SPECTROSCOPIC PROFILING, EXTRACTION, ISOLATION AND CHEMICAL COMPOSITION OF BIOACTIVE MOLECULES OBTAINED FROM URARIA PICTA (PRISHNIPARNI)

Mahesh Kumar Ahirwar Research scholar, Department of chemistry, Govt. Dr. Shyama Prasad Mukherjee science and commerce college Kolar, Bhopal (Madhya Pradesh)
Dr. Asha Verma Professor, Department of chemistry, Govt. Dr. Shyama Prasad Mukherjee science and commerce college Kolar, Bhopal (Madhya Pradesh)
Vivekanand Ahirwar Research scholar, Department of chemistry, Govt. Dr. Shyama Prasad Mukherjee science and commerce college Kolar, Bhopal (Madhya Pradesh)

ABSTRACT
Medicinal plants have long been recognized with significant therapeutic potential in recently study focuses on the isolation and structural characterization of bioactive constituents from Uraria Picta is a well-known medicinal plant widely used in traditional herbal systems due to its diverse therapeutic properties. The present study focuses on the spectroscopic profiling and isolation of bioactive chemical constituents from different extracts of Uraria picta. Various solvents such as methanol, ethanol, and aqueous media were used for extraction to ensure maximum recovery of phytochemicals. Preliminary phytochemical screening revealed the presence of important secondary metabolites including alkaloids, flavonoids, phenolic compounds, tannins, and glycosides. The crude extracts were subjected to chromatographic techniques for the separation and purification of individual compounds. Further characterization of isolated compounds was carried out using advanced spectroscopic methods such as UV-Visible spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy, and Nuclear Magnetic Resonance (NMR) spectroscopy. The spectroscopic analysis confirmed the structural identity of several bioactive molecules responsible for antioxidant and pharmacological activities. The results indicate that Uraria picta is a rich source of biologically active compounds with potential applications in pharmaceutical and nutraceutical industries. This study provides a scientific basis for the traditional use of the plant and highlights its significance for future drug development.

KEYWORDS- Uraria Picta, Isolation, Spectroscopic Technique, TLC, FTIR, UV-Visible, ^{1}H-NMR, NMR Mass Spectroscopy, Hepatoprotective properties.

1. INTRODUCTION
Uraria picta is an important medicinal plant belonging to the family Fabaceae and is widely distributed in tropical and subtropical regions. It holds a significant place in traditional systems of medicine such as Ayurveda, where it is used for the treatment of various ailments including inflammation, fever, wounds, and general weakness. The therapeutic potential of Uraria picta is primarily attributed to the presence of diverse bioactive chemical constituents. In recent years, there has been growing scientific interest in the identification and characterization of plant-based bioactive compounds due to their potential applications in pharmaceuticals, nutraceuticals, and drug development. Medicinal plants are known to contain a wide range of secondary metabolites such as flavonoids, phenolic compounds, alkaloids, glycosides, and tannins, which exhibit significant biological activities including antioxidant, antimicrobial, and anti-inflammatory effects. Spectroscopic profiling plays a crucial role in the analysis and structural elucidation of these bioactive molecules. Advanced analytical techniques such as UV-Visible spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy, and Nuclear Magnetic Resonance (NMR) spectroscopy are widely used for the identification and confirmation of chemical structures. These methods provide detailed information about functional groups, molecular frameworks, and chemical environments of isolated compounds. The determined the free radical scavenging activity, total phenolic and flavonoid contents in leaves, stem and roots of U. picta. Powdered samples of leaves, stem and roots were subjected to successive extraction with solvents of increasing polarities i.e. Ethanol, Water: Ethanol (Aqua- alcoholic) (20: 80) and Water (aqueous) using soxhlet apparatus. Total phenol, flavonoid and antioxidant activity were determined by using Folin-

Ciocalteau method, aluminum chloride colorimetric technique and DPPH free radical scavenging methods respectively. The results exhibited the maximum phenolic (1.991±0.299%) and flavonoid (2.865±0.11%) contents in ethanolic extract of leaves. For stem, the highest phenolic content (1.208±0.115%) and highest flavonoid content (22.189±2.7%) were detected in aqueous and ethanolic extracts respectively. For roots, both the maximum phenolic (3.554±0.004%) and flavonoid (0.497±0.507%) contents were found in Aqua- alcoholic extract of roots. The ethanolic extracts of leaves and stem and aqueous extract of roots were found to contain the lowest IC50 and hence, the maximum antioxidant activity. Based on the findings, it can be concluded that among the different extracts of leaves, stem and roots of U. picta, the ethanolic extracts of leaves and stem and aqueous extract of roots exhibited the more promising antioxidant activity due to the presence of phenolic and flavonoid compounds. **(Mohan et al., 2019).** The aimed to assess the variation in rhoifolin content in aerial parts of Uraria picta collected from eight locations of Madhya Pradesh for its quality standardization and selection of superior chemotype. Methods **(Saxena et al., 2016).** The synthesized silver nanoparticles (AgNPs) using honey as a bio reductant and coated with oligochitosan derived from the depolymerization of low-molecular-weight chitosan. The synthesis employed eco-friendly methods, with characterization performed via UV–Vis spectroscopy, FTIR, TEM, EDX, XRD, and LCHRMS. AgNPs synthesized with Ceibapentandra honey exhibited an average particle size of 11.71 nm, demonstrating high antibacterial activity when coated with oligochitosan **(Fiddaroini et al., 2025).** The produced silver nanoparticles (AgNPs) using five different aqueous plant extracts, namely, *Berberis vulgaris, Brassica nigra, Capsella bursa-pastoris, Lavandulaangustifolia* and *Origanum vulgare* in this study. The present work demonstrates the influence of plant extract composition (antioxidant and total phenolic content) on the size and morphology of the produced AgNPs. The biosynthetic procedure was rapid and simple and was easily monitored via colour changes and ultraviolet and visible (UV-Vis) spectroscopy. Subsequently, measurement of zeta potential (ZP), photon cross-correlation spectroscopy (PCCS), X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM) and selected area electron diffraction (SAED) analysis were employed to characterize the as-synthesis nanoparticles **(Salayová et al., 2021).**

