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Isolation and Characterization of Chrysin from Methanol Extract of *Urtica dioica* and Its Antibacterial Activity against Fish Pathogenic Bacteria

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ABSTRACT

Chrysin is a flavone of plant origin, possessing numerous biomedical properties, such as antimicrobial, anti-inflammatory, antidiabetic, anxiolytic, hepatoprotective, anti-aging and anticonvulsant activities. This study aimed to isolate this compound from methanol extract of *Urtica dioica*. *U. dioica*, commonly known as stinging nettle, is a well-studied medicinal herb from the family Urticaceae. Its leaves have long been used in traditional medicine as an herbal remedy for various diseases. Silica gel column chromatography was used to analyse the methanol extract under increasing methanol and chloroform gradients. This led to isolation of a pure compound (UD1). Spectroscopic analysis revealed that compound is a flavonoid. The structure of compound UD1 was elucidated using MS, NMR and HRMS and was compared with the NMR data of the already isolated compounds from the plant. UD1was characterized as 5,7-dihydroxy-2-phenyl-4H-chromen-4-one commonly known as chrysin. This is the first time that this compound has been isolated from the methanolic extract of the whole aerial part (leaves and stem) of the plant. The antibacterial assay was checked for this compound at a concentration of 2mg/mL and 1mg/ml against five pathogenic bacteria of fish and it was seen that the compound has an overall moderate antimicrobial activity against *S. aureus* and *B. subtilis* at this concentration suggesting its potential as an alternative to conventional antibiotics amid rising resistance among many bacterial strains.

Key Words: *Urtica dioica*; *Methanolic extract; Column chromatography; NMR; Antimicrobial activity; Minimum inhibitory concentration*

INTRODUCTION

Aquaculture is the fastest growing food producing sector over recent decades with an annual increase of 6.7% per year between 1990-2020 (FAO, 2022). Even while aquaculture has grown significantly worldwide, it is still susceptible to problems like microbial disease outbreaks brought on by intensified culture, which can result in a partial or complete loss of productivity (Bondad–Reantaso *et al*., 2005).

In order to evade economic distress related to bacterial diseases caused by these sanitary issues, aquaculture frequently uses a number of veterinary medications to prevent or treat these diseases, but their widespread use has contributed to the emergence of resistant bacterial strains (Chenia and Vietze 2012). In recent years, antibiotic resistance has significantly developed, posing an ever-growing therapeutic challenge. Using plant-based antibiotic resistance inhibitors is one way to lessen the resistance (Gowri *et al.,* 2016). It is well known that plants produce a wide range of phytoconstituents to defend themselves against different types of infections. Thus the isolation of these phytoconstituents and their use

in aquaculture have shown these have target sites different from those utilized by antibiotics are anticipated to be effective against infections that are resistant to drugs (Ahmad and Beg 2001).

U. dioica is a perennial plant which grows across a wide range of tropical and temperate wasteland environments, demonstrating remarkable ecological adaptability (Grauso *et al*., 2020). Stinging nettle possesses many therapeutic properties, like anti-inflammatory effects, relief from asthma symptoms, astringent and depurative actions, galactogogue activity, diuretic and nutritive benefits, as well as general stimulatory effects (Niaz *et al*., 2024). Owing to these medicinal qualities, both its aqueous and alcoholic extracts have been traditionally used for more than a thousand years to treat conditions such as rheumatism, eczema, arthritis, gout, and anaemia (Orčić *et al.,* 2014).

Now, it is a known fact that the biological activity of medicinal plants originates from their chemical constituents. Although many natural compounds contribute to these effects, their combined interactions often enhance overall activity through synergistic mechanisms (Veličković *et al.,* 2017). Chemical profiling of stinging nettle has revealed a diverse array of health-promoting compounds, including terpenes, flavonoids, alkaloids, steroids, minerals, vitamins, fatty acids, carotenes, polyphenols, amino acids, and numerous other bioactive molecules (Đurović *et al*., 2024).

Ongoing scientific efforts aim to develop safer, more accessible, and affordable therapeutic products. As part of this effort, medicinal plants are gaining increased interest for their potential in treating various diseases. Thus, we investigated whether *U. dioica* yields isolable flavonoids like chrysin for promising therapeutic properties (Immaculate *et al.,* 2023).

Chemically, chrysin—also known as 5,7-dihydroxyflavone—is a flavone with a typical 15-carbon backbone. It comprises fused A and C rings, along with B phenyl ring attached at position 2 of the C ring, and carries hydroxyl groups at the 5 and 7 positions on the A ring. Chrysin is biosynthesized from phenylalanine, which is first converted to cinnamic acid by phenylalanine ammonia-lyase, followed by a series of enzymatic steps that yield the final product (Mani *et al*., 2018). It occurs naturally in substances such as gums, honey, propolis, mushrooms, and passion flowers, and exhibits a broad range of biological activities, such as neurotrophic, anti-amyloidogenic, hepatoprotective, cardioprotective, neurotrophic, anti-amyloidogenic, and antimicrobial effects. Chrysin has a molecular formula of $C_{15}H_{10}O_4$, a molecular weight of 254.24 g/mol, and a melting point of 285.5°C (Bansal *et al.,* 2022).

The objective of this study is to isolate and characterize chrysin from the methanolic extract of *Urtica dioica* and to evaluate its antibacterial activity against pathogenic bacteria affecting fish.

