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Original Article

Assessment of Phytochemical and *in vitro* Antioxidant Potential of *Zizyphus nummularia* Stem Bark

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ABSTRACT

The study was designed with the aim of qualitative and quantitative assessment of phytochemicals as well as to ascertain the efficacy of hydroalcohol extract of *Zizyphus nummularia* stem bark for free radical scavenging and antioxidant potential. Antioxidant activity was investigated using DPPH assay, ferric reducing power, and by determining total antioxidant capacity of extract. Findings reflect the promising antioxidant activity of stem bark. Stem bark was also found to be rich source of phenolics as indicated by total phenolic content. Phenolics and flavonoids are assigned as good natural antioxidants and may contribute for the antioxidant efficacy of stem bark. Therefore, *Z. nummularia* may further be explored as a potential candidate for developing the newer and safer natural antioxidants.

Keywords: Zizyphus nummularia; antioxidant; DPPH, flavonoids, phenolic.

1. INTRODUCTION

Zizyphus nummularia belongs to the family Rhamnaceae is a small, thorny bush majorly found in dry and arid region; distributed in North western India.[1,2] It is one of the important plant used as traditional folk medicine. Plant is abundant with secondary metabolite such cyclopetide alkaloids, phenolics and flavonoids that are beneficial for mankind.[3]

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In recent years, phytochemical exploitation of *Z. nummularia* led to the isolation and characterization of various pharmacologically active compounds with anthelmintic, analgesic, antipyretic, anti-inflammatory, sedative-hypnotic, and anti tumor potential.[4-7] Apart from these uses plant is traditionally known for its cooling, digestible, tonic, astringent, laxative, aphrodisiac, and anti-emetic property of fruit.[1] Fruit is also reputed as antidote in aconite poisoning.[8] Traditionally, bark powder finds its application in eye disorders and in treatment of tuberculosis.[9] Reactive oxygen species or free radicals are found crucial in various human diseases as overproduction of free radicals and/ or inadequate antioxidant defense leads to chemical alterations in biomolecules thereby causing modification in structure and function.[10] Antioxidants may find helpful in improving the pathological conditions. Demand is increasing for developing the more effective and harmless natural antioxidants.

The study was undertaken for assessment of antioxidant potential of *Z. nummularia* stem bark in search of newer and safer natural antioxidant. Antioxidant activity of plant is exploited by using three methods: DPPH free radical scavenging, ferric reducing capacity and total antioxidant capacity.

2. MATERIAL AND METHOD

2.1. Plant material

The stem bark of *Zizyphus nummularia* was collected from Sikandarabad, UP, India. The plant material was authenticated by Principal Scientist, NBPGR, Pusa, Delhi. The voucher specimen (NHCP/NBPGR/2016-4/5633) was preserved in NBPGR.

2.2 Preparation of extract

Air dried plant material was reduced to moderate coarse powder and extracted with a mixture of alcohol and water (1:1). The extract was decanted, filtered with Whatman No. 1 filter paper and concentrated at reduced pressure below 40°C using rotary evaporator to obtain dry extract. This alcoholic extract were designated as HAZN and taken up for biological screening.

2.3 Preliminary phytochemical investigation

Preliminary phytochemical screening was performed using standard procedures. The extracts obtained from different solvents were subjected to identification tests for the detection of various organic phytoconstituents such as alkaloids, glycosides, saponins, flavonoids, tannins, and steroids.[11,12]

2.4 Total Phenolic content

Folin–Ciocalteu method[13] was used to evaluate the total phenolic content in test extract iusing Gallic acid as standard. 0.5 mL crude extract was diluted up to 10 mL with distilled water, followed by addition of 1.5 mL of Folin–Ciocalteu reagent and 4 mL 20% (w/v) sodium carbonate and allowed to stand for further 30 minutes at room temperature. The absorbance was measured at 765nm using a UV-VIS spectrophotometer. The total phenolic content is expressed as milligrams of gallic acid equivalent (GAE) per gram of dry extract.

2.5 Total Flavonoid content

0.5 mL crude extract solution was mixed with 0.5 mL of 2% methanolic $AlCl_3$ solution and incubated for an hour at room temperature. The absorbance was determined using spectrophotometer at λmax = 415 nm. The same procedure was repeated for the standard solution of quercetin and the calibration line was constructed. The content of flavonoids in extracts was expressed in terms of quercetin equivalent (mg of QU/g of extract).[14]

2.6 Anti oxidant activity by DPPH assay

1, 1-diphenyl-2-picryhydrazyl (DPPH) free radicals scavenging activity was assessed by the standard method.[15] Different concentrations of extracts were mixed with 3 mL of ethanolic solution of DPPH (DPPH, 0.004%), incubated for 30 min at room temperature; and the reduction in DPPH free radical was measured by reading the absorbance at 517nm using UV-Visible Spectrophotometer. Initially, absorption of blank sample containing the same amount of ethanol and DPPH solution was prepared and measured as control. Gallic acid was used as standard. Percentage inhibition was calculated using equation (1), whilst IC_{50} values were estimated from the % inhibition versus concentration plot, using a non-linear regression algorithm.

