

Chemical composition, antioxidant and antibacterial activities from methanolic extract of *Crataegus monogyna* Jacq. (Rosaceae)

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ABSTRACT

The monogynous hawthorn or *Crataegus monogyna* Jacq (Rosaceae) is a medicinal plant used since antiquity in traditional medicine, recognized by its therapeutic virtues. This study aims to estimate the total phenols and flavonoids contents and to evaluate the antioxidant and antibacterial activity *in vitro* from the methanolic extract of the *C. monogyna* leaves. Antioxidant activity was investigated with six methods, DPPH, ABTS, galvinoxyl scavenging activities, FRAP, CUPRAC and phenanthroline assays, Total phenols and flavonoids contents were studied by Folin–Ciocalteu and aluminum colorimetric methods. The antibacterial activity of the methanolic extract was evaluated against *Staphylococcus aureus*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Streptococcus Sp* in the base of inhibition zone diameter using agar diffusion method. The findings showed a significant antioxidant activity by the methods of DPPH ($IC_{50}=28.00\pm 0.97\mu\text{g/ml}$), CUPRAC ($A_{0.5}=28.79\pm 2.10\mu\text{g/ml}$), ABTS ($IC_{50}=10.12\pm 0.60\mu\text{g/ml}$), phenanthroline ($A_{0.5}=36.84\pm 1.85\mu\text{g/ml}$), GOR ($IC_{50}=20.76\pm 0.40\mu\text{g/ml}$) but this activity is absent with FRAP assay. The amount of TPC was very high in this extract ($201.47\pm 0.55\mu\text{g EAG/mg}$), while the value of flavonoids was moderate ($75.48\pm 5.77\mu\text{g EQ/mg}$). The methanolic extract of *C. monogyna* leaves showed a significant antibacterial potency. The present study has demonstrated that *C. monogyna* extract possess potent antioxidant capacity and a good antibacterial activity, which could be include in medicinal use.

KEY WORDS

Crataegus monogyna; phytochemical screening; antioxidant capacity/Antibacterial activity.

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INTRODUCTION

Crataegus monogyna Jacq, which belongs to the Rosaceae family, is known as “Hawthorn”. This medicinal herb is a small tree and shrubs naturally growing in Europe, Asia and the north of Africa and populates the mountains of the Mediterranean basin (Vikrant & Narender, 2012). *Crataegus monogyna* is frequently used for the treatment of circulatory and respiratory system disorders, in-

somnia and stress, nervousness, sleep disorders, stomachache and sore throat. Both the fruits and flowers of hawthorns are well known in herbal folk medicine as a heart tonic, migraines, irritability and confusion (Camejo-Rodriguez et al., 2003; Novais et al., 2004; Neves et al., 2009). *Crataegus monogyna* leaves have multiple interests in industry, food and cosmetology (Mohand, 2006).

The aerial parts of this medicinal herb possess several compounds like simple fatty acids, terpe-

noid and polyphenolic contents. Globally, flavonoids, particularly flavonols and flavones, are abundant in flower buds, while proanthocyanidins are found in higher amount in unripe fruits, but the highest level of flavonoids (rutin, vitexin, vitexin-211-O-rhamnoside, and hyperoside) are found in the leaves (Sokół-Łętowska et al., 2007; Zhang et al., 2001).

As medicinal plant, *C. monogyna* demonstrated strong antioxidant and antibacterial activities throughout several studies. Belkhir et al. (2013) investigated the chemical composition of the phenolic extracts prepared from leaf, fruit, and syrup of *C. monogyna*. These extracts, which contain hyperoside and procyanidins as main compounds, showed high *in vitro* antioxidant and antiradical activity. Moreover, due to the high antioxidant activity of hawthorn leaf extracts, *C. monogyna* has been employed as ingredient for potential dermatological products (João et al., 2013). Also, hawthorn aerial parts extracts showed a moderate antibacterial activity, especially against Gram-positive bacteria such as *Micrococcus flavus*, *Bacillus subtilis*, and *Lysteria monocytogenes*. In addition, Tunisian *C. azarolus* and *C. monogyna* extracts exhibited potent antimicrobial ability in comparison to ampicillin and oxytetracyclin, this activity was recorded against Gram-positive *S. aureus* and *S. faecalis* bacteria, whereas the lowest activity was against the Gram-negative *Salmonella* strain (Barros et al., 2012; Belkhir et al., 2013).

