



Original Article

Phytochemical Analysis, GCMS Identification, and Estimation of Antioxidant Activity of Iraqi *Vitex negundo* L.

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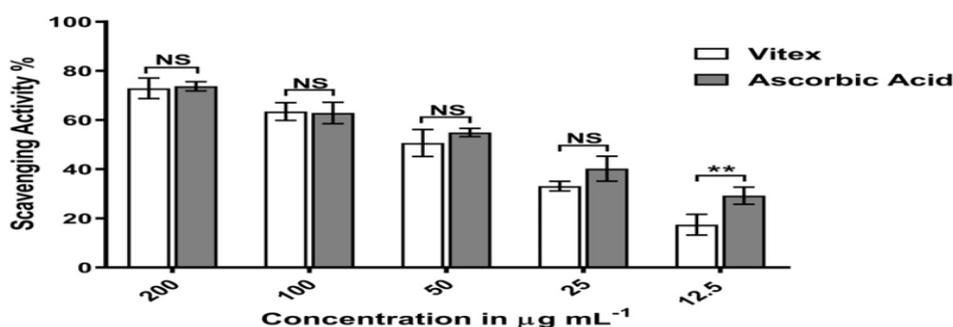
Pinene

Sabinene

ABSTRACT

Vitex negundo Linn, known as chaste tree, belongs to Verbenaceae family. It is a woody medicinal tree; an aromatic and flowering-ornamental plant has different ethnobotanical and pharmacological uses. The aims of this study included phytochemical analysis, detection of two flavonoids by TLC, GC/MS analysis of hexane extract, and the antioxidant estimation in leaves of Iraqi *Vitex negundo* activity. The results showed the important phytochemicals in hydroalcoholic and hexane extracts. In addition, the main constituents obtained by GC MS were Sabinene 3.23% and Pinene 0.87% as monoterpenes, alpha-Farnesene 0.5%, Pregnan-3,11-diol-20-one 1.55%, and alpha-Tocopherol 1.55%. Those Iraqi *Vitex negundo* L. were considered as an important source for chemical constituents with a significant antioxidant activity at low concentration with IC₅₀ 26.7, as compared with ascorbic acid.

GRAPHICAL ABSTRACT



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Introduction

Vitex negundo Linn, known as chaste tree, belongs to Verbenaceae family. It is a woody medicinal tree, native to the Mediterranean regions, while now it is cultivated all over the world as an aromatic and flowering-ornamental plant. *Vitex negundo* is considered as one of the common species among the genus (vitex), which consists 250 species such as *Vitex negundo*, *Vitex agnus-castu*, *Vitex trifolia*, *Vitex polygama*, *Vitex leucoxydon* Linn, *Vitex mollis*, and *Vitex altissima* Linn [1]. *Vitex* tree is characterized as the beautiful little deciduous a medium sized about 3-4 m in high with leaves have approximately 5 leaflets in a hand shape (palmate) arrangement and rounded fruit, black when ripe, with 3-4 mm, while the flower characterized with the aromatic brilliant color varies from violet-deep purple [2]. Furthermore, an ethnobotanical uses of vitex was mainly to reduce breast tenderness, menstrual cycle pain, and amenorrhea symptoms. Likewise, it is regulating the menstrual cycle, regulate hormones related to fertility, and diuretic effects [3]. Most pharmacological effects of this species were as anti-inflammatory, reduce the level of serum prolactin hyperprolactinemia and mastodynii, anti-oxidant, GIT disturbance, analgesic effects, antimicrobial, and anti-tumor activity [4]. *Vitex negundo* contains various potentially bioactive phytochemicals such as flavonoids, flavonoids glycosides, phenolic compounds, terpenes, and phyto steroids. Terpenoids are considered as one of the most abundant class of the secondary metabolites in vitex plants. Chemically, they consist of five - carbon isoprene units to form the building blocks of hemiterpenes (C₅), monoterpenes (ten carbon atoms), sesquiterpenes (fifteen carbon atoms), diterpenes (twenty carbon atoms), and triterpenes (thirty carbon atoms) [5]. Moreover, previous literatures revealed that *V. negundo* contains a large number of terpenes in leaves oil such as caryophyllene epoxide, δ -guaiene, and ethyl-hexadecenoate, while in flowers oil contain α -selinene, (*E*)-nerolidol, caryophyllene epoxide, and germacren-4-ol [6, 7].

