PHENOLIC COMPOUND PROFILE AND EVALUATION OF BIOLOGICAL ACTIVITIES OF THE CRUDE EXTRACT AND SOME BIOACTIVE COMPOUNDS OF *Helianthemum lippii* AERIAL PARTS

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Abstract. This study aimed to investigate the antioxidant and antibacterial capacity of crude extract and bioactive compounds from the aerial parts of *Helianthemum lippii*.

The antioxidant activities of the crude extract and fractions (dichloromethane, ethyl acetate, n-butanol, and anthocyanins) were assayed by Diphenyl 2-pycril hydrazine (DPPH), reducing power (RP), Total Antioxidant Capacity (TAC) and linoleic acid/β -carotene bleaching assay. Their antibacterial activity against six bacterial strains was evaluated by Agar diffusion and a modified broth macro-dilution method was used to measure the minimum inhibitory concentration (MIC). Its phenolic content was also verified by quantitative estimations

The results demonstrated this plant and its fractions have an excellent capacity antioxidant, as dichloromethane fraction exhibited the highest antioxidant activity in tested DPPH, RP assays, and TAC with IC_{50} and effective concentration (EC_{50}) values of 2.404 µg/mL, 0.592 µg/mL, 1.58 mg Ascorbic acid/g of extract respectively. In addition, dichloromethane fraction showed promising antibacterial activity, with strong efficiency against six strains bacteria with the inhibition zone reaching even 26.67 mm, while the MIC was very similar in all fractions and much better than the crude extract so that it ranged in values from 0.1563 to 0.9375 mg/mL. Finally, it is worth noting this plant and its fractions is a promising source of medicinal uses that could be applied in the treatment of many diseases caused by oxidative stress and bacterial infection.

Keywords: *Helianthemuml lippii* (L.) Dum. Cours.; dichloromethane; ethyl acetate; n-butanol; antioxidant activity; antibacterial activity.

INTRODUCTION

Medicinal plants are widely recognized for their therapeutic and nutritional benefits, both of which help in the treatment of various illnesses and the development of new pharmaceuticals, according to a World Health Organization survey [5, 37]. Interest in medicinal plants' antioxidant and antibacterial capabilities has significantly increased in recent years. These qualities are now widely acknowledged to be crucial for guarding against diseases including cancer, coronary artery stenosis and the oxidative stress-induced aging process [17].

The world health organization (WHO) estimates that there are almost 20.000 medicinal plants in 91 countries, including 12 countries with high levels of biodiversity. The initial steps in employing physiologically active molecules from plant resources are extraction, pharmacological screening, isolation and characterization of bioactive material, toxicological evaluation and clinical evaluation [13].

The biological activity of polyphenol compounds, such as flavonoids, tannins and anthocyanins, include those that are anti-inflammatory, antibacterial, antiviral, antithrombotic cardioprotective, antiallergenic, antiviral, anticarcinogenic, hepatoprotective and vasodilatory [4]. They function as antioxidants in a variety of ways, including as free radical scavengers, reducing agents, complexes of pro-oxidant metals, inhibitors of the generation of singlet oxygen and activators of antioxidative defense enzyme activities [1, 27], making them a potentially effective replacement for medical sectors in the treatment of many disorders.

Algeria has a varied plant flora, with 3139 species dispersed across 150 botanical groupings, 653 of which are indigenous [37]. This investigation focuses on the Saharan desert's indigenous *Helianthemum lippii* plant.

Helianthemum lippii L is belonging to the Cistaceae family and contains 08 gener and 200 species that are widespread in the Mediterranean regions [3]. Worldwide, this genus includes 70 species in Algeria and Pakistan. *H.lippii* is widely used in traditional medicine due to its notable pharmacological effects medicinal and it is the host plant for various species of desert truffles, which are valuable for food, and economic development, as well as the development of rural and local communities [19]. Further, it has biological properties that are promising for the treatment of many diseases and is used as a pain reliever such as menstrual pain and uterine diseases in women, thanks to properties tremendous as an antioxidant, antimicrobial, and anti-inflammatory, Further, this plant is characterized by being rich in secondary metabolites as it has been proven in many studies [23].

To the best of our knowledge, there is no previous study to isolate bioactive compounds from *Helianthemum lippii* (L.) Dum Cours. extract and evaluate their biological properties. Hence, this research aimed to isolate bioactive components from *H. lippii* extract and evaluate their antioxidant and antibacterial activity *in vitro*.

MATERIAL AND METHODS

Chemicals

This investigation employed many analytical-grade compounds; Folin-Ciocalteu reagent, gallic acid, ascorbic acid (Vitamin C), quercetin, tannic acid, sodium carbonate, aluminium chloride, 2,2-diphenyl-1-picrylhydrazyl (DPPH), the potassium dihydrogen phosphate (KH_2PO_4), dibasic potassium phosphate (K_2HPO_4), potassium ferricyanide ($C_6N_6FeK_3$), linoleic acid ($C_{18}H_{32}O_2$), b-carotene ($C_{40}H_{56}$), chloroform ($CHCl_3$), tween 40 ($C_{62}H_{122}O_{26}$), ferric chloride ($FeCl_3$), gallic acid ($C_7H_6O_5$), trichloroacetic acid (TCA) and dimethyl sulfoxide (DMSO). All were obtained from Sigma-Aldrich.

Plant materials

In March 2020, during the flowering season, the aerial portions of the *Helianthemum lippii* (L.) Dum.Cours. were collected in Southeast Algeria, specifically in the Elhamadin-district (33°35'00"N6°56'33"E) of El-Oued. Professor Atef Chouikh recognized the plant material (Faculty of Natural Science and Life, El Oued University). The aerial section was thoroughly cleaned with flowing water to remove dust and other foreign objects. After that, it was powdered, dried, and put away for later use.

Preparation of aqueous extract

Powdered *H. lippii* aerial parts weighing 10 g were soaked in 100 mL of distilled water at room temperature for 24 hours. Subsequently, the mixture was filtered using filter paper. After extraction, the material was thoroughly dried at 40°C. The extract was weighed and stored for upcoming analysis at 4 °C in the refrigerator [30].

