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Original Research Article

Isolation, Spectroscopic Identification and Assessment of Antioxidant Activity of Ferulic acid from the Leaves of *Urtica dioica*

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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 the leaves of *Urtica dioica* using spectroscopic techniques, and to evaluate the antioxidant activity of the extract through various in-vitro assays. ***Method:*** Ferulic acid was isolated from the leaves of *Urtica dioica* and characterized using spectroscopic techniques such as UV-Visible spectroscopy, FTIR, 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, as oxidative stress is a significant contributor to inflammatory conditions. ***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 *Urtica dioica*, particularly ferulic acid. These results support the plant's traditional use and highlight its potential in managing oxidative stress-related disorders.

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

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 (Jan et al., 2017). 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 (Carvalho et al., 2017). 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, sample characteristics, and target analytes (Santos-Buelga et al., 2012). Plant-derived medicinal components are widely employed in medications, cosmetics, and food items (Sovova et al., 2004). *Urtica dioica* L., popularly known as stinging nettle, is a genus of perennial plants in the Urticaceae family (Ahmed and Parsuraman 2014). Each corner of the tall, green quadrangular stem features lacunar collenchymas. 12-20 fibro vascular bundles are conceivable (Corsi and Masini 1997). This plant can grow to a height of

approximately 2 m (6.5 feet) (Petruzzello, 2022). The leaves are oblong or oval, opposite, cordate at the base, finely toothed, dark green above and paler underneath (Testai et al., 2002, Hajhashemi and Klooshani 2013). The stinging trichomes on the stems and leaves transport histamine, acetylcholine, and serotonin-rich fluid (Tuberville et al., 1996). The herb has long been used to treat arthritis, gout, hair loss, diuretic, and anti-inflammatory (Grieve et al., 1971). 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 degenerative diseases to arise and spread throughout the body (Sharifi et al., 2020, Lobo et al., 2010). The process of preventing these antioxidant molecules from oxidizing involves a variety of techniques and actions (Shahidi and Zhong 2015). Oxidative stress, a concept in medical sciences, is a significant factor in common diseases like anti-inflammatory diseases, diabetes, high blood pressure, arthritis (Fonseca et al., 2019, Zahan et al., 2020), preeclampsia, atherosclerosis (Forman and Zhang 2021), acute renal failure (Liguori et al., 2018), 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 (Rodrigo and Rodrigo 2009, Spector 2000). In the present study, we summarize the isolation of specified phenolic acid, characterization and anti-oxidant activity which presents particular aspects for the use of extract obtained by*Urtica dioica* in future to treat multiple diseases as good therapeutic agent.

2. MATERIALS AND METHODS

2.1 Plant Material:

The leaves of *Urtica dioica* were collected from Garhwal region (Dehradun) 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 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.

Name of Plant Material- *Urtica dioica*
Part used-Leaves
Collection site-Dehradun
Authentication Number- BSI/NRC/tech/Herb2021-22/108

2.2 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 F254 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 (Coskun 2016 and Dolan 2006).

Rf Value =(Distance traveled by solute)/(Distance traveled by solvent)

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

2.3 Column chromatography

Hydro alcoholic extract was subjected to silica gel column chromatography for isolation of Phenolic acid (Ferulic acid) 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 (6:4:0.`4) number of elutes were collected. The fractions/elutes collected were concentrated and TLC was performed to identify the presence of single compound (Srivastava et al., 2021 and Shusterman et al., 1997).

2.4 Spectroscopic characterization:-

2.4.1 UV-visible Spectroscopy

The isolated fraction (e) of *Urtica 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 (Patel et al., 2022 and Metha 2012).

2.4.2 FT-IR

To establish the presence of the functional groups in the isolated fraction (e) of *Urtica 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 mixtur

of 2% finely dried sample and then examined under IR-spectrometer. Infrared spectra were recorded in the region of 400 - 4,000 cm-1 (Luciene et al., 2008 and Gordon et al., 1972).

2.4.3 NMR Spectroscopy

NMR spectroscopy was performed for the isolated fraction (e) of *Urtica 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 (Zia et al., 2019 and Keeler 2019).

2.4.4 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 *Urtica dioica* Extract was recorded on mass spectrometer instrument MICROTOF-Q 228888.10348. (Wiley et al., 1995 and Cottrell et al., 1986).

2.5 *In-vitro* Anti-oxidant Activity

2.5.1 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 *Urtica 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 (Athavale et al., 2012 and Baliyan et al., 2022).

