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# Different methods evaluation of antioxidant properties of *Myrtus communis* extract and its fractions

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## Abstract

*Myrtus communis* L. is a plant traditionally used as an antiseptic and disinfectant drug. In this research, the antioxidant activity of *Myrtus communis* was assayed by evaluating radical scavenging activity, reducing power, FRAP method and determination of phenolic compounds. The methanolic extract of leaves of *Myrtus communis* was fractionated by using petroleum ether, chloroform, ethyl acetate and buthanol. In reducing power, different concentrations of samples were mixed with phosphate buffer, ferrocyanate, TCA and ferric chloride. Different concentrations of samples were mixed with DPPH and after 30 min the absorbances were measured. For determination of phenolic content, 500 µl of sample was mixed with Folin-Ciocalteu and sodium carbonate. For determination of flavonoids, 500 µl of sample was mixed with 2 ml of distilled water, $NaNO_2$ and NaOH. In reducing power method, chloroform fraction showed the highest reducing capacity. In the DPPH radical scavenging method, the highest antioxidant capacity was found in buthanol fraction ($IC_{50}$=84.42±1.8 µg/ml). In FRAP method, the highest antioxidant capacity was found in crude extract (5.4±0.3 mg/ml) and buthanol fractions (5.51±0.4 mg/ml), respectively. The highest amount of phenolic compounds was detected in ethyl acetate fraction of *Myrtus communis* (17.5±0.001 µg/g). The highest amount of flavonoids was found in crude extract of *Myrtus communis* (171.9±7.3 µg/ml). Overall, we can suggest that the leaves of *Myrtus communis* can be used as antioxidant and as a food additives to avoid oxidative degradation of foods.

*Keywords:* Antioxidant activity, Flavonoid compounds, Fractions, *Myrtus communis*, Reducing power.

## 1. Introduction

A large range of compounds with biological activities is found in the plant Kingdom. Extracts of spices and herbs possess various ranges of antioxidant, antibacterial and antifungal properties (1). *Myrtus communis* L. (MC) is a plant traditionally used as an antiseptic, disinfectant (2) anti-inflammatory drug (3) and has been used in folk medicine for the treatment of various disorders (3).

The chemical study of this plant has shown several compounds which have been isolated from different parts of the plant such as the leaves (4), the essential oil (5) and the fruits (6). The berry of MC is used to produce the characteristic myrtle liqueur typical of *Sardinia*, in Italy. The fruit of MC is spherical in shape, dark red to violet in color which contains delphinidin, petunidin, malvidin, peonidin , cyaniding-3-mono- and 3, 5 diglucosides (6), along with glycosides of myricetin and quercetin. This fruit is used in the treatment of infectious disease, such as diarrhea and bloody diarrhea. The leaves of MC are used as

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anti-inflammatory agent, as a mouth wash and for the treatment of candidiasis (6,7).

Antioxidant compounds are used as food additives that retard its degradation (8). Also, antioxidant compounds prevent different diseases such as cancer and diabetes. In this work, we have evaluated the antioxidant activities of MC extract and its fractions by DPPH radical scavenging, reducing power, FRAP method and determination of phenolic compounds and flavonoids.

## 2. Materials and methods

### 2.1. Materials

DPPH (2,2-diphenyl-1-picrylhydrazyl radical), quercetin, gallic acid and Folin-ciocalteau reagent were obtained from Sigma Chemical Co., St Louis, MO.

All other reagents were obtained from Merck Chemicals, Darmstadt, Germany Collection of plant materials.

### 2.2.Collection of plant materials

Leaves of MC were collected around Jahrom, Fars province, Iran, and identified by one of us ( Mahmoodreza Moein). The sample was preserved in medicinal plants museum (PM 890) at School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran.

### 2.3. Extraction and fractionation

The leaves were separated, dried at room temperature and grounded into powder.

Aerial parts of MC (1500 g) were dissolved in 630 cc methanol and fractionated by petroleum ether (3×630 cc) to give 188 g of petroleum ether fraction. Then fractionation was followed by using chloroform (3×1666.6 cc), ethyl acetate (3×1000 cc) and buthanol (3×1000 cc), respectively.

### 2.4. Measurement of antioxidant properties

#### 2.4.1. Reducing power method

The reducing power of MC crude extract and its fractions were determined using the method described by Moein *et al* (9). A series of concentrations of MC extract was made (12.5- 200 µg/ml) in 0.2 M phosphate buffer (pH 6.6) containing 1% potassium ferrocyanide. After 20 minutes, 2.5 ml of 10% TCA was added to 5 ml of this mixture and centrifuged at 3000 g for 10 minutes. The supernatant was separated and mixed with 2.5 ml of distilled water containing 0.5 ml of ferric chloride 1%. The absorbance of this mixture was measured at 700 nm using UV-visible spectrophotometer (PG instrument, +80). The intensity of absorbance could be the indicator of antioxidant activitiy of the extract and its fractions (9).

