

# Fourier Transform Infrared Spectroscopy (FT-IR) of Fruits and Leaves Extract of *Ziziphus Nummularia* to Find Out Functional Group (Flavonoids and Phenolic Compounds) of the Active Components by Maceration Process and Hot Continuous Extraction Method by Soxhlet Apparatus

Dr. Pooja Bagdi<sup>1</sup>, Swarnim Swati<sup>2</sup>

<sup>1,2</sup>Trinity Educational Institute, Ramgarh, Jharkhand India

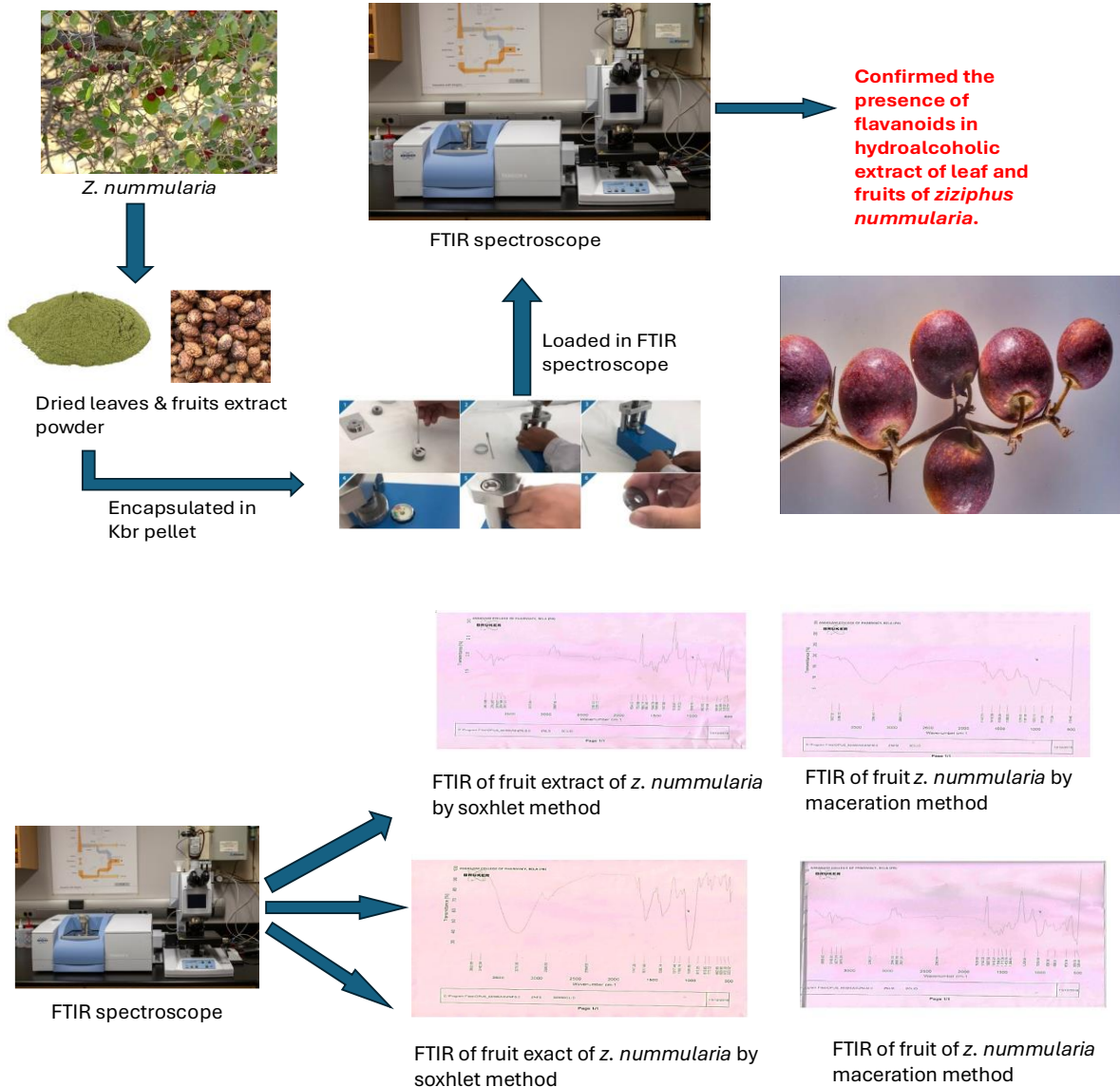
## Abstract

*Ziziphus nummularia* (Burm. f.) Wight & Arn. (Rhamnaceae), commonly known as “Jharberi” in India and “Bairi” or “Karkanrha” in Pakistan, is a thorny shrub reaching 1–2 meters in height and widely distributed across India, Pakistan, and Iran. The leaves and fruits of this species have long been used in traditional medicine to manage mental disorders, frequent colds, influenza, diarrhoea, dysentery, colic, indigestion, and gum inflammation, and also serve as a general tonic. Phytochemical investigations have revealed the presence of numerous bioactive constituents, including flavonoids, alkaloids, glycosides, saponins, tannins, sterols, pectins, triterpenic acids, polysaccharides, fatty acids, and peptide alkaloids such as ziziphin derivatives. These compounds are known to exhibit a wide range of pharmacological activities, including antitumor, anthelmintic, antibacterial, analgesic, and anti-inflammatory effects.

In the present study, Fourier Transform Infrared (FT-IR) spectroscopy was employed to characterize the functional groups present in the leaf and fruit extracts of *Z. nummularia*. The spectral analysis revealed distinctive absorption peaks corresponding to hydroxyl, carbonyl, and aromatic ring vibrations, confirming the presence of phenolic and flavonoid compounds. These functional groups are primarily responsible for the antioxidant and therapeutic properties of the plant.

**Keywords:** *Ziziphus*, Antioxidants, Hydroalcoholic, FTIR, Phenolic compounds, Flavonoids.

Graphical Abstract



