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BIOCHEMICAL STANDARDIZATION OF STEM BARK OF *PTEROCARPUS MARSUPIUM* (ROXB.)

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ABSTRACT

Pterocarpus marsupium (Roxb.) is an important medicinal plant in *diabetes* management. In the present study different stem bark samples (Apical bark, Middle bark and Mature inner bark) were analyzed with respect to phytoconstituents total reducing sugars, total sugars, amylose, amylopectin, starch, crude fibers, crude protein, total polyphenols, water soluble tannins, total flavonoids, total alkaloids, nitrates, total oxalate and total ash value. The concentration of constituents except oxalate and total ash was found higher in the apical stem bark than the middle and mature inner bark. The oxalate and total ash were higher in the mature inner bark than the apical stem bark and middle bark samples. Preliminary Phytochemical analysis indicated presence pharmacologically active phytoconstituents phenols, tannins, flavones, flavonoids, alkaloids, terpenoids, and cardiac glycosides. The saponins were found absent in all the three bark samples.

Keywords: *Pterocarpus marsupium*, Organic constituents, Polyphenols, Tannins, Flavonoids, Alkaloids.

INTRODUCTION

Pterocarpus marsupium (Roxb.) is a deciduous tree, commonly called as Indian Kino tree or Malabar Kino, belonging to the family fabaceae. The bark exudes a red gummy substance called „Gum Kino‟ when injured. Gum Kino is used in the treatment of polyurea and inordinate night sweat and phthisis pulmonalis. The gum is used in the toothache (Chopra, *et al.* 1956). Bark is useful in vitiated condition of *kapha* and *pitta*, *Elephantiasis*, *Erysipelas*, *Urethrorrhea*, *Rectalgia*, *Opthalmopathy*, *Hemorrhages*, *Dysentery*, *Cough* and grayness of hair. Aqueous infusions of the bark possess antidiabetic potential (Anonymous, 1969). *Pterocarpus marsupium* is distributed in deciduous forest throughout the India (Varghese, 1996). Bark is useful in urinary discharge and piles. The gum Kino is externally applied to *Leucorrhoea* (Pullaiah, 1999). Tribal people residing in the Jodhalal forest of Karnataka use stem bark to

treat the wounds, fever, *Stomachache*, *Diabetes* and *Elephantiasis* (Mankani *et al*., 2005).The powdered bark is mixed with *Schleichera oleosa* and taken with cold water to treat dysentery (Mohanta *et al.*, 2006). The juice of the bark is applied in the mouth (Prusti and Behara, 2007). In the present study an attempt has been made to standardize the drug through its biochemical evaluation.

MATERIALS AND METHODS

Materials

Different bark samples (apical rind, middle bark and mature inner bark) of *P. marsupium* were collected from the hilly regions Radhanagari of Kolhapur district. In the winter season the bark was collected in the month of January and summer collection was followed in the month of May. The bark samples were cut into pieces, sun-dried then oven dried at 60^0C. Dried bark samples were ground into powder and stored in an air tight plastic container.

Methods

Reducing sugars

The reducing sugars were estimated by employing arsenomolybdate reagent introduced by Nelson (1944) and were expressed in g 100 g-1 dry tissue.

Total sugars

The total sugars were estimated following the Phenol-sulphuric acid method described by Dey (1990) and expressed in g.100 g^{-1} dry tissue.

Starch

The starch was estimated by employing arsenomolybdate reagent introduced by Nelson (1944) and was expressed in g 100 g-1 dry tissue.

Amylose

Amylose content was estimated according to the method described by Sadasivam and Manickam (1992) and expressed as g.100g^{-1} of dry tissue.

Amylopectin

Amount of Amylopectin was calculated by subtracting the amylose content from the starch content and expressed as g.100g^{-1} of dry weight.

Crude fibers

Crude fiber contents were estimated according to the method described by Maynard (1970) and expressed g.100g^{-1} of dry weight.

Total polyphenols

The total polyphenols contents were determined according to the method of Folin and Denis (1915) and expressed as g.100g^{-1} dry weight.

Water soluble tannins

Method of Schanderl (1970) was employed for determination of water soluble tannins and expressed as g.100g^{-1} of dry weight.

