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RESEARCH ARTICLE

PHARMACOGNOSTIC EVALUATION OF *Bridelia retusa* SPRENG.

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ABSTRACT

Bridelia retusa Spreng belong to family Euphorbiaceae and is being used in the indigenous systems of medicine for the treatment of rheumatism and also used as astringent. The drug part used is the grayish brown roots of this plant. The species is also well known in ayurvedic medicine for kidney stone. The present paper reveals the botanical standardization on the root of *B.retusa*. The Pharmacognostic studies include macroscopic, microscopic characters, histochemistry and phytochemistry. The phytochemical and histochemical test includes starch, protein, saponin, sugar, tannins, glycosides and alkaloids. Percentage extractives, ash and acid insoluble ash, fluorescence analysis and HPTLC.

INTRODUCTION

Bridelia retusa Spreng. belongs to family Euphorbiaceae is distributed in India, Srilanka, Myanmar, Thailand, Indochina, Malay Peninsula and Sumatra (Anonymous, 1992) The plant body is erect and a large deciduous tree. The root is cylindrical in shape. Their length ranges from 30 to 61 cm length and 15 to 31 cm in breadth. (Cooke, 1958). The roots are medicinally important known as Pashanabheda drugs. Literature survey indicated that the species is also known by the name of *B. squamosa* Spreng (Nadkarni *et al*., 1927; Chopra *et al*., 1956; Krishnan, 1992). Bark of the *Bridelia retusa* contain gingili oil which is used as liniment in rheumatism. Roots of *Bridelia retusa* contains bisabolane sesquiterpenes like, (E)-4-(1,5 dimethyl-3-oxo-1-hexenyl) benzoic acid, (E)-4-(1,5 dimethyl-3-oxo-1,4-hexenyl) benzoic acid, (R)-4-(1,5 dimethyl-3-oxo-hexenyl) benzoic acid , 5-allyl-1,2,3- tri methoxy benzene (elemicin), (+)- sesamin and 4-isopropyl benzoic acid (cumic acid) (Anonymous, 1992, Bahl and Seshadri., 1970). For the present investigation *Bridelia retusa* is selected as, it is being sold in the market under the common name asanamul. The drug part used is the roots. The drug asana is useful as rheumatism and astringent (Anonymous, 1992). Review of literature revealed that the asana is used as a nutritive and health promoting properties as well as an antianebic, antianemic, antibacterial, anticonvulsant, antidiabetic, antidiarrheal, antihelminthic, anti-inflammatory, antimalarial and antiviral (Anonymous, 1992; Begun *et al*., 1991; Anonymous, 2001; Dhuley, 1997; Nergard, 2004; Kirtikar *et al*., 1975).

MATERIAL AND METHODS

Collection and Identification of Plant Materials

The plant materials were collected from in and around Pune district of Maharashtra. Efforts were made to collect the plants in flowering and fruiting condition for the correct botanical identification. It was identified with help of Flora of The Presidency of Bombay (Cooke, 1958).

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Microscopic and Macroscopic evaluation

Thin (25µ) hand cut sections were taken from the fresh roots, permanent double stained and finally mounted in Canada balsam as per the plant micro techniques method of (Johansen, 1940).The macroscopic evaluation was studied by the following method of (Trease and Evans,2002 and Wallis,1962).

Histochemical study

The thin transverse sections of fresh root were taken (about 25µ). It was treated with respective reagent for the detection and localization of chemicals in the tissues as per the method of (Krishnamurthy, 1988).

Phytochemical evaluation

Some materials were dried under the shade so as to avoid the decomposition of chemical Constituents, powdered in blender and finally stored in dry air tied containers for phytochemical screening. Ash and percentage extractives were accomplished by following standard pharmacopoeal techniques of (Anonymous, 1955). Fluorescence analysis was carried out as per (Chase and Pratt, 1949). Qualitative phytochemical test were carried out by standard methods of (Harborne, 1973) and (Trease and Evans, 2002). Quantitative phytochemical analysis was determined for proteins, carbohydrates and flavonoid by the methods of Lowry *et al.* 1951; Nelson, 1944 and Boham and Kochipai, 1994 respectively. The phytochemical screening was also detected by the High Performance- Thin Layer Chromatography (HPTLC). HPTLC study was carried out on instrument comprising of Linomat 5 for application using Densitometer- TLC Scanner 3 with "WINCATS" software (Camag, Switzerland). These studies were carried out on pre-coated aluminum fluorescent plates (E. Merck). For HPTLC studies, an extract of methanol (25% GR) solvent system was used and after development, plate was scanned at 254 and 366 nm (Wagner and Baldt, 1994; Reich and Schibii, 2007).

