ESTIMATION OF TOTAL PHENOLIC AND FLAVONOIDS CONTENT AND ANTI-FREE RADICAL SCAVENGER, ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF EXTRACT OF *Matricaria pubescens* (DESF.) SCH. BIP. COLLECTED FROM SOUTH EAST OF ALGERIA

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Abstract. The aim of this study for evaluated of extract of aerial parts of an endemic Saharan African plant *Matricaria pubescens* (Desf.) Sch. Bip. was examined for radical scavenging capacities and antimicrobials activity. The total of phenolic compounds of the extract was found to be 103.5 mg/g of dry extract. The antioxidant activity determined by the DPPH• method revealed that the extract had the highest antioxidant activity on DPPH• free radicals (97.1%). A significant correlation was observed between total polyphenols and the antioxidant activity.

The total antioxidant capacity the inhibition effects of *Matricaria pubescens* extract on linoleic acid emulsion peroxidation were found to be 94.17% at 2 mg/ml. Also, the strongest antibacterial effect of the extract was recorded against *Bacillus subtilus* and *Candida albicans* whereas a mild inhibition was observed against *Escherichia coli*, *Staphylococcus aureus* and *Penicillium expansum*.

Keywords: *Matricaria pubescens* (Desf.) Sch. Bip.; antioxidant activities; DPPH•; antimicrobial activity.

INTRODUCTION

Reactive oxygen species (ROS) such as hydroxyl radicals, hydrogen peroxide and superoxide are involved in many diseases including diabetes, gastric diseases and rheumatism [1]. Many plants, including herbs and spices, have many phytochemicals which are potential sources of natural antioxidants, e.g. phenolic, diterpenes and flavonoids. These compounds are products of secondary metabolism in plants, they have an antioxidant and anti-inflammatory activities [24, 27]. These plants have been widely used to extend the shelf life of foods and in traditional medicine as treatment for many diseases [12, 20].

The antioxidant activity of phenolic compounds in plants is mainly due to their redox properties and chemical structure, which can play an important role in neutralizing free radicals, chelating transitional metals by delocalization or decomposing peroxides [29].

These properties are linked to beneficial health functionality of phenolic antioxidants due to their inhibitory effects against development of many oxidative-stress related diseases.

There is increasing interest in antioxidant, particularly in those intended to prevent the deterioration of fats and of the constituents of foodstuffs and prevent the presumed deleterious effects of free radicals in the human body [11].

The most commonly used antioxidant at present time are BHA, BHT and TBHQ were reported have been suspected of being responsible for liver damage, pulmonary toxicity and carcinogenesis. For thus, it is needful to develop and utilize effective natural antioxidants so that they can protect the human body from free radicals and retard or reduce the progress of many diseases. For that, the research for new molecules which have antioxidant and anti-inflammatory activity is increasing in recent years by moving to the medicinal and aromatic plants [2, 3].

Matricaria pubescens (Desf.) Sch. Bip. is an endemic species well known and particularly found in the northern and central Sahara. Is a small annual plant, 7-15 cm in height up to 40 cm in plant cultivates, with many prostrate stems, which erect. Leaves Hairy dissected and green darkens; involucres of bracts straw yellow with a broad membranous margin, the deeply dissected leaves, with each of the lobes ending with a white tip are slightly fleshy and are between 1 and 3 cm long [19].

Matricaria pubescens is a popular herb used in traditional medicine in Algeria against several diseases, it has antiseptic properties and has been used for instance rheumatism, digestive diseases, diarrhea and aching joint; it was prepared as an infusion or powder and used internally. The crushed stems and leaves are used as a filter for butter goats, providing a delicious smell of butter and aid in maintaining has also been added to the traditional soup and gives food a very pleasant smell. It is not reported as toxic by nomads. The whole plant is collected fresh in spring, and sold in the market in several oases in the south [17].

