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## Phytochemical Investigation of Extracts of *Ziziphus nummularia* (Burm. F. Wight & Arn) Leaves and Fruits

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### Abstract

Hydro-alcoholic extraction of leaves and fruits of *Ziziphus nummularia* were prepared by Soxhlet and Maceration Method. Phytochemical Screening was performed for both of the extracts. Extract were also analyzed by FT-IR and HPLC. The phytochemical screening of various extracts showed the presence of a Flavonoids, glycosides, tannins, Phenolic compounds and Saponins, whereas protein and amino acid are absent in the leaf extract of *Z. nummularia*. However the Saponins are present in leaf extract and are absent in fruit extract of *Z. nummularia*. Among all the extracts, *Ziziphus nummularis* Fruit Maceration (ZNFM) shows highest quantity of quercetin in HPLC analysis.

**Keywords** Phytochemical, Leaves, Fruits, *Ziziphus nummularia*, FT-IR, HPLC

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### Introduction

*Ziziphus nummularia* is a thorny small bush or a shrub 6-8 m in height belonging to family Rhamnaceae found throughout north western India, Pakistan and China [1]. The aerial and root barks, leaves, and fruits of *Ziziphus* species used in Indian system of medicine for the treatment of various diseases such as weakness, liver complaints, obesity, diabetes, skin infections, fever, diarrhea, insomnia and digestive disorders [2].

In the present work, hydro-alcoholic extraction of leaves and fruits of *Ziziphus nummularia* were prepared by Soxhlet and Maceration Method. Phytochemical Screening was performed for both of the extracts. Extract were also analyzed by FT-IR and HPLC.

### Material and Methods

**Plant Material:** *Ziziphus nummularia*- Leaves and Fruits

In Rajasthan *Ziziphus nummularia* is widely distributed in Udaipur was collected from Aravali hill region of Udaipur, Rajasthan.

### Preparation of Extracts

#### a. Hydro-alcoholic extraction of leaves and fruits will be done by continuous Soxhlet Method

The air dried leaves of *Z. nummularia* were powder using grinder. The 10gm powder was extracted with 100 ml of 70 % Hydroalcohol (30:70; Water: Ethanol) in a Soxhlet apparatus at 70°C till exhaustion. The obtained extract was concentrated under reduced pressure at 40°C [3-4].

#### b. Hydroalcoholic extraction of leaves and fruit by Maceration

10 gm of powder leaves and fruit of *Z. nummularia* were blended with 100ml of hydro-alcohol (30:70; water: alcohol) for 5-7 days with agitation at room temperature. After the extract was concentrated using rotator evaporator at 40°C under reduced pressure. Finally the extract were weighted and stored at -20°C till their usage in the different testes [5].



### Phytochemical Screening

The crude extract was subjected to preliminary screening for the evaluation of major phytochemical constituents such as Tannin and Phenolic compounds, Proteins and amino acid, Flavonoids, Saponins and Glycosides as per reported methods [6-7].

### FT-IR Analysis

Dried powder of different solvent extracts of each plant materials were used for FTIR analysis. 1mg of the dried extract powder of *Ziziphus nummularia* was encapsulated in 99 mg KBr pellet, in order to prepare translucent sample discs. The powdered sample of each plant specimen was loaded in FTIR spectroscope (BRUKER), with a Scan range from 400 to 4000 [8-10].

### HPLC Analysis of Extracts

The HPLC (Shimadzu, Kyoto, Japan) instrument was equipped with two LC-10 ATVP pumps, SPD-10AVP UV-Visible detector, Rheodyne injector with a 50  $\mu$ L loop. The results were acquired and processed using Shimadzu LC-solution version 6.42 software for data acquisition and processing.

Moblie phase: 2% v/v acetic acid (solvent A) and methanol/acetonitrile (40/15, v/v) mixture (solvent B) under the following gradient program: 0-8 min 70% A, 8-19 min 60% A and 19-30 min 50% A.

Flow rate: 0.6 ml/min.

Absorption maxima: 270 nm

Injection Volume: 20 $\mu$ l

Column: Waters C18 (150mm  $\times$  3.9 mm I.D., 5  $\mu$ m)

Diluent: ACN

Procedure: 10 mg each sample was accurately weighed and put in to 2 ml tube and add 1 ml of diluents and sonicated for 20 min. filtered with 0.22  $\mu$ m membrane filter and run in HPLC.

