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Research Article

Pharmacognostical Study and Quality Control Parameters of *Dillenia indica* Linn. and *Dillenia pentagyna* Roxb.: A Boon of Ethnomedicinal Herbs of India

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ABSTRACT

Dillenia indica Linn. and *Dillenia pentagyna* Roxb. are two plants which are found to be widely growing plants in many forest regions of India. The quality parameters are set for assuring the standards of plant species. Pharmacognostical and physicochemical parameters are developed which ensures the quality of drug as well as differentiate both plant species. Bark of both plants differentiated morphologically by its colour as well as texture while leaves of both plants differentiated by its size, shape. Microscopically, bark can be differentiated by presence of stone cells, cork, pigments and raphides of Ca-oxalate and leaves based on its trichomes, raphides etc. Physicochemical parameters (extractive values, ash values, foreign matter, moisture content) were determined to ensure quality of plants. Phytochemical screening is performed to have an idea about active phytoconstituents present in plants. Results of heavy metal and microorganism revealed that plants are safe to use further for separation of phytoconstituents. Phytochemical screening of different extracts of bark and leaves revealed the presence of sterols, flavonoids, phenolics etc.

Keywords: Folklore medicine, Dillenia indica, Dillenia pentagyna, WHO.

INTRODUCTION

Herbal medicines which can be used freely by the local community and are well known through long usage by local population in terms of its composition, treatment and dosage are indigenous herbal medicines. If medicines in this category enter in market, they have to meet requirements of safety and efficacy as per national regulations[1]. As the folklore medicines are evolved by the individual and ethnic experiences, it needs further investigations in stipulations of diverse branches of medical science to endeavor the issues like that of standardization, identification, pharmacology etc[2]. The isolation, identification of active principles and pharmacological studies of the active phytoconstituents may be considered and studied elaborately to treat effectively for various types of diseases. Although, a great amount of ethno medicinal research work has been undertaken in various pockets of tribal and rural population scattered throughout the country, more efforts are needed to enhance the utility as well as to explore concealed areas of these plants at global level.

Dillenia indica Linn. and *Dillenia pentagyna* Roxb. are two plants which are found to be widely growing and collected from Dang forest of Gujarat, India. The quality parameters need to be set for assuring the standards of collected species. It is therefore essential to follow internationally recognized guidelines for assessing their identity, quality and purity. Now a days many sophisticated modern research tools for evaluation of the plant drugs are available but pharmacognostical method is still one of the simplest and cheapest methods to start for establishing the correct identity of the source materials. The World Health Organization has described a series of tests for assessing quality of medicinal plant materials[3]. The present research work was conceived to standardize the bark and leaves of *D. indica* and *D. pentagyna* as per the WHO guidelines to determine the correct identity and purity of the plant part and for the detection of adulterant as well. Botanical authentication and physicochemical parameters developed ensures the quality of drug.

MATERIALS AND METHODS

Collection of plant samples: Bark and leaves of *Dillenia indica* and *Dillenia pentagyna* were collected from following places WAGHAI botanical garden, Dist. Dang, Gujarat, India.

Foreign matter in medicinal plant material: Foreign matter of bark and leaves of *D. indica* and *D. pentagyna* were perform using procedure described as per WHO[4].

Preparation of samples: The leaves and bark were dried under shade for 3 days. Bark powder was prepared using pulverizer and leaves using grinder individually. Powder was passed through 60# sieve to get uniform size of particles. It was stored in airtight containers and used for

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Table 1 Foreign organic matter in bark and leaves of both plants

Plant species	Part of plant	% w/w of foreign matter
D. indica	Bark	8.2 %
	Leaves	3 %
D. pentagyna	Bark	6.5 %
	Leaves	4 %

