Antioxidant and antibacterial activities of extracts from *Conyza bonariensis* growing in Yemen

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Abstract: This study aims to examine the antioxidant and antibacterial activities and phenolic contents of *Conyza bonariensis* growing in Yemen. The whole plants of *C. bonariensis* were ultrasonically extracted by ethanol. The antioxidant activity of the extract was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and β-carotene bleaching (BCB). The effectiveness of the extract on the growth inhibition of some indicators of foodborne illness bacteria were investigated by agar well diffusion assay. The total phenols (TP), total flavonoids (TF), total tannins (TT), and total anthocyanins (TA) were determined by Folin-Ciocalteu method, aluminium chloride method, Folin and Ciocalteu method, and pH-differential method, respectively. The extract of *C. bonariensis* possessed TP 144.1 mg/g, TF 143 mg/g, TT 0.99mg/g, and TA 0.97mg 100g, with 94.57% inhibition of DPPH and 92.47% inhibition of BCB, and strong inhibitory effects against tested bacteria, which was approximate to those of peel extract of *Punica granatum*.

Keywords: Antioxidant, antibacterial, *Conyza bonariensis, Punica granatum,* total Phenolic

INTRODUCTION

Recently, various extracts of plants have gained special interest as sources of natural antioxidant and antibacterial agents (Madhavi and Salunkhe, 1995). Natural antioxidants are compounds from plant or animal sources. Phenolics are regarded as antioxidants and found in significant quantities in vegetables, fruits, spices and seeds. They also have roles in food industry and in chemoprevention of diseases (Noguchi and Niki, 2000). The oxidation reactions are involved in aging and progression of several diseases, while antioxidant molecules may slow down the aging process, disease progression, and prolong the life span (Gutteridge and Halliwell, 2010).

Conyza bonariensis (L.) Cronq is an annual plant spread widely all over the world, from North America to Europe. The plant *C. bonariensis* grows spontaneously in central Yemen and is used in folk medicine headache, dental pain, treat rheumatism, cystitis and nephritis (Soheir *et al*., 2012). In addition it was approved in medicine as a hemostatic, possibly an anthelminthic, pungent tonic, astringent to control bleeding and as a diuretic (Lenfeld *et al*., 1986).

This research aims to assess the antioxidant and the antibacterial activities of *Conyza bonariensis* growing in Yemen, and its (TP (TF), (TT) and (TA) compared to extracts of *Punica granatum* which is contained high phenolic contents and possesses strong antioxidant and the antibacterial activities (Zarei *et al*., 2010).

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MATERIAL AND METHODS

Chemicals and machine

Tert-butylhydroquinone (TBHQ), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid (Vitamin C), Folin-Ciocalteu reagent, gallic acid, quercetin reagent, β-carotene, and linoleic acid were from Sigma (USA). Ethanol, dimethylsulfoxide (DMSO), aluminium chloride, potassium acetate, potassium chloride, sodium acetate and Tween 20 were from Sinopharm chemical regent co. ltd. Spectrophotometer (Shanghai-Techcomp, UV 2300), balance (Shanghai-Mettle Toledo, AB 204-N), rotary evaporator (Shanghai-Biochemical Equipment), water bath (Shanghai-Hengzi), pH metter (Shanghai-Mettler Toledo), incubation (Shanghai-Hengzi), and ultrasonic (Wuxi-Kejie Ultrasonic Electronic Equipment Co. Ltd, KJ-300) were also used in the study.

Plant materials

The whole plants of *C. bonariensis* and peels of *Punica granatum* (L.) were collected in September 2012 from Taiz region (Yemen). The identification of plant materials were carried out by the Agricultural Research Authority (Taiz).

Preparation of extracts

The plant samples were air-dried in shade, twenty grams from plant were extracted with 600ml of 90% ethanol in an ultrasonic device at room temperature. The ethanol extract was filtered and the residues were re-percolated for three times and solvent was removed using a rotary evaporator. Dried extracts were kept refrigerated until use.

