International Journal of Medical Toxicology & Legal Medicine Volume 26, No.3 and 4, 2023
e-ISSN: 0974-4614
p-ISSN: 0972-0448

Isolation and Characterization of flavonoids Luteolin and Quercetin from the Leaves of *Piper betle var Magahi*

Singh Nadkar Narayan Singh[1], Amita Mahajan[2], Alok Mukharjee[3*]
1Research Scholar, University School of Pharmaceutical Sciences, Rayat- Bahra University, Kharar, Mohali, Panjab 140103, India
2Rayat-Bahra University, Kharar, Mohali, Panjab 140103, India
3United Institute of Pharmacy, Allahabad 211002, India
*Corresponding author: Dr Alok Mukharjee, E-mail: alokmukerjee2107@gmail.com

Received: 17.09.2023 Revised: 08.10.2023 Accepted: 23.11.2023

ABSTRACT

Background and Objectives: Plants are a rich source of diverse chemical compounds with potential therapeutic properties. Phytopharmaceuticals offer a vast array of bioactive constituents that can be used to develop new drugs or as alternative treatments to conventional medicines. Plant-derived compounds often exhibit diverse pharmacological activities, such as anti-inflammatory, antimicrobial, antioxidant, anticancer, analgesic, and immunomodulatory properties.

Methods: Isolated ethanolic extract from *Piper betle var Magahi*was dissolved in 5.0 ml of 2.0 N hydrochloric acid, 0.04% (w/v) ascorbic acid as an antioxidant was added to 50mL of semi solid sample then heated at 90ºC for 2 hours. Allowed to cool and sonicated for 5 minutes to remove the dissolved oxygen. The extract was cooled, filtered using Buchner filter. By using column chromatography, HPTL, NMR, IR spectroscopy and mass spectroscopy process of isolation was completed.

Result: Isolated sample were compared with standard one and also compared with previous reported data of isolated Luteolin and Quercetin.

Conclusion: The result of present study established the presence of biologically active compounds Luteolin and Quercetin in the leaves of *Piper betle variety Magahi*. The data suggest that the significant amount of biologically active compound present in the leaves of *Piper betle varity Magahi*.

Keywords: Flavonoids, Luteolin, Quercetin, Mass spectroscopy, Chromatography.

1. INTRODUCTION

Plants are a rich source of diverse chemical compounds with potential therapeutic properties. Phytopharmaceuticals offer a vast array of bioactive constituents that can be used to develop new drugs or as alternative treatments to conventional medicines. Integrating traditional knowledge with modern scientific research can lead to the development of effective and safe treatments. Phytopharmaceuticals have been used to treat a wide range of ailments, including common health issues like cough, cold, and indigestion, as well as chronic conditions like diabetes, cardiovascular diseases, inflammatory disorders and gastrointestinal disorder. Plant-derived compounds often exhibit diverse pharmacological activities, such as anti-inflammatory, antimicrobial, antioxidant, anticancer, analgesic, and immunomodulatory properties.

This broad spectrum of actions makes them valuable for managing complex diseases and promoting overall health. Phytopharmaceuticals serve as a valuable source of lead compounds in drug discovery. Researchers often isolate active constituents from plants to create new drugs or modify existing ones. The isolation and characterization of phytopharmaceuticals often involve a combination of different analytical techniques, including NMR (Nuclear Magnetic Resonance), IR (Infrared Spectroscopy), MS (Mass Spectrometry), and HPTLC (High- Performance Thin-Layer Chromatography). The first step is the extraction which can be achieved by various extraction methods, like maceration, percolation, or soxhlet extraction. Present research involves the method of soxhlet extraction.

After extraction, the crude extract is often fractionated using various techniques like, column chromatography, or preparative thin layer chromatography to separate different compounds present in the extract. NMR spectroscopy is a key technique used for structural elucidation and characterization of isolated compounds. The characteristic

absorption bands in the IR spectrum can helps to confirm the presence of specific functional groups, providing additional structural information. Mass spectrometry is employed to determine the molecular weight and fragmentation pattern of the isolated compounds. By combining the information obtained from these different analytical techniques, researcher gain a comprehensive understanding of the phytopharmaceuticals present in the plant extract.

2. MATERIALS AND METHODS

2.1 Collection and Authentication of Plants Material

Magahi Pan Leaf was collected from the Magahi Pan Research Institute Islampur Nalanda, in the month of July 2021. The plant was authenticated by Dr. Shivnath Das a senior scientist in the Magahi Pan Research Institute, Islampur Nalanda, with voucher specimen No. T-01512.

2.2 Preparation of Ethanolic Extract

For the obtainment of the ethanolic extract, first of all leaves were washed properly with tap water and then dried in a hot air oven at 40°C for two days. The dried leaves were grind into powder using a scientific mixing grinder. These powered leaves were used for further soxhlet extraction using ethanol as a solvents. 100 grams of powered leaves were subjected to continuous soxhlet extraction with ethanol as a solvent. It takes almost 10 hours to completely exhaust the crude leaves powder. The requirements for solvent in this process are less. The obtained ethanolic extract was filtered using whatman No -1 filter paper and then dried properly.

2.3 Acid Hydrolysis of Isolated Ethanolic extract

Isolated ethanolic extract (semi solid 50mL) was dissolved in 5.0 ml of 2.0N hydrochloric acid and 0.04% (w/v) ascorbic acid as an antioxidant was added to 50mL of semi solid sample then heated at 90ºC for 2 hours and allowed to cool and sonicated for 5 minutes to remove the dissolved oxygen. The extract was cooled filtered using Buchner filter. The extract was kept in airtight amber bottles and stored in freezer.

2.4 Thin layer chromatography (HPTLC) procedure

The complete CAMAG TLC equipment consists of a fully automatic sample Linomat V sample applicator and a developing chamber. A CAMAG TLC scanner is allowing densitometric evaluation of chromatograms and CATS 4 software for interpretation of data. About 10 mg of the extracts of ethanolic extract of *Piper betle varMagahi* was taken and dissolved in respective solvents and volume was made up to10 ml in standard flask. (1000µg/ml).The fraction were run on silica gel 60 F 254 pre coated aluminum plate of 0.2mm thickness.

