Egypt. J. Chem. **Vol. 64,** No. 6 pp. 3005 - 3017 (2021)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Egyptian *Myrtus communis* L. Essential oil Potential role as *in vitro* Antioxidant, Cytotoxic and α-amylase Inhibitor

Faten M. Ibrahim[a]*, RashaFouad[a], Salwa EL-Hallouty[b],Saber F. Hendawy[a], Elsayed A. Omer[a], Reda S.Mohammed[b]*

[a] *Medicinal and Aromatic Plants Research Department, National Research Centre; 33-Elbohouth St, (Former El-Tahrir St.), Dokki – Giza-Egypt. P.O.12622 ID: 60014618*

[b] *Department of Pharmacognosy, National Research Centre; 33-Elbohouth St, (Former El-Tahrir St.), Dokki – Giza-Egypt. P.O.12622, ID: 60014618*

Abstract

Myrtus communis L. (MC) family Myrtaceae, is cultivated in Egypt as an ornamental plant. The essential oil (EO) isolated from different parts of the plant reported for many pharmacological activities. Myrtus black and white (MB &MW) are two varieties of *Myrtus* diverse from each other in the color of berries; the EO was prepared from the fresh aerial parts of both varieties through hydrodistillation. The EO was screened for its free radicals scavenging activity by five different methods (DPPH, ABTS, NO, SOR, LP) and with Vit. C & BHT as reference standard. It was noticed that the scavenging activity of MB at all used conc. (125 to 1000 µg/mL) was more potent than MW. Screening of the cytotoxic activities of the EO of MB and MW on five cancer cell line (PC3, MCF-7, A549, HepG-2, HCT-116)& one normal cell line (Rpel-1) revealed that both EO of the two varieties had activity against PC3&MCF-7 with IC_{50}; 4.7±0.15, 14.2±0.35, 45.2±0.67, 50.5±0.6 compared with Doxrubacin standard 24±0.26, 26.1±0.4, respectively. MB& MW oil are safe on Rpel-1 (normal cell line). The α-amylase enzyme inhibition activity of the oils was investigated using acarbose as reference inhibitor. Both MB&MW have activity more than the standard at 125µg/ml 36.24±0.146; 32.14±.188, 23.16±0.190 and also at the highest conc. (1000 µg/mL) 96.22±0.140, 89.48±.106, 88.81±0.690, respectively. Study of the chemical constituents of the EO by GC/MS analysis resulted in the identification of 25 compounds from both species constituting 99.21% & 99.96% of the total peak area of the identified compound, respectively. The major compounds are α–Pinene (49.33%, 31.10 %); 1,8 Cineole (9.97 %, 19.21 %); Myrtenyl acetate (30.7%, 40.80 %); α –Terpineol (2.18%, 1.73%) and D-Limonene (1.90% , 2.61%) for MB & MW, respectively.

keywords *Myrtus communis*, free radical scavenging activity, α- amylase inhibitor, cytotoxic activity, cell lines, GC/MS analysis

1. Introduction

Myrtaceae (myrtle family) comprises about 142 genera and 5500 species and it is a family of flowering plants which are distributed all over the world mainly in tropical and southern temperate regions [1]. One of the characteristics of this family is the presence of oil glands in the leaves that produce essential oils. The various plant species belonging to this family are sources of several valuable essential oils, produced by distillation and the main components of the oils are based on the isoprenoid [2]. Myrtle (MC) is a well-known medicinal plant that has been used worldwide in traditional medicine, myrtus genus grows as evergreen shrubs or small trees, which may grow up to 5-m tall, MC is cultivated in Egypt as an ornamental plant. There are two varieties of myrtle; one with black berries and the other with white berries [3]. The leaves of both varieties are very fragrant, so that they are used in the perfumery and cosmetic industries in many countries[4]. The plant had many medicinal uses as antiseptic, disinfectant, hypoglycemic agent [5] and as flavouring in food industries [6,7]. The chemical composition of myrtle essential oil has been extensively studied in many countries of the Mediterranean region [7-19], The EO from leaves, flowers and fruits tends to vary in composition and it

*Corresponding author e-mail: redamohammed2015@gmail.com; **:**fatenmibrahim@gmail.com
Receive Date: 17 January 2021, Revise Date: 15 February 2021, Accept Date: 24 February 2021
DOI: 10.21608/EJCHEM.2021.57354.3245

is mainly used for the treatment of lung disorders, it also has antibacterial and antioxidant activities [11,20]. The major components of the EO from the leaves of MC growing in Turkey were1,8-cineole, linalool, myrtenyl acetate and myrtenol [10].The EOs of MC was used in the packaging of loquat Fruit either by packing together with the leaves of MC or exposing to leaf vapour to preserve the fruit of loquat from postharvest problems which are browning and chilling injury[21].The hydroaloholic extactof the leaves and berries of MC were reported for many biological activities including antioxidant this is due to presence of phenolic compounds, flavonoids tannins and anthocyanins, the later constituent was present in higher percentage in the species with black berreies than that with white berrei[22]. Different exhausted parts of myrtle berries (fruit, pericarp seed), left after hydroalcoholic infusion, provide a rich source of phytochemicals with high antioxidant activity this feature can be used in the by product in feed formulations [23]. So the aim of the current study is screening the radical scavenging activity of the (EO) of two varieties of myrtle plants for their possible use in food industries as antioxidant or as preserving substance which can be fumed to the different stored fruits either used in local market or for export outside the country; the cytotoxic and the ant diabetic activities of the EO are also evaluated to insure its safety to human health. The composition of the EO is also studied to identify the components of the two species which are responsible for their activities.

2. Material and methods

2.1.Plant material

The aerial parts of *MC* (white and black) were collected in March 2018 from the Experimental Farm of SEKEM Company located in Bilbase, Sharqia Governorate (66 km east of Cairo). The plants grow as hedge. They were identified by Mrs. Terase Labib, plant taxonomist of Al-Orman Garden. A voucher specimens were deposited in the Herbarium of National Research centre M 163 (MB) and M 164 (MW).

2.2.Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), potassium ferricyanide, ferrozine, 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonicacid (ABTS), 2, 4, 6-tripyridyl-s-triazine (TPTZ) and Ammonium thiocyanate were purchased (Merck, Germany), polyoxyethylenesorbitanmono laurate (Tween-), Ascorbic acid (Vit C, 3-(2-pyridyl)-5,6-bis(4-phenyl-sulfonic acid)-1,2,4-triazine (ferrozine), nicotinamide adenine dinucleotide (NADH), butylatedhydroxy toluene (BHT), were purchased from Sigma-Aldrich (St. Louis, MO, USA) .Hepatocellular carcinoma cell line, **(**HePG-2**)**; colon cell line (HCT-116**)**; lung carcinoma cell line (A549); breast cancer cell, **(**MCF-7**)**; prostate cell line and **(**PC3), were performed and identified by the Bioassay-Cell Culture Laboratory, National Research Centre(El-BohouseSt., Dokki, Cairo, Egypt). Rpel-1 was kindly provided by Professor Stig Linder, Oncology and Pathology department, Karolinska Institute (Stockholm, Sweden). Reagents for α- amylase activity: 0.02 M Sodium phosphate buffer, 1% Starch , 2 N Sodium hydroxide, Sodium potassium tartrate tetrahydrate, α-amylase(EC 3.2.1.1), and 3,5- dinitrosalicylic acid (DNS)color reagent; all chemicals were from Sigma-Aldrich (St. Louis, MO, USA). All solvents were of analytical grade.

2.3. *Extraction of essential oils*

The essential oil of both varieties was extracted from the fresh aerial parts of seven years shrubs of myrtle plant. One kg of fresh aerial parts of both plants were subjected to hydro-distillation using Clevenger-type apparatus for three hours as mentioned in Egyptian pharmacopeia [24]. The resulted essential oil from each variety was separately dehydrated with anhydrous sodium sulphate and kept in the deep freezer for GC-MS analysis.

