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PHYSIOCHEMICAL STANDARDIZATION AND PHYTOCHEMICAL SCREENING OF *Urtica dioica L.* LEAVES GROWING IN ZAKHO, KURDISTAN REGION, IRAQ

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ABSTRACT:

The research aims to assess different pharmacognostic standards on the *Urtica dioica* L. leaves with the determination of the total phenol and flavonoid contents using different extracts. *U. dioica* L. is a species grown commonly in Kurdistan region, Iraq used among the population as a folklore medicine treating different diseases. These standards include; percentage value of extracts using different solvents, phytochemical screening of bioactive compounds, fluorescence analysis using different chemicals under UV and visible light, and physiochemical properties were performed for moisture content, total solid, total ash, water soluble ash, acid insoluble ash, sulphated ash, crude fat and crude fibers. The percentage extracts yielded 22% and 20% of water and ethanol respectively while n- hexane had the lowest one (2%) using the Soxhlet extraction method. The screening of phytochemicals identified the presence of phenols, flavonoids, carbohydrates, proteins, tannins, alkaloids, saponins and terpenoids. The Kjeldahl method's protein assay revealed a high concentration of protein and the analysis of elements by atomic absorption spectroscopy shows the abundance of calcium, magnesium and iron among the six measured elements. Our findings revealed that *U. dioica* L. leaves are a good source of proteins, minerals, and exhibit a potential source of phytochemicals and these findings will be useful in the identification and development of pharmacopeial standards.
KEYWORDS: *Urtica dioica* L. Stinging nettle, Physiochemical evaluation, Preliminary phytochemical assessment.

1. INTRODUCTION

Plants are significant sources of medical components, which play an important role in plant adaptation to their environments (Cardoso et al., 2019). In recent years, medicinal plants, which are considered a fundamental basis for folk medicine, have become the subject of considerable pharmacological research, as a result of growing awareness of the importance of these medicinal plants. Furthermore, they are considered potential sources of novel compounds with therapeutic effects, as well as source of compounds for developing drugs (Salmerón-Manzano et al., 2020)

Urtica dioica L. 'stinging Nettle' is a herbaceous perennial flowering plant native to Europe, the Middle East, Northern Africa as well as North America, that belongs to the *Urticaceae* family and the species *Urtica*. It has spread widely across the world's temperate areas (Krystofova et al., 2010). In Kurdistan region of Iraq, this herb is known as Kazink, common Nettle in English , Gerrais in Arabic, Indian name is Bichu Butti, 'Vrishchhiyaa shaaka Punjabi' in Sanskrit, Shisuun in Kumaon, and Anjuraa in the folk language of Uranian (Bahmani et al., 2014). The leaves and stems of *U. dioica* L. contain small hairs called "trichomes", when these trichomes directly come into contact with skin, they lead to produce a savage burn because they inject a fluid containing histamine, acetylcholine, serotonin, and formic acid. This fluid will perforate the skin causing dermatitis and if these poisons are infused into the skin, it will cause painful anger and a burning sensation for up to 12 hrs (Mishra & Kharel, 2010).

Nettle plant parts, leaves and roots have been utilized as a blood purifier, nasal, diuretic, rheumatism of muscles and joints, menorrhagia, kidney disease , eczema, seasonal allergies, iron deficiency, arthritis, menstrual haemorrhage jaundice, haematuria and diarrhoea (Bhusal et al., 2022; Joshi et al., 2014). *U. dioica* plant produces a variety of important medicinal chemical compounds such as polyphenols, tannins, phytosterols, saponins, flavonoids, essential fats, proteins, chlorophyll, amino-acids carbohydrate, carotenoids and it is high-nutrient, easy - to - digest food rich in minerals, provitamin A and vitamin C (Al-Tameme et al., 2015; Krystofova et al., 2010; Kataki et al., 2012 ; Rafajlovska et al., 2013). Therefore, the aim of this research study, which is the first to be reported in Kuristan Region / Iraq, is to determine the physiochemical properties and phytochemical screening of *U. dioica* leaves.*†

Figure 1.*Urtica dioica* L.

2. MATERIALS AND METHODS

Plant collection: *U. dioica* L. plant leaves were collected in (April to May 2021) from barware village, Zakho City, Kurdistan region Iraq. *U. dioica* leaves were cleaned up with tap water and then

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washed with distilled water and air-dried in a dark place for about 10 to 15 days. Then, they were grounded to powder and kept in dark containers and keep it in a fridge until the day of use.

2.1 Preparation of *Urtica dioica* leaves extracts

The *U. dioica* leaves powder were extracted using a sequential extraction process (Pathmanathan et al., 2010), with slight modification by employing a variety of solvents, including water, ethanol, ethyl acetate, chloroform, and hexane.

Ten grams of powder of *U. dioica* leaves was placed in a thimble in Soxhlet and soaked overnight, then extracted by 100 ml of water for 10 hours at 90◦C. The extract was collected into conical flask separately and concentrated under reduced pressure and low temperature using rotary evaporator.

The extracts were kept in the refrigerator until they were used for the assay. The residue was dried before being used for next extraction, followed by extractions using ethanol, ethyl acetate, chloroform, and hexane at temperatures that were below the boiling points of each solvent. The percentage of extraction yield was calculated using the following equation:

$$\text{Percentage yield \%} = \frac{Wt}{W0} \times 100 \quad (1)$$

Where Wt represents the weight of the dry isolated sample in (gm) and W_0 represents the weight of plant powder

2.2 Quantitative analysis of bioactive compounds from Urtica dioica leaves

2.2.1 Isolation of flavonoids from *U. dioica* leaves: An amount of (10 gm) powdered *U. dioica* leaves was mixed with 50 ml 80 % methanol, kept stirring overnight at ambient temperature. After filtering the methanolic extract, adding 25 ml of 1 % lead acetate to the filtrate. The mixture was then filtered by a Buchner funnel and the filtrate was pulled out. After that, the precipitate was mixed with 30 ml of concentrated hydrochloric acid (HCl) and 25 ml acetone. The mixture was then filtered, finally was evaporated. (Yadav & Kumar, 2012).

$$\text{Percentage yield \%} = \frac{Wt}{W0} \times 100 \quad (2)$$

Where Wt represents the weight of the dry isolated sample in (gm) and W_0 represents the weight of plant powder.

2.2.2 Isolation of alkaloids from *U. dioica* leaves: Ten grams of powdered *U. dioica* leaves were mixed with 50 ml of (10 %) ethanolic acetic acid and a magnetic stirrer was used for 24 hours at room temperature. Then, the mixture was filtered and the filtrate was concentrated to approximately quarter of its original volume using a rotatory evaporator under vacuum at 40 °C. The PH was also changed to 9 using
ammonium hydroxide to precipitate the alkaloids. After placing the mixture in a separating funnel, 20 ml of the chloroform was added and stirred. The organic part was taken away and this procedure was carried out three times (Al–Maliki, 2012).

$$\text{Percentage yield \%} = \frac{Wt}{W0} \times 100 \quad (3)$$

Where Wt represents the weight of the dry isolated sample in (gm) and W_0 represents the weight of plant powder.

2.2.3 Isolation of glycosides from *U. dioica* leaves: Ten grams of powdered *U. dioica* leaves were mixed within 50 ml of (2 %) acetic acid solution and the mixture was digested by boiling water bath for eight hours with stirring. The residue was removed; the filtrate was put in separating funnel and then extracted with n-butanol that had been saturated with NaCl (20 ml of 3M NaCl was added to 100 ml n-butanol). The organic layer was vaporized at 40 °C in a rotary evaporator under vacuum (Harborne, 1984)(George et al., 2002).

$$\text{Percentage yield \%} = \frac{Wt}{W0} \times 100 \quad (4)$$

Where Wt represents the weight of the dry isolated sample in (gm) and W_0 represents the weight of plant powder.

2.2.4 Isolation of phenols from *U. dioica* leaves: Ten grams of powder *U. dioica* L. leaves were mixed with 50 ml (2 % HCl) and then the mixture placed in the water bath at (60 ◦C) for eight hours. After that, it was filtered via Buchner funnel, then the residue was removed. Diethyl ether was added in an equal quantity to the supernatant, and the solution was placed in a water bath for 50 minutes at 32 °C. The mixture was then condensed using a rotator evaporator under vacuum at 40 °C (Al–Maliki, 2012).

$$\text{Percentage yield \%} = \frac{Wt}{W0} \times 100 \quad (5)$$

Where Wt represents the weight of the dry isolated sample in (gm) and W_0 represents the weight of plant powder.