2. Materials and Methods

2.1 Preliminary Thin layer chromatography:-

Thin-layer chromatography (TLC), a solid–liquid adsorption chromatography technique, was employed for the separation of analytes. Pre-coated TLC plates of silica gel 60 F_{254} with a layer thickness of 0.2 mm were used as the stationary phase, and different solvent systems served as the mobile phase. Samples were applied manually onto the plates as small spots using capillary tubes and allowed to air-dry. The plates were then placed in a TLC chamber previously saturated with the respective solvent system and developed at room temperature. The mobile phase ascended the plate by capillary action, carrying the applied analytes upward at different rates. Separation was achieved based on differential adsorption and migration, which depend on the polarity of the analytes, the stationary phase, and the solvent system **(Ozleme*t al.*, 2016 and Belee*t al.*, 2011).**

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

Preliminary thin-layer chromatography (TLC) analysis of the methanolic extract of *Uraria picta* was carried out using various solvent systems. Among the systems tested, Toluene: Ethyl acetate: Acetic acid (5:4.5:0.5) showed the maximum number of well-resolved spots. The suitability of the mobile phases was further evaluated using a standard flavonoid marker. Based on the resolution and spot clarity observed, Toluene: Ethyl acetate: Acetic acid (5:4.5:0.5) was selected as the optimal mobile phase and subsequently employed for column chromatography of *Uraria picta* methanolic extract.

2.2 Isolation of compound by column chromatography

1. **Column size -** Glass column, 250 x 30 mm
2. **Stationary Phase -** Silica gel (60 to 120 mesh size)
3. **Elution mode -** Gradient elution

4. **Mobile Phase -** Toluene: Ethyl acetate: Acetic acid (5:4.5:0.5) for *Uraria picta* (UP)
5. **Extracts –** *Uraria picta* (UP)
6. **Visualized by -** Short UV (254nm), long UV (365nm) and Visible light

Identification of fractions: Visualized by Short UV (254nm), long UV (365nm) and visible light.

2.3 Column chromatography

The methanolic extract of *Uraria picta* was subjected to silica gel column chromatography for the isolation of flavonoids. A vertical borosilicate glass column (30 mm internal diameter × 250 mm length) was used for the separation. Prior to packing, the column was thoroughly rinsed with acetone and completely dried. Wet packing was carried out using silica gel (60-120 mesh) as the stationary phase, with toluene employed to prepare the slurry. Approximately 3 g of the methanolic extract was carefully loaded onto the top of the packed column. Separation was achieved by gradient elution using Toluene: Ethyl acetate: Acetic acid (5:4.5:0.5, v/v/v) as the mobile phase. Eluted fractions were collected sequentially, concentrated, and analyzed by thin-layer chromatography (TLC) to monitor separation and identify fractions containing different compounds**(Srivastava *et al.*, 2021 and Mukherjee *et al.*, 2024).**

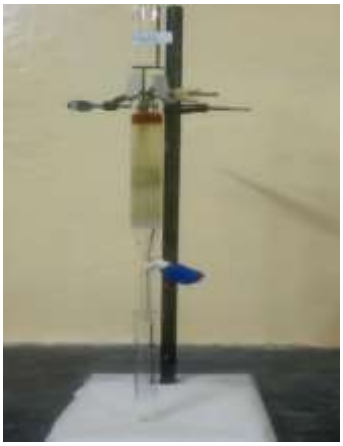


Figure 1 Isolation of components by column chromatography

2.4 Spectroscopic characterization: -

2.4.1 UV-visible Spectroscopy

The isolated fraction (F) of the UP extract was subjected to UV–Visible spectrophotometric analysis. The fraction was scanned over a wavelength range of 200–800 nm using a UV–Visible spectrophotometer (Shimadzu UV-1700), and the characteristic absorption peaks were detected and recorded. **(Patel *et al.*, 2022 and Picollo*etal.*, 2019)**.

