RESEARCH ARTICLE

Isolation and Characterization of Flavonoid (Rutin) from *Atalantia racemosa* Wight for Potential Anti-inflammatory Activity

Pournima Anil Shelar*, Prashant Kumar Dhakad, Ritu Gilhotra

Gyan Vihar School of Pharmacy, Suresh Gyan Vihar University, Jaipur, India

Received: 27th Jun, 2025; Revised: 5th Aug, 2025; Accepted: 19th Aug, 2025; Available Online: 25th Sep, 2025

ABSTRACT

The medicinal plant *Atalantia racemosa* is effective against a wide range of illnesses. Several secondary metabolites found in the plant are thought to be responsible for its medicinal properties. The purpose of this research is to determine whether or not an ethanolic extract of *Atalantia racemosa* stems and leaves has anti-inflammatory properties. According to the results of this research, the ethanolic stem extract has powerful effects. Therefore, the ethanolic extract of the stem was used to isolate the component. Then, FTIR, NMR, and mass spectra were used to analyse this isolated molecule. Rutin, or 3, 3', 4', 5, 7-pentahydroxyflavone-3-rutinoside, was determined to be the isolated component based on these spectral analyses. Anti-inflammatory effect of ethanolic stem extract is thus attributed to Rutin.

How to cite this article: Pournima Anil Shelar, Prashant Kumar Dhakad, Ritu Gilhotra. Isolation and Characterization of Flavonoid (Rutin) from *Atalantia racemosa* Wight for Potential Anti-inflammatory Activity. International Journal of Drug Delivery Technology. 2025;15(3):1237-44. doi: 10.25258/ijddt.15.3.44

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Plants used for traditional medicine were the mainstay of healthcare in most underdeveloped countries. In natural medicine, plants are now the mainstay for creating novel medications and treating a wide range of illnesses. Traditional plant-based medicines are relied upon by the majority of people around the globe, particularly in more remote places, due to their accessibility, social acceptability, and affordability. Plants contain a wide variety of secondary metabolites, some of which are essential phytocomponents like alkaloids and flavonoids[1-3]. Plants are rich in flavonoids and phenolic components, which have several medicinal uses[4]. *Atalantia racemosa,* (Rutaceae), constitutes a notable plant with medicinal properties. It is usually referred to as wild lemon in English, atavijambir in Sanskrit, and ran-limbu or makad limbu in Marathi[5].

The tribes of Tamil Nadu utilize the medicinal properties of *Atalantia racemosa*, employing its roots, stems, leaves, and fruits to treat a wide range of ailments[6]. Essential oils from the fruit and leaves are used to treat paralysis, chronic rheumatism, and inflammation[7]. Fruits, bark, and leaves are used to treat coughs and gastrointestinal issues, while extracts from the leaves and seedsare effective against inflammation, chronic rheumatism, paralysis, and diarrhea. Its volatile oil is also used in aromatherapy[8].

MATERIALS AND METHODS

Collection of Plant

Atalantia racemosa, a plant species noted for its medicinal properties, was sourced from Kas Pathar in Satara district, Maharashtra, during April 2021.

Preparation of Plant Extracts

Plant material was dehydrated & crushed. During the extraction procedure, the dried powder was first treated with petroleum ether to remove the fat content & allowed for Soxhlet extraction by ethanolas solvent. Extracts were concentrated using a rotary evaporator under low pressure, ensuring the elimination of solvents without compromising the quality of the phytochemicals[9].

Preliminary Testing for Phytochemicals

To find the phytocomponents in the extracts, phytochemical screening was performed[10-12].

Experimental Animals

Experiments were carried out using Wistar Albino rats from animal house and standard room conditions were maintained for 7 days prior to study, with rats fed a standard rodent food and given unlimited access to water. The experimental conditions were maintained by keeping temperature at 22± 2°C and the relative humidity at 55± 5%[13]. When required, rats were fed nothing for twelve hours before an experiment. Laboratory animals for investigation was legalized by Animal Ethical Committee of Invitox R and D Institute, Undri, Pune (IRDI/IAEC/M03/05/2024-25).

Acute Toxicity Study

Wistar albino rats were used in this study in accordance with OECD guidelines, with three rats per group and the ethanolic extracts of leaves and stems were administered by oral gavage. These animals were observed for 24hrs and daily for 7 days[14].