Standard (10mg) was taken and dissolved in methanol. This was transferred into a standard flask and volume was made up to 100 ml to prepare 100µg/ml solution. Silica gel 60 F254 coated on HPTLC aluminum sheet were used as an adsorbent (Stationary phase). The extract were applied point wise from 1000µg/ml sample solution. 10µl of the sample was applied on HPTLC aluminium sheet as a different track in the form of 6 mm wide bands by using a Camag semi-automatic Linomet 5 spotter at a distance of 12 mm. Nitrogen gas was also suppled for simultaneous drying of bands and then using drier for completely drying of bands. Mobile phase used for extract were developed on v/v basis to saturate the chamber. 10 ml mobile phase was placed in each flat bottomed CAMAG twin chamber 30 minute before the development of the HPTLC plate.

2.5 Fourier Transform Infrared (FT-IR) spectrum analysis

FAR-MID-NIR-FTIR spectrophotometer (Model Frontier) was used for the sample analysis. The sample (1-2 mg) was crushed in KBr (3-4mg) pellets using FTIR (Fourier transform Infrared spectroscopy)

2.6 NMR spectral analysis

Bruker advance III HD NMR 500 MHz instrument was used for the sample analysis. First samples were dissolved in glass vials using DMSO D6 as a solvents. After closing the lid, glass vials were shaken properly to ensure proper dissolution of samples in given solvent then dissolved samples were transferred to NMR tube. NMR tubes were wipe out properly with tissue paper so no water molecule or anything remain present on the NMR tube.

2.7 Mass spectroscopy Analysis

For mass spectroscopy Agilent 6550 UHD accurate mass QTOF-MS was used. The detailed analysis conditions was given below.

Mass spectroscopy parameters

Table 1

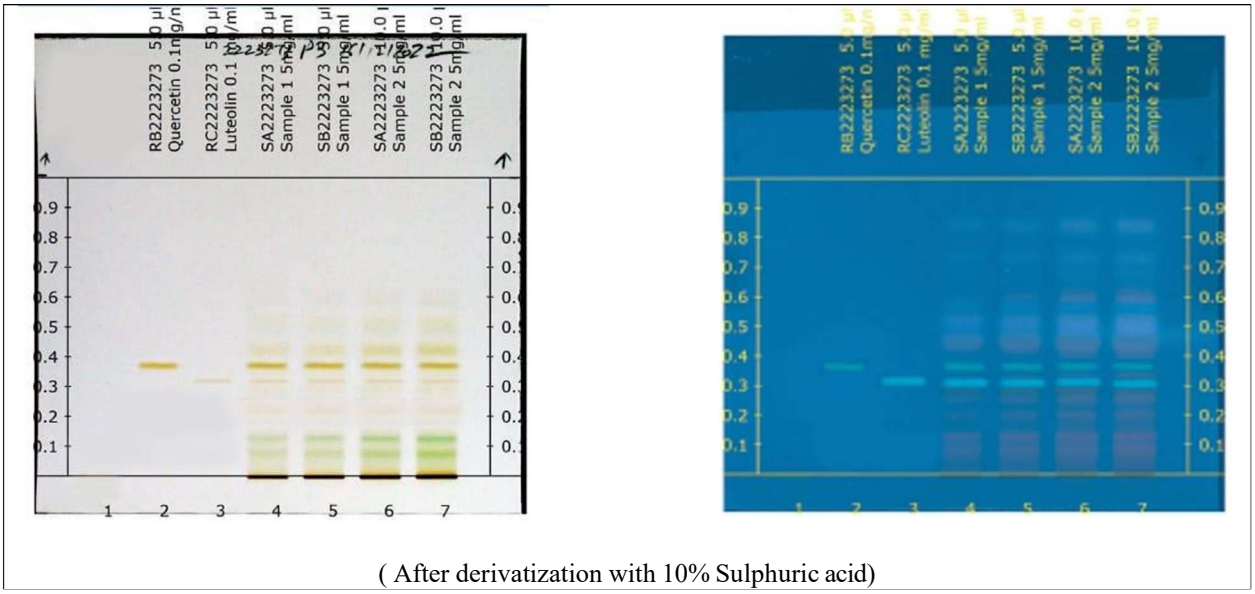
MS Ion source	Dual AJS ESI
Polarity	Negative and Positive
MS Scan range	at m/z 60 to 1600
Ion source Parameters Standard Electrospray (ESI)	
Nebulizer Gas Temperature	350ºC
Sheath Gas Temperature	300ºC
Drying gas	8.1/min
Nebulizer Gas	40 psig
Sheath Gas Flow	101/min
Capillary Voltage	2500V
Nozzle Voltage	500V

3. RESULT AND DISCUSSION

3.1 HPTLC chromatogram

The TLC developing was set as twin through chamber were examined in various solvents and best resolution was obtained with the cyclohexane: dichloromethane: ethyl acetate: formic acid in the ratio of 20:5:8:1 v/retention factor (Rf) was calculated using the formula

Rf =Distance moved by the solute/ Distance moved by the solvent. The Rf value reported to quercetin and luteolin was 0.39 and 0.32



(After derivatization with 10% Sulphuric acid)

