



RESEARCH ARTICLE

PHARMACOGNOSY

**PHYTOCHEMICAL, NUTRITIONAL AND ANTIOXIDANT ACTIVITY
EVALUATION OF FRUITS OF *ZIZIPHUS NUMMULARIA* BURM. F.****DEEPIKA GUPTA, SONIA MANN, ISHA JAIN AND RAJINDER K. GUPTA***

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110075, India**ABSTRACT**

In the present work, we have investigated nutritional, phytochemical content and antioxidant activity of ripe fruits of *Ziziphus nummularia* Burm. f., one of the underutilized plants distributed in drier parts of North Western India. Fruits were quantitatively analyzed for their total phenolic, flavonoid and flavonol content colorimetrically. Nutritional properties including moisture, fats, carbohydrates, proteins, ash content and mineral content in the fruits were also estimated. The antioxidant properties were evaluated using free radical scavenging, ferric reducing antioxidant power and metal chelating assays. The effect of using different methods of extraction and different solvents on yield of phytochemicals and antioxidant activity was compared and in most cases, acetone extracted sample showed higher yields and strong antioxidant activity. The study demonstrated that *Z. nummularia* fruits are good source of nutritional and antioxidant components and thus hold potential for nutraceutical development.



KEYWORDS

Ziziphus nummularia Burm. f., Antioxidant activity, Polyphenolics, Proximal analysis, Mineral analysis.

INTRODUCTION

Higher consumption of fruits and vegetables has proven to reduce the risk of chronic diseases such as cancer, cardiovascular disease, cataract, brain and immune dysfunction. This positive influence could be attributed to the non-nutritive phytochemicals such as carotenoids, alkaloids, vitamins, minerals, flavonoids and other phenolics. Many of these phytochemicals possess antioxidant activity and protect body against free radical damage. Reactive oxygen and nitrogen species contribute towards the degenerative damage of the body by creating oxidative stress. Body has a natural defense mechanism to cope up with this oxidative stress but problem arises when the free radicals outweigh the defense mechanism, damaging essential biomolecules such as proteins, DNA and lipids.

Epidemiological and laboratory studies show a strong correlation between the antioxidant properties of plant derived polyphenolic compounds and their health promoting and/or disease preventing effects. Polyphenols are secondary plant metabolites which constitute a large group of structurally diverse compounds including flavonoids, lignins, and tannins. The antioxidant activity of phenolics can be attributed to their potential to act as reducing agents, hydrogen donors, free radical scavengers, and metal chelators which are generally based on the number and location of hydroxyl groups present as well as the presence of a 2-3 double bond and 4-oxo function.

In view of the growing interest in these compounds, there is a need to identify and quantify these important compounds in fruits and vegetables so as to evaluate the potential nutritional and health benefits of a particular food. Thus, investigation and characterization of various phytochemicals present in the edible and non-commercial fruits of underutilized plant *Z. nummularia* and their use as potential source of natural antioxidants was the aim of the present study in order to understand their nutritional and other health benefits. *Z. nummularia* Burm. f. (syn. *Ziziphus rotundifolia* Lamk, *Rhamnus nummularia* Burm. f.) belongs to the buckthorn family Rhamnaceae. It is a thorny shrub with pale purplish and velvety stems and branches. Fruits are red, edible drupes, locally known as “Konkanber”, “Badber” or “Jhar Beri”.

Z. nummularia has been recognized as an underutilized plant worthy of further research and development emphasis from government and research organizations under the Indian National Genetic Resources Programme and International Centre for Underutilized Crops, University of Southampton, UK, since it forms an integral part of the life of the locals as a source of nutrition and other purposes. Various parts of *Z. nummularia* have been used in folklore medicine curing diarrhoea, dysentery, cough and biliousness [1]. Singh et al. [2] analyzed the chemical composition, phenolics, protein precipitation capacity of *Z. nummularia* but no published data could be retrieved pertaining to

the antioxidant potential of its fruits. Roots, leaves, bark, and seeds have been the subject of study in all the research work till date and fruits have been completely neglected so far as potential subject of study and analyses. Recently, aerial parts of *Z. nummularia* plant were evaluated for their DPPH, hydroxyl, superoxide free radical scavenging and reducing capacity after extraction by cold percolation method [3]. Standardized products of the fruit have not been developed yet and local communities and traditional people have used it for medicinal purposes without having exact information about its constituents. Thus, delving deep into the composition and bioactivities of fruits of *Z. nummularia* can lead to a better understanding and appreciation of the pharmaceutical, nutraceutical and medicinal value that these fruits offer and an increased consumption of the fruits by the general public.

