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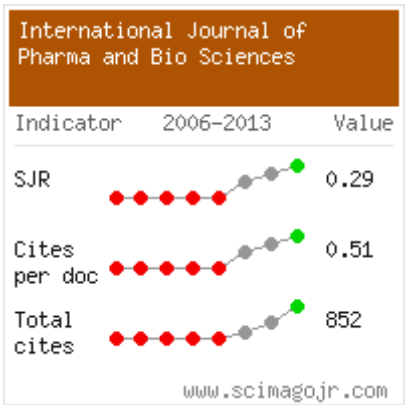

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PHARMA COGNOSTIC EVALUATION AND DETAILED PHYTOCHEMICAL ANALYSIS OF *HYMENODICTYON OBOVATUM* BARK, AN ENDEMIC PLANT.

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ABSTRACT

Hymenodictyon obovatum Wall. in Roxb. (Rubiaceae) is a large endemic tree found in dry deciduous forests of Western Ghats. Bark of this plant is medicinal and used in Ayurvedic and Traditional system of medicine. The bark is febrifuge, antiperiodic, astringent, antimicrobial and also used to cure night blindness. Because of its medicinal properties, inner bark of this plant was subjected to study different pharmacognostical analysis. Microscopic studies have shown acicular and prismatic oxalate crystals, helical and pitted xylem vessels and biseriate medullary ray. The trail was made to study the difference between hot and cold bark extracts, which is found useful to know the importance of compound quality and exaction. Detailed phytochemical analysis and TLC was carried out and observed the presence of steroids, flavonoids, alkaloids, coumarin glycosides, tannins, phenols, vitamins and enzymes. The florescent compound found in TLC is predicted to be Scopoletin by GC-MS study.

KEYWORDS : Pharmacognosy; *Hymenodictyon obovatum*; Scopoletin; phytochemical analysis

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INTRODUCTION

Genus *Hymenodictyon* (Rubiaceae) has three species *Hymenodictyon orixence* Wall., *Hymenodictyon obovatum* Wall.and *Hymenodictyon flaccidm* Wall. in India[1]. Of these *H. orixence and H. obovatum* are found in Karnataka. *H. obovatum* is endemic to Western Ghats, extending from Konkan to Travancore[2]. In Karnataka *H. obovatum* is distributed in Belgaum, Chikmagalur, Hassan, Mysore, North Kanara, Shimoga and South Kanara[3]. Both *H. orixence and H. obovatum* are having medicinal properties as the former is mentioned in Ayurveda and Traditional system of medicine. The bark of this plant is a febrifuge, antiperiodic and the leaves are used to combat feverish heat and chickenpox[3]. Similarly the inner bark of *H. obovatum* is astringent[4,5] used as a substitute for quinine[5,6] and for night blindness[7]. It has also been proved that the bark of *H. obovatum* has antimicrobial properties[8]. In view of this a detailed pharmacognostic and phytochemical study was made. *Hymenodictyon obovatum* Wall. is called as Gandele in Kannada. Morphologically it is a large deciduous tree, 6-8m high; leaves crowded at the apices of twigs, 6-12 X 4-8.5 cm, obovate or elliptic, base cuneate, apex abruptly acuminate, glabrous above, more or less pubescent beneath. Flowers are creamy – yellow, small, with a pungent odour, in sessile clusters along the rachis of dense cylindric pubescent panicled spikes. Calyx is broadly campanulate, *c* 0.2mm long, pubescent outside; teeth triangular, acute. Corolla is *c* 0.2mm long, pubescent outside; tube very short; limb broadly campanulate, *c* 0.2mm long, ellipsoid. Seed wings narrowed at both the ends.Plant flowers and fruits from June to December.