2.2.5 Isolation of saponin from *U. dioica* leaves: One gram of powdered *U. dioica* L. was dissolved in 100 ml of (20 %) ethanol, stirred for half an hour in a flask and then heated for 4 hours at 45 °C with continuous stirring. The solution was filtered by a Whatman N 1 filter papers, and the precipitate was re-extracted with an additional 100 ml of (25 %) ethanol for 4 hours. The mixed extracts were concentrated using a rotary evaporator at 40 °C to achieve about 40 ml. After that, the concentrate was transported to a separator funnel and extracted two times with 20 ml of diethyl ether. The ether layer has been removed, but the aqueous layer was retained and re-extracted with 30 ml of butanol, and then thoroughly washed with 10 ml of (5 %NaCl) and the remainder of the solution was evaporated. Following that, the sample was dried in an oven at 40°C (Farhan et al., 2012) .

$$\text{Percentage yield \%} = \frac{Wt}{W0} \times 100 \quad (6)$$

Where Wt represents the weight of the dry isolated sample in (gm) and W_0 represents the weight of plant powder.

2.2.6 Isolation of tannin from *U. dioica* leaves: The isolation of tannin has been agreed upon via the solvent method utilizing the cold extraction method. Weighted (10 gm) of powdered *U. dioica* leaves were mixed with 50 ml of 70% acetone solution at (60 °C) for one hour. Then, the mixture was filtered and the residue was re-extracted with an equal volume of 70 % of acetone. After that, the solvent was removed using a rotary evaporator under a vacuum at 40 °C to form dark green of tannin and finally weighted (Ukoha et al., 2011).

$$\text{Percentage yield \%} = \frac{Wt}{W0} \times 100 \quad (7)$$

Where Wt represents the weight of the dry isolated sample in (gm) and W_0 represents the weight of plant powder.

2.2.7 Isolation of crude Fat from *U. dioica* leaves: One gram of powdered *U. dioica* leaves were dissolved with 100 ml petroleum ether (40 to 60 °C) in a Soxhlet device and extracted for 8 hours, followed by evaporation at 40 °C utilizing a rotary evaporator. Finally, the fat weight was decided (Krishna et al., 2014)

$$\text{Percentage yield \%} = \frac{Wt}{W0} \times 100 \quad (8)$$

Where Wt represents the weight of the dry isolated sample in (gm) and W_0 represents the weight of plant powder.

Isolation of crude fiber from *U. dioica* leaves: One gm of defatted sample was dissolved with 200 ml of (1.25 % conc.H_2SO_4) with boiling stone, and boiled for 30 minutes. In order to neutralise the mixtures, they were filtered and thoroughly washed with boiling distilled water, and then transported once more to beaker to be boiled in 200 ml of (1.25 % NaOH) for 30 mins. Once again it was filtered, and the precipitate was then washed with boiling distilled water to neutralize. After that, the precipitate was transferred to pre-weighted crucible (W1), then placed in an oven for two hours at 130 °C and cooled down in a desiccator and weighted (W2). Following that, the crucible and its components were placed in a muffle furnace at 600 °C for 30 minutes to finish the combustion of organic materials. It was then desiccated and weighed to a steady weight (W3). The formula was used to calculate the percentage of fiber (Krishna et al., 2014; Bhargava et al., 2013)

Yield percent % = $\frac{(W2-W1)-(W3-W1)}{W0} \times 100$ (9)

Where W1 represents the pre-weighted crucible, W2 represents the weighted sample in an oven and W3 represents the weight on the ignition,

2.2.8 Isolation of total protein content from *U. dioica* leaves: The micro Kjeldahl technique has been used to calculate total protein concentration. One gram of dried powdered *U. dioica* plant was put in the digestion flask, together with 10 grams of potassium sulphate, 0.5 gram of copper sulphate, and (25 ml) of conc.H_2SO_4. After that, digestion was carried out by putting the flask in a slanted position and was heated just under the boiling point of acid for 5 to 15 minutes. The temperature was increased until the acid began to boil rapidly. To restrict air movement, a funnel was installed on top of the flask. The mixture was heated until it was clear. The contents were cooled and then diluted with 200 ml of distilled water. The reaction was then made extremely alkaline by adding 0.5 g of granulated zinc with 50 ml of 40 % NaOH. The compounds were combined and connected to the distillation device, and the receiving flask was filled with 25 mL of (0.1 N) sulphuric acid, following the reaction's conclusion. The mixture was withdrawn from the flask, chilled, and titrated with a 0.1 N caustic soda solution (NaOH) utilizing methyl red as an indicator that the reaction has ended till the color changed from pink to light yellow (Alasmary et al., 2019) .

Protein (%) = $\frac{V \times N2 \times 6.25 \times 0.1\ N(NaOH)}{\text{weight of sample} \times 1000} \times 100$ (10)

Where:
V: volume used in titration
N2 atomic weight of nitrogen
W: weight of sample (gm)
Protein factor: 6.25

2.3 Phytochemical screening of Urtica dioica leaves

2.3.1 Flavonoid test

Alcoholic potassium hydroxide reagent:
One millilitre of (5N) potassium hydroxide KOH in alcohol was added to 1ml for each extract. The formation of yellow precipitate indicates the presence of flavonoids (Joshi et al., 2011)

Lead acetate test:
Few drops of 10 % lead acetate were mixed with 1ml of each extract. The appearance of yellowish precipitate indicates having of flavonoids (Singh & Kumar, 2017) .

Ammonia test:
Five millilitres of diluted Ammonia solution were added to 1ml of each extract and then conc. H_2SO_4 was added in the side of test tube. The formation of a yellow colour indicates the presence of flavonoids (Kumar et al., 2013).

Conc.H2SO4 test:
Few drops of conc.H_2SO_4 were added to 1ml of each extract, the appearance of an orange color indicates the presence of flavonoids (Tyagi, 2017).

2.3.2 Carbohydrates test

Molish's test (General test):
One millilitre of each extract and 5 drops of α-naphthol in alcohol was added to a test tube whilst being vigorously shaken. Then 1 ml of conc.$H_2SO_4$was slowly added to the tube wall, forming a violet ring which indicate the presence of carbohydrates (Singh, 2012).

- Seliwanoff's test (Keto sugar):
One millilitre for each extract was put in a test tube and 3 ml of Seliwanoff's reagent (50 mg of resorcinol dissolve in 33 ml conc. hydrochloric acid and make it 100 ml with distilled water) were added and incubated on water bath for 1 minute. The appearance of the rose colour indicates the formation of keto sugar (Basumatary, 2016).
- Bial's test (pentose sugar):
Two millilitres of each extract in a test tube were added to 1 ml of Bial's reagent (0.25 gm for both $FeCl_3.6H_2O$ and Orcinol dissolved in concentrated HCl and complete the volume to 500ml by distilled water), shaken well and then put in a boiling water bath for 5min. The green colour indicates pentose sugar (Ahmad, 2008).
- Polysaccharide test:
One millilitre of each extract in a test tube was added to 1 ml of Iodine solution (0.25 gm Iodine powder with 15 gm potassium iodide dissolve in 500 ml of distilled water). The establishment of blue, reddish-brown, and purple colours indicates the presence of starch, glycogen, and dextrin respectively (Nayak et al., 2015).

2.3.3 Glycosides test

Before hydrolysis:
Benedict's reagent: Equal volume of benedict's reagent (1.7 gm $CuSO4.5H_2O$ mixed with 17.3 gm sodium citrate and 10 gm NaOH dissolve in 100 ml distilled water) and extracts were taking a test tube. The mixture was kept for 10 mins in boiling water bath. A red precipitate appears to show the existence of reducing sugar (Kumar et al., 2012a).

Fehling's reagent: One millilitre of each extract was added to 1 ml of Fehling's reagent (solution A: 3.4 gm of hydrated copper sulphate $CuSO_4.5H_2O$ dissolved in 50 ml distilled water, solution B: 17.3 gm sodium potassium tartrate with 5.2 gm sodium hydroxide (NaOH) dissolved in 50 ml distilled water) and both solutions were mixed) and boiled in a water bath for 10 minutes. A reddish precipitate indicates the production of reducing sugars (Singh & Kumar, 2017).