Fig. 1. Image of HPTLC plates

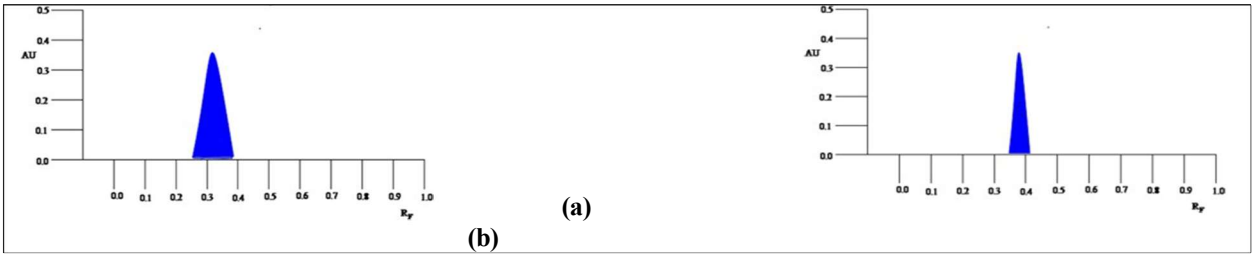


Fig. 2. Chromatogram of standard luteolin (a) and Quercetin (b)

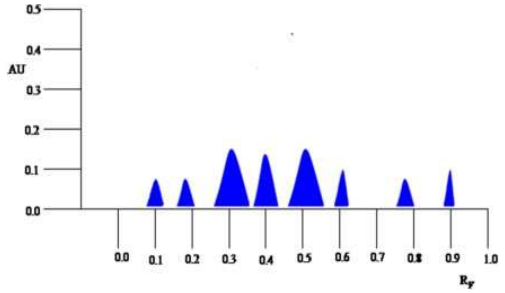


Fig. 3. Chromatogram of ethanolic extract of *Piper betle variety Magahi*

3.2 Fourier Transform Infrared (FT-IR) spectrum analysis of sample 01 and Sample 02

3.2.1 IR spectra of sample 01

FT-IR spectrum of sample 01 is well represented through figure 5 and their cross ponding peak position were explained as follows. The absorption peak at around 3490cm^{-1} was observed for –OH stretching in sample 01 which indicate OH functional group in flavonoids. The absorption peak at 1616 cm^{-1} was observed for C=O stretching of carbonyl group in sample 01. The absorption peak position at around 3114cm^{-1} represents –OH stretching of carboxylic acid. The peak position at 1470cm^{-1}, 1640cm^{-1} and 2950cm^{-1} represents the C-H stretching of aromatic group, C=C stretching of aromatic and C-H stretching of aliphatic group respectively. Various absorption bands due to stretching vibration of carbon–carbon and carbon-oxygen can be observed at 1000cm^{-1} and 1600cm^{-1}. They are particularly useful in identifying the presence of aromatic ring and oxygen contains functional group. Extreme right in figure 5 indicates absorption peak below 1000cm^{-1}that is often represented as "fingerprint region" and contains a complex pattern of absorption bands and provide information about the specific molecular structure and arrangement of atoms in the molecule. In table 2 observed peak position and reported peak position of inter atomic bond presents in sample 01 were compared.

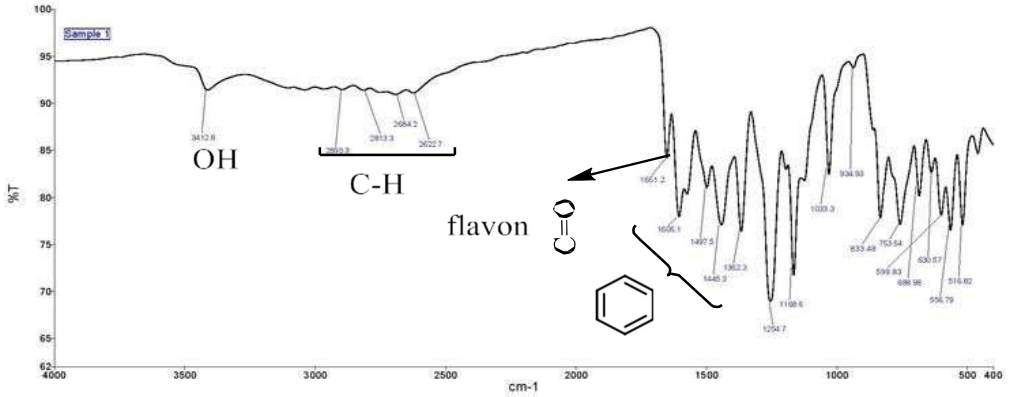


Fig. 4. FT-IR spectra of isolated sample 01

Table 2.

Sl No	Inter atomic bond	Peak position observed in cm^{-1}	Reported by JOLANTA PATORA and BARBARA KLIMEK (2002)
1	O-H stretching vibration	3412.8cm^{-1}	3490-3400cm^{-1}
2	C-H vibrations	2893.3-2627.7 cm^{-1}	2923-2617cm^{-1}
3	C=O in flavon	1651.2cm^{-1}	1655cm^{-1}
4	Aromatic ring	1605.1-1445.3cm^{-1}	1610-1490cm^{-1}
5	C=C (stretching aromatic)	1640 cm^{-1}	1642cm^{-1}
6	C-H bending of aromatic hydrocarbon	934.93cm^{-1}833.48cm^{-1}, 753.54cm^{-1},688.98cm^{-1} 630.57cm^{-1},556.79cm^{-1} 516.82cm^{-1}	

3.2.2 FT-IR spectra of sample 02

The FT-IR spectrum of isolated sample 02 is shown in figure 6 and their corresponding peak position were listed in table3.The broad absorption peak at around 3290 cm^{-1} was assigned to the OH stretching vibration of phenols. The multiple peaks in this region indicates more OH groups present in the molecule which confirm the given molecule is flavonoids. The carbonyl group (C=O) such as ketonic functional group can produce absorption bands at around at 1668 cm^{-1} which is close to the reported value. The absorption peak position at 1612cm^{-1}, 1516cm^{-1} and 1429cm^{-1} are assigned to the C-C,C=O, and C=C aromatic stretching vibrations respectively. The OH bending vibration of phenols were observed at 1359cm^{-1}. The absorption peak at 1315cm^{-1} and the peak at the lower frequency between 950cm^{-1} and 600 cm^{-1} were assigned to the C-H bending vibration of aromatic hydrocarbons. C-O stretching vibrations of aryl ether and phenols were observed at 1240 cm^{-1} and 1210 cm^{-1} respectively. The C-CO- stretching and bending vibrations of ketone were observed 1163 cm^{-1} which confirm that the isolated compounds is quercetin. C-H Stretching: aliphatic and aromatic C-H stretching vibrations will result in absorption bands in the 2800-3100 cm^{-1} range.C-C and C-O Stretching: In the 1000-1600 cm^{-1} range, you may observe various absorption bands due to stretching vibrations of carbon-carbon (C-C) and carbon-oxygen (C-O) bonds. These can be particularly useful for identifying the presence of aromatic rings and oxygen-containing functional groups.

