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Gas Chromatography-Mass Spectrometry and *In vitro* Ethnopharmacological Investigations of the Solvent Ethyl Acetate Extract of *Urtica dioica* L. Leaf from Wokha, Nagaland, India

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Abstract
Urtica dioica L, a plant species belonging to the Urticaceae family, has been documented from the Wokha district of Nagaland, India. The highest level of antibacterial activity observed in the solvent ethyl acetate extracted plant with regards to the zone of inhibition was 17.33.±1.52, which was exhibited against *Salmonella typhimurium* at 100 µg/mL concentration. The potent IC_{50} values in ABTS assay was found to be 23.69±0.32 µg/mL for the ethyl acetate extract. Similarly, the IC_{50} values for the HRSA assay were determined to be 24.66±0.38 µg/mL in ethyl acetate extract. The solvent extract of ethyl acetate exhibited the IC_{50} values for α-glucosidase, measuring 16.60±0.63. A Gas Chromatography-Mass Spectrometry study was conducted to identify the component present in the solvent ethyl acetate leaf extract of *Urtica dioica*. A total of thirty bioactive phytochemical components were detected in the ethyl acetate extract of *Urtica dioica*. This findings may potentially facilitate the creation of innovative pharmaceutical agents.

Keywords: *Urtica dioica*, GC-MS, α-glucosidase, antioxidant, antibacterial, Wokha

1. Introduction
Plants possess significant potential as a valuable reservoir of natural components and biologically active chemicals. Throughout the course of history, medicinal plants and herbs have been extensively utilized in culinary practises as both flavour enhancers and natural preservatives. *Urtica dioica* L, a perennial plant belonging to the Urticaceae family, and its several subspecies exhibit growth patterns in both temperate and tropical regions in Europe, Asia, North and South America, as well as Africa. It belongs to a family Urticaceae of 40 genera and approximately 500 species. The plant is an herbaceous species that typically reaches a height of 0.6-1.2 metres. According to research findings, it has been observed that the aerial components of nettle possess a crude fat content ranging from 2.5% to 3.6%, crude protein content ranging from 18% to 34%, crude fibre content of 9%, total ash content of 16%, and carbohydrate content of 37% in dry matter [1, 2]. The leaves include a variety of vitamins, including vitamins C, B, and K, as well as carotenoids such as β carotene, hydroxy β carotene, lutoxanthin, lutein epoxide, and violaxanthin [3, 4]. It thrives in damp soil that is weakly acidic or weakly basic and rich in nitrogen. The utilization of the plant in traditional medicine spans several centuries, serving as a remedy for a diverse range of ailments, including arthritis, rheumatism, and eczema [5]. The consumption of plant-derived antioxidants has the potential to mitigate oxidative damage resulting from the presence of free radicals and active oxygen. Tarasevičienė *et al.* [6], have identified a number of compounds from the nettle herb, such as quercetin, trans-ferulic acid, beta-sitosterol, erucic acid, dotriacontane, ursolic acid, scopoletin, and rutin. According to Ogla *et al.* [7], the sole plant species of *U.dioica* possesses choline acetyltransferase, which is responsible for the synthesis of acetylcholine. Despite being classified as a weed in the field of agriculture, its multifaceted applications span across human and animal nutrition, medicinal practises, and crop preservation. The indigenous systems of medicine in India, particularly Ayurveda, Siddha, and Unani, have a long-standing history spanning several centuries. Various sections of medicinal plants, which comprise over 80,000 species, have been utilized as traditional remedies within different systems of Indian medicine for the treatment of diverse ailments [8]. In the region of Nagaland, a significant proportion of the population relies on traditional and herbal treatments as opposed to western synthetic pharmaceuticals for addressing a wide range of illnesses.

Prior to the development of modern pharmaceuticals, humans frequently relied on the utilization of plants as primitive forms of medication. In this study, a conventional botanical species with therapeutic properties was chosen. The field of herbal studies encompasses the process of isolating and elucidating the chemical structures of chemicals found in plants, with the purpose of comprehending and assessing their therapeutic capabilities. Gas chromatography-mass spectrometry (GC-MS) is widely regarded as a very effective and efficient method for the detection of a diverse range of compounds. This technique is particularly adept at identifying alcohols, alkaloids, nitro compounds, long chain hydrocarbons, organic acids, steroids, esters, and amino acids [9]. Furthermore, GC-MS offers the advantage of requiring just a minimal volume of plant extracts for analysis. Therefore, the current study employed of our research is to enhance the antioxidant, antibacterial, and antidiabetic characteristics of various solvent extracts obtained from nettle, as well as the GC-MS technology to detect and identify bioactive chemicals derived from the ethyl acetate solvent extracts obtained from *U. dioica*. The purpose of this is to promote the organic preservation of food products and to serve medicinal purposes while minimizing the utilization of chemicals.