2.4. In-vitro biological study

2.4.1. *Free radical scavenging characters*

2.4.1.*1. DPPH radical scavenging activity*

DPPH• (0.1mM) was prepared in methanol, successive concentration from the essential oils and standard materials; BHT and Ascorbic Acid were prepared as 125, 250, 500, and 1000 µg/ ml in methanol. 1ml of DPPH• solution was added to 3 ml of each concentration, and then the mixture was shaken vigorously and was allowed to stand in dark at room temperature for 30 min. Control was assayed with the same procedure, except methanol was used instead of the sample **[**25]. The absorbance was read at 517 nm in a spectrophotometer (Jasco, serial No. C317961148, Japan). DPPH radical scavenging activity was calculated according to the following equation:

DPPH• scavenging effect (%)=[(A_0-A_1)/A_0) ×100].

Where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the sample of Essential oil**.**

2.4.1.2. *ABTS radical cation scavenging activity*

The principle of the assay was based on the previously described method **[**26] and its modification [27]. ABTS radical cation scavenging activity of the essential oils at different concentration was estimated, and was compared with two standard

materials; BHT and Ascorbic Acid at the same concentrations. Reaction was prepared by adding 0.2 ml of peroxidase (4.4 units/ ml), 0.2 ml of H_2O_2 (50 µM), and 0.2 ml of ABTS (100 µM) and 1ml methanol mixed well and kept in the dark for 1 hour to form a bluish green complex. After dark incubation period, 1ml of essential oils, BHT and Ascorbic Acid at different concentrations were added. Control was prepared by the same procedure without samples. The absorbance at 734 nm was measured to represent the ABTS radical cation scavenging activity and then was calculated as follows:

ABTS radical cation scavenging activity (%) = [1-(A_{sample} /$A_{control}$)] x 100.

2.4.1.3. ***Nitric Oxide radical scavenging activity***

Using sodium nitroprusside (SNP) the **NO•** radical scavenging activity of tested material was determined and was compared with two standard materials; BHT and Ascorbic Acid. Aqueous solution of SNP generate NO• free radical at physiological pH 7.4 which interact with oxygen to produce the nitrite ion (NO), the later measured by the Greiss reagent [28]. Greiss reagent constitutes 1% sulfanilamide in 5% ortho-H_3PO_4 and 0.1% naphthylethylene diamine dihydrochloride. The reaction mixture consists of 2ml of essential oils and standard compounds at different concentrations and SNP (10 mM) in phosphate buffered saline pH 7.4. All the constituents were incubated at 25ºC for 150 min. After incubation, 1ml samples of reaction mixtures were removed and were diluted with 1 ml Greiss reagent. The absorbance of these solutions was measured at 540 nm against the corresponding blank solution. All the previous free radical scavenging methods were compared with two standard BHT and Ascorbic Acid.

2.4.*1*.4. *Superoxide anion scavenging activity (SOR)*

Measurement of superoxide anion (O_2^-) scavenging activity of essential oils was based on the method described by [29] with slight modifications [30]. **O_2^-** was generated in 3ml of Tris-HCl buffer (16 mM, pH 8.0) containing 1ml of NBT (50 µM) solution, 1 ml NADH (78 µM) solution and 1 ml from extracts and polysaccharide or standard materials solution at different concentrations. The reaction was started by adding 1 ml of PMS solution (10 µM) to the mixture. The reaction mixture was incubated at 25ºC for 5 min, and was read at 560 nm in a spectrophotometer. Control was prepared with the same procedure without sample. **O_2^-** scavenging was calculated using the following formula:

The O_2^- scavenging % = [(A_0-A_1)/A_0] × 100.

Where A_0 was the absorbance of the control, and A_1 was the absorbance of polysaccharide or standards samples.

2.4.1.5. ***Lipid Peroxidation (LP) Ammonium Thiocyanate assay***

The ability of essential oil to inhibit lipid peroxidation was determined according to the reported method [31] with same modifications, and was compared with BHT and Ascorbic Acid to evaluate it as standard. A pre-emulsion was prepared by mixing 175 µg Tween 20, 155 µL linoleic acid, and 0.04M potassium phosphate buffer (pH 7.0). 1 mL of oil sample in 99.5% ethanol was mixed with 4.1 mL linoleic emulsion, 0.02 M phosphate buffer (pH 7.8) and distilled water (pH=7.9).The mixed solutions of all samples (21 mL) were incubated in screw cap-tubes under dark conditions at 40˚C at certain time intervals. 0.1 mL of this mixture was pipetted and added with 9.7 mL of 75% ethanol and 0.1 mL of 30% ammonium thiocyanate sequentially. After 3 min, 0.1 mL of 0.02 M ferrous chloride in 3.5% HCl was added to the reaction mixture. The peroxide level was determined by daily reading of the absorbance at 500 nm in a spectrophotometer. The inhibition of lipid peroxidation in percentage was calculated by the following equation:

Lipid Peroxidation Inhibition (%) = [(A_0 - A_1) / A_0] x 100.

Where A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of essential oil or standard compounds.

2. 4.2. Cytotoxic evaluation

2.4.2.1 .*Cell culture*

Cytotoxic effect of the volatile oils of both samples as well as doxorubicin (reference drug) were accomplished on the following human cancer cell lines: hepatocellular carcinoma cell line, HePG-2; colon cell line, HCT-116; lung carcinoma cell line, A549; breast cancer cell, MCF-7; prostate cell line and PC3. Culture was maintained in DMEM medium (in case of A549 and PC3), RPMI medium (in case of HCT-116, HepG2 and MCF-7), DMEM F12 medium (in case of Rpel-1) and supplemented with 10% fetalbovine serum at 37 ºC in 5 %CO_2 and 95% humidity, cells were sub-cultured using trypsin versene 0.15 %.

All the following procedures were done in a sterile area using a Laminar flow cabinet biosafety class II level (Baker, SG403INT, Sanford, ME, USA). Cells were suspended in DMEM medium HCT116, 1% antibiotic-antimycotic mixture (10,000U/ml Potassium Penicillin, 10,000µg/ml Streptomycin Sulfate and 25µg/ml Amphotericin B) and 1% L-glutamine at 37ºC under 5% CO_2. Cells were batch cultured for 10 days, then seeded at concentration of $10x10^3$ cells/well in fresh complete growth medium in 96-well microtiter plastic plates at 37ºC for 24 h under 5% CO_2 using a water jacketed

Carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA). Media was aspirated, fresh medium (without serum) was added and cells were incubated either alone (negative control) or with different concentrations of sample (extract) to give a final concentration of (100-50-25-12.5-6.25-3.125-1.56 and 0.78 µg/ml).

2.4.2.2. *Cell viability assay*

Cell viability was assessed by the mitochondrial dependent reduction of yellow MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) to purple formazan **[**32] After 48 h of incubation, medium was aspirated, 40ul MTT salt (2.5µg/ml) were added to each well and incubated for further four hours at 37ºC under 5% CO_2. To stop the reaction and dissolving the formed crystals, 200µL of 10% Sodium dodecyl sulphate (SDS) in deionized water was added to each well and incubated overnight at 37ºC [33]. The absorbance was then measured using a microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) at 595nm and a reference wavelength of 620nm. A statistical significance was tested between samples and negative control (cells with vehicle) using independent t-test by SPSS 11 program. DMSO is the vehicle used for dissolution of plant extracts and its final concentration on the cells was less than 0.2%. A probit analysis was carried for IC_{50} and IC_{90} determination using SPSS 11 program. The percentage of change in viability was calculated according to the formula:

(Reading of extract / Reading of negative control) -1) x 100

After 24 h of seeding 20000 cells per well in case of A-549, HCT-116 and PC3, 10000 cells per well in case of HepG2, RPel-1 and MCF-7 cell lines (in 96 well plates), the medium was changed to serum-free medium containing a final concentration of the extracts of 100 µg/ml in triplicates. The cells were treated for 48 h. 100 µg/ml. doxorubicin was used as positive control and 0.5 % DMSO was used as negative control. Cell viability was determined using the MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay as described **[**32]**.** percentage cytotoxicity was calculated according to the following equation

= (1- (av(x) / (av(NC)* 100

Where Av: average, X: absorbance of sample well measured at 595 nm with reference 690 nm, NC: Absorbance of negative control measured at 595 nm with reference 690.

