Journal of Chemical Health Risks

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JCHR (2025) 15(3), 1016-1027 | ISSN:2251-6727

A Bioactive Approach from Plant to Potential: Investigating the Isolation, Characterization, and Antioxidant Activity of *Urtica dioica*

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(Received: 16 March 2025 Revised: 20 April 2025 Accepted: 22 May 2025)

KEYWORDS

Isolation, Phytochemicals, Anti-inflammatory, Antioxidant, Stinging nettle

ABSTRACT

Introduction: This study focused on the comprehensive isolation, characterization, and assessment of antioxidant activity of *Urtica dioica* extracts. Commonly known as stinging nettle, *Urtica dioica* is a medicinal plant with a long history of use as a natural remedy for arthritis and inflammation.

Objective The objective of this study was to isolate and characterize ferulic acid from *U. dioica* using spectroscopic techniques, and to evaluate the antioxidant activity of the extract through various in vitro assays.

Method: Ferulic acid was isolated from *U. dioica* and characterized using spectroscopic techniques such as UV-Visible spectroscopy, FT-IR, NMR, and Mass spectrometry to confirm its purity and structural identity. Antioxidant activity was evaluated using in vitro assays including DPPH radical scavenging, reducing power assay, and hydrogen peroxide scavenging activity.

Results: Spectroscopic analysis confirmed the successful isolation and structure of Ferulic acid. The in vitro assays revealed significant antioxidant activity of the extract, including effective free radical scavenging, metal ion binding, and lipid peroxidation prevention.

Conclusions: The findings confirm the presence of potent antioxidant constituents in *U. dioica*, particularly ferulic acid. These results support the plant's traditional use and highlight its potential in managing oxidative stress-related disorders.

1. Introduction

Significant scientific attention has been focused on the identification of variables among low-cost herbs that can be beneficial in enhancing human health in recent years [1]. Phenolic compounds, a family of secondary metabolites in plants, are widely distributed and exhibit diverse structural variations. They can be monomers, aglycones, glycosides, or polymerized structures [2]**.** They differ in stability and distribution. Extracting and isolating phenolics is challenging, so a single standardized procedure cannot be recommended for all plants. Procedures must be optimized based on study objectives, s ample characteristics, and target analytes [3]. Plant-derived medicinal components are widely employed in medications, cosmetics, and food items [4]. *Urtica dioica L.*, popularly known as stinging nettle, is a genus of perennial plants in the Urticaceae family [5]. Each corner of the tall, green quadrangular stem features lacunar collenchymas. 12-20 fibro vascular bundles are conceivable [6]**.** This plant can grow to a height of approximately 2 m (6.5 feet) [7]. The leaves are oblong or oval, opposite, cordate at the base, finely toothed, dark green above and paler underneath [8], and oblong or oval, opposite, cordate at the base, finely toothed, dark green above and paler beneath[9]. The stinging trichomes on the stems and leaves transport histamine, acetylcholine, and serotonin-rich fluid [10]**.** The herb has long been used to treat gout, hair loss, and moderate bleeding. It is also an aphrodisiac, diuretic, and anti-inflammatory [11]. Antioxidants are substances that, in very little amounts, naturally occur in food or in the human body, delay, regulate, or stop oxidative processes that degrade food quality or cause

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JCHR (2025) 15(3), 1016-1027 | ISSN:2251-6727

degenerative diseases to arise and spread throughout the body [12,13]. The process of preventing these antioxidant molecules from oxidizing involves a variety of techniques and actions [14]. Oxidative stress, a concept in medical sciences, is a significant factor in common diseases like anti-inflammatory diseases, diabetes, high blood pressure, arthritis [15,16], preeclampsia, atherosclerosis [17], acute renal failure [18], memory loss, Alzheimer's, and Parkinson's. It occurs when cells produce reactive oxygen species (ROS), which can lead to poor cell function, aging, or disease if there is an imbalance between pro-oxidants and antioxidants [19,20]. In the present study, we summarize the isolation of specified phenol, characterization and anti-oxidant activity which presents particular aspects for the use of extract obtained by *U. dioica* in future to treat multiple diseases as good therapeutic agent.

2. Material and Method

2.1 Plant Material

The leaves of *Urtica dioica* were collected from Garhwal region in Uttarakhand in the month of September, 2021. Plant was authenticated by S.K Singh, Scientist E and HoD, Botanical Survey of India, Dehradun, Uttarakhand. A voucher specimen (Ref no BSI/NRC/tech/Herb 2021-22/108) has been deposited in the herbarium of our institute, Botanical Survey of India, Dehradun, Uttarakhand, for future reference.