MATERIALS AND METHODS

Bark of *Hymenodictyon obovatum* were collected from Halligeri village of Dharwad District, Karnataka State and identified with the help of floras[9, 10]. The herbaria and dried powder specimens are deposited in the Department of Botany (KUD/BOT/2013/H2; KUD/BOT/2013/P2), Karnatak University, Dharwad. Fresh material was used for microscopic observations like anatomical and histochemical analysis. Free hand sections were taken and stained with the standard reagents used in pharmacognostic studies [11, 12]. Powder and maceration studies were also performed[13]. Photomicrography was done by using compound binocular microscope at different magnifications (Carl Zeiss Axio Imager M_2 model; 5X and 10X) with inbuilt analogue camera (ProgRess C5- JENOPTIK), using ProgRes® CapturePro 2.8- JENOPTIK software optical system. The inner bark was dried, powdered, sieved and used for chemical analysis. Physicochemical studies like total ash, water soluble ash, acid insoluble ash and sulphated ash were carried out. Solubility test (water and alcohol) and successive solvent extraction (hexane, chloroform, acetone, alcohol and water) were done for both hot and cold extracts[13, 14]. The moisture content was detected by loss on drying method[15]. Nutritive contents, nutritive value and minerals contents were determined[16]. Detailed phytochemical analyses were performed using standard methods[13]. Fluorescence analysis and behavioral studies of the powdered bark material were observed in the visible and UV light at 254 nm and 365 nm wavelength by treating the powder with different reagents[17]. Thin layer chromatography was carried out with aluminium plate (5 x 9 cm) pre-coated with silica gel 60/UV 254, plate no.25425A-P00 (S. D. Fine) for all the extracts and Rf was calculated. Different combinations of solvent system were tried for best separation of constituents[18]. GC-MS for Acetone extract was carried out[19]. All the reagents of analytical grade were procured from Hi-Media, Mumbai, India. Cell size is represented as RDS (radius), DST (distance) and PRM (prism). Standard deviation was calculated as mean of three replicates for physico-chemical parameters using SPSS version 16.0, statistical package. Data is represented in table 1.

RESULTS

(i) Macroscopy

The bark is around 4 cm thick, externally rough and mottle colored. Exfoliations are rectangular flakes, grey-black in color and slightly rough textured. Inner bark is smooth and lenticulate.

Blaze is pinkish red in color. Transverse section of the bark has brownish grey color on outer side and salmon grey color with crystalline texture (Fig. 1-A, B, C) on the inner side.

(ii) Organoleptic charecters

Powder of inner bark is brown in colour, slightly fibrous, rough in texture and it has characteristic woody odour and bitter in taste.

(iii) Microscopy

Radial (Fig. 1- D, E, F and Fig. 2- H) and tangential (Fig. 2- J) sections of bark show presence of rectangular, horizontally arranged, non-lignified cork cells consisting of phellem, phellogen and phelloderm, followed by 2-3 layered polygonal parenchyma, large sclereids of various size and shape. The cortical cells have some of the cells filled with circular starch grains, calcium oxalate crystals, lignified cells and oil globules. The cortex also contains longitudinally arranged rectangular biseriate medullary rays. Transverse sections (Fig. 1-G) and longitudinal sections (Fig. 2-I) show 3-5 layered cork cells followed by brown thick walled sclereids, parenchymatous biseriate medullary rays embedded with fusiform initials, phloem fibres, companion cells, sieve tubes, numerous stone cells with narrow lumen.

(iv) Powder and Maceration analysis

Powder and maceration microscopy is an important parameter to identify and distinguish the drug from its substitute and adulterants. Study shows the presence of acicular (DST 30.27-70.71 µm) and prismatic (DST 20.87-45.15 µm) calcium oxalate crystals (Fig. 2-M & N), oil globules (RDS 5.45-7.58 µm), pigmented cells (DST 58.14-149.59 µm) (Fig. 2-R), stone cells (PRM 31.43 - 47.14 - 60.03 µm) (Fig. 2-S,T), starch grains (RDS 2.69-5.78- 13.95µm) (Fig. 2-X), axial and ray parenchyma (RDS 45.57 X 71.82µm) (Fig. 2-Y), helical (DST 104.2 X 47.05µm – 152.4 X 57.09µm) and pitted (DST 185.9 X 31.95 – 216 X 39.92µm) xylem vessels (Fig. 2-K & L) and sclereids of different shapes (DST 91.09 X 30.27 – 245.5 X 68.08µm) (Fig. 2-O,P,Q,U,V,W,Z&Z1).

(v) Histochemical study

Histochemical study of section and powder analysis showed the presence of cellulose, lignin, chitin, starch, resin and calcium oxalates.

(vi) Physico-chemical analysis

The acetone and alcohol cold extracts showed higher extractive values whereas for hot extracts the water and chloroform extracts showed higher extractive values. Solubility value was high for cold alcoholic extract whereas it was low for water soluble extract. Total ash, acid insoluble ash, water soluble ash, sulphated ash and nutritional contents were calculated and are presented in Table 1.

(vii) Mineral analysis

Mineral composition of *H. obovatum* powder revealed presence of micro elements like Iron, Zinc, Manganese and Copper in terms of ppm. The macro elements such as calcium, nitrogen, potassium, magnesium, sulphur, phosphorous and sodium are estimated in terms of percentage value (Table 2).

(viii) Fluorescence analysis

Though in most of the reagents the colour characteristics are similar, treatment with lead acetate, NaOH, ferric chloride, petroleum ether and glycerin showed diagnostic colour for powder (Table 4).