2.4.2 FT-IR

To confirm the presence of functional groups in the isolated fraction (F) of UP extract, FT-IR spectroscopy was performed. The sample was dried, finely ground with KBr, and analysed using a Thermo Nicolet Model 6700 spectrometer. A KBr disc (200 mg) was prepared by mixing 2% of the finely dried sample with KBr, and then examined using the IR spectrometer. Infrared spectra were recorded in the range of 400–4,000 cm^{-1} **(Lucien*et al.*, 2008 and Dutta *et al.*, 2017).**

2.4.3 NMR Spectroscopy

NMR spectroscopy was performed on the isolated fraction (F)of UP extract to determine the structure of the compounds present. Nuclear Magnetic Resonance (NMR) analysis was carried out using a JNM EC-500 NMR spectrometer **(Zia *et al.*, 2019 and Marion*et al.*, 2013).**

2.4.4 Mass Spectroscopy

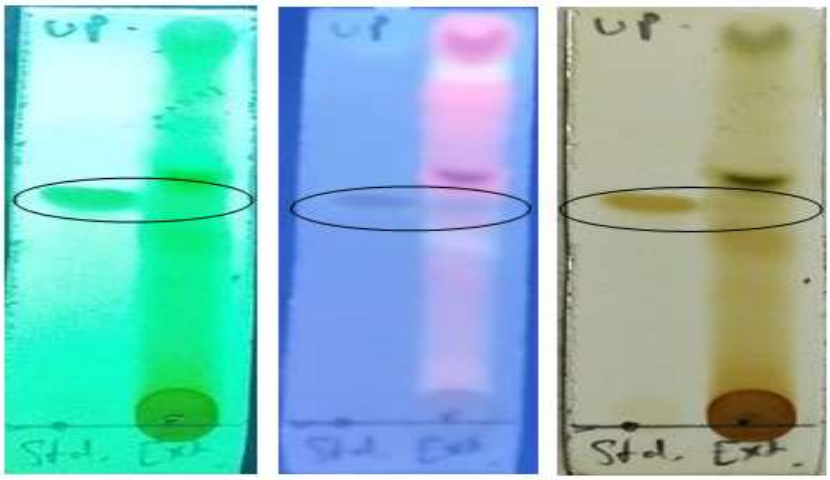
Mass spectrometry converts molecules into ions, which are then separated and sorted based on their mass-to-charge ratio (m/z). The molecular weights of the isolated fraction (F) of UP extract was determined using a micro TOF-Q mass spectrometer (Instrument ID: 228888.10348) **(Wiley *et al.*, 1995 and Torgerson*et al.*, 1974).**

3. Results

3.1 Preliminary TLC preparation for the estimation of active constitutes – TLC of *Uraria Picta* extract

1. **Flavonoid for *Uraria Picta*:-**

Mobile Phase- Toluene: Ethyl acetate: Acetic acid (5:4.5:0.5)



Short-UV (254 nm) **Long-UV (365 nm)** **Visible Light**

Figure 2 TLC estimation by UV lamp for UP with Std. flavonoid (Std.= Standard, UP= *Uraria picta*)

Table 1 TLC of *Uraria picta* extract

S. No.	Solvent system	No. of spots	Colour of spots at Wavelength (365nm)	Colour of spots at Wavelength (254)	Rf value (Extract)	Rf value (Std. flavonoid)
`1.	Toluene: Ethyl acetate: Acetic acid (5:4.5:0.5)	12	Dark Blue (Std.)	Dark Green (Std.)	-	Kaempferol= 3.2/5=0.64
			Light Pink (UP)	Green (UP)	0.9/5=0.18	
			Pink	Green	2.3/5=0.46	
			Yellow	Dark Green	2.6/5=0.52	
			Light Pink	Green	3.0/5=0.60	
			Light Blue	Green	3.2/5=0.64	
			Dark Pink	Dark Green	3.6/5=0.72	
			Pink	Green	4.1/5=0.82	
			Pink	Green	4.4/5=0.88	
			Yellow	Green	4.6/5=0.92	
			Dark Pink	Dark Green	4.7/5=0.94	
			Pink	Green	4.8/5=0.96	

Thin-layer chromatography (TLC) analysis of the *Uraria picta* (UP) extract using different solvent systems selected from the literature revealed optimal separation in Toluene: Ethyl acetate: Acetic acid (5:4.5:0.5). Under these conditions, the UP extract exhibited clearly resolved bands comparable to those of the standard flavonoid. The Rf value of the UP extract was found to be 0.64, which corresponded exactly with the Rf value of the standard kaempferol (0.64).

3.2 Column Chromatography

The fractions (elutes) obtained from silica gel column chromatography of the *Uraria picta* (UP) extract were analyzed by thin-layer chromatography (TLC) to assess the presence of different phytochemical constituents. Distinct TLC profiles were observed among the collected fractions, indicating successful separation of compounds. The UV-Visible spectra of the fractions were subsequently recorded and used for further characterization.

