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ORIGINAL PAPER

Evaluation of antibacterial and antioxidant activities of *Cissus rotundifolia* (Forssk.) leaves extract obtained by ultrasonic-assisted extraction conditions

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Abstract
In this study, the antibacterial and antioxidant activities of *Cissus rotundifolia* leaves extract were evaluated. The active compounds of plant leaves were extracted by using ultrasonic-assisted extraction conditions. Yield, total phenolic compounds and antioxidant activities were determined. In addition to six strains of gram-positive and negative bacteria were used for testing antibacterial activities of the plants' extracts. The obtained results demonstrated that extraction of active compounds by using ultrasonic-assisted extraction was more efficient. The dry yield and total phenol compounds of extract were 29.97% and 82.87 mg GAE/g DE, respectively. The antibacterial activities of the extracts were tested against two types of bacteria, Gram-positive strains: *Bacillus subtilis*, *Staphylococcus aureus*, and *Streptococcus agalactiae*, and Gram-negative strains: *Escherichia coli*, *Salmonella typhimurium*, and *Pseudomonas aeruginosa*. The results showed that leaves extract was more effective against Gram-negative bacterial strains. The concentrations of minimum inhibitory and minimum bactericidal of the leaves extract against *S. typhimurium*, *E. coli* and *P. aeruginosa* (Gram-negative bacteria) were at concentrations of 10, 20 and 20 mg/mL, respectively. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2-azino-bis-(3-ethylbenzothioline-6-sulphonic acid)-diammonium salt (ABTS) scavenging activities at a concentration of 2 mg/mL were 90.94% and 98.20%, respectively, while the IC_{50} was achieved at concentrations of 0.475 and 0.790 mg/mL, respectively. The leaves extract has high antibacterial and antioxidant activities; therefore, it could be a rich source of natural antibacterial and antioxidants for several food and therapeutic applications.

Keywords *Cissus rotundifolia* · Total phenolic compounds · Antibacterial · Bacterial strains · Antioxidant activity

Introduction

Wild plants have returned to the forefront of attention recently because of the results of scientific research confirming their high nutritional value and their content of antioxidant and antimicrobial compounds. Wild vegetables are the cheapest source of protein, vitamins, minerals, and essential amino acids in the food system of many people [1]. In addition the consumption of wild vegetables plays an important role as food supplements contributing to the provision of human food requirements. Therefore, leafy vegetables are functional foods because they contain biologically active ingredients that help prevent numerous diseases [2]. Phenols compounds represent the main fraction of antioxidants present in the food system, where Mokrani and Madani [3] reported that their total dietary ingestion could exceed 1 g/day, which is higher than that of all other compounds of phytochemicals and known dietary antioxidants. Polyphenols are reducing agents, and jointly with other nutritional reducing agents, such as carotenoids and vitamins (E and C) may protect cell ingredients against oxidative damage, therefore reduces the risk of various degenerative diseases related to oxidative stress. Several studies have reported that polyphenols limit the development of cancers, neurodegenerative diseases, diabetes, cardiovascular, and osteoporosis [4, 5].

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Many diseases such as pneumonia, tuberculosis and diarrhoeal diseases (cholera, typhoid, and dysentery) are a consequence of microbial pathogenesis and amongst the leading causes of morbidity and death worldwide. Despite the discovery of antibiotics in the resistance to these diseases, the resistance of microbes to these drugs over time and random use sometimes led to increased concerns of the relevant international health organizations from the development of the injuries and exacerbated to become more dangerous [6]. Recently, the random use of antibiotics led to increased types of bacteria resistant to antibiotics and caused health problems in different regions of the world, which are difficult to treat [7]. Because of this situation, many studies have been conducted in order to obtain antimicrobial compounds from plant extracts [8–10]. Therefore, return to natural sources as alternative treatment strategies to reduce antibiotic usage is important to obtain the necessary prevention or drug from these infections and to achieve the purpose without any adverse effects.