2. Materials and Methods

2.1 Study site

(A) (B) (C)

Fig 1: Map of study area; A. India, B. Nagaland, C. Wokha Town (Source: Google).

Wokha is situated at coordinate's 26.1°N 94.27°E [10], with an average altitude of 1,313 m (4,793 feet). The region exhibits a warm temperate climate. The summer temperature in Wokha exhibits a variation between 16.1 °C and 32 °C. The minimum temperature throughout the winter season is 2 °C. The mean annual temperature in Wokha is recorded as 17.8 °C. The mean annual precipitation is recorded as 1940 mm.

2.2 Plant Material and its descriptions

The plant species known as nettle, scientifically classified as *U. dioica* L., is a member of the Urticaceae family. It has been found to possess a wide range of therapeutic properties, as documented by [11]. *U.dioica* is a perennial herb characterised by the presence of opposite, cordate, and pointed leaves. The period of flowering and fruiting occurs between the months of June and October. Flowers exhibit monoecism, wherein individual flowers include either male or female reproductive structures, however both genders can coexist on the same plant. The plant exhibits the presence of stinging trichomes on both its stem and leaves. If the plants are touched without the use of protective gloves, they can induce skin irritation.

Fig 2: *Urtica dioica* L.

The leaves exhibit a simple structure, arranged in an opposite manner, with coarse teeth along the edges. The veins on the underside of the leaves are raised and have a few stinging hairs. The lower surface of the leaves is lighter in colour and

mostly lacks hair, while the upper surface is also mostly hairless. The petioles, which are 0.7-7 cm long, have grooves on the upper side and along the sides, and they are covered in stinging hairs. The stipules, which are pale green, measure 5-15 mm in length and 2-4 mm in width. They are paired, have an entire margin, pointed, erect, and hairy. The length between nodes ranges from 0.5-8.8 cm. The stem measures between 2-14 mm in thickness, with the lower portion being smooth, naked, and exhibiting a reddish purple coloration. The flowers exhibit a vibrant green coloration as they undergo the process of blooming. The inflorescence of the plant is in the form of a panicle, measuring 2-8 cm in length, and emerges from the top leaf axils. The flower clusters are unisexual. The fruits exhibit a single seed and measuring approximately 1-1.5mm in length, 0.7-0.9mm in width, and 0.3mm in thickness. The seed exhibits a tan coloration and occupies the entirety of the fruit. The rhizomes have a cylindrical shape with a tapering structure, occasionally displaying branching patterns. They possess a diameter of around 6 mm at the upper end. The root exhibits a greyish-brown coloration and displays an unusual twisted shape. It has a thickness of around 5mm and is characterised by distinct longitudinal furrows. When observed in cross-section, the root appears hollow, while its cut surface appears white [12, 13].

2.3 Plant collection and extraction

The present study utilized an ethnomedicinal and traditional perspective to gather information on the herb *U.dioica* traditional and medicinal usage by practitioners of Ayurveda. The information was acquired from the Botanical Survey of India (BSI) [14], specifically from the specimen with the barcode CAL0000013393, collected by Smith and Cane and literature article [15], was also reviewed. Fresh harvested leaf that was pathogen-free was carried out in January from the Wokha district located in the Nagaland district, India. The leaf underwent an initial purification process, subsequent desiccation in a shaded environment, and was afterwards transformed into granules with a coarse texture. The dried leaves were pulverised into a fine powder and then underwent solvent extraction using hexane, ethyl acetate, and methanol. The ratio of solvent to sample mass was 1:3, chosen based on the polarity of each solvent. The extraction method utilized a specific quantity of 750 g powder of *U. dioica*. The application of Whatman filter sheets was employed as a method to purify the extracts via the process of solvent evaporation. The extracted yield was calculated and subjected to a chilling process to ensure its preservation and integrity for any future research experiments.

2.4 Yield of leaf extracts

The extract yield is determined by calculating the ratio of the mass of the produced extract to the dry mass of the plant material that is subjected to treatment. The variables used in this study are denoted as follows:

% yield = [(weight of dried extract) / (weight of dried plant sample)] x 100

2.5. Assessment of antibacterial efficacy

2.5.1 Used microorganisms, inoculum preparation and method

The primary objective of the current investigation was to assess the antimicrobial efficacy of different solvent leaf extracts derived from *U. dioica* against a diverse range of five bacterial strains. The present investigation scrutinizes three discrete strains of gram-negative bacteria, namely *Salmonella typhimurium*, *Klebsiella pneumoniae* and *Shigella flexneri* and two kinds of gram-positive bacteria, specifically *Enterococcus faecalis*, and *Staphylococcus aureus*. The preservation of stock culture was carried out by storing it on nutrient agar slopes under a controlled temperature of 4 °C. A stock culture specimen was introduced into test tubes that were filled with Mueller Hinton broth (MHB). The tubes were then incubated at a temperature of 37 °C without any agitation for a duration of 24 hours. This process was carried out to obtain viable cultures that could be used for experimental purposes. The optical densities of all cultures were standardized to 2.0 x 106 colony forming units (CFU/mL) by diluting them with fresh MHB. The assessment of the extracts' inhibition activity was carried out utilizing the agar well diffusion technique [16]. The evaluation of the zone of inhibition was carried out by determining its diameter following a period of incubation of 24 hours at a temperature of 37 °C.

