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# ANTIOXIDANT, ANTIBACTERIAL AND ANTITUMOR PROPERTIES OF DIFFERENT EXTRACTS OF MELISSA LEAVES AND BARBERRY ROOTS

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**ABSTRACT:** Ethyl acetate and ethanol 80% extracts from *Melissa officinalis* (L.) (leaves) and *Berberis vulgaris* (roots) plants were studied in terms of phenolic compounds, for their antioxidant, antibacterial and antitumor properties. Total phenolics and total flavonoids were measured using Folin-Ciocalteu reagent and aluminium chloride ($AlCl_3$), respectively. The active ingredients of phenolics and flavonoids were identified using high performance liquid chromatography (HPLC). The major phenolic compound was identified by HPLC for melissa ethanolic extract was Rosmarinic acid (40.24 µg $g^{-1}$ extract) and for ethanolic barberry extract was Catechol (40.2 µg $g^{-1}$ extract). Total antioxidant capacity of the extracts was estimated by different methods including DPPH (1,1-diphenyl-2-picrylhydrazyl radical), β-carotene/linoleic bleaching, and ferric reducing antioxidant power (FRAP). Extracts exhibited antioxidant activity that was comparable to the standard antioxidants TBHQ and gallic acid. Antibacterial activity of extracts was determined using pathogen gram-positive and gram-negative bacteria by disc assay method. Antitumor activity of extracts was determined using 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method. The highest activity of extracts for antioxidants, antibacterial and antitumor was Melissa leaves ethanolic extract. Therefore, these extracts could be used as preservative ingredients in the food and/or pharmaceutical industries. Results from this study could be used for improving natural antioxidants and bioactive factors to promote human health.

**Key words:** *Melissa officinalis* (L.), *Berberis vulgaris*, phenolic compounds, HPLC, antioxidant activity, antibacterial activity, antitumor activity.

## INTRODUCTION

Barberry occurs in central and southern Europe, northwest Africa and western Asia. In natural medicine, Barberry leaves are also used being harvested dried and used for medicinal purposes. Barberry fruits are very rich in vitamin C. Barberry species are rich in polyphenolic constituents and have shown significant free radical-scavenging activity (**Charehsaz *et al.*, 2015)**. It has antimicrobial and amoebicidal properties and is used either in the form of the pure compound or as a component of plant extract.

Catechol, the main compound in barberry extract known as pyrocatechol or 1, 2-dihydroxybenzene, is an organic compound with the molecular formula $C_6H_4(OH)_2$. It is the ortho isomer of the three isomeric benzenediols, presence of two ortho hydroxyl groups ensure its antioxidant activity.

Lemon balm (*Melissa officinalis*) is a plant grow and cultivated in some parts of Iran. The leaves are used in Iranian folk medicine for many diseases, however, food preservatives. Historically lemon balm has been said to possess calming tranquilizing, anti-gas, fever-reducing, antibacterial, spasmolytic **(Nasri and Rafieian-Kopaei, 2013)**. Hypotensive, memory-enhancing, menstrual-inducing, and thyroid-related effects; antiviral and antioxidant activities; antifungal, antiparasitic, and antispasmolytic activities; flatulence; asthma; bronchitis; amenorrhea; cardiac failure; arrhythmias; ulcers; and wounds

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(**Akhlaghi *et al.*, 2011; Setorki *et al.*, 2013)**. Besides, it has been said that it is effective in treatment of headaches, indigestion, colic, nausea, nervousness, anemia, vertigo, syncope, malaise, insomnia, epilepsy, depression, psychosis, and hysteria (**Wölbling and Leonhardt, 1994)**. These effects as it rich in phenolic compounds, sesquiterpenes, tannins, and essential oils. The study was to investigate Melissa's therapeutic, nutritive and industrial effects. It is commonly used for its anti-herpes, anti-viral, anti-HIV, antioxidant, antimicrobial, anticancer, antitumor, anti-stress, anti-anxiolytic, antidepressant, anti-Alzheimer, anti-cardiovascular diseases, memory improving, concentration, and anti-inflammatory effects. It was said to be good for insomnia and dyssomnia.

The main and dominant compound in Melissa extract was Rosmarinic acid, it consists of two phenolic rings with ortho-position hydroxyl group for each ring and carboxylic group in between **(Cao *et al.*, 2005)**. So it has higher antioxidant activity by the construct of hydrogen atom of ortho position on phenolic rings (**Cao *et al.*, 2005; Mariappan *et al.*, 2012)**, besides, it has other biological activities.

Antioxidant compounds such as flavonoids, tannins, coumarins, curcumanoids, xanthons, phenolics, lignans and terpenoids are found in various plant parts (*e.g.* fruits, leaves, seeds and oils). For this reason, there is growing interest in separating these plant antioxidants and using them as natural antioxidants. Processing of fruits, vegetables and oilseeds results in high amounts of waste materials such as peels, seeds, stones and oil seed meals **(Jeong *et al.*, 2004)**. Extraction yield is dependent on both solvent and method of extraction **(Pinelo *et al.*, 2004)**. Water and aqueous mixtures of ethanol, methanol and acetone are commonly used in plant extraction **(Goli *et al.*, 2005)**. **Wang and Helliwell (2001)** reported that aqueous ethanol was superior to methanol and acetone for extracting flavonoids from tea. However, in another study, water was found to be a better solvent than methanol 80% or ethanol 70% for extracting tea catechins **(Sun and Ho, 2005)**.

## MATERIALS AND METHODS

Lemon balm leaves (*Melissa officinalis*) and Barberry roots (*Berberis vulgaris*) were obtained from local Egyptian markets. *Tert*-butyl hydroquinone (TBHQ), 1, 1-Diphenyl-2 picrylhydrazyl (DPPH˙), 2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), β-carotene, gallic acid, quercetin, Dimethyl sulfoxide (DMSO), 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and trypan blue dye were purchased from Sigma (St. Louis, MO, USA). Fetal bovine serum (FBS), DMEM (Dulbecco's Modified Eagle Medium), RPMI-1640 (Roswell Park Memorial Institute), HEPES buffer solution (4-(2-hydroxyethyl) -1-piperazine ethane sulfonic acid), L-glutamine, gentamycin and 0.25% Trypsin-EDTA were purchased from Lonza (Belgium). Bacterial strains of *Staphylococcus aureus* ATCC 6538, *Salmonella enteritidis* PT4 were obtained from Egyptian Culture Collection (Cairo, Ain Shams University, MERCIN). Mammalian cell lines: Huh-7 cells (human hepatocellular cancer cell line) (HCC) and MDA-MB-231 (human breast Carcinoma) (BCC) were obtained from the American Type Culture Collection (ATCC, Rockville, MD). All other chemicals used were analytical grade.

### Carcinoma Cell Line Propagation

The cells were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50µg/ml gentamycin. The cells were maintained at 37ºC in a humidified atmosphere with 5% $CO_2$ and were sub cultured two to three times a week.

