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Research article

Stem bark characteristics for determination of pharmacognostical properties of *Holarrhena antidysenterica* (Roth) Wall. ex A.DC. and *Wrightia tomentosa* Roem. et Schulta.

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Abstract: *Holarrhena antidysenterica* a small tree used in the treatment of dysentery, diarrhea, helminthic, astringent and haemostatic disorders. *Wrightia tomentosa* a medium size tree widely used in the treatment of stomach ache, tooth ache, fever, hemorrhage, arthritis, snake bite and scorpion stings. Both the species belongs to the family Apocynaceae. The present study was undertaken to investigate stem bark of *Holarrhena antidysenterica* and *Wrightia tomentosa* for their pharmacognostical properties such as organoleptic, macroscopic, microscopic, powder characteristics, physico-chemical analysis, phytochemical screening, fluorescence studies and microbial limit assay for standardization of herbal drugs as recommended in Ayurvedic Pharmacopeia of India. Physicochemically, the loss on drying, chloroform soluble extractive, total ash, acid insoluble ash and water-soluble ash (2.83%, 5.98%, 12.13%, 1.33%, 10.89% respectively) were comparatively more in the stem bark of *Wrightia tomentosa* while water and methanol soluble extractives (23.33%, 28.45%) were more in stem bark of *Holarrhena antidysenterica*. The preliminary phytochemical screening was carried out in aqueous, methanolic and chloroform extracts which revealed the presence of alkaloid, resin and steroid in all extracts while starch was absent in all extracts. Tannin was observed in all tested extracts of *Holarrhena antidysenterica* but absent in all extracts of *Wrightia tomentosa.* The fluorescence study was carried out in ultraviolet light (366 nm) and day light for determining the chemical nature of drugs. The microbial profile of stem bark of both plants was developed to check the purity of the drugs. Findings revealed through pharmacognostical parameters of stem bark of both plants can help in identification of the plant with high medicinal properties and a suitable diagnostic tool for standardization as well as identification of adulteration.

Keywords: *Holarrhena antidysenterica* - *Wrightia tomentosa* - Pharmacognosy - Ayurveda.

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INTRODUCTION

Medicinal plants are the unique gift of nature to the human kind (Sharma 2017). Since ancient times, vast numbers of Indian medicinal plants have been used globally. In India, herbal medicines have been used for thousands of years and their worldwide use increased during the last few decades which are evidenced by rapidly growing global and national markets of herbal drugs (Mehra *et al*. 2014, Bajpai *et al*. 2016, Srivastava & Shukla 2018, Gogoi *et al*. 2019). However, ayurvedic products are not regular in the market due to lack of infrastructures, skilled manpower, reliable methods and stringent regulatory laws. Development of reliable, specific and sensitive quality control methods by using a combination of classical and modern instruments for analysis and good coordination between the quality of raw materials, in- process materials and the final products

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are the prime need of ayurvedic and alternative medicine system. The quality control of the herbal drugs can be ensured by pharmacognostical studies (Kulkarni 2013, Odewo *et al*. 2020). Pharmacognostical characteristics of two selected medicinal plants of the same family will make them identifiable which are otherwise adulterant and substituent to each other. So, the present study will help to identify and authenticate both the plants.

Holarrhena antidysenterica (Roth) Wall. ex A.DC synonymsally called *Holarrhena pubescens* (Buch.-Ham.) Wall. ex G. Don is a tall shrub or small tree found throughout in deciduous forests and open wastelands of India. It is commonly known as *Kutaja* in Hindi (Gupta 2008, Nishteswar & Hemadri 2010, Chaudhary *et al*. 2016). Stem bark is used in the treatment of diarrhea, piles, skin diseases and biliousness, fever, jaundice, stone in bladders, amoebic dysentery and vaginitis, antidontalgic, antidropsical, colic, dyspepsia, chest affections disease, spleen properties (Chopra *et al.* 1982, Shwetha 2011, Anupbhusal *et al.* 2014, Bajpai *et al*. 2016) and anti-malarial property (Gaur 1999, Dua *et al.* 2013). Literature reveals the presence of different chemical compounds such as alkaloids holamine, kurchamine, holaphyllidine, holaromine, mitiphylline, holadysenterine and non-alkaloidal constituent's kurchinin, kurchinicin, holarrheno (Khan 2013).

Wrightia tomentosa Roem. et Schulta. also called *Wrightia arborea* (Densst.) Mabb. is a small to medium size deciduous tree, found all parts of India. It is commonly known as *Dhudhi* in Hindi (Gupta 2008, Nishteswar & Hemadri 2010, Chaudhary *et al*. 2016). Traditionally the stem bark preparation and root bark of this plant is used in the treatment of menstrual, renal complaint, urinary stones, snake bite and scorpion stings whereas dried bark showed the antipyretic property. Dried stem bark of *Wrightia tomentosa* is used as an adulterant for the stem bark of *Holarrhena antidysenterica* which is morphological similar plants (Mokkhasmit *et al.* 1971, Umapriya *et al.* 2011). *Wrightia tomentosa* contains distinct types of phyto-constituents such as conessine dihydrate, holarrhine, kurchicine and conkurchine alkaloids, flavonoids, phlobatanins, simple phenolics, tannins, β-sitosterol, lupeol, α- amyrin and reducing sugar (Khyade & Vaikos 2014). The stem of both plants are used in the preparation of the wood toys for children playing in Chitrakoot area. The people of Guna district area used the stem wood of these plants as a fuel in cooking food. Large scale consumption of these plants they belong to the endangered category so it is necessary to conserve it by the pharmacognostical study and phytochemical analysis in different parts of both plants. Present communication deals with the detailed comparative pharmacognostical characteristics of stem bark of *Holarrhena antidysenterica* (HASB) and stem bark of *Wrightia tomentosa* (WTSB).

