RESEARCH ARTICLE

Phytochemical Profiling and Evaluation of *In-vitro* Anticancer and Antioxidant Activities of *Urtica dioica L.*

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ABSTRACT

Much research has been conducted in recent decades to screen for and investigate the antioxidant and anticancer properties of medications based on isolated natural products or medicinal plant extracts. In order to evaluate the antioxidant, phytochemical, and *in-vitro* anticancer characteristics of *Urtica Dioica L.* (UDSE-02) seed ethanolic extracts, the current study was conducted. Using conventional methods, flavonoids, alkaloids, glycosides, phytosterols, and glycoside compounds were detected. Through spectral and physical examination, the structure of the isolated chemical was verified. The 2,2'-diphenyl-1-picrylhydrazyl (DPPH) free radical assay was used to evaluate the ethanolic extracts' and isolated compounds' ability to scavenge free radicals. The MTT assay was used to assess an *in-vitro* anticancer investigation employing the MCF-7 Brest cancer cell line. In terms of antioxidant activity, our results showed that the ethanolic extracts had a strong *in-vitro* capacity to scavenge free radicals. The IC50 values of the extracted components from *Urtica Dioica L.* seeds were 154 µg/mL and 53 µg/mL (UDSE-02), respectively, as compared to the standard. In the MTT experiment, UDSE-02 extract showed a considerable and dose-dependent growth inhibition of Brest cancer cells at doses over 100 µg/mL, with an IC50 of 72 µg/mL, in contrast to cisplatin (IC50 = 6 µg/mL), were isolated compounds showed a fruitful result with IC50 of 34µg/mL. Our findings demonstrate the ethanolic extracts and isolated compounds may be a promising anticancer agent and an efficient antioxidant.

Key words: *In-vitro*, Anticancer, Antioxidant activities, Cell Line, *Urtica Dioica.L*.

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INTRODUCTION

Plant-based natural phytochemicals have drawn a lot of attention for their potential to treat a variety of clinical diseases in humans, including cancer. The Greek word for plant is "phyto," and there are numerous "families" of phytochemicals that have a number of beneficial effects on human health[1]. Stinging nettle, or *Urtica dioica L.*, is a common wild vegetable that has been used for thousands of years. This perennial herbaceous plant, belonging to the Urticaceae family, is found almost everywhere, however it is more prevalent in North America, Europe, North Africa, and some parts of Asia.

U. dioica also represents a pharmacologically relevant source of bioactive compounds. In ethano pharmacology, it is considered a plant with therapeutic beneficial properties, able to cure as well as prevent diseases. It has long been used to treat diabetes, hepatic and gastrointestinal issues, and hypertension. However, a comprehensive examination of its pharmacological profile and chemical constituents has only lately been carried out. More precisely, the primary bioactive compounds found in *U. dioica* are lignans, flavonoids, terpenoids, sphingolipids, steroids, and various alkaloids. Based on the information now available, *U. dioica* has been demonstrated to have anti-inflammatory, anti-diabetic, analgesic, antioxidant, hypotensive, and antiproliferative qualities. Because of its anti-inflammatory and antioxidant qualities, *U. dioica* has also been investigated as a treatment for benign prostatic hyperplasia, both alone and in combination. Urtica is a member of the dicotyledonous plant family, which is notable for its fiber content[2-5].

Carotenoids, anthocyanins, phenolics, and tocopherols are just a few of the antioxidant compounds found in fruits and vegetables. The healthcare system has profited from the usage of around 20% of all plants in pharmaceutical research, which has helped treat dangerous illnesses like cancer. It is possible for plants to synthesize a wide variety of bioactive compounds. Vegetables and fruits store large amounts of phytochemicals that may shield against free radical damage[6]. Plantscontaining beneficial phytochemicals may supplement the needs of the human body by acting as natural antioxidants. Various studies have shown that many plants are rich source of antioxidants. For example, plant-based phenolic chemicals including flavonoids, tannins, and lignins, as well as vitamins A, C, and E, all have antioxidant qualities. Eating fruits and vegetables has been linked to several health benefits due to its high nutritional content and therapeutic qualities. By preventing or postponing oxidation brought on by reactive oxygen species (ROS), antioxidants improve the shelf life

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and quality of foods by reducing oxidative damage. Ascorbic acid, beta-carotene, and many phenolics all have important roles in reducing inflammation, delaying the aging process, and avoiding some types of cancer. Increased intake of fruits and vegetables has been promoted by a number of organizations and health care systems worldwide. Oxidation activities in the cells of living organisms are essential for the creation of energy[7-9]. Reactive oxygen species and reactive nitrogen species are produced during normal metabolism and include superoxide radicals, hydroxyl radicals, singlet oxygen, hydrogen peroxide, peroxyl radicals, peroxynitrite anion, and nitric oxide. It is well recognized that these reactive species harm biomolecules such nucleic acids, proteins, and lipids. Overproduction of these reactive species has been linked to a number of illnesses and conditions, including diabetes, cancer, aging, cardiovascular, inflammatory, andneurodegenerative diseases[10-13].

