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Analysis of Phytochemical Content, Toxicological Assessment, And Evaluation Of Antioxidant Activity In Urtica Dioica L

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ABSTRACT

This study explores the antioxidant potential of Urtica dioica extracts using different extraction methods, namely infusion and Soxhlet. Antioxidant activity was assessed through DPPH, FRAP, and TAC assays, alongside a phytochemical analysis focused on secondary metabolites such as polyphenols, flavonoids, flavonols, and tannins. Toxicity evaluations were also conducted to ensure safety.

The phytochemical investigation employed the Folin–Ciocalteu method for total polyphenols, the $AlCl_3$ method for flavonoids and flavonols, and the vanillin method for tannins. Acute toxicity studies followed the OECD 423 protocol, using both aqueous and ethanolic extracts.

Among all extracts, the ethyl acetate fraction demonstrated the highest polyphenol content (6.613 ± 1.24 mg GAE/g extract), while the aqueous extract recorded the lowest (1.98 ± 0.18 mg GAE/g extract). Similarly, the ethyl acetate extract was richest in flavonoids (45.194 ± 1.04 mg QE/g extract) and flavonols, confirming its superior secondary metabolite content. Conversely, the aqueous extract showed the highest tannin concentration (13.829 ± 0.17 mg CE/g extract).

In antioxidant evaluations, the ethyl acetate extract showed the strongest activity in all assays. It registered 78.94 ± 1.84 µg/ml in the DPPH test and 203.27 ± 0.17 µg/ml in the FRAP assay. TAC analysis yielded an IC50 of 18.56 ± 0.001 mg/ml for DPPH, 27.15 ± 0.021 mg/ml for FRAP, and 5.938 mg EAA/g in the TAC test. In contrast, the aqueous extract displayed the weakest antioxidant activity (IC50 = 78.11 ± 0.008 mg/ml for DPPH, 50.37 ± 0.008 mg/ml for FRAP, and 0.128 mg EAA/g in TAC).

These findings indicate that the ethyl acetate extract, due to its richness in phenolic compounds, exhibits superior antioxidant properties. Lastly, acute toxicity assessments at 2000 mg/kg showed no clinical signs of toxicity for either aqueous or ethanolic extracts, confirming their safety for further applications.

Keywords: *Urtica dioica, antioxydant activity, toxicity, phytochemecal content.*

1. INTRODUCTION

For a long time, humanity has recognized and utilized aromatic and medicinal plants to treat various ailments. They are used either in their natural form as condiments in traditional pharmacopoeia or to extract the active principles sought after by pharmaceutical, cosmetic, and agri-food industries. Even today, science confirms the various virtues of aromatic plants, their essential and vegetable oils, and their crude extracts.

According to the World Health Organization (WHO), in some developing countries in Asia, Africa, and Latin America, 80% of the population relies on traditional medicine, especially in rural areas, due to the proximity and affordability of such care, and primarily because of the lack of access to modern medicine for these populations (Zeggwagh et al., 2013).

Due to its wealth, unique geographical location, and floristic diversity, Morocco serves as a genuine phytogenetic reservoir, allowing it to occupy a privileged position among Mediterranean countries with a long medical tradition and traditional expertise in medicinal plants for various pathologies.

A medicinal plant is not only a plant but can also be a tree, a shrub, a fungus, a vegetable, a root, an alga; all plant species possessing therapeutic virtues. These natural phytotherapeutic remedies can often be more cost-effective, efficient, and safer than many medications (with fewer side effects) (Bousta and Ennabili, 2011).

The valorization of natural resources is becoming increasingly important. Thus, the WHO recommends the evaluation of the safety and efficacy of herbal medicines for standardizing their use and integrating them into conventional healthcare systems, especially because they are compatible with human nature and have fewer side effects.

The objective of our work is to study the antioxidant activity of *Urtica dioica* extracts obtained through various extraction methods (infusion and Soxhlet) by conducting tests using DPPH, FRAP, and TAC, as well as quantifying secondary metabolites (polyphenols, flavonoids, flavonols, and tannins), and assessing toxicity.

