Phytochemical analysis, antioxidant and antibacterial activities of various extracts from leaves and stems of *Chrozaphora tinctoria*

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Abstract

The purpose of this study was to evaluate the amounts of phenolics, flavonoids, and tannins, and to determine the antioxidant and antibacterial activities of aqueous, acetone, methanol, and ethanol extracts of stems and leaves of *Chrozaphora tinctoria* L. from Zabol, Iran. After collection and extraction of *C. tinctoria* leaves and stems, the total phenolic, flavonoid, and tannin concentrations were determined spectrophotometrically. The antioxidant activity was determined using 1,1-diphenyl-2-picrylhydrazyl radical-scavenging assay, iron-reducing-power assay, β-carotene-bleaching assay, and antioxidant-capacity assay. Antibacterial activity was determined using disc diffusion assay. The results of this study indicated that *C. tinctoria* leaf extracts had higher antioxidant activity than in stem extracts. Methanol and acetone extracts had greater antioxidant and antibacterial activity than aqueous extracts. *C. tinctoria* has antiradical and antimicrobial activity that may be useful in pharmacy and phytotherapy.

Key words: antioxidant activity, antimicrobial activity, *Chrozaphora tinctoria*, phenolics, tannins.
Abbreviations: DPPH, 1,1-diphenyl-2-picrylhydrazyl.

Introduction

Recently, there has been much research emphasis on the antioxidant and antibacterial properties of plants. Plants with these attributes are good resources for general health maintenance and well-being (Sharifi-Rad et al. 2015a). Many microorganisms are responsible for drug resistant infections, and therefore, alternative chemotherapeutic options are necessary and have been the focus of many studies worldwide (Rad et al. 2013; Sharifi-Rad et al. 2014a; Sharifi Rad et al. 2014b).

In recent years, medicinal plant therapy has been shown to be useful for treatment of many human and animal diseases. Since free-radicals and reactive oxygen species are mechanistically involved in the pathogenesis of chronic-degenerative diseases (Sharifi-Rad et al. 2014c), there has been an increasing interest on study of new phytochemicals able to inhibit the production of oxidants at sensitive target sites and to defend cells and tissues from injury. One of the most effective approaches for discovering new antioxidants is the screening of plant extracts used in traditional medicine.

Although plants are much utilized in traditional healing systems, only in some cases have their therapeutic potentials in human been substantiated. The need of herb-based medicines, cosmetics, food supplements, pharmaceuticals and health products is progressively increasing worldwide, because, in some cases, natural products (i) are non-toxic or have low toxicity, (ii) have few side effects and (iii) are available at affordable costs (Sharifi-Rad et al. 2014c).

Free radicals are molecules or molecular fragments that contain an unpaired electron. Free radicals are generally reactive and can cause deleterious effects. In living systems, free radicals are generated as byproducts of normal cellular redox processes and they can be produced by UV radiation and various environmental pollutants.

Excessive production of free radicals can result in oxidative stress, and this is believed to be a fundamental mechanism for a number of human disorders (e.g., neurological, inflammatory, carcinogenesis and psychiatric disorders) (Das Sarma et al. 2010; Rakesh et al. 2010; Sen, Chakraborty 2011).

Dietary antioxidants play an important role in neutralizing excess free radicals, converting them to non-radical products and/or scavenging intermediate radicals (Miguel 2010; Guinot et al. 2010; Maruthappana, Sakthi 2010). Flavonoids and other phytochemical polyphenolics are well known to exhibit antioxidant properties, and these antioxidant properties have been suggested to be responsible for the positive health effects of these compounds (Salah-Fatnassi et al. 2010; Salas et al. 2010; Djeradi et al. 2014).