After hydrolysis:
Benedict reagent: Five drops of concentrated HCL were added to 5 ml of each extract. The mixture was put in a boiling water bath for 20 minutes, and the solution was neutralized by adding 2M NaOH. The equal volume of Benedict reagent was added into the solution and heated in water bath for 15 minutes. A red precipitate implies the presence of glycosides (Kumar et al., 2012a).

Fehling's reagent: One millilitre of each extract in a test tube was added to 5 ml of diluted H_2SO_4 and placed in water bath for 15 minutes. It was then cooled and 20% of sodium hydroxide was added. A 5 ml of Fehling's reagent was added and heated for 5 minutes. A reddish precipitate indicates glycosides (Singh & Kumar, 2017).

2.3.4 Cardiac glycosides test

Two millilitres of acetic anhydride and a few drops of 5 % Ferric chloride were added to 1 ml of each extract. Then, 1 ml of concentrated sulphuric acid was added. The violet ring indicates the presence of cardiac glycosides (Khan et al., 2011).

2.3.5 Tannin test (Braymer's test)

One millilitre of each extract was added to 3 ml of distilled water and boiled. Then 1ml of 1 % ferric chloride ($FeCl_3$) was added. The appearance of blue-green or bluish-black indicates the presence of tannins (Chooranam, 2017).

2.3.6 Phlobatannins (olive oil test)

One millilitre of each extract was diluted with distilled water and shaken strongly. A few drops of olive oil were added with strong shaking. The appearance of foam indicates the Phlobatannins (Gul et al., 2017).

2.3.7 Alkaloids test

- Wagner's test
2 drops of Wagner's reagent (1.27 gm of iodine with 2 gm KI and distilled water to create a final size 100 ml) were added to each acidified extract along the test tube's sidewalls. The formation of a reddish-to-brown precipitates indicates the presence of alkaloids (Sorescu et al., 2018).
- Dragendroff's test:
A few millilitres of each extract were mixed with1ml of Dragendroff's reagent. The appearance of reddish to brown

precipitates indicate the existence of alkaloids (De Silva et al., 2017).

Hager's test:

1-2 millilitres of Hager's reagent (saturated of aqueous picric acid solution) were added to 1 ml of each extract. The creamy white precipitate indicates the presence of alkaloids (De Silva et al., 2017).

Tannic acid test:

A few drops of 10 % of tannic acid solution were added to 1 ml of each acidified extract. The appearance of buff colour precipitate indicates the presence of alkaloids (Ray et al., 2013).

2.3.8 Proteins and amino acid test

Ninhydrin test (free amino acid):

One millilitre of 1 % ninhydrin reagent was added in 1 ml of each extract, then boiled in boiling water bath for 10 minutes. The purple colour indicates the formation of amino acids (Shaikh & Patil, 2020)

Biuret test (peptide bond):

Two millilitres of each extract were mixed with 5 drops of 2 % copper sulphate solution and then 1ml of 95 % ethanol was added. Finally, pellets of KOH were added. The pink coloured ethanolic layer indicates the presence of peptide bonds (Shaikh & Patil, 2020)

Xanthoproteic test:

A few drops of concentrated HNO_3 were added to 1 ml of each extract. The yellow or orange colour indicates the existence of benzene rings (Tiwari et al., 2011).

2.3.9 Saponin test

Aqueous mercury chloride:

One millilitre of 5 % aqueous mercury chloride was added to one millilitre of each extract. The presence of saponin is indicated by the development of a white precipitate (Faizy et al., 2021).

Foam test:

A tiny amount for each extract was added in 3 ml of distilled water. After that, it was shaken vigorously and left for 1 minute. Saponins were detected by the formation of foam (Sorescu et al., 2018).

2.3.10 Phytosterols test

Salkowski's test:

In a clean and dry test tube, equal volumes of each extract and concentrated sulphuric acid (H_2SO_4) were mixed, shaken well, and was let to set. The red colour on the top layer indicates the presence of phytosterols (Tiwari et al., 2011).

Libermann- Burchard's test (acetic anhydride test):

In a dry and clean test tube, 1 ml of each extract were dissolved in chloroform and mixed with 1ml of acetic anhydride. 2-3 drops of conc. H_2SO_4 were added and shaken vigorously. The formation of pink colour, which turns into dark green, indicates the presence of phytosterols (Auwal et al., 2014).

2.3.11 Terpenoids test

Two millilitres of chloroform were mixed with 5 ml of each extract and then put in a water bath until evaporation, which was later cooled down. Three millilitres of conc. H_2SO_4 were gently added on the side of the test tube. The appearance of a reddish-brown precipitate indicates that terpenoids were created (Indumathi et al., 2014).

2.3.12 Triterpenoids test

1 ml of concentrated sulphuric acid and 1 ml of $CHCl_3$ were added to 2 ml of each extract. The red or purple colour indicates the formation of triterpenoids (Ilahi et al., 2021).

2.3.13 Resins test (Turbidity)

Equal volumes of each extract and 4 % hydrochloric acid were mixed. The appearance of turbidity indicates the formation of resins (Shaikh & Patil, 2020).

2.3.14 Coumarins test (NaOH test)

One millilitre of each extract was mixed with 2 ml of 10 % of sodium hydroxide. The formation of yellow colour indicates the presence of coumarins (Kumar et al., 2013).

2.3.15 Phenolic test

Ferric chloride test:

A few drops of 5 % ferric chloride solution were added to 1ml of each extract. The dark green or bluish- black colour indicates the formation of phenols (Shaikh & Patil, 2020).

Lead acetate test:

One millilitre from each extract was diluted with 5 ml of distilled water, and 3 ml of 10 % lead acetate have been added. The appearance of phenolic compounds was indicated by a white precipitate(Shaikh & Patil, 2020).

Ellagic acid test:

Equal volumes of 5 % glacial acetic acid and 5 % sodium nitrite were mixed with 1 ml of each extract. The solution was turn into a cloudy brown precipitate indicating phenols were formed (Sheel et al., 2014).

2.3.16 Anthocyanins test (HCL test)

Two millilitres of each extract were mixed with 2 ml of 2N HCL. Following that, few millilitres of ammonia were added. The appearance of blue -violet colour indicates the formation of anthocyanins (Savithramma et al., 2011).

2.3.17 Quinone test

One millilitre of conc.H_2SO_4 was added to 1 ml of each extract. The occurrence of Quinone's is indicated by the formation of a red colour (Roghini & Vijayalakshmi, 2018).

2.3.18 Anthraquinones test

A few drops of 10 % ammonia solution were added to 1 ml of each extract. The presence of a pink precipitate indicates the formation of anthraquinones (Roghini & Vijayalakshmi, 2018).

2.4 Physiochemical standardization

2.4.1 Moisture content

Two grams of powdered *U. dioica* leaves were weighed in a dry crucible and placed in an oven at 105 °C for 6 hours. After cooling in a desiccator, the weight was calculated. The procedure was repeated till the differences between the two successive weights were no more than 5mg. The percentage of water loss after drying was used to determine the moisture content (Iram, 1995;Al-Saleem et al., 2018).

$$\% \text{ Moisture content} = \frac{Wt}{W0} \times 100 \quad (11)$$

Where Wt represents weight of losses sample in (g) and W0 represent weight of sample powder (g).

The determination of total solid content was calculated by this formula:

$$\text{Total content (\%)} = 100 - \text{moisture (\%)} \quad (12)$$

2.4.2 Total Ash value

Two grams of powdered *U. dioica* leaves were weighed and put in a silica crucible where the powdered material was distributed in an equal layer. The crucible material was ignited at a constant weight by sequentially increasing the temperature to 450 °C in a muffle furnace for 6 hours until the color white appeared, indicating the lack of carbon. A desiccator was used to cool the residual ash. (Thendral & Lakshmi, 2017).

The total ash content in units of air-dry material was determined as follows:

$$\% \text{Total Ash value} = \frac{\text{weight of ash}}{\text{weight of sample}} \times 100 \quad (13)$$

2.4.3 Water soluble ash

A crucible containing total ash was heated for 5 mins with 25 ml of distilled water. An insoluble material was placed on an ashless filter-paper which was washed with hot water and ignited about 15 minutes at 450 °C in a muffle furnace. The weight of water soluble ash had been determined by subtracting the weight of ash from the weight of water-insoluble materials (Trishala et al., 2019).