Total phenols (TP)

The TP was determined spectrophotometrically according to the Folin-Ciocalteu's method (Arnnok *et al*., 2012). Using a six-point calibration curve, the total phenolics were determined by a comparison of the values obtained with the calibration curve of gallic acid (fig. 1). The results were expressed as mg gallic acid equivalents (gallic acid/g extract).

Total flavonoids (TF)

Flavonoids in the examined plant extracts were determined spectrophotometrically using aluminium chloride according to the reported method (Lin and Tang, 2007). Quercetin was used as a standard. The concentration of flavonoids was in (mg/ml) on the calibration line and the total flavonoid was expressed as mg/g of dry extracts (fig. 2).

Total tannins (TT)

The TT were estimated by Folin and Ciocalteu method (Tamilselvi *et al*., 2012). Using a five-point calibration curve, the TT were determined by a comparison of the values obtained with the calibration curve of gallic acid (fig. 3), total tannins values are expressed in terms of gallic acid equivalent (mg/g of dry extracted).

Total anthocyanin (TA)

The TA was determined by the pH-differential method (Guisti and Wrolstad, 2001). Was calculate the absorbance (A) of the diluted sample as follows: $A=(A_{510}–A_{700})$ pH 1.0 – $(A_{510}–A_{700})$ pH 4.5.

Calculate anthocyanin pigment concentration as follows: Monomeric anthocyanin pigment (mg/L) = $(A \times MW \times DF \times 1000)/(\varepsilon \times 1)$, and it was converted to mg of total anthocyanin content per 100 g sample.

Antioxidant activity of extracts

Determination of antioxidant activity (AA) using DPPH radical scavenging method

In this assay, the (AA) of plant extracts was evaluated by measuring the bleaching of the purple-colored ethanolic solution of DPPH (Burits and Bucar, 2000). The antioxidant activity of six different concentrations (0.2, 0.1, 0.05, 0.02, 0.01 and 0.005mg/ml) of plant extracts was measured in terms of hydrogen donating or radical scavenging ability (Brand et al., 1995). The inhibitory concentration (IC_{50}) value represented the concentration of the plant extracts that caused 50% inhibition.

Determination of antioxidant activity using β-Carotene bleaching (BCB) assay

The (AA) of extracts was evaluated by the β-carotene according to method (Lu and Foo, 2000). The measurement was carried out every 30 min intervals. While TBHQ and Vitamin C. both were at 200 ppm, and were used as standards.

Microbial strains and media

Shigella dysenteriae CMCC 51302, *Escherichia coli* ATCC 25922, *Salmonella typhimurium* CMCC 50013, *Streptococcus pyogenes* ATCC 12344 and *Staphylococcus aureus* ATCC 25923 were provided by the Microbiology Lab in School of Food Science and Technology, Jiangnan University, Wuxi 214122, P. R. China. Each culture was activated by transferring a loopful into nutrient broth (4 ml) followed by incubation at 37ºC±1ºC for 16 h. The optical density of each active culture was adjusted at 615(nm) using fresh broth to give a standard inoculums of 10^8 (CFU)/ml.

Determination of antibacterial activity

It was studied by the agar well diffusion method (Smania *et al*., 1999). Briefly, Agar media were perforated with 6 mm-diameter holes, aseptically cut and filled with 100 µl of plant extracts. The extracts were used in the concentration of 5, 10, and 20 mg/ml from extract of dimethylsulphoxide (DMSO). Streptomycin and Penicillin were used as a reference antibacterial, whereas DMSO was the negative control. The plates were incubated at 37ºC±1ºC for 21 h and then examined to verify inhibition. A positive result was defined as inhibition zone of 9 mm or more around the holes.

STATISTICAL ANALYSIS

Statistical methods were used of three simultaneous assays to calculate means and standard deviations. Statistical analysis (SPSS, 16) was applied to the data to determine differences ($P<0.05$) performed by ANOVA.

RESULTS

Total phenolics and total flavonoids

The content of polyphenols was 144.1 and 134.4 mg/g in *C. bonariensis* and *P. granatum*, respectively (table 1). The results indicated significant differences (P□0.05) among extracts, *C. bonariensis* contained higher phenol content compared to *P. granatum* extracts.

