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PHYTOCHEMICAL STUDY AND EVALUATION OF THE ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF THE METHANOLIC EXTRACT OF *URTICA DIOICA*

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ABSTRACT

The plant world is an excellent source of active ingredients, which gives it significant antibacterial activity, often sought after in alternative medicine and the food industry for food preservation. Our work focuses on the study of essential oil extraction and study of the biological activities of a medicinal plant, *Urtica dioica* (Urticaceae). The phytochemical screening study of the aerial part of the plant confirmed the presence of alkaloids, tannins, saponosides, flavonoids. And the absence of coumarins and volatile oil. The results obtained show that the water content of the studied plants was observed with 13.74%. The extraction yield in methanol-water mixture is low compared to that of water which is 20.8%. The results obtained for total polyphenol contents (mg EAG/g RS) in the two extracts are of the order of: 26.02(EM); 19.26 (AQE). The flavonoid content is around 86.43 (EAq); 10.26 (EM). Condensed tannin content is around 22.29 (EAq); 26.17 (EM).The percentage inhibition of the DPPH radical only reached 05% inhibition with the aqueous extract and even for the methanolic extract the activity did not exceed 19% inhibition of the DPPH radical.

The antimicrobial activity was determined on four bacterial strains, according to the disc diffusion method, the four strains studied show sensitivity towards plant extracts, the results indicate that the two extracts have a good antimicrobial activity against the strains tested (*Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Proteus mirabilis*) and which act differently on the strains tested.

Keywords: Medicinal plant, phytochemical screening, antibacterial activity, *Urtica dioica*. DPPH, Percentage Inhibition

Introduction

Medicinal plants have been used since antiquity as remedies for the treatment of various diseases because they contain components rich in therapeutic principles (Khalid *et al*., 2019; Miara *et al*., 2013; Boukef, 1986; Boughrara, 2016; Battandier, 1884; Daira *et al.*, 2016). These active ingredients can be involved in the development of many drugs acting on various diseases, such as cardiovascular and pulmonary diseases, certain types of cancer, immune and inflammatory diseases, etc.

According to the World Health Organization (WHO), almost 80% of populations depend on traditional medicine. Most plants, however, are used empirically and without scientific validation of efficacy and toxicity (Abad *et al*., 2000; Al Dissi *et al*., 2001).

The use of medicinal plants can provide direct answers to certain health problems, but before being able to recommend their use, it is necessary to scientifically evaluate the pharmacological activity of the medicinal plant chosen. In addition, it is imperative to also verify the absence of toxicity of the plants used (Hseini *et al*., 2007).

The use of local medicinal plants in response to health problems can be perceived as an alternative to drugs, particularly in southern countries where these drugs are often expensive, not easily accessible, and sometimes counterfeited. Among the medicinal plants, the most recommended, is the nettle "*Urtica dioica* L.", a wild plant widespread in the Mediterranean basin and considered a weed (Bertrand and Jeanne, 2008; Daoudi *et al*., 2015a; Bhuwan *et al.*, 2014; Aguirre Duran *et al.*, 2022; Gülçin *et al*., 2004).

Through our research work, we have evaluated the phenolic compounds, the antioxidant and antibacterial activities of the aqueous extract and the methanolic extract of *Urtica dioica* L. and its fractions, to promote medicinal and aromatic plants. Spontaneously from the region of El Tarf.

This research work is part of the research axis "Valorisation of natural resources", of the research laboratory "Functional and Evolutionary Ecology", University Chadli Bendjedid -El Tarf.

Materials and Methods

Vegetal Material

The goal of this study is to determine the value of a medicinal plant, *Urtica dioica* L. that grows naturally in the region of Bouhajar, prefecture of El Tarf, North East Algeria.

The plant studied was chosen mainly based on the results of an ethnopharmacological survey, carried out by a doctoral student whose research axis is the "Valorization of

Natural Resources," research laboratory "Functional and Evolutionary Ecology," Chadli Bendjedid University-El Tarf. This survey was conducted among traditional therapists, herbalists, rural women's cooperatives, and the local population in the El Tarf region, where nettle has been reported to be frequently used in the treatment of various pathologies, including stomach aches, rheumatic pain treatment, colds, coughs, and liver failure.