The percentage of water- soluble ash of air-dried material was determined as follows:

$$\% \text{ Value of water- soluble ash} = \frac{W2 - W3}{W1} \times 100 \quad (14)$$

Where W1 represent the weight of sample powder (g), W2 is the weight of total ash, and W3 represents the weight of water insoluble ash (gm)

2.4.4 Acid insoluble ash

A crucible containing total ash was heated for 5 minutes with 25 ml of (2N) hydrochloric acid, sealed with a watch glass which was later washed with hot distilled water and the rinsed materials were put again into a crucible. The acid-insoluble substance was collected on ashless filter paper and washed with hot water till the filtrate became neutral. The filter paper with acid-insoluble substance was added to the existing crucible, and then ignited in the muffle furnace at 550 °C for 1 hour. Finally, the silica crucible was placed in a desiccator to cool down and be measured (Subakanmani & Umadevi, 2012; Chooranam, 2017).

$$\% \text{ Value of acid insoluble ash} = \frac{Wt}{W0} \times 100 \quad (15)$$

Where Wt represents the weight (gm) of the residue of acid insoluble ash and W0 represents the weight of the sample (gm).

2.4.5 Sulphated ash

Two grams of powdered *U. dioica* leaves were placed in silica crucible and burned at 450 ° C. in a muffle furnace until completely charred. The silica crucible containing ash was placed in desiccator to cool down. After that, 1 ml of sulphuric acid was added to the crucible and it was gently heated until white vapors were no longer produced. Next, ignition took place at 800 °C until the black particles disappeared. The crucible is taken from muffle furnace and put in desiccator to cool down and be weighed to determine the sulphated ash content (Subakanmani & Umadevi, 2012).

$$\% \text{ Value of sulphated ash} = \frac{Wt}{W0} \times 100 \quad (16)$$

Where Wt is the weight of sulphated ash in gm and W0 is the weight of dry sample powder in gm.

2.4.6 Determination of extractive value

Water soluble extractive:

Five grams of powdered *U. dioica* leaves were macerated with 100 ml of water in a closed conical flask for 24 hrs. For the first 6 hours, the conical flask was shaken, and then left to set for 18 hours. The content of the flask was filtered using Buchner funnel and then 25 ml of the filter was transferred to crucible. Later it evaporated and dried in the oven at 105 °C and was finally weighed. The percent of water soluble-extractive was estimated using the air - dry powder as a reference (Krishna et al., 2014).

$$\text{Percentage of water extractive value} = \frac{W3-W1}{W2} \times 100 \quad (17)$$

Where W1 represents the weight of empty crucible, W2 represents the weight of sample powder (gm) and W3 represents the weight of crucible with extract (gm).

Alcohol soluble extractive:

Five grams of powdered *U. dioica* leaves were macerated with 100 ml of different solvents (95% Ethanol, Ethyl acetate, Chloroform, Petroleum ether and n-Hexane) for 24 hours in a closed conical flask. For the first 6 hours, the conical flask was shaken, and then left to set for 18 hours. The content of the flask was filtered using Buchner funnel and then 25ml of the filter were transferred to the crucible. Later it evaporated to became dry, again dried in the oven at 105 °C and finally weighed. The percentage of alcohol soluble extractive was calculated (Krishna et al., 2014).

$$\text{Percentage of alcohol extractive value} = \frac{W3-W1}{W2} \times 100 \quad (18)$$

Where W1 represents the weight of empty crucible, W2 represents the weight of sample powder (gm) and W3 represents the weight of crucible with extract (gm).

2.4.7 Fluorescence analysis

The fluorescence properties of powdered plant material with various chemical reagents were measured using daylight and ultraviolet light. 1 mg of powdered *U. dioica* leaves were placed in a beaker and handled with different reagents to determine the existence of fluorescence characteristics under ultra-violet (UV) lamp. Fluorescence analysis was performed using Kokoski procedures. This plant material was submitted to fluorescence analysis in daylight and UV light (short -UV; 254nm and long -UV; 365nm) (Hanani & Saidah, 2019)

2.4.8 Digestion and determination of element

Half gram of powdered *U. dioica* leaves was digested with 10 ml of concentrated H_2SO_4 and left overnight, then 10 ml of H_2O_2 was added and the sample was heated till it became clear. After that, it was transferred to a 50 ml volumetric flask and the volume was complete with the addition of deionized water. The sample was analyzed using flammable atomic absorption spectroscopy (FAAS) to estimate the concentration of Cu, Mn, Mg, Ca, Zn, and Fe (Rafiee et al., 2015).

3. RESULTS

3.1 Extraction yields of U. dioica L.

Five different solvents with varying degrees of polarity (n-hexane, ethyl acetate, chloroform, ethanol and water) were used to extract *U. dioica* L. leaves to make a complete evaluation of this plant. The findings showed that solvent gradients have an impact on the percentage yield of several extracts of *U. dioica* L. leaves. Table 1 shows that the amount yielded of water extract was more than other extracts (ethanol, chloroform, ethyl acetate, and n- hexane respectively). Among organic solvents applied, ethanol produced the maximum extraction yield whereas n-hexane produced the lowest for extracting bioactive compounds from *U. dioica* L. leaves. Figure 2 Shows the colours for different extracts of *U. dioica* L. leaves.

Table 1: The percentage yield and physical states of *U. dioica* L. leaf extracts using different solvents.

Type of extract	Percentage (%w/w)	Physical state
Water	22.696 ± 0.0071	Sticky, Dark brown
Ethanol	20.206 ± 0.0097	Sticky, Dark green
Ethyl acetate	5.466 ± 0.0071	Sticky, Dark green
Chloroform	3.973 ± 0.0071	Sticky, Dark green
n-Hexane	2.003 ± 0.0071	Sticky, Yellowish brown

Note: each test will carry out replicate (Mean ±SE).

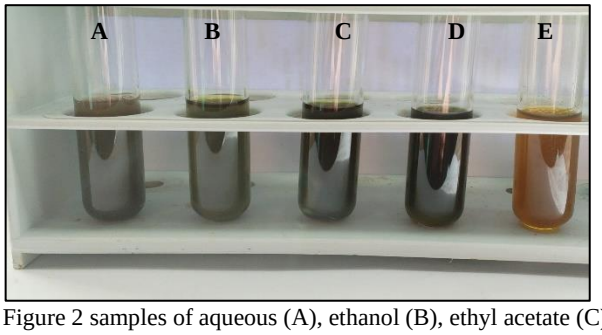

Figure 2 samples of aqueous (A), ethanol (B), ethyl acetate (C), chloroform (D), hexane (E) extracts of *U. dioica* leaves.

3.2 Quantitative analysis of U. dioica L.

The leaves of *U. dioica* L. were quantitatively analysed for total flavonoids, alkaloids, phenols, glycosides, saponins, tannins, proteins, and. carbohydrates using standard methods. The quantitative determination of different bioactive compounds (primary and secondary metabolites) in *U. dioica* L. leaves is shown in Table 2. Phenolic and carbohydrates acids were observed in the highest percentage (44.913 ± 0.0071%) and (39.2166 ± 1.3774%) respectively, followed by total protein and

tannins (11.13 ± 0.0094%) and (10.316 ± 0.0071%) while total glycosides and saponins are (4.6 ± 0. 0.0046%) and (4.006 ± 0.0054%). On the other hand, total alkaloids and flavonoids showed the least percentage (2.87 ± 0.0046%) and (2.126 ± 0.0071%).

Table 2. quantitative analysis (bioactive compound) of *U. dioica* L. leaves.

Bioactive compounds	Percentage (% w/w)	Physical state
Total Flavonoids	2.126 ± 0.0071	Sticky, Green
Total Alkaloids	2.87 ± 0.0046	Sticky, Brown
Total Phenols	44.913 ± 0.0071	Sticky, Brown
Total Glycosides	4.6 ± 0.0046	Powder, Greenish to yellow
Total Saponins	4.006 ± 0.0054	Powder, Light yellow
Total Tannins	10.316 ± 0.0071	Sticky, Dark green
Total Protein	11.13 ± 0.0094	--------
Carbohydrate	39.2166 ± 1.3774	---------

Note: each test will carry out replicate (Mean ±SE).

