



Antioxidant Potential of *Pistacia vera* L. fruit hull, *Anchusa strigosa* flowers and *Llex paraguariensis* A. St.-Hil. leaves extract

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ABSTRACT

The macerated methanolic extract of *P. vera* L., *A. strigosa* and *I. paraguariensis* A. St.-Hil. were used for evaluating their antioxidant activity. The results of present study showed that the IC₅₀ value for *Pistacia vera* L., *Anchusa strigosa* and *Llex paraguariensis* A. St.-Hil. extracts were 5.85 ± 0.11, 43.75 ± 1.05 and 8.98 ± 0.65 µg/ml respectively compared to 1.48 ± 0.05 µg/ml of ascorbic acid against DPPH radical. The IC₅₀ values for β-carotene bleaching (BCB) assay for *P. vera* L., *A. strigosa* and *I. paraguariensis* A. St.-Hil. extracts were 390.1 ± 7.5, 425.8 ± 6.5 and 410.2 ± 9.0 µg/ml respectively compared to 9.5 ± 0.4 µg/ml of rutin. The results of β-carotene bleaching (BCB) assay showed that the plant extract exhibits weak activity compared to rutin. In conclusion, the present study indicates that these plants and their phytochemical constituents can be exploited in future extensively for controlling oxidative stress and ailments.

Keywords: Natural Products, Redox properties, Oxidative stress, Antioxidant potential, DPPH radical scavenging activity, β-carotene bleaching assay.

INTRODUCTION

Antioxidants are compounds that scavenge the free radicals which are responsible for aging. Research studies suggest that free radicals are highly reactive forms covalent bonds with certain enzymes and cause tissue damage¹. Natural products and phytochemicals like flavonoid or polyphenolic compounds can scavenge the free radicals by their redox properties which allow them to act as reducing agents, hydrogen donor, chelator

and singlet oxygen scavenger^{2,3}. These can prevent cell damage and aging process. Dietary antioxidants and supplements are responsible for protecting our system from many diseases (heart failure, hepato- and renal-toxicity) and progression of the disease^{4,5}.

Pistacia vera L., is a plant member of Anacardiaceae family and native to Asia. Pistachio nut is mostly produced in Iran and some other countries². The chemical constituents of pistachios include fatty acids and flavonoids such as luteolin and



vitamin-E (tocopherol) in addition to the presence of poly-phenols (catechins) which have been shown to be protective agents against cancer, cardio-vascular disease and hypolipodemic activity⁶. Seifaddinipore *et al.*, (2018) have reported the cytotoxic effect and anti-angiogenesis potential of pistachio hull⁷ against breast cancer cells.

Anchusa strigosa Banks *et al.*, Sol. (*Boraginaceae*) is a perennial herb that is very common and localized in Jordan, Iraq and Palestine, it is known as hem-hem in Jordan^{8,9}. The active constituents are the alkaloids, pyrrolizidine, polyphenols, oil, proteins and aliphatic hydrocarbons. The flower herbal tea used as an analgesic, diuretic, sedative, diaphoretic and anti-arthritis⁸⁻¹⁰.

In South America, infusion of dried leaves of *Ilex paraguariensis*, is used for different medical purposes¹¹. The phytochemical constituents are mainly caffeine, phenolic acid and saponins. It shows a psychostimulant effect and used as an analeptic in Brazil. It is also used in the management of obesity and cardio-vascular problems¹²⁻¹⁴. Methylxanthines, like caffeine, phenolic acids, and saponins are the main constituents of this plant. It is commonly used as everyday as a potential substituent for coffee in the South America. This encouraging information and research guided us to carry further research on the antioxidant potential of methanolic extracts of some plant's parts, such as the hull (leathery cover) of *Pistacia vera* L. (pistachio) fruit, *Anchusa strigosa* flowers and *Ilex paraguariensis* A. St. Hil. (yerba mate) leaves.

EXPERIMENTAL

MATERIALS AND METHODS

Ascorbic acid, methanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), β -carotene, rutin and other solvents were purchased from Sigma Aldrich, USA, and used without any further purification. The cold maceration technique was used for extractions¹⁵.

Identification and herbarium

Plant material like hull (leathery cover) of fresh *Pistacia vera* L. (pistachio) fruit, flowers of *Anchusa strigosa* and *Ilex paraguariensis* A. St. Hil. (Yerba mate) leaves were collected from the same source during Dec. 2015 till Sep. 2017

and identified from Al-Salt, Jordan, planted at the vicinity of Al-Ahliyya Amman University, Amman, Jordan. Herbarium specimens have been deposited in the Faculty of Pharmacy and Medical Sciences, Al-Ahliyya Amman University after identification by taxonomist Prof. Dawood Al-Eisawi, The University of Jordan, Amman, Jordan. The plant parts were immediately dried, crushed, grinded and stored at -5°C until required for the preparation of extracts.