The leaf is the plant part used for phytochemical analyses, and it was harvested in February 2022 in the Bouhadjar, El Tarf, and North East Algeria regions. The drying was carried out at room temperature, away from light and humidity, for two weeks, at the level of the "Functional and Evolutionary Ecology" research laboratory, Chadli Bendjedid University-El Tarf, in order to avoid the degradation of the active ingredients and the development of molds (Catier and Roux, 2007).

After drying, the nettle leaves were crushed using an electric grinder and stored carefully in kraft bags in a dry place.

Phytochemical Screening

The phytochemical screening was carried out on the dried and powdered leaves of *Urtica dioica.* The analyses focused on the detection of seven active compounds of secondary metabolism. The characterization of its chemical groups was carried out according to the methods described by Ronchetti and Russo (1971), Hegnauer (1973), Wagner *et al*. (1984), Bekro *et al.* (2007), Bouquet (1972), Harborne (1998).

The active compounds analyzed are:

- Alkaloids: we adopted the method described by Harborne (1998) and Békro *et al*. (2007), based on revelations by Mayer's reagent.
- Saponosides: an alveolar foam reveals the presence of saponins, calling this the foam test (Crespin, 1992)
- Flavonoids: a colorimetric reaction in a basic medium is a sign of the presence of flavonoids (Harborne, 1998).
- Tannins: a colorimetric reaction in the presence of a few drops of a 1% FeCl3 solution makes it possible to detect the presence of tannins (Hadduchi *et al*., 2016; Békro *et al.,* 2007).
- Coumarins: we adopted the method described by Harborne (1998), Hadduchi *et al*. (2014) and Bouquet (1972).
- Volatile oils: according to Harborne (1998) and Békro *et al*. (2007), a white precipitate is formed in the presence of volatile oils, after the maceration reaction.
- Polyphenols: a colorimetric reaction in the presence of a few drops of 2% ferric chloride (FeCl3) was the sign of the presence of polyphenols (Chaouche *et al*., 2015; Hadduchi *et al*., 2014).

Preparation of Raw Plant Extracts

Extraction by maceration (aqueous extract)

The crude extract is obtained by maceration, which consists of simple contact between the solid support (dried and crushed nettle leaves) and a solvent.

This operation consists of soaking 5 grams of the powdered leaves of *Urtica dioica* in 100 ml of distilled water for 24 hours. The masticate obtained is filtered using Whatman filter paper. The recovered solution is dried in a ventilated oven at 60°C for 24 hours in order to obtain a powder or paste, which is stored at 4°C.

The yield of the extract per maceration is calculated using the following formula:

$$\text{Yield}(\%) - m_0 / m_1 \times 100$$

m_0: Mass in grams of the evaporated crude extract.

m_1: Mass in grams of the initial dry plant matter (5g).

Extraction by Soxhlet (Methanolic Extract)

A solid-liquid extraction, using 20 g of dry powder from the leaves of Urtica dioica, was placed in a "Soxhlet" type cellulose cartridge and heated to 150 °C.

The organic solvent used for the extraction operation is 70% methanol, contained in the glass flask. The principle is to use heat to dissolve the active ingredient found in nettle leaves in an organic solvent. This operation lasts for 5 hours for the realization of 06 cycles, until the total exhaustion of the active ingredient (Luque de Castro and Priego-Capote, 2010; López-Bascón and Luque de Castro, 2020).

The crude methanolic extract is then filtered and then evaporated to dryness under reduced pressure, at a temperature of 45 °C using a BÜCHI R-215 type Rotavapor.

The yield of the extracts obtained by solid-liquid extraction, using the Soxhlet extractor, was calculated using the following formula:

$$\text{Yield}(\%) - m_0 / m_1 \times 100$$

m_0: Mass in grams of the evaporated crude extract.

m_1: Mass in grams of the initial dry plant matter (20g).

Dosage of phytochemicals

Assay of total polyphenols

The determination of total polyphenols is carried out according to the method of Singleton (1999), modified by Dewanto *et al*. (2002), and taken up by Medjeldi *et al*. (2018). To 100 µl of the extracts obtained (methanolic and aqueous), 400 µl of Folin Ciocalteu's reagent (diluted 10 times in ultrapure water) was added. The reaction mixture is stirred and left to stand for 5 minutes. A blue color appears and is intensified and stabilized by adding 500µl of sodium carbonate (Na2CO3) at 7.5%. After vortexing, the mixture is incubated for 1 hour in the dark and at room temperature. A Speccord 200 Plus Analytik Jena double-beam UV-Visible spectrophotometer is used to measure the optical density (OD) at = 725 nm.