(ix) Preliminary phytochemical analysis

Hot (Soxhlet) and cold (Percolation) extracts of five different solvents like hexane, chloroform, acetone, alcohol and water showed the presence of carbohydrate, proteins, saponins, enzymes, vitamins, organic acids, tannins, phenolic compounds, alkaloids, flavonoids, steroids. And absence of glycosides, volatile oils, fats and oils were confirmed. Tests do not show any difference in the constituents of hot and cold extracts (Table 3).

(x) TLC Profile

TLC for five different cold and hot extracts was carried out. Cold extracts exhibited higher number of bands than that of hot extracts except for acetone extract (Table 5).

Table 1
Physicochemical values of H. obovatum stem powder

Parameter		
1. Ash Value (% w/w):		
a. Total ash	5.483 ± 0.015	
b. Acid insoluble ash	0.500 ± 0.010	
c. Water soluble ash	3.476 ± 0.025	
d. Sulphated ash	8.983 ± 0.015	
2. Extractive value (% w/w):	Cold extraction	Hot extraction
a. Hexane	2.940 ± 0.055	2.993 ± 0.011
b. Chloroform	1.606 ± 0.050	1.996 ± 0.005
c. Acetone	8.613 ± 0.057	3.983 ± 0.028
d. Alcohol	8.570 ± 0.020	7.950 ± 0.050
e. Water	6.160 ± 0.010	8.966 ± 0.057
3. Solubility Test (% w/w):		
a. Alcohol	6.500 ± 0.050	8.576 ± 0.023
b. Water	14.96 ± 0.055	13.07 ± 0.072
4. Nutritive content (%)		
a. Ash	5.483 ± 0.015	
b. Moisture	4.016 ± 0.005	
c. Fat	1.563 ± 0.005	
d. Fiber	1.043 ± 0.003	
e. Protein	5.103 ± 0.005	
f. Carbohydrate	86.808	
5. Nutritive value in cal per 100 g powder	**381.709**	

Table 2
Mineral analyses of H. obovatum stem powder

Sl. no.	Mineral content	Quantity
1	N %	0.816
2	P %	0.026
3	K %	0.468
4	Na %	0.093
5	S %	0.016
6	Ca %	2.250
7	Mg %	0.127
8	Zn ppm	12.57
9	Fe ppm	603.55
10	Mn ppm	7.38
11	Cu ppm	3.58

Table 3
Detailed qualitative chemical analysis of H. obovatum for five successive cold and hot extracts.

Test for Hot and cold extract.	Test	Pet. ether extract	Chloroform extract	Acetone extract	Ethanol extract	Aqueous extract
Test for Carbohydrate	Molish's test	-	+	+	+	+
Reducing sugar	(a) Fehling's test	-	-	+	+	+
	(b) Benedict's test	-	-	0.5%	1.0%	0.5%
Monosaccharides	Barfoed's test	-	-	-	+	+
Pentose sugar	(a) Bial's reagent	-	-	-	+	+
	(b) HCl test	-	-	-	+	-
Hexose sugar	(a) Selwinoff's test (for Ketohexose like fructose)	-	-	+	+	+
	(b) Tollen's phloroglucinol test for galactose.	-	-	+	+	+
Non- Reducing sugars	(a)	-	-	-	-	-