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

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

2.5.2 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 *Urtica 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. (Quisumbing 1978 and Jayanthi et al., 2011).

2.5.3 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. (Ruch et al., 1989 and Ali et al., 2020 Aliquot of 0.1 mL of extract of *Urtica dioica l*eaves (20-100 µg/mL) was transferred into 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 –

A) TLC of *Urtica dioica* Hydro alcoholic extract For Phenolic compound (Ferulic acid)

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

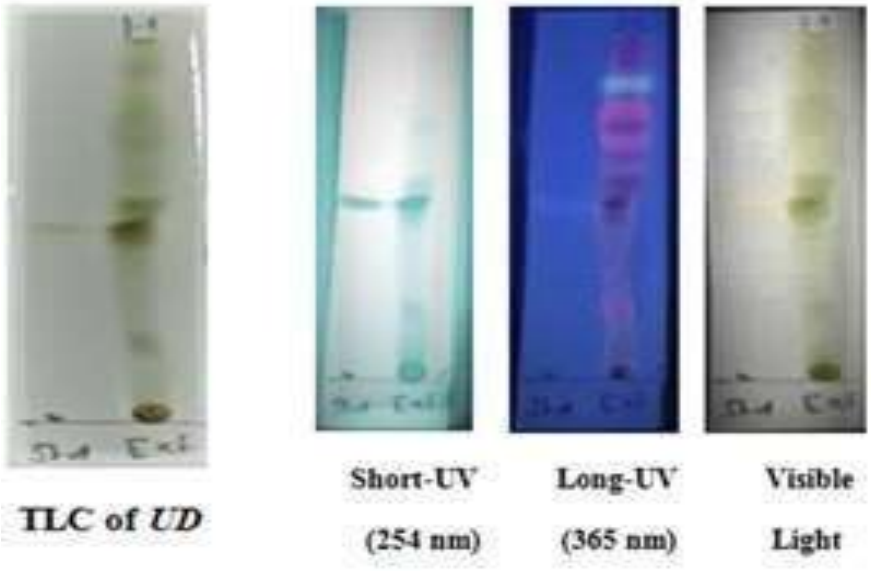


Figure 1: TLC estimation by UV lamp for *Urtica dioica* with Std. Phenolic compound (Ferulic acid)

Table 1: TLC of *Urtica dioica* Hydro alcoholic extract

S. No.	Solvent system	No. of spots	Color of spots at Wavelength (254 & 365nm)	Rf value (*Urtica dioica* Extract)	Rf value (Std. Ferulic acid)
`1.	Toluene: Ethyl Acetate: Acetic acid (6:4:0.4)	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

TLC of *Urtica 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 (6:4:0.4) that were clearly visible bands of *Urtica dioica* Extract with Std. Phenolic compound (Ferulic acid). The Rf values of *Urtica dioica* Extract with Std. Phenolic compound (Ferulic acid) were found to be 0.49 and 0.49.

3.2 Column Chromatography

The fractions/elutes obtained from silica gel column chromatography of *Urtica 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.

3.2.1 Column Chromatography of *Urtica dioica* Hydro alcoholic extract –

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

S. No.	Eluent composition	Fraction collected	Remarks
1	Toluene: Ethyl Acetate: Acetic acid (6:4:0.4)	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
7		09 (g)	Dark Brownish coloured mixture of compound
8		10 (h)	Light Brownish coloured mixture of compound