Chrozophora tinctoria (L.) A. Juss. (Euphorbiaceae) has a distribution range from Spain and northwestern Africa eastward to the Arabian Peninsula and northwestern India (Flora of Pakistan, 2015). The plant inhabits the dry areas coastal areas, sand dunes, and grows in the southeast, northwest, Sistan and Baluchestan of Iran. In Iran, *C. tinctoria* is used to treat fever, warts and as an emetic and cathartic drug (Delazar et al, 2006). Root ashes are given to children for cough. Previous research has shown this plant contains flavonoid glycosides (Delazar et al. 2006; Abdallah et al. 2015) and an essential oil (Dastagir et al. 2014). Crude extracts of *C. tinctoria* have shown phytotoxic allelopathic effect (Kumbhar, Dabgar 2012), antibacterial activity (Dastagir et al. 2012), and antioxidant activity (Shahwar et al. 2010). A phytochemical screen has shown the plant to test positive for alkaloids, flavonoids, tannins, anthraquinones and saponins (Begum et al. 2015).

The main purpose of our current study was to determine the total phenolic, flavonoid, and tannin concentrations in various extracts of the leaves and stems of *C. tinctoria*, to evaluate the antioxidant and antibacterial activity of the extracts.

Materials and methods

Preparation of plant extracts

C. tinctoria leaf and stems were collected in May-June 2014 from the area of Hamun Lake of Zabol (31°1'43" N, 61°30'4" E), Sistan and Baluchestan Province, Iran. The plant was identified at the Department of Botany of Shahid Beheshti University of Medical Sciences, Tehran, Iran, where a voucher specimen was deposited. Leaves and stems were separated before drying. The plant parts were air dried in an oven at 60 °C for 72 h, and plant extracts were obtained by magnetic stirring of 2.5 g of powdered dry matter with 50 mL of solvent (water, ethanol, methanol and acetone) for 40 min at room temperature (24 ± 3 °C). The extracts were kept for 24 h at 4 °C, filtered through Whatman No. 2 filter paper, evaporated to dryness under vacuum and stored at 4 °C until assays.

Total phenol concentration

Total phenol concentration was measured as described by Dewanto et al. (2002). An aliquot of diluted extract was added to 0.5 mL of distilled water and 0.125 mL of Folin–Ciocalteu reagent. The mixture was shaken and allowed to stand for 10 min, before addition of 1.25 mL of 5% Na_2CO_3. The solution was then adjusted with distilled water to a final volume of 4 mL and mixed thoroughly. Absorbance at 760 nm was read versus a prepared blank after incubation in the dark. The total phenol concentration of plant parts was expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE per g DW) from a calibration curve with gallic acid.

Total flavonoid concentration

Colorimetric assay was used for measurement of total flavonoid concentration according to Dewanto et al. (2002). An aliquot of diluted sample or standard solution of (+)-catechin was added to 50 mL of $NaNO_2$ solution (5%) and mixed for 5 min before addition of 0.15 mL $AlCl_3$ (10%). After 5 min, 0.5 mL of NaOH were added. The final volume was adjusted to 2.5 mL with distilled water and mixed thoroughly. Absorbance was determined at 510 nm against a blank. The total flavonoid concentration was expressed as milligrams of catechin per gram of dry weight (mg CE g^{-1} DW) against the calibration curve of (+)-catechin, from 0 to 400 mg mL^{-1}.

Total tannins

Total tannin concentration was determined based on the method of Sun et al. (1998). To 50 µL of diluted sample, 2 mL of 4% vanillin solution in methanol and 1.5 mL of concentrated HCl were added. The mixture was allowed to stand for 25 min, and absorption was measured at 500 nm against methanol as a blank. The amount of total condensed tannins is expressed as mg (+)-catechin g^{-1} DW.