MATERIAL AND METHODS

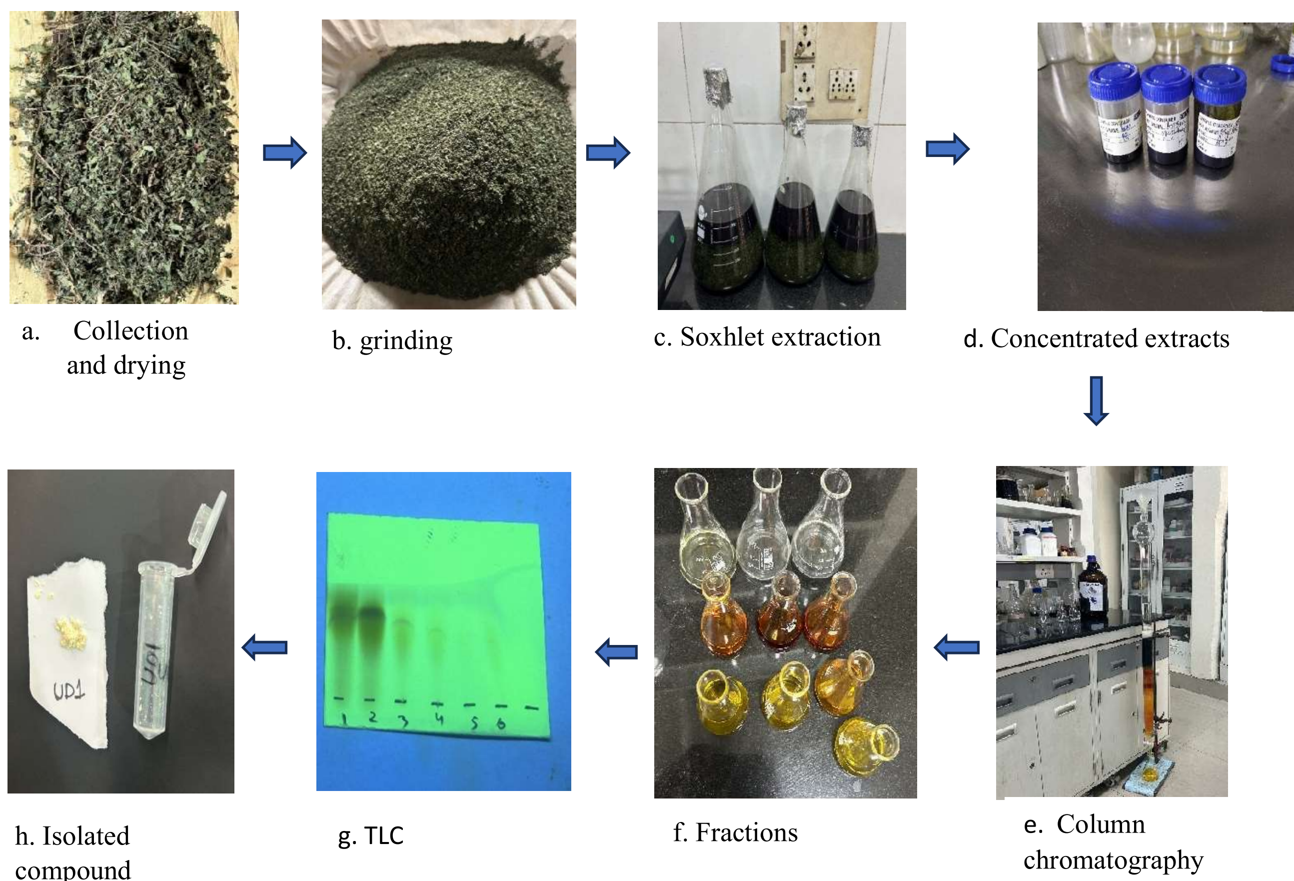

Fig. 1. Pictorial representation of the methodology

Plant material collection and preparation

The young plants (stem and leaves) of *U. dioica* were collected from Mt. Apharwat area of Gulmarg, Kashmir on 02 August 2023. Geographical coordinates of the site sample are as Latitude:34.0484° and Longitude as 74.3805°. The plant was identified by Akhtar H. Malik, Curator, Taxonomist, Centre for Biodiversity and Taxonomy (CBT), Department of Botany, University of Kashmir vide voucher specimen No. 9076-KASH Herbarium, dated: 10-08-2023. Stem and leaves of *U. dioica* were washed with tap water and then shade dried for 7 days. A coarse powder of the material was prepared using mortar and pestle for extraction.

Soxhlet Extraction

A total of 3 kg of dried plant powder was subjected to Soxhlet extraction in batches of 100 g resulting in a total of 30 extraction batches. For each batch, 100g of powdered plant material was loaded into a cellulose thimble and placed in a1L capacity Soxhlet extractor connected to 2L round bottom flask and a reflux condenser. Each batch was extracted using 1000 mL of methanol in a Soxhlet apparatus at the boiling point of 65°C. Extraction

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was carried out for 8 h, corresponding to approximately 10–12 siphon cycles per batch. Each cycle had an average duration 40-50 minutes. The process was repeated in successive batches until the entire 3 kg of powdered material was extracted. A total of 30L of solvent was used for the extraction. The methanolic extracts obtained from all batches were pooled and concentrated under reduced pressure using a rotary evaporator to obtain the crude methanolic extract. The concentrated extract was further dried to constant weight and stored at 4 °C until further analysis. The total yield of the crude methanolic extract was 600 g (20% yield)