Extractions of chemical compounds from the aerial part of *H. lippii*

Extraction ethyl acetate and n-butanol phase of flavonoids

To extract the flavonoids, 30 g of the plant material was macerated in 300 mL of MeOH for 24 hours. The solvent was then filtered and evaporated twice. For the first extraction, 150 mL each of hot water and ethyl acetate were used after the solvent had been filtered and evaporated. Two 150 mL injections of n-butanol were used for the second extraction in the aqueous phase. The two organic flavonoid phases, n-butanol and ethyl acetate, were rotated and evaporated to produce the two flavonoid phases [9].

Extraction of anthocyanins

Using 50 mL of 0.1% HCl (v/v) in methanol and stirring at room temperature for 20 hours, *H. lippii* was extracted in the dark. *H. lippii* was used in doses of 12 grams. After filtering, an additional 50 mL of 0.1% HCl (v/v) in methanol was used to wash the material. Combinations of filtrates were dried at 30°C in a rotary evaporator. The residue was dissolved using deionized water that contained 0.01% (v/v) HCl. The mixture was dried, then centrifuged at 3000 rpm with the supernatant being used for analysis [26].

Extraction tannins

The dried plant was macerated in 300 mL of water/acetone (7V/3V) for three days at room temperature. Dichloromethane and ethyl acetate (2 × 180 mL) were used to extract the aqueous layer after the acetone was removed through filtering. The organic phase was dried and tannin extract was produced [38].

Determination of Total Phenolic content (TPC)

The polyphenols were identified using the Folin-Ciocalteu method. Per gram of extract, to 1mL of 10% Folin-Ciocalteu reagent with 0.2mL of the aqueous extract of *H.lippii* was added. The addition of 800µL of saturated sodium carbonate (75 g/L) was made after 4 minutes. After 2 hours of incubation at room temperature, the absorbance was measured at 765 nm. To ensure that the results could be replicated, the tests were run three timesthe total phenolic content was reported as mg equivalent of gallic acid [24]. The total phenolic content was expressed in mg equivalent of gallic acid per gram of extract, as follows $y = 0.0104x+0.0819$, $R^2= 0.9925$

Determination of Total Flavonoids content (TFC)

We employed a colorimetric technique with aluminum chloride ($AlCl_3$) to determine the total flavonoids concentration of the *H. lippii* extract. Added 1mL of the sample with 1mL of $AlCl_3$, and on the other hand with 1mL of the standard. At 430 nm, the absorbance was measured, after 30 minutes against the prepared reagent blank. In order to determine the results, a linear calibration equation using quercetin as the standard was utilized. The results were represented as milligrams of quercetin per gram of extract [16].

Determination of Total Hydrolyzable Tannins contain (HTC)

The total hydrolyzable tannin concentration was determined using the Folin-Ciocalteu colorimetric technique described Poudel and Rajbhandari [35]. An aliquot of 1mL of tannic acid in distilled water of each concentration was added to a 10 mL test tube that already had 8.4 mL of distilled water, after that 0.5 mL of Folin-Ciocalteu reagent, and followed by 0.1 mL of sodium carbonate solution (7%) in it. After 30 minutes of incubation, absorbance was measured at 700 nm in comparison to a blank. Three times each were added to each test. The same procedure used for the standard was used to measure the absorbance for each concentration of the extract. The total amount of tannin in the extracts is expressed as the number of tannic acid equivalents (TAE) per gram of dry extract (mg/g).

Determination of Total Condensed Tannins contain (HTC)

According to Poudel and Rajbhandari [35] the condensed tannins contain (HTC) were calculated. Added 3 mL of freshly made vanillin reagent (4% vanillin in methanol) and 1.5 mL of strong hydrochloric acid were pipetted into an aluminum foil-wrapped tube with the sample. The mixture was thoroughly mixed before the hydrochloric acid was added. The reaction's absorbance against water was

evaluated at 500 nm after 15 minutes at 20 to 2°C. Catechin was utilized to create the calibration curve.

Determination of total Antocyanins

The pH differential method was used to determine the quantity of total anthocyanins, according to Brito, Areche [7]. The sample, with a concentration of 1 mg/mL in water, underwent dilution in 400 µL using two distinct buffers: a 0.025 M potassium chloride buffer with a pH of 1.0 and a 0.4 M sodium acetate buffer with a pH of 4.5. Subsequently, the absorbance at 510 and 700 nm was measured after a 30-minute incubation period. The following equation is used to compute the anthocyanin content, which is given as mg cyanidin 3-glucoside equivalents per gram of extract (C3GE/g extract): TAC = (A×MW×DF×100)/MA where; **A** = $(A_{510}–A_{700})pH_1 – (A_{510}–A_{700})pH_{4.5}$; **MW** : molar weight (449.2 g/mol); **DF**: dilution factor (10); **MA**: molar absorptivity of cyanidin 3-glucoside (26.9 $l·cm^{-1}·mol^{-1}$).

1. Antioxidant activity

DPPH free-radical scavenging activity

The 1,1-diphenyl-2-picrylhydrazyl solution is made by dissolving 2.4 mg of DPPH in 100 mL of methanol. The same volume of each phenolic extract (or ascorbic acid as control) and of the prepared DPPH solution was mixed. The reaction mixture is stirred briefly, then allowed to cool to ambient temperature for 30 minutes while being kept in the dark. At 517 nm, the absorbance of the reaction medium is measured and contrasted with that of the control (methanol-distilled water) [28].

Reducing Power Assay

The extract's reducing power was identified using Oyaizu's [32] methodology. They prepared everything in distilled water and combined phosphate buffer (2.5 mL, 0.2 M, pH 6.6) with varied extract strengths (mg/mL) of water and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 minutes. Additions of trichloracetic acid (2.5 mL, 10% aqueous solution) were made in aliquots and the mixture was then centrifuged at 3000 rpm for 10 minutes. After separation 2.5 mL of supernatant, 2.5 mL of purified water and 0.5 mL of fresh $FeCl_3$ solution were combined. Then the absorbance was calculated at 700 nm. Ascorbic acid was utilized as a positive control. In this procedure, the higher the absorbance, the greater the reducing power.

