International Journal for Pharmaceutical Research Scholars (IJPRS)

V-3, I-2, 2014 ISSN No: 2277 - 7873

RESEARCH ARTICLE

Phytochemical and Antioxidant Screening of *Suaeda vera* L. growing in Libya

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Manuscript No: IJPRS/V3/I2/00313, Received On: 17/06/2014, Accepted On: 27/06/2014

ABSTRACT

The ability of the plants to generate phytochemicals is environment factor. *Suaeda vera* is one of halophyte plants belonging to family Chenopodiaceae that grown in high salted area on the coast of Mediterranean sea in Libya. The abnormal ability of such plant to overcome and get along with the high salted environment, probably gives a strong signal to how such plant deals with the oxidative stress combined with the high salts contents of the soil. Phytochemical screening of *Suaeda vera* qualitatively and quantitatively revealed the ability of this plant to biosynthesis of phenolic compounds in high quantity. Ethyl acetate extract of *Suaeda vera* showed high quantities of phenolic constituents particularly, flavonoids, with strong ability to scavenging the DPPH free radical with IC50 equal to 188.12±3.12 µg/ml. preliminary antimicrobial activity have been done with a great sign for the resistance of the G +ve and G –ve microbial as well as fungus strains for the *Suaeda vera* extracts.

KEYWORDS

Phytochemical screening, Antioxidant activity, Halophytes, *Suaeda vera*

INTRODUCTION

The Chenopodiaceae (goosefoot family) is a large family including about 102 genera, and 1400 species of low growing plants. Most grow naturally in soils containing much salt (Halophytes)[1]. The abnormal ability of such plants to overcome and get along with the high salted environment, probably gives a strong signal to how such plants deal with the oxidative stress combined with the high salts contents of the soil. Halophytes can be avoiding the dangers of reactive oxygen species (ROS) by different ways. High percentage of phenolic particularly flavonoids secondary metabolites is one of these ways that enable halophytic plants overcome oxidative damage[2].

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Investigation of other Suaeda species such as Suaeda maritime[3], revealed the presence of phenolic tannins and flavonoids in addition to alkaloids as main constituents. Alkaloids like cholines and trigonellines[4], along with Coumarins and triterpenoidal saponins were isolated from *Suaeda soecies*[5]. In Libya, *Suaeda vera* species is widely distributed along the north region near the Mediterranean sea. No evidence literatures are concerning with the phytochemicals as well as biological or pharmacological effects of *Suaeda vera* species.

The chemistry of the flavonoids are predictive of their free radical scavenging activity as the reduction potentials of flavonoids and the consequently radical form, are lower than those of alkyl peroxyl radicals and the superoxide radical, which therefore means the flavonoids may inactivate these radical species and prevent the deleterious consequences of their reactions[6].

Thousands of publications have investigated the antioxidant activities of flavonoids and how they can contribute to the treatment of several diseases. Considering these publications, they indicate that biological and pharmacological effects of flavonoids may depend upon their behaviour as either antioxidants or as prooxidants[7].

The present work investigate the relation between the oxidative stress combined by high salt contents of *Suaeda vere* and the capacity to production of phenolic and flavonoids in addition to estimation of *Suaeda vera* extracts to scavenging the free radicals. The antibacterial and antifungal activities of *Suaeda vera* extracts were estimated as well.

MATERIALS AND METHOD

Methods

Plant Collection and Preparation of Extracts

Fresh herbs of *Suaeda vera* were collected from the coast of the Mediterranean sea near Benghazi, Libya and identifies by comparison with standard sample in the herbarium of faculty of science, Benghazi University.

The plant was washed with tap water and left for drying in the open air place. 100 gram of the dried *Suaeda vera* powders was gradually extracted by continuous soxhlation with petroleum ether, chloroform, ethyl acetate and ethanol (500 ml), respectively.

All fractions were evaporated to dryness using rota vapour (IKA-WERKE, GMBH & Co. Kg, D-79219 Staufen, Germany). The different fractions were reconstituted in their extraction solvent to give the required concentration needed in this study.

