Research Article

In vitro Analysis of Antioxidant and Antimicrobial Activity of Iraqi *Bryonia dioica*

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Received: 10-02-2017; **Revised:** 06-03-2017; **Accepted:** 20-03-2017.

ABSTRACT

Bryonia dioica is used as a medicinal plant in traditional medicine. This study was performed to investigate the phytochemical, antioxidant and antimicrobial potentials of *Bryonia dioica* by using different in-vitro methods. 1, 1 Diphenyl 2 picryl hydrazyl (DPPH) was used for determination of antioxidant potential of ethanolic extract. Antibacterial analysis carried out using agar well diffusion method for different concentrations of aerial parts extract of plant. Qualitative phytochemical analysis of different metabolites was performed using specific chemical tests on ethanolic extract after extraction by 80% ethanol using soxhlet apparatus. Preliminary phytochemical investigation of *Bryonia dioica* indicated the presence of various chemical compounds including alkaloids, Glycosides, steroids, Tannins, Carbohydrates and flavonoids. The results exhibited that *Bryonia dioica* extract has a valuable antibacterial activity against *E.coli*, *K. pneumoniea*, and *P. valgaris*. In addition it has significant antioxidant activity especially in concentrations of 100 and 150 and 200 mg ml^{-1} at which plant extract shows similar reading as that of ascorbic acid. The experimental data verified *Bryonia dioica* displayed remarkable antioxidant activity. Furthermore, ethanolic extract has considerable antibacterial activity that comparable to the tested antibiotics especially against *P. valgaris*.

Keywords: Antibacterial, Antioxidant, *Bryonia dioica*, Phytochemistry.

INTRODUCTION

Plants have played an important function in the treatment of many diseases from ancient time [(1)]. *Bryonia dioica*, A climbing perennial herb with tuberous roots which developing in temperate Europe, North Africa, and western Asia[2]. it has been used in diverse system of traditional medication for the treatment of ailments of human beings[3]. *Bryonia dioica* is used for both internal and external uses[4]. It is taken orally in small amounts for various inflammations, bronchial complications, asthma, intestinal ulcers, hypertension[5] and arthritis[6]. Topically, it is applied as a rubefacient to muscular and joint pains and pleurisy[7]. Reports shows that the plant is used in folk medicine as a drastic purgative, emetic, bitter tonic and anti-diabetic agent[8]. In Europe it is considered to be a cicatrizing agent[9]. In Iraq it has been reported that the plant is used in folk medicine to treat bronchitis and as anti-diabetic agent, also their leaves and seeds traditionally used for treatment of fevers[10]. *B. dioica* Jacq. may be suggested as a new potential source of natural antioxidant and there was a good correlation between total phenol content and antioxidant capacity of the extracts[11]. The roots have been used as an antiphlogistic, detoxicant and anodyne, while the whole plant was used for the treatment of sclerosis, nose cancer and uterus tumors[12]. There are many reports indicated that *B. dioica* are efficient sources for antibacterial compounds[13]. In addition, many phytochemical studies revealed the presence a variety of bioactive ingredients[14, 15]. This study was designed for determination of antioxidant and antibacterial potential of Iraqi *B. dioica* Jacq. and thus interpret the scientific basis for the traditional uses of the plant. As well phytochemical investigation of secondary metabolites was performed.

Plant Material

Whole plant was collected from south of Baghdad. The plant was authenticated by the National Herbarium at Abu-Graib, the plant leaves were dried in the shade for few days at room temperature and then powdered and weighed.

Extraction

Powdered plant leaves were defatted with petroleum ether for 24 hours then dried at room temperature. Defatted plant materials then extracted with 70% ethanol using soxhlet apparatus until exhaustion for 16 hours. Alcoholic extract was evaporated under reduced pressure at a temperature not exceeding 40°C to produce crude extract.

Phytochemical Analysis

Chemical tests were carried done using the ethanolic extracts of the plant and standard procedures[16, 17] were carried out to identify the active constituents as listed in the Table 1.

Antibacterial Analysis

Bacterial strains were subculture in Meuller Hinton Agar plates for every 15 days and maintained on nutrient agar slants at 40C, for determination of antibacterial activity of

Bryonia dioica extracts against both Gram-negative and Gram-positive bacteria.

