GC/MS Analysis of Essential Oil and *In Vitro* Comparative Study of Antioxidant and Antibacterial Activities of Essential oil and Ethanol Extract of *Conyza bonariensis*

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Abstract— The aim of this study was to describe the chemical composition of essential oil of *Conyza bonariensis* and to identify the antioxidant and antibacterial activities of essential oil and ethanol extract and find out the total phenols from *Conyza bonariensis* . Were extracted essential oils by hydro distillation and analysed by gas chromatography mass spectrometry (GC–MS), While the plant extract was extracted using 90% ethanol, by microwave. The antioxidant activity of the essential oil and ethanol extract were determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and β-Carotene bleaching (BCB). While the total phenols was determined by Folin-Ciocalteu method. The effectiveness of the extract on the growth inhibition of some of bacteria were investigated by agar well diffusion assay. Were identified 45 constituents in essential oil. The major component was sesquiterpene accounting for 51.14% of the total oil composition. The principal components were Aromadendrene oxide - (2) (17.38%), Cedren - 13- ol, 8 (17.30%), Caryophyllone oxide (7.23%), alpha – Bisabolol (2.32%), Isoaromadendrene epoxide (1.69 %), Globulol (1.49%), Thymol (2.86 %), Tetracyclo(6.3.2.0(2.5).0(1.8)tridecan-9-ol,4,4-dimethyl(8.41%),1-Naphthalenol,decahydro-1,4a-dimethyl-7-)1-methylethylidene- (1R-(1.alpha., 4abeta, 8a. Alpha)(7.20%), Butanoic acid , 3,7- dimethyl - 2,6-octadianyl ester (5.87%), n- hexadecanoic acid (4.27%), 4-Penten-2- 0l (3.76%), phenol, 4 - methoxy - 2,3,6 – trimethyl (3.02 %). The essential oil of C. bonariensis exhibited a strong antioxidant potential displaying at (0.001 mg/ml) 92.12 % DPPH and 1.74(µg/ml) of IC50, while exhibited the extract 54.06 % of DPPH and 4.86(µg/ml) of IC_{50}.While the total phenols was 156 mg/ml. The results of the essential oil of *C. bonariensis* showed significant antibacterial activity against many enteric pathogens tested as *Staphylococcus aureus*, Streptococcus pyogenes and Shigella dysenteriae with inhibitory zone of (16.9± 0.17, 15.9± 0.51 and 115.7± 0.4) mm at 5 mg / ml respectively. While inhibitory zone of ethanol extract was (15.1± 1.15, 13.0± 0.017 and 12.5± 0.2) mm at 5 mg / ml respectively.

Index Terms — HPLC Gas chromatography mass spectrometry; *Conyza bonariensis*; Essential oil; Antioxidant; Antibacterial

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1 Introduction

Aromatic and medicinal plants were used for centuries as remedies for human diseases because they contain chemical components of therapeutic value [1]. In the last few years, plant products and their modified derivatives have been rich sources for clinically useful drugs. According to the World Health Organization [2], more than 80% of the world's population rely on traditional medicine for their primary health care needs [3]. Essential oils are important components of *Conyza bonariensis,* and these oils contain large quantities of terpenes and aromatic compounds. They found a high correlation between the altitude where aromatic plants grow and their essential oil yield. However, altitude seems to affect the essential oil content of only oil-rich and oil-intermediate aromatic plants and it does not seem to influence oil poor plants [4].

There has been increased development of antimicrobial drug resistance to presently available antimicrobials [5]. Plant extracts and essential oils can be used as potentially useful sources of antimicrobial compounds. Since ancient times, the crude herbal extracts of aromatic plants have been in use for food preservation and they have also been considered very valuable for their usage as antimicrobials [6 - 7].

The aim of the present study is to find out the chemical compounds of the essential oil obtained from *Conyza bonariensis* and the determination of the antioxidant and antibacterial activities of essential oils and ethanol extract and compare their activities.

2 Materials and Methods

2.1 Instrumentations

Tert-butylhydroquinone (TBHQ), ascorbic acid (Vitamin C), 2,2-diphenyl-1-picrylhydrazyl (DPPH), were from Sigma (USA) and D-101 macroporous resin was from Tianjin Dajun Co., Ltd. Spectrophotometer (Shanghai-Techcomp, UV 2300), balance (Shanghai-Mettle Toledo, AB 204–N), rotary evaporator (Shanghai-Biochemical Equipment) and Microwave (Beijing- Xianghu Science & Technology.XH-200A) were used.

