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Research Article

Phytochemical investigation of H*elianthemum lippii* l. aerial Dum.Cours part and evaluation for its antioxidant activities

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Abstract: Objective: The aim of this study is to determine the phytochemical contents and the antioxidant activity of Helianthemum lippii (L.) Dum.Cours. crude extract.
Methods: For preliminary phytochemical analysis, standard procedures were applied, while identification and quantification of individual phenolic compounds were performed by HPLC analysis. The Folin–Ciocalteu method was used to evaluate the total phenolic acid content of the plant extracts, The total flavonoid content was determined using the aluminum chloride colorimetric assay. The FTIR spectroscopy method was used to examine the chemical makeup of the organic extracts. The antioxidant activities were assessed using the 1,1-diphenyl-2-picrylhydrazyl and reducing power assays.
Results: Chemical analysis revealed the presence of numerous secondary metabolites, such as polyphenols, flavonoids, tannins, saponins, anthocyanins, cardiac glycosides, leuco anthocyanins steroids, terpenoids, alkaloids, and mucilage. For the HPLC analysis, we obtained 65 peaks and we identified 6 major elements of bioactive compounds. The total concentration of polyphenols and flavonoids was varied respectively 183.12±2.84 mg gallic acid eq/g dry wt and 72.00±1.03 mg quercetin eq/g dry wt /mg. The general concentration of condensed tannin and hydrolyzable tannin compounds were expressed in terms of catechin equivalent (5.88±1.58 mg Ca eq/g dry extract) and gallic acid (2.818±0.138 mgTA eq/g dry wt) respectively. FTIR spectroscopy investigation indicated several characteristic peak values in the extract with diverse functional groups such as amide, alcohol, and phenol groups. Concerning the antioxidant activity, we found that this extract has high inhibitory percentages equivalent to IC50 3.085±0.001 for DPPH and 1.724±0.021 for reduction power (µg/mL).
Conclusion: Our study proved that the aqueous extract of the H lippii is very rich in secondary metabolites; in addition, it has a tremendous anti-oxidant capacity, which leads us forward to introduce it for medical use.

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1. INTRODUCTION

Medicinal plants and their therapeutic properties are widely used around the world for a variety of disorders (Ahmad *et al*., 2014; Mohammed, Kına, *et al*., 2021; Sevindik *et al*., 2017). Herbal medicine is one of the most important branches of traditional medicine all over the world; it is made up of phytochemicals that help people to restore and improve physiological balance. The

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knowledge of these medicinal properties has been passed down through generations (Chaouche *et al*., 2020). Secondary metabolites form a series of active substances for the treatment of some human diseases (Ali-Rachedi *et al*., 2018; Uysal *et al*., 2021).

Phytochemicals with anticarcinogenic and antibacterial capabilities are currently gaining popularity 1-3 in a variety of fields, including agriculture, food, and preventive and therapeutic medicine. Plants biosynthesize a diverse spectrum of phytochemical compounds, which are primarily employed in the discovery and development of novel medications (chemotherapeutics). The food sector, on the other hand, is getting more interested in plants that can be eaten and that contain health-promoting chemicals, notably those high in phenols, which have antioxidant characteristics (Mubashar Sabir *et al*., 2015). Polyphenols compounds possess a tremendous capacity for natural antioxidants, which have the potential to scavenge free radicals and protect cells from oxidative damage (Rotta *et al*., 2017). As a result, Polyphenols or plant extracts high in phenols could be used as active biocapacity elements diets to aid in the prevention of oxidative stress-related disorders.

As a result, researchers exploring food additives, nutraceuticals, and diet supplements are looking for new plant sources of antioxidants inclusive edible and non-edible plants are high in phenols (Ćetković *et al*., 2004; Rotta *et al*., 2017). Plant phenols play an important role act as free radical scavengers, reducing Fe+3/Fe+2, inhibiting peroxidation, and having an anti-inflammatory effect through reducing cytokine expression has been linked to a lower risk of cardiovascular and chronic disease, as well as certain cancer. All of this has been proven in previous studies (Farahpour, 2014; Girola *et al*., 2015).

This study was designed specifically for the purpose of conducting a phytochemical screening of *Helianthemum lippii*, and to determine the total phenolic, flavonoid, and total tannins content, and FT-IR spectrum is used to validate the presence of organic functional groups. In order to verify the antioxidant capacity of the extract, we used methods DPPH and reduction power.