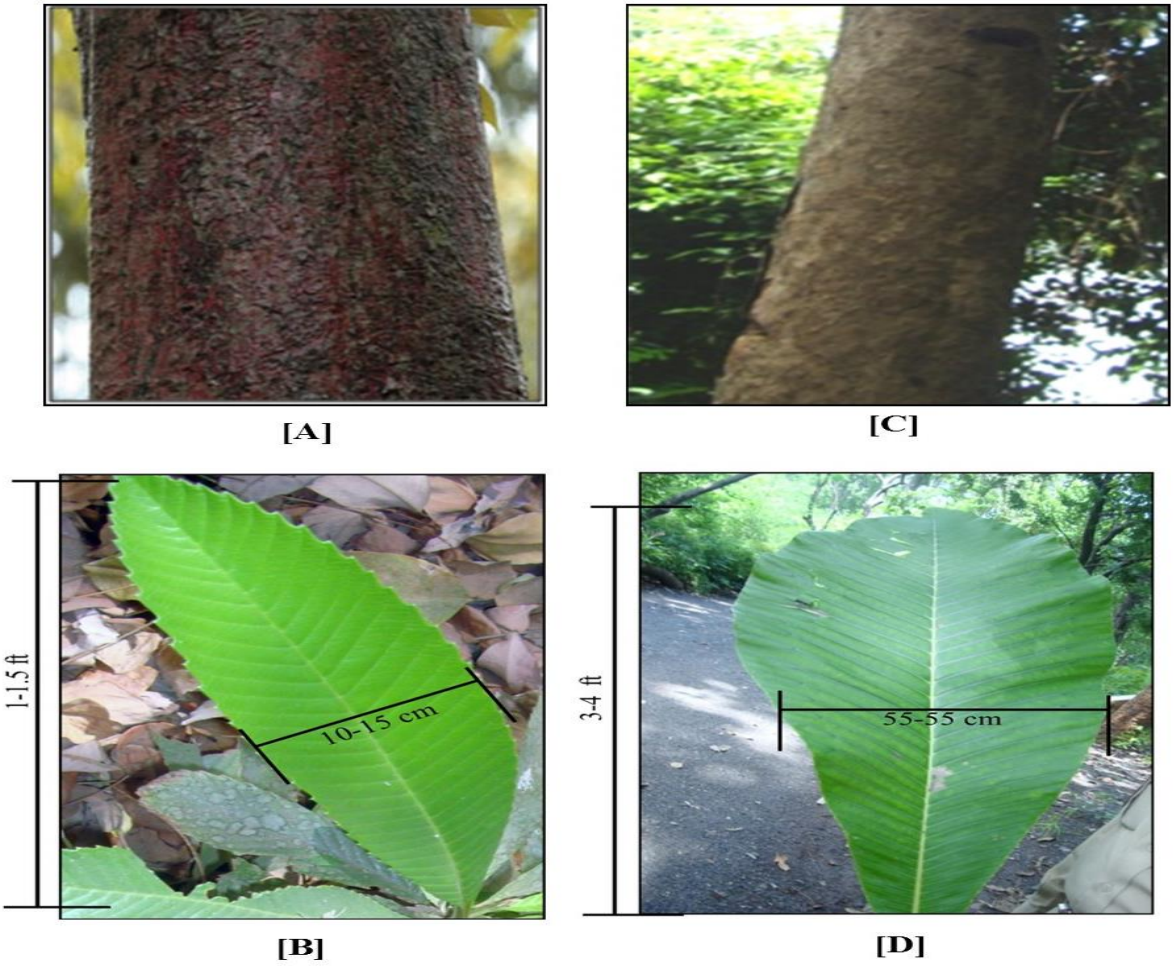

Fig. 1: Morphology of D. indica (A) bark and (B) leaf and D. pentagyna (C) bark and (D) leaf