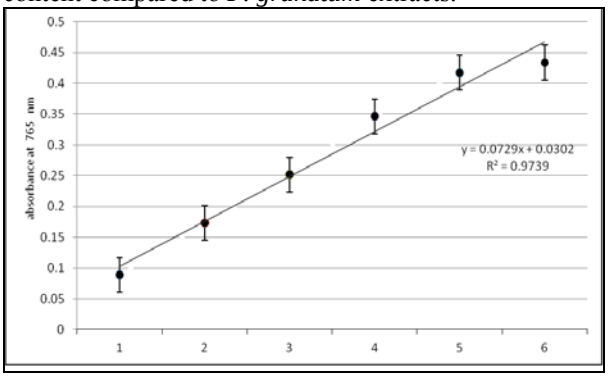

Fig. 1: Calibration curve for gallic acid (mg/g of dry extract)

There were significant differences ($P<0.05$) as the extract of *C. bonariensis* had higher flavonoid content, while the extract of *P. granatum* containd less flavonoids

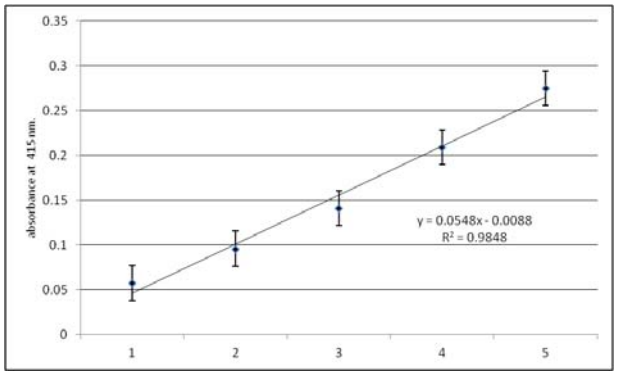

Fig. 2: Calibration curve for quercetin (mg/g of dry extracts.)

Total tannins and total anthocyanins

From the data presented in table 1, it is apparent that the tannin content of extracts varied significantly (P<0.05). Tannins were low in *P. granatum* (0.91 mg/g) and high in *C. bonariensis* (0.99 mg/g), which also had the highest level of total phenolics.

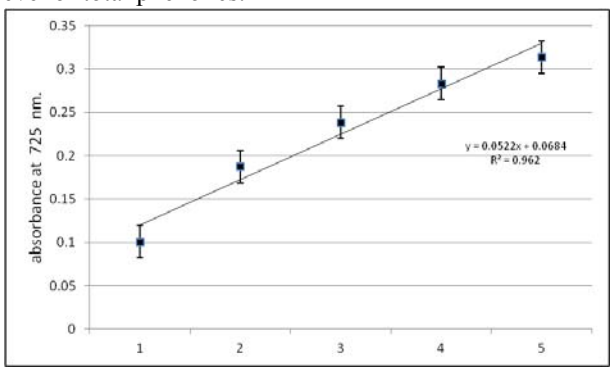

Fig. 3: Calibration curve for gallic acid (mg/g of dry extracted)

The present study found that there were significant differences (P<0.05) among study samples. The highest anthocyanins content was in *C. bonariensis* (0.97mg/100g), where the smallest was in *P. granatum* (0.63mg/100g).

Antioxidant activity using (DPPH) radical scavenging

Extracts of *C. bonariensis* and *P. granatum* inhibited antioxidant activities of 94.57% and 92.92% scavenging DPPH, respectively at a concentration of 0.05 mg/ml (table 2).

Antioxidant activity using β-Carotene bleaching assay

There were no significant differences (P □ 0.05) among the study samples (table 2). Results showed that extracts of *C. bonariensis* and *P. granatum* were better inhibitors of β -carotene bleaching than reference antioxidants like TBHQ (table 2).

The DPPH radical scavenging IC_{50} values of extracts were summarized in table 2. Extracts of *C. bonariensis* and *P. granatum* exhibited strong DPPH radical scavenging with IC_{50} values at 4.93 and 4.95 µg/ml, respectively. Two extracts tested in the DPPH assay had good antioxidant properties (fig. 4).