INTRODUCTION

Herbal medicines have long been used for the treatment of diabetes mellitus. This is because such herbal plants have hypoglycaemic properties and other beneficial effects. Herbal medicines have the advantage of usually having no or less side-effects 3. Most of these plants have antioxidant activities 4 and hence, prevent or treat hard curable diseases, other than having the property of combating the toxicity of toxic 5 or other drugs 6,7.

Recent studies suggest that several plant products including polyphenolic substances (e.g. flavonoids and tannins) and various plant or herb extract exhibit potent antioxidant activity 8.

Recently there has been an upsurge of interest in the therapeutic potential of plants as antioxidants in reducing free radical induced tissue injury. Butylated hydroxyanisole (BHA) and Butylated hydroxytoluene (BHT) are commercially available but unsafe for use. Natural antioxidants, phenols and

flavonoids from tea, wine, fruits, vegetables, and spices used as nutritional supplements. Food may also enhance antioxidant levels because food contains a lot of antioxidant substances. Fruits and vegetables are loaded with key antioxidants such as vitamin A, C, E, beta-carotene and important minerals, including selenium and zinc 9.

### **Characterization of *Ziziphus nummularia***

*Ziziphus nummularia*, also called Jharberi, is species of *Ziziphus* native to the western India and south-eastern Pakistan and south Iran 10. *Ziziphus nummularia* leaves and fruits are used for mental retardation, preventing frequent attacks of colds and influenza, treating diarrhoea, dysentery and colic, indigestion, inflammation of gums and tonic 1. The unripe fruits of the plant are prescribed in the management of vomiting, burning sensations and as tonic, while dried fruits are useful as an anticancer, sedative, stomach ache and in treatment of anaemia, bronchitis, burns, chronic fatigue, diarrhoea, hysteria, loss of appetite and pharyngitis 11.

