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Original Article

PHYTOCHEMICAL SCREENING, ANTIOXIDANT POTENTIAL AND CYTOTOXIC ACTIVITY OF MELASTOMA MALABATHRICUM LINN. FROM DIFFERENT LOCATIONS

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ABSTRACT

Objective: The initial study was to screen the phytochemical content of methanol extract of *M. malabathricum* from seven different locations. The other objective was to evaluate the total phenolic content (TPC), Total flavonoid content (TFC), antioxidant potential and cytotoxic activity (on Hepatoma G2 cells) of these extracts and to determine the relationship between TPC and other parameters.

Methods: The preliminary phytochemical screening for the presence of the secondary metabolite was carried out according to standard procedures. The TPC, TFC and antioxidant activity were determined using Folin-Ciocalteu method, aluminium chloride (colorimetric) methodand1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity respectively.

Results: Qualitative phytochemical screening showed the presence of tannins, analkaloid, steroids, flavonoid, phenols, terpenoids and fixed oil but tested negative for the presence of glycoside and saponins. The samples were found to have high TPC and antioxidant activity. The Bachok Kelantan sample (L7) showed highest phenolic content (671.51±50.07 mg of GAE/g) as well as highest DPPH free radical scavenging activity (80.81% and IC_{50} 102 µg/ml). The highest cytotoxic activity against HepG2 cells (IC_{50} 1.4 µg/ml) was shown by KualaTerengganu, Terengganu sample (L1). The Spearman correlation showed that, there is a strong positive correlation between TPC and antioxidant activity (r = 0.714) as well as strong negative correlation between MTT IC_{50} and TPC (R =-0.649) of *M. malabathricum* from different locations. Moreover, there is a weak positive correlation between TFC and antioxidant activity (R = 0.286, p= 0.535). Also, there is poor correlation between TFC and cytotoxicity (R =-0.216, p= 0.64).

Conclusion: The phenolic compounds are associated with the cytotoxic and antioxidant activities of *M. malabathricum,* whereas flavonoids are poorly and weakly associated with cytotoxic and antioxidant activities of *M. malabathricum* respectively. The total phenolic content, mean flavonoid content and mean antioxidant activity of *M. malabathricum* from different locations were significantly different across seven locations ($p<0.05$).

Keywords: *Melastoma malabathricum,* Total phenolic content, Flavonoid, Antioxidant, Cytotoxicity.

INTRODUCTION

Melastoma malabathricum L. is a member of *Melastomataceae*, locally known as senduduk. The plant is about 1.5 to 5 m height,is available throughout Malaysia and commonly used in traditional medicine for treatment of various diseases such as wounds, to othache and sore [1,2]. Scientific investigations have shownthat*M. Malabathricum* has the positive therapeutic activity such as antiulcer [3], anticoagulant [4], hepatoprotective [5], antibacterial as well as anticancer [6]. An earlier study has shown that *M. malabathricum* has a promising effect against various cancer cell lines. It was reported that methanol, chloroform and aqueous extracts inhibit the proliferation of MCF-7, Hela, Caov-3, HL-60, CEM-SS, MDA-MB-231 cancer cell lines, with the effects being associated with antioxidant and polyphenolic content of the plant [7]. It was reported that extracts of leaves of *M. malabathricum* exhibited hepatoprotective activity through antioxidant activity [5, 8, 9]. In addition to cancer and hepatic ailments, several other pathologic conditions such as aging, and atherosclerosis diabetes are caused by free radicals, which are toxic to the cells [10]. Antioxidants are compounds or molecules that prevent effects of oxidation in tissues and protect cells from the toxic effect of free radicals [11]. Presently antioxidants obtained from natural sources play a vital role in combating several diseases and improving the quality of life, because of their safety and efficacy compared to synthetic antioxidants [12].

