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Phytochemical profiling of *Melastoma malabathricum* Linnaeus (Melastomataceae): an ethnomedicinally important plant of Eastern Himalaya

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Abstract

Melastoma malabathricum Linnaeus of Melastomataceae is traditionally used as folk medicine in the treatment of bacterial dysentery, diarrhea, hemorrhoids, leucorrhoea, wounds, cuts, small pox, piles and diabetic conditions in different parts of the world. but sporadic report are available in the literature regarding the study of TLC profiling based analysis of diversity of phytochemicals present in the bark and leaf extracts. Qualitative phytochemical analysis of leaves and stem bark extracts reflected the presence of steroid, phenol, flavonoid, and saponin. This information will be useful in isolation of more phytochemicals of medicinal importance from the said plant.

***Key words*:** *Melastoma malabathricum* , Phytochemical screening, TLC Profiling.

INTRODUCTION

Traditionally used medicinal plants are supposed to be the richest sources of bioactive phytochemicals those act upon a large variety of mechanisms to combat different types of diseases. *Melastoma malabathricum* Linnaeus of Melastomataceae is one such medicinal plant which is commonly found in tropical and temperate Southeast Asian countries, locally known to India as *Lutki, Futki, Chulest* or *Dantrangi* (Bengali; Das & Ghosh 2009). This small shrub has dark purple-magenta flowers. Different parts (viz. leaves, stem, stem bark, flower and fruits) of the species are used as folk medicine for the treatment of dysentery, diarrhea, hemorrhoids, leucorrhoea, wounds and cut mainly in India, Malaysia, Indonesia and other parts of the world (Joffry *et al* 2012). Crude plant extracts are also used in infection during confinement, to prevent scarring of smallpox, piles and toothache (Begum & Nath 2000; Ong & Norzalina 1999). Multiple pharmacological bioactivities of the plant extracts viz. antibacterial (Dutta Choudhury *et al* 2011; Grosvenor *et al* 1995; Maji *et al* 2010; Wiart *et al* 2004; Johnny *et al* 2010; Thatoi *et al* 2008), antifungal (Wiart *et al* 2004; Johnny *et al* 2010) antiviral (Lohenzic-le-Devahat *et al* 2002), anticoagulant (Manicam *et al* 2010), platelet-activating factor inhibitory (Jantan *et al* 2005), wound healing (Sunilson *et al* 2008), antiulcer (Hussain *et al* 2008), antidiarrheal (Sunilson *et al* 2009), antivenom (Uawonggul *et al* 2006), anti-inflammatory (Zakaria *et al* 2006),

antinociceptive (Susanti *et al* 2008) and antipyretic activity (Zakaria *et al* 2006) have been experimentally validated.

In Northeastern states of India, the Meitei community of Manipur uses the bark and leaves of *M. malabathricum* for treating skin infections, leukorrhea, diarrhea, and dysentery. The Naga tribe of Manipur also use the fresh and dry leaves of *M. malabathricum* to treat cuts and wounds (Jamir *et al*. 2010), stomach disorder, and fever (Ringmichon *et al*. 2010). The Lakher and Pawi community of Mizoram uses the decoction of leaves in the treatment of diarrhoea and dysentery (Sharma *et al* 2001; Khumbongmayum *et al* 2005). The central part of Himalayan region uses the juice of tender shoots against microbial diseases (Negi *et al* 2002) and in healing wounds (Mazumder *et al* 1978).

Several groups of plant secondary metabolites and bioactive constituents like anthocyanin (e.g., malvidin-3,5-diglucoside) , cyanidin- (Cy-) 3-glucoside , Cy-3,5-diglucoside) , *â*-sitosterol and melastomic acid (5-hydroxylup-20(29) -en-28-oic acid) have been isolated from flowers , fruit and root of *M. malabathricum* respectively (Lowry 1968; Lowry 1976; Manzoor-I-Khuda *et al* 1981). 1-octyl docosanoate , 11-methyl-1- tricontanol (Dinda & Saha 1986), 32-methyl-1-tritriacontanol, ursolic acid, p-hydroxybenzoic acid, gallic acid, Kaempferol-3-*O*-*â*-D-xyloside, quercetin-3-*O*-*á*-L-rhamnosyl-(1*'!*2)-*â*-D-galactoside and flavan-3-ol, 4-methylpeonidin-7-*O*-*â*-D-glucoside, have been isolated from leaves of *M. malabathricum* (Mohandoss & Ravindran 1993; Dinda & Saha 1988).