Table 2: Pharmacognostical differences of bark of *D. indica* and *D. pentagyna*

	D. indica	*D. pentagyna*
Size	broken irregular pieces having thickness of 1.5 to 2 cm	broken irregular pieces having thickness of 0.9 to 1.3 cm
Colour	Upper surface: Reddish brown Lower surface: Reddish brown	Upper surface: Dark brown Lower surface: Brownish
Surface	Rough with lenticels and longitudinal striations	Rough with lenticels and longitudinal striations
Shape	Re curved/ Curved	Flat
Fracture	Fibrous	Fibrous to granular
Cork	Reddish brown polygonal shaped parenchymatous cells	Dark brown in colour polygonal shaped
Fibres	Long and narrow phloem fibres	Long and narrow phloem fibres
Stone cells	Rectangled shaped thick walled lignified pitted stone cells (21-40.3µ in Length ; 12.4-20 µ in width)	Rectangled shaped thick walled lignified pitted stone cells (52.3-46.3µ Length ; 15.6-23 µ in width)
Cortex	Parenchymatous cells of cortex brownish pigments	Parenchymatous cells of cortex yellow to orange pigments
Ca-oxalates	Needle shaped acicular Raphides	Needle shaped acicular Raphides

pharmacognostical, physicochemical and phytochemical studies.

Pharmacognostical studies

Macroscopical study: Freshly collected bark and leaves of *D. indica and D. pentagyna* were studied and identified by comparing their morphological characters mentioned in the literature.

Microscopical study: Powder of bark and leaves of *D. indica and D. pentagyna* were taken and unstained and stained slides were prepared to study presence of microscopical characters[5].

Physico-chemical evaluation of bark and leaves of *D. indica and D. pentagyna:* Proximate parameters has been performed for evaluation of collected plant species which