Fingerprint Region: The region below 1000 cm^-1 is often referred to as the "fingerprint region" and contains a complex pattern of absorption bands that can provide information about the specific molecular structure and the arrangement of atoms in the molecule.

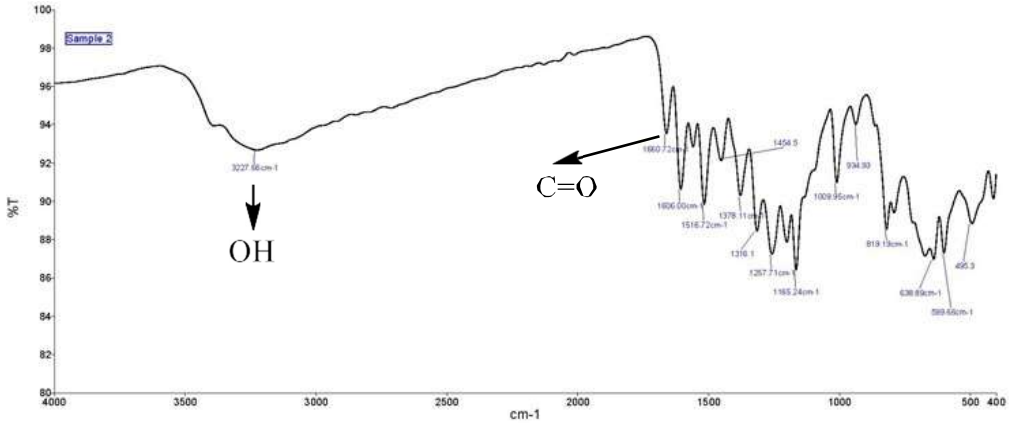


Fig. 5.FT-IR spectra of isolated sample02

Table 3

Sl No	Inter atomic bond	Peak position observed cm^{-1}	Reported by MACIEJ HENECZKOWSKI et al (2001)
1	O-H stretching vibration of Phenol	3227.66cm^{-1}	3300 cm^{-1}
2	C=O aryl ketonic stretch	1660.72cm^{-1}	1662cm^{-1}
3	C=C aromatic stretch	1554.5cm^{-1}	1560 cm^{-1}
4	O-H bending of phenols	1378.11cm^{-1}	1400 cm^{-1}
5	C-H bond in aromatic hydrocarbon	1316.1cm^{-1}	1320 cm^{-1}
6	C-H bending of aromatic hydrocarbon	934.93cm^{-1},819.19cm^{-1}, 638.89 cm^{-1},599.66 cm^{-1}, 495.30 cm^{-1}	

3.3 NMR spectrum of sample 01 and Sample 02

3.3.1 ^{1}H and NMR spectrum of sample 01

The following observation can be made in sample 01. Multiple signals which include multiplets and multiplets cluster reported in the range of (6.0ppm to 8.5 ppm) indicating the presence of neighboring proton in the aromatic

ring. Peaks in the range of 9ppm to 12ppm indicate the hydrogen in hydroxyl group (-OH). The series of peaks in the range 100ppm to 160ppm corresponds to the various carbon atoms in the aromatic ring. The peaks in the range of 160- 190ppm indicates the carbonyl carbon present in the sample 01.