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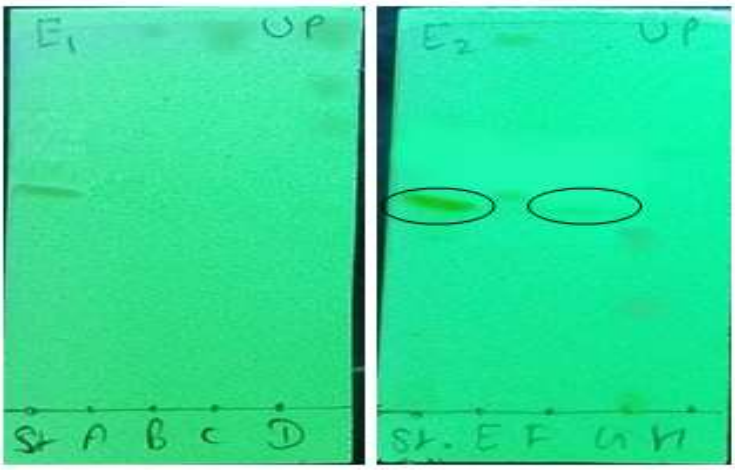
3.2.1 Column Chromatography of UP extract –

Table 2 Fraction collected from Column Chromatography ofUPExtract

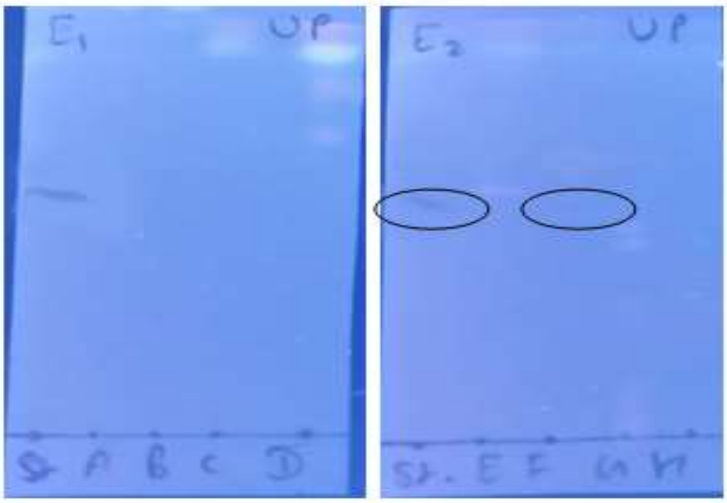
Sr. No.	Eluent composition	Fraction collected	Remarks
1	Toluene: Ethyl acetate: Acetic acid (5:4.5:0.5)	01 (A)	Creamy coloured mixture of compound
2		02 (B)	Light yellowish coloured mixture of compound
3		03 (C)	Greenish coloured mixture of compound
4		04 (D)	Yellowish coloured mixture of compound
5		05 (E)	Light yellowish coloured mixture of compound
6		06 (F)	Light Greenish coloured mixture of compound
7		07 (G)	Light yellowish coloured mixture of compound
8		08 (H)	White coloured mixture of compound

3.2.2 TLC of all collected fractions-

A) TLC of all collected fractions of UP extract-



(a) Short-UV (254 nm)



(b) Long-UV (365 nm)

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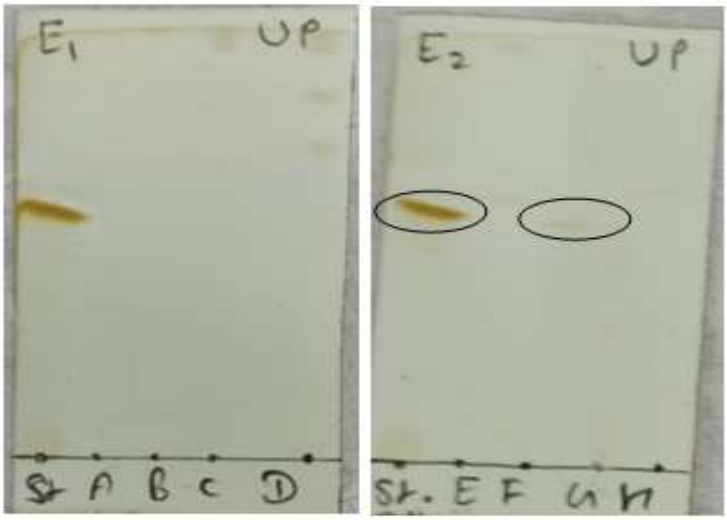

(c) Visible Light

Figure 3 TLC estimation by UV lamp for UP fractions after column chromatography with Std. flavonoid a) Short-UV (254 nm), b) Long-UV (365 nm), c) visible light.
(Std.= Standard, UP= *Uraria picta*)