2. MATERIAL AND METHODS

Plant Material

The aerial part of *Urtica dioica* was harvested on April 11, 2021, in the Khenifra region. The leaves were dried in the shade for 15 days, at the laboratory's ambient temperature, shielded from light, heat, and humidity.

Animals

Mice were used for the oral toxicity assessment.

Breeding was carried out in the animal facility of the Faculty of Medicine and Pharmacy of Rabat, at an average temperature of 24°C, with a relative humidity of 70%.

Preparation of Extracts

Aqueous Extract by Infusion

The leaves of *Urtica dioica* were crushed using a grinder to obtain plant powder. The aqueous extract is prepared by infusion following these steps:

• In a 2000 mL volumetric flask: pour 1000 mL of boiling distilled water over 50g of nettle powder and cover. Let it infuse for 30 minutes.

• The mixture is subjected to vacuum pressure using a rotary evaporator to remove the distilled water.

• Freeze at -60°C and lyophilize (photo 5) using a lyophilizer (photo 4), then recover the aqueous extract.

Preparation of Extracts using Soxhlet

The preparation of extracts from our plant using three different solvents (hexane, ethanol, and ethyl acetate) was carried out by solid-liquid extraction using a Soxhlet apparatus. This process allows for the extraction of non-volatile secondary metabolites from the plant (crude extracts). It enables the recovery of diverse chemical families such as alkaloids, flavonoids, and tannins. Depending on the polarity of the solvent used, one can obtain apolar, low-polarity, moderately polar, or polar compounds.

Principle

This is a classic method for solid-liquid extraction. The sample quickly comes into contact with a portion of pure solvent, which helps shift the transfer equilibrium towards the solvent.

The collected mixture is subjected to vacuum pressure using a rotary evaporator to separate the solvent and the chemical compounds present in the extract. The principle of this apparatus is based on vacuum distillation.

Protocol

The plant material (30 g of nettle powder) is placed in a cartridge that is inserted into a Soxhlet-type extractor, equipped at its base with a flask into which the appropriate organic solvent (hexane, ethyl acetate, and ethanol) is introduced.

The solvent is brought to a boil (heated), the vapor passes into the side tube and condenses in the condenser, the solvent gradually fills the extraction chamber containing the solid, takes up some of the product to be extracted, and the solution is then automatically siphoned into the flask once the extraction chamber is full. The cycle repeats indefinitely.

The collected mixture is subjected to vacuum pressure using a rotary evaporator (photo 3), and then the extracts are recovered.

Choice of Solvent

For this study, we conducted a triple extraction of the same material, the first one using hexane (nonpolar), the second one using ethyl acetate (moderately polar), and ethanol (polar).

Extraction Yield

The extraction yield is defined as the ratio between the mass of the extract obtained after extraction and the initial mass of the plant material used (in grams). The yield is expressed as a percentage and is given by the following formula:

Yield (%) = (mass of the extract obtained in g) / (mass of the dry matter in g) × 100

Determination of Phenolic Compounds

Determination of Total Phenols

Principle

The Folin-Ciocalteu reagent consists of a mixture of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$).

In a basic environment, this reagent oxidizes the oxidizable groups of polyphenolic compounds. The reduction products (blue tungsten oxide (W_8O_{23}) and molybdenum oxide (Mo_8O_{23})) are formed. The quantity of polyphenols for each sample is determined by projecting the optical density value onto a standard curve of a standard polyphenol, prepared under the same conditions.

Protocol

The total polyphenol content of the four *Urtica dioica* extracts was determined by the method described by (Poh-Hwa et al), with some modifications.

200 µl of each extract was mixed with 1000 µl of Folin-Ciocalteu reagent and 800 µl of 7.5% sodium carbonate (Na_2CO_3). The mixture was incubated in the dark for 30 minutes, and the absorbance was determined relative to a blank at 765 nm. The experiment was conducted in triplicate. The concentration of total polyphenols was expressed in milligrams of gallic acid equivalent per gram of extract (mg GAE/g of extract) and was determined from the standard curve equation of gallic acid (Poh-Hwa et al., 2011).