### Preparation of the Extracts

The extracts were prepared as recommended by **El-Maati *et al.* (2016)**. The residue of ethanol 80% extracts after evaporation was freeze-dried (Thermo-Electron Corporation-Heto power dry LL300 Freeze Dryer). The dried residues were weighed to determine the dried yield then stored at -20°C for further analysis.

### Determination of Phenolic Compounds

Phenolic compounds were determined as reported by **AOCS (1990) and Škerget *et al.* (2005)**. The absorbance was measured at 760 nm. Total phenolic content expressed as gallic acid equivalent (GAE) was calculated using the following linear equation based on the calibration curve:

$$y = 0.0201x + 0.0439$$
$$R^2 = 0.9968$$

Where y is the absorbance and x is the concentration (mg GAE $g^{-1}$ extract).

## Determination of Total Flavonoids

Total flavonoid content was determined by the method of **Ordon *et al.* (2006)**. Total flavonoid content expressed as quercetin equivalent (QE) was calculated using the following equation based on the calibration curve:

$$y = 0.0144x - 0.0092$$
$$R^2 = 0.9985$$

Where y is the absorbance and x is the concentration (µg QE).

## Separation and Identification of Chemical Compounds of Extracts by HPLC

HPLC Agilent 1200 series equipped with a quaternary pump, autosampler, column compartments ET at 35°C, multiwavelength detector set at 330nm, 280nm for detection flavonoid and phenolic compounds, degasser, the column used for fraction Zorbax OD. 4.6x250nm and the flow rate of the mobile phase during the run was 1 ml/min.

## Chromatographic Analysis of Phenolic Compounds

The phenolic compounds of barberry and Melissa ethanolic extracts were fractionated and identified by HPLC according to the method described by **Goupy *et al*. (1999)**.

## Chromatographic Analysis of Flavonoid Compounds

Flavonoid compounds of barberry and Melissa ethanolic extracts were estimated according to the method described by **Mattila *et al*. (2000)**.

## DPPH Radical-Scavenging Activity

The electron donation ability of the obtained extracts was measured by bleaching of the $DPPH^{\cdot}$ purple colored solution according to the method of **Hanato *et al.* (1988)**. One hundred µL of each extract (10 mg extract/10 ml solvent) was added to 3.9 ml of 0.1 mM $DPPH^{\cdot}$ dissolved in ethanol and methanol according to the solvent used for extraction. After incubation period of 30, 60 and 120 min at room temperature, the absorbance was determined against a control at 517 nm **(Gulcin *et al.*, 2004)**. Percentage of antioxidant activity of free radical $DPPH^{\cdot}$ was calculated as follow:

$$\text{Antioxidant activity (Inhibition) (\%)} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

Where $A_{control}$ is the absorbance of the control reaction and $A_{sample}$ is the absorbance in the presence of plant extract. TBHQ was used as a positive control. Samples were analyzed in triplicate.

## β-Carotene/Linoleic Acid Bleaching

The ability of extracts and synthetic antioxidants to prevent the bleaching of β-carotene was assessed as described by **Keyvan *et al.* (2007**). Antioxidant activity was calculated as follows:

$$\text{Antioxidant activity (\%)} = [1 - (A^0_{sample} - A^{120}_{sample})/ (A^0_{control} - A^{120}_{control})] \times 100$$

## Ferric Reducing Antioxidant Power (FRAP)

Reducing power of extracts was measured by method of **Gülçin *et al.* (2010)**. The reduction of $Fe^{+3}$ to $Fe^{+2}$ was determined by measuring absorbance of the Prussian blue complex. Distilled water was used as blank and gallic acid and TBHQ for control. Absorbance of this mixture was measured at 700 nm using a UV spectrophotometer. Decreased absorbance indicates ferric reducing power capability of sample.

## Disc Assay Test

Antibacterial activity was assayed as described by **Bauer *et al.* (1966)** using Nutrient agar media (lab-lemco powder) poured into Petri dishes. four tested extracts were applied at one concentration (10 mg/ml) to Petri dishes containing nutrient agar infected with pathogenic Gram-positive bacteria (*S. aureus*) and Gram-negative bacteria (*S. enteritidis*), incubated at 37°C for 3, 6, 12, 18 and 24 hr., and the diameter of the resulting inhibition zones are recorded. The disc was saturated with 20 µl of extract solution. Negative control was exactly prepared as the treatments except that extract was replaced by the solvent that is used in solubility dimethyl sulfoxide (DMSO). Positive controls were exactly prepared as the treatments except gallic acid and commercial and synthetic

antibiotic (tetracycline). It was observed that negative control samples did not produce any inhibition zones (data not shown).

### Cytotoxicity Evaluation Using MTT Viability Assay

The tumor cell lines were suspended in medium at concentration $5x10^4$ cell/well in Corning® 96-well tissue culture plates, then incubated for 24 hr., two extracts (Melissa Et 80% and B et 80 extracts) were then added into 96-well plates (three replicates) to achieve twelve concentrations for each extract. Six vehicle controls with media or 0.5% DMSO were run for each 96 well plate as a control. After incubating for 24 hr., the numbers of viable cells were determined by the MTT test. Briefly, the media was removed from the 96 well plate and replaced with 100 µl of fresh culture RPMI 1640 medium without phenol red then 10 µl of the 12 mM MTT stock solution (5 mg of MTT in 1 ml of PBS) to each well including the untreated controls. The 96 well plates were then incubated at 37°C and 5% $CO_2$ for 4 hours. An 85 µl aliquot of the media was removed from the wells, and 50 µl of DMSO was added to each well and mixed thoroughly with the pipette and incubated at 37°C for 10 min. Then, the optical density was measured at 590 nm with the microplate reader (Sun Rise, TECAN, Inc, USA) to determine the number of viable cells and the percentage of viability was calculated as:

$$\text{Vaibility (\%)} = [(O_{Dt}/O_{Dc})] \times 100\%$$

$$\text{Inhibition (\%)} = 100 - \text{Viability (\%)}$$

Where $O_{Dt}$ is the mean optical density of wells treated with the tested sample and $O_{Dc}$ is the mean optical density of untreated cells.

The relation between surviving cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified compound. The 50% inhibitory concentration ($IC_{50}$), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose response curve for each concentrate using Graphpad Prism software (San Diego, CA. USA) (**Mosmann, 1983; Elaasser *et al,* 2015**).

## RESULTS AND DISCUSSION

In foods containing lipids, antioxidants delay the onset of oxidation or slow the rate at which it proceeds. These substances can occur as natural constituents of foods, but they can also be intentionally added to products or formed during processing. Their role is not to enhance or improve the quality of foods, but they do maintain food quality and extend shelf life. Antioxidants for use in food processing must be inexpensive, nontoxic, effective at low concentrations, stable, and capable of surviving processing (carry-through effect); color, flavor, and odor must be minimal (**Giese, 1996**).

