EVALUATION OF ANTI-OXIDANT ACTIVITY AND DETERMINATION OF PHENOLIC CONTENT OF LEAVES EXTRACTS OF *BOUGAINVILLEA GLABRA* 'SNOW WHITE'

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Summary

Our aim of the present study was to evaluate the anti-oxidant activity and determination of the phenolic content in *Bougainvillea glabra* 'Snow White'. The anti-oxidant activity was evaluated against DPPH free radical, ABTS free radical scavenging and superoxide scavenging using BHT (Butylated Hydroxy Toluene) as a standard. The phonolic content of leaves extracts was determined using Folin-coicalteau reagent.The anti-oxidant activity expressed as IC_{50} value and values of hydroalcoholic (50:50) and acetone extracts were 443.55 and 570.83 µg/ml respectively for DPPH free radical scavenging, 220.086 and 181.50 µg/ml respectively, for ABTS free radical scavenging and 155.96 and 173.83 181.50 µg/ml respectively for NBT superoxide scavenging activity. Total phenolic content was expressed as tannic acid equivalent and 1.437 and 1.284 mg/0.005 g of dried extract of hydroalcoholic (50:50) and acetone extract respectively. The correlation between total phenolic content and anti-oxidant activity was determined to be R^2= 0.9362. The results were analysed statistically by regression method. The findings of the study suggested that Acetone and hydroalcoholic extract (50:50) of *Bougainvillea glabra* 'Snow White' was shown effective antioxidant activity. The greater amount of phenolic component leads to the stronger antioxidant activity.

Keywords: Anti-oxidant, *Bougainvillea*, Superoxide scavenging, Total phenolic content.

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Introduction

Free radicals are included many disorders in humans including atherosclerosis, arthritis, ischemia, central nervous system injury, cancer, reperfusion injury of many tissues, gastritis and AIDS [1-2]. Antioxidants can be effective both exogenous and endogenous, whether synthetic or natural, in prohibition of the free radical synthesis by promotion of their decomposition and suppression of such diseases [3-4]. The depletion of immune system antioxidants and free radicals due to environmental pollutants, like chemicals, radiation, toxins, and spicy foods as well as physical stress and mental stress, cause change in gene expression and induce abnormal proteins. Oxidation is one of the most important way for producing free radicals in living systems. Catalase and hydro-peroxidase enzymes used as natural antioxidants in human body and convert hydrogen peroxide and hydro peroxides to non radical forms. Due to depletion of immune system natural antioxidants in different maladies, consuming antioxidants as free radical scavengers may be necessary [5-7]. Natural antioxidants which found from herbal sources is presently growing interest in world [8-10]. Epidemiological and *in vitro* studies on medicinal or herbal plants and fruits and vegetables strongly support the idea that plant constituents with antioxidant activity are exerting protective effects against oxidation stress in biological systems [11-13]. Phenolic compounds with antioxidant activity, which are widely distributed in many vegetables and fruits tea are believed to account mainly for the antioxidant capacity of many plants [14-16].

Therefore, the aim of that study was to evaluate the antioxidant activity of *Bougainvillea glabra* 'Snow White' using three different methods, and to evaluate total phenolic content of the plant.

Material and Methods

Plant material:

The plant material (leaves) was collected from the Balaji Nursery, Jagatpura, Jaipur (Rajasthan), India. The botanical identity of this plant was confirmed by the Dr. N.S. Shekhawat, Head of the Department of Botany, Jai Narayan Vyas University, Jodhpur, (Raj.), India. The voucher

specimen (JNU/Phcog./004/2009) was deposited in the museum of the Department of Pharmacognosy, Jaipur National University, Jaipur-302025, (Raj.), India.

Chemicals and instrument:

All chemicals and solvents used in the study were analytical grade. Solvents viz. petroleum ether, benzene, chloroform, acetone, ethanol (95%), methanol, n-butanol and reagents were procured from Ranbaxy Fine Chemicals Ltd., Mumbai, India. DPPH (1, 1- Diphenyl-2-Picryl Hydrazyl) and ABTS (2, 2-Azino bis (3-ethyl Benzo Thiazoline-6-Sulphonic acid) were obtained from Sigma Chemicals (St. louis, Mo, USA). Ethylene Di-amine tetra acetic acid (EDTA), sodium carbonate and sodium hydroxide were obtained from Ranbaxy Fine Chemicals Ltd. India. NBT (Nitro blue tetrazolium chloride) was obtained from Himedia Laboratories Ltd. Mumbai, India. Tannic acid, Butylated hydroxy toluene (BHT), Potassium per sulphate and Folin Ciocalteau reagent were purchased from Merck India Ltd, Mumbai-18. UV spectrophotometer (Shimadzu 1800) was used for evaluation of anti-oxidant activity.

