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

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PEER REVIEW

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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. malabathricum* 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.

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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 IC₅₀ 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 IC₅₀ 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 IC₅₀ 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

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treatment of wounds, post-natal care, and prevention of scars from small pox infection, stomach ulcers, dysentery and diarrheal[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) CO₂ 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 5×10⁴ 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:

$$\% \text{Inhibition} = \frac{(\text{Abs. Control} - \text{Abs. Sample})}{(\text{Abs. Control})} \times 100$$

where Abs.=Absorbance.

Experiments were performed in three replicate. Results were expressed as percentage growth inhabitation of control. The IC₅₀ 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 IC₅₀. The IC₅₀ 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
<i>n</i> -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
<i>n</i> -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).

Table 3

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

Extracts	% Inhibition at different concentrations ($\mu\text{g/mL}$)				
	6.25	12.5	25	50	100
Methanol	14.69 \pm 0.75	21.31 \pm 1.75	25.35 \pm 1.01	31.12 \pm 0.67	38.31 \pm 2.14
Chloroform	11.52 \pm 1.69	18.72 \pm 1.08	25.92 \pm 2.01	36.88 \pm 0.84	46.10 \pm 1.34
<i>n</i> -hexane	7.48 \pm 1.71	15.56 \pm 0.66	19.59 \pm 1.07	26.78 \pm 1.95	32.84 \pm 1.11

Values have been expressed as mean \pm standard deviation ($n=3$).

A dose-dependent response was observed between the concentration of all extracts and their growth-inhibition activities. Almost all extracts exhibited increased activity after prolonged treatment. The results obtained showed that methanol leaf extract appeared to be cytotoxic with IC_{50} value of 7.14 $\mu\text{g/mL}$ (Figure 1) followed by methanol flower extract with IC_{50} value of 33.63 $\mu\text{g/mL}$ (Figure 2) respectively after 72 h of treatment. All results obtained showed a significant difference between solvents and plant parts with P -values less than 0.05 except stems with undetermined IC_{50} (Figure 3).

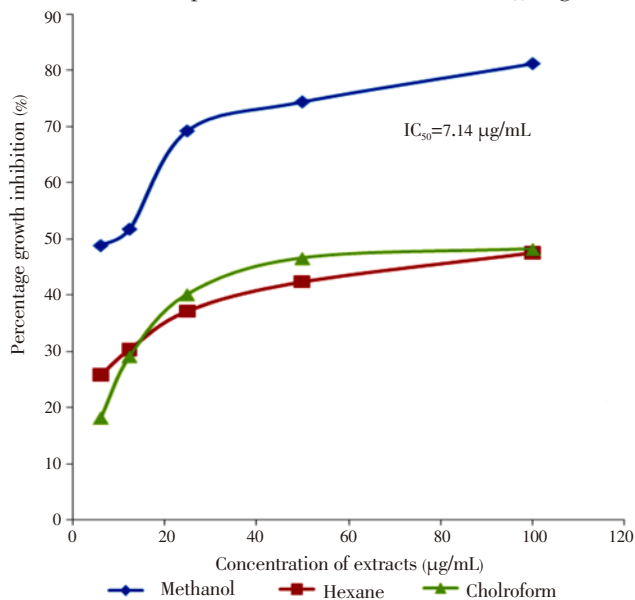


Figure 1. Growth inhibition of leaf extracts on MCF-7 cell line after 72 h of treatment.

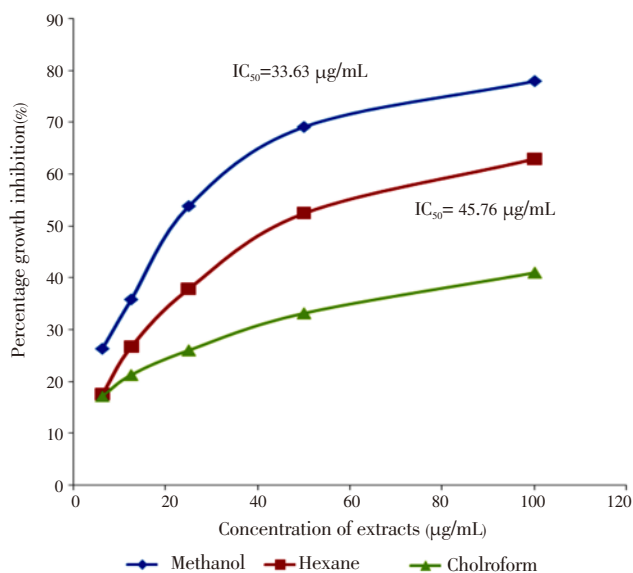


Figure 2. Growth inhibition of flower extracts on MCF-7 cell line after 72 h of treatment.

