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DETERMINATION OF PHENOLIC, FLAVONOID, ANTIOXIDANT, AND ANTIBACTERIAL ACTIVITY OF *MESEMBRYANTHEMUM CRYSTALLINMU*M FROM LIBYAN COASTS

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***Abstract*— The halophyte plant *Mesembryanthemum crystallinum* (family: Aizoaceae), widely used in the traditional medicine, was chosen for this study. There is no much information about the antioxidant activities of this plant growing in the East Libya coasts. The most of studies conducted confirmed the responses of this plant to the abiotic stresses. Methanolic extract was screened by applying general chemical tests for flavonoids, glycosides, reducing sugars, tannins, and saponins, proteins, and free amino acids. Preliminary phytochemical screening indicated the presence of phenolic compounds and flavonoids in the methanolic extracts of the plant. We demonstrate a high antioxidant activity in the methanolic extract of the plant by using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method in concentration dependence manner. In term of antimicrobial activity the methanolic extract of plant revealed a broad spectrum activity against 3 strain of gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa* and *Neisseria gonorrhoeae)* and 3 strain of gram positive bacteria (*Bacillus subtilis, Staphylococcus aureus* and *Streptococcus faecalis.* The medicinal properties can be attributed in part to the presence of polyphenolics compounds in the plant extracts.**

Keywords— *Mesembryanthemum crystallinum*m, phenolic, flavonoid, antioxidant, and antimicrobial**.**

I. INTRODUCTION

Global use of herbs as food or for therapy was known since the early days of human kind. They continue to be used throughout the world for health promotion and treatment of disease especially on developing countries and Africa. According to whom apparitional 25% of drugs presented worldwide were derived from plants. Herbs matter than drugs are after used in health care became they are affordable and there is belief that herbs. Since, natural products are inherently safe and efficacy they can be obtained over a wide range of doses. Oxidative stress is well known to be involved in the pathogenesis of lifestyle-related diseases, including atherosclerosis, hypertension, diabetes mellitus, ischemic diseases, and malignancies. Oxidative stress has been defined as harmful because oxygen free radicals attack biological molecules such as lipids, proteins, and DNA. The human body is constantly under oxidative stress arising from exogenous origins (e.g., ultraviolet rays) and endogenous origins (at the cellular level where mitochondria are involved). One of the mechanisms by which anticancer agents and radiation therapy exert their effects is through apoptosis of cancer cells. Oxidative stress is also involved in the problem of resistance to these treatments (Tsajimoto Y, *et al.* 1993). A large number of medicinal plants and their purified constituents have been shown to have beneficial therapeutic potential. Natural antioxidants may function as reducing agents, free radical scavengers, complexes of pro-oxidant metals, and quenchers of singlet oxygen (Hanasaki Y. *et al.* 1994). Currently there is considerable interest in new natural antioxidants to replace the synthetic ones that are used in foods and cosmetics.

Halophytes are known for their ability to overcome and quench these toxic ROS, since they are equipped with a powerful antioxidant system

(Hernandez JA, *et al*. 2000) (Qiu-Fang Z, et al. 2005).

Mesembryanthemum crystallinum (family: *Aizoaceae*) is found on a wide range of soil types, from well-drained sandy soils (including sand dunes), to loamy and clay soils. It can tolerate nutritionally poor or saline soils. As with many introduced species it also grows in disturbed sites such as roadsides, rubbish dumps and homestead yards. The plant is covered with large, glistening bladder cells or water vesicles, reflected in its common names of common ice. The species of *Mesembryanthemum* in general were traditionally used as emollient, leaf juice used to soothe inflammation of the mucous membranes of the respiratory or urinary system. In Europe, the fresh juice has been used to treat water retention, painful urination and soothe lung inflammation (Bouftira I, *et al*. 2012). The leaves of plant contains several flavonoids (include; rutin, hyperoside, catechin, ferulic acid and catechol tannins). Flavonoids are a group of naturally occurring polyphenolic compounds ubiquitously found in plants (Carlo Di G, et al. 1999).

Numerous 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 (Mihaela I. et al, 2009) (Cao G. et al. 1997).

The increasing number of antibiotic resistant bacteria motivates prospective research toward discovery of new antimicrobial active substances. There are, however, controversies concerning the cost-effectiveness of such research with regards to the description of new substances with novel cellular interactions, or description of new uses of existing substances to overcome resistance. Therefore, there is an urgent need to search for new antimicrobial compounds with these chemical structures and novel mechanisms of actions.

The antimicrobial properties of the plant extracts and photochemical can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency (Almagboul A. Z. *et al*. 1985) (Artizzu N. *et al.* 1995)

Many plants have been used because of their antimicrobial activities which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, the phenolic compounds which are part of the essential oils (Jansen A.M. *et al*. 1987) as well as in tannin (Saxena G. *et al.* 1994).