One of the top causes of death, cancer has a substantial impact on everyone's health because it is very expensive to treat those who are afflicted. Lung cancer was the leading cause of mortality, followed by colorectal, breast, stomach, and liver cancer. Nowadays, cancer is treated using a variety of therapeutic approaches, such as chemotherapy drugs, surgery, and/or radiation therapy. While the chemotherapeutic medicines used to treat cancer can help prolong the life of cancer patients and provide them with temporary comfort, certain anticancer drugs have negative side effects. As a result, natural compounds have been the focus of the hunt for substitute possible anticancer medicines. The anticancer effectiveness of natural bioactive substances has been confirmed by numerous investigations and help to trigger various biological reactions that may contribute to the fight against cancer cells, and some of the stimuli regulate the activity of proteins and enzymes that have a specific function in the biology of cancer. Phenolic and flavonoid molecules are among the natural substances with anticancer properties that have been shown to prevent cancer cells from spreading and invading other areas.

MATERIALS AND METHODS

Chemicals, Reagents and Cell Lines

All the other chemicals were of the highest analytical grade and were procured from certified suppliers. All are obtained from Hi-Media Laboratories Private Limited, Invitrogen, and Sigma, Mumbai, India. The Center for Cellular and Molecular Biology (CCMB), located in Hyderabad, Telangana, India, provided the MCF-7 cell lines used in the investigation.

Sample Collection and Authentication

The plants *Urtica dioica L.* were gathered from Andhra Pradesh's Visakhapatnam district. The Director of the Botanical Survey of India has verified the botanical identity. For future use, the voucher specimen has been turned in and is kept in the herbarium.

Plant Material Processing

To obtain a course powder with the appropriate particle size, *Urtica dioica L.* seeds were gathered, shade-dried at room temperature, and then size-reduced. After passing through mesh size 80, the powdered medication was kept in an airtight container. This powdered substance underwent a series of solvent extraction processes. For additional studies, the powdered form was utilized.

Preparation of Seed Powder and Extract

The seeds of Urtica *dioica L*. were completely cleaned with running tap water to get rid of any dirt or debris that had stuck, then rinsed with sterile distilled water and allowed to dry in the shade. for 14 days. Materials were ground with the help of mixer into a fine powder. Plant material (Seeds 10gms) were extracted with 250mL of Ethanol at 60°C for 6 hours in Soxhlet extractor. The ethanolic extracts were filtered with filter paper (Whatmann No. 1). After being dried off at room temperature, the filtrate was kept for later examination.

Phytochemical Screening of the Extracts

Using the previously reported standard methodologies, phytochemical screening was carried out[14]. *Urtica dioica L.* water and ethanolic extract samples were examined for Qualitatively tested for different phytochemical constituents namelyFlavonoids, Alkaloids, Glycoside, Phytosterols and Glycosides compounds by following the standard procedure.

Isolation of Plant Extract

Seed powder of *Urtica dioica* are an6-10 x 2-4 cm long, acuminate, cuneate at base, oblong-lanceolate, and shiny above. The dried portions of *Urtica dioica* seed powder were ground into a powder, weighed out to be 200g, and then put into Soxhlet extraction instruments. The powdered material was defatted with petroleum ether. Ethanol and water were used to extract the defatted powder after it had dried at room temperature. The dry extract residue was obtained by evaporating the solvents. A small portion of crude ethanol extracts is dissolved in chloroform and the solution is spotted on TLC plates. Then these plates are run by specific solvents system and viewed individually under UV light and also with the 10% H_2SO_4 reagent. A portion of ethanol extracts (2g) is subjected to column chromatography (silica gel, 2x90 cm). Finally, the column is eluted with the starting 2:8 to 4:6 proportions solvents system of n-hexane and ethyl acetate to give a one major

