



Phytochemical Profiling and Antioxidant Activity of Aqueous Leaf Extract of *Vitex negundo*

Govindarajan Umamaheswari¹, Kuppusamy Selvam^{1*} and Palanisamy Prakash¹

¹Department of Botany, Periyar University, Periyar Palkalai Nagar, Salem 636 011, Tamil Nadu, India.

Authors' contributions

This work was carried out in collaboration among all authors. Author GU credited contribution not specified, methodology, formal analysis, visualization, writing-original draft preparation. Author PP validation, investigation and author KS credit contribution not specified. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i23B31418

Editor(s):

(1) Dr. Q. Ping Dou, Barbara Ann Karmanos Cancer Institute, Wayne State University, USA.

(2) Dr. Paola Angelini, University of Perugia, Italy.

(3) Dr. Ana Cláudia Coelho, University of Trás-os-Montes and Alto Douro, Portugal.

Reviewers:

(1) Murat Aydin, Adiyaman University, Turkey.

(2) Olga del Rosario Valdés Iglesias, Institute of Marine Science, Cuba.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/67365>

Received 05 February 2021

Accepted 11 April 2021

Published 15 April 2021

Original Research Article

ABSTRACT

Natural products from plants, animals and minerals are the basis for treating human diseases. The present investigation explains that the antioxidant potential of aqueous extract of *vitex negundo* leaves. Phytochemical constituents, total phenolic and flavanoid content of aqueous analysis and vitro methods such as 1,1-diphenyl-2picrylhydrazyl (DPPH), 2,2-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radical scavenging assays and GC_MS studies are reported. In addition, TPC and TFC of the extract were evaluated. It was observed that the leaf extract total phenol (14.3 mg) and flavanoids (8.5 mg) Quercetin equivalent (Q/g) high level of Phenolic and flavonoid content that might have accounted for the strong activity observed ABTS 62% of inhibition $\mu\text{g}/\text{mL}$ almost equivalent to that of standard vitamin C and DPPH value of $66.32 \mu\text{g}/\text{mL}$ almost equivalent to that of standard vitamin C results revealed that leaves of *vitex negundo* have Since this investigation is a preliminary study, a detailed study of the antioxidant mechanisms of specific phenol components is an absolute necessity. Many phytochemical constituents which may be responsible for many pharmacological activities.

Keywords: DPPH; ABTS; *Vitex negundo*; phenolic; flavonoid; GC-MS.

1. INTRODUCTION

Medicinal plants have been a major source of therapeutic agents since ancient times to cure human disease. The revival of interest in natural drugs started in last decade mainly because of the wide spread belief that green medicine is healthier than synthetic products. The major advances in the modern medicine, the development of new drugs from natural products is still considered as important [1]. Traditional therapeutics based on herbal medicinal principles is time tested and widely accepted across various cultural and socioeconomic strata. However, there is lack of precise guidelines to study the herbal compounds and till date a very meager portion of this tremendous potential drug-repertoire has been scientifically screened. Hence, there is a real need for scientific evidence based validation of these agents. Almost all human pathogenic bacteria capable of obtaining rapid resistance to the antimicrobial drugs, as a result multiple drug resistant pathogenic bacteria have evolved hence we are unable to treat the infectious diseases [2]. Antioxidant refers to a compound that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions and which can thus prevent or repair damage done to the body cells by oxygen. They may act on one or more of the following mechanisms: reducing activity, free radical-scavenging, potential complexing of pro-oxidant metals and quenching of singlet oxygen. Epidemiological studies have shown that many phytonutrients of fruits and vegetables might protect the human body against damage by ROS. The consumption of natural antioxidant phytochemicals was reported to have potential health benefits [3].

Vitex negundo its belongs to the Verbenaceae family. It is a woody, aromatic deciduous shrub growing like small trees. In this study, to explain all the beneficial properties along with the advantages of antioxidant and biological properties. The decoction of leaves is used for the treatment of inflammation, eye-disease, toothache, leucoderma, enlargement of the spleen, ulcers, cancers, catarrhal fever, rheumatoid arthritis, gonorrhoea, sinuses, scrofulous sores, bronchitis and as tonics, vermifuge, lactagogue, emmenagogue, antibacterial, antipyretic, antihistaminic, analgesic, insecticidal, ovicidal, feeding deterrence, growth inhibition and morphogenetic

agents [2]. The objective of this study was to evaluate the potential of aqueous leaves used in the present work an extraction and phytochemicals screening, total phenol and flavanoid and antioxidant activity of the leaves of *Vitex negundo* has been undertaken.