This current study aims to estimate the total phenols and flavonoids contents and to evaluate the antioxidant and antibacterial activity *in vitro* from the methanolic extract of the *C. monogyna* leaves.

MATERIAL AND METHODS

Plant material

Crataegus monogyna leaves were collected from the city of Mahmoud Bouchtâta in “Skikda” province (Eastern Algeria) during the month of December 2018. The plant collection and use were in accordance with all the relevant guidelines. The plant has been identified at the department of Animal Biology, Faculty of Nature and Life Sciences Mentouri Brothers University Constantine1, Algeria, following the description of Quézel & Santa (1962). The leaves were shade-dried, powdered,

and stored in a tightly-closed container at ambient temperature in darkness for further use.

ABBREVIATIONS. DPPH: 2,2-diphenylpicrylhydrazyl. FRAP: ferric reducing/antioxidant power. GAE: gallic acid equivalent. DMSO: dimethyl sulfoxide.

Extracts preparation

Crataegus monogyna leaves were prepared by maceration in hydro-alcoholic solution (methanol/water: 70:30: v/v) for 24 h at room temperature. This operation was repeated thrice with renewal solvent. The obtained extract was concentrated in Rotary Evaporator (Buchi.R-210) at a temperature equal to 40 °C in order to obtain the crude dried extract.

Determination of total phenolic content (TPC)

TPC was determined using the modified Folin-Ciocalteu method of Singleton & Rossi (1965). 20µL sample (1mg/mL) was mixed with 100µL Folin-Ciocalteu reagent (diluted ten-fold) and 75µL (75 g/L) sodium carbonate. Absorbance was measured at 740nm in the microplate reader after 2h incubation in darkness at room temperature. Results were expressed as micrograms of gallic acid equivalents per milligrams of extract (µg GAE/mg) obtained from a calibration curve of 25–500µg/mL of gallic acid.

Determination of total flavonoid content (TFC)

TFC was determined using the modified method of Topçu et al. (2007). 130 µL of methanol were transferred into a micro-plate (96-wells) containing 50 µL of each extract and then 10 µL of potassium acetate (1 M) and 10 µL of aluminum nitrate at 10% were added. The Incubation was at room temperature for 40 min. The absorbance was measured at 415 nm in the micro-plate reader. The results were expressed as mg of quercetin equivalent per gram (mg/QE/g) of extract.

Antioxidant activity

Antioxidant activity of extract was evaluated by employing six different models: DPPH, galvinoxyl and ABTS scavenging activities, phenanthroline, cupric and ferric reducing power assays.

DPPH Free Radical Scavenging

DPPH scavenging activity was determined spectrophotometrically by the method described by (Blois, 1958). The test was realized in a 96-well micro-plate. 160 μL of prepared DPPH methanolic solution (0.1mM) was mixed with 40 μL of sample solutions at different concentrations. The absorbance of the reaction mixture was measured after incubation (30 min). The scavenging activity of DPPH was calculated using the following equation and the results were given as 50% inhibition concentration (IC_{50}).

$$\text{Inhibition \%} = (\text{A control} - \text{A sample}) / (\text{A control}) \times 100$$

The results were compared to the standard antioxidant BHA and BHT.

Cupric ion reducing antioxidant capacity (CUPRAC)

Cupric reducing antioxidant capacity was determined according to the method described by (Apak et al., 2004) with slight modifications. 50 μL CuCl_2 (10mM), 50 μL neocuproine (7.5mM) and 60 μL of ammonium acetate buffer (1 mol/L, pH 7.0) solutions were added to each well, in a 96 well plate containing 40 μL extracts at different concentrations. Absorbance was measured at 450nm after 1h. Results were given as absorbances ($A_{0.5}$ $\mu\text{g/mL}$) comparing with standards BHT, BHA and α -Tocopherol.