Antioxidants not only extend shelf life of the products, but also reduce raw material waste, reduce nutritional losses, antimicrobial agents and widen the range of fats that can be used in specific products (**Coppen, 1983**). By extending maintaining quality and increasing the number of oils that can be used in food products, antioxidants allow processors to use more available and less costly oils for product formulation.

Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tert-butyl hydroquinone (TBHQ) are widely used in the food industry because they are more effective and less expensive than natural antioxidants **(Pitchaon *et al.*, 2007)**. Their safety, however, has been questioned. TBHQ is banned in Japan and certain European countries and BHA and BHT are reported to be carcinogenic. Hence research into safer and more effective natural antioxidants is under way and several natural sources are being examined **(Shahidi, 1997)**.

### Yield, Total Phenolic and Total Flavonoids of Extracts

The yield of extracts was 0.78 to 16.26 g extract/100 g plant (Table 1). Variation in the yields of extracts is attributed to differences in polarity of compounds present in plants, such differences have been reported by **Jayaprakasha *et al.* (2001)**.

The Folin-Ciocalteu method measures the reduction of the reagent by phenolic compounds *via* the formation of a blue complex that can be

**Table 1. Yield, total phenolic and flavonoids of Barberry and Melissa extracts**

| | Ethyl acetate extract | | Ethanol 80% extract | |
|---|---|---|---|---|
| | **Barberry (B EA)** | **Melissa (M EA)** | **Barberry (B Et 80%)** | **Melissa (M Et 80%)** |
| **Extract Yield g/100 g plant** | 0.78 | 6.95 | 16.26 | 13.07 |
| **Total phenolic compounds (TPC) mg GAEg$^{-1}$ extract** | 60.97±1.33 | 31.55±0.75 | 113.71±1.11 | 141.12±1.04 |
| **Total flavonoids (TF) mg QEg$^{-1}$ extract** | 6.78±0.86 | 12.99±1.01 | 54.08 ±1.36 | 19.05±0.86 |

measured at 760 nm against gallic acid equivalent (GAE) as a standard **(Imeh and Khokhar, 2002)**. The amount of total phenolics showed in Table 1 as mg GAE $g^{-1}$ extract. Flavonoids possess a broad spectrum of chemical and biological activities, including radical-scavenging properties. For this reason, extracts were analyzed for total phenolic, flavonoid contents. Table 1 present the flavonoid contents of extracts as mg quercetin equivalent/g extract (mg QE $g^{-1}$). The results revealed that ethanol 80% extraction was better than ethyl acetate extracting for phenolic compounds and flavonoids. This might be due to the polarity and good solubility during extraction **(Siddhuraju and Becker, 2003; Kequan and Liangli, 2004; Wieland *et al.*, 2006; Zhang *et al.*, 2011)**.

## Phenolic and Flavonoid Compounds in Extracts Identified By HPLC

As shown in Figs. 1, 2, 3 and 4 and Tables 2, 3, 4 and 5, Luteolin was the dominant flavonoid with 24.1 mg/ml following with 12.41 mg/ml to Myrecetin in M Et 80% extract, where in B Et 80% extract; Kampferol was the highest with 23.14 mg/ml., the phenolic compounds in two extracts Rosmarinic acid and catechol were the highest compounds in M Et 80% and B Et 80% extracts with about 40 mg/ml respectively.

Generally, Melissa Et 80% extract was higher than Barberry Et 80% in phenolics and flavonoids.

## Antioxidant Activity of Plant Extracts

As mentioned by **Frankel and Meyer (2000)** and **Huang *et al.* (2005)** no single method is adequate for evaluating the antioxidant capacity of foods or extracts, since different methods can yield widely diverging results. Several methods based on different mechanisms must be used.

## DPPH$^{\cdot}$ Tadical-Scavenging Activity

The effect of antioxidants on DPPH radical-scavenging is thought to be due to their hydrogen-donating ability, DPPH$^{\cdot}$ is a stable free radical and accepts an electron or hydrogen radical to become a stable molecule (**Gulcin *et al.*, 2004)**. Free radicals involved in the process of lipid peroxidation are considered to play a major role in numerous chronic pathologies such as cancer and cardiovascular diseases (**Dorman *et al.*, 2003**). DPPH$^{\cdot}$ is a model of a stable lipophilic radical. A chain reaction of lipophilic radicals is initiated by lipid autoxidation. Antioxidants react with DPPH$^{\cdot}$, reducing the number of DPPH$^{\cdot}$ free radicals to the number of their available hydroxyl groups. Therefore, the absorption at 517 nm is proportional to the amount of residual DPPH$^{\cdot}$ **(Juan *et al.*, 2005)**. It is visually noticeable as a discoloration from purple to yellow. The scavenging activity of extracts against DPPH$^{\cdot}$ was concentration-dependent. The results of DPPH radical-scavenging activities of M EA, B EA, M Et 80 and B Et 80% extracts were represented in (Fig. 5) where M Et 80 extract was the higher antioxidant activity about 40.91% following by B Et80% with 19.98% compared with TBHQ and GA 90.82% after 2 hours. The results clearly indicate that both extracts exhibited antioxidant activity. The antioxidant activity of extracts is mainly ascribable to the concentration of phenolic compounds in extracts **(Heim *et al.*, 2002)**.

The results of the DPPH$^{\cdot}$ free radical scavenging assay suggest that components involving the extracts can scavenge free radicals *via* electron- or hydrogen-donating mechanisms and thus should be able to prevent the initiation of deleterious free radical mediated chain reactions

