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PHYTOCHEMICAL, PHARMACOLOGICAL STUDY ON EAST LIBYAN ORIGINATED *GLOBULARIA ALYPUM*

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***Abstract*—Peoples looking for unconventional therapies such as herbal medicine when conventional medicine fails to cure chronic diseases. Therefore, herbal drugs gained popularity in the treatment of several diseases. Since there is no phytochemical, pharmacological study and a limited scientific data available on the antioxidant and antimicrobial effects on Libyan *Globularia alypum (G alypum)* species, the aim of this study intended to investigate the antimicrobial and antioxidant properties of the extracts of aerial part of *G alypum*. A modified Kirby-Bauer disc diffusion method was used to study the antimicrobial effect of the prepared samples and the Antioxidant activity was examined according to scavenging effects using stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. Our results showed significant antibacterial activity against gram positive (*Staphylococcus aureus, Bacillus subtilis*) and showed a moderate effect against gram-negative (*Escherichia coli, Pseudomonas aeruginosa*) and fungi *Aspergillus flavus* and *Candida albicans.* In addition it had strong scavenging effects on the DPPH radical that possessed a high antioxidant activity compared to gallic acid. *Globularia alypum* can be consider as a significant source of compounds with antimicrobial and antioxidant activity and thus further studies should be performed.**

***Keywords*— *Globularia Alypum*, phytochemical screening, antioxidant, antimicrobial**

I. INTRODUCTION

Natural products have served as important sources of drugs since ancient times and a significant part of today's drugs were somehow derived from natural sources. In recent years, a renewed interest in obtaining biologically active compounds from natural sources has been observed.

As medicinal plants are used as sources of pharmaceuticals; ingredients of traditional medicines are of value in new drug discovery. Therefore, herbal drugs are gaining popularity in the treatment of several diseases .There is an intense interest in plant polyphenols, as witnessed by numerous papers devoted to various aspects of these compounds [1]. Phenolic compounds are antioxidants with redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers [2and 3].

Around the middle of 20th century, major advances in antibacterial drug development and other means of infection control helped human beings triumph over many infectious diseases. Bacteria respond to antibiotics by developing various modes of resistance as fast as we use antibiotics. Antimicrobial resistance in bacteria is one of the biggest global concerns at present [4].

Three types of antibacterial resistance strategies have been suggested. A drug efflux pump has been observed in several bacteria which prevent

accumulation of antibiotics. A second mechanism is deactivation or destruction of the antibiotics by hydrolytic enzymes in the periplasmic space and a third strategy for antibiotic resistance appears to be reprogramming of the target macromolecules to reduce the affinity of antibiotics for their RNA [5, 6, and 7]. Resistance has led to need for development of alternate sources of antibiotics to control microbial infections.

As peoples looking for unconventional therapies such as herbal medicine when conventional medicine fails to cure chronic diseases, G alypum has been used in traditionally medicine for several thousands of years in countries of the Mediterranean basin for the treatment of many diseases.

As there is no phytochemical, pharmacological study and a limited scientific data available on the antioxidant and antimicrobial effects on Libyan Globularia alypum species. Therefore, the aim of this study intended to determine the chemical composition of extracts from aerial part of G. alypum Investigate the antimicrobial and antioxidant properties of the plant.

II. METHODS

A. **Plant collection and preparation** –

The aerial parts of *Globularia alypum* plant were collected during August 2016 from Massa area, about 185 km east of Benghazi / Libya. The plant was identified by department of Pharmacognosy (Faculty of Pharmacy, University of Benghazi). The plant was left to dry at open air. Prior the extraction the dried plant was powdered finely by using blender to be used for extraction, phytochemical screening, and antimicrobial as well as for antioxidant studies.

B. **Extraction of the plant** –

The methanol (70%) extract was obtained through exhaustive cold maceration of the dried plant. The solvent was evaporated under reduced pressure (at 40 C°). The residue was dried saved for further chemical and/ or biological examination.

C. **Phytochemical qualitative screening** –

The phytochemical screening of methanol extract was done to identify the main groups of chemical constituents such as carbohydrate, phenols and tannins, flavonoids, saponins, *glycosides,* steroid and terpenoids using standard qualitative methods as described by [8].

D. **Antimicrobial study**–

To investigate the antimicrobial effect of the tested extract; a modified Kirby-Bauer disc diffusion method [9] was used.

A series of bacterial and fungal strains used for antimicrobial susceptibility testing were (Staphylococcus aureus and bacillus subtilis) as representatives of Gram-positive bacteria; Gram-negative bacteria (Escherichia coli, Pseudomonas aeruginosa), and fungi (Aspergillus flavus and Candida albicans).

The plates were inverted to be incubated for 24-48 hours in case of bacteria at 35-37 oC and at 250 C for 48 hours in case of fungi. Amphotericin B and tetracycline were used as standard (positive controls) for fungi and bacteria respectively while 10 µl of Dimethyl sulfoxide (DMSO) was used as negative control.

E. **The measurment of antioxidant activity**–

The Antioxidant activity of methanolic extract of Globularia alypum was examined according to scavenging effects using stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) method [10]. Newly prepared DPPH solution should be save at 4°C in a dark place. 25 ml of methanol is then used to dissolve 3.2 µl of (DPPH) with steering regularly up to 30 minutes. Methanolic solutions of DPPH (90 µl) were added to 10 µl of plants extracts in each different concentration to be incubated for 30 minutes at 37°C at room temperature. After incubation the absorbance was measured at 490 nm using multiplate reader (Bio-Tek Elx800™, Instruments, Inc.,USA).

