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**Research Article**

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# Study of eight medicinal plants for antioxidant activities

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**ABSTRACT**

*There is now an expansion of interest in phytochemicals as a new source of natural antioxidants to be used in foods and pharmaceutical preparations to substitute synthetic antioxidants, which are being restricted due to their potential health risks and toxicity. Eight Libyan medicinal plants belonging to different families were extracted successively with three solvents of different polarities using microwave technique. The antioxidant activity of these plant extracts were evaluated using 2,2, Diphenyl-1-picryhydrazyl (DPPH) radical scavenging method. Among the twenty four crude extracts, six showed strong antioxidant activity ($IC_{50}$ ranging between 4.55 to 21.55 µg/ml), six of the extracts exhibited moderate antioxidant activity ($IC_{50}$ values ranged between 40.7 to 94.4 µg/ml), and four crude extracts exhibited weak antioxidant activity ($IC_{50}$ more than 200 µg/ml). The $IC_{50}$ values of quercetin and ascorbic acid, used as standards in this assay were found to be 3.35 and 15.35 µg/ml, respectively. The antioxidants that are present in the eight Libyan medicinal plants studied in this paper could be used to inhibit or prevent the deleterious consequences of oxidative stress. The antioxidant effect could be related to the free radical scavengers like polyphenols, flavonoids and phenolic compounds.*

**Keywords:** Libyan Medicinal Plants; Antioxidant activity; DPPH assay.

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## INTRODUCTION

A lot of researches now are taken place everywhere in the world to evaluate the safety, therapeutic use and the composition of the traditional medicine. The Mediterranean climate in Libya favors the growth of a great number of plant species, some of which have various medicinal and antioxidant properties. There are more than hundred species used by local people in Libyan folk medicine for medicinal purposes. Recently, free radicals have attracted a great deal of attention for researchers. The free radicals are mainly derived from oxygen and nitrogen, and are produced in our body systems, exposure to physicochemical conditions or could bee related to some diseases. Free radicals can cause adverse effects on lipids, proteins and oligonucleotides including DNAs and RNAs and also involved in aging and a many human diseases [1]. The use of plant extracts and phytochemicals with antioxidant activity can be of great significance in the treatment of many diseases [2, 3]. One of most the important role played by natural products as therapeutic agents are through their antioxidant activity. Plants are able to produce a large number of antioxidants to manage the oxidative stress resulted from the sunbeams and oxygen, that make the plants as good sources for the new compounds with antioxidant activity. Antioxidants can be of immense therapeutic importance in the treatment of free radical-linked pathogenesis like cancer, cardiovascular disease, atherosclerosis, and ageing [4]. Antioxidants have been established to be the most effective way to eliminate adverse effects caused by free radicals as antioxidants can scavenge them or endorse their decomposition [5]. In the present study many plants were chosen to investigate their aerial parts for antioxidant activity using DPPH assay including *Cistus*

*incanus, Cistus parviflorus, Helianthemum lippii, Arbutus pavarii, Capparis spinosa, Rhamnus alaternus, Quercus coccifera* and *Globularia arabica*

## EXPERIMENTAL SECTION

**Chemicals:** Petroleum ether, *n*-hexane, ethyl acetate, methanol. 2, 2, Diphenyl-1-picryhydrazyl (DPPH), quercetin and ascorbic acid were purchased from Sigma Aldrich Co, UK.

**Preparation of extracts:** In the present study the different crude extracts of the studied plants that traditionally used by local people in Libya for various disorders were collected from AL-Jabal Al-Akhdar and AL-Jabel, Al-Garbi, Libya. The whole aerial parts of each plant (**Table1**) were collected during the spring season. They were identified and authenticated by the experts at the Department of Botany, Faculty of Sciences, Tripoli University, Tripoli, Libya. The dried and grinded collected plants were extracted successively using microwave assisted closed extraction system (Milestone start E 2450 MHz, Italy) with three different solvents of three different polarities. The microwave power was set at 500W. Five grams of each powdered plant were extracted for 10 min using petroleum ether or *n*-hexane, 15 min with chloroform or ethyl acetate and 20 min with methanol. The crude extracts were evaporated to dryness using rotary vacuum evaporator and stored at -20 °C.