DPPH test

The radical scavenging ability of the obtained extracts was measured by bleaching a purple solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical according to the method of Hatano et al. (1988). The extracts (5, 10, 50 and 100 µg mL^{-1}) were added to 0.5 mL of 0.2 mmol L^{-1} DPPH–methanol solution. After incubation for 45 min at room temperature (25 ± 3 °C), the absorbance was determined versus a blank at 517 nm. The percentage inhibition of free radical DPPH was calculated from

$$(A_{blank} - A_{sample} / A_{blank}) \times 100,$$

where A_{blank} is the absorbance of the control reaction and A_{sample} is the absorbance in the presence of plant extract. The concentration of extract that caused 50% inhibition (IC_{50}) was calculated from the regression equation for the concentration of extract and percentage inhibition. Butylated hydroxy-toluene (BHT) was used as a positive control.

Total antioxidant capacity

Total antioxidant capacity was determined based on the reduction of Mo (VI) to Mo(V) by the extract and subsequent formation of a green phosphate–Mo(V) complex at acid pH (Prieto et al. 1999). An aliquot (0.1 mL) of plant extract was added to 2 mL of reagent solution (0.6 mol L^{-1} H_2SO_4, 28 mmol L^{-1} Na_3PO_4 and 3 mmol L^{-1} ammonium molybdate). The tubes were incubated in a thermal block at 95 °C for 80 min. Once the mixture had cooled to room temperature (25 ± 2 °C), the absorbance of each solution was measured at A_{695} nm versus a blank.

Iron reducing power

The reducing power of the extracts was determined according to the method of Oyaizu (1986). Different concentrations of *C. tinctoria* extracts (200 µL) were mixed with 500 µL of 200 mmol L^{-1} Na_3PO_4 buffer (pH 6.6) and 3 mL of 1% potassium ferricyanide. The mixture was incubated at 45 °C for 30 min; then, 3 mL of 10% trichloroacetic acid (w/v) were added, and the mixture was centrifuged at 650 rpm for 15 min. The upper layer (300 µL) was mixed with 300 µL deionized water and 100 µL of 0.1% ferric chloride, and absorbance was measured at A_{700} nm, higher absorbance indicates higher reducing power. Ascorbic acid was used as the standard. The extract concentration that gave 0.5 absorbance (IC_{50}) was calculated from a graph of absorbance at A_{700} nm against extract concentration.

β-Carotene bleaching

In the β-carotene-linoleate system, β-carotene undergoes rapid discoloration in the absence of antioxidants. The β-carotene bleaching test was carried out as described by Koleva et al. (2002). Briefly, a solution of 2 mg β-carotene was prepared in 20 mL chloroform, and 4 mL of this solution were pipetted into a 250-mL round-bottom flask, with 40 mg linoleic acid and 400 mg Tween 40. The chloroform was removed under reduced pressure in a rotary evaporator at 40 °C, and then the mixture was shaken with 100 mL of aerated distilled water.

Aliquots of 1500 µL of this emulsion were transferred into a series of test tubes containing 100 µL of the extracts or methanol (control), then incubated at 45 °C for 120 min. Butylated hydroxytoluene was used as the reference antioxidant. Absorbance was measured at A470 nm immediately after addition of the emulsion to each tube and then at 120 min. The capacity of the extracts to protect against oxidation of β-carotene was determined from

$$100 \times (A_0 - A_1 / A_1 - A_2),$$

where A_0 is the absorbance of the sample at 120 min, A_1 is the absorbance of the control at 0 min, and A_2 is the absorbance of the control at 120 min. The results are expressed as IC_{50} (µg mL^{-1}).

Assay of antimicrobial activity with dick diffusion test

Crude extracts were screened for antibacterial activity against four human pathogenic Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative bacteria (*Klebsiella pneumoniae* and *Pseudomonas aeruginosa*).