Anti-inflammatory Activity

Carrageenan Induced Edema Model

5 groups of 6 rats were made such as Group I: 3ml/kg DW p. o., Group II: Carrageenan0.1 ml (1%), Group III: indomethacin 3mg/kg p.o., Group IV and V: Treatment groups which receives ethanolic extract of stem and leaves

**Author for Correspondence: pournima.shelaragcop@gmail.com*

Table 1: Anti-inflammatory activity by Carrageenan induced inflammation

Groups	Mean Paw edema at different time intervals					% edema inhibition in different time intervals			
	0 min	30 min	60 min	120 min	180 min	30 min	60 min	120min	180 min
Group I	3.46 ± 0.17*	3.57 ± 0.63*	3.63 ± 0.76*	3.61 ± 0.086*	3.37 ± 0.16*				
Group II	4.16 ± 0.025*	8.09 ± 0.087**	7.89 ± 0.032**	7.76 ± 0.048**	7.66 ± 0.085**	-	-	-	-
Group III	5.01 ± 0.089*	7.91 ± 0.087**	7.44 ± 0.154**	6.31 ± 0.056**	4.55 ± 0.086**	2.22%	5.70%	18.68%	40.60%
Group IV	5.02 ± 0.125*	7.89 ± 0.087***	7.27 ± 0.185***	6.40 ± 0.023***	4.89 ± 0.079***	2.47%	7.85%	17.52%	36.16%
Group V	5.13 ± 0.056*	7.94 ± 0.096***	7.17 ± 0.251***	6.48 ± 0.089***	5.12 ± 0.065***	1.85%	9.12%	16.49%	33.15%

Mean ± SEM (n=6), one way ANOVA followed by Dunnet t test. Nonsignificant, p≥0.05. *P<0.05, **P<0.01, ***P<0.001 compared with arthritic control.

Table 2: Anti-inflammatory activity in Histamine induced inflammation

Groups	Mean Paw in different time intervals					% edema inhibition in different time intervals			
	0 min	30 min	60 min	120 min	180 min	30 min	60 min	120 min	180 min
Group I	3.48 ± 0.34*	3.60 ± 0.09*	3.65 ± 0.012*	3.59 ± 0.89*	3.46 ± 0.74*				
Group II	4.71 ± 0.078*	8.88 ± 0.087**	8.731 ± 0.032**	8.59 ± 0.25**1	8.42 ± 0.085**	-	-	-	-
Group III	5.14 ± 0.267*	8.69 ± 0.16**	7.13 ± 0.086**	6.40 ± 0.056**	4.61 ± 0.154**	2.13%	18.32%	25.49%	45.24%
Group IV	4.57 ± 0.056*	8.80 ± 0.76***	7.61 ± 0.63***	6.425 ± 0.185***	4.98 ± 0.048***	0.90%	12.82%	25.26%	40.85%
Group V	4.94 ± 0.125*	8.78 ± 0.087***	7.96 ± 0.089***	7.485 ± 0.086***	5.43 ± 0.17***	%0.56	8.82%	12.92%	35.51%

Mean ± SEM (n=6), one way ANOVA followed by Dunnet t test. Nonsignificant, p≥0.05. *P<0.05, **P<0.01, ***P<0.001 compared with arthritic control.

Table 3: Analysis of flavonoid by TLC

Spot	Rf Value
Sample	0.41
Std. (Rutin)	0.42

Table 4: FTIR of Isolated compound

Reference Wave numbers (cm^{-1})	Wave numbers (cm^{-1})	Functional group
3460-3500	3423.62	OH carbohydrates, polyphenols
2850-2925	2930.05 2856.69	CH and CH_2 stretching alkane
1727	1726.74	C-O streching
1638-1660	1655.30	Aromatic ketone (C=O)
1500-1400	1458.18	Aromatic ring(C=C)
1200-1300	1233.86 1205.30	C–O–C asymmetric stretch in sugar units. Aromatic ether linkages (like Ar–O–C from the glycosidic bond)
1205-1124	1149.75	C-O streching tertiary alcohol
840-790	829.97	C-H out of plane bending in aromatic ring.

respectively (200mg/kg p.o.). The size of the edema for all groups was measured before and after the carrageenan injection using a digital caliper. The difference between size of edema at 0 hours (before the injection) and subsequent intervals: 30, 60, 120 & 180 minafter carrageenan injection were measured[15].

Histamine Induced Edema in Rats

Extract's capability to lessen inflammation was evaluated using histamine, which mediate the inflammatory process. In this case, we found the same clustering trend as in the carrageenan experiment. After administering standard and plant extracts orally to rats for one hour, a 0.1 ml injection of a 1% histamine solution was injected into right hind paw of the rats to initiate an inflammation. Aforementioned method was used to ascertain the variable paw edema[16]. The percentage of paw edema that was inhibited was calculated[17].

Isolation by Thin-layer Chromatography

The extract was applied as spots on a stationary phase (a plate coated with silica gel) by capillary tubes, approximately 1 cm from base of the plate. A suitable mobile phase solvent mixture consisting of Ethyl acetate: formic acid: glacial acetic acid: water (100:11:11:27) subsequently, a sufficiently-sized tank containing solvent was used to place the plate, and the solvent was allowed to rise through plate. Formation of spots in thin coating is caused by solvent rising through mixture as it separates the components at different rates. Once the solvent has covered approximately three quarters of the plate and is almost at the top edge, dried at sixty to one hundred degrees Celsius. Using TLC, the spot was located. The sample and solvent went different distances, and the result is a ratio of that two[18].