2. MATERIALS AND METHODS

2.1 Collection of Plant and Preparation of Extraction

Vitex negundo Roxb (Verbeaceae) leaves were collected from Boduvarayan malai of Kalvarayan hills, Eastern Ghats, Tamilnadu, India Latitude $N 11 47'$ and $12 33'$ and Longitude $E 77 02'$. The plant was authenticated by BSI (Botanical survey of India) specimen number is PU/SRC/1/18/2020/PP/124. The leaves extract was washing, shade dried and powdered using mixer grinder. The powdered material (25 g) was extraction with 100 ml of Aqueous using a maceration method and Whatman No. 1 filter paper using filtration.

2.2 Preliminary Studies on Phytochemical Screening of *Vitex negundo*

Qualitative analyses of the phytochemicals present in the Aqueous leaf extracts were determined using the method described [4].

2.3 Total Phenol Content

Total Phenol content was estimated using the aqueous leaf extract of *Vitex negundo* as determined by [5]. Added 0.5 ml of extract (5mg/ml in ethanol) inserted into a test tube containing 2.25 cml of methanol. After that 0.22 ml of Folin-ciocalteu reagent was added and the mixture was stirred for 1 min and allowed to stand for 8 min. Then 2.0 ml of sodium carbonate (7.5% W/V) was added and the mixture was incubated for 120 min at 25°C. The absorbance of extract and a prepared blank as ethanol was measured at 756 nm.

2.4 Estimation of Total Flavonoid

Total flavonoid content was measured by an aluminum chloride colorimetric method as described [6]. Total flavanoid content was done on the basis of a standard curve of quercetin prepared in 80% ethanol and results expressed as mg quercetin equivalent/g dry (mg QE/g).

2.5 In vitro Antioxidant Activity of *Vitex negundo*

2.5.1 DPPH (1, 1-diphenyl - 2 - picrylhydrazyl hydrate radical scavenging activity)

The DPPH radical scavenging activities of the aqueous extracts of *Vitex negundo* parts were evaluated by the method of [7]. A different extract of samples (0.1ml) at various concentrations (125, 250, 500 µg/ml) was mixed with 1ml of 0.2 mM DPPH dissolved in methanol. The reaction mixture was incubated for 20 min in the dark. The control contained all the reagents without the sample and was used as blank. The DPPH radical scavenging activities were determined by measuring the absorbance at 517 nm using a spectrophotometer (Hitachi U-20). Vitamin C was used as positive control. The antioxidant activities of plant extracts were expressed as which was defined as the concentration (µg/ml) of extracts required to inhibit the formation of DPPH radicals by 50 per cent.

2.5.2 ABTS•+ scavenging effects (2, 2-Azino-bis-3-ethyl benzthiazoline-6-sulphonic acid)

The antioxidant effect of the different crude extracts of *Vitex negundo* parts were evaluated by the method [8] ABTS•+ radical cations (ABTS•+) were produced by reacting ABTS•+ solution 7mM with 2.45mM potassium persulphate. The mixture was incubated at room temperature in the dark for 12 to 16 h to yield a dark-colored solution containing ABTS•+ radicals and diluted. The different concentration of (25, 50, 75, 150µg/ml) extracts were added to 1 ml of ABTS•+ solution. The absorbance was read at 734 nm after 6 minutes in a spectrophotometer (Hitachi U-20). Vitamin C was used as the standard. Appropriate solvents blanks were run in each assay.

2.6 Gas Chromatography - MS Analysis

Gas chromatography (GC) analysis was carried out using Agilent 6890 N gas chromatography equipped with mass selective detector coupled to front injector type 1079. The chromatograph was fitted with DB 5 MS capillary column (30 m x 0.25 mm i.d., film thickness 0.25 µm). The injector temperature was set at 280°C and the oven temperature was initially at 45°C then programmed to 300°C at the rate of 10°C/min and finally held at 200°C for 5 min. Helium was

used as a carrier gas with the flow rate of 1.0 ml/min. 1ml of the sample (diluted with acetone 1:10) was injected in the split mode in the ratio of 1:100. The percentage of sample was calculated by the GC peak area. GC-mass spectrometry (GC-MS) analysis of sample was performed using Agilent gas chromatography equipped with JEOL GC MATE-II HR Mass Spectrometer. GC conditions were the same as reported for GC analysis and the same column was used. The mass spectrometer was operated in the electron impact mode at 70 eV. Ion source and transfer line temperature was kept at 250 °C. The mass spectra were obtained by centroid scan of the mass range from 40 to 1000 amu. The compound was identified based on the comparison of their retention indices (RI), Retention time (RT), mass spectra of WILEY, NIST library data of the GC-MS system and literature data [9].

