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HPTLC comparision of leaf and heart wood of *Pterocarpus marsupium* ROXB – An endangered medicinal plant

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Abstract

Pterocarpus marsupium Linn. (Family: Fabaceae) commonly known *Asana* or *Vijoysara* is an important medicinal plant, widely distributed throughout India. Heartwood of the plant is traditionally used for various diseases like diabetes, angina, cancer, cardiotonic, brain tonic etc. Many researchers were isolated several active principle like flavonoids, terpenoids and reported its pharmacological activity. The count of this tree species is declining in the wild and therefore it has been placed in the red data book. It is exploited mainly for its medicinal heart wood. Hence use of leaf in place of heart wood would be beneficial for surviving of the plant. On this background the present study was undertaken to compare the phytochemical aspects of leaf and heart wood of *Pterocarpus marsupium* Linn. Alcohol extract of both leaf and heart wood of the plant were analyzed through HPTLC. Study resulted out that leaf sample showed more or equal spot on HPTLC finger printing. That indicates that leaf may having similar active potency like heart wood. That may provide the base for further study to use the leaf as a substitute of heart wood *P. marsupium*.

Keywords: *Asana*, Leaf HPTLC, Heartwood, Comparison.

Introduction

The medicinal plants are extensively utilized throughout the world in different systems of health management. Hence the numbers of plant species are individually under attack to accomplish the global demand. *Pterocarpus marsupium* Roxb, commonly known as *Asana* or *Vijaysara* is such a plant species.[1] This is a deciduous tree and well-known drug in Ayurvedic system of medicine. The plant is commonly available in deciduous and evergreen forest of Central, Western, and Southern region of India.[2] It is found mostly in the states of Gujrat, MP, Bihara, Orissa.[3, 4] The wood and bark of *Asana* are known for their anti-diabetic activity.[5, 6] Traditionally, the plant material has been used as a cooling external application for inflammations and internally as antipyretic, anti-helminthic, aphrodisiac, and in biliousness, mental aberrations and ulcers.[7] The flowers are used in fever and the gum is locally applied in leucorrhoea and passive haemorrhage. The bruised leaves are considered useful as an external application for boils, sores and skin diseases. The wood of the tree is useful in making water glasses of the diabetic patients.[8] Heartwood of *P. marsupium* has anti cataract activity[9] and was found to be effective on glycogen content of tissue and the key enzyme of carbohydrate metabolism[10]. Ethanol extracts of *P. marsupium* exhibited significant anti-ulcer and antioxidant properties in rats. Water extract of heartwood showed antioxidant activity.[11] Also methanol extract of bark showed maximum activity against *Pseudomonas aeruginosa*, *Streptococcus pyrogens* and *Staphylococcus aureus*.[7, 12] Several chemical constituents like pterostilben, epicatechin, pterosupin, marsupin etc. have identified and isolated from the plant.[13]

The count of this tree species is declining in the wild and therefore it has been placed in the

red data book. It is exploited for its timber and its medicinal bark, heartwood and latex[14]. Hence use of leaf in place of heart wood would be valuable for existing of the plant. Only few studies are available on leaf of *Asana* like physiochemical and phytochemical study[15], *in vit*ro free radical scavenging effect[15], antimicrobial study[14]. But regarding the HPTLC comparison no studies are available. On this background present study was conducted to compare the preliminary phytochemical property of leaf with heart wood of *P. marsupium* through HPTLC. Alcohol extract of both leaf and heart wood of the plant were analyzed which may provide the base for further study to use the leaf as a substitute of heart wood *P.marsupium.*

Materials and Methods

Plant materials

Leaves and heart wood sample of *P. marsupium* were collected from Ayra Vaidya Sala, Kottakkal, Kerala, India, during June 2014. The flora of presidency of Madras (Gamble, 1935) was used for identification and further authentication of plant materials were done in Centre for Medicinal Plant Research (CMPR), AVS, Kottakkal. Collected materials were washed thoroughly using running tap water, rinsed in distilled water and shade dried in open air and grounded in to powder.

Preparation of extract

10 g of coarsely powdered drug was taken in 250 ml round bottom flask. Then powder was extracted with 50 ml alcohol by refluxing for 30 min. afterwards the material was filtered and concentrated under vacuum and was made up to 5 ml.