Extraction:

The plant material was dried in shade and crush in the grinder. The dried powder was weighed in sufficient quantity. Then dried powdered material was initially defatted with pet. ether (60-80 ^{0}C) in a soxhlet apparatus for 72 h according to successive solvent extraction. The pet. ether extract was dried and collected. The mark was dried and successively extracted with acetone and hydro-alcohol (50:50) for 72 h. The extracts were filtered while hot and the solvent was removed by distillation under reduced pressure.

Determination of antioxidant activity:

DPPH free radical scavenging:

1, 1-diphenyl-2-picryl hydrazyl (DPPH) was used for evaluation of antioxidant potential of the different extracts of *Bougainvillea glabra* 'Snow White' [17]. Each herbal extract (hydro-alcoholic and acetone) of different concentrations were added at an equal volume, to methanolic solution of 0.1 mM DPPH (0.39 mg/10 ml methanol). The absorbance was recorded at 517nm after 15 min at room temperature. It was reproduced for 3 times and BHT (Butylated hydroxy

toluene) was used as standard. IC_{50} values were demonstrating the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

Percentage inhibitions of DPPH radical were determined by the following formula.

$$\% \text{ Reduction} = \frac{A_0 - A_1}{A_0} \times 100$$

Where A_0 = absorbance of control

A_1 = absorbance of the extract/ standard

ABTS free radical scavenging:

The ABTS free radical was used for evaluation of anti-oxidant activity of plant extracts. ABTS solution 2mM (0.0548g / 50 ml water) and potassium per sulphate 70 mM (0.0189g / 1ml water) were prepared. 200 µl of Potassium per sulphate and 50 ml of ABTS were taken and mixed in volumetric flask and used after 2 hrs. To the 0.5 ml of various concentrations (100- 600 µg/ml) of extracts (Acetone and hydroalcoholic (50:50)) separately, 0.3 ml of ABTS solution and 1.7 ml of phosphate buffer pH 7.4 was added. For control studies, methanol was taken and the absorbance was measured at 734 nm. The experiment was reproduced in triplicate [18-19].

Percentage inhibitions of ABTS radical by test compound were determined by the following formula.

$$\% \text{ Reduction} = \frac{A_0 - A_1}{A_0} \times 100$$

Where A_0 = absorbance of control

A_1 = absorbance of the extract/ standard

Superoxide scavenging activity:

The method was based on the capacity of the sample to inhibit blue formazan formation by scavenging the superoxide radical generated in riboflavin-light-NBT system. This was performed by the reduction of nitro blue tetrazolium (NBT). In this experiment the superoxide anion was generated in 3 ml of phosphate buffer (100 mM, pH 7.4) containing 0.1 ml of NBT (5 mg in 5 ml phosphate buffer) solution, 0.2 ml of EDTA (402 mg in 10 ml phosphate buffer) and 0.1 ml of different concentrations of the extract. The reaction was influenced by adding 0.1 ml of riboflavin (5 mg in 25 ml phosphate buffer) solution to the mixture. The tubes were uniformly illuminated with an incandescent lamp for 15 min and absorbance was measured at 590 nm. The percentage inhibition of superoxide generation was measured by comparing the absorbance values of control and those of the test compound [20-21]. The antioxidant activity was evaluated by this formula:

$$\% \text{ Reduction} = \frac{A_0 - A_1}{A_0} \times 100$$

Where A_0 = absorbance of control

A_1 = absorbance of the extract/ standard

Total phenolic content determination:

The amount of total phenolics in extract was determined with Folin–Ciocalteu reagent with slight modification using tannic acid as a standard. Briefly, 1.0 ml (solvent water) of extracts (acetone and hydroalcoholic (50:50)) solution (5 mg/ml) was added in a 100 ml volumetric flask that contained about 60 ml distilled water. Then, 5.0 ml of Folin–Ciocalteu reagent was added and the content of the flask was mixed thoroughly. After 1 - 8 min, 15.0 ml Na_2CO_3 (20 %) was added and the volume was made up to 100 ml using distilled water. The mixture was allowed to

stand for 2 h with intermittent shaking. The absorbance was measured at 760 nm using a UV-Vis spectrophotometer (Shimadzu 1800). The total phenolic content was determined as mg of tannic acid equivalent (TAE) using an equation obtained from the standard tannic acid calibration graph [22-23].