Cissus rotundifolia (Forssk.) Vahl, is a wild plant spread in different regions of Yemen. This plant is an evergreen climber that belongs to the species of *Cissus* in the Vitaceae family (grape family), its leaves are cooked and eaten directly for nutritional and therapeutic purposes of some diseases such as liver diseases, malaria, and otitis [11]. *C. rotundifolia* plant commonly used in the treatment of many infections, and due to the lack of sufficient studies on antioxidants and antimicrobial agents, especially the bacteria, an extract has been prepared from plant leaves by ultrasonic-assisted extraction conditions and estimate the antibacterial effects of different species of Gram-positive and Gram-negative bacteria. As well as evaluation of antioxidant activities of leaves extract.

Material and methods

Plant material

Leaves of *C. rotundifolia* were collected from Haifan directorate, southern of Taiz city, Yemen (coordinates 13°16′06″ N and 44°18′16″ E) during spring season 2017. The leaves were divided into small pieces (less than 10 mm), and then dried at room temperature (25 ± 2 °C) in a shaded place by the air stream for 2 weeks and packaged in plastic bags emptied from the air by vacuum, and then the samples were transported to the laboratory to complete the drying process by using an oven (Boxun GZX-9240, Shanghai, China) at 50 °C until weight stability.

Microorganisms and chemicals

The clinical bacterial strains were supplied by the laboratory of Nutrition, School of Food Science and Technology, Jiangnan University. 1,1-diphenyl-2-picrylhydrazyl (DPPH) 2,2-azino-bis-(3-ethylbenzothioline-6-sulphonic acid)-diammonium salt (ABTS), Folin–Ciocalteu reagent, and 3,4,5-trihydroxybenzoic acid (gallic acid) were purchased from Sigma-Aldrich, China. All other reagents and chemical were of high purity and analytical grade.

Preparation of the extract

In order to obtain the crude extract of *C. rotundifolia* leaves, the optimal conditions obtained by AL-Bukhaiti et al. [11] were used (solvent type, acetone; solvent concentration, 40%; time, 40 min; power, 150 W; temperature, 40 °C and sample to solvent ratio, 1:50 w/v). Ultrasound-assisted extraction was performed by using an ultrasonic cleaning bath (YIJING YQ-920 D, Shanghai Yi Jing ultrasonic instrument Co., Ltd. Kunshan, China). The extract was dried using rotary evaporator and the drying was completed in the oven at 40 °C until weight stability. Dry extract was then collected and kept in glass containers and stored at − 18 °C until used.

Yield calculation

The yield of *C. rotundifolia* extract was estimated by calculating dry extract as a percentage of the substrate used for the extraction process according to the following equation:

$$\text{Yield } (\%) = \frac{\text{dry extract (g)}}{\text{sample used (g)}} \times 100 \quad (1)$$

Determination of the total phenolic compounds (TPC)

Total phenolic compounds were determined by using the Folin–Ciocalteu method reported by AL-Bukhaiti et al. [11] with slight modifications. 200 µL (2 mg/mL) of the extract and 5 mL of 1:10 diluted Folin–Ciocalteu reagent were mixed and left for 5 min. 4 mL of saturated sodium carbonate solution (70 g/L) was added to the mixture and then incubated at room temperature (25 ± 2 °C) for 105 min in the dark. The absorbance of the mixture was recorded at 765 nm using the respective solvent as a blank sample (UV-1800 PC spectrophotometer). The gallic acid

was used as a standard for a calibration curve, and the findings were expressed as mg of gallic acid equivalents per gram of the dry weight extract (mg GAE/g DE).

Antibacterial activities of the leaves extract

Bacterial strains

The antibacterial activities of *C. rotundifolia* extract were determined according to the method described by Tu et al. [12] with some modifications. Two kinds of bacteria were used in the experiments such as Gram-positive strains (*Bacillus subtilis* WB 800, *Staphylococcus aureus* 6538, and *Streptococcus agalactiae* CICC 10465) and Gram-negative strains (*Salmonella typhimurium* 50013, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* CMCC 10104). The test strains were incubated in the Nutrient broth medium (Beijing Land Bridge Technology Co., Ltd., China) for 18 h at 37 °C to obtain the active cultures.