The extracts were dissolved in dimethylsulfoxide to final concentrations of 50 and 12.5 mg mL^{-1}. Sterile discs (6 mm in diameter) were impregnated with 20 mL of the extracts (50 and 25 mg mL^{-1}, respectively). Negative controls were prepared on discs impregnated with dimethylsulfoxide (solvent control). Gentamicin (10 UI) was used as the positive reference for all bacterial strains. Inocula of bacterial strains were prepared from 18-h cultures, and suspensions were adjusted to 0.5 at 570 nm with a spectrophotometer. Petri dishes were prepared with 20 mL of Mueller-Hinton agar; the inocula were spread on top of the solidified medium and allowed to dry for 60 min. The discs with extract were then applied, and the plates were left for 30 min at room temperature to allow diffusion of the extract, before incubation for 24 h at 37 °C. The diameter of the inhibition halo was evaluated in millimeters (Freney et al. 2002).

Statistical analysis

All assays were performed in triplicate. Data were analyzed with the statistical software package SPSS v. 11.5 (IBM Corporation, Armonk, NY, USA). Analysis of variance (ANOVA) and Duncan's multiple range method were used to compare any significant differences between solvents and samples. Values were expressed as means ± standard deviations (SD). Differences were considered significant at $p < 0.05$.

Results

The total polyphenol, flavonoid and tannin concentrations in *C. tinctoria* leaf and stem extracts are shown in Table 1.

Table 1. Total polyphenol (GAE g^{-1} DW), flavonoid (mg CE g^{-1} DW) and tannin (mg (+) catechin g^{-1} DW) concentration in different *Chrozophora tinctoria* extracts. Values followed by different letters are significantly different at $p < 0.05$

Solvent	Polyphenols		Flavonoids		Tannins	
	leaf	stem	leaf	stem	leaf	stem
Water	1.6 ± 0.00 d	0.9 ± 0.01 d	2.5 ± 0.01 c	0.2 ± 0.06 c	2.8 ± 0.03 d	0.2 ± 0.00 c
Ethanol	2.2 ± 0.02 c	1.4 ± 0.03 c	3.8 ± 0.06 b	0.8 ± 0.03 b	3.1 ± 0.01 c	0.9 ± 0.01 b
Methanol	3.6 ± 0.07 b	2.8 ± 0.1 b	3.9 ± 0.01 b	0.8 ± 0.04 b	4.8 ± 0.00 b	0.9 ± 0.04 b
Acetone	4.9 ± 0.62 a	3.3 ± 0.0 a	8.6 ± 0.04 a	1.1 ± 0.02 a	6.3 ± 0.01 a	1.5 ± 0.06 a

Table 2. Scavenging activity, expressed as median inhibitory concentration (IC_{50} μg mL^{-1}), in the DPPH test with *Chrozophora tinctoria* extracts and butylated hydroxytoluene. Values followed by different letters are significantly different at $p < 0.05$

Part	Solvent				Butylated hydroxytoluene
	Water	Ethanol	Methanol	Acetone	
Leaf	>1000	57.2 ± 0.0	26.4 ± 0.2	29.9 ± 0.0	10.23 ± 0.01
Stem	>1000	>1000	375.9 ± 0.4	167.5 ± 0.1	

In all extracts obtained by different solvents, the total polyphenol, flavonoid and tannin concentrations were higher in leaf extracts than in stem extracts. Acetone extract had the highest and the aqueous extract had the lowest concentration of polyphenols, flavonoids and tannins. Polyphenol concentration ranged from 1.6 to 4.9 mg GAE g^{-1} DW in the leaf extracts and 0.9 to 3.3 mg GAE g^{-1} DW in the stem extracts. Flavonoid concentration ranged from 2.5 to 8.6 mg CE g^{-1} DW in leaf extracts and 0.2 to 1.1 mg CE g^{-1} DW in stem extracts. Tannin concentration ranged from 2.8 to 6.3 mg CE g^{-1} DW in leaf extracts and 0.2 to 1.5 mg CE g^{-1} DW in stem extracts.

