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Research Article

PHYTOCHEMICAL SCREENING, ESTIMATION OF TOTAL PHENOLIC, FLAVANOID CONTENT AND ESTIMATION OF ANTIOXIDANT ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *VITEX NEGUNDO, VITEX TRIFOLIA* AND *VITEX PARVIFLORA*

Dharmendra Kumar Ojha*, Alok Pal Jain

RKDF College of Pharmacy, Sarvepalli Radhakrishnan University, Bhopal, Madhya Pradesh, India

**Corresponding author: dhar005.mendra @gmail.com*

ABSTRACT

The objectives of this study are to screen the phytochemicals, estimate the content of phenolic and flavonoid compounds and determine the antioxidant capacity of the leaves hydroalcoholic extract of *Vitex negundo, Vitex trifolia* and *Vitex parviflora*. Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenol and flavonoids were determined by the well-known test protocol available in the literature. The hydro alcoholic extract of bark of leaves extract of *Vitex negundo, Vitex trifolia* and *Vitex parviflora* was studied for antioxidant activity on *In vitro* models namely 1,1-diphenyl, 2-picryl hydrazyl (DPPH) assay. Phytochemical analysis revealed the presence of phenols and flavonoids in all the three plants. TPC of hydroalcoholic extract of *Vitex negundo, Vitex trifolia* and *Vitex parviflora* showed the content values of 0.872, 0.784 and 0.463 mg/100mg of dried extract respectively followed by TFC were found 0.974, 0.657, 0.652 mg/100mg of dried extract in hydroalcoholic extract of *Vitex negundo, Vitex trifolia* and *Vitex parviflora* respectively. DPPH radical scavenging assay measured hydrogen donating nature of extracts. Under DPPH radical scavenging activity, the inhibitory concentration 50% (IC_{50}) value of *Vitex negundo, Vitex trifolia* and *Vitex parviflora* hydroalcoholic leaves extracts were found to be 50.33µg/ml, 70.57µg/ml and 73.05µg/ml as compared to that of ascorbic acid.

Keywords: *Vitex negundo, Vitex trifolia* and *Vitex parviflora,* Hydroalcoholic extract, TPC, TFC, Antioxidant activity, DPPH.

1. INTRODUCTION

The recent development of functional foods and pharmaceutical products based on medicinal and food plants has brought improvements to all aspects of life, including the alleviation of physical disorders, the reduction in the use of synthetic antibiotics, and the increase in life expectancy [1, 2]. Indeed, these plants have long been used as safe, effective and sustainable sources of natural antioxidants or free radical scavengers, particularly phenolic compounds, such as phenolic acids, flavonoids, tannins, stilbenes, and anthocyanins. Those phenolics are mostly regarded to confer upon the antioxidant activity of medicinal and food plants, making a marked contribution in the fight against many pathological conditions such as cancer, diabetes, aging, cardiovascular, and other degenerative diseases [2-5]. *Vitex negundo*, also known as the Chinese Chaste tree is classified under the kingdom Plantae and is a member of the Verbanaceae family. This family consists of 250 species in which most of them have medicinal value [6]. It is a woody, aromatic shrub commonly found throughout the Indian subcontinent on riverbanks, moist localities and deciduous forests. The shrub grows to 2-4m in height. *Vitex negundo* has been used to cure several ailments such as asthama, cancer, fever, head ache, wounds, antidote for snake bite, Rheumatism [7]. The plant *Vitex trifolia* L., (Verbenaceae) is commonly known as common chaste tree (English), *nochi* (Kannada) and *jalanirgundi* (Sanskrit). Leaves are commonly used as poultice for rheumatic pains, in inflammations, sprains and fever. Roots are used to treat febrifuge, painful inflammations, cough and fever. Flowers are used in treating fever and fruits in amenorrhoea [8]. This plant is known to possess various active constituents viz., essential oil [9], halimane-type diterpenes, vitetrifolins [10] and several pharmacological properties have been studied viz., antipyretic [11], antibacterial [12], against asthma and allergic diseases [13].

Vitex parviflora (V. parviflora), locally known in the

Philippines as "molave" or mulawin", is a medium-sized to fairly large tree. It is most commonly found in regions with wet and dry seasons. It is used as medicinal herb in the Philippines. Ayta communities in Pampanga, Philippines utilize leaf and stem of *V. parviflora* as insect repellent [14]. In the province of Zamboanga, Philippines, its barks and roots are used to treat toothache, irregular menstruation, goiter, ulcer, anemia and acidity [15]. Keeping this in view, the present study has been conducted to evaluate the comparative phytochemical and antioxidant activity of Hydroalcoholic extracts of *Vitex negundo, Vitex trifolia* and *Vitex parviflora* which are traditionally well known for their various activities.

2. MATERIAL AND METHOD

2.1. Collection of plant material

Leaves of *Vitex negundo, Vitex trifolia* and *Vitex parviflora* were collected from local area of Bhopal (M.P.) in the month of December, 2020. Plant material (leaves) selected for the study were washed thoroughly under running tap water and then were rinsed in distilled water; they were allowed to dry for some time at room temperature. Then the plant material was shade dried without any contamination for about 3 to 4 weeks. Dried plant material was ground using electronic grinder. Powdered plant material was observed for their colour, odour, taste and texture.

