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Anticancer, antioxidant and antimicrobial activities of *Suaeda fruticosa* related to its phytochemical screening

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Abstract

Concerning anticancer evaluation of methanolic extract of *Suaeda fruticosa* arial parts demonstrated the utmost anticancer activity with IC_{50} value of 50 µg/ml against human lung carcinoma (LU-1), the same extract displayed a preeminent activity with IC_{50} ≥ 50 µg/ml against hormone dependent prostrate carcinoma (LnCaP). The analyzed root extract exhibited IC_{50} ≤ 65 µg/ml against hormone dependent prostrate carcinoma (LnCaP) whereas, the same extract demonstrated IC_{50} ≤ 55 µg/ml against human lung carcinoma (LU-1). The highest content of total phenolic (0.52-0.67 mg gallic acid equiv./g) and total flavonoids (0.44 mg/g D/W) content was detected in the arial part of *Suaeda fruticosa*. MIC value of 50-85 µg ml^{-1} has been observed gainst *Aspergillus fumigates, Aspergillus niger, Fusarium solani* and *Mucor sp* in comparision with 1-2.5µg/ml of Terbinafine used as a standard fungicide. MIC value of 80 µg/ml and 35 µg ml^{-1} of *Suaeda fruticosa* arial parts and root extract against bacterial pathogen *Klebsiella pneumonia* and 50 and 90 µg ml^{-1} against *Enterococcus* has been measured. DPPH radical scavenging activity of *Suaeda fruticosa* with IC_{50} values of ≤ 100 µg/ml and ≤ 50 µg ml^{-1} was observed whereas, hydrogen peroxide scavenging activity with IC_{50} values of ≤ 12.5 µg/ml for arial parts and ≤ 25 µg ml^{-1} for the root extract of *Suaeda fruticosa* has been shown with galllic acid (R2= 0.819) and ascorbic acid (R2= 0.728). These data suggested that the methanolic extract of *Suaeda fruticosa* could be potential candidates for natural antioxidants and anticancer. The findings demonstrated the remarkable potentiality of *Suaeda fruticosa* as valuable source of antioxidant and anticancer capacities.

Keywords: Suaeda fruticosa, Human lung carcinoma, Hormone dependent prostrate carcinoma, DPPH

Introduction

Natural products represent an important source of drugs in a number of therapeutic fields, such as infection, cardiovascular and cancer treatment. They possess compounds with an enhanced biological properties has been achieved by exploiting structural multiplicity of natural product libraries. Organic chemists devote considerable efforts to the isolation and characterisation of secondary metabolites from natural sources. Diseases such as respiratory infections, HIV/AIDS, cancers, diarrhoeal diseases, tuberclosis malaria, etc., are leading killers and extensive efforts are being made to improve their control using natural resources like plants, fungi and some beneficial bacteria. There is therefore a general need for new antibiotics, chemothrapeutic agents and agrochemicals that are highly effective, possess low toxicity and have no environmental impact. The use of natural products derived from meicinal plants continues to offer opportunities for innovation in drug and agrochemical discovery.

Suaeda fruticosa Forssk (Chenopodiaceae) commonly called 'Alkali seepweed' a Shruby halophyte, has been collected from natural high saline and arid habitat of District Mardan, Pakistan. In general halophytes are known for their ability to resist and quench toxic reactive oxygen species (ROS) because they are equipped with powerful antioxidant enzyme systems [1]. While ethnomedicinal applications have been reported for all parts and essential oil of Suaeda fruticosa, the leaves are associated with the most prevalent traditional use. Due to its diverse and enduring ethnomedical indications, and the broad range of therapeutic and nutritional claims made in promotional literature, a substantial number of biological and chemical studies have been performed on Suaeda fruticosa dating back more than 70 years. Highest DPPH scavenging ability was found in *Suaeda mollis* with the lowest IC_{50} value (2.5 µg/ml), followed by *Suaeda pruinosa, Suaeda fruticosa and Suaeda maritima* whereas, dichloromethane extract of *Suaeda fruticosa* exhibited the highest anticancer activity against human lung carcinoma (A-549) and colon adeno-carcinoma cell