	(b)	-	-	-	+	+
Non- Reducing	(a) Iodine test	-	-	-	-	-
Polysaccharides (Starch)	(b) Tannic acid test	-	-	-	-	-
Gums		-	-	+	+	+
Mucilage	(a) Ruthenium red test	+	+	+	+	+
Test for Proteins	(a) Biuret test	-	-	-	+	-
	(b) Millon's test	-	-	+	+	+
	(c) Xanthoprotein test	-	-	-	+	+
	(d) Test for protein containing sulphur	-	-	+	+	+
	(e) Precipitation test					
	(1) absolute alcohol					
	(2) 5% $CuSO_4$					
	(3) $HgCl_2$ Solution	-	-	-	+	+
	(4) 5% ammonium sulphate	-	-	+	+	+
		-	-	-	+	+
		-	-	+	+	+
Test for Amino acids	(a) Ninhydrin test	-	-	+	+	+
	(b) Test for tyrosine	-	-	+	+	+
	(c) Test for tryptophan	-	-	+	+	+
	(d) Test for Cysteine	-	-	+	+	+
Fats and oils	(a) Sudan red III test	-	-	-	-	-
	(b) Filter paper test	-	-	-	-	-
	(c) Solubility test	-	-	-	-	-
	(d) Saponification test	-	-	-	-	-
	(a) Salkowski test	-	-	+	+	+
Steroid test	(b) Liebermann- Burchard reaction	-	-	+	+	+
	(c) Liebermann's reaction	-	-	+	+	+
Volatile oils		-	-	-	-	-
Test for glycosides	(a) Kedde's test	-	-	-	-	-
(A) Test for cardiac glycosides	(b) Legal's test	-	-	-	-	-
	(c) Deoxysugars	-	-	-	-	-
	(d) Liebermann's test	-	-	+	+	+
(B)Test for Anthraquinone glycosides	(a) Borntrager's test for anthraquinone glycosides	-	-	-	-	-
	(b) Modified borntrager's test for C-glycosides	-	-	-	-	-
	(c) Hydroxy- antraquinone	-	-	-	-	-
(C) Test for Saponin glycosides	(a) Foam test	-	-	-	-	+
(D) Test for Cyanogenetic	(a) Guinard test	-	-	-	-	-
	(b) Mercurous nitrate test	-	-	-	-	-
	(c) guaiacum resin test	-	-	-	-	-
	(a) Aromatic test	-	-	-	-	-
	(b) Alkalinity test	-	-	+	+	+
	(c) Florescence test	-	-	+	+	+
	(a) Shinoda test	-	-	+	+	+
	(b) Lead acetate test	-	-	+	+	+

glycosides	(c) NaOH test	-	-	+	+	+
(E) Test for Coumarin glycosides						
(F) Test for Flavonoids						
Test for Alkaloids:	(a)Dragendorff's test	-	-	-	-	-
	(b) Mayer's test	-	-	+	+	+
	(c) Hager's test	-	-	+	+	+
	(d) Wagner's test	-	-	+	+	+
Tests for Tannins and Phenolic compound	(a) 5% $FeCl_3$ solution	-	-	-	+	+
	(b) Acetic acid solution	-	-	+	+	+
	(c) Potassium dichromate	-	-	+	+	+
	(d) $NH_4OH+AgNO_3$	-	-	+	+	+
	(e) Dilute HNO_3	-	-	+	+	+
Test for Enzymes	(a) Catechol test	+	+	+	+	+
(1) Oxidase	(b) Guaicum test	+	+	+	+	+
	(a) Catechol test	-	-	-	-	-
	(a) H_2O_2 test	+	+	+	+	+
	(a) TTC test	+	+	+	+	+
(2) Peroxidase	(b) Methylene blue test	+	+	+	+	+
(3) Catalase						
(4) Dehydrogenase						
Test for Organic acids	(a) Oxalic acid	-	-	-	-	-
	(b) Tartaric acid	-	-	-	-	-
	(c) Citric acid	+	+	+	+	+
	(d) Malic acid	-	-	-	-	-
Test for Vitamins	(a) Antimony trichloride test					
(1) Vitamin A		-	-	-	+	+
	(a) Sodium nitroprusside	+	+	+	+	+
(2) Vitamin C	test					
	(b) Ferrous sulphate test	+	+	+	+	+
(3) Vitamin D		-	-	+	+	+

Table 4
Fluorescence analysis of H. obovatum at different wavelengths

Treatment	Visible light	U. V. light at 254nm	U.V. Light 365nm
	Fruit	Fruit	Fruit
P + NaOH (Aqueous)	Arylide yellow	Apple green	Lime yellow
P + NaOH (Alcoholic)	Wine red	Dark brown	Alga green
P + 1N HCl	Cream	Cream	White
P + 50% H_2SO_4	Light Arylide yellow	Light green	White
P + 50% HNO_3	Yellow	Yellow	Brown
P + Lead acetate + NaOH	Wine red	Green	Willow green
P + HNO_3	Yellow	Yellow	Brown
P + acetic acid	Arylide yellow	Light green	White
P + $FeCl_3$	Greenish brown	Door country green	Apple green
P + HNO_3 + NH_3	Brown	Door country green	Light green
P + H_2SO_4	Light brown	Door country green	White
P + Pet. Ether	Cream	Light cream	Florescent white
P + Methanol	School bus chrome	Light green	White
P + Water	School bus chrome	Door country green	White
P + Benzene	Cream	Light green	Tan
P + Glycerin	School bus chrome	Light green	Florescent tan