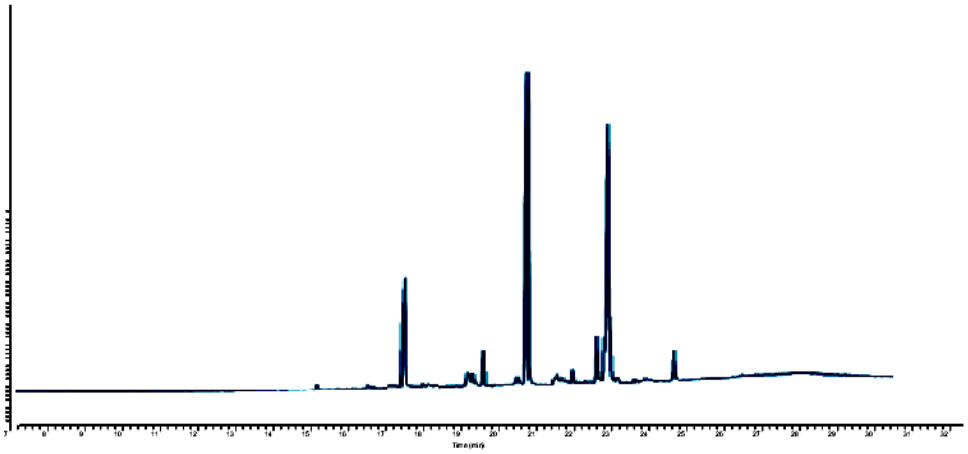


**Fig. 1. Phenolic compounds in Melissa Et 80% extract**

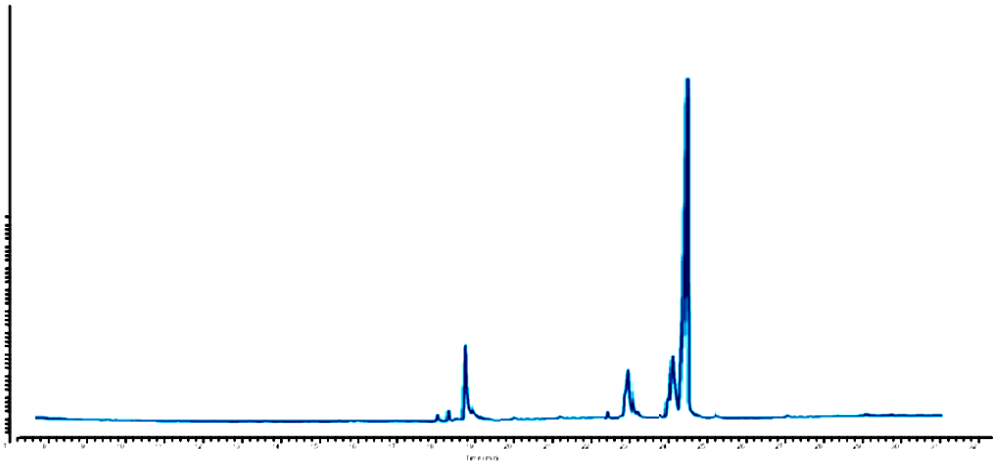


**Fig. 2. Phenolic compounds in Barberry Et 80% extract**

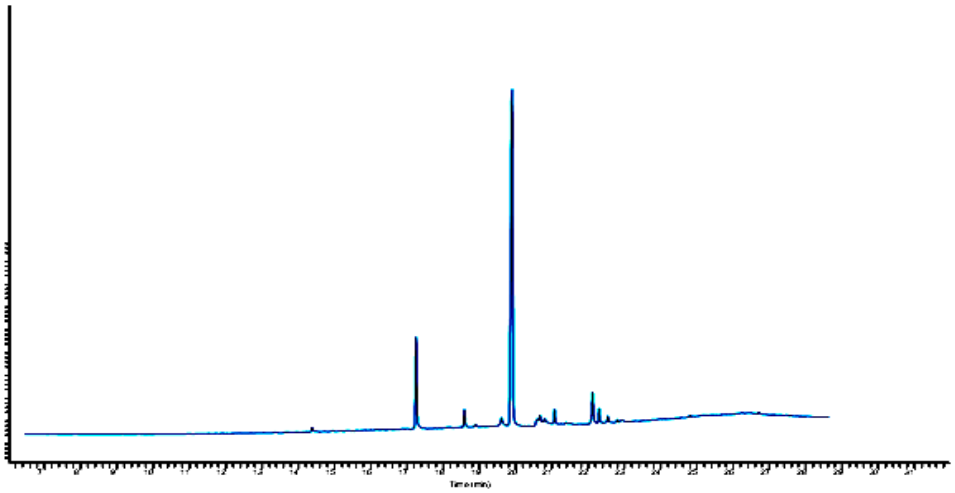


**Fig. 3. Flavonoid compounds in Melissa Et 80% extract**

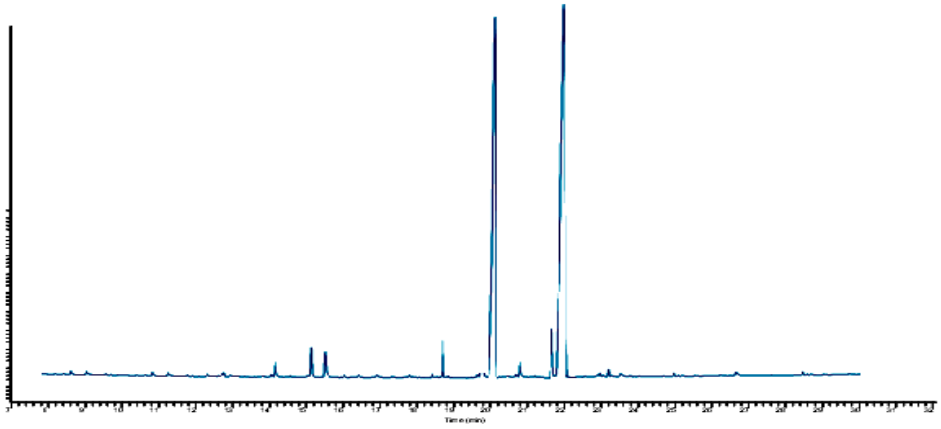


**Fig. 4. Flavonoid compounds in Barberry Et 80% extract**

**Table 2. Concentration of phenolic compounds in Melissa Et 80% extract**

| Peak No. | Retention time | Compound | Concentration µg/g extract |
|---|---|---|---|
| **1** | 17.8 | Ellagic acid | 8.25 |
| **2** | 20.7 | Rosmarinic acid | 40.23 |
| **4** | 23.2 | Gallic acid | 28.69 |
| **5** | 25.0 | Caffeic | 5.26 |

**Table 3. Concentration of phenolic compounds in Barberry Et 80% extract**

| Peak No. | Retention time | Compound | Concentration µg/g extract |
|---|---|---|---|
| 1 | 18.8 | Resorcinol | 3.96 |
| 2 | 23.2 | Gallic acid | 10.2 |
| 3 | 24.3 | catechol | 40.2 |
| 4 | 24.9 | caffeic | 33.5 |

**Table 4.Concentration of Flavonoids compounds in Melissa Et 80% extract**

| Peak No. | Retention Time | Compound | Concentration µg/g extract |
|---|---|---|---|
| 1 | 17.0 | Myrecetin | 12.41 |
| 2 | 18.5 | Apigenin | 5.6 |
| 3 | 20.0 | Luteolin | 24.1 |
| 4 | 21.0 | Rutin | 1.65 |

**Table 5. Concentration of Flavonoids compounds in Barberry Et 80% extract**

| Peak No. | Retention time | Compound | Concentration µg/ g extract |
|---|---|---|---|
| **1** | 18.8 | Apigenin | 8.06 |
| **2** | 20.3 | Luteolin | 19.68 |
| **3** | 22.7 | Kampferol | 23.14 |