2. MATERIAL and METHODS

2.1. Preparation of Plant Material

The aerial parts of the *H. lippii* were collected during the flowering period in March 2020 in Southeastern Algeria (exactly in the region of Elhamadin- province of El -Oued), the plant material was identified by Professor Atef CHOUIKH (Faculty of Natural Science and Life, El Oued University). To eliminate dust and other foreign particles, the aerial section was cleaned under running tap water. Then, it was dried, ground, and stored for future use.

2.2. Chemicals

This investigation employed only analytical-grade compounds, Folin-Ciocalteu reagent, Gallic acid, Quercetin, Tannic acid and Ascorbic acid (Vitamin C), Sodium carbonat, Aluminium chlorid, ferric chloride, Potassium ferricynide, trichloroacetic acid, vanillin, hydrochloric acid, 2,2_-diphenyl-1-picryl-hydrazyl (DPPH), and all other reagents of phytochimical analytical Chemicals were received from Sigma Aldrich Co (St. Louis, MO, USA).

2.3. Preparation of Aqueous Extract

H. lippii aerial parts powder was steeped for roughly 10 g in 100 mL distilled water and left at room temperature for 24 hours in the dark. After that, it was filtered with filter paper. After extraction, the sample was dried extensively at 40°C. The extract was weighed and refrigerated at 4°C for further analysis in the future (Murugan & Parimelazhagan, 2014).

2.4. Phytochemical Screening

The extract was tested to detect the different compounds such as; phenols, cardiac glycosides, tannins (catechetical tannins. and gallic tannins), alkaloids, steroids, saponins, flavonoids, triterpenoids, leuco anthocyanins, anthocyanins, and of mucilages employing usual standard procedures for phytochemical analysis (screening)(Matos, 1997). The presence of phytochemicals is indicated by a (+) while the absence of phytochemicals is indicated by a (-).

2.5. Analyze Qualitative by HPLC

Using scanning equipment and high-performance liquid chromatography, the active components were discovered (HPLC). For the investigation of phenolic chemicals in crude extract, we utilized HPLC with UV-Vis type Shimadzu LC20 AL equipped with the universal injector (Hamilton 25l), an analytical column was a Shim-pack VP-ODSC18 (4,6mm, 250mm, 5m), and UV-VIS detector SPD 20A type (Shimadzu). The reverse-phase chromatography studies were conducted using non-polar aliphatic residues, and the mobile phase was comprised of gradient elution of a combination of acetonitrile and acetic acid (0.1%). The injection volume was 0, 45µL and the flow rate was 1mL/min. The monitoring wavelength was 268nm, and the sample and standard injection volume were 20µL. The retention duration and UV absorbance of various compounds were compared to those of the standards to identify them.

2.6. Quantification of Phytochemical Compounds

2.6.1. Estimation of total phenolics

Total phenolic content was calculated using the Folin–Ciocalteu method (Li *et al*., 2007). (0.2 mL) of the sample of the aqueous extract of *Helianthemum lippii* and 1mL of Folin–Ciocalteu reagent were added diluted of 1:10 and added 800 µL of saturated sodium carbonate (7,5%) after 4 min. The absorbance was measured at 765 nm after 2 hours of incubation at room temperature. The tests were repeated three times to confirm that the results were consistent. The total phenolic content of the extract was measured in milligrams of gallic acid equivalent per gram of extract.

2.6.2. Estimation of total flavonoids

1 mL of the $AlCl_3$ solution is mixed with 1 mL of the sample, and on other hand with 1 mL of the standard. The absorbance was measured at max = 430 nanometers after 10 minutes against the prepared reagent blank (Ahn *et al*., 2007). The results were expressed in milligrams of Quercetine per gram of extract.

2.6.3. Estimation of total hydrolysable tannins

The Folin-Ciocalteu colorimetric method was used to calculate the total hydrolysable tannin concentration. A 10 mL test tube containing 8.4 mL distilled water, 0.5 mL Folin-Ciocalteu reagent, and 0.1 mL 7 percent Na_2CO_3 solution was filled with an aliquot of 1 mL tannic acid in distilled water of each concentration. Absorbance was measured at 700 nm against a blank after incubation for 30 minutes. All of the tests were repeated three times. Tannic acid equivalents (TAE) per gram of dry extract (mg/g) were used to assess the total tannin content of the extract (Poudel & Rajbhandari, 2020).

2.6.4. Estimation of condensed tannins

The extract's tannin concentration was determined using spectrophotometry (Broadhurst & Jones, 1978).The calibration curve was created using catechin. 0.5 mL sample pipetted into an aluminum foil tube, mixed with 3.0 mL vanillin reagent (freshly made 4 percent w/v vanillin in methanol), and 1.5 mL concentrated hydrochloric acid, and thoroughly mixed. The absorbance was measured against water at 500 nm after 15 minutes of reaction at 20-2°C.

