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In Vitro Anti-MRSA and Antioxidants Activities of Different Aerial Part Extracts of *Cakile maritima*

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ABSTRACT

Background: *The increased prevalence of antibiotic resistance, as a result of improper and prolonged antibiotic use, may render the current antimicrobial agents insufficient to control, at least, some bacterial infections. The aerial part of Cakile Maritima was extracted with Soxhlet apparatus using Petroleum ether, DCM and Methanol respectively for 24 hours. The solvents were evaporated under reduced pressure.* ***Methods****: To evaluate antimicrobial activity, the agar diffusion assay was used against a Gram-positive bacteria (Staphylococcus aureus, MRSA and Streptococcus pyrogens), three Gram-negative bacteria (Escherichia coli, Pseudomonas aeruginosa, and klebsiella peunmonia).* ***Results****: Different extracts of Cakile maritima with different concentrations demonstrated an antimicrobial activity against tested pathogenic bacteria. The antioxidant activity of the extracts were tested utilizing DPPH as the radical reagent and ascorbic acid as reference.* ***Conclussion****: The methanolic extract showed effective free radical scavening.*

Keywords: ***Halophytes, Antioxidant, total phenols, MRSA.***

INTRODUCTION

The development of bacterial resistance to the deployed antibiotics increases the need to search for new antibacterial agents. Multiple drug resistance in human pathogenic microorganisms has been developed due to the uncontrolled use of commercial antibacterial agents deployed in the treatment of infectious diseases. Researchers have become interested to search for new antimicrobial substances from various sources as novel antimicrobial chemotherapeutic agents. This was attributed to that the cost of synthetic analogues is high and they produce adverse effects compared to plant derived drugs [1,2]. The excessive production of free radicals leads to numerous diseases and accelerate aging. The antioxidants of low molecular weight are regarded as possible protection agents reducing oxidative damage of the human body when the internal enzymatic reactions insufficient [3]. Therefore, the need of the new alternative products having antioxidant properties is growing [4].

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Cakile maritima (sea rocket, Brassicaceae) is a halophytic plant that is commonly growing in sand hills. The plant has found many applications in folk medicine. C. maritima is thought to have diuretic, antiscorbutic, digestive and purgative properties [5,6]. Antifungal activity of phytoalexins and glucosinolates, two natural compounds present in Cakile maritima was also identified [7]. The aim of the present study was to determine the antibacterial, radical scavenging and antioxidant activities of different extracts of this halophytic species, associated with demonstrating their biological activities.

METHODOLOGY

Methods

The Aerial parts of *cakile maritime* were collected from shores of Tajura, Libya, identified and confirmed by a taxonomist. The absorbance of the reaction mixture was measured with Analytic Jena spectrophotometer, Germany. Mueller–Hinton agar (MHA) was used as base medium for the screening of antibacterial activity, Mueller–Hinton broth (MHB) for preparation of inoculums and both were purchased from Merck, Germany. Unless otherwise stated, all other chemicals and reagents used throughout this study were of purest analytical reagent grade.

Extraction of cakile maritime

Aerial parts of the plant were cut into small pieces and dried completely in shade at room temperature. The plant materials were crushed and blended to fine powder in an electronic grinder and stored in polyethylene bag. The powdered plant materials were extracted with Soxhlet apparatus using Petroleum ether, DCM and Methanol respectively for 24 hours. Each filtrate was evaporated under reduced pressure in rotary evaporator. The dried extracts were stored until use.

Scavenging properties of extracts against DPPH:

Qualitative estimation: The DPPH free radical scavenging assay was carried out for the evaluation of the antioxidant activity. This assay measures the free radical scavenging capacity of the investigated extracts. DPPH is a molecule containing a stable free radical. In the presence of an antioxidant which can donate an electron to DPPH, the purple colour, typical for free DPPH radical decays. The crude extracts were tested for scavenging properties against DDPH (Fluka). In this experiment, the samples were made in the final concentration of 1 mg mL-1 in EtOH. 10 µL samples were spotted onto a silica gel TLC 60 F254 (Merck, Germany) along with vitamin C reference prepared in a similar way. The spots were thereafter sprayed with 0.2% DPPH dye in EtOH and incubated (37 ºC, 30 min.) after which the colours observed were matched with that of the vitamin C reference. Extracts showing the white on purple colour of the vitamin C reference were regarded as antioxidant (8).