HPTLC test

HPTLC of ethanol extract of both leaf and heart wood of *Asana* were compared in two different solvent systems viz. 1. Toluene: Ethyl acetate: formic acid (5: 5: 1) 2. Toluene: Ethyl acetate (9.3: 0.7)

***Procedure*[17]:** Samples were applied on the plate using Camag automatic TLC sampler 4 attached to camag HPTLC system. The samples (2µl) each were spotted on aluminum backed pre-coated silica gel plate 60_F-254 plate (5×10 cm) in the form of bands with width 8 mm by using Hamilton syringe (100µl). Then the plate was developed in the two different solvent systems in a twin trough chamber to a distance of 8 cm.

Visualization: After making dry in air the plates were examined under UV 254 nm and under UV 366 nm. R_f value and the colour of the resolved bands were recorded. Photographs of the plates were captured using camag TLC visualizer. For second solvent system the plate was sprayed with Liebermann-Burchard reagent and heated at 105^o till the colour of the spots/ bands appeared without charring. Again the R_f value and the colour of the resolved bands were recorded. It was calculated by using the formula (Distance travelled by solute / Distance travelled by solvent)

Result

In Solvent system 1 [Toluene: Ethyl acetate: formic acid (5: 5: 1)] HPTLC profile of ethanol extract of leaf under UV light at 254 nm showed 3 spots with R_f values of 0.60, 0.66, 0.76 (Figure-1.2,Table-1). But samples of heart wood showed 5 spot with R_f values of 0.05, 0.63, 0.67, 0.71, 0.84 (Figure-1.2,Table-1).When the plates were viewed at 366 nm, the leaf sample showed 10 spots with R_f values of 0.08, 0.20, 0.25, 0.29, 0.37, 0.50, 0.62, 0.66, 0.74,0.82 (Figure-1.1,Table-1). The spots with R_f values 0.66 was observed under both 254 nm and 366 nm. At 366 nm sample of heart wood showed 8 spots with R_f values of 0.08, 0.34, 0.63, 0.66, 0.74, 0.77, 0.84, 0.90 (Figure-1.1,Table-1). The spots with R_f values 0.63 and 0.84 were observed under both 254 nm and 366 nm. The spots with R_f values of 0.08, 0.66. 0.74 were observed in both leaf and heartwood sample.

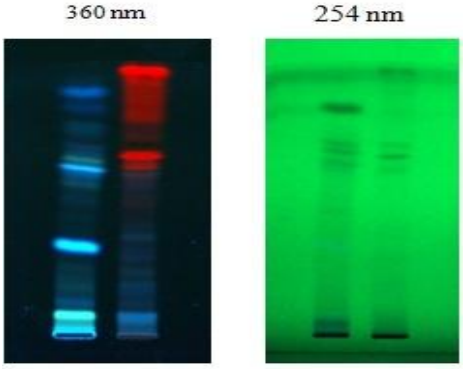
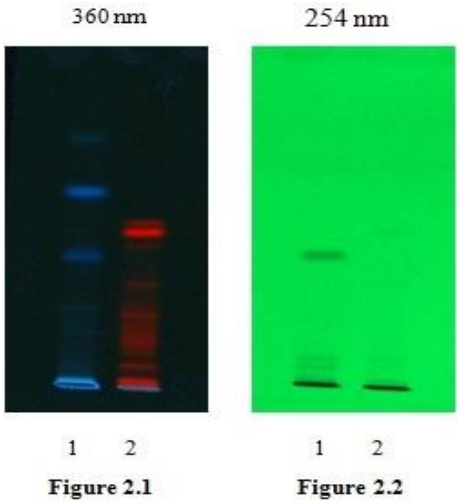


Figure 1.1 Figure 1.2

Figure 1: HPTLC profile of ethanol extract of leaf and heart wood of *Asana* in solvent system Solvent system 1 [Toluene: Ethyl acetate: Formic acid (5: 5: 1)] **Figure 1.1** HPTLC profile of ethanol extract of leaf (1) and heart (2) wood of *Asana* at 366nm. **Figure 1.2** HPTLC profile of ethanol extract of leaf (1) and heart (2) wood of *Asana* at 254nm.