lines (DLD-1, Caco-2 and HT-29) with specificity against DLD-1 (IC_{50}=10 µg/ml) [2]. *Suaeda fruticosa* aqueous extract showed hypoglycaemic (41%) and antihyperglycaemic (53%) effects in the hyper-cholesterolaemic and insulin-resistant sand rat [3]. Furthermore, the plant led to a decrease in plasma levels of insulin by 31%. Previous work revealed that essential oil of *Suaeda fruticosa* inhibited the visible growth of *Staphylococcus aureus, Staphylococcus epidermidis, Micrococcus luteus, Escherichia coli* and *Salmonella typhimurium* at minimum inhibition concentration of 0.5 and 0.8 mg/ml [4]. Methanolic extracts of *Suaeda fruticosa* leaves at a concentration of 0.5mg/ml and 0.35mg/ml were found to be active against Gram-positive (*Bacillus subtilis, Staphylococcus aureus*) and Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) microorganisms, in addition to two fungal strains (*Candida tropicalis* and *Candida albicans*) [5].

Materials and Methods

Collection and Extraction

Arial parts and root of *Suaeda fruticosa* were collected in summer from arid and high saline fields of District Mardan, Pakistan (34° 05' to 34° 32' north latitudes and 71° 48' to 72° 25' east longitudes, total area of the District is 1632 square kilometres). The plant was identified by Dr. Rizwana Aleem Qureshi. A voucher specimen (SK1520/2008) was deposited in the National Herbarium of Pakistan, Quaid-i-Azam University, Islamabad. The arial parts and roots were rinsed with distilled water and air dried for 12 days. The plant parts were ground into powder, then soaked 300g of the powder in 80% methanol (600ml for 6-8 days, 3X) and incubated at room temperature (25 °C). The mixture was filtered twice, using whatman-41 filter paper. The extracts were dried by removing the methanol using a rotary film evaporator yielding a dark brown colored semisolid mass 15 g (1.84%). The extract was kept at -20 °C.

Chemicals

DPPH (1,1-diphenyl-2-picryl-hydrazyl), methanol, ferric chloride, chloroform, H_2SO_4, HCl, benzene, NH_4OH, potassium ferro-cyanide, sodium chloride, ethanol, Folin-Ciocalteau, Na_2CO_3, Gallic acid, hydrogen peroxide and ascorbic acid were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Preliminary phytochemical screening

Phytochemical screening of *Suaeda fruticosa* were performed to detect the presence of different class of compounds, such as alkaloids, flavonoids, saponins, steroids, terpenes, Coumarins, Anthraquinone, phlobatannins, Cardiac glycosides and tannins [6] and [7].

The total phenolic contents (TPC)

Total phenolic content of *Suaeda fruticosa* was determined by the Folin-Ciocalteu colorimetric method using gallic acid as a standard, and the absorbance was measured at 765 nm in a spectrophotometer (HITACHI. Model: U-1100 573 ˟ 415). Results were expressed as gallic acid equivalent (GAE) mg/g of dried fraction. Data for each fraction was recorded in triplicate [8] and [9].

Tannins determination

Tannin content was determined by using method [10]. Absorbance at 120 nm was recorded in a spectrophotometer (HITACHI, Model: U-1100 573 ˟ 415) within 10 min and tannins contents were expressed as percentage of the dried fraction.

Determination of the total Flavonoids content

Total Flavonoids content was determined according to the protocol of [11]. The absorbance was measured immediately at 510 nm in a spectrophotometer (HITACHI, Model: U-1100 573 ˟ 415). Flavonoids were estimated as mg/g of dried fraction. All samples were run in triplicate.