Agar diffusion method

The nutrient agar was prepared according to the manufacturer's specification (Beijing Land Bridge Technology Co., Ltd., China) and sterilized in the autoclave at 121 °C for 20 minutes. The antibacterial activity of *C. rotundifolia* extract was determined by agar diffusion method according to Ma et al. [13] with slight modifications. The dry extract was dissolved in distilled water and 10% dimethyl sulfoxide (DMSO) (v/v) to obtain a stock solution of 50% concentration. Different dilutions have been prepared (25%, 12.5%, 6.25%, 3.125%, 1.56%, 0.78% and 0.39%). 10 mL of sterilized nutrient agar was placed in the petri plates until solidification. The grown cultures of test bacteria (5×10^5 CFU/mL) were added to the medium and the holes were made (6 mm in diameter). 50 µL of each concentration were added and the plates were incubated at 37 °C for 24 h. The antibacterial activity was calculated by determining the inhibition zone (mm) including the holes diameters.

Minimum inhibitory concentration (MIC) and Minimum bactericide concentration (MBC)

MIC was determined according to the method used by Elshikh et al. [14] with some modifications. From the stoke solution, different dilutions were prepared to obtain a serial final concentrations ranging from 1 to 12% in nutrient broth medium. 100 µL of the nutrient broth medium and 100 µL of each concentration were mixed. Bacterial suspension (5×10^5 CFU/mL) was added to all holes except negative control. The mixture was incubated for 24 h at 37 °C and resazurin (0.015%) was added to all holes (30 µL per hole). the observation of color change compared to negative control was performed by using ALISA at OD_{600}. MIC defines as the lowest concentration of extracts or compounds that visibly inhibited the bacterial growth (no turbidity), while MBC defines as the lowest concentration of rotundifolia extract with initial inoculum bacteria killed.

Evaluation of antioxidant activities

DPPH radical-scavenging activity (DPPH-RSA)

The DPPH radical-scavenging activity (DPPH-RSA) was assessed as described by Vora et al. [15] with some modifications. DPPH solution was prepared (0.1 mM) in methanol (95%). 100 µL of the dry extract solution of various concentrations (0.4, 0.8, 1.2, 1.6 and 2 mg/mL) were mixed with 3.5 mL of DPPH stock solution. In the control sample, distilled water was used instead of the sample solution. The reactions were carried out at room temperature (25 ± 2 °C) for 30 min. Reduction in the absorbance was measured by UV-1800PC spectrophotometer at 517 nm. The inhibition percentage was calculated according to the following formula:

$$\text{DPPH} - \text{RSA}\,(\%) = \frac{(\text{Acontrol} - \text{Asample})}{\text{Acontrol}} \times 100 \quad (2)$$

ABTS·+ radical-scavenging activity

ABTS radical scavenging activities of *C. rotundifolia* extract were determined by the method of Zhang et al. [16] with modifications. ABTS·+ solution was prepared by the reaction of 7 mM ABTS stock solution (in distilled water) with 2.45 mM potassium persulfate. The mixture was placed in the dark for 16 h at room temperature and diluted with methanol (98%) to an absorbance of 0.7 ± 0.2 at 734 nm before use. 100 µL from each extract concentration (0.4, 0.8, 1.2, 1.6 and 2 mg/mL) were mixed with 3.5 mL of ABTS·+ working solution and the mixtures were left at room temperature for 30 min in the dark. The blank sample was prepared by the same method, except that distilled water was used instead of the sample. The absorbance was then measured at 734 nm using a UV-1800PC spectrophotometer after incubation time, and the ABTS·+ scavenging ability was calculated using the following equation:

$$\text{ABTS}\ (\%) = 1 - \frac{(\text{Asample})}{(\text{Ablank})} \times 100 \quad (3)$$

