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Determination of total phenolic and flavonoid content of *Ziziphus nummularia* leaf and fruit extract by maceration and hot continuous process by Soxhlet apparatus

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Abstract
Medicinal plant recognized as a valuable source of natural antioxidants due to the presence of diverse bioactive phytochemicals. Among these, phenolic and flavonoid compounds play a crucial role in protecting biological system against oxidative stress by neutralizing free radicals. The present study aimed to evaluate the total phenolic content and total flavonoid content of *Ziziphus nummularia* plant extracts using established spectrophotometric methods. Total phenolic content determined by the Folin-Ciocalteu method, while total flavonoid content was estimated using the aluminum chloride colorimetric assay. Gallic acid and quercetin were employed as reference standards for phenolic and flavonoid estimation, respectively. Absorbance measurements were carried out using a UV- visible spectrophotometer, and the results were expressed as gallic acid equivalents and quercetin equivalents. The findings revealed the *Ziziphus nummularia* possesses a significant amount of phenolic and flavonoid constituents, indicating its strong antioxidant potential. The presence of these phytochemicals supports the traditional medicinal use of the plant and suggests its possible application as a natural source of antioxidants in pharmaceutical and nutraceutical formulations. Further studies are recommended to isolate individual compounds and explore their biological activities in detail.

Keywords: Phenolic content, flavanoids, *Ziziphus nummularia*, antioxidant activity, folin- ciocalteu method, gallic acid, quercetin

Introduction
Diabetes mellitus is a complex set of metabolic disorders characterized by chronic hyperglycaemia and disturbances of carbohydrate, fat, and protein metabolism [1] resulting from defects in insulin secretion, insulin action, or both [2, 3]. Recent evidence suggests that oxidative stress may contribute to the pathogenesis of Type-2 Diabetes mellitus by increasing insulin resistance or impairing insulin secretion [4, 5]. Globally, the number of people suffering from diabetes is increasing at an alarming rate [6].

The consequences of oxidative stress in diabetes are widespread and multifaceted. Beta-cell dysfunction and apoptosis occur due to the damaging effects of ROS, further compromising insulin production and secretion. Oxidative stress also contributes to insulin resistance, impairing the ability of insulin to facilitate glucose uptake into cells [7].

The global information on ethnobotanicals includes about 800 medicinal plants used for controlling diabetes mellitus. Several plants, including vegetables, are commonly consumed in India and other parts of the world; and many of these are purported to possess antidiabetic potential [8]. Moreover, recently, diet and spice therapies have become the major approaches being proposed for the treatment and control of diabetes; and a considerable amount of work has been carried out in this regard with Momordica charantia, Allium sativum, Azadirachta indica, and Ocimum sanctum [9].

A potent scavenger of these free radical species may serve as a possible preventive intervention for free radical mediated diseases. 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 [10].

Ziziphus nummularia (Burm. f.) Wight and Arn. (*Rhamnaceae*), commonly known as "Jharberi," and as Bairi or Karkanrha in Pakistan, is a branched thorny shrub with a height of 1-2 m found in Pakistan, India, and Iran. This plant is known for its palatable and vitamin C-rich fruits.

Phytochemical analysis of *Ziziphus nummularia* leaves and fruits confirm the presence of phytoconstituents such as flavonoids, alkaloids, glycosides, pectin, polysaccharides, peptide alkaloids, saponins, sterols, tannins, triterpenoic acids, fatty acids, ziziphin N, O, P, Q and dodeca acetyl Prodelphinidin B3 [11]. *Ziziphus nummularia* is reported to possess antitumor [12], anthelmintic [11], antibacterial [13], analgesic and anti-inflammatory properties [14].

The natural product based traditional knowledge has become a recognized tool in search for new sources of drugs and the ethno botanical survey can bring out many different clues for the development of drugs to treat various human diseases. The major benefits of using plant derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment. Wide arrays of plants representing active principles of numerous chemical compounds have demonstrated activity consistent with their possible use in the treatment of diabetes. Among these are polysaccharides, glycosides, alkaloids, peptidoglycan, flavonoids, tannins, anthraquinones, steroids, glycopeptides, terpenoids and inorganic ions. The introduction of these indigenous herbal compounds in the management of diabetes mellitus will greatly simplify the management and make it less expensive. So, the identification of potential anti-diabetic and antioxidant agents using mechanism-based studies holds great promise for elucidating mechanisms and devising more specific and effective treatments for diabetes mellitus. One of the approaches used in drug discovery is the ethno medical data approach, in which the selection of a plant is based on the prior information on the medicinal use of the plant. The present work was aimed at investigation of anti-diabetic and anti-oxidant potential of *Ziziphus nummularia*, a well-known medicinal plant.