Gallic acid was used as standard. Every determination was performed in triplicate. Percentage inhibition of the radical scavenging activity of test samples was calculated [11]. Extracts concentration providing 50% inhibitions (IC50) was calculated from the graph in which inhibition percentage was plotted against extracts concentration.

III. RESULT

A. **Phytochemical screening:**

The presence of bioactive constituents were assessed by the qualitative phytochemical screening of the methanol extract and results of screening was represented in (Table 1).

B. **Antimicrobial Activity:**

The antimicrobial activity of methanolic extract of Globularia alypum was studied against gram positive bacteria and gram negative bacteria (Staphylococcus aureus, and Bacillus subtilis) and (Escherichia coli, and Pseudomonas aeruginosa) respectively. While, the antifungal effect of extract was done against Aspergillus
flavus and Candida albicans. Diameter of zone of inhibition (mm) and potency percentage of the tested samples was recorded in (Table 2).

Table - 1 Phytochemical screening tests for the methanolic extract of *Globularia alypum*

Test	Constituents	Aerial parts
Sudan III	**Steam volatile substances**	+
Lieberman-burchard's test	**Sterols and/or triterpenes**	++
Fehling's solutions test **Benedict's reagent test**	**Carbohydrates and/or glycosides**	++
Shinoda test	**Flavonoids (free and combined)**	++
Ferric chloride test	**Tannins (condensed)**	++
Dragendoff's test **Mayer's test** **Wagner's test**	**Alkaloids**	--
Froth Test	**Saponins**	--
Borntrager's test	**Anthraquinones**	-
Keller-killiani test	**Cardiac glycosides**	-

C. **Antioxidant activity:**

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 IC_{50} ± SD, which is the concentration in mg/ml causing 50% inhibition of the free radical.

The results indicated that the methanolic extract of *Globularia alypum* showed significant scavenging effects on the DPPH radical, compared to gallic acid

Table- 2 The antimicrobial activity of the methanolic extract of *Globularia alypum* aerial part, (a) against Bacteria (b) against fungi

(a)

	Diameter of zone of inhibition (mm) (Potency %)			
	G^+ bacteria		**G- bacteria**	
	S. aureus	***B. subtilis***	***E. coli***	***Ps. aeruginosa***
(Standard) Tetracycline	19 100%	29 100%	30 100%	30 100%
methanolic extract	17 99%	25 86%	18 60%	17 57%

(b)

	Diameter of zone of inhibition (mm) (Potency %)	
	Fungi	
	A. flavus	***C. albicans***
(Standard) Amphotericin B	17 100%	19 100%
methanolic extract	10 59%	14 74%

Table -3 The antioxidant activity of *Globularia alypum* in terms of IC_{50}±SD

Sample	DPPH % Inhibition*
Methanolic extract of *Globularia alypum*	0.0543 ± 0.02
Gallic acid	0.03± 0.002

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

IV. DISCUSSION

From the results displayed in table1, it seems that the investigated plant organs (aerial parts) responded to the applied tests almost equally and confirmed the presence of , carbohydrates and/or glycosides, sterols and/or triterpenes, condensed tannins and, free and combined flavonoids (phenolic compound). It was recognizable that sample was more enriched in flavonoids and tannins (strongly positive response). Meanwhile, saponins, anthraquinones, cardiac glycosides and alkaloids are undetected in any of the tested organs consequently it could be measured as absent. In this sight it was reported that three new phenolic compounds in addition to three phenyl ethanoid glycosides were isolated from the aerial parts of *Globularia alypum* and showed strong antioxidant effect in vitro [12].

Results illustrated in table 2 revealed that there was a significant antibacterial activity of studied *Globularia alypum* against representatives of Gram positive (*Staphylococcus aureus*, *Bacillus subtilis*) and showed a moderate effect against Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) and fungi *Aspergillusflavus* and *Candida albicans.* Our result was comparable with that obtained from previous study in which the extract essential oil of *G alypum* were demonstrated antibacterial activity against all clinical pathogens tested bacteria (7 Gram negative and 5 Gram positive bacteria) [13]. In addition there was another study confirmed that *G. alypum* extracts were active against Gram-positive bacteria, particularly those of roots which were the most efficient against *Bacillus subtilis* and *Staphylococcus aureus* [14].

According to obtained result it has been found that the methanolic extract of *G. alypum* had significant scavenging effects 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 [14]. In addition there was a study found a positive correlation between the total phenolic content and the strongest antioxidant activity of the methanolic extract of *G. alypum* [15].

V. CONCLUSION

The phytochemical screening showed that the aerial part of *Globularia alypum* plant extract contain a mixture of phytochemicals as sterols , reducing sugars, flavonoids (free and combined), condensed tannins and phenolic compounds

The results of antimicrobial activity of the methanolic extract revealed that the methanolic extract showed broad spectrum antibacterial activity when used against 4 strains of bacteria (2 Gram positive) and (2 Gram negative). Therefore it can be consider as a good candidate for further microbiological and chemical analysis.

The antioxidant effect by using the DPPH assay showed that the plant has potent antioxidant activity which can be an excellent choice for biological and chemical analysis and further study should be done for the isolation of the therapeutically active compounds with anticancer potency and also for further pharmacological evaluations.

Conflict of interest: The authors declare no conflict of interest

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