Innovare
Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 2, 2015

Original Article

COMPARATIVE EVALUATION OF TOTAL PHENOLIC CONTENT, TOTAL FLAVONOID CONTENT AND DPPH FREE RADICAL SCAVENGING ACTIVITY OF DIFFERENT PLANT PARTS OF *VITEX NEGUNDO* L.

HEMLATA SINGH[1*], ANUPMA DIXIT[1], R. A. SHARMA[1], ARCHNA SHARMA[2]

[1*]Plant Physiology and Biochemistry Laboratory, Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India, [2]R. L. Saharia Govt College, Kaladera, Jaipur, Rajasthan, India.
Email: hemlatasingh86@gmail.com

Received: 06 Oct 2014 Revised and Accepted: 02 Nov 2014

ABSTRACT

Objective: The study was aimed to comparatively evaluate Total Phenolic Content, Total Flavonoid content and DPPH free radical scavenging activity of the methanolic extracts of different plant parts (leaves, stems, flowers, fruits and roots) of *Vitex negundo*.

Methods: In this study, quantitative estimation of total phenolic and flavonoid contents were performed by using Folin–Ciocalteu method and aluminum chloride colorimetric method respectively. The free radical scavenging activities of the methanolic extracts of different plant parts were examined using established DPPH (2,2-Diphenyl-1-Picrylhydrazyl) assay.

Results: The highest Total Phenolic Content was found in flowers (71.21 ± 0.855 mg Gallic Acid Equivalent/ g dry weight of extract) while Total Flavonoid content was observed in leaves (27.32 ± 0.205 mg Quercetin Equivalent/g dry weight of extract). The highest free radical scavenging activity was recorded in flowers with IC_{50} value (82.25 ± 0.174 µg/ml) thus the most potent antioxidant part of the plant. Free radicall scavenging activity of the extracts was also found to increase in a dose dependent manner.

Conclusion: These findings suggest that methanolic extracts of different plant parts of *Vitex negundo* contain significant amount of phenols and flavonoids and also have significant antioxidant activity therefore it can be used in formulation of many antioxidant products and can be used as an important source of natural antioxidants.

Keywords: *Vitex negundo*, Total Phenolic content, Total Flavonoid content, DPPH Free radical scavenging activity, Antioxidant Activity.

INTRODUCTION

Free radicals are defined as any atoms or molecules possessing unpaired electrons. There are many reports regarding the participation of free radicals in the development of various diseases like cancer, diabetes, cardiovascular diseases, autoimmune disorders, neurodegenerative diseases, aging etc. Antioxidants are agents which scavenge the free radicals and prevent the damage caused by reactive oxygen species (ROS) such as superoxide anion, hydroxyl, hydroperoxyl, peroxyl, alkoxyl radicals. [1]. All biological systems have antioxidant defense mechanism that protects against oxidative damages. However, this natural antioxidant mechanism can be inefficient; hence dietary intake of antioxidant compounds is important [2]. Synthetic antioxidants like Butylated hydroxyanisole (BHA) and Butylated hydroxytoulene (BHT) have restricted use in foods as they are carcinogenic [3]. Therefore, the importance of search for natural antioxidants, especially of plant origin, has greatly increased in recent years. [4].

Medicinal plants contain a wide variety of free radical scavenging molecules such as phenols, flavonoids, vitamins, terpenoids that are rich in antioxidant activity [5]. *Vitex negundo* Linn. belongs to family Verbenaceae is commonly known as five leaved chaste trees (English), Nirgundi (Hindi) [6]. It thrives in humid places or along water courses in wastelands and mixed open forests and has been reported to occur in Afghanistan, India, Pakistan, Sri Lanka, Thailand, Malaysia, eastern Africa and Madagascar [7]. Although all parts of *Vitex negundo* are used as medicine in the indigenous system of medicine, the leaves are the most potent for medicinal use. It is used for treatment of eye-disease, toothache, inflammation, leucoderma, enlargement of the spleen, skin-ulcers, in catarrhal fever, rheumatoid arthritis, gonorrhoea, and bronchitis. They are also used as tonics, vermifuge, lactagogue, emmenagogue, antibacterial, antipyretic and antihistaminic agents[8]. Fruit is nervine, cephalic and emmenagogue; dried fruits act as a vermifuge; flowers are cool and astringent [9]. Root is used in dyspepsia, colic, rheumatism, worms, boils and leprosy [9, 10]. *Vitex negundo* contains many polyphenolic compounds, terpenoids, glycosidic iridoids and alkaloids [11, 12, 13]. Even though some reports are available on the antioxidant potential of *Vitex negundo*, but they are mainly concern on its leaves part. However, less attention was also paid on the antioxidant potential of other different plant parts of *Vitex negundo*. Hence in the present investigation, an attempt has been made to Comparatively evaluate total phenolic and flavonoid content and DPPH free radical scavenging potential of methanolic extracts of leaves, stems, flowers, fruits and roots of *Vitex negundo*.