Previous studies have reported that the antioxidant property of plant closely related to the presence of phenolic acids and flavonoids. Therefore, present study designed to evaluate the total phenolic and flavonoid content of *Ziziphus nummularia* extracts using established analytical methods.

Materials and Methods

Collection of Plant Materials, Identification and Authentication *Ziziphus nummularia* plant were collected from the Udaipur region of Rajasthan and were identified by the Department of Pharmacy, Bhupal Nobles' College of Botany, Bhupal Nobles' University, Udaipur (Rajasthan). Leaves of *Ziziphus nummularia* were collected during March-April, 2016 and fruits were collected during the month of Nov- Dec, 2016. Leaves and fruits were washed thoroughly with distilled water to remove the dust particles. Selected medicinal plant parts (Crude drug) were cut into small pieces, cleaned and shade dried at room temperature and then subjected to physical evaluation with different parameters. These selected medicinal plant parts were subjected to size reduction to get coarse powder, separately, in a mechanical grinder and then passed through sieve no. 40 to get desired particle size and stored in well-closed glass jars. The uniform powder was subjected to standardization for different parameters.

Determination of Phenolic content

The most active dietary antioxidants belong to the family of phenolic and polyphenolic compounds. Phenolic antioxidants are reported to quench oxygen- derived free radicals as well as the substrate-derived free radicals by donating a hydrogen atom or an electron to the free radical and the antioxidant activity of phenolics in several systems has indicated that they were as active as Buthylated hydroxyaniscle (BHA) or Buthylated hudroxytoluane (BHT) antioxidant effects of Z. *nummularia* have been attributed to the main phenolic components- rosmarinic acid, a caffeic acid derivative, and carnosic acid. Polyphenol antioxidants have protective effects against different diseases, including cardiovascular, inflammatory, and neurological diseases, as well as cancers [15].

3.5.1 Preparations of reagents

a) Folin-Ciocalteu Reagent: 10ml of Folin Ciocalteu reagent is dissolve in 90 ml of Distilled water.
b) 6% Na2CO3: 6g of Na2CO3 is dissolve in100 ml of distilled water.

3.5.2 Principle

Phenolic Quantification Assay is based on Folin-Ciocalteu method. The FC reagent contains phosphomolybdic/ phosphotungstic acid complexes. The method relies on the transfer of electrons in alkaline medium from phenolic compounds to form a blue chromophore constituted by a phosphotungstic/ phosphomolybdenum complex where the maximum absorption depends on the concentration of phenolic compounds. The reduced Folin-Ciocalteu reagent is detectable with a spectrophotometer in the range of 690 to 710 nm. The reaction temperature has been used to reduce the time necessary to attain the maximum colour (T= 37ºC). Generally, gallic acid is used as the reference standard compound and results are expressed as gallic acid equivalents (mg/ml).

The F-C assay has been used as a measure of total phenolics in natural products, but the basic mechanism is an oxidation/reduction reaction. In the original F-C assay, the carbonate buffer is used for pH adjustment and the end-point of the reaction was attained after 120 min at room temperature, which makes its implementation for routine analysis difficult. The proposed method was performed in a 96-well microplate format and it was applied to several phenolic compounds and food products (wines, beers, infusions, juices) [16].