The scavenging activity in the DPPH test with *C. tinctoria* extracts and butylated hydroxytoluene (BHA) are shown in Table 2. The methanol extract had the maximum and the aqueous extract had the minimum antiradical activity in the leaf extracts. The acetone extract had the maximum antiradical activity and the aqueous and ethanol extracts had the minimum antiradical activity in the stem extracts. Acetone, ethanol and methanol extracts of *C. tinctoria* had more efficient antiradical activity than butylated hydroxytoluene.

Total antioxidant activity of leaf and stem extracts of *C. tinctoria* is shown in Fig. 1. The acetone extract of leaves and stems of *C. tinctoria* had the highest total antioxidant activity and aqueous extract had the lowest total antioxidant activity. Antioxidant activity ranged from 22.5 to 59.9 mg GAE g^{-1} DW in leaf extract and 4.9 to 6.5 mg GAE g^{-1} DW in stem extract.

The iron reducing power and the β-carotene bleaching activity of extracts are summarized in Table 3. The reducing power shows the potential of *C. tinctoria* to reduce ferric ion, and this depended both on the plant part (stems or leaf) and the solvent type. The reducing power of the leaf extract in this plant was higher than stem extract. The ethanol extract had maximum activity, followed by methanol and then acetone extracts in leaves and methanol, ethanol and then acetone in stems. The addition of *C. tinctoria* extract to the β-carotene linoleate system prevented bleaching. In terms of inhibition of β-carotene bleaching, the methanol extract had the highest activity and aqueous extract had the lowest activity. The bleaching activity (IC_{50}) of *C. tinctoria* leaves ranged from 345.9 to >1000 μg mL^{-1}, and 625.9 to >1000 μg mL^{-1} in leaf and stem extracts, respectively.

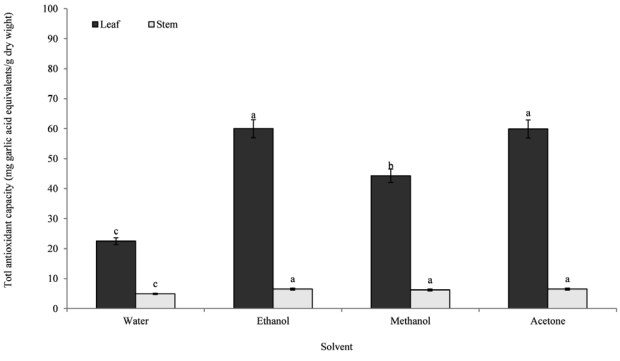

Fig. 1. Total antioxidant capacity (expressed as mg gallic acid equivalents/g dry weight) of Chrozophora tinctoria extracts. Values followed by different letters are significantly different at $p < 0.05$.

The antibacterial activities of *C. tinctoria* extracts against human pathogenic bacteria *S. aureus, B. subtilis, K. pneumoniae* and *P. aeruginosa* are shown in Table 4. The acetone leaf extract of *C. tinctoria* had the highest antimicrobial activity against *S. aureus, B. subtilis* and *P. aeruginosa*, while the aqueous extract had the lowest antimicrobial activity against these human pathogenic bacteria. The methanol leaf extract had the highest antimicrobial activity against *K. pneumoniae*. There was a dose dependence in antibacterial activities with 1000 μg per disc concentration of each solvent showing maximum effects on the bacteria, but no antimicrobial activity at concentrations of 200 μg per disc.

Discussion

In this study, total phenolic, flavonoid and tannin concentrations, antioxidant activity and antimicrobial activity were determined in *Chrozaphora tinctoria* extracts obtained by different solvents. These parameters usually depend on the type and polarity of solvent. (Medini et al. 2014). In the present study, acetone extract of *C. tinctoria* had the maximum concentration of polyphenols, flavonoids and tannins. This is is similar to results of Medini et al. (2014) obtained using *Limonium delicatulum*, where acetone extract had the highest yield of total polyphenols, while methanol extract gave the highest yield of tannins. Several researchers have examined the effects of solvents for extraction of natural products; solvent polarity can have a large effect on extraction of different phytochemical classes (Yusha'u 2011; Stanković, Topuzović 2012; Daoud et al 2015). Eloff (1998) showed that acetone as an extraction solvent was superior compared to ethanol, methanol, dichloromethane, methanol/chloroform/water, and water,