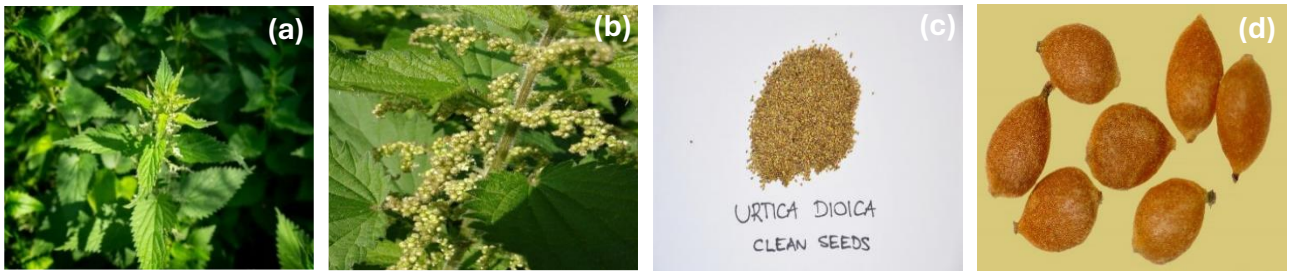


Figure 1: Urtica dioica L plant (a) whole plant, (b) Fruits branching, (c) Seeds, (d) Fruit

Table 1: Fluorescence study of *Urtica dioica L.* powdered leaves

S. No.	Samples	Treatment With chemical reagents	Under-ordinary light	Under UV light (254 nm)
1	Seeds powder	Powder with water	Dull white	White
2	Seeds powder	Powder with 1N NaOH	Light brown	Dark brown
3	Seeds powder	Powder with 50% H_2SO_4	Light green	Green
4	Seeds powder	Powder with 1 N HCl	Light yellow	Yellow

compounds (250mg) and reaming two compounds small quantity (2mg and 3mg). Identification and analytical, standard spectroscopic techniques for examining the structure of ethanol extract from natural products [*Urtica dioica* seed powder (UDSE-02)] include infrared spectroscopy (IR) and nuclear magnetic resonance (NMR), which are frequently coupled with mass spectrometry (MS).

Radical-scavenging Activity-Antioxidant Activity In vitro (DPPH Assay)

The ability of DPPH antioxidants to decolorize 1, 1-diphenyl-2-picrylhydrazyl serves as the foundation for evaluation. To put it briefly, DPPH (100 µg/mL, 0.2 mmol/L) solution was combined with MeOH solution of the extract or standard compounds at varying concentrations of 5, 10, 30, 40, 50, and 100 µg/mL[15-18]. After shaking the reaction mixture vigorously and letting it sit at room temperature for 30 minutes, the absorbance of the leftover DPPH was measured at 517 nm. Vitamin C, or ascorbic acid, was once a conventional medication. The following formula was used to determine the extract's scavenging activity against the stable DPPH*: The inhibition percentage is

$$\% \text{ Scavenging} = \frac{\text{AB} - \text{AA}}{AB} \times 100$$

where AA stands for the test sample's absorption and AB for the blank sample's.

In vitro Anticancer Activity

Figure 2: *Urtica dioica L*- Seeds powder

Table 2: Preliminary Phytochemical Screening ethanol Extract of Seeds of *Urtica dioica L*

Test	Ethanol extract *(Urtica dioica L)*
Alkaloids	+
Glycosides	+
Carbohydrates	-
Phenolic Compounds	+
Phytosterols	+
Saponins	-
Tannins	-
Proteins and amino acids	-
Flavanoids	+
Terpenoids	-

Table 3: The outcomes of extracts' and pure phenolic compounds' antioxidant activities

S. No.	Test Samples	IC50 (µg/mL)
1	Ethanol exctract of *Urtica dioica L.*	118 µg/mL
2	UDSE-02	48 µg/mL

Table 4: The results of *in vitro* anticancer activity of extracts and pure phenolic compounds

S. No.	Test Samples	IC50 (µg/mL)
1	Ethanol extract of *Urtica dioica L*	64 µg/mL
2	UDSE-02	28 µg/mL

The 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide (MTT) assay was used to evaluate the *in vitro* antiproliferative activity of plant extracts against the breast cancer cell line MCF-7, as has been done before[19-20].

In 96-well microtiter plates, cell suspensions were plated at a density of 1×105 per milliliter. The plates were then incubated for 24 and 48 hours at 37°C in an incubator with 5% CO2. Then, different amounts of plant extracts, isolated compounds, and the negative control (DMSO) (25, 50, 75, 100, and 200 µg/mL) were added to each well to replace the medium.