The quantification of polyphenols is carried out using a range of gallic acid standards in a final concentration varying from 0.031 to 0.5 mg/mL in an aqueous medium.

The polyphenol contents are expressed in milligrams of gallic acid equivalent per gram of fresh material (mg EAG/g MF) (Ghedadba *et al*., 2015; Kouamé *et al*., 2021; Miliauskas *et al.*, 2004; Medjeldi *et al*., 2018).

Dosage of Flavonoids

The flavonoid assay is carried out according to the method reported by Kumaran (2006) and Medjeldi *et al*., (2018). A 100µl dose of the extracts (methalonic and aqueous) is mixed with 400µl of distilled water and 30µl of

NaNO2 (5%). After 5 min of rest, 60 µl of the freshly prepared AlCl3 solution (10%) was added. The mixture is incubated for 6 minutes. Then, 200µl of NaOH (1M) was added to the reaction mixture, which was adjusted to 1M using distilled water. After incubation for 1 hour in the dark and at room temperature, the absorbance is read at 510 nm. The control tank is filled with the extraction solvent (distilled water and methanol).

A range of the catechin standard at final concentrations ranging from 0 to 60µg/ml is used for the quantification of flavonoids. The flavonoid contents are expressed in milligrams of catechin equivalent per gram of fresh material (mg EC/g MF) (Kouamé *et al*., 2021; Boulanouar and Gherib, 2014).

Dosage of condensed tannins

Condensed tannins are analyzed by the colorimetric method (Medjeldi *et al.,* 2018). A 100µl dose of the suitably diluted methanolic extract is mixed with 600µl of freshly prepared vanillin (4%) and 300µl of concentrated HCl. The solution obtained is homogenized by stirring, then kept at rest for 30 minutes in the dark. Absorbance is measured at 500 nm.

The catechin standard range is prepared at final concentrations varying from 0 to 60 µg/ml. Tannin levels are expressed in milligrams of catechin equivalent per gram of dry matter (mg EC/g MF) (Kouamé *et al.,* 2021).

Evaluation of antioxidant power

- **Scavenging of the free radical DPPH:**

The methodology adopted in our work was described by Iwashima *et al.* (2005); Rigane *et al*. (2011); Medjeldi *et al.* (2018); El-Haci *et al*. (2011), which involves trapping the free radical DPPH

The estimate of the anti-radical activity is determined according to the method described by El-Haci *et al.,* (2011). 975µl of a methanolic solution of DPPH (4%) are added to 25µl of the extract at different concentrations. After vigorous stirring, the mixture is kept in the dark for 1 hour. The absorbance is measured at 517 nm referring to a control without extract. Methanol is used as a control.

The expression of the anti-radical activity is expressed as a percentage of inhibition, calculated according to the following formula:

PI% = [(DO control – DO extract) / DO control] x 100

PI%: percentage inhibition

DO control : Absorbance of control solution (DPPH)

DO extract: Absorbance of the antioxidant solution (extract)

Linear regression of the curve: PI% = f [Cextract] makes it possible to determine the IC50, which corresponds to the concentration of the extract responsible for the 50% inhibition of the DPPH radical. The IC50 is expressed in units of concentration of the extract.

- **Total antioxidant capacity**

This test is based on the reduction of molybdenum (VI) to molybdenum (V) by the plant extract at acid pH. The phosphate/molybdenum (V) complex formed is green in color. The total antioxidant capacity (% TAC) was determined by the phospho molybdenum test Medjeldi *et al.* (2018). Absorbance is measured at 695 nm against a test tube containing 1 ml of reagent solution and 0.1 ml of solvent. Total antioxidant activity was expressed relative to ascorbic acid.

Antibacterial activity

Preparation of the Culture Medium:

The culture medium used in this study is the "Muller-Hinton" medium, prepared as follows: Dissolve 38 g of Muller-Hinton agar in one liter of distilled water. Boil with stirring constantly, until completely dissolved. Autoclave the medium for 15 minutes at 121°C (Harrar, 2012).