2.2 Extraction of Plant

On the basis of last publication extraction of dried leaves powder of *Urtica dioica* was done with petroleum ether and by placing in a thimble of Soxhlet apparatus using Hydroalcoholic solvent system at 40-60 ^{o}C temperature of the heating mantle for 8-10 hours [21]. Further evaluation of that extract like isolation of phenols and characterization is carried out in this paper.

2.3 Preliminary Thin layer chromatography

Thin-layer chromatography is a “solid-liquid adsorption” chromatography. In this method stationary phase was TLC plates of silica gel 60 F_{254} pre coated with layer thickness of 0.2 mm using different solvent system comprising with std. Phenol. In this method, the mobile phase travels upward through the stationary phase. Spots were applied manually using capillary tube, plates were air dried using and TLC chamber were developed at room temperature with respective solvent system. The solvent travels up the thin plate soaked with the solvent by means of capillary action. During this procedure, it also drives the mixture priorly dropped on the lower parts of the plate with a pipette upwards with different flow rates. Thus the separation of analytes was achieved. This upward travelling rate depends on the polarity of the material, solid phase, and of the solvent [22].

$$\mathbf{R_f\ Value} = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

Solvent system developed in preliminary TLC for *U. dioica* extract in which the maximum spots were visible in Toluene: Ethyl acetate: Acetic acid (6:4:0.4) mobile phase with std. Phenol. So that Toluene: Ethyl acetate: Acetic acid (6:4:0.4) solvent was taken as mobile phase for column chromatography.

2.4 Column chromatography

Hydro alcoholic extract was subjected to silica gel column chromatography for isolation of Phenol from *Urtica dioica* extract. A vertical glass column made of borosilicate material was used for chromatography. The column was rinsed with the acetone and was completely dried before packing. Column was packed using wet packing technique using silica gel (60-120) as the adsorbent. Slurry was prepared using toluene and was poured in to the column. 1gm of extract was added over the top of the column. Gradient elusion technique was followed for column chromatography. The column was eluted with Toluene: Ethyl Acetate: Acetic acid (6:4:04) number of elutes were collected. The fractions/elutes collected were concentrated and TLC was performed to identify the presence of single compound [23].

2.5 Spectroscopic characterization:-

2.5.1 UV-visible Spectroscopy

The isolated fraction (e) of *U. dioica* Extract was scanned from 200 to 800 nm wavelength using UV-Visible Spectrophotometer (Shimadzu UV-1700) and the characteristic peaks were detected and recorded [24].

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2.5.2 FT-IR

To establish the presence of the functional groups in the isolated fraction (e) of *U. dioica* Extract, FT-IR spectroscopy was performed using Perkin Spectrum BX spectrophotometer. The samples were dried and ground with KBr pellets and analyzed on Thermo Nicolet model 6700 spectrum instrument. A disk of 100 mg of KBr was prepared with a mixture of 2% finely dried sample and then examined under IR-spectrometer. Infrared spectra were recorded in the region of 400 - 4,000 cm-1 [25].

2.5.3 NMR Spectroscopy

NMR spectroscopy was performed for the isolated fraction (e) of *U. dioica* Extract to identify the structure of the compound present in the isolated fraction. NMR spectroscopy for this purpose was Fourier Transform Nuclear Magnetic Resonance spectroscopy, Model AVNACENEO500 Ascend Bruker BioSpin International AG, Switzerland [26].

2.5.4 Mass Spectroscopy

Mass spectrometry converts molecules into ions and according to their mass and charge the ions can be separated and sorted. The mass spectrometer used for the identification of the molecular weight of isolated fraction (e) of *U. dioica* Extract was recorded on mass spectrometer instrument MICROTOF-Q 228888.10348. [27].

2.6 *In-vitro* Anti-oxidant Activity

2.6.1 DPPH Radical Scavenging Activity

a) Preparation of DPPH reagent

0.1mM solution of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) in methanol was prepared.

b) Preparation of Sample/Standard

Freshly 1 mg/ml methanol solution of extracts of *U. dioica*/ standard was prepared. Different volume of extracts/standard (20 – 100µl) was taken from stock solution in a set of test tubes and methanol was added to make the volume to 1 ml. To this, 2 ml of 0.1mM DPPH reagent was added and mixed thoroughly and absorbance was recorded at 517 nm after 30 minutes incubation in dark at room temperature.

c) Preparation of control

For control, 3 ml of 0.1mM DPPH solution was taken and incubated for 30 min at room temperature in dark condition. Absorbance of the control was taken against methanol (as blank) at 517 nm [28].