MATERIALS AND METHODS

Collection of Sample Material and Extraction:

Ripe fruits of *Z. nummularia* were collected in Delhi, India, in February 2009. The samples were identified and authenticated by Dr. H. B. Singh and deposited under accession number NISCAIR/RHMD/Consult/-2009-10/1206/10 at Raw Materials Herbarium & Museum, NISCAIR, CSIR, New Delhi, India.

Air-dried ripe fruits of *Z. nummularia* after seed removal were powdered and extracted by two methods viz soxhlet extraction and modified acetone extraction [4]. In Soxhlet extraction, samples were sequentially extracted with three solvents viz petroleum ether, dichloromethane and methanol. In acetone extraction procedure, 5 g sample was extracted with 30 ml of 80% acetone containing 0.2% formic acid using a homogenizer for 10 min, kept overnight on orbital shaker and then centrifuged at 13,000 rpm for 20 min at 4 °C. Different extracts were concentrated under vacuum and stored at 4 °C.

Determination of Nutritional Attributes:

Proximal Analysis:

Z. nummularia fruit powdered sample were dried in an oven at 105 °C overnight for 17 h to obtain moisture content by weighing the samples before and after drying [5]. The ash content was analyzed by weighing the samples before and after burning at 500 °C for 24 h [6]. Macro Kjeldhal method was used for estimation of total nitrogen and crude protein content (N x 6.25) [7]. The fat content of the seeds was determined by Soxhlet extraction, using petroleum ether as a solvent [7]. Total carbohydrate was estimated using the formula:

Total carbohydrates (% fresh weight) = {100 - moisture (%) - protein content (% fresh weight) - crude fat (% fresh weight) - ash (% fresh weight)} [6].

Mineral Analysis:

The mineral components of the *Z. nummularia* fruit were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES). 1 g of crushed *Z. nummularia* fruits was digested by 5 ml of concentrated nitric acid in microwave. After digestion, sample was cooled and volume made up to 25 ml with double distilled water. Set plasma conditions for analysis were: Argon on 151/min, auxiliary 0.21/min, nebulizer flow at 0.851/min, RF power on 1300 W and chiller at 15 °C. Set of standards were run and then samples were analyzed against the standard.

Phytochemical Analysis:

Concentration of crude alkaloids [8], saponins [9] and tannins [10] in the dried powdered samples were determined. The total phenolic content of various extracts was determined by the Folin-Ciocalteu method as described by Slinkard and Singleton [11]. Total phenolics values of extracts are calculated using standard curve of Gallic acid (0-50 µg/ml, $y=0.0121x$, $R^2=0.9979$) and expressed in terms of Gallic acid equivalent (µg/mg of dry extract).

Total flavonoid content of extracts was estimated spectrophotometrically according to Jia et al. [12]. The flavonoid content was expressed as mg of rutin equivalents (RE) per g of dry weight of the fruit extract and calculated from calibration curve of Rutin (0-500 µg/ml, $y=0.0012x$, $R^2=0.9998$).

For estimation of the flavonol content, method of Miliauskas et al. [13] was followed. Rutin (0-150 µg/ml) was used as the standard for calibration curve ($y=0.0083x$, $R^2=0.9937$) and the flavonol content was expressed in mg of rutin equivalents (RE) per gram of dry weight of the fruit extract.

Determination of Antioxidant activity:

The stable DPPH radical was used for determination of free radical scavenging activity of the extracts according to the modified method of Blois [14]. The total antioxidant capacity of extracts was determined as ABTS radical scavenging activity. ABTS radicals are generated through a chemical oxidation reaction with potassium persulfate [15]. The FRAP assay

was carried out as described by Benzie & Strain [16] and FRAP values were calculated as µg of trolox equivalents (TE) /mg extract. The chelating activity of fruit extracts on ferrous ions was estimated using the method of Dinis et al. [17]. Ascorbic acid, Trolox and Na₂EDTA were used as positive controls.

Statistical Analysis:

All data are presented as means ± SD. The mean values were calculated based on the data taken from at least three independent experiments conducted on separate days using freshly prepared reagents. The statistical significances were achieved when $p < 0.05$.

RESULTS AND DISCUSSION

Proximal analysis:

Nutritional studies have demonstrated potential benefits of ripe fruits of *Z. nummularia*. Proximal values were calculated and are depicted in the Table 1.