P: Powder sample

Table 5
Observation of TLC of H. obovatum for different extracts

Extract/Solvent combination	Rf Value	
	Hot extract	Cold extract
Hexane extract	0.128, 0.028, 0.1, 0.24, 0.65, 0.74, 0.85,	0.128, 0.028, 0.1, 0.24, 0.28, 0.65,0.74, 0.85, 0.94, 0.95
Hexane : Acetone (8: 2v/v)	0.94, 0.95	
Chloroform extract	0.041, 0.069, 0.125, 0.208, 0.291, 0.36,	0.041, 0.069, 0.125, 0.166, 0.139, 0.36, 0.47, 0.56, 0.611,
Hexane : ethyl acetate: acetone (6: 1: 3v/v)	0.47, 0.902, 0.958, 0.97	0.69, 0.77, 0.79, 0.902, 0.93, 0.94, 0.95, 0.97, 0.98
Acetone extract	0.069, 0.142, 0.208, 0.357, 0.458, 0.638,	0.069, 0.142, 0.208, 0.357, 0.458, 0.638, 0.68, 0.81, 0.83,
Hexane : Acetone (6: 4v/v)	0.68, 0.81, 0.83, 0.88, 0.94, 0.95	0.88, 0.94, 0.95
Alcohol extract	0.083, 0.166, 0.263, 0.33, 0.146, 0.55,	0.083, 0.166, 0.263, 0.33, 0.146, 0.55, 0.625, 0.76, 0.87, 0.98
Dichloromethane : Acetone (7: 3v/v)	0.625, 0.76, 0.87, 0.95, 0.98	
Aqueous extract (Hexane: Acetone 5: 5v/v)	0.125	0.13

Habit and Anatomy of Hymenodictyon obovatum

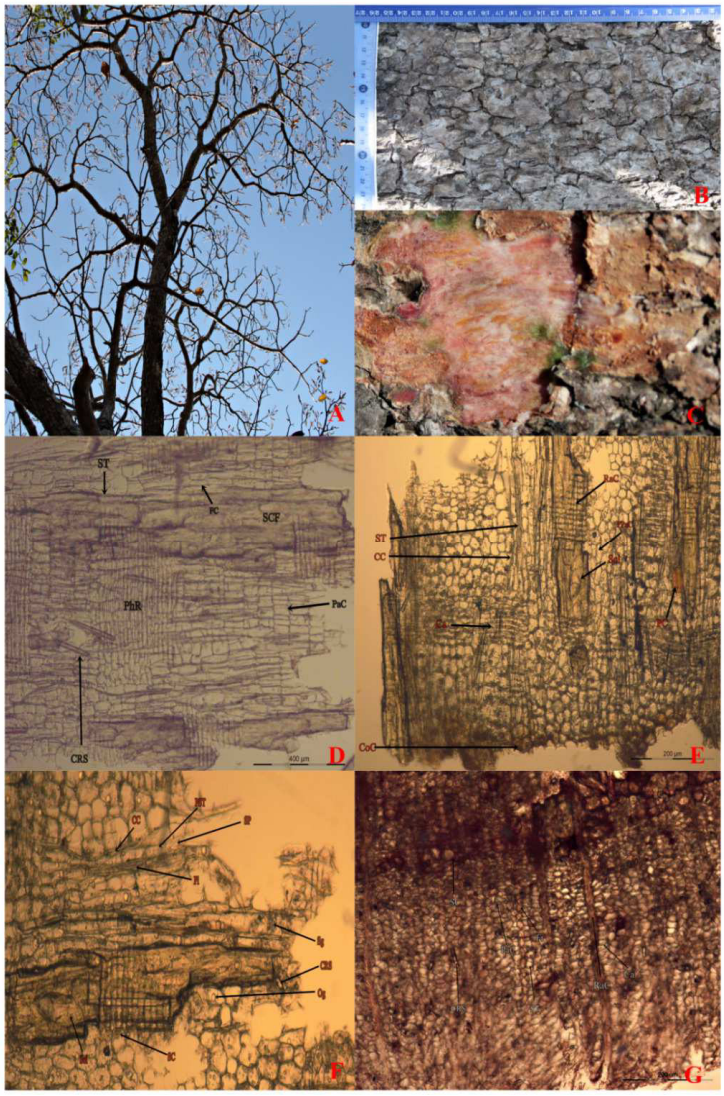

Figure. 1
A- Habit of Hymenodictyon obovatum, B- Bark, C- Blaze of bark, B- D, E, F- Radial sections of bark, G- Transverse section.

ST- Sieve tube, FC-Fusiform cells, SCF-Sclerenchyma fibre, PhR-Phloem ray, PaC-Parenchyma cell, CRS-Crystals, CC-Companian cell, Scl- Sclerenchyma cell, PPaC- Pitted parenchyma cell, RaC- Ray cell, PC-Pigmented cell, CoC-Cork cell, PST-Pitted sieve tube, SP-sieve plate, Fi-Fibres, SC-Stone cell, Og-Oil globule, Ca-cambium

Anatomy, powder and maceration study of Hymenodictyon obovatum.