Isolation of the Compound

The fractionation of the methanolic solvent extract was performed in order to isolate chrysin by silica 120 column chromatography. Column chromatographic separation was performed using Merck 7734 silica gel (60–120 mesh), and TLC experiment was carried out with pre-coated Merck silica gel 60 PF254 aluminium sheets; the spots were visualized under UV light. The extract prepared (600g) was mixed with silica 60 to make a slurry. The column was loaded with silica of mesh size 120 and at top loaded with the slurry. The solvent gradient used was Chloroform: Methanol (100:0, upto 0:100) and 60 fractions were collected and were then analysed by TLC and those with same spots were pooled together to give over all 8 subfractions due to similarity in TLC profile. The subfraction having the compound was again run on column till pure bioactive compound was isolated. Structure elucidation of the compound was performed by Mass spectroscopy, NMR and HRMS.

Antibacterial assay

The antibacterial activity of the compound was checked by the Agar well diffusion method (Vignesh *et al.*, 2021c) against five fish pathogenic bacteria (*E. coli, B. subtilis, S. aureus, P. mirabilis, K. pneumoniae)*. The fish bacterial strains were procured from the Advanced Research Lab, Department of Zoology, University of Kashmir. Five strains of bacteria: *E. coli* (OP268610.2), *K. pneumonia* (OP268611.2), *S. aureus* (OP268597.2), *P. mirabilis* (OP268587.2) and *B. subtilis* (OP268612.2) were taken for checking the antibacterial activity of our isolated compound.

Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) of each extract was evaluated using the broth dilution technique as outlined by (Andrews *et al.,* 2001). Each tube with broth and compound was inoculated with 100 µL of a 24-hour-old bacterial suspension standardized to 1.5×10^6 CFU/ml. After inoculation, the tubes were sealed and incubated at 37°C for 24 hours. Following incubation, bacterial growth was assessed by checking for turbidity, and the optical density at 600 nm was measured using a UV–Visible double-beam spectrophotometer.

Statistical Analysis

All experiments were performed in triplicate, and the results were presented as the mean (± SD). Prior to analysis of variance (ANOVA), preliminary test (Shapiro Wilks test), ensured normality of the data. Pairwise comparisons were made using Tukey's HSD test ($p < 0.05$).

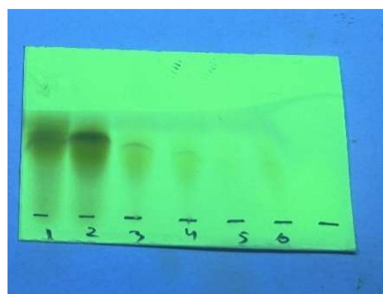
RESULTS AND DISCUSSION

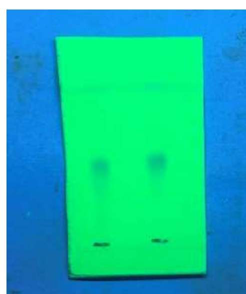
The fractionation of the methanolic solvent extract was performed in order to isolate bio active compounds by silica 120 column chromatography. 60 fractions were collected and were analysed by TLC as depicted in Table 1. Fractions having the same TLC were pooled together into 8 subfractions

SF1-SF8. These 8 subfractions were again analysed on TLC. The subfraction 1 and 2 were seen with spot, they were selected for further fractionation and in the solvent gradient 90:10 (chloroform: methanol) the compound named UD1 was isolated. Fig.2. shows the UV active compound on TLC under UV spectrophotometer.

Table 1. Isolation of chrysin from methanolic extract

Chloroform: Methanol (%)	**Fractions**	**Subfractions**	**Subfraction with spot on Tlc**	**Compound isolated**
100:0	1-2			
95:5	3-5	SF1	Yes	UD1
90:10	6-10	SF2		
85:15	11-15	SF3		
80:20	16-20			
75:25	21-25	SF4		
70:30	26-30			
60:40	31-35	SF5		
50:50	36-40	SF6		
40:60 30:70	41-45 46-50	SF7		
0:100	50-60	SF8		





a **b**

Fig. 2 (a, b) Shows the TLC profile of UD1

Fig. 2.a shows the TLC of sub fraction SF1 and SF2, showing the presence of the compounds and Fig. 2.b shows the TLC of isolated compound UD1. The Rf valve is 0.4 (RF= $\frac{20\text{mm}}{50\text{mm}}$ = 0.4). The UD1 was chrysin which was validated by NMR, mass spectroscopy, HRMS.