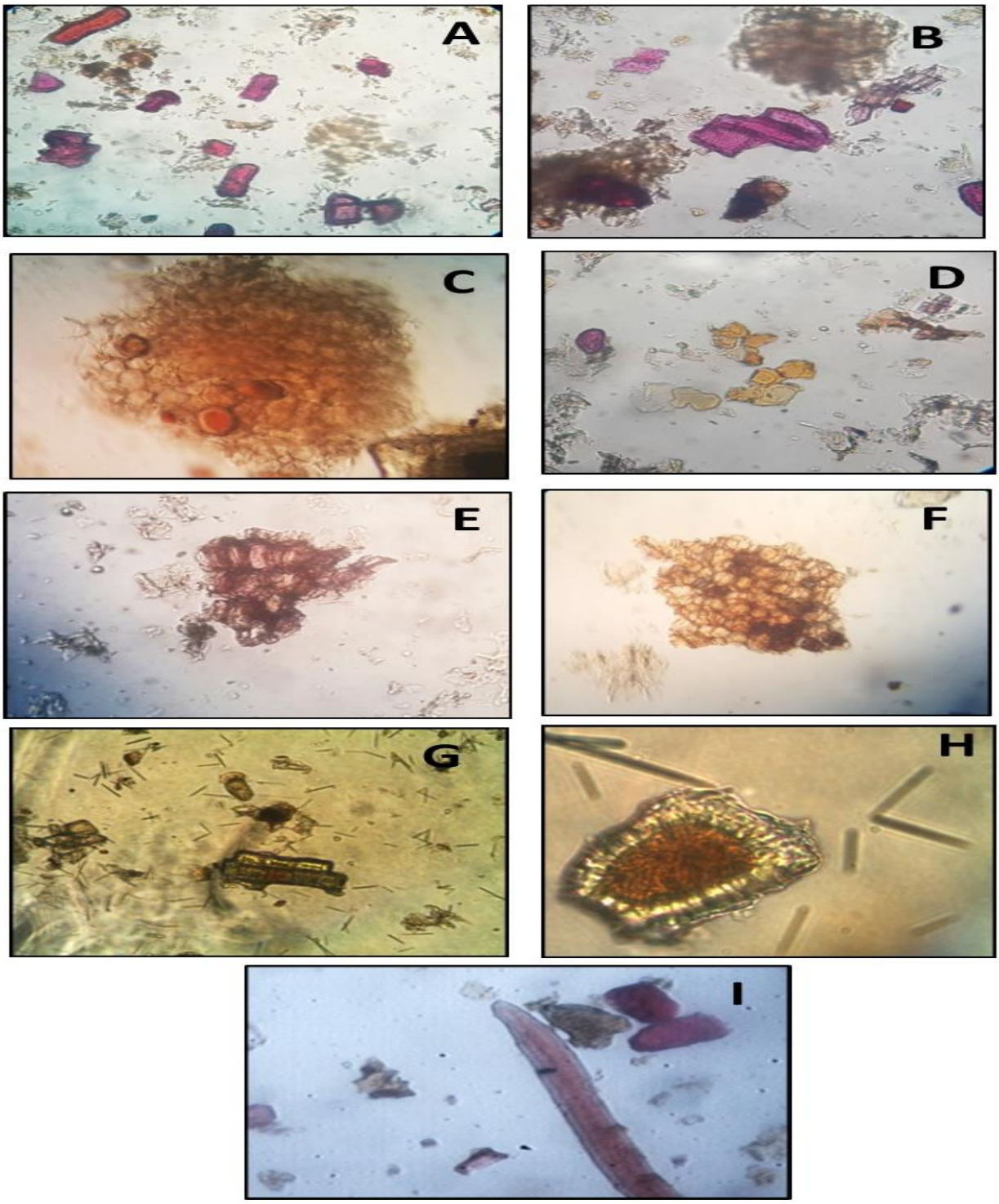

*Fig. 2**:** Microscopy of bark of D. indica and D. pentagyna [A- Stone cell (DIB) ; B- Stone cells (DPB); C- brownish pigments of DIB; D- yellowish pigment of DPB; E-Reddish brown cork (DIB) F- dark brown cork of DPB] [G- Raphides & stone cells; H-Raphides stone cells in (**45X**); I- Phloem fibre in both species]*