Characterization of Isolated Compound

Isolated compound was further characterized by ^{1}HNMR, ^{13}CNMR and MASS. The compound was determined by their functional groups, molecular weights and finally its structure was elucidated from the data obtained.

FT-IR Spectroscopic Analysis

This absorption leads to different molecular vibrations, including stretching, bending, twisting, and scissoring. The resulting spectrum, which plots absorbance or transmittance against wave number (cm^{-1}), serves as a unique fingerprint for each compound.

^{13}C NMR Spectroscopy

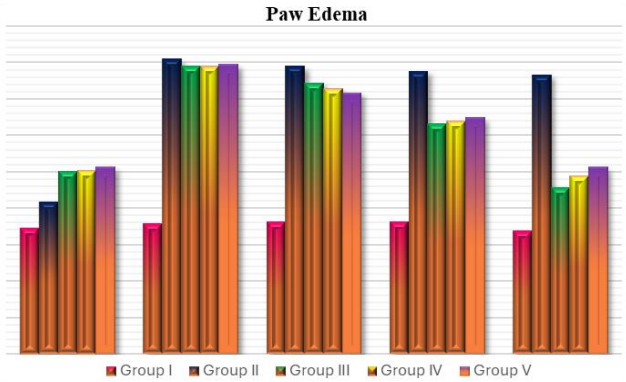

Figure 1: Mean paw edema by Carrageenan induced model

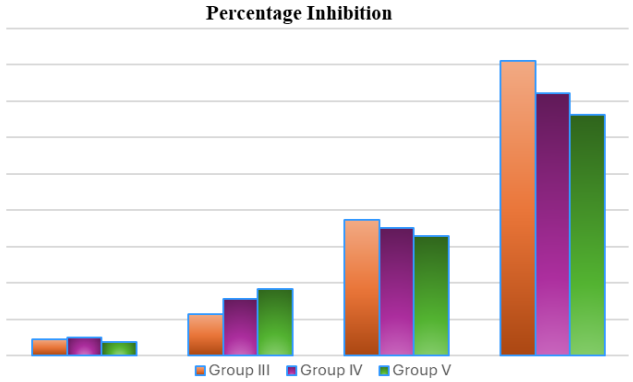

Figure 2: Anti-inflammatory activity in Carrageenan induced model

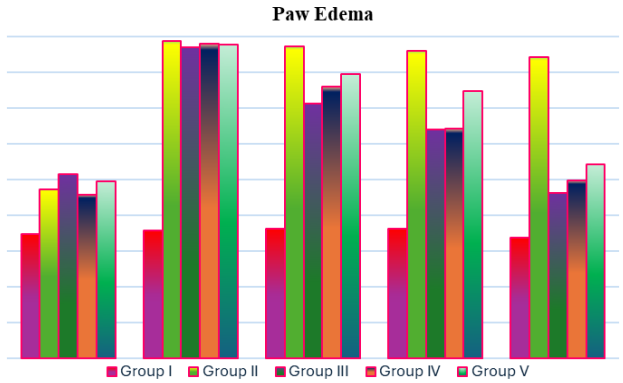

Figure 3: Mean paw edema by Histamine induced model

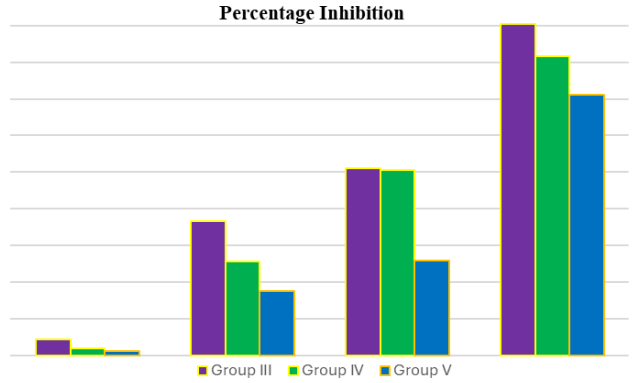

Figure 4: Anti-inflammatory activity in histamine induced inflammation

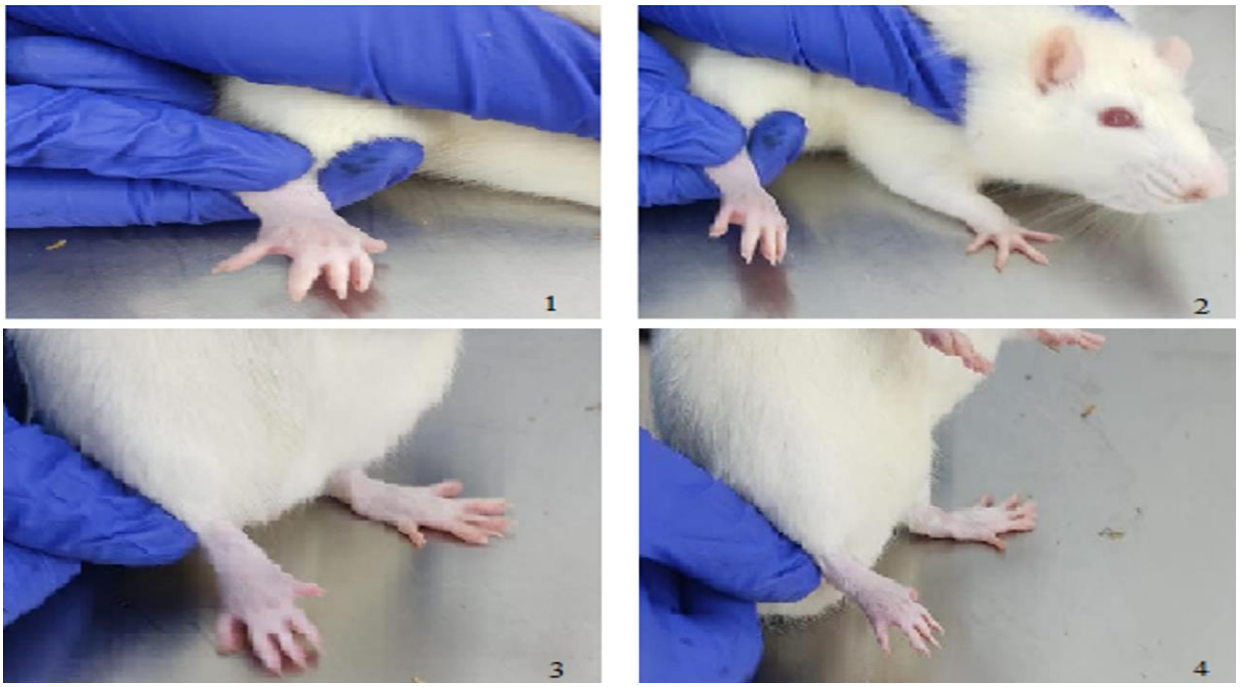

Figure 5: Development and recovery of inflammation

Table 5: ^{13}C NMR data and their assignment for isolated compound

Location of Carbon	Isolated compound	Standard (Rutin)[20, 21]
2	157.12	158.5
3	133.72	135.6
4	177.83	179.4
5	161.65	162.5
6	99.16	99.9
7	164.51	166.0
8	94.10	94.8
9	157.20	159.3
10	104.42	105.6
1’	122.09	123.1
2’	115.68	117.6
3’	145.20	145.8
4’	148.85	149.7
5’	116.69	116.1
6’	122.85	123.5
1”	103.61	104.7
2”	76.32	75.7
3”	76.86	77.2
4”	71.00	71.4
5”	78.12	78.1
6”	68.70	68.6
1”’	101.59	102.4
2”’	72.29	72.0
3”’	74.53	72.2
4”’	73.72	73.9
5”’	70.82	69.7
6”’	18.15	17.9

The working premise of nuclear magnetic resonance (C^{13} NMR) is that carbon-13 nuclei, when exposed to a powerful external magnetic field, absorb RF energy and undergo a spin transition. Resonance frequencies of ^{13}C atoms vary based on their electronic environment, resulting in a spectrum where each peak corresponds to a unique carbon environment in the molecule.

