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Antioxidants, Total Phenolic Content and Antimicrobial Evaluation of *Myrtus communis* Leaf and Stem Extract

***Zuhair Radhi Addai and Methaq Sattar Abood**

Department of Biology, Faculty of Education for Pure Sciences, University of Thi-Qar, Iraq

Abstract : Medicinal plants are a source for a wide variety of natural antibacterial and antioxidants. The aim of this study was to investigate the antioxidant and antibacterial capacities of *Myrtus communis* leaf and stem. Methanol extracts of *Myrtus communis* leaf and stem were assessed for its antimicrobial activity. The antibacterial activity was determined using paper disc method against two bacteria namely *Staphylococcus aureus* and *Bacillus cereus*. The sensitivity in terms of zones of inhibition of both extract was determined. Gentamicin was used as a standard drug for the study of antibacterial activity. The antioxidant activity was determined by measuring total phenolic content (TPC), ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH). The result showed that the methanol extracts of *Myrtus* leaf and stem were effective against both the bacteria tested. The leaf extract of *Myrtus* exhibited a higher antibacterial activity than the stem extract of *Myrtus*. The acetone extract of *Myrtus* leaf showed the largest antioxidant TPC, FRAP and DPPH compared with stem extract. A marked antimicrobial and antioxidant activity of *Myrtus communis* leaf and stem extracts was observed which may be attributed to the presence of phenolic compounds and other phytochemicals. The plants can be used to control infectious diseases and prevent oxidative damage.
Key Words: *Myrtus communis,* Antioxidants, Phenolics, Antibacterial activity.

Introduction

There are various studies emphasizing that free radicals contribute to the development of many diseases, including hemorrhagic shock, arthritis, ageing, atherosclerosis, ischemia, Alzheimer and Parkinson's disease, gastrointestinal disorders, tumor promotion and carcinogenesis[1]. Antioxidants are substances that play an important role in delaying or preventing degenerative diseases caused by oxidative damage of living cell components caused by free radicals[2].

Antioxidant are compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions by free radicals and reducing oxidative damage to human preservation and human health by combating damage caused by oxidizing agents. There are two categories of antioxidants namely, synthetic and natural antioxidant[3]. Therefore, development and utilization of more effective antioxidant of natural origins become more desirable[4]. Recently, a greater attention is focused on natural antioxidants derived from various plant sources. Many phytochemicals with various bioactivities, including antioxidant, anti-inflammatory and anticancer activities, have been reported in plants[4].Myrtus species have been reported as very rich in phenolic acids, flavonoids[5], tannins[6], anthocyanin pigments and fatty acids[8]. Previous studies on *M. communis* L. aerial parts have also revealed the presence of several specific chemical compounds, for example, the essential oils, phenolic acids, flavonoids and tannins in leaf and flowers[9].

However, little researches have undertaken the antioxidant activity of myrtle leaf and stem[9].The aim of this work was to evaluate the antioxidants and antibacterial activity acetone and methanol extract from *M. communis* L. leaf and stem

Materials and Methods

Extraction of Antioxidant

Plant materials were extracted using the methods described previously[10]. Briefly, 0.1 g dried plant powder and 10 ml 50% aqueous acetone were stirred for 1 h in a 25-mL universal bottle at 1,000 rpm using a magnetic stirrer (IKA, Staufen, Germany). Samples were then centrifuged at 4,750 g for 10 min using a mini centrifuge (Thermo-line, China) and the supernatants were used for further analyses.

Total Phenol Content (TPC)

The determination of antioxidant activity through TPC was carried out according to the method of [10]. About 100 µLleaf and stem extracts was added with 0.4 mL distilled water and 0.5 mL diluted Folin-Ciocalteu reagent. The samples *Myrtus* leaf and stem extracts with Folin-Ciocalteu reagent were left for 5 min before 1 mL 7.5% sodium carbonate (w/v) was added. The absorbances were taken at 765 nm wavelength with spectrophotometer after 2 hours. Calibration curve of gallic acid was set up to estimate the activity capacity of samples. The result was expressed as mg of gallic acid equivalents per 100 g of fresh sample (mg GA/100 g of FW).