In the present work, the halophyte plant *Mesembryanthemum crystallinum* (family: *Aizoaceae*), widely used in the traditional medicine, under quantitative estimation for the general phenolic and specific flavonoids contents. Antioxidant and antibacterial activities were also investigated.

II. METHODS

1.Plant collection and Extract preparation –

The aerial parts of *Mesembryanthemum crystallinum* plant were collected during July 2016 from Deryana town, about 30 km east of Benghazi / Libya. The department of Pharmacognosy (Faculty of Pharmacy, University of Benghazi) identified the plant which then was dried at open air after washed with tap water. Prior the extraction the dried plant was gradually by using mortar and then finely powdered by using blender. The powdered plant was used for extraction, phytochemical screening, antioxidant, antimicrobial study.

200 gram of the plants dried 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 rotatory evaporator. The different fractions were reconstituted in their extraction solvent to give the required concentration needed in this study.

100 gram of dried plant was extracted by methanol (70%) through exhaustive cold maceration. The solvent was evaporated under reduced pressure (at 40 C°). dried extractives saved for biological examination.

2.preliminary phytochemical screening of the plant extracts-

Preliminary screening of the different extracts of Mesembryanthemum crystallinum 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 B. *et al*.2012).

A. Quantitative Estimation of Total Phenolic Constituents:

Total phenol contents of different extracts were determined by the modified Folin-ciocalteu method according to (Omoruyi B E. et al. 2012). An aliquot of 0.5 ml of each extract (1 mg/ml) was mixed with 2.5ml Folin- Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 2ml (75% w/v) of sodium carbonate (Na2CO3). The tubes were vortexed for 15 s and allowed to stand for 30min at 40 °C for colour development. Absorbance was then measured at 765nm 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.

B. Quantitative estimation of total flavonoids:

Total flavonoid contents of different extracts were determined by method described by Ordonez et al. based on the formation of a flavonoid-aluminium complex (Ordonez AAL. *et al*. 2006). 0.5ml of various solvent extracts (1mg/ml) was mixed with 0.5ml of aluminium chloride prepared in (2% in ethanol). The resultant mixture was incubated for 60min at room temperature for yellow colour development which indicated the presence of flavonoid. Absorbance was measured at 420nm 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.

C. Quantitative estimation of total flavonols:

Total flavonoid contents of different extracts were determined by method described by (Omoruyi B E. *et al*. 2012). The reaction mixture consisting of 2 ml of the sample, 2 ml of aluminium chloride prepared in (2% in ethanol) and 3 ml of sodium acetate solution (50 gm/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 Y = 0.217×, R2 = 0.9582 where × is the absorbance and Y is the quercetin equivalent.

3. Scavenging activity of 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) radical:

The effect of methanolic extract on DPPH radical was estimated using the method of (Hosny M. *et al.* 2002), with some modification. 1.9ml of DPPH-ethanol solution (300µM) was mixed with 0.1ml of different concentrations (6.5–500µg/ml) of plant extract. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30min. The absorbance of the mixture was measured spectrophotometrically at 517nm. Gallic acid was used as standard drug. The percentage of free radical scavenging was calculated according to the following equation: % scavenging = (1-Sample absorbance517/blank absorbance517) X 100.

4. Antibacterial Activity:

The antimicrobial activity of the methanolic plant extract was performed against selected bacterial strains of standard properties. The tested Gram positive bacteria were [*Bacillus subtilis ATCC 6051, Streptococcus faecalis ATCC 19433 and Staphylococcus aureus ATCC 12600*]. The Gram negative bacteria included [*Escherichia coli ATCC 11775, Pseudomonas aeruginosa ATCC 10145 and Neisseria gonorrhea* ATCC 19424]. The prepared extract (aerial parts) was separately tested against the selected strains at concentration of 20 mg/ml adopting the disc agar diffusion method. Discs impregnated with tetracycline as antibacterial standards.

5. Statistical analysis:

The experimental results were expressed as mean ± standard deviation (SD) of three replicates.

III. RESULT

1.RESULTS OF PHYTOCHEMICAL SCREENING AND QUANTITATIVE ANALYSIS:

The result of the preliminary phytochemical screening give a clear evidence for the presence of phenolics, flavonoids, tannins, saponins and alkaloids in addition to carbohydrates and sterols. The tests also revealed that the absence of anthraquinones.

Table 1: Results of phytochemical screening for the different extracts of *M. crystallinum*

Constituents	Plant Extracts			
	Petroleum ether	Chloroform	Ethyl acetate	Ethanol
Phenolics	-	+	++	+++
Flavonoids	-	+	++	+++

Tannins	-	-	++	+++
Anthraquinones	-	-	-	-
Alkaloids	-	-	-	-
Saponins	-	-	+	+
Carbohydrates	-	-	++	++
Sterols	+++	+	-	-

Absent (-ve), Low in abundance(+), Moderate in abundance(++), High in abundance(+++).