3.2.2 TLC of all collected fractions-

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

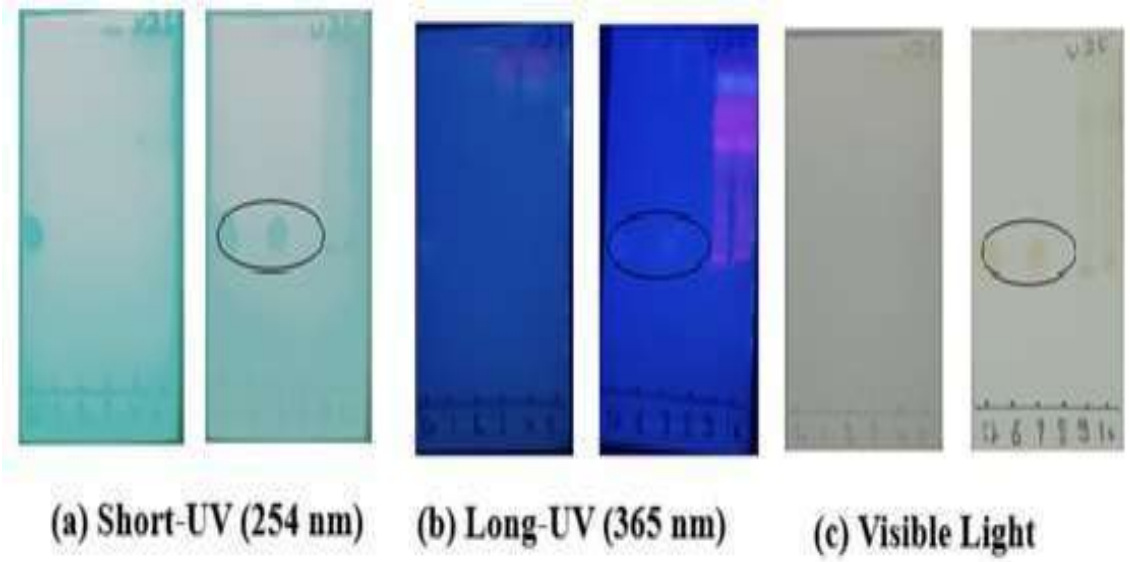


Figure 2: TLC estimation by UV lamp for *Urtica dioica* fractions after column chromatography with Std. Phenolic compound (Ferulic acid). a) Short-UV (254 nm), b) Long-UV (365 nm), c) visible light.

3.2.3 TLC of fractions (a, b, c, d, e, f, g & h) of *Urtica dioica* Hydro alcoholic extract –

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

Sr. No.	Fraction	Solvent system	No. of spots	Color of spots at Wavelength (254 & 365nm)	Rf value (*Urtica dioica* Extract)	Rf value (Std. Ferulic Acid)
1.	A	Toluene: Ethyl Acetate: Acetic acid (6:4:0.4)	-	-	-	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	

Rf value Resulted after performing the TLC estimation was also done for the confirmation of active constituent in fraction (e) of *Urtica dioica* Hydro alcoholic extract with mobile phase Toluene: Ethyl Acetate: Acetic acid (6:4:0.4) by comparing with Std. Phenolic compound (Ferulic acid).

3.3 Spectroscopic characterization:-

3.3.1 Active constitutes estimation By UV-Spectroscopy-

UV-Spectra of isolated fraction (e) of *Urtica 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 *Urtica dioica* Hydro alcoholic extract was found to be 327 nm.

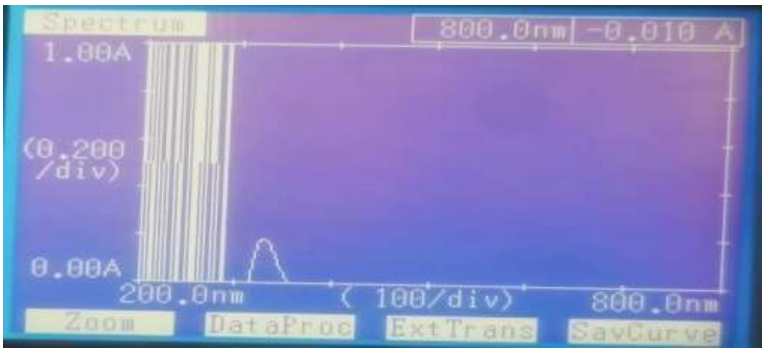


Figure 3: Active constitutes estimation By UV- Spectra of e fraction of *Urtica dioica* Hydro alcoholic extract after column chromatography

3.3.2 Active constitutes estimation by FTIR – Spectroscopy

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

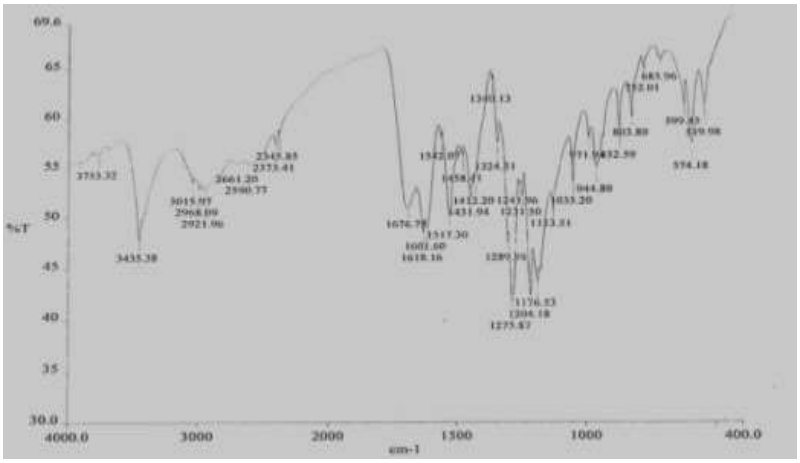


Figure 4: **IR spectra of the isolated Fraction (e) of *Urtica dioica* Hydro alcoholic extract**