Total Antioxidant Capacity (TAC)

The total antioxidant activity of the fractions was assessed using the phosphomolybdate technique using Ascorbic Acid AA as a standard. An aliquot of an extract was mixed with 0.3 mL of the extract and 3 mL of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixture was incubated in a tube for 90 minutes at 95 °C. Using a spectrophotometer, the solution's absorbance at 695 nm in comparison to a blank was calculated. Using the standard AA graph, the ascorbic acid equivalents in mg per gram of extract were calculated [20].

Linoleic acid/β-carotene bleaching assay (BCB)

The anti-lipid peroxidation properties of the samples were evaluated using the linoleic acid/β-carotene technique. A spectrophotometer was used to measure the zero time absorbance at 470 nm as soon as the sample was added to each tube. The tubes were then incubated at 50 degrees celsius in a hot water bath. After two hours, the absorbance readings were assessed once more at 470 nm. Gallic acid was utilized as a positive control. A blank without β-carotene was made so that background subtraction could be performed [20].

The inhibition percentage of bleaching (I bleaching percent) was calculated using the formula below:

I bleaching (%) = [(Absorbance after 2h of assay)/(Initial absorbance)]× 100

2. Antibacterial activity

The agar well-diffusion method was employed to assess the efficacy of the crude extract and the fractions isolated from *H. lippii* against six pathogenic bacteria, including three Gram-positive bacteria (B.S: *Bacillus subtilis* ATCC 6633 ; S.A: *Staphylococcus aureus* ATCC 6538; and L.I: *Listeria innocua* CLIP 74915) and three Gram-negative bacteria (E.C: *Escherichia coli* ATCC 8737; P.A: *Pseudomonas aeruginosa* ATCC 9027 and S.T: *Salmonella typhimurium* ATTCC 14028). The Petri dishes were incubated for 24 hours at 37 °C. The inhibitory zone was characterized by a millimeter inhibition diameter. Three repitition were used for the antibacterial tests [4].

Determination of minimal inhibitory concentration (MIC)

Using a modified broth macro-dilution approach, the MIC of *H. lippii* crude extract and its fractions was assessed against the six previous bacteria. Each bacterial strain was divided into two sets of sterile test tubes (13 x 100 mm) with cotton plugs and 0.2 mL of bacterial inoculum. For 24 hours, each test tube was kept at 37 °C [36].

3. Statistical analysis

All measurements were taken in triplicate and all data were expressed as means ± standard deviations. Using IBM SPSS Statistics 26, one-way analysis of variance (ANOVA) and the Duncan multiple range tests were used for the statistical analysis.

RESULTS

Extracts yield

The aqueous extract of aerial parts of *H. lippii* exhibited the content (9.830±0.244%). Regarding the distribution of secondary metabolites, we see a high anthocyanin production (5.665±0.433%), followed by low contents of both butanol fractions (0.995± 0.0001%), ethyl acetate (0.75±0.006%) and tannins (0.71±0.002%) (Table 1).

Total Phenolic Content (TPC), Total Flavonoid Content (TFC), Hydrolyzable Tannins (THC), Condensed Tannins (TCT) and Anthocyanin Content (TAC).

H. lippii aqueous extract possesses high levels of total phenolic and flavonoids contents with 183.12±2.84 mg GA eq/g Ex and 72.00±1.03 mg Q eq/g Ex content respectively, while, low levels content of hydrolyzable tannins, condensed tannins and anthocyanin with 2.818±0.138 mg TA eq/g Ex, 5.88±1.58 mg Ca eq/g Ex and 4.256±0.590 mg C-3-G eq/g Ex respectively (Table 2).

Antioxidant activity

The assay principles and experimental settings employed in the methods used to determine antioxidant activity vary and as a result, the contributions of different antioxidants to the overall antioxidant potential vary. To examine the antioxidant properties of crude extract and bioactive components, DPPH, reducing power, TAC and BCB tests were used. The findings are shown in (Fig. 1).

DPPH radical scavenging activity

In DPPH assay, the highest DPPH scavenging activity of tannins with an IC_{50} of 2.404± 0.24 µg/mL, followed by the fraction anthocyanin IC_{50} = 2.868± 1.67 µg/mL, then *Helianthemum lippii* aqueous extract with an IC_{50} of 3.085±1.28 µg/mL. On the other hand, the fraction ethyl acetate fraction and butanolic fraction has a low antiradical activity with an IC_{50} of 4.546±0.041 and 5.061±0.033 µg/mL respectively.

Reducing power

The use of EC_{50} parameters is an index to compare and to the importance of the reducing power capacity of the *H. lippii* and the bioactive substances. The higher reducing power was observed in tannins, fraction ethyl acetate fraction and butanolic fraction (0.592±0.054, 1.058±0.031 and 1.172±0.04 µg/mL respectively). The EC_{50} concentration ranged between 1.633±0.076 in anthocyanin to 1.724±0.021 µg/mL in *H. lippii*. These capacities of all our samples were less than that of ascorbic acid 0.225±0.032 µg/mL.

Table 1: Yields of some bioactive compounds from *H.lippii*

Bioactive compound	Yields (%)
Aqueous extract	9.830±0.244
Ethyl acetate fraction	0.750±0.006
Butanolic fraction	0.995±0.0001
Dichloromethane fraction	0.710±0.002
Anthocyanins	5.665±0.433

Table 2. Phenolic compounds of the aqueous extract of *H.lippii*.