RESULTS AND DISCUSSIONS

Macroscopic evaluation (Figure I and II)

Tree: 2-3 ft. in height.

Roots: The root is cylindrical in shape. Their length ranges from 30 to 61 cm length and 15 to 31 cm in breadth.

Leaves: Leaves alternate, leathery, with straight parallel veins, margins without teeth.

Inflorescence: Spicate or terminal on leafless branch lets flowers in the same inflorescence with usually the central pistillate ones surrounded by staminate ones.

Flower: Flowers are small, greenish, in clusters about 8 mm in diameter in the leaf axils, actinomorphic, whitish to greenish, sessile, sepals 5, petals 5, stamens 5 and ovary 2- or 3-locular.

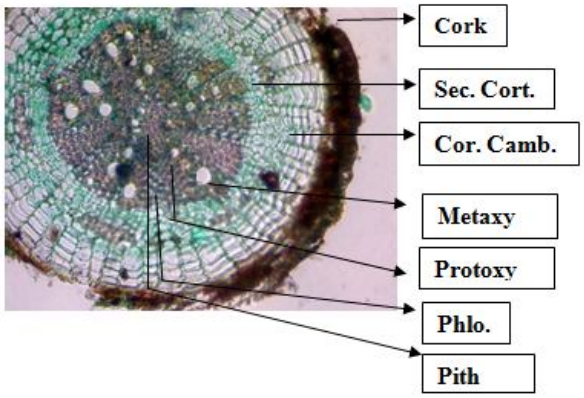
Fruit: A drupaceous about 8 mm in diameter, containing one or two seeds.

Figure I: Habit of *B.retusa*

Figure II: Roots of *B.retusa*

Microscopic characters

The transverse section of root is circular in outline. It shows cork, secondary cortex and a region showing anomalous secondary growth. The cork is 4-7 layered and is made up of thick walled cells. Next to the cork lies a single layer of cork cambium. Secondary cortex made up of parenchymatous cells. Some cells contain calcium oxalate crystals. Endodermis and pericycle are indistinct. Vascular bundle is arranged in rings and is conjoint collateral and open type. The central portion is occupied by pith. (Figure III).



(Sec. Cort= Secondary; Cor. Camb= Cork Cambium; Protoxy= Protoxylem; Metaxy= Metaxylem; Phlo= Phloem).

Figure III: Transverse section of root of *B.retusa* (10x X 3.3x)

Histochemical Screening

Histochemical screening showed the presence of starch, protein, fat, Saponin, tannin, sugars and alkaloids (Table I).

Table I: Histochemical study of

Test	Reagents	Color	Tissue
Starch	I_2KI	Blue	Cork, Sec.cort
Tannin	Acidic $FeCl_3$	Light brown	Cork, Sec.cort
Saponin	Conc. H_2SO_4	Yellow	Cork, Sec.cort.
Fat	Sudan III	Pink	Cork, Sec.cort
Sugar	20% aq. NaOH	Yellow	Cork, Sec.cort.
Glycosides	Guignard's Test	Brown	Cork, Sec.cort
Alkaloids	Mayer's Reagent	Colorless	Cork, Sec.cort.
	Wagner's Reagent	Dark brown	Cork, Sec.cort
	Dragendorff's Reagent	Dark Brown	Cork, Sec.cort.
	Tannic acid	Buff color	Cork, Sec.cort
	Hager's Reagent	Yellow	Cork, Sec.cort.

Abbreviations: I_2KI: Potassium iodide, $FeCl_3$: Ferric chloride, Conc. H_2SO_4: Concentrated sulphuric acid, NaOH: Sodium hydroxide.
Sec. Cort: Secondary Cortex.

Phytochemical Study

It contains the total ash 8 % and acid insoluble ash is 7.5 % (Table II). The values of percentage extractives were higher in Distilled water and lower in Petroleum ether solvent (Table III). Fluorescence analysis was carried out to check the purity of the drug.

Table II: Ash and Acid Insoluble Ash of *B.retusa* Spreng.

Parameter	Results
Total Ash	8 % dry wt.
Acid Insoluble Ash	7.5 % total ash

Table III: Percentage extractives of *B.retusa* Spreng.