Table 4: FTIR- Spectrum Frequency Range of the isolated Fraction (e) of *Urtica 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
		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 *Urtica 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 1458.41 cm-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 1H NMR Spectroscopy

1H NMR spectra of isolated fraction (e) of *Urtica 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 *Urtica dioica*

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

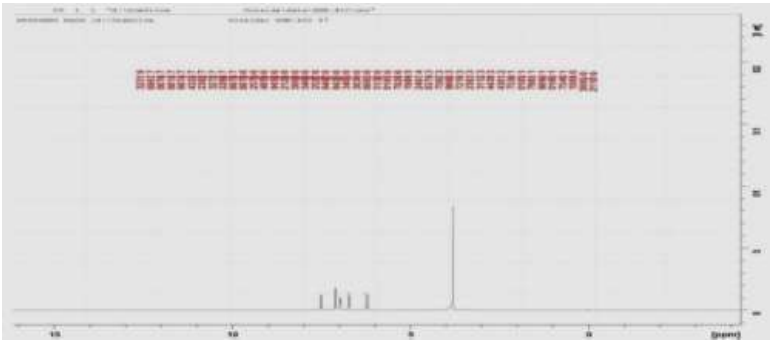


Figure 5: ^{1}H-NMR spectra of the **isolated compound Fraction (e) of *Urtica dioica* Hydro alcoholic extract**

3.3.4 Mass Spectroscopy-

A mass spectrum of isolated Fraction (e) of *Urtica dioica* Hydro alcoholic extract was recorded on Mass Spectroscopy. Mass spectra of isolated Fraction (e) of *Urtica dioica* 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 Urtica dioica Hydro alcoholic extract-

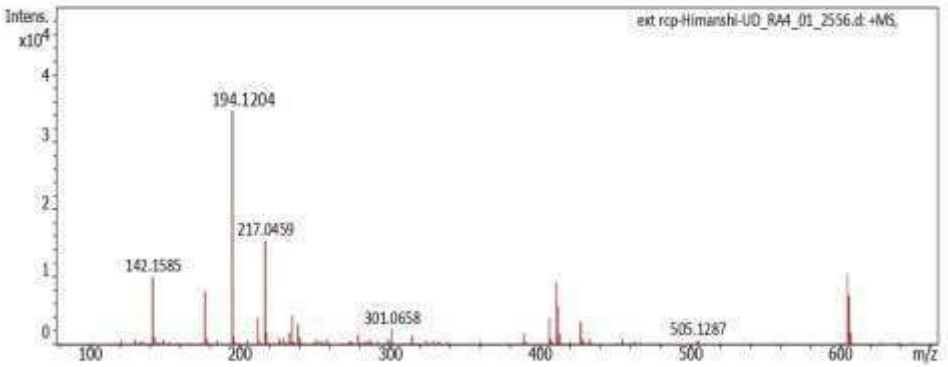
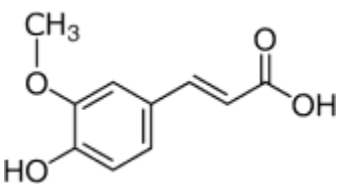


Figure 6: **Mass spectra of the isolated Fraction (e) of *Urtica dioica* Hydro alcoholic extract**



Ferulic Acid

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

3.4 *In-Vitro* Anti-oxidant activity

3.4.1 DPPH Assay

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

Concentration (µg/ml)	Absorbance of Ascorbic acid	Absorbance of extract of *Urtica dioica*	% Inhibition of Ascorbic acid	% Inhibition of extract of *Urtica 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**	