2.6 Evaluation of Antioxidant Activity

2.6.1 The ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) antioxidant assay [17].

The preparation of a stock solution involved the combination of potassium per sulphate ($K_2S_2O_8$) (140 mM, 440 µL) with deionized water (25 mL), resulting in the formation of ABTS (7mM). The amalgamation of the stock solution with extracts and standard ascorbic acid was performed at different concentrations spanning from 5-320 µg/mL. The resultant amalgamation was permitted to undergo incubation at ambient temperature for a period of 25 min, following which the degree of light absorption was gauged at a wavelength of 680 nm. The potential of botanical extracts to eradicate ABTS radical was assessed through the utilisation of the subsequent formula:

$[(A_0 - A_1)/A_0] \times 100$

The variables A_0 and A_1 have been assigned the roles of control and test, respectively.

2.6.2 The activity of scavenging hydroxyl radicals [18].

Different concentrations (ranging from 5-320 µg/mL) of solvent extracts derived from *U. dioica* leaves exhibited a combination of different elements separately with 1ml of iron-EDTA solution, which was composed of 0.13% ferrous ammonium sulphate and 0.26% EDTA. Additionally, 0.5 ml of EDTA solution (0.018%) and 1 ml of DMSO (0.85% v/v in 0.1 M phosphate buffer, pH 7.4) were added to the mixture. The study was initiated by the addition of 0.5 ml of ascorbic acid (0.22%) to the mixture, followed by incubation in a temperature-controlled water bath range of 80-90 °C for a duration of 15 min. After the incubation period, the reaction was concluded by the addition of 1 mL of Trichloroacetic acid (TCA), with a concentration of 17.5% (w/v) that had been previously cooled to a low temperature. A solution was prepared by combining 75.0 g of ammonium acetate, 3 mL of glacial acetic acid, and 2 mL of acetyl acetone, followed by dilution with distilled water to a final volume of 1 L. After the addition of three millilitres of Nash reagent, the resulting solution was exposed to duration of 15 min at ambient temperature. The experimental control was implemented by utilizing the reaction mixture in the absence of any sample. The measurement of colour intensity was conducted via

Spectrophotometry at a wavelength of 412 nm with reference to a reagent blank. The determination of the activity of scavenging hydroxyl radicals in percentage involves the formula:

HRSA (%) = [(OD of control - OD of test)/ (OD of control)] ×100

2.7 *In vitro* activity of antidiabetic agents.

2.7.1 α-glucosidase assay [19].

A substrate solution of p-nitrophenyl glucopyranoside (pNPG) was formulated utilizing a 100 mM phosphate buffer with a pH of 6.8. The extracts were pre-incubated with varying concentrations (10-320 µg/mL) and subsequently combined with a 200 µL solution of α-glycosidase for a period of 10 min. The enzymatic reaction was commenced by introducing 400µL of 5.0 mM pNPG, which was solubilized in 100 mM phosphate buffer, and consequently placed in an incubator set at ambient temperature for approximately 20 min. Subsequently, a volume of 1 mL of sodium carbonate (0.1 M) was added. The spectrophotometric analysis of the yellow-hued reaction mixture derived from pNPG involved measuring its absorbance at a wavelength of 405 nm, which facilitated the quantification of 4-nitrophenol liberation. Voglibose, a compound, was utilized as a positive control to evaluate the inhibitory activity of α-glycosidase through the utilization of plant extracts. The calculation of the percentage of inhibition was performed by the subsequent formula:

% Inhibition = [(Abs Control - Abs Sample) / Abs Control] x 100

2.8 Gas Chromatography Mass Spectrum (GC-MS) Analysis

The ethyl acetate solvent extract of *U. dioica* leaf was subjected to GC-MS analyses using the SHIMADZU QP2020 instrument The system was fitted with a fused silica column, namely the SH-RXi-5Sil MS capillary column, which had dimensions of 30 m in length, 250 µm in diameter, and 0.25 µm in thickness. The carrier gas employed in this study was pure helium gas with a purity of 99.99%. It was maintained at a constant flow rate of 1.20 mL/min. In order to perform GC-MS spectrum detection, a method utilizing electron ionisation energy was employed, with high ionization energy of 70 eV (electron Volts). The scan period was set at 0.30 seconds, and the detected fragments ranged from 50 to 500 m/z. A volume of 1 µL was employed for the injection (with a split ratio of 10:0), while the injector temperature was consistently maintained at 250 °C. The column oven temperature was initially set to 50 °C for duration of 5 min. Subsequently, the temperature was increased at a rate of 6 °C per min until it reached 280 °C. The identification of phytochemical in the test samples was conducted by comparing their retention time (in min), peak area, peak height, and mass spectral patterns with the spectral database of genuine compounds maintained by the National Institute of Standards and Technology (NIST) library 60.