2.7 Statistical Analysis

Statistical analysis was done using SPSS 16.0 software (SPSS Inc. Chicago, USA). Differences among the samples were evaluated by using Analysis of Variance (ANOVA) and Duncan's Multiple Comparison method. A significant difference was assumed at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Qualitative Analysis

Therapeutic propensity of the plants can be assessed by performing initial qualitative screening to ensure the presence of phytochemicals. In the conducted study bioactive constituents that confer biologically dynamic nature to the plants were screened and the results confirmed the existence of phytochemical screening of *Vitex negundo* was assessed and the results are shown in (Table 1). It was observed that the maximum groups of phytochemicals were present in high polar solvents, especially in methanol extracts of both the plants. Alkaloids, flavonoids, saponins, tannins, triterpenes, anthraquinones and phenolics were observed from the aqueous extract of *Vitex negundo* similarly, in *L. chinensis* the presence of flavonoids, saponins, tannins, triterpenes and phenolics in methanol extract. In this study the solvents were unable to resolve the presence of phytochemicals on the polarity basis and most of these phytochemicals were in different fractions. Similar results were recorded in other studies.

Table 1. Phytochemical analyses of aqueous leaf extract of *Vitex negundo*

Phytoconstituents	Aqueous leaf extract
Alkaloids	+
Saponins	-
Steroids	-
Flavonoids	++
Tannins	+
Phenolic	++
Glycosides	+
Terpenoids	+
Carbohydrates	-

(++ = Strong; + = Present; - = Absent)

Table 2. Quantities analysis of aqueous leaf Total Phenol and Flavonoids content

S. No	Solvents	Total Phenol (GAE mg/g)	Flavonoids (Q mg/g)
1	Aqueous	14.5±0.32	8.5±0.25

3.2 Quantities Analysis of Aqueous Leaf Extracts of *Vitex negundo*

The total phenol (14.3 mg GAE/g) and flavonoids (8.5 mg QE/g) Quercetin equivalent (Q/g) were found to be higher in aqueous leaf extract of *V. negundo*. Phenolic compounds are powerful antioxidants and act in a structural-depend manner; they can scavenge reactive oxygen species (ROS) and chelate transition metals which play vital role in the initiation of deleterious free radical reactions [10]. Obviously, the total phenol content could be regarded as an important indication of antioxidant properties of the plant extract [11]. The major phenolic and flavonoids compounds present in have been reported to possess many useful properties, including antimicrobial, anti-inflammatory, antioxidant, anti-allergic, hepatoprotective, antiviral and anti-carcinogenic activities.

3.3 Antioxidant Potential

In the present investigation, the commonly accepted assays DPPH and ABTS were used for the evaluation of antioxidant activity of plant extracts. The total phenolic contents and flavonoid contents were also determined. Herbal plants considered as good antioxidant since ancient times. The natural antioxidant properties of plant depend on the total phenols and flavonoids content. [11] Plants are important

source of potential compounds for the development of new therapeutic agents. Plant phenolics are widely distributed in the tissues of plants as well as play a vital role in the highly effective free radical scavengers and antioxidant activity [12]. It has been reported that the antioxidant activity of phenol is mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers [13].

3.3.1 DPPH antioxidant assay

Herbal drugs comprising antiradical constituents are acquiring importance in inhibition and treatment of stress related disorders. The free radical scavengers like polyphenolics are well identified for their therapeutic activity in disorders such as cancer, diabetes and skin [14]. DPPH with an IC₅₀ value of 28.32 µg/ mL almost equivalent to that of standard vitamin C (IC₅₀ value 19.47 µg/mL). The percentage of inhibition was 98.11% and 99.36% for the leaf extract and vitamin C respectively at 150 µg/mL concentration. The observed antioxidants of extract may be due to the neutralization of free radicals (DPPH), either transfer of hydrogen atom or transfer of an electron. In support of our work, similar results have been observed using the whole plants *A. benthamii* and significant DPPH activity has also been documented against *A. densiflora* root extract [15] also observed different extracts of *Azima tetraantha* [16] leaves showed good dose dependent free

radical scavenging activity. Methanol solvents generally used for antioxidant ability assays, are strongly hydrogen bond – accepting, therefore the hydrogen - abstracting reaction occurs very slowly [17]. Three extracts of *Andrographis paniculata* were demonstrated for *in vitro* antioxidant and antimicrobial activity using two assays. In the DPPH and total reducing capacity method. All the three extracts of *A. paniculate* showed potent antioxidant activity with half maximal inhibitory concentration (IC₅₀) values, ranging from 223.3µg/ml, 69.32µg/ml and 82.23 µg/ml. The dichloromethane extract showed the lowest IC₅₀ followed by other two extracts [18]. Presence of phenolics and flavonoids impart the scavenging capabilities to the plant. Phenolics and flavonoids are greatly extracted in the polar solvents which show good scavenging abilities as they donate electron or hydrogen to stabilize DPPH free radicals (Fig. 1).