^{1}H NMR Spectroscopy

In organic and biomolecular chemistry, one of the most effective methods for structure elucidation is ^{1}H NMR spectroscopy. It aids in the identification of functional groups, molecular connections, and spatial layout by providing comprehensive information about hydrogen atoms (protons) in a molecule.

Mass Spectrometry

The molecular mass, content, and structure of chemical substances can be determined using mass spectrometry (MS), an extremely sensitive analytical technique. Ionising molecules, sorting the resulting ions according to m/z & then detecting them to create a mass spectrum are the main steps in mass spectrometry[19].

RESULTS AND DISCUSSION

Plant Material

A. racemosa plant was collected from Kas platue, Satara (Maharashtra). The botanical specimen was placed in Botanical Survey of India's repository in Pune, accompanied by a voucher specimen no. Ref. No.BSI/WRC/Iden. Cer./2021/2505210007300.

Table 6: ^{1}H NMR data and their assignment for isolated compound

Isolated compound	Standard (Rutin)	Assignment
0.97	1.12	d, 3H, CH_3
3.08-3.55	3.38-3.56	M, 8H, rutinoside protons and CH_2O
3.68	3.62	dd, 1H, CH-CH_3 rhamnose
3.87	3.82	d,1H, CH-CH_2O glucose
4.40	4.51	d, 1H, OCHO rhamnose
5.13, 5.32	5.10	d, 1H, OCHO glucose
6.21	6.20	d, 1H, Ar-H
6.40	6.39	d, 1H, Ar-H
6.87	6.89	d, 1H, Ar-H
7.55	7.61-7.67	m, 2H, Ar-H

Preliminary Testing for Phytochemicals

The phytochemical examination of extracts showed a variety of compounds, including glycosides, alkaloids, saponins, terpenoids, and flavonoids.

Acute Oral Toxicity Study

The ethanolic stem and leaf extract was safe and effective up to 2000 mg/kg. For this reason, the in vivo actions were evaluated at a dosage of 200 mg/kg.