3. Results and Discussion

3.1 Yield (%) solvent extracts leaf of *U. dioica*

The method of plant extraction offers valuable insights into the effectiveness of pharmaceuticals and allows for the measurement of active constituents within a certain amount of plant material extracted using a prescribed solvent. The study aimed to investigate the extractive value of the solvents hexane, ethyl acetate, and methanol in the leaf of *U. dioica*. The results indicated that the extractive values were found to be 0.26%, 2.66%, and 1.73%, respectively.

3.2 Antibacterial activity by agar well diffusion method

The abundance of antimicrobial agents notwithstanding, the prevalence of microorganisms that have developed resistance to these drugs is a cause for concern. Consequently, there is a pressing need for further investigation into alternative therapeutic options that are both more efficacious and less hazardous than extant treatments. The current investigation assessed the antibacterial effectiveness of several solvents in *U. dioica* extracts against both Gram-positive bacterial strains (*S. aureus* and *E. faecalis*) and Gram-negative bacterial strains (*K. pneumoniae*, *S. flexneri*, and *S. typhimurium*). The results obtained from our analysis suggest that the extracts tested showed varying levels of antibacterial activity against nearly all of the microorganisms studied. Significantly, the ethyl acetate extract exhibited the highest degree of efficacy compared to the other solvent extractions that were assessed. The plant extract exhibited a peak activity (zone of inhibition) of 17.33±1.52 mm against *S. typhimurium* when present at a concentration of 100 µL/mL in an ethyl acetate extract. The study's results also revealed that *S.typhimurium* exhibited the lowest level of activity, as demonstrated by the similar zone of inhibition measuring 10.00±0.57 (mm) when exposed to methanol extracts at a concentration of 50 µL/mL. However, the hexane and methanol extracts, when applied at a concentration of 50 µL/mL, demonstrated minimal or no antimicrobial activity against most of the tested bacteria, as shown by the lack of a zone of inhibition. The findings derived from the present study indicate that the ethyl acetate extract exhibited significantly higher antibacterial efficacy when compared to the hexane and methanol extracts. Additionally, the findings of the investigation demonstrated that the ethyl acetate extract shown a greater degree of antibacterial efficacy against gram-negative bacteria in comparison to gram-positive bacteria, as evidenced by the data presented in Tables 1, 2, and 3. The observed antibacterial properties can be attributed to its significant concentration of hydroxycinnamic acids, namely chlorogenic acid, caffeic acid, and rosmarinic acid, as well as flavonoid quercetin [20].

Table 1: Antibacterial activity (Zone of inhibition in mm) of *U.dioica* hexane extract.

Bacteria	Antibacterial activity Hexane extract (in mm)			
	50(µL /mL)	75(µL /mL)	100(µL /mL)	Streptomycin(10µg/disc)
E. faecalis	NA	13.00±1.73	14.33±0.57	18.33±3.51
S. aureus	NA	11.66±0.57	15.00±2.00	15.33±3.51
K. pneumoniae	NA	11.66±0.57	13.00±2.00	15.33±1.52
S. flexneri	NA	13.66±2.08	11.00±1.73	13.33±1.52
S. typhimurium	11.00±1.73	15.33±1.52	16.33±2.51	16.66±2.08

Values and mean of three different experiments were performed in triplicate and data are expressed as Mean ±SD. NA= no activity.

Table 2: Antibacterial activity (Zone of inhibition in mm) of *U.dioica* ethyl acetate extract.

Bacteria	Antibacterial activity Ethyl acetate extract (in mm)			
	50(µL /mL)	75(µL /mL)	100(µL /mL)	Streptomycin(10µg/disc)
E. faecalis	12.33±0.70	13.66±0.57	15.66±1.15	17.66±1.52
S. aureus	13.00±0.70	13.33±2.08	15.66±0.57	23.33±2.08
K. pneumoniae	12.33±0.00	13.66±1.15	14.66±0.57	18.33±2.08
S. flexneri	10.66±0.70	13.66±0.57	14.66±1.52	16.33±1.15
S. typhimurium	12.00±1.41	13.66±0.57	17.33±1.52	20.66±0.57

Values and mean of three different experiments were performed in triplicate and data are expressed as Mean ±SD.