3.3.2 ABTS antioxidant assay

It exhibited potent scavenging effects against ABTS with an IC₅₀ value of 28.32 µg/ mL almost equivalent to that of standard vitamin C (IC₅₀ value 19.47 µg/mL). The percentage of inhibition was 68.11% and 99.36% for the leaf extract and vitamin C respectively at 150 µg/mL concentration show in (Fig. 2). Further the

antioxidant activity of the extract by this assay implies that action may be either by inhibiting or scavenging properties of antioxidant towards this radical have been reported in earlier studies [15]. These results clearly indicate that the leaves of *V negundo* can be used to discover new bioactive natural products that may serve as a lead to the development of new pharmaceuticals and also as a good source of antioxidants. [16] studied antioxidant properties using DPPH and ABTS assay of leaves extracts, that is, methanol, chloroform, ethyl acetate, and aqueousness of *P. aculeate* L., and found that different phytochemicals, present in the leaves, are responsible for the high antioxidant potential [19].

3.3.3 GC-MS analysis of aqueous leaf extract of *Vitex negundo*

The GC-MS results reveals the major peak found in the aqueous leaf extract, and the NIST library was referred for the identification of each compound, based up on the peak area and the retention time in the chamber. Bio active compounds present in the Aqueous leaf extract of *Vitex negundo* are show in (Table 3). The major compound namely 1. Propanoic acid ethyl ester 2.Acetic acid 3.n-Hexadecanoic acid in this compound identified different biological activities (Figs. 3 & 4).

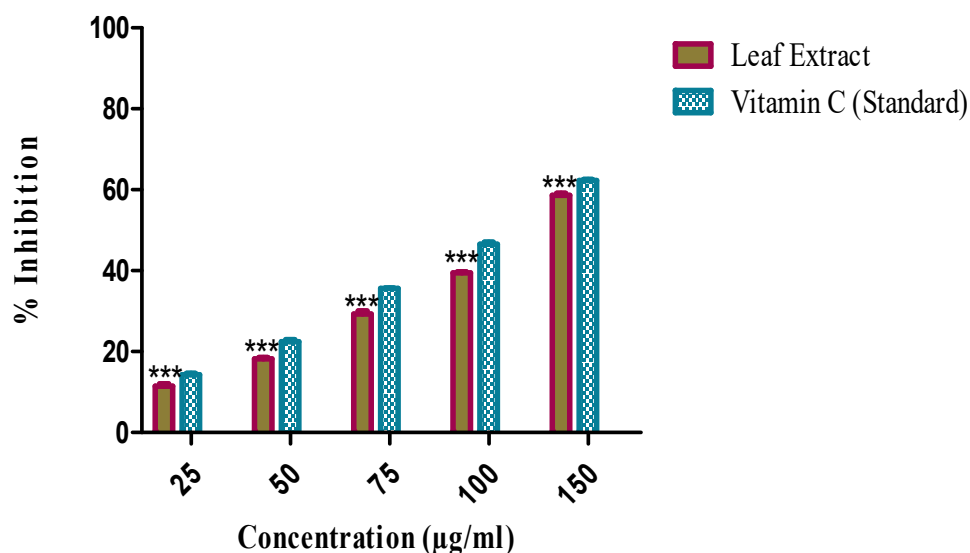


Fig. 1. *In-vitro* antioxidant activity of using aqueous extract of *Vitex negundo* leaf DPPH radical scavenging activity