Plants are potential sources of natural bioactive compounds such as antioxidants [11]. The methanolic extract of the flower of *M. Malabathricum* was found to have strong DPPH free radical scavenging activity. Naringerin and kaemferol-3-0-(2",6"-di-0-p-trans-coumaroyl) glucoside isolated from the flowers of *M. malabathricum* were found to be active DPPH free radical scavengers and they inhibit the proliferation of MCF 7 [13]. Other than these isolated constituents, the plant contains several phytochemical constituents such as alkaloid, coumarin, glycoside, flavonoid, terpenoid, saponin, phenol, steroid and tannins [14–16].

The environment often influences the chemical content and activity of the plant. Similar plants may vary in their chemical contents and activity due to the nature of the soil constituents and the weather of the environment. This study was carried out to evaluate the TPC, TFC, antioxidant potential through DPPH free radicals cavenging activity and cytotoxicity of methanol extract of *M. malabathricum* from seven different locations, and to determine their relationship.

MATERIALS AND METHODS

Chemicals and standards

Gallic acid, 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical, quercetin, Folin–Ciocalteu phenol reagent, sodium carbonate solution, methanol, ethanol, HCl, H_2SO_4, iodine, zinc, ferric chloride, aluminium nitrate, potassium acetate and potassium iodide were obtained from Merck (Germany). The HepG2 and Chang liver cells were sourced from ATCC (Manassas, VA, USA), whereas other items are T75 flask (Costar), 10% fetal bovine serum (Gibco, USA), RPMI-1640 media (Gibco, USA), dimethylsulfoxide (Sigma, USA), hydrogen peroxide (Sigma, USA), penicillin and streptomycin (Nacalai-Tesque, Japan).

Plant materials

M. malabathricum Leaves were collected in April from the seven different localities around the northeast coast of Peninsula Malaysia. These are Kuala Terengganu, Kemaman, Jertih; from the state of

Terengganu, as well as Tumpat, Bakong Luar, Jeram Perdah, Bachok; from state of Kelantan, and coded as L1, L2, L3, L4, L5, L6 and L7, respectively. The samples were identified by Science Officer; Noorhaslinda Haron and deposited with the specimen voucher 00245 in the Herbarium Unit, Department of Agriculture and Biotechnology, Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin (UniSZA). The plant samples were washed with tap water, rinsed with distilled water and dried at 50 °C.

Preparation of extracts

The dried samples of the leaves from the different locations were grinded into powder form; each plant sample was weighed and soaked in methanol at the ratio of 1:10 (w/v) for 3d. Extracts were then decanted and filtered through filter paper. The filtrate was concentrated in a rotary evaporator at 40 °C, and the extracts were dried at 50 °C and kept in a freezer at 4 °C for further use.

Determination of percentage yield (%)

The percentage yield of the extract was determined gravimetrically using the dry weight of extract (x) and soaked samples material (y) using the formula:

$$Percentage\ yield(\%) = \left(\frac{x}{y}\right) \times 100$$

The extraction yield was calculated for each extract, and the result was expressed as a percentage yield (%).

Preliminary qualitative phytochemical screening

The qualitative evaluation of the presence of groups of metabolites was accomplished for seven different locations of *M. Malabathricum* leaves according to the established procedures [17-21].

Saponins

The extract (50mg) is diluted with distilled water and made up to 20ml. The suspension is shakens in the graduated cylinder for 15min. A 2cm layer foam indicates the presence of saponins [17].

Tannins

An amount of extract weighing 50mg is dissolved in distilled water, and 3ml of 10% solution of lead acetate was added. Formation of bulky white precipitate indicated the presence of tannins[18].

Alkaloids

A fifty mg of an extract is stirred with dilute HCl and filtered. A few ml of filterate is taken into a test tube and to the filtrate, afew drops of Wagner's reagent are added. A reddish-brown precipitate indicated the presence of alkaloids[19].