Reducing power assay

The reducing power of *C. rotundifolia* extract was determined according to the procedure described by Lou et al. [17] with some modifications. Briefly, 2 mL from each extract concentration (0.4, 0.8, 1.2, 1.6 and 2 mg/mL) were mixed with 2 mL of phosphate buffer (pH 6.6, 0.25 mM) and 2 mL of 1% potassium ferricyanide $K_3[Fe(CN)_6]$. The mixtures were incubated in water bath at 50 °C for 20 min. The mixtures were incubated for 20 min at 50 °C, and then 2 mL of 10% TCA was added. The centrifuge process was carried out for mixtures at 1200 rpm for 10 min, and then 2 mL of incubated mixtures with 2 mL of distilled water and 0.4 mL of 0.1% ferric chloride ($FeCl_3$) were mixed. The resulting mixtures were left for 10 min, and the absorbance was measured at 700 nm using a UV-1800PC spectrophotometer. Increased absorbance of the reaction mixtures indicates a higher reducing power.

Statistical analysis

The statistical analysis was carried out with three replications. The values were expressed as mean ± SD, and significant differences at ($P < 0.05$) between mean values were evaluated by ANOVA using SPSS version 20.0.0 (SPSS IBM, Chicago, IL, USA).

Results and discussion

Yield

The yield result is illustrated in Table 1. The yield strongly relates to the plant raw materials and extraction conditions applied, where the highest yield (29.97 ± 0.51%) was achieved under optimal extraction conditions. The result obtained in this study was higher than that found by Vu et al. [18] from banana peel (*Musa cavendish*). The use of ultrasound energy helps to increase yields as these waves destroy cell walls, and thus facilitate solvent penetration in cells and the extracted of active compounds.

Table 1 Yield, total phenolic compounds of Cissus rotundifolia extract obtained ultrasonic-assisted extraction conditions (n = 3, mean ± SD)

Yield (%)	TPC (mg Gallic acid/ g DE)
29.97 ± 0.51	82.87 ± 1.1

TPC total phenolic compounds

Total phenolic compounds (TPC)

In this work, diverse optimal extraction conditions were used to extract phenolic compounds from *C. rotundifolia*. Result showed that the extract contained phenolic compounds (82.87 mg GAE/g DE) as shown in the Table 1. The phenol content of *C. rotundifolia* extract was higher than the range from 13.53 to 29.39 mg GAE/100 g DE of eggplant extracts [3]. This result, also higher than the total phenols content of *Citrus limon* residues which were 15.74 and 15.08 mg GAE/g DW, obtained under optimal conditions by using ultrasound-assisted and microwave-assisted extraction, respectively, found by Dahmoune et al. [19], and more than the total phenol content of six species of plants that ranged between 4.76 to 56.21 mg GAE/g DW [20]. Generally, phenolic content of natural extracts depends on plant species and extraction conditions used.

Antibacterial activities

Effect of extract concentration on the bacteria

The phenolic compounds lead a major role in the antibacterial activities of the plants' extracts. Table 2 and Fig. 1 show the antimicrobial inhibition zones, including the diameter (6 mm) of the Petri plate of six bacterial strains (Gram-positive and Gram-negative bacteria). The results showed that the *C. rotundifolia* extract had a broad spectrum and was able to inhibit the growth of the tested bacteria strains between the concentration ranges of 3.125 to 25%. The results showed a positive relationship between the inhibition zones and the concentrations of the plants' extract. The highest inhibition zones was achieved at a concentration of 25% as shown in Table 2, which were 18 mm, 17.33 mm and 17 mm against *S. typhimurium*, *P. aeruginosa* and *S. agalactiae*, respectively, with non-significant differences ($P^{<}$ 0.05). Followed by inhibition zones (14.33 mm), (14.33 mm) and

Table 2 Average inhibition zones of C. rotundifolia extract against tested organisms (n = 3, mean ± SD)