Quantification of Flavonoids

Principle

The quantification of flavonoid content in the plant's leaves is estimated using the aluminum chloride ($AlCl_3$) method. The method is based on the oxidation of flavonoids by this reagent ($AlCl_3$). The comparison of the observed absorbance to that obtained from a known concentration standard allows for the evaluation of the total flavonoid content.

Protocol

A volume of 0.5 ml of each extract was mixed with 0.5 ml of 2% aluminum chloride (AlCl3). After one hour of incubation at room temperature, the absorbance was measured at 420 nm. The total amount of flavonoids was expressed in milligrams equivalent to Quercetin per gram of each extract (mg QE/g extract), based on the Quercetin calibration curve (Ordoñez et al., 2006).

Determination of Flavonols

Principle

The determination of flavonols is based on the same principle as that of total flavonoids.

Protocol

2ml of each extract or the standard (Quercetin) was mixed with 2ml of an $AlCl_3$ solution (20mg/ml) and 6ml of a sodium acetate solution (50mg/ml). After 2.5 hours of incubation, the absorbance was read at 440 nm. The total amount of flavonols was expressed in milligrams equivalent to Quercetin per gram of each extract (mg QE/g extract), based on the Quercetin

calibration curve (Ordoñez et al., 2006).

Quantification of Tannins

Principle

Condensed tannins are determined by the vanillin method. This method is based on the ability of vanillin to react with the units of condensed tannins in the presence of acid.

Protocol

A volume of 50µl of each extract or the standard (Catechin) was mixed with 1.5ml of a methanolic solution of vanillin (4%) and 750µl of concentrated hydrochloric acid. The mixture was incubated in the dark for 20 minutes at room temperature, and the absorbance was read against a blank at 500nm. The tannin content was expressed in micrograms equivalent to Catechin per gram of extract (µg EC/g extract), based on the Catechin calibration curve (Price et al., 2002).

Antioxidant Activity

DPPH Method

Principle

The antioxidant activity of our extracts was determined with respect to the DPPH radical (2,2-diphenyl-1-picrylhydrazyl). This violet-colored free radical has a maximum absorbance at 517 nm. When reduced, it forms the yellow compound 2,2-diphenyl-1-picryl hydrazine.

Protocol

50µl of each concentration or methanol (negative control) was added to 2 ml of a 0.0023% DPPH solution, also prepared in methanol (60 mL). After homogenization, the mixture was incubated in the dark for 20 minutes at room temperature, and then the absorbance was measured using a spectrophotometer at 517nm [6]. The inhibition percentage (I%) was calculated using the formula:

I% = (A control - A sample) / A control × 100

Where:

▪ I%: DPPH inhibition percentage

▪ A control: Absorbance of the negative control

▪ A sample: Absorbance of the sample.

Calculation of IC_{50}

IC_{50} or the inhibitory concentration of 50% is the concentration of the tested sample required to reduce 50% of the DPPH radical. The lower the IC_{50} value, the more potent the extract is against free radicals. IC_{50} values are calculated graphically by linear regressions of plots showing inhibition percentages at different sample concentrations.

FRAP Method

Principle

This test is based on the reduction reaction of (Fe^{3+}) in the potassium ferrocyanide complex to (Fe^{2+}). The reaction is revealed by the change in color from yellow ferric iron (Fe^{3+}) to green-blue ferrous iron (Fe^{2+}), and the intensity of this coloration is measured using a spectrophotometer at 700 nm (Şahin et al., 2004).

Protocol

0.5ml of each sample.

➢ 2.5ml of phosphate buffer (0.2M and pH=6.6).

➢ 2.5ml of 1% potassium ferricyanide complex (K3Fe(CN)6).

➢ The mixture was incubated in a water bath at 50°C for 20 minutes.

➢ After incubation, 2.5ml of 10% trichloroacetic acid was added to the mixture to stop the reaction.

➢ Then, 2.5ml of each tube was mixed with 2.5ml of distilled water and 0.5ml of 0.1% iron chloride ($FeCl_3$).

➢ The absorbance was measured at a wavelength of 700 nm with the spectrophotometer calibrated using distilled water.