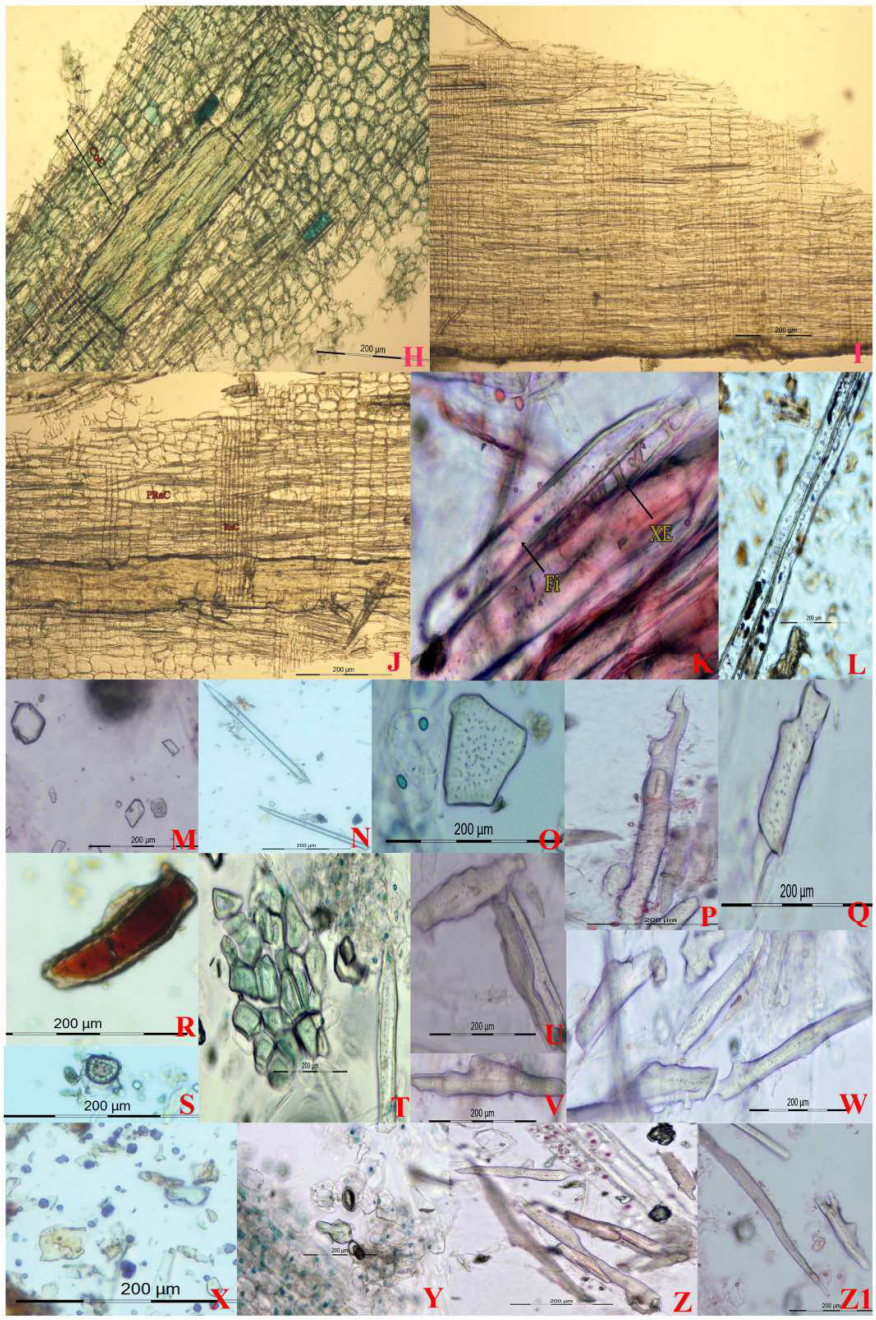

Figure. 2

H- Radial section of Hymenodictyon obovatum stem, I- longitudinal section, J- Tangential section, K-section showing fiber and helical xylem element, L- Pitted xylem element, M-Prismatic calcium oxalate crystals, N-Acicular calcium oxalate crystals, O,S- Stone cell, P,Q,U,V,W,Z,Z_1 – Different shaped sclereids, Y-Axial and ray parenchyma. CoC-Cork cell, PRaC-Phloem ray cell, RaC- Ray cell, Fi- Fiber, XE- Xylem element.