Anti-inflammatory Activity

Carrageenan Induced Edema Model

In this study, Group II (arthritic control) demonstrated a significant increase in paw edema across all measured time intervals, confirming the successful induction of inflammation. In contrast, the test groups (Group III, IV, and V) showed a time-dependent reduction in paw edema, indicative of anti-inflammatory activity.

Group III showed the highest inhibition of paw edema at 180 minutes 40.60%, suggesting a potent anti-inflammatory effect followed by Group IV with 36.16% inhibition, while Group V demonstrated 33.15% inhibition.

In this model, the development of edema peaks quickly due to increased vascular permeability and dilation caused by

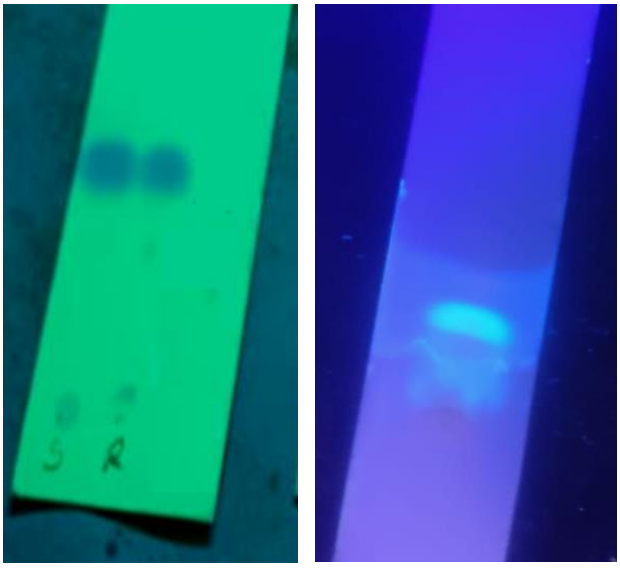

Figure 6: Thin layer chromatography of isolated compound

histamine release. At 180 minutes post-administration Group III (Standard) exhibited the highest reduction in paw edema (45.24% inhibition), indicating the most significant anti-inflammatory activity followed by Group IV (Ethanolic stem extract) followed with 40.85% inhibition, and Group V (Ethanolic leaf extract) showed 35.51% inhibition.

TLC

Characterization of Isolated Compound

Isolated compound was characterized by using IR, Proton NMR, ^{13}C- NMR, and Mass spectrometry.

FT-IR Spectroscopic Analysis

FTIR analysis of the isolated compound revealed several characteristic absorption bands that support existence of multiple functional groups commonly found in flavonoid glycosides. A broad absorption band at 3423.62 cm^{-1} corresponds to O–H stretching vibrations, representing existence of hydroxyl groups, which are abundant in both flavonoid core and the sugar moieties of rutin.