MATERIALS AND METHODS

Plant materials

The different plant parts (leaves, stems, flowers, fruits and roots) of *Vitex negundo* were collected from the Rajasthan University Campus. The plant was identified and authenticated in the Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India and a voucher specimen number RUBL21107 was deposited at the herbarium, Department of Botany. The plant parts were rinsed with tap water, dried under shade at room temperature and ground to fine powder by employing an electrical grinder and stored in air tight containers.

Chemicals and reagents

2,2-Diphenyl-1-picrylhydrazyl (DPPH) and Quercetin were purchased from Sigma Chemical Co. (St., Louis, USA)., Ascorbic acid, Folin Ciocatteu's (FC) phenol reagent and methanol were purchased from Thermo Fisher Scientific India Pvt. Ltd Powai, Mumbai. Gallic acid, anhydrous sodium carbonate, aluminum chloride, and potassium acetate were purchased from Sisco Research Laboratories (SRL) PVT. Ltd. (Mumbai, India). All chemicals used were of analytical grade.

Preparation of extracts

10 gm each of the dried powder plant material (leaves, stems, fruits, flowers and roots) was soxhlet extracted with methanol for 24 hours. The extract was filtered with Whatman filter paper no 1 and

the filterate was concentrated to dryness. The dry crude extracts were weighed and stored in air-tight bottles with necessary markings for identification and kept in refrigerator 4°C for future investigation.

Determination of total phenolic content

Total Phenolic content was determined spectrophotometrically using Folin–Ciocalteu reagent by the method of McDonald *et al.* 2001 with modifications [14]. 1 ml of the plant extract was mixed with 5 ml Folin-Ciocalteu reagent (diluted tenfold) and Sodium carbonate solution in Distilled Water (4 ml, 0.7 M). The mixtures were allowed to stand for 1 hour at room temperature. The absorption was measured at 765 nm using UV-visible spectrophotometer against a blank which were composed of the same reagents except test extract. The standard calibration curve was prepared using 50, 100, 150, 200, 250 µg/ml solutions of Gallic Acid in methanol (R^2= 0.999) Total Phenolic content in the plant extracts were expressed as Gallic Acid Equivalents (mg of GAE/g dry weight of extract) and were calculated by the formula:

$$T = (C \times V) / M$$

Where, T=Total content of phenolic compounds, milligram per gram dry weight of plant extract, in GAE; C=the concentration of Gallic Acid established from the calibration curve, milligram per milliliter; V=the volume of extract, milliliter; M=the weight of methanolic plant extract, gram.

Determination of total flavonoid content

The Total Flavonoid Content in the crude extracts was determined by using the Aluminum Chloride Colorimetric method of Chang *et al.* 2002 with some modifications [15]. 1 ml of the plant extract was mixed with 3 ml methanol, 0.2 ml of 10% aluminum chloride, 0.2 ml potassium acetate (1M) and 5.6 ml of distilled water. Then the solution was incubated for 30 minutes at room temperature. The absorbance was measured at 415 nm using UV-visible spectrophotometer against a blank. The Standard calibration curve was prepared using 25, 50, 100, 200 µg/ml solutions of Quercetin in methanol (R^2= 0.996). The Total Flavonoid Content of plant methanolic extracts was expressed in Quercetin Equivalent (mg of QE/ g dry weight of extract) and was calculated by the following Formula:

$$T = (C \times V) / M$$

Where; T = total content of flavonoid compounds, mg per gram dry weight of plant extract, in Quercetin equivalent, C = concentration of Quercetin established from the calibration curve in mg/ml, V = volume of extract in ml and M = weight of methanolic plant extract in gram.

Determination of DPPH free radical scavenging activity

The free radical scavenging activities of the plant parts extracts were determined by using the modified method of Blois (1958) [16]. 1 ml each of the different concentrations of extracts or standard (Ascorbic acid) was added to 1 ml of 0.3 mM DPPH (in methanol). The mixture was vortexed and then incubated in a dark chamber for 30 min after which the absorbance was measured at 517 nm against a DPPH control containing only 1 ml of methanol in place of the extract. The Percentage scavenging activity was expressed as percentage inhibition of DPPH free radical by the sample and was calculated by using the following expression [17].