Concentration (%)	Average diameter of zone of inhibition (mm)					
	S. aureus	*S. agalactiae*	*B. subtilis*	*S. typhimurium*	*E. coli*	*P. aeruginosa*
25	15.67 ± 0.47	17.00 ± 00	16.67 ± 0.47	18.00 ± 0.82	15.67 ± 0.47	17.33 ± 0.47
12.5	11.67 ± 0.47	14.33 ± 0.47	13.67 ± 0.47	14.00 ± 0.82	13.33 ± 0.47	14.33 ± 0.47
6.25	9.67 ± 0.47	10.00 ± 00	12.00 ± 00	10.00 ± 00	11.33 ± 0.47	11.00 ± 00
3.125	8.33 ± 0.47	8.67 ± 0.47	9.67 ± 0.47	8.67 ± 00	10.33 ± 0.47	9.67 ± 0.94

(14 mm) at the concentration of 12.5% against *S. agala*ctiae, *P. aeruginosa* and *S. typhimurium*, respectively, with non-significant differences. The concentration of 6.25% achieved the highest inhibition activity (12 mm), (11.33 mm) and (11 mm) against *B. subtilis*, *E. coli* and *P. aeruginosa*, respectively, as shown in Table 2, with non-significant differences between inhibition of *E. coli* and *P. aeruginosa*. The concentration of 3.125% gave the highest result of inhibition (10.33 mm) against *E. coli* followed by *B. subtilis* and *P. aeruginosa* with Inhibition zone (9.67 mm) for both of them and the statistical analysis results in Table 3 showed no significant differences between the three inhibition zones. Within the Gram-positive bacteria, *S. agalactiae* was the more sensitive to concentrations of 25 and 12.5% compared to *B. subtilis*, which was more sensitive to concentrations of 6.25 and 3.125%. While *S. typhimurium* (Gram-negative