After incubation for 24 and 48 hours, the previously published tetrazolium-based colorimetric test was utilized to identify the inhibition of cell growth in the plant extracts under study. Following a 4-hour incubation period with 10µL of MTT in each well, 100µL of DMSO was added, and a plate reader (TECAN, Manne Dorf, Switzerland) was used to measure absorbance at 570 nm.

Each extract's cytotoxic potential was determined using the following formula for extracts made in various solvents:

$$\% \text{ Inhibition } = \frac{1 - \text{Abs.}_{\text{treated}}}{\text{Abs.}_{\text{control}}} \times 100$$

Analysis of Data

The Microsoft Excel Package was used to analyze both quantitative and graphical data. The mean standard deviation was used to represent the outcomes of each set of experiments, which were carried out in triplicate. Phytochemical qualitative data were examined.

The mean ± SEM was used to display the data. Every experiment was conducted in triplicate. P values were considered significant if they were less than 0.05. The GraphPad Prism tool (Graph Pad program, San Diego, USA)

was used to conduct statistical analyses. A P-value of less than 0.05 was deemed statistically significant.

RESULTS AND DISCUSSION

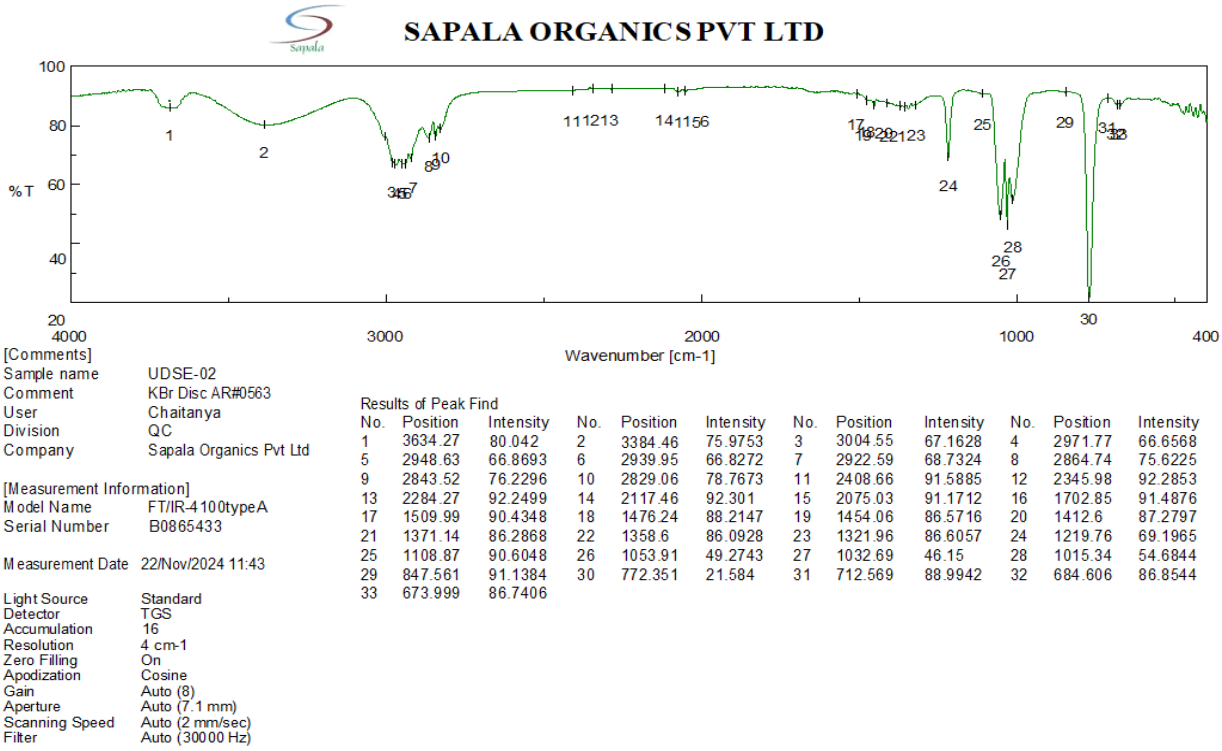
Organoleptic Study/Macroscopical characters of Seeds of Urtica dioica L.

Fluorescence Analysis

The powdered form of seed part of *Urtica dioica L.* treated with 10% NaOH, 50% H2SO4, 1N HCl, and water. The fluorescence of these extracts was detected and recorded using UV light (254 nm) as well as regular visible light. The fluorescence of these extracts was detected and recorded using UV light (254 nm) as well as regular visible light.