Statistical Analysis:

Values are expressed as Mean±SEM. Statistical Difference in mean were analyzed using one way ANOVA followed by Dunnett's test $p< 0.05$ was considered significant.

Results

According to the DPPH assay, both the extracts (hydroalcoholic (50:50) and acetone) of *Bougainvillea glabra* 'Snow White' exhibited a noticeable concentration-dependent anti free radical effect but differed in their inhibiting activities. High scavenging activity was observed for hydroalcoholic (50:50) extract [IC_{50}=443.55 µg/ml], and comparatively low savenging activity was observed for acetone extract [IC_{50} = 570.83 µg/ml] (Figure 1). And IC_{50} for standard compound (BHT), was less than 40 µg/ml (Table 1).

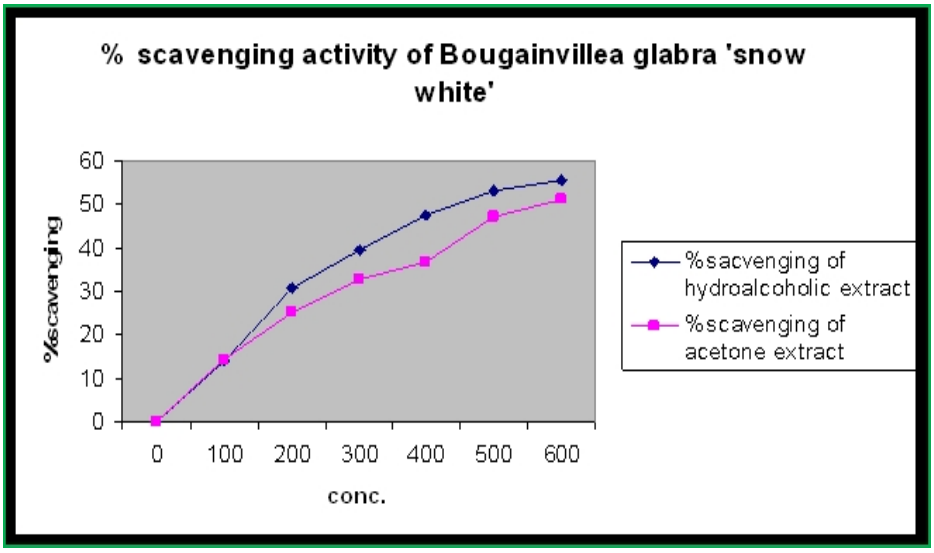

Figure 1. % DPPH free radical scavenging activity of Bougainvillea glabra 'Snow White', where IC50= Inhibitory concentration. Where IC50hydroalcoholic = 443.55 µg/ml. and IC50Acetone = 570.83 µg/ml

Table 1 DPPH Free Radical Scavenging Activity of hydro-alcoholic (50:50) and acetone extract of *Bougianvillea glabra* 'Snow White'

Sr. No.	Concentration (µg/ml)	Hydro alcoholic extract (50:50)		Acetone extract		BHT(0.04 mg/ml) Abs. = 0.6620
		Abs.	% scavenging	Abs.	% scavenging	% scavenging
1	Blank (only DPPH)	1.5029	0	1.5029	0	55.95±0.954
2	100	1.2923	14.01±0.923	1.2863	14.41±0.941	
3	200	1.0403	30.78±0.956*	1.1242	25.19±0.655*	
4	300	0.9106	39.41±0.944*	1.0123	32.64±0.532*	
5	400	0.7889	47.50±0.744*	0.9523	36.63±0.765*	
6	500	0.7027	53.24±0.988*	0.7923	47.28±0.611*	
7	600	0.6700	55.41±0.911*	0.7346	51.12±0.954*	

Results are expressed as Mean±SEM. $p<0.05$ (Dunnett's test), compared to blank, some values are $p<0.01$*

The extracts possessed stronger antioxidant effect compare to the DPPH free radical scavenging. Both the extracts (hydroalcoholic (50:50) and acetone) of *Bougainvillea glabra* 'Snow White' were shown concentration-dependent anti free radical effect but differed in their inhibiting activities. Hydroalcoholic (50:50) extract was shown high anti-oxidant activity [IC_{50}=181.50 µg/ml], and acetone extract was comparatively shown low scavenging activity [IC_{50} = 220.086 µg/ml] (Figure 2). And IC_{50} for standard compound (BHT), was less than 40 µg/ml (Table 2).