Reducing power assay

The reducing power was determined according to the method of Oyaizu (1986). Different concentrations of extracts were mixed with 0.2 M phosphate buffer (pH 6.6) and 1% of potassium ferricyanide $\text{K}_3\text{Fe}(\text{CN})_6$ after incubation at 50° for 20 min; 10% of trichloroacetic acid and 10 μL ferric chloride FeCl_3 (0.1%) were added. The results were given as absorbance $A_{0.5}$. The $A_{0.5}$ value is the effective concentration at which the absorbance is 0.5 and is obtained by the equation described for DPPH assay. Ascorbic acid and α -Tocopherol were used as standards for comparison.

ABTS radical scavenging assay (acid 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic)

The ABTS activity is determined by the ABTS cation decolorization assay as described by Re et al, (1999). 40 μL of each fraction at different con-

centrations was placed in micro-plate (96-wells) and then 160 μL of ABTS^{++} solution was transferred, the absorbance was measured at 734 nm after 6 min. The ABTS radical cation (ABTS^{++}) was produced by mixing two aqueous solutions 7 mM stock solution of ABTS with 2.45 mM potassium persulfate and allowing the mixture to stand in dark at room temperature (25 °C) for 12 h, the absorbance of the solution thus obtained is adjusted by Ethanol or H_2O to 0.700 ± 0.020 at 734 nm before use. The scavenging rate and IC_{50} value were calculated using the equation described for DPPH assay. α -Tocopherol and butylated hydroxyanisol (BHA) were used as standards for comparison.

Phenanthroline test

This activity was determined by the method of Szydłowska-Czerniaka et al. (2008). 30 μL of phenanthroline was deposited into micro-plate (96-wells) containing 10 μL of methanolic extract. Then 50 μL of FeCl_3 (0.2%) and 110 μL MeOH were transferred. The mixture was incubated in the dark for 20 min at 30 °C. The absorbance was measured at 510 nm. Butylated hydroxyanisol (BHA) were used as standards for comparison.

Galvinoxyl radical scavenging assay (GOR)

This activity (GOR) was determined by the method of Shi et al. (2001). 160 μL of galinoxyl (0.1 mM) were placed in micro-plate (96-wells) containing 40 μL of methanolic extract. After 120 min of incubation at room temperature the absorbance was measured at 428 nm. BHT and butylated hydroxyanisol (BHA) were used as standards for comparison.

Statistical Analysis

Results are reported as mean value \pm SD of three measurements; the IC_{50} and $A_{0.50}$ values were calculated by linear regression analysis, and one-way analysis of variance ANOVA to detect significant differences ($p < 0.05$) using XLSTAT.

Antibacterial activity

The antibacterial activity of the methanolic extract was evaluated using gram-positive bacteria *Streptococcus* sp. and *Staphylococcus aureus* and

gram-negative bacteria: *Pseudomonas aeruginosa* (ATCC75/2.5), *Escherichia coli* (ATCC235) and *Proteus mirabilis*. These pathogenic bacteria were obtained from microbiology laboratory of Abderrezak Buhara hospital at "Skikda" province. Bacterial species were cultured overnight at 37 °C in nutrient agar medium for 24 h. Subsequently, sterilized paper discs (6 mm) were impregnated with 20 µL of extract (2.5mg/ml) and placed on plates containing Mueller–Hinton agar spreaded previously with 1 ml of each bacterial suspension (10⁸ CFU / ml). Negative control was prepared using DMSO and Gallic acid was used as positive control. All the plates were incubated at 37 °C for 24 h. Antibacterial activity was determined by measuring the zone of inhibition in millimeters. All of the measurements were done in duplicate (Bauer et al., 1966).