In Solvent system 2 [Toluene: Ethyl acetate (9.3: 0.7)] HPTLC profile of ethanol extract of leaf under UV light at 254 nm showed 3 spots with R_f values of 0.05, 0.09, 0.49 (Figure-2.2, Table-2). The samples of heart wood also showed 3 spot with same R_f values of 0.05, 0.09, 0.49 (Figure-2.2,Table-2).When the plates were viewed at 366 nm, the leaf sample showed 8 spots with R_f values of 0.03, 0.08, 0.15, 0.25, 0.34, 0.46, 0.51, 0.53 (Figure-2.1,Table-2). At 366 nm sample of heart wood showed 6 spots with R_f values of 0.18, 0.21, 0.26, 0.43, 0.63, 0.80 (Figure-2.1,Table-2). After derivatisation with Liebermann-Burchard reagent, at 254nm the leaf sample showed 3 spots with R_f values of 0.06, 0.10, 0.49 (Figure-3.2, Table-2). Whereas heartwood sample showed 2 spots with R_f values of 0.05 and 0.40. At 366 nm leaf showed 8 spots with R_f values of 0.03, 0.08, 0.11, 0.25, 0.34, 0.46, 0.51, 0.53 (Table-2 and Figure-3.1). But heartwood

sample showed spots with R_f values of 0.05, 0.09, 0.21, 0.26, 0.31, 0.43, 0.63, 0.80.



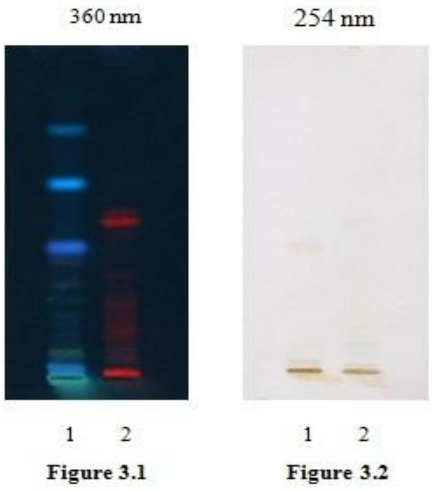


Figure 2: HPTLC profile of ethanol extracts of leaf and heart wood of *Asana* in solvent system Solvent system 2 [Toluene: Ethyl acetate (9.3: 0.7)] **Figure 2.1**, HPTLC profile of ethanol extract of leaf (1) and heart (2) wood of *Asana* at 366nm. **Figure 2.2**, HPTLC profile of ethanol extract of leaf (1) and heart (2) wood of *Asana* at 254nm.

Figure 3: HPTLC profile of ethanol extracts of leaf and heart wood of *Asana* in solvent system Solvent system 2 [Toluene: Ethyl acetate (9.3: 0.7)] **Figure 3.1**, HPTLC profile of ethanol extract of leaf (1) and heart (2) wood of *Asana* at 366nm after derivetization. **Figure 3.2**, HPTLC profile of ethanol extract of leaf (1) and heart (2) wood of *Asana* at 254nm after derivatization.

Table 1: HPTLC profile of ethanol extract of leaf and heart wood of *Asana* in solvent system Solvent system 1 [Toluene: Ethyl acetate: formic acid (5: 5: 1)]

Sample	254 nm		366nm	
	Rf	**Colour**	**Rf**	**Colour**
Heartwood	0.05		0.08	Lightgreen fluorescence
	0.63		0.34	Skyblue fluorescence
	0.67		0.63	,,
	0.71	Dark	0.66	Lightgreen fluorescence
	0.84		0.74	Light blue fluorescence
			0.77	Light Skyblue fluorescence
			0.84	Light Skyblue fluorescence
			0.90	Skyblue fluorescence
Leaf	0.60		0.08	Skyblue fluorescence
	0.66	Dark	0.20	Light blue
	0.76		0.25	Light blue
			0.29	Light blue
			0.37	Light blue
			0.50	Light red
			0.62	Red fluorescence
		Dark	0.66	Light red
			0.74	Red fluorescence
			0.82	

Table 2: HPTLC profile of ethanol extracts of leaf and heart wood of *Asana* Solvent system 2 [Toluene: Ethyl acetate (9.3: 0.7)]