Steroids

About 100mg of dried extract was dissolved in 2ml of chloroform. Sulphuric acid was carefully added to form a lower layer. A reddish brown colour at the interface was an indicative of the presence of steroidal ring [20,21].

Phenols

To 1ml of an alcoholic solution of the sample, 2ml of distilled water followed by a few drops of 10% aqueous ferric chloride solution were added. Formation of blue or green colour indicated the presence of phenols [20,21].

Terpenoid

2ml of chloroform and 1ml of conc. H_2SO_4 was added to 1 mg of extract and observed for reddish brown colour that indicated the presence of terpenoid [20,21].

Fixed oils

A small amount of dried extract is pressed between two filter papers, oil stains on the paper indicate the presence of fixed oils[17].

Flavonoids

In a test tube containing 0.5ml of alcoholic extract of the samples, 5 to 10 drops of diluted HCl (1%) and small amount of Zn was added and the solution was boiled for few minutes. The appearance of reddish pink or dirty brown colour indicated the presence of flavonoids [20,21].

Glycosides

A small amount of alcoholic extract of samples was dissolved in 1ml water, and then aqueous sodium hydroxide was added. Formation of a yellow colour indicated the presence of glycosides [20, 21].

Determination of total phenolic content (TPC)

The total phenolic content of *M. malabathricum*leaves were determined according to the method ofSchalbert *et al.*, 1989 [22] with slight modification. The extracts of the leaves were diluted to 100µg/ml concentration. The Folin-ciocalteau reagent was also diluted at the ratio of 1:10 before use. A 1.25 ml portion of Folin-Ciocalteau reagent were added to 0.25 ml mixture above and allowed to incubate at room temperature for 2 min and then 1 ml of sodium carbonate solution (75 % w/v) was added. The solutions were mixed and kept in a dark place for 30 min. The absorbances of the total phenols was determined spectrophotometrically at 760 nm and the gallic acid aqueous solutions in the range of 2.5-160µg/ml were used as a standard.

Total flavonoid content (TFC)

Flavonoid content of methanol extracts of *M. malabathricum*leaves were determined according to the method of Moreno *et al.,* 2000 [23] with slight modifications. A0.25 ml aliquot of the diluted extract at 200µg/ml was mixed with solutions comprising 50 µl of 10% aluminium chloride, 50 µl of 1 M aqueous potassium acetate and 2.15 ml of 95% ethanol. After incubation for 40 min at room temperature, the absorbance was recorded spectrophotometrically at 415 nm. Total flavonoid was calculated from the calibration curve using quercetin as a standard and expressed as mg/g.

Determination of DPPH free radical scavenging activity

The DPPH free radical scavenging activity of methanol extracts of *M malabathricum* leaves was estimated, by adapting the method of Miser-Salihoglu *et al.*, 2013 [24] with minor modification. A 20 µl of extract at different concentrations (1000, 500, 250, 125, 62.5, 31.25, 15.625, 0 µg/ml respectively) was added in 96 well microplate and then a 200 µl of 0.1 mM solution of DPPH was added to the wells. Quercetin was used as standard while DMSO 20 µl served as blank negative control (reaction mixture without test extract). The mixtures were shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbances were measured at 517 nm using microplate reader. Each sample was assayed in triplicate,and the percentage of inhibition was calculated. The IC_{50} values were also calculated as the concentration of a test sample required to give 50% radical scavenging activity (DPPH). The results were compared with that of the quercetin standard.

$$\text{Percentage inhibition}\ (\%) = \left(\frac{\text{Acontrol} - \text{Asample}}{\text{Acontrol}}\right) \times 100$$

Where A = Absorbance

Cell line and culture

The HepG2 (human hepatocellular carcinoma cell) and Chang liver (normal cell line) cells were cultured in a sterile T75 flask (Costar) containing D-MEME and RPMI-1640 media respectively, supplemented with 10% fetal bovine serum and antibiotics (100.0 unit/ml penicillin and 100.0µg/ml streptomycin) in 5% CO_2 humidified air at 37 °C. Exponentially growing cells were used for all the experiments [25].