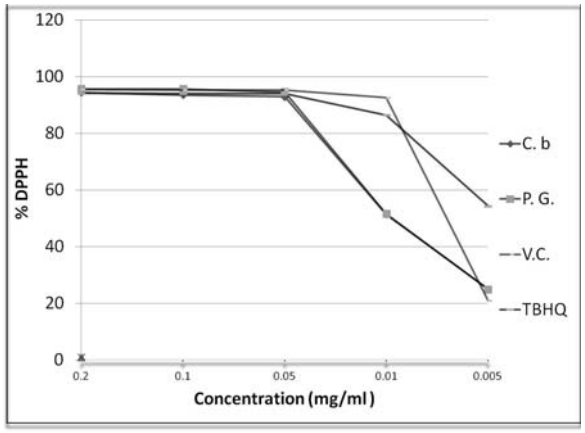

Fig. 4: DPPH radical scavenging activities of extracts from *C. bonariensis* and *P. granatum* and reference antioxidants (VC and TBHQ)

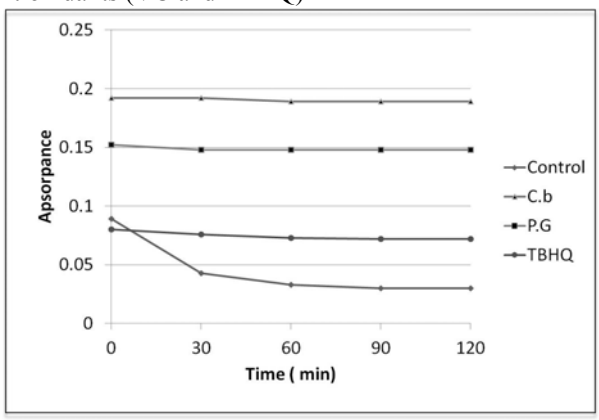

Fig. 5: Antioxidant activity of extracts from *C. bonariensis* and *P. granatum* and standard antioxidants in β-carotene-linoleate bleaching system

The BCB absorbance of *C. bonariensis* and *P. granatum* for 120 min were 0.188 and 0.152, respectively, whereas they were 0.192 and 0.148 at zero time, respectively (fig. 5).

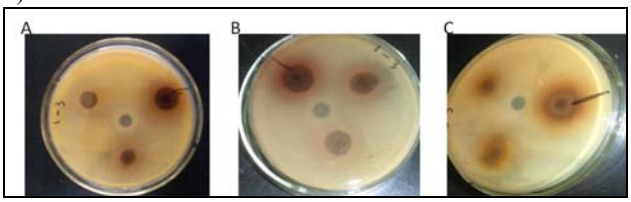

Fig. 6: Antibacterial activity of extracts: (A) *C. bonariensis* on *S. aureus*, (B) *C. bonariensis* on *S. typhimurium*, and (C) *P. granatum on S. aureus*, using 5, 10, and 20 mg/ ml of each extract.

Antibacterial activity

Presents antibacterial activities of the two plants tested at concentrations of 5, 10, and 20 mg/ml (table 3). It showed that gram positive bacteria (*Staphylococcus* and

Streptococcus) were more susceptible to extracts from *C. bonariensis* and *P. granatum*. *S. aureus* was the most sensitive organism to *C. bonariensis* extracts at 20 mg/ml than *S. pyogenes*. Gram negative bacteria were susceptible to extracts from *C. bonariensis* than *P. granatum*. Extracts of *C. bonariensis* and *P. granatum* were inhibitory for *S. typhimurium* and S. *dysenteriae*. *E. coli* was more strongly inhibited by extract of *C. bonariensis* than that of *P. granatum*.

Extracts of *C. bonariensis* appeared to be the most effective with the zone of inhibition sizes ranging from14.6 to 19.8 mm at 20 mg/ml, as shown in fig. 6.