Table 1: Chemical tests used for identification of active constituents

constituent	Test
Alkaloids	**Mayer's Test**: 10 ml of alcoholic extract was mixed well with 5 ml of 1% HCL on a steam bath then added the reagent to produce white precipitate. **Wagner's test:** 10 ml of alcoholic extract was mixed well with 5 ml of 1% HCL on a steam bath then added the reagent to produce reddish brown colour precipitate. Both tests results indicate the presence of alkaloids
Flavonoids	**Lead acetate**: 5ml of alcoholic extract mix with 1 ml of 10%Lead acetate to produce a yellowish- white precipitate indicating a positive test for flavonoids.
Steroids	**Liebermann-Burchard**: 3 ml of extract sterried with chloroform, acetic anhydride and few drops of sulphuric acid to yeild dark pink or red colour indicate the presence of steroids. **H2SO4**: 2 ml of extract were treated with sulphuric and acetic acids.to develop a greenish color to give an indication for the presence of steroids
Anthraquinones	**Borntrager's test**: 3ml of extract was shaken with 3 ml of benzene. Then, filtration was performed and 5 ml of 10% ammonia solution was added to the filtrate. The mixture was shaken and the development of a pink, red or violet color in the ammonical (lower) phase indicates the presence of anthraquinones.
Tannins	**FeCl3 solution (5%w/v):** 3 ml of distilled leaves extract (in water) mixed with 3ml of FeCl3 solution (5%w/v) to produce dark green or blue black precipitate indicate the presence of tannins.
Cardiac glycoside	**Keller-kiliani test**: Alcoholic extracts (2ml) +1ml glacial acetic acid+ FeCl3+con.H2SO4. Formation of green-blue colour indicates the presence of cardiac glycoside.

Well diffusion assay was carried out and all plates were left to set and incubated at 37 ^{0}C for one day. The bacterial activity was expressed in term of average diameter of the clear zone around each well in (mm). Control is consisting of sterile distal water with 0.1 % DMSO. Tested bacteria include both gram positive (*Staphylococcus aureus*) and gram negative (*E.coli, Pseudomonas aeruginosa, Salmonella typhi, Proteus vulgaris and Klebsiella pneumonie*). The strains were obtained from collage of sciences / University of Baghdad in Al-gadreeah. The results were compared with the standard antibiotics, Amoxiclave (10 mg ml^{-1}), Cefuroxime (10 mg ml^{-1}), Cefixime (10 mg ml^{-1}) and Cefpodoxime (10 mg ml^{-1}). While extracts concentrations were (100, 125, 150, 175, and 200 mg ml^{-1}).

Antioxidant Activity (DPPH radical scavenging assay)

Antioxidant activity assessment includes UV Spectrophotometric detection [18]. Control solution (DPPH with methanol) was prepared in addition to the sample solution. Different concentrations of ascorbic acid were also prepared in methanol (0.025, 0.05, 0.1, 0.15 and 0.2 mg/ml). Then, 3 ml of each solution of ascorbic acid were mixed with 1 ml of 4×10^{-2} µ/ml (DPPH with methanol) solution and incubated for 30 min at 37 ^{0}C temperature in dark. Absorbance of each solution was taken at 517 nm. Different concentrations of the given sample and control were designed (25×10^{-3}, 50×10^{-3}, 100×10^{-3}, 150×10^{-3} and 200×10^{-3} mg/ml). 3 ml of each solution of given sample was mixed with 1 ml of 4×10^{-2} µ/ml (DPPH with methanol) solution and incubated at 37°C in dark. The absorbance of each solution was measured against blank at 517 nm and the antioxidant present of extract and Ascorbic acid was calculated by using formula:

Scavenging activity % = (AC-AS) / AC*100

AC: absorbance of control

AS: absorbance of different concentrations of ascorbic acid and given sample with DPPH solution.

RESULTS AND DISCUSSIONS

Phytochemical analysis

Result shows positive results for most of tested components which indicate the presence of alkaloids, flavonoids, anthraquinones, tannins and steroids as shown in Table 2.

Table 2: Qualitative phytochemical analysis of *Bryonia dioica* leaves extract.