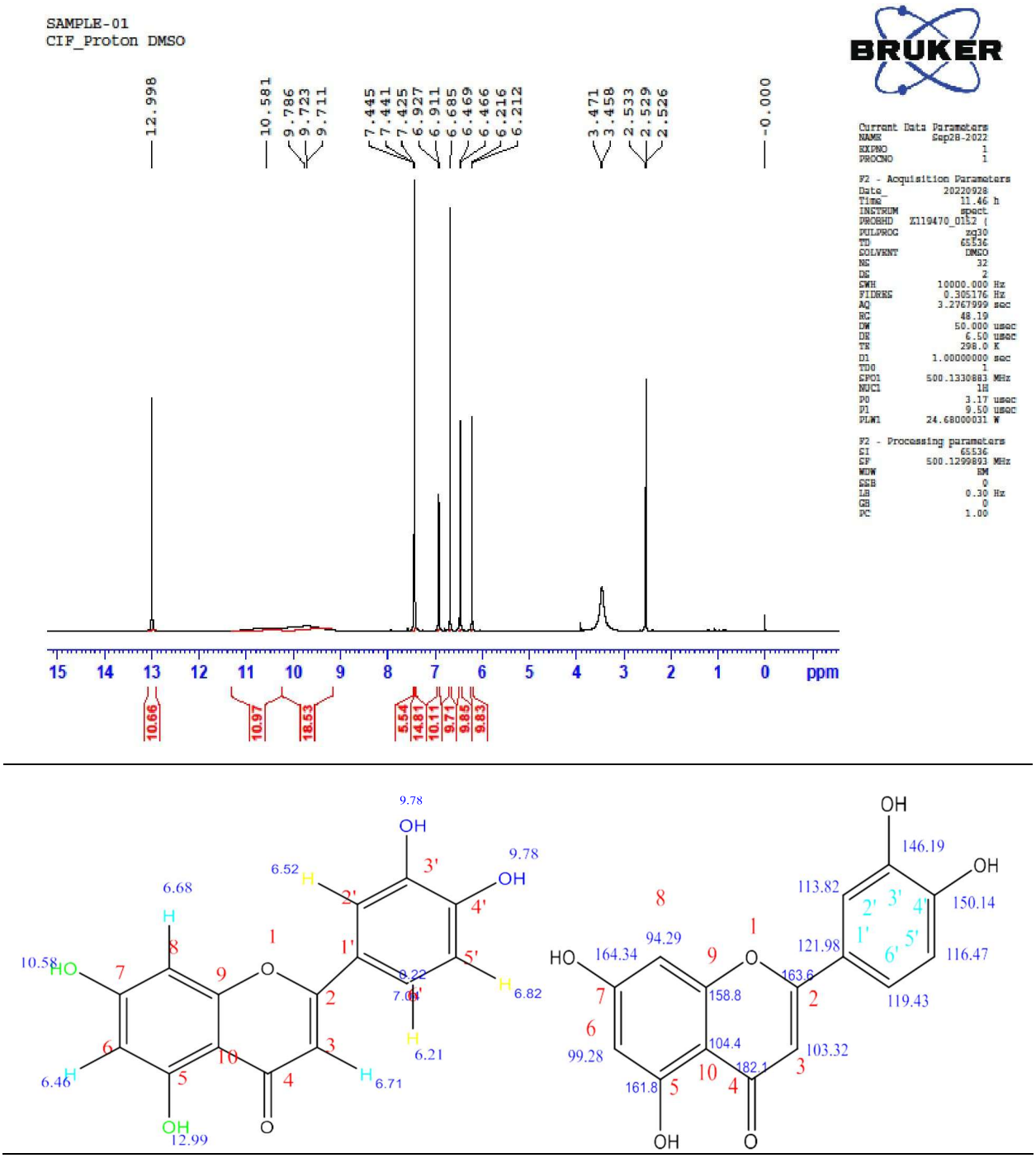


Fig. 7[1]H NMR spectra of isolated sample 01

Table 4: ^{1}H and ^{13}C NMR chemical shifts (ppm) for sample 01

^{1}H	Chemical shift (ppm)		^{12}C	chemical shift(ppm)
3H	6.71C2	163.9		
			C3	102.8
5H	12.99C4	181.6		
6H	6.46C5	161.4		
7H	10.58		C6	98.80
8H	6.68C7	164.80		
C8	93.80			
C9	157.30			
	C10	103.30		
2'H	6.52C1'	121.50		
3'H	9.78C2'	113.30		
4'H	9.78C3'	145.70		
5'H	6.82C4'	149.70		
6'H	6.21C5'	116.00		
C6'	119.00			

3.3.2 ^{13}C NMR spectrum of sample 01

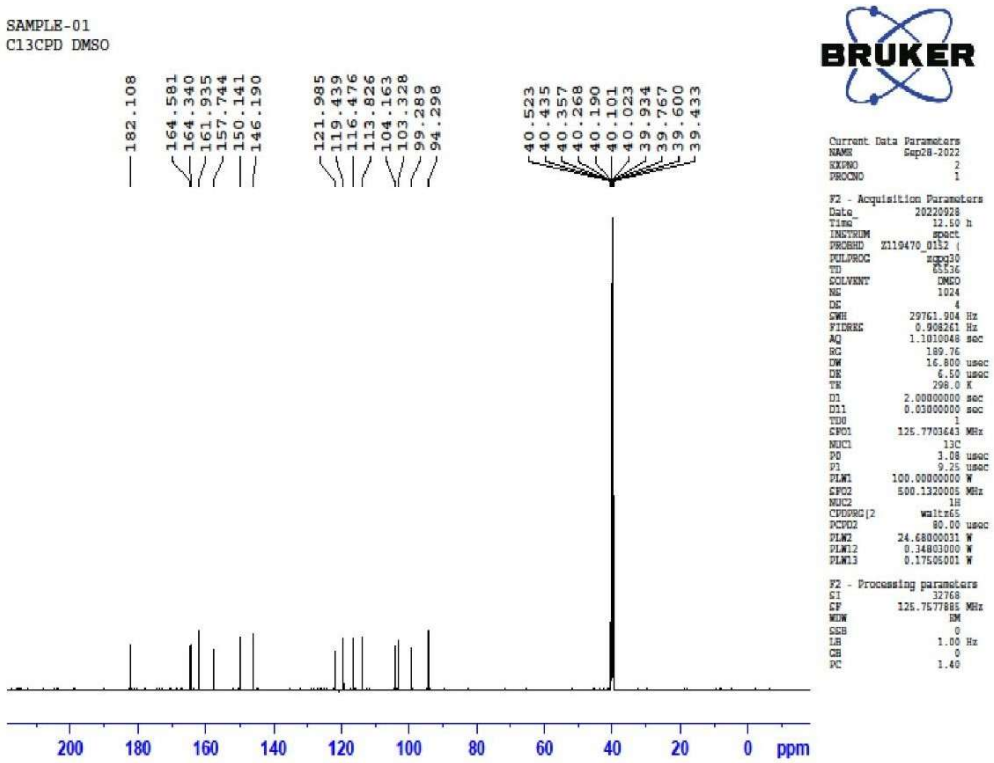


Fig. 8: ^{13}C NMR spectra of sample 01

3.3.3 ^{1}H NMR spectrum of sample 02

Some significant observation can be made in sample 02.Multiple signals in the aromatic range of 6.0 to 8.58ppm can be noted in the given spectrum. These signals are indicating the presence of protons in the aromatic range, multiple peaks in the range of 9ppm to 12ppm indicate the protons in the hydroxyl group. The ^{13}C NMR spectrum showed carbonyl group at 176.20 ppm that represents the carbon environment.