MATERIALS AND METHODS

Collection and powder preparation of plant material

The stem bark of *Holarrhena antidysentrica* was collected from the Sati Anusuiya' area of Chitrakoot forest of Satna district and stem bark of *Wrightia tomentosa* was collected from Holipur village forest of Budhni tehsil of Sehore district of Madhya Pradesh, India. The plant was identified by Dr. R.L.S. Sikarwar, Department of Auyveda Sadan, Arogyadham, Deendayal Research Institute (DRL), Chitrakoot, Satna (M.P.). The stem bark was washed under running water to eliminate dust and other foreign particles and dried in Tray dryer at 35±2^{o}C and grinded with electric grinder. The powder was sieved (No. 43) and stored in air tight containers separately for protection from moisture and microbes for further use.

Macroscopic, microscopic and powder characteristics

Systematic recording of macroscopic, microscopic and powder characteristics of stem bark of both selected plants were recorded using binocular compound microscope attached with camera Lucida (Kokate 1994, Ahmad *et al.* 2013).

Physico-chemical evaluation

The percentage yield of physico-chemical values (w/w) like loss on drying (LOD) at 105^{o}C, methanol soluble extractive (MSE), water soluble extractive (WSE) and chloroform soluble extractive (CSE), total ash (TA), acid insoluble ash (AIA), water soluble ash (WSA) were carried out in triplicate as per as the standard methods (Thimmaiah 1999, Anonymous 2008).

Phytochemical screening

Powdered material was extracted with three solvents such as water, methanol and chloroform using cold maceration procedure and was tested for the presence or absence of various types of phytochemical constituents by using standard methods (Harborne 1984, Sadasivam & Manickan 1996, Anonymous 2010).

Fluorescence study

The colour of the powdered samples change with respect to different chemicals and reagents and was

observed in daylight and ultraviolet light at 366 nm as per the standard method (Chase & Pratt 1949, Kokoski *et al.* 1958, Tiwari *et al.* 2014).

Microbial analysis / Microbial limit test (MLT)

Microbial limit test (MLT) was carried out for the determination of microbial load of *Staphylococcus aureus* g^{-1}, *Salmonella* sp. g^{-1}, *Pseudomonas aeuroginosa* g^{-1}, *Escherichia coli*, Total bacterial count (TBC) and Yeast & Mould (Y & M) in the powder sample of stem bark of *Holarrhena antidysentrica* and *Wrightia tomentoa* as per standard methods (Anonymous 1998, Anonymous 2008, Ahirwar *et al.* 2019). Specified agar and enrichment media (Make- Himedia) were used in this test.

RESULTS AND DISCUSSION

Organoleptic identification

Powder colour of the stem bark of both plants *viz*. *Holarrhena antidysentrica* and *Wrightia tomentosa* was appeared brown (Fig. 1). The powder odour of stem bark of *Wrightia tomentosa* was characterized in terms of odour while stem bark of *Holarrhena antidysentrica* was found odourless. The powder of stem bark of *Holarrhena antidysentrica* was bitter in taste, while stem bark of *Wrightia tomentosa* was tasteless.

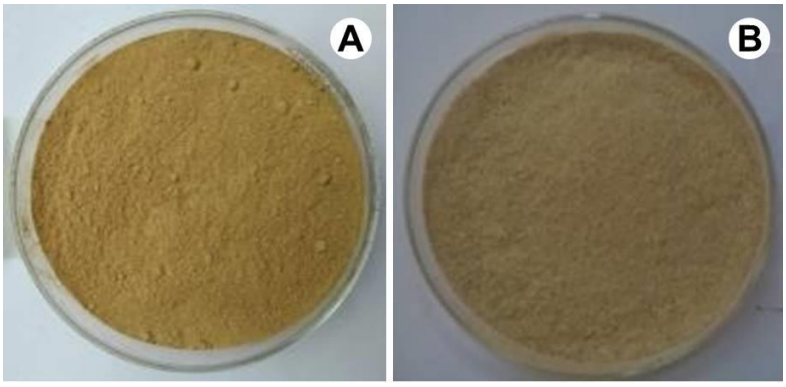

Figure 1. Powder of stem bark: A, *Holarrhena antidysenterica* (Roth) Wall. ex A.DC.; B, *Wrightia tomentosa* Roem. et Schulta.