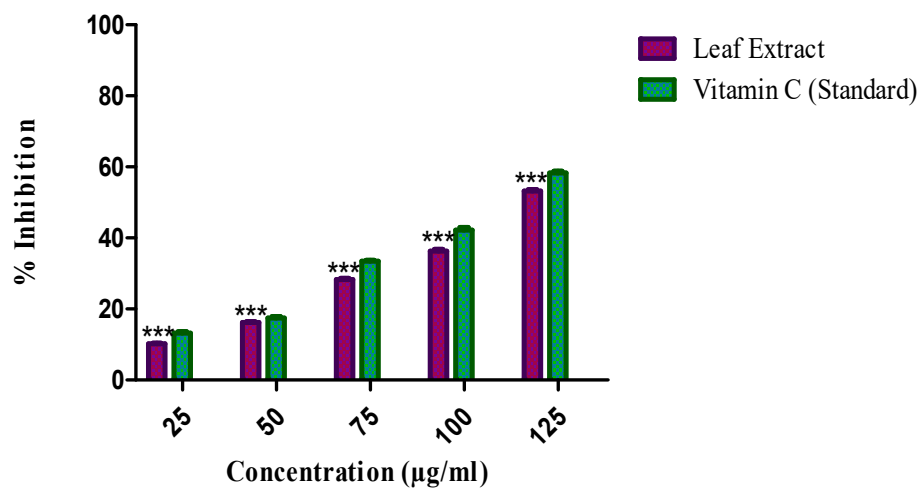


Fig. 2. *In-vitro* antioxidant activity of using aqueous extract of *Vitex negundo* leaf ABTS radical scavenging activity

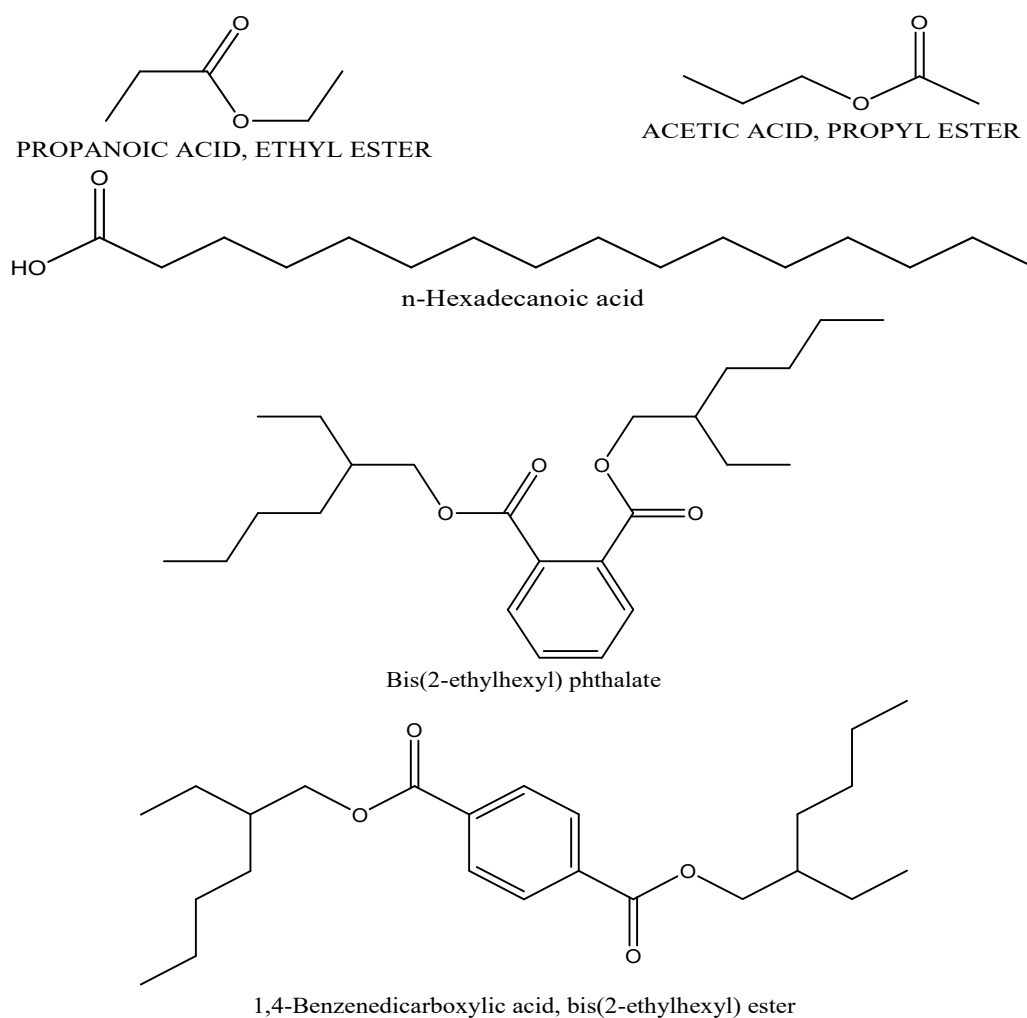
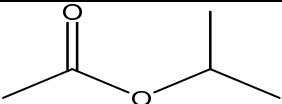
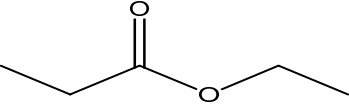
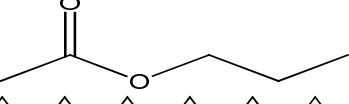