TPC (mg GA eq/g Ex)	TFC (mg Q eq/g Ex)	HTC (mg TA eq/g Ex)	TCT (mg Ca eq/g Ex)	TAC (C-3-GE/g Ex)
183.120±2.840	72.000±1.030	2.818±0.138	5.880±1.580	4.256±0.590

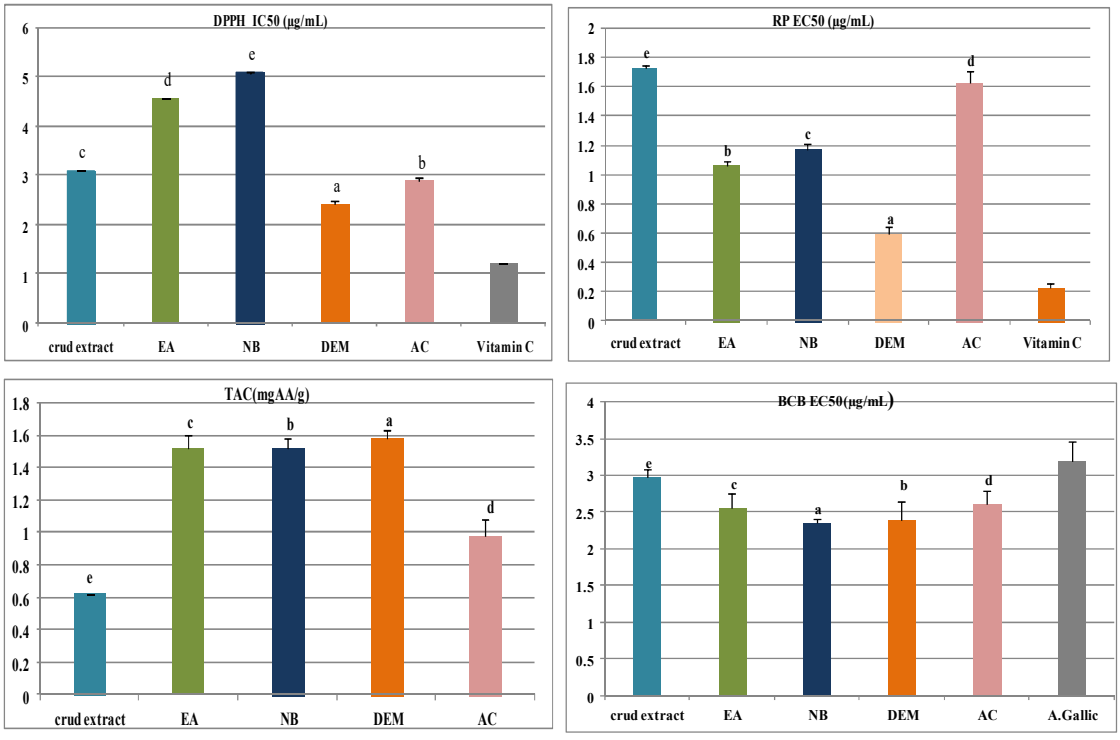

Figure 1. Antioxidant capacities of *Helianthemum lippii* and their fractions; a-e: means Values with different letters in the same column are significantly different (P<0.05). Waller Dunkan (p < 0.05).

Total antioxidant capacity

The highest antioxidant activity corresponded by tannins with 1.58 mg AA eq/g dw, ethyl acetate fraction and butanolic fraction (1.518 mg AA eq/mg and 1.522 mg AA eq/g), then anthocyanin 0.97 mg/g and lastly crud extract with 0.618 mg AA eq/g equivalents of ascorbic acid.

Linoleic acid/B-carotene bleaching assay (BCB)

Lastly, the ability of a substance to prevent b-carotene from being oxidized by free radicals produced during linoleic acid peroxidation was evaluated using the BCB method. The outcomes were similar and excellent in all samples and were better than the standard gallic acid (EC_{50} = 3.19±0.37 µg/mL). n-Butanol fraction recorded the maximum activity with a value (EC_{50} = 2.34±0.06 µg/mL), followed by the anthocyanin fraction EC_{50} = 2.39±0.26 µg/mL, then ethyl acetate fraction EC_{50} = 2.54±0.50 µg/mL. On the other hand, the fraction dichloromethane and the *H. lippii* it has less activity compared to the other sample's activity with an EC_{50} of 2.60±1.18 µg/mL and 2.9715±0.012 µg/mL respectively.

Antibacterial activity

The inhibition of three Gram-negative and three Gram-positive bacteria by the crude extract of *H. lippii* and its fractions is described in (Table 4). All of the data demonstrate that our samples investigated have a broad spectrum of activity, working in varying degrees on both Gram-positive and Gram-negative bacteria (Fig. 2). We observe that the proportionality of this activity to concentration. Additionally, none of the negative controls (DMSO) inhibited the development of the bacteria.

The zones of inhibition against different bacterial species were between 8 and 26.67 mm (Fig. 2 and Table 4). In the majority of the bacterial strains, the dichloromethane fraction had the best antibacterial and showed the highest activity, followed by ethyl acetate, which also produced excellent starch. Whereas crude extract, fraction anthocyanins and fraction n-butanol, which had shown moderate activity.

Minimum inhibitory concentration (MIC)

The investigation has also been expanded in order to ascertain the MIC for our samples against six different bacterial strains. According to Table 5, the fractions had unquestionably stronger and more effective antibacterial properties than the crude plant extract. The minimum concentration of these fractions found to be similar and nearly identical in all six bacterial strains in the current study was 0.1563 to 0.9375 mg/mL. Additionally, it was much lower than crude extract, which had a concentration of 2.8125 mg/mL.