3.5.3 Procedure: 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) and 10 mL of sample (10 mg/mL) was put into a test tube. The mixture was allowed to stand at room temperature for 5 min. 75 µL of 6% (w/v) Na2CO3 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 100 g plant extract.

$$\mathrm{TP}\% = \frac{R \times F \times V \times 100}{W}$$

Where, R = Result obtained from the standard curve

- D.F = Dilution factor
- V = Volume of stock solution 100 = For 100 g dried plant
- W = Weight of plant used in the experiment

Determination of Flavonoid content

Flavonoids are the largest group of naturally occurring phenolic compounds, which occurs in different plant parts both in Free State and as glycosides. They are found to have many biological activities including antimicrobial, mitochondrial adhesion inhibition, antiulcer, antiarthritic, antiangiogenic, anticancer, protein kinase inhibition, etc. The flavonoids have two benzene rings separated by a propane unit. The flavones and flavonols are the most widely distributed of all the phenolics. Flavonoids are particularly beneficial, acting as antioxidants and giving protection against cardiovascular disease, certain forms of cancer and age-related degeneration of cell components. Their polyphenolic nature enables them to scavenge injurious free radicals such as super oxide and hydroxyl radicals [17].

3.6.1 Prepration of Reagents

a) **5% Sodium Nitrite:** 5gm of Sodium nitrite is dissolve in 100 ml of distilled water.
b) **10% Aluminium Chloride:** 10g of Aluminium Chloride is dissolve in100 ml of distilled water.
c) **1M NaOH:** 4g of NaOH is dissolved in 100ml water

3.6.2 Procedure

Total flavonoid content was measured with the aluminium chloride colorimetric assay. 1ml of aliquots and 1ml standard quercetin solution (100, 200, 400, 600, 800, 1000 µg/ml) was positioned into test tubes and 4ml of distilled water and 0.3 ml of 5% sodium nitrite solution was added into each. After 5 minutes, 0.3 ml of 10% aluminium chloride was added. At 6th minute, 2 ml of 1 M sodium hydroxide was added. Finally, volume was making up to 10 ml with distilled water and mix well. Orange yellowish colour was developed. The absorbance was measured at 510 nm spectrophotometer using UV-visible (1800) Shimadzu, Japan Instrument. The blank was performed using distilled water. Quercetin was used as standard. The samples were performed in triplicates. The calibration curve was plotted using standard quercetin.

Results

Total Phenolic content: Figure (1.3) depicts the standard curve of Gallic acid. Total Phenolic content of leaves and fruits extract of Z. *nummularia* is 2.13±0.003 and 1.30±0.001 mg GAE/gm by maceration process. However, in soxhlet process the total phenolic content was 0.74±0.0005 and 0.75±0.007 mg GAE/gm of dried plant part respectively (2.1). Total phenolic content are expressed as mg gallic acid equivalent (GAE).

Total Phenolic contents have been implicated as natural antioxidants in fruits and leaves. They contribute to quality and nutritional value and provide health beneficial effects.

Total Flavonoid content

Standard curve of quercetin was prepared and shown in figure (1.4). Flavonoid content of all extracts was measured and expressed as mg quercetin equivalents (QE). Total flavonoid content of all leaves and fruits extract of Z. *nummularia* was 1.02±0.0005 and 0.66±0.0011 mg/gm by maceration process. However, in soxhlet process the total flavonoid content was 0.59±0.0015 and 3.21±0.0017 mg QE/gm of dried leaves and fruits respectively. The highest flavonoid content was found in fruits extract prepared by soxhlet method (Table 2.2)

Discussion and Conclusion

Phenolic compounds are secondary metabolites that are present generally in plants. These compounds possess many medicinal properties and have shown to have anti-inflammatory, anti-carcinogenic antioxidant effects [18].

Flavonoids are polyphenolic compounds known for their high antioxidant properties and free radical scavenging ability [19].

The phenolic acids and flavonoids present in the hydroalcoholic extract of leaves and fruits of Z. *nummularia* may be the major contributors for the antioxidant activity of these extracts.

The presence of these bioactive compounds supports the medicinal importance of

Z. *nummularia* and suggest its potential use in the development of natural antioxidant formulations.

Conclusions

The present investigation confirms that *Ziziphus nummularia* is a rich in phenolic and flavonoid compounds. These phytoconstituents may be responsible for the antioxidant property of the plant. Further studies are recommended to isolate and characterize individual bioactive compounds and evaluate their pharmacological activities.