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2.2. Plant collection and identification.

The whole plants of *C. bonariensis* were collected from Taiz region (Yemen). Identification was carried out by the Agricultural Research Authority (Taiz).

2.3. Preparation of essential oil.

The *C. bonariensis* was air-dried in shade, (200 g) were chopped to small pieces and subjected to hydro - distillation (1000 ml distilled water 200 g plant material, in 2000 ml round bottom flask) using a Clevenger type apparatus for 3h. Separating water from oil by diethyl ether, and remove diethyl ether by drying. The oil was collected, and stored in a sealed vial at – 4°C until analysis [8].

2. 4. Analysis of the essential oil by GC-MS.

GC-MS analyses were performed with a Varian 1200 L gas chromatograph equipped with a DB-5 relatively nonpolar capillary column (30 m length, 0.25 mm film thickness, 0.25 mm internal diameter). [9] (kumar et al., 2011). Analytical conditions: injector and transfer line temperatures 220 and 260°C, respectively; oven temperature programmed from 50°C to 220°C at 15°C per minute and a hold at 220°C for 25 min., carrier gas helium at 1 ml/min. Mass spectra electron impact mode (Ionization potential 70 eV), ion source temperature 200°C and mass range 35 – 500 Da. Identification of the components of essential oil was based on retention indices (RI) relative to n-alkanes and computer matching with the Wiley spectral libraries.

2. 5. Preparation of ethanol extract using microwave-assisted extraction (MAE).

5 grams of plant was extracted with 150 ml of 90% ethanol, in microwave at 80 ºC, extraction power 300 w and extraction time 45 min under different MAE conditions. MAE was performed on microwave apparatus using vessel system. After extraction, the vessel was allowed to cool at room temperature. The ethanol extracts were filtered and the solvents were removed using a rotary evaporator. Dried extracts were kept refrigerated until use.

2. 6. Total Phenols (TP).

The TP was determined spectrophotometrically according to the Folin–Ciocalteu's method [10]. Briefly, the sample extract (0.2 ml) was mixed with 5 ml of deionized water, 10 ml of 7% (w/v) sodium carbonate, and 1 ml of the Folin-Ciocalteu reagent. After incubation at room temperature for 90 min, the absorbance of the reaction mixture was measured at λ 765 nm against a blank containing the same mixture solution without the sample extract. 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 of dry extract).

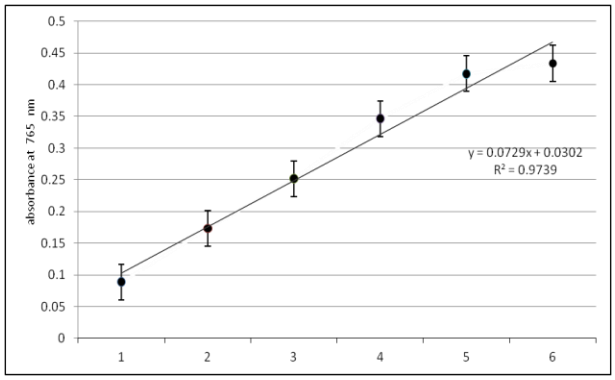

Fig. 1. Calibration curve for Gallic acid (mg/g of dry extract)

2. 7. Determination of antioxidant activity using the DPPH radical scavenging method

In this assay, the antioxidant activity of essential oil and ethanol extract were evaluated by measuring the bleaching of the purple-colored ethanolic solution of DPPH [11] The antioxidant activity of six different concentrations (0.2, 0.1, 0.05, 0.01, 0.005, and 0.001 mg/ml) of essential oil and ethanol extract were measured in terms of hydrogen donating or radical scavenging ability [12]. The mixtures were well shaken and kept at room temperature in the dark for 30 min., then absorbance (A) was measured at 517 nm using a spectrophotometer. Vitamin C and TBHQ were used as positive controls, while ethanol was the blank. Inhibition (I %) of DPPH radical was calculated using the equation,

$$I\ (\%) = A_{blank} - A_{sample} / A_{blank} \times 100 \qquad (1)$$

The inhibitory concentration (IC_{50}) value represents the concentration of essential oil and plant extracts that caused 50% inhibition.