Assessment of cell viability

The assessment of cell viability on Hep G2 and Chang liver cells was determined by 3-(4,5-dimethylthiazolzyl-2-yl)-2,5-diphenyl-

tetrazolium bromide (MTT) assays. Briefly, human cell lines were seeded in 96-well microplate plates at concentration 1.0 x 10^5cells/well. After incubation for 24 h, either the methanol extracts of the seven locations or hydrogen peroxide was added at 100µg/ml, followed by two-fold dilutions. The treated plates were then incubated for a prolonged period of 72 h. Then, 20.0µl of MTT solution (5.0 mg/ml) was added to each well and was incubated at 37 °C for 4 h. All medium was removed from each well and replaced with 100.0 µl dimethylsulfoxide. The solubilized formazan produced by metabolically active cells was measured by scanning the 96-well plates at 570 nm with reference at 630 nm wavelengths using Infinate M200 microplate reader from Tecan, Switzerland. The IC_{50} (50% inhibition concentration) values were determined as the concentration of an extract to result in 50% growth inhibition of the cancerous cells. Data was expressed as mean±SE of three or more experiments.

Statistical analysis

All the experiments were carried out in triplicate and expressed as means±SD. The means were compared by one-way ANOVA, and the values were considered to be significantly different at $P<0.05$. Furthermore, Spearman's Correlation was also carried out to estimate the inter relation ship between TPC, TFC, MTT Assay IC_{50} & DPPH % Inhibition of free radicals of different locations *M. malabathricum*.

RESULTS

Percentage yield

The percentage yields of extracts of *M. malabathricum* leaves from different locations is shown in table 1, and L7 was found to have the highest percentage yield followed by L3 while L5 gave the lowest percentage yield.

Table 1: Percentage yield for *M. malabathricum*leaves extracts fromseven different locations

Sample	Percentage yield (%)
Kuala Terengganu (L1)	7.55
Kemaman,Terengganu (L2)	10.26
Jertih,Terengganu (L3)	10.74
Tumpat,Kelantan (L4)	8.21
Bakong Luar,Kelantan (L5)	5.94
Jeram Perdah,Kelantan (L6)	8.57
Bachok,Kelantan (L7)	18.29

Preliminary phytochemical screening

Preliminary qualitative phytochemical test of methanol extracts for seven locations of leaves of *M. Malabathricum* indicates the presence of alkaloid, steroids, phenols, fixed oil, terpenoids and tannins,but tested negatively for the presence of saponins,as well as glycoside and these results, are shown in table 2.

All samples show similar profile for all preliminary qualitative phytochemical screening sex cept for the test of tannins, which is more prominent in L1, L2, L3 and L7.

Total phenolic content (TPC)

TPC for all samples were determined as gallic acid equivalent (GAE) in mg per g dry weight, from theequation$Y = 0.0033x+0.1244$ with$R^2 = 0.9843$. The sample L7 was found to have the highest TPC (671.51±50.07 mg of GAE/g) followed in sequence by L3, L4, L5, L1, L2 and then L6with the lowest TPC value and the results are shown in table 3.

Table 2: Qualitative analysis for phytochemical constituents of leaves of *M. Malabathricum* from seven locations

Tests	L1	L2	L3	L4	L5	L6	L7
Saponins	-	-	-	-	-	-	-
Tannins	++	++	++	+	+	+	++
Alkaloids	+	+	+	+	+	+	+
Steroids	+	+	+	+	+	+	+
Phenols	+	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+	+
Fix oil	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+
Glycoside	-	-	-	-	-	-	-

+indicates presence of the phytoconstituent; − indicates absence of the phytoconstituent

Table 3: Results of total phenolic content of methanol extract *M. malabathricum* from seven different locations