Alkaloid content

Alkaloid content was determined using 10% acetic acid followed by concentrated ammonium hydroxid. Alkaloid content was expressed as percentage of the dried fraction [12].

Saponins estimation

Saponin contents were calculated as percentage of the dried fraction using method [13].

Assay for antibacterial activity

Antibacterial activity of *Suaeda fruticosa* crude extracts was determined by the agar well diffusion method [14].

Assay for antifungal activity

The agar tube dilution method was used for determination of antifungal activity of methanolic extracts of *Suaeda fruticosa* [15].

DPPH Radical Scavenging Activity

The antioxidant activity was assessed in DPPH radical scavenging system using gallic acid and ascorbic acid as a positive control, and the decrease in absorbance was determined at 517 nm in a spectrophotometer (HITACHI, Model: U-1100 573 ˟ 415) [16], [17] and [18].

$$\text{DPPH scavenging effect (\%)} = 100 - \left[\frac{A0 - A1}{A0} \times 100\right]$$

Where *A*0 was the absorbance of the control reaction and *A*1 was the absorbance in the presence of the sample [19].

Hydrogen peroxide-scavenging activity

The ability of the extracts to scavenge hydrogen peroxide was determined according to the method of [20]. Absorbance of the hydrogen peroxide activity was recorded at 230 nm in a spectrophotometer (HITACHI, Model: U-1100 573 ˟ 415). Hydrogen per oxide scavenging ability (in triplicate) was calculated by following equation:

Hydrogen peroxide scavenging activity (%) = 1- *A*1/ *A*0 X 100

*A*0 was the absorbance of the control and *A*1 was the absorbance of the sample.

Reducing power assay

The reducing powers of the extracts were determined according to the method described by [21]. The Extract which have reduction potential, react with potassium ferricyanide (Fe3+) to form potassium ferrocyanide (Fe2+), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm in a spectrophotometer (HITACHI, Model: U-1100 573 ˟ 415).

Potassium ferricyanide + Ferric chloride $\xrightarrow{\text{Antioxidant}}$ Potassium ferrocyanide + ferrous chloride

Assay for Cellular Cytotoxicity

The standard protocol used for the assessment of cellular toxicity measures the ability of cultured cells to proliferate in the presence of a test sample, and subsequently quantitates total protein content with sulforhodamine B (SRB) dye as a measure of the percentage of surviving cells [22] and [23]. The cytotoxic potential of the crude methanolic extract was determined in the LU-1 (human lung carcinoma) and LnCaP cell line at the highest concentration of 80 µg/ml in DMSO. Cells (3 x 10-4 cells/mL) were seeded in 96-well plates, and six two-fold serial dilutions of test samples in 10% DMSO (10 µl) were added to each well. The plates were incubated for 72 h at 37°C, after which cell viability was determined with SRB staining following the standard protocol [24]. EC_{50} values were determined as the concentration of sample required to inhibit cell growth by 50% relative to a control treated with 0.5% DMSO, and represent the average of triplicate values obtained from three independent experiments. Colchicine (Sigma-Aldrich; purity > 96 by HPLC) was used as the positive control compound.

Aims of the study

The problems like emergence of new diseases and development of drug resistant pathogenic microorganisms, is forcing the scientists to explore different natural resources for the safe and potent agents to meet the challenges of the twenty first century. For the purpose *Suaeda fruticosa* has been selected to be evaluated for cancer cytotoxicity, antioxidant potential and antimicrobial screening.