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

The antioxidant activity (AA) of essential oil and ethanol extract were evaluated by the β-carotene, according to the method [13]. One milliliter of β-carotene solution (0.2 mg/ml in chloroform) was pipetted into a round-bottom flask containing 0.02 ml of linoleic acid and 0.2 ml of Tween 20. The mixture was then evaporated at 40°C for 10 min using a rotary evaporator to remove the chloroform. Then, the mixture was immediately diluted with 100 ml of distilled water and agitated vigorously to form an emulsion. 5 ml aliquot of the emulsion was subsequently transferred into test tube containing 0.2 ml of extract. The tube was then gently mixed and placed in a water bath for 2 h at 50 °C. The absorbance was measured at 470 nm at initial time (t = 0) against a blank, consisting of an emulsion with β-carotene. Solvent in the 5 ml of emulsion was used as a control. The measurement was carried out at every 30 min intervals, while TBHQ (standards) was at 200 ppm. AA was calculated based on a formula:

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$$AA = [1- (A_0 – A_t) / (A^o_0 - A^o_t)] \times 100 \quad (2)$$

Where, (A_0) and (A^o_0) are the absorbance values measured at initial time of the incubation for samples or standard and control, respectively, while (A_t) and (A^o_t) are the absorbance values measured in the samples or standard and control at t = 120 min.

2. 9. 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 37 ºC ± 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 108 (CFU) /ml.

2. 10. Determination of antibacterial activity.

The antibacterial activity was studied by the agar well diffusion method [14]. The bacterial suspension was spread uniformly on the agar surface. Agar surface was perforated with 6 mm-diameter holes, aseptically cut and filled with 100 µl of essential oil and ethanol extracts. The essential oil and ethanol extracts were used in the concentration of 5mg/ml of (DMSO). Streptomycin and Penicillin were used as a reference antibacterial, whereas DMSO was the negative control. The plates were incubated at 37 ºC ± 1ºC for 21 h and then examined to verify inhibition. A positive result was defined as an inhibition zone of 9 mm or more around the holes.

2. 11. Statistical analysis.

Statistical methods were used to calculate means and standard deviations of three replications. Statistical analysis (SPSS, 20) was applied to the data to determine differences ($P < 0.05$) performed by ANOVA.

3 Results and Discussion

3. 1. Chemical composition of the essential oils.

The essential oil yield by the steam distillation for 3 h, obtained from fresh leaves, and roots of the *C. bonariensis*, collected during the flowering phase in Taiz locations in southern Yemen, the yield was 0.4% (w/w).

The technique, GC–MS, is a useful tool in modern food, medicine and biological research aiming at the separation and identification of components of organic mixtures, and this method has already been applied successfully in the analysis of various essential oils [15].

To the best of our knowledge, the composition of ethanol extract of *C. bonariensis* has not been reported earlier detailed and therefore the results of the present study may be considered as the first report on the composition of the ethanol extracts of this unique and endemic species. However, it is noteworthy that the composition of the essential oils and extracts of a particular species of plant may differ depending upon the harvesting season, extraction method, and geographical location of the plant materials, and that those from the different parts of the same plant may also differ widely [16].