Table 3. Reducing power and β-carotene bleaching activity (IC_{50}, μg mL^{-1}) of aerial parts of *Chrozophora tinctoria*. N.D., not determined. Values followed by different letters are significantly different at $p < 0.05$

Solvent	Reducing power		β-carotene bleaching	
	leaf	stem	leaf	stem
Water	>1000 a	>1000 a	>1000 a	>1000 a
Ethanol	62.3.6 ± 0.2 d	532.3 ± 0.1 b	459.7 ± 0.3 c	795.4 ± 0.2 c
Methanol	108.9 ± 0.1 c	229.9 ± 0.4 d	345.9±0.7 d	652.9 ± 0.8 d
Acetone	254 ± 0.0 b	359.2 ± 0.9 c	652.3 ± 0.1 b	856.3 ± 0.2 b
Ascorbic acid	52.5 ± 0.0 e		ND	
Butylated hydroxytoluene	ND		84.3±0.1 e	

both in terms of number of components extracted as well as the number of antibacterial substances extracted. However, in terms of antioxidant activity and polyphenolic concentration, methanol has been shown to be a superior extraction solvent compared to acetone or ethyl acetate for several medicinal plants (Miliauskas et al., 2004). Similarly, Hayouni et al. (2007) studied extraction of *Quercus coccifera* and *Juniperus phoenicea* fruits with different solvents and found that polar solvents were more efficient in extracting polyphenols than non-polar solvents and that the more polar extracts had greater antioxidant and antibacterial activities. On the other hand, polar organic solvents like acetone are better than water for extraction of polyphenols (Kratchanova et al. 2010).

Polyphenols have the highest antioxidant activity among secondary metabolites in plants. Phenolics are oxidized by a wide range of free radicals forming stable radical intermediates (Koolen et al. 2013; Farasat et al. 2014). Polyphenols have reducing power and act as electron donors, singlet and triplet oxygen quenchers as well as metal chelators (Delazar et al. 2006; Shawkatul et al. 2015). In the present study, as in many other studies (Da-Silva et al. 2011; Stanković, Topuzović 2012; Iqbal et al. 2015) phenolic concentration in leaf extracts was higher than in stem extracts. The antioxidant activities in the present study are comparable to these observed by Shahwar et al. (2010), who found that ethyl acetate extract of *C. tinctoria* had greater antioxidant activity (DPPH radical-scavenging, total antioxidant activity, inhibition of lipid peroxidation) than petroleum ether, chloroform, or methanol/butanol extracts. Similarly, the ethyl acetate extract had the highest concentration of total phenols.

Although phenolics increase the antioxidant activity of plant extracts, there does not seem to be a "linear" correlation between total polyphenol concentration and antioxidant activity of the same extract; antagonistic and synergistic interactions of polyphenolics with each other, and the presence of glycosylated flavonoids and anthocyanins can increase or decrease the antioxidant activity of a plant extract (Koolen et al. 2013; Daoud et al. 2015; Wong-paz et al. 2015).

The antioxidant activity of flavonoids (such as flavones, flavanols and condensed tannins) is due to their free OH groups, especially 3-OH. They suppress the formation of reactive oxygen species, inhibit metabolic processes, nucleic acid biosynthesis, platelet aggregation, and mast

Table 4. Chemical properties of cow manure vermicompost used in the present experiments. Values followed by different letters are significantly different at $p < 0.05$