Tested Component	Type of Test	Result
Alkaloids	Mayer	+Ve
	Wagner	+Ve
Flavonoids	Lead acetate	+Ve
Steroids	Liebermann-Burchard	+Ve
	H2SO4	+Ve
Tannins	FeCl3	-Ve
Anthraquinones	Borntrager	+Ve
Cardiac glycoside	Keller-kiliani	-Ve

The presence of these groups provides a scientific indication for the claimed ethenomedical uses of *Bryonia dioica* in the treatment of mentioned diseases.

Antibacterial Activity

The obtained results listed in table 3, results exhibited a valuable antimicrobial activity for leaves extract against three of the tested microorganisms (*E.coli, K.pneumoniea* and *P. valgaris*). Maximum zone of inhibition against E.coli at concentration 200 mg ml^{-1}, *K.pneumoniea* readings ranged from 43 mm to 45.66 mm at concentrations 100 mg ml^{-1} and 200 mg ml^{-1} respectively. Finally, leaves extract exhibited higher readings against *P. valgaris* compared to all tested antimicrobial agents with zone of inhibition equal to 52 mm at 200 mg ml^{-1} as shown in figure 1. Results attributed to the effects of present compounds which can act by different mechanisms. In this study the assumption is that the mixture consists of a mixture of active components with other constituents with minor activity that achieves a synergistic effect. However, this supposition should be confirmed by further analysis [19].

Table 3: Antimicrobial sensitivity assay for Bryonia dioica leaves extract compared to the tested antibacterial drugs.

Sample		Diameter of Zone of inhibition (mm)					
		E.coli	***S.aureas***	***K.pneumoniea***	***S. typhi***	***P. aeruginosa***	***P. valgaris***
B. dioica leaves extract (mg ml^{-1})	100	22.39 ± 2.52*	N/A	43 ± 1.73*	N/A	N/A	30.66 ± 1.15*
	125	52.33 ± 2.52*	N/A	45.33 ± 1.53*	N/A	N/A	31.33 ± 1.15*
	150	56 ± 1.73*	N/A	41 ± 3.06*	N/A	N/A	36.33 ± 0.58*
	175	60 ± 1.00*	N/A	46 ± 1.00*	N/A	N/A	45.33 ± 1.53*
	200	60.33 ± 1.53*	N/A	45.66 ± 0.58*	N/A	N/A	52 ± 2.00*
Amoxiclave		74.33 ± 1	50.66 ± 1	74.66 ± 1	22.33 ± 1	44 ± 1	35 ± 1
Cefuroxime		76.66 ± 1	64.66 ±1	65 ± 1	17.33 ± 1	44 ± 1	31.66 ± 1
Cefixime		76 ± 1	67.66 ± 1	73.66 ± 1	19.33 ± 1	43.66 ± 1	28.66 ±1
Cefpodoxime		76.66 ± 1	66.66 ± 1	73.33 ± 1	15 ± 1	42 ± 1	22.66 ± 1
Date presented as: mean ± Standard deviation, N/A: Not applicable							

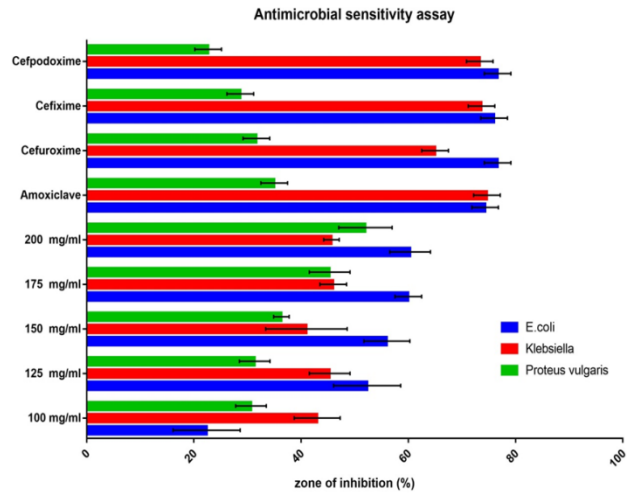

Figure 1: comparison between different antimicrobial agents and *Bryonia dioica* plant extract at different concentrations (bars represent 95% confidence interval and any 2 bars that intercept indicate non-significant difference).

Results indicated that *Bryonia dioica* extract is highly active against *E.coli* with MIC 143.9 mg ml^{-1} compared to its activity against *K.pneumoniea* MIC is 186 mg ml-1 and *P. valgaris* that have the higher MIC which equal to 227.3 mg ml-1. Table 4 and Figure 2.