Percentage antioxidant activity of sample/standard was calculated by using formula:

% Inhibition = [(Ab of control- Ab of sample/ Ab of control x 100]

2.6.2 Reducing power assay

Preparation of standard solution

3 mg of ascorbic acid was dissolved in 3 ml of distilled water/solvent. Dilutions of this solution with distilled water were prepared to give the concentrations of 20, 40, 60, 80 and 100 µg/ml.

Preparation of extracts

Stock solutions of extract of *U. dioica* were prepared by dissolving 1mg of dried extracts in 1 ml of methanol to give a concentration of 1mg/ml. Then sample concentrations of 20, 40, 60, 80 and 100 µg/ml were prepared.

Protocol for reducing power

According to this method, the aliquots of various concentrations of the Ascorbic acid as a standard and extracts (20 to 100µg/ml) in 1.0 ml of deionised water were mixed with 2.5 ml of (pH 6.6) phosphate buffer and 2.5 ml of (1%) potassium ferricyanide. The mixture was incubated at 50°C in water bath for 20 min after cooling. Aliquots of 2.5 ml of (10%) tri chloro acetic acid were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution 2.5 ml was mixed with 2.5 ml distilled water and a freshly prepared 0.5 ml of (0.1%) ferric chloride solution. The absorbance was measured at 700 nm in UV spectrometer (Systronic double beam-UV-2201). A blank was prepared without adding extract. [29].

2.6.3 Hydrogen peroxide scavenging activity

The ability of the extract to scavenge hydrogen peroxide (H_2O_2) was determined according to the method of Ruch et al. [30]. Aliquot of 0.1 mL of extract of *U. dioica* leave (20-100 µg/mL) was transferred into

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the eppendorf tubes and their volume was made up to 0.4 mL with 50 mM phosphate buffer (pH 7.4) followed by the addition of 0.6 mL of H_2O_2 solution (2 mM). The reaction mixture was vortexed and after 10 min of reaction time, its absorbance was measured at 230 nm. Ascorbic acid was used as the positive control. The ability of the extracts to scavenge the H_2O_2 was calculated using the following equation:

% Inhibition = [(Ab of control- Ab of sample/ Ab of control x 100]

3. Results

3.1 Preliminary TLC preparation for the estimation of active constitutes

TLC of *U. dioica* Hydro alcoholic extract

For Phenol:-
Mobile Phase- Toluene: Ethyl acetate: Acetic acid (6: 4: 0.4)

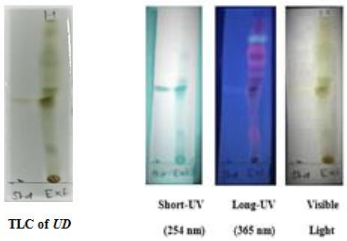

Figure 1: TLC estimation by UV lamp for *U. dioica* with Std. Phenol
(Std.= Standard, *UD* = *U. dioica*)

TLC of *U. dioica* extract was performed on different solvent systems (solvent system was selected on the basis of literature survey). TLC performed in Toluene: Ethyl Acetate: Acetic acid (6:4:04) that were clearly visible bands of *UD* Extract with Std. Phenol. The Rf values of *UD* Extract with Std. Phenol were found to be 0.49 and 0.49 (Table 1).

3.2 Column Chromatography

The fractions/elutes obtained from silica gel column chromatography of *U. dioica* Hydro alcoholic extract were tested for the detection of various phyto compounds using TLC. The collected fractions/elutes were taken properly and do the UV spectrum (Table 2).

3.2.1Column Chromatography of *UD* Hydro alcoholic extract -

3.2.2 TLC of all collected fractions-

A) TLC of all collected fractions of *UD* Hydro alcoholic extract -

(a) Short-UV (254 nm)(b) Long-UV (365 nm) (c) Visible Light

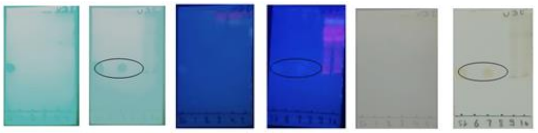

Figure 2: TLC estimation by UV lamp for *U. dioica* fractions after column chromatography with Std. Phenol. a) Short-UV (254 nm), b) Long-UV (365 nm), c) visible light.
(Std.= Standard, *UD* = *U. dioica*)

TLC of fractions (a, b, c, d, e, f, g & h) of *UD* Hydro alcoholic extract -

Rf value Resulted after performing the TLC estimation was also done for the confirmation of active constituent in fraction (e) of *U. dioica* Hydro alcoholic extract with mobile phase Toluene: Ethyl Acetate: Acetic acid (6:4:04) by comparing with Std. Phenol (Table 3 and Figure 2).