DISCUSSION

Polyphenols are some of the most occurring phytochemicals in plants. Moreover, these phenolic contribute to quality and nutritional value. Also contribute to color and sensory characteristics of fruits and vegetables and also play an important role in providing protection against *in vivo* and *in vitro* oxidation, and have plant defense mechanisms to counteract reactive oxygen species (ROS) and prevent damage of micro organisms. Phenolic compounds are antioxidant working as a free radicals scavenger (Shahidi and Wanasundara, 2003). The phenolics and flavonoids are considered as important indicators of antioxidant capacity.

Some studies indicated of *P. granatum* extract had phenol content between 124.43 and 91.2 mg/g (Ahmed, 2012; Mutahar *et al*., 2012), respectively. *C. bonariensis* extracts were 167.9 mg/g (Durre *et al*., 2012).

There is a strong correlation between antioxidant activity and total phenols in table 2. These findings suggest that total phenols are a good predictor of antioxidant activities. Investigations reported that phenolic concentrations varied from 5 to 46% of peel *P. granatum* extracts (Li *et al*., 2006; Negi and Jayaprakasha, 2003). The variability of total phenolic in this study could be partially attributed to differences in solvents used for extracting peels, geographic sources of samples and varieties.

Phenolic contents are affected by plant species, maturity at harvest, post harvest, soil conditions and growing conditions (Nasır *et al*., 2011). The results of the extract *P. granatum* were consistent with the previous study, which the TF in the extract of *P. granatum* was 59.44 mg/g (Ahmed, 2012).

Tannins are natural polyphenols ubiquitously distributed in plants, such as vegetables, fruits and seeds (Elías *et al*., 2009). Commercially tannins are used in the wine industry for a multitude of reasons: to stabilize the color of red wines, ensure palate balance and complexity in wines, inhibit lacasse in botrytis-infected fruit and to serve as fining agents to reduce protein concentrations (Luz *et al*., 2008). These results of extract *P. granatum* were also reported in a previous study (Hakime *et al*., 2012). Anthocyanins are responsible for the red and blue colors in some plants (Concepcion *et al*., 2003).

There were significant differences ($p<0.05$) among in the extracts of C. *bonariensis* and *P. granatum*. That was probably due to the presence of high polyphenolic content. Also could be related to the presence of hydroxyl and carbonyl groups (Galati and Brien, 2004; Payet *et al*., 2005). And also due to the fact that radical-scavenging capacity is directly related to the hydrogen donating ability of compounds (Lucarini *et al*., 1990). The antioxidant efficiency of the extracts tested was basically dependent on their concentrations. The extracts of *C. bonariensis* and *P. granatum* maintained stability and the strength of antioxidative activities were related to the Phenolic concentrate.

The antioxidant activity of extracts was also assessed by the ability to prevent β-Carotene from oxidation by linoleic acid. The oxidation of linoleic acid generates peroxyl free radicals due to the abstraction of a hydrogen from diallylic methylene groups (Kumaran and Joel Karunakaran, 2006). The BCB antioxidant activities are stable with time. Extracts of *C. bonariensis* and *P. granatum* exhibited strong BCB activities, possibly due to

Table 1: Total phenols, flavonoids, tannins and anthocyanins in extracts of *C.bonariensis* and *P.granatum* (means±SD)

Plant	Total phenols (mg GAE/g)	Flavonoids (mg quercetin/g)	Tannins (mg GAE/g)	Anthocyanins (mg/100g)
C. bonariensis	144.1^{a}±5.32	134.0^{a}±5.87	0.99^{a} ±0.02	0.97^{a} ±0.02
P. granatum	134.4^{b}±3.24	79.3^{b}±0.36	0.91^{b}±0.02	0.63^{b}±0.02

Table 2: Antioxidants activity by DPPH scavenging and β-carotene bleaching of *C. bonariensis* and *P. granatum* extracts (means ± S.D)

Extraction	DPPH scavenging test		β-carotene bleaching test
	Inhibition of DPPH (%) (0.05 mg/ml)	IC_{50} (µg/ml)	Inhibition of BCB (%) (5 mg/ml)
C. bonariensis	94.57^{b}±0.16	4.93^{b}±0.15	92.4^{a}±3.15
P. granatum	92.92^{c}±0.10	4.95^{b}±0.05	90.77^{a}±0.21
VC	95.01^{a}±0.12	5.21^{c}±0.05	6.37^{c}±3.12
TBHQ	95.12^{a}±0.09	2.14^{a}±0.04	84.60^{b}±2.25

Values are means of three independent analyses.