Total Antioxidant Capacity (TAC) Method

Principle

This technique is based on the reduction of molybdenum Mo(VI) present in the form of molybdate ions MoO42- to molybdenum Mo(V) MoO2+ in the presence of the extract to form a green complex of phosphate/Mo(V) at an acidic pH.

Protocol

A volume of 0.2 ml of each extract was added to a mixture of sulfuric acid (0.6M), ammonium molybdate (4mM), and sodium phosphate (28mM). The mixture was then incubated in a water bath at 95°C for 90 minutes. The absorbance was determined at 695nm against a blank. The results were expressed in milligrams equivalent of ascorbic acid (mg EAA/g extract), with the test conducted in triplicate (Prieto et al., 1999).

Evaluation of Acute Toxicity

The evaluation of acute oral toxicity of the aqueous and ethanol extracts was conducted following the protocol established by the Organisation for Economic Co-operation and Development (OECD 423). For each extract, three non-pregnant and nulliparous female mice weighing between 20 and 30g were fasted for 4 hours.

Then, the two extracts were dissolved in water and administered orally using an esophageal tube at a dose of 2000mg/kg. The dose was estimated based on the body weight of each mouse. After the administration of the extracts, the animals were observed for 30 minutes and for 14 days. During this period, variations in body weight, mortality, and clinical signs (aggressiveness, restlessness, sedation, paralysis, prostration, etc.) were noted.

3. RESULTS AND DISCUSSIONS

Extraction yield

The extraction yields of organic extracts were determined relative to 30g of crushed nettle leaves (Table 1).

Table 1 : Organic extract yields

Extrait	Weight of plant material(g)	Empty bottle weight(g)	Weight of full bottle(g)	R%
Hexane	30	41,84	45,51	**12,23**
Acétate d'éthyle	30	42,20	42,84	**2,13**
Ethanol	30	42,65	43,17	**1,733**

In this experiment, we aimed to assess the extraction efficiency of three different solvents, namely Hexane, ethyl acetate, and Ethanol, when applied to a 30-gram sample of *Urtica dioica*. The results revealed significant variations in extraction performance among these solvents. Hexane demonstrated the highest extraction efficiency at 12.23%, indicating its effectiveness in extracting compounds from the plant material. ethyl acetate, on the other hand, exhibited a significantly lower efficiency of 2.13%, implying that it was less adept at extracting compounds compared to Hexane. Ethanol, with an extraction efficiency of 1.733%, was found to be the least effective among the solvents tested.

Quantification of Phenolic Compounds

Quantification of Total Polyphenols

The quantification of total polyphenols was performed based on a calibration curve created using a standard solution (gallic acid) at different concentrations. Optical density measurements for the extract were conducted at 765 nm. The concentration of total phenols was determined for the four extracts using the Folin-Ciocalteu reagent. The analysis results were obtained by comparing the absorbance values of the extract solutions to standard solutions of gallic acid. The results for the polyphenol content of each extract are presented in the following table.

Table 2: Polyphenol content in mg eq gallic acid/1g extract.

Extrait	Teneur (en mg EAG/1g d'extrait)
Aqueux	1,98±0.18
Hexane	3,58±0.14
Acétate d'éthyle	6,613±1.24
Éthanol	2 ,92±0.11

In Table 2, we examine the polyphenol content of extracts obtained using different solvents, expressed in milligrams equivalent to gallic acid per one gram of extract. The results provide valuable insights into the efficiency of various solvents in extracting polyphenolic compounds. The Aqueous extract displayed the lowest polyphenol content at 1.98 mg eq gallic acid per gram, indicating that aqueous extraction may not be the most optimal method for polyphenol extraction from the source material. In contrast, Hexane extraction yielded a notably higher polyphenol content of 3.58 mg eq gallic acid per gram, signifying its superior ability to extract these compounds compared to the Aqueous extract. Ethyl acetate, on the other hand, demonstrated the highest polyphenol content among all the solvents tested, with an impressive value of 6.613 mg eq gallic acid per gram, establishing it as a highly effective solvent for polyphenol extraction. Ethanol extraction, with a moderate polyphenol content of 2.92 mg eq gallic acid per gram, falls in between Aqueous and the superior ethyl acetate in terms of efficiency. These findings underscore the significance of solvent choice in polyphenol extraction, with ethyl acetate proving to be the most promising for obtaining high polyphenol yields from the source material.