Preliminary Phytochemical Screening of Ethanol Extract of Seeds of Urtica dioica L.

Table 2 presents the findings from the phytochemical assay. Using conventional methods, a preliminary phytochemical screening of the plant's stem bark extracts was conducted to



[Comments]
Sample name UDSE-02
Comment KBr Disc AR#0563
User Chaitanya
Division QC
Company Sapala Organics Pvt Ltd

[Measurement Information]
Model Name FT/IR-4100typeA
Serial Number B0865433

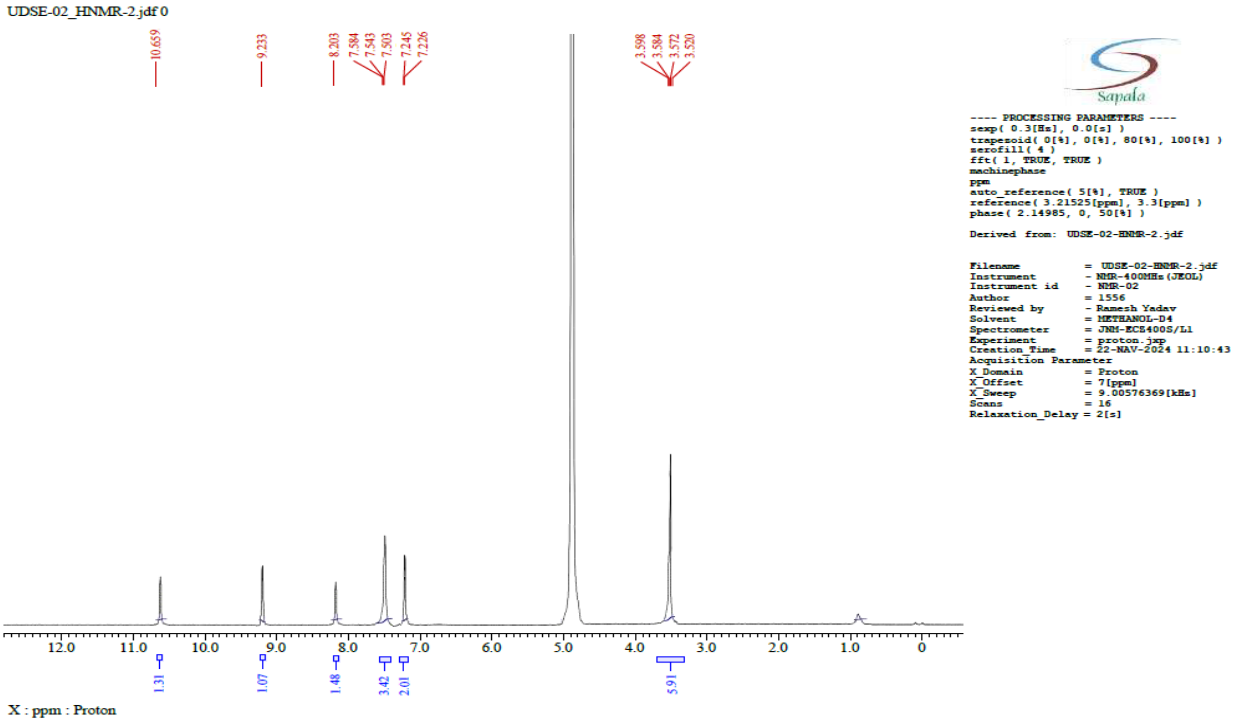
Measurement Date 22/Nov/2024 11:43

Light Source Standard
Detector TGS
Accumulation 16
Resolution 4 cm-1
Zero Filling On
Apodization Cosine
Gain Auto (8)
Aperture Auto (7.1 mm)
Scanning Speed Auto (2 mm/sec)
Filter Auto (30000 Hz)

Results of Peak Find

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3634.27	80.042	2	3384.46	75.9753	3	3004.55	67.1628	4	2971.77	66.6568
5	2948.63	66.8693	6	2939.95	66.8272	7	2922.59	68.7324	8	2864.74	75.6225
9	2843.52	76.2296	10	2829.06	78.7673	11	2408.66	91.5885	12	2345.98	92.2853
13	2284.27	92.2499	14	2117.46	92.301	15	2075.03	91.1712	16	1702.85	91.4876
17	1509.99	90.4348	18	1476.24	88.2147	19	1454.06	86.5716	20	1412.6	87.2797
21	1371.14	86.2868	22	1358.6	86.0928	23	1321.96	86.6057	24	1219.76	69.1965
25	1108.87	90.6048	26	1053.91	49.2743	27	1032.69	46.15	28	1015.34	54.6844
29	847.561	91.1384	30	772.351	21.584	31	712.569	88.9942	32	684.606	86.8544
33	673.999	86.7406									