3.3 Spectroscopic characterization:-

3.3.1 Active constitutes estimation By UV-Spectroscopy-

UV-Spectra of isolated fraction (e) of *U. dioica* Hydro alcoholic extract was recorded with a Shimadzu 1700 double beam-UV-VIS spectrophotometer. UV spectra of the isolated fraction was recorded in solvent as Toluene: Ethyl acetate: Acetic acid (6:4:0.4) over a scanning range of 200-800 nm and λmax of isolated compound were determined. The Blank was Toluene: Ethyl acetate: Acetic acid (6:4:0.4). The wavelength of isolated fraction (e) of *UD* Hydro alcoholic extract was found to be 327 nm.

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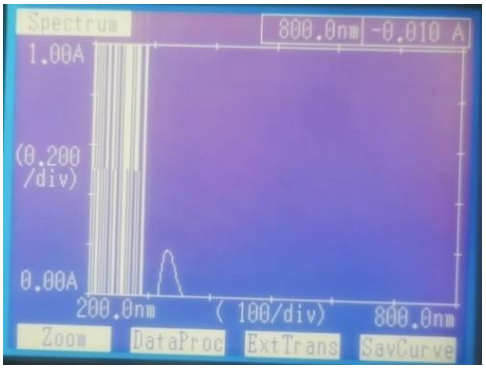

Figure 3 Active constitutes estimation By UV-Spectra of e fraction of *U. dioica* Hydro alcoholic extract after column chromatography

3.3.2 Active constitutes estimation By FTIR – Spectroscopy

(A) IR spectra of the isolated Fraction (e) of *U. dioica* Hydro alcoholic extract

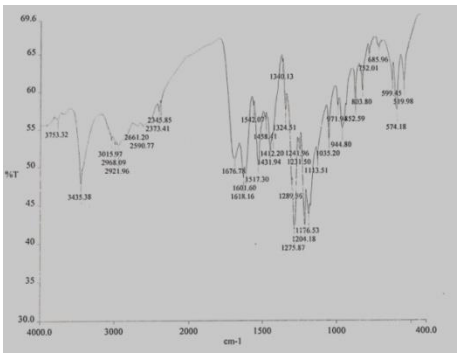

Figure 4: IR spectra of the isolated Fraction (e) of *U. dioica* Hydro alcoholic extract

Table 1: TLC of *U. dioica* Hydro alcoholic extract

S. No.	Solvent system	No. of spots	Color of spots at Wavelength (254 & 365nm)	Rf value (Extract)	Rf value (Std. Phenol)
`1.	Toluene: Ethyl Acetate: Acetic acid (6:4:04)	15	Light Florescence (Std) Purple Pink Light Purple Brown Dark Brown Light Florescence (Std) Brown Purple Dark Purple Pink Purple Pink Sky Blue Dark Purple	- 0.08 0.14 0.40 0.45 0.47 0.49 0.54 0.57 0.62 0.71 0.77 0.80 0.85 0.91	0.49

Table 2: Fraction collected from Column Chromatography of *U. dioica* Hydro alcoholic extract

S. No.	Eluent composition	Fraction collected	Remarks
1	Toluene: Ethyl Acetate: Acetic acid	01 (a)	White creamy coloured mixture of compound
2		02 (b)	Light Greenish coloured mixture of compound
3		03 (c)	Dark Yellowish coloured mixture of compound
4		04-06 (d), (d1,d2, d3)	Yellowish coloured mixture of compound
5		07 (e)	Creamy coloured mixture of compound
6		08 (f)	White creamy coloured mixture of compound

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7	(6:4:04)	09 (g)	Dark Brownish coloured mixture of compound
8		10 (h)	Light Brownish coloured mixture of compound

Table 3: Rf values of all collected fractions of *U. dioica* after column chromatography

S. No.	Fraction	Solvent system	No. of spots	Color of spots at Wavelength (254 &365nm)	Rf value (Extract)	Rf value (Std. Phenol)
1.	A	Toluene: Ethyl Acetate: Acetic acid (6:4:04)	-	-	-	0.49
2.	B		02	Fluorescence Pink	0.96 0.97	
3.	C		03	Fluorescence Pink Pink	0.89 0.94 0.96	
4.	d1		03	Pink Pink Fluorescence	0.89 0.94 0.96	
5	d2		02	Fluorescence Purple	0.95 0.98	
6	d3		01	Fluorescence	0.97	
7	E		02	Light Fluorescence Fluorescence	0.49 0.97	
8	F		01	Fluorescence	0.97	
9	G		08	Light Brown Pink Purple Dark Pink Light Purple Pink Sky Blue Dark Purple	0.50 0.52 0.63 0.74 0.78 0.82 0.89 0.94	
10	H		08	Light Brown Pink Purple Dark Pink Light Purple Pink Sky Blue Dark Purple	0.50 0.52 0.63 0.74 0.78 0.82 0.89 0.94	