2.4.2.3. *Determination of IC_{50} values*

In case of highly active extracts possessing ≥ 75 % cytotoxicity on different cancer cell lines and human normal cell line, different concentrations were prepared for dose response studies. The results were used tocalculate the IC_{50} values of each extract using probit analysis and utilizing the SPSS computer program (SPSS for windows, statistical analysis software package / version 9 / 1989 SPSS Inc., Chicago, USA).

2.4.2.4.*Selectivity Index* (SI)

The selectivity index (SI) indicates the cytotoxic selectivity (i.e. safety) of the extract against cancer cells versus normal cells (RPel-1)

SI= IC_{50} of plant extract in a normal cell line/ IC_{50} of the same plant extract in cancer cell line.

2.4.3. Determination of α-amylase inhibitory activity as a carbohydrate hydrolyzing enzyme

α-Amylase (EC 3.2.1.1**)** catalyzes the hydrolysis of α-1,4 glycosidic linkages in starch and other related carbohydrates. In particular, α-amylase participates in glucose digestion and is considered as a key enzyme that can control postprandial hyperglycemia. The principle of the assay depends on the fact that reducing sugars have the property to reduce many reagents; chemically they form an aldehyde or ketone in basic solution, and the aldehyde group of glucose converts DNS to its reduced form (3-amino-5-nitrosalicylic acid); the formation of the latter results in a change in the amount of light absorbed at a wavelength of 540nm. By using this method, the percentage of α-amylase inhibitory activity values of each essential oil was calculated as follow:

α-Amylase activity (% inhibition) = (Abs-Control – Abs-sample) / Abs-Control × 100)

A volume of 0.3ml of enzyme solution (3U) was premixed with extracts at various concentrations (125, 250, 500, 1000 µg/ml); thereafter, 0.5ml of starch was added to start the reaction. The reaction mixture was carried out at 37°C for 5min and terminated by the addition of 2ml of DNS reagent. The mixture was then heated for 15min at 100°C and diluted with 10ml of bidistilled water in an ice bath. Theα-amylase activity was determined by measuring the absorbance at 540 nm [**3**4].

2.5. Chemical composition of the Essential Oils

2.5. 1. *Condition of GC-MS analysis*

GC-Ms analysis of the essential oil samples was carried out using gas chromatography-mass spectrometry instrument stands at the Department of Medicinal and Aromatic Plants Research, National Research Center with the following specifications. Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a THERMO mass spectrometer detector (ISQ Single

Quadrupole Mass Spectrometer). The GC-MS system was equipped with a TG-WAX MS column (30 m x 0.25 mm i.d., 0.25 μm film thickness). Helium as carrier gas at a flow rate of 1.0 ml/min and a split ratio of 1:10; temperature program: 60 ºC for 1 min; rising at 3.0 ºC /min to 240 ºC and held for 1 min. The injector and detector temperature were held at 210 ºC. Diluted samples (1:50 hexane, v/v) of 0.2 μL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. Most of the separated compounds were identified using the analytical method mass spectra authentic chemicals, Wiley spectral library collection and NSIT library and or published data [35].

2.5.2. *Statistical analysis*

Statistical analyses were carried using SPSS 16. Data were expressed as mean ± SD. Variables were compared using one-way ANOVA; post hoc Duncan's test and the significance of differences among means were determined at $p \leq 0.05$.

3. Results

3.1.***Free radical scavenging characters***

The results of antioxidant screening of the EO by five different methods (DPPH, ABTS, NO, SOR, & LP) revealed that there is an increase inthe % of scavenging activity as the conc. of the sample increased this mean it is a dose dependent. It was noticed that at all conc. used of EO of the two plant species they exerted their antioxidant activities which were more than the used standard at lower conc 125, 250 µg/mL while it has nearly the same or close activity to the standard at 1000µg/mL

3.1.1.DPPH

The DPPH scavenging activity for MB &MW at 1000µg/mL were 95.26±0.074; 92.80±0.097compared with Vit C; BHT 98±0.182 , 96.44±0.193 respectively, while at lower 750µg/mL, 500µg/mL, both MB & MW have nearly close activity to the standard; at 250, 125 µg/mL, the scavenging activity of both MB &MW were more than the used standard (Table:1a;) The $IC_{50}$of the EO of MB & MW were measured and were found to be 187±1.0 & 313±1.1 commpared with Vit.C & BHT 402±1.5, 248±2.1 respectively.

3.1.2. ABTS

The scavenging activity for MB &MW at 1000µg/mLwere 94.30±0.20; 91.26±0.25 compared with Vit C,BHT 98.03±0.31; 96.44±0.33 respectively, while at lower conc750µg/mL, 500µg/mL, they have close activity to the standard ; at 125µg/mL the scavenging activity of both MB &MW were more than the used standard (Table: 1b;) The IC_{50} of the EOs of MB &MW were 31.7±1.3 & 138±2.0 µg/ml compared with Vit.C & BHT 391±0.6, 105±0.5 respectively.

3.1.3. NO

The EO of Myrtus volatile oils of MB & MW inhibited the NO• liberation from SNP through its effect as nitric oxide radical scavenger. The scavenging activity for MB & MW at 1000µg/mL compared with Vit.C & BHT were 94.21±0.018, 91.78±0.014, 93.27±2.73, 95.88±1.89 respectively; while at lower conc 750-------250 µg/mL both MB & MW oils have close activity to the standard (Table 1c).The $IC_{50}$of the EO of MB & MW were measured and was found to be 112.36±0.8 &116.45±0.65 compared with Vit.C & BHT 31.98± 2.3, 39.67±1.6 respectively.

3.1.4. SOR

The EO of MB & MW inhibited the generation of O_2^- radical in PMS-NADH-NBT system compared with two standard compounds Vit.C & BHT. The scavenging activity of the EOs at 1000µg/mL for MB & MW were 96±0.023; 91.81±0.024 compared with Vit. C& BHT 100.00±0.00, 100.00±0.00 , respectively. The scavenging activity of the EO at all used conc 750------125 µg/mL were less than the used standard (Table:1d) The IC_{50} of the EOs of MB & MW were found to be 142.56±0.49 & 208.67±1.2 commpared with Vit.C & BHT 91.00±0.78, 96.35±0.53 respectively.

3.1.5. Lipid Peroxidation(LP)

Inhibition of lipid peroxidation in ammonium thiocyanate system of the EO of MB & MW and Vit.C & BHT as standard. The scavenging activity of the EOs for MB &MW at 1000µg/mL were 95.36±0.138 and 91.65±0.032 compared with and Vit. C & BHT 96.57±0.593 and 96.34±0.055 respectively, the LP scavenging activity of MB is more active than MW at lower conc 750-------125 µg/mL (Table:1e).The $IC_{50}$of the EOs of MB, MW , Vit.C & BHT were measured and were found to be 108.59±0.9& 245.±2.0, 183.97±5.0, 97.±0.59 respectively . From Table 1 (a, b, c ,d & e) which showed screening of the antioxidant activity of the EOs of the two species of MC it was concluded that the scavenging activity of MB at all used conc 125-------1000 µg/mL was more potent than MW.

3.2. Cytotoxic activity

Screening of the cytotoxic activities of the EO of MB and MW revealedthat theyhave good activity against PC3 & MCF-7 with IC_{50} of 4.7±0.15, 14.2±0.35; 45.2±0.67 and 50.5±0.5 respectively. Compared with Dox. standard 24±0.26 and 26.1±0.4. The essential oils of MB &MW have activity against A549; HepG-2 & HCT-116 cell line; but more potent and selective to PC3 and MCF7 Table 2, 3

Both MB & MW oil has no cytotoxic activity on Rpel-1 (normal cell line) indicating its safety with IC_{50} 54.7±0.18 and 70.9±0.46 respectively.

