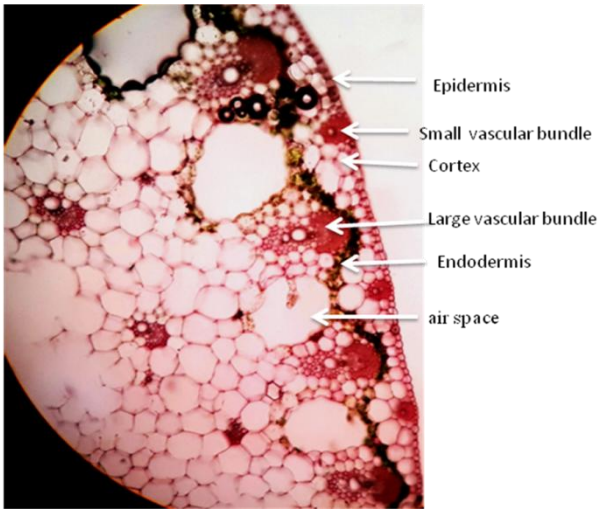

Fig.9 T.S. through the petiole of *Curcuma zedoaria*

Fig.10 Crude drug from *Curcuma amada* Rhizome

Fig.11 Crude drug from *Curcuma aromatica* Rhizome

Fig.12 Crude drug from Curcuma *longa* Rhizome

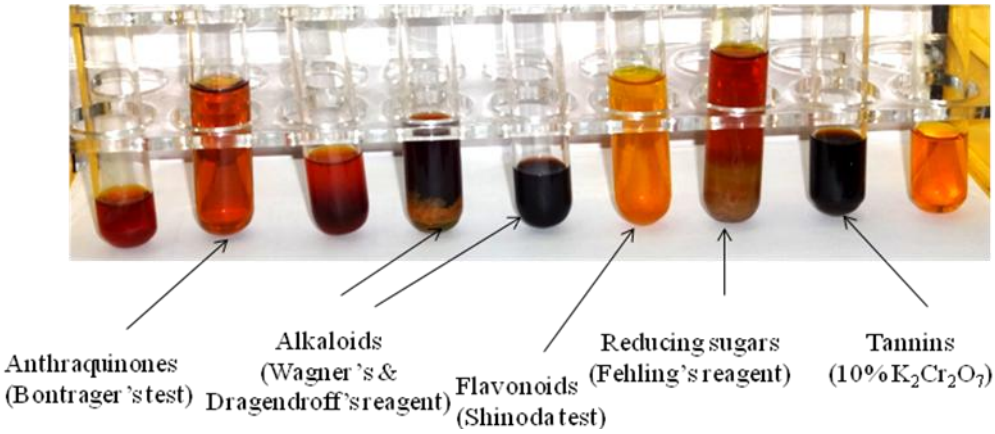

Fig.13 Microchemical colour reaction test of ethanolic extracts of *Curcuma longa* (Rhizome)

Total ash value is very diverse among the investigated species. Lowest ash value was recorded in *Curcuma zedoaria* (3.91 %) and the highest value was observed in *Curcuma aromatica* (5.49%).

These results indicate that the selected plant species are rich in mineral contents. Acid insoluble and water soluble ash determination is important, because it indicates the quality and purity of the crude drug (Dev *et al.*, 2015). Hence the physical parameters indicate the therapeutic potential of the crude drugs obtained from the selected plant species.

The phytochemical groups like alkaloids, flavonoids, proteins, saponins are present among the investigated species. A variety of biological activities like antioxidant, CNS stimulatory, antihelminthic, anti-hypertensive, anti-malarial, anti-diabetic, anti-rheumatic, anti-cancerous, anti-inflammatory, antimicrobial effects of various types of phytochemical groups have been critically studied by many workers earlier (Sinha *et al.*, 2013; Obouayeba *et al.*, 2014; Kumar *et al.*, 2012; Hegde *et al.*, 2010; Pal *et al.*, 2020; Prakash *et al.*, 2009). Flavonoids from different plant sources have been reported to have anti-inflammatory, anti-arthritic and anti-oxidant activities. Plants containing high amount of flavonoids were found to have more therapeutic values (Nagarkar *et al.*, 2013). Through phytochemical screening it has been found that the selected plant species are of possessing adequate quantity of flavonoids.

From the above mentioned findings following key to the identification of the investigated taxa may be deduced

1. Trichomes are restricted to the upper epidermal surfaces …….. 2
2. Epidermal cells are irregular in shape *C. amada*
2. Epidermal cells are polygonal in shape *C. caesia*

1. Trichomes are present on both the epidermal surfaces …….3
3. Epidermal cells are hexagonal in shape……..... 4
4. In petiole T.S., number of vascular bundle 50 and number of air space 15 *C. aromatica*
4 In petiole T.S., number of vascular bundle 57 and number of air space 16 …….. *C. longa*
3. Epidermal cells are polygonal in shape *C. zedoaria*

This study will be very useful in proper identification of the selected plant species with the key described. It will be also useful for proper identification of crude drugs obtained from these selected genera of *Curcuma* L. along with detection of drug adulterants. Thus it will be a tool in maintaining the quality of crude drugs obtained from the selected plant species. Moreover, the pharmacognostic studies of the investigated taxa highlight the presence of some important phytochemical groups which indicate promising curative potentials of these medicinal plants which need further investigation to explore the novel bioactive compounds. Thus it can be concluded that the crude drug of the selected plant species are good candidates for bioprospecting.

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