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Adulteration And Its Mitigation By Phytochemical And Anatomical Screening Of Family Liliaceae

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Abstract- *Endangered medicinal plants have been used as traditional and alternative medicines from last several decades. Deforestation and extinction of many medicinal plant species may leads to scarcity of these plants and was resulted in adulteration. In India, of the 8000 species of medicinal plants harvested from the wild, approximately 960 are in the active trade. Adulteration in market sample is one of the greatest drawback in promotion of herbal medicinal plant products. Future of herbal medicine totally depends upon the correct identification, standardization and quality assurance. Comparative anatomical study and phytochemical screening may be used for its correct authentication and prevention of adulteration. In order to ensure the use of only genuine and uniform material of such herbal drugs, work on plant identifying features assumes vital significance. Preliminary phytochemical analysis showed that saponins, flavonoids, glycosides, steroids were present in the plant material while comparative anatomical study would serve as standard reference for identification of the different plant species of Chlorophytum Ker. Gawl and Asparagus L.*

Key Words - *Adulteration, Herbal plant, Phytochemicals, Saponins.*

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I. Introduction

Adulteration in market sample is one of the greatest drawback in promotion of ayurvedic medicinal plant products. In health aspect, adulteration and substitution is burning issue of herbal industries (Vines, 2004, Canter *et al.,* 2005). Herbal plants have been used as alternative drugs with no side effect. Scientific method for evaluation of adulteration has been done by microscopic and phytochemical analysis of medicinal plant products i.e; leaf, stem, root and flower parts. Present paper deals with phytochemical and anatomical evaluation of nine plant species of genus *Chlorophytum* ker. Gawl. and *Asparagus* L. belonging to family Liliaceae. They are considered as 'wonder drug' due to its aphrodisiac properties (Kirtikar and Basu, 1975, Oudhia, 2001), anti-diabetic, anti-stress, anti-inflammatory, anti-cancerous, anti-oxidant, anti-tumerous, anti-ageing and anti-microbial properties.

Although 215 different species of *Chlorophytum* ker. Gawl.(Li *et al.,* 1990) and 300 species of *Asparagus* L. have been reported throughout the world, but only few find medicinal relevance, out of which prominent are *Chlorophytum borivilianum* Santapau and Fernandes, *Chlorophytum tuberosum* Baker. and *Asparagus recemosus* willd.

II. Method And Material- A. Collection And Identification

Different species of *Chlorophytum* Ker. Gawl. and *Asparagus* L. were collected from different regions of Varanasi and Lucknow of Utter Pradesh. Tubers of *Chlorophytum borivilianum* Santapau and Fernandes and *Chlorophytum tuberosum* Baker. were obtained from National Botanical Research Institute (NBRI) Lucknow. Seed and two seedlings of *Asparagus recemosus* willd. and *Gloriosa superba* L.were collected from Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow. While *Chlorophytum laxum* R. Br., *Chlorophytum comosum* Jacq., *Asparagus fulcatus* L., *Asparagus retrofractus* L., *Asparagus densiflorus* (Kunth.) Jessop. *Asparagus setaceous* (Kunth.) Jessop. were collected from different nurseries of Lucknow and Varanasi.

b. Anatomical analysis: transverse section of fresh roots and stems of these plants obtained after double staining, permanent slides were prepared after dehydration in different grades of alcohol and mounting in D.P.X. sections were studied and photomicrographed for detailed observation.

c. Phytochemical studied: Fresh leaves of plant samples were washed thoroughly 2-3 times in running water and then by distilled water. 120 ml of acetone, ethyl acetate, 80% methanol , benzene, petroleum ether and

hexane were added with 5g of washed leaves and kept at room temperature for 24 hours. After it resulted filtrate was used for phytochemical analysis.

i. Test of Carbohydrates - 1 ml of extract, 0.5 ml of fehling solution (A) and 0.5 ml of fehling solution (B) was added respectively and boiled for 5 minutes (Shalini *et.al.*, 2012), formation of brick red precipitate will confirm presence of carbohydrate.

ii. Test of Saponins - 1ml of extract and 4ml of distilled water was added to make up 5ml volume. The suspension is shaken for 2 minutes (Shalini *et.al.*, 2012). Formation of foam will confirm the presence of saponins.

