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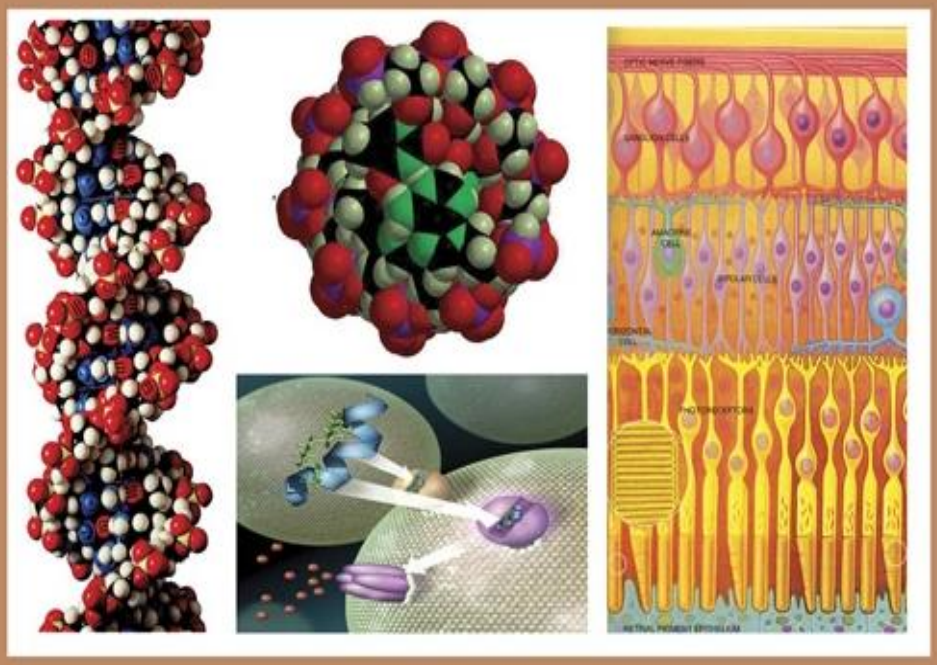

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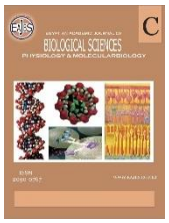

Phytochemical Screening of Methanolic Extract of *Urtica dioica* L.: Antioxidant and Antimicrobial Power for Food Safety

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ABSTRACT

In the present study, a phytochemical screening, antioxidant and antimicrobial activity in vitro were studied for the methanolic extract of the leaves of the plant *Urtica dioica* L. The DPPH method was used to evaluate the antioxidant activity, and the agar disk diffusion method determined the antibacterial power. The results of the qualitative phytochemical analysis show a high content of phenolic compounds characterized by a higher presence of saponins, and a medium richness in tannins with a low content of flavonoids, free quinines and sterols. On the other hand, the absence of alkaloids is observed. For the results of antioxidant activity, the percentage of inhibition of DPPH is 82.76%, 77.85%, and 74.83% for the concentrations 25mg/ml, 12.5mg/ml, and 6.25mg/ml respectively. The lowest percentage of inhibition is noticed for the concentration of 3,15mg/ml with 29, 84%. Our plant shows positive antioxidant activity with an IC50 of 4.69mg/ml. In addition, the antimicrobial activity revealed the most significant effect for *Pseudomonas aeruginosa* strain with an inhibition zone of 9mm to 10mm, with concentrations of 400mg/ml, 300 mg/ml and 200 mg/ml, respectively. Our results suggest that the methanolic extract of *Urtica dioica* L, as a pleasant natural antioxidant and antimicrobial agent, can be applied in the preservation of food products while conducting further studies on the bioactive molecules of this plant to determine their mechanism of action.