Ferric Reducing Antioxidant Power (FRAP)

The determination of antioxidant activity through FRAP was carried out according to the method of [10]. FRAP reagent was prepared fresh as using 300 mM acetate buffer, pH3.6 (3.1 g sodium acetate trihydrate, plus 16 mL acetic acid made up to 1:1 with distilled water); 10 mM TPTZ (2,4,6-tris (2-pyridyl)-*s*-triazine), in 40 mMHCl and 20 mM $FeCl_3$•6H2O in the ratio of 10:1:1 to give the working reagent. About 1 ml FRAP reagent was added to 100 µL *Myrtus* leaf and stem extracts and the absorbances were taken at 595 nm wavelength with spectrophotometer after 30 minutes. Calibration curve of Trolox was set up to estimate the activity capacity of samples. The result was expressed as mg of Trolox equivalents per 100 g of fresh sample (mg TE/100 g of FW).

DPPH Radical Scavenging Activity

The determination of antioxidant activity through 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging system was carried out according to the method of [10]. Stock solution was prepared by dissolving 40 mg DPPH in 100 ml methanol and kept at -20°C until used. About 350 mL stock solution was mixed with 350 ml methanol to obtain the absorbance of 0.70±0.01 unit at 516 nm wavelength by using spectrophotometer (Epoch, Biotek, USA). About 100 µL *Myrtus* leaf and stem extracts with 1 ml methanolic DPPH solution prepared were kept overnight for scavenging reaction in the dark. Percentage of DPPH scavenging activity was determined as follow: DPPH scavenging activity (%) = $[(A_{blank} - A_{sample}) / A_{blank}] \times 100$.Where A is the absorbance

Antibacterial Assay

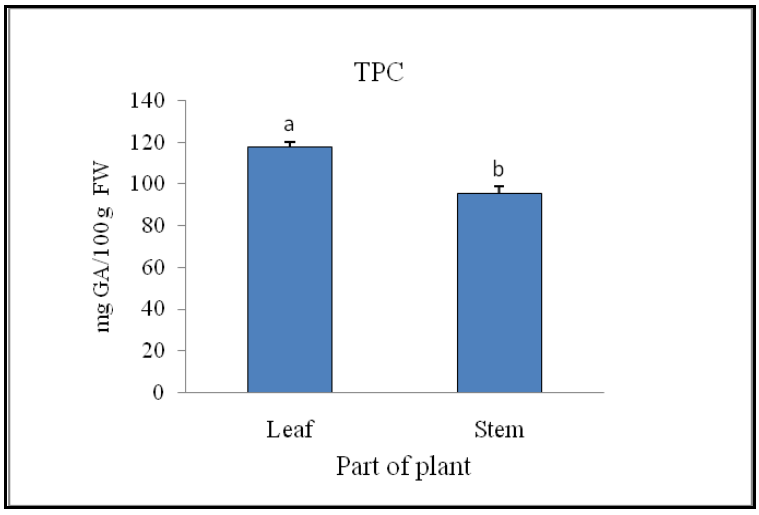
Staphylococcus aureus and *Bacillus cereus* were used in experiment. Mueller Hinton agar was used in antibacterial assay. Plant extracts were dissolved in methanol to obtain a concentration of 40µg/10µL. Antibacterial assays were conducted using the disc diffusion method as previously described by[11]. Negative controls were prepared using the same solvent employed to dissolve the plant extract. Gentamicin discs (10 µg/disc, Oxoid, UK) were used as control and positive controls. Zones of inhibition around the discs were measured in mm. The experiment was repeated in triplicate and the mean of diameter of the inhibition zones was calculated.

Statistical Analysis

Data were expressed as the means of three independent experiments. Statistical comparisons of the results were performed by one-way ANOVA using SPSS ver.19. Significant differences ($P<0.05$) among the medicinal plants were analyzed by Duncan 'triplicates range test.