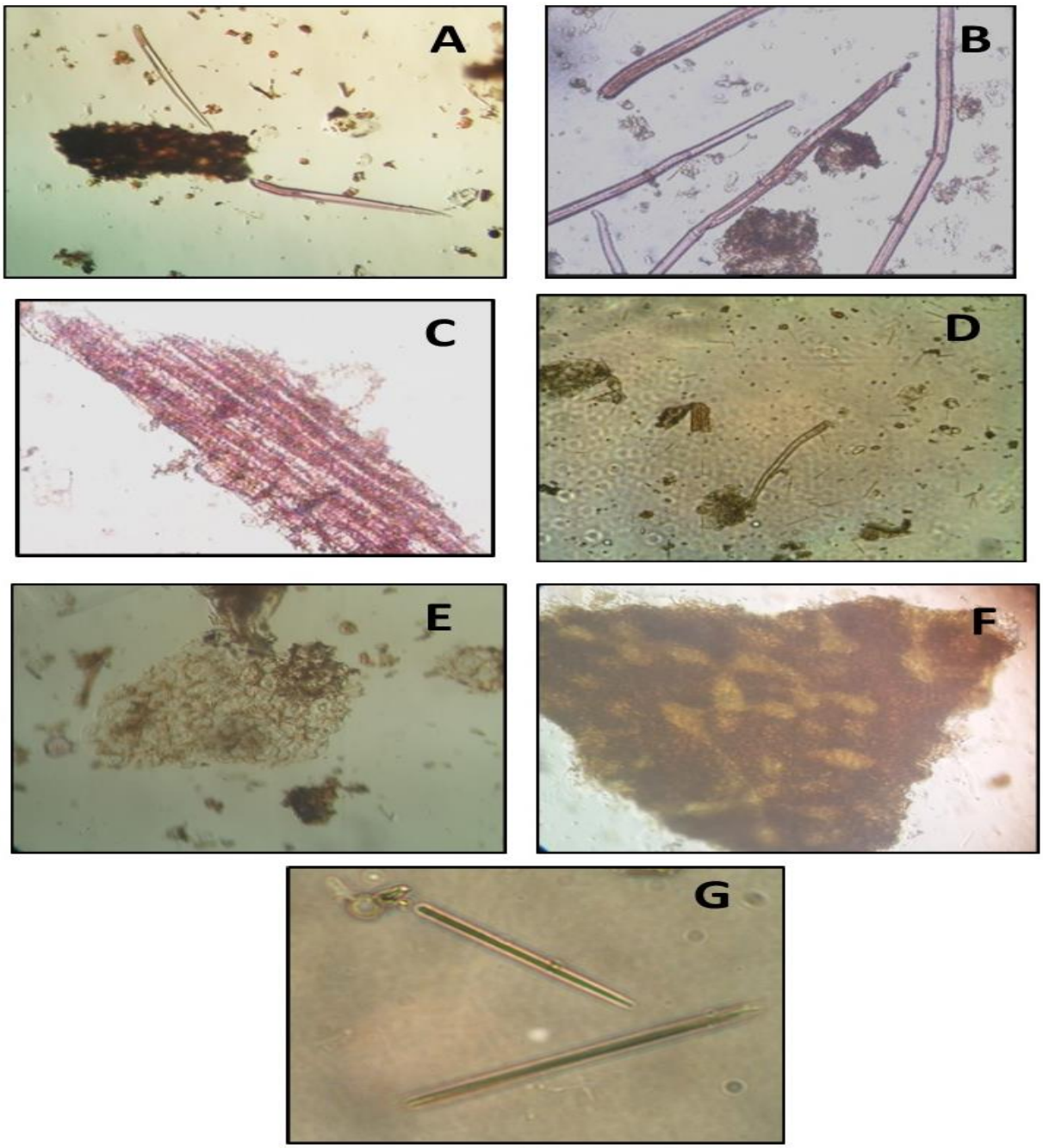

Fig. 3: Microscopy of leaf of D. indica and D. pentagyna [A- Lignified and unlignified trichomes D. pentagyna; B- lignified Trichomes in D. indica] [C- Pitted Xylem vessels; D- Raphides of Ca-oxalates; E- Anomocytic stomata; F- surface view of leaf; G- Raphides of Ca-oxalates in both species (45X)]

includes *Loss on drying, ash values and extractive values* were determined by the procedure described by WHO[8].

Determination of arsenic and heavy metals in raw material: Collected raw materials such as bark and leaves of both plants has been analyzed for heavy metal analysis using AAS. It has been have analysed for presence of Lead (as Pb), Cadmium (as Cd) as well as Arsenic (as As) as per AOAC official Method 999.11[9]. AOAC official Method 971.21 was followed for determination of Mercury (as Hg) in plant material. Results obtained were compared with limits set by WHO for each element[10].

Determination of microorganisms in raw material: Collected raw materials such as bark and leaves of both plants has been analyzed for yeast and mould count, total viable count and presence of *E. coli*. Results obtained were compared with limits for each microorganism.

Phytochemical screening of bark and leaves of *D. indica and D. pentagyna:* Different fractions has been prepared with increasing polarity of different solvents and checked for presence of phytoconstituents by performing phytochemical screening. Successive solvent extraction was done using petroleum ether, ethyl acetate extract, methanolic extract and water and taken for performing preliminary chemical tests. Each prepared extracts by successive extraction were analyzed for its physical properties. These were further subjected to the following

Table 3: Pharmacognostical differences of leaves of *D. indica* and *D. pentagyna*