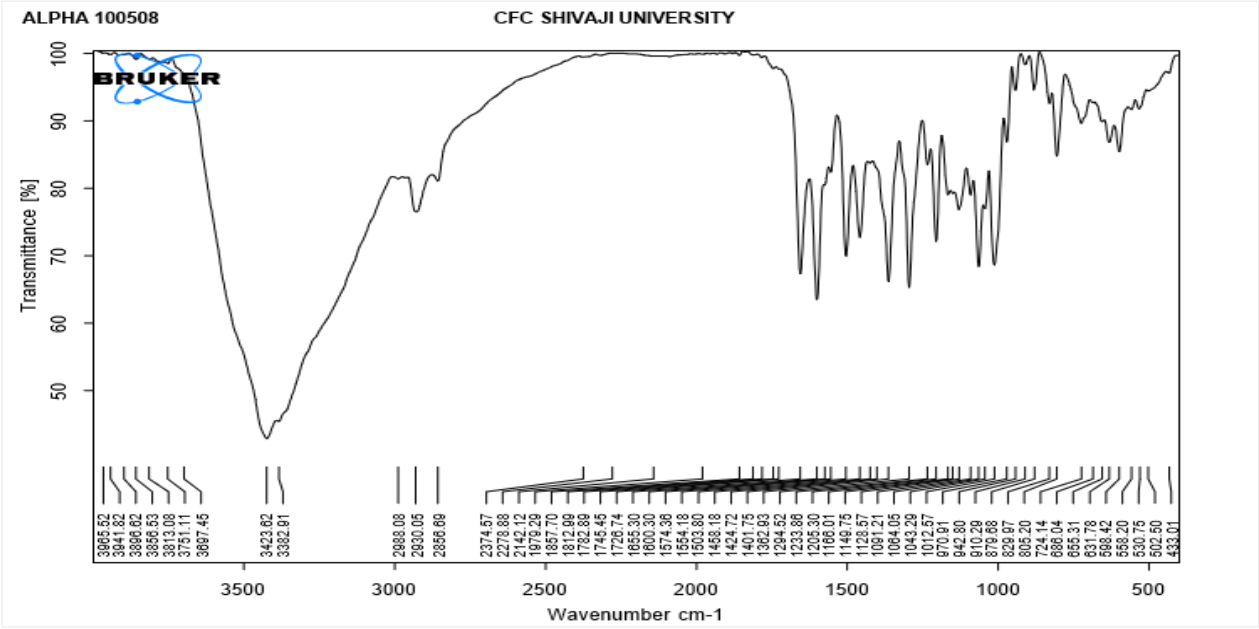

Figure 7: FTIR spectrum of isolated compound

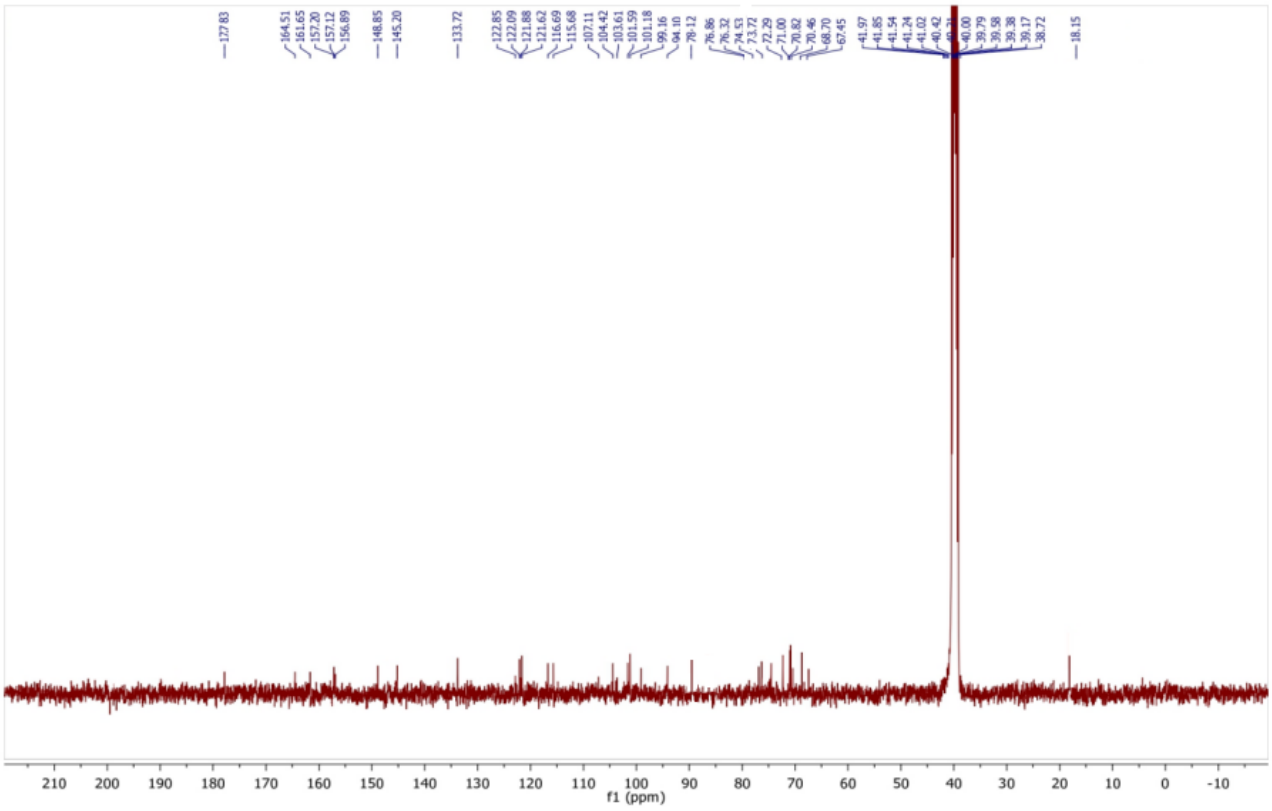

Figure 8: ^{13}C-NMR spectrum of isolated compound

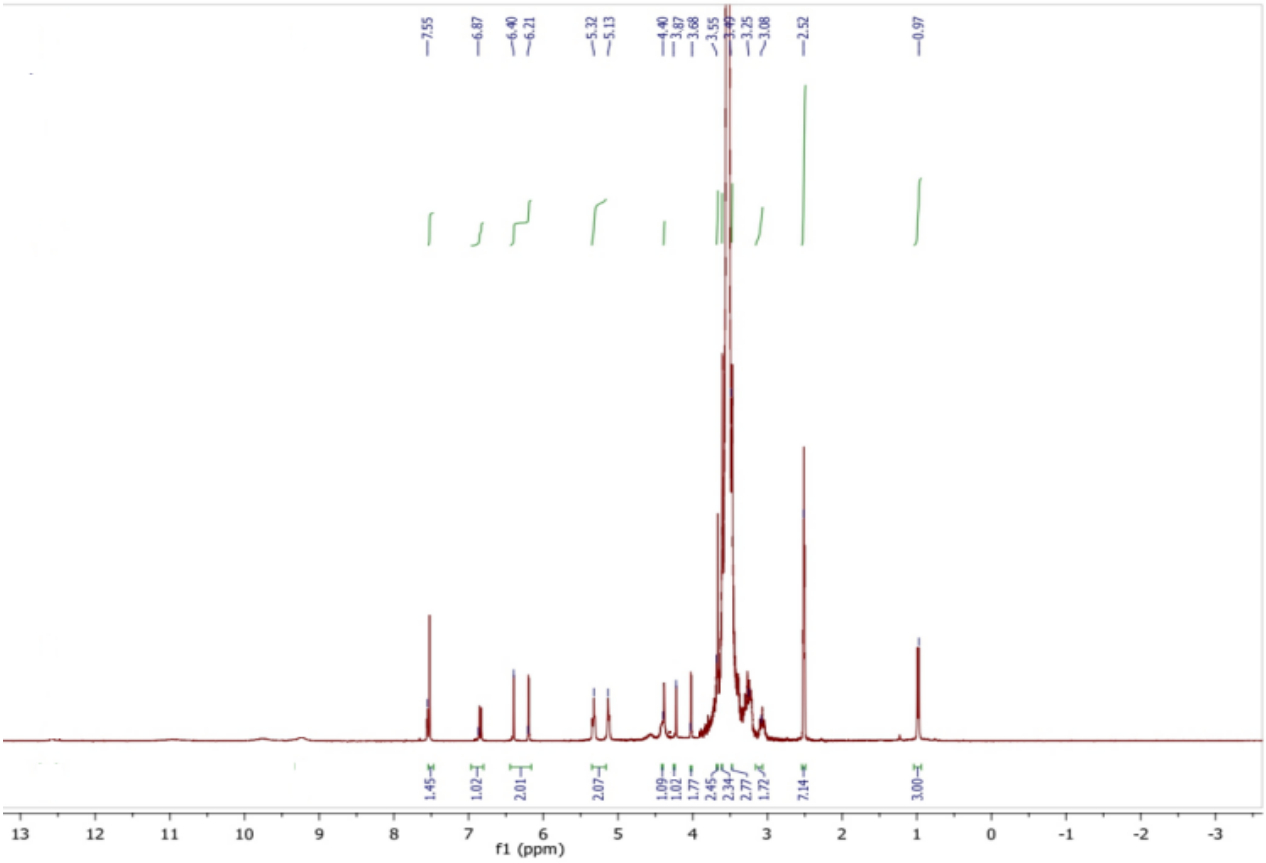

Figure 9: ^{1}H NMR spectrum of isolated compound

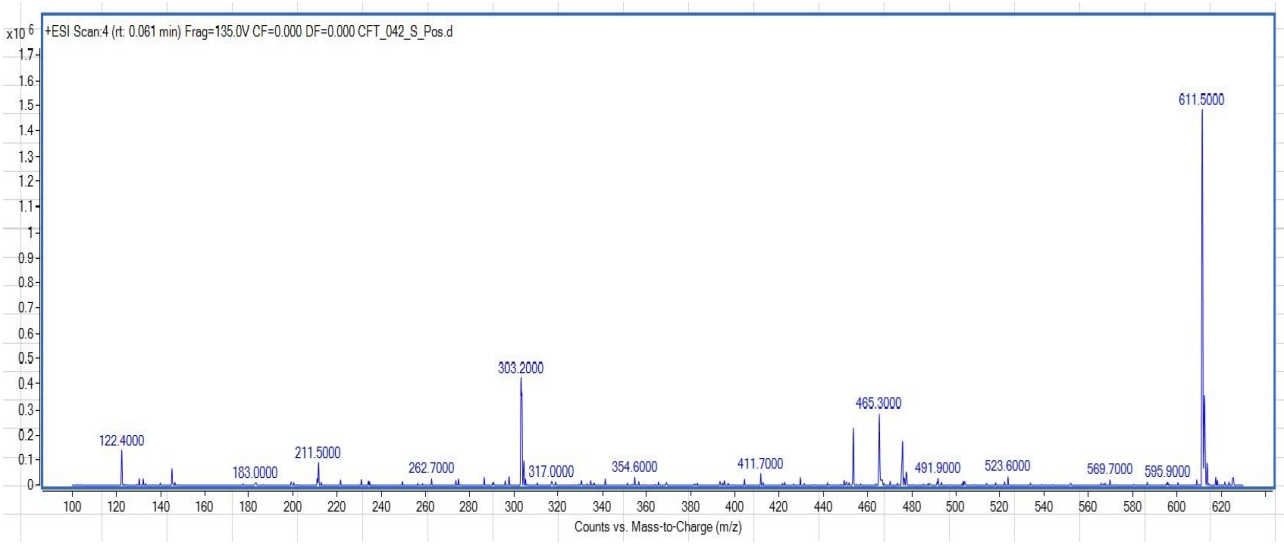

Figure 10: Mass spectrum of isolated compound

The peaks observed at 2930.05 cm^{-1} and 2856.69 cm^{-1} are assigned to C–H and CH_2 stretching vibrations, typical of aliphatic chains or sugar units. The strong peak at 1726.74 cm^{-1} recommends existence of a carbonyl (C=O) group, consistent with aromatic ketone structure of flavonoids, particularly the chromone ring of quercetin (the aglycone of rutin). Another important peak at 1655.30 cm^{-1} confirms this assignment.