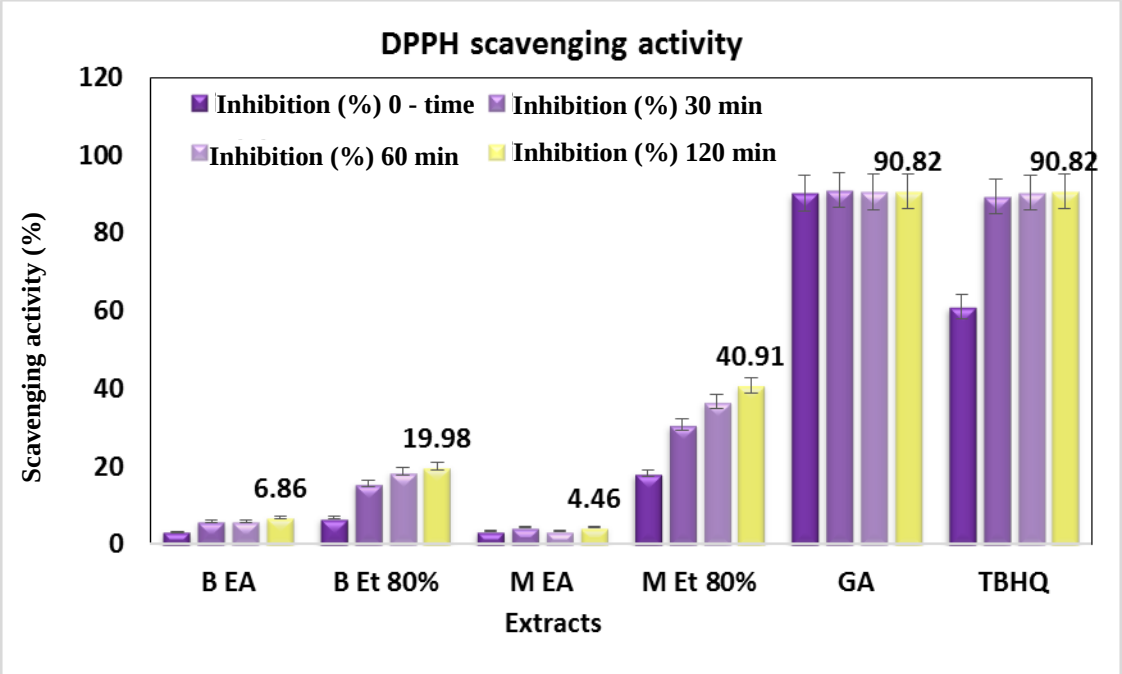



**Fig. 5. Scavenging activity of Barberry and Melissa extracts against DPPH radical compared with gallic acid and TBHQ**

in susceptible matrices. This further shows the capability of the extracts to scavenge different free radicals in different systems, indicating that they may be useful therapeutic agents for treating radical-related pathological damage.

## β-Carotene/Linoleic Acid Bleaching Assay

Synthetic free radical-scavenging (DPPH˙) model is valuable tools to indicate the potential antioxidant activity of plant extracts; however, these systems do not use a food or biologically relevant oxidizable substrate, so no direct information on an extract's protective action can be obtained **(Dorman *et al.*, 2003)**. Therefore, it was considered important to assess the extracts in a β-carotene/linoleic acid lipid–water emulsion assay despite its reported limitations **(Koleva *et al.*, 2002; Ley and Bertram, 2003)**. In this assay, oxidation of linoleic acid produces hydroperoxide-derived free radicals that attack the chromophore of β-carotene, resulting in bleaching of the reaction emulsion. An extract capable of retarding/inhibiting the oxidation of β-carotene may be described as a free radical scavenger and primary antioxidant (**Liyana-Pathirana and Shahidi, 2006**).

As can be seen in Fig. 6, extracts can inhibit the bleaching of β-carotene by scavenging linoleate-derived free radicals. The order of decreasing efficacy at a dose of 200 µg $ml^{-1}$ was TBHQ>M Et 80%>B Et 80%. The results had revealed comparable scavenging ability 49% and 29% for M Et 80% and B Et 80% to the synthetic antioxidants TBHQ 92.02% and

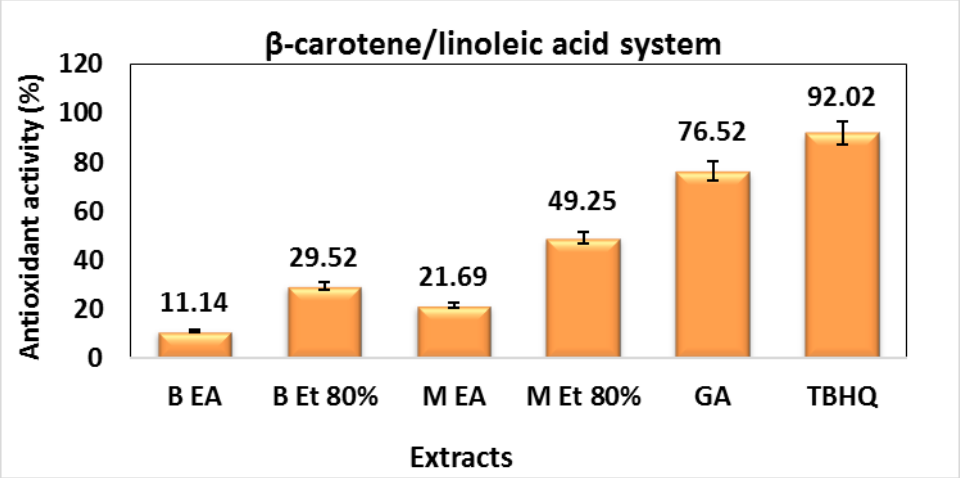



**Fig. 6. Antioxidant activity of plant extracts in β-carotene/linoleic acid system compared with gallic acid and TBHQ**

gallic acid (GA) 76.52%. It has been suggested that the polarity of an extract is important in water–oil emulsions, in that non-polar extracts are more effective antioxidants than polar extracts owing to a concentrating effect within the lipid phase **(Koleva *et al.*, 2002)**. Thus, it would be expected that the less polar extracts would be more potent. This phenomenon was not observed in the case of extracts studied here, a finding which has also been reported previously **(Koleva *et al.*, 2003)**. According to the data on β-carotene/linoleic acid bleaching, the extracts can scavenge free radicals in a complex heterogeneous medium. This suggests that the extracts may have potential use as antioxidant preservatives in emulsion-type systems.