	D. indica	*D. pentagyna*
Size	Around 1-1.5 ft in length and 10-15 cm wide	Around 3-4 ft in length and 45-55 cm wide
Colour	Upper surface: Greenish Lower surface: Pale greenish	Upper surface: Dark Greenish Lower surface: Pale greenish
Surface	Glabrous, shiny and hairy	Pale and Hairy
Shape	Lanceolate	Obovate
Apex	Acute	Obtuse
Margin	Serrate	Dentate
Venation	Reticulate	Reticulate
Base	Symmetrical	Symmetrical
Trichomes	Lignified trichomes (550-1360µ in Length)	Lignified and unlignified trichomes (250-760µ in length)
Stomata	Anomocytic	Anomocytic
Xylem vessels	Broad Pitted vessels	Broad Pitted vessels
Surface view	Palisade cells filled with chlorophyll	Palisade cells filled with chlorophyll

Table 4: Physico-chemical parameters of bark and leaves of *D. indica* and *D. pentagyna*

Sr. No	Quality Parameters	Literature Value	Samples % w/w *D. indica**		*D. pentagyna**	
			Bark	Leaves	Bark	Leaves
1.	Loss on Drying	-----	18.20	7.856	19.157	4.56
2.	Ash value					
	a. Total ash value	-----	10.242	7.11	11.87	9.23
	b. Acid insoluble	-----	3.55	1.47	1.92	1.577
	c. Water soluble	-----	5.265	4.578	6.45	4.496
3.	Extractive value					
	a. Water soluble	7.60-26.59	14.55	7.12	12.98	6.78
	b. Alcohol soluble	9.68-24.6 (DIB)	21.70	17.14	17.15	10.74`

**Number of readings (N) =3*

Table 5: Heavy metals in bark and leaf of *D. indica* and *D. pentagyna*.

Sr. no.	Test name	Limits (ppm)	Detection limit (D.L.)* (ppm)	Results (ppm) *D. indica*		*D. pentagyna*	
				Bark	Leaf	Bark	Leaf
1	Lead (Pb)	10	-----	4.10	5.25	3.7	4.12
2	Cadmium (Cd)	3	-----	0.032	0.041	0.043	0.029
3	Arsenic (As)	3	0.005.	B.D.L. **	B.D.L.	B.D.L	B.D.L.
4	Mercury (Hg)	1	0.02	B.D.L.	B.D.L.	B.D.L.	B.D.L.

*D.L. * Detection limit; B.D.L. **- Below Detection limit*

Table 6 Presence of microorganisms in *D. indica* and *D. pentagyna*

Sr. no.	Test name	Detection Limit cfu/gm (Lohar DR, 2007)	Result *D. indica* Bark	Leaf	*D. pentagyna* Bark	Leaf
1	Total plate count	10^5	1570 cfu	1530 cfu	1245 cfu	1140 cfu
2	Yeast and mould count	10^3	< 10 cfu	< 10 cfu	< 10 cfu	< 10 cfu
3	E. *coli*/ gm	10	Absent	Absent	Absent	Absent

cfu/gm = colony forming unit/ gram

chemical tests separately for the presence of various phytoconstituents viz. alkaloids, flavonoids, saponins, carbohydrates, steroids and terpenoids, anthraquinone glycosides, coumarins, carotenoids, tannins and phenolic compounds[5].

RESULTS AND DISCUSSION

Herbal medicines are defined (as per WHO) on the basis of assessment of quality. The quality assessment includes pharmacopoeial assessment like official pharmacopoeias. Authentication of medicinal plants, foreign matters, organoleptic evaluation, microscopy, physicochemical parameters, microorganisms, chromatographic profiling as well as market components are important aspects for

Table 7: Phytochemical screening of *D. indica* bark & leaves

Tests	*Extracts Dillenia indica* bark				*Dillenia indica* leaf			
	Pet. Ether	Ethyl acetate	Methanolic	Aqueous	Pet. Ether	Ethyl acetate	Methanolic	Aqueous
Alkaloids	-	-	-	-	-	-	-	-
Carbohydrates	-	-	+	+	-	-	+	+
Sterols	++	-	-	-	++	-	-	-
Saponins	-	-	-	+	-	-	-	-
Phenolics	-	-	++	++	-	-	+	+
Tannins	-	-	+	+	-	-	+	+
Flavonoids	-	-	+++	++	-	-	++	+

determining quality.

