Available online on 30.08.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

Phytochemical Investigation and Determination of Total Phenols and Flavonoid Concentration in Leaves Extract of *Vitex trifolia* Linn

Geeta Parkhe[1]*, Deepak Bharti[1]

[1]Department of Biotechnology, SRK University, Bhopal, MP, India

ABSTRACT

Medicinal plants have bioactive compounds which are used for curing of various human diseases and also play an important role in healing. Secondary constituents contain alkaloids, flavonoids, phenol, saponin, steroids and tannins. Medicinal plants have anticancer, antimicrobial, antidiabetic, antidiuretic and anti-inflammation activities etc. The increasing interest in powerful biological activity of secondary metabolites outlined the necessity of determining their contents in medicinal plants. *Vitex trifolia L.* (*V. trifolia*, syn. *Vitex rotundifolia*), a member of the family Verbenaceae, is known to produce a variety of diterpenoids and iridoids that display antioxidant, tracheospasmolytic, cytotoxic and trypanosidal activities. The plant species is a deciduous shrub native to Southeast Asia, Micronesia, Australia and East Africa. This plant can be commonly found along the banks of water bodies like canals, rivers and ponds and hence locally known as "Neer Nochi" ("Neer" means water). The leaves of *V. trifolia* are consumed to improve memory, relieve pain, remove bad taste in mouth and cure fever. The leaf extract is anti-cancerous while fruits are good for amenorrhea. The aim of the present study is to examine leaf of *V. trifolia* for phytochemical profile. Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenolics and flavonoids were determined by the well-known test protocol available in the literature. Quantitative analysis of phenolic and flavonoids was carried out by Folins Ciocalteau reagent method and aluminium chloride method respectively. Phytochemical analysis revealed the presence of phenols, flavonoids, tannins, saponins, alkaloids, fixed oil and fats. The present study concluded that the crude extract of *V. trifolia* is a rich source of secondary phytoconstituents which impart significant antioxidant potential. The findings of the present study will be helpful to phytochemists, pharmacologists and pharmaceutical industries.

Keywords: *Vitex trifolia,* Verbenaceae, Phytochemical, Folins ciocalteau reagent

Article Info: Received 11 June 2019; Review Completed 05 Aug 2019; Accepted 20 Aug 2019; Available online 30 Aug 2019

Cite this article as:

Parkhe G, Bharti D, Phytochemical Investigation and Determination of Total Phenols and Flavonoid Concentration in Leaves Extract of *Vitex trifolia* Linn, Journal of Drug Delivery and Therapeutics. 2019; 9(4-A):705-707
http://dx.doi.org/10.22270/jddt.v9i4-A.3554

***Address for Correspondence:**

Geeta Parkhe, Department of Biotechnology, SRK University, Bhopal, MP, India

INTRODUCTION

Drugs from the plants are easily available, less expensive, safe and efficient and rarely have side effects[1]. According to World Health Organization (WHO), medicinal plants would be the best source to obtain variety of drugs. Large sections of the population in developing countries still rely on traditional practitioners and herbal medicines for their primary care[2]. Medicinal plants are plants in which one or more of their organs contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. WHO consultative group that formulated this definition stated also that, such a description makes it possible to distinguish between medicinal plants whose therapeutic properties and constituents have been established scientifically and plants that are regarded as medicinal but which have not yet been subjected to a thorough scientific study[3]. Such plants should be investigated to better understand their properties, safety and efficacy. The medicinal properties of plants are due to some chemical constituents that produce certain pharmacological action on the humans. The qualitative analysis of phytochemicals of a medicinal plant is reported as vital step in any kind of medicinal plant research. Screening of plants constituents accurately can be done by employing chromatographic techniques[4]. Quantification usually employs the use of gravimetric and spectroscopic methods with several advanced approaches now available[5]. *Vitex trifolia* is basically a sea side shrub from the family Lamiaceae or Verbenaceae. The *Vitex* genus family is comprised of about 250 species of shrubs and trees; it's widely cultivated in warm temperate and subtropical regions[6]. *V. trifolia* is a shrub or shrubby tree that may grow up to 6 m. Its origin is unknown and several varieties have been described in distant countries as India and Mexico and Northern Sudan[7]. Several *Vitex* species are used as folk

remedies in Mexico. *Vitex. mollis* is reported as a remedy to alleviate dysentery, as well as an analgesic and anti-inflammatory medicine; other folk uses include the treatment of scorpion stings, diarrhea and stomach ache[8]. Antimalarial, antimicrobial, and antifungal activity has been reported for *V. gaumeri*, *V. agnuscastus* and *V. negundo*, respectively; *V. negundo* is also used as an anti-inflammatory agent, and is an important medicinal plant found throughout India[9]. *V. trifolia* extracts from leafs and roots are the most important in the field of medicine and drug, its leaves[10] and seeds[11]. are widely used externally for rheumatism and inflammations of joints and are also reported to have insecticidal properties and decoction of its leaves is taken as diuretic, expectorant, vermifuge, tonic and febrifuge. The chemical components of the essential oil of leaf isolated from *Vitex negundo* and other species while *Vitex gaumeri* is used to treat colds and coughing spells[12, 13]. It is well known that a considerable number of plant species, besides their popular use as medicine in many countries, In India some species are present *Vitex glabrata, Vitex leucoxylon, Vitex penduncularis, Vitex pinnata,* and *Vitex trifolia*[14]possess insecticidal activities.