Dried plant material was packed in air tight container and stored for phytochemical and biological studies.

2.2. Chemical reagents

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals and solvent used in this study were of analytical grade.

2.3. Defatting of plant material

A 75.6 gram of shade dried leaves of *Vitex negundo,* 52.3 gram of *Vitex trifolia* and 80.4 gram of *Vitex parviflora* was coarsely powdered and subjected to extraction with petroleum ether using soxhlation method. The extraction was continued till the defatting of the material had taken place.

2.4. Extraction by soxhlation process

Defatted dried leaves of *Vitex negundo, Vitex trifolia* and *Vitex parviflora* were exhaustively extracted with hydroalcoholic solvent (ethanol: water: 80:20) using soxhlation method by soxhlet apparatus [16]. The extract was evaporated above their boiling points. Finally the percentage yields of the dried extracts were calculated.

2.5. Phytochemical screening of the extract

The extract was subjected to qualitative analysis for the various phytoconstituents like alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, proteins, amino acids and flavonoids [17-19].

2.6. Total phenol determination

The total phenolic content was determined using the method of Olufunmiso *et al* [20]. A volume of 2ml of each extracts or standard was mixed with 1 ml of Folin Ciocalteau reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was allowed to stand for 15 min under room temperature. The colour developed was read at 765 nm using UV/visible spectrophotometer. The total phenolic content was calculated from the standard graph of gallic acid and the results were expressed as gallic acid equivalent (mg/100mg).

2.7. Total flavonoids determination

The total flavonoid content was determined using the method of Olufunmiso *et al* [20]. 1ml of 2% $AlCl_3$ solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; the absorbance of the reaction mixture was measured at 420 nm using UV/visible spectrophotometer. The content of flavonoids was calculated using standard graph of quercetin and the results were expressed as quercetin equivalent (mg/100 mg).

2.8. Antioxidant activity of hydroalcoholic extracts of *Vitex negundo, Vitex trifolia* and *Vitex parviflora* using DPPH method

DPPH scavenging activity was measured by the spectrophotometer with slightly modification of method [21]. Stock solution (6 mg in 100ml methanol) was prepared, 1.5 ml of DPPH solution was taken and volume was made up to 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test and standard solution of ascorbic acid of different concentrations (10- 100 µg/ml) were put in a series of volumetric flasks. Absorbance at zero time was taken

for each concentration. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm. The percentage inhibition of free radical DPPH was calculated from the following equation: % inhibition = [(absorbance of control-absorbance of sample)/ absorbance of control] × 100%.

3. RESULTS AND DISCUSSION

The percentage yields of Pet ether and hydroalcoholic extract obtained from *Vitex negundo, Vitex trifolia* and *Vitex parviflora* are depicted in the table 1. Preliminary phytochemical studies of the extracts were done according to the published standard methods. Phyto-chemical analysis revealed the presence of phenols and flavonoids in leaves Hydroalcoholic extracts of *Vitex negundo, Vitex trifolia* and *Vitex parviflora* (Table 2). The total phenolic content (TPC) was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: $Y = 0.011X+0.011$, $R^2= 0.998$, where X is the gallic acid equivalent (GAE) and Y is the absorbance. Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: $Y=0.040X + 0.009$, $R^2=0.999$, where X is the quercetin equivalent (QE) and Y is the absorbance. TPC of hydroalcoholic extract of *Vitex negundo, Vitex trifolia* and *Vitex parviflora* showed the content values of 0.872, 0.784 and 0.463 mg/100mg of dried extract respectively followed by TFC which were found 0.974, 0.657, 0.652 mg/100mg of dried extract in hydroalcoholic extract of *Vitex negundo, Vitex trifolia* and *Vitex parviflora* respectively (Table 3). DPPH radical scavenging assay measured hydrogen donating nature of extracts. Under DPPH radical scavenging activity, the inhibitory concentration 50% (IC_{50}) value of *Vitex negundo, Vitex trifolia* and *Vitex parviflora* hydroalcoholic leaves extracts were found to be 50.33µg/ml, 70.57µg/ml and 73.05µg/ml as compared to that of ascorbic acid (17.68µg/ml) (table 4).