The purpose of the present work is to determine the antimicrobial and anti-free radical scavenger activities of the ethanolic extracts from aerial parts of *Matricaria pubescens* (Desf.) Sch. Bip.

MATERIAL AND METHODS

Plant material. *Matricaria pubescens* was collected along the south east of Algeria, the plant material was shade dried at room temperature.

Preparation of extract. For preparation of ethanol extraction 25 g of *Matricaria pubescens*, was added to 250 ml ethanol at room temperature under dark

condition, this mixture was stirred by magnetic stirrer during 24h. Then the extract was filtered through Whatmann N°.1 filter paper, then the resulting pellet was extracted twice following the same protocol and filtered, the combined filtrates of extract were evaporated to dryness using Buchi Rotary evaporator (model R-200) under vacuum at temperature less then 40°C, the prepared extract were stored at 4°C until further analysis [8].

Determinations of the total phenolic content. The total content of phenolic was measured using Folin-Ciocaltau reagent [22]. Distilled water (7 ml), 0.5 ml of sample and 0.5 ml Folin-Ciocaltau reagent were added to 25 ml volumetric flask, the content were mixed and allowed to stand for 3 min at room temperature, next 2 ml of a 20% sodium carbonate solution was added. The samples were vortexes and then left to stand at room temperature during 30 min. Absorbance of the clear supernatants was measured at 750 nm using a spectrophotometer (UV 2550 Shimadzu); giants a blank consisting of all reagents and solvents without extracts. The content of total phenolic was calculated using a standard curve as above prepared using gallic acid and expressed as micrograms of gallic acid equivalents (GAE) per gram of extract.

Estimation of total flavonoids content. Total flavonoids were measured by a colorimetric assay according to [10]. An aliquot of diluted sample or standard solution was added to a 75 µl of $NaNO_2$ solution (5%), and mixed for 6 min before adding 0.15 ml $AlCl_3$ (10%). After 5 min, 0.5 ml of NaOH was added. The final volume was adjusted to 2.5 ml with distilled water and thoroughly mixed.

Absorbance of the mixture was determined at 510 nm against the blank where the sample was omitted. Total flavonoids content was expressed as mg Quercetin per gram of extract. All samples were analyzed in triplicate.

Free radical scavenging capacity in DPPH• radical assay. The free radical scavenging capacity of extract solution was evaluated with DPPH• assay assessed by the method of [2] 0.5 ml of test sample variable concentration was added to 1 ml of DPPH• solution (0.1 mM), after vortexing, the mixture was incubated for 10 min room temperature in the darkness and the absorbance at 517 nm was measured (UV 2550 Shimadzu). Triplicate measurements were carried out. The DPPH• radical scavenging activity was calculated using following formula. BHA and Trolox was the reagent used as standards.

$$\text{DPPH}^{\bullet} \text{ radical scavenging activity } \% = [(1-A_1/A_0) \times 100]$$

Were A_0 is the absorbance of the control, A_1 is the absorbance of the sample or standard.

Antioxidant activity in linoleic acid emulsion system. The total antioxidant activity of the prepared extract and the standards was determined according the ferric thiocyanate method described by [21]. Briefly, a mixture consisting of 0.5 ml of sample or standards (BHA and BHT) which contained different concentration was mixed with 2.5 ml linoleic acid emulsion (was prepared by homogenizing 0.2804 g of linoleic acid, 0.2804 g of Tween 20 as emulsifier and 50 ml phosphate buffer 0.02 M, pH 7.0) and 2.0 ml of phosphate buffer (0.2 M, pH 7.0). Then the mixture was incubated at 37°C. At several intervals during incubation 0.1 ml of aliquots were taken. The degree of oxidation was measured by sequentially adding 0.1 ml of each tube was transferred to other tube containing 4.7 ml of 75% ethanol and 0.1 ml of (30% w/w) ammonium thiocyanate followed by the addition of 0.1 ml of 0.02 M ferrous chloride (prepared in 3.5% hydrochloric acid). After the mixture had rested for 3 min, the peroxide value was determined by monitoring absorbance at 500 nm. A control was performed with linoleic acid but without the samples. The degree of oxidation was measured for every 24 h until a day after the absorbance of the control reached its maximum; every assay was done in triplicate.