Sterilization of Equipment:

Distilled water, culture medium, test tubes used in the preparation of bacterial solutions, and Wattman paper discs (06 mm diameter) wrapped in aluminum foil were sterilized in an autoclave at 121 °C for 15 minutes (Harrar, 2012).

Preparation of Dilutions:

The dilutions of the extracts were prepared according to the method described by Zellagui *et al.* (2012), which consists of dissolving the methanolic extract in dimethyl sulfoxide (2% DMSO) to obtain a stock solution of 10%. The aqueous extract is dissolved in sterile distilled water (Daoudi *et al*., 2015b).

Preparation of Discs:

The discs are made from Wattman #01 paper (6 mm in diameter), using a die cutter with a diameter of 06 mm according to current antibiotic disc measurements. These discs are then impregnated with 15 µl of the extracts to be tested and deposited in the previously prepared and inoculated media. Negative and positive controls were also used, containing discs impregnated with sterile distilled water and antibiotic solutions. After incubation at 37°C for 24 hours, any zones of inhibition formed around the discs will be measured. Each test was repeated three times under the same experimental conditions (Gupta *et al.*, 2010; Daoudi *et al*., 2015b).

The effect of the antimicrobial product on the target is assessed by measuring an inhibition zone and a function of the inhibition diameter. The strain will be classified as susceptible, very susceptible, extremely susceptible, or resistant.

The reading is done by measuring the diameter of the zone of inhibition around each disc using a caliper or a ruler in millimeters (mm).

Non sensible (-) or resistant: diameter < 8mm.

Sensible (+): diameter between 9 to 14 mm.

Very sensible (++): diameter between 15 to 19 mm.

Extremely sensible (+++): diameter > 20 mm.

Preparation of the Strains Tested

Bacterial strains:

The microbial strains used in the biological tests are pathogenic, obtained from the microbiology laboratory, Hospital El Hadi Benjedid El-Taref, Algeria. They have been isolated clinically and correspond to the species mentioned below, whose sensitivity to antibiotics is already known by the antibiogram test according to the recommendations of the

antibiogram committee of the French Society of Microbiology (Bonnet *et al.,* 2010; Gülçin *et al.,* 2004):

Gram(-) bacteria: - *Streptococcus aureus,*

- *Klebsiella Pneumoniae,*

- *Escherichia coli,*

Gram(+) bacteria: - *Portus mirabilis*.

Results and Discussion

Phytochemical screening results (Phytochemical tests)

Phytochemical tests were carried out on the dried and crushed leaves of *Urtica dioica*, using specific revelation reagents. The phytochemical screening made it possible to highlight the presence of secondary metabolites in the plant tissues of our plant. The results of these tests are shown in table 01:

Table 01: Results of the phytochemical screening of the *Urtica dioica* plant

Compounds	Abundance
Saponosides	+
Alkaloids	+
Coumarins	-
volatile oil	-
Flavonoids	+++
Tannins	+++
Polyphenols	+++

These phytochemical tests indicate the presence in the *Urtica dioica* plant of chemical compounds of secondary metabolism, namely: flavonoids, saponosides, tannins, polyphenols, and alkaloids, with a total absence of coumarins and volatile oil.

The phytochemical results of neighboring species of the same genus *Urtica* show the presence of flavonoids and tannins but not alkaloids. These are *Urtica urens, Urtica piluliferae,* and *Urtica membranacea* (Daoudi *et al*., 2015a; Farag *et al*., 2013).

III-2- Yields of extractions of crude compounds:

The yield of aqueous and methanolic extractions carried out on the dry powder of Nettle leaves is shown in Table 4. This yield was calculated on the basis of the ratio between the weight of the extract and that of the initial plant material.

Table 02 : Yields of aqueous and methanolic extracts of *Urtica dioica*.

Vegetal Material	Extract	yield
Urtica dioica	Aqueous (EA)	20.6
	70% Methanol (EM)	08

The results obtained show that the yield of the extraction using methanol is lower than that with water. It should be noted that the yield is influenced by the degree of polarity of the solvent in relation to the compounds to be extracted, on the one hand. On the other hand, the yield is also influenced by the geographical origin of the plant, the phenological stage, as well as environmental factors such as temperature, humidity, and the nature of the soil (Bruneton, 1999).