(B) TLC of fractions (A, B, C, D, E, F, G, H, I and J) of UP extract -

Table 3Rfvalues of all collected fractions of GA after column chromatography

Sr. No.	Fraction	Solvent system	No. of spots	Colour of spots at Wavelength (365nm)	Colour of spots at Wavelength (254 nm)	Rf value (Extract)	Rf value (Std. flavonoid)
1.	A	Toluene: Ethyl acetate: Acetic acid (5:4.5:0.5)	-	-	-	-	Kaempferol= 3.2/5=0.64
2.	B		01	Pink	Light Green	4.8/5=0.96	
3.	C		02	Yellow Pink	Green Green	4.7/5=0.94 4.8/5=0.96	
4.	D		04	Pink Pink Yellow Pink	Light Green Light Green Green Green	4.1/5=0.82 4.4/5=0.88 4.7/5=0.94 4.8/5=0.96	
5.	E		02	Pink Fluorescence	Green Green	3.6/5=0.72 4.6/5=0.92	
6.	F		02	Light Blue Fluorescence	Green No spot	3.2/5=0.64 4.6/5=0.92	
7.	G		04	Pink Pink Yellow Pink	Light Green Light Green Green Green	0.9/5=0.18 2.3/5=0.46 2.6/5=0.52 3.0/5=0.60	
8	H		-	-	-	-	

The Rf values obtained from thin-layer chromatography (TLC) were used to confirm the presence of active constituents in the isolated fraction (F) of the *Uraria picta* extract. TLC analysis using Toluene: Ethyl acetate: Acetic acid (5:4.5:0.5) as the mobile phase showed spots corresponding to those of the standard flavonoid. Fraction F was collected separately, transferred to a watch glass, dried, and weighed. The yield of fraction F from the *Uraria picta* extract was found to be 37 mg. The dried fraction was stored in freeze for further analysis.

3.3 Spectroscopic characterization: -

3.3.1 Active constitutes estimation by UV-Spectroscopy-

The UV spectra of the isolated fractions (F) from *Uraria Picta*(UP) methanolic extract was recorded using a Shimadzu 1700 double-beam UV-Visible spectrophotometer. The spectra were obtained using

the solvents Toluene: Ethyl acetate: Acetic acid (5:4.5:0.5) for UP, over a scanning range of 200–800 nm. The λ max values of the isolated compound were determined. The isolated fraction (F) from the UP extract showed three absorption peaks at 365 nm, 266 nm, and 212 nm.

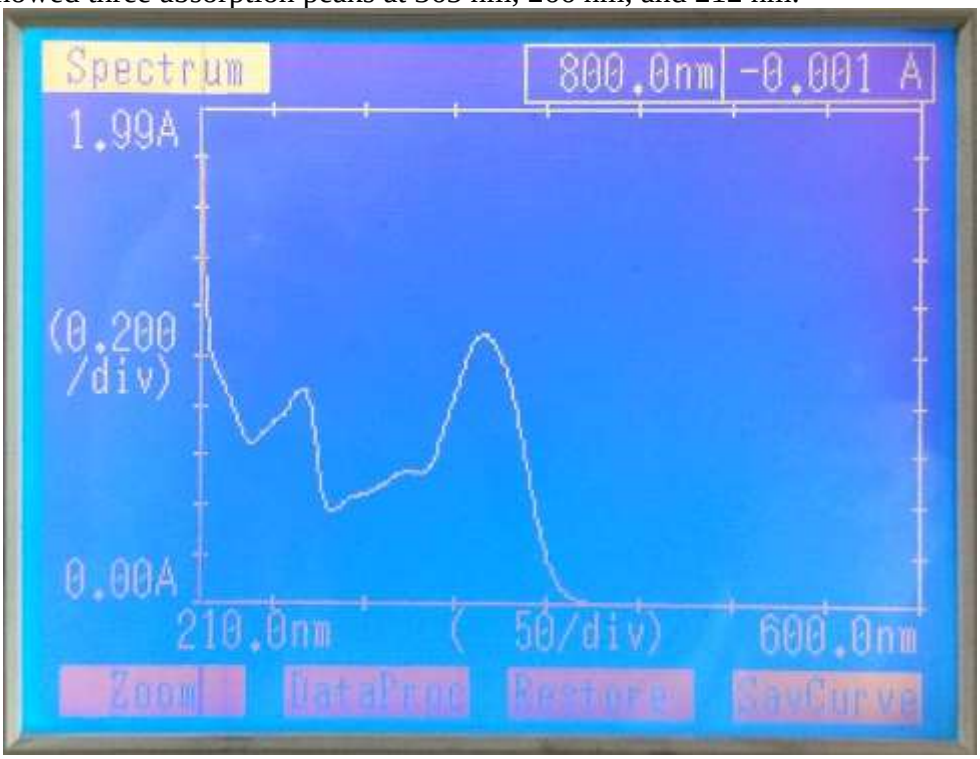

Figure 4 Active constitutes estimation by UV- Spectra of isolated fraction (F) of UP extract after column chromatography