## Results and Discussion

### Extraction

Maceration and Soxhlet method was used for the extraction of fruit and leaves of *Z. nummularia*. After extraction each extract was weighted.

**Table 1:** Weight of each extract after extraction

S. No.	Method	Part Used	Weight Taken	Final Weight of Extract
1.	Maceration	Leaves	10 gm	0.5141 gm
		Fruits	10 gm	1.0451 gm
2.	Soxhlet	Leaves	10 gm	0.5760 gm
		Fruits	10 gm	3.8953 gm

### Preliminary Phytochemical Screening of *Z. nummularia* leaf Extract

On preliminary phytochemical analysis of *Z. nummularia* (Maceration and Soxhlet Process) (Hydroalcoholic leaf extract) showed the presence of Flavonoids, Saponins, Glycosides, Tannins, and Phenolic compound. Protein and amino acid are absent in the both extract.

S. No.	Chemical Test	Results			
		Maceration Process		Soxhlet Process	
		Leaf Extract	Fruit Extract	Leaf Extract	Fruit Extract
1.	Flavonoids (Lead acetate Test)	+ve	+ve	+ve	+ve
2.	Tannins (Ferric Chloride Test)	+ve	+ve	+ve	+ve
3.	Glycosides (Con. H <sub>2</sub> SO <sub>4</sub> Test)	+ve	+ve	+ve	+ve
4.	Saponins (Foam Test)	+ve	-ve	+ve	-ve
5.	Proteins (Biuret test)	-ve	-ve	-ve	-ve
6.	Amino acid (Ninhydrin Test)	-ve	-ve	-ve	-ve
7.	Phenols (Ferric Chloride Test)	+ve	+ve	+ve	+ve



**FT-IR Analysis of Extracts**

The FTIR spectrum of leaf and fruit extracts (prepared in hydroalcohol; 70:30; alcohol:water) of *Z. nummularia* given in Figures 1 to 4. The data on the peak values and the probable functional groups (obtained by FTIR analysis) present in the leaf and fruit extracts of *Z. nummularia* presented in Tables 3.