2.7. Characterization of Heluintium liippii L. Aqueous Extract by Fourier Transforms Infrared (FTIR)

Fourier transform infrared spectroscopy analysis: The FT-IR spectrum was produced using a spectrophotometer (Shimadzu-00463 model) with a resolution of 4 cm^{-1} and 64 coadded scans in the spectral region of 4000-400 cm^{-1}. The surface chemistry and organic functional group are confirmed by the FT-IR spectrum.

2.8. Antioxidant Activity

2.8.1. DPPH free-radical scavenging activity

2.4 mg of DPPH• is dissolved in 100 mL of methanol to make the 1,1-diphenyl-2-picrylhydrazyl solution. 1mL of each phenolic extract (or ascorbic acid as a control) is added to 1 mL of the DPPH• solution previously produced. The reaction mixture is quickly agitated and then maintained at room temperature for 30 minutes in the dark to complete the reaction. The reaction medium's absorbance is measured at 517 nm (Mansouri *et al*., 2005).

2.8.2. Reducing power assay

The reducing power of the extract was determined using Oyaizu's methods (Oraiza, 1986). In distilled water, the extract was mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and 1 percent potassium ferricyanide water solution (2.5 mL, K3 [Fe (CN) 6]) at different concentrations (mg/mL). The mixture was incubated at 50°C for 20 minutes; the mixture was centrifuged for 10 minutes at 3000 rpm after Aliquots of trichloracetic acid (2.5mL, 10% aqueous solution) were added. A freshly produced FeCl3 (0.5mL, 0.1%) solution was combined with the supernatant (2.5mL) and purified water (2.5mL) At 700 nm, the absorbance was measured. A positive control was employed, which was ascorbic acid.

2.9. Statistical Analysis

All of the experiments were carried out in threes. The data were analyzed in Microsoft Excel and are presented as mean ± standard deviation (n = 3). Graphpad Prism 7 for Windows was used to calculate the IC_{50} and EC_{50} values.

3. RESULTS

3.1. Phytochemical Screening

The results of the phytochemical tests reveal the richness of the extract by various active constituents. The main active constituents of *H.lippii* are polyphenols, flavonoids, tannins, saponines, anthocyanins, cardiac glycosides, leuco anthocyanins steroids, terpenoids, alkaloids, and mucilage (Table 1).

Table 1. Phytochemical screening of *H.lippii* aqueous extract.

Phytochemical compounds		*Helianthemum lippii* (aqueous extract)
Polyphenols		(+)
Alkaloids	Mayer	(-)
	Wagner	(+)
Tannins	Catechin	(+)
	Gallic	(+)
Flavonoids		(+)
Saponines		(+)
Anthocyanins		(+)
leuco anthocyanins		(+)
Cardiac glycosides		(+)
Steroids and terpenoids		(+)
Mucilage		(+)

(−) Absence of phytochemicals compounds.
(+) Presence of phytochemicals compounds.

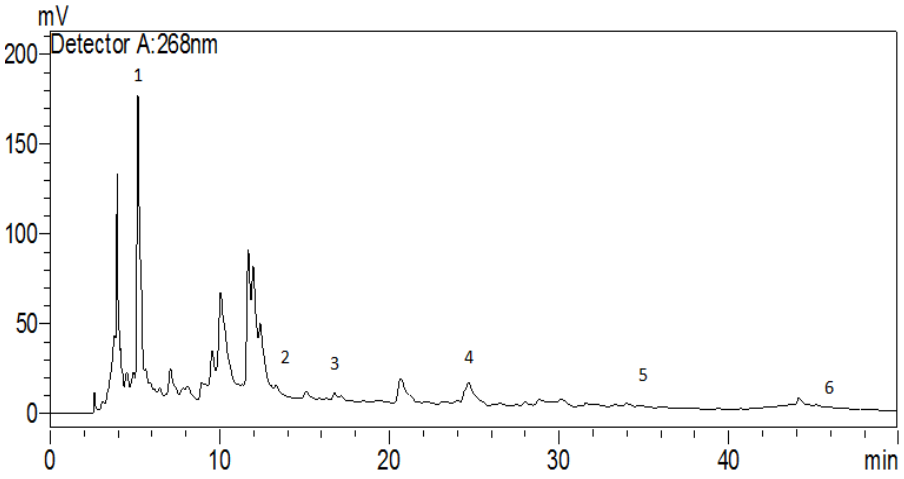
3.2. Analysis Qualitative by HPLC

The results of the separation of the aqueous extract by the use of HPLC are illustrated in the Helianthemum lippii chromatogram (Figure 1). Where we have identified six phenolic compounds out of 65 peaks. The analysis revealed that gallic acid (9495.115305 µg/g) was the most abundant element with a high amount of chlorogenic acid (7107.242096 µg/g), a moderate quantity of quercetin (1118. 647803 µg/g), a little amount of Naringin (738.1908251 µg/g), p-coumaric acid (663.776139 µg/g), and caffiec acid (444.8195465 µg/g) were detected in *H.lippii*. The results are represented in Table 2.