% Inhibition of DPPH

$$= \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \text{ X } 100$$

The IC_{50} value is the concentration (in µg/mL) of sample (extract/standard) that provided 50% inhibition of the DPPH radical. Linear graph of concentration Vs percentage inhibition was prepared and IC_{50} values were calculated. The lower the IC_{50} value indicates high antioxidant capacity.

Statistical analysis

All the experiments done in triplicates and results were shown as mean ± Standard Deviation (S. D.). Linear regression analysis was used to calculate the IC_{50} values. Microsoft Excel 2007 software was used for all statistical analysis.

RESULTS AND DISCUSSION

In the present study, the total phenolic and flavonoid content and free radical scavenging activity of the methanolic extract of different plant parts of *Vitex negundo* was comparatively evaluated. Phenols and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources, and they have been shown to possess significant antioxidant activities [18]. The high amount of phenols and flavonoids in extracts may explain their high antioxidative activities.

Total phenolic content

Phenolic compounds are a class of antioxidant agents which act as free radical terminators [19]. Phenolic compounds are very important plant constituents because of their scavenging ability due to their hydroxyl group; they are also powerful chain breaking antioxidants and have been associated with antioxidant activity [20]. Total Phenolic Content in methanolic extracts of different Plant parts of *Vitex negundo* were determined by using the Folin-Ciocalteu method and were expressed as Gallic Acid Equivalent per gram dry weight of plant extract.

The Maximum Phenolic Contents was found in flowers (71.21±0.855 mg GAE/ g dry weight of extract) while lowest Phenolic Contents in stems (49.09±0.618 mg GAE/g dry weight of extract). Total Phenolic Content of the different plant parts extracts decreased in the following descending order: Flowers > Fruits > Leaves >Roots > Stems. The results of Total Phenolic Content are shown in Table 1.

Total flavonoid content

Flavonoids play an important role in antioxidant system in plants. The antioxidative properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper and inhibition of enzymes responsible for free radical generation [21].

Aluminium chloride colorimetric method was used to determine the total flavonoid contents in methanolic extracts of different Plant parts of *Vitex negundo* and was expressed as Quercetin Equivalent per gram of dry weight of extract. The highest Flavonoid Contents was found in leaves (27.32±0.205 mg QE/ g dry weight of extract) while lowest in roots (0.76±0.135 mg QE/g dry weight of extract). Total Flavonoid content of the different plant parts extracts decreased in the following descending order: Leaves >Fruits > Stems> Flowers >Roots. The results of Total Flavonoid Content are shown in table 1.

Table 1: Total phenolic content and total flavonoid content in different plant parts of *Vitex negundo*

Plant parts	Total Phenolic content (mg GAE /g dry weight of extract)	Total Flavonoid content (mg QE /g dry weight of extract)
Leaves	52.56±0.250	27.32±0.205
stems	49.09±0.618	6.36±0.433
Flowers	71.21±0.855	5.62±0.085
fruits	69.95±0.270	12.99±0.235
roots	52.22±0.501	0.76±0.135

Each value in the table is represented as mean ± S. D. (n = 3).

DPPH free radical scavenging assay

The DPPH assay is extensively used for the evaluation of free-radical scavenging potential of antioxidative compounds. DPPH is a stable nitrogen-centered free radical the color of which changes from violet to yellow upon reduction by either the process of hydrogen or electron donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers [22]. In the present study, the crude methanolic extracts of different parts of *Vitex negundo* and Ascorbic acid ($IC_{50} = 16.33$ ±

0.325 µg/mL) were shown to serve as potent antioxidant agent or hydrogen donor that can scavenge radicals. It was found that the radical-scavenging activities of all the extracts increased with increasing concentration.

A lower IC_{50} value indicates higher antioxidant potential. In DPPH assay, the methanolic flower extract showed highest Free Radical Scavenging activity (IC_{50} = 82.25 ± 0.174µg/ml) thus the most potent antioxidant part of the plant while leaves show lowest Free Radical Scavenging activity (IC_{50} = 291.72 ± 1.165µg/ml). The DPPH radical scavenging activity of the plant parts extracts was decreased in the following order as Flowers>Roots> Fruits >Stems>Leaves.