$$\%\ inhibition = \left(\frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control}\right) X\ 100$$

2.7 Reducing power assay

Various concentration of extracts were prepared in 1ml of distilled water was mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5 mL, 1%). The mixture was incubated at 50◦C for 20 min. A portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 RPM for 10 min. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%) and the absorbance was measured at 700 nm. Gallic acid was used as the reference material. All the tests were performed in triplicate and the graph was plotted with the average of three observations.[16]

2.8 Total antioxidant capacity

The total antioxidant capacity of extract was evaluated by the phosphomolybdenum reduction assay method according to the procedure described by Prieto.[17] 1mL of various concentrations of extract were combined with 1 mL of reagent solution (0.6M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) and incubated at 95°C for 90 min. The absorbance of the reaction mixture was measured at 695 nm against reagent blank using a spectrophotometer. The total antioxidant capacity was expressed as the number of equivalent of ascorbic acid.

3. RESULTS

3.1 Phytochemical screening

Preliminary phytochemical screening has its key role in identification of bioactive secondary metabolites, which are accountable for the pharmacological potential of the drug.[18] Investigating the crude extract of stem bark revealed the presence of alkaloids, flavonoids, tannins, and glycosides in hydroalcohol extract of the stem bark (Table 1).

Table 1: Qualitative phytochemical investigation of *Z. nummularia* stem bark

Bioactive Group	Hydroalcohol extract
Alkaloids	+
Flavonoids	-
Tannins	+
Glycosides	+
Steroids	-
Carbohydrates	+
Proteins	-

3.2 Total phenolic and flavonoid content

The linear regression line of gallic acid (y=0.173x – 0.002, R^2 = 0.995) was used to calculate the TPC and expressed as gallic acid equivalents. TPC in hydroalcohol extract of *Z. nummaria* stem bark was found to be 15.89±0.15 mg GAE/g extract.

TFC was determined using quercetin as standard and expressed as quercetin equivalent, using regression equation of quercetin (y = 37.3x – 0.073, R^2 = 0.993). The TFC in HAZN was found to be 4.72±0.10 mg QE/g extract (Table 2).

Table 2: Quantitative estimation of TPC and TFC

Parameter	Hydroalcohol extract of stem bark (mg/g extract)
Total Phenolic content (Gallic acid equivalent)	15.89±0.15
Total flavonoid content (Quercetin equivalent)	4.72±0.10

3.3 Antioxidant activity by DPPH assay

hydroalcohol extract of stem bark exhibited promising DPPH radical scavenging activity with IC_{50} value 0.0152 mg/mL when compared to 0.00195 mg/mL for gallic acid used as standard. The scavenging potential of the crude extract was found to be concentration dependent as illustrated in Figure 1. 98.35% inhibition was reported at concentration 100 µg/mL which is comparable to standard gallic acid activity (93.74%) produced at 4 µg/mL concentration (Table 3).

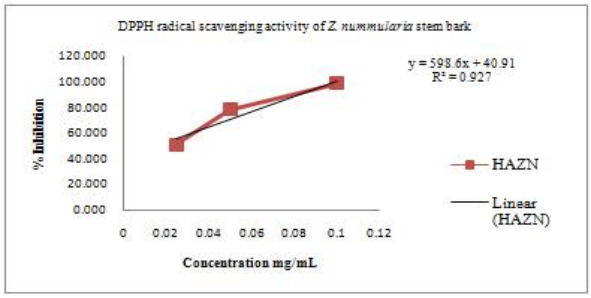

Fig 1: DPPH assay of hydroalcohol extract of stem bark (HAZN)

3.4 Reducing power assay

Reduction of the ferric cyanide complex (Fe^{3+}) to the ferrous cyanide form (Fe^{2+}) by natural agents indicate their potential as reducing agents. Dose dependent reducing action of HAZN is illustrated in Figure 2. Reducing power was found to increase with increase in concentration. Results are summarized in Table 3.