Phytochemical Screening of the Suaeda Vera Extracts

Preliminary screening of *Suaeda vera* different extracts were performed to investigate the presence or absence of the different phytochemical constituents such as phenolics, flavonoids, tannins, saponins and alkaloids using standard procedures described by Alex *et al*[8]. The qualitative results showed in table 1.

Quantitative Estimation of Total Phenolics

Total phenol contents of *Suaeda vera* different extracts were estimated by the modified Folin-ciocalteu method according to Hamdoon *et al*[2]. An aliquot of 0.5 ml of each extract (1 mg/ml) was mixed with 2.5 ml Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 2 ml (7.5 % w/v) of sodium carbonate (Na_2CO_3). The tubes were vortexed for 15 s and allowed to stand for 30 min at 40 °C for colour development. Absorbance was then measured at 765 nm using spectrophotometer (Spectro UV-VIS double, 110V, 60Hz, Serial No. Double 001158, Labomed, Inc. U.S.A.). Total phenolics content of different extracts were expressed as mg/g tannic acid equivalent using the following equation from the calibration curve: Y = 0.4879×, R2= 0.9064, where × is the absorbance and Y is the tannic acid equivalent in mg/g. The experiment was conducted in triplicate and the results were expressed as mean ± SD values.

Quantitative Estimation of Total Flavonoids

Total flavonoid contents of *Suaeda vera* extracts were determined by method described by Ordonez *et al*. based on the formation of a flavonoid-aluminum complex[9]. 0.5 ml of various solvent extracts (1 mg/ml) was mixed with 0.5 ml of aluminum chloride (2% in ethanol).

The resultant mixture was incubated for 60 min at room temperature for yellow colour development which indicated the presence of flavonoids. Absorbance was measured at 420 nm using UV–VIS spectrophotometer. Total flavonoid content was calculated as quercetin equivalent (mg/g) using the following equation based on the calibration curve: Y = 0.217x, R2 = 0.9582, where × is the absorbance and Y is the quercetin equivalent.

Quantitative Estimation of Total Flavonols

Total flavonols of *Suaeda vera* different extracts were determined by method described by Omoruyi *et al*[10].

The reaction mixture consisting of 2 ml of the sample, 2 ml of aluminium chloride prepared as (2% in ethanol) and 3 ml of sodium acetate

solution (50 g/l) was allowed to incubate for 2.5 h at 20°C. Absorbance at 440 nm was measured. Total flavonol content was calculated as mg/g of quercetin equivalent from the calibration curve using the equation: Y = 0.217×, R2 = 0.9582, where × is the absorbance and Y is the quercetin equivalent.

Scavenging Activity of 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) Radical

Scavenging activity of the *Suaeda vera* extracts was measured by exerting the effect of extracts on DPPH stable free radical according to method described by Hosny M. *et al*[11], with some modification. 1.9 ml of DPPH-ethanol solution (300 µM) was mixed with 0.1 ml of different concentrations (6.5–500 µg/ml) of various extracts. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Quercetin and Butylated hydroxyl anisole (BHA) were used as standard antioxidants. The percentage of free radical scavenging was calculated according to the following equation: % scavenging = (1-Sample absorbance517/blank absorbance517) X 100.

Antimicrobial Screening

Microorganisms

Bacterial strains include *Staphylococcus aureus* (Gram-positive), *Escherichia coli, Klebsiella pneumoniae,* and *Proteus mirabilis* (Gram-negative). In addition to *Candida albicans* (fungal strain) were used in this study. These microorganisms were kindly provided by Microbiology Laboratory of AL-Jamhoria Hospital - Benghazi, Libya. The bacterial strains were maintained in Nutrient Agar and Blood Agar, and the fungal strains were maintained in Subouraud Agar. The strains were subcultured from microbial pathogens in urine of patients with Acute Urinary Tract Infection, and in throat swab of Patients with Respiratory Tract Infections.

Antibacterial Assays

The antibacterial activity of the plant extract was determined by Agar Well Diffusion Method described by Perez *et al*[12], and Vikas Dhingra *et al*[13]. Briefly, nutrient agar and blood agar were used as the culture medium for this assay. The molten nutrient agar or blood agar was dispensed in pre-sterilized petridishes (25 ml each), the plates were allowed to solidify by cooling.

