The Pharmaceutical and Chemical Journal, 2017, 4(1):88-93

Available online www.tpcj.org

Research Article

ISSN: 2349-7092
CODEN(USA): PCJHBA

In vitro Anti-diabetic activity of Hydro-alcoholic Extracts of Leaves and Fruits of *Ziziphus nummularia* (Burm. F. Wight & Arn)

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Abstract Hydro-alcoholic extraction of leaves and fruits of *Ziziphus nummularia* were prepared by Soxhlet and Maceration Method. Total Phenolic Contents of extracts were determined. Extract were also analyzed by FT-IR and HPLC. *In vitro* anti-diabetic activity of Extracts of Leaves and Fruits of *Ziziphus nummularia* (Burm. F. Wight & Arn) was evaluated. Among all extracts, leaves extract of Maceration process utilize higher glucose hence have higher anti-diabetic activity.

Keywords *Ziziphus nummularia*, Leaves, Fruits, Total Phenolic Content, Anti-diabetic activity

Introduction

A floristic survey of ethnomedicinal plants occurring in the tribal area of Rajasthan was conducted to assess the potentiality of plant resources for modern treatments [1]. Folk medicines, mainly based on plants, enjoy a respectable position today, especially in the developing countries, where modern health service is limited. Safe, effective and inexpensive indigenous remedies are gaining popularity among the people of both urban and rural areas, especially in India and China [1].

Ziziphus nummularia is a thorny small bush or a shrub 6-8 m in height belonging to family Rhamnaceae found throughout north western India [2]. 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 [3].

In this study, *Ziziphus nummularia* were prepared by Soxhlet and Maceration Method and screened for phenolic content and anti-diabetic activity.

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 ofUdaipur, 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 % Hydroalchohol (30:70; Water: Ethanol) in a Soxhlet apparatus at 70˚C till exhaustion. The obtained extract was concentrated under reduced pressure at 40˚C [4-5].

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 [**6**].
Phytochemical screening, Total phenolic content, FTIR analysis and HPLC analysis of these extracts have been reported in our previous method. [7]

UV-vis spectroscopic Analysis of Extracts
The spectra for phenolic compounds and flavonoids typically lie in the range of 230-290 nm.

Thin Layer Chromatography of Extracts
Large number of solvent systems such as n-Hexane: Ethyl acetate: formic acid (10:5:1), Benzene: Ethyl acetate (1:0.5) and Methanol: HCl (9:1) were tried to achieve a good resolution. Finally, the solvents Methanol: HCl (9:1) gives the best result. Many of other solvent system were investigated before developing the the solvent system but none of other gave the satisfactory results.

Total Phenolic Content
The total phenolic content of extracts was determined using to the Folin-Ciocalteu method. Briefly, 0.75 mL of Folin–Ciocalteu reagent (1:9; Folin-Ciocalteu reagent: distilled water) and10 mL of sample (10 mg/mL) were put into a test tube. The mixture was allowed to stand at room temperature for 5 min. 75 µL of 6% (w/v) Na_2CO_3 was added to the mixture and then mixed gently. The mixture was homogenized and allowed to stand at room temperature for 90 min. Total polyphenol content was determined using a spectrophotometer at 725 nm. The standard calibration (10–100 µg/mL) curve was plotted using gallic acid. The total phenolic content was expressed as Gallic Acid Equivalents (GAE) in milligrams per 100g plant extract [8-9].

***In-Vitro* evaluation of Anti-Diabetic activity of *Z. nummularia* extracts**
Anti-diabetic activity of *Z. nummularia* extracts was determined by previously reported method [10-12].

Preparation of Standard Curve of D-Glucose
a) 3,5-dinitrosalicylic acid [DNS]: About 1g of DNS is dissolved in 50ml of distilled water. To this solution add about 30g of sodium potassium tartarate tetrahydrate in small lots, the solution turns milky yellow in colour. Then add 20ml of 2N NaOH, which turns the solution to transparent orange yellow colour. The final volume is made to 100 ml with the distilled water. This solution is stored in an amber coloured bottle.
b) D-Glucose working solution: 180 mg of maltose is weighed and made up to 100ml with distilled water.
Standard solution of maltose (0.2, 0.4, 0.6, 0.8 and 1 ml) added into 5 separate test tubes and A test tube containing a blank solution was prepared. The volume of each test tube was made upto 2 ml. DNS reagent (1 ml) was added to each test tube and covered with aluminum foil. Test tubes were heated in a boiling water bath for 5 minutes then it was cooled to room temperature. Distilled water (9 ml) was added to each test tube. The absorbance of solution was recorded at 540 nm.