RESULTS AND DISCUSSION

Total phenolic compounds (TPC) and flavonoids contents (TFC)

From the results shown in Table 1, we can see that the hydroalcoholic extract *C. monogyna* leaves is very rich in polyphenolic compounds with value to 201.74±0.55 µg GAE/mg. But the same extract possesses moderate flavonoids content with value 75.48±5.77 µg QE/mg. In similar study Hamdaoui et al. (2015) have found low levels of phenols and flavonoids in the methanolic extract of *C. monogyna* leaves with values 34.82 ± 0.63, mg EAG/g of phenols and 17.79 ± 0.55 mg EC/g of flavonoides. In this case several factors can influence the content of phenolic compounds. Recent studies have shown that extrinsic factors (such as geographical and climatic factors), genetic factors, the degree of maturation of the plant and the duration of storage have a strong influence on the content of polyphenols (Aganga & Mosase, 2001). In addition, quercetol

heterosides, rhamnosides, flavanic polymers, procyanidin oligomers, chlorogenic acid and caffeic acid are the major types of phenols found in *C. monogyna* leaves (Bruneton, 1993; Fong & Bouman, 2002; Mohand, 2006).

Antioxidant activity

In this study, the antioxidant activity of *Crataegus monogyna* extract was evaluated by using DPPH scavenging, galvinoxyl and ABTS radicals scavenging, phenanthroline, ferric reducing power and CUPRAC assays. The results of antioxidant activity are shown on Table 2 and expressed in terms of IC₅₀ and A_{0.5}.

According to the obtained values of the IC₅₀, it can be concluded that the methanolic extract has good anti-free radical activity IC₅₀ rich to 28.00 ± 0.97µg / ml. This activity is four times lower than that of the BHA IC₅₀ standard (6.14 ± 0.41 µg / ml). Moreover, the antioxidant capacity detected by the ABTS method was significant with IC₅₀=10.12±0.60µg/ml and close to that registered in the standard. Furthermore, the antiradical ability assessed employing the radical galvinoxyl showed considerable activity with IC₅₀ 20.76±0.40 µg/ml. Similarly, the measurement of antioxidant capacity via a Fe (III)-phenanthroline and cuivre-neocuproine (CUPRAC assay) of the same extract showed important activity with A_{0.5}(36.84±1.85µg/ml, 28.79±12.10, respectively) but this methanolic extract no activity recorded using FRAP assay. In this context, different antioxidant capacities were determined in the aerial parts of *C. ambigua* Becker subsp. *ambigua*, *C. caucasica* C. Koch, *C. meyeri* Pojark., *C. monogyna* var. *monogyna* species using FRAP, CUPRAC and ABTS assays. Values was CUPRAC>ABTS>FRAP and all the samples exhibited high antioxidant activity (Özyürek et al, 2012). In addition, Bahorun et al. (1994) have shown that the leaf extracts of *C. monogyna* dem-

Methanolic extract	TPC (µg GAE/mg) *	TFC (µg QE/mg) **
	201.74±0.55	75.48±5.77

Table 1. Total phenolics and flavonoids contents of methanol extract of *Crataegus monogyna* leaves. *Total phenolics is expressed as µg Gallic acid equivalents/mg of extract. **Total flavonoids are expressed as µg Quercetin equivalents/mg of extract.

Leaf samples	CUPRAC reducing (A _{0.50} µg/mL)	FRAP reducing (A _{0.50} µg/mL)	Phenanthroline (A _{0.50} µg/mL)	ABTS radical (IC _{0.50} µg/mL)	DPPH radical (IC _{0.50} µg/mL)	Galvinoxyl radical GOR (IC _{0.50} µg/mL)
methanolic extract	28.79±12.10	na*	36.84±1.85	10.12±0.60	28.00±0.97	36.84±1.85
BHT	9.62±0.87	/	2.24±0.17	1.59±0.03	6.55±0.59	3.32±0.18
BHA	3.64±0.19	/	0.93±0.07	1.03±0.00	6.14±0.41	5.38 ±0.06
Ascorbic acid	/	6.77±1.15	/	/	/	/
α-Tocopherol	/	34.93±2.38	/	/	/	/