Macroscopy, microscopy and powder characteristics

The dried stem bark of *Holarrhena antidysentrica* appears in small recurved pieces of different sizes and thickness, outer surface was dark brownish, longitudinally wrinkled and showed horizontal lenticles (Fig. 2A–C). Whereas, dried stem bark of *Wrightia tomentosa* appears recurved, outer surface of bark was pale yellow to greenish-brown and inner surface appeared brownish-white in colour and fibrous. The fracture was hard and rigid fibrous (Fig. 2D–F).

Transverse section (T.S.) of stem bark of *Holarrhena antidysentrica* shows periderm, secondary phloem and wide cortex (Fig. 3). Periderm made up of thin-walled and rectangular shape cork cells consisting 2 to 3 layers of phellogen. Phelloderm paranchymatous cells contain prism and starch grains are very few in numbers were recorded. Cortex cells showed groups of lignified pitted stone cells of varying shapes and sizes. Occasionally groups of non- lignified pericylic fibres, sclereids and stone cells were observed. Medullary rays were found bi- or tri-seriate in various regions. Phloem parenchyma contains calcium oxalate crystals and abundance starch. Phloem fibres were not found. Similar characters were also reported by Akhtar *et al*. (2011) and Rao (2013). Whereas, T.S. of stem bark of *Wrightia tomentosa* showed multilayered cork cells arranged radially and suberized through phelloderm and ground tissue. Calcium oxalate crystals and starch grains were seen. Stone cells scattered everywhere in the section. Medullary rays were uniseriate and a few bi or triseriate rays observed. Phloem fibres were not observed.

Powder of stem bark of *Holarrhena antidysentrica* shows transversely cut thin-walled cork cells, stone cells in groups of various size and shape, paraenchymatous cells with prism and stone cells containing crystals of calcium oxalate, thick-walled cortical parenchyma filled with starch grains, medullary rays associated with phloem parenchyma containing starch grains and crystals, laticiferous canal, fibres and some sclereids were found (Fig. 4). Findings on similar plant part were also reported by Anonymous (2006), Zope (2016). While, powder of stem bark of *Wrightia tomentosa* showed transversely cut thick-walled cork cells, prismatic crystals, parenchyma containing starch grains, stone cells single or in the group with and without crystals, sclereids, crystal fibres of various thickening and reticulated vessels were observed. Medullary rays were uniseriate and a few bi or triseriate (Fig. 5).

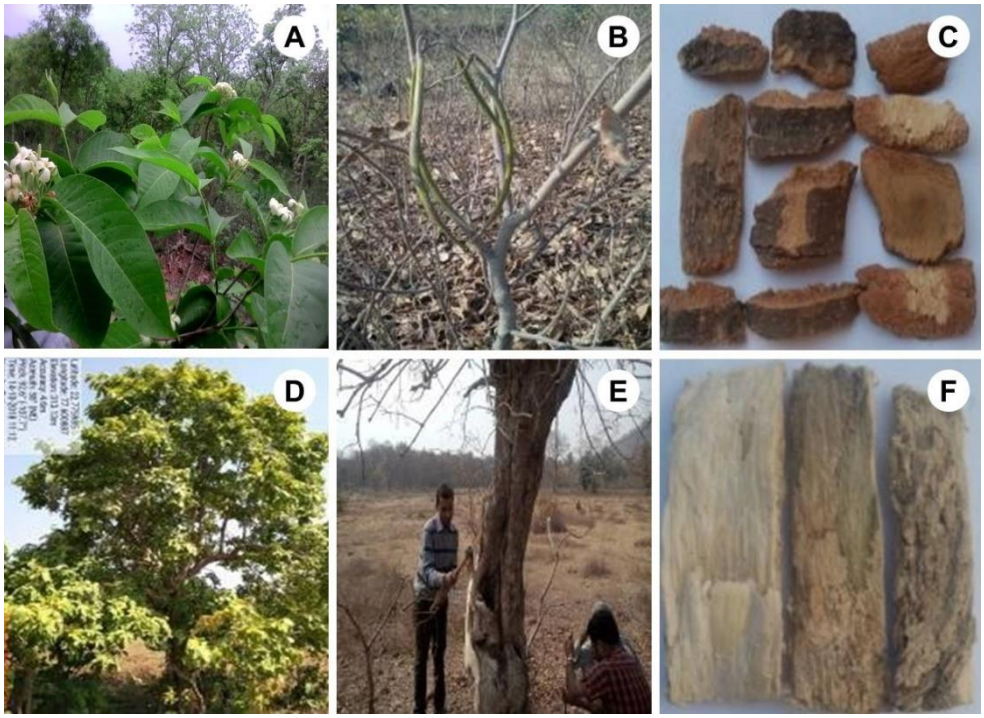

Figure 2. Macroscopic characteristics of *Holarrhena antidysenterica* (Roth) Wall. ex A.DC. (**A–C**) and *Wrightia tomentosa* Roem. et Schulta. (**D–F**): **A & D**, Plant habit; **B & E**, Stem; **C & F**, Dry stem.