Table 2. Retention time and the concentration of phenolic compounds identified in *Helianthemum lippii* aqueous extract.

Phenolic compound	Retention Time (min)	Concentrction (µg/g extract)
Gallic Acid	5.29	9495.115305
Chlorogenic Acid	13.392	7107.242096
Caffiec Acid	16.277	444.8195465
p-Coumaric Acid	23.817	663.776139
Naringin	34.788	738.1908251
Quercetin	45.047	1118.647803

Figure 1. HPLC chromatogram's of the extract of *Helianthemum lippii:* 1: Gallic Acid; 2 Chlorogenic Acid; 3: Caffiec Acid; 4: p-Coumaric Acid; 5: Naringin; 6: Quercetin.



3.3. Quantification of Phytochemical Compounds

The total phenol and total flavonoid content were determined in comparison with a standard which is equivalent (mg GA eq/g dry extract) gallic acid and quercetin equivalent (mg Q eq/g dry extract) respectively, using the following equations based on the calibration curve: y = 0.0104x+0.0819, R^2= 0.9925 for total phenol and y =0.0096x+0.0521, R^2= 0.994 for total flavonoid.

Whereas, total condensed tannin and total hydrolyzable tannin compounds were calculated by Catechin equivalent (mg Ca eq/g dry extract) and gallic acid (mgTA eq/gdry wt) respectively, using the following equations based calibration curve: y = 0.0005x-0.0052, R^2 = 0.9885 for condensed tannin and y = 0.0482x – 0.0522, R^2 = 0.9377 for total hydrolysable tannin compounds. The results were represented in Table 3.

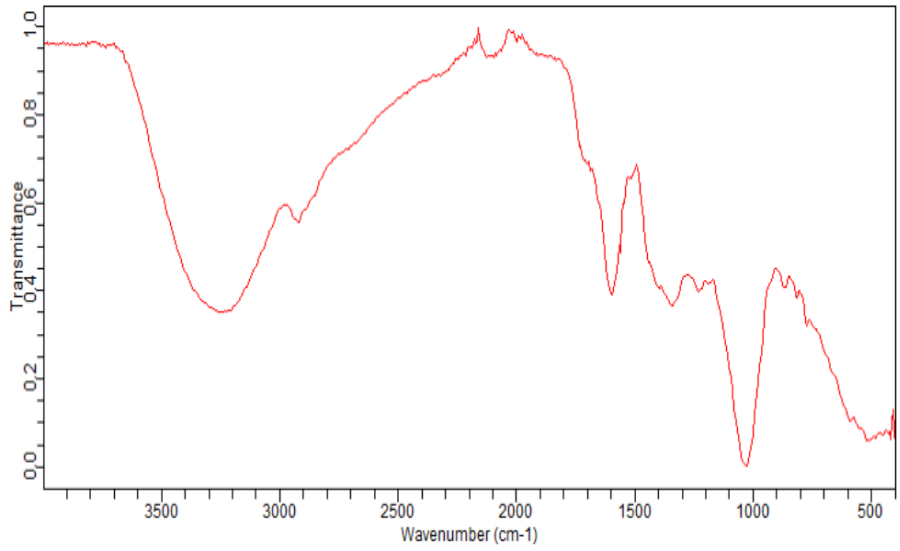
Table 3. Quantitative analysis of total phenolic, flavonoid and total tannins compounds of *Helianthemum lippii* aqueous extract.

Extract	Total phenolic (mgGAE/g extract)	Total flavonoid (mgQE/mg extract)	Condensed tannin (mg Ca eq/g dry wt)	Total hydrolysable tannin (mgTA eq/gdry wt)
H.lippii	183.12±2.84	72.00±1.03	5.88±1.58	2.818±0.138

3.4. Characterization of *Helianthemum lippii* Aqueous Extract by Fourier Transforms Infrared (FTIR)

Based on the infrared radiation region's highest value, the FTIR spectrum was utilized to determine the active component's functional group. The most prominent FTIR peak values and functional groups are shown in the results (Figure 2). The presence of a hydroxyl group at 3201.07 cm^{-1} was discovered in the profile of FTIR spectra of aqueous extract of *H. lippii.* The stretching vibration of C=C is responsible for the steep peak at 1599.49 cm^{-1}. Finally, our plant's C-O function produces a sharp band at 1078 cm^{-1}.