The significant scavenging activity of the extracts may be attributed to the presence of phenolic and flavonoid content. These results indicated that methanolic extracts of different Plant parts of *Vitex negundo* have significant Free Radical Scavenging activity and thus exhibited the significant antioxidant potential. The IC_{50} values are reported in table 2 and graphs of DPPH radical-scavenging activities of different plant parts extracts are shown in fig. 1-5.

Table 2: IC_{50} values of different Plant parts of *Vitex negundo* of DPPH radical Scavenging Assay

Plant parts	IC_{50} values (µg/ml)
Leaves	291.72 ± 1.165
Stems	288.38± 0.289
Flowers	82.25 ± 0.174
Fruits	93.88 ± 0.378
Roots	90.36 ± 0. 461
Ascorbic acid	16.33 ± 0.325

Each value in the table is represented as mean ± S. D. (n = 3).

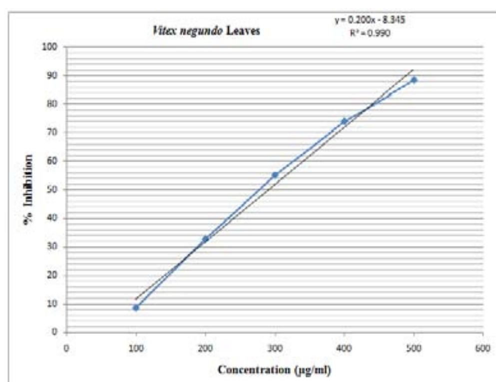

Fig. 1: DPPH radical scavenging activity of *Vitex negundo* leaves extract

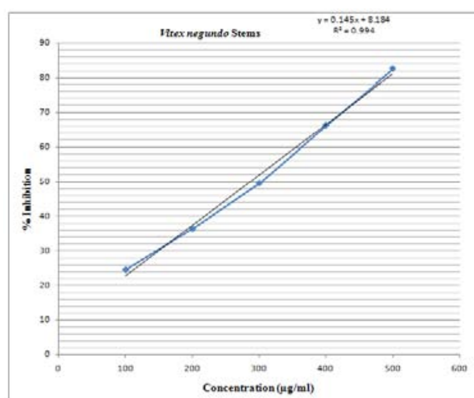

Fig. 2: DPPH radical scavenging activity of *Vitex negundo* stems extract

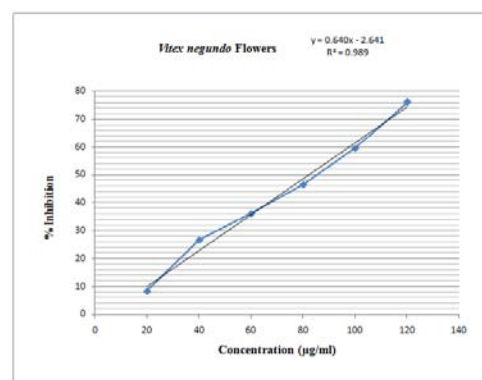

Fig. 3: DPPH radical scavenging activity of *Vitex negundo* Flowers extract

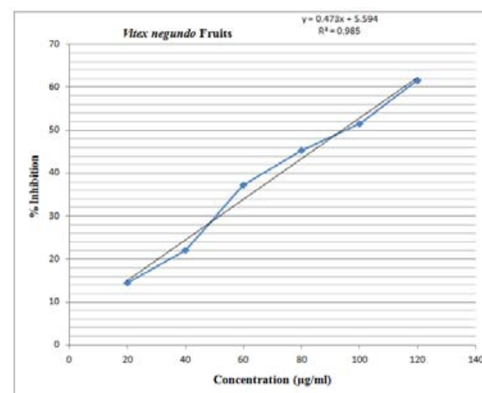

Fig. 4: DPPH radical scavenging activity of *Vitex negundo* Fruits extract

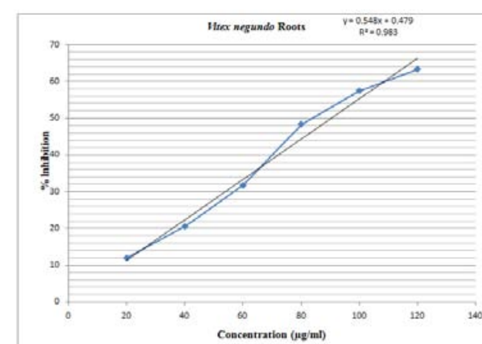

Fig. 5: DPPH radical scavenging activity of *Vitex negundo* roots extract

CONCLUSION

In the present study comparative evaluation of Total Phenolic Content, Total Flavonoid content and DPPH free radical scavenging activity of the methanolic extracts of different plant parts of *Vitex negundo* were performed. From our results, it was concluded that the methanolic extracts of different plant parts of *Vitex negundo* contain the significant amount of phenolic and flavonoids content and exhibits the significant antioxidant activity through the scavenging of free radicals. The antioxidant potential of plant parts extracts may be attributed to the presence of phenolic as well as flavonoid content. Hence further investigation for identification and proper isolation of the natural antioxidant compounds from plant extracts may help to develop new drug against free radical mediated diseases.