The selectivity index showed the toxicity or safety of the used extract as cytotoxic. It was found that for *in vitro* studies, SI value less than 1 is classified as non-selective (toxic), from 1 to 10 is

weakly selective and SI above 10 is considered safe (non-toxic) [36,37]. This indicate that both oil sample MB & MW were weakly selective as cytotoxic but they were still away from the margin to be toxic to normal cell.

Table 1. DPPH, ABTS, NO, SOR and Lipid peroxidation scavenging activity of the EOs of MB, MW compared with two reference standard Vit.C & BHT

Concentration	Vit.C	BHT	MB	MW
a.DPPH				
125µg/mL	20±0.257^{e}	32.26±0.164^{e}	40.54±0.071^{e}	35.12±0.054^{e}
250µg/mL	36±0.116^{d}	59.55±0.165^{d}	61.49±0.096^{d}	49.42±0.039^{d}
500µg/mL	61±0.110^{c}	69.46±0.264^{c}	72.34±0.088^{c}	60.30±0.029^{c}
750µg/mLl	80±0.217^{b}	88.30±0.221^{b}	86.37±0.086^{b}	77.71±0.49^{b}
1000µg/mL	98±0.182^{a}	96.44±0.193^{a}	95.26±0.074^{a}	92.80±0.097^{a}
		b. ABTS		
125µg/mL	19.53±0.44^{e}	45.26±0.90^{e}	54.62±0.040^{e}	46.44±0.22^{e}
250µg/mL	39.59±0.41^{d}	69.55±0.63^{d}	67.32±0.30^{d}	60.26±0.15^{d}
500µg/mL	61.57±0.66^{c}	77.46±0.98^{c}	73.54±0.09^{c}	71.60±0.27^{c}
750µg/mL	80.61±0.65^{b}	88.30±0.38^{b}	87.70±0.37^{b}	79.64±0.23^{b}
1000µg/mL	98.03±0.31^{a}	96.44±0.33^{a}	94.30±0.20^{a}	91.26±0.25^{a}
125µg/mL	19.53±0.44^{e}	45.26±0.90^{e}	54.62±0.040^{e}	46.44±0.22^{e}
		c. NO		
125µg/mL	64.39±1.61^{e}	65.99±2.00^{e}	53.89±0.008^{e}	50.18±0.008^{e}
250µg/mL	78.68±1.70^{d}	77.92±2.00^{d}	70.20±0.017^{d}	68.10±0.049^{d}
500µg/mL	86.15±1.95^{c}	88.72±2.00^{c}	79.51±0.02^{c}	77.74±0.017^{c}
750µg/mL	90.25±2.00^{b}	91.75±1.55^{b}	90.36±0.012^{b}	85.40±0.035^{b}
1000µg/mL	93.27±2.73^{a}	95.88±1.89^{a}	94.21±0.018^{a}	91.78±0.014^{a}
		d. SOR inhibition		
125µg/mL	64.73±2.20^{e}	60.31±1.69^{e}	51±0.030^{e}	46.51±0.034^{e}
250µg/mL	74.58±3.00^{d}	76.41±0.59^{d}	78±0.028^{d}	68.70±0.034^{d}
500µg/mL	88.74±3.20^{c}	87.51±1.60^{c}	81±0.020^{c}	74.90±0.017^{c}
750µg/mL	94.37±2.75^{b}	93.75±1.75^{b}	86±0.063^{b}	80.43±0.043^{b}
1000µg/mL	100.00±0.00^{a}	100.00±0.00^{a}	96±0.023^{a}	91.81±0.024^{a}
		e. Lipid peroxidation		
125µg/mL	49.51±0.0.756^{e}	56.42±0.029^{e}	53.34±.121^{e}	35.35±0.070^{e}
250µg/mL	67.95±0.0.590^{d}	69.78±0.032^{d}	68.80±.206^{d}	47.37±0.033^{d}
500µg/mL	78.98±0.540^{c}	77.92±0.028^{c}	85.38±.193^{c}	75.68±0.062^{c}
750µg/mL	87.28±0.014^{b}	88.57±0.105^{b}	90.25±.148^{b}	83.37±0.061^{b}
1000µg/mL	96.57±0.593^{a}	96.34±0.055^{a}	95.36±.138^{a}	91.65±0.032^{a}

Data presented as mean ± SD. ANOVA one-way was used for data analysis (n=3, $p<0.05$). Different lower case letters within the same column designates significant differences.

The Selectivity Index = $\frac{IC_{50}\text{ Normal cell line}}{IC_{50}\text{ of cancer cell line}}$

MB :SI
PC3 = 54.9/4.7= 9. 8
MCF-7 = 54.9/ 50.5=1.09

MW: SI
PC3 = 54.9/14.2=3.9
MCF-7 =54.9/45.2=1.2

Table2: Death percentage of different cancer cell lines resulted from application of the EO of MB

Conc. Ppm	Cancer cell line					Normal cell line
	A549	PC3	MCF-7	HepG-2	HCT-116	Rpel
0.78	0.00±0.00^{h}	15.65±0.051^{h}	1.01±0.010^{h}	0.35±0.26^{h}	0.00±0.00^{h}	0.95±0.043^{h}
1.65	0.123±0.025^{g}	22.45±0.10^{g}	1.83±0.118^{g}	0.81±0.030^{g}	0.00±0.00^{g}	1.03±0.025^{g}
3.125	0.343±0.030^{e}	37.43±0.300^{f}	2.01±0.020^{f}	1.28±0.015^{f}	0.72±0.21^{f}	1.11±0.30^{f}
6.25	0.520±0.01^{e}	88.29±0.100^{e}	2.25±0.05^{e}	1.42±0.020^{e}	0.96±0.015^{d}	1.25±0.20^{e}
12.5	0.670±0.021^{d}	88.52±0.66^{d}	19.25±0.025^{d}	1.52±0.208^{d}	1.32±0.20^{c}	1.29±0.010^{d}
25	0.867±0.02^{c}0	88.75±0.051^{c}	70.10±0.256^{c}	1.77±0.021^{c}	2.12±0.025^{c}	30.8±0.04^{c}
50	5.26±0.049^{b}	92.25±0.12^{b}	71.51±0.118^{b}	9.07±0.049^{b}	8.64±0.032^{b}	77.31±0.185^{b}
100	44.2±0.300^{a}	98.82±0.031^{a}	72.78±0.252^{a}	70.46±0.211^{a}	63.12±0.123^{a}	78.80±0.04^{a}

Data presented as mean ± SD. ANOVA one-way was used for data analysis (n=3, *p<0.05*). Different lower case letters within the same column designate significant differences.

Table 3: % of Death of different cancer cell line on using the EO of MW

Conc. ppm	Cancer cell line					Normal cell line
	A549	PC3	MCF-7	HepG-2	HCT-116	Rpel
3.125µg/ml	0.00±0.00^{c}	0.00±0.00^{f}	0.00±0.00^{f}	0.00±0.00^{e}	0.00±0.00^{c}	0.00±0.00^{d}
6.25	0.00±0.00^{c}	10.76±0.321^{e}	6.45±0.18^{e}	0.00±0.00^{e}	0.00±0.00^{c}	0.00±0.00^{d}
12.5	0.00±0.00^{c}	28.50±0.300^{d}	15.66±0.305^{d}	1.50±0.361^{d}	0.00±0.00^{c}	0.00±0.00^{d}
25	0.00±0.00^{c}	75.60±0.300^{c}	61.53±0.251^{c}	3.50±0.300^{c}	0.00±0.00^{c}	19.60±0.360^{c}
50	9.63±0.305^{b}	87.60±0.400^{b}	71.63±0.404^{b}	8.66±0.416^{b}	6.36±0.416^{b}	49.10±0.793^{b}
100	77.4±0.360^{a}	92.53±0.30^{a}	84.33±0.351^{a}	78.66±0.342^{a}	46.62±0.311^{a}	67.66±0.351^{a}

Data presented as mean ± SD. ANOVA one-way was used for data analysis (n=3, *p<0.05*). Different lower case letters within the same column designate significant differences.