These results do not align with the study on the antioxidant activity of nettle (*Urtica dioica*) ethyl acetate extract conducted by (Ghaima et al., 2013), which reported a total polyphenol content of 48.3 mg GAE/g DW. Similarly, the result for the total polyphenol content in the aqueous extract of *U. dioica* leaves found by (Gülçin et al., 2004) was 17.9 mg equivalent of gallic acid/ml. However, our results are consistent with the findings of the work conducted by (Duda et al., 2009); the values for total polyphenol content obtained for the aqueous extract of *U. dioica* leaves were lower than those of the methanolic extract.

Since the aerial part of our plant was harvested on April 11, 2021, this may explain the lower total polyphenol content in *Urtica dioica* leaves. This is in line with a study on the influence of harvest timing on the chemical composition of *Urtica dioica*, which found that the highest total phenolic content (TPC) was in the leaves harvested in August. TPC in terms of gallic acid equivalent ranged from 3.28 mg GAE/g DW in May to 19.07 mg GAE/g DW (Paulauskienė et al., 2021).

Flavonoid Quantification

Flavonoid quantification was carried out using the $AlCl_3$ method with Quercetin as the standard. The flavonoid contents are expressed in milligrams of quercetin equivalent per gram of plant extract and are presented in the following table.

Table 3 : Quercetin flavonoid content in mg eq/1g extract. Extract Aqueous hexane Ethyl acetate Ethanol.

Extract	Content (in mg EQ/1g extract)
Aqueous	8,87±0.14
Hexane	35,514±0.001
ethyl acetate	45,194±0.03
Ethanol	20,90±0.04

In Table 3, we examine the quercetin flavonoid content in various extracts, measured in milligrams equivalent (mg eq) per gram of extract. This data provides important insights into the efficiency of different solvents for extracting quercetin, a bioactive compound with numerous health benefits.

The Aqueous extract displayed the lowest flavonoids content at 8.87 mg eq per gram of extract, suggesting that aqueous extraction might not be the most effective method for quercetin extraction from the source material.

In contrast, Hexane extraction yielded a significantly higher quercetin content of 35.514 mg eq per gram, indicating its superior ability to extract this valuable flavonoid. This substantial increase in quercetin content compared to the Aqueous extract highlights the importance of solvent selection in optimizing flavonoid extraction.

Ethyl acetate, meanwhile, exhibited the highest flavonoids content among all the solvents tested, with a remarkable value of 45.194 mg eq per gram of extract. This demonstrates that ethyl acetate is exceptionally effective for quercetin extraction, outperforming both Aqueous and Hexane extractions.

The ethanol extract had a flavonoids content of 20.90 mg eq per gram. Although not as efficient as ethyl acetate, it still offers a significant improvement over aqueous extraction.

Flavonol Quantification

The calibration curve for quercetin demonstrates a proportional relationship between concentration and absorbance, confirming the validity of the Beer-Lambert law within the concentration range employed. The results of the quantitative analysis of flavonol contents in different nettle leaf extracts are expressed in milligrams of quercetin equivalent per gram of plant extract and are presented in the following table.

Table 4 : Quercetin flavonol content in mg eq/1g extract. Aqueous extract Hexane Ethyl acetate Ethanol

Extract	Content (in mg EQ/1g extract)
Aqueous	3,684±0.1
Hexane	15,70±0.2
ethyl acetate	41,865±0.005
Ethanol	8,79±0.3

In Table 4, we investigate the quercetin flavonol content within different extracts, expressed in milligrams equivalent (mg eq) per gram of the extract. These findings provide valuable insights into the efficiency of various solvents for extracting quercetin, a potent flavonol with a range of health-promoting properties.

The Aqueous extract exhibited the lowest quercetin flavonol content at 3.684 mg eq per gram of extract, indicating that aqueous extraction may not be the most effective method for obtaining quercetin flavonols from the source material.

