

PRELIMINARY PHYTOCHEMICAL SCREENING AND EXTRACTION OF POLYPHENOL FROM STEMS AND LEAVES OF A LEBANESE PLANT *MALVA PARVIFLORA* L.

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ABSTRACT

In this study, the primary phytochemical screening of the stems and leaves of a Lebanese plant *Malva parviflora* L. have been done. Also, an extraction and a partial purification of the phenolic compounds of the two parts of this plant have been realized and their antioxidant activity has been evaluated using two different tests, DPPH and the H₂O₂ test. The obtained results of the phytochemical screening showed the presence of flavonoids, tannins, phenols, saponins, alkaloids and resin in both parts of *M. parviflora*. Also, our results demonstrated that the total phenolic content (TPC) was found at higher levels in the stems and leaves of this plant and this amount was at 40 mg/ml. The DPPH test demonstrated higher antioxidant potential at 4 mg/ml of *M. parviflora*. Also, the H₂O₂ test showed that this maximal antioxidant activity was at 2.5 mg/ml of *M. parviflora*.

Keywords: Phytochemical screening, Extraction of polyphenol, Lebanese plant *Malva Parviflora*.

INTRODUCTION

Many studies have highlighted several pharmacological properties in medicinal plants or their isolated constituents including antioxidant, anti-diabetes, antibacterial, antiviral and anti-ulcer activities^{1,3}. Plants have formed the basis of traditional medicine systems that have been in existence for thousands of years. Even in modern times, plant-based systems continue to play an essential role in health care. It has been estimated by the WHO that approximately 80% of the world's population from developing countries rely mainly on traditional medicines for their primary health care. Plant products also play an important role in the health care for the remaining 20% in developing countries, and for those in industrialized countries as well⁴.

Medicinal plants (fruits, vegetables, medicinal herbs, etc.) are a source for a wide variety of natural products, such as the phenolic acids and flavonoids which are very interesting for their antioxidant properties². In addition to their ability to act as an efficient free radical scavengers⁵ their natural origin represents an advantage to consumer in contrast to synthetic antioxidants which their use is being restricted due to their carcinogenicity⁶. Epidemiological studies have shown that many of these antioxidant compounds possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, or antiviral activities to a greater or lesser extent^{7,9}. The intake of natural antioxidants has been associated with reduced risks of cancer, cardiovascular disease, diabetes, and diseases associated with ageing⁹.

Phenolics inhibit carcinogenesis by affecting the molecular events in the initiation, promotion, and progression stages¹⁰. They modulate the secretion of protein kinases in tumor cell proliferation and induce the expression of anticarcinogenic enzymes or inhibit induction of cancer promoting enzymes^{1,13}.

Antioxidant activity is a fundamental important property for human life. Many of the biological functions, including antimutagenicity, anticarcinogenicity, and antiaging, among others, originate from this property^{7,10}.

Malva parviflora L. belongs to the family Malvaceae that includes trees, shrubs and herbs. In Lesotho, dried powder or an infusion made from leaves and roots of *M. parviflora* have been used to clean wounds and sores. A hot poultice made from leaves is also used to treat wounds and swelling and is incorporated into a lotion to treat bruised and broken limbs¹⁴. The leaves of *M. parviflora* have been used by the Xhosa people of South Africa for drawing swollen, inflamed purulent wounds¹⁵. However, if ingested it could be toxic as *M. parviflora* is reported to cause mortality in foraging livestock, such as sheep, horses and cattle. Sheep are the most often affected

and develop clinical signs including staggering, trembling arched back and labored breathing^{15,16}. This toxicity may be due to the presence of malvalic acid, an unsaturated fatty acid previously referred to as halphen acid¹⁵⁻¹⁷.

The present study is aimed to identify and to quantify the phenolic compounds contained in the stems and leaves of an endemic Lebanese plant, *M. parviflora*, and to evaluate their antioxidant capacity using two tests the DPPH and H₂O₂. Furthermore, spectrophotometric analyses were employed for the determination of total phenolics concentrations of the stems and leaves of this plant.