3.3. *In vitro* anti-diabetic activity (α-amylase inhibitor)

Screening of the reducing ability of MBoil on α-amylase carbohydrate hydrolysing enzyme activity as compared with acarbose (as standard inhibitor reference) revealed that the inhibition of α-amylase appears to be dose dependent. In case of MB:the highest inhibiting activity was observed at 1000, 750& 125µg/mL 96.22±.140; 83.20±162 & 36.24± 146 compared with acarbose standard 88.81± 0.69; 78.95±0.917 & 23.16±0.190, respectively; at conc. 250----500 µg/mL the reducing activity of the oil was very close to the standard. As well as in case of MW*:* the highest inhibiting activity was observed at 1000 &125µg/mL; 89.48±.106, 32.14±.188 compared with acarbose standard 88.81±0.690 and 32.14±.188 respectively. both MB &MW have activity more than the standard at 125µg/ml 36.24±146; 32.14±188 and 23.16±0.190. The activity of the two species on α-amylase inhibitor can be summarized in Table 4. The IC_{50} for MB & MW EOs were measured and was found to be 249.5± 1.6 & 358±2.4 compared with Acarbose 222.5± 3.0, respectively.

Table 4. α- Amylase inhibition activity of the EOs of MB and MW compared with Acarbose as standard

Concentration	Acarbose	MB	MW
125µg/mL	23.16±0.190^{e}	36.24±.146^{e}	32.14±.188^{e}
250µg/mL	57.399±0.611^{d}	51.40±.218^{d}	47.57±.538^{d}
500µg/mL	69.58±0.511^{c}	68.62±.297^{c}	59.11±.455^{c}
750µg/mL	78.95±0.917^{b}	83.20±.162^{b}	69.64±.396^{b}
1000µg/mL	88.81±0.690^{a}	96.22±.140^{a}	89.48±.106^{a}

Data presented as mean ± SD. ANOVA one-way was used for data analysis (n=3, *p<0.05*). Different lower case letters within the same column designate significant differences.

3.4. GC/MS analysis

The main compounds of the essential oil extracted from the aerial parts of the two varieties of myrtle (*Myrtus communis* L.) as analyzed by GC/MS are shown in Table (5) Fig.(1). GC/MS analysis of the essential oil of two varieties (MB, MW**)** resulted in the identification of 25 compounds from both species constituting 99.21% & 99.96% of the total peak area of the identified compound, respectively. The major compounds were identified as α –pinene 49.33%, 31.10 %; myrtenyl acetate 30.7%, 40.80 %; 1,8 cineole 9.97 %, 19.21 %; α –terpineol 2.18%, 1.73% and D-limonene 1.90%, 2.61% for MB & MW**,** respectively. The two species differ in the presence or absence of minor compounds i.e in case of MB, tran-pinocarveol 0.07% is detected in the GC/MS of MB only. On the other hand α –myrcene 0.04%, α–phellandrene 0.04% & α –terpinene 0.06% are detected in the GC/MS of MW only. The oxygenated compounds constituted (45.59 and 44.49). The nonoxygenated compounds constituted (52.4 and 36.86) for MB & MW respectively

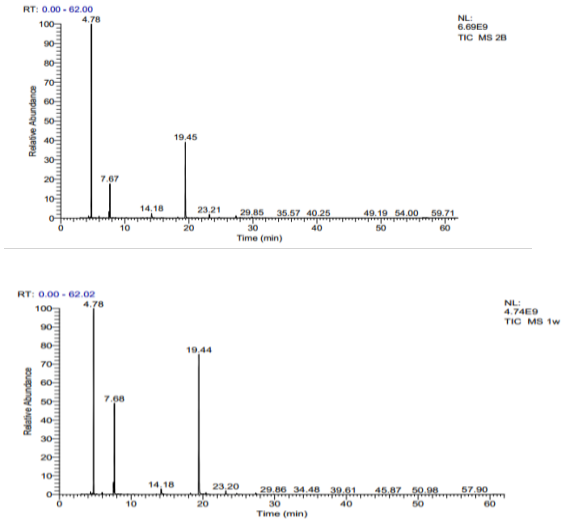

Fig. 1. Total ion chromatogram of the volatile oil of the aerial parts of MB (A) and MW (B)

4. Discussion

Free radicals are active chemical species involved in biological processes whose high existence can give rise to several diseases (cancer, cardiovascular disease, etc.) [38]. In healthy biological system, it is essential to predict the balance between antioxidants and free radicals [39]. Many people especially in their middle age suffer from the previous diseases, consumption of plants with high contents of antioxidant compounds will diminish the negative effects of free radicals as a result of their electronic and molecular structures **[**40].

Antioxidant play a significant role in protecting the vital cells in the human body against damage of the exposure to reactive oxygen species (ROS). It is well known that ROS have the potential to cause dangerous health problems, including cancer [41,42], supply the body with antioxidants can eliminate ROS quickly than body chemicals can though pairing with them. Medicinal plants are potential sources of natural compounds with biological activities and therefore attract the attention of researchers worldwide. There are different mechanisms involved in the antioxidant process which include inhibition of chain initiation, complexting with transition metal ion that act as catalyst in lipid peroxidation, decomposition of peroxides radicals , and radical scavenging [43].

Essential oils (EOs) are complex, volatile, and odorous mixture with monoterpenes, sesquiterpenes, and several aromatic compounds as their main components**,** the antioxidant activity of essential oils is considered to be an important aspect in food and pharmaceutical research, as synthetic food additives are nowadays replaced with plant-based natural ingredients, due to their safety, effectiveness and consumer acceptance **[**44**]**. The EO of the two studied species have strong antioxidant activities proved by five different mechanisms, so it has the ability to neutralize ROS, so prevent the damage of protein, lipid and DNA which are supposed to be the main reason for cell aging, oxidative stress-originated diseases (cardiovascular and neurodegenerative diseases) and cancer. Antioxidants have been extensively studied for their ability to prevent or treat cancer in humans [45].

Additionally there was a reported data about the regular intake of natural antioxidants which was associated with reduced risks of cancer [46**].** The EO of the two Myrtle species are rich in different constituents mainly of monoterpene or sesquterpene or other constituents which were previously reported for their activity as antioxidant or cytotoxic; *α* - Pinene one of the major constituents in the essential oil of MB & MW , is a monoterpene compound, present naturally in the essential oils of many plants which was reported for different biological activities as antioxidant, anticancer, anti-inflammatory [47-50], antibacterial and anti-nociceptive activities **[**51, 52]. Both *α*-pinene and *β*-pinene were reported to have antioxidant properties through the use of different assay methods [53-55].