3.3 Qualitative phytochemical screening of U. dioica L.

The phytochemical active compounds of *U. dioica* L. leaves for all extracts were qualitatively analyzed and the findings are shown in Table 3. In these screening methods, flavonoids, phenols, terpenoids, glycosides, alkaloids, tannins, protein and carbohydrates were found in water and ethanolic extract except for protein and alkaloids are absent in ethanolic one. In ethyl acetate, chloroform and n- hexane extracts, phenols, flavonoids, terpenoids, tannins, and carbohydrates, saponins, alkaloids were found in these extracts except cardiac glycoside, protein and saponin in ethyl acetate extract. Cardiac glycoside and glycoside, were found to be absent in chloroform extract while protein, amino acids, alkaloids were not found in n- hexane extract.

3.4 Physiochemical analysis of U. dioica L.

The physical properties such as moisture content, total solid, the ash values, and extractive values of *U. dioica* L. leaves were determined. The outcomes of physiochemical parameters which are based on the standard procedures are shown in Tables 4 and 5 respectively. The moisture content, total solid, total ash, water soluble ash, acid insoluble ash and sulphated ash were found to be 9.5 ± 0.577 %, 90.5 ± 0.577%, 17.35 ± 000 %, 3.695 ± 0.0028%, 1.72 ± 0.0115 %, and 24.09 ± 0.0057 % respectively (Table 4). On the other hand, the content of crude fat and fibers were found to 1.2033 ± 0.0118 % and 7.0066 ± 0.0054%.

Table 3 Qualitative phytochemical screening for *U. dioica* L. leaves.

Phytochemicals	Test	Water	Ethanol	Ethyl acetate	Chloroform	n-Hexane
Flavonoids	Alcoholic KOH reagent Lead acetate reagent Ammonia test Conc.H_2SO_4 test	+	++	+	+	+
Carbohydrates	Molish's reagent Seliwanoff's reagent Bials reagent Iodine reagent Benedict's reagent Fehling's reagent	++ + - ++ + +	++ + + ++ ++ ++	+ - - ++ - -	+ + + ++ + +	+ - - ++ - -
Glycosides	Benedict's reagent Fehling's reagent	+	+	-	+	-
Cardiac glycosides	Acetic anhydride	++	++	-	-	+
Tannins	Braymer's reagent	++	++	+	+	+
Phlobatannins	Olive oil test	+	+	+	+	+
Alkaloids	Wagner's reagent Dragendroff's reagent Hager's reagent Tannic acid test	+	-	+	+	-
Proteins and Amino acids	Ninhydrin test gent oteic	+ + +	++ - +	- - -	+ + -	- - -
Saponin	Aqueous Mercury chloride Foam teat	++	+	-	++	+
Phytosterols	Salkowski's reagent Liebermann-Burchard's reagent	-	+	+	+	+
Terpenoids	Chloroform test	+	+	+	-	+
Tri-terpenoids		+	+	-	-	+
Resins	Turbidity	+	+	-	-	-

Coumarins	Sodium hydroxide test	+	-	-	+	-
Phenolic	Ferric chloride reagent Lead acetate reagent Ellagic acid test	++	+	+	+	+
Anthocyanins	Hydrochloric acid test	+	+	-	-	-
Quinones	Conc.H_2SO_4	+	+	-	-	-
Anthraquinone	Ammonia solution	-	-	-	-	-

.Note: (++) High concentration, (+) low concentration, (-) absent.

Table 4. Physiochemical analysis of *U. dioica* L. leaves.

Quality parameter	percentage (% w/w)
Moisture content	9.5 ± 0.577
Total solid	90.5 ± 0.577
Total ash	17.35 ± 000
Water soluble ash	3.695 ± 0.0028
Acid insoluble ash	1.72 ± 0.0115
Sulphated ash	24.09 ± 0.0057
Crude Fat	1.2033 ± 0.0118
Crude Fibers	7.0066 ± 0.0054

Note: each test will carry out replicate (Mean ±SE).

However, the extractive values of the *U. dioica* L. leaves using water and different solvents like ethanol, ethyl acetate, chloroform, hexane and petroleum ether were shown in Table 5 showing that the greatest extractive yield was found in water while n- hexane has the least extractive value.

Table 5 percentage the extractive value from *U. dioica* L. leaves.

Extract	Color of extract	Percentage (% w/w)
Water	Brown	6.7933 ± 0.0380
Ethanol	Dark green	1.0333 ± 0.0271
Ethyl acetate	Dark green	0.8666 ± 0.0543
Chloroform	Brown	0.8 ± 0.0942
n-Hexane	Dark yellow	0.2 ± 000
Petroleum ether	brown	0.6 ± 0.1632

Note: each test will carry out replicate (Mean ±SE).

3.5 Mineral elements analysis of U. dioica L. leaves.

Six mineral elements concentrations (Cu, Mn, Ca, Zn, Fe and Mg) were measured in *U. dioica* L. leaves as shown in Table 6. In our results, the highest amount of calcium was found to be (4115 ± 124.71 mg. g^{-1}) in the nettle leaves whereas magnesium content (669.75 ± 2.7424 mg. g^{-1}) was about six-fold lower. Regarding the trace elements, the highest content was reached by iron (195.2 ±0.3464 mg. g^{-1}) followed by zinc (34 ± 0.2886mg. g^{-1}) and manganese (28.8 ± 0.577 mg. g^{-1}) respectively. The lowest concentration was identified for copper (7.05 ± 0.8373 mg. g^{-1}).

Table 6. Mineral elements contents in *U. dioica* L. leaves.

Elements	Concentration (mg. g^{-1})
Cupper (Cu)	7.05 ± 0.8371
Manganese (Mn)	28.8 ± 0.5773
Calcium (Ca)	4115 ± 124.71
Zinc (Zn)	34 ± 0.2886
Iron (Fe)	195.2 ± 0.3464
Magnesium (Mg)	669.75 ± 2.7424

3.6 Fluorescence analysis of U. dioica L.

U. dioica L. leaf powder was subjected to various chemical reagents and studied under visible (daylight) and UV light (short and long wavelengths) for fluorescence investigation. In this study, *U. dioica* L. leaf powder showed various colouration characteristic under visible and UV light (short and long wavelength) using different chemical reagents such as 1N NaOH in methanol, 10 % $K_2Cr_2O_7$, 50 % HNO_3, 10 % $FeCl_3$, 5 % H_2O_2 as shown in Table 7.

Table 7. Fluorescence behaviour of *U. dioica* L. leaf powder treated with different solvents.

U. dioica L. Powdered	Visible/day light	UV 254 nm	UV 365nm
Powder plant	Green	Light green	Dark green
Ethanol	Green	Brown	Dark brown
Methanol	Light green	Light yellow	Dark green
Chloroform	Green	Light yellow	Dark yellow
Acetone	Green	Light brown	Dark brown
n-Hexane	Green	Green	Brown
Petroleum ether	Dark green	Dark green	Dark brown
Water	Green	Light brown	Dark brown
P + 1N NaOH in methanol	Green	Light brown	Dark brown
P + 1N HCl	Light brown	Violet	Dark brown
P+HNO_3(1:1)	Light brown	Violet	Black
P + H_2SO_4 (1:1)	Green	Dark green	Blue
P+50%H_2SO_4	Light yellow	Yellow to brown	blue
P+50%HNO_3	Light brown	Violet	Dark brown
P+10%$K_2Cr_2O_7$	Orange	Purple	Red
P + $AgNO_3$	Light green	Dark green	Black
P + 5% H2O2	Cloudy white	Purple	Black

P+ 5% NaOH	Green	Dark green	Black
P + HCl	Light green	Yellow	Brown
P + Aqueous 10%$FeCl_3$	Brown	Violet	Purple
P + Glacial acetic acid	Dark green	Violet	Dark brown
Ammonia	Light green	Dark green	Black
Iodine solution	Maroon	Red	Purple
P + 1N NaOH in methanol	Green	Light brown	Dark brown

Note: each test will carry out replicate (Mean ±SE);
p= plant.

4. DISCUSSION

Stinging nettle, commonly known as *U. dioica* L., *is* a member of the Urticaceae family used as a folk remedy to treat many diseases. Traditional medicine has used the roots and leaves of this plant for a variety of conditions, including diuretic blood purifiers, liver problems, stomach aches, eczema and anaemia. In our society (Kurds), Kurdistan Region, Iraq, *U. dioica* L., known as Kazink, is considered as a herb and used as a tea choice. Additionally, the leaves of this plant are cooked and mixed with onion and consumed as a meal. The purity, quality, and authenticity of the plant can all be measured using the protocols of quality control (Devkota et al., 2022; Karlsen, 2017). Regarding the quality control of *U. dioica*, there isn't much information available. Consequently, the initial goal of this study, the first to be done on this plant, is to establish the quality control methods of *U. dioica* L. which is grown in the Kurdistan region, Iraq. These methods involve extraction using different solvents, phytochemical screening, physiochemical analysis and fluorescence analysis.