MATERIALS AND METHODS

Plant Materials

Fresh plant was gathered from different regions in Lebanon on spring season between March and May in 2011 and the biological authentication was carried out by Professor George Tohme, president of C.N.R.S of Lebanon. Stems and leaves of *M. parviflora* were left on air at room temperature for two weeks to be very well dried. After that, they were crushed up and ground to get homogeneous fine powder by a grinder and then kept in a dark place at room temperature till their use in the different studies.

Chemicals

All the chemicals used were of analytical grade. Absolute ethanol, petroleum ether, n-hexane, hydrochloric acid, meta phosphoric acid, ethyl acetate, sodium hydroxide, potassium hydroxide, potassium iodide, ferric chloride, potassium ferric cyanide, acetic anhydride, bismuth nitrite, acetic acid, anhydrous sodium sulfate, sulphuric acid, were purchased from BDH England. Sodium carbonate, hydrogen peroxide and ammonium sulfate were purchased from Unichem India. Ascorbic acid, Gallic acid, Folin-Ciocalteu reagent and 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) were purchased from sigma Aldrich, USA and phosphate buffer solution (PBS) from Gibco, UK.

Phytochemical screening

The preliminary phytochemical screening of various active compounds from the stems and leaves of *M. parviflora* were accomplished.

1. Detection of tannins

This test has been done according to¹⁸ [Aiyegoro and Okoh 2010]. 10 ml of the ethanolic extracts (70%) of the stems and leaves of the plant were filtered by filter paper. Many drops of ferric chloride reagent (FeCl₃) 1% have been added to the different filtered extracts. The presence of tannins is indicated by a blue color.

2. Detection of resins

The method of ¹⁹ [Al-Balany 2004] has been used. 10 ml of each previous filtered extracts were taken and 20ml of HCl 4% were added. The appearance of turbidity indicates the presence of resins in the extracts.

3. Detection of coumarins

The method of ²⁰ [Geisman 1962] was used. 5 ml of each previous filtered extracts were putted in a test tube and covered by a filter paper saturated in NaOH. Then the test tube was putted in water bath, boiled for 10 min. After that, the filter paper was taken and exposed to UV light. The presence of coumarin is indicated by a green bright yellow color.

4. Detection of saponins

The method of ²¹ [Shihata 1951] was used. The test tubes containing each the previous filtered extracts were shaken for 5 min using a vortex. The appearance of big foamy indicates the presence of saponins.

5. Detection of alkaloids

This test has been done according to¹⁸ [Aiyegoro and Okoh 2010]. 0.2 g of the powder of the two parts of the plant was dissolved in 10 ml of HCl 1%. Then, they were transferred to a water bath for few minutes. After, 1 ml of the filtrated extract was treated with 2-4 drops of Dragendorff's reagent. The presence of alkaloids is indicated by the appearance of an orange reddish precipitation.

6. Detection of phenols

The method of ²² [Aljumaily 2004] was used. In beakers, 5 ml of each previous filtered extracts were taken and 1ml of FeCl₃ 1% and 1 ml of K₃(Fe(CN)₆) 1% were added. The appearance of fresh radish blue color indicated the presence of polyphenols.

7. Detection of terpenoids

This test has been done according to¹⁸ [Aiyegoro and Okoh 2010]. 1 ml of acetic anhydride and 2 ml of concentrated sulphuric acid were added to beakers containing 1 ml of previous filtered extracts. The presence of reddish brown on the interface indicated the presence of terpenoids.

8. Detection of volatiles oils

According to the ²³ [Indian herbal pharmacopoeia 1998], 10 ml of previous filtered extracts were filtered by filter paper till saturation and then exposed for UV light. The presence of volatile oil is indicated by the appearance of a bright pinkish color on the filter paper.

9. Detection of flavonoids

The method of ²⁴ [Jaffer et al. 1983] has been used. Two solutions A and B from both parts of the plant extract were prepared. The solution A contains 5 ml of ethanolic extract previously prepared. The solution B consists of 5 ml of ethanolic solvent added to 5 ml of KOH 50%. Then the two solutions A and B were mixed together. The presence of flavonoids is indicated by the appearance of yellow color.