Figure 3: IR spectrum of UDSE-02: (Isolated compound from ethanol extract-Seed powder of *Urtica dioica L.*)



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machinephase
ppm
auto_reference(5[%], TRUE)
reference(3.21525[ppm], 3.3[ppm])
phase(2.14985, 0, 50[%])

Derived from: UDSE-02-HNMR-2.jdf

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Instrument id - NMR-02
Author = 1556
Reviewed by - Ramesh Yadav
Solvent = METHANOL-D4
Spectrometer = JNM-ECS400S/L1
Experiment = proton.jxp
Creation_Time = 22-NAV-2024 11:10:43
Acquisition Parameter
X_Domain = Proton
X_Offset = 7[ppm]
X_Sweep = 9.00576369[kHz]
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Figure 4: ^{1}H NMR spectrum of UDSE-02 (Isolated compound from ethanol extract-Seed powder of *Urtica dioica L.*)

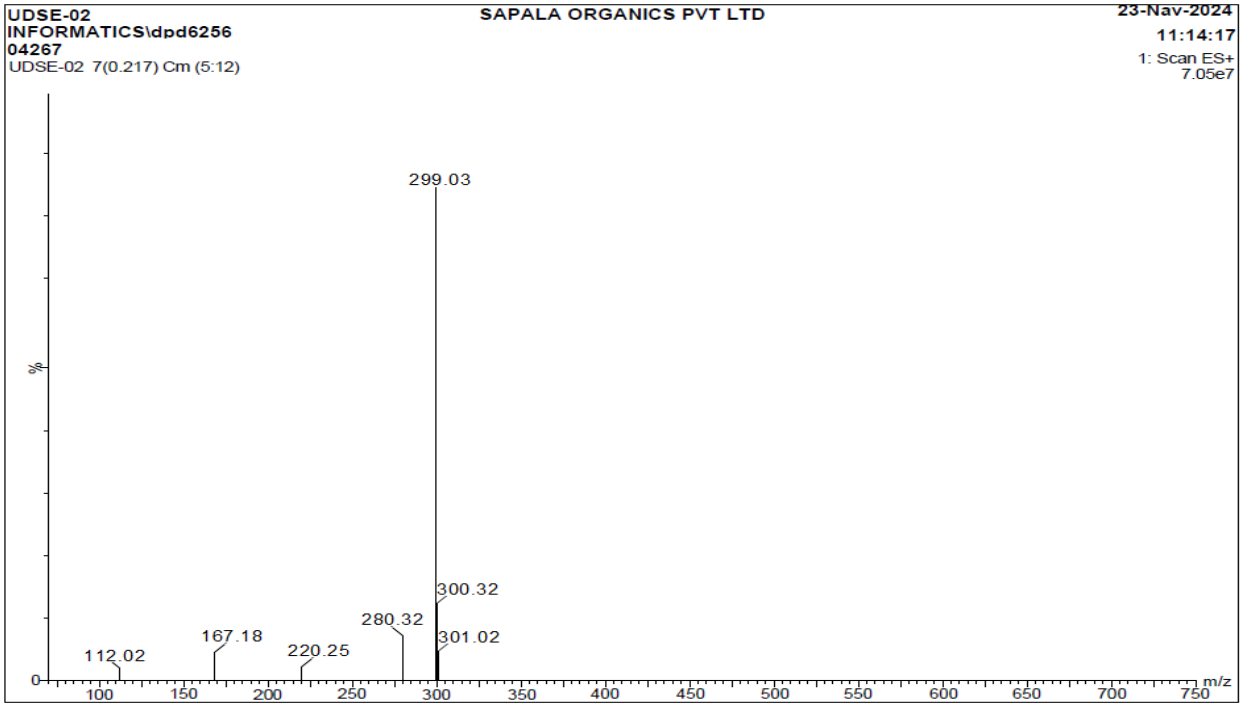


Figure 5: MASS spectrum of UDSE-02: (Isolated compound from ethanol extract-Seed powder of *Urtica dioica L.*)