Table 4: MIC of Bryonia dioica for each microorganism

Organism	MIC (mg ml^{-1})
E.coli	143.9 ± 14.8
S. aureas	N/A
Klebsiella	186 ± 11.53
S. typhi	N/A
P. aeruginosa	N/A
Proteus vulgaris	227.3 ± 9.342

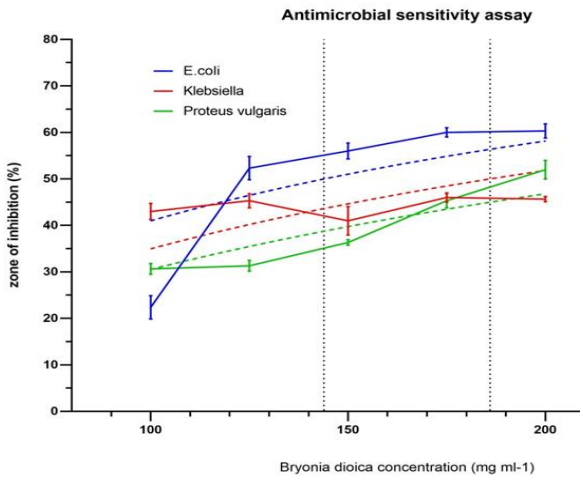

Figure 2: *Bryonia dioica* showing it dose response relationship, different MIC (dotted vertical lines) and none-linear fitted module.

Antioxidant Activity

DPPH is a stable free radical has colour changes from violet to yellow upon reduction by either the process of hydrogen- or electron- donation [20]. Antioxidant assay results showed interesting values especially at concentrations of 100, 150 and 200 mg/ml of plant extract at which there are non-significant difference in scavenging activity percentage from that of ascorbic acid with scavenging activity of 100%. Antioxidant activity is highly related to the increasing of the concentration. Results suggested that *Bryonia dioica* as a new potential source of natural antioxidant including phenolic compounds.

Table 5: Effect of concentration on scavenging activity % in *Bryonia dioica* extract and ascorbic acid.

Concentration (mg/ml)	Mean ± SE (%)		LSD value
	Sample	Ascorbic acid	
25	66.27 ± 2.74	95.70 ± 0.36	5.457 *
50	68.70 ± 0.56	90.70 ± 0.41	5.136 *
100	100 ± 0.00	100 ± 0.00	0.00 NS
150	100 ± 0.00	100 ± 0.00	0.00 NS
200	100 ± 0.00	100 ± 0.00	0.00 NS
LSD	4.195 *	3.822 *	---
** (P<0.01), NS: Non-significant.			

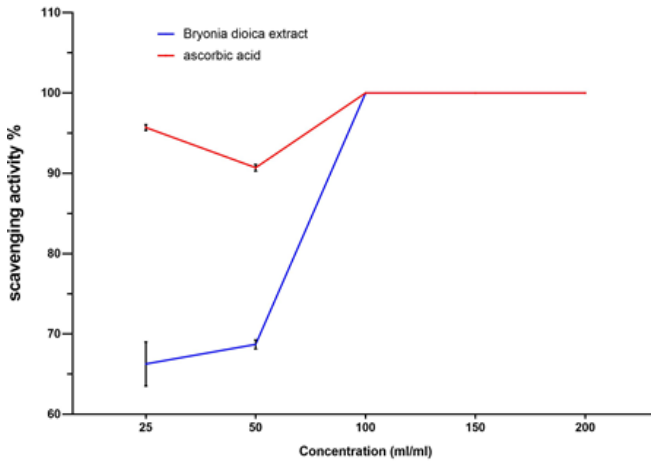

Figure 3: scavenger activity of *Bryonia dioica* extract and ascorbic acid

CONCLUSION

Investigations of Iraqi *Bryonia dioica* leaves extract revealed the presence of alkaloids, flavonoids, steroids, anthraquinoin and terpenoids. Plant considered an important pool of antioxidant components including poly phenolic compounds. Results exhibited a significant antimicrobial activity for leaves extract against three gram negative microorganisms including E.coli, *K.pneumoniea* and *P. valgaris.*

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Source of Support: Nil, **Conflict of Interest:** None.