Table 3: Antibacterial activity (Zone of inhibition in mm) of *U.dioica* methanol extract.

Bacteria	Antibacterial activity / Methanol extract (in mm)			
	50(µL /mL)	75(µL /mL)	100(µL /mL)	Streptomycin(10µg/disc)
E. faecalis	11.33±1.52	10.66±2.08	13.66±1.52	16.33±1.52
S. aureus	11.66±0.57	12.33±1.15	13.33±0.57	17.00±3.60
K. pneumoniae	10.66±1.15	11.33±1.52	12.33±1.52	14.33±1.15
S. flexneri	NA	10.66±1.52	11.33±2.08	13.33±2.08
S. typhimurium	10.00±0.57	11.33±2.51	14.66±1.15	19.33±1.15

Values and mean of three different experiments were performed in triplicate and data are expressed as Mean ±SD. NA= no activity

3.3 Antioxidant activity

3.3.1 ABTS and HRSA assay

Nettle extracts exhibit a capacity to neutralize reactive oxygen species (ROS). It has provided evidence that the methanolic and ethanolic extracts obtained from leaves possess antioxidant properties when tested against the DPPH [21]. The *Urtica* root extracts of methanol and direct-ethanol exhibited a free radical scavenging activity of 46.71% and 45.03%, respectively, at a concentration of 500 µg/ml [22]. The findings (table 4) of ABTS assay of the present study revealed that the ethyl acetate extract demonstrated the most significant antioxidant properties at a high dose of 136.81±0.41. The ABTS scavenging activities of the hexane and methanol extracts were found to be 89.97±0.37 and 113.49±0.36, respectively. The IC_{50} values of hexane, ethyl acetate, methanol, and the reference standard Ascorbic acid were documented as 41.15±0.49, 23.69±0.32, 28.68±0.20 and 19.12±0.25 µg/mL, respectively, in the study. The *U. dioica* plant exhibited varying antioxidant activity values when subjected to different extraction methods, as determined by DPPH and ABTS tests. Carvalho *et al.* [23], conducted a comparative analysis which revealed greater antioxidant properties in all assays, namely DPPH (2.89 g/100 g lyophilized), ABTS (2.60 TEAC), and FRAP (3.81 TEAC), as compared to the aerial parts of *U. membranacea* and *U. urens*. The findings of HRSA (table 4), revealed that the scavenging activities of the hexane, ethyl acetate, and methanol extracts were 118.09±0.42, 192.99±0.80, and 161.84±0.65, respectively. Notably, the ethyl acetate extract exhibited the most significant inhibition. The investigation ascertained the IC_{50} values of hexane, ethyl acetate, methanol, and the reference standard Ascorbic acid to be 31.93±2.15, 24.66±0.38, 26.09±1.92 and 18.13±1.33 µg/mL, respectively. The antioxidant properties of this substance have the potential to accelerate the healing process, minimize the prominence of scars and blemishes, while augmenting the efficacy of anti-aging properties by minimizing the appearance of wrinkles and age-related pigmentation.

Table 4: The IC_{50} values of *in vitro* antioxidant and antidiabetic assays of different solvent leaves extracts of *U. dioica*. Each value was obtained by calculating the average and data are expressed as mean of triplicates ± SD.

S. No.	Antioxidant assays	Hexane	Ethyl acetate	Methanol	Standard (Ascorbic acid)
1	ABTS	41.14±0.49	23.69±0.32	28.68±0.20	19.12±0.25
2	HRSA	31.93±2.15	24.66±0.38	26.09±1.92	18.13±1.33
3	α-glucosidase assay	29.74±0.95	16.60±0.63	19.93±0.37	6.07±0.96 (Voglibose)

3.4 Antidiabetic activity

3.4.1 α- amylase and α- glucosidase assay

Diabetes mellitus is a significant metabolic disorder which possesses the capacity to affect the central nervous system through various functional and morphological mechanisms [24]. A majority of the studies indicate the advantageous effects in managing hyperglycemia. Table 4 displays a comparative investigation of the inhibitory action exhibited by plant extracts and a conventional medication in relation to α-glucosidase. Chemical drugs such as voglibose, miglitol, and acarbose are utilised as inhibitors of α-glucosidase and α-amylase enzymes. One significant issue associated with these drugs pertains to their comparatively reduced hypoglycemic effect in comparison to other oral antidiabetic medications such as sulfonylureas. It has been suggested that conventional medications such as *U.dioica* exhibit a low level of inhibition on α-amylase, but a high level of inhibition on α-glucosidase [25]. The present study employs Voglibose as a standard medication for inhibiting α-glucosidase, and our results support previous research.