Diagram, Tables and Graphs

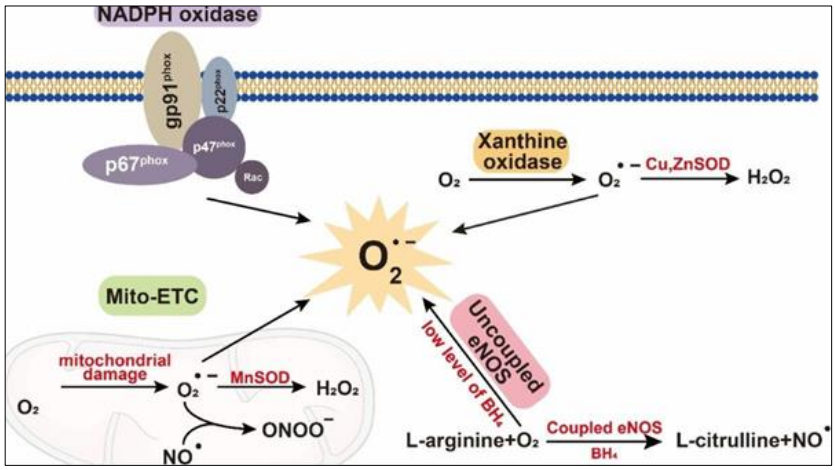

Fig 1.1: Oxidative Stress, Free Radical Generation, and Cellular Damage Pathway

Fig 1.2: *Ziziphus nummularia* (JharBer) plant with Leaves and Fruits

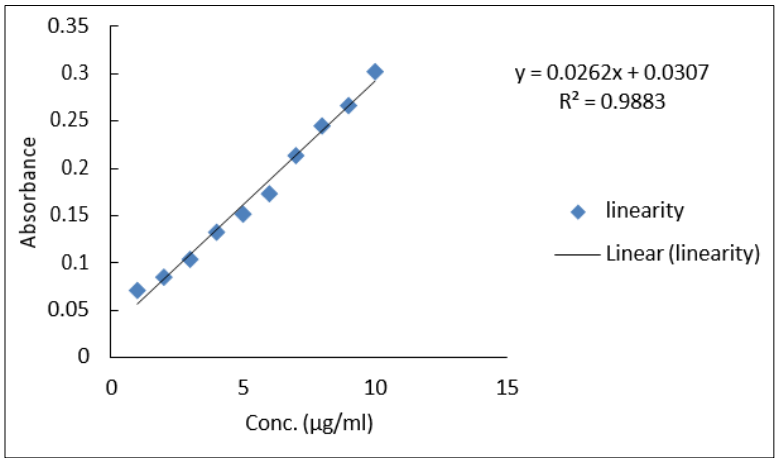

Fig 1.3: Standard curve of Gallic acid

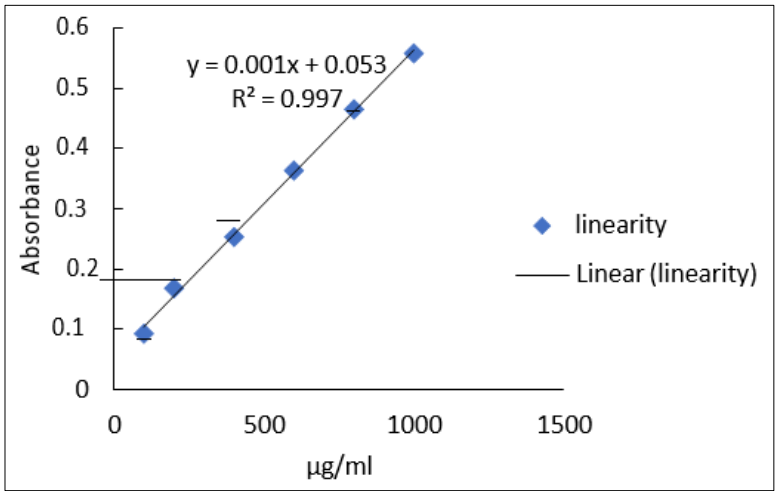

Fig 1.4: Standard curve of Quercetin

Table 2.1: Total Phenolic content of *Z. nummularia* Extracts

S.no	Extraction Process	Extract	Total phenolic content mg GAE/gm
1.	Maceration	Leaves Extract	2.13±0.003
		Fruits Extract	1.30±0.001
2.	Soxhlet	Leaves Extract	0.74±0.0005
		Fruits Extract	0.75±0.007

Data are presented as mean±SE of each triplet test

Table 2.2: Total Flavonoid content of *Z. nummularia* Extracts

S. No	Extraction Process	Extract	Total flavonoids mg QE/gm
1.	Maceration	Leaves Extract	1.02±0.0005
		Fruits Extract	0.66 ±0.0011
2.	Soxhlet	Leaves Extract	0.59±0.0015
		Fruits Extract	3.21±0.0017

Data are presented as mean±SE of each triplet test.