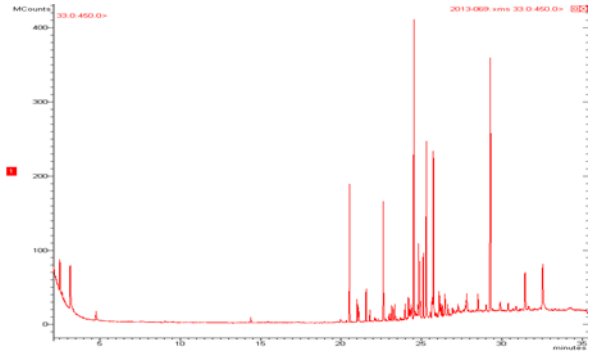

Fig. 2. GC-MS Chromatogram of essential oil in *C. bonariensis*

The essential oil from *C. bonariensis* was obtained by hydro distillation and analyzed by gas chromatography mass spectrometry (GC- MS). The essential oils shown in Fig.2. Led the analysis of the essential oils of *C. bonariensis* by GC–MS to the identification and quantification of a total of 44 major components between 25 and 32.5 min, representing 100% of the essential oil Table 1. The oil composition is dominated by the presence of sesquiterpene accounting for 51.14% of the total oil composition. The principal components of the essential oil were Aromadendrene oxide - (2) (17.38%), Cedren - 13- ol, 8 (17.30%), Caryophyllone oxide (7.23%), alpha – Bisabolol (2.32%), Isoaromadendrene epoxide (1.69%), Globulol (1.49%), Thymol (2.86%), Tetracyclo (6.3.2.0(2.5). 0 (1.8) tridecan-9-ol, 4,4-dimethyl (8.41%), 1 - Naphthalenol, decahydro-1,4a - dimethyl-7-) 1 - methylethylidene) - (1R-(1. alpha., 4abeta, 8a. Alpha) (7.20%), Butanoic acid, 3,7- dimethyl - 2,6-octadianyl ester (5.87%), n- hexadecanoic acid (4.27%), 4-Penten-2-0l (3.76%), phenol, 4 - methoxy - 2,3,6 – trimethyl (3.02%).

The essential oil composition of various members of this genus has been reported and they contain monoterpene and sesquiterpene in their volatile fractions. A fair amount of variation in composition was observed in different parts of this plant. The chemical class distribution of essential oil is given in Table 1. It can be seen that the essential oil consisted mostly of sesquiterpenoids and monoterpenoids, and some organic compounds (*e.g.* Ethyl acetate and 2-Butanol) [17,18], but these oils are considered very rich sesquiterpene [19], which are C15 compounds formed by the assembly of three isoprenoid units (*e.g.* Cedren - 13- ol, 8, Caryophyllene oxide, Aromadendrene oxide - (2), alpha – Bisabolol, Globulol, Isoaromadendrene epoxide, Verrucarol) Fig.3, which have an important role as antioxidants, antibacterial and antifungal and against some diseases [20-17]. Also essential oils in Table 1 contain monoterpenoids (*e.g.* Thymol) has a resistance to the effects of inhi-

bition of bacteria and fungi and antioxidant activity due to its ability to donate H-atoms from phenol hydroxyl groups, which could react with peroxyl radicals to produce stabilized phenoxyl radicals [21].

Table 1. The GC–MS analysis of the essential oil of *C. bonariensis*

Peak No.	Name of the compounds	R. Time	Area %
1	Ethyl acetate	2.523	1.95
2	4-Penten-2-0l	3.17	3.67
3	2-Butanol	4.78	0.59
4	Acetic acid	19.968	0.33
5	2H-pyran-3-ol,6-ethenyltetrahydro-2,2,6,trimethyl	20.328	0.33
6	Eicosane	20.328	0.08
7	Butanoic acid, 3,7- dimethyl - 2,6-octadianyl ester	20.53	5.78
8	Phenol,5-mrthyl-2-(methylethyl)-,acate	20.998	0.92
9	2,6-octadien-1-ol,3,7 - dimetjyl	21.081	1.07
10	Butanoic acid , 3 - methyl - , 1 - ethenyl,1,5dimethyl - 4-hexenyl ester	21.559	1.37
11	Butanoic acid, 3 - methyl -, 1 - ethenyl-1,5dimethyl - 4 hexenyl ester	21.739	0.31
12	2H- Benzopyran, 3,5,6,8atetrahydro - 2,5,58a - tetramethyl-, trans	22.089	0.17
13	3-Buten - 2 - one, 4 - (2,6,6, - trimethyl - 1 - cyclohexen - 1 -yl)	22.14	0.09
14	Caryophyllone oxide	22.624	5.59
15	Alpha - Caryophllene	22.973	0.36
16	7,11-Hexadecadienal	23.116	0.63
17	12-Oxabicycle (9.1.o)dodeca-3,7 - diene, 1,5,5,8 - tetramethyl - (IR-(1R , 3E.7E.11R)	23.225	0.77
18	Caryophylleny alcohol	23.354	0.76
19	2-Pentadacanone , 6,10,14-trimethyl	23.574	0.64
20	Octadecane	23.668	0.79
21	Octadecane	23.727	0.09
22	Globulol	24.183	1.15
23	Globulol	24.267	0.24
24	1H-Cycloprop (e)azulen-4-ol, decahydro-1,1,4,7-tetramethyl-, (1 ar-(1a. alpha, 4 bete, 4a. Beta, 7 alpha. 7a. Beta, 7b. Alpha))	24.346	0.45
25	Thymol	24.398	0.20
26	3 - Oxabicyclo (4.2.0) oct - 5 - ene , endo - 8- methyl - exo - 8 - methyl - exo - 8 - (2 - propenyl)	24.552	1.19
27	Thymol	24.809	2.86
28	Alpha - Bisabolol	24.901	2.32
29	1 - Naphthalenol, decahydro-1,4a - dimethyl-7-) 1 - methylethylidene) - (1R-(1. alpha., 4abeta, 8a. Alpha))	25.292	7.20
30	Tetracyclo(6.3.2.0 (2.5).0 (1.8)tridecan-9-ol,4,4-dimethyl	25.736	8.41
31	Aromadendrene oxide - (2)	26	17.38
32	Isoaromadendrene epoxide	26.092	1.00
33	Menthol, 1 - (butyn – 3 – 1 - 1 - yl) -, (1S.2S.5R)	26.182	0.47
34	Diethyl Phthalate	26.28	0.544
35	Caryophyllene oxide	26.449	1.64
36	4-tert - Butyl - o- phenylene diecetate	26.64	0.33
37	Cedren - 13- ol, 8	27.81	1.11
38	Benzaldehyde. 3.4.5 - tiimethoxy	28.506	0.94
39	Caryophylleno oxide	29.018	0.54
40	Cedren - 13- ol, 8	29.273	17.188
41	Isoaromadendrene epoxide	29.9	0.69
42	Verrucarol	30.394	0.60
43	Phenol, 4 - methoxy - 2,3,6 - trimethyl	31.432	3.02
44	N- hexadecanoic acid	32.53	4.27