The inhibitory activity of the plant's hexane, ethyl acetate, and methanol extracts was noteworthy, displaying a dose-dependent increase within 10-320 µg/mL range. The study determined the concentrations required for 50% inhibition of hexane, ethyl acetate, and methanol found to be 29.74±0.95, 16.60±0.63, and 19.93±0.37 µg/mL, respectively. In comparison, the standard Voglibose exhibited an IC_{50} value of approximately 6.07±0.96 µg/mL, indicating its superior efficacy relative to the other extracts under investigation (Table 4). Metformin treatment has been associated with the occurrence of lactic acidosis in patients who have cardiac and liver diseases. Research has demonstrated that sulfonylureas are associated with inducing hypoglycemia in elderly individuals with diabetes. Chemical medications have been

found to exhibit adverse effects. For instance, thiazolidinedione (TZD) has been associated with obesity and fat accumulation [26].

As such, scholars priorities the exploration of alternative medicinal sources, such as herbal remedies, that exhibit reduced adverse effects. Furthermore, the pursuit of novel anti-diabetic therapies derived from botanical sources is highly appealing due to their minimal adverse effects, affordability, widespread availability, and notable efficacy.

3.5 Gas Chromatography Mass Spectrometry (GC-MS) analysis of *U. dioica*

Bioactive are chemical substances that are commonly known as secondary metabolites. The compounds present in the solvent ethyl acetate extract of *U. dioica* was identified through a method that involved comparing the retention times and mass spectral data of the compounds with those of standard compounds. Additionally, computer correspondence with the Wiley and NIST libraries was utilised for identification purposes. Figure 3 and table 5 exhibit the bioactive chemical compounds' identification, which is determined by factors such as peak area, retention duration, molecular weight, molecular structure, and molecular formula. A total of thirty bioactive components were detected in the ethyl acetate leaf extract of *U. dioica*.

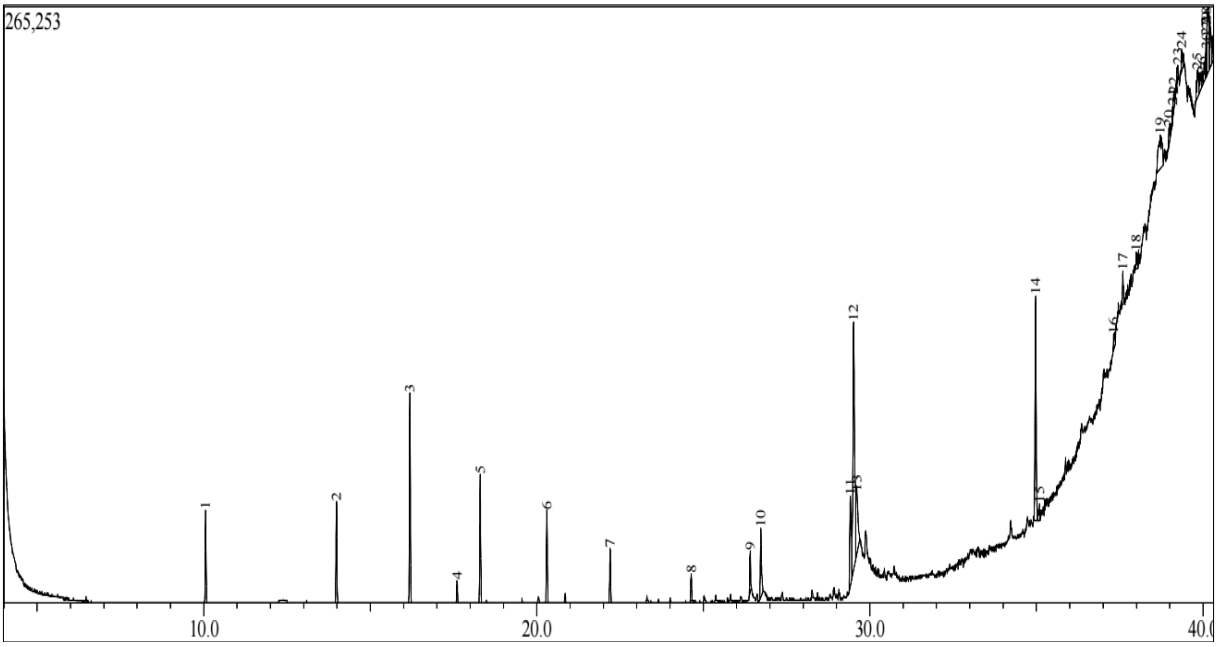
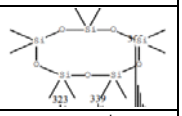
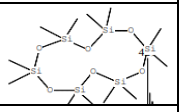
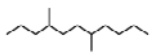
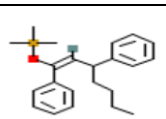
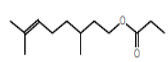
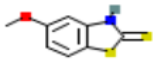
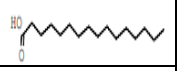
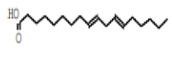
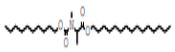
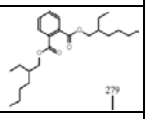
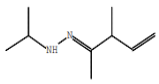
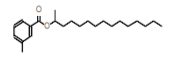
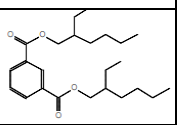
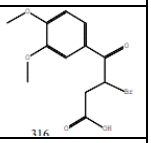
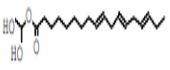
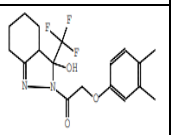
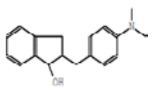
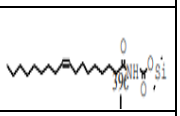
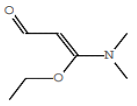
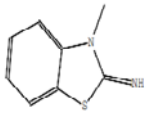
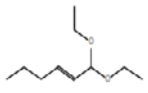
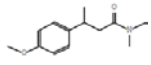