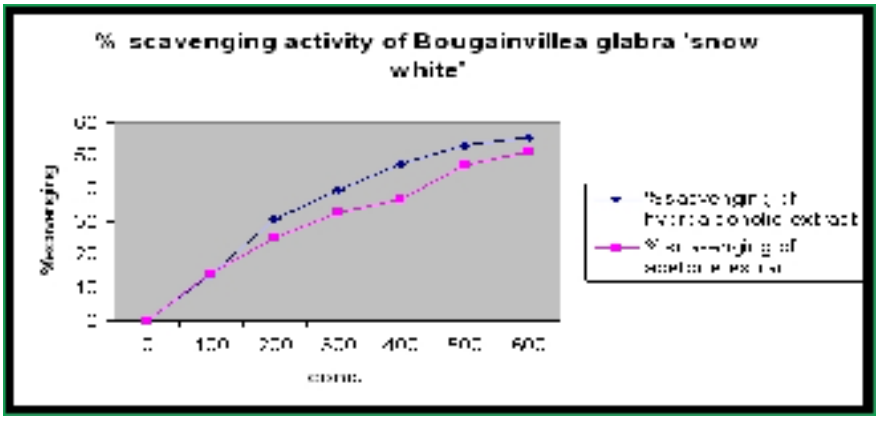

Figure 2. % ABTS free radical scavenging activity of Bougainvillea glabra 'Snow White, where IC50= Inhibitory concentration. Where IC50hydroalcoholic = 181.50 µg/ml and IC50Acetone = 220.086 µg/ml.

Table 2 ABTS Free Radical Scavenging Activity of hydro-alcoholic (50:50) and acetone extract of *Bougianvillea glabra* 'Snow White'

Sr. No.	Concentration (µg/ml)	Hydro alcoholic extract (50:50)		Acetone extract		BHT(0.04 mg/ml) Abs. = 0.212
		Abs.	% scavenging	Abs.	% scavenging	% scavenging
1	Blank (Only ABTS)	0.716	0	0.716	0	70.37±0.926
2	100	0.474	33.70±0.945*	0.490	31.48±0.931*	
3	200	0.331	53.70±0.977*	0.371	48.14±0.951*	
4	300	0.265	62.96±0.916*	0.305	57.40±0.775*	
5	400	0.225	68.51±0.765*	0.246	65.55±0.968*	
6	500	0.196	72.59±0.822*	0.218	69.62±0.959*	
7	600	0.150	79.11±0.933*	0.166	76.70±0.977*	

Results are expressed as Mean±SEM. $p<0.05$ (Dunnett's test), compared to blank, some values are $p<0.01$*

The scavenging property of the both extracts (acetone and hydroalcoholic 50:50) was good but the scavenging property of acetone extract was more compare to the hydroalcoholic extract (50:50). Acetone extract was shown high superoxide scavenging activity [IC_{50}=155.96 µg / ml], and hydroalcoholic extract (50; 50) was comparatively shown low scavenging activity [IC_{50} = 173.83 µg/ml] (Figure 3). And IC_{50} for standard compound (BHT), was less than 40 µg/ml (Table 3).