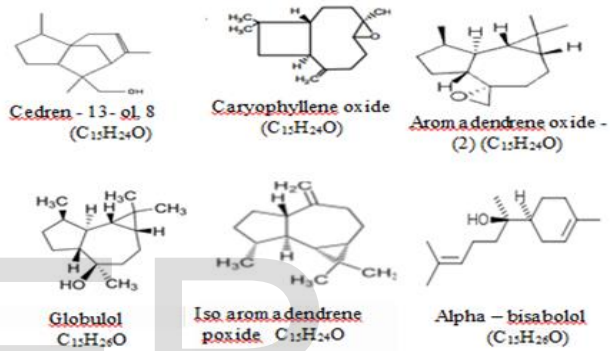

Fig. 3. Chemical structures of sesquiterpene (common compounds) in the essential oil of *C. bonariensis*

3. 2. Total phenolics

Polyphenols contribute to color and sensory characteristics of fruits and vegetables and play an important role in providing protection against in vivo and in vitro oxidation. Phenolic compounds are a class of antioxidant agents which act as free radical terminators [23]. The phenolic and flavonoid contents are considered as important indicators of antioxidant capacity. The content of polyphenols was (156± 0.44) mg/g in *C. bonariensis*. Some studies indicated of C. bonariensis extract was 167.9 mg/g [24]. There is a strong correlation between total phenols and antioxidant activity in Table 2. These findings suggest that total phenols are a good predictor of in vitro antioxidant activity.

3. 3. Antioxidant activity using (DPPH) radical scavenging.

The antioxidant activity depends on the chemical composition. Antioxidant activities of essential oils can be attributed to not only the presence of a high percentage of the main constituents, but also the presence of other constituents in small quantities or the synergy among them. Some essential oils, poor in phenolic compounds might also possess antioxidant potentials [25]. They also serve in plant defense mechanisms to

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counteract reactive oxygen species (ROS) in order to survive and prevent molecular damage and damage by microorganisms, insects, and herbivores [26]. Furthermore, some researchers demonstrated that essential oils, which contain oxygenated sesquiterpenes and/or monoterpenes, possess greater antioxidative properties[27,28]. In this study, was determined the ability of essential oils and ethanol extracts of *C. bonariensis* to scavenge DPPH radical on the basis of their concentrations providing 50% inhibition (IC_{50}).

Table 1 Indicates that the essential oil of *C. bonariensis* was significantly rich in caryophyllene oxide, thymol, alpha - bisabolol and globulol, which may act as radical-scavenging agents. The antioxidative activity of *C. bonariensis* in Table 2. Shown significant differences ($P < 0.05$), where essential oil, ethanol extracts, V.C and TBHQ at 0.001mg/ml were (92.12 ± 0.25, 74.06 ± 0.31, 90.01 ± 0.06 and 92.09 ± 0.17) % respectively. The current results further show that the antioxidant activity of essential oil can be attributed to the synergistic activities for compounds such as Caryophyllene oxide, Cedren, alpha – Bisabolol, Thymol, Aromadendrene oxide and Globulol.