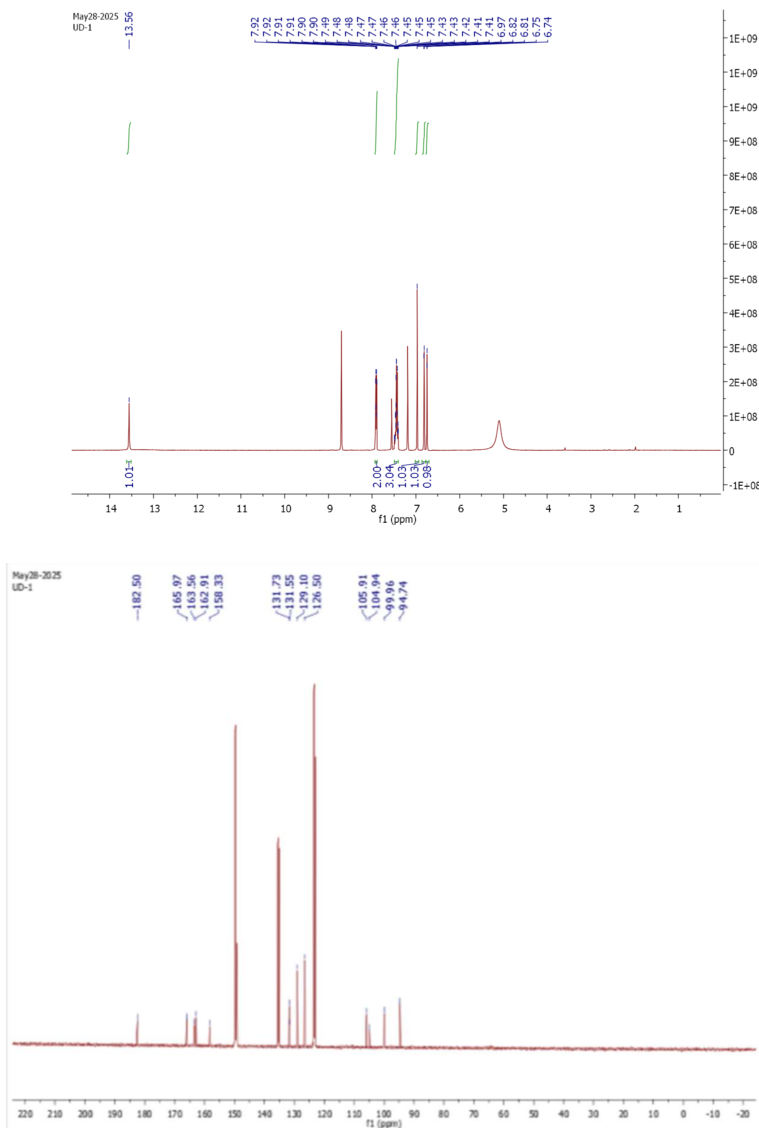


Fig. 3 shows the HNMR (above) and CNMR (below) of the isolated compound UD1 respectively. The structure of chrysin was confirmed by detailed analysis of its 1H and 13C NMR spectra recorded in pyridine-d_5 and are as 1H NMR (400MHz, Pyridine-d 5): δ 13.56 (s, 1H), 7.92 – 7.90 (m, 2H), 7.49 – 7.41 (m, 3H), 6.97 (s, 1H),6.81 (d, J = 2.1 Hz, 1H), 6.75 (d, J = 2.1 Hz, 1H); 13C NMR (101 MHz, Pyridine-d 5): δ 182.50(–C=O), 165.97 (–C), 163.56 (–C), 162.91 (–C), 158.33 (–C), 131.73 (–CH), 131.55 (–C),129.10 (2× –CH), 126.50 (2×–CH), 105.91 (–CH), 104.94 (–C), 99.96 (–CH), 94.74 (–CH).

The **^{1}H NMR** spectrum (400 MHz, pyridine-d_5) displays a highly de-shielded singlet at δ 13.56 (1H), which is assigned to the 5-hydroxyl proton. This pronounced downfield shift is attributed to strong intramolecular hydrogen bonding between the 5-OH group and the C-4 carbonyl functionality, a characteristic feature of flavones bearing a 5-hydroxy substitution. The aromatic protons of the monosubstituted B ring resonate as multiplets at δ 7.92–7.90 (2H, H-2′ and H-6′) and δ 7.49–7.41 (3H, H-3′, H-4′, and H-5′), consistent with a phenyl ring attached at C-2 of the flavone nucleus. A singlet observed at δ 6.97 (1H) is attributed to H-3, a diagnostic proton of the flavone core. Additionally, two meta-coupled aromatic protons appear as doublets at δ 6.81 and 6.75 (J = 2.1 Hz, each 1H), corresponding to H-8 and H-6 on ring A, respectively, confirming hydroxyl substitution at the C-5 and C-7 positions.

The ^{13}CNMR spectrum (101 MHz, pyridine-d_5) exhibits fifteen carbon resonances, in accordance with the molecular structure of chrysin. The conjugated carbonyl carbon (C-4) is observed at δ 182.50. Signals in the downfield region at δ 165.97, 163.56, 162.91, and 158.33 are assigned to oxygenated quaternary carbons within the flavone framework, including C-5, C-7, C-9, and C-2. The aromatic carbons of the B ring resonate at δ 131.73 (CH), 129.10 (2 × CH), and 126.50 (2 × CH), while the quaternary ipso carbon linking the B ring to the flavone core appears at δ 131.55. Resonances at δ 105.91 (CH) and 104.94 (C) are attributed to C-3 and C-10, respectively. The upfield signals at δ 99.96 (CH) and 94.74 (CH) correspond to C-6 and C-8 of ring A, reflecting their proximity to electron-donating hydroxyl groups.

The further validation of the compound was performed by its mass spectrometry (Fig. 4) and its mass came to be 254 which was further validated by HRMS which shows ion peak at m/z 255.0656 $[M+H]^+$ indicating a molecular formula of C15H10O4 (Fig.5).

As the compound now was identified as chrysin, its antibacterial activity was seen at a concentration of 2mg/mL and 1mg/ml against 5 pathogenic bacterial strains of fish as depicted in table 2.