The lipid peroxidation inhibition (LPI) % was calculated as:

$$\text{LPI } \% = (1- A_E/A_C) \times 100$$

Where A_E is the absorbance in the presence of extract after 48h, A_C is the absorbance of control after 48h.

Reducing power. Reducing power of extract was determined by the method prescribed by [16]. Briefly, an aliquot of 1.0 ml of extracts at various concentrations was mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml potassium ferricyanide solution [$K_3Fe(CN)$] (1% w/v). The mixture was incubated at 50° C for 20 min. after incubation, 2.5 ml of 10% (w/v) trichloroacetic acid were added and the mixture was centrifuged at 1750 tour/min for 10 min. from upper layer, 2.5 ml solution was mixed with 2.5 ml distilled water and 0.5 ml $FeCl_3$ (0.1%) in a test tube. and the absorbance was measured at 700 nm against blanks that contained all reagents except the sample extracts, The assay was replicated for each sample three times. L- Absorbic acid is used as a positive control.

Antimicrobial activity assay. In this study The number of strains used (03 bacterial strains + one yeast one fungi) is sufficient for the antimicrobial activity., and Its purpose is to experiment with new effective compounds in the Saharan plants; because this plant is widely used by desert tribes in North Africa and has a good reputation in large-scale as antiseptic plant [19].

The antibacterial activity of extracts was assessed by the agar disk diffusion assay [7] against three pathogenic bacteria's including *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923), one pathogenic yeast *Candida albicans* (IPA 200) and one fungi *Penicillium expansum*.

The pathogenic strains were first grown on Muller Hinton medium at 37°C for 24 h prior to seeding onto the nutrient agar. A sterile filter disc with 6 mm in diameter (Whatman paper N°. 3) was placed on the infusion agar seeded with bacteria's, and 20 µl of

extract was dropped onto each paper disc, the treated Petri dishes were kept at 37°C (five days for *Candida* and *Penicillium* are and 24 hours for bacteria's strains). The antimicrobial activity was assessed by measuring the zone of growth inhibition surrounding the discs.

RESULTS

Total phenolic and flavonoid contents. The total polyphenols and flavonoid contents based on the absorbance values. The amount of total polyphenols was 103.5 mg/g of dry extract and for the flavonoids was 77.5 mg/g of dry extract.

DPPH$^{\bullet}$ radical-scavenging activity. The obtained extract of *Matricaria pubescens* shows a great effect on the DPPH$^{\bullet}$ (Fig. 1). The percent inhibition of DPPH$^{\bullet}$ was 94.63%; While Trolox and BHA give 71.17% and 92.2% respectively at concentration 250 µg/ml. The IC_{50} is 55 µg/ml was recorded for the extract and 30 µg/ml, 16 µg/ml were recorded for Trolox and BHA respectively. The results indicated that the standards (Fig. 1) higher antioxidant activity than the extract low concentration, whereas at high concentration showed higher activity.

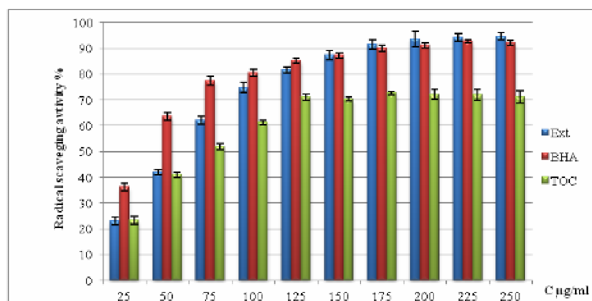

Figure 1. Radical scavenging activities of different concentrations of ethanol extract and standard antioxidants by free radical DPPH.