Table 4 FTIR- Spectrum Frequency Range of the isolated Fraction (e) of *U. dioica* Hydro alcoholic extract

Sr. No.	Fraction	Frequency Range	Group Absorption (cm-1)	Appearance	Group	Compound Class
1	E	3550-3200 (cm-1)	3435.38	Strong, Broad	O-H stretching	Hydroxyl Group
		3100-3000 (cm-1)	3015.97	Medium	C-H stretching	Alkene

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		3000-2840 (cm-1)	2968.09	Medium	C-H stretching	Alkane
		3000-2840 (cm-1)	2921.96	Medium	C-H stretching	Alkane
		2000-1650 (cm-1)	1676.78	Weak	C-H bending	Aromatic compound
		2000- 1600 (cm-1)	1618.16	Medium	C-O stretching	Carbonyl group
		1600-1300 (cm-1)	1458.41	Medium	C-H bending	Methyl group
		1440-1395 (cm-1)	1412.20	Medium	O-H bending	Carboxylic acid
		1600-1400 (cm-1)	1431.94	Strong	C=C stretching	Benzene Ring
		1390-1310 (cm-1)	1324.51	Medium	O-H bending	Phenol
		1210-1163 (cm-1)	1176.53	Strong	C-O stretching	Ester
		1400- 1100 (cm-1)	1113.51	Weak	C-C stretching	Alkane
		980-960 (cm-1)	971.94	Strong	C=C bending	Alkene
		840-790 (cm-1)	803.80	Medium	C=C bending	Alkene
		730-665 (cm-1)	685.96	Strong	C=C bending	Disubstituted

The IR Spectra of isolated fraction (e) of *U. dioica* Hydro alcoholic extract showed that -OH group Strong, Broad peak appeared at 3435.38 cm-1, the C-H stretching peak of Alkene at 3015.97 cm-1, C-H stretching peaks of Alkane at 2968.09 & 2921.96 cm-1. The C-H bending peak of Aromatic compound at 1676.78 cm-1, Carbonyl group C-O stretching peak at 1618.16 cm-1, C-H bending peak of Methyl group at 1458.41cm-1,O-H bending peak of Carboxylic acid at 1412.20 cm-1, C=C Stretching peak of Benzene Ring at 1431.94 cm-1, O-H bending peak of Phenol at 1324.51 cm-1, C-O stretching peak of Ester at 1176.53 cm-1, C-C stretching peak of Alkane at 1113.51 cm-1 and C=C bending peak of Alkene at 971.94 & 803.80 cm-1. The C=C stretching peak of disubstituted at 685.96 cm-1.

3.3.3 ^{1}H NMR - Spectroscopy-

^{1}H NMR spectra of isolated fraction (e) of *U. dioica* Hydro alcoholic extract was recorded on NMR Spectrometer. Tetramethylsilane used as an internal standard. The signals are denoted with the symbols s, d, t, and m for singlet, doublet, triplet, and multiplet, respectively.

(A) 1H NMR spectra of the isolated compound (Fraction (e)) of *U. dioica* –

In ^{1}H NMR spectra of isolated fraction (e) of *UD* Hydro alcoholic extract showed that ^{1}H-3 protons appeared at 3.79 (s) ppm, ^{1}H-1 proton appeared at 6.39 (d) ppm, ^{1}H-1 proton appeared at 6.72 (dd) ppm, ^{1}H-1 proton appeared at 7.08 (dd) ppm, ^{1}H-2 protons appeared at 7.20-7.32 (7.27 (dd) ppm, 7.28 (dd) ppm) and ^{1}H-2 protons appeared at 7.62-7.79 (7.67 (d) ppm. 7.70 (d) ppm)

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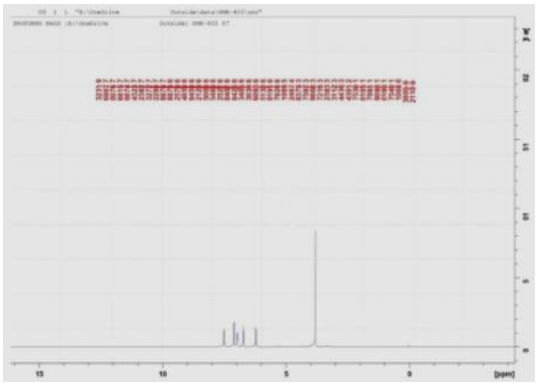


Figure 5: ^{1}H-NMR spectra of the **isolated compound (Fraction (e)) of *U. dioica* Hydro alcoholic extract**

3.3.4 Mass – Spectroscopy-

A mass spectrum of isolated Fraction (e) of *UD* Hydro alcoholic extract was recorded on Mass Spectroscopy. Mass spectra of isolated Fraction (e) of *UD* Hydro alcoholic extract showed molecular ion [M^{+}] peaks at mlz 194.1204 which corresponds to the molecular formula $C_{10}H_{10}O_4$ according to their fragments.