MATERIALS AND METHODS

Plant material

The leaves of *Vitex trifoli*a were collected from ruler area of Bhopal (M.P.) in the month of Feb, 2018. The leaves plant sample were separated and washed with sterile distilled water to remove the adhering dust particles and other unwanted materials. The leaf was air dried under room temperature. The dried plant samples were cut and grinded to make it in powder form. The powdered samples were stored in clean, dry and sterile container for further use.

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 used in this study were of analytical grade.

Defatting of plant material

Powdered leaves of *V. trifolia* were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether using maceration method. The extraction was continued till the defatting of the material had taken place.

Extraction by maceration process

100gm of dried plant material were exhaustively extracted with different solvent using maceration method. The extract was evaporated above their boiling points. Finally the percentage yields were calculated of the dried extracts. The extracts were evaporated above their boiling points and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts[15].

Qualitative phytochemical analysis of plant extract

The *V. trifolia* extracts obtained was subjected to the preliminary phytochemical analysis following standard methods by Khandelwal and Kokate[16, 17]. The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavonoids, glycosides, saponins, alkaloids, fats or fixed oils, protein and amino acid and tannins.

Total phenol determination

The total phenolic content was determined using the method of Olufunmiso *et al*[18]. 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).

Total flavonoids determination

The total flavonoid content was determined using the method of Olufunmiso *et al* [18]. 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/100mg).

RESULTS AND DISCUSSIONS

The crude extracts so obtained after each of the successive maceration extraction process were concentrated on water bath by evaporation the solvents completely to obtain the actual yield of extraction. The yield of extracts obtained from the leaves of the plants using chloroform, ethyl acetate, ethanol and water as solvents are depicted in the Table 1.

Table 1 Results of percentage yield of leaf extracts

S. No.	Solvents	Percentage Yield (%)
1.	Chloroform	3.2
2.	Ethyl acetate	4.9
3.	Ethanol	8.9
4.	Aqueous	6.5

The results of qualitative phytochemical analysis of the crude powder of leaf of *V. trifolia* were shown in Table 2. Ethanolic and aqueous extracts of *V. trifolia* showed the presence of alkaloids, glycosides, flavonoids, saponins, phenols, proteins and amino acids and carbohydrate.

The determination of the total phenolic content, expressed as mg gallic acid equivalents and per 100 mg dry weight of sample. The total flavonoids content of the extracts was expressed as percentage of quercetin equivalent per 100 mg dry weight of sample. TPC of ethanolic and aqueous extract of *V. trifolia* showed the content values of 0.214 and 0.174 respectively. The total flavonoid content of *V. trifolia* ethanolic and aqueous extract showed the content values of 0.125 and 0.085 respectively Table 3 and Figure 1.

Table 2 Result of phytochemical screening of extracts of *V. trifolia*

S. No.	Constituents	Chloroform extract	Ethyl acetate extract	Ethanol extract	Aqueous extract
1.	**Alkaloids**				
	A) Wagner's Test:	-Ve	-Ve	+Ve	+Ve
	B) Hager's Test:	-Ve	-Ve	+Ve	+Ve
2.	**Glycosides**				
	A) Legal's Test:	-Ve	-Ve	+Ve	+Ve
3.	**Flavonoids**				
	A) Lead acetate Test:	-Ve	+Ve	+Ve	+Ve
	B) Alkaline Reagent Test:	-Ve	+Ve	+Ve	+Ve
4.	**Saponins**				
	A) Froth Test:	-Ve	+Ve	+Ve	+Ve
5.	**Phenolics**				
	A) Ferric Chloride Test:	-Ve	+Ve	+Ve	+Ve
6.	**Proteins and Amino Acids**				
	A) Xanthoproteic Test:	-Ve	+Ve	+Ve	+Ve
7.	**Carbohydrate**				
	A) Fehling's Test:	-Ve	-Ve	+Ve	+Ve
8.	**Diterpenes**				
	A) Copper acetate Test:	-Ve	+Ve	-Ve	+Ve

Table3Estimation of total phenolic and flavonoids content of *V. trifolia*

S. No.	Extracts	Total phenolic content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
1.	**Chloroform**	-	-
2.	**Ethyl Acetate**	0.178	0.098
3.	**Ethanol**	0.214	0.125
4.	**Aqueous**	0.174	0.085