Table 2. Antioxidant activity (A_{0.50} µg/mL and IC_{0.50} µg/mL) of *Crataegus monogyna* leaves; *na: no activity.

onstrated excellent antioxidant ability. In previous study, the ethanolic extract of *C. monogyna* leaves exhibited moderate antioxidant activity using ABTS radical scavenging (Serhat et al., 2014). Further, *C. monogyna* fruits and aerial parts reveal higher DPPH scavenging capacity (3.61 ± 0.01 and 3.34 ± 0.38 µg/mL, respectively) and higher ferric reducing antioxidant power (85.65 ± 0.09 and 95.05 ± 0.15 µmol TE(trolox equivalents)/g, respectively) (Simirgiotis, 2013). On the contrary Hamdaoui et al. (2015) found that the methanolic extract of *C. monogyna* leaves has a weak activity with IC₅₀ 196.70 ± 13.96 µg/ml. As it is well known, the antioxidant activity increases with high total phenols and flavonoids content, however no correlation registered both between FRAP/DPPH and FRAP/TPC/TFC. This low relationship between the antioxidant assays and phenols or flavonoids content can be due to the different antioxidant capacity. The FRAP assay is based on the capability of the substance to reduce Fe³⁺ to Fe²⁺ while the DPPH assay the hydrogen donating capacity to scavenge DPPH radicals (Barreira et al., 2013).

Antibacterial Activity

Methanolic extract of *C. monogyna* leaves showed significant antibacterial capacity particularly against *Staphylococcus aureus*. As results, *C. monogyna* extract exhibited the highest inhibition zone against *Staphylococcus aureus* (inhibition zone of 14 mm), against *Proteus mirabilis* (13 mm),

Bacteria species	<i>Crataegus monogyna</i> Jacq. leaves 2.5mg/ml
<i>Staphylococcus aureus</i>	Diameters of Inhibition zone (mm) 14
<i>Proteus mirabilis</i>	13
<i>Pseudomonas aeruginosa</i> (ATCC75/2.5)	12
<i>Escherichia coli</i> (ATCC235)	11
<i>Streptococcus</i> sp.	10

Table 3. Antimicrobial activity of *Crataegus monogyna* expressed as the diameter of the inhibition zone in mm. Resistant (-): < 8mm. Sensitive (+): 9–14 mm. Very sensitive (++) : 15–19 mm. Extremely sensitive (+++) : >20 mm (Ponce et al., 2003).

Pseudomonas aeruginosa (12 mm) (Table 3). Both *E. coli* and *Streptococcus* sp. were moderately sensitive with inhibition zone of 11 mm and 10 mm, respectively, so this medicinal herb registered a good antibacterial capacity against both Gram- and Gram+ bacteria. As it is well known, many parameters can elucidate the different antibacterial abilities of diverse plants like extraction solvent, total phenols and flavonoids contents and the character of utilized bacteria (Elias et al., 1991; Fauchere, 2002; Klervi, 2005). In this case a few studies highlighted the antibacterial potential of *C. monogyna* which showed various results. Djeddi & Boutaleb (2014) indicated that the methanol extract of *C. monogyna* showed a strong antimicrobial activity against *A. baumannii* (20.00 mm) and a moderate

effect against *E. coli* (10.66 mm). In contrary, Benmalek et al. (2013) found that the extract of *C. oxyacantha* ssp *monogyna* leaves had a larger inhibiting activity on the growth of *E. coli* and *Pseudomonas aeruginosa*.

CONCLUSIONS

The findings of this current study show a significant antioxidant capacity and a potent antibacterial activity from the methanolic extract of *Crataegus monogyna* leaves. This medicinal plant registered a high levels of phenols and a moderate flavonoids content. As a whole, *C. monogyna* can be used as easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry but further researches are indispensable to evaluate other biological activities and to identify its chemical profile.

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