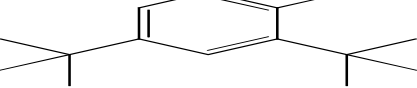
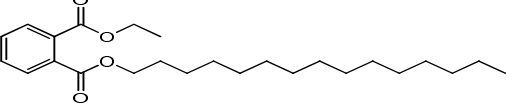


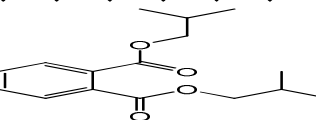
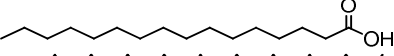

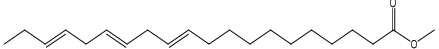
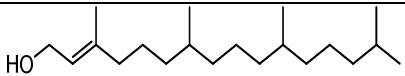
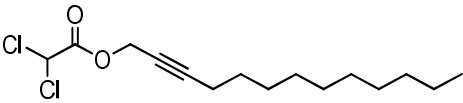
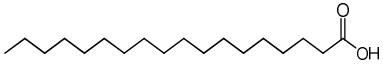
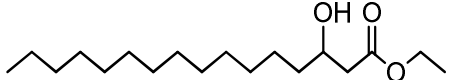
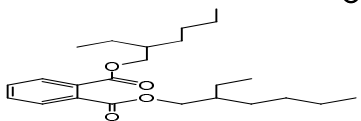
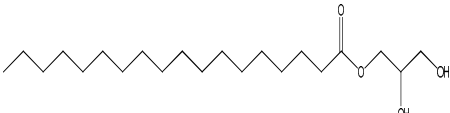
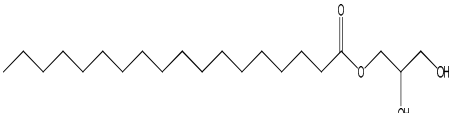
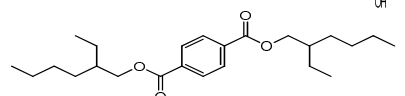


Fig. 3. GC-Mass spectroscopy analysis of identification of Major compounds structure

Table 3. GC MS Analysis of identification of compounds of aqueous leaf extract of *Vitex negundo*

S. No	Bioactive compounds name	Structure	Molecular Formula	Molecular, Weight
1	Acetic Acid, 1-Methylethyl Ester		C5H10O2	102
2	Propanoic Acid, Ethyl Ester		C5H10O2	102
3	ACETIC ACID, PROPYL ESTER		C5H10O2	102
4	Tetradecane		C14H30	198
5	PHENOL, 2,4-BIS(1,1-DIMETHYLETHYL)-		C14H22O	206
6	Phthalic acid, ethyl pentadecyl ester		C25H40O4	404
7	Heptadecane		C17H36	240
8	Heneicosane		C21H44	296
9	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester		C16H22O4	278
10	n-Hexadecanoic acid		C16H32O2	256
11	n-Nonadecanol-1		C19H40O	284
12	11,14,17-Eicosatrienoic acid, methyl ester		C21H36O2	320

S. No	Bioactive compounds name	Structure	Molecular Formula	Molecular Weight
13	Phytol		C ₂₀ H ₄₀ O	296
14	Dichloroacetic acid, tridec-2-ynyl ester		C ₁₅ H ₂₄ Cl ₂ O ₂	306
15	Octadecanoic acid		C ₁₈ H ₃₆ O ₂	284
18	Ethyl 3-hydroxyhexadecanoate		C ₁₈ H ₃₆ O ₃	300
20	Bis(2-ethylhexyl) phthalate		C ₂₄ H ₃₈ O ₄	390
21	1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetramethyl-6-ethyl)octadecanoic acid, 2,3-dihydroxypropyl ester		C ₃₃ H ₅₆	452
22	Octadecanoic acid, 2,3-dihydroxypropyl ester		C ₂₁ H ₄₂ O ₄	358
23	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester		C ₂₄ H ₃₈ O ₄	390