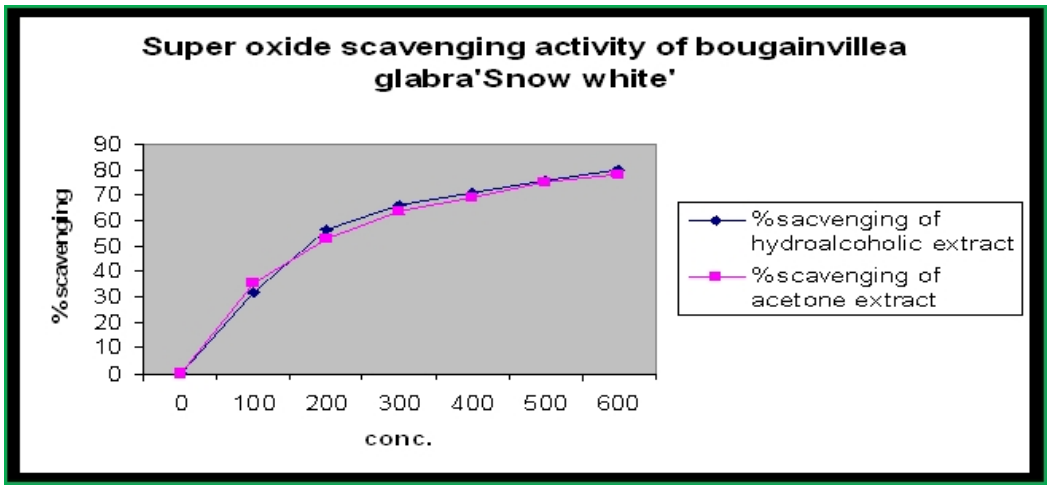

Figure 3. % NBT superoxide scavenging activity of Bougainvillea glabra 'Snow White', where IC50= Inhibitory concentration. Where IC50hydroalcoholic = 173.83 µg/ml and IC50Acetone = 155.96 µg/ml.

Table 3 NBT Superoxide Scavenging Activity of hydroalcoholic (50:50) and acetone extract of *Bougianvillea glabra* 'Snow White'

S. No.	Concentration (µg/ml)	Hydro alcoholic extract (50:50)		Acetone extract		BHT(0.04 mg/ml) Abs. = 0.186
		Abs.	% scavenging	Abs.	% scavenging	% scavenging
1	Blank (only NBT)	0.836	0	0.836	0	78.03±0.954
2	100	0.567	32.08±0.927*	0.541	35.26±0.871*	
3	200	0.365	56.35±0.887*	0.396	52.60±0.755*	
4	300	0.286	65.89±0.764*	0.302	63.87±0.862*	
5	400	0.242	71.09±0.644*	0.258	69.07±0.965*	
6	500	0.205	75.43±0.788*	0.210	74.85±0.924*	
7	600	0.166	80.05±0.811*	0.186	77.74±0.954*	

Results are expressed as Mean±SEM. $p<0.05$ (Dunnett's test), compared to blank, some values are $p<0.01$*

Total phenolic content was expressed as mg/g tannic acid equivalent using the following equation based on the calibration curve: $y = 0.006$ x, $R^2 = 0.9362$, where x was the absorbance and y was the tannic acid equivalent (mg/g) (Figure 4) (Table 4). Total phenolic content was 1.437 and 1.284 mg/0.005 g of dried extract of hydroalcoholic (50:50) and acetone extract respectively (Table 5).