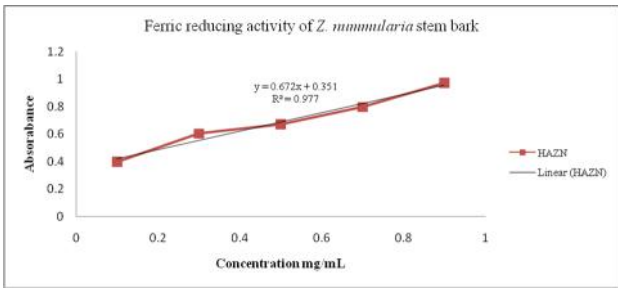

Fig 2: Ferric reducing power of HAZN

3.5 Total antioxidant capacity

The total antioxidant capacity was measured by phosphomolybdate method and is expressed as number equivalent of ascorbic acid per gram of extract. HAZN extract was found to be 4.42 mmol ascorbic acid equivalent/mg extract. A direct correlation was found to exist between the concentration of the extract used and the spectrophotometrically measured phosphomolybdenum complex (R^2>0.98) (Figure 3). A calibration curve was also plotted for ascorbic acid (y=4.835x+0.017; R^2>0.97). The total antioxidant capacity Results for antioxidant profile of HAZN have been summarized in Table 5.

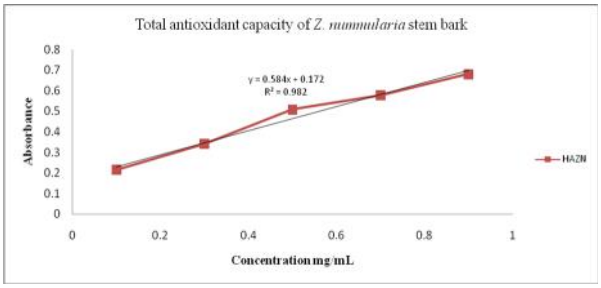

Fig 3: Total antioxidant capacity of HAZN

Table 3: Antioxidant Profile of HAZN

Results / Methods	IC_{50} (µg/mL)		mmol AA/ extract		mgConcentration µg/mL (Absorbance 0.5)	
	Std.	Test	Std.	Test	Std.	Test
DPPH	**1.95**	**15.2**	-	-	-	-
Total Antioxidant-Capacity	-	-	-	**4.42**	-	-
Reducing Power	-	-	-	-	**56.69**	**221.7**

4. DISCUSSION

Free radical mediated reaction may involve themselves in pathogenesis of allied diseases and aggravate tissue damage. A potent broad spectrum scavenger of these species may serve as a possible preventive intervention for free radical mediated cellular damage and diseases.[19] There is an urgent call for the development and utilization of natural

antioxidants over synthetic ones due to lesser side effects, higher solubility and dietary value of former.[20]

Presence of Alkaloids, tannins, glycosides, and phenols was indicated by preliminary phytochemical investigation. Further, the drug was explored for evaluating the total phenolic conten and total flavonoid content. Total phenolic content was found to be very high. Plant phenols are of great interest, can be categorized into various classes and flavonoids, have potent antioxidant potential.[21] Flavonoids are also reputed for their free radical scavenging potential and find its use as antioxidant, antibacterial, anti-inflammatory and antidiabetic.[22]

DPPH radical scavenging assay is most convenient and standard method for estimating the radical scavenging potential of the extract under study. DPPH is a stable pink colored radical change to yellow on scavenging, which is also indicated by decrease in absorbance at 517 nm. Dose dependent scavenging was observed with both test extract as well as standard gallic acid. % inhibition increased with increase in concentration of HAZN and found to be maximum at concentration 100 μg/mL i.e. 98.3% activity was observed.

Free radical chain reaction can be terminated by reducing the free radicals into more stable products, which may not participate in further reaction. Reducing ability of an antioxidant may be illustrated by reduction of Fe^{3+} into Fe^{2+} in the reaction mixture by the crude extract under study The reducing power is associated with the ability of an antioxidant to donate an electron.[23] Yellow colored reaction mixture turned to various shades of green to blue, depending on the reducing power of the compounds. Strong reducing agents, however, formed Perl's Prussian blue color and absorbed at 700 nm.[24] It was investigated that reducing power of HAZN increased with increase in concentration, which is indicated by increase in absorbance.

phosphomolybdenum method gives the total antioxidant capacity of extract irrespective of nature and mechanisms of the drug.[17] A green colored complex phosphomolybdenum V is formed from Mo (VI) by accepting an electron from the extract. Total antioxidant capacity can be expressed as ascorbic acid equivalent per gram extract. Many phenolics and flavonoids may produce their antioxidant activity either by donating an electron or hydrogen atom to the free radical.[25]

The observed activity may be due to the presence of above detected phytoconstituents. From the findings it can also be concluded that phenolics and flavonoids may be responsible for eliciting the antioxidant potential of the extract and may also find helpful in reducing the oxidative stress. However, further clinical studies are needed to be identifying the bioactive compound and to re-confirm the findings.