DISCUSSION

The great range of pharmacological activities of medicinal plants are mostly attributed to their phytochemical components [11]. Biologically active substances are recognized to be chemical components in plants or crude extracts. Secondary metabolites components include some chemical compounds. They are directly responsible for antioxidant, antibacterial, antifungal and anticancer activities [18]. The secondary metabolites in our samples all exhibited antioxidant and antibacterial qualities through different pathways. The yield of chemical groups that are more frequently employed in medicine was calculated in this study using specific extractions of *H. lippii* aerial sections. The results collected are displayed in (Table 1). The plant's aerial parts, which produced higher yields, were distinguished by the presence of flavonoids, tannins and anthocyanins. Before, both the medicinal and physiological effects of these substances were understood. The genotypic factors that affect the accumulation of these chemicals in the plant may be partially responsible for these variations in secondary metabolite distribution [15]. In addition, additional studies have shown that biotic (species, organs and physiological stages) and abiotic (salinity, luminosity, water scarcity and edaphic factors) difficulties that are typically present in the desert zone may increase phenolic metabolism as a response to oxidative stress [22]. This assertion was supported by the results of Benhamou et *al*. (2009) [6]. Using three different DPPH, reducing power, total antioxidant capacity and BCB tests, the aqueous extract and bioactive elements of *H. lippii* aerial parts were evaluated. Our results showed strong antioxidant activity in all the samples. Tannins and anthocyanins were more effective at scavenging DPPH free radicals than crude extract and other fractions. This result may be explained by the fact that the antioxidant activity of the aqueous extract is lower than that of the bioactive components that have been extracted due to the additive or synergistic effects of polyphenols. Additionally, not all antioxidants are included in the total phenolic content of crude extract [33]. The DPPH and reduction power tests revealed that tannins had the strongest antioxidant activity. It is well known that a substance's antioxidant activity is inversely correlated with the number of hydroxyl groups it contains [2]. The reducing power test is a useful method for evaluating possible antioxidant activity. The dichloromethane fraction once more had the highest reducing activity. Usually, the existence of different reductants is connected to the reducing characteristics [12]. The Fe^{3+}/ferricyanide complex can be reduced to the ferrous form by receiving an electron from the reductants (antioxidants) present in the solution [15, 25]. Reductants rupture the chain of free radicals by giving an atom of hydrogen, which has the effect of being an antioxidant. Because some peroxide precursors react with reductions due to the presence of phenolic components, the generation of peroxide is inhibited. Different *H. lippii* extract fractions may have reductive capacity that is the cause of their antioxidant activity. The antioxidant capacity of *H. lippii* as a whole and various fractions were discovered in the following order: anthocyanin fraction > butanolic fraction > ethyl acetate fraction > aqueous

extract (Fig. 1). The assay functions on the theory that a substance with antioxidant activity, such as an extract, converts Mo (VI) to Mo (V), which produces green phosphate Mo (V). The tannins in this fraction have a high antioxidant activity, which could be attributed to the presence of phenolic chemicals. It is believed that the biological mechanisms underlying the antioxidant and radical scavenging effects of tannins are only now beginning to be understood [10].

Table 4. Antibacterial activity of the crude extract of *H.lippii*, and different fractions determined by agar well diffusion assay

Sample	Con (µg/mL)	Zone inhibition (mm)					
		B.S	S.T	L.I	E.C	P.A	S.A
H. lippii	1000	16.30±0.321***	17.67±0.057***	13.37±0.050**	14.66±0.057**	18.00±0.000***	15.00±0.000***
Crude	750	14.33±0.321**	14.33±0.057**	12.67±0.057**	12.33±0.057**	17.00±0.000***	14.67±0.057**
Extract	500	13.33±0.057**	12.667±0.057**	11.30±0.057**	11.60±0.050**	15.33±0.057***	13.33±0.154**
	250	10.06±0.110**	12.00±0.100**	9.99±0.208**	11.00±0.000**	14.33±0.057	12.00±0.100**
EA	1000	24.10±0.520***	17.60±0.057***	18.01±0.100***	18.260±0.046***	16.00±0.000***	17.00±0.200***
	750	18.33±0.286***	17.00±0.100***	17.50±0.050***	16.667±0.050***	15.00±0.000***	15.67±0.057***
	500	18.09±0.600***	16.00±0.100***	11.00±0.100**	15.667±0.050***	14.00±0.000**	13.67±0.057**
	250	16.33±0.321***	14.667±0.057	08.00±0.100*	14.33±0.115**	14.00±0.000**	11.00±0.000**
NB	1000	16.00±0.100***	16.00±0.100***	13.00±0.000**	17.00±0.100***	15.00±0.000***	17.33±0.057***
	750	15.60±0.115***	14.66±0.057**	12.67±0.057**	13.00±0.000**	14.33±0.057**	17.00±0.100***
	500	15.00±0.100***	14.00±0.200**	11.67±0.05**	12.667±0.050**	14.00±0.100**	15.33±0.115***
	250	12.60±0.115**	13.00±0.264**	11.33±0.057**	8.633±0.057**	13.33±0.115**	14.00±0.100**
DCM	1000	26.67±1.040****	18.00±0.100***	14.00±0.000**	17.00±0.000***	22.00±0.000***	24.00±0.100***
	750	26.33±0.050****	18.20±0.200***	12.66±0.570**	16.66±0.057***	14.67±0.700**	16.33±0.115***
	500	25.66±0.120****	15.66±0.152***	11.66±0.070**	16.00±0.000***	14.00±0.000**	15.00±0.100***
	250	23.30±0.286	14.00±0.100***	11.33±0.057**	14.00±0.100**	11.00±0.000**	14.66±0.057
AC	1000	19.66±0.404***	16.00±0.100***	14.00±0.000**	13.667±0.05**	15.50±0.050***	16.33±0.057***
	750	17.33±0.057***	15.00±0.100***	13.67±0.05**	13.00±0.000**	14.67±0.050**	14.00±0.000**
	500	17.00±0.017***	14.33±0.152**	10.00±0.000**	12.667±0.050**	14.50±0.050**	13.67±0.050**
	250	14.33±0.057**	13.667±0.152**	8.00±0.100*	11.33±0.057**	11.67±0.667**	12.00±0.100**
DMSO	--	07^{-}	07^{-}	07^{-}	07^{-}	07^{-}	07^{-}
Amoxicilin	10	30.00±0.02	32.00±0.020	22.00±0.056	25.00±0.030	27.00±0.050	30.00±0.010
Cephalexin	30	32.00±0.02	27.00±0.030	19.00±0.030	26.00±0.050	32.00±0.040	22.00±0.030

B.S: *Bacillus subtilis* ATCC 6633; **L.I**: *Listeria innocua* CLP 74915; **E.C**: *Escherichia coli* ATCC 8737; **P.A**: *Pseudomonas aeruginosa* ATCC 9027; **S.A**: *Staphylococcus aureus* ATCC 6538; **S.T**: *Salmonella typhimurium* ATTCC 14028; **DMSO**: Dimethyl sulfoxide. Inhibition zones> to 25 mm were delared as very strong (****), inhibition zones from 15 mm to 25 mm as strong (***), from 8 to 15 as moderated (**) and ≤ 8 as week activities (*), no activitity ($^{-}$) [20].