According to Toubal *et al.* (2019), the extraction yield obtained is 1.62±1.25% for the aqueous extract and 4.8±0.18% for the alkaloids, from the whole plant of *Urtica dioica* L., harvested in the city of Dellys (Algeria). Bensella (2015) obtained a polyphenol yield from the roots of stinging nettle harvested in Dellys that was significantly higher, i.e., a value of 8.34%.

A study by Jakubczyk *et al.* (2015) on *Urtica dioica*, harvested in Poland, gave a yield of methanolic extract from the aerial part of 17.45%.

However, Krajewska and Mietlińska (2022) found an aqueous extract yield of more than 50% from the leaf powder.

These yields, which are clearly higher than those obtained in our results, are mainly due to the extraction techniques, a soxhlet apparatus in our case, which requires a high temperature for several hours, thus degrading sensitive active ingredients such as polyphenols (Taylor, 2009; Joshi *et al*., 2014; Chrubasik *et al*., 2007).

This observation is in accordance with certain studies which show that the yield rates of aqueous extracts of polyphenols are higher than those of extracts made from organic solvents (Andersen and Markham, 2006).

Total polyphenol content:

The total polyphenol content is determined from the linear regression equation of the gallic acid calibration curve: Y = 2.1034 X, with a regression coefficient from the straight line (R^2 = 0.9839) close to 1, thus proving the reliability of this curve in the determination of total phenols (Fig. 04).

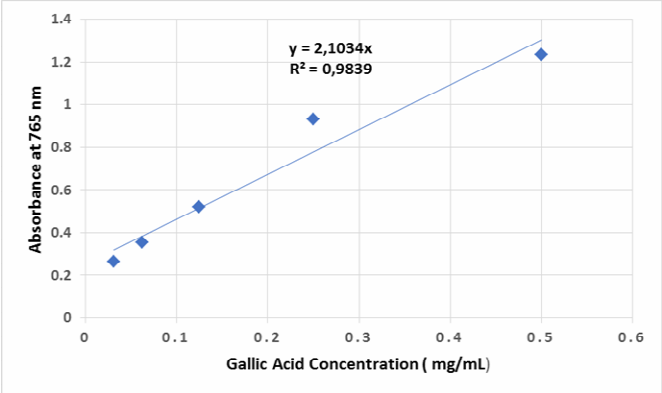

Fig. 1: Gallic Acid Calibration Curve

The result is expressed in milligrams of gallic acid equivalent per dry residue (mg GAE/g RS).

The contents of total polyphenols (mg GAE/g RS) in the two methalonic and aqueous extracts are respectively 26.02 (ME) and 19.26 (AQE).

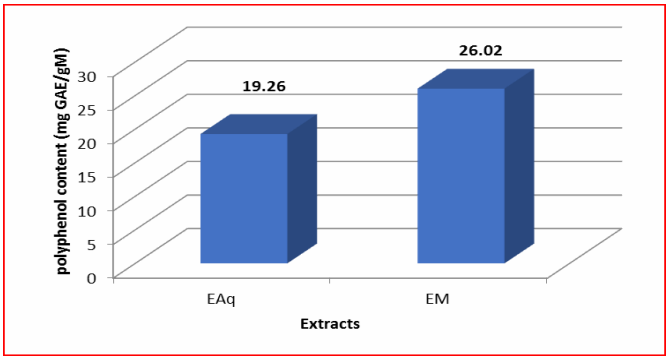

Fig. 2 : The content of total phenols in the two extracts of the leaves of *Urtica dioica* (Aqueous and methalonic)

In the light of these results, we found that the methanolic extract of the *Urtica dioica* plant is richer in total polyphenols than that of the aqueous extract, although the yield is greater for the aqueous extract.

When we compare our findings to those of Asgarpanah and Mohajerani (2012) on *Urtica dioica* leaves from North West Iran, we find that the contents are comparable, with concentrations of 32 ± 0.148 mg GAE per gram of methanoic extract.