Chemical constituents are present in leaves and fruits of *Ziziphus nummularia* are Glutamine synthetase, nitrate reductase and glutamine dehydrogenase, Ascorbic acid (vitamine C), nummularine-T, nummularine-M and N, Nummularine E, nummularine S, frangufoline, nummularine R, sterols and/or triterpenes, alkaloids, flavonoids, tannins and saponins 12, nummularine-P (I), mauritine-D, jubanine B, nummularine O (I), Jubanine-A, -B, and mauritine-C, N-desmethyl-jubanine-B (I) (Miana and Shah 1985), nummularogenin, (25S)-3  $\beta$ -hydroxy-5  $\beta$ -spirostane-2,12-dione (I), nummularine B, nummularine M (I), nummularine N (II), Zizynummin, sitosterol, stigmasterol, betulinic acid, oleanolic acid, ceanothic acid,  $\beta$ -D-glucosides of sitosterol and stigmasterol, n-octacosanol and quercetin-3-O-galactoside 13.

### **Plant Material and Authentication**

The leaves and fruits of *Ziziphus nummularia* used in this study were obtained from cultivated plants grown in Mohanlal Sukhadia University Udaipur Rajasthan.

As the plant material was not collected from the wild, no special permissions or licences were required. All procedures adhered to institutional and national ethical guidelines for the use of plant materials in research.

Fresh leaves and fruits of *Ziziphus nummularia* (Burm. f.) Wight & Arn. were collected from the botanical garden of Government Science College, Udaipur, Rajasthan, India (approximate coordinates: 24.5854° N, 73.7125° E).

### **FT-IR (Fourier Transform Infrared Spectroscopy)**

Fourier Transform Infrared (FT-IR) spectroscopy was performed to identify the major functional groups present in the hydroalcoholic extracts of *Ziziphus nummularia* leaves and fruits. This technique detects the vibrational transitions of molecular bonds, providing a characteristic absorption pattern for each functional group.

For sample preparation, dried hydroalcoholic extracts were finely powdered, and approximately 10 mg of each sample was homogenized with 100 mg of spectroscopic-grade potassium bromide (KBr). The mixture was compressed into translucent pellets and analyzed using a Shimadzu IR Affinity-1 spectrophotometer (Japan). Spectra were recorded in the range of 4000–400  $\text{cm}^{-1}$  with a resolution of 4  $\text{cm}^{-1}$ .

The infrared spectrum of each extract exhibited two main regions: the functional group region (4000–1500

cm<sup>-1</sup>), which provides information about stretching vibrations of specific functional groups, and the fingerprint region (<1500 cm<sup>-1</sup>), which is unique for each compound and useful for molecular identification. Distinct absorption bands corresponding to hydroxyl (–OH), carbonyl (C=O), and aromatic (C=C) vibrations confirmed the presence of phenolic and flavonoid compounds in the samples. These functional groups are known contributors to the antioxidant and therapeutic properties of *Z. nummularia*.

### Result and Discussion

The FT-IR spectra for phenolic compounds and flavonoids typically lie in the range of 230-290 nm. The results of UV- VIS spectroscopic analysis confirms the presence of flavonoids in the hydroalcoholic extract of leaves and fruits of *Z. nummularia*. The data on the peak values and the probable functional groups obtained by FT-IR analysis present in the leaves and fruits extract are shown in Tables 1.1,1.2,1.3,1.4. The FT-IR spectrum of leaves and fruits extracts (prepared in hydroalcoholic; 70:30; alcohol: water) of *Z. nummularia* are given in Figures 2.1,2.2,2.3,2.4.