Results and Discussion

Several groups of polyphenols (anthocyanins, tannins, flavanones, isoflavones, resveratrol and ellagic acid) are currently used in nutraceuticals industries and functional foods [25]. Preliminary phytochemical screening carried out on this specie showed the presence of numerous constituents' classes, such as flavonoids, saponins, tannins, steroids and terpenes. Since several flavonoids and tannins isolated from medicinal plants have been discovered for their significant role in antibacterial, antifungal and anti-inflammatory activities. It is, therefore, possible that the present activities observed with this extract in the study may be attributable to its total phenolic, total flavonoids and tannins contents. Total phenolic content was shown in the range of 0.52-0.67 mg/g of the *Suaeda fruticosa* extract using standard curve of gallic acid (R2= 0.783). The phenolic compounds may contribute directly to the antioxidant action as the present results coincide with those of total antioxidant capacity. To better understand the antioxidant potential of *Suaeda fruticosa* extracts of root and arial parts were evaluated for radical scavenging activity against DPPH. Fig.1 illustrated a significant decrease in the concentration of DPPH due to scavenging activity of the extract. DPPH radical scavenging activity of *Suaeda fruticosa* arial parts and root extract with IC_{50} values of ≤ 100 µg/ml and ≤ 50 µg ml^{-1} respectively with galllic acid (R2= 0.871) and ascorbic acid (R2= 0.780) was shown in table. 5 whereas, hydrogen peroxide scavenging activity with IC_{50} values of ≤ 12.5 for arial parts and ≤ 25 µg ml^{-1} for the root extract of *Suaeda fruticosa* has been shown in the same table with galllic acid (R2= 0.819) and ascorbic acid (R2= 0.728).

Table 1 Preliminary phytochemical screening of methanolic extract of *Suaeda fruticosa* arial parts and root

Metabolites	*Suaeda* Arial	*Suaeda* Root
Tannins	+	+
C.Glycoside	+	-
P.Batannins	+	-
Flavonoids	+	+
Terpenoids	+	-
Alkaloids	+	+
Ant.Quinone	-	-
Cumarins	+	-
Steriods	-	+
Saponins	+	+

Table 2 Tannins, total flavonoids content (TFC), total phenolic content (TPC), alkaloid and saponins content of methanolic extract of *Suaeda fruticosa* arial parts and root

Metabolites	*Suaeda Arial*	*Suaeda Root*
Tannins (mg/g. D/W)	0.78±0.013	0.49±0.005
Total flavonoids (mg/g. D/W)	0.44±0.002	0.32±0.001
Alkaloids (mg/g. D/W)	0.85±0.095	0.62±0.068
Saponins (mg/g. D/W)	1.09±0.141	0.68±0.076
Total phenolic content (TPC)	0.67±0.014	0.79±0.034

Data are expressed as mean±SEM (n = 3) of three independent experiments
All data expressed as (mg/g. Dry Weight)

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Figure 3 showed the reducing powers of the methanol extracts of *Suaeda fruticosa* root and arial parts along with gallic acid (R2= 0.943) and ascorbic acid (R2= 0.851) are as a function of their concentration. With regards to reducing power, higher reducing activities can be attributed to higher amounts of polyphenolics and the reducing capacity of a compound may reflect its antioxidant potential [26]. It has been reported that the reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom [27]. The total flavonoids content were 0.44 mg/g D/W, alkaloids 0.85 mg/g. D/W, saponins 1.09 mg/g D/W and tannins 0.78 mg/g D/W in *Suaeda fruticosa* arial parts shown in Table 2.

The methanolic extracts of *Suaeda fruticosa* arial parts and root were significantly active against the fungal pathogens studied. The arial parts of *Suaeda fruticosa* showed the broadest spectrum of activity against *Aspergillus fumigates, Aspergillus niger, Fusarium solani* and *Mucor sp* with MIC value of 50-85 $\mu g\ ml^{-1}$ than the root extract shown in table 3. Terbinafine 1-2.5μg/ml is used as a standard fungicide. MIC value of 35 and 80 $\mu g\ ml^{-1}$ of *Suaeda fruticosa* arial parts and root extract against bacterial pathogen

Table 3 Antifungal activities (expressed in MIC) of methanolic extracts of *Suaeda fruticosa* arial parts and root