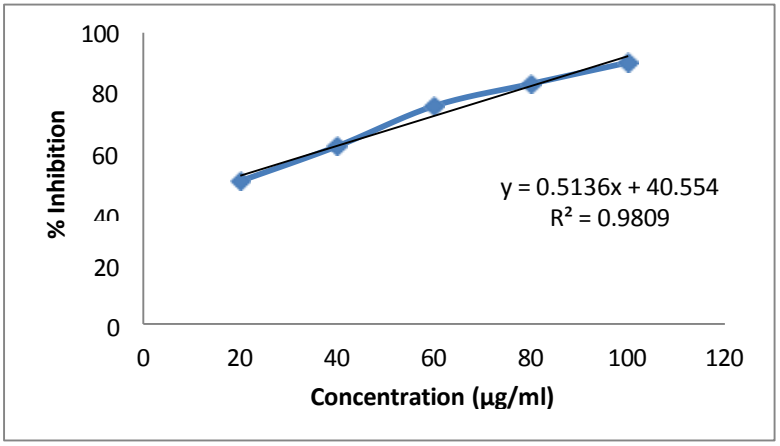


Figure 7: Graph represents the Percentage Inhibition *Vs* Concentration of Ascorbic acid

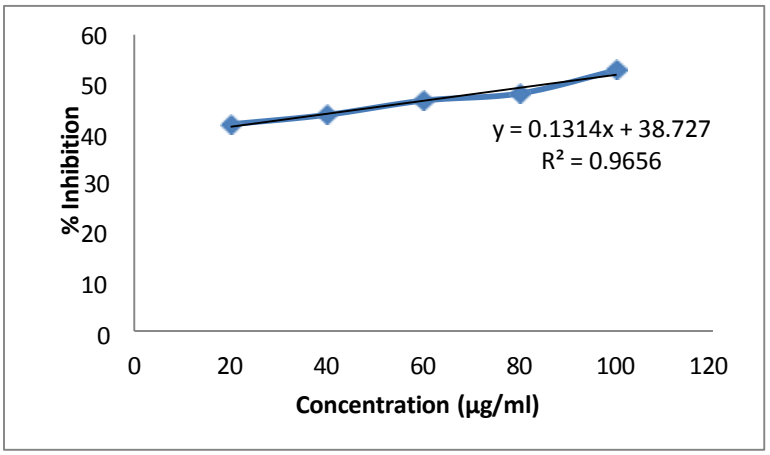


Figure 8: Graph represents the Percentage Inhibition *Vs* Concentration of Hydro alcoholic extract of *Urtica dioica*

3.4.2 Reducing power scavenging activity

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

Concentration (µg/ml)	Absorbance of Ascorbic acid	Absorbance of Hydro-alcoholic extract of *Urtica 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

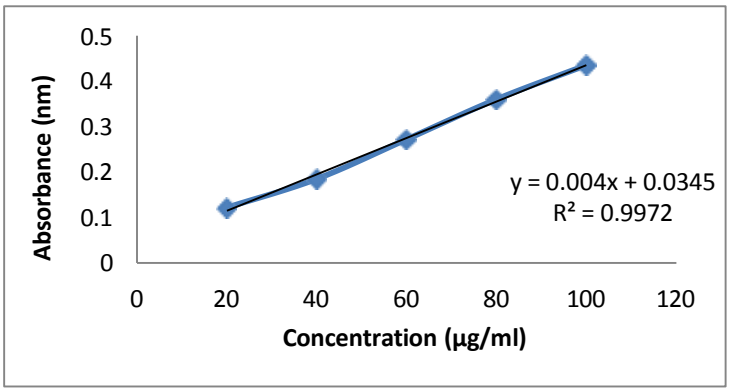


Figure 9: Graph represents the Absorbance *Vs* Concentration of Ascorbic acid

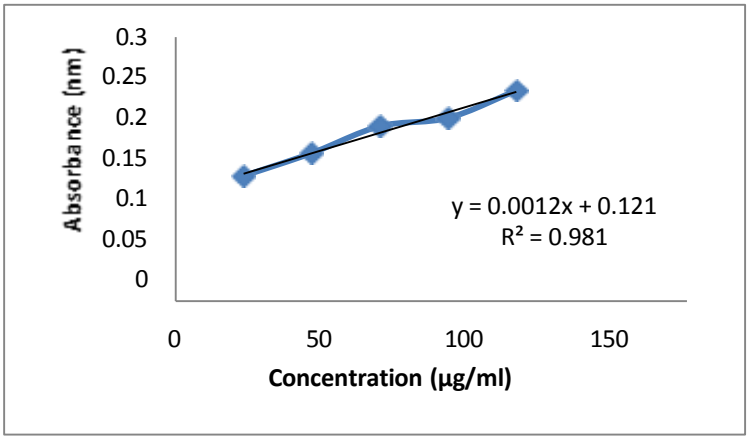


Figure 10: Graph represents the Absorbance *Vs* Concentration of Hydro-alcoholic extract of *Urtica dioica*