Table .5: Results of GC/MS analysis of the EO of the aerial parts of two varieties of *Myrtus communis L.*(myrtle) MB and MW) as studied with GC/MS

No.	Compounds	R_t	MB	MW	BP	M^+	Area (%)	
							MB	MW
1	Propanoic acid, 2-methyl-2-butyl ester	4.33	+	+	71	144	0.54	0.54
2	α-Thujene	4.58	+	+	93	136	0.21	0.16
3	α –Pinene	4.78	+	+	93	136	49.33	31.10
4	Camphene	5.22	+	+	93	136	0.09	0.06
5	β –Pinene	5.97	+	+	93	136	0.50	0.35
6	α–Myrcene	6.26	-	+	--	----	------	0.04
7	Butanoic acid, 2 methyl-2-methylpropyl ester	6.66	+	+	57	158	0.09	0.05
8	α–Phellandrene	6.82	-	+	93	136	--	0.04
9	α–Terpinene	7.14	-	+	93	136	---	0.06
10	D-Limonene	7.54	+	+	68	136	1.90	2.61
11	1,8 Cineole	7.67	+	+	43	154	9.97	19.21
12	β –Ocimene	8.14	+	+	93	136	0.17	0.15
13	γ-Terpinene	8.56	+	+	93	136	0.09	0.11
14	à-Terpinolene	9.53	+	+	93	136	0.11	0.09
15	Linalool	10.22	+	+	71	154	0.19	0.13
16	Fenchyl alcohol	11.01	+	+	81	154	0.05	0.03
17	Tran-Pinocarveol	11.88	+	-	92	152	0.07	------
18	Borneol	13.19	+		95	154	0.06	0.04
19	4-Terpineol	13.51	+	+	71	154	0.12	0.10
20	α -Terpineol	14.18	+	+	59	154	2.18	1.73
21	Myrtenyl acetate	19.45	+	+	91	194	30.35	40.48
22	α -Terpinenyl acetate	20.44	+	+	121	196	0.15	0.56
23	Caryophyllene trans	23.20	+	+	93	204	1.44	1.00
24	Humulene	24.72	+	+	93	204	0.27	0.28
26	Caryophyllene oxide	29.85	+	+	79	220	0.43	0.1

Total identified	98.31	99.02
Total non identified	0.71	0.52
Non oxygenated compounds	54.82	36.57
Oxygenated compounds	45.18	63.43
Monoterpenids	96.17	97.64
Sesquiterpenes	2.14	1.38

R_t: Retention time, B.P.: Base peak ,M^+: Molecular weight,

Different Myrtaceae plants containing 1,8-cineole, linalool and *α*-terpineol were reported for *In vitro* cytotoxic activities against liver and colon carcinoma with IC_{50} values of 0.36- 0.69 µg [56]. Terpinen-4-ol was found to induce *in vitro* cytotoxic inhibition of colorectal cells growth in a dose-dependent manner [57]. Myrcene showed strong inhibitory activities against HCT-116 and Hep-G2 carcinoma withIC_{50}=1.27 and 0.93 µg, respectively) when evaluated by MTT assay [31] finding support our results where both EO sample of MB&MW varieties exhibited strong antioxidant activities through the use of five different assay methods (DPPH, ABTS, NO, SOR& LP). On the other hand the cytotoxic activity for both varieties was observed only for PC3&MCF-7 cell line. Based on the protocol of the American National Cancer Institute (NCI) which recommends that crude extracts of plant origin should be considered significant for IC_{50} values ≤ 30 µg/ml as well as IC_{50} values ≤ 4 µg/ml for pure substance**s [**58]. This evaluation is also in accordance with reported data which classified the IC_{50} values as follows: IC_{50} ≤ 20 µg/ml = highly active, IC_{50} 21-200 µg/ml =moderately active, IC_{50} 201-500 µg/ml = weakly active andIC_{50}> 501 µg/ml is inactive **[**59]. So the EO of the MB & MW varieties considered to

be highly active in case of PC3 cell line and moderately active in case of MCF-7 cell line.

Diabetes, is a metabolic disease that causes elevation of blood sugar; normally the insulin moves sugar from the blood into your cells to be stored in liver as glycogen used for energy but in case of diabetes there is not enough insulin due to β-cell destruction or the body can't use the produced insulin to do its action ; many complication were associated with diabetes including keto acidosis and nonketotic hyperosmolar coma. Serious long-term complications include heart disease, stroke, kidney failure, foot ulcersand damage to the eyes [60].

There is a strong relation between antioxidants and diabetes; antioxidant defends and preserves the function the β-cell against oxidative stress. It was reported that there is a strong association between the dietary antioxidants intake and protection against diabetes as the diet-derived antioxidants are important in the prevention and management of various diseases[61].In our study the *in vitro* activity of the essential oils as α-amylase inhibitor was investigated. This enzyme is carbohydrate-hydrolyzing enzymes, it is commonly used to modulate postprandial hyperglycemia. The mechanism of action of acarbose as synthetic α-amylase inhibitor enzymes take place through inhibition of enzymatic breakdown of complex carbohydrates so the absorption of glucose was delayed into the bloodstream and reduce postprandial hyperglycemia [62, 63]. However their side effects and failure to alter the course of diabetic complication limited their use. Additionally the inhibition of α - amylase enzymes may be related to the nature of compounds detected in the EOs of two varieties of MB & MW species ; terpenoides; sesquterpenes and others ;it was reported earlier that presence of phenolic monotepernes and sesquiterpenes, limonene, α-pinene and β-pinene have high inhibitory effects against α-amylase and α-glucosidase activities[61, 62], this explain the inhibitory action of the EOs of the two varieties of the plants as α - amylase inhibitor. This is the first report to study the effect of EOs of MC as α-amylase inhibitor. There are many EOs isolated from different plants with terpenoid compounds as main constituents reported for various pharmacological activities; the EOs of *Eucalyptus camaldulensis* Dehnh proved scavenging of reactive oxygen species and inhibitor to α-amylase and α – glucosidase.[63]**;** *Mentha piperita* EOs collected in Guatemala showed significant cytotoxic activity against Caucasian gastric adenocarcinoma (AGS), Human malignant melanoma (A375), and epidermal cell line (A431) cell lines with IC_{50} 0.35, 0.40, and 0.23 µL/mL, respectively[64]; *Osmarinus officinalis* L. EO showed potent cytotoxic activity against AGS, A375, and A431 cell lines with IC_{50} of 0.21, 0.24, and 0.41 µL/mL, respectively, it also prove, antioxidant, antibacterial, , antimutagenic activities [64]; The EO obtained from the leaves of *Salvia. officinalis* L proved anticancer activity against melanoma cell line (M14), A375, and human caucasian metastatic melanoma (A2058) with IC_{50} values of 8.2, 12.1, and 11.7 µg/mL, respectively [65]; The EOs of two species of *Teucrium (T. pseudochamaepitys* and *T. flavum*) which demonstrates significant antioxidant activity and moderate cytotoxic effects on the HEP-2 cell line than that of *T. flavum*. [66]. Furthermore; several EOs obtained from plants belonging to family laminaceae with thymol, carvacrol, 1,8-cineole, and limonene as major chemical constituents demonstrated cytotoxic activity against different cancer cell lines [67].

Conclusion

The essential oil (EO) isolated from the aerial parts of the two varitiesof *Myrtus communis* L (MB & MW) exhibited antioxidant, cytotoxic and α-amylase inhibiting activities . Myrtenyl acetate and α -pinene were identified as the major compounds in both varities. The EO of both myrtle species have cytotoxic activity against PC3 & MCF-7 and have moderate cytotoxic effect against other tested cell line (A549, HepG-2 and HCT-116) it also showed no toxic effect on normal cell line (Rpel-1) which indicate its safety. Furthermore the α-amylase inhibiting activity indicated that at all used conc. of both species have inhibitory activity to α-amylase enzyme which prove their possible use as antidiabetic.

Acknowledgment

The authors extend special appreciation to the National Research Centre, Dokki, Giza, Egypt for offering the facilities for this study

References

1.Wilson, P.G. Myrtaceae. In Kubitzki K (Ed.). Families and genera of vascular plants. Springer-Verlag, Berlin, 10 (2011).

2.Chaverri, C. and Ciccio, J. F. Essential oils from *Eucalyptus deglupta* (Myrtaceae) cultivated in Costa Rica. *American Journal of Essential Oils and Natural Products*..6 , 2, 01-06 (2018).

3.Sumbul, S.; Ahmad, M.A.; Asif, M.; Saud, I.and Akhtar, M. Evaluation of *Myrtus communisL*. berries (common myrtle) in experimental ulcer models in rats. *Hum Exp Toxicol*. ,29 ,11, 935–44 (2010).

4.Romeilah, R.M. Asian Journal of Biochemistry Chemical Compositions, Antioxidant, Anticancer Activities and Biological Effects of *Myrtus communis* L. and *Origanum vulgare* Essential Oils. *Asian J. Biochem*. 11, 2, 104-117.(2016).