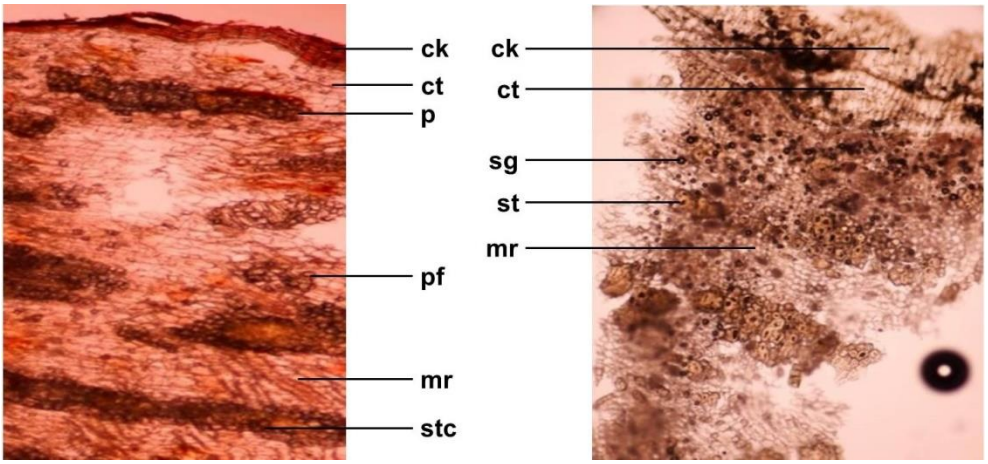

Figure 3. Transverse Section (T.S.) of stem bark: **A,** *Holarrhena antidysenterica* (Roth) Wall. ex A.DC.; **B,** *Wrightia tomentosa* Roem. et Schulta. [ck - Cork, ct - Cortex, mr - Medullary ray, p - Prism, pf - Pericyclic fibres, stc - Stone cells, sg - Starch grain]

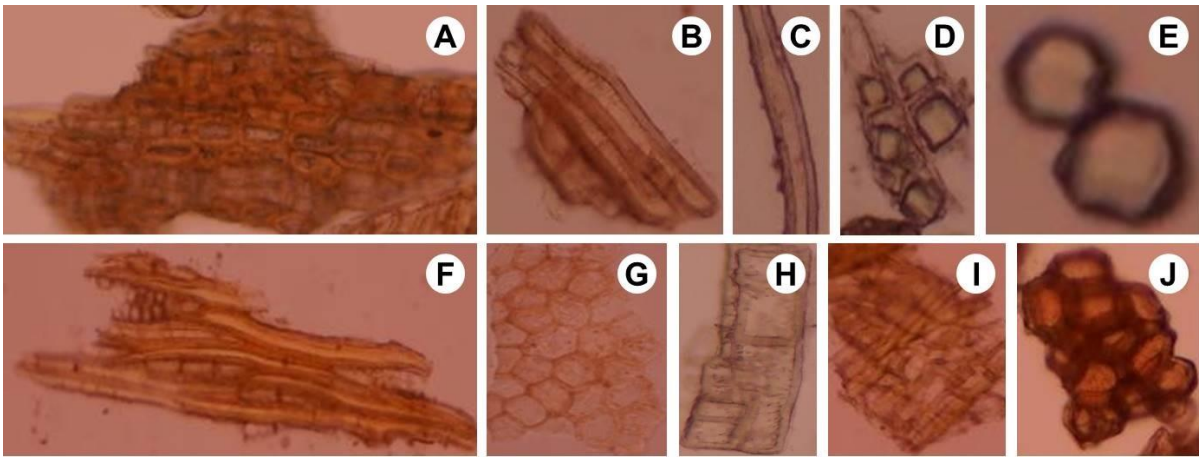

Figure 4. Distinct powder characteristics of stem bark of *Holarrhena antidysenterica* (Roth) Wall. ex A.DC.: **A**, Group of stone cells; **B**, Sclereids; **C**, Fibres; **D**, Crystal fibres; **E**, Calcium oxalate crystals; **F**, Medullary rays associate with phloem parenchyma filled with starch grains, crystals and laticiferous canal; **G**, Cortical parenchyma filled with starch grains; **H**, Simple pitted vessels; **I**, Cork cells in sectional view; **J**, Cork cells in surface view.

