

Secondary Metabolites and Bioactivities of *Melastoma Malabathricum* (L.) Smith: An Anti-Diarrheal Plant of Bangladesh

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Abstract

Melastoma malabathricum (L.) Smith is a naturally growing plant of Bangladesh and is investigated for its secondary metabolites, cytotoxic and antibacterial activities. Among the secondary metabolites alkaloid, flavonoid, sterol, tannin and glycoside found to be present while saponin is absent in its leaf. Ethanolic extract of *M. malabathricum* leaf showed significant cytotoxicity with the LC₅₀ value of 19.38µg/ml. In the antibacterial screening, the highest zone of inhibition record was 8 mm (at 100mg/ml) and the lowest was 5 mm (at 50mg/ml). These significant cytotoxic and antibacterial activities also validate its ethno-medicinal tradition.

Keywords. Antibacterial, cytotoxic, ethno-medicinal tradition, secondary metabolites.

1. Introduction

Plants are the natural militant as they face so many obstacles in this earth [1]. During this military they produce and store versatile compounds in them [2]. The more the plant face obstacles the diverse its compounds [3]. Thus, plants become the outstanding harbor of foresaid compounds under different conditions which are in fact secondary metabolites [4]. As these are the arsenal of plants against various living and nonliving beings; these compounds adapt bioactive nature [5]. Due to this nature such compounds master healing skill and appraise its reservoir as a medicinal plant. This is why; medicinal plants have various biological effects which are the prognostic of potent and powerful drugs [6].

Melastoma malabathricum or 'futki' [7] is such an unexplored antidiarrhoeal plant of Bangladesh which naturally grows under versatile ecological conditions [8,9]. The appreciable medicinal properties the plant has drawn the attention of the researchers in recent times. Leaves of the plant contain phytochemical constituents strongly associated with its ethno medicinal uses [10]. It is a traditional medicine for alleviating urinary problems, leucorrhea, urinary tract infection (presence of sperm, blood or pus in urine), indigestion, rabies and diarrhea in Bangladesh as well as other countries of the world as flatulence, infection, small pox, toothache (Malaysia), leucorrhea (Java) [11].

Although a number of investigations have executed on *Melastoma malabathricum* with promising results; it is yet to be explored here in Bangladesh. Moreover, scientific investigations reveal that, similar species of different geographical and environmental conditions has significant variation in their metabolite contents as well as biological activities [12]. This impelled to investigate *Melastoma malabathricum* leaf from Bangladesh for its secondary metabolites and bioactivities.

2. Materials and methods

2.1 Collection of leaf sample:

Leaf sample was collected from *Melastoma malabathricum* plant, grown naturally in the premises of Botanical Garden, Department of Botany, University of Chittagong, Bangladesh. The plant was identified and a voucher specimen was kept in the department. The collected leaf sample (≈ 500 g) was cleaned from undesirable materials, chopped, air dried in shade at room temperature and finally ground to a coarse powder.

2.2 Extraction

About 100 g powder was macerated with ethanol (1:5) in a sealed container for 5 days at room temperature with occasional shaking. Extract was filtered through Whatman No.1 filter paper and evaporated to dryness under vacuum below 50°C to get about 3 g blackish extract. The extract thus obtained was kept at 4°C for future use.

2.3 Assessment of secondary metabolites

Alkaloid detecting reagents were prepared following Cromwell [13] and assessed according to Aplin and Cannon [14]. Flavonoids, tannins and sterols were determined following Wall [15], Farnsworth [16] and Bhattacharjee and Das [17], respectively. Glycosides, saponins and resins in the extract were assessed qualitatively according to Ghani [18].

2.4 Bioassays

Cytotoxic activity of the extract was determined by brine shrimp nauplii lethality assay according to Meyer [19] and antimicrobial activity was determined by disc diffusion method. Each set of experiment was replicated three times and their mean values were taken.

2.5 Test Microorganisms

A total of eight human pathogenic microorganisms were used in the study. They include three Gram-positive bacteria; (*Bacillus subtilis*, *Bacillus cereus*, and *Staphylococcus aureus*) and five Gram-negative bacteria; (*Escheretia coil*, *Vibrio cholerae*, *Shigella dysenteriae*, *Shigella sonnei*, and *Salmonella paratyphi*).

3. Result and discussion

In the present work, leaf extract of *M malabathricum* L. was examined qualitatively for its alkaloid content using Dragendorff's (D), Wagner's (W), Mayer's (M), Hager's (H) and Tannic acid (T) reagents. The relative abundance of other secondary metabolite contents in the extract was expressed by '+' sign in different degrees signifying the abundance. Absence of any secondary metabolite was indicated by '-' sign. Results are given in Table 1. Qualitative assessment of alkaloid in the leaf extract indicated its high (3+ and 4+) presence. Among the other metabolites flavonoids, sterol, tannin and glycoside are present while saponins are absent.

Table 1. Secondary metabolite constituents of *M. malabathricum* L. leaf

Secondary metabolites									
Alkaloids					Flavonoids	Sterols	Tanins	Glycosides	Saponins
D	H	M	T	W	+	+	+	+	-
4+	4+	3+	4+	4+					

D=Dragendorff's, W=Wagner's, M=Mayer's, H=Hager's and T= Tannic acid reagents

Medicinal value of a plant is associated with the bioactive compounds found within it [20]. It is proven that, medicinal plants exert antidysentric and antidiarrhoeal properties due to their tannins, alkaloids, saponins, flavonoids, sterols and/or triterpenes and reducing sugars constituents [21, 22]. Presence of tannins in a plant is the indication of its astringent, cytotoxic or antineoplastic, antiviral, antibacterial activities; and capabilities to make the intestinal mucosa more resistant, reduce the secretion, aid wound healing and burns [23-26]. Flavonoids are renowned to exert antidiarrhoeal activity by inhibiting intestinal motility and hydro-electrolytic secretion [27]. Hence, the presence of various phytochemicals encouraged to test its cytotoxic activity which

has been considered as a convenient probe for the assessment of pharmacological potentialities of plant extracts and prescreening assay for antimicrobial, antitumor, antimalarial, antifungal, and insecticidal activities [28]. A number of novel natural products have been isolated using this bioassay [19, 29, 30].