iii. Test of Flavonoides - 1ml of extract, little amount of magnesium and 1ml of conc. sulphuric acid were added along the side of test tube (Avijit *et.al.*, 2002). Formation of yellow colour will confirm the presence of flavonoides.

iv. Test of Steroids - 1ml of extract and 2ml of chloroform was mixed gently. Then 2ml of conc. H_2SO_4 was carefully added along the wall of test tube (Shalini *et.al.*, 2012). Formation of reddish brown colouration at the junction of two layers will confirm the presence of steroids (Siddiqui and Ali, 1997).

v. Test of Glycosides - 1ml of extract and few drops of glacial acetic acid were added for a minute and cooled. The content was gently transferred to another test tube having 1 ml of sulphuric acid (Sreenivasa *et. al.*, 2015). Formation of reddish ring at the junction of two layers will confirm the presence of glycosides.

Table 1: Observation of phytochemical screening of plant species of *Chlorophytum* Ker. Gawl. and *Asparagus* L.

S. No.	Extract	Test of significance	*Chlorophytum borivilianum*	*Chlorophytum tuberosum*	*Chlorophytum comosum*	*Chlorophytum laxum*	*Asparagus racemosus*	*Asparagus densiflorus*	*Asparagus setaceous*	*Asparagus fulcatus*	*Asparagus retrofracts*
1.	Acetone	i. Carbohydrates	++	++	++	++	++	++	++	++	+
		ii. Saponins	+	+	-	+	++	-	-	-	-
		iii. Flavonoides	++	++	+	+	++	+	++	++	+
		iv. Steroides	++	++	+	+	++	-	+	-	+
		v. Glycosides	++	++	-	-	+	-	-	-	-
2.	Ethyl acetate	i. Carbohydrates	++	++	++	++	++	++	++	++	++
		ii. Saponins	++	++	++	++	++	++	++	++	+
		iii. Flavonoides	++	+	+	+	++	-	++	-	-
		iv. Steroides	++	++	+	+	+	-	-	-	-
		v. Glycosides	++	++	-	-	+	-	-	-	-
3.	80% Methnol	i. Carbohydrates	++	++	++	++	+	+	+	+	+
		ii. Saponins	+	+	-	++	+	+	-	-	-
		iii. Flavonoides	++	++	+	+	++	+	+	+	+
		iv. Steroides	++	++	+	+	++	-	+	+	+
		v. Glycosides	+	+	-	-	+	-	-	-	-
4.	Benzene	i. Carbohydrates	++	++	++	++	++	+	+	+	++
		ii. Saponins	+	+	+	+	+	+	++	+	+
		iii. Flavonoides	++	++	+	+	+	-	-	-	-
		iv. Steroides	++	+	-	-	-	-	+	+	-
		v. Glycosides	+	++	-	-	-	-	-	-	-
5.	Petro-ether	i. Carbohydrates	++	++	++	++	++	+	+	+	+
		ii. Saponins	+	+	+	++	+	+	+	-	+
		iii. Flavonoides	-	-	+	+	+	+	-	+	-
		iv. Steroides	+	+	-	-	+	-	-	-	-
		v. Glycosides	+	+	-	-	+	-	-	-	-
6.	Hexane	i. Carbohydrates	++	++	++	+	++	+	+	+	+
		ii. Saponins	++	++	+	+	++	+	+	+	-
		iii. Flavonoides	+	+	+	+	++	-	-	-	-
		iv. Steroides	++	++	-	-	+	-	-	-	-
		v. Glycosides	++	++	-	-	++	-	-	-	-

(++) present in appropriate amount, (+) present in less amount, (-) absent.

Table -2:1 Root differentiation and identification chart of different species of *Chlorophytum* Ker. Gawl.