INTRODUCTION

Nowadays, the scientific community pays special attention to biological preservatives in food products (Valková *et al.*,2022), as sustainable and healthy production is challenging for the food industry (Rahman *et al.*,2022). Plant extracts continue to be used for food preservation for a long time (Bhusal *et al.*,2022). These natural compounds have low health toxicity and biodegradability and therefore are safe for the environment (Habeeb *et al.,* 2022). Plant bioactive compounds present an amazing range of structural diversity and biological activities, such as pharmaceuticals and alternatives to agricultural chemicals (Kim *et al.*,2021). Algeria has an important heritage of endemic medicinal and aromatic plants (Ayari-Guentri *et al.*,2022). Our study focused on the nettle (*Urtica dioica* L.; UD), an indigenous plant belonging to the Urticaceae family (Taheri et al., 2022). Although it is considered a weed in agriculture, its uses are multiple, in human and animal food, medicine and crop protection (Jeszka-Skowron *et al.,* 2022). In this regard, our work aims to enhance the antioxidant and antibacterial activity of the methanolic extract of the nettle to ensure the organic preservation of food products, reducing the use of chemicals.

MATERIALS AND METHODS

Plant Material:

In this study, we are interested in the aerial part of the UD plant. The plant is collected at the level of the commune of Belarbi Wilaya of Sidi-Bel-Abbés during the period of flowering: February 2022. The leaves were dried at room temperature and protected from light; then reduced to powder and sieved, then stored in a sealed glass bottle protected from light.

Bacterial Strains:

The microbial material comprises four pathogenic strains: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Neisseria gonorrhoeae* ATCC 49226.

Methanolic Extraction:

The extraction was performed by the continuous solid-liquid method in a Soxhlet extractor according to the method described by Boubekeur et al. (2022). Briefly, 20g of the plant is introduced into the Soxhlet for a hot extraction with 250 ml of methanol. The result of the filtration is placed under rotary evaporation at 40°C, and the solubilized active principles are well preserved in the cold. The yield of the extract is defined as the ratio between the mass of the extract obtained and the dry mass of the plant material to be treated.

$$Y = \frac{W1}{W2} * 100$$

Y: Yield of extract in (%), W 1: Weight of extract in (g), W2: Weight of sample in (g)

Phytochemical Screening:

The screening of the different chemical groups (polyphenols, alkaloids, terpenes and saponins) was carried out according to the methods described in the work of (Kayani et al., 2007; N'Guessan et al.,2009; Gul,2012).

The reaction with ferric chloride (FeCl3) allowed for the characterization of the polyphenols; 2 ml of ethanol was added to 2 ml of the methanolic extract, and a few drops of FeCl3 caused the appearance of a greenish coloration which indicates the presence of phenols. The cyanidin reaction sought flavonoids. In the presence of 1 ml of concentrated hydrochloric acid and some magnesium chips, the flavonoids are responsible for releasing hydrogen and a coloration ranging from orange to purple-red.

To detect the tannins' presence, we added a few drops of 1% FeCl3 (ferric chloride) to the methanolic extract. The color turns blue-black in the presence of gallic tannins and greenish blue in condensed tannins.

Reducing sugars were detected in the methanolic extract by Fehling's reagent. A volume of 5 ml of crude extract was added to 5 ml of Fehling's liquor. The formation of a brick-red precipitate after 2-3 min of

heating in a water bath at 70°C indicates a positive reaction. The presence of saponins is determined quantitatively by the foam test. The residues were taken up in 5 ml of distilled water and then introduced into a test tube. The latter was shaken vigorously. The formation of a stable foam (height greater than 1 cm), persisting for one hour, indicates the abundant presence of saponins.

Mayer's reagent detects alkaloids. Adding a few drops of this reagent to 2 ml of the petroleum ether extract solution results in forming of a white or white-yellow precipitate in the presence of an alkaloid.

Quinone substances were sought by adding a few drops of 1/10 NaOH to the petroleum ether extract. The presence of free quinones is confirmed by a color change of the aqueous phases to yellow, red, or purple.

The Liebermann reaction sought sterols. The residue was dissolved in 1 ml of acetic anhydride at a high temperature; we added 0.5 ml of concentrated sulfuric acid to the triturate. The interphase appearance of a purple or violet ring, turning blue and then green, indicates a positive reaction.