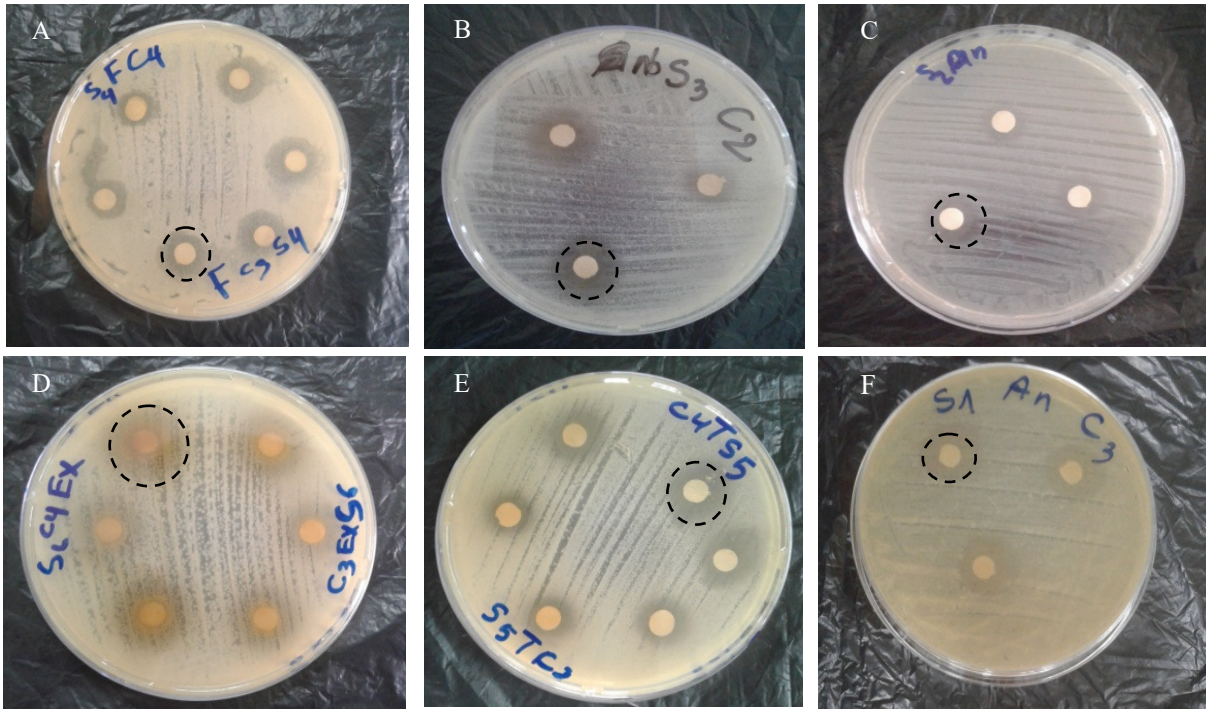

Figure 2. Zones of inhibition of *Helianthemum lippii* and their fractions against various bacterial strains. **A:** Zones of inhibition for fraction ethyl acetate fraction against *Pseudomonas aeruginosa*, **B:** Zones of inhibition for fraction n-butanol against *Escherichia coli*, **C:** Zones of inhibition for fraction anthocyannins against *Listeria innocua*, **D**: Zones of inhibition for aqueous extract of *Helianthemum lippii* against *Salmonella typhimurium*, **E**: Zones of inhibition for fraction dichloromethane against *Staphylococcus aureus*, **F**: Zones of inhibition for fraction anthocyannins against *Bacillus subtilis*.

Table 5. Minimum inhibitory concentration (MIC) in mg/mL of crude aqueous extract and fractions obtained from *H. lippii* on growth of different bacteria strains.

Tested samples	Tested Microorganism					
	B.S	**S.T**	**L.I**	**E.C**	**P.A**	**S.A**
Crude Extract	2.8125^{c}	2.8125^{c}	2.8125^{d}	2.8125^{c}	2.8125^{c}	2.8125^{c}
EA	0.4685^{a}	0.2343^{a}	0.4685^{c}	0.4685^{b}	0.4685^{b}	0.2343^{b}
NB	0.4685^{a}	0.2343^{a}	0.1563^{a}	0.4685^{b}	0.1563^{a}	0.1563^{a}
DCM	0.4685^{a}	0.4685^{b}	0.2343^{b}	0.4685^{b}	0.1563^{a}	0.2343^{b}
AC	0.9375^{b}	0.2343^{a}	0.2343^{b}	0.2343^{a}	0.4685^{b}	0.2343^{b}

a-d: means within a column row with different letters were significantly different ($p<0.05$). Waller Dunkan ($p < 0.05$). Fractions: **DCM**: dichloromethane, **EA**: ethyl acetate; **NB**: n-butanol, **AC**: anthocyannins