TLC Profile

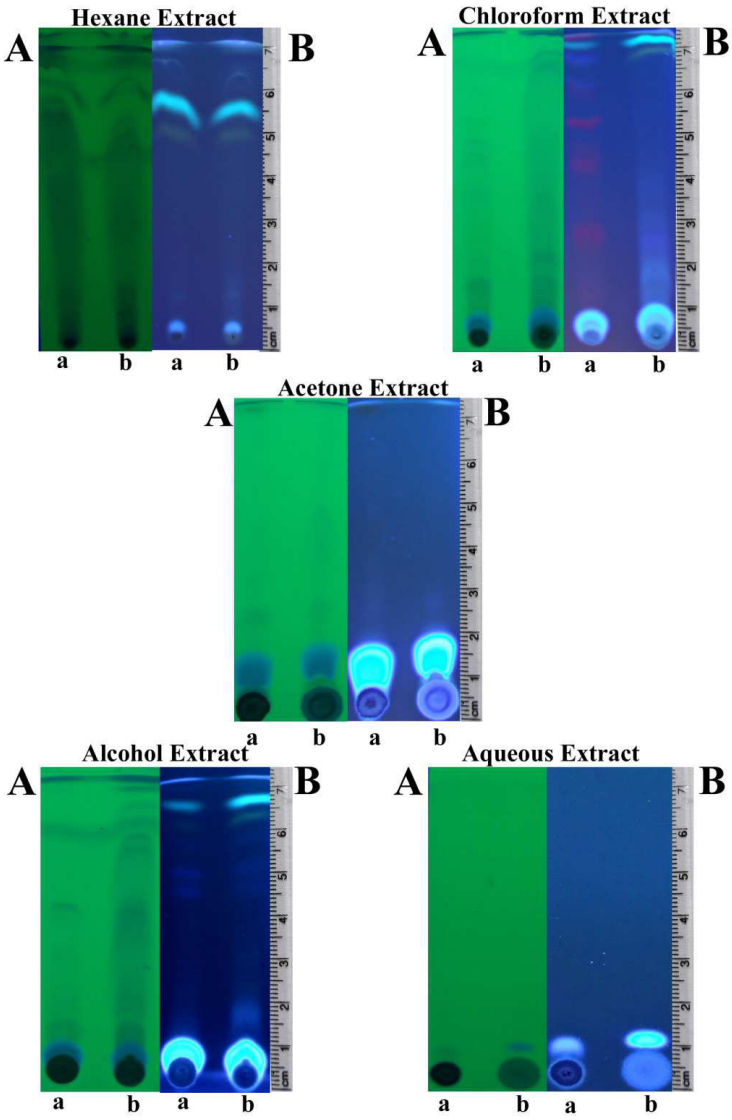

Figure. 3
TLC profile of Hexane, Chloroform, Acetone, Alcohol and Aqueous extracts. A- UV at 254nm, B- UV at 365nm. a- cold extract, b- hot extract.

DISCUSSION

Traditional system of medicine uses a majority of crude drug of plant origin. Pharmacognostical and physico- chemical parameters are considered as reliable tool in identification and evaluation of crude drug. World Health Organization[14] has considered the macro and microscopic characters as reliable, accurate and inexpensive analytical technique and should be carried out before any further studies are undertaken. The real problem in identification is when the herbal drugs are in powder form or in the form of small pieces. Morphological and microscopic studies of stem bark act as a reliable source for identification and to check adulteration. Some salient anatomical features of *H. obovatum* are helical and pitted xylem vessel, acicular prismatic crystals and uni to biseriate medullary rays. Physico chemical studies like ash value are useful in indicating extraneous matter adhering to plant material. Extractive value and solubility value helps to know the net yield of dry sample in different solvents. Moisture indicates the quality and preliminary

phytochemical screening indicates the detailed chemical groups and compounds present in crude sample. Depending on system of medicine mode of extraction varies. Some systems prefer cold (infusion method) and some hot (decoction, soxhlet) extraction method. Both extractions have their own importance. Cold extraction method is used to retain heat labile compounds and hot for heat resistant compounds. Solvents like methanol (polar) and aqueous (highly polar) are preferred as they dissolve almost all the compounds. It is found that the activity of the sample gets affected by the mode of extraction. As some compounds are thermo labile they get destroyed when heated whereas some heat resistants get reformed when heated. This is confirmed by the work of [20]. Although Preliminary phytochemical analysis did not show any difference for hot and cold extracts (Table 3) the TLC profile showed differences (Fig C). The cold extracts showed more bands as compared to hot extracts except for acetone extract which is a clear indication that there are thermolabile compounds which probably get denatured on heating. Quinoidin, berberin and paracin are some of the known compounds of *H. excelsum*[21]. Scopoletin is a major fluorescent compound which was isolated from the related genus *Hymenodictyon orixense*[22], which has a molecular weight of 192. The fluorescence compound observed in TLC of all the extracts of *H. obovatum* is also probably Scopoletin as the GC-MS analysis of the extracts showed that the compound has a same Molecular weight of 192, which needs further confirmation.

