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## Phytochemical screening of stem bark of valuable medicinal tree of tropical forest-*Pterocarpus marsupium* (Roxb.)

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**Abstract**

*Pterocarpus marsupium* has known traditional and ethnobotanical uses since past thousands of years. The present study was carried out to screen the stem bark samples for different phytochemicals using different solvents. Bark samples were collected from different forest divisions of Madhya Pradesh and processed. The powdered samples were subjected to extraction with eight solvents of increasing polarity i.e. distilled water, ethanol, methanol, ethyl acetate, chloroform, benzene, hexane and petroleum ether. These extracts were evaluated for phytochemicals qualitatively as well as quantitatively. Results indicated the extraction of phytochemicals better in polar solvents i.e. distilled water, ethanol and methanol. Moreover, the extracts of these solvents were found to contain saponins, tannins, alkaloids, flavonoids, terpenoids, steroids and phenols of pharmacological importance. Quantitative estimation of the totalphenol (%), flavonoids (%) and alkaloids ( $\mu\text{g}/100\text{g}$ ) revealed high range of variation within as well as between the sampling sites. This indicates influence of genotype, environment and GxE interaction on the phytochemicals.

**Keywords:** *Pterocarpus marsupium*, stem bark, phytochemicals, qualitative and quantitative screening

**Introduction**

Since ancient times, various herbs, shrubs, climbers, trees are being used in treatments of various ailments. As per the reports of the World Health Organization (WHO), medicinal plants are being used two to three times more than conventional drugs as remedial measures in curing various ailments (Evans, 1994) <sup>[5]</sup>. *Pterocarpus marsupium* (Roxb.) is one of such valuable medicinal trees of tropical forest having multifarious medicinal uses. Its medicinal significance has been reported in Indian traditional systems of medicine like Ayurveda, Unani and Homeopathy (Badkhane *et al.* 2000) <sup>[2]</sup>. *P. marsupium*, commonly known as Bijasalar Vijaysar (in Hindi), Asana (in Sanskrit), Malabar Kino or Indian Kino (in English) and by many other names, is a deciduous tree of fabaceae family. It is multipurpose leguminous tree having yellow flowers. It's flowering and fruiting period is March to June (Yadav and Sardesai, 2002) <sup>[22]</sup>. Fruit is circular, flat, winged pod. Seed is convex and bony (Warrier, 1995) <sup>[21]</sup>. The tree is indigenous to India, Nepal and Sri Lanka (Gamble, 1935; Matthew, 1983) <sup>[7, 13]</sup>. It is distributed in deciduous forest of the country (Varghese, 1996) <sup>[20]</sup> and reported in the forests of Madhya Pradesh, Chhattisgarh, Maharashtra, Andhra Pradesh, Bihar, Karnataka, Kerala, Orissa, Rajasthan, Tamil Nadu, Uttar Pradesh and West Bengal (Sanjappa, 2000) <sup>[19]</sup>. It attains height up to 15-20 meter and has dark brown to grey bark having swallow cracks. The bark exudes a red gummy substance called 'Gum Kino' when injured (Patil and Gaikwad, 2011) <sup>[16]</sup>. Almost each and every plant part of the *P. marsupium* is reported to have uses in curing various diseases. It has long history of numerous ethno medicinal uses. Leaves are used for curing the boils, sores, skin diseases and stomach pain; flowers in fever; Gum-Kino for diarrhea, dysentery, leucorrhoea etc. (Anonymous, 2001; Pullaiah, 1999) <sup>[1, 17]</sup>. The heartwood of *P. marsupium* is known to possess astringent, anti-inflammatory, anti-diabetic and anodyne properties (Kirtikar, 1987) <sup>[10]</sup>. The heartwood is an important source of Pterostilbene (Mathew *et al.* 1977; Dama *et al.* 1982) <sup>[12, 4]</sup> which has numerous clinical applications (Estrela *et al.* 2013; McCormack and McFadden 2013) <sup>[5, 14]</sup>. Decoction of bark and resin is used for the treatment of tumors of the gland, urethral discharges and as abortifacient (Basu, 1975) <sup>[3]</sup>.

Due to unsustainable extraction coupled with low natural regeneration and population fragmentation, *P. marsupium* is now listed as 'Vulnerable' in the IUCN red data list (IUCN, 2017) <sup>[9]</sup>.

At Tropical Forest Research Institute, Jabalpur (Madhya Pradesh) steps have been initiated for the conservation of this species including genetic improvement (Mishra *et al.* 2020)<sup>[15]</sup>, assessment of natural regeneration, genetic and phytochemical variations, etc.