Process to Remove Egg Shell Membrane
An average size hen's egg has been taken and broke carefully the shell at one point with the help of glass rod. All egg yolk and egg white (albumin) were removed from the egg hole with the help pf glass rod leaving behind empty egg shell. The empty egg shell was placed in 100ml of 50% HCl solution and egg membrane was separated out and washed with water.

Glucose Diffusion inhibition through Egg shell membrane:
In this model sealed dialysis tube or membrane is replaced by the egg shell membrane into which 1ml of a solution of glucose and sodium chloride (0.15M) was introduced and the appearance of glucose in the external solution was measured. The model used in the present experiment consisted of a dialysis membrane into which 1% plant extract

and 1ml of 0.15M sodium chloride (NaCl) containing 0.22mM D-glucose was added in test solution. In control 1 ml of 0.15M sodium chloride containing 0.22mM D-Glucose was introduce in a membrane without any extract. The membrane was sealed at each end placed in a 50ml centrifuge tube containing 50ml of 0.15M sodium chloride. The membrane was placed on a magnetic stirrer and kept at room temperature. The movement of glucose into the external solution was monitored after 1, 2, 3, 4, 5, 6, 24 and 27 hours. After each withdrawal of sample add equal quantity of DNSA reagent and heat it for 10-15 and observe at 540 nm on UV-Vis spectrophotometer.

Results and Discussion

The result of UV-VIS spectroscopic analysis confirms the presence of flavonoids in the Hydroalcoholic extract of *Z. nummularia.*

Table 1: Absorbance and λmax of Hydroalcoholic extract of *Z. nummularia*

S. No.	Extraction Process	Extract	Scaninng Range (nm)	λ_{max} (nm)	Absorbance (µg/ml)
1.	Maceration	Leaf Extract	200-400	279	0.641
		Fruit Extract	200-400	278	0.737
2.	Soxhlet	Leaf Extract	200-400	275	0.701
		Fruit Extract	200-400	277	0.683

TLC of *Z. nummularia* fruit and leaf extract having R_f values by Maceration process of 0.83, 0.78 and by Soxhlet process is 0.83, 0.84 respectively. TLC of all extracts showed the TLC spot and R_f value have been found to similar to Flavonoids (Quercitins and rutin).

Total Phenolic Content

Flavonoids are plant secondary metabolites widely distributed in the plant kingdom. More than 6000 Phenolic compound have been identified in plants. Figure 5 shows the standard calibration curve of Gallic acid (100µg/ml stock solution) for the determination of total flavonoid content in the hydroalchoholic extracts. From the respective standard curves, concentration values of the all extracts were obtained and total Phenolic content (TPC) was calculated by using the following formula:

$$TPC = \frac{R\ x\ D.F\ x\ V}{W} x\ 100$$

Where, R: Result obtained from the standard curve, D.F: Dilution factor, V: Volume of stock solution, 100: For 100 g dried plant and W: Weight of plant used in the experiment.

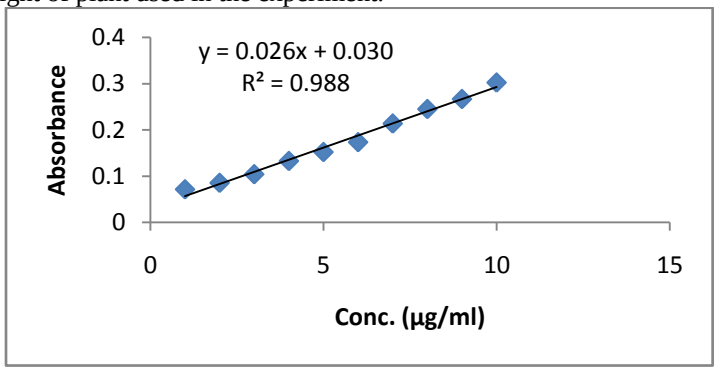

Figure 1: Standard curve of Gallic acid

Table 2: Total Phenolic component of *Z. nummularia* Extracts

S. No.	Extraction Process	Extract	Total Phenolic content in mg/100g
1.	Maceration	Leaf Extract	2.137±0.003
		Fruit Extract	1.306±0.001
2.	Soxhlet	Leaf Extract	0.747±0.0005
		Fruit Extract	0.755±0.007

***In-vitro* evaluation of Anti-Diabetic activity of *Z. nummularia* extracts**

The results of the glucose diffusion inhibitory test are given in Table 3 & 4. They have been compared in Figures 3 and 4. All extracts showed significant inhibitory activity but leaf extract of soxhlet process shows maximum inhibition to the diffusion of glucose and fruit extract of Maceration process shows least inhibition to the diffusion of glucose.