Extraction and partial purification of phenols

The phenolic extraction was done according to²⁵ [Djeridane et al. 2006] with some modifications. 1 g of dried plant material (stems and leaves) from the used plant was crushed and transferred by grinder to powder. Maceration during 48 h with 50 ml of ethanol 70 % at room temperature has been done. Then, 2 h in shaker into a water bath has been accomplished. After, a filtration step has been done and the removal of ethanol was done under reduced pressure in a rotary evaporator at 40 °C. The remaining aqueous solution was defatted twice with petroleum ether and n-hexane in order to remove the lipids. Then lyophilized solution was extracted with ethyl acetate by separating funnel, with existent of aqueous solution, ammonium sulphate (20%), and meta-phosphoric acid solution (2%). The upper phase ethyl acetate fraction was dried by adding a sufficient amount of anhydrous sodium sulphate, and then evaporated to dryness using a rotary evaporator. The precipitate was dried, dissolved in 5 ml of absolute methanol and kept at - 20°C.

Total phenolic content

Determination of total phenolic extracts quantities was determined using the Folin-Ciocalteu reagent method ²⁶ [Lister and Wilson, 2001] with slight modification. 100 µl of various concentrations plant extracts which made five concentrations (5, 10, 15, 20, 40 mg/ml for *M. parviflora*) to the plant parts alone according of the plant dissolved in 1ml distilled water with 0.5 ml of Folin-Ciocalteu reagent (1/10 dilution) and 1.5 ml of Na₂CO₃ 2% (w/v) were added and mixed well. The blend was incubated in the dark at room temperature for 15 min. The absorbance of blue-colored solution of all samples was measured at 765 nm using a Gene Quant 1300 UV-Vis spectrophotometers. The results were expressed in mg of Gallic acid equivalent of g dry weight of plant powders.

Evaluation of Antioxidant activity

DPPH radical scavenging activity

Scavenging activity of DPPH antioxidant assay was measured according to methods of ²⁷ [Chew et al. 2009] with slight modification. 1 ml of different concentration of diluted phenolic extracts of the plant parts in methanol was added to 1mL of DPPH (0.15 mM in methanol) and at the same time we prepared a control that consists on 1ml DPPH with 1 ml methanol. The reaction mixtures were mixed very well by hand and then incubated in the dark at room temperature for 30 min and the absorbance was measured at 517 nm by a Gene Quant 1300 UV-Vis spectrophotometer. The results were expressed in mg ascorbic acid equivalents per g dry weight of plant extracts. The DPPH scavenging ability of plant extracts was calculated using the following equation:

$$\% \text{ Scavenging activity} = \frac{[(\text{Abs control} - \text{Abs sample}) / (\text{Abs control})] \times 100}$$

The Abs control is the absorbance of DPPH + methanol; Abs sample is the absorbance of DPPH radical + sample. Also, three controls have been prepared.

Scavenging activity of hydrogen peroxide (H₂O₂) radical

The H₂O₂ scavenging of the partial purified phenolic extracts was determined according to the method of ²⁸ [Ruch et al. 1989]. A solution of H₂O₂ (40 mM) was prepared in PBS (pH 7.4) and concentration was determined spectrophotometrically (Gene Quant 1300 UV-Vis) at 230 nm. Different concentrations of extracts from stems and leaves of the plant (0.5, 1, 1.5, 2 and 2.5 mg/ml of *M. parviflora*) in distilled water were added to a H₂O₂ solution (0.6 ml, 40 mM) and the absorbance of H₂O₂ at 230 nm was determined after 10 min against a blank solution containing the plant extracts without H₂O₂.

The percentage scavenging of H₂O₂ was calculated using the following equation:

$$\% \text{ Scavenged [H}_2\text{O}_2] = \frac{[(\text{Abs control} - \text{Abs sample}) / \text{Abs control}] \times 100}$$

Statistical Analysis

All analyses were carried out in triplicates. The result of scavenger activity and total phenolic compounds were performed from the averages of all samples reading Mean ± SD used Excel 2003.