A significant absorption at 1458.18 cm^{-1} is ascribed to aromatic C=C stretching, confirming aromatic nature of the compound. The region between 1200–1300 cm^{-1}, especially at 1233.86 cm^{-1} and 1205.30 cm^{-1}, reveals C–O–C asymmetric stretching typical of glycosidic linkages and aromatic ether (Ar–O–C) structures, further supporting existence of a sugar moiety bonded to a flavonoid.

Band at 1149.75 cm^{-1} is indicative of C–O stretching in tertiary alcohols, which may be due to hydroxyl groups attached to the sugar or aromatic rings. Finally, peak at 829.97 cm^{-1} corresponds to C–H out-of-plane bending vibrations, typically associated with substituted aromatic

rings, which is consistent with the multi-substituted phenyl rings found in rutin.

NMR

The NMR study includes ^{13}C NMR, Proton NMR. The spectrum of both NMR and its interpretation is given below.

^{13}Carbon NMR

The ^{13}C-NMR spectrum of the isolated compound shows a carbon resonance pattern that closely aligns with that of the standard compound Rutin, indicating significant structural similarity (Fig. 7 & Table 5).

In aglycone region (carbons 2–10), the observed chemical shifts (e.g., C-2 at 157.12 ppm, C-4 at 177.83 ppm, C-7 at 164.51 ppm) are nearly identical to those of Rutin, confirming the presence of the flavonol scaffold. The chemical shifts such as C-3′ (145.20 ppm) and C-4′ (148.85 ppm) match closely with Rutin, suggesting a similar substitution pattern on the aromatic ring. The sugar regions also show strong correlation: the rhamnose unit (1‴–6‴) and glucose unit (1″–6″) present signals consistent with their standard counterparts. For instance, C-1″ (103.61 ppm) and C-6‴ (18.15 ppm) fall within expected ranges, indicating intact glycosidic linkages.

Proton NMR

The proton NMR data of the isolated compound exhibit features highly characteristic of Rutin, a glycosylated flavonoid with a quercetin core and a disaccharide unit (rutinose).

The methyl doublet at 0.97 ppm corresponds well with the CH_3 group of rhamnose, a deoxy sugar. Multiplet signals in the 3.08–3.55 ppm range are typical of overlapping sugar protons, confirming the presence of both glucose and rhamnose units. The anomeric protons at 4.40 ppm (rhamnose) and 5.13, 5.32 ppm (glucose) indicate glycosidic linkages and confirm the disaccharide connectivity. The aromatic signals between 6.21 and 7.55 ppm are consistent with the flavonoid aglycone, particularly quercetin, which is known to possess such substitution patterns. Compared to the standard (Rutin), the chemical shifts in the isolated compound are nearly identical, indicating structural similarity with minimal deviation that might result from experimental conditions (e.g., solvent or concentration). These data collectively suggest that the isolated compound is a flavonoid glycoside[19, 22].

Mass Spectroscopy

Mass spectrum exhibited a base peak [M+] at m/z 611.5000 for the isolated rutin component. Based on the evidence, the chemical formula is $C_{27}H_{30}O_{16}$.

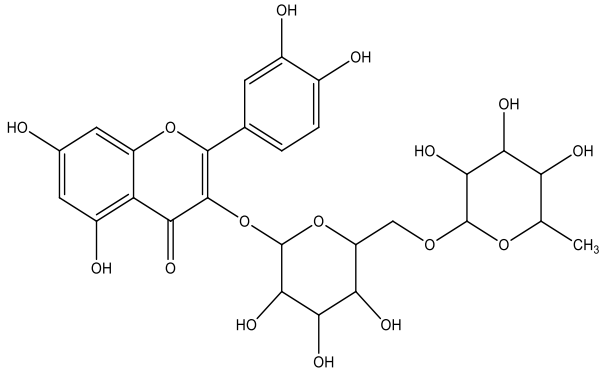

Figure 11: Structure of isolated compound

CONCLUSION

It was found that ethanolic stem extract had significantly more anti-inflammatory effect than ethanolic leaf extract in carrageenan & histamine-induced paw edema model. Thin layer chromatography as separation techniques were used to isolate bioactive component from stem ethanolic extract. To investigate molecule's structure, the FTIR, ^{13}C-NMR, ^{1}H NMR & MS spectroscopy methods were employed. According to the NMR data, the isolated molecule is most likely a Rutin, which could have several bioactive effects. A flavonoid with the molecular formula $C_{27}H_{30}O_{16}$, rutin is also named as 3, 3′, 4′, 5, 7-pentahydroxyflavone-3-rutinoside.

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