Quantitative estimation: Determination of the free radical scavenging activity of the different extracts was carried out using a modified quantitative DPPH (8). Various concentrations of the sample extracts in methanol were prepared (1000, 500, 250, and 100 µg•ml1). Ascorbic was used as a positive control at concentrations of 100, 50, 25, and 10 µg.ml-1. Blank samples were run using 1 ml methanol in place of the test extract. One ml of 0.2 mM DPPH in methanol was added to 1 ml of the test solution, or standard, plus 1 ml of methanol for dilution and allowed to stand at room temperature in a dark chamber for 30 min. The change in colour from deep violet to light yellow was then measured at 517 nm. Inhibition of free radical in percent (IP%) was calculated according to the following equation: IP %= [(A0-A1)/A0] x 100, with A0 being the absorbance of the control reaction (containing all reagents except for the extract) and A1 the absorbance of the extract. Measurements were carried out in triplicates. From a plot of concentration against %IP, a linear regression analysis was performed to determine the IC50 value for plant extract.

Determination of Total Phenolic Content: Total phenol contents of different extracts were determined by the modified Folin-ciocalteu method according to (9), a aliquot of 0.5 ml of each extract (1 mg.ml-1) was mixed with 2.5 ml Folin-Ciocalteu reagent (previously diluted with distilled Water 1:10 v/v) and 2ml (75% w/v) of sodium carbonate (Na_2CO_3). The tubes were vortex for 15s and allowed to stand for 30min at 40°C for color development. Absorbance was then measured at 765 nm using spectrophotometer. The same procedure was repeated for the standard solution of gallic acid and the calibration plot was generated. Based on the measured absorbance, the concentration of total phenol was determined (mg.ml-1) and the content of phenolics in extracts was expressed in terms of gallic acid equivalent (mg of GA/g of extract).

Antimicrobial assays

The cup cut diffusion method: Antimicrobial activity was determined using cup cut agar diffusion method (10, 11,12) with reference to McFarland standard. The bacterial cultures were grown in the nutrient broth (Merck, Germany) at 37 oC. Mueller-Hinton agar (Oxoid, UK) plates were all lawn with the investigated bacteria. All plates were incubated at 37oC for overnight after they all have been left for 1hr for the extract diffusion from the performed wells into the agar. Tested microorganisms used in this study were all ATCC references. These were, *S. aureus* (ATCC 29213), E. Coli (ATCC 259222) and *Pseudomonas aeruginosa* (ATCC 10231). The *Streptococcus pyrogens*, MRSA and *Klebsiella peunmonia* were clinical isolates obtained from Centre for control of the disease. The assessment of antibacterial activity was based on measurement of the diameter of the inhibition of zone formed around the well.

The minimum inhibitory concentration (MIC): An eight test tubes micro dilution method was employed, using Nutrient broth as described by (13). Different concentrations of the dilution extract, ranging from 500-3.9 mg.ml-1 were prepared in the test tubes in a total volume of 1 ml of bacterial suspensions that were inoculated in to the test tubes and incubated at 37 oC for 24 hr. The bacterial growth was determined for the turbidity. The lowest concentration that inhibited the growth of bacteria was considered the minimum inhibitory concentration (MIC) of each extract.

RESULTS AND DISCUSSION

A rapid evaluation for antioxidants using TLC (Thin Layer Chromatography) was screening and DPPH staining methods demonstrated only methanolic extract having a free radical scavenging capacity. The intensity of the yellow color depends on the amount and nature of radical scavenger present in the samples. The 15, 31, 62, 125, 250 and 500 µg/ml of the extract 20, 38.7, 36.8, 52.4, 72.2 and 84.9 % free radical scavenging of DPPH, while 125 µg/ml of ascorbic acid exhibited 93.4. It is well known that there is a strong relationship between the antioxidant activity and total phenol content, as the scavenging ability for free radicals increases with the number of hydroxyl groups [14]. Therefore, the content of total phenolic was carried out based on the absorbance values of the methanolic extract, reacted with Folin–Ciocalteu reagent and compared with the standard solutions of gallic equivalents as described above. Data obtained from the total phenolic method support the key role of phenolic compounds in free radical scavenging. As assumed, amount of the total phenolics was 13.4 mg GA/g.