RESULTS AND DISCUSSION

The obtained results from the phytochemical screening showed that both the leaves and stems of *M. parviflora* contain the polyphenol, flavonoid, tannin, alkaloid, resin and saponin [Table 1].

Antioxidants are substances that delay the oxidation process, inhibiting the polymerization chain initiated by free radicals and other subsequent oxidizing reactions²⁹. It has been proposed that the health beneficial effects of polyphenols could result from either their antioxidant functions and/or independently from these properties e.g. by acting as modulators of cellular signaling processes^{30,32}. However, many of the health beneficial functions of polyphenols, including antimutagenicity, anticarcinogenicity and anti-aging, among others, have been discussed in relation to their antioxidant properties³⁰.

Table 1: phytochemical screening of the stems and leaves of *M. parviflora*

Active compounds	Leaves	Stems
Phenols	++	++
Flavonoid	++	+
Saponins	+	+
Alkaloid	+	+
Resin	+	+
Tannins	+	+
Coumarines	-	-
Volatile oil	-	-
Terpenoids	-	-

+++ = high amount after added of reagent immediately; ++ = moderate amount after 5 minutes of reagent added; + = low amount after 10 minutes of reagent added and - = absent of active compound after 20 minutes

Table 2: The amount of TPC in the leaves and stems of *M. parviflora*

Plant parts	Total phenolic content (mg GAE/g dry weight) in <i>M. parviflora</i>				
	5	10	15	20	40
Stems	0.11±0.002	1.7±0.005	4±0.002	6±0.005	8.6±0.005
Leaves	0.87± 0.002	2.2±0.004	5.7±0.00	6.5±0.001	9.8±0.003

Values are the average of triplicate experiments and values expressed as mean ± sd

The TPC in leaves and stems of the plant have been evaluated. As shown in table 2, both leaves and stems contain high amounts of TPC. This amount has increased with the concentration of the extracts. Our results demonstrated that in [40mg/ml] of *M. parviflora* there was [9.8 mg] gallic acid [Table 2].

A number of reports on flavonoids, triterpenoids, and polyphenols indicated that they possess antioxidant and free radical scavenging activity³³. These phytoconstituents may exert multiple biological effects against tumors, heart disease, AIDS, and different pathologies due to their free radical scavenging activities. Realizing these facts, our study was carried out to evaluate the

antioxidant power of the stems and leaves of a Lebanese plant *M. parviflora*. An easy, rapid and sensitive method for the antioxidant screening of plant extracts is free radical scavenging assay using DPPH stable radical spectrophotometrically. In the presence of an antioxidant, DPPH radical obtains one more electron and the absorbance decreases³⁴.

Our obtained results showed that both leaves and stems of this plant have exerted a high antioxidant power at different concentrations. The DPPH test demonstrated that [4 mg/ml] of both stems and leaves of *M. parviflora* have significantly increased the % of scavenger activity by 73 % and 79 % respectively [Fig. 1].

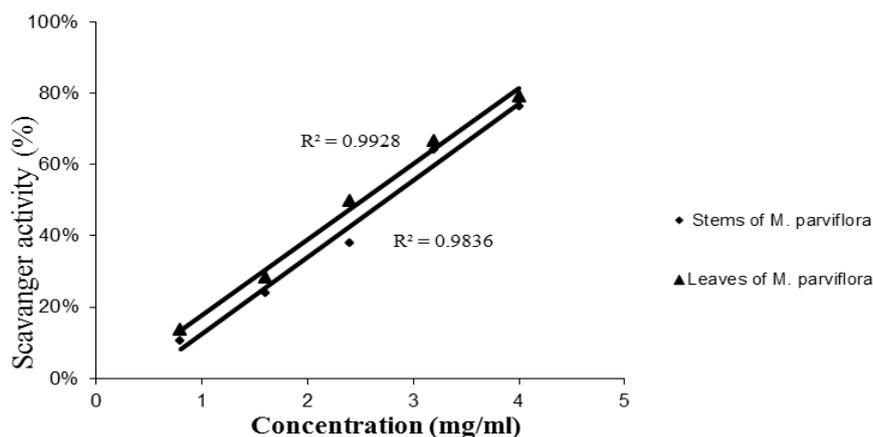


Fig. 1: Positive correlation between the concentration of leaves and stems of *M. parviflora* and the DPPH test