WHO is emphasizing on Pharmacognostical, Physicochemical and Phytochemical evaluation of crude drugs. As bark and leaves of *D. indica* and *D. pentagyna* were collected from forest region, developed quality parameters are set and reported as per WHO guidelines. The developed parameters mentioned here ensure quality, purity and authenticity of both plant species which can be used for further analysis.

Identification and authentication of collected plant species: Plant authentication was done by Dr. Jasrai, Botanist, School of Botany, Gujarat University, Ahmedabad, Gujarat. Collected plant species were identified by comparing morphological description in mentioned in literature[12].

Foreign organic matter in medicinal plant material: % of foreign matter has been mentioned in Table 1.

Pharmacognostical studies

Morphological characteristics of collected plant species: Bark of *Dillenia indica* is usually reddish brown to dark brown in colour externally and internally, found broken irregular pieces having thickness of 1 to 2 cm and containing longitudinal striations and cracks on the surface. Fracture is somewhat fibrous (Figure 1a). Upper surface of leaf is greenish and lower surface is pale green, size of leaf approx. 1-1.5 ft in length and 10-15 cm wide, margin is serrate, reticulate venations, acute apex, symmetrical base, prominent midrib (Figure 1b). Bark of *Dillenia pentagyna* is dark brown in colour, broken irregular pieces are available having thickness of 0.9 to 1.3 cm containing longitudinal striations and cracks on the surface. Fracture is somewhat fibrous to granular. (Figure 1c). Upper surface of leaves is greenish and lower surface pale green in colour, size of leaf approx. 3-4 ft in length and 45-55 cm wide, Obovate shape having dentate margin and obtuse apex, reticulate venations, prominent midrib, asymmetrical base. (Figure 1d)Powder of bark of *D. indica* is reddish brown in colour having fibrous texture and *D. pentagyna* is brownish to dark brown in colour having coarse texture without any distinct odour and taste. Leaf powder of *D. indica* is greenish in colour, slightly fibrous powder having slightly bitter taste and no distinct odour. Leaf powder of *D. pentagyna* is pale greenish in colour powder having slightly bitter taste and no distinct odour.

Microscopical study of powder of D. indica and D. pentagyna

Microscopical characters of Dillenia indica and Dillenia pentagyna bark powder: Sclereids are brachy-sclereid (stone cells) type, and they are isodiametric and polyhedral; their walls are heavily thick and lignified (Figure 2A & B). Rectangle shaped thick walled lignified pitted stone cells of *D. indica* with size of 21-40.3 µ in Length and 12.4-20 µ in width (Figure 2A). Rectangle shaped thick walled lignified pitted stone cells of *D. pentagyna* with size of 52.3-46.3µ Length and 15.6-23 µ in width (Figure 2B). Pigments are also one of differentiating factor in both species where *D. indica* bark contains brownish pigments (Figure 2C) while *D. pentagyna* bark shows yellowish pigments (Figure 2D). Bark of *Dillenia indica* contain reddish brown polygonal shaped parenchymatous cork cells (Figure 2E) and *Dillenia pentagyna* found to contain brownish coloured polygonal shaped Parenchymatous cork cells (Figure 2F). Under microscopic observation the powder shows fragments of cork cells in surface and tangential view. Both species found to contain needle shaped (acicular) raphides (Figure 2G & 2H) of calcium oxalate crystals as well as phloem fibres. (Figure 2I). Major morphological and microscopical differences of bark of both species mentioned in Table 2.