***(A)** Mass spectra of the isolated Fraction (e) of **U. dioica Hydro alcoholic extract**-*

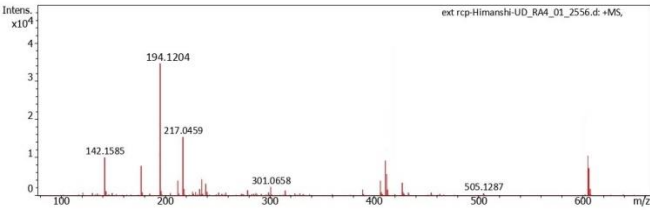
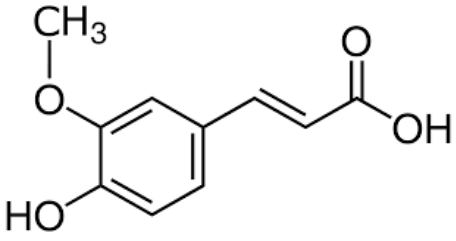


Figure 6 Mass spectra of the isolated Fraction (e) of *U. dioica* Hydro alcoholic extract



Ferulic acid [31]

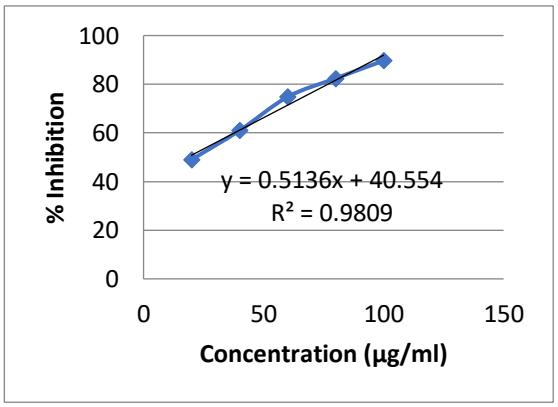
.IUPAC NAME: (2E)-3-(4-HYDROXY-3-METHOXYPHENYL) PROP-2-ENOIC ACID

3.4 Anti-oxidant activity

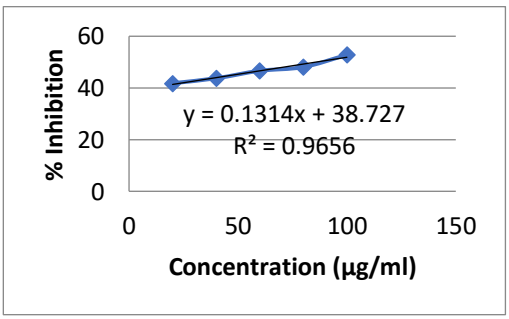
3.4.1 DPPH Assay

Table 5: DPPH radical scavenging activity of Ascorbic acid & Hydroalcoholic extract of *U. dioica*

Concentration (µg/ml)	Absorbance of Ascorbic acid	Absorbance of extract of *U. dioica*	% Inhibition of Ascorbic acid	% Inhibition of extract of *U. dioica*
20	0.469	0.535	49.021	41.815
40	0.359	0.517	60.978	43.739
60	0.232	0.491	74.782	46.630
80	0.162	0.477	82.391	48.076
100	0.095	0.434	89.673	52.782
Control	0.920			
IC50	**18.421**		**86.106**	



Graph 1: Graph represents the Percentage Inhibition *Vs* Concentration of Ascorbic acid



Graph 2: Graph represents the Percentage Inhibition *Vs* Concentration of Hydro alcoholic extract of *U. dioica*