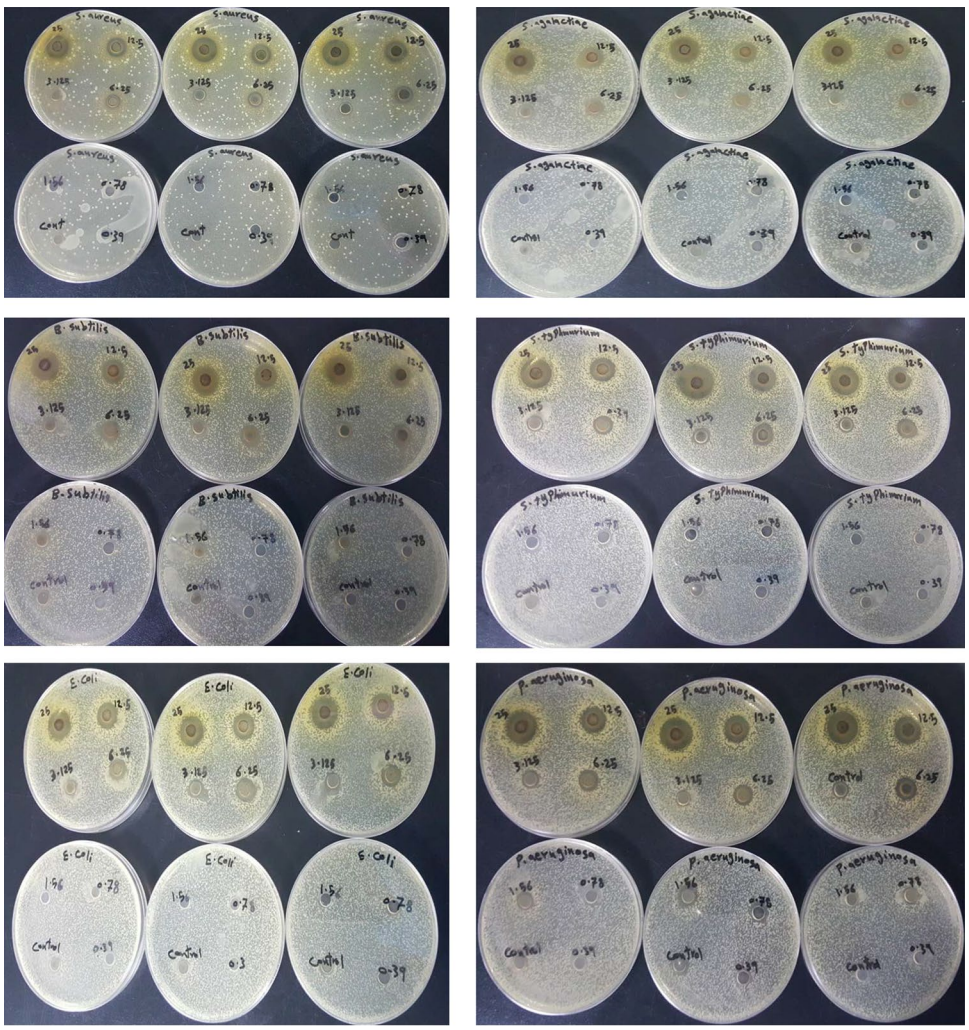

Fig. 1 Antimicrobial inhibition zones of *C.rotundifolia* extract against test organisms

Table 3 Inhibition zones (mm) obtained by the industrial antibiotic treatment (n = 3, mean ± SD)

Bacterial	Ampicillin (10 µg)	Streptomycin (10 µg)	Augmentin (20/10 µg)	Tetracycline (30 µg)	Erythromycin (15 µg)
Bacillus subtilis (WB 800)	18	17	14	30	16
Staphylococcus aureus (6538)	23	16	18	27	30
Streptococcus agalactiae (CICC 10465)	16	15	9	22	12
Salmonella typhimurium (50013)	17	17	16	30	27
Pseudomonas aeruginosa (CMCC 10104)	16	17	16	31	27
Escherichia coli (ATCC 25922)	16	18	18	30	0

bacteria) was more sensitive to concentration of 25%, on the contrary, *P. aeruginosa* bacteria was more sensitive to concentrations of 12.5%. *E. coli* was sensitive more than all strains at an extract concentration of 3.125%.

Tian et al. [10] reported that the antibacterial activity of phenolic acids mainly depended on the presence of a carboxyl group (–COOH). In addition, a number of hydroxyl groups in the molecules might affect the antimicrobial activity of phenolic compounds, which may explain the inhibition on Gram-positive bacteria. Generally, the difference in antibacterial potential may be due to variations in chemical compositions, which may be influenced by the extraction methods, as well as plant geographical origin [21], in addition to the variability in the experimental conditions amongst the methods used [22].

By comparing the inhibition zones of *C. rotundifolia* extract at the highest concentration (25%) with those obtained by the antibiotic treatment (Table 3), at this concentration, the inhibition zones were 17 mm, 16.67 mm, and 15.67 mm compared with Ampicillin (against Gram-positive bacteria) which were 16 mm, 18 mm, and 23 mm against *S. agalactiae*, *B. subtilis*, and *S. aureus*, respectively. While the inhibition zones were 18 mm, 15.67 mm, and 17.33 mm, compared with Streptomycin (against Gram-negative bacteria) which were 17 mm, 16 mm and 16 mm against *S. typhimurium*, *E. coli* and *P. aeruginosa*, respectively. From the results of Table 3, it is clear that *C. rotundifolia* extract result was close to the result of synthetic antibiotics, which enhances the possibility of this extract used as an antibiotic against the studied bacteria strains, particularly Gram-negative bacteria.

On the other hand, it is clear from Tables 2 and 3 that the effect of *C. rotundifolia* extract achieved higher inhibition results than Augmentin (20/10 µg) with *S. agalactiae*, *B. subtilis*, *S. typhimurium* and *P. aeruginosa*. In addition, the inhibition zones of *C. rotundifolia* extract were higher than inhibition zones of Erythromycin (15 µg) against *S. agalactiae*, *B. subtilis*, and *E. coli*. While the Tetracycline (30 µg) achieved better results from plant extract with all tested bacteria strains.