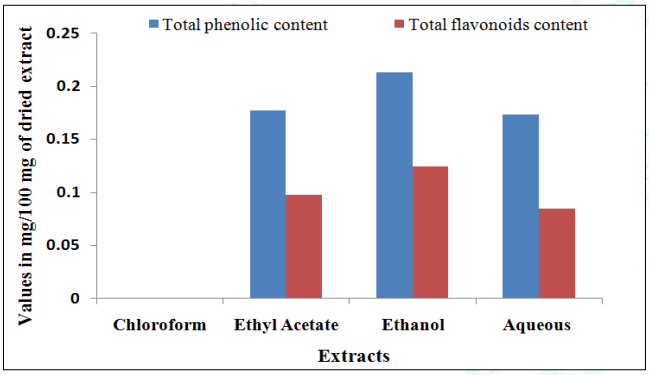

Figure 1 Graph of estimation of total phenolic and flavonoids content of *V. trifolia*

CONCLUSION

Qualitative and quantitative analysis of phenolics and flavonoids from leaves extract of *V. trifolia* was performed. The observed level of phytoconstituents revealed that *V. trifolia* is a rich source of antioxidant compounds. Currently available synthetic antioxidants are suspected to cause or prompt negative health effects, hence strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. Moreover, the plant parts may be used as an alternative source for flavonoids and phenols for traditional remedies. Further phytochemical studies are also required to isolate and characterize active ingredients that are responsible for its antioxidant and others activity and to explore the existence of synergism if any, among the compounds.

REFERENCES

1. Yadav RNS, Agarwala M. Phytochemical analysis of some medicinal plants. J Phytology 2011; 3(12):10-14.
2. WHO. Traditional medicine-growing needs and potentials. WHO Policy Perspectives Med, 2002.
3. WHO. Traditional medicine and modern health care progress report by the direct general world health organization, Document No A-144110, 1991.
4. Sneader W. The discovery of aspirin: A reappraisal, BMJ (Clinical research ed., 2000; 321(7276):1591-1594.
5. Aguoru CU, Ameh SJ, Olasan O. Comparative phyochemical studies on the presence and quantification of various bioactive compounds in the three major organs of okoho plant (cissus popunea guill & perr) in Benue State, North Central Nigeria, West Africa, European J Adv Res Biol Life Sci 2014; 2(2):16-22.
6. EL-Kousy S, Mohamed M, Mohamed S. Phenolic and biological activities of *Vitex trifolia* aerials parts, Life Sci J 2012; 9(2): 670-677.
7. McMillan X. A Concise Dictionary of Plants Cultivated in the United States and Canada. In: Bayley, L.H. (Ed.). Hortorium. Cornell University, New York, 1976; pp. 1161-1162.
8. Argueta A, Cano LM, Rodarte ME. Atlas de las Plantas de la Medicina Tradicionally III. Instituto Nacional Indigenista 1994; 537-538.
9. The Wealth of India Adictionary of India-Raw Materials and Industerial Products- Raw material Series (Publication and Information Directorate, Council of Scientific & Industrial Research, New Delhi) 1976 reprint 1998; X: 520-525.
10. Dharmasiri MG, Jayakody JRAC, Galhena G. Anti-inflammatory and analgesic activity of mature fresh leaves of *Vitex negundo*. J Ethnopharmacol 2003; 87 (2-3): 199-206.
11. Chawla AS, Sharma AK, Handa SS, Dhar KL. Chemical investigation and inflammatory activity of *Vitex negundo* seeds. J Nat Prod 1992; 55 (2): 163-167.
12. Ekundayo O, Laakso I, Holopainen M, Hiltunen R, Oguntimein B, Kauppinen V. The chemical composition and antimicrobial activity of the leaf oil of *Vitex agnus-castus* L.J Essent Oil Res 1990; 2 (3): 115-119.
13. Damayanti M, Susheela M, Sharma GJ. Effect of plant extracts and systemic fungicide on the pineapple fruit-rotting fungus, Ceratocystis paradoxa. Cytobios 1996; 86 (346): 155-165.
14. Meier B, Berger D, Hoberg E, Sticher O, Schaffner W. Pharmacological activities of *Vitex agnus-castus* extracts *in vitro*. Phytomedicine 2000; 7(5): 373-81.
15. Mukherjee PK. Quality control of herbal drugs. 2nd Ed. Business Horizons; 2007.
16. Khandelwal KR, Practical pharmacognosy technique and experiments. 23rd Ed. Nirali Prakashan; 2005.
17. Kokate CK. Practical pharmacognosy. 4th Ed. Vallabh Prakashan; 1994.
18. Olufunmiso OO, Afolayan AJ, Phenolic content and antioxidant property of the bark extract of *Ziziphus mucronata* willd. Subsp. mucronata willd, BMC Complement Alter Med 2011; 11:130.