Materials and Methods

Plant's collection

Vitex negundo plant was collected in February 2021 from AL-Zawraa Gardens in Iraq. The authentication of plant was done by Prof. Dr. Ibrahim Salih Abass, in Pharmacognocny Department, College of Pharmacy, Mustansiriyah University, Baghdad, Iraq. The leaves's part was dried in shade and ground to a fine powder by mechanical grinder.

Chemicals

Ethyl acetate, n-hexane, acetic acid, and chloroform (95-97%) were purchased from Merck (Darmstadt, Germany), while the standard kampferol ($\geq 98\%$), and apigenin ($\geq 97\%$) were purchased from Hyperchem Company (China) and DPPH (China).

Extracts preparation

50 g of *V. negundo* leaves was defatted with hexane by Soxhlet for 12 hours, and then the residue was dried and extracted with 90% methanol. Both extracts were concentrated under the reduced pressure and stored in glass containers for further analysis.

Preliminary phytochemical screening

The hydroalcoholic leaves extract was screened by different chemical tests to investigate the major secondary metabolites in this plant [8-10].

Screening of Flavonoids

Alkali test depends on using ethanolic KOH (2 mL) mixed with 3 ml of alcoholic extract. The yellow color is indicated the flavonoids presence.

Screening of tannins

Few drops of crude extract were mixed with 1% aqueous ferric chloride change of dark green to the blue color can predict the tannin presence.

Screening of saponin

Froth assay was used to screen saponin by shaking distilled water vigorously with 5 mL of crude extract for approximately fifteen minutes.

A persistent froth (1 cm) can be indicator for the saponins presence.

Screening of terpenoids

Salkowski test were used as screening of terpenoids confirmed by using 4 ml of the hexane and alcoholic extracts were treated with 4 mL extract was added to 2 mL of chloroform and 2 mL of concentrated sulphuric acid, the positive result gives a reddish- brown color.

Screening of sterols and steroids

Dried alcoholic and hexane extract were treated with 1.5 mL of the equal quantity of acetic anhydride and chloroform according to Liebermann reaction, and then the drops of concentrated H₂SO₄ was added gradually. The appearance of the green color indicates the sterol presence.

Screening of reducing sugar

Fehling test (solution I and II) was done by the addition of 20 mL of diluted sulphuric acid (H₂SO₄) to 4 mL of the dried extract in a test tube and boil for 10 min, and then it was cooled and 4 mL of Fehling solution and 10% potassium hydroxide were added, the red to blue precipitate was considered as the positive results.

Screening of alkaloids

Approximately few drops of the crude extract were dissolved individually in few volumes of 1% hydrochloric acid, and then it was filtered. After that, the filtrate was reacted with Mayer's reagent; a positive result for alkaloids was indicated by the presence of white precipitate.

Analytical thin layer chromatography (TLC)

Approximately 2 mg of hydroalcoholic extract were suspended in about 1 mL of absolute methanol, applied on plate of analytical TLC was precoated with a silica gel GF254, and then developed with chloroform-ethyl acetate-acetic acid (5:5:0.1) for detecting the constituents, as compared with the standard.

GC-MS analysis condition

The GC-MS identification was carried out to the hexane extract. Chromatograph analysis was done on Agelint (7820A) USA Gas Chromatography/ Mass Spectrometer (GC-MS) at the Ministry of Industry and Minerals- Ibn Al-Baitar research center-Baghdad. The conditions were as follow: carrier gas was helium column, the split ratio was 2.0, the column was used Agelint HP-5ms Ultra Inleit (30 m × 250 μm × 0.25 μm), 250-300 °C, column temperature, rate 10 °C/min, and the injection volume 1 μl.

Anti-oxidant analysis

In this work, we used 1,1-Diphenyl-2 picryl-hydrazyl; (DPPH assay), using a colorimetric technique defined by Anna Floe gel and coworkers by using a methanolic dilution of DPPH. About 2.95 mL of DPPH solution (approximately 1 Mm DPPH in eighty percent of methanol) was assorted with 0.05 mL of sample or standard (different concentrations ranging from 12.5 to 200 mcg per 1 mL methanol). The assay was completed in triplicate, and the preparing mixture was incubated in the dark condition for about half an hour with aluminum foil paper over it. The absorbance decrease was observed at 517 nm [11, 12].