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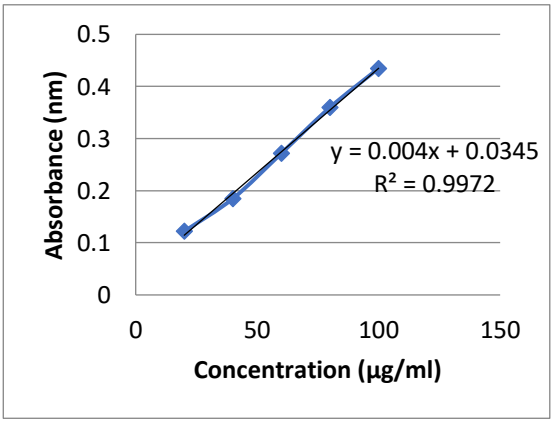
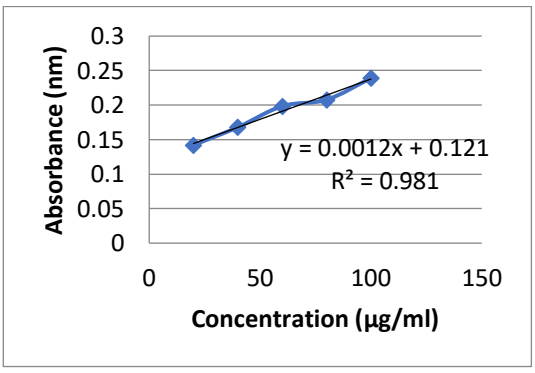
3.4.2 Reducing power scavenging activity

Table 6: Reducing power scavenging activity of Ascorbic acid& Hydro-alcoholic extract of *U. dioica*

Concentration (µg/ml)	Absorbance of Ascorbic acid	Absorbance of Hydro-alcoholic extract of *U. dioica*
20	0.122	0.142
40	0.185	0.168
60	0.272	0.198
80	0.360	0.207
100	0.435	0.238



Graph 3: Graph represents the Absorbance *Vs* Concentration of Ascorbic acid

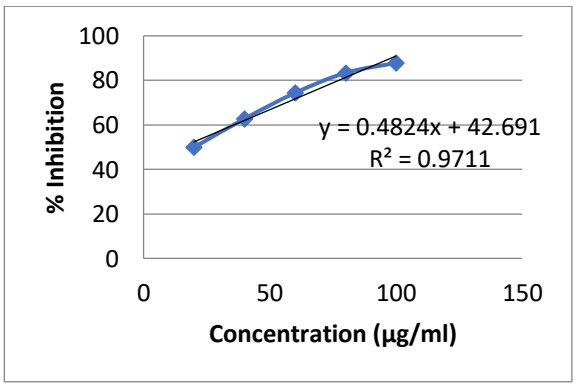


Graph 4: Graph represents the Absorbance *Vs* Concentration of Hydro-alcoholic extract of *U. dioica*

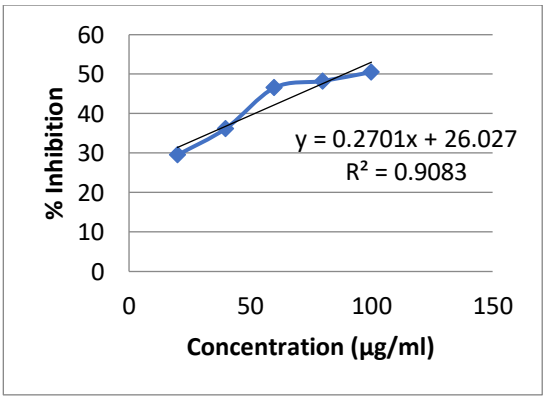
3.4.3 Hydrogen peroxide scavenging activity

Table 7 Hydrogen peroxide scavenging activity of Ascorbic acid & Hydro-alcoholic extract of *U. dioica*

Concentration (µg/ml)	Absorbance of Ascorbic acid	Absorbance of Hydro-alcoholic extract of *U. dioica*	% Inhibition of Ascorbic acid	% Inhibition of Hydro-alcoholic extract of *U. dioica*
20	0.618	0.870	49.902	29.522
40	0.460	0.787	62.744	36.267
60	0.316	0.659	74.340	46.566
80	0.205	0.638	83.360	48.275
100	0.150	0.611	87.838	50.525
Control	1.235			
IC50	15.165		88.814	



Graph 5: Graph represents the Percentage Inhibition *Vs* Concentration of ascorbic acid



Graph 6: Graph represents the Percentage Inhibition *Vs* Concentration of Hydro-alcoholic extract of *U. dioica*