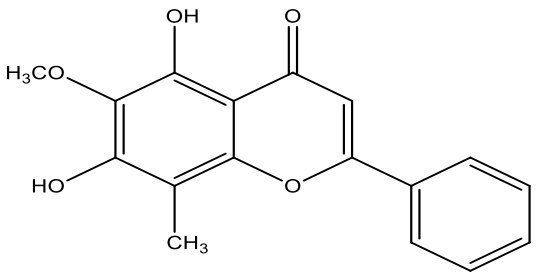
identify the presence or lack of secondary metabolites. Flavonoids, alkaloids, glycosides, phytosterols, and glycoside compounds were found in the different extracts with differing degrees of intensity, according to the qualitative analysis. The ethanolic extract contains the bulk of these secondary metabolites, as seen in Table 2.

Successive Solvent Extraction

For the preparation of the extracts, a Using various solvents in ascending order of polarity, sequential solvent extraction was performed. The order of the solvents used was ethanol and Water. Packed accurately weighed about 100 gm seed powder of *Urtica dioica L.* in muslin cloth and started extraction with Soxhlet apparatus. Extractions are carried out with solvents of increasing polarity order like ethanol and water at temperatures 62°C, 77°C, 78°C and 100°C respectively. The obtained extracts are filtered through filter paper and solvent was evaporated to dryness at their respective temperatures finally obtained semi solid mass.

Characterization of Isolated Compounds (Seed Powder of Urtica dioica L.)

Identification and analytical, Typical spectroscopic techniques for examining the structure of natural materials,



Chemical Formula: $C_{17}H_{14}O_5$
Exact Mass: 298.08

Figure 6: Isolated Phytomolecule: Phenoplic compound (UDSE-02)

such as ethanol extracts and *Urtica dioica* seed powder (UDSE-02) comprise Nuclear magnetic resonance (NMR) and infrared spectroscopy (IR) are frequently used in conjunction with mass spectrometry (MS).

Physical and Chemical Characterization

The UDSE-02 was isolated as light dark brown colour powdered. The M.P. of UDSE-02 is 193-195ºC. It gave a following phytochemical test like Phenolic compounds.

Spectral Characterization of UDSE-02

IR spectrum of UDSE-02 (Ethanolic Extract-II): IR bands was observed at 3634(-OH Str, Phenolic-OH), 3384(-OH Str, Phenolic ring-OH), 3004(CH Str in aromatic ring), 2971(CH str in aliphatic group), 1702(CO Str in Ketone group), 1509(C-C Str, Cyclic ring), 1454(C=C Str, Phenyl), 1053(C-O Bend). 1H-NMR spectrum of UDSE-02 (Ethanolic Extract-II): The ^{1}H NMR spectra was observed at 10.659(1H, Phenolic proton Ar-OH), 9.733(1H, Phenolic proton Ar-OH), 8.203(1H, Aromatic proton), 7.654-7.503(3H, t in aromatic proton Ar-H), 7.245-7.226(2H, d aromatic proton Ar-H), 3.528-3.600(6H, metoxy protons on aromatic ring) respectively.

Mass spectrum of UDSE-02 S (Ethanolic Extract-II): This compound have shown the base peak at [M]- ion at 298.08, [M+1]-ion at 299.03, and [M+2]-ion at 300.32 respectively.

In vitro Antioxidant Activity

A test for *Urtica dioica L.* ethanol extracts' antioxidant capacity, such UDSE-02. The *Urtica dioica L.* ethanol extract To check for biomolecules in seeds, free radical scavenging was employed. With an unpaired nitrogen atom, 1,1-diphenyl-2-picryhydrazil is a valuable free radical. As 1,1-diphenyl-2-picryhydrazil interacts with the antioxidant's hydrogen atoms, the color changes from purple to yellow.

Table 3 displays the extract's and pure phenolic components' antioxidant activity data.