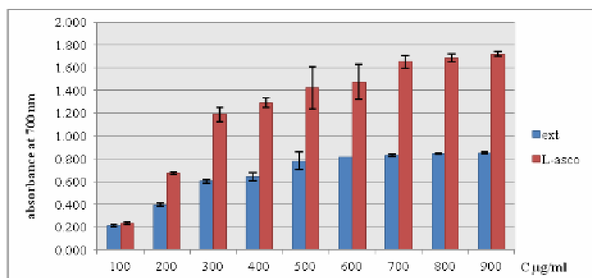

Figure 2. Reducing power of water and ethanol extracts and standard antioxidants

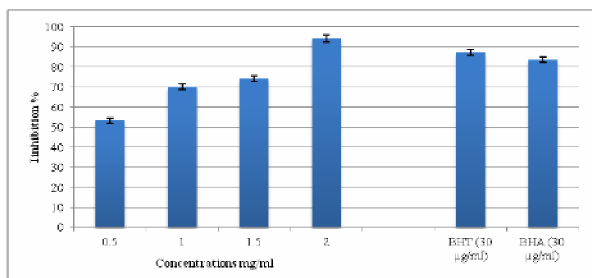

Figure 3. Anti- free radical scavenger activity determined in linoleic acid system

Reducing power assay. Increased absorbance of the reaction mixture indicates increased reducing power. Figure 2 shows the reducing power of different concentration extracts of *Matricaria pubescens* (100-900 µg/ml) compared to standard L-ascorbic acid. The reducing power increased with increasing concentration, which had less effect on the extract. The EC_{50} value of extract and L-ascorbic acid were 235 µg/ml and 215 µg/ml, respectively.

Anti- free radical scavenger activity in linoleic acid emulsion system. The antioxidant effects of the extract and standards on the peroxidation of linoleic acid were investigated. The results showed that extract inhibited 94.17% peroxidation of linoleic acid after incubation for 48h. The values were significantly higher than those of BHT (90.5%) and BHA (83.2%).

Table 1. Diameters of inhibitions (mm) of antimicrobial activities of extract of *Matricaria pubescens*

Micro-organisms		Diameters of inhibitions (mm)
Gram Positive Bacteria	*Bacillus subtilis*	18.1 ± 0.4
	Staphylococcus aureus	7.3 ± 0.6
Gram Negative Bacteria	*Esherichia coli*	8.1 ± 0.2
Yeast	*Candida albicans*	19.5 ± 7
Fungi	*Penicillium expansum*	8.7 ± 4

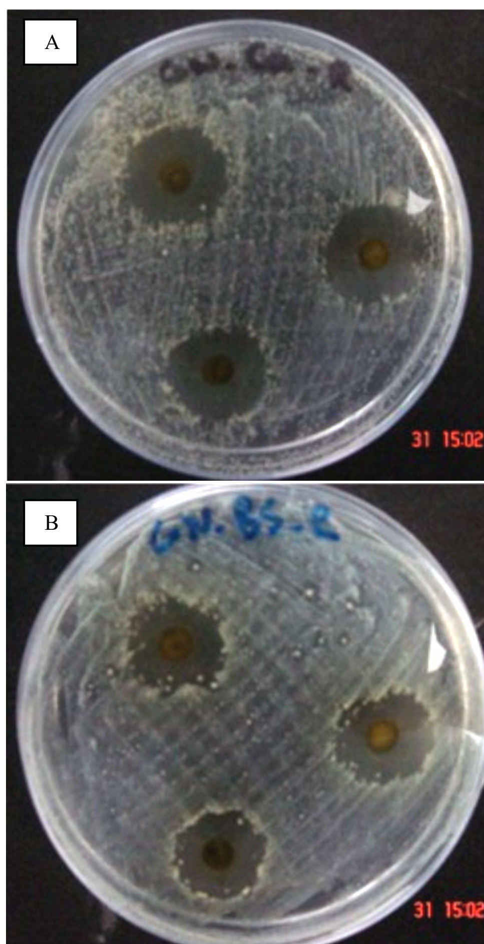

Figure 4. Antimicrobial diagram present a clear zone around each of the paper discs: **A** - *Bacillus subtilis* and **B** - *Candida albicans*