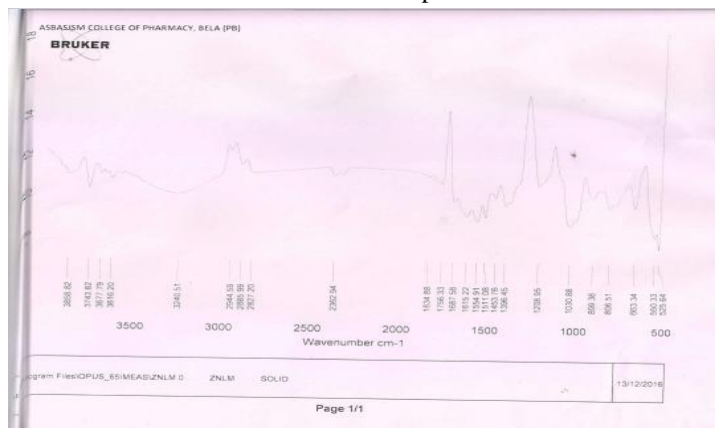


Figure 1: FT-IR of Leaf extract of *Z. nummularia* by Maceration Process

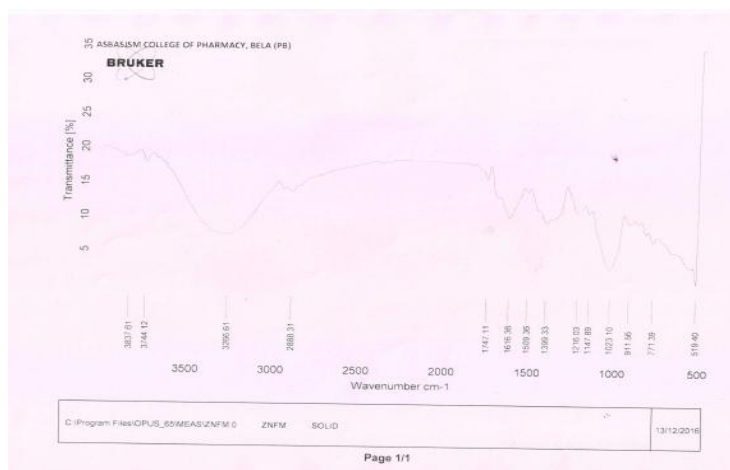


Figure 2: FT-IR of Fruit extract of *Z. nummularia* by Maceration Process

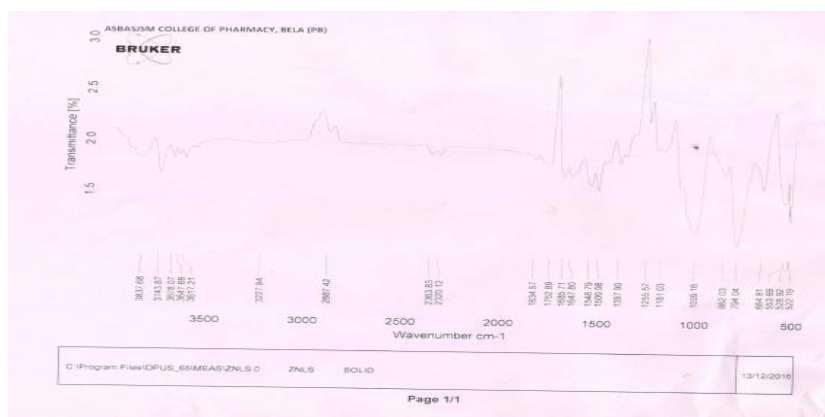
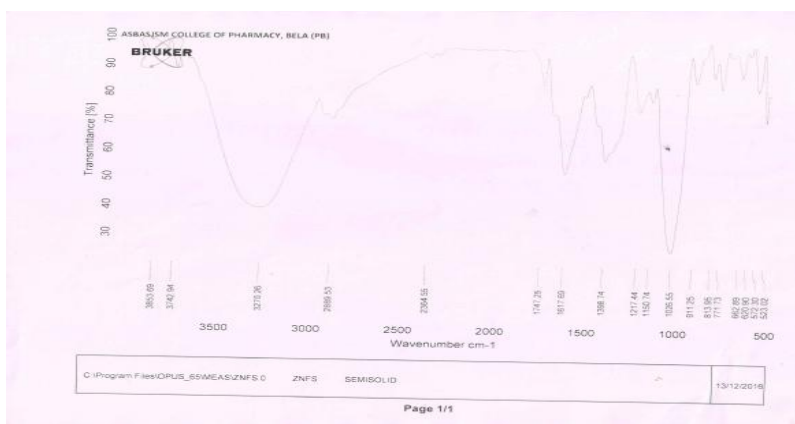


Figure 3: FT-IR of Leaf extract of *Z. nummularia* by Soxhlet Process



Figure 4: FT-IR of Leaf extract of *Z. nummularia* by Soxhlet ProcessTable 3: FT-IR analysis of Leaf extract of *Z. nummularia* by Maceration Process

S. No.	Extraction Process	Observed Frequency (cm <sup>-1</sup> )	Observation	Functional Group		
1.	Maceration (Leaf Extract)	3616.20	O-H stretch, Free hydroxyl	Alcohols, Phenols		
		3240.51	O-H stretch, H-Bonded	Alcohols, Phenols		
		2944.59, 2885.99	C-H stretch,	Alkanes		
		2827.20	H-C=O:C-H	Aldehydes		
		1756.33, 1687.58	C=O stretch	Carboxylic acids		
		1615.22	N-H bend	Primary amines		
		1511.08	N-O	Nitro compounds		
		1453.76	C-C stretch (in rings)	Aromatics		
		1208.95	C-N stretch	Aliphatic amines		
		806.51, 663.34	C-Cl	Alkyl halides		
		550.33, 525.64	C-Br or I	Alkyl halides		
		2.	Maceration (Fruit Extract)	3266.61	O-H stretch, H-Bonded	Alcohols, Phenols
				2885.99	C-H stretch	Alkanes
1747.11	C=O stretch			Carboxylic acids		
1616.36	N-H bend			Primary amines		
1509.36	N-O			Nitro compounds		
1399.33	C-H stretch (in rings)			Aromatics		
1216.03	C-N stretch			Aliphatic amines		
1147.89	C-H wag (CH <sub>2</sub> X)			Alkyl halides		
1023.10	C-N stretch			Aliphatic amines		
911.56, 771.39	C-Cl stretch			Alkyl halides		
519.40	C-Br or I stretch			Alkyl halides		
3.	Soxhlet (Leaf Extract)			3678.07, 3617.21	O-H stretch, Free hydroxyl	Alcohols, Phenols
				3227.84	O-H stretch, H-Bonded	Alcohols, Phenols
		2887.42	C-H stretch	Alkanes		
		1752.89, 1685.71	C=O stretch	Carboxylic acids		
		1647.80	N-H bend	Primary amines		
		1548.79, 1509.98	N-O	Nitro compounds		
		1255.57	C-O	Alcohols, ethers, esters, carboxylic acids		
		1181.03, 1009.16	C-N stretch	Aliphatic amines		
		862.03, 794.04	C-Cl	Alkyl halides		
		661.81, 553.69, 528.92, 522.19	C-Br or I	Alkyl halides		
		4.	Soxhlet	3270.26	O-H stretch, H-Bonded	Alcohols, Phenols