Indeed, the polyphenolic content varies qualitatively and quantitatively from one plant to another. This can be attributed to several factors:

- Climatic and environmental factors, such as geographical location, drought, attacks, diseases, and so on (Chrubasik *et al*., 1997; Collier and Chesher, 1956).
- The genetic heritage, the harvest period, and the stage of development of the plant
- The extraction method and the quantification method can also influence the estimation of the content of total polphenols (Aguirre Duran *et al*., 2022).

Total flavonoid content:

The flavonoid content is determined from the linear regression equation of the catechin calibration curve: Y = 0.0115 X, with a straight line regression coefficient (R^2 = 0.9967) close to 1, thus proving the reliability of this curve in the determination of flavonoids (Fig. 03).

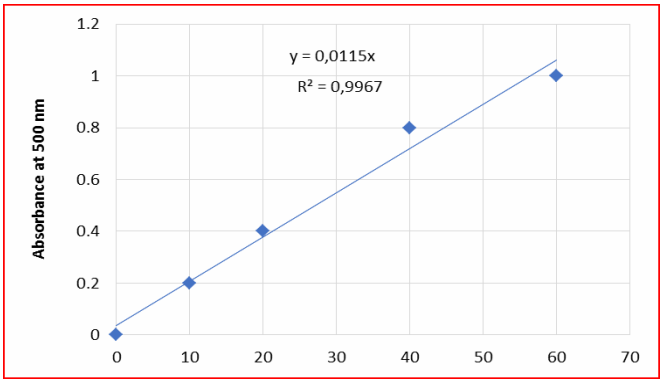

Fig. 3: Calibration curve of catechin for the total flavonoid assay.

The results obtained, expressed in milligram equivalent of catechin per gram of the extract (mg ECT/g extract), in the two aqueous and metallonic extracts are respectively 86.43 (EAq) and 10.26 (ME).

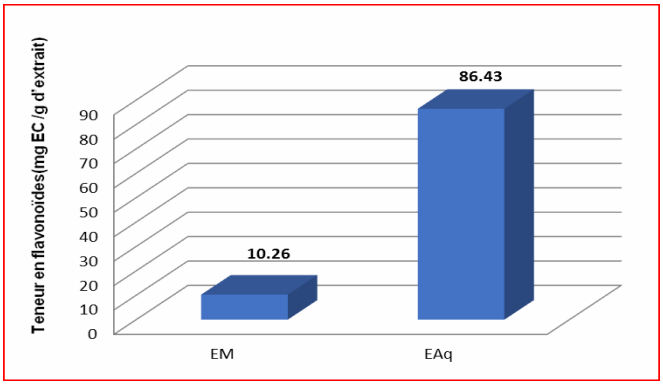

Fig. 4: The content of total flavonoids in the two leaf extracts of *Urtica dioica* (aqueous and methalonic).

According to the histogram, the concentration of total flavonoids is low in the metallonic extract compared to the aqueous extract. The results obtained from the harvests carried out at the level of the region of El Tarf are similar to the research work carried out by Chaurasia and Wichtl (1987).

Contents of condensed tannins:

The tannin content is determined from the linear regression equation of the catechin curve: Y = 0.0177 X, with a straight line regression coefficient (R^2 = 0.9966) close to 1, thus proving the reliability of this curve in the determination of tannins (Fig. 05).

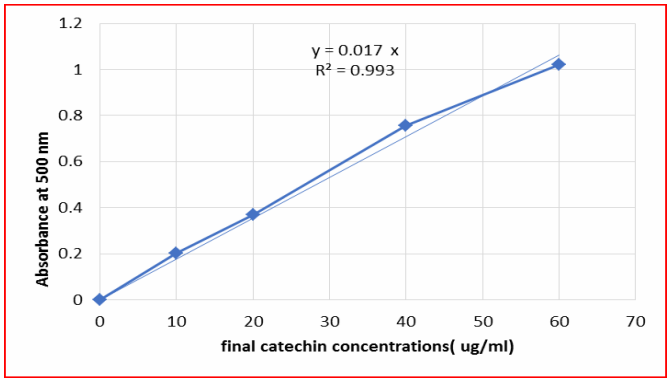

Fig. 5: Catechin calibration curve for tannin assay.