The yield of the extracted compounds using Soxhlet extraction technique showed that water had the highest yield. Ethanol is the most effective organic solvent that gives the maximum yield due to its ease of entry into the cellular membrane for the extraction of the intracellular components (Wang et al., 2010; Bandar et al., 2013) while the non- polar solvent n- hexane gave the lowest extraction yield. Although, extraction is considered as a key step in extracting bioactive compounds from plants, extraction yield is influenced by many factors; type of extraction method, the solvent used and the chemical nature of these bioactive compounds (Ri et al., 2019). Comparing our results for the percentage yields with those of other authors showed considerable agreement. Maobe and his group showed that ethanol percentage yield is higher than n- hexane and ethyl acetate according to the following yields; 1.82, 0.8 and 0.67 respectively (Maobe et al., 2013). Additionally, Bandar et al. (2013) conducted a comparison study between different techniques by using different solvents for the extraction of the bioactive compounds from the Lebanese *U. dioica* L.This group concluded that the Soxhlet method had the highest extraction yield and in the term of solvent applied, ethanol was the most effective solvent and their results are in agreement with our results as shown that the water gave the highest yield followed by ethanol, acetone, dichloromethane and n- hexane respectively. On the other hand, our results are in firm disagreement with Joshi & Uniyal, 2017 group as they mentioned that ethyl acetate extract has the highest yield of 4.5 %, ethanol extract 3.25 % whereas water extract was found to be 1.95 % respectively.

The comparison of our results regarding screening of phytochemical active compounds with the results of other authors showed fair agreement. The phytochemical screening of *U. dioica* originating from Tunisia showed that polyphenols, flavonoids, alkaloids and tannins are more abundant in hydroethanolic fraction than water, ethyl acetate and n-hexane fractions (Zouari et al., 2017). Other results were obtained by Joshi & Uniyal (2017) who noted the presence of phenols, tannins and flavonoids in ethanol and ethyl acetate extracts with absence of alkaloids while in water and ethanol extracts, carbohydrates, protein, amino acids, glycosides and saponin are found. The phytochemicals which are reported by Kannan group showed the presence of flavonoids, tannins, phenols and alkaloids in water and ethanol extracts while saponin is absent (Kannan et al., 2013). The results presented by (Muttalib & Naqishbandi, 2013) show that chloroform extract contains flavonoids only, tannins, saponins and alkaloids were absent. On the other hand, Sayed-Ahmed group confirm the presence of phenols and terpenoids while flavonoids, alkaloids, saponins and glycosides are not found in n- hexane extract (Sayed-ahmad et al., 2014).

The physicochemical studies of plant material were used to confirm the authenticity of the herb material. Although these studies are effective and cheap tools for the identification of adulteration (Hanani & Saidah, 2019). These analyses play a key role in the establishment of quality control protocols for medicinal plants. Therefore, the outcomes of physiochemical parameters are based on standard methods. The findings revealed that the percentage of loss due to drying or moisture content was 8.5 %. The lower the moisture content, the less chance of bacterial, fungal, or yeast growth. Our finding are in consistence with the results published by Đurović et al., 2017 that a percentage of moisture less than 12 % is considered to be acceptable quality and sufficient for the analysis (Đurović et al., 2017). Also, the leaves powder contained an ash value of 17.35 % which is considered as a critical quantitative tool for determining the validity and purity in medicinal plant. The water-soluble ash was found to be 3.695 ± 0.005 % which is used to evaluate the percentage of inorganic compounds present in plant while the acid- insoluble ash was found to have a low percentage of 1.72 ± 0.02 %, an induction of contamination with earthy substance mainly silica. In this research, the extractive value of water was the highest followed by the ethanolic alcohol value as this value provides information on the plant's phytoconstituents and their composition depending on the nature of these plants and the type of solvent (Kumar et al., 2012a; Mukhi et al., 2016).

The fluorescence properties of crude powdered plant and different extracts were investigated under UV radiation of long (365 nm) and short (254 nm) wavelengths, as well as under visible light. The most important characteristic of fluorescence is that UV radiation causes fluorescence in various natural compounds that would not be visible in natural daylight (Adham, 2015).The powdered *U. dioica* L. leaves emitted various colour radiations when exposed to various reagents and viewed under UV and normal light. The colour change in the crude powder and each extract was distinct and repeatable, indicating the solvent characteristics of phytoconstituents therefore, the fluorescence test is considered as an alternative method for identifying the suspicious sample when physical and chemical methods for identifying and distinguishing plant materials from their adulterants are insufficient. This analysis may be helpful in the detection of adulterants (Kumar et al., 2012b).

Our results showed that *U. dioica* L. contains high concentrations of calcium, magnesium and iron followed by zinc, manganese and copper respectively. These variations either in the plant's chemical composition, or the contents of selected minerals are determined by the harvest time, the soil type and the plant being grown in the shade or exposure to the sun (Đurović et al., 2017). Our results are consistent with the findings published by Đurović et al., 2017; Jaja et al., 2022; Kara, 2009; Rafajlovska et al., 2013) showing that calcium is the major macro-elements in the *U. dioica* L. followed by magnesium while other elements copper, zinc, and manganese were within the physiological levels. Iron is

considered the most significant trace element in *U. dioica* L. a higher concentration of both zinc and iron makes it suitable for use in diets for people who are in the risk for osteoporosis or cardiovascular disorders.

5. CONCLUSION:

Our research revealed that among the several solvents used to extract U. dioica L. leaves, water extract gave the highest yield in terms of percentage, while n-hexane provided the lowest yield. The phytochemical analysis of Urtica leaves showed differences in the content of bioactive compounds among five solvents used which indicated the presence of terpenoids, phenols, alkaloids, tannins, saponins, proteins, carbohydrates, and flavonoids that have potential as medicinal agents. The fluorescence analysis of powdered *U. dioica* L. leaves using a variety of chemical reagents, each reagent produced different colour under UV light and visible.

Our research also presented the analysis of physiochemical such as moisture, total ash, sulphated ash, total solid, water soluble and acid insoluble ash, total protein, crude fibre and crude fat. Atomic absorption spectroscopy was used to determine the elements in *U. dioica* L. leaves, and the results showed that calcium, iron, and magnesium were the highest concentrations among other elements. These findings maintain the traditional applications of this plant in medicine and demonstrated that this herb has the potential to be exploited as a source of multi-resistant drugs in the future.

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REFERENCES

A. Alasmary, F., Assirey, E. A., El-Meligy, R. M., Awaad, A. S., El-sawaf, L. A., Allah, M. M., & Alqasoumi, S. I. (2019). Analysis of Alpina officinarum Hance, chemically and biologically. *Saudi Pharmaceutical Journal*, *27*(8), 1107–1112.

Adham, A. N. (2015). Comparative extraction methods, phytochemical constituents, fluorescence analysis and HPLC validation of rosmarinic acid content in *Mentha piperita*, *Mentha longifolia* and *Osimum basilicum*. *J. Pharmacogn. Phytochem*, *3*(6), 130–139.

Al–Maliki. (2012). Isolation and Identification of Phenols and an Alkaloidic Compound from *Matricaria chamomilla* Plant Flowers and Study of Their Medicinal Activity Against the Pathogenic Bacteria of Skin Infections. *Journal of Thi-Qar University*, *3*, 1–17.

Ahmad, S. (2008). *Introduction of plant constituents and their tests*.

Al-Saleem, M. S., Awaad, A. S., Alothman, M. R., & Alqasoumi, S. I. (2018). Phytochemical standardization and biological activities of certain desert plants growing in Saudi Arabia. *Saudi Pharmaceutical Journal*, *26*(2), 198–204.

Al-Tameme, H. J., Hadi, M. Y., & Hameed, I. H. (2015). Phytochemical analysis of *Urtica dioica* leaves by fourier-transform infrared spectroscopy and gas chromatography-mass spectrometry. *Journal of Pharmacognosy and Phytotherapy*, *7*(10), 238–252.