S.No.	Plant part	Plant name			
		Chlorophytum borivilianum	***Chlorophytum tuberosum***	***Chlorophytum comosum***	***Chlorophytum laxum***
1.	Cortex and structure of vascular tissue	Cortex thin walled, parenchymatous, thick walled xylem vessels,vascular tissue radial, exarch.	Cortex thick walled sclerenchymatous, lignified pitted xylem, vascular tissue radial, exarch.	Cortex thick walled sclerenchymatous, cork cell filled with few colour, lignified pitted xylem, vascular tissue radial, exarch.	Cortex thick walled sclerenchymatous,vascular tissue radial, exarch.
2.	No. of xylem	No. of xylem ranges between 8-10.	No. of xylem ranges between 12-15	No. of xylem ranges between 30-40	No. of xylem ranges between 25-30
3.	Raphides and scleried	Raphides and scleried are present large in amount.	Raphides and scleried are present less in amount.	Raphides are large, but scleried less in amount.	Raphides and scleried are present lesser in amount.

4.	Endodermis, pericycle and pith	Endodermis and pericycle are single layered, parenchymatous pith occupy less place.	Endodermis and pericycle are single layered, parenchymatous pith occupy less place.	Endodermis and pericycle are single layered parenchymatous pith occupy larger space.	Endodermis and pericycle are single layered parenchymatous pith occupy larger space.

Table -2:2 Stem differentiation and identification chart of different species of *Chlorophytum* Ker. Gawl.

S.No.	Plant part	Plant name			
		Chlorophytum borivilianum	***Chlorophytum tuberosum***	***Chlorophytum comosum***	***Chlorophytum laxum***
1.	Epidermis & cortical cell (Hypodermis)	Epidermis single layered , cortical cell is 4 layered (sclerenchymatous).	Epidermis single layered ,cortical cell is 5 layered (sclerenchymatous).	Epidermis single layered , cortical cell is 4 layered (sclerenchymatous).	Epidermis single layered , cortical cell is 3 layered (sclerenchymatous).
2.	Ground tissue	It extends from just below hypodermis , thin walled and parenchymatous.	It extends from just below hypodermis , thin walled and parenchymatous.	It extends from just below hypodermis , thin walled and parenchymatous.	It extends from just below hypodermis , thin walled and parenchymatous.
3.	Vascular tissue	Vascular bundles are 25-30 in number, conjoint, collateral, endarch, closed, arranged in 2 rows.	Vascular bundles are 15-20 in number, conjoint, collateral, endarch, closed, arranged in 4 rows.	Vascular bundles are 30-35 in number, conjoint, collateral, endarch, closed, arranged in 6 rows.	Vascular bundles are 20-25 in number, conjoint, collateral, endarch, closed, arranged in 5 rows.
4.	Pith	Absent	Absent	Absent	Absent

Table -2.3 Root differentiation and identification chart of different species of *Asparagus* L.

S.No.	Plant part	Plant name				
		Asparagus recemosus	***Asparagus densiflorus***	***Asparagus setaceous***	***Asparagus falcatus***	***Asparagus retrofractus***
1.	Epidermis and cortical tissue	Epidermis single layered, outer wall of cell proliferate to form multicellular hair. Cortical cell is undifferentiated and consists of loosely walled parenchymatous cells.	Epidermis single layered, outer wall of cell proliferate to form multicellular hair in few places. Cortex occupy 3/4 portion comprises thick walled (central) and thin walled (peripheral) tissue.	Epidermis single layered , multicellular hair is not seen, Cortex occupy 3/4 portion comprises thick walled (central) and thin walled (peripheral) tissue.	Epidermis single layered, multicellular hair is absent, Cortex occupy 3/4 portion comprises thick walled (central) and thin walled (peripheral) tissue.	Epidermis single layered, multicellular hair is absent, Cortex occupy 3/4 portion comprises thick walled (central) and thin walled (peripheral) tissue.
2.	Number and structure of vascular bundle	Vascular bundles are 12-15 in number, xylem alternating with phloem in exarch and radial manner.	Vascular bundles are 18-20 in number, xylem alternating with phloem in exarch and radial manner.	Vascular bundles are 15-18 in number, xylem alternating with phloem in exarch and radial manner.	Vascular bundles are 20-25 in number, xylem alternating with phloem in exarch and radial manner.	Vascular bundles are 18-20 in number, xylem alternating with phloem in exarch and radial manner.
3.	Endodermis , pericycle and pith	Endodermis is well distinct as compare to pericycle and parenchymatous pith occupy small area.	Endodermis and pericycle are well developed and parenchymatous pith occupy very small area.	Endodermis and pericycle both are well distinct and parenchymatous pith occupy small area.	Endodermis is well distinct as compare to pericycle and parenchymatous pith is completely absent.	Endodermis is well distinct as compare to pericycle and parenchymatous pith occupy very small area.