#### 2.4.2. DPPH radical scavenging

The DPPH scavenging assay was performed according to procedures described by Moein *et. al* (9). Samples for the experiments (1 mg) were dissolved in 1 ml of methanol and further diluted to appropriate concentrations (12.5-400 µg/ml) before being transferred to a 96-well microplate. Negative controls were prepared with 20 µL of methanol and 200 µL DPPH in triplicates. Each well contained 200 µL of DPPH (100 mM) and 20 µL of crude extract (or fraction). The microplates were incubated at 25 °C for 30 min and the absorbance was measured at 495 nm using a microplate reader (Biotek, ELX 800) (9).

Quercetin was used as the antioxidant standard. The obtained data were used to determine the concentration of the sample required to scavenge 50% of the DPPH free radicals ($IC_{50}$). The percent inhibition was plotted against the concentrations of the extract and the $IC_{50}$ was obtained from the fitted linear curve. A lower $IC_{50}$suggests a more potent antioxidant. The results were expressed as the mean±SD of the three replicates (Figure 1).

#### 2.4.3. FRAP (ferric-reducing antioxidant power) assay

TPTZ (2, 4, 6-tripyridyl-S-triazine) solution (10 mmol/ L) in HCl (40 mmol /L), $FeCl_3$ (20 mmol/ L), acetate buffer (0.3 mol/ L, pH 3.6) are the solutions needed for this method. Acetate buffer, $FeCl_3$ and TPTZ were mixed before using. The mixture was then heated to 37 ° C. Twenty µl of each sample and 180 µl of FRAP reagent were mixed in a 96-well microplate reader and then incubated at 37 °C for 10 minutes. The absorbance of the resulted complex was measured at 593 nm (10).

#### 2.4.4 Content of phenolic compounds

The content of total phenolic compounds in MC extract was determined by a modification in Folin-ciocalteu method (4). For the preparation of calibration curve 0.5 ml aliquots of (0.024, 0.075, 0.105 and 0.3 mg/ml) gallic acid solutions were mixed with 2.5 ml of Folin-ciocalteu reagent (di-

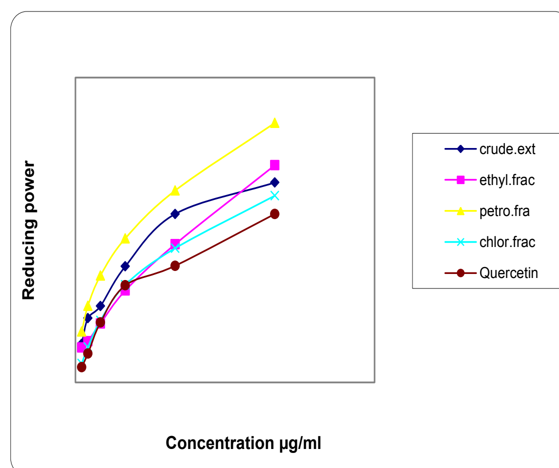


Figure 1. Reducing power of *Myrtus communis* fractions compared with quercetin as a standard.
crude.ext: crude extract, ethyl.frac: ethyl fraction, petro.frac: petroleum ether fraction, chlor frac: chloroform fraction.

luted ten –folds) and 2 ml (75 g/l) sodium carbonate. The absorption was read after 30 min at 765 nm and the calibration curve was drawn. Half of one ml of the plant extract (10 g/l) was mixed with the same reagents as described above and after 1 hour, the absorption was measured for the determination of plant phenolics. All tests were performed in triplicate. Total content of phenolic compounds in plant extract was expressed as gallic acid equivalents (GAE) and was calculated by the following formula:

$$C= c.v/m$$

Where: C is the total content of phenolic compound of mg/g of plant extract, in GAE; c is the concentration of gallic acid established from the calibration curve, mg/ml; v is the volume of extract, ml; m is the weight of plant extract, g (4).

### *2.4.5. Determination of total flavonoid content.*

The total flavonoid content of MC was determined by using of a slightly modified colorimetric method described previously (11). Half of one ml aliquot of appropriately diluted sample solution was mixed with 2 ml of distilled water and subsequently with $NaNO_2$ 15% solution. After 6 min, 2 ml of NaOH 4% solution was added to the mixture. Immediately, water was added to bring the final volume to 5 ml, then the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was determined at 510 nm versus a prepared water blank (5). Quercetin was used as a standard compound for the quantification of the total flavonoids. All values were expressed as milligram of quercetin equiv per 1 gram of extract. Data was recorded as mean±SD for three replicates.