On the other hand, Hexane extraction resulted in a substantially higher quercetin flavonol content of 15.70 mg eq per gram, highlighting its superior ability to extract this important flavonol compared to the Aqueous extract. This significant increase in quercetin flavonol content underscores the critical role of solvent selection in optimizing flavonol extraction.

Ethyl acetate, remarkably, displayed the highest quercetin flavonol content among all the solvents tested, with an impressive value of 41.865 mg eq per gram of extract. This indicates that ethyl acetate is exceptionally effective for quercetin flavonol extraction, surpassing both Aqueous and Hexane extractions.

Ethanol extraction, although not as efficient as ethyl acetate, still offered a notable improvement over Aqueous extraction, with a quercetin flavonol content of 8.79 mg eq per gram.

Tannin Quantification

The calibration curve for catechin confirms a proportional relationship between concentration and absorbance, thus validating the Beer-Lambert law within the range of concentrations used. The results obtained with vanillin are depicted in the following graph:

The results of tannin content for each extract are expressed in milligrams of catechin equivalent per gram of plant extract and are presented in the following table.

Table 5: Tannin content in mg EC/1g extract.

Extract	Content (mg EC /1g extract)
Aqueous	13,829±0.17
Hexane	4,175±0.24
ethyl acetate	8,004±0.17
Ethanol	2,252±0.44

In Table 5, we delve into the tannin content within different extracts, quantified in milligrams equivalent (mg EC) per gram of the extract. These findings offer critical insights into the efficiency of various solvents for extracting tannins, a group of polyphenolic compounds with diverse applications, including in the food and beverage industry, and as antioxidants.

The Aqueous extract revealed the highest tannin content at 13.829 mg EC per gram of extract, indicating that aqueous extraction is particularly effective for extracting tannins from the source material. This result aligns with the common practice of using water-based extraction methods for tannin-rich substances.

Conversely, Hexane extraction yielded a substantially lower tannin content of 4.175 mg EC per gram, highlighting its limited capacity for tannin extraction. Hexane's lower tannin yield emphasizes the critical role of solvent selection in optimizing tannin extraction, as it appears to be less efficient for this specific compound.

Ethyl acetate, while not as effective as Aqueous extraction, still managed to produce a noteworthy tannin content of 8.004 mg EC per gram, which suggests its suitability for tannin extraction in situations where water-based methods are not applicable.

Ethanol extraction showed the lowest tannin content among all the solvents, with a value of 2.252 mg EC per gram. While it is the least efficient of the solvents tested, it still offers an improvement over Hexane extraction, making it a potential choice when the use of more polar solvents is necessary.

Antioxidant Activity

The antioxidant activity of the extracts was assessed using three different methods: the free radical scavenging technique (DPPH), the ferric reducing antioxidant power (FRAP), and the total antioxidant capacity (TAC).

DPPH Method

The antioxidant activity of various *Urtica dioica* extracts concerning the DPPH free radical was evaluated using a spectrophotometer by monitoring the reduction of this radical, which is accompanied by a change in color from violet (DPPH•) to yellow (DPPH-H), measurable at 517 nm.

The results leading to 50% inhibition of the DPPH free radical and that of the negative control are represented below (Table 6):

Table 6: IC_{50} of extracts and BHT obtained by the DPPH method.

Extract	IC_{50}(DPPH) in µg/ml
Aqueous	78,11±0,008
Hexane	41,96 ± 0,005
ethyl acetate	18,56±0,001
Ethanol	23,7±0,021
BHT(T-)	4,20±0,02

In Table 6, we examine the IC_{50} values of different extracts and butylated hydroxytoluene (BHT), measured using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. IC_{50} represents the concentration at which a substance effectively scavenges 50% of the DPPH radicals, thus indicating its antioxidant activity. The lower the IC_{50} value, the stronger the antioxidant activity of the substance.

The Aqueous extract displayed an IC_{50} of 78.11 ± 0.008 µg/ml, suggesting moderate antioxidant activity. Hexane extraction produced a lower IC_{50} of 41.96 ± 0.005 µg/ml, indicating better antioxidant activity compared to the Aqueous extract. This may be attributed to the extraction of different compounds with antioxidant properties by Hexane.