After solidification holes/wells (cups) of 6 mm diameter were punched into the agar with the help of flamed cork borer. Five wells were prepared for each plate. Of these five, three holes were filled with 0.01 ml of the plant extract and the fourth hole was filled with 0.01 ml of standard antibiotic solution (Gentamycin, 500 µg/ml) and the fifth hole was filled with blank (extracting solvent alone).

The petridishes were incubated at 37° C for 24 hrs. Diameter of the inhibition zone formed around each hole (well/cup) were measured. Testing was carried out for each bacterium two times.

Antifungal Assays

The antifungal activity of the plant extract was determined by Streak Plate Method according to method described by Orzechowski[14]. Subouraud agar medium about 15-20 ml were poured in petridish containing 0.2 ml of the plant extract. They were mixed thoroughly. Medium was allowed for solidification. With the help of inoculation loop dipping in the particular fungal strain, streak was made in petridish on the solidified Subouraud agar medium. They were incubated at room temperature. After 48 hours, observations were recorded. Testing was carried out for the fungal strain two times.

RESULTS

Result of Phytochemical Screening

The result of the preliminary phytochemical screening for the *Suaeda vera* extracts give a clear resulted to the presence of phenolics, flavonoids, tannins and alkaloids in addition to carbohydrates and sterols. The tests also revealed that the absence of anthraquinons in all extracts. Table 1 showed these resulted in a qualitative manner.

Table 1: Phytochemical screening of *Suaeda vera* extracts in qualitative manner

Constituents/ extracts	Ethanol extract	Ethyl acetate extracts	Chloroform extract	Petroleum ether extracts
Phinolics	+++	++++	+++	-ve
Flavonoids	++	+++	++	-ve
Tannins	+++	++++	-ve	-ve
Anthraquinon	-ve	-ve	-ve	-ve
Alkaloids	+++	-ve	-ve	-ve
Saponins	+	-ve	-ve	-ve
Carbohydrates	++	-ve	-ve	-ve
Sterols	-ve	-ve	++	++++

Key: −ve (Absent), + (Low in abundance), ++ (Moderate in abundance), +++ (High in abundance).

Table 2: Quantitative estimation of total phenolic, flavonoids and flavonols in Suaeda vera extracts

Extracts	Total phenolic	Total flavonoids	Total flavonols
Ethanol extract	164.42 ± 4.31	66.83±6.62	34.50±3.12
Ethayl acetate extract	371.77 ± 0 .99	98.08 ± 4.15	44.91±1.09
Chloroform extract	242.48 ± 2.63	76.49 ± 5.78	35.18±2.84
Petrollium ether extract	3.67 ± 4.29	0.93±1.30	Nd

The results are expressed as a mean ± SD of three independent measures.

Table 3: Scavenging activity of *Suaeda vera* extracts and stander antioxidant controls (the results expressed IC_{50} in µg of extracts /ml)

Extracts and controls	IC_{50} of DPPH inhibition in µg/ml			
	Ethanol extract	Ethyl acetate extract	Chloroform extract	Petrollium ether extract
Suaeda vera	Nd	188.12±3.12	392.29±5.09	Nd
Quercetin	13.10±5.99			
Butaylated hydroxy anisole	9.54± 1.33			

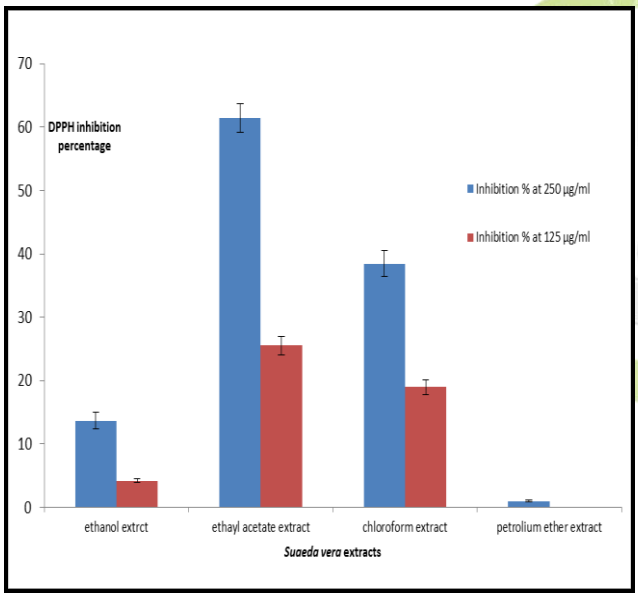
The results are expressed as a mean ± SD of three independent measures.