### *2.5. Statistical analysis*

Means±SD of samples were calculated. The $IC_{50}$ values were calculated by linear regression. The data were analyzed for statistical significance using one way ANOVA followed by Tukey

Table 1. Antioxidant effects of *Myrtus communis* evaluated by different methods in comparison with antioxidant standards.

| Samples of *Myrtus communis* | DPPH radical scavenging (IC50 µg/ml)(mean±SD) | FRAP mg/ml (mean±SD) | Total phenolic content mg/g (mean±SD) | Total flavonoid content µg/g (mean±SD) |
|---|---|---|---|---|
| Crude extract | 130.44±12.1 | 5.4±0.3 | 0.92±0.001 | 171.9±7.3 |
| Petroleum ether fraction | 108.3±10.2 | NA | 6.45±0.002 | 47.4± 6 |
| Chloroform fraction | 111.14±11.1 | NA | 8.86±0.001 | 150. 8± 5.7 |
| Ethyl acetate fraction | 85.6±5.7 | 4.25±0.05 | 17.5±0.001 | 46.3± 3.3 |
| Buthanol fraction | 84.42±1.8 | 5.51±0.37 | ND | ND |

ND: non determined, NA: non active.

post test. P values less than 0.05 were considered significant.

## 3. Results

Results of this study indicate that the reducing power of petroleum ether fraction is higher than the other fractions (Fig 1).

The $IC_{50}$ of MC buthanol fraction is less than the other fractions (84.42±1.8 µg/ml), (Table 1). The amount of phenolic compounds is higher in ethyl acetate fraction (17.5±0.001 mg/g), (Table 1). But the highest amount of flavonoids is detected in crude extract of MC (171.9±7.3 µg/g), (Table 1). In the FRAP method, the highest antioxidant potential was found in butanol fraction (5.51 ±0.37 mg/ml).

## 4. Discussion

The leaves of MC possess a refreshing smell, a very intensive and strongly bitter taste (5). As described in previous studies, myrtle (*Myrtus communis*) possesses the highest antioxidant activity in inhibition of lipid peroxidation (8).

In the present study, the antioxidant properties of MC extract and its fractions were evaluated by DPPH radical scavenging method, reducing power, FRAP method, determination of flavonoids and phenolic compounds. In previous reports the antioxidant activities of MC extracts were shown and were comparable with standard antioxidants such as BHT and alpha- tocopherol. Also, MC concentrations up to 160 ppm, are superior to alpha tocopherol in this matter, $P<0.05$ (1). In the DPPH radical scavenging assay, the purple color of DPPH in exposure to antioxidant compounds, changes to yellow. The yellow color intensity of DPPH, shows more antioxidant activity.

DPPH radical scavenging assay determines hydrogen donating ability of the samples (2). In the DPPH radical scavenging method, the $IC_{50}$ of buthanol (84.42±1.8 µg/ml) and ethyl acetate (85.6±5.7) fractions of MC are less than other fractions, (Table 1). It was reported that the $IC_{50}$ of subfraction E of *Salvia mirzayanii* (84.2±0.98 µg/mL) is similar to the $IC_{50}$ of MC buthanol fraction (12). While an earlier report indicated that the $IC_{50}$ of the ethyl acetate fraction was less than buthanolic fraction and crude extracts (13).

The reducing power of MC extract and its fractions increased by an increase in their concentrations. The reducing power of petroleum ether fraction is higher than the other fractions (Fig 1). The amount of phenolic compounds is higher in the ethyl acetate fraction (17.5±0.001mg/g) $P<0.05$, (Table 1). But the highest amount of flavonoids is detected in the crude extract of MC (171.9±7.3 µg/g), $P<0.05$, (Table 1).

In another study, a low correlation ($R=0.048$) between phenolic contents and flavonoids has been established (12). This could be explained by the existence of some chemical groups such as amino acids and proteins that can also react with the Folin- Ciocalteu reagent (12). Also, it is reported that more antioxidant properties of plant extracts (or fractions) may be related to its higher content of phenolic compounds (13).

In FRAP assay, standard curve was depicted between 1000-10000 µmol concentrations of $FeSO_4$. The results were illustrated as µmol of $FeSO_4$, iron equivalents per g of sample.

In the FRAP assay, the ferric-tripyridyl triazine (Fe3+-TPTZ) complex reduces to blue ferrous (Fe2+ -TPTZ) complex by antioxidants. This reduction developes at low pH and changes the color of complex at 593 nm (10). In the FRAP method, the antioxidant potential of crude extract, ethyl acetate and buthanol fractions were found to be 5.4±0.3 mg/ml, 4.25±0.05 mg/ml and 5.51±0.4 mg/ml, respectively, (Table 1). In other words in the FRAP method, buthanol fraction possesses the highest antioxidant potential.

## 5. Conclusion

Different fractions of MC possess antioxidant potential especially polar fractions such as n-buthanol and ethyl acetate. Overall we can suggest that MC extract and its fractions can be used as antioxidant compounds for retardation of oxidation processes.

## Conflict of interest

None declared.

## 6. References


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