Activities that vary previous research has suggested that there are some qualitative and quantitative differences between the extracts and fractions. The extracts and fractions may contain a variety of secondary metabolites. Another hand is anticipated about the susceptibilities of microorganisms as well. The chemical is bioactive, so. The discovery of a variety of potentially active secondary metabolites in the crude extract and fractions may account for the relatively broad spectrum of antibacterial activity. There is evidence for the antimicrobial effects of flavonoids, phenols, tannins and alkaloids [31]. This inhibiting action may be due to tannins and flavonoids, which have a long history of use as antibacterial agents. According to this investigation, tannins have antibacterial properties against both Gram-positive and Gram-negative microorganisms. By preventing the bacteria from adhering to surfaces and consuming sugar and amino acids, tannic acid inhibits the bacterium's ability to multiply [34]. The ability of tannins to penetrate the bacterial cell wall and reach the inner membrane interferes with the metabolism and ultimately kills the cell. Tannic acid's ability to block the NorA efflux pump is regarded to be the primary mechanism by which it exerts its antibacterial effects. It is possible that the action of flavonoids, which are hydroxylated phenolic compounds that exist as a C6-C3 unit connected to an aromatic ring, can be understood. Their activity is due to their capacity to combine with extracellular and soluble proteins, as well as bacterial cell walls [29]. According to Campos, et al. [8] the extracts and/or fractions from this species are primarily composed of phenolic compounds such as flavonoids, phenolic acids and terpenes, all of which have antibacterial activity, which is consistent with the findings of this investigation. So we can say the results obtained in this work indicate that this species of plants is a promising source of compounds with antimicrobial activity.

This study found that *Helianthemum lippii* and its bioactive components have promising antibacterial and antioxidant activities and it represents a step forward in the development of an antimicrobial phytomedicine with a broad spectrum of activity derived from *Helinthemum lippii* isolated constituents. As the present study showed, the dichloromethane fraction of *Helianthemum lippii* presented very high antioxidants activity with DPPH, assay reduction power and TAC, in addition, the dichloromethane fraction showed promising antibacterial activity and was the better, followed by ethyl acetate and n-butanol, further research including purification and isolation of the polyphenols from this fraction would be worthwhile in order to establish their real potential as phytotherapeutic agents.

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REFERENCES

[1] Alia, F., Chouikh, A., Djahra, A.B., Bousbiai Brahim, A., Sadok, N., Tliba, A., (2021): Comparative study of some physicochemical and biological properties of effect host species variation on the relationship saharan parasitic plant *Cistanche violacea* (desf.) beck. Notulae Scientia Biologicae, 13(4): 11054-11054.

[2] Al-Jaber, N.A., Mujahid, T.G., Al-Hazmi, H.M., (1991): Flavonoids from *Atriplex farinosa*. Journal of King Saud University, 3(2): 163-167.

[3] Alsabri, S.G., Rmeli, N.B., Zetrini, A.A., Mohamed, S.B., Meshri, M.I., Aburas, K.M., Bensaber, S.M., Mrema, I.A., Mosbah, A.A., Allahresh, K.A., (2013): Phytochemical, anti-oxidant, anti-microbial, anti-inflammatory and anti-ulcer properties of *Helianthemum lippii*. Journal of Pharmacognosy and Phytochemistry, 2(2): 86-96.

[4] Belhaoues, S., Amri, S., Bensouilah, M., (2020): Major phenolic compounds, antioxidant and antibacterial activities of *Anthemis praecox* link aerial parts. South African Journal of Botany, 131: 200-205.

[5] Benchikha, N., Chelalba, I., Debbeche, H., Messaoudi, M., Begaa, S., Larkem, I., Amara, D.G., Rebiai, A., Simal-Gandara, J., Sawicka, B., (2022): *Lobularia libyca*: Phytochemical profiling, antioxidant and antimicrobial activity using *in vitro* and in silico studies. Molecules, 27: 3744.

[6] Benhammou, N., Bekkara, F.A., Panovska, T.K., (2009): Antioxidant activity of methanolic extracts and some bioactive compounds of *Atriplex halimus*. Comptes Rendus Chimie, 12(12): 1259-1266.

[7] Brito, A., Areche, C., Sepúlveda, B., Kennelly, E.J., Simirgiotis, M.J., (2014): Anthocyanin characterization, total phenolic quantification and antioxidant features of some Chilean edible berry extracts. Molecules, 19(8): 10936-10955.

[8] Campos, F.R., Bressan, J., Jasinski, V.G., Zuccolotto, T., Da Silva, L.E., Cerqueira, L.B., (2016): Baccharis (Asteraceae): Chemical constituents and biological activities. Chemistry & Biodiversity, 13(1): 1-17.

[9] Chouikh, A., Chemsa, A.E., Aounallah, C., Aounallah, I., Fatma, A., (2020): Phytochemical study, nutritive value, antioxidant and anti-inflammatory activities of phenolic extracts from desert plant *Calligonum comosum* l'hér. Algerian Journal of Biosceinces, 1(2): 68-75.

[10] Chung, K.T., Wei, C.I., Johnson, M.G., (1998): Are tannins a double-edged sword in biology and health? Trends in Food Science & Technology, 9(4): 168-175.