Solvent Used	Extract (%)
Distilled Water	16%
Absolute Alcohol	8.4%
Petroleum ether	7%
Benzene	9.2%
Chloroform	7.2%
Diethyl ether	7.3%
Acetone	7.6%

The powder drug was observed in visible light as yellowish brown in color. The powder was then observed in ultraviolet light as Grayish black. It was treated with reagent like nitrocellulose, 1 N sodium hydroxide, 1 N sodium hydroxide in nitrocellulose and dry for 30 minutes and then it was observed under ultraviolet light and it emits the color as shown in (Table IV). Qualitative analysis of the root drug indicated the presence of proteins, reducing and non-reducing sugars,

saponin, fats, tannin, glycoside and alkaloids in the plant (Table V). The quantity of Starch is higher than protein, flavonoid, reducing and non reducing sugar (Table VI). In HPTLC study, the methanolic extract is ultrasonic for 15 minutes and filtered. The filtrate is used as an application for Quercetin. For each application 10µl and 5µl extracts were used and loaded on instrument comprising of Linomat 5 for application using Densitometer- TLC Scanner 3 with "WINCATS" software (Camag, Switzerland). These studies were carried out on pre-coated aluminum fluorescent plates (E. Merck). The plates were scanned at 254 and at 366 nm (Wagner and Baldt, 1994; Reich and Schibii, 2007).

Table IV: Fluorescence analysis of *B.retusa* Spreng.

Treatments	Color Emits
Powder as such	Yellowish brown
Powder as such in UV-light	Grayish black
Powder + Nitrocellulose	Greenish green
Powder + 1 N NaOH in Methanol	Dark blackish green
Powder + 1 N NaOH in Methanol dry for 30 min. + Nitrocellulose.	Blackish green

Table V: Phytochemical study of *B.retusa* Spreng.

Compound	Reagents	Results
Water Extracts		
Starch	I_2KI	+ve
Protein	Millan's reagent	+ve
Tannins Acidic	$FeCl_3$	+ve
Saponin	Distilled water	+ve
Steroids	Liebermann – Burchard's Test	+ve
Anthraquinone's	Benzene + 10% NH_4OH	+ve
Sugars	Benedict's reagent	+ve
Fats	Sudan III	+ve
Alcoholic extracts		+ve
Alkaloids		
	a. Mayer's Reagent	+ve
	b.Wagner's Reagent	+ve
	c. Dragendorff's Reagent	+ve
	d. Tannic acid	+ve
	e.Hager's Reagent	+ve
	f. Folin-Phenol Reagent	+ve
Glycosides	Benzene	+ve

Table VI: Quantitative estimation of *B.retusa* Spreng.

Quantitative estimation	(mg / g)
Protein	2.26
Reducing Sugar	1.65
Non - Reducing Sugar	1.69
Starch	2.82
Flavonoid	0.07

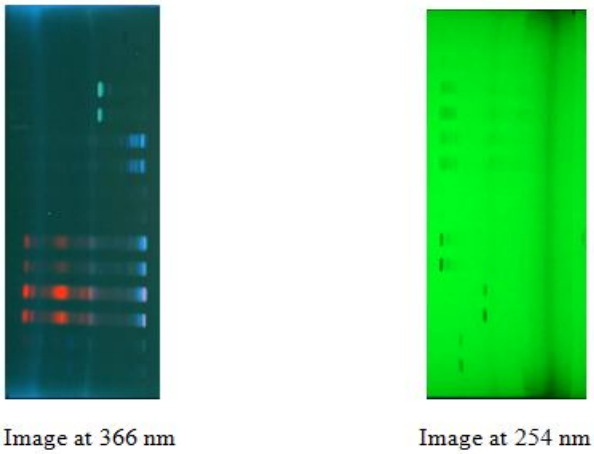
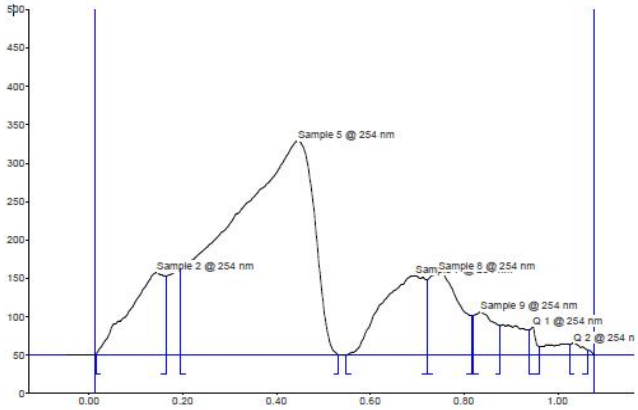


Figure – IV- Detection of flavonoid by HPTLC techniques



Graph I- Showing the peak for Quercetin for 10 µl plant extract

Conclusions

The plant *B.retusa* showed the correct taxonomy which is helpful for the standardization of drug. Findings of the present investigation will be useful for the correct botanical identification and authentication of the drug. *B.retusa* widely used as Pashanbheda

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Table VII: Showing the peak values for Quercetin for 10 µl plant extract.