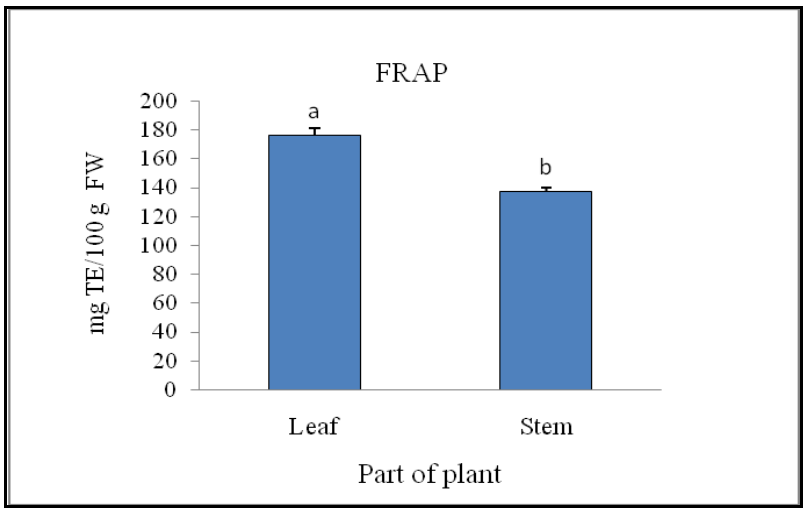
Results and Discussion

A large number of methods have been developed to evaluate antioxidant capacity of food and dietary supplements, herbal extracts or pure compounds. Nevertheless, few of them have been used widely due to the difficulty of measuring total antioxidant capacity owing to limitations associated with methodological issues and free radical sources[12]. A comparison between leaf and stem of *Myrtus* in terms of the total phenolic content (TPC) and antioxidant activity (FRAP and DPPH) is illustrated in Fig. 1, 2 and 3. The leaf showed different trends with regard to total phenolic content. The TPC was higher in leaf (117.54 mg GAE/100 g DW) than in stem (95.67 mg GAE/100 g DW). The high contents of total phenolic compounds in this species contribute to important antioxidant activity of[13]. Indeed, the phenolic fraction of plant extracts has been linked to their antioxidant capacity and antimicrobial activities[14]. Phenolic acid and flavonoids are a group of polyphenolic components synthesized by plants with known properties, which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes, anti-inflammatory action, reduce blood-lipid and glucose and to enhance human immunity[15].Antioxidant capacity is widely used as a parameter to characterize nutritional health food or plants and their bioactive components. Recently, interest has considerably increased in finding naturally occurring antioxidant to replace synthetic antioxidants, which were restricted due to their side effects such as carcinogenesis[16]. Two different and complementary assays: the DPPH• (2,2-di-phenyl-1-picrilhydrazyl) free radical scavenging and the FRAP (Ferric Reducing Antioxidant Power) were used to evaluate *in vitro* antioxidant activities of the obtained *Myrtus* leaf and stem. Based on DPPH and FRAP tests, leaf extract present the higher antioxidant activities than stem extract. Concerning FRAP test, for *Myrtus* leaf and stem extract, the higher ferric reducing antioxidant Power was observed in fruits and stem 176.65and 137.58 mg TE/100 g DW, respectively. Furthermore, leaf exhibited the highest antioxidant activities according to both used tests, DPPH and FRAPS (Figure 2 and 3). For acetone extract, the free radical scavenging varied 83.68% in leaf to 71.79% in stem. Comparing antioxidant activity from this study and other published data is difficult due to the fact that content of antioxidant compounds can be influence by extracting solvent, cultivar and location. As reported by [17] antioxidant compounds content in *Myrtus* at different part of plant.