MIC and MBC

The antimicrobial assay showed that leaves extract is able to inhibit the growth of all evaluated bacteria. Minimal inhibitory and minimal bactericidal concentrations (MIC and MBC) of the *C. rotundifolia* leaves extract are presented in Table 4 and Fig. 2. Generally, strains of Gram-negative bacteria were more sensitive than Gram-positive bacteria for *C. rotundifolia* extract, particularly *E. coli* and *P. aeruginosa* and *S. typhimurium* strains, where the MIC and MBC were achieved at 10, 20 and 20 mg/mL, respectively. This result differs from those reported by Rashed et al. [23] and Vieitez et al. [24], which the Gram-positive bacteria was more sensitive than Gram-negative bacteria when treated with the *Lavandula pubescens* extract. Therefore, the *C. rotundifolia* extract with antimicrobial potential could be used in some foods to preservation.

Table 4 Minimum inhibitory concentration (MIC), Minimum bactericide concentration (MBC) and antioxidants activities (IC50 of DPPH and ABTS) of Cissus rotundifolia extract obtained ultrasonic-assisted extraction conditions (n = 3, mean ± SD)

Bacterial strains	MIC (mg/mL)	MBC (mg/mL)
Staphylococcus aureus	30	40
Streptococcus agalactiae	40	50
Bacillus subtilis	30	30
Salmonella typhimurium	20	20
Escherichia coli	10	10
Pseudomonas aeruginosa	20	20
IC_{50} DPPH (mg/mL)	0.475	
IC_{50} ABTS (mg/mL)	0.79	

DPPH 1, 1-diphenyl-2-picrylhydrazyl; *ABTS* 2,2-azino-bis-(3-ethylbenzothioline-6- sulphonic acid)-di-ammonium salt

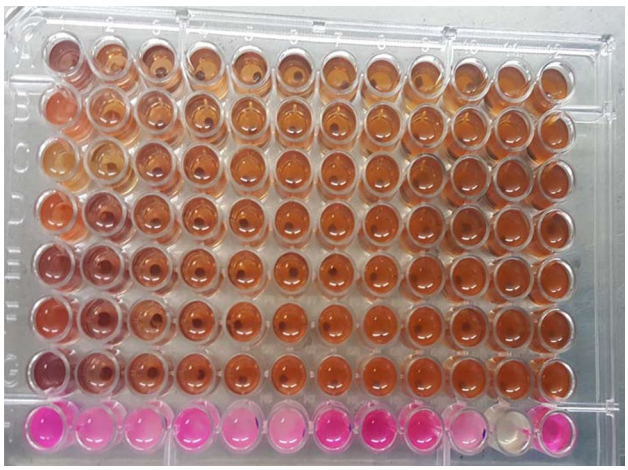

Fig. 2 MIC of the *C. rotundifolia* leavesextract obtained by ultrasonic-assisted extraction conditions

Antioxidants activities

DPPH· radical-scavenging activity

Recently, attention has been paid to natural antioxidants, and plants are considered the most important source, which produces a wide range of secondary metabolites with antioxidative activities that have therapeutic potential [25]. This