Remarkably, ethyl acetate exhibited a significantly lower IC_{50} of 18.56 ± 0.001 µg/ml, signifying a potent antioxidant capacity. This result suggests that ethyl acetate is highly effective in scavenging free radicals, which is crucial for protecting cells and tissues from oxidative damage.

Ethanol extract also demonstrated notable antioxidant activity with an IC_{50} of 23.7 ± 0.021 µg/ml, although it was slightly less effective than ethyl acetate. These findings suggest that both ethyl acetate and Ethanol extracts have the potential to serve as natural antioxidants.

These results underline the significant differences in antioxidant activity among the extracts, with ethyl acetate showing the most promising potential for natural antioxidants.

These IC_{50} results are nearly the same as those found by (Belmaghraoui et al., 2018), which showed that the ethyl acetate and ethanol extracts are more active than the aqueous extract. The IC_{50} values were estimated as follows: 301.32 ± 1.73 µg/ml for the aqueous extract, 298.84 ± 4.25 µg/ml for the ethyl acetate extract, and 94.73 ± 3.95 µg/ml for the ethanol extract, compared to 28.6 ± 0.41 µg/ml for ascorbic acid (standard).

Similarly, this is consistent with the results of the DPPH test conducted by (Loshali et al., 2021), which showed that the ethyl acetate extract is more active than the ethanol extract. However, the result of the study on the aqueous nettle extract conducted by (Fattahi et al., 2014) is not consistent with our findings, as the IC_{50} for the DPPH radical was 1.2 mg/ml.

FRAP Method

The FRAP test is a method based on the reduction of ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) through electron transfer in the presence of an antioxidant. The absorbance is measured at 700 nm. The results of this study are provided below (Table 7).

Table 7: IC_{50} of extracts and BHT obtained by the FRAP method.

Extract	IC_{50}(FRAP) in µg/ml
Aqueous	50,37±0,008
Hexane	36,22±0,005
ethyl acetate	27,15±0,021
Ethanol	33,10±0,005
BHT(T-)	7,2±0,02

In Table 7, we assess the IC_{50} values of various extracts and butylated hydroxytoluene (BHT) using the FRAP (Ferric Reducing Antioxidant Power) method. The IC_{50} represents the concentration required to reduce 50% of the ferric ions (Fe^{3+}) to the ferrous ions (Fe^{2+}), serving as an indicator of the antioxidant capacity of these substances. A lower IC_{50} value signifies stronger antioxidant activity.

The Aqueous extract displayed an IC_{50} of 50.37 ± 0.008 µg/ml, indicating a moderate level of antioxidant activity. Hexane extraction produced a lower IC_{50} of 36.22 ± 0.005 µg/ml, suggesting better antioxidant activity compared to the Aqueous extract. This may be due to the extraction of different compounds with antioxidant properties by Hexane.

Ethyl acetate, remarkably, exhibited a substantially lower IC_{50} of 27.15 ± 0.021 µg/ml, indicating a robust antioxidant capacity. This result suggests that ethyl acetate is highly effective in reducing ferric ions, demonstrating its potential for protecting against oxidative damage.

Ethanol extract also showed notable antioxidant activity with an IC_{50} of 33.10 ± 0.005 µg/ml, although it was slightly less effective than ethyl acetate. These findings indicate that both ethyl acetate and Ethanol extracts have significant potential as natural antioxidants when measured using the FRAP method.

Method TAC

The antioxidant activity of the extracts was determined using the phosphomolybdenum method, which is based on the reduction of Mo(VI) to Mo(V) and the formation of a green complex at an acidic pH. The total antioxidant capacity is reported in milligrams of ascorbic acid equivalent per gram of extract.

The results of this study are presented in the table below (Table 8).

Table 8: Antioxidant activity by TAC expressed as mg eq ascorbic acid/1g extract.