Localities	TPC (mg of GAE/g of extract)
Kuala Terengganu (L1)	$339.19±45.49^{ab}$
Kemaman, Terengganu (L2)	$278.59±48.99^{a}$
Jertih, Terengganu (L3)	$567.47±91.63^{cd}$
Tumpat, Kelantan (L4)	$501.82±35.72^{bcd}$
Bakong Luar, Kelantan (L5)	$479.60±17.76^{bc}$
Jeram Perdah, Kelantan (L6)	$216.97±30.75^{a}$
Bachok, Kelantan (L7)	$671.51±50.07^{d}$

Data are expressed as mean±SD (n = 3 in each locality); means were compared by Scheffe test ($p<0.05$). The mean Phenolic Content of *M. Malabathricum* from different locations is significantly different $p<0.05$.

Total flavonoid content

The TFC of methanol extract *M. malabathricum* leaves from different locations were determined as quercetin equivalent (QE) in mg per g dry weight, from the equation$Y = 0.0052x+0.0883$ with $R = 0.9963$. All samples were found to contain flavonoid with the locationL2 recording highest TFC whereas L5 recorded lowest TFC,and the overall results are presented in table 4.

Table 4: Total flavonoid content of *M. malabathricum* from seven different locations

Locations	TFC (QE mg/g of extract)
Kuala Terengganu (L1)	$79.84±1.45^{b}$
Kemaman, Terengganu (L2)	$102.92±12.77^{c}$
Jertih, Terengganu (L3)	$38.49±2.94^{a}$
Tumpat, Kelantan (L4)	$41.70±5.80^{a}$
Bakong Luar, Kelantan (L5)	$35.93±1.47^{a}$
Jeram Perdah, Kelantan (L6)	$42.34±2.00^{a}$
Bachok, Kelantan (L7)	$76.96±1.16^{b}$

Data are expressed as mean±SD (n = 3 in each locality); means were compared by Dunnet's C test ($p<0.05$). The mean flavonoid content of *M. Malabathricum* from different locations is significantly different $p<0.05$

Antioxidant (DPPH free radical scavenging activity)

The DPPH free radical scavenging activity of methanol extracts of*M. Malabathricum* leaves from different locations, as well as the standard quercetin, were determined and compared using the IC_{50} values and the highest percentage of DPPH free radical inhibition. The results of IC_{50} are shown in table 5 whereas percentage inhibition of DPPH free radical scavenging activity at various concentrations is shown in fig. 1, and it can be noticed that among

the locations tested; L7 was found to have lowest IC_{50} value and highest percentage inhibition of free radicalsor the highest antioxidant activity, which is shown in table 6. The results also show that L6 has percentage inhibition of free radical(antioxidant activity) below 50%, which is the lowest antioxidant activityamong seven samples, and its IC_{50} is above 1 mg/ml.

MTT Assay

The methanol extract of *M. malabathricum* from seven different locations possessed cytotoxic activity on HepG2 cell,and this is shown fig. 2. The L1 possessed the highestcytotoxic activity on Hep G2 cell with IC_{50}valuesof 1.4 µg/ml, whereas L3 has the lowest activity with IC_{50} of 10.0 µg/ml. Each sample demonstrated cytotoxic effect at low concentration, and they kill 50% of Hep G2 at low concentration. The IC_{50} value for each sample has been presented in table 7.