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4. Discussion

The preliminary TLC of *U. dioica* Hydro alcoholic extract was conducted on various solvent systems, with the Toluene: Ethyl acetate: Acetic acid (6:4:0.4) solvent system being chosen based on literature survey. The Rf values were 0.49 and 0.49 for *UD* and Std. Phenol, respectively. The active compounds were isolated from column chromatography using the mobile phase of Toluene: Ethyl acetate: Acetic acid for *U. dioica*, resulting in Fractions 01, 02, 03, 04-06, 07, 08, 09, and 10 (**Table 2**). The TLC estimation confirms active constituents in fractions e of *U. dioica* with mobile phase Toluene: Ethyl acetate: Acetic acid (6:4:0.4) by comparing with Std. Phenol (**Fig 2, Table 3**). The collected fractions of *UD* were analyzed using UV spectra, revealing a wavelength of 327 nm, and the λmax of these fractions was determined over a scanning range of 200-800 nm (**Fig 3**). The IR Spectra of isolated fraction (e) of *UD* Hydro alcoholic extract showed that -OH group Strong, Broad peak appeared at 3435.38 cm-1, the C-H stretching peak of Alkene at 3015.97 cm-1, C-H stretching peaks of Alkane at 2968.09 & 2921.96 cm-1.TheC-H bending peak of Aromatic compound at 1676.78 cm-1 of , Carbonyl group C-O stretching peak at 1618.16 cm-1, C-H bending peak of Methyl group at 1458.41cm-1,O-H bending peak of Carboxylic acid at 1412.20 cm-1, C=C Stretching peak of Benzene Ring at 1431.94 cm-1, O-H bending peak of Phenol at 1324.51 cm-1,C-O stretching peak of Ester at 1176.53 cm-1, C-C stretching peak of Alkane at 1113.51 cm-1 and C=C bending peak of Alkene at 971.94 & 803.80 cm-1. The C=C stretching peak of disubstituted at 685.96 cm-1. (**Fig 4, Table 4**). In ^{1}H NMR spectra of isolated fraction (e) of *U. dioica* Hydro alcoholic extract showed that ^{1}H-3 protons appeared at 3.79 (s) ppm, ^{1}H-1 proton appeared at 6.39 (d) ppm, ^{1}H-1 proton appeared at 6.72 (dd) ppm, ^{1}H-1 proton appeared at 7.08 (dd) ppm, ^{1}H-2 protons appeared at 7.20-7.32 (7.27 (dd) ppm, 7.28 (dd) ppm) and ^{1}H-2 protons appeared at 7.62-7.79 (7.67 (d) ppm. 7.70 (d) ppm) (**Fig 5**). The mass spectrum of the isolated fraction (e) of *UD* Hydro alcoholic extract, recorded using Mass Spectroscopy, revealed molecular ion [M+] peaks at mlz 194.1204, corresponding to the molecular formula C10H10O4 (Fig 6). From this physical, chemical and spectral investigation were confirmed the presence of Ferulic acid in fraction (e) of *U. dioica* Hydro alcoholic extract.

DPPH radical scavenging activity of Hydro alcoholic Extract of *U. dioica* exhibited percent inhibition 52.78 % and its IC50 value was found to be 86.106 µg/ml. Ascorbic acid was used as a reference compound which exhibited percent inhibition 89.67 % and showed IC50 value of 18.421 µg/ml. Similarly, Hydrogen peroxide scavenging activity of Hydro alcoholic extract of *U. dioica* exhibited percent inhibition 50.52 % and its IC50 value was found to be 88.814 µg/ml. Ascorbic acid was used as a reference compound which exhibited percent inhibition 87.83 % and showed IC50 value of 15.165 µg/ml. The reducing capacity of a compound indicates its potential antioxidant activity. Comparing it to dietary antioxidants like ascorbic acid, compounds with reducing power act as electron donors, reducing oxidized intermediates in lipid per oxidation processes, acting as primary and secondary antioxidants.

5. Conclusion and Future Perspectives:-

The study examined the Hydro alcoholic extract of plant *U. dioica*, revealing the presence of Ferulic acid in fraction (e). Ferulic acid has been shown to have a number of biological functions, particularly in oxidative stress, inflammation, vascular endothelial damage, fibrosis, apoptosis, and platelet aggregation. Many studies have demonstrated that ferulic acid can inhibit the PI3K/AKT pathway, ROS generation, and aldose reductase activity. Ferulic acid's anti-inflammatory activity is mostly related to PPAR, CAM, NF-kB, and p38 MAPK signaling pathways. It has anti-inflammatory properties in addition to antioxidant properties. It can eliminate too many reactive oxygen species (ROS) or eliminate free radicals and the enzymes that produce them in order to prevent oxidative damage and lessen inflammatory responses. The study provides insights into its composition and structure, and emphasizes *U. dioica* medicinal characteristics and potential for producing anti-arthritic and anti-inflammatory medicines, which might be incorporated into pharmaceutical formulations for long-term health management. Further research is needed to explore its applications in developing novel pharmaceuticals or functional foods with enhanced

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antioxidant, anti-inflammatory properties. This contributes to understanding bioactive components in *U. dioica.*

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