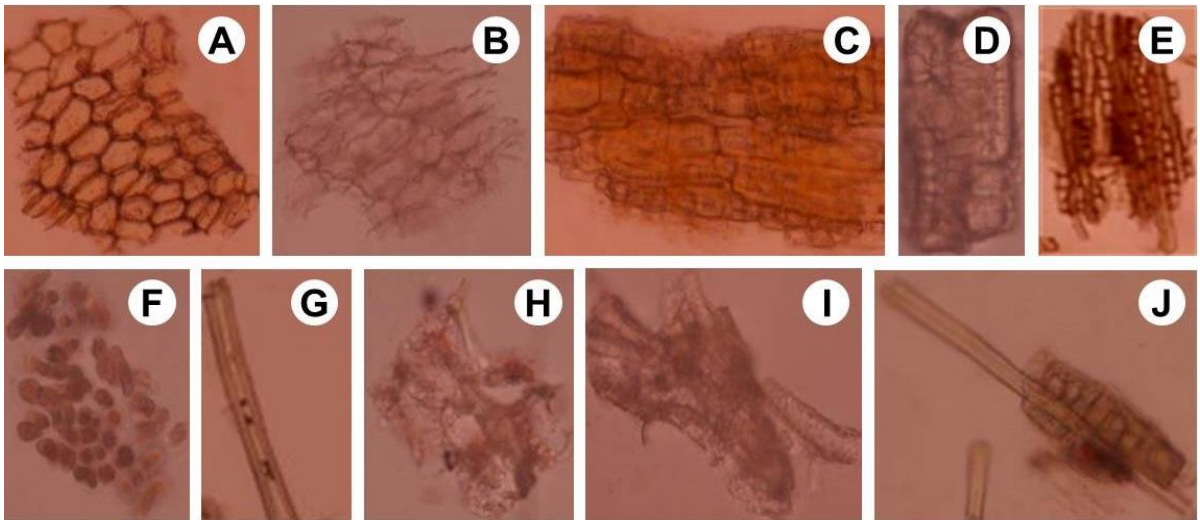

Figure 5. Distinct powder characteristics of stem bark of *Wrightia tomentosa* Roem. et Schulta.: **A**, Cork cell in surface view; **B**, Sectional view of cork cell; **C**, Stone cell with prismatic crystals; **D**, Scleried cells; **E**, Medullary rays; **F**, Starch grains; **G**, Fibres; **H**, Parenchyma with starch grains; **I**, Reticulated vessels; **J**, Crystal fibre.

Physico-chemical evaluation

The value of LOD and AIA of stem bark of *Holarrhena antidysentrica* was under the prescribed limit reported by Anonymous (2003), Anonymous (2006) but TA and WSE values were less than the standard limit (Table 1). The standard limit of MSE, CSE and WSA values are not defined by Ayurvedic Pharmacopeia of India while ethanol-soluble extractive value reported by API which is not less than 27%. Similar findings of ASE, WSE and AIA were also reported by Gupta (2008) but TA value is slightly more than the standard limit. Similar results of WSE, ASE, TA, AIA of stem bark of *Holarrhena antidysentrica* were also reported by Zope (2016) but in contrast, findings of LOD was not in conformity. While all the physico-chemical values of WTSB were not defined by the API. The value of LOD determines that the samples were free from moisture and well dried. Less value of LOD could prevent bacterial, fungal and yeast growth. Extractive values determine the nature of soluble of different phytoconstituents. Higher MSE value indicates the possibility of the considerable amount of polar compounds. Higher total ash value showed the inorganic compositions such as carbonates, phosphates, silicates and silica of calcium, sodium, potassium and magnesium. AIA value indicates low impurity of silicates. WSA value determines more insoluble inorganic composition in water. The ash values check the authenticity, quality and purity of drugs.

Table 1. Percentage yields of physico-chemical analysis of stem bark of *Holarrhena antidysenterica* (Roth) Wall. ex A.DC. (HASB) and *Wrightia tomentosa* Roem. et Schulta. (WTSB).

Physico-chemical parameters	**HASB (n= 3 ± SD)**	**API Value (%)**	**WTSB (n= 3 ± SD)**	**API value (%)**
LOD at 105^{o}C	2.62 ± 0.131	<8.0	2.83 ± 0.238	ND
WSE value	23.33 ± 1.152	>27.0	12.48 ± 0.352	ND
MSE value	28.45 ± 0.499	>21.0	19.50 ± 0.215	ND
CSE value	2.15 ± 0.107	ND	5.98 ± 0.074	ND
TA value	8.48 ± 0.04	<7.5	12.13 ± 0.029	ND
AIA value	0.62 ± 0.017	<8.0	1.33 ± 0.015	ND
WSA value	6.75 ± 0.191	ND	10.89 ± 0.038	ND

Note: Results expressed as mean ± SD (n=3); ND = Not develop.

Phytochemical screening

Ganapathy *et al.* (2009) reported similar phytochemical screening results of chloroform and ethanolic extracts of stem bark of *Holarrhena antidysentrica* except flavonoid which was found absent in methanolic extract (Table 2). Similarly, Zope (2016) reported similar findings in stem bark of *Holarrhena antidysentrica.* Similar findings were also reported by Khyade & Vaikos (2011) in bark of *Wrightia tomentosa.* These phytochemicals are mainly secondary metabolites which make the plant medicinally more beneficial and useful. Presence of these phytochemicals directly related with medicinal properties of plants. Carbohydrates and proteins makes the plant nutritionally rich whereas, presence of alkaloids, flavonoids, glycosides, tannins, steroids and resins are responsible for different therapeutic and pharmacological properties such as antidiarrheal, antidysentry, antidiabetic, antioxidant, anticancer, anti-inflammatory activity of plants. Findings validate that plant samples can be used as a potential source in the industries both for the preparation of medicines as well as

nutritionally rich food products.

Table 2. Preliminary phytochemical studies of different extracts of stem bark of *Holarrhena antidysenterica* (Roth) Wall. ex A.DC. (HASB) and *Wrightia tomentosa* Roem. et Schulta. (WTSB).