Table 5: Results of 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH) scavenging activity of methanol extract of *M. malabathricum* from seven different locations and standard quercetin

Sample	IC_{50} (µg/ml)
Kuala Terengganu (L1)	148
Kemaman, Terengganu (L2)	388
Jertih, Terengganu (L3)	210
Tumpat, Kelantan (L4)	210
Bakong Luar, Kelantan (L5)	692
Jeram Perdah, Kelantan (L6)	>1000
Bachok, Kelantan (L7)	102
Q (Quercetin)	30

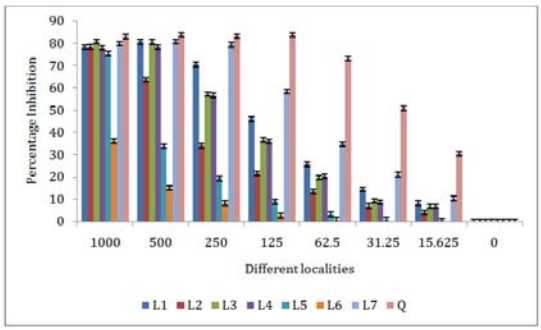

Fig. 1: Percentage inhibition of DPPH free radical by methanol extracts of *M. malabathricum* from seven different locations and Quercetin

Table 6: The highest percentage inhibition of DPPH free radical scavenging activityby extracts of *M. malabathricum* from different locationsand quercetin

Locations	DPPH % Inhibition
Kuala Terengganu (L1)	80.69 ± 0.20^{cd}
Kemaman, Terengganu (L2)	78.46 ± 0.13^{bc}
Jertih, Terengganu (L3)	80.77 ± 0.14^{cd}
Tumpat, Kelantan (L4)	78.35 ± 1.06^{bc}
Bakong Luar, Kelantan (L5)	75.41 ± 2.08^{b}
Jeram Perdah, Kelantan (L6)	36.13 ± 1.61^{a}
Bachok, Kelantan (L7)	80.81 ± 0.86^{cd}
Q (Quercetin)	83.84 ± 0.34^{d}

Data are expressed as mean±SD (n = 3 in each locality); means were compared by Dunnet's C test ($p<0.05$). The mean antioxidant of *M. malabathricum*from different locations is significantly different $p<0.05$

DISCUSSION

The present study showed that methanolic extracts of *M. Malabathricum* leaves from the seven locations had similar phytochemical content among them. All the tested samples contained flavonoid, phenols, tannins, terpenoids, steroids and alkaloids. This is in agreement with screening by earlier researchers [3, 16], but these earlier reports, as well as work of others [7, 9], mentioned the presence of saponin which is not present in our study. Furthermore, our screening did also not detect glycosides which were also not detected in an earlier study [26].

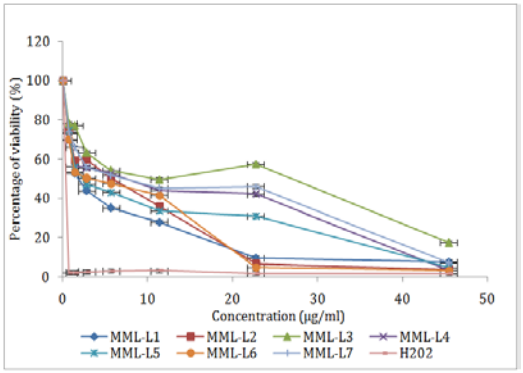

Fig. 2: MTT assay of methanol extract of *M. malabathricum* from seven different locations on HepG2 cell

Table 7: IC_{50} (µg/ml) of MTT assay of methanol extract of *M. malabathricum* from seven different locations on HepG2 cell

Sample	IC_{50} (µg/ml)
Kuala Terengganu (L1)	1.4
Kemaman, Terengganu (L2)	5.6
Jertih, Terengganu (L3)	10.0
Tumpat, Kelantan (L4)	7.5
Bakong Luar, Kelantan (L5)	2.2
Jeram Perdah, Kelantan (L6)	2.8
Bachok, Kelantan (L7)	7.5

The screening which indicated high tannin content as well as positive results for phenol demonstrates a high total phenolic content for these samples which is similar to findings of earlier researchers [3, 12, 15], who reported that *M. malabathricum* contains several chemical compound and high TPC.