DPPH Radical Scavenging Activity:

The method described by Popovici et al. (2009) was used. Different concentrations between 25 and 3.125 mg/ml of the studied samples and control (ascorbic acid: reference antioxidant). One hundred microliters of various concentrations of the methanolic extract were added to 1,950 ml of a 0.004% methanolic solution of DPPH (2,2-diphenyl-1-picrylhydrazyl, $C_{18}H_{12}N_5O_6$), after incubation for 30 min in the dark at room temperature; absorbances are measured at 517 nm against the corresponding blank. The antioxidant activity is estimated according to the following equation:

% Antioxidant activity = [Abs control - Abs sample/Abs control] x 100.

With: Abs=Absorbance

The 50% inhibitory concentration of DPPH activity (IC50) of each extract was subsequently calculated from the equation determining the inhibition percentage as a function of inhibitor concentration. It was expressed in µg / ml and compared with ascorbic acid. The IC50 values of the different extracts were estimated using the linear regression curve: y= ax + b (Bentabet *et al.,* 2014).

Antibacterial Activity:

The antibacterial activity was evaluated by the disc diffusion method on the agar medium described by Bammou *et al.* (2015). Bacterial strains were subcultured by the streak method, then incubated at 37°C to obtain fresh colonies for inoculum preparation. Bacterial cultures on Mueller-Hinton agar, aged 24 hours, were suspended in sterile physiological water (0.9%). The turbidity of the inoculum was adjusted to 0.5 Mc Farland, which corresponds to an inoculum of 10^8 CFU/ml. The extracts of our plant were dissolved in 50% methanol to prepare the different concentrations, knowing that the attention of the mother solution of each section is 400 mg/ml. The concentrations of extracts used were: 100%, 75%, 50% and 25%. Sterilized 6 mm diameter discs of Wattman paper N°1, impregnated with 10 µL of extracts, were placed on the surface of a medium previously seeded by swabbing. After incubation at 37°C for 24 h, the zones of inhibition formed around the discs were measured with a caliper (Celikel *et al.*, 2008). Each trial was repeated three times under the same experimental conditions.

Statistical Analysis:

For each test, three repetitions were performed. Our results were designed and treated using Microsoft Excel 2016 software.

RESULTS AND DISCUSSION

The annual food losses worldwide are estimated at 40%, of which microbial degradation and organoleptic quality are the major causes (Beya et al., 2021). In addition, the use of chemical products to combat these losses has favored the appearance of resistant pathogenic strains and the problem of chemical residues, which have repercussions on health and environmental problems (Sapper and Chiralt, 2018). On the other hand, in the review article by Bensid *et al.* (2020), preservatives of plant origin are generally harmless to health. Several

bioactive compounds naturally present in plants have antioxidant and antimicrobial properties but are little exploited (Alirezalu *et al.*, 2020).

Extract of the Plant:

In the present study, the methanolic extract of UD is characterized by a strong odor with a dark green color, its average yield calculated based on a mass of 20g was about 31.5%. The yield varies from plant to plant due to the richness of each species in secondary metabolites compatible with the solvent (Lachguer *et al.,* 2021).

Phytochemical Screening:

The results of the phytochemical screening of the studied plant showed a diversity of secondary metabolites produced, and we noticed the presence of several families of compounds, except for reducing compounds and alkaloids. The results of the qualitative phytochemical analysis are presented in Table 1.

These results agree with those published previously (Kalia et al.,2014; Rawat *et al.,*2019), relating to the richness of the plant in phenolic compounds, flavonoids and tannins. According to the results presented, we observe a high content of phenolic compounds characterized by a more significant presence of saponins, a medium richness in tannins with a low content of flavonoids, free quinines and sterols. Furthermore, the absence of alkaloids is observed, which is consistent with what has been reported in the literature (Saklani *et al.,*2012).

Table 1: Qualitative phytochemical analysis of the methanolic extract of UD.