Effectiveness of antioxidant activity and scavenging ability is inversely correlated with their IC_{50} values. The IC_{50} value of antioxidant activity of the essential oil is found to be 1.74 ± 0.15 µg/ml, while in ethanol extract, V.C and TBHQ were 4.86 ± 0.28, 6.21 ± 0.08 and 2.36 ± 0.26 µg/ml respectively. Fig. (4). Such results can be explained by the different chemical composition of the oil. The highest percentage of a phenolic compounds present in the essential oil can be responsible for the higher ability to scavenge free radicals such as H•, measured by DPPH method. The presence of available hydrogen atoms from phenol represents a good barrier against the primary oxidative process.

Table 2. Antioxidant activity by DPPH scavenging and BCB of essential oil and ethanol extracts from *C. bonariensis* and reference antioxidants (V.C and TBHQ) (means ± S.D.).

Type	DPPH scavenging test		β -carotene bleaching	Total phenols
	Inhibition of DPPH (%) (0.01mg/ml)	IC_{50} (µg/ml)	BCB % [1 mg /ml]	[mg (GAE) /g]
Essential oil	92.12a± 0.25	1.74a±0.15	91.45a ± 0.34	--
Ethanol extracts	74.06c±0.31	4.86c±0.28	90.08c ± 0.06	156 ± 0.44
V.C	90.01b±0.06	6.21d±0.08	---	--
TBHQ	92.09±0.17	2.36b±0.26	91.13a ± 0.23	--

The essential oil and ethanol extract of *C. bonariensis* in the DPPH assay had good antioxidant properties Fig. 4. The antioxidant efficiency of the essential oil and ethanol extract tested were basically dependent on their concentrations. The essential oil and ethanol extract of *C. bonariensis* maintained stability and the strength of antioxidative stress in different concentrations, but the essential oil was stability better compared to ethanol extract, V.C and TBHQ.

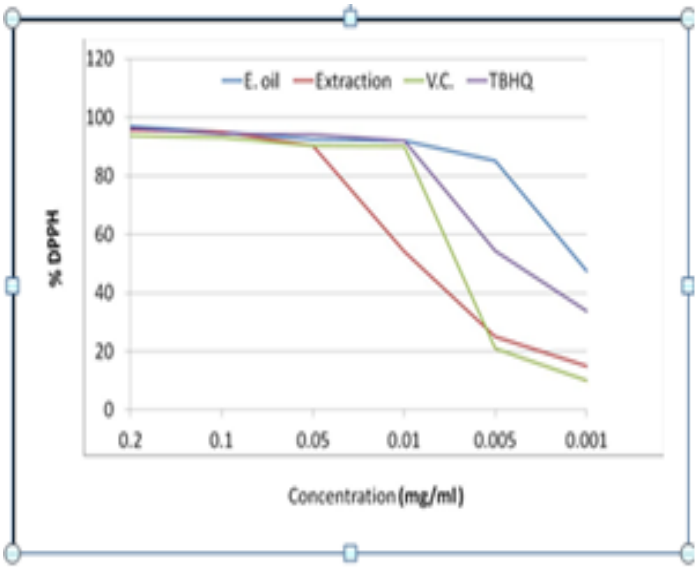

Fig. 4. DPPH radical scavenging activities of essential oil and ethanol extracts from *C. bonariensis* and reference antioxidants (V.C and TBHQ).

3. 4. Antioxidant activity using β-Carotene bleaching assay.

In the BCB assay, the oxidation of linoleic acid generates peroxyl free radicals due to the abstraction of a hydrogen atom from diallylic methylene groups of linoleic acid [29]. The free radical will then oxidize the highly unsaturated β-carotene. The presence of antioxidants in the fraction will minimize the oxidation of β-carotene by hydroperoxides. Hydroperoxides formed in this system will be neutralized by the antioxidants from the fractions. Thus, the degradation rate of β-carotene depends on the antioxidant activity of the fractions.