Table 3: Antibacterial activities of the ethanolic extracts of *C.bonariensis* and *P.granatum* by agar well diffusion assay

Bacteria	Inhibition zone (mm)[1]					
	Concentration	Extraction		Concentration	Reference[2]	
		C. bonariensis	*P. granatum*		Streptomycin	Penicillin
E. coli	5mg/ml	11.0±0.30	NA*	5µg/ml	12.3±0.01	ND**
	10mg/ml	12.0±0.30	9.9±0.17	50µg/ml	15.7±0.86	ND
	20mg/ml	14.6±0.23	11.8±017	100µg/ml	18.9±1.31	ND
S. dysenteriae	5mg/ml	12.0±0.01	NA	5µg/ml	12.0±0.01	ND
	10mg/ml	12.8±0.17	10.4±0.51	50µg/ml	15.3±0.58	ND
	20mg/ml	15.0±0.70	11.4±0.75	100µg/ml	17.7±0.58	ND
S. typhimurium	5mg/ml	11.7±2.1	10.6±1.20	5µg/ml	11.3±0.58	ND
	10mg/ml	13.3±2.51	11.7±1.15	50µg/ml	13.7±1.23	ND
	20mg/ml	15.8±1.01	12.7±1.15	100µg/ml	17.0±2.0	ND
S. pyogenes	5mg/ml	12.5±0.87	9.3±0.35	5µg/ml	ND	16.7±0.51
	10mg/ml	14.2±0.68	10.8±0.1	50µg/ml	ND	17.3±1.2
	20mg/ml	16.2±1.15	12.7±0.62	100µg/ml	ND	18.7±0.98
S. aureus	5mg/ml	14.1±0.41	10.3±1.20	5µg/ml	ND	11.7±1.15
	10mg/ml	16.6±0.98	11.7±0.77	50µg/ml	ND	15.7±0.63
	20mg/ml	19.2±0.51	13.0±0.00	100µg/ml	ND	15.7±0.98

[1]Values (diameter in mm, including diameter of 6 mm) are expressed as mean ± standard deviation, analyzed individually in triplicate.[2]Reference Streptomycin(gram-negative bacteria), Penicillin (gram-positive bacteria); *NA=not activity; ND** =not done Inhibition zones: <9 mm, no active; 9-12 mm, less active; 13-18 mm, active; >18 mm, strong active.

their oil components, such as tocopherols, phytosterols, and phenolic compounds. The synthetic antioxidant TBHQ had a stronger antioxidant activity when compared to VC (fig. 5).

Confirmed the scientific literature on the importance of antibacterial activity of the extracts, which are designed to provide extended shelf life safety food microbial. In this study the antibacterial activity of two plant extracts was assessed by diffusion method agar well. All extracts tested showed antibacterial abilities against *S. aureus, S. pyogenes, S. dysenteriae* and *S. typhimurium*.

The results may support the use of *C. bonariensis* and *P. granatum* in traditional medicines.

Gram negative bacteria were more resistant than gram-positive bacteria, which were also reported by (Ahmad and Beg, 2001). The resistance towards antibacterial substances by gram-negative bacteria were related to the lipopolysaccharides in their cell wall (Gao *et al*., 1999; Alzoreky and Nakahara, 2003). The results of the present study are encouraging as extracts of *C. bonariensis* showed significant antibacterial activity against many enteric pathogens tested.

CONCLUSION

This study showed that the two plants used in traditional medicine in Yemen have antioxidant and antibacterial activities. The types and contents of bioactive components varied among different plants. The characterization of the active components of those plants may lead to full utilization of these plants by the local folks.

ACKNOWLEDGEMENTS

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