Results represented in table (1) showed the results in a qualitative manner. Quantitative analysis results for the total phenolic and flavonoids of different plants extracts showed in table (2).

Table 2: Results of quantitative estimation of total phenolics, flavonoids and flavonols

Extracts	Total Phenolics	Total Flavonoids	Total Flavonols
Ethanol Extract	414.71 ±0.23	146.18 ±2.52	4.16 ±3.13
Ethyl acetate Extract	119.31 ±0.66	56.32 ±1.97	0.81 ±1.09
Chloroform Extract	33.40 ±2.91	23.76 ±1.99	0.32 ±0.45

Result were expressed as mean ± slander deviation

2. RESULTS OF ANTIOXIDANT

The ability of plant to scavenging the free radical was determined by using the DPPH method. The antioxidant activity data were presented in (table 3), in terms of IC50 ± SD, which is the concentration in mg/ml causing 50% inhibition of the free radical.

Table -3 The antioxidant activity of *Mesembryanthemum crystallinum*m in terms of IC_{50}±SD

Sample	DPPH %Inhibition *
Methanolic extract of *M Crystallinum* 20 µg/ml	27.21±0.02
Gallic acid	30±0.02

*Values are presented as mean ± SE of 3-test sample observations, P <0.05 for all values

Conc. (µg/mL)	Radical scavenging activity (%)
0.61	1.5
2.44	1.9
9.77	12.0
39.06	56.6
156.25	57.7
625.00	58.9
2500.00	65.6

Table -4 Radical scaveneging activity of different concentrations of methanolic extract of *M crystallinum*m

3. RESULTS OF ANTIBACTERIAL

The antimicrobial activity of methanolic extract of *M. crystallinum* was studied against gram positive bacteria and gram negative bacteria (Staphylococcus aureus, *Streptococcus faecalis* and Bacillus subtilis) and (Escherichia coli, *Neisseria gonorrhoeae* and Pseudomonas aeruginosa) respectively.

Table- 5 The antimicrobial activity of the methanolic extract of *Mesembryanthemum crystallinum*m aerial part, against Bacteria

microorganism	*Gram reaction*	Inhibition zone diameter (mm/mg sample)	
		Tetracycline	Methanolic extract
Escherichia coli	G –ve	30	11
Pseudomonasaeruginosa	G –ve	30	12
Neisseria gonorrhoeae	G –ve	31	13
Bacillus subtilis	G +ve	29	22
Staphylococcus aureus	G +ve	28	18
Streptococcus faecalis	G +ve	31	16

IV. DISCUSSION

The result of phytochemical screening revealed the presence of different phytochemical constitutes. Generally, phenolic, flavonoids and tannins represented in the chloroform, ethyl acetate, and ethaolic extracts with a special concern to the later one. sterols are detected in petroleum ether extracts of plants, and low in abundance in chloroform extract. Carbohydrates found in moderate amount in ethyl Acetate and ethanol extracts. Furthermore, Phytochemical screening tests revealed the absence of anthraqinoun and alkaloids in all plant extracts (table 1). On the other hand, quantitative estimation of the phenolics and flavonoids in the extracts of our present plant give a clear evidence for the high quantities of these by-products percentage (table 2). The present results confirmed the result obtained from the study carried out on the plant showed that, the plant extract had antibacterial activity against *Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa* Vander W. E. and Pretorius A. J. C. (2001). In addition it was similar to the results of *In vitro* study which found that the fresh leaf juice and aqueous extract of dried leaf showed antimicrobial activity against *Staphylococcus aureus* and *Pseudomonas aeruginos*. The antibacterial activity of the (crude extract) was attributed to the presence of different flavonoids as well as tannins (Vander W. E. and Pretorius A. J. C. 2001).

According to finding it has been found that the methanolic extract of *Mesembryanthemum crystallinum*m had significant scavenging activity on the DPPH radical that possessed a high antioxidant activity compared to gallic acid. The antioxidant effect of extract could be explained due to the presence of phenolic compound as reported that there is a linear relationship has been found between the total phenolic content and the antioxidant activity of the studied extracts (Oth mane T. et al. 2016). Additionally, it was concluded that , the antioxidant activity of phenolic compounds is principally due to their redox properties, which make them act as reducing agents, hydrogen donors, and singlet oxygen quenchers (Scalfi L. *et al*.2000). These results may be give a reason for the activity of the plant as antibacterial and antioxidant.

V. CONCLUSION

The finding of the present study confirmed that the aerial part of *Mesembryanthemum crystallinum*m plant extract contain phenolic and flavonoid compounds.

The plant can be considered as an good choice of antioxidant as it has a potent ability to scavenging a free radicals in addition to broad spectrum antibacterial activity when used against 6 strains of bacteria.

Further study should be done for the isolation of the therapeutically active compounds with anticancer potency and also for further pharmacological evaluations, microbiological and chemical analysis.

Conflict of interest: The authors declare no conflict of interest

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