Present study was undertaken with aim to screen the stem bark samples of *P. marsupium* collected from different forest divisions of Madhya Pradesh for major phytochemicals and also to assess the variations for selected phytochemicals i.e. the total phenolic, flavonoids and alkaloids.

## Material and Methods

### Collection of material and processing

Stem bark samples were collected from six different sites in Madhya Pradesh (Table 1). Number of trees sampled per location varied from 20 to 12 as indicated in Table 1. The bark samples were cleaned for foreign particles and dust followed by shade drying and 48 hrs hot air oven drying at 40°C. Dried bark samples were ground into fine powder.

### Qualitative phytochemical analysis

100 mg of powdered bark material of each tree was kept overnight in 25 ml of different solvents *viz.* distilled water, ethanol, methanol, ethyl acetate, chloroform, benzene, hexane and petroleum ether. Filtered extracts were subjected to preliminary phytochemical testing for different phytochemicals by standard methods as described by Harborne (1998).

### Test for Saponin

0.5ml filtrate was taken into a test tube and 5ml distilled water added to it, shaken vigorously, persistent frothing indicates the presence of saponins.

### Test for Tannins

2ml filtrate was taken into a test tube and 2ml FeCl<sub>3</sub> was added to it. Blue-black precipitation or brown coloration indicates the presence of tannins.

### Test for Alkaloids

2ml filtrate was taken into a test tube and 1% of 2ml HCl was added to it. Ceramic coloration indicates the presence of alkaloids.

### Test for Flavonoids

1ml filtrate was taken into a test tube and few drops of 1% liquor ammonia solution added to it. Yellow coloration indicates the presence of flavonoids.

### Test for Terpenoids

2ml filtrate was taken into a test tube and add 1ml chloroform and then transfer Conc. H<sub>2</sub>SO<sub>4</sub> through the wall of test tube. Reddish brown coloration indicates the presence of terpenoids.

### Test for Steroids

2ml of acetic anhydride was added to 2ml extract of each sample followed by careful addition of 2ml H<sub>2</sub>SO<sub>4</sub>. The colour changed from violet to blue or green indicate the presence of steroids.

### Test for Phenols

2ml filtrate was taken in test tube and 1ml of 1% FeCl<sub>3</sub> added to it. Brown precipitate or haziness indicates the presence of phenols.

## Test for Cardiac glycosides

2ml filtrate was taken into a test tube and add 1ml glacial acetic acid + FeCl<sub>3</sub> to it and then transfer Conc. H<sub>2</sub>SO<sub>4</sub> through the wall of test tube. Green blue color indicates the presence of cardiac glycosides.

## Quantitative phytochemical analysis

**Estimation of total phenolic compounds:** The total phenolic content of the extracts was determined by using Folin-Ciocalteu reagent. 100mg of the extract of the sample was weighed and dissolved in 100 ml of distilled water. 1 ml of this solution was transferred to a test tube, then 0.5 ml 2N of the Folin-Ciocalteu reagent and 1.5 ml 20% of Na<sub>2</sub>CO<sub>3</sub> solution was added. Final volume was made up to 8 ml with distilled water followed by vigorous shaking and finally allowed to stand for 2 hours after which the absorbance was taken at 765 nm. These data were used to estimate the total phenolic content using a standard calibration curve obtained from various diluted concentrations of gallic acid.

**Estimation of total flavonoids:** 100µl of the sample extracts in methanol (10 mg/ml) was mixed with 100 µl of 20% aluminum trichloride in methanol and a drop of acetic acid, and then diluted with methanol to 5ml. The absorption at 415 nm was read after 40 minutes. Blank samples were prepared from 100 ml of sample extracts and a drop of acetic acid, and then diluted to 5ml with methanol. The absorption of standard rutin solution (0.5 mg/ml) in methanol was measured under the same conditions. All determinations were carried out in triplicates (Kumaran and Karunakaran 2006)<sup>[11]</sup>.

**Estimation of total alkaloids:** 5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

## Results and Discussion

Qualitative analysis of extracts of bark of *P. marsupium* revealed the presence of saponin, tannins, alkaloids, flavonoids, terpenoids, steroids and phenols mostly in polar solvents (distilled water, ethanol and methanol). Earlier, Ramya *et al.* (2008)<sup>[18]</sup> and Patil & Gaikwad (2011)<sup>[16]</sup> also reported the presence of alkaloids, glycosides, flavonoids, flavonols, phenols and terpenoids in the stem bark of this species. Contrary to the present findings, they did not find the saponins in bark samples screened.