Table -2.4 Stem differentiation and identification chart of different species of *Asparagus* L.

S.No.	Plant part	Plant name				
		Asparagus recemosus	***Asparagus densiflorus***	***Asparagus setaceous***	***Asparagus falcatus***	***Asparagus retrofractus***
1.	Epidermis and cortical tissue	Epidermis is not well distinct, cortex sclerenchymatous erom central while thinner towards periphery.	Epidermis is distinct, tangentially elongated to form multicellular hair. Cortex composed of collenchymatous and parenchymatous cell.	Epidermis is not well distinct. Cortex composed of 2-3 layered collenchymatous and parenchymatous cell.	Epidermis is distinct, tangentially elongated to form multicellular hair. Cortex composed of collenchymatous and parenchymatous cell.	Epidermis is distinct. Cortex composed of collenchymatous and parenchymatous cell.
2.	Vascular tissue	No. of vascular tissue are 15-18, conjoint, collateral, endarch and closed, small vessels of	No. of vascular tissue are 10-15, conjoint, collateral, endarch and closed, protoxylem and	No. of vascular tissue are 20-25, conjoint, collateral, endarch and closed, small	No. of vascular tissue are 20-25, conjoint, collateral, endarch and closed, vascular	No. of vascular tissue are 25-28, conjoint, collateral, endarch and closed, protoxylem and

		protoxylem and two bigger vessels of metaxylem arranged in two rows.	metaxylem arranged in two rows i.e; larger in centre while shorter in periphery.	vessels of protoxylem and two bigger vessels of metaxylem arranged in two rows.	tissue are arranged in two rows.	metaxylem arranged in two rows.
3.	Pith	Very small and parenchymatous pith is present in centre region	Absent	Very small and parenchymatous pith is present in centre region.	Parenchymatous pith is present in centre region.	Very small and parenchymatous pith is present .

Keys for anatomical identification of plant *Chlorophytum* Ker. Gawl.

A. Number of xylem strand 10-15, pith less developed in root.
B. Cortex 4 layered, vascular bundles 20-25 in stem……..…….....*C. borivilianum*
BB. Cortex 5-6 layered, vascular bundles 15-16 in stem...…….*C. tuberosum*
AA. Xylem strands 30-40, pith well developed in root.
B. Raphides plenty……..*C. comosum*
BB. Raphides scanty……..*C. laxum*

Keys for anatomical identification of plants *Asparagus* L.

A. Pith absent in root……...*A. fulcatus*
AA. Pith present.
B. Root hair present .
C. Cortex cell loosely arranged……*A. racemosus*
CC. Cortex cell compactively arranged……..*A. densiflorus*
BB. Root hairs absent.
C. Vascular bundles 20-23 in stem……..*A. setaceous*
CC. Vascular bundles 26-28 in stem……....*A. densiflorus*

III. Result And Discussion

Preliminary phytochemical screening of extract revealed the presence of saponins , flavonoides, steroids and glycosides are in significant amount *Chlorophytum borivilianum*, *Chlorophytum tuberosum* and *Asparagus recemosus* in comparison of other species of *Chlorophytum* Ker. Gawl. and *Asparagus* L. Anatomical differentiation of these species reveals exact identification and can be employed to prevent misconception and adulteration.

From medicinal point of view *Chlorophytum borivilianum* contains Arundinoside A (a spirostane steroidal saponin), Arundinaside B (an aliphatic glucosides) and sapoginins (Bordia et al, 1995) and these chemical constituents make safed musli as potent drug having aphrodisiac agent (Marais and Reilly, 1978), as remedy of arthritis and natal and post natal problems. In case of *Asparagus recemosus*, three steroidal saponins i.e; Racemosides A(1), B(2), C(3) and new flavonoides were reported from roots and fruits which give potency to satawar antidysenteric property. Its meticulous use as a galactogogue to enhance breast milk secretion and producing thousand of healthy ova is well known (Thakur and Dixit, 2005).