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area%
1	0.01	1.1	0.15	108.0	15.23	0.16	103.6	5872.0	10.31
2	0.20	113.5	0.45	279.0	39.32	0.53	0.0	36648.6	64.35
3	0.55	0.1	0.70	103.9	14.65	0.72	98.4	6888.4	12.10
4	0.72	98.7	0.75	108.4	15.29	0.82	52.4	5031.1	8.83
5	0.82	52.1	0.84	56.9	8.02	0.88	39.1	1812.6	3.18
6	0.94	133.7	0.95	37.4	5.28	0.96	11.4	388.6	0.68
7	1.03	14.3	1.04	15.7	2.21	1.07	7.5	310.1	0.54

Analytical studies (Quercetin)

The HPTLC analysis showed that, the Quercetin from the *B.retusa* root samples gave yellow bands in visible light and blue bands after derivatization in fluorescence light. The plates were scanned at 254 and 366 nm. The table indicates the starting Rf values and end Rf values (Figure IV; Graph-I; Table VII)

REFERENCES

Anonymous, 1992. "The Wealth of India" CSIR, New Delhi. lA, pp.414 - 415.

Anonymous; Medicinal Plants more on Safed Musali. Agriculture and Industry Survey. 2001, 38-39.

Anonymous. Pharmacopoeia of India, Government of India, Ministry of Health Manager, Publications Delhi, 1st Edn, 1955, 370 & 864.

Nadkarni, A.K., K. M. Nadkarni's Indian Materia Medica. Popular Book Depot., Lamington Road, Bombay, 3rd Edtn. 1927, 1, 208-209.

Bahl and Seshadri., 1970. Advances in research in "Indian Medicine", "Pashanbedi" drugs for urinary calculus, Udupa, K.N.(Eds), : pp 77-98.

Begun, F.P., Knoll, C.E, Gottlieb M & R.K Lawson., 1991. Chronic effects of focused electrohydraulic shock waves on renal function and hypertension. *J Urol* 145: 635-639.

Boham A.B, Kocipai, A.C., 1994. Flavonoid and condensed tannins from Leaves of *Hawaiian vaccininum, vaticulum* and *vicalycinium.* Pacific Sci. 48:458-463.

Nergard, C. S., D. Diallo, T. E. Michaelsen, K. E. Malterud, H. Kiyohara, T. Matsumoto, H. Yamada, B. S. Paulsen; *Journal of Ethnopharmacology*, 2004, 91, 141-152.

Chase, C. R., R. Pratt; *Jour. Of Amer. Phar. Asso. (Sci. ed.),* 1949, 38, 324-330.

Johansen, D. A., Plant Microtechnique, McGraw-Hill Book Co. Inc. New York. 1940, 151-154, 182-203.

Dr. Krishnan R.S. The Wealth of India- A dictionary of Indian raw materials and industrial products. Revised Edn. Publication and Information Directorate, CSIR. Marg, New Delhi, 1992, 3 (Ca-Ci), 482-483.

Reich, E., A. Schibii; High Performance- Thin Layer Chromatography for the analysis of medicinal plants, Thieme medical publishers. Inc., 2007, 129-160, 206-210, 224-240.

Trease, G. E., W. C. Evans; Trease and Evans Pharmacognosy, 15th Ed. W. B. Saunders Edinburgh London, New York, Philadelphia St. Louis Sydney Toronto. 2002, 3-4, 528-533, 538-547.

Wagner, H., S. Baldt; Plant Drug Analysis: A Thin Layer Chromatography Atlas. Springer – Verlag, Berlin., 1996, 129, 144, 155, 157, 176, 178, 206.

Harborne J.B. Phytochemical Methods, Chapman and Hall International Edition, London. 2nd edition, 1973, 5-8.

Dhuley J. N. *Journal of Ethnopharmacology,* 1997, 58, 15-20.

Kirtikar, K. R., B. D. Basu; Euphorbiaceae, *Bridelia.* In: Kirtikar, K.R.; Basu B.D. (Eds.) Indian Medicinal Plants. L.M. Basu Publishers. Allahabad, India, 1975, 2508-2509.

Krishnamurthy, K. V., Methods in the Plant Histochemistry, Viswanadhan Pvt. Limited, Madras, 1988, 1 – 77.

Nelson N. *Jour. of Biol. Chem*., 1944, 153, 375-380.

Lowry, O. H., N. J. Rosebrough, A. L. Farr, R. J. Randall; *Jour. of Biol. Chem.*, 1951, 193, 265-275.

Chopra, R. N., S. L. Nayer, I. C. Chopra; Glossary of Indian medicinal Plants CSIR. New Delhi. 1956, 218.

Cooke T.; Flora of Presidency of Bombay, B.S.I. Calcutta, 1958, 3, 280-289.

Wallis, T. E.; A Text Book of Pharmacognosy, reprinted edition, Churchill, Livingstone. London, 1967, 578 – 617.

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