## Ferric Reducing Antioxidant Power (FRAP)

Antioxidant compounds cause the reduction of ferric ($Fe^{+3}$) form to the ferrous ($Fe^{+2}$) form because of their reductive capabilities. Prussian blue-colored complex is formed by adding $FeCl_3$ to the ferrous ($Fe^{+2}$) form. Therefore, reduction can be determined by measuring the formation of Perl's Prussian blue at 700 nm (**Chang *et al.*, 2002**). In this assay, yellow color of the test solution changes to green or blue color depending on the reducing power of antioxidant samples. A higher absorbance indicates a higher ferric reducing power.

$$Fe(CN)_6^{-3} + \text{Reductive agent} \rightleftharpoons Fe(CN)_6^{-4}$$

$$Fe(CN)_6^{-4} + FeCl_3 \longrightarrow Fe_4[Fe(CN)_6]_3$$

As shown in Fig. 7, M Et 80% extract showed ferric reducing power with the increased concentration as standard antioxidants (GA and TBHQ). According to results of the present study, both ferric reducing power and total phenolic content of extract were higher than those of microwave extract. Total phenolic content and ferric reducing power are related with each other. Fe (III) reduction is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action (**Dorman *et al.*, 2003**). The extracts that contained high amount of phenolic and Flavonoids compounds (Tables 1, 2, 3, 4 and 5) showed relatively high antioxidant activity. In general, extracts showed ferric reducing power compared with TBHQ (Fig. 7). It has been proven that ferric reducing power of plant extracts is mainly ascribable to the concentration of phenolic compounds in the plant (**Heim *et al.*, 2002**). The ferric reducing power of all extracts were from 0.562 to 1.243 compared with GA (2.57) and TBHQ (2.89).

Considering the results of all four assays, phenolic compounds can explain high antioxidant capacity **(Fernandez-Pachon *et al.*, 2004; Mullen *et al.*, 2007)**, Although some authors have reported that there is no correlation between the content

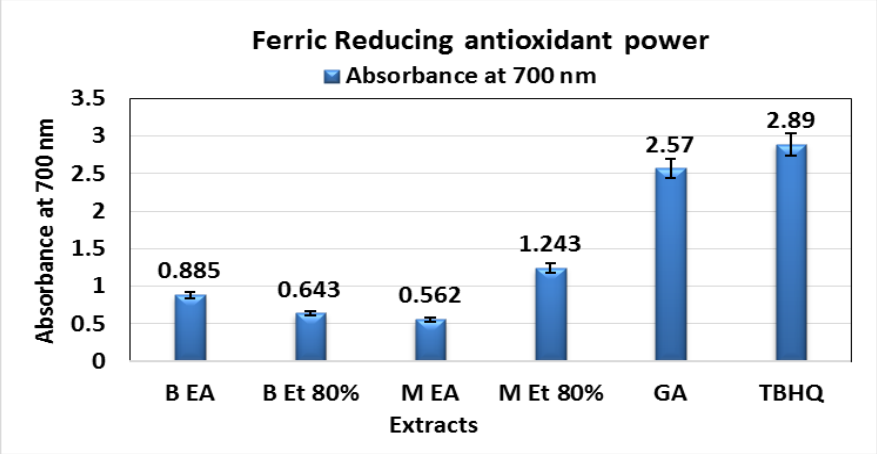



**Fig. 7. Absorbance of ferric reducing power of Barberry and Melissa extracts against gallic acid and TBHQ**

of these main antioxidant compounds and radical-scavenging capacity. The results obtained in this study do not support this claim. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet, triplet oxygen or decomposing peroxides and reductive heavy metals with two or more valence states **(Osawa, 1994)**.

## Disc Assay of Lemon Balm and Barberry Extracts Against Tested Bacteria

As shown in Fig. 8, Tetracycline and gallic acid used as positive controls in this assay. The results indicated that the largest inhibition of extracts was at 12 hr., for M Et 80% 14 mm followed by B Et 80% with 13 mm compared with positive control 18mm (GA) and 22 mm (Tetracycline) against *S. aureus*.

In Fig. 9 the results indicated that the largest inhibition of extracts was at 12 hr., for M Et 80%14 mm followed by B Et 80% with 11 mm compared with positive control 16mm (GA) and 24 mm (Tetracycline) against *S. enteritidis*.

These results agreed with **Abdel-Naime *et al.* (2019)** who studies ethanol extract against *Staphylococcus aureus*. **Jafari and Sani (2016)**, determined antibacterial activity of essential oil from *Melissa officinalis* leaves obtained by hydro-distillation Antibacterial activity were evaluated by micro-dilution and disk-diffusion methods. Results showed that *Bacillus cereus* has largest inhibition zones of 25.88mm and lowest MIC and MBC *vice-versa*, *S. enterica*. Our results showed significant antibacterial activity for this herbal essential oil, which suggests its capacity as a natural food preservative. **Dashti *et al.* (2014)** determined the antibacterial activity of aquatic and ethanolic *Berberis vulgaris* extracts using agar diffusion and MIC. 40 µg/ml of each extract added to the dishes. The results showed a significant antibacterial effect against tested bacteria. Especially, *Pseudomonas aeruginosa* (MIC=16 µg/ml), *Proteus vulgaris* (MIC=32 µg/ml) and *Escherichia coli* (MIC=32 µg/ml) were the most inhibited. This indicated that these extracts have good antibacterial activity and it might be used as natural food preservatives.

## Cytotoxicity Evaluation Using MTT Viability Assay

Cancer is a collective term used to describe a group of different diseases that are characterized by the loss of control of cell growth and division, leading to a primary tumor that invades and destroys adjacent tissues. It may also spread to other regions of the body through a process known as metastasis, which is the cause of 90% of cancer deaths. Cancer remains one of the most difficult diseases to treat and is responsible for approximately 14.5% of all deaths worldwide. This incidence is increasing due to the aging of the population in most countries, including those under development.