**Table 1.1 FT-IR analysis of Leaves extract of *Z. nummularia* by Maceration Process**

Frequency, cm-1	Observed Peak value	Bond	Functional group
3640-3610	3616.20	O-H stretch, Free hydroxyl	Alcohols, Phenols
3500-3200	3240.51	O-H stretch, H-Bonded	Alcohols, Phenols
3000-2850	2944.59 2885.99	C-H stretch	Alkanes
2830-2695	2827.20	H-C=O:C-H	Aldehydes
1760-1665	1756.33 1687.58	C=O stretch	Carboxylic acids
1650-1580	1615.22	N-H bend	Primary amines
1550-1475	1511.08	N-O	Nitro compounds
1500-1400	1453.76	C-C stretch (in rings)	Aromatics
1250-1020	1208.95	C-N stretch	Aliphatic amines
800-600	806.51 663.34	C-Cl	Alkyl halides
≤667	550.33 525.64	C-Br or I	Alkyl halides

**Table 1.2: FT-IR analysis of Fruits extract of *Z. nummularia* by Maceration Process**

Frequency, cm-1	Observed Peak value	Bond	Functional group
3500-3200	3266.61	O-H stretch, H-Bonded	Alcohols, Phenols
3000-2850	2885.99	C-H stretch,	Alkanes
1760-1665	1747.11	C=O stretch	Carboxylic acids
1650-1580	1616.36	N-H bend	Primary amines
1550-1475	1509.36	N-O	Nitro compounds

1500-1400	1399.33	C-H stretch (in rings)	Aromatics
1250-1020	1216.03	C-N stretch	Aliphatic amines
1300-1150	1147.89	C-H wag (CH <sub>2</sub> X)	Alkyl halides
1250-1020	1023.10	C-N stretch	Aliphatic amines
800-600	911.56 771.39	C-Cl stretch	Alkyl halides
≤667	519.40	C-Br or I stretch	Alkyl halides

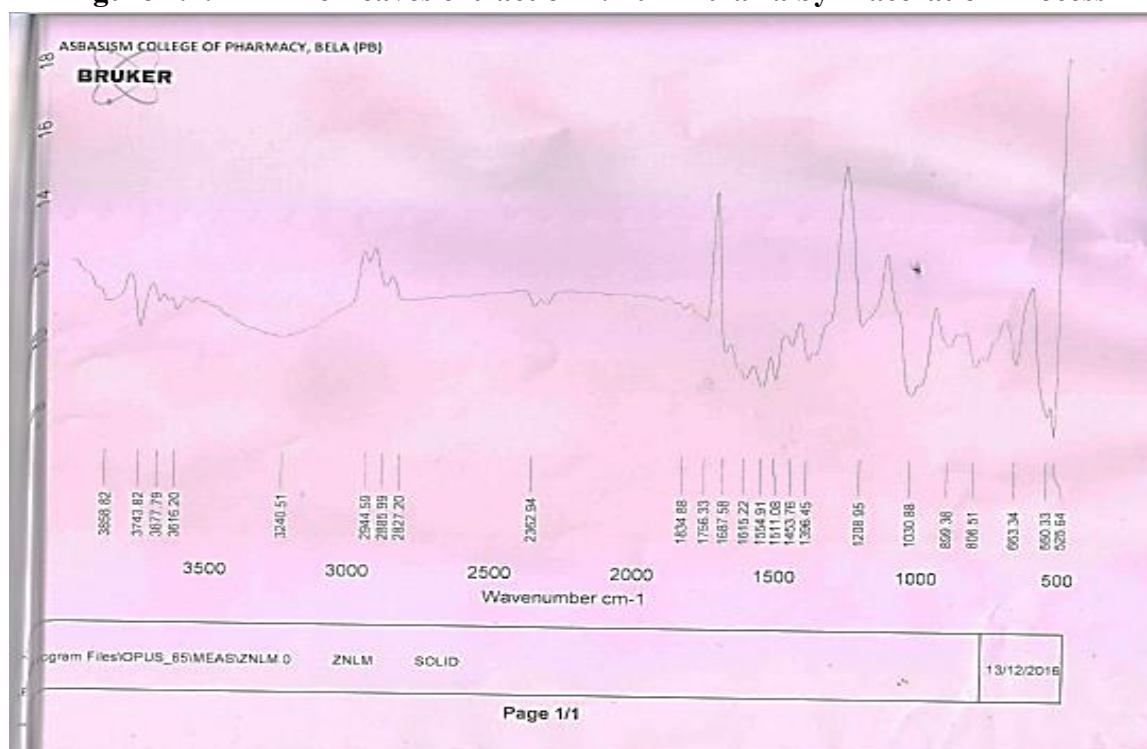
**Table 1.3 FT-IR analysis of Leaves extract of *Z. nummularia* by Soxhlet Process**