Name of phytoconstituents	AE		ME		CE	
	HASB	WTSB	HASB	WTSB	HASB	WTSB
Alkaloids						
(i) Mayer's test	+	+	+	+	+	+
(ii) Wagner's test	+	+	+	+	+	+
(iii) Dragendorff's test	+	+	+	+	+	+
(iv) Hager's test	+	+	+	+	+	+
Carbohydrate						
(i) Anthrone's test	-	-	-	+	-	-
(ii) Fehling's test	+	+	+	+	+	+
(iii) Molish's test	+	+	+	+	+	+
Proteins						
(i) Bieuret's test	+	-	+	+	+	-
(ii) Millon's reagent	-	-	+	+	-	-
(iii) Ninhydrin test	-	-	-	-	-	-
(iv) Xanthoproteic test	+	-	-	-	-	-
Resins	+	+	+	+	+	+
Saponin (Foam test)	+	+	-	-	-	-
Starch test	-	-	-	-	-	-
Flavonoid (Shinoda's test)	-	-	-	+	-	-
Steroid (Salkowski's test)	+	+	+	+	+	+
Glycoside (Borntrager's test)	+	-	-	-	+	-
Tannins (Lead acetate test)	+	+	+	-	-	-

Note: +, Present; -, Absent; AE, Aqueous extract; ME, Methanolic extract; CE, Chloroform extract.

Fluorescence study

The results of the fluorescence studies of stem bark of *Holarrhena antidysenterica* (Roth) Wall. ex A.DC. (HASB) and *Wrightia tomentosa* Roem. et Schulta. (WTSB) have been presented in table 3. Similar fluorescence results were also reported by Ganapathy *et al.* (2009) in bark of *Holarrhena antidysentrica* and by Khyade & Vaikosh (2014) in bark of *Wrightia tomentosa* which determined the presence of different types of cells, tissues and phytoconstituents.

Table 3. Fluorescence studies of stem bark of *Holarrhena antidysenterica* (Roth) Wall. ex A.DC. (HASB) and *Wrightia tomentosa* Roem. et Schulta. (WTSB).

Powder + Reagents	Colour observation in day light		Colour observation at 366 nm	
	HASB	WTSB	HASB	WTSB
Powder as such	Light green	Cream	Whitish green	Creamish white
P + NH_3 solution	Brown	Brown	Light brown	Cream
P + Conc. HCl	Dark brown	Light green	Black	Green
Powder + 1N HCl	Yellowish brown	Cream	Brown	Green
P + Conc. H_2SO_4	Black	Light green	Green	Green
P+ Glacial acetic acid	Light yellow	Cream	Light yellow	Creamish white
P + Iodine solution	Light brown	Cream	Brown	Cream
P + 50% KOH	Brown	Light yellow	Green	Green
P + 1N NaOH in methanol	Dark brown	Light yellow	Green	Creamish white
P + Conc. methanol	Yellowish brown	Cream	Light yellow	Creamish white
P + Conc. HNO_3	Brick-red	Yellowish orange	Black	Black
P + 1N NaOH in water	Reddish brown	Black	Dark brown	Dark Brown
P + 50% HNO_3	Brick	Turmeric	Reddish	Dark yellow
P + 50% H_2SO_4	Brown	Cream	Dark green	Green
P + 50% methanol	Yellowish brown	Cream	Creamish white	Creamish white
P + 50% NaOH	Reddish brown	Pale yellow	Black	Green
P + 50% NH_3	Light brown	Cream	Yellow	Creamish white

Note: P, Powder.

Microbial analysis / Microbial limit test (MLT)

The microbial profile of stem bark of *Holarrhena antidysentrica* and *Wrightia tomentosa* was determined as described by Lohar (2007) and found satisfactory. The average total bacterial count (cfu g^{-1}) of stem bark of *Holarrhena antidysentrica* and *Wrightia tomentosa* was 729 cfu g^{-1} and 186 cfu g^{-1} and with Yeast and Moulds

were 29 cfu g^{-1} and 62 cfu g^{-1} counts. Specific pathogenic bacteria, *i.e. Salmonella* sp., *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* were completely absent. Bacterial growth was found absent in all the blank petridishes of each sample which is as per the standard limit of WHO reported by Anonymous (1998) and API (Anonymous 2008) (Table 4). Results indicate that the powder of samples was free