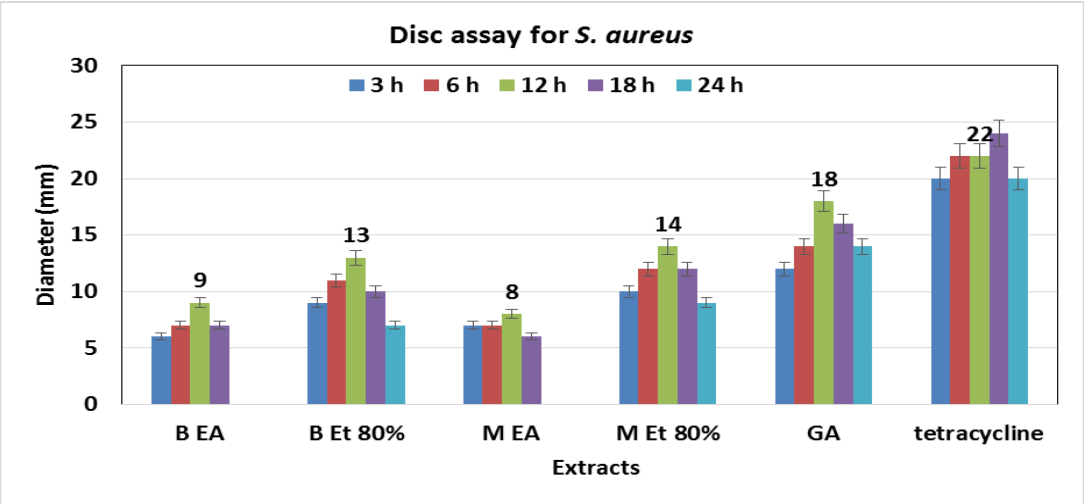



**Fig. 8. Disc assay of Melissa and Barberry extracts against *S. aureus***

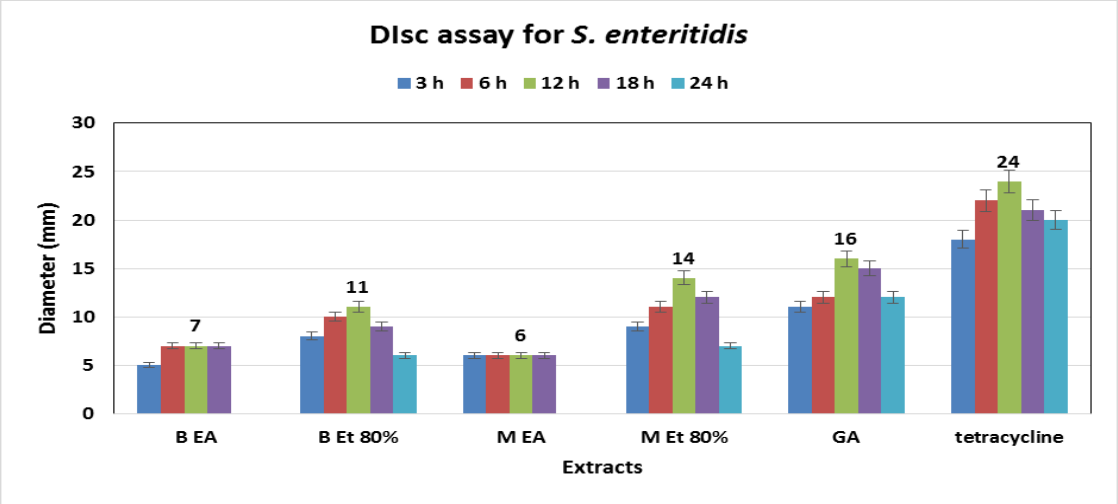
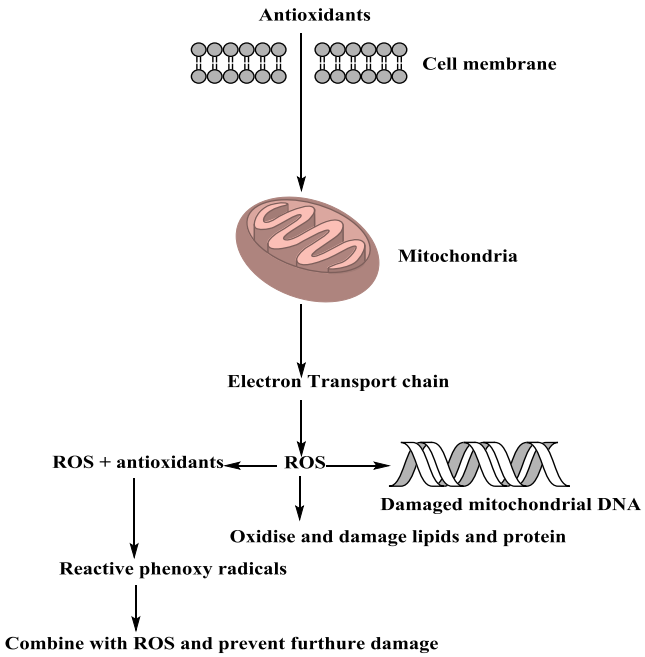



**Fig. 9. Disc assay of Melissa and Barberry extracts against *S. enteritidis***

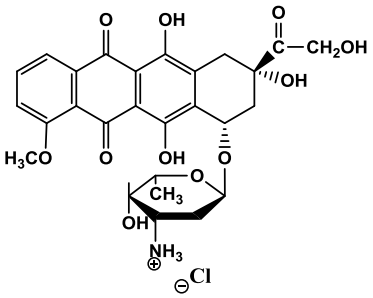
Hydroxyl radicals that are highly damaging to mitochondrial DNA are produced by superoxide anions and hydrogen peroxide (Scheme 1). Damaged mitochondrial DNA inhibits the expression of electron transport protein, leading to the accumulation of reactive oxygen species (ROS) (**Van Houten *et al.*, 2006**). The free radicals generated by damaged mitochondrial membranes, when combined with antioxidants, produce reactive phenoxy radicals that combine with ROS and prevent further damage of DNA.

This is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, colored (dark purple) formazan product. The cells are then solubilized with an organic solvent (*e.g.* isopropanol) and the released, solubilized formazan reagent is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells.

As shown in Figs. 10 and 11, The M Et 80% and B Et 80 extracts showed a good inhibition against hepatocellular carcinoma cell (HCC) and