3.3.2 Active constitutes estimation By FTIR – Spectroscopy

(A) FTIR spectra of the isolated fraction (F) of UP extract

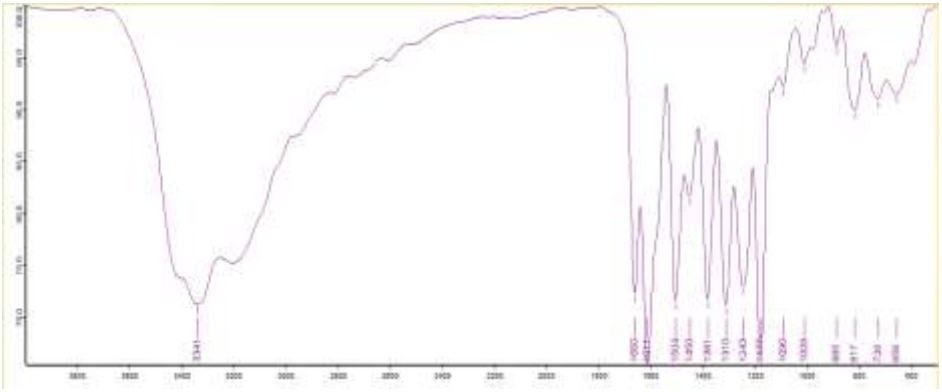

Figure 5FTIR spectra of the isolated fraction (F) **of UP extract**

Table 4 FTIR- Spectrum Frequency Range of the isolated fraction (F) **ofUP extract**

Sr. No.	Fraction	Frequency Range	Group Absorption (cm^{-1})	Appearance	Group	Compound
06	F	3550-3200 (cm^{-1})	3441	Strong, broad	O-H stretching	Hydroxyl group
		2000-1650 (cm^{-1})	1660	Weak	C-H bending	Aromatic compound
		1650-1500 (cm^{-1})	1611	Medium	C=C stretching	Cyclic alkene

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1650-1500 (cm^{-1})	1504	Medium	C=C stretching	Cyclic alkene
1480-1400 (cm^{-1})	1450	Medium	C-H bending	Alkane
1420-1330 (cm^{-1})	1381	Medium	O-H bending	Alcohol
1390-1310 (cm^{-1})	1310	Medium	O-H bending	Alcohol
1275-1200 (cm^{-1})	1243	Strong	C-O stretching	Ether
1205-1124 (cm^{-1})	1177	Strong	C-O stretching	Alcohol
1124-1087 (cm^{-1})	1090	Strong	C-O stretching	Alcohol
1000-650 (cm^{-1})	885	Strong	C=C bending	Substitute
1000-650 (cm^{-1})	817	Strong	C=C bending	Substitute
780-600 (cm^{-1})	726	Strong	C=C bending	Alkene
780-600 (cm^{-1})	656	Strong	C=C bending	Alkene

The FTIR spectrum of the isolated fraction (F) from the *Uraria picta* (UP) extract revealed the presence of various functional groups. O-H stretching appeared as medium peaks at 3441 cm^{-1}. A C-H bending peak corresponding to Aromatic compound was observed at 1660 cm^{-1}, while C=C stretching peaks of Cyclic alkene were noted at 1611 and 1504 cm^{-1}. C-H bending peak appeared at 1450 cm^{-1}. O-H bending peaks for Alcohol were detected at 1381 cm^{-1} and 1310 cm^{-1}, respectively. C–O stretching peak of ether were observed at 1243 cm^{-1}. C-O stretching peaks of Alcohol were found at 1177 cm^{-1} and 1090 cm^{-1}. C=C bending peaks of substituted compounds appeared at 885 and 817 cm^{-1}, while a strong C=C bending peaks of alkenes were noted at 726 and 656 cm^{-1}.

3.3.3 1H NMR - Spectroscopy-

^{1}H NMR spectra of the isolated fractions (F) from *Uraria Picta*(UP)extract was recorded using an NMR spectrometer. Tetramethyl silane (TMS) was used as the internal standard. The signals in the spectra are denoted as s (singlet), d (doublet), t (triplet), and m (multiplet), corresponding to the respective splitting patterns observed.

***(A) 1H NMR spectra** of the isolated fraction (F) of UP –*

In the ^{1}H-NMR spectrum of the isolated fraction (F) from *Uraria picta* (UP), the following proton signals were observed: H-1 proton appeared 0.806 (d) ppm, H-1 proton appeared at 1.185 (d) ppm, H-1 proton appeared at 2.457-2.461 (d) ppm, H-1 proton appeared at 3.298 (d) ppm, H-1 proton appeared at 5.241 (dd) ppm, H-1 proton appeared at 6.139-6.145 (d) ppm, H-1 proton appeared at 6.389-6.394 (d) ppm, H-1 proton appeared at 6.867-6.890 (dd) ppm, H-1 proton appeared at 7.988 (dd) ppm and H-1 proton appeared at 7.992-8.009 (dd) ppm.