**Free radical scavenging activity**

The free radical scavenging activity of methanol extract was evaluated using 1,1-diphenyl-2-picryl-hydrazil (DPPH) [6], where the stock solution of methanolic extract (1 mg/mL) was prepared. Aliquot of 400µl of 0.1µM of DPPH solution was added to 1 mL *cuvett.* Extract solutions at different doses (1 to 50 µg) were added**.** A volume of 600 µl of ethanol was added and the mixture was shaken vigorously and allowed to stand in dark place at room temperature for 5 min. Then the absorbance was measured at 517 nm in UV-Visible-NIR spectrophotometer (Varian Cary 5000, USA). The radical scavenging activity of the tested samples were calculated according to the equation one and expressed as percentage of inhibition [7, 8]:

Percent of DPPH inhibition= $[(A_A - A_B)/A_B] \times 100$ Equation 1

$A_A$ and $A_B$ are the absorbance values of the test and the blank samples, respectively.

Percent inhibition *versus* concentration curve was plotted and the concentration of sample required for 50% inhibition was determined and expressed as $IC_{50}$ values using a non-linear regression algorithm. The data were presented as mean values ± standard deviation (n = 3).

**Statistical analysis**

All experimental measurements were carried out in triplicate and are expressed as average of three analyses ± standard deviation.

## RESULTS

Among twenty four crude extracts, the methanol extract of *Arbutus pavarii* was the most active with an $IC_{50}$ value of 4.55 µg/ml followed by the methanol extracts of *Cistus parviflorus*, *Globularia arabica*, *Cistus incanus*, *Quercus coccifera*, and ethyl acetate extract of *Arbutus pavarii* with $IC_{50}$ values of 4.75, 7.65, 17.75, 18.65 and 21.55 µg/ml, respectively. Methanol extracts of *Rhamnus alaternus*, *Helianthemum lippii*, *Capparis spinosa*, and the ethyl acetate extracts of *Quercus coccifera*, *Globularia arabica*, *Capparis spinosa* exhibited moderate antioxidant activity with $IC_{50}$ values of 40.7, 45.2, 57.75, 63.75, 74.75 and 94.4 µg/ml, respectively. The ethyl acetate extracts of *Rhamnus alaternus*, *Cistus incanus*, *Cistus parviflorus* and *Helianthemum lippii* showed weak antioxidant activities with $IC_{50}$ values over 200 µg/ml. The $IC_{50}$ values of quercetin and ascorbic acid were found to be 3.35 and 15.35 µg/ml, respectively.

## DISCUSSION

Natural products especially medicinal plants produce and contain a variety of second metabolites that able to perform various functions, and many of them exhibited interesting and useful biological activities [9]. It`s well established that natural products have been a source of lead compounds for the development of some of the most effective drugs available for the treatment of deferent human disease [10]. Investigations have revealed that daily consumption of antioxidants can reduce the incidence of various ailments [11].

**Table 1: The percentage extraction yield the selected Libyan medicinal plants in this study**

| Plant name | Family | Area of collection | Percentage yield of extraction (%) | |
|---|---|---|---|---|
| *Cistus Incanus* Durand & Barratte | *Cistaceae* | Jabal Al-Akhdar | Methanol | 10.34 |
| | | | Ethyl Acetate | 5.33 |
| | | | *n*-Hexane | 10.60 |
| *Cistus Parviflorus* ,Lam | *Cistaceae* | Jabal Al-Akhdar | Methanol | 8.77 |
| | | | EtAc | 4.68 |
| | | | *n*-Hexane | 16.25 |
| *Helianthemum Lippii* | *Cistaceae* | Jabel Al-Garbi | Methanol | 12.90 |
| | | | Chloroform | 8.40 |
| | | | Petroleum ether | 6.6 |
| *Arbutus Pavarii*, Pamp | *Ericaceae* | Jabal Al-Akhdar | Methanol | 19.80 |
| | | | Ethyl Acetate | 4.60 |
| | | | *n*-Hexane | 15.60 |
| *Capparis Spinosa* Linn | *Capparaceae* | Jabal Al-Akhdar | Methanol | 2.40 |
| | | | Ethyl Acetate | 11.90 |
| | | | *n*-Hexane | 9.60 |
| *Rhamnus Alaternus* , Durand &Barratte | *Rhamnaceae* | Jabal Al-Akhdar | Methanol | 8.73 |
| | | | Ethyl Acetate | 4.80 |
| | | | *n*-Hexane | 16.40 |
| *Quercus Coccifera* Durand &Barratte | *Fagaceae* | Jabal Al-Akhdar | Methanol | 9.00 |
| | | | Ethyl Acetate | 1.80 |
| | | | *n*-Hexane | 1.60 |
| *Globularia Arabica* | *Globulariaceae* | Jabal Al-Akhdar | Methanol | 2.40 |
| | | | Ethyl Acetate | 6.20 |
| | | | *n*-Hexane | 3.80 |