Result of Quantitative Analysis

Determination of total phenolic, flavonoids and flavonols were measured for the *Suaeda vera* different extracts. The results showed in table 2, as mg/gram of the extracts. The results are expressed as a mean ± SD of three independent measures.

Result of Antioxidant Activity (DPPH Scavenging Activity)

Antioxidant activity of the *Suaeda vera* extracts are expressed as the ability of these extract to inhibit the DPPH stable free radical purple colour. IC_{50} of the different extracts of *Suaeda vera* in addition to quercetin and butaylated hydroxy anisole (positive controls), are expressed in the table 3. Antioxidant potency comparison between different extracts at 250 µg/ml and 125 µg/ml are showed in Figure 1.



The results are expressed as a mean ± SD of three independent measures

Figure 1: Comparison between *Suaeda vera* extract for the inhibition of the DPPH at 250 and 125 µg/ml

Result of Antimicrobial Screening

Preliminary antimicrobial screening of *Suaeda vera* extracts showed no evidence for any inhibition zone against all microbial strains under this investigation. The results may revealed the safety of *Suaeda vera* for the microorganisms and will allow us in the future to see how it safe for higher animals and humans.

DISCUSSION

Natural products are of great interest in the process of drug discovery, and have long been sources of drugs. In addition, large proportion (30–40%) of the pharmaceuticals available in modern medicine is directly or indirectly derived from natural sources[6].

The variations in kinds and quantity of bi-products between different plants are environmental dependent factor, since plants be forced to biosynthesis of these compounds in response to some sort of environment conditions. A clear example of this phenomenon is reflected in halophytes, to overcome the oxidative stress produced under the influence of high salt concentrations of the soil[15].

On this basis, this work focused on the investigation of the phytochemicals of *Suaeda vera* and the power of these chemicals in the quenching of the free radicals. Results of phytochemical screening mentioned in table 1, and presence of high percentage of phenolic compounds such as tannins and flavonoids in the different extracts (table 2) supports the ability of *Suaeda vera* to overcome high salinity of the soil.

Result in table 2 for the quantities of phenolic, flavonoids and flavonols in the *Suaeda vera*, represent a high percentage of the ethanol, ethyl acetate and chloroform extracts with 16 %, 37 % and 24 %, respectively for total phenolic contents and 6.6 %, 9.8 % and 7.6 %, respectively for total flavonoids and 3.4 %, 4.4 % and 3.5 %, respectively for total flavonols.

Antioxidant activity of *Suaeda vera* summarized in table 3, showed a noticeable quenching effect of ethyl acetate and chloroform extracts for the DPPH radical compared to slandered antioxidant compounds (quercetin and Butaylated hydroxyl anisole). Furthermore, the inhibition effect of the different extracts for the colour of the DPPH free radical at 250 and 125 µg/ml, were showed in Figure 1. The rich phenolic ethyl acetate extract showed 61 and 26

% inhibition for the DPPH at concentration of 250 and 125 µg/ml, respectively. Chloroform extract showed moderate effect at the same concentration with 38 and 19 %, inhibition respectively. The lowest antioxidant activity appeared in ethanol and petroleum ether extract that, contain the lowest amount of phenolic compound compared to other extracts.

All suaeda vera extracts showed no killing effects on all tested microbial strains which may give sense for the safety of *Suaeda* for the simple organisms. Further studies for the LD50 in addition to measuring the safety of *Suaeda* for humans and higher animals are point for our future research interest.

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