Fig 3: GC-MS chromatogram of solvent ethyl acetate leaf extract of *U. dioica.*

Table 5: The list of 30 phytoconstituents identified from solvent ethyl acetate leaf extract of *U. dioica* through GC-MS studies.

S. No	Name of compounds	Molecular formula	Molecular weight	Retention time(min)	Peak area %	Structure
1	cyclopentasiloxane, decamethyl-	$C_{10}H_{30}O_5Si_5$	370	10.059	3.27	
2	cyclohexasiloxane, dodecamethyl- (d6)	$C_{12}H_{36}O_6Si_6$	444	13.988	3.87	
3	undecane, 4,7 dimethyl-	$C_{13}H_{28}$	184	16.191	7.87	
4	1,3-diphenyl-1-((trimethylsilyl)oxy)-1(z)-heptene	$C_{22}H_{30}OSi$	338	17.607	0.75	
5	octadecane	$C_{18}H_{38}$	254	18.301	4.75	
6	octadecane	$C_{18}H_{38}$	254	20.303	3.62	
7	octadecane	$C_{18}H_{38}$	254	22.202	2.09	
8	6-octen-1-ol, 3,7-dimethyl-, propanoate	$C_{13}H_{24}O_2$	212	24.635	0.92	

9	3(hydroxymethyl)2thioxobenzothiazoline	$C_8H_7NOS_2$	197	26.409	1.4	
10	hexadecanoic acid	$C_{16}H_{32}O_2$	256	26.724	3.28	
11	9,12-octadecadienoic acid (z,z)-	$C_{18}H_{32}O_2$	280	29.413	5.87	
12	oleic acid	$C_{18}H_{34}O_2$	282	29.51	17.25	
13	dl-alanine, n-methyl-n-decyloxycarbonyl-, pentadecyl ester	$C_{30}H_{59}NO_4$	497	29.61	5.66	
14	1,2-benzenedicarboxylic acid	$C_{24}H_{38}O_4$	390	34.974	10.77	
15	4-penten-2-one, 3-methyl-, isopropylhydrazone	$C_9H_{18}N_2$	154	35.09	0.8	
16	m-toluic acid, 2-pentadecyl ester	$C_{23}H_{38}O_2$	346	37.315	0.79	
17	1,3benzenedicarboxylic acid, bis(2-ethylhexyl) ester	$C_{24}H_{38}O_4$	390	37.594	1.18	
18	benzenebutanoic acid,.beta.bromo-3,4dimethoxygamma.-oxo-	$C_{12}H_{13}BrO_5$	316	37.99	0.91	
19	linolenic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (z,z,z)-	$C_{21}H_{36}O_4$	352	38.716	5.04	
20	2-pyrazoline,3,4-tetramethylene-5-hydroxy-5-trifluoromethyl-1-(3,4dimethylphenoxyacetyl)-	$C_{18}H_{21}F_3N_2O_3$	370	38.975	1.23	
21	2-(4-dimethylamino-benzyl)-indan-1-ol	$C_{18}H_{21}NO$	267	39.085	0.29	
22	n-oleoyl-l-glycine,trimethylsilyl ester	$C_{23}H_{45}NO_3Si$	411	39.115	1.32	
23	2-propenal, 3-(dimethylamino)-3-ethoxy-	$C_7H_{13}NO_2$	143	39.243	0.7	
24	2(3h)-benzothiazolimine, 3-methyl-	$C_8H_8N_2S$	164	39.36	1.18	
25	2-hexene, 1,1-diethoxy-	$C_{10}H_{20}O_2$	172	39.822	1.95	
26	3-(4-methoxyphenyl)-n,n-dimethylbutanamide	$C_{13}H_{19}NO_2$	221	39.965	2.93	