On the other hand and in order to confirm the antioxidant power of these two plants another known test has been used in our study, the H_2O_2 test. Hydrogen peroxide is an important reactive oxygen species because of its ability to penetrate biological membranes. However, it may be toxic if converted to hydroxyl radical in the cell³⁵. Scavenging of H_2O_2 by the plant extracts may be attributed to their phenolics, which donate electron to H_2O_2 , thus reducing it to water. Our results demonstrated that the extracts from both plants were capable of scavenging hydrogen peroxide in a concentration dependent manner. The % of scavenger activity of [2.5 mg/ml] of stems and leaves of *M. parviflora* was 81 % and 88 % respectively [Fig. 2]. These results showed that the studied plants possess

higher antioxidant activity and by consequence, they can be considered as good sources of natural products that may be employed in the treatment of the different diseases associated to the oxidative stress.

In order to determine the concentration of the extracts at which 50 % of the initial DPPH were decreased, the IC_{50} was studied. Our results showed that the IC_{50} of DPPH was different between leaves and stems of both plants. As shown in table 3 and in table 4, the IC_{50} of stems was [2.74 mg] for *M. parviflora*. Also, the IC_{50} of leaves was [2.52 mg] for *M. parviflora*. At the same time, the IC_{50} of H_2O_2 was [1.33 mg] for the stems of *M. parviflora* and 0.8 mg for the leaves of the plant.

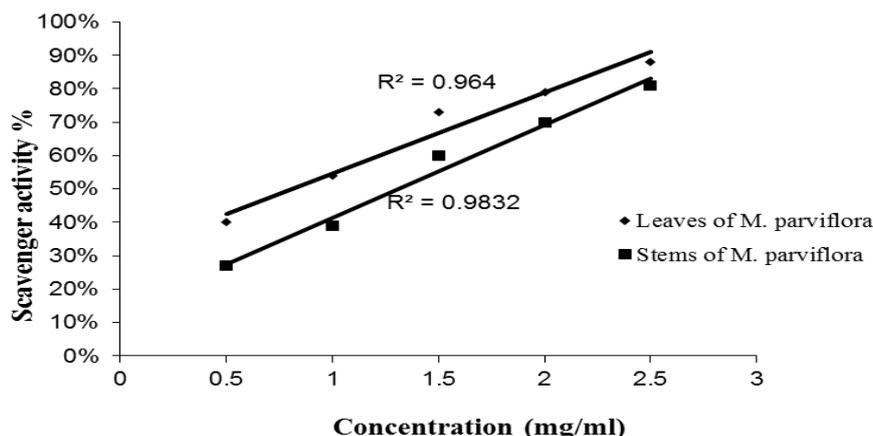


Fig. 2: Positive correlation between the concentration of leaves and stems of *M. parviflora* and the H_2O_2 test

Table 3: IC_{50} values of stems and leaves of the plant for the DPPH test

Antioxidant of DPPH	IC_{50}
Ascorbic acid	2.5 $\mu\text{g.mL}^{-1}$
Stems extracts of <i>M. parviflora</i>	2.74 mg
Leaves extracts of <i>M. parviflora</i>	2.52 mg

Table 4: IC_{50} values of stems and leaves of the plant for the H_2O_2 test

Antioxidant of H_2O_2	IC_{50}
Stems extracts of <i>M. parviflora</i>	1.33 mg
Leaves extracts of <i>M. parviflora</i>	0.80 mg

CONCLUSION

The present study suggested that *M. parviflora* could be a potential source of natural antioxidant and thus could be useful as therapeutic agents in the preventing and slowing the progress of aging, age-associated oxidative stresses-related degenerative diseases. In a future studies, we will isolate and identify the components in the ethanolic extracts responsible for eliciting such pharmacological effects.

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