Result and Discussion

The phytochemical analysis revealed a positive result for the presence flavonoids, tannins, reducing sugar, terpenoids, steroids, and saponin, while negative result were found for alkaloids.

TLC

TLC was applied to detect apigenin and Kaempferol in hydroalcoholic extract, as compared with the standard at 0.5 and 0.7, respectively (Figure 1).

Hexane extract identification by GC/MS

Analysis and determination of bioactive chemical components identified in leaves of Iraqi *Vitex negundo* hexane extract shows the different components and groups of terpenes and steroids, as displayed in Figure 2. Table 1 presents the major components identified in hexane extract of *Vitex negundo* leaves. The results reveal the

percentage of the main constituents were Sabinene 3.23% and Pinene 0.87% as monoterpenes, alpha-Farnesene 0.5% as a sesquiterpene, in addition to the other important

terpenes Pregnan-3,11-diol-20-one 1.55% and alpha-Tocopherol 1.55%.

The GC-Mass spectra of the main known chemical constituents are shown in Figure 3.

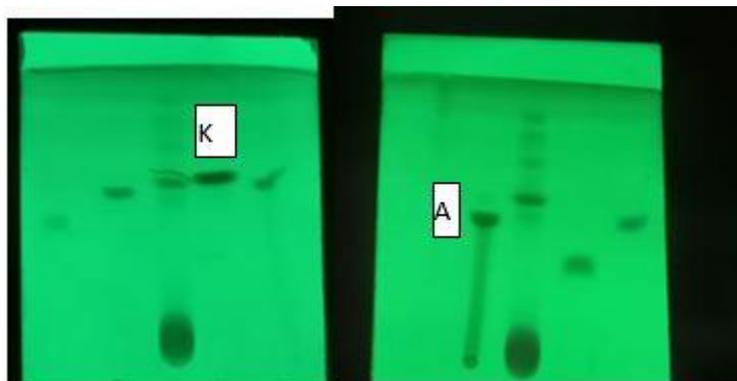


Figure 1: TLC analysis for crude extract compared with apigenin (A) and kaempferol (K) reference standard