Table 1
Proximate composition of *Z. nummularia* fruits on dry weight basis (g /100 g)

Ash	Moisture	Crude Fat	Total Protein	Total Carbohydrate
1.65±0.03	10.3±0.06	0.9±0.06	43.896±0.14	43.254±0.08

Data are mean±SD values of triplicate determinations.

Moisture content and dry matter analysis during nutrition reporting is very important because it directly affect its nutritional content, its stability and storage. In our studies, *Z. nummularia* fruits were found to be rich in proteins and carbohydrates. Moisture content

was low. Crude fat and ash content were found to be very low. ICP-OES studies demonstrated *Z. nummularia* fruits to be highly rich in K followed by Ca and Na (Table 2). *Z. nummularia* fruits were also found to be an important source of microelement Zn

Table 2
Mineral composition of *Z. nummularia* fruits

Analyte	Concentration (ppm)
K	1692±1.23
Ca	87±0.51
Mg	82±0.21
P	53±0.15
Na	37.91±0.36
Zn	28±0.12
Sr	0.646±0.05
Ti	0.555±0.11
Se	0.370±0.09
As	0.366±0.12
Mn	0.195±0.18
Co	0.037±0.02
Ni	0.011±0.05
Cu	0.009±0.08

Data are mean±SD values of triplicate determinations.

Phytochemical Analysis:

The phytochemical content of *Z. nummularia* were analyzed and the values of tannins, saponins and crude alkaloids were

determined on dry weight basis (g/100g) (Table 3). High quantity of alkaloids and crude tannins was found in *Z. nummularia* fruit.

Table 3
Phytochemical composition of *Z. nummularia* fruits on dry weight basis (g /100 g)

Alkaloids	Saponins	Tannins
37.37±0.14	29.85±0.12	1.476±0.42

Data are mean±SD values of triplicate determinations.

Phytochemicals, especially phenolics are suggested to be the major bioactive compounds for health benefits. Plant extracts which contain different classes of polyphenols, are very attractive not only in modern phytotherapy but

also in the food industry. Therefore, in this study, we investigated the total phenolic content, total flavonoid content and flavonol content of various extracts of fruits of *Z. nummularia* (Table 4).

Table 4
Total polyphenolic content of different extracts of *Z. nummularia* fruits

Extracts	Total phenolic content ($\mu\text{g GAE/ mg extract}$)	Total flavonoid content ($\mu\text{g RE/ mg extract}$)	Total flavonol content ($\mu\text{g RE/ mg extract}$)
Petroleum ether (Soxhlet extraction)	9.97 \pm 0.18	97.63 \pm 0.73	35.37 \pm 0.39
Dichloromethane (Soxhlet extraction)	33.94 \pm 0.42	105.96 \pm 0.13	39.17 \pm 0.52
Methanol (Soxhlet extraction)	22.09 \pm 0.76	12.3 \pm 0.15	1.66 \pm 0.02
Acetone (Liquid extraction)	54 \pm 1	24.97 \pm 0.18	3.41 \pm 0.06

Data are mean \pm SD values of triplicate determinations. GAE, Gallic acid equivalent; RE, Rutin equivalent.

The total phenolic content was found to be the highest in acetone liquid extraction sample as compared to the Soxhlet extracted samples using three different solvents. Among the three solvents used in Soxhlet extraction, dichloromethane fraction had the highest total phenolic content followed by methanolic fraction and petroleum ether fraction had the lowest. Thus, liquid extraction procedure using acetone as the solvent is a better extraction method than the soxhlet extraction procedure for obtaining better yield of phenolics in *Z. nummularia*.

The highest flavonoid content was reported in case of dichloromethane fraction followed by petroleum ether fraction and the lowest was found in methanol fraction. The total flavonoids exceeded the total phenolic content values in case of both dichloromethane and petroleum ether fractions whereas they constituted approximately 50% of the total phenolic content in methanol fraction and acetone liquid extraction sample; depicting that the fruits contain more low polarity flavonoids containing large number of non-polar prenylated or methylated –R groups instead of hydroxyl groups which got fractionated mainly in the

petroleum ether and dichloromethane fractions during soxhlet extraction. The flavonol content of the extracts were in order of methanol < acetone < petroleum ether < dichloromethane.

