

**PHYTOCHEMICAL EVALUATION AND HIGH-PERFORMANCE THIN LAYER CHROMATOGRAPHY PROFILING: *SAPINDUS EMARGINATUS* VAHL. AND *MORINDA PUBESCENS* J.E.SM. BARKS EXTRACTS**

JEDAGE HD\*, MANJUNATH KP

Department of Pharmacognosy, K.L.E. University, College of Pharmacy, Vidyanagar, Hubli, Karnataka, India.  
Email: haribajedage@gmail.com

Received: 23 Jul 2016, Revised and Accepted: 30 Aug 2016

**ABSTRACT**

**Objective:** To identify the flavonoids high-performance thin layer chromatography (HPTLC) profiles from barks of *Sapindus emarginatus* Vahl. (Sapindaceae) and *Morinda pubescens* J.E.Sm. (Rubiaceae) by ethanol extracts.

**Methods:** The barks of *S. emarginatus* Vahl. and *M. pubescens* J.E.Sm. extracted each separately with ethanol. Both these ethanol extracts were subjected to preliminary phytochemical analysis. Based on phytochemical studies, the extracts obtained were subjected to HPTLC profiles for identify and confirmation flavonoids, both these samples were compared with standard (Rutin). HPTLC analysis performed with silica gel G 60 F254 plates with mobile phase ethyl acetate:n-butanol:formic acid:water (5:3:1:1). Detection of flavonoid compound was performed by scanning the developed plate at 254 nm.

**Results:** Result of these both extracts shows positive tests for flavonoids. Ethanol extracts barks of *S. emarginatus* Vahl. and *M. pubescens* J.E.Sm each were showed bands of different  $R_f$  values with range 0.50-0.90 and standard showed bands of 0.50  $R_f$  values.

**Conclusion:** It can be concluded that rutin constituents of each bark extract are presents and have effective components which can be utilized as a useful herb for alleviation of various illness and disorders.

**Keywords:** *Sapindus emarginatus* Vahl., *Morinda pubescens* J.E.Sm. Ethanol extracts, High-performance thin layer chromatography, Rutin.

© 2016 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>) DOI: <http://dx.doi.org/10.22159/ajpcr.2016.v9s3.14265>

**INTRODUCTION**

According to the WHO, over 70-80% of the world populations still rely mainly on plant derived medicines [1-4]. Nowadays, researchers worldwide are engaged in searching for alternative drugs for various diseases such as hepatotoxicity, nephrotoxicity, cardiotoxicity, and neurotoxicity (without side effects), only a few significant and effective agents are available in traditional medicine therapy [5-7]. But hindered or difficult the acceptance of the alternative medicines in the developed countries, because the lack of documentation and stringent quality control. There is a need for documentation of research work carried out on traditional medicines. With this backdrop, it becomes extremely important to make an effort toward standardization of the plant bark material to be used as a medicine such as *Sapindus emarginatus* Vahl. and *Morinda pubescens* J.E.Sm.

The plant of *S. emarginatus* Vahl. is belonging to family Sapindaceae, commonly known as Soap nut tree (Ritha) [8], and the plant of *M. pubescens* J.E.Sm belonging to family Rubiaceae, commonly known as Brim stone tree, Wild ach root (Bartondi) [9,10]. Both barks and other parts of these plants are used (ethnobotanical) to treatment of various diseases in tribal and rural area, etc. Very few attempts have been made to isolate and characterization compound from the fruits and leaves.

Therefore, the present work was planned to the bark extracts of the plants were subjected to a systemic phytoconstituents isolation and the isolated phytoconstituents compounds analysis by biochemical method, i.e., phytochemical tests and chromatography method, i.e., high-performance thin layer chromatography (HPTLC).

**METHODS****Collection**

The barks of *S. emarginatus* Vahl. and *M. pubescens* J.E.Sm were collected from Western Ghat regions of (Satara - District) Maharashtra and (Belgaum - District) Karnataka state.

**Authentication**

The plant material is identified and authenticated by the Botanist Dr. Harsha Hegde, Scientist 'C' Regional Medical Research Centre, Indian Council of Medical Research, Belgaum. The voucher specimen has been deposited at the same herbaria with accession no: RMRC-989 (*S. emarginatus* Vahl.) and RMRC-990 (*M. pubescens* J.E.Sm.).

**Extraction**

The 175 g each dried bark of *S. emarginatus* Vahl. and *M. pubescens* J.E.Sm. powders (40 meshes) is extracted separately with 750 ml of 90% ethanol [11]. The both extracts a solvent was evaporated in rota evaporator, and this extract concentrated on water bath. This ethanol extract part of was named *S. emarginatus* Vahl. (EESe) and *M. pubescens* J.E.Sm. (EEMp).

