Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.apjtb.com

Document heading doi:10.12980/APJTB.4.2014C658

Cytotoxicity screening of *Melastoma malabathricum* extracts on human breast cancer cell lines *in vitro*

Nurfariza Ahmad Roslen, Nur Aizura Mat Alewi, Hadji Ahamada, Mohammad Syaiful Bahari Abdull Rasad*

Department of Biomedical Science, Kulliyyah of Allied Health Sciences, IIUM, 25200 Kuantan, Pahang, Malaysia

PEER REVIEW

Peer reviewer

Dr. Mohd. Arifin Bin Kaderi, PhD, IARC Postdoctoral Fellow, International Agency for Research on Cancer (WHO-IARC), 150 cours Albert Thomas, 69372 Lyon, France.
Tel: +33 (0)4 7273 8354
Fax: +33 (0)4 7273 8388

Comments

This is an interesting research work in which the authors had demonstrated the *in vitro* cytotoxic activity of the methanol extract of the leaves of *M. malabatricum* on the MCF-7 cancer cell-line, as well as moderate cytotoxic activity of the methanol and chloroform extract of the flowers. This appears to provide interesting data that these extracts of the plant may become an interesting anticancer agent against breast cancer. The promising potential of this plant extract should be further studied.

(Details on Page 548

ABSTRACT

Objective: To screen the cytotoxic activity of *Melastoma malabathricum* (*M. malabathricum*) against human breast cancer cell line (MCF-7) *in vitro*.

Methods: A three steps extraction protocol using *n*-hexane, chloroform and methanol as the solvents systems was carried out on leaves, stems and flowers of *M. malabathricum*. Dimethyl sulfoxide was used in extracts dilution and serial dilutions were conducted to obtain five different extract concentrations (100 μg/mL, 50 μg/mL, 25 μg/mL, 12.5 μg/mL and 6.25 μg/mL). The evaluation of cell growth was determined using methylene blue assay.

Results: Methanol extract from the leaves showed significant anticancer activity against MCF-7 cell lines with the IC_{50} value of 7.14 μg/ml while methanol and chloroform extract from the flowers exhibited a moderate activity towards MCF-7 cell line with the IC_{50} value of 33.63 μg/mL and 45.76 μg/mL respectively after 72 h of treatment.

Conclusions: The extracts from leaves and flowers of *M. malabathricum* showed promising anticancer activity toward human breast cancer cell lines with the lowest IC_{50} at 7.14 μg/mL while the extracts from stems showed less growth inhibition activity.

KEYWORDS

Melastoma malabathricum, Cytotoxicity, Human breast cancer, MCF-7

1. Introduction

Malaysia is well known as a tropical rainforest country that has abundant sources of medicinal plants. *Melastoma malabathricum* (*M. malabathricum*) belongs to the family Melastomataceae and has many common names including *senduduk* in the native language of Malaysia. *M. malabathricum* is a small shrub commonly found in cleared land, wasteland and roadside throughout the Southeast Asian countries and other parts of the globe. Melastomataceae is a family of plants with a total of more than 4000 species in the world.

In the Southeast Asian region alone, the genus *Melastoma* comprises of 22 species, 2 subspecies and 3 varieties. It is a meter tall shrub with lots of branches and can grow up to a height of about 3 to 6 m[1]. As described by Sirat *et al*., there are 3 varieties of *M. malabathricum* classified by the colors of their petals *i.e.* pink-magenta petals, white petals, and purple-magenta petals[2]. The attractive flowers produced by *M. malabathricum* have 5 petals with the pink-magenta as the commonest variety found in Malaysia and Indonesia. The fruits of *M. malabathricum* are soft, dark purple with numerous orange seeds inside. The seeds are tasteless and can be eaten and stain the tongue to black.

Traditionally, it is believed that many parts of *M. malabathricum* have been used in herbal remedies for the treatment of various human ailments. The Malay population has used the leaves and roots of *M. malabathricum* for the

*Corresponding author: Mohammad Syaiful Bahari Abdull Rasad, Department of Biomedical Science, Kulliyyah of Allied Health Sciences, International Islamic University Malaysia (IIUM), 25200 Kuantan, Pahang, Malaysia.
Tel: +609-5705253
Fax: +609-5706776
E-mail: syaiful@iium.edu.my

Foundation Project: Supported by International Islamic University Malaysia (EDW B11-024-0502 and EDW B11-216- 0694).

Article history:
Received 19 Jan 2014
Received in revised form 27 Jan, 2nd revised form 2 Feb, 3rd revised form 7 Feb 2014
Accepted 13 Mar 2014
Available online 5 Apr 2014

treatment of wounds, post-natal care, and prevention of scars from small pox infection, stomach ulcers, dysentery and diarrhea[3]. Previous studies have shown that the family Melastomataceae demonstrates various bioactivities including antiviral and cytotoxic activities[4], anti-oxidant and anti-cancer activities[2]. In addition to its anti-hypertensive effect[3], it has anti-nociceptive, anti-inflammatory and anti-pyretic properties[5], as well as antibacterial and antifungal activity[6,7]. The plant has many attributes, multiple uses and considerable potentiality.