Eight solvents of different polarity were used for extraction of the bark sample (Table 2). It is cleared that distilled water, ethanol and methanol are better solvents for extraction of the major phytochemicals. Specifically, qualitative tests of phenols, flavonoids and alkaloids were better with these three polar solvents compared to others. The qualitative results were better in distilled water for saponins while flavonoids and alkaloids were found better with methanol. Ethanol was seen better for tannins and steroids.

Quantitative estimation of the total phenolic compounds, flavonoids and alkaloids was carried out and estimates were presented in Table 3. Critical perusal of the Table 3 revealed that range of phytochemicals quantified is high between the

genotypes of a sampling location and also between sampling sites. This suggests that environmental, genotypic and their interactions may have profound effect on the phytochemical content.

Considering the overall findings of the present study, it is apparent that *P. marsupium* possesses major phytochemicals in its stem bark and these phytochemicals may be contributing

for the medicinal properties of the stem bark of this species. Quantification of flavonoids, alkaloids and phenolic compounds revealed that variation exists between the trees and sites also. This may be due to the genotypic and/or environmental effect and their interaction. This also revealed the scope for selection of promising trees having higher amount of phytochemical contents.

**Table 1:** Details of the sampling sites of *P. marsupium* in Madhya Pradesh

Sl. no	Name of Site	Name of Forest Division	No of trees sampled	Forest Types (Major type)	Forest Types Sub-groups
1.	Semariya	Rewa	20	Tropical Dry Deciduous Forests	Northern Dry Mixed Deciduous Forests
2.	Lamta	Balaghat	20	Tropical Moist Deciduous Forests	Northern Tropical Moist Mixed Deciduous Forests
3.	Bahoriband	Katni	20	Tropical Dry Deciduous Forests	Southern Tropical Dry Mixed Deciduous Forests
4.	Chada	Dindori	20	Tropical Dry Deciduous Forests	Tropical Dry high-level Sal Forest
5.	Sara	Mandla	19	Tropical Dry Deciduous Forests	Southern Tropical Dry Deciduous Mixed Forests
6.	Barha	Jabalpur	12	Tropical Dry Deciduous Forests	Southern Tropical Dry Deciduous Teak Forests

**Table 2:** Qualitative analysis of phytochemical in stem bark samples of *P. marsupium* collected from different forest division of Madhya Pradesh

Tests	Appearance	PH	Ethanol	Methanol	D/W	Hexane	Ethyl acetate	Petroleum Ether	Benzene	Chloroform
Saponin	Transparent	6.3	+	+	++	-	-	-	-	-
Tannins	Pink	6.1	++	+	++	-	-	-	-	-
Alkaloids	Transparent	6.5	+++	+++	++	-	-	-	-	-
Flavonoids	Brown	6.2	++	+++	+++	-	-	-	-	-
Terpenoids	Brown	6.6	+	+	+	-	-	-	-	-
Steroids	Dark Brown	6.4	++	+	+	-	-	-	-	-
Phenols	Pinkish	6.1	+++	++	++	-	+	-	-	-
Cardiac Glycosides	Yellow	6.3	+	-	-	-	-	-	-	-

Where, '+++': Present in high concentration, '++': Present in moderate concentration, '+': Present in low concentration and '-' Absent.

**Table 3:** Estimates of total flavonoids, alkaloids and phenolic compounds in stem bark samples of *P. marsupium* collected from different forest division of Madhya Pradesh

Name of Sites	Name of Forest Division	Phenol %		Flavonoids %		Alkaloids ( $\mu\text{g}/100\text{g}$ )	
		Range	Average	Range	Average	Range	Average
Semariya	Rewa	10.63-20.94	16.18	6.75-11.91	10.16	2.40-10.20	5.95
Lamta	Balaghat	16.12-22.53	19.26	5.72-18.49	9.09	9.63-16.99	13.57
Bahoriband	Katni	14.57-23.09	18.94	8.10-18.76	11.32	2.36-21.58	5.68
Chada	Dindori	13.14-21.07	17.36	5.75-20.25	10.35	0.04-9.98	5.09
Sara	Madala	12.80-18.87	16.66	6.32-17.56	10.09	10.86-18.70	14.98
Barha	Jabalpur	15.48-23.36	17.72	8.64-13.11	10.62	12.78-17.85	14.79

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