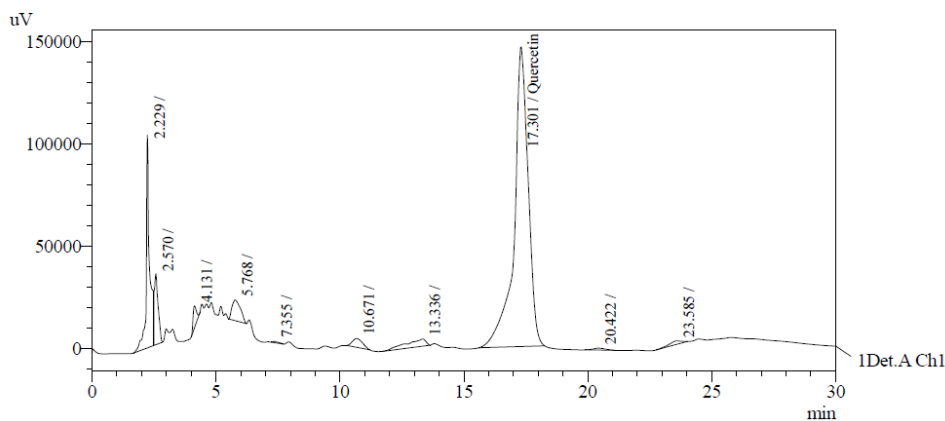
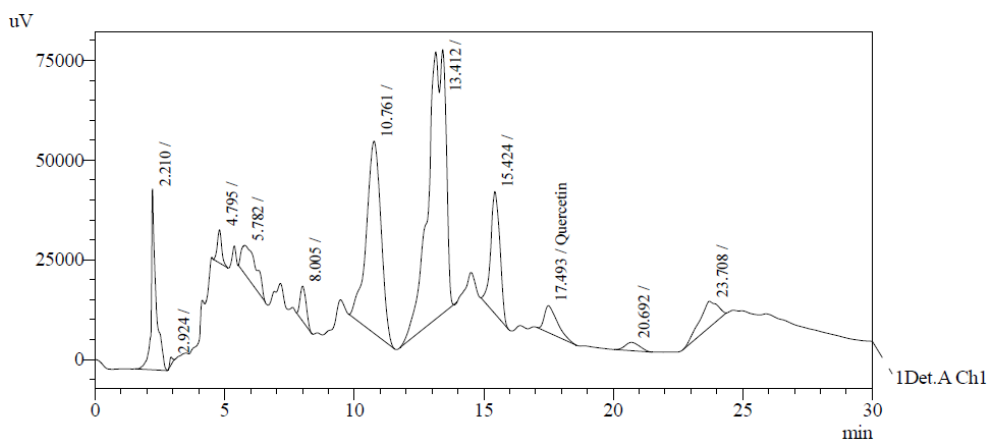
(Fruit Extract)	2889.53	C-H stretch,	Alkanes
	1747.25	C=O stretch	Carboxylic acids
	1617.69	N-H bend	Primary amines
	1509.36	N-O	Nitro compounds
	1398.74	C-C stretch (in rings)	Aromatics
	1217.44	C-N stretch	Aliphatic amines
	1150.74	C-H wag (CH <sub>2</sub> X)	Alkyl halides
	1026.55	C-N stretch	Aliphatic amines
	911.25, 813.95, 771.73	C-Cl stretch	Alkyl halides
	662.89, 620.90, 572.30,	C-Br or I stretch	Alkyl halides
	523.02		

### HPLC Analysis of Extracts

According to HPLC analysis of all extract the area under curve (AUC) of ZNLS, ZNFM, ZNFM and ZNLM was  $16.66229 \pm 0.932986$ ,  $12.97792 \pm 0.162205$ ,  $533.4347 \pm 7.685162$  and  $45.17005 \pm 0.324734$  respectively. Above all these extract ZNFM shows highest quantity of quercetin in HPLC analysis.