Micro-organisms	Tested materials (MIC μg ml^{-1}) ±SEM		
Fungai	*Suaeda Arial*	*Suaeda Root*	*Terbinafine (μg/ml)
Aspergillus fumigatus	80±2.16	≥80±2.16	2.5±0.099
Aspergillus niger	≤60±4.27	≤ 70±1.55	3.0±0.075
Aspergillus flavus	≥ 70±1.56	75±0.167	≥ 2.0±0.024
Fusarium solani	≤ 85±2.11	≤ 100± 0.938	2.5±0.022
Mucor Sp	50±1.04	≥ 60±4.27	≤ 2.0±0.091

Data are expressed as mean±SEM (n = 3) of three independent experiments
*Terbinafine 1-2.5μg/ml is used as a standred fungicide

Table 4 Antibacterial activities (expressed in MIC) of methanolic extracts of *Suaeda fruticosa* arial parts and root

	Minimal Inhibition Concentration (MIC μg ml^{-1}) ±SEM	
Tested materials	*Klebsiella pneumoniae*	*Enterococcus*
Suaeda Arial	90±0.401	≥ 80±0.372
Suaeda Root	≤ 35±0.168	≥ 50±0.388
*Penicillin	≤ 3.0±0.084	2.0±0.144
*Chloramphenicol	1.5±0.063	≤ 2.5±0.213

Data are expressed as mean±SEM (n = 3) of three independent experiments
*Penicillin and Chloramphenicol 1-3.5μg/ml is used as a standred antibiotics

Table 5 Cytotoxicity against (LU-1) and (LnCaP) and antioxidant activities of methanolic extracts of *Suaeda fruticosa* arial parts and root expressed as IC_{50} (μg ml^{-1})

Micro-organisms	Tested materials IC_{50} values (μg ml^{-1}) ±SEM	
Anticancer assays	*Suaeda Arial*	*Suaeda Root*
*[1]LU-1	50±0.168	≤65±0.199
*[2] LnCaP	≤ 50±0.144	≥65±0.154
Antioxidant assays		
DPPH Radical Scavenging Activity	≤ 100±1.431	≤ 50±0.210
Hydrogen peroxide-scavenging activity	≤ 12.5± 0.099	≤ 25±0.184

Data are expressed as mean±SEM (n = 3) of three independent experiments
*[1]Human lung carcinoma
*[2]Human prostrate carcinoma
Colchicine with IC_{50} values 0.02±0.002 is used as Standard anticancer drug

Table 6 Correlations established between the concentration of extracts with antioxidant Cytotoxicity assays IC_{50} µg ml^{-1} (df = 3)

Assays	Cytotoxicity Assays		Antioxidant Assays		
Extract	***1LU-1**	***2LnCaP**	**DPPH**	**HPOX RSA**1**	**RPA**2**
Arial parts	y = 0.8324x +100.54	y = -0.61x + 85.7	y = 0.001x + 0.36	y = 0.12x + 50.1	y = 0.001x + 0.667
R^2 = 0.986	R^2 = 0.805	R^2 = 0.85	R^2 = 0.60	R^2 = 0.614	
Root extract	y = 0.7314x + 92.7	y = 0.781x + 99.8	y = 0.001x + 0.40	y = 0.14x + 49.9	y = 0.001x + 0.715
R^2 = 0.962	R^2 = 0.805	R^2 = 0.77	R^2 = 0.61	R^2 = 0.817	

****1Hydrogen peroxide Radical scavenging activity (HPOX RSA)**
****2Reducing Power Activity**
***1Human lung carcinoma**
***2Human prostrate carcinoma**