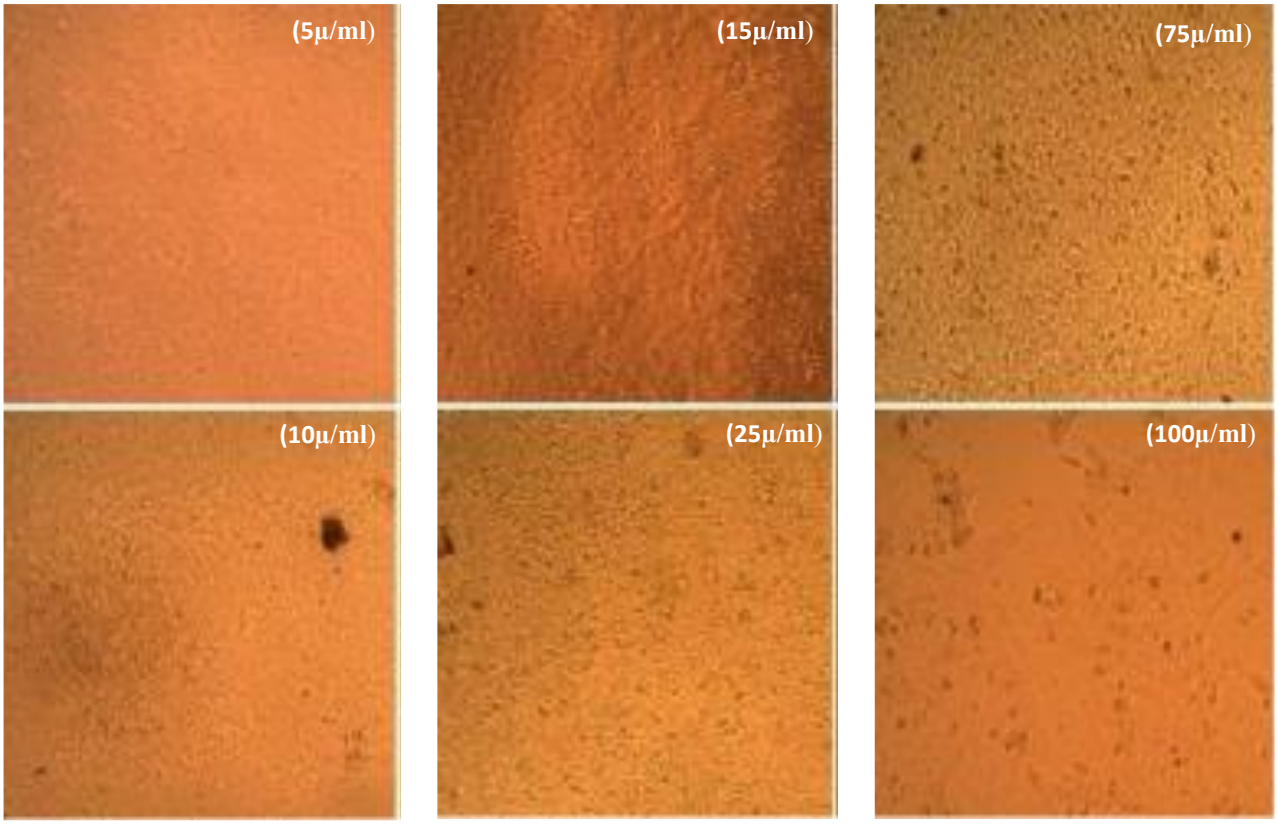


Figure 7: Morphological changes of MCF-7 cells treated with the pure phenolic compound-UDSE-02 at difference concentrations (5,10,15, 25, 75 and 100µ/ml) for 48 h

The results showed that the Inhibitory Concentration 50% value of the extract was lesser than the pure compound, but this difference was significantly different from the 50% is the Inhibitory Concentration value in relation to pure substances. These findings demonstrate that the pure compounds' antioxidant activity is higher.in order for the use of pure substances to be very efficient and successful.

In vitro Anticancer Activity-MTT Assay

Because of its strong ability to scavenge free radicals, we investigated the ethanolic extract's potential for cytotoxicity in a cancer cell line *in vitro*. The 3-(4) 5-Dimethyl-thiazol-Zyl)−2,5 biphenyl tetrazolium bromide (MTT) assay was used to examine the cytotoxic characteristics of the extracted pure compounds and extracts. The findings showed that there was a dose-dependent increase in cell lysis. Cisplatin is the chemotherapeutic drug utilized in this experiment to treat the MCF-7 cancer cell line, shown potent IC50= 64µg/mL, 72µg/mL(ethanol extract). However, the pure phenolic compounds are showed higher IC50= 28µg/mL, 64µg/mL values.

CONCLUSION

The cytotoxic and antioxidant qualities of pure phenolic components and extracts from *Urtica dioica* are being reported for the first time in this work. *L. Urtica dioica's* numerous benefits make it a fascinating natural wonder. When compared to other extracts, the ethanolic extract's biological activities may be explained by the presence of phenolic chemicals. According to current studies, the plant is utilized to treat a number of illnesses. It draws attention to every facet of the plant and gives researchers the motivation they need to continue working on creating its different formulations, which may eventually benefit both people and animals.

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