Metabolites tested	Results
Phenolic compounds	+++
Flavonoids	+
Tannins	++
Reducing compounds	-
Alkaloids	-
Saponins	+++
Free quinines	+
Sterols or tritepenes	+

Absence (-), Low grade (+), Medium grade (++), High grade (+++).

Previously published results indicated that changes in the chemical composition and distribution of bioactive compounds occur with the plant's maturity. Many factors affect the absence of specific metabolites, such as climate, plant age, and especially the time of plant harvesting (Repajić *et al.,* 2021).

DPPH Radical Scavenging Activity:

For the results of antioxidant activity in Figure 1, the percentage of DPPH inhibition is 82.76%, 77.85%, and 74.83% for the concentrations 25mg/ml, 12.5mg/ml, and 6.25mg/ml respectively. On the other hand, the lowest percentage of inhibition is noticed for the concentration of 3,15mg/ml with 29, 84%. In this context, the variation of the percentage of inhibition according to the concentration of the extract is distinguished. In the present study, it seems that the free radical's inhibition percentage increases with the concentration increase; we can say that there is a very highly significant correlation between the concentration and the percentage of inhibition at 82%.

The IC_{50} of the extract shows a positive antioxidant activity of the plant, which was already reported in the study of Adhikari *et al.* (2015) and Mhamdia *et al.* (2022). The oxidation mechanisms are often radical reactions with molecular oxygen. The initiation phase is followed by a propagation phase and then the termination phase, where different free radicals formed recombine.

This leads to various molecules (hydrocarbons, acids) degrading food quality. The presence of antioxidants such as polyphenols and flavonoids is essential for the stability of the products; these termination agents (radical scavengers) block both the initiation and propagation phases (Marc *et al.,* 2004).

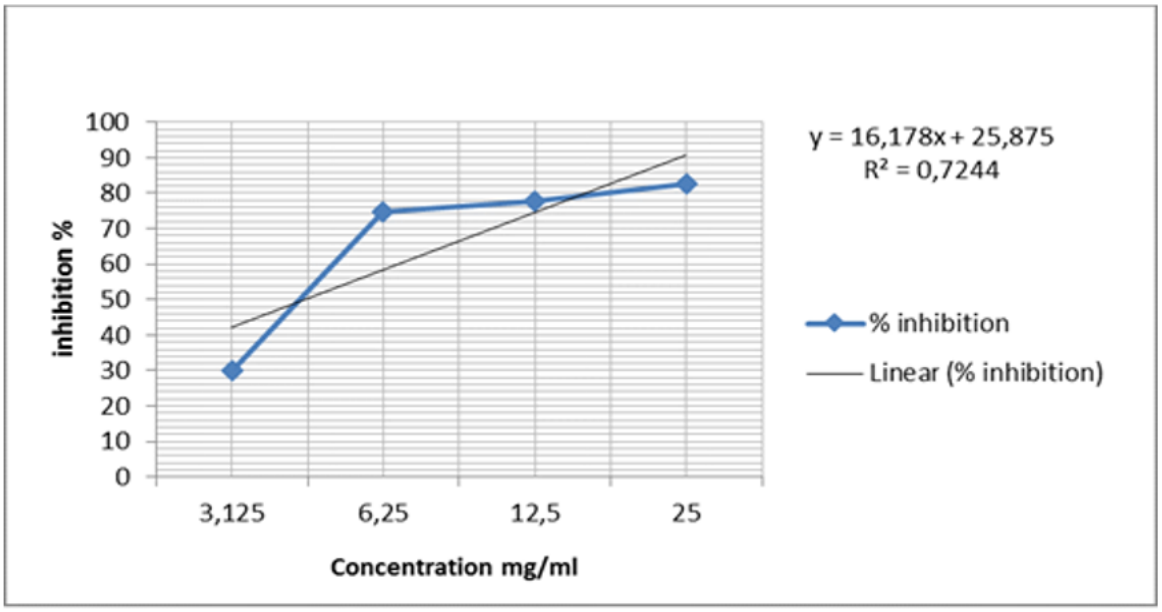

Fig. 1: Calibration of anti-radical activity by DPPH assay, λ = 517 nm, DPPH= 0.747. IC50= 4.69 mg/ml**.**

In this perspective, Kregiel *et al.* (2018) defend the use of *U. dioica* as a functional ingredient to decrease lipids' oxidation, increasing the color's stability during storage.