Frequency, cm <sup>-1</sup>	Observed peak value	Bond	Functional group
3640 – 3610	3678.07 3617.21	O-H stretch, free hydroxyl	Alcohols, phenols
3500 – 3200	3227.84	O-H stretch, H-bonded	Alcohols, phenols
3000 – 2850	2887.42	C – H stretch	Alkanes
1760 – 1665	1752.89 1685.71	C = O stretch	Carboxylic acid
1650 – 1580	1647.80	N – H bend	Primary amines
1550 – 1475	1548.79 1509.98	N - O	Nitro compounds
1320-1000	1255.57	C - O	Alcohols, ethers, esters, carboxylic acids
1250 – 1020	1181.03 – 1009.16	C-N stretch	Aliphatic amines
800 – 600	862.03 794.04	C - Cl	Alkyl halides
≤667	661.81 553.69 528.92 522.19	C-Br or I	Alkyl halides

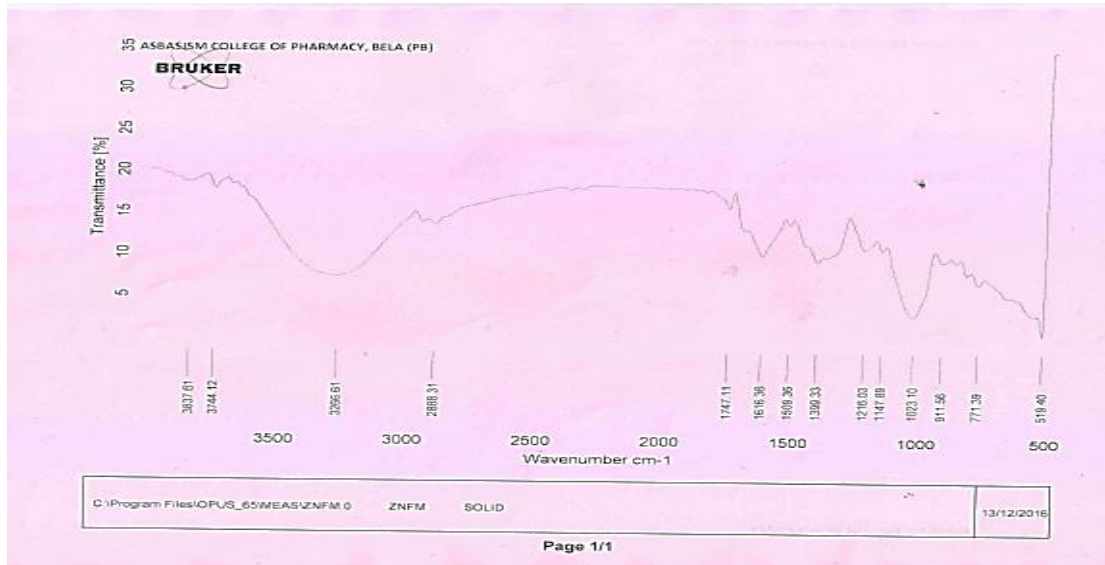
Frequency $\text{cm}^{-1}$	Observed peak value	Bond	Functional group
3500-3200	3270.26	O-H stretch, H-Bonded	Alcohols, Phenols
3000-2850	2889.53	C-H stretch,	Alkanes
1760-1665	1747.25	C=O stretch	Carboxylic acids
1650-1580	1617.69	N-H bend	Primary amines
1550-1475	1509.36	N-O	Nitro compounds
1500-1400	1398.74	C-C stretch (in rings)	Aromatics
1250-1020	1217.44	C-N stretch	Aliphatic amines
1300-1150	1150.74	C-H wag ( $\text{CH}_2\text{X}$ )	Alkyl halides
1250-1020	1026.55	C-N stretch	Aliphatic amines
800-600	911.25 813.95 771.73	C-Cl stretch	Alkyl halides
$\leq 667$	662.89 620.90	C-Br or I stretch	Alkyl halides