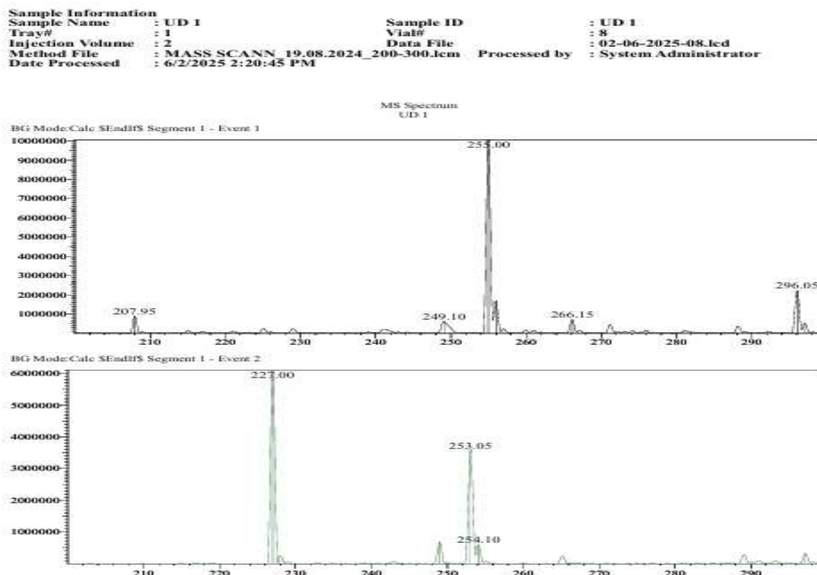


Fig. 4. shows the mass spectroscopy of the compound UD1

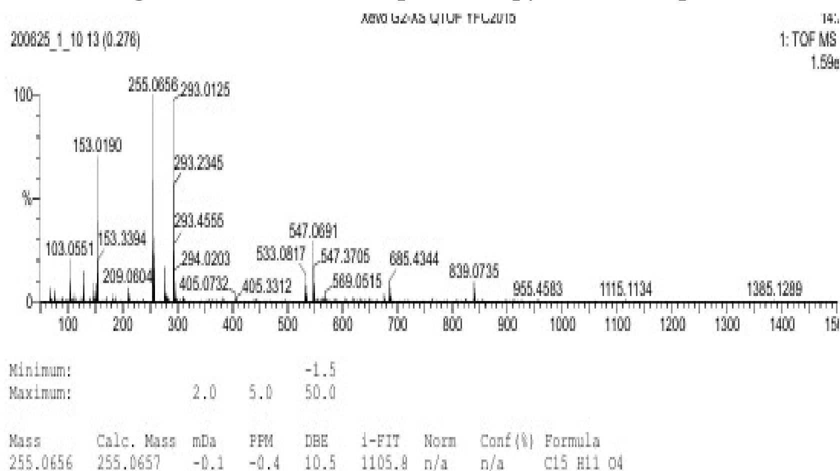


Fig. 5. Shows the HRMS of the compound UD1

Table 2. Showing the antibacterial assay of the compound

Zone of inhibition in mm				
Bacteria	Ciprofloxacin(+ive)	Chrysin(2mg)	Chrysin(1mg)	2% DMSO(-ive)
E. coli	19.87±0.13	8.00±0.00^{a}	8.00±0.00^{a}	8.00±0.00
B. subtilis	26.20±0.20	12.27±0.30^{a}	9.33±0.92^{b}	8.00±0.00
S. aureus	22.47±0.29	13.63±0.13^{a}	9.66±0.47^{b}	8.00±0.00
P. mirabilis	27.33±0.33	8.00±0.00^{a}	8.00±0.00^{a}	8.00±0.00
K. pneumoniae	26.53±0.29	8.00±0.00^{a}	8.00±0.00^{a}	8.00±0.00

The experiment was carried out in triplicates and the data is present as mean ± standard deviation.

From the above table 2 and fig. 6, it is very clear that the compound was active against *S. aureus* and B. subtilis only with a Zone of inhibition (ZOI) of about 13.63±0.13 mm and 12.27±0.30 mm respectively which is considered to have moderate activity against these two bacteria. Other bacteria were seen to be resistant against the compound. ZOI were measured along with the well diameter which is 8mm.