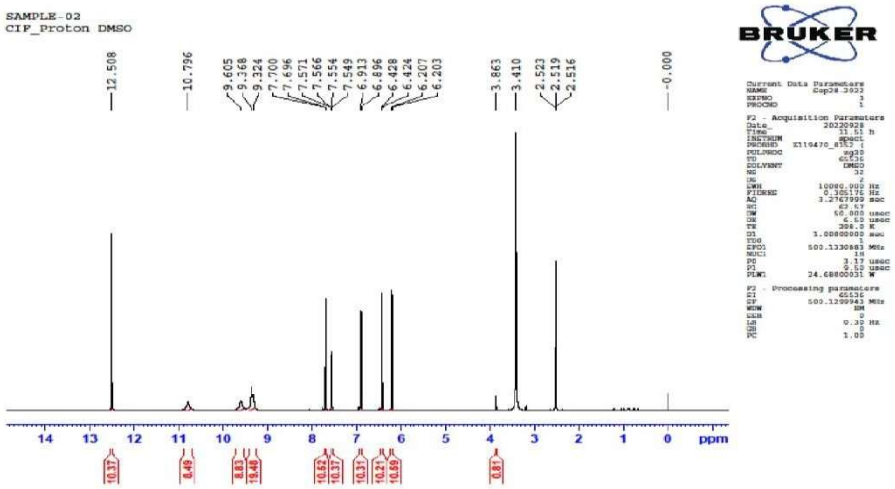
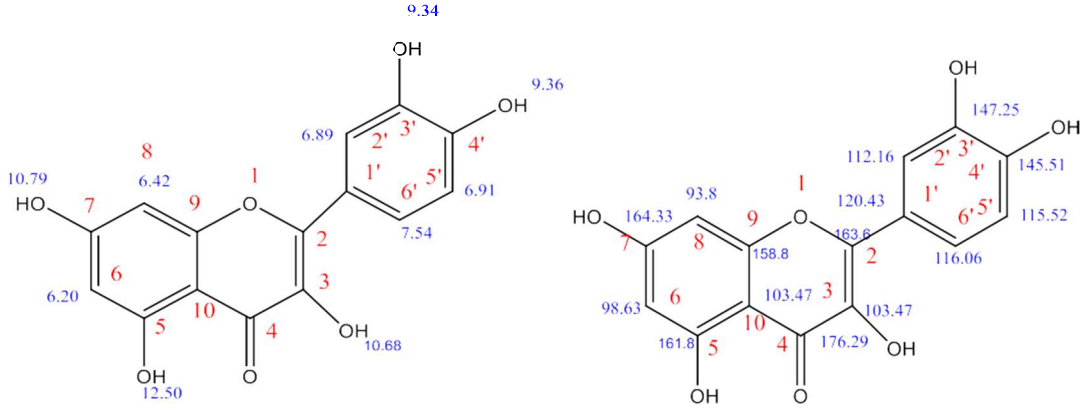


Fig. 9: ^{1}H NMR spectra of sample 02



(A) (B)

Fig. 10: ^{1}H and ^{13}C NMR spectra of sample 02 and chemical shift in (ppm)

3.3.4 ^{13}C NMR spectrum of sample 02:

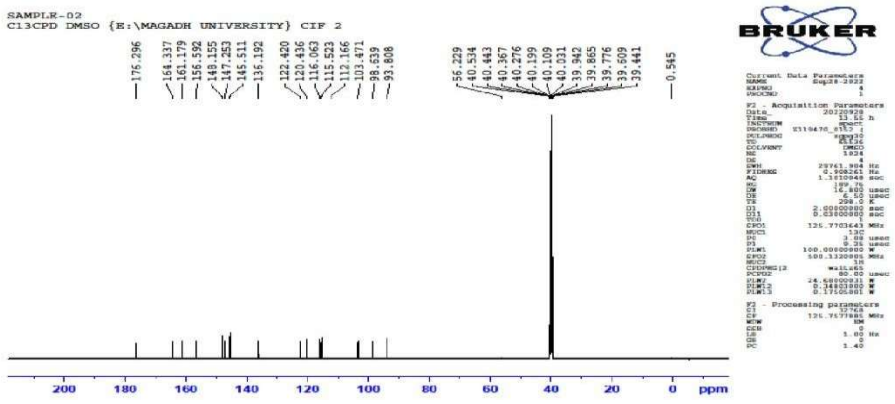


Fig. 11: ^{13}C NMR spectra of sample 02.

Table 5: ^{1}H and ^{13}C NMR chemical shift (ppm) for sample 02

^{1}H	Chemical shift (ppm) ^{12}C	chemical shift(ppm)	
3H	10.68	C2	163.6
		C3	103.47
5H	12.50	C4	176.29
6H	6.20	C5	161.8
7H	10.79	C6	98.63
8H	6.42	C7	164.33
C8	93.80		
C9	158.80		
C10	103.47		
2'H	6.89	C1'	120.43
3'H	9.34	C2'	112.1
4'H	9.36	C3'	147.51
5'H	6.91	C4'	145.51
6'H	7.54	C5'	115.51
		C6'	116.06

NMR studies were carried out to confirm the position of proton in sample 02. The isolated compounds displayed a better and sharp 1H NMR and C13 NMR spectra. The 1H NMR spectrum of the isolated compounds showed aromatic hydrogen group from 6.18 -7.66ppm. The phenolic OH group shows deflection from 9.36 -12.48ppm respectively.
The 13C NMR spectrum showed carbonyl group at 176.2ppm aromatic carbon group from 93.8 -164.3 ppm . The cross ponding 1H NMR and 13C NMR peak position for isolated compounds were shown in figure 2

3.4 Mass Spectroscopy of sample 01 and sample 02

3.4.1 Mass spectroscopy of sample 01

Mass spectroscopy:
Mass spectroscopy is a power full analytical technique used to identify and quantify compounds based on their mass to charge ratio. When applied to quercetin a flavonoid with numerous health benefits MS can provide detailed information about its structure and composition.
Isolated plant compounds was analyzed by the mass spectroscopy the chromatogram of the sample 01 was shown in Figure 11