Table 3: Percentage Glucose movement into external media via Egg Shell Membrane

Time Interval (Hrs)	Control without Extract	Maceration Process		Soxhlet Process	
		Leaf Extract	Fruit Extract	Leaf Extract	Fruit Extract
0	0±0	0±0	0±0	0±0	0±0
1	11.25±0.003	3.692±0.003	4.338±0	0.360±0.003	5.408±0.002
2	16.952±0	5.678±0	7.148±0	0.545±0	6.617±0
3	20.367±0.002	6.364±0.005	8.455±0.005	0.800±0.003	7.802±0.002
4	22.426±0	7.475±0	10.784±0.004	0.896±0	10.702±0.0005
5	44.035±0.002	8.006±0.001	12.254±0	0.919±0.001	13.390±0.003
6	49.836±0.005	8.545±0.003	13.014±0.001	0.964±0.002	14.183±0.003
24	83.905±0.0005	10.351±0.0005	14.885±0.001	1.123±0.0005	19.787±0.001
27	95.506±0.002	10.326±0.001	19.362±0.004	1.131±0.005	25.187±0.003

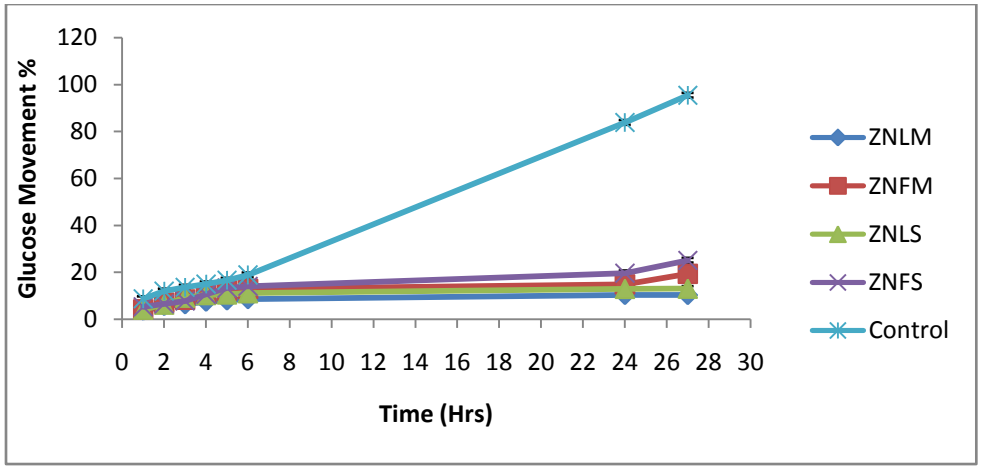

Figure 2: Effect of all extracts of Ziziphus nummularia on the diffusion of glucose out of a hen's egg dialysis membrane

Table 4: Concentration of Glucose into external media after specified time Intervals

Time Interval (Hrs)	Control without Extract	Maceration Process		Soxhlet Process	
		Leaf Extract	Fruit Extract	Leaf Extract	Fruit Extract
0	0±0	0±0	0±0	0±0	0±0
1	0.968±.003	0.317±0.003	0.373±0	0.360±0	0.465±0.002
2	1.459±0	0.488±0	0.615±0	0.545±0.003	0.569±0
3	1.753±.002	0.547±0.005	0.727±0.005	0.800±0	0.671±0.002
4	1.930±0	0.643±0	0.928±0.004	0.896±0.003	0.921±0.0005
5	3.790±0.002	0.689±0.001	1.054±0	0.919±0	1.152±0.003
6	4.289±0.005	0.735±0.003	1.120±0.001	0.964±0.001	1.220±0.003
24	7.222±0.0005	0.890±0.0005	1.281±0.001	1.123±0.002	1.703±0.001
27	8.220±0.002	0.888±0.001	1.666±0.004	1.131±0.0005	2.168±0.003

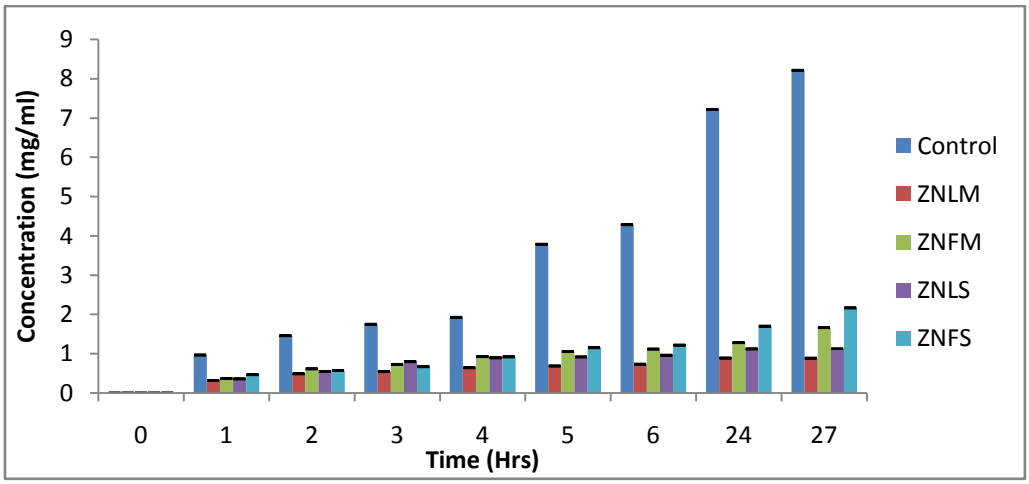

Figure 3: Concentration of Glucose (mg/ml) in external media after Specifies time Interval