The results obtained, expressed in milligram equivalent of catechin per gram of the extract (mg ECT/g extract), in the two aqueous and metallonic extracts are 22.29 (EAq) and 26.17 (EM) (Figure 5).

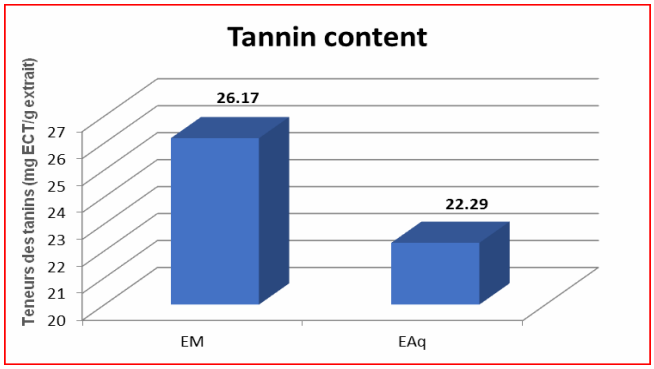

Fig. 6: Tannin content in the two *Urtica dioica* extracts (aqueous and metallonic).

Our results are consistent with data on tannin content from an aqueous extract of *Urtica dioica* presented by (Boudlioua and Ferraguena, 2018), where the authors gave the figure of 18.06 mg/g of dry extract.

Generally, in the diet, they may benefit human health and induce a more astringent taste sensation, although at higher concentrations they inhibit digestive enzymes and reduce the bioavailability of enzymes, iron, and vitamin B12 (King-Thom, 1998). Alternatively, tannins have shown potential antiviral, antibacterial, antiparasitic, and anticarcinogenic effects (Lu, 2014). In the end, all these differences are only the result of the influence of biotic and abiotic factors on the synthesis of these secondary metabolites.

Indeed, environmental factors, namely temperature, humidity, light intensity, supply of water, minerals, and CO_2, and influence the growth of a plant and secondary

metabolism production. (Ramakrishna and Ravishankar, 2011).

Evaluation of antioxidant activity by DPPH:

The percentage inhibition of the DPPH radical The decrease in the absorbance of the DPPH radical caused by antioxidants is due to the reaction between the antioxidant molecules and the DPPH radical, which results in the scavenging of the radical by donation of hydrogen. This is visualized as discoloration from purple to yellow (Duh *et al*., 1999; Chang *et al*., 2002; Gülçin *et al*., 2015; Ebrahimzadeh *et al*., 2015).

The absorbance measurement was carried out by spectrophotometer at 517 nm. From the values obtained, we calculated the percentages of inhibition using the formula cited in the materials and methods section.

IC_{50} is defined as the concentration of the crude extract of the secondary metabolites necessary for the reduction of 50% of the DPPH radical.

Table 03 mentions the percentage inhibition of the DPPH radical of the different extracts of *Urtica dioica*.

Table 03: The percentage of DPPH inhibition of extracts of *Urtica dioica* L.

	Methanolic extract	Aquous extract
PI	**19.03**	**05.26**

It clearly appears in Table 3, representing the percentages of DPPH inhibition of the two nettle extracts (aqueous and metallonic), that the percentage reduction of 50% of the DPPH radical was not achieved. We obtained percentages of 5.26% for the aqueous extract and 19.03 for the metallonic extract.

According to Gordon (1990), the percentage of inhibition increases with the substitution of monophenols with the menthydroxyl group in the ortho position.

Albayrak *et al.* (2012) recorded a percentage of inhibition not exceeding 40% of the DPPH radical for extracts of nettle by infusion and by decoction, with concentrations ranging up to 2 mg/mL. These results are in agreement with those recorded in our research work. Similarly, the methanolic extract of this plant gave, for the same authors, a percentage of inhibition of the DPPH radical of the order of 21.18%.

In Turkey, Deliorman-Orhan *et al*. (2012) obtained a maximum percentage inhibition of the DPPH radical of 21.4 0.2%, from the decoction of nettle leaves.

Evaluation of Antimicrobial Activity:

The evaluation of the antibacterial activity of the extracts of the plant *Urtica dioica* and its fractions was carried out by the disk diffusion method, referring to the scale of the estimation of the antimicrobial activity given by Ponce *et al*., (2003) and Fontanay (2015) (Table 4).