**Phytochemical test**

The EESe and EEMp were done phytochemical tests for identification constituents [12,13].

**Solvents and chemicals**

All chemicals and solvents used were of analytical grade and obtained from (Rutin from Sigma - Germany).

**HPTLC development**

The technique for separating or identifying the components in a mixture. The developments methods for HPTLC (Table 1).

The experiment was evaluated by given formula:

$$\text{Evaluation : } R_f = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

## RESULTS AND DISCUSSION

### Phytochemical tests

The results of these barks of *S. emarginatus* Vahl. and *M. pubescens* J.E.Sm. ethanol extracts were confirmed the presence of flavonoids, tannins, phenols, etc. (Table 2).

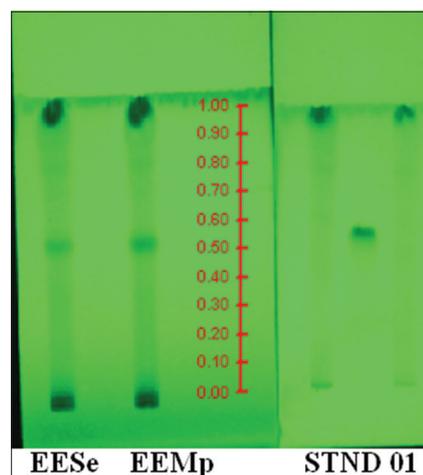
### HPTLC

The EESe was showed 04 spots,  $R_f$  values is 0.50, 0.70, 0.80, and 0.90 at shortwave 254. Moreover, EEMp was showed 03 spots;  $R_f$  values are 0.50, 0.80, and 0.90 at shortwave 254.

Both these test samples of extracts were compared with standard compound rutin was showed 01 spot,  $R_f$  values are 0.50 at shortwave 254.

Here, HPTLC's  $R_f$  values were showed confirmed the presence of rutin constituents in EESe and EEMp (Fig. 1).

The rutin constituent is flavonoid in nature, flavonoids constituents having an antioxidant activity [16] (more effective in superoxide



**Fig. 1:** Ethanol extracts of bark of *Sapindus emarginatus* Vahl. and bark of *Morinda pubescens* J.E.Sm. with standard compound 01 at Shortwave 254 nm

scavenging and nitric oxide scavenging activity etc.) with their large contribution to decrease the reactive oxygen species formation in various diseases. It is also used in various diseases as treatments of hepatotoxicity, nephrotoxicity, neurotoxicity, diabetes, cancer, Alzheimer's, etc. [5,17-21].

## CONCLUSION

It can be concluded that rutin constituents are confirmed in each both barks of ethanol extracts and have effective components which can be utilized as a useful herb for alleviation of various illness and disorders.

These study simple but reliable standards will be useful to manufacturers; he can utilize them for identification and selection of the active pharmaceutical ingredients or raw material for the drug production.

## ACKNOWLEDGMENTS

The authors are thankful to K.L.E. University, Belgaum and Principal, K.L.E. University's College of Pharmacy Hubli - 31, Karnataka, India, for providing the necessary facilities to carry out the work.

## REFERENCES

- Sharma SK, Sharma SM, Saini V, Mohapatra S. Hepatoprotective effect of *Abutilon indicum* on carbon tetra chloride induced hepatotoxicity. *Glob J Pharm Res* 2013;2(1):1608-12.
- Rang HP, Dale MM, Ritter JM, Flower RJ. Anti-inflammatory and immunosuppressant drugs. 6<sup>th</sup> ed. London: Churchill Livingstone, Elsevier Publications; 2008. p. 226-45.
- Dharmasiri MG, Jayakody JR, Galhena G, Liyanage SS, Ratnasooriya WD. Anti-inflammatory and analgesic activities of mature fresh leaves of *Vitex negundo*. *J Ethnopharmacol* 2003;87(2-3):199-206.
- Kumara NK. Identification of Strategies to Improve Research on Medicinal Plants Used in Sri Lanka. Sri Lanka: University of Ruhuna; 2001. p. 12-4.
- Nema AK, Agarwal A, Kashaw V. Hepatoprotective activity of *Leptadenia reticulata* stems against carbon tetrachloride-induced hepatotoxicity in rats. *Indian J Pharmacol* 2011;43(3):254-7.
- Bataller R, Brenner DA. Liver fibrosis. *J Clin Invest* 2005;115(2):209-18.
- Subramoniam A, Pushpangadan P. Development of phytomedicines for liver diseases. *Indian J Pharmacol* 1999;31(3):166-75.
- Nadkarni KM. *Indian Materia Medica*. 3<sup>rd</sup> ed., Vol. I. Mumbai: Popular Prakashan Pvt. Ltd.; 1976. p. 1102-3.
- Magadi RG. In: Komala BM, editor. Botanical and Vernacular Names of South India Plants. 1<sup>st</sup> ed. Bangalore: Divyachandra Prakashan; 2001. p. 282.
- Ghorband DP, Biradar SD. Folk medicine used by the tribes of Kinwat forest of Nanded district Maharashtra India. *Indian J Nat Prod Resour* 2012;3(1):118-22.