Figure 2. Infrared spectrum of *Helianthemum lippii* aqueous extract.



3.5. Antioxidant Activity

We measured the relative antioxidant ability of our sample by using DPPH and Reducing power assays. The extract of *H.lippii* has a very high antioxidant capacity through the values of IC50 and EC50 values for DPPH and reduction power activities which were respectively 3.085±0.001µg/mL and 1.724±0.021µg/mL Table 4.

Table 4. Antioxidant activities of aerial part of *Helianthemum lippii.*

Extracts/standards	DPPH· (IC_{50}: µg/mL)	Reduction Power (EC_{50}: µg/mL)
H.lippii	3.085±0.001	1.724±0.021
Ascorbic acid	1.219±0.005	0.225±0.032

4. DISCUSSION

Medicinal plants contain active ingredients, according to extensive research. This bioactivity is caused by a variety of antioxidant-rich phytochemicals found in medicinal plants. The phytochemical content and antioxidant activity of crude extract from *H. lippii* species are studied in this paper. An examination of the Phytochemical indicated the presence of phenols,

cardiac glycosides, tannins (catechetical tannins and gallic tannins), flavonoids, steroids, triterpenoids, alkaloids, saponins, anthocyanins, leuco anthocyanins, and mucilages. These substances are biologically active and have the potential to improve *H. lippii*'s antioxidant capabilities. This plant extract has antioxidant properties due to because of the existence of terpenoids and flavonoids.

Gallic acid was found to be the most abundant phenolic ingredient in the *H.lippii* extract, which was quantified by HPLC, followed by chlorogenic acid, caffiec acid, p-coumaric acid, naringin, and quercetin. These compounds were also demonstrated to have the ability to scavenge free radicals (Rotta *et al*., 2017).

The study has shown that the *H. lippii* contains a high quantity of phenolic and flavonoid compounds and a slight amount of tannins. The antioxidant activity of this plant is due to the presence of phenolic and flavonoid content imoprtant. Many investigations have discovered that phenolic compounds also have biological effects, as for example anti-inflammatory properties, in addition to antioxidant, antimicrobial,antiulcerogenic, and even cancer-fighting capabilities (Alarcón *et al*., 2008; Carro *et al*., 2016; Głód *et al*., 2015 ;Granato *et al*., 2013; Kına *et al*., 2021; Mohammed, *et al*., 2021; Pehlivan *et al*., 2021; Valdés, 2015).

There is a lot of interest these days in measuring the ability of plant or food extracts to function as antioxidants to see if they have any therapeutic qualities. One of the methods utilized to assess antioxidant activity was the DPPH assay, which is one among the most strong, simple, and reliable in vitro methods for sequestering free radicals. The total phenolic content of plant extracts is frequently linked to antioxidant activity (Ghani *et al*., 2019). They can react with hydroxyl, superoxide anion, and lipid peroxyl radicals, which are all active oxygen radicals to early detection and prevention of lipid peroxidation. The hydroxyl groups in phenolic acids are responsible for their high scavenging activity (Bruck de Souza *et al*., 2020; Cai *et al*., 2003; Miliauskas *et al*., 2004). Because a compound's reducing power is connected to its ability to move electrons, it can be used to predict its prospective antioxidant activity. This can be linked to the polyphenols in the extract's propensity to donate electrons (Aluko, 2017).

Fourier Transform Infrared Spectroscopy (FTIR) is a non-destructive characterization technique that uses infrared light to irradiate the sample, and the absorbed energy gives a specific spectrum depending on the chemical composition of the sample. The absorption signals for diverse wavenumber ranges in the FTIR spectrum produced in this investigation revealed varied typical peak values with various functional groups in the extract such as alcohol, amide, and amino acids compounds.

5. CONCLUSION

According to this study, the crude extract of H.lippii is extraordinarily rich in phytochemicals and antioxidants of high medicinal importance, verifying and validating its use in the treatment of a variety of human illnesses. Consequently, the extract’s chemical composition, mainly phenolic compounds, may have a synergistic impact. As a result, our findings pave the way for additional detailed examinations of the extract’s mechanisms of action at a higher level of cellular and organism complexity for its medicinal evaluation.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship contribution statement

Ibtissam Laib: Investigation, Visualization, Conceptualization Ideas; formulation or evolution of overarching research goals and aims, design of methodology, and Writing the manuscript. **Ali Boutlelis Djahra**: Supervision, Methodology, and Validation.

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