**Scheme 1. Mechanism of antioxidants against oxygen species**



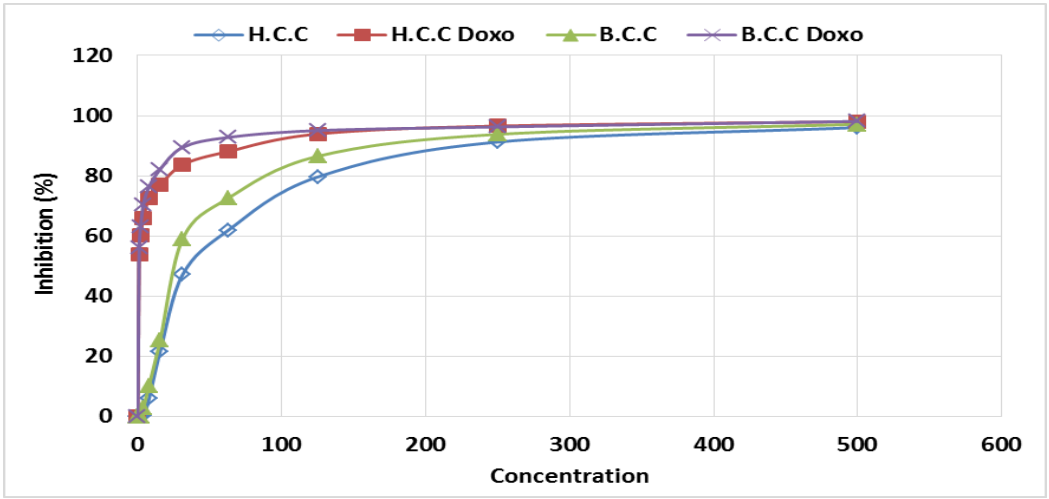




**Fig.10. Evaluation of cytotoxicity of Melissa extract against hepatocellular and breast carcinoma cell**

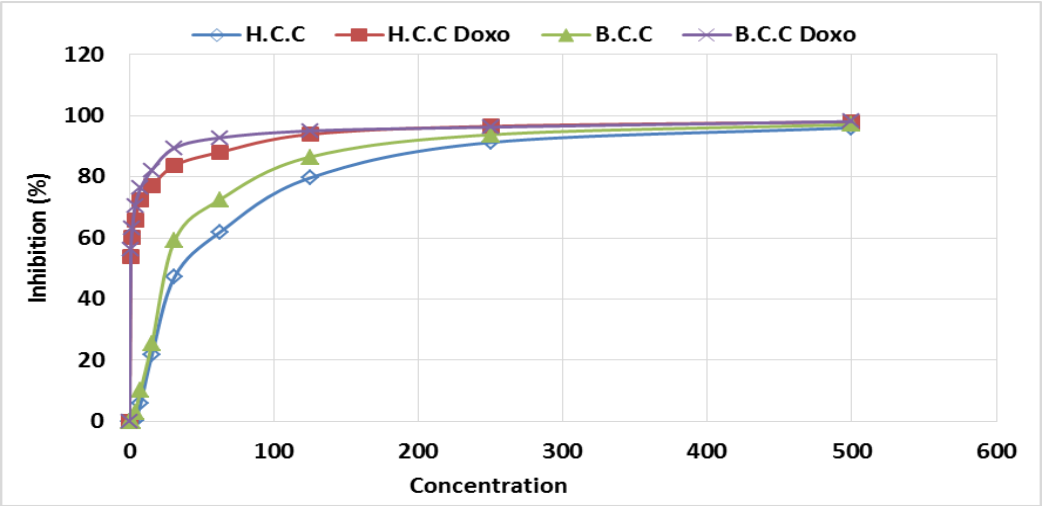



**Fig. 11. Evaluation of cytotoxicity of Barberry extract against hepatocellular and breast carcinoma cell**

breast carcinoma cell (BCC). The best result against HCC was M Et 80% with $IC_{50}$ = 27 ±1.9 µg/ml compared with Doxorubicin (HCC $IC_{50}$ = 0.71 ±0.06 µg/ml). B ET 80% has $IC_{50}$ of HCC = 37.1 ± 2.7 µg/ml. Also, M Et 80% extract had the best inhibition against BCC with $IC_{50}$= 26.8 ± 1.5 µg/ml against Doxorubicin (BCC $IC_{50}$ = 0.6 ± 0.02 µg/ml). B ET 80% has $IC_{50}$ of BCC = 48 ± 3.2 µg/ml.

All these results depended on the total phenolic compounds of extracts, total flavonoid (Table 1) and HPLC identified of phenolic and Flavonoid compounds (Tables 2, 3, 4 and 5). Phenolic compounds and flavonoids other may exert local anti-carcinogenic effects in the intestine where, in addition to acting as intraluminal antioxidants, they may induce Phase II xenobiotic metabolizing enzymes, suppress the production of biologically active prostaglandins by inhibiting the arachidonic acid cascade and inhibit mitosis by inhibiting intracellular protein kinases. Although briefly under suspicion as a natural carcinogen, the ubiquitous flavonol quercetin is now regarded as a possible protective factor against cancers of the alimentary tract (**Formica and Regelson, 1995**).

Doxorubicin is anthracycline antibiotic that has hydroxymethyl in the $C_{13}$ substituent, which delays the action of cytosolic aldoketoreductase and prevent the conversion to the less active and chronically cardiotoxic doxorubicinol. This gives the longer duration of action compared to analogs that have $CH_3$ at this position (e.g., daunorubicin). Doxorubicin is highly lipophilic and concentrates in the lymph nodes, muscle, liver, fat, skin, and bone marrow. Elimination is triphasic, and the terminal shelf-life of the drug ranges from 30 to 40 hours. Most of an administered dose is excreted in the feces (**Mordente *et al.*, 2009**). Doxorubicin is treated a wide range of neoplastic disorders, including solid tumors and hematologic cancers in thyroid gland, ovary, stomach, breast, and bladder.

## Conclusion

Melissa and Barberry extracts showed varying degrees of antioxidant, antibacterial and antitumor activity in different test systems in a dose-dependent manner. Furthermore, the pattern of activity of the extracts within the assays also differed. As observed, extracts with antioxidant capacity were in parallel to their higher phenolic contents. It can be concluded that the obtained extracts using higher phenolic compounds were more effective radical scavengers than those obtained using lower. M Et 80% was better than B Et 80% in phenolic compounds and flavonoids. So, for use in the food industry, ethanol would be a more appropriate solvent. Furthermore, it is notable that M Et 80% exhibited the strongest antioxidant capacity in all assays used expect β-

carotene linoleic emulsion assay. Overall, M Et 80% showed comparable activity to TBHQ and GA. Therefore, these extracts could be used as preservative ingredients in the food or pharmaceutical industries provided that any resulting organoleptic effects were acceptable. However, further research is required before such use can be proposed with confidence

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# الخواص المضادة للأكسدة والبكتريا والاورام لمستخلصات مختلفة من اوراق المليسيا وجذور البرباريس

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يهدف البحث إلى دراسة مستخلص الايثايل اسيتات والايثانول ٨٠% لكلا من أوراق المليسيا وجذور البرباريس من حيث محتواها من المركبات الفينولية وخواصها المضادة للأكسدة والبكتريا والأورام، تم تقدير المركبات الفينولية والفلافونودية الكلية بواسطة جوهر فولن وكلوريد الألومنيوم على الترتيب، تم التعرف علي المركبات الفينولية والفلافونودية النشطة بواسطة HPLC، فكان المركب الأساسي في المستخلص الإيثانولي للمليسيا هو حمض الروزمرينيك ٤٠.٢٣ ميكرو جرام لكل جرام مستخلص والكاتيكول ٤٠.٢ ميكروجرام لكل جرام للمستخلص الايثانولي للبرباريس، تم تقدير الخواص المضادة للاكسدة بعدة طرق مختلفة شملت طريقة DPPH وهي للشقوق الحرة (١.١ ثنائي فينايل -٢، بكريل هيدرازيل)، وطريقة قصر لون مستحلب البيتا كاروتين واللينوليك، وطريقة اختزال الحديديك بقوة مضادات الأكسدة، وأظهرت نتائج المستخلصات نشاطا مضادا للاكسدة مقارنة بمضادات اكسدة صناعية مثل TBHQ وحمض الجاليك، تم تقدير النشاط المضاد للبكتريا للمستخلصات بواسطة بكتريا مرضية موجبة وسالبة لجرام بواسطة طريقة فحص القرص تم تقدير النشاط المضاد للأورام بواسطة طريقة MTT، استحوذ المستخلص الايثانولي لاوراق المليسيا على أعلى النتائج المضادة للأكسدة والبكتريا والأورام، لذا يمكن استخدام تلك المستخلصات كمواد حافظة للأطعمة او مواد تستخدم في التصنيع الدوائي، ويمكن استخدام بيانات هذه الدراسة لتحسين مضادات الأكسدة الطبيعية والعوامل النشطة بيولوجيًا لتعزيز صحة الإنسان.

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