**Table 5:** HPLC analysis of Extracts

Extract Name	Id	Quercetin ( $\mu\text{g/ml}$ )
<i>Ziziphus nummularis</i> Leaf Soxhlet	ZNLS	$16.66229 \pm 0.932986$
<i>Ziziphus nummularis</i> Fruit Soxhlet	ZNFS	$12.97792 \pm 0.162205$
<i>Ziziphus nummularis</i> Fruit Maceration	ZNFM	$533.4347 \pm 7.685162$
<i>Ziziphus nummularis</i> Leaf Maceration	ZNLM	$45.17005 \pm 0.324734$



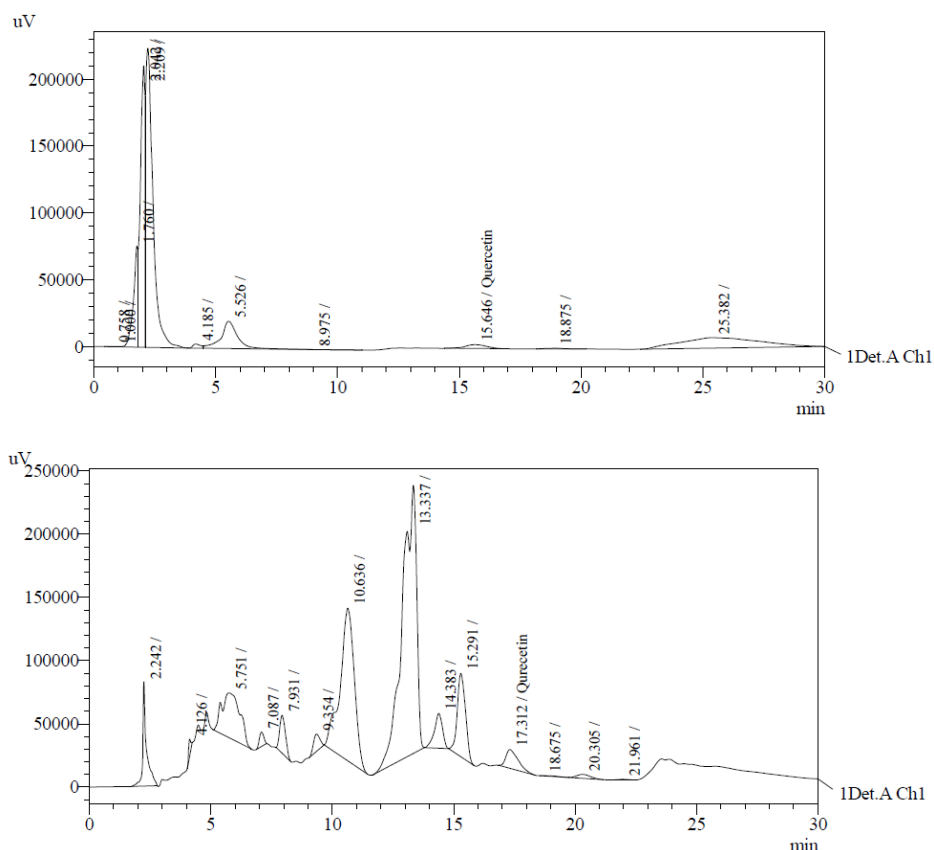


Figure: 3 Chromtogram of different extraction sample (A) ZNLS, (B) ZNFM (C) ZNFS (D) ZNLM

## Conclusion

The phytochemical screening of various extracts showed the presence of a Flavonoids, glycosides, tannins, Phenolic compounds and Saponins, whereas protein and amino acid are absent in the leaf extract of *Z. nummularia*. However the Saponins are present in leaf extract and are absent in fruit extract of *Z. nummularia*. FT-IR analysis all Extracts was carried out. In FT-IR analysis each extract showed the characteristic peak in the range of 400-4000. Among all the extracts, *Zizyphus nummularis* Fruit Maceration (ZNFM) shows highest quantity of quercitin in HPLC analysis.

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