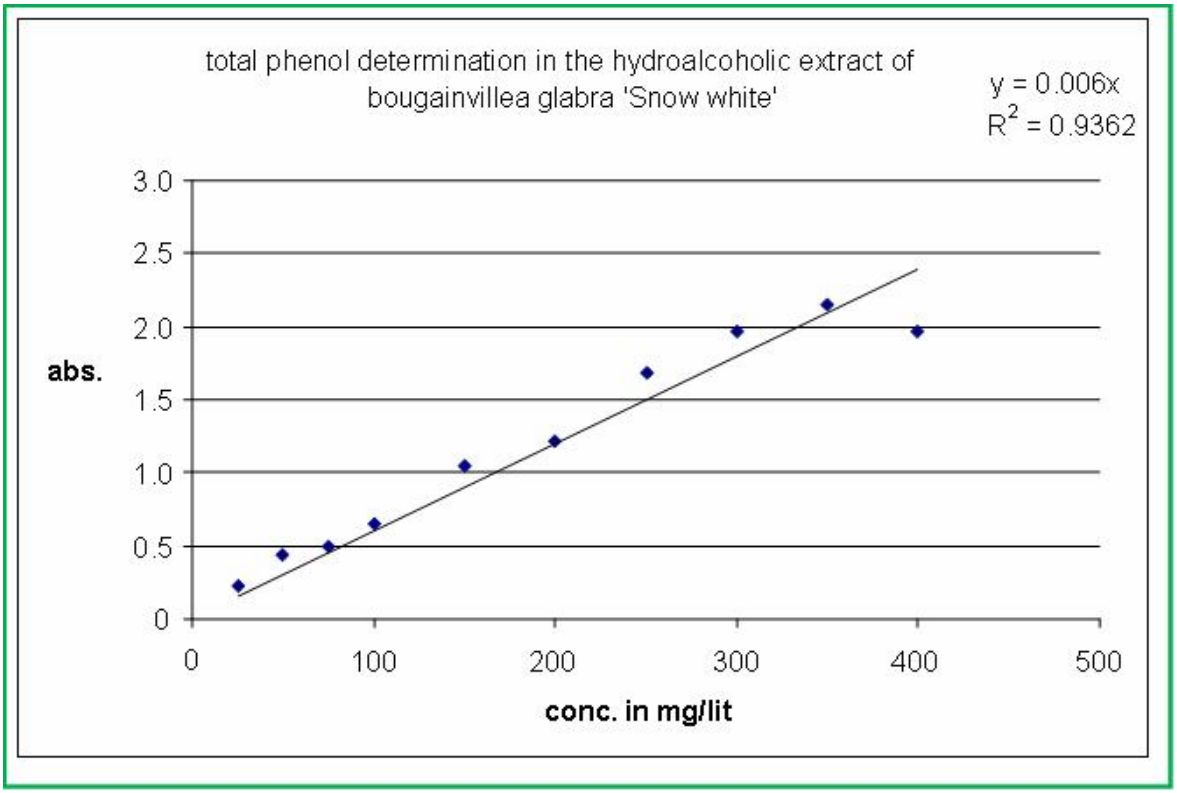

Figure 4. Calibration curve of tannic acid

Table 4 Absorbance of tannic acid at different concentrations

S. no.	Concentration (mg/ml)	Absorbance
1.	25	0.22
2.	50	0.44
3.	75	0.5
4.	100	0.65
5.	150	1.05
6.	200	1.21
7.	250	1.68
8.	300	1.96
9.	350	2.15
10.	400	1.97

Table 5 Total phenolic content of hydroalcoholic (50:50) and acetone extract of *Bougianvillea glabra* 'Snow White'

Extracts	Absorbance	Total phenolic content (mg/0.005 gm)
Acetone extract	2.141	1.284 ± 0.01353*
Hydro alcoholic extract (50:50)	2.396	1.437 ± 0.01513*

Results are expressed as Mean±SEM. $p<0.05$* (Dunnett's test)

Discussion

The extracts and essential oils of many plants have been investigated for their antioxidant activity [24-26]. Secondary metabolites such as polyphenols are not required for plant development and growth, but are involved in plant communication and defense [27-28]. Polyphenols interact with pathogens, herbivores, and other plants; they protect from ultraviolet radiation and oxidants, repel or poison predators and attract beneficial insects or microbes [29-30]. DPPH is relatively stable nitrogen centered free radical that easily accepts an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH radicals react with suitable reducing agents as a result of which the electrons become paired off forming the corresponding hydrazine. The solution therefore loses colour stoichometrically depending on the number of electrons taken up [31].

The decolorization of ABTS+ radical is an unambiguous way to measure the antioxidant activity of phenolic compounds. Recently Awika et al. [32], found positive correlations between the determination of phenolic antioxidant using the oxygen radical absorbance capacity (ORAC), ABTS+, and the 1, 1-diphenyl-2, 2picrylhydrazyl (DPPH) assays. Thus monitoring the antioxidant activity by ABTS+ radical scavenging assay gives good prediction of their ORAC and DPPH radical scavenging capacity.

Superoxide dismutase, catalyse the dismutation of the highly reactive superoxide anion to oxygen and hydrogen peroxide [33]. Superoxide anion is the first reduction product of oxygen [34]. This is measured in terms of inhibition of generation of O_2.

Phenolic compounds are considered as a major group of compounds that contributed to the antioxidant activities of plant materials because of their scavenging ability on free radicals due to their hydroxyl groups [35-36].

Polyphenols are the major plant compounds with antioxidant activity. This activity is believed to be mainly due to their redox properties [37], which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides.

Conclusion

The results from this study strongly suggest that phenolics are important components of these plants, and some of their pharmacological effects could be attributed to the presence of these valuable constituents. In addition, the antioxidant activity may be due to enzymatic and other non-enzymatic antioxidants, which needs further analysis.

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