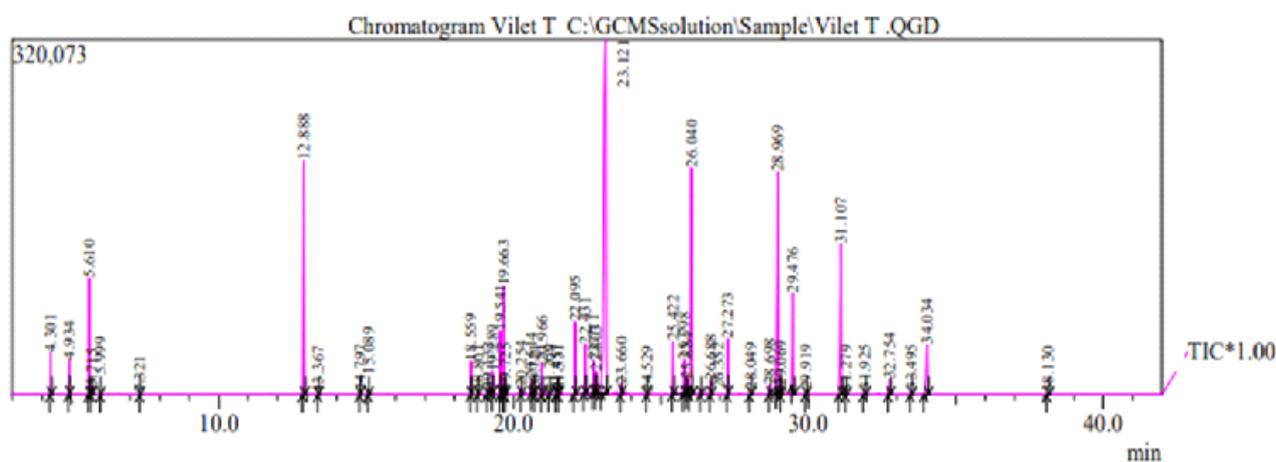


Figure 2: GC/MS chromatogram of *Vitex negundo* leaves

Table 1: The main chemical constituents of *Vitex negundo* leaves

Peak No.	Name	Retention time	Area (%)
2	Pinene	4.934	0.87
3	Thujene, Sabinen	5.608	3.23
11	P-Mentha-[1(7),8]-diene 2-hydroperoxide	18.558	0.07
14	(Z,E)-alpha-Farnesene	19.292	0.50
19	Coprostan-16 beta.-ol	20.617	0.41
25	Alpha-Cholestan-16-ol	22.092	0.09
32	Pregnan-3,11-diol-20-one	25.425	1.55
33	2-Bromocholestanone	25.800	1.12
43	Alpha-Tocopherol	29.475	3.89
48	Beta-Amyrene	32.750	0.77

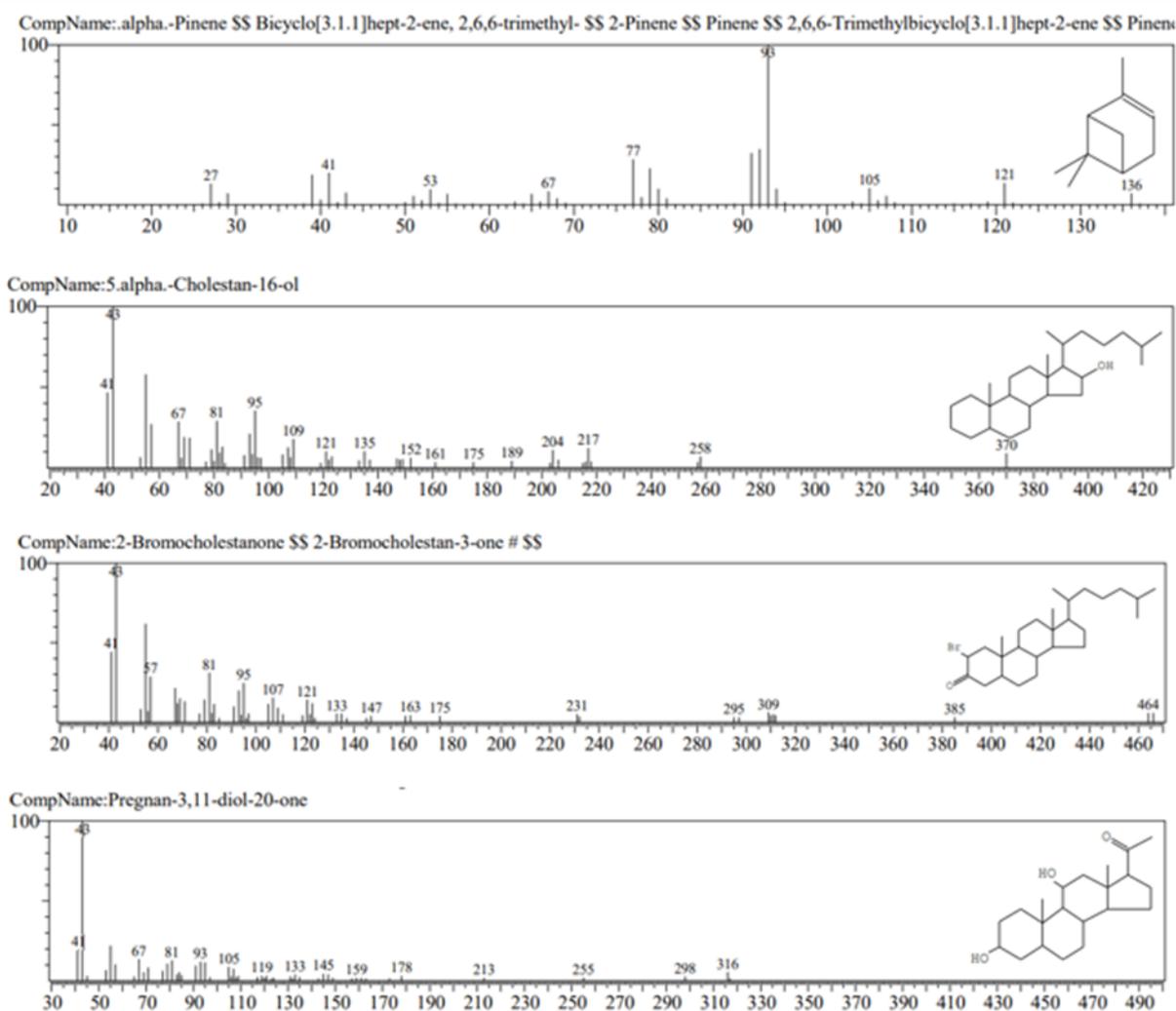


Figure 3: The GC-Mass spectra of the main known chemical constituents in hexane extract