Conclusion

Various trials have been performing to check the *in-vitro* Inhibition of *Ziziphus nummularia* extracts. Glucose diffusion with marketed dialysis membrane does not give better results but hen's egg shell membrane shows subsequently gives better results. Hence Egg Shell membrane is selected for the *in vitro* Glucose diffusion Model.

Total Phenolic content of leaves and fruit extracts of *Z. nummularia* is 2.137±0.003 and 1.306±0.001 by Maceration process. However, in soxhlet process the total phenolic content was 0.747±0.0005and 0.755±0.007 mg/100g of dried plant respectively. Among all extracts, leaves extract of Maceration process utilize higher glucose hence have higher anti-diabetic activity.

References

1. Katewa, S. S., Chaudhary, B. L., & Jain, A. (2004). Folk herbal medicines from tribal area of Rajasthan, India. *Journal of Ethnopharmacology*, *92*(1), 41-46.
2. Srivastava SK and Chauhan JS. Chemical examination of the whole plant of *Zizyphus nummularia*. *Planta Medica*. 32(4): 384-7, 1977.
3. Kirtikar KR and Basu BD. Indian Medicinal Plants. Allahabad: Published by Basu LM; 2(2):1361-1363, 1984.
4. James Redfern, Malcolm Kinninmonth, Dariel Burdass, and Joanna Verran (2003); Using Soxhlet Ethanol Extraction to Produce and Test Plant Material (Essential Oils) for Their Antimicrobial Properties. *J Microbiol Biol Educ*. 2014 May; 15(1): 45–46.
5. Vikas Thakur, ManjushaChoudhary, Ankur Garg,Nitesh Choudhary, Ashwani Jangra, Vikaas Budhwar (2015); Evaluation of a Hydroalcoholic Extract of the Leaves from the Endangered Medicinal Plant gloriosa superba linn. (colchicaceae) for its Potential Anti-diabetic Effect. *Achieves of Medicine*. 2015; 7:6-8.
6. Mahbubeh Setorki (2016); Effect of hydro-alcoholic extract of Ziziphus spina-christi against scopolamine-induced anxiety in rats. *Bangladesh J Pharmacol*. 2016; 11: 421-427.
7. Bagdi P, Rathore V (2016). Phytochemical Investigation of Extracts of *Ziziphus nummularia* (Burm. F. Wight & Arn) Leaves and Fruits. The Pharmaceutical and Chemical Journal, 2016, 3(4):220-226
8. Mariela González, Bernardo Guzmán, Roxana Rudyk, Elida Romano & María A.A. Molina. (2003) Spectrophotometric Determination of Phenolic Compounds in Propolis. Lat. Am. J. Pharm. 22 (3): 243-8
9. Mammadov, R., Ili, P., Ertem Vaizogullar, H., & Afacan Makascı, A. (2011). Antioxidant activity and total phenolic content of Gagea fibrosa and Romulea ramiflora. *Iranian Journal of Chemistry and Chemical Engineering (IJCCE)*, *30*(3), 57-62.
10. Amanpreet K.Sidhu, Sonal J.Wani, Poonam S.Tamboli, Sucheta N.Patil. *In-vitro* Evaluation of Anti-Diabetic Activity of Leaf and Callus Extracts of *Costus pictus*. International Journal of Science and Research (IJSR); ISSN (Online): 2319-7064.

11. Arunachalam Sathiavelu, Sundaramoorthy Sangeetha, Rastogi Archit, Sathiavelu Mythili. *In vitro* anti-diabetic activity of aqueous extract of the medicinal plants Nigella sativa, Eugenia jambolana, Andrographis paniculata and Gymnema sylvestre. International Journal of Drug Development & Research. April-June 2013;5:2. ISSN 0975-9344.
12. Ali Fattahi, Fatemeh Niyazi,Behzad Shahbazi, Mohammad Hosein Farzaei and Gholamreza Bahrami. Antidiabetic Mechanisms of Rosa canina Fruits: An *in vitro* Evaluation. Journal of Evidence-Based Complementary & Alternative Medicine 2017, Vol. 22(1) 127-133.