Auwal, M. S., Saka, S., Mairiga, I. A., Sanda, K. A., Shuaibu, A., & Ibrahim, A. (2014). Preliminary phytochemical and elemental analysis of aqueous and fractionated pod extracts of *Acacia nilotica* (*Thorn mimosa*). *Veterinary Research Forum: An International Quarterly Journal*, *5*(2), 95.

Bahmani, M., Zargaran, A., & Rafieian-Kopaei, M. (2014). Identification of medicinal plants of Urmia for treatment of gastrointestinal disorders. *Revista Brasileira de Farmacognosia*, *24*(4), 468–480.

Bandar, H., Hijazi, A., Rammal, H., Hachem, A., Saad, Z., & Badran, B. (2013). Techniques for the extraction of bioactive compounds from Lebanese *Urtica dioica*. *American Journal of Phytomedicine and Clinical Therapeutics*, *1*(6), 507–513.

Basumatary, A. R. (2016). Preliminary phytochemical screening of some compounds from plant stem bark extracts of *Tabernaemontana divaricata* Linn. used by Bodo Community at Kokrajhar District, Assam, India. *Archives of Applied Science Research*, *8*(8), 47–52.

Bhargava, V. V, Saluja, A. K., & Dholwani, K. K. (2013). Detection of Heavy Metal Contents and Proximate Analysis of roots of *Anogeissus latifolia*. *Journal of Pharmacognosy and Phytochemistry*, *1*(6), 61–65.

Bhusal, K. K., Magar, S. K., Thapa, R., Lamsal, A., Bhandari, S., Maharjan, R., Shrestha, S., & Shrestha, J. (2022). Nutritional and pharmacological importance of stinging nettle (*Urtica dioica* L.): A review. *Heliyon*, e09717.

Cardoso, J. C., Oliveira, M. E., & Cardoso, F. de C. I. (2019). Advances and challenges on the in vitro production of secondary metabolites from medicinal plants. *Horticultura Brasileira*, *37*(2), 124–132.

Chooranam, V. (2017). Pharmacognostical and preliminary phytochemical screening of *aavaarai*.. *Asian J Pharm Clin Res*, *10*(10), 111–116.

De Silva, G. O., Abeysundara, A. T., & Aponso, M. M. W. (2017). Extraction methods, qualitative and quantitative techniques for screening of phytochemicals from plants. *American Journal of Essential Oils and Natural Products*, *5*(2), 29–32.

Devkota, H. P., Paudel, K. R., Khanal, S., Baral, A., Panth, N., Adhikari-Devkota, A., Jha, N. K., Das, N., Singh, S. K., & Chellappan, D. K. (2022). Stinging nettle (*Urtica dioica* L.): Nutritional composition, bioactive compounds, and food functional properties. *Molecules*, *27*(16), 5219.

Đurović, S., Pavlić, B., Šorgić, S., Popov, S., Savić, S., Petronijević, M., Radojković, M., Cvetanović, A., & Zeković, Z. (2017). Chemical composition of stinging nettle leaves obtained by different analytical approaches. *Journal of Functional Foods*, *32*, 18–26.

Faizy, H. S., Esmail, L. S., & Mahdi, H. S. (2021). Phytochemicals analysis in Watercress (*Nasturtium officinale*) plant extracts. *IOP Conference Series: Earth and Environmental Science*, *761*(1), 12042.

Farhan, H., Rammal, H., Hijazi, A., Hamad, H., Daher, A., Reda, M., & Badran, B. (2012). In vitro antioxidant activity of ethanolic and aqueous extracts from crude *Malva parviflora* L. grown in Lebanon. *Asian J Pharm Clin Res*, *5*(3), 234–238.

George, H. L., Davies, F. S., Crane, J. H., & Schaffer, B. (2002). Root temperature effects on 'Arkin'carambola (*Averrhoa carambola* L.) trees: II. Growth and mineral nutrition. *Scientia Horticulturae*, *96*(1–4), 67–79.

Gul, R., Jan, S. U., Faridullah, S., Sherani, S., & Jahan, N. (2017). Preliminary phytochemical screening, quantitative analysis of alkaloids, and antioxidant activity of crude plant extracts from *Ephedra intermedia* indigenous to Balochistan. *The Scientific World Journal*, *2017*.

Hanani, E., & Saidah, N. (2019). Pharmacognostical, Preliminary Phytochemical Evaluation and Flavonoids Content of *Paederia foetida* Linn. *Research Journal of Pharmacognosy and Phytochemistry*, *11*(2), 81–86.

Harborne, J. B. (1984). Methods of plant analysis. In *Phytochemical methods* (pp. 1–36). Springer.

Ilahi, F., Hanifa, D., & Batubara, I. (2021). Evaluation and Determination of Total Antioxidant in Anting-Anting (*Acalypha indica* L.) Leaf Extract. *IOP Conference Series: Earth and Environmental Science*, *757*(1), 12061.

Indumathi, C., Durgadevi, G., Nithyavani, S., & Gayathri, P. K. (2014). Estimation of terpenoid content and its antimicrobial property in *Enicostemma litorrale. Int J ChemTech Res*, *6*(9), 4264–4267.

Iram, N. (1995). *20503. Instituto de Racionalización de Materiales*. Yerba.

Jaja, N., Codling, E. E., Rutto, L. K., Timlin, D., & Reddy, V. R. (2022). Poultry Litter and Inorganic Fertilization: Effects on Biomass Yield, Metal and Nutrient Concentration of Three Mixed-Season Perennial Forages. *Agronomy*, *12*(3), 570.

Joshi, B. C., Mukhija, M., & Kalia, A. N. (2014). Pharmacognostical review of *Urtica dioica* L. *International Journal of Green Pharmacy (IJGP)*, *8*(4).

Joshi, B. C., & Uniyal, S. (2017). Establishment of quality control protocols and antioxidant activity of *Urtica dioica* L. *Journal of Conventional Knowledge and Holistic Health*, *1*(1), 1-6p.

Joshi, B., Sah, G. P., Basnet, B. B., Bhatt, M. R., Sharma, D., Subedi, K., Janardhan, P., & Malla, R. (2011). Phytochemical extraction and antimicrobial properties of different medicinal plants: *Ocimum sanctum (Tulsi), Eugenia caryophyllata (Clove), Achyranthes bidentata (Datiwan)* and *Azadirachta indica (Neem*). *Journal of Microbiology and Antimicrobials*, *3*(1), 1–7.

Kannan, D., Mehra, R. S., Dubey, S., Tiwari, S., Maheshwari, U., & Bisht, V. S. (2013). Evaluation of phytochemical constituents, antibacterial activities, cytopathic and cytotoxic effects of extracts of *Tylophora indica*, *Curcuma amada* and *Urtica dioica*. *J Rec Adv In App Sci*, *28*(1), 1–11.

Kara, D. (2009). Evaluation of trace metal concentrations in some herbs and herbal teas by principal component analysis. *Food Chemistry*, *114*(1), 347–354.

Karlsen, J. (2017). Quality control and instrumental analysis of plant extracts. In *The Medicinal Plant Industry* (pp. 99–106). Routledge.

Kataki, M. S., Murugamani, V., Rajkumari, A., Mehra, P. S., Awasthi, D., & Yadav, R. S. (2012). Antioxidant, hepatoprotective, and anthelmintic activities of methanol extract of *Urtica dioica* L. leaves. *Pharmaceutical Crops*, *3*(1), 38–46.

Khan, A. M., Qureshi, R. A., Ullah, F., Gilani, S. A., Nosheen, A., Sahreen, S., Laghari, M. K., Laghari, M. Y., Shafiq-Ur-Rehman, Hussain, I., & Murad, W. (2011). Phytochemical analysis of selected medicinal plants of Margalla hills and surroundings. *Journal of Medicinal Plant Research*, *5*(25), 6017–6023.

Khare, C. P. (2008). Indian medicinal plants*: an illustrated dictionary*. Springer Science & Business Media.

Krishna, T. P. A., Krishna, T. P. A., Kumuthakallavalli, R., Raj, V. N. S., Juliet, S., Rani, T. S., Darsana, U., Nair, S. N., & Ravindran, R. (2014). Physico-chemical evaluation and biochemical quantification of crude drug powder (stem) of *Chassalia curviflora* (Wall. ex Kurz.) Thwaites; A folk medicinal plant. *Journal of Pharmacognosy and Phytochemistry*, *3*(4), 121–124.