5.Elfellah, M.S.; Akhter, M.H. and Khan, M.T..Antihyperglycaemic effect of an extract of *Myrtus communis* in Streptozotocin-induced diabetes in mice. *J. Ethnopharmacol*.11, 275-281(1984).

6.Chalchat, J.; Garry, R. and Michet, A. Essential Oils of Myrtle of the Mediterrenean litoral. *J. Essent. Oil Res*., 10, 613-617 (1998).

7.Bradesi, P.; Tomi, F.; Casanova, J. and Costa, J. A. Bernardini, Chemical Composition of Myrtle Leaf Essential Oil from Corsica (France). *J. Essent. Oil Res*.9, 283-288 (1997).

8.Boelens, M.and Jimenez, R. The chemical composition of Spanish Myrtle Oils. Part II. *J. Essent. Oil Res*, 4 349-353(2002).

9.Asllani, U. Chemical Composition of Albanian Myrtle Oil, *J. Essent. Oil Res*. 12, 140-142(2000).

10.Ozek, T.; Demirci, B.and Baser, K. Chemical Composition of Tukish Myrtle Oil. *J. Essent. Oil Res*., 12, 541-544(2000).

11.Gardeli, C.; Papageorgiou, V.; Mallouchos, A.; Theodosis, K.andKomaitis M. Essential oil composition of *Pistacia lentiscus L*. and *Myrtus communis L*.: Evaluation of antioxidant capacity of methanolic extracts, *Food Chem*., 107, 1120-1130(2008).

12.Jerkovic, I.; Radonic, A. and Borcic, I. Comparative Study of Leaf, Fruit and Flower Essential Oil of Croatian*Myrtus communis* During a One Year Vegetative Cycle, *J. Essent. Oil Res*., 14, 266-270(2002).

13. Farah, A.; Afifi, A., Fechtal, M., .Chhen, A, Satrani, B.; Talbi, M.andChaouch,A. Fractional distillation effect on the chemical composition of Moroccan myrtle essential oils. *Flav. Frag. J*.21 , 351-354 (2006).

14. Messaoud, C.; Zaouali, Y.; Salah, A.; Khoudja, M. and Boussaid, M. *Myrtus communis* in Tunisia: variability of the essential oil composition in natural populations, *Flav. Frag. J*. 20, 577-582(2005).

15.Jamoussi, B.; Romdhane, M., Abderraba, A.; Hassine, B. and Gadri, A., Effect of harvest time on the yield and composition of Tunisian myrtle oils, *Flav. Frag. J*. 20, 274-277(2005).

16.Wannes, W.A; Mhamdi, B.; Marzouk,, B. Variations in essential oil and fatty acid composition during*Myrtus communis* var. italica fruit maturation. Food Chem., 11, 621-626 (2009).

17.Tuberoso, C.; Barra, A.; Angioni, A.; Sarritzu, E. and Pirisi F. Chemical composition of volatiles in Sardinian Myrtle *(Myrtus communis L*.) alcoholic extracts and essential oils. *J. Agric. Food Chem*.54, 1420-1426(2006).

18.Tuberoso, C.I.G.;Melis,M.; Angioni,A.; Pala, M. and Cabras,P. Myrtle hydroalcoholic extracts obtained from different selections of *Myrtus communis L*. *Food Chem*., , 101, 806-811(2007).

19.Paula, C. P.; . Cebola, M.J.; Bernardo-Gil, M.G.; Evolution of the Yields and Composition of Essential Oil from Portuguese Myrtle (*Myrtus comunis* L.) through the Vegetative Cycle; *Molecules* ,14, 8, 3094-310 (2009).

20.Yadegarinia,D.; GachkarL.;Rezaee,M. B.;Taghizadeh, M.;Astaneh,S.D.A. andRasooli,I. Biochemical activities of Iranian *Mentha piperita L*.*Phytochemistry*. 67, 12, 1249-55(2006).

21. Bahadırlı, N. P.; , lu, I K. and Wan, C. Exposure to Volatile Essential Oils of Myrtle (*Myrtus communis*L.). Leaves for Improving the Postharvest Storability of Fresh Loquat Fruits. *Journal of Food* Quality., Article ID 8857669 (2020)

22. Hennia, A.; Miguel, M G. and Nemmiche, S. Antioxidant Activity of *Myrtus communis* L. and *Myrtus nivellei* Batt. & Trab. Extracts: A Brief Review. *Medicines*, 5, 89 (2018).

23. Correddu, F.; Maldini, M.; Addis, R.; Petretto, G. L. ; Palomba, M.; Battacone, G.; Pulina, G.; Nudda, A.; , and Pintore, G. *Myrtus communis* Liquor By product as a Source of Bioactive Compounds. *Foods*, 8, 237(2019**)**

24. Egyptian Pharmacopoeia .General Organization for Governmental Printing Office, Ministry of Health, Cairo, Egypt, , 31-33, (1984).

25.Yamaguchi.T.; Takamura, H.; Matoba,T. and Terao, J. HPLC method for evaluation of the free radical-scavenging activity of foods by using 1, 1-diphenyl-2-picrylhydrazyl. *Bioscience, biotechnology, and biochemistry*. 62, 6, 1201-1204 (1998).

26. Miller, M.J.; Sadowska-krowicka, H.; Chotinaruemol, S.; Kakkis, J.L. and Clark, D.A. Amelioration of chronic ileitis by nitric oxide synthase inhibition. *J. Pharmacol Exp Ther*, 264, 264:11–16 (1993).

27. Arnao, M.B. Cano, A. and Acosta, M. The hydrophilic and lipophilic contribution to total antioxidant activity. *Food Chem*, 73, 239–244 (2001).

28. Marcocci, I. J.J.; Marguire, M.T. and Packer, D. L. The nitric oxide scavenging properties *Ginkgo biloba* extract. *Biochem. Bioph. Re.s Comm*, 201, 748–755 (1994).

29. Liu, D.; Diorio, J.; Tannenbaum, B. and Meaney M.J. et al., Maternal Care, Hippocampal Glucocorticoid Receptors, and Hypothalamic-Pituitary-Adrenal Responses to Stress, Science.277, 5332, 1659-1662 (1997).

30.Gülçin**,** I. Antioxidant activity of food constituents: an overview**.** *Arch Toxico*, 86, 3, 345-91(2012).

31. Gülçin¸ I.; Huyut, Z.; Elmastas, M. and Aboul-Enein H.Y. Radical scavenging and antioxidant activity of tannic acid.*Arabian Journal of Chemistry*.3, 43–53(2010).

32.Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods*, 65, 55-63(1983).

33.Thabrew, M. I.; Hughes, R.D. and Mc Farlane, I.G. Screening of hepatoprotective plant components using a HepG2 cell cytotoxicity assay. *J. Pharm Pharmacol*. 49, 1132–1135(1997).

34. El Souda, S.S.M.; Mohammed, R.S.; Ibrahim, M. F.; and Matloub,A A. *Harpullia pendula* Planch leaves: phenolics, in vitro antioxidant and a-amylase inhibitory activity, *Egyptian Pharmaceutical journal*, 16, 2, 103 (2017).

35. Adams, R.P. Identification of essential oil compounds by gas chromatography/mass spectroscopy. Carol Stream, Illinois, USA: Allured Publishing Corporation, 2009.

36. Ondo J.; Lekana-Douki, J.; Bongui, J.; ZangEdou, E.; Zatra, R.; Toure-Ndouo, F.; Elomri, A.; Lebibi, J. and Seguin, E. *In vitro* antiplasmodial activity and cytotoxicity of extracts and fractions of *Vitex madiensis*, medicinal plant of Gabon. *Tropical Med Int Health*. 17, 3, 316–21(2012).

37. Ogbole, O.O.; Ajaiyeoba, E.O.; Adeniji, J.A.; Kamdem, S.R. Sajan, S. and Muhammad, I.C. Bioassay-guided isolation of poliovirus-inhibiting constituents from *Zephyranthes candida*. *Pharm Biol*, 53, 6, 882–7(2015).