Although a number of compounds have been isolated from different parts of the plant, considering the ethnomedicinal importance, the bark remained neglected and from leaves few compounds have been isolated .So to develop a better insight into the phytochemical diversity of the plant, we focused on total TLC profiling of bark and leaves of the plant using a number of organic solvents like petroleum ether, ethyl acetate and methanol.

MATERIALS AND METHODS

Collection of Plant Material

The fresh leaves and stem barks of *Melastoma malabathricum* Linnaeus (MM) of Melastomataceae were collected from their natural habitat in Karimganj district, Southern Assam of India. The plant was basically identified by comparing with the previously authenticated specimens available at the Herbarium of Assam University, Department of Life Science, Assam University, Silchar, Assam. Collected species (Voucher Specimen number-1507) has been deposited in Assam University Herbarium.

Preparation of Plant Extracts

Collected fresh leaves and stem bark of MM were air dried at room temperature and powdered with the help of a grinder. Both the leaf and stem bark extracts of MM were prepared sequentially with petroleum ether (P), ethyl acetate (E) and methanol (M) using Soxhlet's method of hot extraction. Resulted six plant extracts were then concentrated with rotary evaporator.

Protocol for Preliminary Phytochemical Screening

Qualitative Preliminary Phytochemical Screening of the prepared crude extracts were performed to study the presence or absence of the chemical constituents such as alkaloids, steroids, flavonoids, reducing sugar and tannin using following procedure:

Alkaloids: The plant extract was treated with 2 % HCl and heated on water bath. On cooling, the mixture was filtered and treated with few drops of Mayer's reagent. The sample was then observed for the presence of turbidity or yellow precipitation (Siddiqui & Ali 1997).

Steroids: 20 mg of dried extract was treated with 2.5 ml of acetic anhydride and 2.5 ml of chloroform. Then to that concentrated solution of H_2SO_4 was added slowly and red-violet colour was observed for terpenoid and green-bluish colour for steroids (Siddiqui & Ali 1997).

Flavonoids: 4 ml of plant extract solution was treated with 1.5 ml of 50 % methanol. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red color was observed for flavonoids and orange color for flavones (Siddiqui & Ali 1997).

Tannins: To 0.5 ml of plant extract 1ml of water and 1-2 drops of ferric chloride solution was added. Blue color was observed for gallic tannins and green black for catecholic tannins (Iyengar 1995).

Reducing Sugar: To 0.5 ml of plant extracts solution, 1ml of water and 5-8 drops of Fehling's solution was added at hot and observed for brick red precipitate (Siddiqui & Ali 1997).

TLC Analysis of the Fractions

Thin Layer Chromatography (TLC) of crude plant extracts were done with analytical plates over silica gel-G (TLC-grade; Merck India). Seven different solvent systems Viz; PE: EA= 9:1, 17:3, 4:1 and 13:7; PE:EA: M=18:1:1, 15:3:2 and 6:3:1 (where, PE=Petroleum ether; EA=Ethyl Acetate and M=Methanol) were used as developing solvent system and each chromatograms were visualized by iodine vapour exposures. The retention factor (R_f) values of the separated compounds in TLC experiments were calculated by using the following formula:

Retention factor (R_f) = Distance travelled by the solutes from the baseline / Distance travelled by the Solvents from the same baseline

RESULT

The preliminary qualitative screening confirms the presence of diverse type of phytochemicals like Reducing sugar, Flavonoid, Steroid, Saponin and Tannin in all six crude extracts as detailed in Table1.