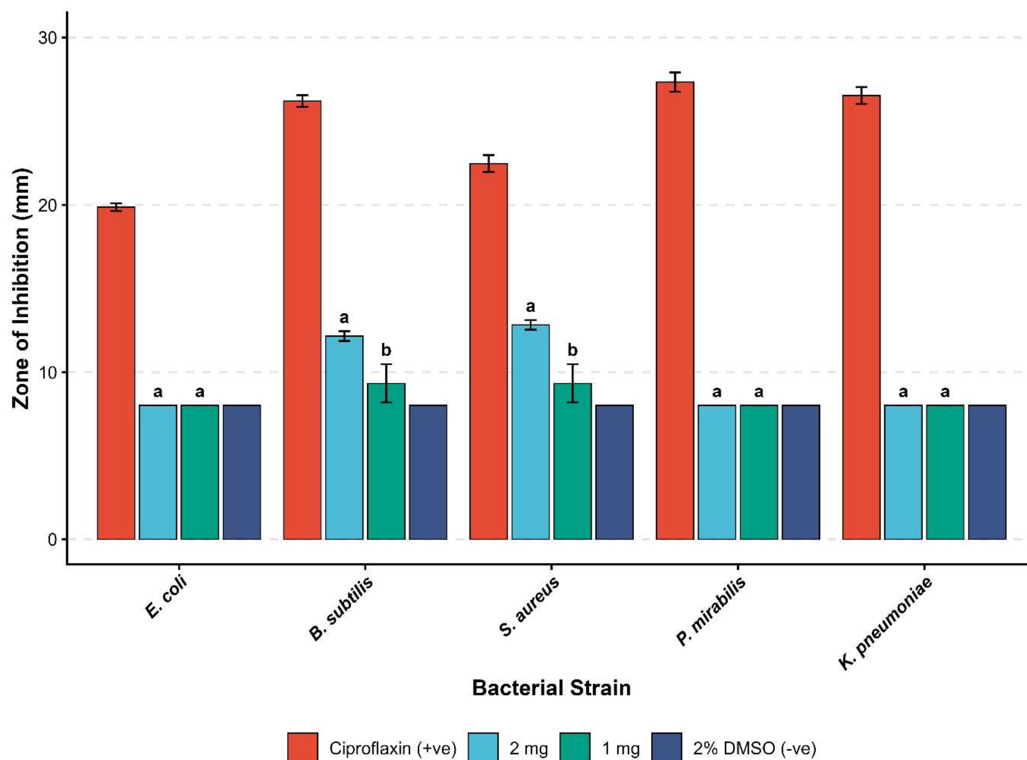


Fig.6 Bar graph depicting the Zone of inhibition of chrysin against the pathogenic bacteria

A two-way ANOVA was performed to evaluate the effects of treatment concentration and bacterial strain on antibacterial activity. The analysis revealed a significant main effect of treatment, F (3, 40) = 4385.42, p < 0.001, and a significant main effect of bacterial strain, F (4, 40) = 975.20, p < 0.001.

Furthermore, a significant interaction between treatment and bacterial strain was observed, F (12, 40) = 295.14, p < 0.001, indicating that the effect of treatment varied among bacterial species (Table 3).

Table 3. 2-way Anova results of Extract x Bacteria

Term	*df*	sumsq	meansq	F-value	*p*
Treatment	3	2315.27	771.76	4385.42	< .001***
Bacteria	4	686.53	171.05	975.20	< .001***
Treatment × Bacteria	12	623..18	51.93	295.14	< .001***
Residuals	40	7.04	0.176		

P<0.001*** shows data is significant

Post hoc comparisons using Tukey's HSD (Table 4) test were conducted to further examine differences between concentrations within each bacterial strain. Concentration 2 showed a significant increase in antibacterial activity compared to concentration 1 for *Bacillus subtilis* (mean difference = 2.833, *p* = 0.0146) and *Staphylococcus aureus* (mean difference = 3.5, p = 0.0070). No significant differences between concentrations were observed for *Escherichia coli*, *Proteus mirabilis*, or *Klebsiella pneumoniae* (*p* > 0.05).

Table 4: Shows Pairwise Comparisons using Tukey's HSD

Bacteria	Difference	SE	t-ratio	p-value	Sig.
E. coli	0	0	1	0.3739	ns
B. subtilis	2.833	0.687	4.123	0.0146	Sig.
S. aureus	3.5	0.687	5.093	0.0070	Sig.
P. mirabilis	0	0	1	0.3739	ns
K. pneumoniae	0	0	1	0.3739	ns

Where ns indicates non-significant and sig. indicates significant

Table 5. Showing the MIC of chrysin against the selected bacteria

S.no.	Bacteria	MIC(μg/mL)	
		Ciprofloxacin	Chrysin
1	*B. subtilis*	3.125	50
2	*S. aureus*	3.125	50

MIC of the UD1 was checked only against 2 bacteria (*B. subtilis and S. aureus*) against which it showed ZOI. It was seen to be 50 µg against both these bacteria as in (table 5).

The present study focuses on the isolation and characterization of chrysin from *U. dioica* and its antibacterial activity. Chrysin is a dietary flavone that was first isolated from *Passiflora caerulea* by (Wolfman *et al.,* 1994). Subsequent studies reported its extraction from several other natural sources, including *P. incarnata*, mushrooms, and *Pleurotus ostreatus* (Jayakumar *et al.,* 2009). Brown *et al*., (2007) extracted chrysin from *P. incarnata* and evaluated its anxiolytic activity under in vitro conditions. More recently, Đurović *et al.* (2023; 2024) isolated chrysin from the leaf extract of *U. dioica*, showing that nettle extract obtained through Soxhlet extraction contains approximately 0.04 ± 0.00 mg/L of chrysin. In the present study, we successfully isolated chrysin from *Urtica dioica*. The concentration of purified chrysin obtained from the methanolic extract was 0.1mg/L in solvent. Structural identification and confirmation were achieved using several analytical techniques, including ^{1}H NMR, ^{13}C NMR, MS, and HRMS. A previous report by (Saric *et al*.,2004) optimized TLC conditions for chrysin detection, and our results were further validated by comparison with published spectral and structural data (Liu *et al.,* 1993; 2001; Larit *et al.,* 2012). Reported chrysin yields from other natural sources vary widely, including 2.92–20.4 mg/L from *Oroxylum indicum* root bark (Zaveri *et al.,* 2007); 400 mg/kg from ethanolic extracts of *Pleurotus ostreatus*; and 500 mg/kg in ethanolic extracts of *Cytisus multiflorous* (Pereira *et al.*, 2012).