CONFLICT OF INTERESTS

Declared None.

REFERENCES

1. Sannigrahi S, Mazumder UK, Pal D, Mishra SL. Hepatoprotective potential of methanol extract of *Clerodendrum infortunatum* Linn. Against CCl_4 induced hepatotoxicity in rats. Indian J Exp Biol 2009;l5(20):394-9.
2. Willett WC. Balancing life-style and genomics research for disease prevention. Sci 2002;296:695-8.
3. Ito N, Fukushima S, Tsuda H. Carcinogenicity and modification of carcinogenic response by BHA, BHT and other antioxidants. Crit Rev Toxicol 1985;15:109-50.
4. Jayaprakasha GK, Jaganmohan Rao L. Phenolic constituents from lichen Parmotrema stuppeum (Nyl.) Hale and their antioxidant activity. Z Naturforsch 2000;55C:1018-22.
5. Cai YZ, Sun M. Antioxidant activity of Betalins from amaranthacea. J Agric Food Chem 2003;51:2288-94.
6. Khare CP. Indian medicinal plants: an illustrated dictionary. New York: Springer Science and Business Media; 2007. p. 710.
7. De Padua LS, Bunyapraphatsara N, Lemmens RHMJ. Medicinal and Poisonous Plants, Plant Resources of South East Asia. Backhuys Publishers Leiden; 1999.
8. Tiwari OP, Yamini BT. Antioxidant properties of different fractions of *Vitex negundo.* Linn Food Chem 2007;100:1170-6.
9. Nadkarni KM. Indian Materia Medica. Vol.1. Bombay Popular Prakashan; 2002. p. 1278-80.
10. Anonymous. The Wealth of India, Raw Materials. Vol.10. NewDelhi: Council of Scientific and Industrial Research; 2003. p. 158-60.
11. Singh P, Mishra G, Jha KK, Kumar V, Khosa RL. Chemical composition and antimicrobial activity of essential oil of leaves of *Vitex negundo* L. Int J Chem Tech Res 2010;2(3):1688-90.
12. Tandon VR. Medicinal uses and biological activities of *Vitex negundo*. Nat Prod Rad 2005;4(3):162-5.
13. Venkata SSN, Kantamreddi y, Nagendra L, Kasapu VVVS. Phytochemical analysis of some important Indian plant species. Int J Pharm Bio Sci 2010;1(4):351-7.
14. McDonald S, Prenzler PD, Autolovich M, Robards K. Phenolic content and antioxidant activity of olive extracts. Food Chem 2001;73:73-84.
15. Chang C, Yang M, Wen H, Chern J. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Analy 2002;10:178-82.
16. Blois MS. Antioxidant determination by use of Stable free radicals. Nat 1958;29:1199-200.
17. Gulcin I. Antioxidant activity of L-Adrenaline: an activity structure insight. Chem Biol Interact 2009;179(2-3):71-80.
18. Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Fazelian M, Eslami B. *In vitro* antioxidant and free radical scavenging activity of *Diospyros lotus* and *Pyrus boissieriana* growing in Iran. Pharmacogn Mag 2009;4(18):123-7.
19. Shahidi F, Wanasundara PKJPD. Phenolic anti-oxidants. Crit Rev Food Sci Nutr 1992;32:67-103.
20. Sharma SK, Gupta VK. *In vitro* antioxidant studies of *Ficus racemosus* Linn. Pharmacogn Mag 2008;4:70-4.
21. Benavente-Garcia O, Castillo J, Marin FR, Ortuno A, Del-Rio JA. Uses and properties of citrus flavonoids. J Agric Food Chem 1997;45(12):4505-15.
22. Dehpour AA, Ebrahimzadeh MA, Nabavi SF, Nabavi SM. Antioxidant activity of methanol extract of *Ferula assafoetida* and its essential oil composition. Grasas Aceites 2009;60(4):405-12.