Table 2. Cytotoxic effect of ethanolic extract of *M. malabathricum* L. leaf on *Artemia salina* nauplii

Dose ($\mu\text{g/ml}$)	Log dose	Total	Survived	Dead	Lethality (%)	Actual (%)	Probit
5	0.7	10	9	1	10	0.1	3.72
10	1.00	10	8	2	20	0.2	4.16
15	1.18	10	7	3	30	0.3	4.48
20	1.30	10	6	4	40	0.4	4.75
50	1.7	10	1	9	90	0.9	6.28
75	1.86	10	0	10	100	0.98	6.96
100	2.00	10	0	10	100	0.98	6.96
200	2.30	10	0	10	100	0.98	6.96

The cytotoxic activity of the ethanol extract of *M. malabathricum* leaf was determined by the brine shrimp nauplii lethality bioassay using seven concentrations of the extract ranging from 5 to 100 $\mu\text{g/ml}$, each with 10 nauplii, which died progressively in greater number with the increase of the concentration of the extract and exposure time (up to 24 hours) at the rate of 0.1, 0.2, 0.3, 0.4, 0.9 and 0.98% (Table 2). In the present work, percent and probit were calculated using statistical software 'Biostat 2009' and the leaf extract showed LC₅₀ value of 19.38 $\mu\text{g/ml}$ (Table 3). Chi square value is insignificant at 5% i.e. the data between concentration and lethality is homogenous.

Log (LC ₅₀)	LC ₅₀ ($\mu\text{g/ml}$)	95% Confidence limit ($\mu\text{g/ml}$)	Regression equation	Chi square	
				Calculated	Tabulated
1.29	19.38	14.07-26.47	$Y=2.46+1.83$	1.54	12.6

Table 3. Calculation of LC₅₀ value, regressions equation and confidence limit.

While screening cytotoxicity activity of *M. malabathricum* leaf extract; Lohezic-Le Devehat [31] found similar (<25 $\mu\text{g/ml}$) cytotoxicity activity of the plant from Indonesia while Khatun [32] found a bit higher (53.84 $\mu\text{g/ml}$) from Bangladesh. The cytotoxicity of plant material is predicted as an indication of the presence of bioactive compounds in the plant extract [33]. Crude extract having LC₅₀ value less than 250 $\mu\text{g/ml}$ could be considered significantly active and potential for further investigation[34]. Plant extract in the present work had shown LC₅₀ value less than 250 $\mu\text{g/ml}$ (19.38 $\mu\text{g/ml}$) which exhorted for its further investigation.

It has been found that, plants with antimicrobial activity also deserves antidiarrhoeal vigor [21]. Results of the ethanolic extract of *M. malabathricum* also exhibited promising activity against the selected human

pathogenic bacteria. These pathogens cause a variety of diseases including diarrhoea and gastroenteritis in human [35].

The antimicrobial activity (Table 4) of *M. malabathricum* determined by disc diffusion method at low concentration (2mg/ml) showed no inhibitory effect, but at increased concentrations (25mg/ml, 50mg/ml and 100mg/ml) the extract showed inhibition zone up to 8 mm. In case of *Escheretia coli* it was 6 mm at 25mg/ml, 7mm at 50mg/ml and 8 mm at 100mg/ml. *Bacillus cereus*, *Shigella dysenteriae*, *Staphylococcus aureus* and *Salmonella paratyphi* had no marked inhibition zone at 25mg/ml but they showed inhibition (5 mm to 8 mm) at 50mg/ml and 100mg/ml. However, response of five bacteria namely *E. coli*, *Vibrio cholerae*, *S.dysenteriae*, *S. sonnei* and *S.paratyphi* was highest (8 mm).

Alwash et al., [10] found similar results found in present work; whether other investigators i.e. Grosvenor [36], Wiart [37] and Thatoi [38] whom observed varied results during their studies regarding the antibacterial activity of the ethanolic extract of *Melastoma malabathricum* L. leaf .

Table 4. Antibacterial activity of the ethanolic extract of *Melastoma malabathricum* L. leaf

Test organisms	Diameter of zone of inhibition (mm)			
	2 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml
<i>E. coli</i>	-	-	7	8
<i>B. cereus</i>	-	-	6	7
<i>B. subtilis</i>	-	5	6	7
<i>V. cholerae</i>	-	-	7	8
<i>S. dysenteriae</i>	-	-	-	8
<i>S. sonnei</i>	-	6	7	8
<i>S. aureus</i>	-	-	5	7
<i>S.paratyphi</i>	-	-	6	8

4. Conclusion

On the basis of the results evaluated as above, it may be concluded that, the plant *Melastoma malabathricum* have promising medicinal properties. It possesses important therapeutic phytochemicals with cytotoxic and antibacterial properties which can be a *vital* source of antidiarrheal drugs in the future.

Conflict of Interests

The author declares no conflict of interests regarding the publication of this paper.

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