Total flavonoids

Total flavonoids were estimated by the method of Luximon-Ramma *et al.* (2002) and expressed as g.100g^{-1} of dry weight.

Total alkaloids

The total alkaloid contents in the bark samples were measured using 1, 10-phenanthroline method described by Singh *et al.* (2004) with slight modifications.

Crude protein content

Crude protein contents were calculated by multiplying the total nitrogen content by factor 6.25 and expressed as g.100g^{-1} of dry weight.

Nitrate content

The nitrate contents in bark powder were determined using rapid colorimetric method given by Cataldo *et al.* (1975) and expressed µg of NO_3.g^{-1} dry weight.

Oxalic acid content

The oxalic acid contents were estimated according to the method given by Abaza et al. (1968) and expressed as g.100g^{-1} of dry weight

Total ash content

Total ash content was determined as described in Indian Pharmacopeias (1996) and expressed as $g.100g^{-1}$ of dry weight.

RESULTS AND DISCUSSION

Qualitative analysis of stem bark of *Pterocarpus* is depicted in the table No. 1. The Phytoconstituents phenols, tannins, flavones, flavonoids, alkaloids, terpenoids, cardiac glycosides are present in all bark samples but their intensity is higher in the apical stem bark, moderate in the middle stem bark and low in the mature inner bark. Saponins are absent in the all bark samples. Quantification of the different phytoconstituents is shown in the Table No. 2. The concentration of the biochemical moieties except oxalate and total ash was found higher in the apical bark than the middle and mature inner bark. The reducing sugars were higher in the apical bark (2.68%) while lowest in mature inner bark (1%). These are in the range of reducing sugar content in Black locust bark (0.9% to 3.2%, (Siminovitch *et al*., 1953). Total sugar content in the middle bark was moderate (8.41%) but higher than the mature inner bark (6.27%) and lower than the apical bark (10.13%). Sugar content reported in *P. marsupium* was lower than the *Saraca asoca* (15%), *Polylthia liongifolia* (33%) and *Saraca declinata* (11- 12%) by Khatoon *et al.* (2009). Amylopectin and amylose contents were recorded maximum in apical bark (15.68 and 0.65% respectively) and lowest in mature inner bark (8.25 and 0.48% respectively) and in middle bark their values are moderate. Higher amount of starch was estimated in apical bark (16.47%) than middle (14.45%) and mature inner bark (8.73%) which is much lower than the *Polyalthea longifolia* (65-70%), *Saraca asoca* (52-55%) and *Saraca declinata* (51-52%) reported by Khatoon *et al*., (2009) and higher than the starch content in the bark tissue of six tree species- Ash tree (6.9%), Alder (2.8-2.9%), Oak (2.5-2.7%) , Maple (0.5- 0.6%) and Birch (0.3-0.4%) estimated by Essiamllh and Eschrich (1985).Mature inner bark contained less crude fibers (17.70%) while no large difference was observed between apical (22.15%) and middle bark (20.65%).The crude fiber content estimated in our study are lower than the crude fiber content recorded by Prajapati (2008) in bark *Mallotus phillippinensis* (36.26%), and *Dalbergia sisso* (35.93%). Crude protein content was also higher in apical stem bark (19.58%) than middle (17.11%) and mature inner bark (12.43%). The Crude protein values in the *P. marsupium* bark are lower than black locust varied (21.43% to 21.58%) as reported by Jones and Philips (1937) and are higher than the Poplar bark (2.2%) reported by Enzman *et al*., (1969).

Total polyphenol content in the in the mature inner bark (9.74%) was lower than the apical (11.99%) and middle bark (11.09%). Sarkar *et al*., (2005) reported 47.19% and 40.80% total polyphenols in the bark of *Khaya senegalensis* and *Pterocarpus erinaceus.* Polyphenol content determined in our study are lower than the phenol content reported by Sarkar *et al*., (2005). No large difference was noticed in the tannin content of middle and mature inner bark (2.51 and 2.29% respectively) but, lower than the apical stem bark (3.31%). *Acasia nilotica* bark tannin content varied from 10.6% to 20% (Sureh *et al*., 2009). Tannin content in the Oak bark was 12-16% (Hathaway, 1958). The bark of *Labumum vulgare* and *Pettrria ramentacea* contained 4.18% and 5.11% tannin respectively (Alibalic and Murko, 1991). Tannin estimated in *Pterocarpus marsupium* bark samples in our study are lower than tannin in above plants. The middle and mature inner bark flavonoid content was not largely differing from each other (0.480 and 0.434% respectively) but was lower than the apical stem bark (0.88%). The flavonoids estimated in our study are much lower than reported in *Pinus massoniana* (27.1%) by Gui *et al*., (2005). The total alkaloid content among the