Table 1.4: FT-IR analysis of Fruits extract of *Z. nummularia* by Soxhlet Process

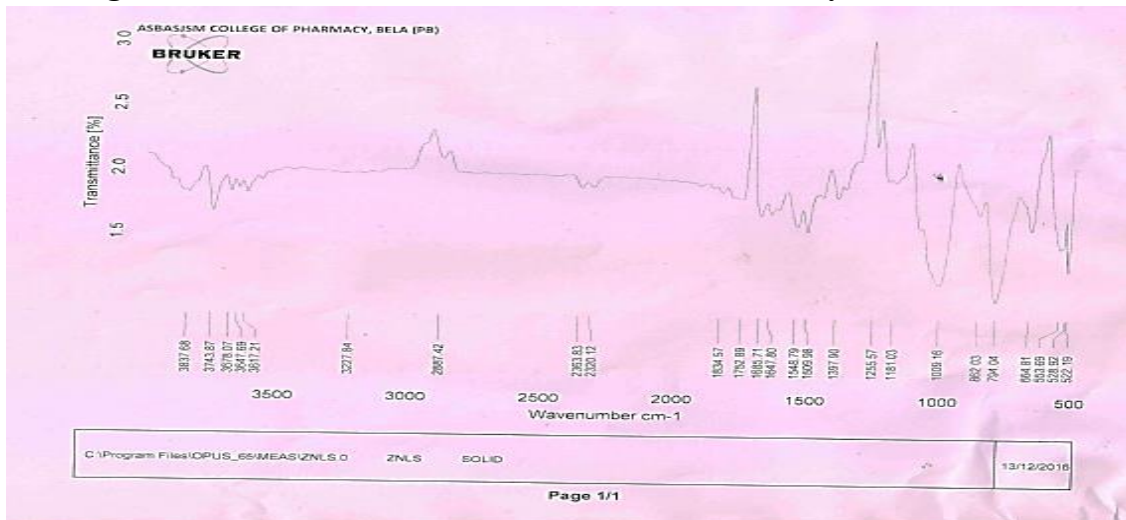
Figure 2.1: FT-IR of leaves extract of *Z. nummularia* by Maceration Process



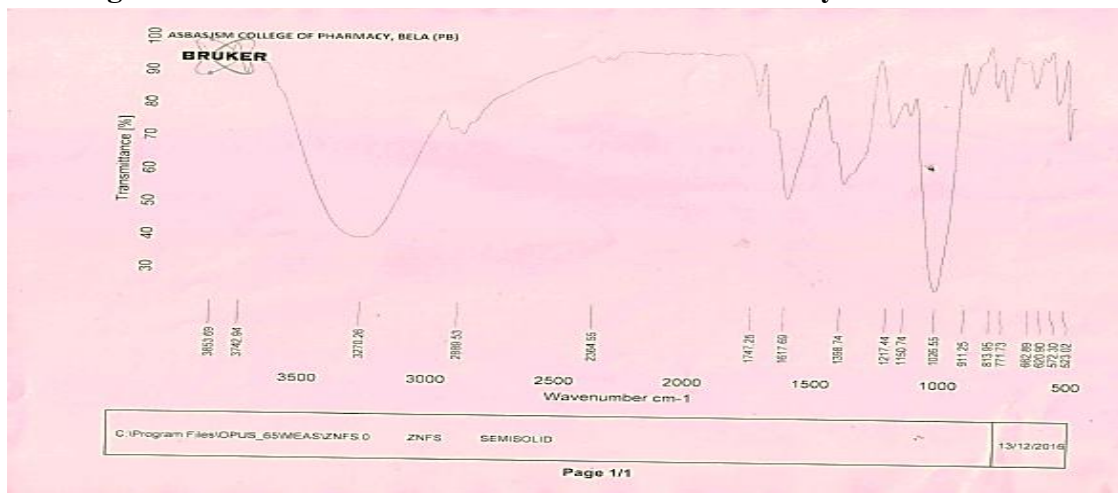
**Figure 2.2: FT-IR of fruits extract of *Z. nummularia* by Maceration Process**