In the present study, essential oil showed a higher ability to prevent the bleaching of β-carotene than that of ethanol extract in *C. bonariensis* and this antioxidant capacity can possibly be due to other oil components, e.g. phenolic compounds (Caryophyllene oxide, Cedren, alpha – Bisabolol, Thymol, Aromadendrene oxide and Globulol), which were investigated in this study. The BCB mean for essential oil was 91.45 %. While the ethanol extract was 90.08%. It can be observed that the synthetic antioxidant, TBHQ has a stronger antioxidant activity when compared to ethanol extract Table 2.

The Fig.5 shows no significant difference in the stability of both the essential oil, ethanol extract and TBHQ. Although, the stability of the essential oil higher than in the ethanol extract within 2 h.

3. 5. Antibacterial activity.

Antimicrobial activity of the essential oils and ethanol extract has been evaluated in vitro against five bacterial species included two Gram-positive bacteria (*Streptococcus pyogenes* and *Staphylococcus aureus*), three Gram-negative bacteria (*Escherichia coli* , *Shigella dysenteri*aeand and *Salmonella typhimurium*) Table 3.

Table 3. Antibacterial activities of the essential oil and ethanol extracts of *C. bonariensis* by agar well diffusion assay.

		Inhibition zone (mm)[1]				
Extraction	Conc.	Escherichia coli	Shigella dysenteriae	Salmonella typhimurium	Streptococcus pyogenes	Staphylococcus aureus
Essential oil	5	13.5± 0.2	15.7± 0.4	14.0± 1.2	15.9± 0.51	16.9± 0.17
Ethanol extract	mg/mL	10.7± 0.6	12.5± 0.2	12.3± 0.3	13± 0.017	15.1± 1.15
Streptomycin2	5	13.2± 0.18	12.0±0.66	12.5±0.50	ND	ND
Penicillin[2]	µg/mL	ND	ND	ND	14.5± 0.77	13.9± 0.50

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

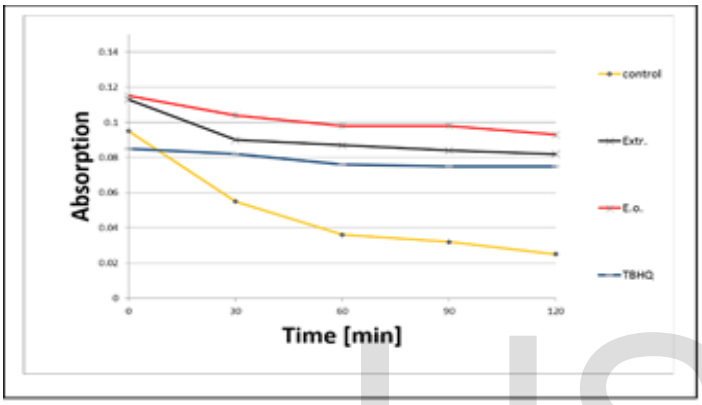

Fig. 5. Antioxidant activity of essential oil and ethanol extracts from C. bonariensis and reference antioxidants (TBHQ) in β-carotene-linoleate bleaching system.

This samples studied in this work showed antimicrobial activity against the test microorganisms. The most active essential oils was, whereas, the least active ethanol extract. It showed that the essential oil was the most active on Gram-positive bacteria compare with ethanol extract, while the Gram-negative bacteria were less inhibition. The results of the present study are encouraging the essential oil of *C. bonariensis* showed significant antibacterial activity against many enteric pathogens tested as Staphylococcus aureus, *Streptococcus* pyogenes and *Shigella dysenteriae* with inhibitory zone of (16.9± 0.17, 15.9± 0.51 and 115.7± 0.4) mm at 5 mg / ml respectively. While inhibitory zone of ethanol extract was (15.1± 1.15, 13.0± 0.017 and 12.5± 0.2) mm at 5 mg / ml respectively.

4 Conclusion

This study shows that essential oils containing 44 compounds and considered rich of sesquiterpenoids percentage 51.14%. The total identified compounds accounted for about 100% of the oil. The study showed that antioxidant and antibacterial activities were related to the chemical composition of the essential oils from *C. bonariensis*. The results obtained by the use of methods (DPPH), (BBC) and agar well diffusion showed that essential oils from *C. bonariensis* can be considered a good source of natural antioxidants and antibacterial. This may be attributed either to high percentage of the main constituents or to synergy among different oil constituents

5. Acknowledgements

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