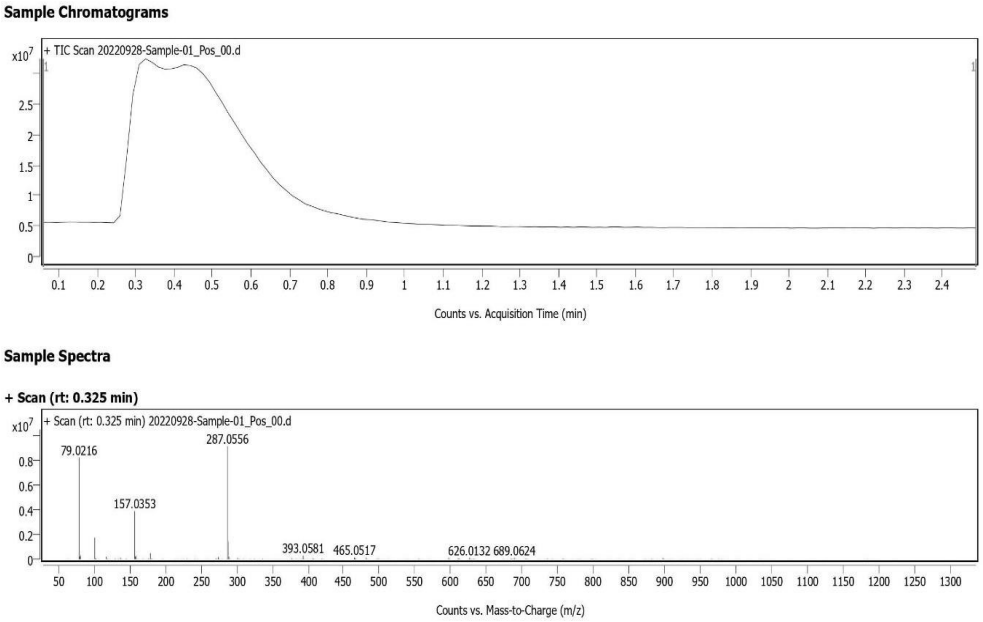


Fig. 11. Total ion chromatogram of isolated sample 01

Flavonoid commonly found in many plants and has several biological activity. The fragmentation pattern of sample 01 when analyzed by mass spectroscopy can provide insight into its structure and name of its substituents.
When sample one was analyzed using mass spectrometry particularly using technique ESI MS (Electron spray Ionization Mass spectrometry the following key fragments are typically observed.
Parent ion [molecular ion [M-H] at m/z 285 the deprotonated molecular ion of sample one the following characteristic fragmentation pattern was observed.

(1) m/z 217 this fragment results from the losss of a C2H2O (68 dalton) indicate cleavage between the A and C ring of the flavone structure.
(2) m/z 151 this fragments cross pond to the A ring of the flavonoid structure indicating the stability of this ring during fragmentation.
(3) m/z 133 often observed due to further fragmentation of the ion at m/z 151
(4) m/z 175 this ion arises from the loss of a $C_4H_6O_3$ unit from the parent ion indicating cleavage within the C ring and loss of substituents.
(5) A common fragmentation pattern for flavonoids when C ring undergoes a RetroDiels Alder reaction result in the formation of the fragments m/z 151 and m/z 133.
(6) Heterocyclic ring cleavage: Fragmentation involving the cleavage of the hetrocyclic ring (C- ring) results in significant ion like m/z 217 and m/z 175.

Interpretation of Fragmentation patterns: The observed fragments can be interpreted to confirm the structure of sample one is of luteolin flavonoid and can be distinguished it from other similar flavonoids. The key fragments at m/z 217, 151,133, 175 are diagnostic for luteolin due to the specific position of the hydroxyl group and the stability of the resulting ions.
These fragments and their pathway provide a clear mass spectrometric fingerprints for luteolin aiding in its identification and structural elucidation in complex mixture.

3.4.2 Mass spectroscopy of sample 02

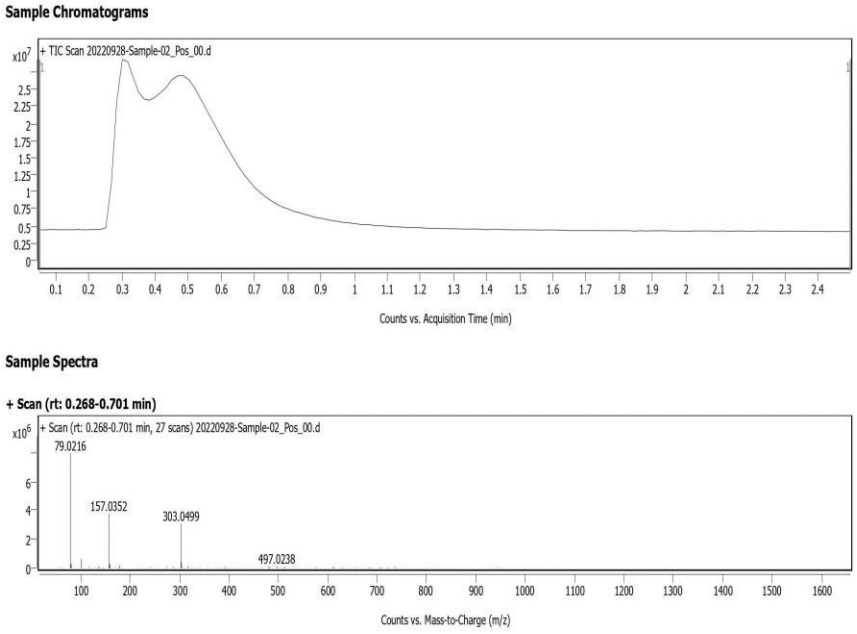


Fig. 11: Total ion chromatogram of isolated sample 02

Ionizations Electro spray ionizations (ESI) of sample two molecules are ionized by applying high voltage producing ions like [M-H] protonated molecule. The ionized molecule are then introduced into the mass analyzer which separate them based on their mass to charge ratio m/z
Characteristic fragments pattern of sample two molecule:

(1) m/z 153, 137,125107 and 95 which are indicative of the breakdown of the flavonoid structure

(2) Interpretation of mass spectra: parent in peak and the peak cross ponding to the molecular ion $[M-H]^+$ provides the molecular weight of the 303

(3) Fragment ions peak at lower m/z value cross pond to fragments of the sample two molecule. The pattern of these peaks helps in elucidating the structure.