CONCLUSION

The parameters studied can be utilized in identification of *Hymenodictyon obovatum* in crude drug form and can be used as a potential source for useful therapeutics. The resulted data will be beneficial for monographs, quantitative and qualitative standardization of genuine drug in herbal preparations. As there is no record on detailed pharmacognostic evaluation of this crude drug, this communication throws light on parameters essential to fix standards for this medicinal material.

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REFERENCES

1. Hooker JD. The Flora of British India, Vols 7, Bishen Singh Mahendra Pal Singh reprint : Dehra Dun, (1978).
2. Ahmedullah M. Nayar MP. Endemic Plants of the Indian Region, Vol1, Peninsular India. Botanical Survey of India: Calcutta, (1986).
3. Manilal. Hortus Malabaricus, Vol 4, University of Kerala: Thiruvananthapuram, (2003).
4. Punekar SA, Lakshaminarasimhan P. Flora of Anshi National Park, Western Ghats, Karnataka. Biosphere Publication: Pune, (2011).
5. Yoganarasimhan SN. Medicinal Plants of India. Vols 2. Interline Publishing Pvt. Ltd., Bangalore: Karnataka, (1996).
6. Talbot WA. Forest Flora of the Bombay Presidency and Sind, Vols 2, Bishen Singh Mahendra Pal: Dehra Dun, (1976).
7. Prashant YM, Vijay VB. Ethno- medicinal Wisdom of tribals of Aurangabad district (M. S.), India. Indian J of Natural Products and Resources, 2(1): 102-109, (2011).
8. Irawwa BG, Reshma MG, Ranjita K, Hegde GR. Antimicrobial Screening of tree barks of *Heterophragma quadriloculare* (Roxb.) K. Schum. and *Hymenodictyon obovatum* Wall. from Kumta and Dharwad, Karnataka, India. Int J Medicinal and Aromatic Plants, 2: 167-171, (2011).
9. Cooke T. The Flora of the Presidency of Bombay, Vols 3, London. (B.S.I. Reprint): Culcutta, (1958).

10. Almeida MR. Flora of Maharashtra, Vol 2, St. Xeviers College: Mumbai, (1996).
11. Johansen OA. Plant Microtechnique, Mc Graw Hill: New York, (1940).
12. Trease GE, Evans WC. Pharmacognosy, Edn 13. Bailliere Tu- idal: London, (1989).
13. Khandelwal KR. Practical Pharmacognosy, Nirali Publication: Pune, (2007).
14. World Health Organization. Quality control methods for medicinal plants, AITBS publishers: New Delhi, (2002).
15. Anonymous. Pharmacopoeia of India, Vol 1, Government of India, Ministry of Health, Controller of Publication: New Delhi, (1996).
16. Indrayan AK, Sudeep Sharma, Deepak Durgapal, Neeraj Kumar, Manoj Kumar. Determination of nutritive value and analysis of mineral elements for some medicinally valued plants from Uttaranchal. Current Science, 89(7): 1252-55, (2005).
17. Kokoshi CJ. Kokoski RJ, Slama FJ. Fluorescence of powdered vegetable drugs under ultraviolet radiation. J of Pharmaceutical Science, 47: 715-717, (1958).
18. Stahl E. Thin layer chromatography; A laboratory hand book, Edn 2, Verlag, Berlin, Haidelberg: Springer, (2005).
19. Janakiraman N, Johnson M, Sahaya Sathish S. GC-MS analysis of bioactive constituents of *Peristrophe bicalyculata* (Retz.) Nees. (Acanthaceae). Asian Pacific J of Tropical Biomedicine 2012; 46-49.
20. Ranjita RK, Hegde GR, Hegde G and Hebbar SS. Antimicrobial screening of Medicinal plants against human Pathogens- A Comparative account of two different methods of Extraction. Int J of Drug Development, 4(1): 82-89, (2012).
21. George Watt. Dictionary of the Economic Products of India, Vol 3, Bishen Singh Mahendra Pal Singh, New Connaught Place (Reprint), Dehra Dun: India, (2006).
22. Swe NN. Scopoletin from *Hymenodictyon orixense* (Roxb.) Mabb. J of Myanmar Academy of Arts & Science, 4: 193-203, (2008).