Table 1. Qualitative phytochemical analysis of crude plant extracts of leaves and stem bark of *Melastoma malabathricum* in different solvent systems [*Here*: '+' = presence;'— = absence; MM = *Melastoma malabathricum;* L = leaves; S = stem bark; P = petroleum ether; E = ethyl acetate; M = methanol]

Extractive	Alkaloid	Reducing Sugar	Flavonoid	Steroid	Saponin	Tannin
MMLP	-	+	-	-	-	-
MMLE	-	+	-	+	+	+
MMLM	-	+	+	+	+	+
MMSP	-	+	-	-	-	-
MMSE	-	+	+	+	+	+
MMSM	-	+	+	+	+	+

TLC profiling of leaf and stem bark extracts of MM showed the presence of diverse type of phytochemicals. The Rf values against different solvent system are presented in Table 2.

Table 2: The retention factor (R_f) values for each of the six different extracts of leaves and stem bark of *Melastoma malabathricum in* different solvent system [*Here*: MM = *Melastoma malabathricum*; L = Leaves; S = Stem bark; P = petroleum ether; E = ethyl acetate; M=methanol]

Extracts	Developing Solvent Systems						
	P-E (9:1)	**P-E (17:3)**	**P-E (4:1)**	**P-E (13:7)**	**P-E-M (18:1:1)**	**P-E-M (16:3:1)**	**P-E-M (15:3:2)**
MMLP	0.35, 0.67, 0.70	0.37, 0.55, 0.72	0.56, 0.62, 0.67	0.43 0.59, 0.69	0.28, 0.63 0.84	0.39, 0.60, 0.77	0.37, 0.44, 0.67
MMLE	0.48, 0.59, 0.57	0.68, 0.59, 0.78	0.56, 0.69, 0.77	0.70, 0.65, 0.55	0.75, 0.67, 0.32	0.45, 0.65 0.70	0.65, 0.77, 0.54
MMLM	0.65, 0.72, 0.58	0.54, 0.71, 0.67	0.74, 0.49, 0.68	0.45, 0.88	0.64, 0.55, 0.71	0.71, 0.63, 0.60	0.78, 0.57, 0.55
MMSP	0.58, 0.68, 0.69	0.52, 0.72, 0.67	0.60, 0.65, 0.73	0.25 0.79, 0.69	0.53, 0.77, 0.80	0.47, 0.69, 0.45	0.55, 0.59, 0.72
MMSE	0.60, 0.78, 0.55	0.63, 0.51, 0.69	0.61, 0.59, 0.67	0.64, 0.73, 0.79	0.64, 0.69, 0.76	0.56, 0.64 0.28	0.66, 0.75, 0.50
MMSM	0.76, 0.67, 0.66	0.72, 0.36, 0.56	0.45, 0.77, 0.78	0.55, 0.75, 0.75	0.58, 0.56, 0.59	0.74, 0.68, 0.79	0.59, 0.67, 0.66

DISCUSSION

Preliminary phytochemical profiling reflects essential information regarding the diversity of different classes of secondary metabolites such as alkaloids, flavonoids, steroids, saponins, tannins, reducing sugars etc. in the plant extracts. In the present study, qualitative tests for all six crude extracts showed significant indication about the presence of various secondary metabolites. Steroids, flavonoids, saponins and tannins were found to be present in the ethyl acetate and methanol extracts of both the leaves and stem bark, whereas, these were found to be absent in the petroleum ether extracts. Reducing sugars were found to be present in all the six extracts, whereas, the alkaloids could not be detected in any of the extracts. These finding gives very important clue to understand the medicinal importance of the plant and hence, these extracts need to be explored for identification of new biologically active phytochemicals. The present findings are in accordance with earlier reports that concerned with different bioactivity of the plant secondary metabolites (Evans & Trease 2002; Cushine & Lamb 2005).

