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A Study of Phytochemical Properties and the Synergistic Effect of *Mesembryanthemum crystallinum* on Some Human Pathogenic Bacteria

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ABSTRACT

Antibiotic resistance is increased as a result of extensive antibiotic use, may render the current antimicrobial agents insufficient to control, at least, some bacterial infections. The aerial part of *Mesembryanthemum crystallinum* was extracted by maceration with methanol (96% v/v) to exhaustion. The solvent was evaporated under reduced pressure. The decoction of the plant is used in traditional folk remedies as vaginal douche to treat vaginitis. To evaluate antimicrobial activity, the agar disc-diffusion assay was used against a Gram-positive bacteria (*Staphylococcus aureus*) and two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). The methanolic extract did not show any inhibitory effect on the tested bacterial strains. Association of antibiotics and the plant extract showed synergistic antibacterial activity especially with Ciprofloxacin, Tetracyclin and Amikacin. The antioxidant activity of the methanolic extract was investigated utilizing DPPH as the radical reagent and ascorbic acid as reference. The methanolic extract showed effective free radical scavenging. The major chemical constituents reported from the plant parts are flavonoids, saponins, steroids, triterpenoids and phenolic compounds which show that this plant part can be a potential candidate to be used as a therapeutic agent.

INTRODUCTION

Traditional medicine system based on natural products continues to play an important role in treatment of many diseases especially the infectious diseases. Different species of bacteria show various degree of antibiotic resistance, so everywhere we need to develop our drugs. Increased prevalence of antibiotic resistance, as a result of extensive antibiotic use, may render the current antimicrobial agents insufficient to control, at least, some bacterial infections and as we know the traditional medicine system based on natural products continues to play an important role in treatment of many diseases especially the infectious diseases, which are the second major cause of death worldwide today [1,2]. Libya is a fertile land with a great number of different plants. *Mesembryanthemum crystallinum* is annual, much branched herb with glittering silvery crystal-like papillae, geographically distributed in South and North Africa, canary, Australia, and southern Europe, usually growing in saline or distributed areas as on rock crevices and coastal sands [3,4]. 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 [5]. Therefore, the need of the new alternative products having antioxidant properties is growing [6]. The present paper estimate the antibacterial, antioxidant and synergistic effect of the methanolic extract of *Mesembryanthemum crystallinum* L and its phytochemical constituents.

MATERIALS AND METHODS

Plant material

The plant materials were collected from aldafneia area. The plant has been classified by the Department of Botany, Faculty of Science at the University of Misurata as ***Mesembryanthus crystallinum L***.

Extraction

The aerial parts of the plant were cut in small pieces and macerated in MeOH (600 ml) for 24 hr for three days. The obtained extract was filtered and evaporated by using water bath, the deried extract was stored in refrigerator at (20 ^{o}C) until used.

Phytochemical screening

The methanolic extract of *Mesembryanthemum crystallinum* was subjected to phytochemical screening to detect different chemical groups of compounds [7,8].

Test for saponins:

To 2ml of methanolic extract 5ml of distilled water was added and then shaken vigorously for 30 second.

Test for steroids:

To 2ml of methanolic extract, 2ml of chloroform, acetic acid, and 1ml of concentrated H_2SO_4 was added.

Test for anthraquinone:

To 2ml of methanolic extract, 2ml of 10% NH_4OH was added.

Test for phenolic compounds:

To 2ml of methanolic extract, one ml of ferric chloride was added.

Test for glycosides:

To 1ml of methanolic extract, few drops of dilute sodium hydroxide was added.

Test for alkaloids:

5ml of methanolic extract was added to 2 ml of HCl, then added 1ml dragendorffs reagent.

Scavenging properties of extracts against DPPH

The crude extracts were tested for scavenging properties against DDPH (Fluka). In present experiment, the samples were made in the final concentration of 1 mg mL -1 in EtOH. 10 µL samples were spotted on pre-coated silica gel TLC plates (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 [9].

Antimicrobial assays:

The agar diffusion assay was performed according to European Pharmacopoeia. One loopful of each test organism (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* was suspended in 3 ml of 0.9% NaCl soln. Nutrient agar was inoculated with a suspension of the respective organism. Sterilized paper discs containing 2 mg extract and the antibiotics were transferred to the prepared petri dishes. Pre-diffusion for 3 h was allowed, and inhibition zones were measured after 24 h incubation at 37 °C [10].

RESULTS

The methanolic extract did not show any inhibitory effect on the tested bacterial strains. Association of antibiotics and the plant extract showed synergistic antibacterial activity especially with Ciprofloxacin, Tetracyclin and Amikacin (**Table 1**).

Table 1: The antimicrobial activities (zones of inhibition) of the methanolic extract of *M. crystallinum* and its synergistic effect on antibiotics

Microorganisms	Extract	Standard antibiotic discs						Methanolic extract and standard antibiotic discs					
		CIP	TET	AMO	AMI	G	B	CIP	TET	AMO	AMI	G	B
E. coli	0	28	19	0	22	18	0	32	20	0	24	20	0
Staphylococcus aureus	0	28	25	12	23	24	24	30	27	12	24	26	26
Pseudomonas aeruginosa	0	30	12	0	0	0	0	33	13	0	0	0	0

CIP: Ciprofloxacin, TET: Tetracycline, AMO: Amoxicillin, AMI: Amikacin, G: Gentamicin, B: Bactrim

The methanolic extract showed effective free radical scavenging after spraying the chromatogram with 0.2% DPPH solution.

The major chemical constituents reported from the plant parts are flavonoids, saponins, steroids, triterpenoids and Phenolic compounds which show that this plant part can be a potential candidate to be used as a therapeutic agent.

DISCUSSION

The antimicrobial activity of medicinal plant extracts have been approved to the presence of bioactive secondary metabolites. These secondary metabolites provides unexploited source of antimicrobial agents which can be used in the future as source for new antibiotics, industrial food preservative or even as anti-toxins [11]. The methanolic extract of *M. crystallinum* L. with different concentrations did not demonstrated antimicrobial activity against the examined bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas auerginosa*), and when combined with different antibiotics demonstrated various degrees of synergistic effects against same examined bacteria. This study indicates that the combination of methanolic extract of *M. crystallinum* and the standard antibiotics leads to discovery and development of new antimicrobial treatment and the provision of more effective treatment regimens. The results indicated the presence of various classes of phytochemical active compounds such as steroids, phenolic compounds, and saponins. The most important one are flavonoids which have many medical uses as antiviral, anti allergic, antithrombotic, antioxidant and anti-inflammatory properties by inhibition of a serious of enzymes which are activated in the course of the inflammatory process so it relieve pain, decrease swelling, redness and fever [12], this behavior may translate the reason for the use of this plant traditionally as douches in treatment of UTI and vaginal infections by relieving the symptoms. The presence of saponins which explain the traditional use of the plant as natural detergents. Saponins also exhibit Antiprotozoal, antifungal, molluscicidal and antiviral effects [13]. The plant demonstrated promising activity as antioxidant so it will be useful to be used as natural alternative antioxidant due to the side effects to the commercial antioxidants.

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