Microscopical characters of Dillenia indica and Dillenia pentagyna Leaf powder: Major differentiating microscopical feature in leaves of both species is presence of unicellular trichomes. Trichomes present in leaf of *Dillenia indica* Linn. are unicellular and lignified (Figure 3A), approx.. 550-1360 µ in Length. Trichomes present in leaf of *D. pentagyna* Roxb. are unicellular lignified and/or unlignified (Figure 4.3B) trichomes with 250-760 µ in length. Vascular tissue of veins contains broad pitted lignified vessels (Figure 3C). The fragment of lamina in surface view is observed. Epidermis composed of polygonal cells with slightly wavy walls (Figure 3E). Both plant species shows the anomocytic types of stomata which is also a characteristic of Dilleniceae family (Figure 3F). Raphides of calcium oxalate crystals, found to be scattered throughout the slide and observed in abundant in both species. (Figure 3D & G). Other microscopical characters like palisade parenchyma cells, epidermal cells etc. which is generally found to be present in leaves. Major morphological and microscopical differences of leaves of both species mentioned in table 3.

Physico-chemical evaluations of bark and leaves of *D. indica* and *D. pentagyna:* Table 4 shows proximate values which include LOD, Ash values and extractive values of bark and leaves of *D. indica* and *D. pentagyna*. The ash and extractive values were determined for prepared powdered samples. (Table 4)

Table 8: Phytochemical screening of *D. pentagyna* bark & leaves

Tests	*Dillenia pentagyna bark*				*Dillenia pentagyna leaf*			
	Pet. Ether	Ethyl acetate	Methanolic	Aqueous	Pet. Ether	Ethyl acetate	Methanolic	Aqueous
Alkaloids	-	-	+	+	-	-	-	-
Carbohydrates	-	-	+	+	-	-	+	+
Sterols	+++	-	-	-	++	-	-	-
Saponins	-	-	-	+	-	-	-	-
Phenolics	-	-	++	++	-	-	+	+
Tannins	-	-	+	+	-	-	+	+
Flavonoids	-	-	+++	++	-	-	++	+

Determination of heavy metals in bark and leaf of *D. indica* and *D. pentagyna*: Presence and amount of heavy metals in bark and leaf of *D. indica* as well as in *D. pentagyna* has been summarized in Table 5. It has been observed that all the heavy metals are below the detection limits required as per AOAC method guideline[9-11].

Determination of microorganism in *D. indica and D. pentagyna:* Results of total plate count, yeast and mould count and E. *coli* of bark and leaves of both plants has been reported and mentioned in Table 6. It has been observed that microorganisms found within the limits specified by WHO. E. *coli* is absent in raw material which indicated plants can be used for further investigation[11].

Phytochemical Screening of bark and leaves of *D. indica* and *D. pentagyna:* Phyto-chemical screening of selected plants was carried out for presence of various phytoconstituents. Results obtained are shown in Table 8 & 9. Bark and leaves of *D. indica* and *D. pentagyna* showed presence of phenolics, flavonoids (flavanone), carbohydrates, steroids and triterpenoids. Saponins are found to be present in bark (less quantity) which is absent in leaves of both plant species. Alkaloids are absent in bark and leaves both plant species.

CONCLUSION

Pharmacognostical, physicochemical and phytochemical parameters are set to ascertain identity, purity and quality of plants. Pharmacognostical parameters mentioned in results will be helpful to identify and differentiate both plant species. The physicochemical evaluation of powdered drugs revealed that the standard quality and purity of herbal drug and it is also give information regarding the authenticity of crude drug. In addition to that, heavy metals like Hg and As was found below detection limit as well as absence of E. *coli* proved that collected plant parts are safe to use for further investigations. The present study concluded that the plants contain variety of phytoconstituents. Phytochemical studies of the prepared extracts showed presence of triterpenoids, phytosterols, carbohydrates, glycosides, flavonoids, tannins & phenolic compounds. Different fractioned were used for phytochemical screening which can further be useful for isolation of various phytoconstituents and for further evaluation of potential treatment of diseases in humans.

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