Impressive results of TLC investigations of all crude extracts of *Melastoma malabathricum* depict the presence of varied numbers of phytochemicals. Different R_f values were observed for each separated phytochemical in different solvent systems which

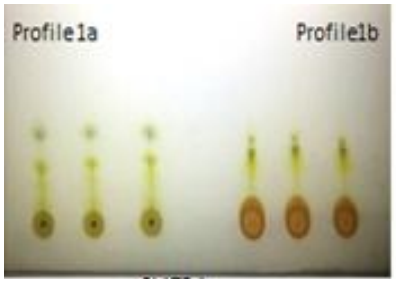

Fig. 1.

Fig. 2.

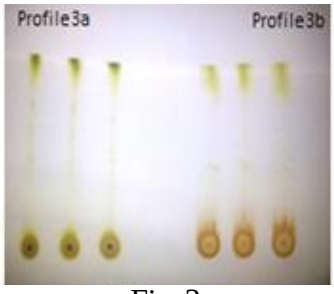

Fig. 3.

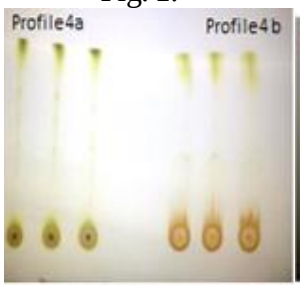

Fig. 4.

Fig. 5.

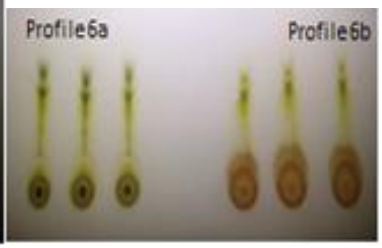

Fig. 6.

Fig. 7.

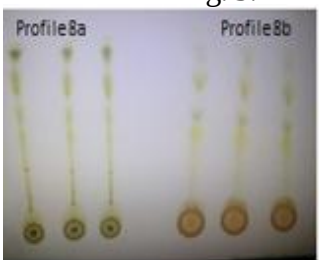

Fig. 8.

PLATE – I: Fig. 1. TLC profiling of petroleumether extracts of stem bark (Profile b) and leaves (Profile a) at PE:EA-9:1; **Fig. 2.** TLC profiling of petroleumether extracts of stem bark(Profile b) and leaves(Profile a) at PE:EA-17:3; **Fig. 3.** TLC profiling of petroleumether of stem bark(Profile b) and leaves (Profile a) at PE:EA-4:1; **Fig. 4.** TLC profiling of petroleumether of stem bark(Profile b) and leaves(Profile a) at PE:EA-13:7; **Fig. 5.** TLC profiling of Ethylacetate extracts of stem bark(Profile b)and leaves(Profile a)at PE:EA:M-18:1:1; **Fig. 6.** TLC profiling of Ethylacetate extracts of stem bark(Profile b) and leaves (Profile a) at PE:EA:M-16:3:1; **Fig. 7.** TLC profiling of methanol extracts of stem bark(Profile b) and leaves at(Profile a) PE:EA-15:3:2; **Fig. 8.** TLC profiling of methanol extracts of stem bark(Profile b) and leaves (Profile a) at PE:EA-16:3:1

helps in proper understanding of their polarity and molecular weight. TLC profiling of the crude extracts also directs the selection of particular mobile phase composition for further column chromatographic separation of pure compounds from the crude plant extracts (Das Talukdar *et al* 2010; Dutta J 2013). In low polar solvent systems, the R_f values of separated compounds are inversely proportional to the polarity of the respective compounds. In the present study, TLC profiling of crude plant extracts of leaves and stem bark in different solvent systems of increasing polarity indicates the presence of diverse group of phytochemicals in *Melastoma malabathricum*. This information will help in selecting the appropriate solvent system for further separation of compounds from these plant extracts.

CONCLUSION

Preliminary phytochemical analysis of the prepared crude plant extracts confirms the presence of steroids, flavonoids and saponins in all the six crude extracts of the leaves and stem bark of *Melastoma malabathricum.* TLC profiling of crude extracts in different solvent systems indicated the presence of diverse group of phytochemicals in the same plant. This indicates that the plant should be explored further phytochemically for isolation and identification of some more phytochemicals of medicinal importance.

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