5. REFERENCES

1. R.N. Chopra, S.L. Nayar, I.C. Chopra. Glossary of Indian Medicinal Plants. New Delhi: CSIR; 1956: 261.
2. K.R. Kirtikar, B.D. Basu. Indian Medicinal Plants, Vol I. BSMP Publishers: Dehradun, India; 1975: 592.
3. Castro-Concha LA, Canche-Chuc I, Miranda- Ham MDL. Determination of antioxidants in fruit tissues from three accessions of *habanero pepper* (Capsicum chinense Jacq). J Mex Chem Soc 2012; 56:15–18.
4. Bachaya HA, Iqbal Z, Khan MN, Sindhu Z, Jabbar A. Anthelminthic activity of *Zizyphus nummularia* (bark) and *Acacia nilotica* (fruit) against *Trichostrongylid nematodes* of sheep. J Ethanopharmacol 2009; 123: 325-329.
5. Rauf A, Ali J, Khan H, Mubarak S, Patel S. Emerging CAM *Ziziphus nummularia* with in vivo sedative-hypnotic, antipyretic and analgesic attributes. 3 Biotech 2016; 6(1): 11.
6. Goyal M, Sasmal D, Nagori BP. Analgesic and Anti-inflammatory activity of ethanolic extract of *Zizyphus nummularia*. Res J Med Plants, 2012; 6(7): 521-528.
7. Kumar A, Sharma S, Kaushik C, Dhobal MP, Joshi BC. Antitumour activity of Lapachol isolated from *Zizyphus nummularia* as adjuvant for radiation therapy. Traditional Medicine & Materia Medica Research Center (TMRC) 2002; 1: 17.
8. Anonymous, The Wealth of India (Raw material, Vol XI: X-Z). New Delhi: Council of Industrial and Scientific Research; 1989: 111-124.
9. K.M. Nadkarni. Indian Materia Medica. Bombay: Popular Prakashan; 1986: 1315-1319.
10. Hoake JB, Pastorino JG. Ethanol, oxidative stress and cytokine-induced liver cell injury. Alcohol 2002; 27: 63–68.
11. K.R. Khandelwal. Practical Pharmacognosy Techniques and Experiments. New Delhi: Nirali Prakashan; 2002.
12. C.K. Kokate. Practical Pharmacognosy. 1st ed. New Delhi: Vallabh Prakashan; 2005: 111.
13. Panday MM, Katara A, Pandey G, Rastogi S, Rawat AKS. An important traditional indian drug of Ayurveda Jatamansi and its substitute Bhootkeshi: Chemical profiling and antioxidant activity. Evid Based Complement Alternat Med 2013; Article ID 142517: http://dx.doi.org/10.1155/2013/142517.
14. Quettier DC, Gressier B, Vasseur J, Dine T, Brunet C, Luyckx MC, et al. Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. J Ethnopharmacol 2000; 72: 35-42.
15. Hirano R, Sasamoto W, Matsumoto A, Itakura H, Igarashi O, Kondo K. Antioxidant ability of various flavonoids against DPPH radicals and LDL oxidation. J Nutr Sci Vitaminol 2001; 47(5): 357-62.
16. Oyaizu M. Studies on product of browning reaction prepared from glucose amine. Japanese J Nut 1986; 44: 307-15.
17. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the

formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal Biochem 1999; 269: 337-41.
18. Liu KC, Yang SL, Roberts MF, Elford BC, Phillipson JD. Antimalarial activity of *Artemisia annua* flavonoids from whole plants and cell cultures. Plant Cell Rep 1992; 11: 637-40.
19. Battu GR, Ethadi SR, Veda PG, Swathi PK, Chandrika K,Rao VA *et al*. Evaluation of antioxidant and anti-inflammatory activity of *Euphorbia heyneana* spreng. Asian Pac J Trop Biomed 2011: S191-194.
20. Chen C, Pearson MA, Gray IJ. Effects of synthetic antioxidants (BHA, BHT and PG) on the mutagenicity of IQ-like compounds. Food Chem 1992; 43: 177-183.
21. Kähkönen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, Heinonen M. Antioxidant activity of plant extracts containing phenolic compounds. J Agri Food Chem 1999; 47: 3954–3962.
22. Bravo L. Polyphenols: chemistry, dietary sources, metabolism and nutritional significance. Nutr Rev 1998; 56:317–333.
23. Yildirim A, Mavi A, Oktay M, Kara AA, Algur OF, Bilaloglu. Comparison of antioxidant and antimicrobial activities of Tilia (*Tilia argentea* Desf ex DC), sage (*Salvia triloba* L.), and Black tea (*Camellia sinensis*) extracts. J Agri Food Chem 2000; 48: 5030–5034.
24. Ferreira ICFR, Baptista P, Vilas-Boas M, Barros L. Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: individual cap and stipe activity. Food Chem 2007; 100: 1511–1516.
25. Amic D, Davidovic-Amic D, Beslo D, Trinajstic N. Structure-Radical Scavenging Activity Relationships of Flavonoids. Crotica Chemical Acta 2003;76: 55-61.

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