[11] David, S.S., (1998): Plant secondary metabolism. Kluwier Acad. Publ., Massachusetts, pp. 354-355.
[12] Duh, P.D., (1998): Antioxidant activity of burdock (*Arctium lappa* linne): Its scavenging effect on free-radical and active oxygen. Journal of the American Oil Chemists' Society, 75(4): 455-461.
[13] Duraipandiyan, V., Ayyanar, M., Ignacimuthu, S., (2006): Antimicrobial activity of some ethnomedicinal plants used by paliyar tribe from Tamil Nadu, India. BMC Complementary and Alternative Medicine, 6(1): 1-7.
[14] El-Waziry, A.M., (2007): Nutritive value assessment of ensiling or mixing Acacia and Atriplex using *in vitro* gas production technique. Research Journal of Agriculture and Biological Sciences, 3(6): 605-614.
[15] Elzaawely, A., Tawata, S., (2012): Antioxidant activity of phenolic rich fraction obtained from *Convolvulus arvensis* L. leaves grown in Egypt. Asian Journal of Crop Science, 4(1): 32-40.
[16] Gheraissa, N., Chemsa, A.E., Elsharkawy, E.R., Cherrada, N., (2022): Phenolic compound profile, and evaluation of biological properties of *Bassia muricata* (L.) Asch. aerial part. International Journal of Secondary Metabolite, 9(3): 335-347.
[17] Haraguchi, F.K., Pedrosa, M.L., Paula, H.D., Santos, R.C.D., Silva, M.E., (2009): Influência das proteínas do soro sobre enzimas hepáticas, perfil lipídico e formação óssea de ratos hipercolesterolêmicos. Revista de Nutrição, 22: 517-525.
[18] Hossain, M.A., Nagooru, M.R., (2011): Biochemical profiling and total flavonoids contents of leaves crude extract of endemic medicinal plant *Corydyline terminalis* L. Kunth. Pharmacognosy Journal, 3(24): 25-30.
[19] Ibtissam, L., Boutlelis, D.A., Boudebia, O., (2023): Green synthesis of silver nanoparticles using *Helianthemum lippii* extracts (hl-nps): Characterization, antioxidant and antibacterial activities, and study of interaction with DNA. Journal of Organometallic Chemistry, 986: 122619.
[20] Islam, E., Islam, R., Rahman, A.A., Alam, A., Khondkar, P., Rashid, M., Parvin, S., (2013): Estimation of total phenol and *in vitro* antioxidant activity of *Albizia procera* leaves. BMC Research Notes, 6(1): 1-7.
[21] Ismail, A., Ktari, L., Ahmed, M., Bolhuis, H., Boudabbous, A., Stal, L.J., Cretoiu, M.S., El Bour, M., (2016): Antimicrobial activities of bacteria associated with the brown alga *Padina pavonica*. Frontiers in Microbiology, 7: 1072.
[22] Ksouri, R., Megdiche, W., Falleh, H., Trabelsi, N., Boulaaba, M., Smaoui, A., Abdelly, C., (2008): Influence of biological, environmental and technical factors on phenolic content and antioxidant activities of tunisian halophytes. Comptes Rendus Biologies, 331(11): 865-873.
[23] Laib, I., Djahra, A.B., (2022): Phytochemical investigation of *Helianthemum lippii* L. aerial dum. Cours part and evaluation for its antioxidant activities. International Journal of Secondary Metabolite, 9(2): 229-237.
[24] Li, H.-B., Cheng, K.-W., Wong, C.-C., Fan, K.-W., Chen, F., Jiang, Y., (2007): Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. Food Chemistry, 3: 771-776.
[25] Liu, J., Wang, C., Wang, Z., Zhang, C., Lu, S., Liu, J., (2011): The antioxidant and free-radical scavenging activities of extract and fractions from corn silk (*Zea mays* L.) and related flavone glycosides. Food Chemistry, 126(1): 261-269.
[26] Longo, L., Scardino, A., Vasapollo, G., (2007): Identification and quantification of anthocyanins in the berries of *Pistacia lentiscus* L., *Phillyrea latifolia* L. and *Rubia peregrina* L. Innovative Food Science & Emerging Technologies, 8(3): 360-364.
[27] Manach, C., Scalbert, A., Morand, C., Rémésy, C., Jiménez, L., (2004): Polyphenols: Food sources and bioavailability. The American Journal of Clinical Nutrition, 79(5): 727-747.
[28] Mansouri, A., Embarek, G., Kokkalou, E., Kefalas, P., (2005): Phenolic profile and antioxidant activity of the algerian ripe date palm fruit (*Phoenix dactylifera*). Food Chemistry, 89(3): 411-420.
[29] Mogana, R., Adhikari, A., Tzar, M., Ramliza, R., Wiart, C., (2020): Antibacterial activities of the extracts, fractions and isolated compounds from *Canarium patentinervium* Miq. Against bacterial clinical isolates. BMC Complementary Medicine and Therapies, 20(1): 1-11.
[30] Murugan, R., Parimelazhagan, T., (2014): Comparative evaluation of different extraction methods for antioxidant and anti-inflammatory properties from *Osbeckia parvifolia* arn. – an *in vitro* approach. Journal of King Saud University-Science, 26(4): 267-275.
[31] Njateng, G.S.S., Du, Z., Gatsing, D., Mouokeu, R.S., Liu, Y., Zang, H.-X., Gu, J., Luo, X., Kuiate, J.-R., (2017): Antibacterial and antioxidant properties of crude extract, fractions and compounds from the stem bark of *Polyscias fulva* hiern (Araliaceae). BMC Complementary and Alternative Medicine, 17(1): 1-8.
[32] Oraiza, M., (1986): Studies on product of browning reaction prepared from glucosamine. Japanese Journal Nutrition, 44: 307-315.
[33] Özkan, G., Sagdiç, O., Baydar, N., Baydar, H., (2004): Note: Antioxidant and antibacterial activities of *Rosa damascena* flower extracts. Food Science and Technology International, 10(4): 277-281.
[34] Pandey, A., Negi, P.S., (2018): Phytochemical composition, *in vitro* antioxidant activity and antibacterial mechanisms of *Neolamarckia cadamba* fruits extracts. Natural Product Research, 32(10): 1189-1192.
[35] Poudel, M., Rajbhandari, M., (2020): Phytochemical analysis of *Ampelopteris prolifera* (retzius) copeland. Nepal Journal of Science and Technology, 19(1): 78-88.
[36] Verma, D.K., Hasan, S.H., Banik, R.M., (2016): Photo-catalyzed and phyto-mediated rapid green synthesis of silver nanoparticles using herbal extract of *Salvinia molesta* and its antimicrobial efficacy. Journal of Photochemistry and Photobiology B: Biology, 155: 51-59.
[37] Zahnit, W., Smara, O., Bechki, L., Bensouici, C., Messaoudi, M., Benchikha, N., Larkem, I., Awuchi, C.G., Sawicka, B., Simal-Gandara, J., (2022): Phytochemical profiling, mineral elements, and biological activities of *Artemisia campestris* L. grown in Algeria. Horticulturae, 8: 914.
[38] Zhang, S.Y., Zheng, C.G., Yan, X.Y., Tian, W.X., (2008): Low concentration of condensed tannins from catechu significantly inhibits fatty acid synthase and growth of mcf-7 cells. Biochemical and Biophysical Research Communications, 371(4): 654-658.

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