Determination of Antioxidant activity:
1,1-Diphenyl-2-picrylhydrazyl radical scavenging activity:

DPPH is a free-radical generating compound and has been widely used to evaluate the free-radical scavenging ability of various antioxidant compounds [18]. Fig. 1 shows the percentage inhibition of DPPH radicals by *Z. nummularia* fruits extracts and standard ascorbic acid. Among soxhlet extracted samples, dichloromethane extract (IC_{50} = 685 \pm 1 $\mu\text{g/ml}$) was more potent than methanolic extract (IC_{50} = 740 \pm 0.08 $\mu\text{g/ml}$) and petroleum ether extract was least active in DPPH free radicals scavenging. The IC_{50} value of acetone liquid extraction sample was the lowest (50.8 \pm 0.2 $\mu\text{g/ml}$) indicating that this extract had the highest antioxidant activity. The extracts were significantly different ($p < 0.05$) from standard ascorbic acid (IC_{50} = 6.47 \pm 0.07 $\mu\text{g/ml}$).

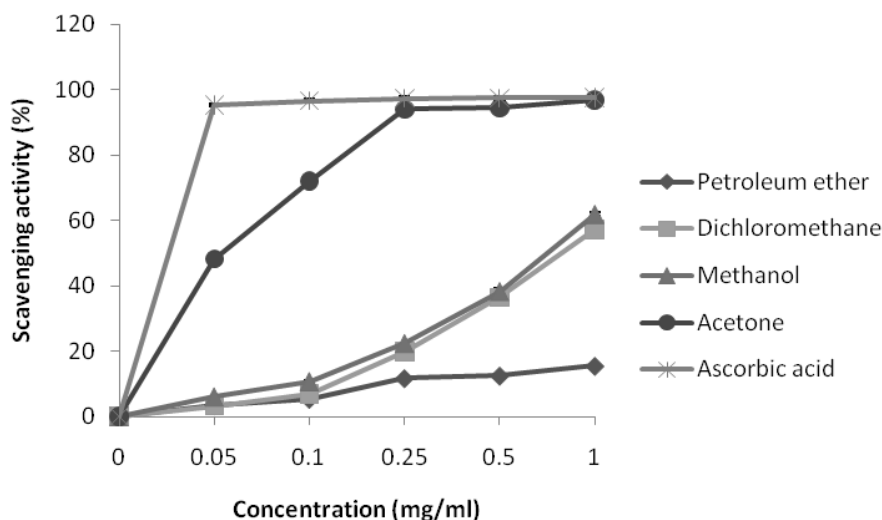


Figure 1
DPPH free radical scavenging activity of extracts of *Z. nummularia* fruits

2,2'-Azino-bis(3-ethylbenzthiazoline)-6-sulfonic free radical cation scavenging activity:

ABTS scavenging assay is applicable for screening both lipophilic and hydrophilic antioxidants. Fig. 2 shows the percentage inhibition of ABTS radical by *Z. nummularia* fruits extracts and standard trolox. The most active extract was acetone liquid extraction sample with an IC_{50} value of $8.75 \pm 0.15 \mu\text{g/ml}$, when compared to the other extracts. Soxhlet extracted samples showed lower activity than acetone liquid extracted sample. Among soxhlet extracted samples dichloromethane fraction was highly active ($IC_{50} = 45 \pm 1 \mu\text{g/ml}$) followed by methanol fraction ($IC_{50} = 95 \pm 0.08 \mu\text{g/ml}$) and PE fraction ($IC_{50} = 262.5 \pm 0.5 \mu\text{g/ml}$). There was

significant difference ($p < 0.05$) in ABTS scavenging activity of the extracts.

Ferric reducing antioxidant power (FRAP):

In this assay, reduction of the ferric-TPTZ to the ferrous complex forms an intense blue colour which can be measured at a wavelength of 593 nm. The intensity of the colour is related to the amount of antioxidant reductants in the samples. The ferric reducing activity of the acetone extract was found to be highest ($103.557 \pm 0.457 \mu\text{g TE/mg}$) followed by dichloromethane extract ($56.08 \pm 0.089 \mu\text{g TE/mg}$), petroleum ether extract ($26.94 \pm 0.07 \mu\text{g TE/mg}$) and methanolic extract ($14.373 \pm 0.264 \mu\text{g TE/mg}$). *Z. nummularia* fruits extracts were significantly different ($p < 0.05$), in their FRAP values.