Antioxidant activity of the crude extracts and standard compound (BHA, BHT) was investigated by the ferric thiocyanate method in the linoleic acid system. The results (Fig. 3) showed that the extracts inhibited 53.3-94.6% peroxidation of linoleic acid after incubation for 48 h with concentration range from 0.5 to 2mg/ml. The values were lower than those of BHT (87.3%) and BHA (83.5%) with 30 µg/ml concentration.

Antimicrobial activity. The antibacterial activities of extract measured by the diffusion method against selected pathogenic bacteria were representing in (Table 1; Fig. 4). The mean inhibition zone for all bacteria treated with extract range from 0 to 20.1 mm when the concentration is 50 µg/ml. mean inhibition zone for *Bacillus subtilis* and *Candida albicans* were 18.1 and 19.5 mm (mean), respectively. However, no inhibition was observed against *Escherichia coli*, *Staphylococcus aureus* and *Penicillium expansum*.

DISCUSSION

Total phenolic and flavonoid contents. The high content of total phenols of the extract might explain the strong antioxidant properties of this plant [25].

Antioxidant activities. Radical scavenging activity is very important, due to the deleterious role of free radicals in foods and in biological systems. Diverse methods are currently used to assess the antioxidant activity of plant phenolic compounds. $DPPH^{\bullet}$ method is based on the reduction of $DPPH^{\bullet}$ in ethanol solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H in the reaction [6, 23]. The reduction capacity of $DPPH^{\bullet}$ radical was determined by the decrease in absorbance induced by plant antioxidants [28]. This result represents the high correlation between the antioxidant activity and the concentration of polyphenols content. These results suggest that the physico-chemical nature of the individual phenolic including flavonoid glycosides in the extracts [5], may be the major contributor in the antioxidant activity [13, 26].

The transformation of Fe^{3+} into Fe^{2+} in the presence of various fractions was measured to as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action [16], and can be strongly depends on the presence of antioxidants, which exert the antioxidant activity by breaking the free radical chain by donating a hydrogen atom [15]. The reducing power of the extracts was measured by direct electron donation in the reduction of $[Fe(CN)_6]^{3-}$ to $[(Fe(CN)_6]^{4-}$. The product was visualized by addition of free Fe^{3+} ions after the reduction reaction, by forming the intense Prussian blue color complex, $[(Fe(CN)_6]^{4-}$, and quantified by absorbance measurement at 700 nm [9, 28].

Also, the results of the test linoleic acid emulsion system could suggest that extract represent a source of natural antioxidants, which are able to act against lipid peroxidation and to scavenge free radicals due to phenolic compounds [14].

Antimicrobial activity. Several studies attributed the inhibitory effect of plant extracts against bacterial pathogens to their phenolic composition [4, 18]. These results suggest that the antibacterial capacity has a good efficiency with crude extract of *Matricaria pubescens*. Purified components may be used as natural antimicrobials in food systems, as well as to prevent the growth of food born bacteria resulting in extension of the shelf life of processed food.

In the present study, we showed the higher amount of total phenolic compounds present in the extract of *Matricaria pubescens* plant, which had significant antioxidant activity, radical-scavenging capacities reducing power and lipid peroxidation inhibition activities compared to some commercial standards. The high phenolic and antioxidant activity correlated well with high antimicrobial activity against some strains. This research proposes to develop innovative strategies of food additives. Further studies into the incorporation and effects of *Matricaria pubescens* phenolics compounds on antioxidant status in animal models are needed to confirm these results.

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