Table 04 : Antimicrobial activity estimation scale (Ponce *et al*., 2003 and Fontanay, 2015).

Antimicrobial activity	Degree of sensibility	The diameter of the inhibition zone
Extremely sensible	+++	More than 20mm
Very sensible	++	15 mm to 19 mm
sensible	+	8 mm to 14 mm
Not sensible	-	Less than 8 mm

According to the results in the table, the metallonic extract of *Urtica dioica* acts differently on the strains tested. The activity of the metallonic extract is higher in contact with *Escherichia coli* at 13 mm and lower in contact with *Klebsiella pneumoniae* and *Staphylococcus aureus* at 8 mm. a total absence of antibacterial activity in the Proteus mirabilis strain with 6mm in diameter.

Table 05: Diameter of inhibition zones of methanolic extracts of Urtica dioica

	Staphylococcus aureus	***Escherichia coli***	***Proteus mirabilis***	***Klebsiella pneumonie***
Pure	8	13	6	8
1/2	8	9	6	8
1/4	8	8	6	8
1/8	8	8	6	8
1/16	8	8	6	8

The work of Albayrak *et al*. (2012) showed that the hydro-methanolic extract exerts an inhibitory effect, resulting in zones of inhibition of around 9 mm with *S. aureus* and *E. coli* ATCC 25922.

The sensibility of E. coli and *S. aureus* was also reported by Farhan *et al*. (2012). These authors indicated that the different concentrations of the hydro-methanolic extract showed different antimicrobial effects depending on the tested microorganism.

Table 06: Diameter of inhibition zones of aqueous extract of Urtica dioica

	Staphylococcus aureus	***Escherichia coli***	***Proteus mirabilis***	***Klebsiella pneumonae***
Pure	8	8	6	6
½	8	8	6	6
¼	8	8	6	6
1/8	8	8	6	6
1/16	8	8	6	6

According to the results in the table, the aqueous extract of *Urtica dioica* acts differently on the strains tested. The activity of the extracts is less important in contact with *Staphylococcus aureus* and *Escherichia coli*, with 8mm in diameter each.

The Manseur (2021) work showed that *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 33962 detect sensitivity to different concentrations of dilutions of the aqueous extract with zones of inhibition varying between 5 and 12 mm.

Conclusion

Medicinal plants still remain a reliable source of active ingredients known for their therapeutic properties. This work was carried out within the framework of the valorization of the natural resources, which are widespread in the region of El Tarf, in the north-east of Algeria. A phytochemical study was conducted in order to identify the active compounds present in "*Urtica dioica,"* a medicinal plant known for its use in traditional medicine and harvested from the Bouhadjar region of El Tarf (North East Algeria). This study was accompanied by a revelation of the biological activities (antioxidant and antibacterial) of the crude extracts.

The phytochemical screening study of the aerial part of the plant confirmed the presence of alkaloids, tannins,

saponosides, flavonoids, and the absence of coumarins and volatile oil.

The results obtained show that the yield from a methanol-water extraction is low (8%) compared to a decoction extraction (20.6%).

The contents of total polyphenols (mg GAE/g RS) in the two extracts are of the order of: 26.02 (ME) and 19.26 (AQE); in flavonoids, around 86.43 (AQE) and 10.26 (ME); and in condensed tannins, around 22.29 (AQE) and 26.17 (ME).

The percentage inhibition of the DPPH radical reaches only 05% inhibition with the aqueous extract and 19% for the methanolic extract.

According to the disk diffusion method, the antibacterial activity was determined for four bacterial strains. The four bacterial strains studied are sensible to extracts from the leaves of the nettle plant. The results indicate that the two extracts (aqueous and metallonic) at different concentrations have antibacterial activity in contact with the strains tested: *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae,* and *Proteus mirabilis*. The extracts have higher activity against *Escherichia coli* at 13 mm and lower activity against *Klebsiella pneumonia* and *Staphylococcus aureus* at 8 mm, as well as a complete lack of antibacterial activity against the Proteus mirabilis strain.

In perspective, it would be very interesting to complete this study with an in vivo experiment and to ensure its total safety in an animal model of choice, taking into consideration the other biological properties of this extract. The use of other extraction methods and other polar solvents.

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