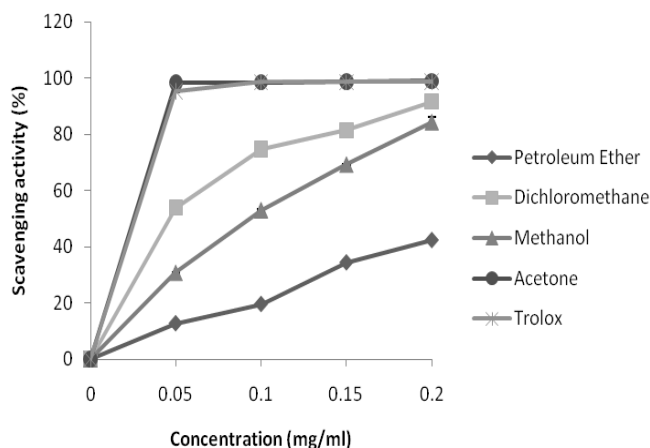


Figure 2
ABTS free radical scavenging of the extracts of *Z. nummularia* fruits

Fe²⁺ - chelating activity:

The transition metal, iron, is capable of generating free radicals from peroxides by Fenton reactions. Thus, minimizing Fe²⁺ concentrations in Fenton reactions by metal chelation affords protection against oxidative damage. In this assay, both extracts and standard Na₂EDTA interfered with the formation of ferrous and ferrozine complex, suggesting that they have chelating activity and capture Fe²⁺ ion before ferrozine (Fig. 3). The metal chelation activity was found to be poor in all the extracts

as compared to their radical scavenging activity and reducing potential. The acetone liquid extraction sample was the most active extract among the four extracts as observed in other assays. The antioxidant effectiveness of many polyphenols in vitro is essentially a result of the ease with which a H atom from an aromatic hydroxyl group is donated to a free radical and the ability of the aromatic structure to support an unpaired electron due to delocalization around the π-electron system [19].

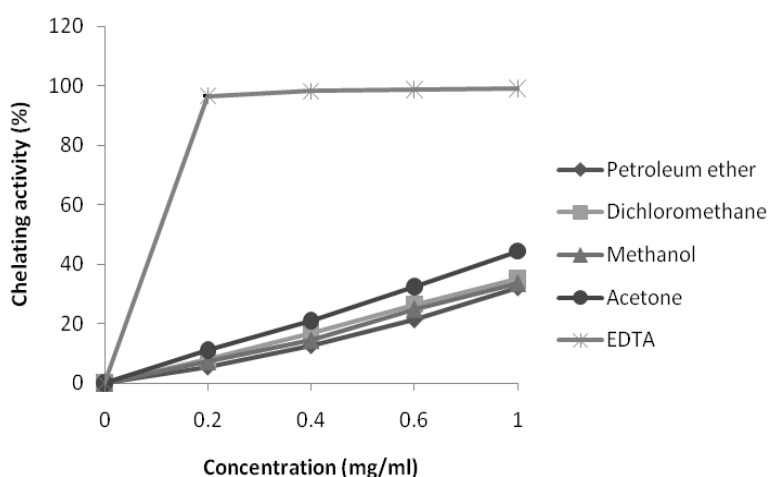


Figure 3
Metal chelation activity for the extracts of *Z. nummularia* fruits



CONCLUSION

Z. nummularia fruits were found to be rich in proteins, carbohydrates and minerals with high amount of phytochemicals and strong antioxidant properties. The antioxidant potential was found to be in good correlation with total phenolic content of the samples with acetone liquid extraction sample showing the highest antioxidant activity followed by dichloromethane and methanol extracts. Antioxidant activity of *Z. nummularia* fruits were evaluated by DPPH radical scavenging assay, ABTS radical scavenging assay, FRAP assay and metal chelation activity assay to find out their potential as good candidate for nutraceutical.

Petroleum ether extract was found to be the least bioactive. All the four extracts were found to be poor metal chelators as compared to

the standard Na₂EDTA. Thus, emphasizing that polyphenolics in *Z. nummularia* fruits mainly exhibit their antioxidant potential via free radical scavenging and electron donation. The results indicate that the fruits of *Z. nummularia* possess high phenolic content and antioxidant properties like berries [20, 21] and consequently, are potential source of dietary polyphenolics in human diet. Therefore, it seems reasonable to consider these fruits as new valuable ingredients for food and nutraceutical applications in the promotion of health as commercial products like jams, jellies, chutney, beverage, sports bar etc. However, further studies on the bioavailability of the plant extracts and their antioxidant status in animal models are needed to evaluate their potential health benefits.

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