**Table 1: Developments for high-performance thin layer chromatography**

Conditions	
Stationary phase	HPTLC precoated, silica gel G 60 F254 (Merck, Germany)
Size	10×10 cm
Mobile phase	Ethyl acetate: n-Butanol: Formic acid: Water (5:3:1:1) for identification of flavonoids
Sample	EESe, EEMp, and STND 01
Sample preparation	Extract dissolved in methanol
Application of sample	Narrow bands of 6 mm length (150 µl/s)
Developing chamber	Twin trough glass chamber
Mode of application	Band
Band size	5 mm
Separation technique	Ascending
Temperature	20±50°C
Saturation time	30 minutes
Scanning wavelength	254 nm
Scanning mode	Absorbance/reflectance
Detection/scanning	CAMAG TLC scanner V, densitometric system with WINCAT software

Documentation or fingerprint [14,15]. HPTLC: High-performance thin layer chromatography, EESe: Ethanol extract part of was named *S. emarginatus* Vahl, EEMp: Ethanol extract part of was named *M. pubescens* J.E.Sm, STND: Standard compound 01

**Table 2: A phytochemical test of ethanol extracts of bark of *Sapindus emarginatus* Vahl. and *Morinda pubescens* J.E.Sm.**

Phytochemical constituents	Chemical test	Observation	Inference
Flavonoids	Shinoda	Magenta color	Present
	Ferric chloride	Violet color	Present
Tannins	Vanillin hydrochloride	Pink-red color	Present
	Gelatin	White ppt	Present
Phenols	Zinc-hydrochloride	Yellow-orange color	Present
	reduction	color	

*M. pubescens*: *Morinda pubescens*, *S. emarginatus*: *Sapindus emarginatus*, EESe: Ethanol extract part of was named *S. emarginatus* Vahl, EEMp: Ethanol extract part of was named *M. pubescens* J.E.Sm

11. Anonymous. Phytochemical Reference Standards of Selected Indian Medicinal Plants. Vol. I. New Delhi: Indian Plants Unit, Indian Council of Medical Research; 2003. p. Appendix – II-343.
12. Khandelwal KR. In: Sethi V, editor. Practical Pharmacognosy. 22<sup>nd</sup> ed. Pune: Nirali Prakashan; 2012. p. 25.1-25.6.
13. Kokate CK. Practical Pharmacognosy. 4<sup>th</sup> ed. Delhi: Vallabh Prakashan; 1994. p. 123-5.
14. Sethi PD. HPTLC Quantitative Analysis of Pharmaceutical formulation. 1<sup>st</sup> ed. New Delhi: CBS Publication and Distribution; 1996. p. 3-30.
15. Chatwal GR, Anand SK. Instruments Method of Chemical Analysis. 5<sup>th</sup> ed. Mumbai: Himalaya Publication House; 2007. p. 2.566-2.699.
16. Hesham RE, Shgeru N. Chemistry of bioflavonoids. Indian J Pharm Educ 2002;36:191-4.
17. Wagner H, Geyer B, Fiebig M, Kiso Y, Hikino H. Isobutrin and butrin, the antihepatotoxic principles of *Butea monosperma* flowers. Planta Med 1986;2:77-9.
18. Sonkar N, Ganeshpurkar A, Yadav P, Dubey S, Bansal D, Dubey N. An experimental evaluation of nephroprotective potential of *Butea monosperma* extract in albino rats. Indian J Pharmacol 2014;46(1):109-12.
19. Anupam KB, Mumtaz SM. Hepatoprotective and nephroprotective activity of hydroalcoholic extract of *Ipomoea staphylina* leaves. Bangladesh J Pharmacol 2013;8(3):263-8.
20. Koti BC, Gore A, Thippeswamy AH, Swamy AH, Kulkarni R. Alcoholic leaf extract of *Plectranthus amboinicus* regulates carbohydrate metabolism in alloxan-induced diabetic rats. Indian J Pharmacol 2011;43(3):286-90.
21. Yadala P, Viswanathswamy AH. *In-vitro* antioxidant and cytotoxic activity of rutin and piperine and their synergistic effect. Int J Pharm Pharm Sci 2016;8(5):78-82.