The anti-bacterial assay showed that compound has moderate anti-bacterial activity against *S. aureus* with a ZOI of about 13.63±0.13 mm followed by *B. subtilis* with a ZOI of about12.27±0.30 mm. Other three bacteria were seen to be resistant. The selective antibacterial activity of the compound against *Gram-positive* bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) may be attributed to fundamental differences in the cell envelope architecture of Gram-positive and Gram-negative organisms. Gram-positive bacteria possess a thick peptidoglycan layer but lack an outer membrane, which facilitates the penetration of small molecules and enables easier access to intracellular or cell wall-associated targets. In contrast, Gram-negative bacteria are protected by an additional outer membrane enriched with lipopolysaccharides (LPS), which acts as an effective permeability barrier and restricts the entry of many antibacterial agents, particularly those that are hydrophobic or moderately polar (Silhavy *et.al*., 2010; Zgurskaya *et.al*., 2015). Ciprofloxacin was taken as positive control and 2% DMSO as negative control. MIC of the compound was checked against bacteria (*B. subtilis and S. aureus*) and was seen to be 50µg/mL against both of these bacteria. These findings were seen to be consistent with earlier studies (Babu *et.al.,* 2006) and were also supported by (Adesina *et.al*., 2024).

CONCLUSION

In conclusion, we have been successful in isolating chrysin from the aerial part of *U. dioica*. From different solvent extracts, methanolic extract was seen to have potent amount of chrysin. As a result, the amount of chrysin obtained was 0.1 mg/ L in solvent and was greater than that of previously isolated chrysin by different researchers. So, the

total amount of purified chrysin obtained was 3mg/ 30 litres of solvent or 3mg/ 3kg of dry plant material giving the total yield of 0.0001%.

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REFERENCES

Bondad-Reantaso, M. G., Subasinghe, R. P., Arthur, J. R., Ogawa, K., Chinabut, S., Adlard, R., and Shariff, M. 2005. Disease and health management in Asian aquaculture. *Veterinary parasitology*, **132**(3-4), 249-272. https://doi.org/10.1016/j.vetpar.2005.07.005

Chenia, H. Y., and Vietze, C. J. A. J. M. R. 2012. Tetracycline resistance determinants of heterotrophic bacteria isolated from a South African tilapia aquaculture system. *Afr. J. Microbiol. Res*, **6**(39), 6761-6768. https://doi.org/10.5897/AJMR10.840

Gowri, J., Sahayaraj, P. A., and Amaladasan, M. 2016. Antimicrobial activity of the leaf, flower and stem extracts of Sphenoclea zeylanica. *International Journal of Applied Sciences and Biotechnology*, **4**(3), 325-329. https://doi.org/10.3126/ijasbt.v4i3.15760

Ahmad, I., and Beg, A. Z. 2001. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. *Journal of ethnopharmacology*, **74**(2), 113-123. https://doi.org/10.1016/S0378-8741(00)00335-4

Grauso, L., De Falco, B., Lanzotti, V., and Motti, R. (2020). Stinging nettle, *Urtica dioica* L.: Botanical, phytochemical and pharmacological overview. *Phytochemistry Reviews*, **19**(6), 1341-1377. https://doi.org/10.1007/s11101-020-09680-x

Niaz, M., Abrar, H., Ashfaq, S., Khan, N., e Baseerat, N., Jadoon, R., & Abrar, A. 2024. Qualitative Phytochemical Analysis of Roots, Stem, and Leaves Extracts of *Uritica dioica*. *Phytopharmacology Research Journal*, **3**(1), 24-30.

Orčić, D., Francišković, M., Bekvalac, K., Svirčev, E., Beara, I., Lesjak, M., and Mimica-Dukić, N. 2014. Quantitative determination of plant phenolics in *Urtica dioica* extracts by high-performance liquid chromatography coupled with tandem mass spectrometric detection. *Foodchemistry*, **143**, 48-53 .https://doi.org/10.1016/j.foodchem.2013.07.097

Veliˇckovi´c, V.; Đurovi´c, S.; Radojkovi´c, M.; Cvetanovi´c, A.; Švarc-Gaji´c, J.; Vuji´c, J.; Trifunovi´c, S and Maškovi´c, P.Z. 2017. Application of conventional and non-conventional extraction approaches for extraction of Erica carnea L.: Chemical profile and biological activity of obtained extracts. *Journal of Supercritical Fluids*, **128**, 331–337. https://doi.org/10.1016/j.supflu.2017.03.023

Đurović, S., Kojić, I., Radić, D., Smyatskaya, Y. A., Bazarnova, J. G., Filip, S., and Tosti, T. 2024. Chemical constituents of stinging nettle (*Urtica dioica* L.): A comprehensive review on phenolic and polyphenolic compounds and their bioactivity. *International Journal of Molecular Sciences*, **25**(6), 3430. https://doi.org/10.3390/ijms25063430

Immacualte, A. A., Vimala, J. R., and Takuwa, D. T. 2023. Development and Validation of an RP-HPLC Method for the Quantification of Selected Anti-Cancer Standards (Rutin, Chrysin, Piperine and Berberine) in Botswana Medicinal Plants.