(4) A prominent peak at m/z 303[$M-H]^+$ in positive ion mode peaks at m/z 285 273 and other represents various ions by interpreting the mass spectra and fragmentation patters

These fragments and their pathway provide a clear mass spectrometric fingerprints for quercetin aiding in its identification and structural elucidation in complex mixture.

4. CONCLUSION

The result of present study established the presence of biologically active compounds Luteolin and Quercetin in the leaves of *Piper betle variety Magahi*. The data suggest that the significant amount of biologically active compound present in the leaves of *Piper betle varity Magahi*. Thus it may be concluded that the leaves of Piper betle variety Magahi may be the potentials source for producing the healthy and highly nutritive products.

Acknowledgments

The author are thankful for the facilities provided by the Venture Center Pune India, Pune University Pune, Birla Institute of Technology Ranchi and Anchrom Laboratorty Mumbai for MS spectroscopic analysis, NMR spectroscopic analysis, FT IR analysis and HPTLC analysis respectively.

Author Contribution

Singh Nadkar narayan singh participated in isolation and characterization of flavonoids Luteolin and Quercetin from the leaves of *Piper betle var Magahi*and writing and editing the original draft. Dr Dr Jitendra Singh[2] Dr Alok Mukharjee[3]participated in interpretation of mass spectroscopic, Nuclear magnetic resonance and infrared spectroscopic data analysis.

Declaration of interest

the author declare that there is no conflict of interest. The author alone are responsible for the accuracy and integrity of the paper content.

REFERENCE

1. Sen, A.K., Sen, D.B. and Maheshwari, R.A., 2019. Extraction, isolation, and quantitative determination of flavonoids by HPLC. In *Herbal Medicine in India: Indigenous Knowledge, Practice, Innovation and its Value* (pp. 303-336). Singapore: Springer Singapore.
2. Akanksha, Kumar, N., Aqil, M., Najmi, A.K., Ismail, M.V., Zehgeer, M.M. and Mujeeb, M., The Novel Approaches to Extraction Techniques, Drug Delivery Technologies, Pharmacological Applications, Patents of the Potent Flavonoid 'Luteolin': An Appraisal. *Chemistry & Biodiversity*, p.e00193.
3. Qasim, Q.A. and Al-Salman, H.N.K., 2023. The isolation characterization and assessment of bioactive flavonoid with special reference to anti-arthritic activity. *Materials Today: Proceedings*, *80*, pp.2567-2572.
4. Heal, H.H., Al-Dallee, Z.T. and Khadim, E.J., 2023, December. Extraction, isolation and identification of luteolin flavonoid from Vitex pseudonegundo leaves. In *IOP Conference Series: Earth and Environmental Science* (Vol. 1262, No. 5, p. 052016). IOP Publishing.
5. Maiti, P., Nand, M., Joshi, T., Ramakrishnan, M.A. and Chandra, S., 2021. Identification of luteolin-7-glucoside and epicatechin gallate from Vernonia cinerea, as novel EGFR L858R kinase inhibitors against lung cancer: Docking and simulation-based study. *Journal of Biomolecular Structure and Dynamics*, *39*(14), pp.5048-5057.
6. Yang, Z., Luo, K., Chen, D., Dou, L., Huang, X. and Kan, J., 2025. Dual Blockade of PI3K and EGFR Pathways by Flavonoids from Idesia polycarpa Maxim Cake Meal: Valorization of Agro-Industrial Waste for NSCLC Therapy. *Foods*, *14*(18), p.3278.
7. Sevastre-Berghian, A.C., Ielciu, I., Bab, T., Olah, N.K., Neculicioiu, V.S., Toma, V.A., Sevastre, B., Mocan, T., Hanganu, D., Bodoki, A.E. and Roman, I., 2023. Betula pendula leaf extract targets the interplay between brain oxidative stress, inflammation, and NFkB pathways in amyloid aβ1-42-treated rats. *Antioxidants*, *12*(12), p.2110.
8. Muller, C.J., Malherbe, C.J., Chellan, N., Yagasaki, K., Miura, Y. and Joubert, E., 2018. Potential of rooibos, its major C-glucosyl flavonoids, and Z-2-(β-D-glucopyranosyloxy)-3-phenylpropenoic acid in prevention of metabolic syndrome. *Critical Reviews in Food Science and Nutrition*, *58*(2), pp.227-246.
9. Trang, N.M., Vinh, L.B., Phong, N.V. and Yang, S.Y., 2024. Traditional uses, phytochemistry, and pharmacological activities of Vernonia cinerea (L.) Less.: an updated review. *Nutrients*, *16*(9), p.1396.

10. Nugraha, A.S., Sangsopha, W., Cahyaningsih, R., Purwoko, R.Y., Firli, L.N. and Wangchuk, P., 2025. Dandelions: Ethnopharmacology, Phytochemistry, and Pharmacological Activities for Human Wellness. In *Exploring Traditional Wild Edible Plants* (pp. 160-193). CRC Press.
11. Tripathi, A., Sachan, A., Singh, M. and Lal, N., 2025. A Comprehensive Review on Bioactive Natural Products of Moringa oleifera Lam.: A'Developmental Tree'of Sub-Saharan Africa. *Traditional Medicine in North East Africa: Research on Traditional Healer Preparations and Herbs*, pp.9-30.
12. Moreno-Vázquez, S.E., Quevedo-Corona, L., Vargas-Díaz, M.E., Cardador-Martínez, A., Nieto-Yáñez, O., Gutiérrez-Rebolledo, G.A. and Garduño-Siciliano, L., 2025. Study of the chloroformic extract of Gnaphalium sp in a model of alloxan induced diabetes in Wistar rats. An alternative use of the "gordolobo" plant within traditional Mexican medicine. *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas*, *24*(1), pp.76-100.