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References

1. Taverna MJ. Epidemiology of diabetes. In: Dermatology and diabetes. Cham: Springer; 2018. p. 1-6.
2. World Health Organization. The world health report: working together for health. Geneva: World Health Organization; 2006.
3. World Health Organization. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: Diagnosis and classification of diabetes mellitus. Geneva: WHO; 1999.
4. Ceriello A, Motz E. Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes, and cardiovascular disease? The common soil hypothesis revisited. Arterioscler Thromb Vasc Biol. 2004;24(5):816-823.
5. Monnier L, Mas E, Ginet C, Michel F, Villon L, Cristol JP, *et al*. Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. JAMA. 2006;295(14):1681-1687.
6. Montonen J, Knekt P, Järvinen R, Reunanen A. Dietary antioxidant intake and risk of type 2 diabetes. Diabetes Care. 2004;27(2):362-366.
7. Darenskaya MA, Kolesnikova LI, Kolesnikov SI. Oxidative stress: pathogenetic role in diabetes mellitus and its complications and therapeutic approaches to correction. Bull Exp Biol Med. 2021;171:179-189. doi:10.1007/s10517-021-05191-7.
8. Fridlyand LE, Philipson LH. Does the glucose-dependent insulin secretion mechanism itself cause oxidative stress in pancreatic beta cells? Diabetes. 2004;53(8):1942-1948.
9. Gidado A, Ameh DA, Atawodi SE. Indian herbs result in hypoglycemic responses in streptozotocin-induced diabetic rats. Nutr Res. 2007;27(3):143-148.
10. Velioglu YS, Mazza G, Gao L, Oomah BD. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. J Agric Food Chem. 1998;46(10):4113-4117.
11. Bachaya HA, Iqbal Z, Khan MN, Jabbar A. Anthelmintic activity of *Ziziphus nummularia* bark and Acacia nilotica fruit against trichostrongylid nematodes of sheep. J Ethnopharmacol. 2009;123(2):325-329.
12. Kumar S, Garg VK, Kumar N, Sharma PK, Chaudhary S, Upadhyay A. Pharmacognostical studies on the leaves of *Ziziphus nummularia* (Burm. f.). Eur J Exp Biol. 2011;1(2):7-15.
13. De Boer HJ, Kool A, Broberg A, Mziray WR, Hedberg I, Levenfors JJ. Antifungal and antibacterial activity of some herbal remedies from Tanzania. J Ethnopharmacol. 2005;96:461-469.
14. Goyal M, Ghosh M, Nagori BP, Sasmal D. Analgesic and anti-inflammatory studies of cyclopeptide alkaloid fraction of leaves of *Ziziphus nummularia*. Saudi J Biol Sci. 2013;20(4):365-371.
15. Mammadov R, Ili P, Vaizogullar HE, Makasci AA. Antioxidant activity and total phenolic content of *Gagea fibrosa* and *Romulea ramiflora*. Iran J Chem Chem Eng. 2011;30(3):57-62.
16. Ainsworth EA, Gillespie KM. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. Nat Protoc. 2007;2(4):875-877.
17. Sulaiman CT, Balachandran I. Total phenolics and total flavonoids in selected Indian medicinal plants. Indian J Pharm Sci. 2012;74(3):258-265.
18. Di Carlo G, Mascolo N, Izzo AA, Capasso F. Flavonoids: old and new aspects of a class of natural therapeutic drugs. Life Sci. 1999;65:337-353.
19. Scherer R, Godoy HT. Antioxidant activity index (AAI) by the 2,2-diphenyl-1-picrylhydrazyl method. Food Chem. 2009;112:654-658.