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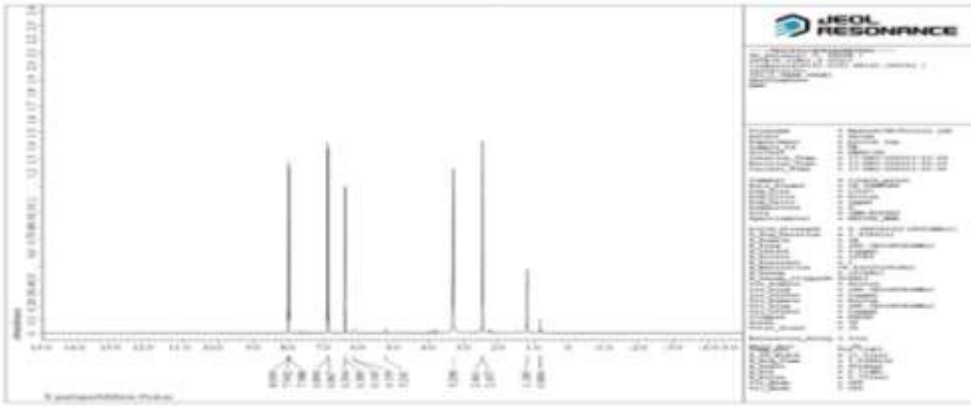


Figure 6 ^{1}**H-NMR spectra of the isolated Fraction (F) of UP**

3.3.4 Mass – Spectroscopy-

Mass spectra of the isolated fraction (F) from *Uraria Picta*(UP)were recorded using a Bruker micro TOF-Q mass spectrometer.

(A) Mass spectra of the ***isolated fraction (F) of UP***-

The mass spectrum of the isolated fraction (F) from *Uraria picta* (UP) showed a molecular ion peak [M^+] at m/z 286.2000, corresponding to the compound 3,5,7-trihydroxy-2-(4-hydroxyphenyl) chromen-4-one. The molecular composition indicated the presence of 15 carbon atoms (C), 10 hydrogen atoms (H), and 6 oxygen atoms (O), resulting in the molecular formula $C_{15}H_{10}O_6$. This identification was further supported by characteristic fragment ions observed at m/z 69, 133, 147, 165, 193, 212, 242 and 258.

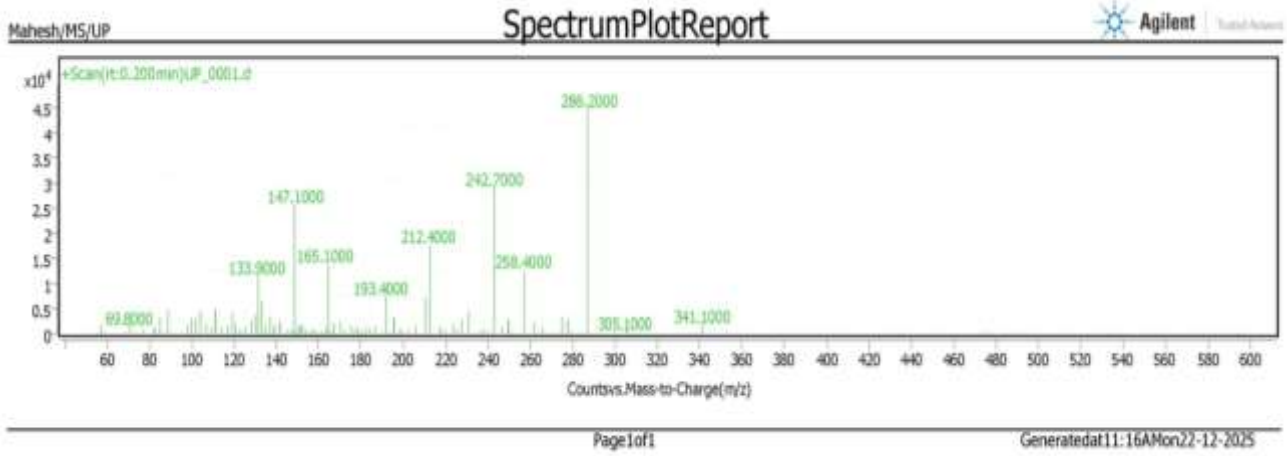
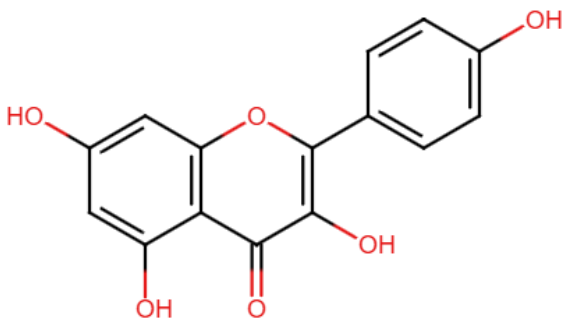


Figure 7: Mass spectra of the**isolated fraction (F) of UP**



3,5,7-trihydroxy-2-(4-hydroxyphenyl) chromen-4-one

4. Discussion:-

Thin-layer chromatography (TLC) is a widely used preliminary analytical technique for the qualitative assessment of phytochemical constituents in plant extracts. In the present study, TLC analysis of the *Uraria picta* (UP) extract was performed using various solvent systems reported in the literature to achieve optimal resolution of phytoconstituents. Among the solvent systems evaluated, Toluene: Ethyl acetate: Acetic acid (5:4.5:0.5) provided the most effective separation, yielding well-defined and clearly resolved bands.