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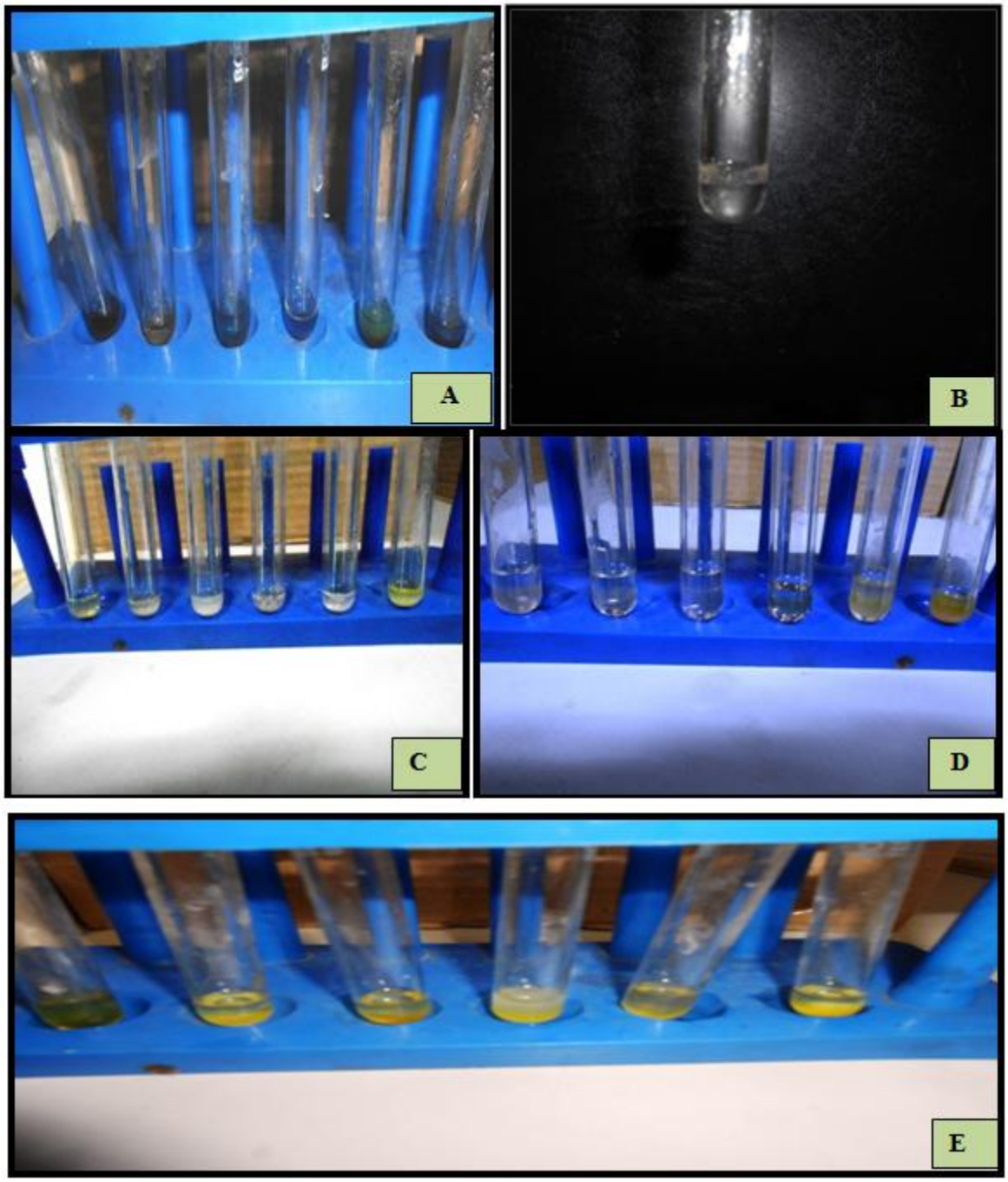
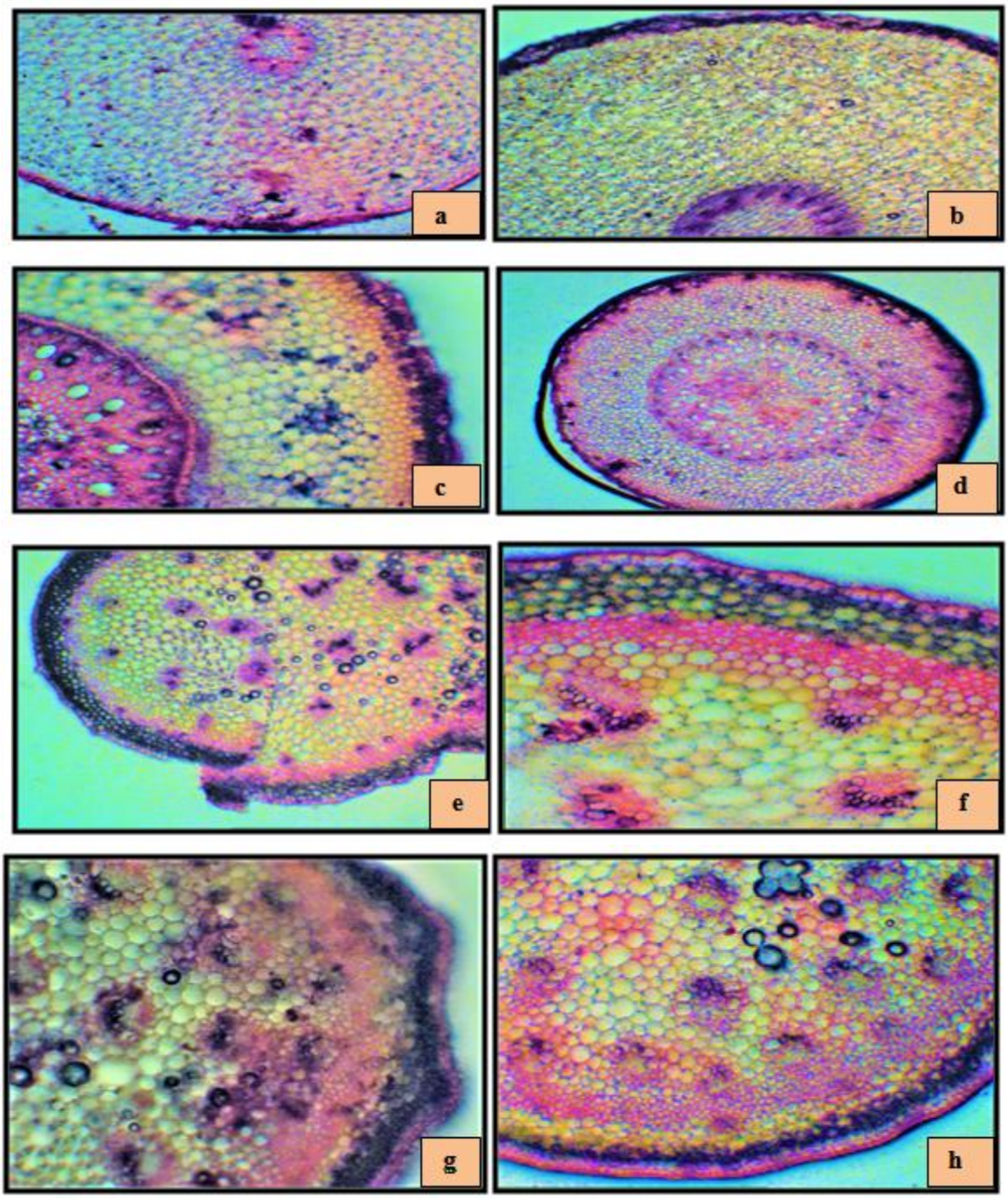
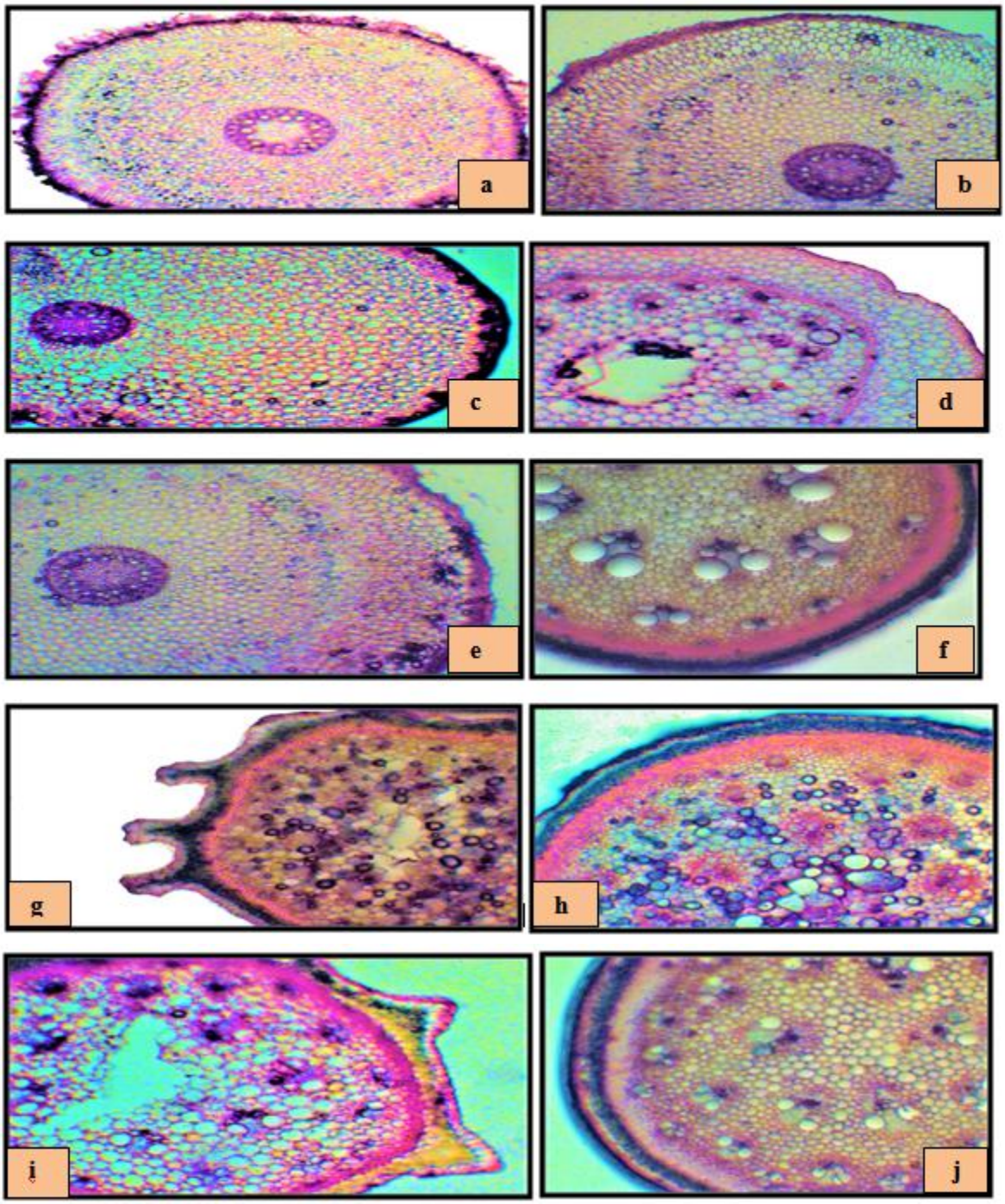


Qualitative estimation of phytochemicals- (A) Carbohydrate (B) Saponins (C) Flavonoides (D) Steroides and (E) Glycosides.



Cross section of root and stem of genus *Chlorophytum* Ker. Gawl. (a- *C. borivilianum* (root), b- *C. tuberosum* (root), c- *C. comosum* (root), d- *C. laxum* (root), e- *C. borivilianum* (stem), f- *C. tuberosum* (stem), g- *C. comosum* (stem), h- *C. laxum* (stem).



Cross section of root and stem of genus *Asparagus* L. (a- *A. recrmosus* (root), b- *A. densiflorus* (root), c- *A. setaceous* (root), d- *A. falcatus* (root), e- *A. retrofractus* (root), f- *A. recemosus* (stem), g- *A. densiflorus* (stem), h- *A. setaceous* (stem), i- *A. fulcatus* (stem), j- *A. retrofractus*.

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