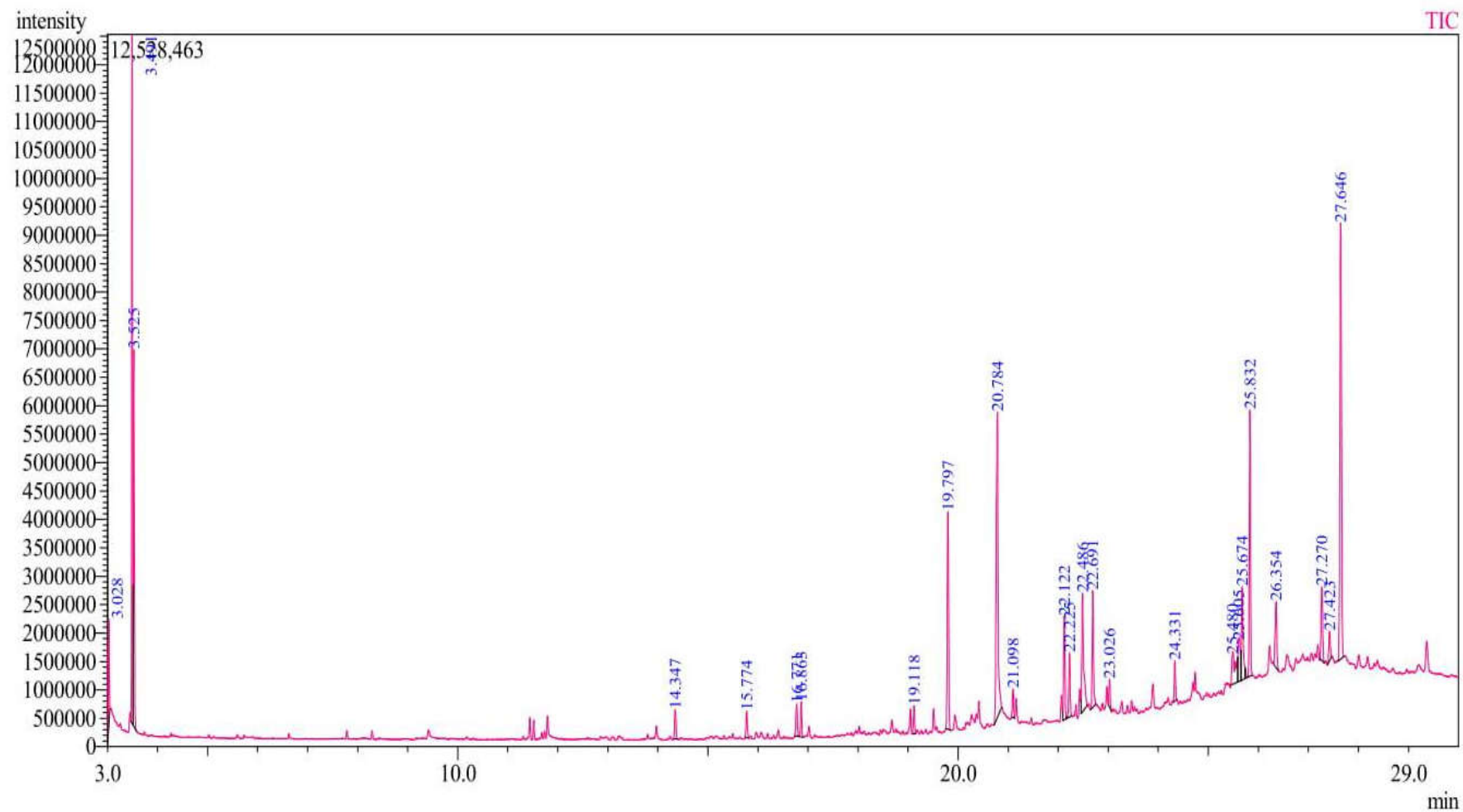


Fig. 4. Gas chromatography analysis of aqueous leaf extract of *Vitex negundo*

4. CONCLUSION

In the present investigation, leaf extract of *Vitex negundo* exhibited outstanding scavenging effects on phytochemical constituent's DPPH, ABTS radicals. In addition, Total phenol and Total flavanoids contents of the extract were evaluated. It was observed that the leaf extract contained high level of phenolic and flavonoid content that might have accounted for the strong activity observed against the free radicals. Since this investigation is a preliminary study, a detailed study of the antioxidant mechanisms of specific phenolic components is an absolute necessity. For further work on the profile and nature of chemical constituents of leaves will provide more information on the active principles responsible for their pharmacological properties. This may also lead to the development of a new generation of drugs that possess both chemotherapeutic and chemo preventive properties which can result in ways of combating the serious problems of diseases. Never the less, based on the above presented results, leaf extract of *Vitex negundo* could be investigated as a possible new source of natural antioxidants in the food and pharmaceutical industry.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENTS