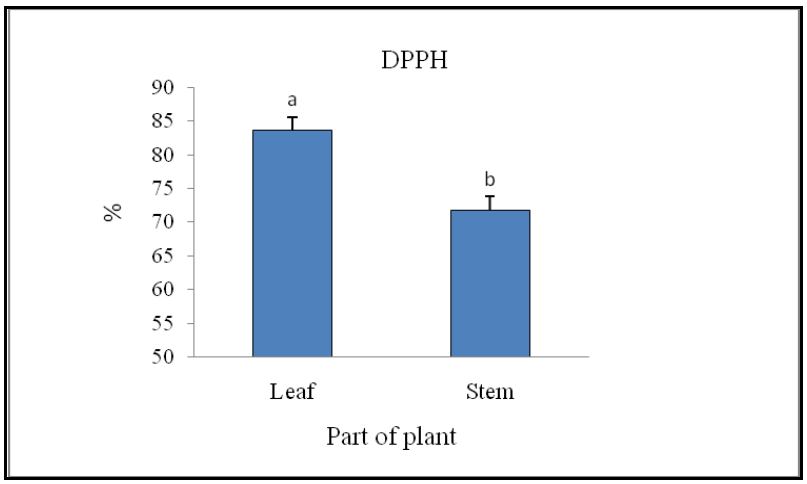
[a-b]Mean with different letters are significantly different (P<0.05)

Figure 1: Total phenol content (TPC) of *Myrtus* leaf and stem extract



[a-b]Mean with different letters are significantly different (P<0.05)

Figure 2: Ferric reducing antioxidant power (FRAP) of *Myrtus* leaf and stem extract



[a-b]Mean with different letters are significantly different (P<0.05)

Figure 3: Radical-scavenging activity (DPPH) of*Myrtus* leaf and stem extract

Antibacterial screening

Natural products may be a particularly rich source of antiinfective agents. The antimicrobial activity on pathogenic strains of Gram-positive *Staphylococcus aureus* and *Bacillus cereus* bacteria of *Myrtus* leaf and stem extracts was evaluated in the present study (Table 1).The antimicrobial activity of the *Myrtus* leaf and stem varied depending on the bacterial species used.

As it can be observed from this Table, all extracts exhibited antibacterial action against *Staphylococcus aureus* and *Bacillus cereus*. The most sensitive organism was *Staphylococcus aureus* and *Bacillus cereus* being the most resistant. The diameter of the zone of inhibition varied ranging from (9mm) to (12 mm) for leaf extract as compared to (6mm) to (8 mm) for stem extract (Table 1). The antimicrobial activity of the *Myrtus* leaf was found highest against *Staphylococcus aureus* while lowest activity was found against *Bacillus cereus.* Furthermore, the antibacterial activity of the leaf and stem extracts could also be associated with their higher total phenolic contents (Fig 1).This result agrees with several other studies that have shown that the inhibitory effect of phenolic compounds from natural extracts are more potent to Gram-positive bacteria than Gram-negative [18]. In our study, the total phenol content of leaf and stem extracts is in well correspondence to the antimicrobial activity against. It has been shown that phenolic compounds have antimicrobial activity[18].

In general, phenolic compounds potentially disturb the function of bacterial cell membranes which causes retardation of growth and multiplication of bacteria. Further phenolic compound involved in adhesion binding, protein and cell wall binding, enzyme inactivation, and intercalation into the cell wall and/or DNA during inactivation of pathogens [19]. Previous studies have suggested that the reactiveportion of antimicrobial phenolic compounds may be the free hydroxylgroup[20].

Table 1: Antibacterial activity of *Myrtus communis* leaf and stem and standard Gentamicin discs.

Test organism	Diameter of zone of inhibition (mm)		
	***Myrtus* leaf**	***Myrtus* stem**	**Gentamicin (10µg/disc)**
Bacillus cereus	8	6	14
Staphylococcus aureus	12	9	17

Conclusion

A marked antimicrobial and antioxidant activity of *Myrtus communis* leaf and stem extracts was observed which may be attributed to the presence of phenolic compounds and other phytochemicals. The plants can be used to control infectious diseases and prevent oxidative damage.

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