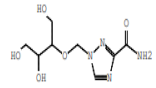
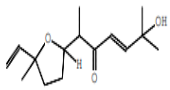
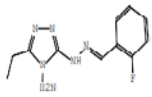
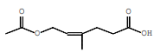
27	2r,3s-1-[1,3,4-trihydroxy-2-butoxymethyl]-1,2,4-triazole-3-carboxamide	$C_8H_{14}N_4O_5$	246	40.09	1.14	
28	(s,e)-6-hydroxy-6-methyl-2-((2s,5r)-5-methyl-5vinyltetrahydrofuran-2-yl)hept-4-en-3-one	$C_{15}H_{24}O_3$	252	40.146	5.52	
29	2fluorobenzaldehyde (4-amino-5-ethyl-4h-1,2,4-triazol-3-yl)hydrazone	$C_{11}H_{13}FN_6$	248	40.235	2.69	
30	4-hexenoic acid, 6-(acetyloxy)-4-methyl-	$C_9H_{14}O_4$	186	40.295	0.96	

Table 6 presents comprehensive data regarding the reliability and legitimacy of the chemicals found in *U.dioica*, including cyclopentasiloxane, decamethyl, cyclohexasiloxane, dodecam ethyl, undecane, 4,7-dimethyl, 1,3-diphenyl-1-((trimethylsilyl)oxy)-1(z)-heptene, octadecane, 6-octen-1-ol, 3,7-dimethyl-, propanoate, hexadecanoic acid, 9,12-octadecadienoic acid (z,z), oleic acid, 1,3-benzenedicarboxylic acid, bis (2-ethylhexyl) ester and (s,e)-6-hydroxy-6-methyl-2-((2s,5r)-5-methyl-5-vinyltetrahydrofuran-2-yl) hept-4-en-3-one, which play a significant role in the plant's pharmacological activities. This information serves to substantiate the effectiveness of *U. dioica* as an active agent in ethnopharmacology. The findings of this investigation align with the information presented in these reports. The GC-MS analysis of the ethyl acetate extract of *U. dioica* leaf revealed that a substantial amount of the compounds has antimicrobial, antibacterial, and antioxidant activities.

Table 6: Bioactive compounds identified in the solvent ethyl acetate extracts of *U. dioica* leaf.

Sl. No	Name of compounds	Biological activity	References
1	cyclopentasiloxane, decamethyl	antimicrobial	[27]
2	cyclohexasiloxane, dodecamethyl- (d6)	hair/skin care products, antiperspirants, and deodorants; antibacterial and antifungal	[28]
3	undecane, 4,7 dimethyl	skin inflammatory disorders	[29]
4	1,3-diphenyl-1-((trimethylsilyl)oxy)-1(z)-heptene	antibacterial	[30]
5	octadecane	antimicrobial	[31, 32]
6	6-octen-1-ol, 3,7-dimethyl-, propanoate	fragrance, flavour	[33]
7	hexadecanoic acid	antioxidant, hypocholesterolemic, nematicide, pesticide	[34]
8	9,12-octadecadienoic acid (z,z)-	antibacterial, antiviral and antioxidant properties	[3]
9	oleic acid	antimicrobial, antioxidant, and apoptotic activity	[36-38]
10	1,2-benzenedicarboxylic acid	antibacterial, antifungal	[39]
11	1,3-benzenedicarboxylic acid, bis(2-ethylhexyl) ester	anticancer/antitumor activity	[40]
12	(s,e)-6-hydroxy-6-methyl-2-((2s,5r)-5-methyl-5 vinyltetrahydrofuran-2-yl)hept-4-en-3-one	cytotoxicity	[41]

4. Conclusion

The extract derived from *U. dioica* demonstrates characteristics of a free radical scavenger, acting as an antioxidant with the ability to alleviate the detrimental impacts of free radicals. This excerpt highlights the potential opportunities for effectively managing and regulating the complexities linked to diabetes by means of dietary supplementation. Additionally, this study suggests that *U. dioica* exhibits a wide variety of compounds that appear to possess the documented biological activity, indicating its importance in the field of pharmaceuticals and nutrition. It is imperative to furnish scientific substantiation for the utilization of traditional remedies and to advance the use of extracts and compounds as nutritional constituents. The significance of preserving this particular plant species cannot be overstated, as it holds potential for yielding pharmacologically active compounds. It is crucial that these activities is to offer significant knowledge and understanding to pharmaceutical companies, enabling them to effectively handle various health disorders prevalent in society. However, further research is necessary to substantiate the significance of abundant chemicals in combating chronic diseases by the utilisation of bioactive components derived from *U. dioica* leaf extract. Consequently, current scientific investigations are being carried out to examine the process of isolating and characterising specific compound.

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