Antibacterial Activity:

For antimicrobial activity, the most significant effect is observed for the *Pseudomonas aeruginosa* strain with a zone of inhibition of 9mm to 10mm, with concentrations of 400mg/ml, 300 mg/ml and 200 mg/ml, respectively (Fig.02). The inhibition zones for *Escherichia coli* and *Staphylococcus aureus* strains allow them to be classified in the resistant category with a diameter lower than 8mm.

Our results agree with the findings of Shale *et al.* (1999), who found that *Escherichia coli* was utterly resistant to the methanolic extract of UD leaves. Furthermore, in the 2012 study by Kukric *et al.* (2012), UD extracts had inhibitory effects on *Pseudomonas aeruginosa*. According to Daoudi *et al.* (2015), the method and solvents used for extraction could be the cause of differences in antimicrobial properties because the extraction method and the solvent's nature can influence the phenolic antibacterial activity compounds of plants.

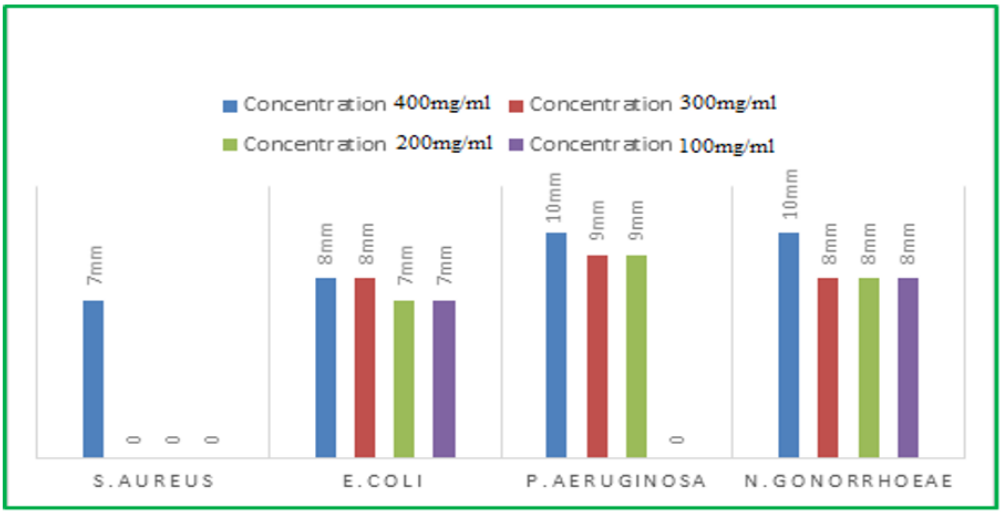

Fig. 2: Inhibitory effect of the methanolic extract on the tested bacterial strains

Therefore, a direct comparison of the results is difficult due to the different products and methods used and the authors' ways of expressing the results. Experimental results of a recent study by Harrison et al. (2022) showed that UD extracts do not possess significant bactericidal activity but can prevent the expression of virulent bacterial phenotypes such as biofilm. These biofilms secrete certain chemicals that protect bacteria from disinfectants and antimicrobial agents (Belmamoun et al.,2022).

CONCLUSION

The results of the present study indicate the considerable potential of radical scavenging by DPPH assay on the methanolic extract of *Urtica dioica* L.; these results are directly related to the qualitative diversity of its compounds. Our study reveals the effect of *Urtica dioica* L. as a natural food preservative since it possesses antioxidant and antimicrobial properties while protecting against oxidative or proliferative degradation of food pathogens. Therefore, it would be helpful to conduct further studies on the bioactive molecules of this plant to determine their mechanism of action.

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