Table 1: Result of percentage yield of leaves of *Vitex negundo, Vitex trifolia* and *Vitex parviflora*

Extracts	% Yield (w/w)		
	Vitex negundo	*Vitex trifolia*	*Vitex negundo*
Petroleum ether	1.52	2.54	2.72
Hydroalcoholic	6.55	5.62	4.57

Table 2: Result of phytochemical screening of leaves Hydroalcoholic extracts of *Vitex negundo, Vitex trifolia* and *Vitex parviflora*

Constituents	*Vitex negundo*	*Vitex trifolia*	*Vitex parviflora*
Alkaloids			
A) Wagner's Test:	-ve	+ve	+ve
B) Hager's Test:	-ve	-ve	-ve
Glycosides			
A) Legal's Test:	-ve	-ve	-ve
Flavonoids			
A) Lead acetate Test:	+ve	+ve	-ve
B) Alkaline Reagent Test:	-ve	+ve	+ve
Saponins			
A) Froth Test:	+ve	+ve	+ve
Phenolics			
A) Ferric Chloride Test:	+ve	+ve	+ve
Proteins			
A) Xanthoproteic Test:	+ve	+ve	-ve
Carbohydrate			
A) Fehling's Test:	+ve	+ve	-ve
Diterpenes			
A) Copper acetate Test:	-ve	+ve	+ve

Table 3: Estimation of total phenolic and flavonoids content of *Vitex negundo, Vitex trifolia* and *Vitex parviflora*

Hydroalcoholic Extracts	Total phenolic content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
Vitex negundo	0.872	0.974
Vitex trifolia	0.784	0.657
Vitex parviflora	0.463	0.652

Table 4: % Inhibition of ascorbic acid and Hydroalcoholic extract of *Vitex negundo, Vitex trifolia* and *Vitex parviflora* using DPPH method

Concentration (µg/ml)	% Inhibition			
	Ascorbic acid	*Vitex negundo*	*Vitex trifolia*	*Vitex parviflora*
10	44.65	20.85	20.85	17.85
20	48.62	27.95	27.95	30.54
40	65.34	32.61	32.61	39.77
60	69.65	45.63	45.63	43.47
80	77.41	50.62	50.62	52.85
100	84.13	67.58	67.58	60.51
IC_{50}	**17.68**	**50.33**	**70.57**	**73.05**

4. CONCLUSION

The results obtained in the present study clearly demonstrate that the extracts of *Vitex negundo, Vitex trifolia* and *Vitex parviflora*, can effectively scavenge various reactive oxygen species/free radicals under *in vitro* conditions. This may be due to the number of stable oxidized products that it can form after oxidation or radical scavenging. However, the *in vivo* safety of all three plants needs to be thoroughly investigated in experimental rodent models prior to its possible application as an antioxidant ingredient, either in animal feeds or in human health foods. The above results showed that *Vitex parviflora* hydroalcoholic bark extract could exhibit antioxidant properties. Further studies, on the use of above plants for their antioxidant role in various systems may provide potential natural antioxidants.

5. REFERENCES

1. Li Q. et al. *Ind. Crops Prod.,* 2017; **108:**512-519.
2. Nollet LM, Gutierrez-Uribe JA. Phenolic Compounds in Food: Characterization and Analysis 1st edn, 167 (CRC Press, 2018).
3. Uritu CM et al. *Pain Res. Manag.* 2018; **43:**780-783.
4. Etkin NL. Plants and Indigenous Medicine and Diet: Biobehavioral Approaches eBook, 336 (Taylor & Francis, 2019).
5. Watson RR, Preedy VR. Fruits, Vegetables, and Herbs: Bioactive Foods in Health Promotion 1st edn. (Elsevier Academic Press, 2016).
6. Meena AK, Uttam S, Yadav AK, Singh B, Rao MM. *Int J Pharm Clin Res*, 2010;**2(1):**01-09.
7. Vishwanathan, Basavaraju. *Plant E J Bio Sci,* 2010; **3(1):**30-42.
8. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal plants. New Delhi: CSIR Publications; 1956. p. 257.
9. Pan JG, Xu ZL, Fan JF. *Zhongguo Zhong Yao Za Zhi.*, 1989; **14:**357-358.
10. Ono M, Ito Y, Nohara T. *Chem Pharm Bull.,* 2001; **49:**1220-1221.
11. Ikram M, Khattak SG, Gilani SN. *J Ethnopharmacol.,* 1987;**19:**185-187.
12. Hossain MM, Paul N, Sohrab MH, Rahman E, Rashid MA. *Fitoter.* 2001; **72:**695.
13. Ikwati Z, Wahyuono S, Maeyama K. *J Ethnopharmacol.,* 2001; **75:**249.
14. Obico JJA, Ragragio EM. *Philipp Sci Lett,* 2014; **7:**179-186.
15. Philippine Traditional Knowledge Digital Library on Health. Philippine plants and natural products used traditionally. Manila: Philippine Traditional Knowledge Digital Library on Health; 2016.
16. Kokate CK. Practical pharmacognosy. 4th Ed. Vallabh Prakashan; 1994.
17. Roopashree TS, Dang R, Rani SRH, Narendra C. *International Journal of Applied Research in Natural Products,* 2008; **1(3):**20-28.
18. Obasi NL, Egbuonu ACC, Ukoha PO, Ejikeme PM. *African Journal of Pure and Applied Chemistry,* 2010; **4(9):**206-212.
19. Audu SA, Mohammed I, Kaita HA. *Life Science Journal,* 2007; **4(4):**75-79.
20. Olufunmiso OO, Afolayan AJ. BMC Complement *Alter Med,* 2011; **11:**130.
21. Geeta P, Deepak B. *Journal of Drug Delivery and Therapeutics,* 2019; **9(4-A):**708-711.