Extraction¹⁵

The plants materials were extracted separately. *Pistacia vera* L. Plant material (30 g) was macerated in 300 ml of methanol. Similarly, 10 g of yerba mate leaves in 150 ml and 10 g of *Anchusa strigosa* flowers in 500 ml of methanol were macerated. The plant material was soaked in methanol and kept for 48 hours. Thereafter, the extract was filtered and evaporated using rotatory evaporator. The dried extracts were kept under nitrogen environment till further use.

Standard and sample solutions

A stock solution of ascorbic acid (1000 μg /ml) in water was prepared. From this stock solution a working standard (100 μg /ml) by diluting the stock solution using water. A series of different standard solutions (0.5-10 μg /ml) of ascorbic acid were prepared for the evaluation of DPPH scavenging activity.

A stock solution of DPPH (0.010 w/v g%) was prepared in methanol and kept under nitrogen environment. A working solution of DPPH (0.002%) was prepared from the stock solution.

The different stock solutions (1 mg/ml) of plant extract were prepared by dissolving appropriate amount of extract from these plants. A series of solutions of plant extract (0.78-800 μg /ml) were prepared for the antioxidant activity in methanol.

DPPH scavenging activity

The plant extracts were analyzed for its free radical scavenging activity using DPPH according to the reported method^{16,17}. In the present study a methanolic solution of 0.002% DPPH was used. Briefly, 1 ml of plant extract of different concentration in methanol and 1 ml of methanolic solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution were mixed and vortexed for 1 min and kept aside

in dark for 30 minute. The absorbance of solutions was measured at 517 nm using methanol as blank. The absorbance of 0.002% DPPH solution mixed with equal volume of methanol was recorded at the beginning of the experiment. DPPH radical scavenging activity was determined using given equation (1) and the IC₅₀ was calculated.

$$\text{DPPH Scavenging activity} = \left[1 - \frac{\text{Abs}_{\text{control}}}{\text{Abs}_{\text{sample}}} \right] \times 100$$

β -carotene bleaching (BCB) assay

β -carotene bleaching assay was conducted according to the earlier procedure¹⁸. Briefly in 50 ml chloroform, 5 mg of β -carotene was dissolved. Forty milligrams of linoleic acid and 400 mg of Tween-20 were weighed and placed in an Erlenmeyer flask. Three ml aliquot of β -carotene prepared earlier in chloroform was added to it and mixed well. After 5 min the chloroform was evaporated with the aid of nitrogen gas at 30°C. The resultant residue was re-dissolved in 100 ml of deionized water. The absorbance of this solution was measured at 470 and 700 nm.

Briefly 1 ml of β -carotene-linoleic acid emulsion was mixed with different solution of plant extract (3.13 $\mu\text{g/ml}$ to 800 $\mu\text{g/ml}$) prepared earlier, capped and incubated at 50°C for 60 minute. Appropriate control samples containing equivalent amount of methanol were prepared and used in the study. The absorbance of all samples and control solutions were recorded after 60 min at 470 and 700nm. Degradation rate (DR) and antioxidant activity was calculated using given formulas.

$$\text{Degradation rate (DR) of } \beta\text{-carotene} = \text{Ln} \left(\frac{A_{\text{initial}}}{A_{\text{sample}}} \right) / 60 \quad (2)$$

$$\text{Antioxidant activity(\%)} = \frac{(\text{degradation rate of control} - \text{degradation of sample}) \times 100}{\text{degradation rate of control}} \quad (3)$$

Statistical analysis

Results are expressed as mean \pm standard deviation (SD). GraphPad Prism 5 (San Diego, CA, USA) for Windows was used for statistical analyses of experimental data.

RESULTS AND DISCUSSION

It is known that phytochemical reduce the risk of chronic diseases by scavenging the free radicals. Antioxidants appear to play a major role as protective agent for controlling the oxidative stress in diseased condition. Research indicates that the

mechanism of different components depends on their structure and functional groups (poly phenolic compounds, flavonoids and related) responsible for scavenging these radicals. The extracts contain several chemical compounds which are collectively showing DPPH scavenging activity. Studies suggest that the free radical scavenging activity depends on type of phenolic compound as well as total phenolic content.

The phenolic compounds scavenge free radicals through several proposed mechanisms, delocalization of electrons and formation of intramolecular hydrogen bonds are the proposed mechanism by which antioxidant scavenge the DPPH radical.