**Figure 2.3: FT-IR of leaves extract of *Z. nummularia* by Soxhlet Process**



**Figure 2.4: FT-IR of fruits extract of *Z. nummularia* by Soxhlet Process**



The results table revealed the presence of polyphenols and flavonoid due to O – H stretching 17.

### **Conclusion**

The FTIR spectrum confirmed the presence of alcohols, phenols, alkanes, aldehydes, carboxylic acids, alkyl halides, aliphatic amines, nitro compounds, ether, and esters in the test plant. All these compounds belong to secondary plant metabolite as per researchers' explanation 18.

The FTIR study that predicted the presence of the groups C – Cl, C – Br, O – H, C=O, C≡N, N-H, C-H stretching. The presence of characteristic functional groups of carboxylic acid, alcohols, phenols, amines, esters, ethers, nitrates, nitrites, organic halogens could be responsible for the various medicinal property of *Z. nummularia*. FTIR analysis of leaves and fruits of *Z. nummularia* showed the presence of phenolic compounds and flavonoids which are responsible for various medicinal properties of the test plant.

### **Consent to Participate:**

Not applicable. The plant material of *Ziziphus nummularia* used in this study was cultivated in a private garden located in Mohanlal Sukhadia University. As the material was not collected from the wild, no special permissions or licenses were required. The study followed all institutional and national guidelines for the ethical use of plant materials in research. The leaves and fruits of *Ziziphus nummularia* used in this study were obtained from cultivated plants grown in Mohanlal Sukhadia University Udaipur Rajasthan.

As the plant material was not collected from the wild, no special permissions or licences were required. All procedures adhered to institutional and national ethical guidelines for the use of plant materials in research. No voucher number was assigned. The plant material was identified and authenticated by Dr. Geeta Swami, Professor, Department of Botany, Meera Girls College, Udaipur, Rajasthan, India. The collected plant materials were cleaned, shade-dried, and powdered for further use. No voucher number was assigned.

The collection and use of *Ziziphus nummularia* plant materials complied with institutional, national, and international guidelines. The plant material was collected from a non-protected area with due permission from the authorities of Government Science College, Udaipur. The species is not listed as endangered or protected under the Wildlife Protection Act (1972). The plant was identified by Dr. Geeta Swami, Professor, Department of Botany, Meera Girls College, Udaipur, Rajasthan. No herbarium and voucher number was prepared at the time of identification.

### **Data Availability Statement:**

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

### **Competing Interests:**

The author declares that there are no competing interests.

### **Acknowledgement**

I would like to thank Principal of B.N. College of Pharmacy Dr. Yuvraj Singh Sarangdevot, Dr. S.S. Sisodia (Head of Dept, Pharmacology B.N. College of Pharmacy), Dr. Jai Singh Vagela (B.N. College of Pharmacy) for providing me laboratory and equipment for lab work and for their encouragement, insightful comments, valuable cooperation, and voluntary help throughout during the thesis work. I would like to thank Ms Swarnim Swati for her valuable guidance and manuscript preparation. I am thankful to Trinity Educational Institute, Ramgarh, Jharkhand India for providing necessary support and flexible schedule that enabled to prepare and publish manuscript while serving as a principal.