Krystofova, O., Adam, V., Babula, P., Zehnalek, J., Beklova, M., Havel, L., & Kizek, R. (2010). Effects of various doses of selenite on stinging nettle (*Urtica dioica* L.). *International Journal of Environmental Research and Public Health*, *7*(10), 3804–3815.

Kumar, A., Jha, K. K., Kumar, D., Agrawal, A., & Gupta, A. (2012a). Preliminary Phytochemical Analysis of Leaf and Bark (Mixture) Extract of Ficus Infectoria Plant. *1*(5), 71–76.

Kumar, R. S., Venkateshwar, C., Samuel, G., & Rao, S. G. (2013). Phytochemical Screening of some compounds from plant leaf extracts of *Holoptelea integrifolia (Planch* .) and *Celestrus emarginata (Grah* .) used by Gondu tribes at Adilabad District ,. *2*(8), 65–70.

Kumar, S. V., Sanghai, D. B., Rao, C. M., & Shreedhara, C. S. (2012b). Histological and physiochemical standardization of *Melia azedarach*. Linn bark. *Asian Pacific Journal of Tropical Biomedicine*, *2*(1), S284–S289.

Maobe, M. A. G., Nyarango, R. M., & Box, P. O. (2013). Fourier transformer infra-red spectrophotometer analysis of Urtica dioica medicinal herb used for the treatment of diabetes, malaria and pneumonia in Kisii region, Southwest Kenya. *World Applied Sciences Journal*, *21*(8), 1128–1135.

Mishra, A., & Kharel, G. P. (2010). Preservation and Quality Evaluation of Sisnu (*Urtica plaviflora*) by making Gundruk like fermented product. *Journal of Food Science and Technology Nepal*, *6*, 114–117.

Mukhi, S., Bose, A., Panda, P., & Rao, M. M. (2016). Pharmacognostic, physicochemical and chromatographic characterization of *Samasharkara Churna*. *Journal of Ayurveda and Integrative Medicine*, *7*(2), 88–99. https://doi.org/10.1016/j.jaim.2015.11.004

Muttalib, L. Y., & Naqishbandi, A. M. (2013). Antimicrobial and preliminary phytochemical investigations of some traditional medicinal plants in iraqi kurdistan. *Journal of Advanced Biomedical & Pathobiology Research Vol*, *3*(3), 52–58.

Nayak, A. K., Pal, D., & Santra, K. (2015). Screening of polysaccharides from tamarind, fenugreek and jackfruit seeds as pharmaceutical excipients. *International Journal of Biological Macromolecules*, *79*, 756–760.

Pathmanathan, M. K., Uthayarasa, K., Jeyadevan, J. P., & Jeyaseelan, E. C. (2010). In vitro antibacterial activity and phytochemical analysis of some selected medicinal plants. *Int J Pharm Biol Arch*, *1*(3), 291–299.

Rafajlovska, V., Kavrakovski, Z., Simonovska, J., & Srbinoska, M. (2013). Determination of protein and mineral contents in stinging nettle. *Quality of Life*, *7*(1–2).

Rafiee, H., Mehrafarin, A., Labbafi, M., Qaderi, A., & Badi, H. N. (2015). Mineral elements and biochemical analysis of *Calendula officinalis* L. affected by bio-stimulators. *Trakia Journal of Sciences*, *13*(1), 27–35.

Ray, S., Chatterjee, S., & Chakrabarti, C. S. (2013). Antiprolifertive activity of allelochemicals present in aqueous extract of *Synedrella nodiflora* (L.) Gaertn. in apical meristems and Wistar rat bone marrow cells. *IOSR Journal of Pharmacy*, *3*(2), 1–10.

Ri, H., Kim, C., Pak, U., Kang, M., & Kim, T. (2019). Effect of different polarity solvents on total phenols and flavonoids content, and In-vitro antioxidant properties of flowers extract from *Aurea Helianthu*s. *ArXiv Preprint ArXiv:1906.12006*.

Roghini, R., & Vijayalakshmi, K. (2018). Phytochemical Screening, Quantitative Analysis of Flavonoids and Minerals in Ethanolic Extract of *Citrus Paradisi*. *International Journal of Pharmaceutical Sciences and Research*, *9*(11), 4859.

Salmerón-Manzano, E., Garrido-Cardenas, J. A., & Manzano-Agugliaro, F. (2020). Worldwide research trends on medicinal plants. *International Journal of Environmental Research and Public Health*, *17*(10), 3376.

Savithramma, N., Rao, M. L., & Suhrulatha, D. (2011). Screening of medicinal plants for secondary metabolites. *Middle-East Journal of Scientific Research*, *8*(3), 579–584.

Sayed-ahmad, B., Hijazi, A., Fayyad, K. H., Rammal, H., Kobeissy, A., & Badran, B. (2014). Extraction, phytochemical screening, chemical quantification and identification of bioactive compounds from Lebanese *Urtica dioica*. *Am J Pharm Tech Res*, *4*(2), 591–604.

Shaikh, J. R., & Patil, M. (2020). Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies*, *8*(2), 603–608.

Sheel, R., Nisha, K., & Kumar, J. (2014). Preliminary phytochemical screening of methanolic extract of *Clerodendron infortunatum*. *IOSR Journal of Applied Chemistry*, *7*(1), 10–13.

Singh, S. (2012). Phytochemical analysis of different parts of *Prosopis juliflora*. *Int. J. Curr. Pharm. Res*, *4*(3), 59–61.

Singh, V., & Kumar, R. (2017). Study of phytochemical analysis and antioxidant activity of *Allium sativum* of Bundelkhand region. *International Journal of Life-Sciences Scientific Research*, *3*(6), 1451–1458.

Sorescu, A.-A., Nuta, A., Ion, R.-M., & Iancu, L. (2018). Qualitative analysis of phytochemicals from sea *buckthorn* and *gooseberry*. *Phytochemicals–Source of Antioxidants and Role in Disease Prevention*, 161–177.

Subakanmani, S., & Umadevi, P. (2012). Physicochemical Characterization and Phytochemical Analysis of Aerial Parts of Hypericum hookerianum. *5*(3), 1387–1391.

Thendral, T., & Lakshmi, T. (2017). Antifungal activity of *Acacia catechu* bark extract against dermatophytes: An in vitro study. *Journal of Advanced Pharmacy Education and Research*, *7*(1), 25–27.

Tiwari, P., Kumar, B., Kaur, M., Kaur, G., & Kaur, H. (2011). Phytochemical screening and extraction: a review. *Internationale Pharmaceutica Sciencia*, *1*(1), 98–106.

Trishala, A., Lakshmi, T., Rajeshkumar, S., Gurunathan, D., & Roy, A. (2019). Physicochemical Profile of *Acacia Catechu* Bark Extract--An Invitro Study. *Indian Journal of Public Health Research & Development*, *10*(11).

Tyagi, T. (2017). Phytochemical screening of active metabolites present in *Eichhornia Crassipes* (Mart.) *Solms* and *Pistia* Stratiotes (L.): Role in ethanomedicine. *Asian Journal of Pharmaceutical Education and Research*, *6*(4), 40–56.

Ukoha, P. O., Cemaluk, E. A. C., Nnamdi, O. L., & Madus, E. P. (2011). Tannins and other phytochemical of the *Samanaea saman pods* and their antimicrobial activities. *African Journal of Pure and Applied Chemistry*, *5*(8), 237–244.

Wang, G.-X., Zhou, Z., Jiang, D.-X., Han, J., Wang, J.-F., Zhao, L.-W., & Li, J. (2010). In vivo anthelmintic activity of five alkaloids from *Macleaya microcarpa* (Maxim) Fedde against Dactylogyrus intermedius in Carassius auratus. *Veterinary Parasitology*, *171*(3–4), 305–313.

Yadav, S., & Kumar, P. (2012). Production, isolation and identification of flavonoids from aerial parts of *Hiptage benghalensis*. *Int J Life Sci Pharma Res*, *2*(3), 1–5.

Zouari Bouassida, K., Bardaa, S., Khimiri, M., Rebaii, T., Tounsi, S., Jlaiel, L., & Trigui, M. (2017). Exploring the *Urtica dioica* leaves hemostatic and wound-healing potential. *BioMed Research International*, *2017*.