Sample	**254 nm**		**366nm**	
Heartwood	**Rf**	**Colour**	**Rf**	**Colour**
	0.05	Dark	0.18	Lightsky blue fluorescence
	0.09		0.21	Light Sky blue fluorescence
	0.49		0.26	Light Sky blue fluorescence
			0.43	Blue fluorescence
			0.63	Blue fluorescence
			0.80	Blue fluorescence
	After derivatized		**After derivatized at 366 nm**	
	Rf	**Colour**	**Rf**	**Colour**
	0.05	Grey colour	0.05	Light sky blue fluorescence
	0.40	Brown colour	0.09	Yellow fluorescence
			0.21	Light sky blue fluorescence
			0.26	Light sky blue fluorescence
			0.31	Pale cream
			0.43	Blue fluorescence
			0.63	Blue fluorescence
			0.80	Blue fluorescence
Leaf	254 nm		366nm	
	Rf	**Bolour**	**Rf**	**Bolour**
	0.05	Dark	0.03	Red fluorescence
	0.09		0.08	Red fluorescence
	0.49		0.15	Red fluorescence
			0.25	Red fluorescence
			0.34	Red fluorescence
			0.46	Red fluorescence
			0.51	Red fluorescence
			0.53	Red fluorescence
	After derivatized		**After derivatized at 366 nm**	
	Rf	**Colour**	**Rf**	**Colour**
	0.06	Grey colour	0.03	Red fluorescence
	0.10	Greycolour Grey	0.08	Red fluorescence
	0.49	colour	0.11	Cream fluorescence
			0.25	Red fluorescence
			0.34	Red fluorescence
			0.46	Red fluorescence
			0.51	Red fluorescence
			0.53	Red fluorescence

Discussion

Thin layer chromatography is an important device by which chemical constituents can be separated from a mixture. The main principle of analysis is adsorption. The components move according to their affinities towards the stationary phase. The components having more affinities for the stationary phase will have a slow move and the ones having lesser affinity will move faster. The Retention factor (R_f Value) is determined. R_f value is the ratio of the distance travelled by the solute to the distance travelled by the solvent front. The R_f values varies between 0 to 1. The ideal values range from 0.3 to 0.8. This value is constant- and specific for every compound for a particular combination of mobile phase and the stationary phase.[16] In present study, in Solvent system 1 [Toluene: Ethyl acetate: formic acid (5: 5: 1)] HPTLC profile of ethanol extract of leaf showed more spot compared to heart wood except under UV light at 254 nm. Here leaf showed 3 spots (Figure-1.2, Table-1), while samples of heart wood showed 5 spot (Figure-1.2, Table-1). At 366 nm leaf sample showed 10 spots (Figure-1.1, Table-1) while sample of heart wood showed 8 spots (Figure-1.1, Table-1). The spots with R_f values of 0.08, 0.66. 0.74 were observed in both leaf and heartwood sample. That indicates the presence of 3 same active compounds in both samples. Similarly in Solvent system 2 [Toluene: Ethyl acetate (9.3: 0.7)] also HPTLC profile of ethanol extract of leaf showed more or equal spot compare to heartwood. Under UV light at 254 nm leaf showed 3 spots (Figure-2.2, Table-2) which was similar to heart wood (Figure-2.2, Table-2). When the plates were viewed at 366 nm, the leaf sample showed 8 spots while heart wood showed 6 spots (Figure-2.1, Table-2). After derivatisation with Liebermann-Burchard reagent, at 254nm the leaf sample showed 3 spots (Figure-3.2, Table-2) but heartwood sample showed 2 spots. At 366 nm leaf showed 8 spots (Table-3.1 and Figure-3.1). But heartwood sample also showed 8 spots (Table-2 and Figure-3.1). That indicates that leaf sample may possess more active compound compare to heart wood. Previous Phytochemical and TLC studies also showed that the leaf extract contain free radical scavenging molecules, such as amino acids, terpenoids, tannins, glycosides, flavonoids, alkaloids, amines.[15]

Conclusion

To face the global demand and scarcity of medicinal plant at present scenario, it becomes obligatory to save the plant. In this regard it is requisite to find out the part of substitute like root, heart wood, bark etc. which may destroy the plant. Present study aimed to compare the chemical compound present in leaf and heart wood of *Asana*. Study resulted out that leaf sample showed more or equal spot on HPTLC finger printing. That indicates that leaf may have similar active potency like heart wood. But it is a preliminary phytochemical study. Further *invitro* or *in vivo* study should be conducted to provide the scientific base to leaf as a substitute of heart wood.

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