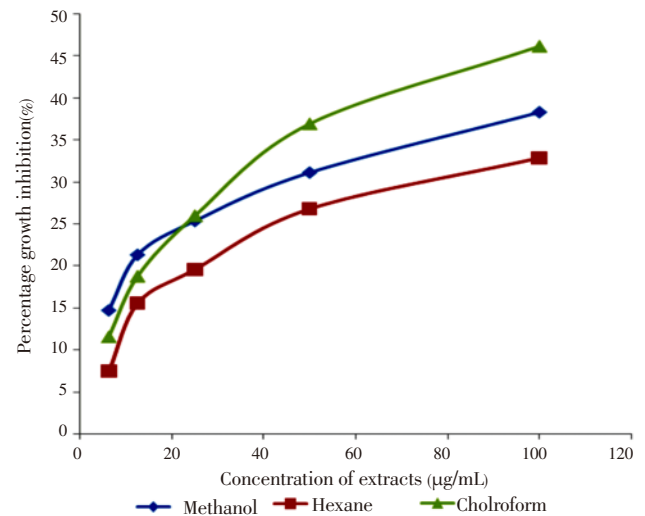


Figure 3. Growth inhibition of stem extracts on MCF-7 cell line after 72 h of treatment.

4. Discussion

This study was aimed at screening for the potential cytotoxicity activity against human breast cancer cell line (MCF-7) *in vitro*. Leaves, stems and flowers were extracted using three solvents, namely, *n*-hexane, chloroform and methanol. The cytotoxicity was determined using methylene blue assay[8]. The results obtained were analyzed by determining the mean absorbance and standard deviation for each concentration. The percentage growth inhibition and half maximal inhibitory concentration (IC_{50}) were determined using SPSS version 17 and Excel (point to point analysis). Significant results were compared using One-way ANOVA and paired t -test. The criterion of cytotoxic and non-cytotoxic was adapted from the guideline set by the National Cancer Institute of America. The screening protocol has indicated that plant extracts with IC_{50} less than 30 $\mu\text{g/mL}$ were considered to be cytotoxic and non-cytotoxic if otherwise[9].

Similar study was reported by Sirat *et al.* that methanol extract of flower from *M. malabathricum* yielded kaempferol-3-O-(2'',6''-di-O-p-trans-coumaroyl) and ethyl acetate extract of the same flower gave naringenin as an isolated compounds[2]. Both compounds were found to be active in inhibiting cell proliferation of MCF-7 with IC_{50} values of 0.28 $\mu\text{mol/L}$ and 1.3 $\mu\text{mol/L}$ respectively. It is important to note that the reported IC_{50} value of this kaempferol-3-O-(2'',6''-di-O-p-trans-coumaroyl) was lower than the positive control used, tamoxifen (0.76 $\mu\text{mol/L}$), a prominent breast cancer drug in the market. It was also mentioned that this cytotoxic activity affects the cells proliferation and morphology and act in a dose-dependent manner. Furthermore, Mohd Joffry *et al.* observed that methanol extract of leaf from *M. malabathricum* showed cytotoxic activity against human cancers cell line (MCF-7) with an IC_{50} value less than 25 $\mu\text{g/mL}$ [3]. This present study contributes to the mounting evidence that the leaf and flower extracts of *M. malabathricum* have potential anticancer activity.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

Breast cancer is one of the most important malignancy among women in the developed and developing countries. It accounts for almost a quarter of all cancers among female. The potential of natural biological products from the tropical rainforest has become subjects of intense research in recent years. As a source of tremendous diversities of fauna, systematic scientific studies on these natural resources may provide discovery towards potential novel pharmaceutical agents against breast cancer.

Research frontiers

The present research work explored the cytotoxic activity of several extracts from various parts of *M. malabathricum* on human breast cancer cell line, the MCF–7. The study seems to provide some initial interesting data on the potential of certain extracts of the plant to inhibit the growth of the cell line *in vitro*.

Related reports

Several extracts of *M. malabathricum* had been previously reported to contain various bioactive phytochemical and had been demonstrated to possess anti-inflammatory, anti-oxidative and wound-healing properties. This may support the findings of the present research, as these pathways are also involved in the development of neoplasia and malignancy.

Innovations and breakthroughs

M. malabathricum, known commonly as *senduduk* in the native language of Malaysia, is a plant used to stop bleeding and for wound healing among rural inhabitants. In the present study, the authors provided initial data on the cytotoxic activity of certain extracts of the plant.

Applications

M. malabathricum is a common shrub, which is ubiquitous throughout tropical and sub-tropical regions. The findings from the present study, if confirmed in further

detailed analyses and proven safe to be developed as chemotherapeutic agent against breast cancer, may provide interesting application of this abundant natural resource from the tropical forest.

Peer review

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. malabathricum* 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.

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