2. Materials and methods

2.1. Preparation of extracts

Leaves, stems and flowers of *M. malabathricum* were cleaned and dried in a drying cabinet (Protech, Malaysia). The air dried powdered leaves (314 g), stems (426.7 g) and flowers (173.2 g) of *M. malabathricum* were extracted respectively with *n*-hexane, chloroform, and methanol in a Soxhlet apparatus. The extracts were concentrated using rotary evaporator (Eyela, UK) and evaporated to dryness in a fume cupboard. The leaves yielded *n*-hexane (7.108 g, 69.8%), chloroform (4.019 g, 57.9%) and methanol (38.967 g, 47.6%) extracts as brown sticky liquid. The stems gave *n*-hexane (2.429 g, 23.8%), chloroform (1.775 g, 25.6%) and methanol (24.965 g, 30.5%) extracts as light green liquid. The flower produced *n*-hexane (0.641 g, 6.4%), chloroform (1.141 g, 16.5%) and methanol (17.913 g, 21.9%) as green sticky liquid. The extracts were dissolved in dimethyl sulfoxide to a final concentration that ranging from 6.25 to 100 μg/mL.

2.2. Cell lines and culture medium

Human breast cancer cell line (MCF-7) was obtained from American Type Cell Culture (ATCC, USA). They were cultured in Dulbecco's Modified Eagle's Medium. All media were supplemented with 10% (v/v) fetal bovine serum, 100 μg/mL of 1% (v/v) penicillin-streptomycin (PenStrep), and 4 mmol/L l-glutamine. The cell cultures were maintained in an incubator containing 5% (v/v) CO_2 at 37 °C.

2.3. In vitro cytotoxicity assay

Confluent stock cultures of cells were harvested with 0.05% (v/v) trypsin-EDTA and plated onto 96-well plates at the cell density of 5×10^4 cells/well. Cell viability before plating was routinely determined by trypan blue exclusion test. The cells were allowed to attach onto the plate by incubation for approximately 24-48 h. When the cells reached confluency between 80%-90%, the medium was replaced with fresh medium containing 0.5% (v/v) fetal bovine serum. The cells were incubated for another 4 h to achieve dormant state. The cells were then treated with different concentrations of methanol, chloroform and *n*-hexane extracts from *M. malabathricum*. One column of wells was treated with only 0.1% of dimethyl sulfoxide that was used to dissolve the crude extracts in the stock solutions; this served as a control. After treatment, the plates were incubated for a further 72 h.

2.4. Cell viability determination

Viability of cells were determined by using methylene blue staining method[8]. Methylene blue only stains viable cells. Briefly, glutaraldehyde was added to each well to a final concentration of 2.5% (v/v) and the viable cells were fixed for 15 min. After washing with 0.15 mol/L sodium chloride (NaCl) and removing the dead cells, the fixed cells were stained with 0.1 mL of 0.05% (w/v) methylene blue solution for 15 min. After washing off the excess dye with 0.15 mol/L sodium chloride solution, dye solution was carried out with 0.2 mL of 0.33 mol/L hydrochloric acid (HCl). The absorbance was then read at 650 nm using Vmax Kinetic Microplate Reader (TECAN, Germany).

2.5. Calculations and statistics

Percentage inhibition of cancer cell line was calculated using the following equation:

%Inhibition=[(Abs. Control-Abs. Sample)/(Abs. Control)]×100

where Abs.=Absorbance.

Experiments were performed in three replicate. Results were expressed as percentage growth inhabitation of control. The IC_{50} values for each of the active extracts were determined by plotting the percentage inhibition values against the respective concentrations of the extracts. A linear equation for the resulting plot was obtained. The y in the equation was substituted with 50 to obtain the x value which represents the IC_{50}. The IC_{50} is the concentration of the extracts, at which growth was inhibited in 50% of the cells population. Statistical significant difference was determined using One-way ANOVA and differences were considered to be significant if P-value is less than 0.05.

3. Results

The growth inhibitions of various extracts from leaves, stems and flowers of *M. malabathricum* were evaluated using methylene blue assay. The determinations of cytotoxicity were done using dose-response curve obtained by non-linear regression analysis. The results showed that methanol leaf extract caused 81.23% of growth inhibition against MCF-7 cell line (Table 1) followed by methanol flower extract (77.97%) and chloroform flower extract (63.06%) at concentration of 100 μg/mL respectively (Table 2). All stem extracts produced very low inhibition against MCF-7 cell line with less than 47% growth inhibition for every concentration used (Table 3).

Table 1

Percentage inhibition of leaf extracts on MCF-7 cell lines after 72 h of treatment.

Extracts	% Inhibition at different concentrations (μg/mL)				
	6.25	12.5	25	50	100
Methanol	48.86±0.75	51.78±0.28	69.26±0.34	74.43±0.61	81.23±1.44
Chloroform	18.12±0.42	29.12±0.69	40.13±0.81	46.59±1.49	48.21±1.03
n-hexane	25.89±0.91	30.42±0.34	37.21±1.13	42.39±0.79	47.57±0.51

Values have been expressed as mean±standard deviation (n=3).

Table 2

Percentage inhibition of flower extracts on MCF-7 cell lines after 72 h of treatment.

Extracts	% Inhibition at different concentrations (μg/mL)				
	6.25	12.5	25	50	100
Methanol	26.44±0.88	35.93±0.37	53.90±1.76	69.15±0.70	77.97±1.25
Chloroform	17.62±1.45	26.78±0.43	37.97±0.72	52.54±1.53	63.06±1.35
n-hexane	17.28±1.65	21.36±1.03	26.09±1.96	33.22±1.41	41.02±0.74

Values have been expressed as mean±standard deviation (n=3).