3.4.3 Hydrogen peroxide scavenging activity

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

Concentration (µg/ml)	Absorbance of Ascorbic acid	Absorbance of Hydro-alcoholic extract of *Urtica dioica*	% Inhibition of Ascorbic acid	% Inhibition of Hydro-alcoholic extract of *Urtica 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	

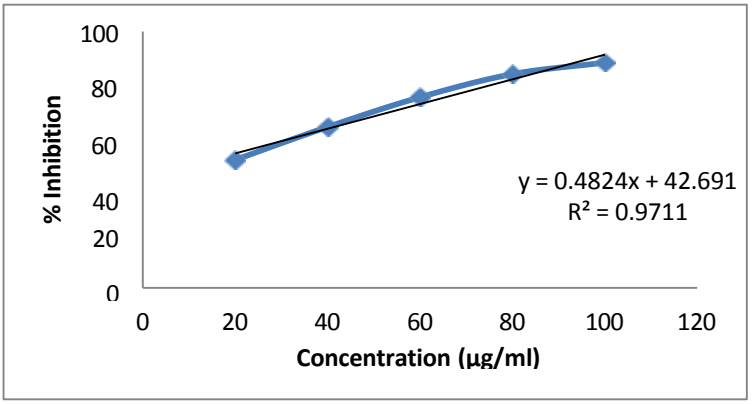


Figure 11: Graph represents the Percentage Inhibition *Vs* Concentration of ascorbic acid

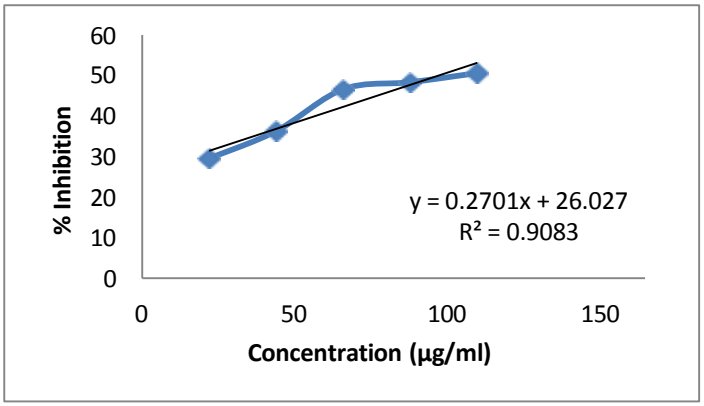


Figure 12: Graph represents the Percentage Inhibition *Vs* Concentration of Hydro-alcoholic extract of *Urtica dioica*

4. DISCUSSION

The preliminary TLC of *Urtica 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 *Urtica dioica* and Std. Phenolic compound (Ferulic acid), respectively. The active compounds were isolated from column chromatography using the mobile phase of Toluene: Ethyl acetate: Acetic acid for UD, 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 *Urtica dioica* with mobile phase Toluene: Ethyl acetate: Acetic acid (6:4:0.4) by comparing with Std. Phenolic compound (Ferulic acid) (Fig 2, Table 3). The collected fractions of *Urtica dioica* 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 *Urtica 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 of Carbonyl group C-O stretching peak at 1618.16 cm-1, C-H bending peak of Methyl group at 1458.41 cm-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 1H NMR spectra of isolated fraction (e) of *Urtica dioica* Hydro alcoholic extract showed that lH-3 protons appeared at 3.79 (s) ppm, lH-1 proton appeared at 6.39 (d) ppm, lH-1 proton appeared at 6.72 (dd) ppm, lH-1 proton appeared at 7.08 (dd) ppm, lH-2 protons appeared at 7.20-7.32 (7.27 (dd) ppm, 7.28 (dd) ppm) and lH-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 *Urtica dioica* 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 *Urtica dioica* Hydro alcoholic extract. DPPH radical scavenging activity of Hydro alcoholic Extract of *Urtica 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 Urtica 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

The study examined the Hydro alcoholic extract of plant *Urtica 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 *Urtica 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 antioxidant, anti- inflammatory properties. This contributes to understanding bioactive components in *Urtica dioica*.

FUNDING
NIL

CONFLICT OF INTEREST
None

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