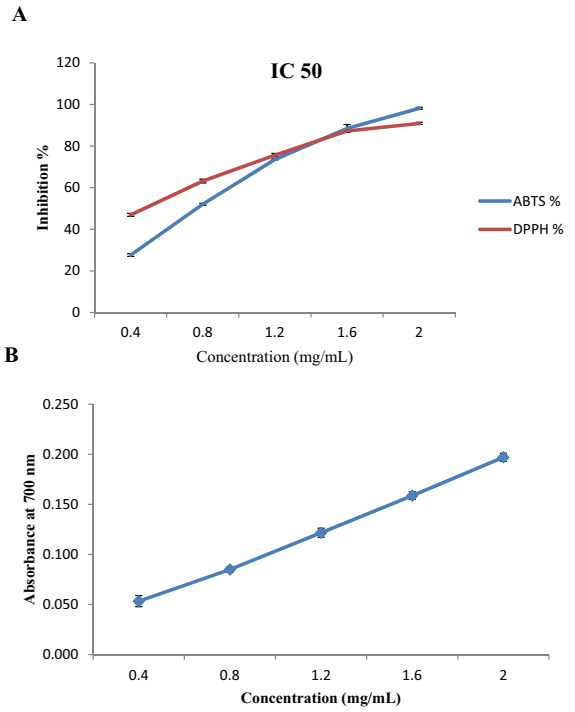

Fig. 3 Antioxidant activities of *C.rotundifolia* leaves extract obtained by ultrasonic-assisted extractionconditions, **a** DPPHradical-scavenging activity and ABTS radical-scavenging activity; **b** Reducing power.Data expressed as mean±SD of triplicate determinations

test is a sensitive method to evaluate the antioxidant activity of plants extracts. It is based on the decolourization of DPPH in the presence of antioxidants in the sample. DPPH assay depends on the presence of natural phenols in the extract and their ability to prevent peroxide formation or donate hydrogen ions and the conversion of fixed free radicals (DPPH.) to non-radicals (DPPH-H) and this changing can be monitored by measuring the bleaching of DPPH. color from violet to yellow by using a spectrophotometer [23]. The dry extract of *C. rotundifolia*, which evaluated in the study exhibited strong inhibition at the incubation time of 30 min (Fig. 3a). The highest percentage of inhibition was 90.94% at a concentration of 2 mg/mL, which is higher than that found by Mokrani and Madani [3] of peach fruit (*Prunus persica* L.) under optimal conditions. The IC_{50} (defined as the concentration of phenols required to inhibit 50% of DPPH) was achieved at a concentration of 0.475 mg/mL. This result is in accord with high phenol content in the dry extract as shown in Table 4.

ABTS·$^{+}$ radical-scavenging activity

The ABTS radical-scavenging activity measures the antioxidant properties of the plant extract. Figure 3a showed that the ABTS radical-scavenging activity was 98.20% at extract concentration 2 mg/mL. The high activity of ABTS scavenging observed in the current study could be attributed to a high content of bioactive components which is similar to the result reported by [26, 27]. On the other hand, the IC_{50} in this assay was achieved at a concentration of 0.79 mg/mL (Table 4). This result indicates a high antioxidant ability of *C. rotundifolia* extract. The low value of the IC_{50} means higher antioxidant activity [26]. The antioxidant activities of plant extracts may be influenced by many factors, such as extraction solvent, methods, and test system [22].

Reducing power assay

The reducing power assay depends on the reduction of ferricyanide (Fe^{+3}) complex to the ferrous (Fe^{+2}) form in the presence of antioxidants in the sample extract. Lou et al. [17] reported that there is a strong correlation between antioxidant activities and iron reducing power. The results are shown in Fig. 3b. It was found that the reducing power of *C. rotundifolia* extract was increased with the increase of the concentration of extract. Therefore, reducing power indicates the potential antioxidant activity of particular compounds. This finding is consistent with several studies indicated that the concentration of the extract increases the reducing power [17, 28], and these results vary according to the plant variety and the concentration used in the measurement.

Conclusion

Recently, interest has grown in the use of plant-derived antibacterial and antioxidants as an alternative source of natural antioxidants and antibacterial compounds. *C. rotundifolia* extract was obtained under ultrasonic-assisted extraction conditions with high total phenol content. The results showed good antibacterial activities against Gram-positive bacteria (*B. subtilis, S. aureus, and S. agalactiae*) and Gram-negative bacteria (*E. coli, S. typhimurium, and P. aeruginosa*), where Gram-negative bacteria were more sensitive for the extract under study. *C. rotundifolia* extract possesses good antioxidant properties for DPPH radical scavenging, ABTS radical scavenging, and reducing power, where the extract showed appropriate IC_{50} concentration with DPPH and ABTS radical scavenging assay.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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