## Reference

1. Kapoor B.B.S. and Arora V., (2014). Ethnomedicinal Plants of Jaisalmer District of Rajasthan used in Herbal and Folk Remedies: International Journal of Ethnobiology. *Ethnomedicine*; 1: 1 – 7.
2. Munck, A., Guyre, P. M. and Holbrook, N. J. (1984). Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. *Endocrine Reviews*, 5(1), 25-44.
3. Nasri, H. and Shirzad, H. (2013). Toxicity and safety of medicinal plants. *Journal of Herbal Medicine Pharmacology*, 2 – 11.
4. Rafieian-Kopaei, M., Baradaran, A. and Rafieian, M. (2013). Plants antioxidants: From laboratory to clinic. *Journal of Nephropathology*, 2(2), 152-153.
5. Nasri, H. (2013). On the occasion of the world diabetes day 2013; diabetes education and prevention; a nephrology point of view. *Journal of Renal Injury Prevention*, 2(2), 31- 37.
6. Nasri, H. (2013). On the occasion of the world diabetes day 2013; diabetes education and prevention; a nephrology point of view. *Journal of Renal Injury Prevention*, 2(2), 31- 37.
7. Velioglu, Y. S., Mazza, G., Gao, L. and Oomah, B. D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of Agricultural and Food Chemistry*, 46(10), 4113-4117.
8. Roshan, B. and Stanton, R. C. (2013). A story of micro albuminuria and diabetic nephropathy. *Journal of Nephropathology*, 2(4), 234 – 251.
9. Schuler, P. (1990). Natural antioxidants exploited commercially. In: *Food antioxidants*. Springer, Dordrecht, 3<sup>rd</sup> Edition, Editor B.J.F. Hudson pp 99-170.
10. S., Chaudhary, B. L., & Jain, A. (2004). Folk herbal medicines from tribal area of Rajasthan, India. *Journal of Ethnopharmacology*, 92(1), 41-46. Katewa, S.
11. Chopra R.N., Nayar S.L. and Chopra I.C., (1965). In: *Glossary of Indian Medicinal Plants*. Medicinal Plants in India, Council of Scientific and Industrial Research New Delhi; 4<sup>th</sup> edition, Editor Ram Nath Chopra, pp 551 – 554.
12. Dwivedi, S. P. D., Pandey, V. B., Shah, A. H., & Eckhardt, G. (1987). Cyclopeptide alkaloids from *Ziziphus nummularia*. *Journal of natural products*, 50(2), 235-237.
13. Sharma S, Nasir A, Prabhu KM, Murthy PS, Dev G (2003): Hypoglycemic and hypolipidemic effect of ethanolic extract of seeds of *Eugenia jambolana* in alloxan induced diabetic rabbits. *J. Ethnopharmacol* 85: 201-206.
14. Kokate, C. K., Purohit, A. P., and Gokhale, S. B. (1995). *A Text Book of Pharmacognocny*. Nirali Prakashan Pune; 4<sup>th</sup> edition 40, 1-20.
15. Guevarra, B. Q. (2005). *A Guidebook to Plant Screening: Phytochemical and Biological*. University of Santo Tomas Publishing House. Manila, Edition revised. 22- 27.
16. R.Ashokkumar\* and M. Ramaswamy, (2014). Phytochemical screening by FTIR spectroscopic analysis of leaf extracts of selected Indian Medicinal plants. *International journal of current microbiology and applied sciences*, pp 395 – 406
17. Sahu N, and Saxena J. (2013). Phytochemical analysis of *Bougaimallea glabra*, Choisy by FTIR and UV – Vis spectroscopic analysis. *Int. J. Pharm Sci. Rev. Res* 21: 196 – 198.
18. Skoog A, Holler E.J. and Crouch SR (2007). *Principles of instrumental analysis* 6<sup>th</sup> edition p. 1039.