Extract	TAC (mg EAA/1g extract)
Aqueous	0,128±1.2
Hexane	1,661±0.04
ethyl acetate	5,938±0.5
Ethanol	3,44±0.11

In Table 8, we explore the antioxidant activity of different extracts, expressed in terms of TAC (Total Antioxidant Capacity) and measured as milligrams equivalent to ascorbic acid (mg EAA) per one gram of extract. This data provides critical insights into the ability of various solvents to extract antioxidant compounds from the source material.

The Aqueous extract displayed the lowest TAC value at 0.128 mg EAA per gram of extract, indicating a relatively low antioxidant capacity. This result implies that aqueous extraction may not be the most efficient method for extracting antioxidants from the source material.

Conversely, Hexane extraction resulted in a significantly higher TAC value of 1.661 mg EAA per gram, highlighting its superior ability to extract antioxidants. This suggests that Hexane was effective in extracting antioxidant compounds, which can be valuable for various applications.

Ethyl acetate, however, exhibited the highest TAC value among all the solvents tested, with a substantial 5.938 mg EAA per gram of extract. This indicates that ethyl acetate is exceptionally effective for extracting antioxidants, outperforming both Aqueous and Hexane extractions.

Ethanol extraction also demonstrated notable antioxidant capacity with a TAC of 3.44 mg EAA per gram, although it was slightly less efficient than ethyl acetate.

Acute Toxicity Evaluation

This study was conducted to assess the acute toxicity of the aqueous extract and the ethanol fraction of the wild plant species *Urtica dioica*. The results of this study, with both extracts administered orally at a dose of 2000 mg/kg, show no clinical signs of toxicity. All animals used in the experiment survived after the 14-day observation period, and their behaviors remained normal. This implies that the lethal dose (LD50) is higher than 2000 mg/kg.

During the 14 days of monitoring, the animals' body weights did not undergo any significant changes compared to the control group. Especially after 10 days, based on these results and following the OECD 423 guidelines, we can conclude that both extracts from nettle leaves are considered non-toxic when administered orally.

Our findings are consistent with recent studies conducted by (Chira et al., 2025) and (Loshali et al., 2021), which reported an LD50 of 5770 mg/kg for hydroalcoholic extracts of *Urtica dioica* leaves administered orally. However, this contradicts the results of (Mukundi et al., 2017), who observed a significant decrease in the weekly weight gain rate compared to the control group and a significant reduction in testicular weight from 1000 mg/kg in extracts of nettle leaves collected in Kenya.

4. CONCLUSION

The present study focuses on comparing the antioxidant activity of four different extracts from the aerial parts, specifically the leaves of the plant *Urtica dioica*, and quantifying phenolic compounds using spectrophotometry for each extract.

The extracts were prepared differently, with the aqueous fraction obtained through infusion, while the hexane, ethyl acetate, and ethanol fractions were obtained using a Soxhlet apparatus with yields of 12.23%, 2.13%, and 1.733%, respectively.

The study of acute oral toxicity of the aqueous and ethanol extracts of *Urtica dioica* showed that they have no toxic effects when administered orally to animals at a dose of 2000 mg/kg, in agreement with other studies in this context.

The results of the antioxidant activity study using DPPH, FRAP, and TAC assays revealed that the ethyl acetate extract is the most active, followed by the ethanol extract, while the hexane and aqueous fractions exhibited lower activities.

The quantification of phenolic compounds showed that the ethyl acetate and ethanol fractions are the richest in flavonoids, whereas the aqueous fraction is rich in tannins.

In parallel, according to previous studies on the biological effects of *Urtica dioica*, the timing of harvest influences the yield and production of secondary metabolites. Since our plant was harvested in April, we can suggest that this is the reason for the lower levels of total polyphenols in the aerial parts of *Urtica dioica*.

Given the high levels of tannins, this suggests the potential of our plant to play a significant role as an antimicrobial agent. It would be interesting to consider future perspectives such as performing additional extractions using different solvents, conducting structural analyses of the constituents present in our plant (GC-MS), and investigating other biological activities (antimicrobial and antibacterial activities) of this plant. This includes exploring its potential in treating challenging diseases like cancer and its incorporation into food and pharmaceutical manufacturing.

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