**Table 2: *In vitro* antioxidant activity of the eight plants extracts using DPPH radical scavenging activity.**

| Plant | Extract | $IC_{50}$ µg/ml |
|---|---|---|
| *Cistus Incanus* Durand & Barratte | MEOH | 17.75±1.5 |
| | Ethyl Acetate | 329.1±8.2 |
| | *n*-Hexane | -ve |
| *Cistus Parviflorus* ,Lam | MEOH | 4.75±5.6 |
| | Ethyl Acetate | 405.5±6.7 |
| | *n*-Hexane | -ve |
| *Helianthemum Lippii* | MEOH | 45.2±2.3 |
| | Chloroform | 916±4.5 |
| | *n*-Hexane | -ve |
| *Arbutus Pavarii*, Pamp | MEOH | 4.55±1.90 |
| | Ethyl Acetate | 21.55±1.1 |
| | *n*-Hexane | -ve |
| *Capparis Spinosa* Linn | MEOH | 57.75±2.3 |
| | Ethyl Acetate | 94.4±4.5 |
| | *n*-Hexane | -ve |
| *Rhamnus Alaternus* , Durand &Barratte | MEOH | 40.7±1.3 |
| | Ethyl Acetate | 218.6±4.4 |
| | *n*-Hexane | -ve |
| *Quercus Coccifera* Durand &Barratte | MEOH | 18.65±1.4 |
| | Ethyl Acetate | 63.75±2.1 |
| | *n*-Hexane | -ve |
| *Globularia Arabica* | MEOH | 7.65±1.40 |
| | Ethyl Acetate | 74.75±2.9 |
| | *n*-Hexane | -ve |
| Quercetin | | 3.35±0.3 |
| Ascorbic Acid | | 15.35±1.4 |

**$IC_{50}$ values are represented as mean's ±SD (n=3).*

Libyan medicinal plants under the current investigation can be considered as good sources of natural antioxidants as their extracts were found to possess high antioxidant activity. In most cases the methanolic extracts of the plants were found to be the most active extract. It is well known that plant polyphenols are good antioxidants and they can be extracted from plants using polar solvents like methanol [12, 13]. Compounds like carotenoids and omega-3 fatty acids are also good antioxidants and can be extracted from plants using non polar solvents like petroleum ether or *n*-hexane [14]. In the present study, most of the extracts that exhibited the strong activities were methanol extracts, while those showed moderate activities were ethyl acetate extracts. All of the non-polar extracts (*n*-hexane or petroleum ether) did not show any antioxidant activity. Therefore, it can be concluded that the antioxidant activity of these plant extracts may be due to their phenolic content and not for carotenoids or unsaturated fatty acids [15, 16]. Further studies needed to identify the active constituents present in these plant extracts.

## CONCLUSION

As free radicals are involved in the etiology of many diseases. Free radicals can adversely affect many important biological molecules, consequently leading to loss of their function. Such unwanted changes in the body leading to serious illness. Antioxidant results in this study can be use to protect against the damage induced by free radicals acting at various levels. Recent research centers in many countries around the world develop novel antioxidants agents to protect important tissues and organs against oxidative injures induced by free radicals. The traditional Libyan medicinal plants are rich sources of natural antioxidants. Higher intake of foods with functional attributes including high level of antioxidants in functional foods is one strategy that is increasing importance in advanced countries and is making its appearance in our country. Synchronized research involving biomedical scientists, nutritionists and physicians be able to make important difference to human health in the coming years.

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