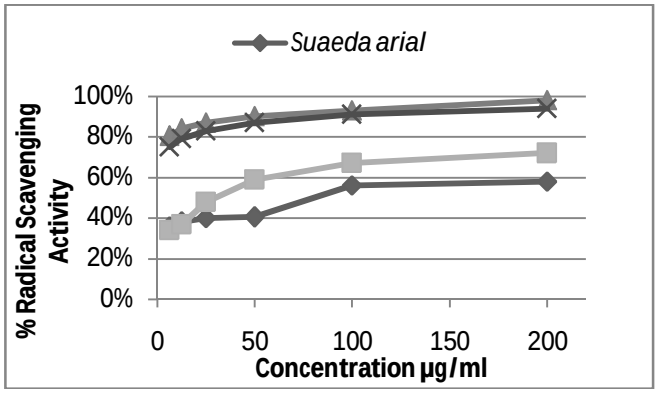

Fig.1 Analysis of DPPH radical scavenging activity of methanolic extract of arial parts and root of *Suaeda fruticosa*

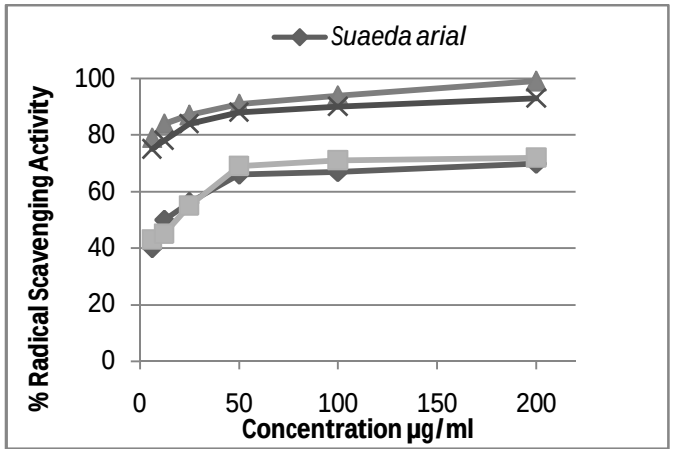

Fig.2 Analysis of Hydrogen Peroxide Radical scavenging activity of methanolic extracts of arial parts and root of *Suaeda fruticosa*

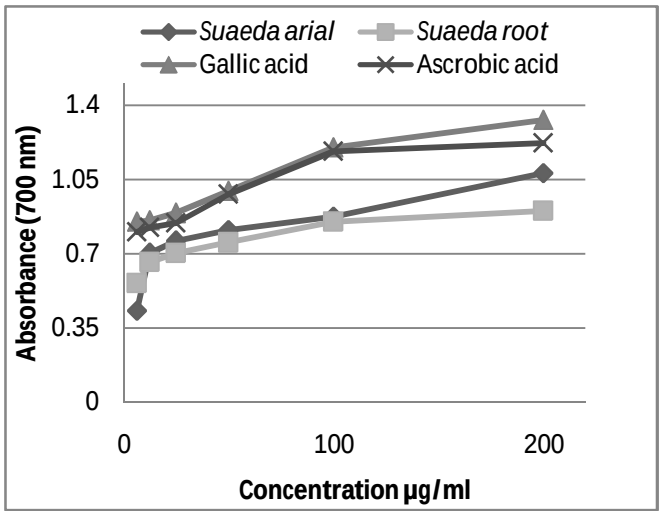

Fig.3 Analysis of Reducing Power ability of methanolic extracts of arial parts and root of Suaeda *fruticosa*

Klebsiella pneumonia and 50-90 µg ml^{-1} against *Enterococcus* has been shown in table 3. Penicillin and Chloramphenicol with MIC value of 1.5-2.5 µg ml^{-1} has been used as a positive control against *Klebsiella* and *Enterococcus* specie.

Cytotoxicity results against LU-1 and LnCaP cell lines are summarized in table 5. *Suaeda fruticosa* arial parts and root extract showed IC_{50} value of 50 and ≤ 65 µg/ml against LU-1 whereas, ≤ 50 and ≥65 µg/ml were calculated against LnCaP cell line. Interestingly, *Suaeda fruticosa* possessed the highest inhibition potential against human lung carcinoma (LU-1) and human prostrate carcinoma (LnCaP) cell lines indicating its ultimate potential for biopharmaceutical uses.

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