Solvent	Concentration (μg $disc^{-1}$)	*Staphylococcus aureus*	*Bacillus subtilis*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*
Water	200	0	0	0	0
	500	0	8.2 ± 0.5 g§	11.5 ± 0.2 g	0
	1000	15.6 ± 0.3 e	18.4 ± 0.1 c	13.9 ± 0.4 f	8.3 ± 0.2 f
Ethanol	200	0	0	0	0
	500	13.2 ± 0.1 f	15.2 ± 0.1 d	9.5 ± 0.4	6.5 ± 0.2h
	1000	15.5 ± 0.3 e	12.8 ± 0.4 f	14.9 ± 0.5 e	12.2 ± 0.1 e
Methanol	200	0	0	0	0
	500	12.9 ± 0.3 g	14.4 ± 0.2 e	15.3 ± 0.2	13.2 ± 0.1 d
	1000	18.9 ± 0.5 c	19.3 ± 0.7 b	19.4 ± 0.1 b	15.5 ± 0.2 c
Acetone	200	0	0	0	0
	500	16.3 ± 0.1 d	12.5 ± 0.2 f	9.5 ± 0.2 h	5.5 ± 0.3 g
	1000	19.9 ± 0.3 b	19.8 ± 0.2 b	17.4 ± 0.3 c	18.7 ± 0.5 b
Gentamicin	10 UI	25.0 ± 0.0a	22.5 ± 0.3 a	23.4 ± 0.2 a	20.5 ± 0.2 a

cell histamine release (anti-inflammatory effect), and they exhibit antimicrobial activities, etc. (Babaa, Malik 2015). Tannins are polyphenols that are known to bind and precipitate microbial proteins and render nutritional proteins unavailable for bacteria. They cause a bitter and dry feeling in the mouth and small concentrations are useful for digestive problems because they bind iron and reduce non-heme iron absorption (Tshivhandekano et al. 2014; Iqbal et al. 2015).

The basis of radical scavenging assay is a decrease in extract absorbance (decrease in the concentration of DPPH radicals) in comparison to a DPPH standard solution (Stanković, Topuzović 2012). A decrease in DPPH radical concentration indicates an increase in antioxidant activity (Daoud et al. 2015). In the DPPH test, the aqueous extract had the lovest antiradical activity presumably because of the reduced water-solubility of radical-inhibiting polyphenolics (Da-Silva et al. 2011).

In this study, acetone and methanol extracts of *C. tinctoria* showed the greatest antibacterial activities, while the aqueous extract had the lowest (Table 4). Dastagir et al. (2012) previously reported that a methanol extract of *C. tinctoria* showed notable antibacterial activity against *Salmonella typhi* and *Escherichia coli* as well as *P. aeruginosa, S. aureus*, and *K. pneumoniae*. Antimicrobial activity is one of the important properties of polyphenolic compounds (Medini et al. 2014). The antimicrobial activity of phenolics is due to their partially hydrophobic nature. They inhibit the hydrolytic enzymes (proteases), microbial adhesion, cell envelope transport proteins etc. (Babaa, Malik 2015). Additionally, flavonols can bind with extracellular and soluble proteins and with bacterial cell walls.

Most antibacterial medicinal plants are effective on Gram-positive bacteria, but few against Gram-negative bacteria, as the construction of these two types of bacteria differs. Gram-negative bacteria have a lipopolysaccharide barrier on their outer membrane, while the Gram-positive bacteria do not (Wei et al. 2015). The present study showed that acetone extract of *C. tinctoria* is effective on both Gram-positive (*S. aureus* and *B. subtilis*) and Gram-negative (*K. pneumoniae* and *P. aeruginosa*) bacteria.

In conclusion, leaf extract of *Chrozaphora tinctoria* displayed higher antiradical activity than the stem extract, and the methanol leaf extract had the maximum antiradical activity. The results of this study confirm that *C. tinctoria* has antioxidant and antimicrobial potential and may be useful in pharmaceutical and phytotherapeutic applications. Additional studies on all parts of this plant, additional bioactivity screening, and phytochemical isolation and structure elucidation should be carried out to more completely discover the medicinal potential of *C. tinctoria*.

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