Mani, R., and Natesan, V. (2018). Chrysin: Sources, beneficial pharmacological activities, and molecular mechanism of action. *Phytochemistry*, *145*, 187-196. https://doi.org/10.1016/j.phytochem.2017.09.016

Zeybek, E., and Kart, A. 2021. Chrysin: potential pharmacological and toxicological effects. *Veteriner Farmakoloji ve Toksikoloji Derneği Bülteni*, **12**(3), 151-160.

https://doi.org/10.38137/vftd.995966

Bansal, A., Srivastava, N., and Nagpal, K. 2022. Development and validation of UV spectrophotometric method for determination of chrysin and its solubility studies. *Journal of Applied Spectroscopy*, **89**(1), 150-158 https://doi.org/10.1007/s10812-022-01338-0

Vignesh, A., Selvakumar, S., and Vasanth, K. 2022. Comparative LC-MS analysis of bioactive compounds, antioxidants and antibacterial activity from leaf and callus extracts of Saraca asoca. *Phytomedicine plus*, *2*(1), 100167. https://doi.org/10.1016/j.phyplu.2021.100167

Andrews, J. M. 2001. The development of the BSAC standardized method of disc diffusion testing. *Journal of Antimicrobial Chemotherapy*, **48**(suppl_1), 29-42.

https://doi.org/10.1093/jac/48.suppl_1.29

Wolfman, C., Viola, H., Paladini, A., Dajas, F., and Medina, J. H. 1994. Possible anxiolytic effects of chrysin, a central benzodiazepine receptor ligand isolated from Passiflora coerulea. *Pharmacology Biochemistry and Behavior*, **47**(1), 1-4. https://doi.org/10.1016/0091-3057(94)90103-1

Jayakumar, T., Thomas, P. A., and Geraldine, P. 2009. In-vitro antioxidant activities of an ethanolic extract of the oyster mushroom, Pleurotus ostreatus. *Innovative Food Science & Emerging Technologies*, **10**(2), 228-234.

https://doi.org/10.1016/j.ifset.2008.07.002

Brown, E., Hurd, N. S., McCall, S., and Ceremuga, T. E. 2007. Evaluation of the anxiolytic effects of chrysin, a Passiflora incarnata extract, in the laboratory rat. *AANA journal*, *75*(5).

Đurović, S., Micić, D., Šorgić, S., Popov, S., Gašić, U., Tosti, T., ... and Zeković, Z. 2023. Recovery of polyphenolic compounds and vitamins from the stinging nettle leaves: thermal and behavior and biological activity of obtained extracts. *Molecules*, **28**(5), 2278. https://doi.org/10.3390/molecules28052278

Đurović, S., Kojić, I., Radić, D., Smyatskaya, Y. A., Bazarnova, J. G., Filip, S., and Tosti, T. 2024. Chemical constituents of stinging nettle (Urtica dioica L.): A comprehensive review on phenolic and polyphenolic compounds and their bioactivity. *International Journal of Molecular Sciences*, **25**(6), 3430.

https://doi.org/10.3390/ijms25063430

Medić-Šarić, M., Jasprica, I., Smolčić-Bubalo, A., and Mornar, A. 2004. Optimization of chromatographic conditions in thin layer chromatography of flavonoids and phenolic acids. *Croatica Chemica Acta*, **77**(1-2), 361-366.

Zschocke, S., Liu, J. H., Stuppner, H., & Bauer, R. 1998. Comparative study of roots of Angelica sinensis and related umbelliferous drugs by thin layer chromatography, high-performance liquid chromatography, and liquid chromatography–mass spectrometry. *Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques*, *9*(6), 283-290.

https://doi.org/10.1002/(SICI)1099-1565(199811/12)9:6<283::AID-PCA419>3.0.CO;2-9

Zaveri, M., & Jain, S. 2007. Gastroprotective effects of root bark of Oroxylum indicum, vent. *Journal of Natural Remedies*, **7**(2), 269-277.

Pereira, O. R., Silva, A. M., Domingues, M. R., & Cardoso, S. M. (2012). Identification of phenolic constituents of Cytisus multiflorus. *Food Chemistry*, **131**(2), 652-659.

https://doi.org/10.1016/j.foodchem.2011.09.045

Silhavy, T. J., Kahne, D., and Walker, S. (2010). The bacterial cell envelope. *Cold Spring Harbor perspectives in Biology*, **2(**5), a000414. doi: 10.1101/cshperspect.a000414

Zgurskaya, H. I., López, C. A., and Gnanakaran, S. (2015). Permeability barrier of Gram-negative cell envelopes and approaches to bypass it. *ACS infectious diseases*, **1**(11), 512-522.

https://doi.org/10.1021/acsinfecdis.5b00097

Babu, K. S., Babu, T. H., Srinivas, P. V., Kishore, K. H., Murthy, U. S. N., & Rao, J. M. (2006). Synthesis and biological evaluation of novel C (7) modified chrysin analogues as antibacterial agents. *Bioorganic & medicinal chemistry Letters*, **16**(1), 221-224.

https://doi.org/10.1016/j.bmcl.2005.09.009

Adesina, A. F., Adewuyi, A., and Otuechere, C. A. (2024). Exploratory studies on chrysin via antioxidant, antimicrobial, ADMET, PASS and molecular docking evaluations. *Pharmacological Research-Modern Chinese Medicine*, **11**, 100413.

https://doi.org/10.1016/j.prmcm.2024.100413