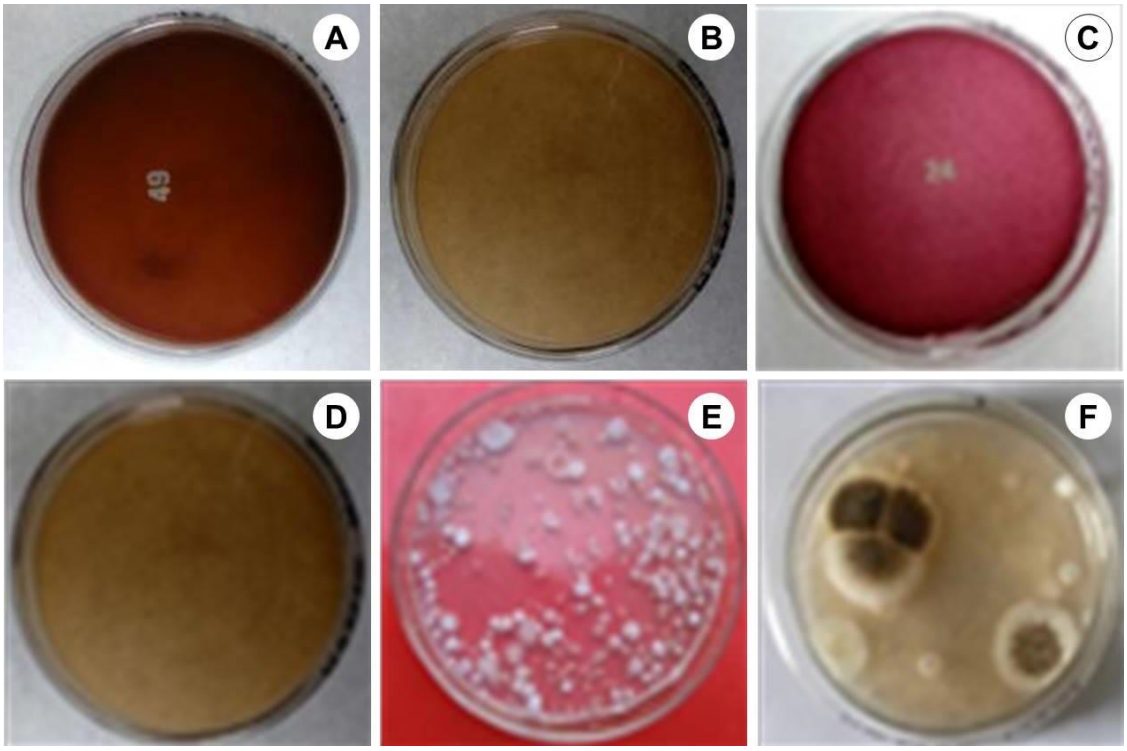

Figure 6. Microbial limit test of stem bark of *Holarrhena antidysenterica* (Roth) Wall. ex A.DC.: **A**, *Salmonella* sp. showing negative result; **B**, *Staphylococcus aureus* showing negative result; **C**, *Escherichia coli* showing negative result; **D**, *Pseudomonas aeruginosa* showing negative result; **E**, Total bacterial count (TBC) showing positive result; **F**, Yeast & Mould (Y & M) showing positive result.

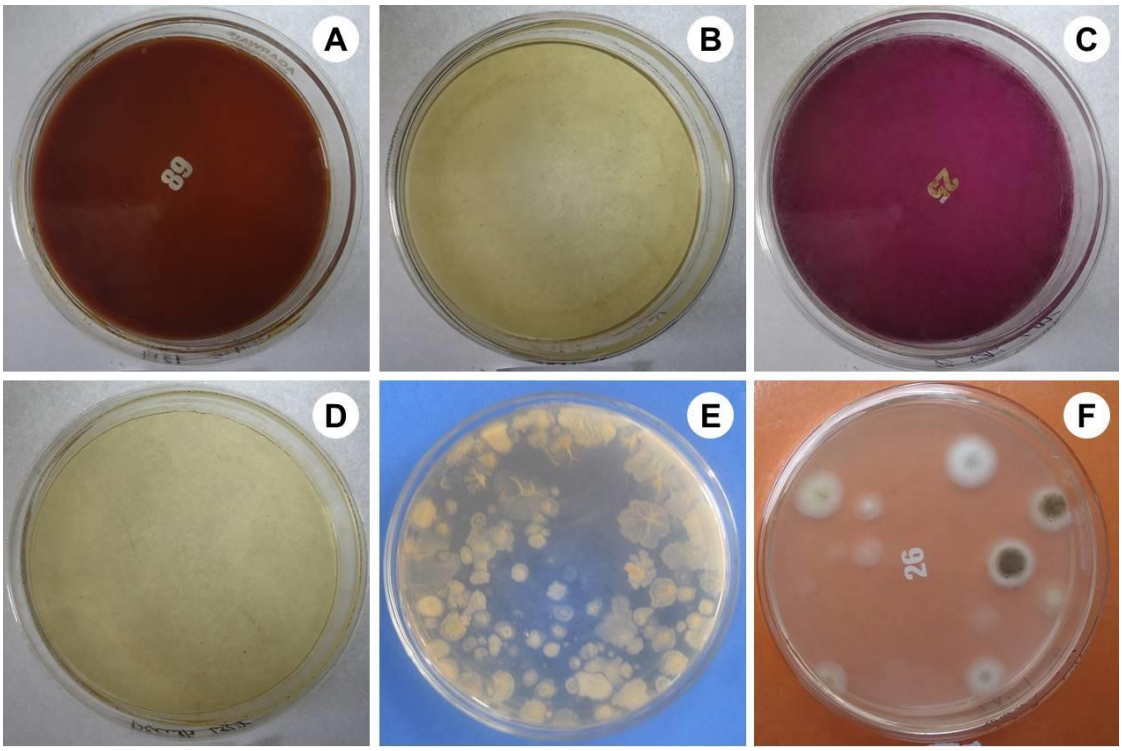

Figure 7. Microbial limit test of stem bark of *Wrightia tomentosa* Roem. et Schulta.: **A**, *Salmonella* sp. showing negative result; **B**, *Staphylococcus aureus* showing negative result; **C**, *Escherichia coli* showing negative result; **D**, *Pseudomonas aeruginosa* showing negative result; **E**, Total bacterial count (TBC) showing positive result; **F**, Yeast & Mould (Y & M) showing positive result.