The authors Acknowledge to Periyar University, Salem to providing University facilities. The authors also thank to Department of Botany, School of Life Sciences, Periyar University, Salem, Tamil Nadu-636 011, India, for providing infrastructural facility.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Joshi V, Namboori K, Vaghani DD. Chemical components of some Indian medicinal plants. *Indian Journal of Chemistry*. 1974;12:226.

2. Rao UK, Rao EV, Rao DV. Phenolic constituents of the bark of *Vitex negundo*. *Indian Journal of Pharmacy*. 1977; 39(2):41.
3. Suganya T, Okonogi, Chowwanapoonpohn S. Study on antioxidant activity of certain plants in Thailand: Mechanism of antioxidant action of guava leaf extract. *Food Chem*. 2007; 103:381–388.
4. Evans WC. *Trease and Evans pharmacognosy*. 14th Edn. Harcourt Brace and company. Asia Pvt. Ltd. 1997; Singapore.
5. Lim YY, Lim TT, Tee JJ. Antioxidant properties of several properties of several tropical fruits: A comparative study. *Food Chem*. 2017;103:1003-1008.
6. Chang C, Yang M, Wen HJ. Estimation of total flavonoid content in propolis by two complementary colorimetric method. *J Food Drug Anal*. 2002;10:178-182.
7. Adams RP. Identification of essential oil compounds by gas chromatography and mass spectrometry; 4th Edn. Allured Publishing Corporation, Coral Stream, IL; 2009.
8. Ganie SA, Haq E, Masood A, Zargar MA. Antioxidant activities of methanolic rhizome extract of *Podophyllum hexandrum* against CCl₄ induced kidney and lung injury in rats. *J Pharmacol Toxicol*. 2010;5:334–342.
9. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *J Agr Food Chem*. 1992;40:945-948.
10. Rajeshwar Y, Senthilkumar GP, Malay AG, Mazumder UK. Studies on in vitro antioxidant activities of methanol extract of *Mucuna pruriens* (Fabaceae) seeds. *Int J Green Pharm*. 2005;13:131-138.
11. Gao MS, Gonzalez SML, Rivero PMD, Pereira CI, Pintado ME, Malcata FX. Infusions of Portuguese medicinal plants: Dependence of final antioxidant capacity and phenol content on extraction features. *J Sci Food Agric*. 2007;87:2638-2647.
12. Bolivara P, Paredesa CC, Hernándezb LR, Juárezc ZN, E. Sánchez-Arreolab, Y. Av-Gaya and Bacha H. Antimicrobial, anti-inflammatory, antiparasitic and cytotoxic activities of *Galium mexicanum*. *J Ethnopharmacol*. 2011;137:141–147.
13. Bravo L. Polyphenols chemistry, dietary sources, metabolism and nutritional significance. *Nutr Rev*. 1998;56:317–333.

14. Sangwan P, Kumar H, Rani R. Wet chemical synthesis, characterization and antibacterial activity of molybdenum oxide nanoparticles against *Staphylococcus epidermidis* and *Enterobacter aerogenes*. Asian J Pharm Clin Res. 2019;12:59-63.
15. Deepak S, Pawar A, Shinde P. Study of antioxidant and antimicrobial activities of *Andrographis paniculata*. Asian Journal of Plant Science and Research. 2014;4(2): 31-41
16. Singh P, Shrivastava RC, Saxena M, Sharma MS, Karchuli J. Tripathi, Phytochemical screening and evaluation of antioxidant activity of *Parkinsonia aculeate* L. (Family-Leguminosae) leaves extract. International Journal of Pharm Tech Research. 2011;3(4):1952–1957.
17. Oyaizu M. Studies on product of browning reaction prepared from glucose amine. J Nutr. 1986;44:307–315.
18. Vinoth B, Manivasagaperumal R. Antimicrobial activity of different extracts of *Azima tetraantha* root. Int J Pharma and Bio Sci. 2015;6(2):613-620.
19. Dhawan D, Gupta J. Research article comparison of different solvents for phytochemical extraction potential from datura metel plant leaves. Int J Biol Chem. 2017; 11(1):17-22.

© 2021 Umamaheswari et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
<http://www.sdiarticle4.com/review-history/67365>