Previous studies reported that major components of essential oils in different vitex species consist of sesquiterpene hydrocarbons mainly was caryophyllene which identified in four vitex species which are: *V. limonifolia* in Thailand [13], *V. polygama*, *V. rufescens*, and *V. megapotamica* in Brazil [14]. Besides caryophyllene, another presented sesquiterpene were δ -cadinene, cis-calamenene, and 6,9-guaiadiene. In addition, the monoterpene hydrocarbons and oxygenated monoterpenes were identified in different regions. The main monoterpene was reported is β -pinene, α -pinene [15], and 1,8-cineole. Moreover, the other terpenoids reported in vitex genus such as abietane-type diterpene, labdane-type diterpenoids, and nor labdane-type

diterpenoids [16], triterpenoids oleanane-type, and Oleanolic acid [17].

Antioxidant

Vitex negundo leaves extract indicate a good antioxidant activity when compared with ascorbic acid as shown in Figure 4 and Table 2, 3 and 4. This activity may belong to the presence of a good concentration of various terpenoids or terpenes in Iraqi plant of vitex leaves. The reported previous studies revealed the antioxidant activities of the phytochemical components [18-20].

The results show a significant antioxidant activity at a low concentration with IC₅₀ 26.7 as compared with ascorbic acid with IC₅₀=34.74.

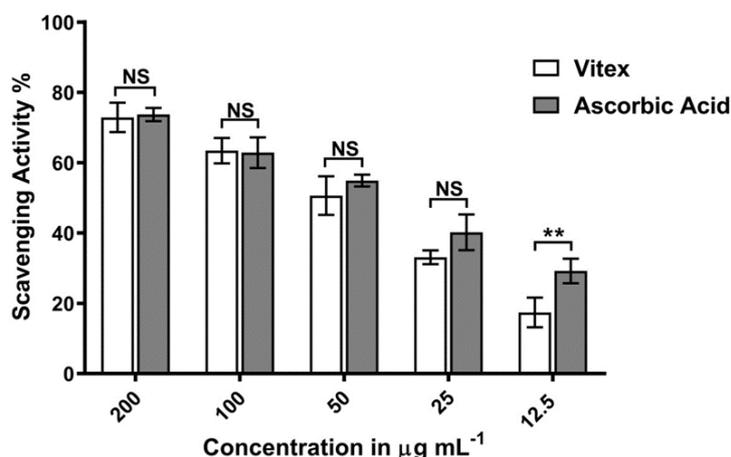


Figure 4: The scavenging activity of *Vitex negundo* leaves extract and ascorbic acid

Table 2: Antioxidant assay of ascorbic acid as a control

	200	100	50	25	12.5
Number of values	3	3	3	3	3
Mean	73.69	62.85	54.90	40.20	29.21
Std. Deviation	1.882	4.357	1.675	5.091	3.506
Std. Error of Mean	1.086	2.515	0.9670	2.939	2.024

Table 3: The scavenging activity of *Vitex negundo* leave extract. Bauhinia leaves extract IC₅₀= 26.70

	200	100	50	25	12.5
Number of values	3	3	3	3	3
Mean	72.88	63.43	50.62	33.10	17.40
Std. Deviation	4.175	3.614	5.480	1.978	4.207
Std. Error of Mean	2.410	2.086	3.164	1.142	2.429

Table 4: The relation of antioxidant activity results of *Vitex negundo* leave extract compared with ascorbic acid

Sidak's multiple comparisons test	Significant	Summary	Adjusted P Value
200	No	ns	0.9997
100	No	ns	>0.9999
50	No	ns	0.6395
25	No	ns	0.1582
12.5	Yes	**	0.0058

Conclusion

Those Iraqi *Vitex negundo* L. were considered as an important source for chemical constituents with a significant antioxidant activity at low concentration with IC₅₀ 26.7, as compared with ascorbic acid.

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Authors' contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

Conflict of Interest

The author declared that they have no conflict of interest.

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