The location L7 has the highest TPC (671.51±50.07 mg of GAE/g) as well as highest antioxidant activity (80.81%), and correspondingly the lowest IC_{50} value. On the other hand, the sample L6 possessed the lowest TPC (216.97±30.75 mg of GAE/g)as well as lowest antioxidant activity (36.13%) with the overall antioxidant activity less than IC_{50}. The results show that there are significant differences in both the phenolic content and antioxidant activity of *M. malabathricum*from different locations.

The results for correlation between different parameters are shown in table 8 and the Spearman correlation showed that there is a moderate negative correlation between the TPC and TFC (R =-0.357, p= 0.432) and although often this is not the case but it has been reported. For example, this finding is in line with the finding of [27], which shows that there is a negative correlation between TPC and TFC of *S. scabrum*. The Spearman correlation showed that there was a strong positive correlation (R = 0.714, p = 0.71) between total phenolic content and antioxidant activity of *M. malabathricum* from the different locations. Additionally, there is weak positive correlation between TFC andantioxidant activity (R = 0.286, p= 0.535). The coefficient of determination or R^2 for TPC with radical scavenging activity is 0.510 whereas R^2 between TFC and scavenging activity is 0.082.

This means that TPC contributed 51.0% whereas TFC contributed 8.2 % to the radical scavenging activity. Therefore,the finding of this study showed that there is a strong association between TPC, as well as DPPH radical scavenging activity,and this is in line with the

finding of [28]. The contribution of compounds other than phenolics such as Vitamin C, tocopherols and even terpenoids to antioxidant activity has been reported [29]. For example, the study of [30] reported the presence of two saponins from *Entada rheedii* with antioxidant activity.

Table 8: Spearman's correlation toestimatethe interrelationship between TPC, TFC, MTT Assay IC_{50} & DPPH % Inhibitionoffree radicals of different locations *M. malabathricum*

	TPC	TFC (mg/g)	DPPH % Inhibitionoffree radicals	MTT Assay(µg/ml) IC_{50}
TPC (mg/g)	1	-0.357	0.71	0.649
TFC (mg/g)		1	0.286	-0.216
DPPH % Inhibitionoffree radicals			1	0.468
MTT Assay (µg/ml) IC_{50}				1

In terms of cytotoxic effect, it is noticed that the sample obtained from Kuala Terengganu (L1) has the highest cytotoxic activity (1.4 µg/ml) against HepG2 cellwhereassample collected from Jertih, Terengganu (L3) has the lowest cytotoxic activity(10.0 µg/ml) against HepG2 cell.

It was also found out that there is a strong negative correlation between TPC and cytotoxicity (R =-0.649,p = 0.115), whereas, there is poorcorrelation between TFC and cytotoxicity (R =-0.216, p= 0.64). Overall, this finding indicates that the phenolic compounds are associated with cytotoxic activity of *M. malabathricum,* which is in line with the finding which showed antiproliferative activity of *M. malabathricum* is associated with its phenolic compounds and antioxidant activity [7]. The latter effect is shown by the moderate negative correlation between MTT IC50 and antioxidant effect (R =-0.468,p = 0.289). This indicates that DPPH free radical scavenging activity of different locations of *M. malabathricum* is moderately contributing toward cytotoxic activity. Some of the correlations showed to be insignificant at p>0.05,and this may beasa result of small samplesize. The present study highlights the differences in composition of methanolic extracts of *M. malabathricum* in terms of content of both phenolic and flavonoid group of chemicals when geographical locations are taken into consideration and also show that this invariably affects the activity that is of concern. These differences may possibly be relatedto the natural climatic differences which occur over aparticular geographical area to be influenced by several microclimatic factors.

CONCLUSION

From the finding of this study we can conclude that *M. malabathricum* can be an important source of therapeutic agent in the treatment of diseases such as cancer and liver diseases as shown by their prominent activity on Hep G2 cells whichis associated with its antioxidant activity and phenolic compounds.

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CONFLICTOF INTERESTS

Authors declare no conflict of interest.

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