38. Pugh, M.B. Stedman's Medical Dictionary *(27th ed.).* Baltimore, *Maryland*, USA: Lippincott Williams & Wilkins, ed. ,65.(2000)

39. Hung, T.M.; Na, M.; Thuong, P.T. Su, N.D.; Sok D. Antioxidant activity of caffeoylquinic acid derivatives from the roots of *Dipsacus asper* Wall. *J. Ethnopharmacol*.,108, 188-192 (2006).

40.Boora, F. Chirisa, E. and Mukanganyama, S. Evaluation of Nitrite Radical Scavenging Properties of Selected Zimbabwean Plant Extracts and Their Phytoconstituents, *Journal of Food Processing*. 7 p, Article ID 918018 (2014).

41.Koldas, S.; Demirtas, I.; Ozen, T.; Demirci, M.A.; Behcet, L.Phytochemical screening, anticancer and antioxidant activities of *Origanum vulgare L.* ssp. viride (Boiss.) Hayek a plant of traditional usage. *J. Sci. Food Agric*.,95 786-798 (2015).

42.Cozzi, R.; Ricardy, R.; Aglitti, T.; Gatta, V.; Petricone, P. and De Salvia, R. Ascorbic acid and β-carotene as modulators of oxidative damage. *Carcinogenesis*,18, 223-228 (1997).

43.Perera, M.G.A.N.; Soysa S.S.S.B.D.P.; Abeytunga, D.T.U. and Ramesh, R. Antioxidant and cytotoxic properties of three traditional decoctions used for the treatment of cancer in Sri Lanka. *Pharmacogn. Mag*. 4, 72-181 (2001).

44.Oktay M.; Gülçin, I. and Küfrevioglu, Ö.I. Determination of in vitro antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. *Lebensm Wiss Technol*, 36, 263–271 (2003).

45. Tuğba, İ. and Medeni, M. The Potential Application of Plant Essential Oils Extracts as Natural Preservatives in Oils during Processing *A Review .J. of Food Sci. and Enginee*..2, 1-9 (2012).

46. Singh, D. K. and Lippman, S. M. Cancer chemoprevention. Part 1: Retinoids and carotenoids and other classic antioxidants, Oncology (Williston Park). (1998).

47 Cai, Y.; Luo, Q.; Sun, M. and Corke, H. Antioxidant activity and phenolic compounds of 112 traditional chinese medicinal plants associated with anticancer. *Life Sci*. 74, 2157–2184 (2004).

48.Bae, G.S.; Park, K.C.; Choi, S.B.; Jo, I.J.; Choi, M.O.; Hong, S.H.;Song, K;. Song, H.J.; Park, S. J. Protective effects of *α*-pinene in mice with cerulein-induced acute pancreatitis. *Life Sci.*, 91, 866–871 (2012).

49. Wang, W.; Wu, N.; Zu, Y.G.; Fu, Y.J.Antioxidative activity of *Rosmarinus officinalis L.* essential oil compared to its main components. Food Chem.108, 1019–1022 (2008).

50.Wang, W.; Li, N.; Luo, M.; Zu, Y.; Efferth T..Antibacterial activity and anticancer activity of *Rosmarinus officinalis L.* essential oil compared to that of its main components. *Molecules*, 17, 2704–2713 (2012).

51. El Hartiti, H.; El Mostaphi.A., Barrahi, M.,; Ben Ali, A.; Chahboun,N. Chemical composition and antibacterial activity of the essential oil of Myrtus communis leaves *Karbala International Journal of Modern Science*. 6 ,251e258. (2020).

52.Him, A.; Ozbek, H.; Turel, I.; Oner, A.C.; Antinociceptive activity of *α*-pinene and fenchone, Pharmacol. 363– 369(2008).

53. Wei, A. and Shibamoto, T. Antioxidant activities and volatile constituents of various essential oils. J. Agric. Food Chem. 55, 1737–1742 (2007).

54.Singh, H.P.; Mittal, S.; Kaur, S.; Batish, D.R.; Kohli, R.K.. Characterization and antioxidant activity of essential oils from fresh and decaying leaves of *Eucalyptus tereticornis*. *J. Agric. Food Chem*. 57, 6962–6966(2009).

55.Ho, C.L. and Su, Y.C. Composition, antioxidant and antimicrobial activities of the leaf essential oil of *Machilus japonica* from Taiwan.*Nat. Prod. Commun*. 7, 109–112 (2012).

56. Aboutabl, E.; Meselhy, K.; Elkhreisy, E.; Nassar, M.; Fawzi, R. Composition and bioactivity of essential oils from leaves and fruits of *Myrtus communis* and *Eugenia supraxillaris* (Myrtaceae) grown in Egypt, *J. Essent. Oil Bear*. 14,192-200, (2011).

57.Hart, P.H.; Brand, C.; Carson, C.F.; Riley, T.V.; Prager, R.H.; Finlay-Jones, J.J. Terpinen-4-ol, the main component of the essential oil of *Melaleuca alternifolia* (tea tree oil), suppresses inflammatory mediator production by activated human monocytes,*Inflammation Research*. , 49,619–626, (2000).

58.Suffness, M. and Pezzuto, J. M.Assays Related to Cancer Drug Discovery. London, Acad. Press.(1990).

59.Srisawat T.; ChumkaewP.,; Heed-Chim W.; Sukpondma Y. K.; Anokwiroon, K.. Phytochemical screening and cytotoxicity of crude extracts of *Vaticadiospyroides*Symington type LS. *Trop, J. Pharm. Res*, , 12 , 71-76 (2013).

60.Rajendirani, D.; Packirisamy, S.; Gunasekaran,K. A Review on role of antioxidant in diabetes. *Asian J Pharm Clin Res*,11 , 2, 48-53(2018).

61. Reshma, M.V.; Namitha, L.K; Sundaresan, A.; Kiran, C.R. Total phenol content, antioxidant activities and α-glucosidase inhibition of sesame cake extracts. *J. Food Biochem*., 22, 1-9(2012).

62.Shinde, J.; Taldone, T.; Barletta, M.; Kunaparaju, N.; Hu, V.; Kumar, S.; Placido, J.; Zito S.W., Alpha-glucosidase inhibitory activity of Syzygiumcumini（Linn.）Skeels seed kernelinvitroand in Goto-Kakizaki（GK） rats, *Carbohydr. Res*., , 343, 1278-128 (2008).

63.Basak, S.F. and Candan, F., Chemical composition and in vitro antioxidant and antidiabetic activities of *Eucalyptus camaldulensis*Dehnh. essential oil, *J. Iran Chem. Soc*., 7, 216-226(2010).

64. Miller, A.B.; Cates, R.G.; O'Neill, K.; Fuentes Soria, J.A.; Espinoza, L.V.; Alegre, B.F., Jose Vicente Martinez, J.V. and Arbizu, D.R..Evaluation of essential oils from 22 Guatemalan medicinal plants for in vitro activity against cancer and established cell lines. *Journal of Medicinal Plants Research*.;12 (3):42-49(2018).

65. Russo, A; Formisano, C.; Rigano, D.; Senatore, F.; Bruno, M. Chemical composition and anticancer activity of essential oils of Mediterranean sage *Salvia officinalis* L. grown in different environmental conditions. *Food and Chemical Toxicology*. 55:42-47(2013).

66. Hammami, S.; Jmii, H.; ElMokni, R.; Khmiri, A.; Faidi, K.; Dhaouadi, H.; El Aouni, M. H.; Aouni, M.; . Joshi, R. K. Essential Oil Composition, Antioxidant, Cytotoxic and Antiviral Activities of *Teucrium pseudochamaepitys* Growing Spontaneously in Tunisia *Molecules*, 20, 20426–20433, (2015).

67. Mesquita, L. S. S. de; Luz, T. R. S. A.; Mesquita, J. W. C. de; Coutinho, D. F.; Amaral, F. M. M. do, Ribeiro, M. N. de S. and Malik, S. Exploring the anticancer properties of essential oils from family Lamiaceae., *Food Review International.*, 35(2):105-131(2019).