The data collected from the cup-cut agar diffusion method displayed that some activity on both G+ve and G-ve bacteria including notorious resistant strains. This was illustrated, for example, towards *S. aureus*, *K. pneumonia* and *S. pyogenus* for all types of the plant extract with variable degree of activity (Table 1) associated with various extract concentrations. The MRSA was clearly succumb to the effect of the methanol extract only. This was wit half fold magnitide of activity compared to ciprofloxacin (Table 1).

In the case of S. sureus, the petroleum ether extract activity (13mm) was about one third of that displayed by the ciprofloxacin (38mm). Similar data was obtained using the dichloroether and methanol extracts with increased activeity towards *K. pneumonia* and *S. pyogenus*. İnactivating ability of all extracts toards the bacterium *K. pneumonia* (20mm) was of abouth half fold of the ciprofloxacin (35mm). More avtivity was noticed against the bacterium *S. pyogenus* (18mm) bacteria that was inactivated by a magnitude of towthird of the ciprofloxacin ability (23mm).

Indeed, various extracts of the plant *cakile maritime* would clearly comprise a variety of antibacterial components that displayed differential inactivating potencies towrds various classes of Gram positive and Gram negative bacteria.

The data obtained from the determination of the minimum inhibitory concentrations (Table 2) displayed various MIC values towars the test bacteria. İn the case of *S .aureus* bacteria, the petroleum ether MIC values was indeed beyound the value 3.9mg.ml-1 displaying the ability of this type of extract to eradicate bacteria at a concentration even below that displayed by the ethanol extract (15.625mg.ml-1). Interestingly, the methanol extract displayed similar activities towards most of the tested bacteria at a value of 62.5mg.ml-1.

Collectively, the plant *cakile maritime* have indeed an antibacterial activity towards various medically important Gram positive and Gram negative bacteria that are well known to cause illness to human being and impact a negative effect on the community. The petroleum ethr extract could be the faraction of the plant that may comprise various antibacterial component. The plant, therefore, can be a remedy for various disorders associated with various bacteria and may be recomended for an alternative therapy especially after displaying another pharmacological effects.

DISCLOSURE STATEMENT

Authors declare that there is no conflict of interest concerning this manuscript.

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Table 1 The diffusion method (Cup cut) result

Microorganism	**Petroleum ether extract mg/ml**		**Dichloromethane extract mg/ml**		**Methanol extract mg/ml**		**Ciprofloxacin as control**
	300	150	300	150	300	150	
S. aurous	1.3cm	0.8cm	1.4cm	1.5cm	1.6cm	0.9cm	3.8cm
E. coli	_	_	_	_	1.2cm	_	3.4cm
P. aeruginosa	_	_	_	_	1.2cm	_	3.5cm
Klebsiella peunmonia	2cm	_	2.5cm	_	2cm	1.2cm	3.5cm
MRSA	_	_	_	_	1.8cm	1.0cm	3.5cm
Streptococcus pyrogens	1.8cm	_	2.4cm	_	2.2cm	_	2.3cm

Table 2 The minimum inhibited concentration

Organi	***S. aureus***		***E. coli***		***P. aeruginosa***		***K. peunmonia***		**MRSA**	***S. pyrogens***	
Conc mg/ml	P. ether	Me.OH	P. ether	Me.OH	DCM	Me.OH	P. ether	Me.OH	Me.OH	P. ether	Me.OH
500	_	_	_	_	_	_	_	_	_	_	_
250	_	_	+	_	_	_	_	_	_	_	_
125	_	_	+	_	_	_	_	+	_	+	_
62.5	_	_	+	+	_	+	+	+	_	+	_
31.25	_	_	+	+	+	+	+	+	+	+	+
15.625	_	_	+	+	+	+	+	+	+	+	+
7.81	_	+	+	+	+	+	+	+	+	+	+
3.9	_	+	+	+	+	+	+	+	+	+	+