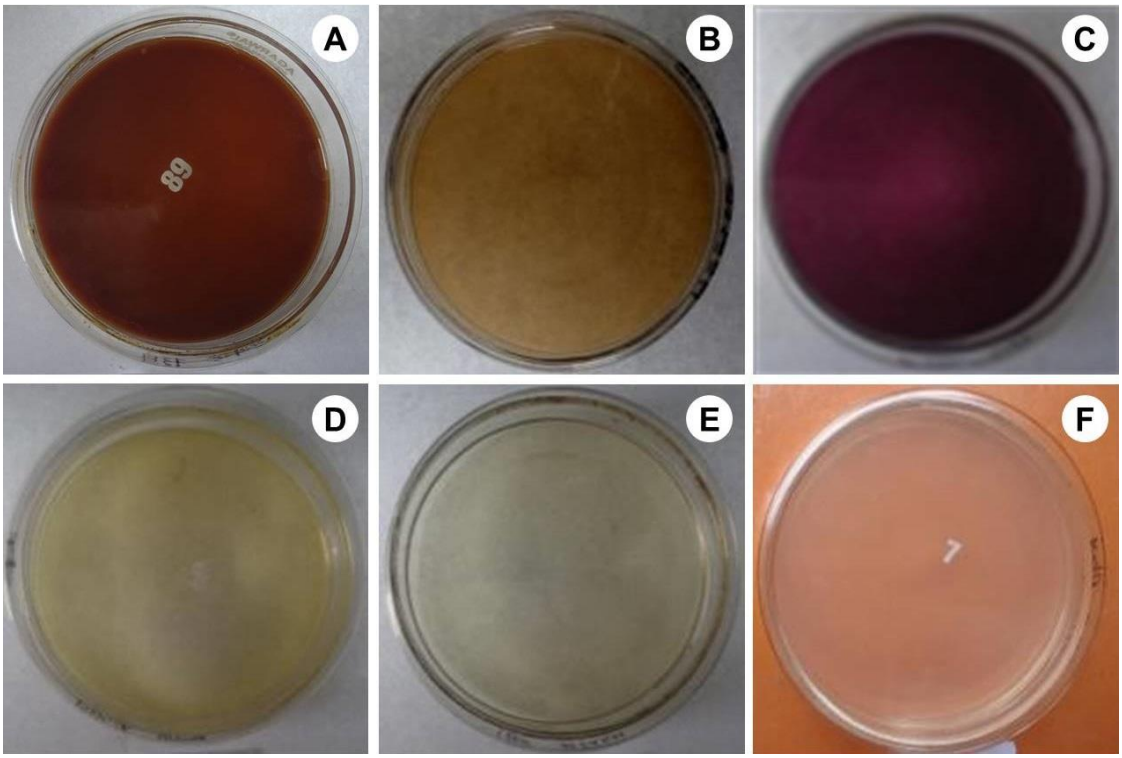

Figure 8. Microbial limit test of blank of stem bark of *Holarrhena antidysenterica* (Roth) Wall. ex A.DC.and *Wrightia tomentosa* Roem. et Schulta.: **A**, Blank of *Salmonella* sp. showing negative result; **B**, Blank of *Staphylococcus aureus* showing negative result; **C**, Blank of *Escherichia coli* showing negative result; **D**, Blank of *Pseudomonas aeruginosa* showing negative result; **E**, Blank of Total bacterial count (TBC) showing negative result; **F**, Blank of Yeast & Mould (Y & M) showing negative result.

from moisture, microbial contamination and thus having good quality and purity for therapeutic use. It indicates that stem bark in powder form can be used as an ingredient in the ayurvedic drug formulations (Fig. 6–8). All these parameters *viz*. organoleptic, macroscopy, microscopy, powder characteristics, physico-chemical analysis, phytochemical screening, fluorescence study and microbial analysis were helpful in differentiation of stem of both plants morphologically, anatomically and chemically.

Table 4. Standard limit of microorganism per pathogen in plant powder sample.

Microorganism	**Permissible limit as per WHO/API**	**Observation/Result**	
		HASB	**WTSB**
Salmonella sp.	None	Absent	Absent
Staphylococcus aureus	Absent	Absent	Absent
Escherichia coli	Absent	Absent	Absent
Pseudomonas aeruginosa	Absent	Absent	Absent
Total Bacteria Count (TBC)	<105 g^{-1}	729 g^{-1}	186 g^{-1}
Total Yeast & Mould Count (Y & M)	103 g^{-1}	29 g^{-1}	62 g^{-1}

CONCLUSION

The present work on comparative pharmacognostical studies of stem bark of *Holarrhena antidysenterica* and *Wrightia tomentosa* was carried out using standard procedures prescribed by API and WHO and findings are helpful in taxonomic description of both the plant species and also useful in adulterant check, identification and authentication of plants. These studies indicated that both drugs are used in preparation of medicines in pharmaceutical industries and also beneficial in the development of monograph of stem bark of both plants.

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