three bark samples was maximum in the apical bark (0.97%) and decreased in the order of apical bark (0.97%) > middle bark (0.83%) > mature inner bark (0.61). Martin and Gandara (1945) estimated 9.3% alkaloid levels in the barks of *Chinchona officinalis*. In the present study alkaloids are reported in the range of 0.5% to 3.0%. Mature inner bark contained maximum total ash content (16.60%) as compared to middle (12.40%) and apical bark (10.40%). This total ash percentage was maximum than the ash content of bark of *Pinus pinea* (2.3%, *Nunes et al*., 1999), *Stryphnodendron adstringens* (1.6%, Audi *et al*., 2004), Poplar bark (2.2%, Enzman *et al*., 1969), Shri Lankan Cinnmon and Chinese cinnamon (3.77% and 2.89%, Al- Numair *et al*. 2007) , *Careya arborea*, and *Shorea robusta* (4.41% and 5.36%, Santra *et al*., 2008).

Higher content of one of the anti-nutritional factor oxalate was higher in the mature inner bark (7.88%) than the middle (6.79%) and apical bark (5.29%). Pandey and Kori (2009) reported that oxalate content in *T. arjuna* bark varied from 7.66 to 20.05%. In the present study the oxalate content are in the range of 5-8%. Highest nitrate content was recorded in apical stem bark (2732) followed by middle (2295) and mature inner bark (1885). Anjana *et al*. (2007) reported maximum 6269 µg/g of nitrate in spinach which is much higher than nitrate content reported in our study.

CONCLUSION

Presence phytoconstituents in the plant parts imparts medicinal potential to crude drug. The bark of *Pterocarpus marsupium* showed presence secondary metabolites in appreciable amount which claims for its use in various ayurvedic preparations. These phytoconstituents may acts as source of pharmacologically active ingredients in ayurvedic formulations. The Lower nitrate and oxalate content points to fearless use of this drug. The present evaluation of various biochemical parameters will be helpful for standardizing the drug for its various pharmacological potentials and to check the adulteration in natural valuable drug.

Table 1: Qualitative analysis of stem bark samples of *P. marsupium*

Sr. No.	Parameter	Samples		
		Apical Bark	Middle Bark	Mature inner Bark
1	Polyphenols	+++	++	+
2	Flavonoids	+++	++	+
3	Tannins	+++	++	+
4	Alkaloids	+++	++	+
5	Flavones	+++	++	+
6	Terpenoids	+++	++	+
7	Saponins	--	--	--
8	Cardiac Glycosides	+++	++	+
9	Sterols	+++	++	+

„+++“= High concentration; „++“=Moderate concentration; „+“= Low concentration and „-“ = Absent

Table 2: Quantitative estimation of phytochemicals

Sr. No.	Parameter	Samples		
		Apical Bark	Middle Bark	Mature inner Bark
1	Reducing Sugars	2.68	1.41	1.00
2	Total Sugars	10.13	8.41	6.27
3	Amylopectin	15.68	13.88	8.25
4	Amylose	0.65	0.57	0.48
5	Starch	16.47	14.45	8.73
6	Crude fiber	22.15	20.65	17.70
7	Crude Protein	19.58	17.11	12.43
8	Total Polyphenol	11.99	11.09	9.74
9	Tannin	3.31	2.51	2.29
10	Flavonoid	0.88	0.48	0.43
11	Total Alkaloid	0.97	0.83	0.61
12	Total Oxalate	5.29	6.79	7.88
13	Nitrate (µg of $NO_3.g^{-1}$ dry tissue)	2732	2295	1885
14	Total ash	10.40	12.40	16.60

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