Under these optimized chromatographic conditions, the UP extract displayed bands that were comparable in appearance and migration behavior to those of the standard flavonoid. Notably, the Rf value of the prominent band observed in the UP extract was 0.64, which exactly matched the Rf value of the reference standard kaempferol (0.64). This close correspondence strongly suggests the presence of kaempferol or a kaempferol-like flavonoid in the UP extract **(Fig 2, Table 1)**. The selection of an appropriate mobile phase is a critical step in the successful isolation of bioactive constituents from plant extracts. Based on the TLC profiling of the *Uraria picta* extract, the solvent system Toluene: Ethyl acetate: Acetic acid (5:4.5:0.5) demonstrated optimal separation and clear resolution of phytochemical bands. Consequently, this solvent system was rationally selected for further purification of the extract using silica gel column chromatography. Application of the optimized mobile phase to column chromatography enabled effective fractionation of the *Uraria picta* extract, leading to the separation of constituents based on their differential affinities toward the stationary phase. A total of eight fractions were collected and designated sequentially as fractions 01 (A), 02 (B), 03 (C), 04 (D), 05 (E), 06 (F), 07 (G), and 08 (H). The systematic elution and collection of these fractions indicate a gradual and controlled separation of compounds with varying polarities **(Fig. 1, Table 2)**. Rf values obtained from TLC analysis were used to confirm the presence of active constituents in the isolated fractions (F) of the *Uraria picta* (UP) extract. TLC was performed using the mobile phase Toluene: Ethyl acetate: Acetic acid (5:4.5:0.5) for UP, with comparisons made against a flavonoid standard **(Fig 3, Table 3)**.

The UV spectra of the isolated fractions (F) from the *Uraria Picta* (UP) methanolic extract were recorded using a Shimadzu 1700 double-beam UV–Visible spectrophotometer. The spectra were obtained using the solvent system Toluene: Ethyl acetate: Acetic acid (5:4.5:0.5) for UP over a scanning range of 200–800 nm. The λ max values of the isolated compound were determined. The isolated fraction (F) from the UP extract showed three absorption peaks at 365 nm, 266 nm, and 212 nm **(Fig 4).**

The FTIR spectrum of the isolated fraction (F) from the *Uraria picta* (UP) extract revealed the presence of various functional groups. O–H stretching vibrations appeared as medium-intensity peaks at 3441 cm^{-1}. A C–H bending peak corresponding to an aromatic compound was observed at 1660 cm^{-1}, while C=C stretching peaks of cyclic alkenes were noted at 1611 and 1504 cm^{-1}. A C–H bending peak appeared at 1450 cm^{-1}. O–H bending peaks corresponding to alcohol groups were detected at 1381 cm^{-1} and 1310 cm^{-1}, respectively. A C–O stretching peak of an ether group was observed at 1243 cm^{-1}. C–O stretching peaks of alcohol groups were found at 1177 cm^{-1} and 1090 cm^{-1}. C=C bending peaks of substituted compounds appeared at 885 and 817 cm^{-1}, while strong C=C bending peaks of alkenes were noted at 726 and 656 cm^{-1} **(Fig. 5, Table 4).**

In the ^{1}H-NMR spectrum of the isolated fraction (F) from *Uraria picta* (UP), the following proton signals were observed: H-1 proton appeared 0.806 (d) ppm, H-1 proton appeared at 1.185 (d) ppm, H-1 proton appeared at 2.457-2.461 (d) ppm, H-1 proton appeared at 3.298 (d) ppm, H-1 proton appeared at 5.241 (dd) ppm, H-1 proton appeared at 6.139-6.145 (d) ppm, H-1 proton appeared at 6.389-6.394 (d) ppm, H-1 proton appeared at 6.867-6.890 (dd) ppm, H-1 proton appeared at 7.988 (dd) ppm and H-1 proton appeared at 7.992-8.009 (dd) ppm**(Fig 6).**

The mass spectrum of the isolated fraction (F) from *Uraria Picta* (UP) showed a molecular ion peak [M^{+}] at m/z 286.2000, corresponding to the compound 3,5,7-trihydroxy-2-(4-hydroxyphenyl) chromen-4-one. The molecular composition indicated the presence of 15 carbon atoms (C), 10 hydrogen atoms (H), and 6 oxygen atoms (O), resulting in the molecular formula $C_{15}H_{10}O_6$. This

identification was further supported by characteristic fragment ions observed at m/z 69, 133, 147, 165, 193, 212, 242, and 258 (**Fig 7**).

5. Conclusion:-

The present study on Uraria picta successfully demonstrates the importance of spectroscopic profiling and systematic isolation of bioactive chemical constituents from this medicinal plant. The use of different solvent extracts enabled the efficient recovery of a wide range of phytochemicals, including flavonoids, phenolic compounds, alkaloids, and glycosides, which are known for their significant biological activities.

The application of chromatographic techniques facilitated the separation and purification of individual compounds, while advanced spectroscopic methods such as UV-Visible, FTIR, and NMR spectroscopy provided detailed insights into their structural characteristics. These analytical approaches confirmed the presence of several biologically active molecules responsible for antioxidant and therapeutic properties Physical, chemical, and spectral investigations confirmed the presence of 3,5,7-trihydroxy-2-(4-hydroxyphenyl) chromen-4-one (kaempferol) in fraction (F) of *Uraria Picta*.

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