DPPH radical on reaction with antioxidant loses its free radical activity and the color of reagent changed to yellow from purple. Thereby decreasing the absorbance at 517 nm. The IC₅₀ is calculated graphically by plotting the percent inhibition versus concentration of antioxidant. In the present study the IC₅₀ value for *Pistacia vera* L., *Anchusa strigosa* and *Ilex paraguariensis* A. St.-Hil. extracts were 5.85 \pm 0.11, 43.75 \pm 1.05 and 8.98 \pm 0.65 $\mu\text{g/ml}$ respectively compared to 1.48 \pm 0.05 $\mu\text{g/ml}$ of ascorbic acid. The *P. vera* L. extract showed potent antioxidant which may be due to the phenolic compounds and flavonoids present in the extract. The results are presented here are comparable to the results reported by Özbek *et al.*,¹⁹. IC₅₀ value for *P. vera* L. hull extract prepared using 40% ethanol was 2.73 mg/ml. The antioxidant activity of *I. paraguariensis* leaves extract might be due to the presence of hydroxycinnamic acid derivatives and flavonols present²⁰. The antioxidant activity of these extracts were statistically comparable to the activity of the plant extract collected from different plant (*viz.* *R. officianalis*, *P. harmala*, *T. polium*, *V. officinalis*, *A. herba-alba*, and *A. palaestinum*) from Jordan and Palestine²¹.

The β -carotene bleaching (BCB) assay indicated the *P. vera* L., *A. strigosa* and *I. paraguariensis* A. St.-Hil. extracts were also capable of controlling the oxidation of β -carotene. The IC₅₀ values for β -carotene bleaching (BCB) assay for *P. vera* L., *A. strigosa* and *I. paraguariensis* A. St.-Hil. extracts were 390.1 \pm 7.5, 425.8 \pm 6.5 and 410.2 \pm 9.0 $\mu\text{g/ml}$ respectively compared to 9.5 \pm 0.4 $\mu\text{g/ml}$ of rutin. The antioxidant investigations are reported in Table 3.

Researchers earlier investigated antioxidant nature of phytochemicals such as catechins, gallic acids, quercetin and rutin; caffeic, chlorogenic, sinapic, ferulic and p-coumaric acids²².

Table 1: DPPH radical scavenging activity and β -Carotene bleaching Assay of *Pistacia vera* L. fruit hull, *Anchusa strigosa* flower and, *Ilex paraguariensis* A. St.-Hil. Leaves extract

Samples	IC ₅₀ (μ g/ml) DPPH radical activity*	β -Carotene bleaching Assay*
<i>Pistacia vera</i> L. hull Ext.	5.85 \pm 0.11	390.1 \pm 7.5
<i>Anchusa strigosa</i> flower Ext.	43.75 \pm 1.05	425.8 \pm 6.5
<i>Ilex paraguariensis</i> A. St.-Hil. leaves Ext.	8.98 \pm 0.65	410.2 \pm 9.0
Rutin	-	9.5 \pm 0.4
Ascorbic acid	1.48 \pm 0.05	-

Values are given as mean \pm SD (n=3)

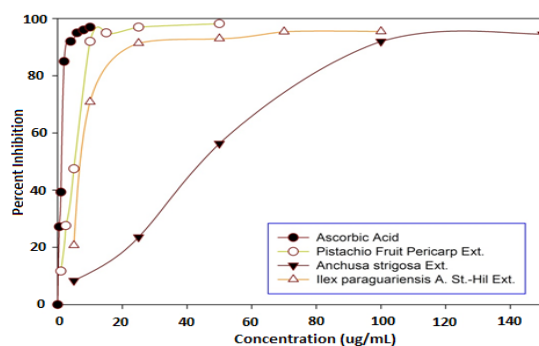


Fig. 1. DPPH radical scavenging activity of *Pistacia vera* L. fruit hull, *Anchusa strigosa* flowers and *Ilex paraguariensis*

CONCLUSION

The methanolic extract of *P. vera* L. and *I. paraguariensis* A. St.-Hil. extracts showed excellent antioxidant comparable to ascorbic acid. *A. strigosa* flower extract showed moderate activity against DPPH radical. The extract shows remarkable inhibition of β -carotene bleaching suggesting to study in detailed profile of phenolic compounds quantitatively to evaluate the antioxidant potential of these herbal plants used frequently and can be exploited in future extensively for controlling oxidative stress and ailments. As these plant extracts are having significant antioxidant activity hence these can also be exploited for the preservation of food, snack foods and beverages industry.

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CONFLICTS OF INTEREST

There are no conflicts of interest.

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