Mohankumar et al /J. Pharm. Sci. & Res. Vol. 9(10), 2017, 1677-1681

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Essential Oil Composition of *Vitex negundo*, Acetylcholine Esterase Inhibition Activity and Molecular Docking Studies against Bacterial Proteins

Mohankumar, S. Raju, J. Dharani and S. Ravi*
Department of Chemistry, Karpagam University,
Karpagam Academy of Higher Education, Coimbatore- 641021,Tamilnadu, India.

Abstract

The composition of the essential oil from *Vitex negundo* was determined by GC-MS analysis. It showed the presence of six compounds out of which caryophyllene (21.58 %) and epiglobulol (47.13 %) are the major compounds. The molecular docking studies was carried out for the essential oil constituents against the bacterial proteins 1UAG, 2X5O, 3UDI and 3TYE. Caryophyllene and Epiglobulol have showed very good docking scores thereby supporting the antibacterial potential of this oil. The Acetylcholine esterase activity of the essential oil from *Vitex negundo* showed an IC_{50} value of 13µg/ml. This indicates that the essential oil from *Vitex negundo* exhibits a strong AChE inhibition activity.

Key words *Vitex negundo*; Lamiaceae; Acetylcholine esterase inhibition; docking.

INTRODUCTION

A man cannot die of disease in an area where *Vitex negundo*, *Adhatoda vasica* and *Acorus calamus* are found. The plant holds great promise as a commonly available medicinal plant and it is indeed no surprise that the plant is referred to in the Indian traditional circles as 'sarvaroganivarini' – the remedy for all diseases. *Vitex negundo* belongs to the family Lamiaceae, usually grows from three to nine feet tall, but under cultivation can develop to 20 feet tall. This species is globally distributed in Indo-Malaysia, cultivated in America, Europe, Asia and West Indies. It is often found growing next to streams and it loves water [2]. The decoction of leaves is used for treatment of inflammation, eye-disease, toothache, leucoderma, enlargement of the spleen, ulcers, cancers, catarrhal fever, rheumatoid arthritis, gonorrhea, sinuses, scrofulous sores, bronchitis and as tonics. As vermifuge, lactagogue, antibacterial, antipyretic, antihistaminic, analgesic, insecticidal, ovicidal, growth inhibition and morphogenetic agents. antigenotoxic, antihistamine, CNS depressant activity and anti-fertility effects were reported from the leaves of *Vitex negundo* Linn [3]. *Vitex negundo Linn*. has shown promise as an effective bio-control agent. The extracts of *Vitex negundo* possess inhibitory, deterrent or lethal activity on biological agents that cause disease and damage to other organisms.

MATERIAL AND METHODS

Plant material

The aerial part from the plant *Vitex negundo* was collected from the Coimbatore district during the month of December 2016 and authenticated by Dr. G.V.S. Murthy, Botanical Survey of India, Southern Regional Centre, Coimbatore.

Extraction of essential oil

Fresh leaves of *Vitex negundo* was cut down into small parts (500 g) and exposed to hydro distillation for 3 hours using a Clevenger type apparatus. The obtained essential oil being collected was dried over anhydrous sodium sulphate to absorb the small amount of water present along the essential oil (0.8gm). The essential oil was then stored at 4ºC until use.

Acetylcholine esterase inhibition activity

Acetylthiocholine iodide (ATCI), 5, 5‟-thiobis-2-nitrobenzoic acid (DTNB), Acetylcholine esterase enzyme was purchased from sigma Aldrich. Acetylcholine esterase activity was carried out for the essential oil obtained from the leaves of *Vitex negundo*. Spectrophotometric assay was used to determine the inhibitory potential of the compounds against acetylcholine esterase enzyme isolated from red blood cells. Acetyl thiocholine iodide was used as a substrate. 2.81ml of phosphate buffer of pH 8 was taken in each test tube. The test sample solutions of different concentrations of 2µg, 4µg, 6µg, 8µg, 10µg were added and 30µl of enzyme were added. The mixture was allowed standing for 10min. The coloring reagent DTNB (dithiobisnitro benzoic acid) was added which produces the yellow anion of 5-thio-2-nitro benzoic acid and then substrate 30µl followed by incubation for 20 min. The absorbance was measured at 412nm. The percentage inhibition in enzyme activity can be calculated as follows:
% inhibition = Absorbance (control) – Absorbance (test) / Absorbance (control) × 100

Molecular docking

For the AutoDock docking calculation, default parameters were used and 10 docked conformations were generated for each compound. The energy calculations were done using genetic algorithms. The outputs were exported to Chimera 1.10 and Discovery studio 4.5 for visual inspection of the binding modes and interactions of the compounds with amino acid residues in the active site. A computational ligand-target docking approach was used to analyze structural complexes of the proteins 1UAG, 2X5O and 3UDI and 3TYE (target) with the compounds present in the essential oil (ligand) in order to understand the structural basis of this protein target specificity. Finally, docking was carried out by PyRx, AutoDock Vina option based on scoring functions.

RESULT AND DISCUSSION

Pharmaceutical properties of aromatic plants are in the part attributed to essential oils that are known as flavoring additives to cosmetics, disinfection agents and medicinal means for a long time. Ingredients of the essential oil from *Vitex negundo* are determined by collecting the plant from the same locality.

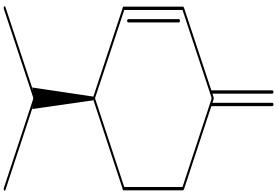
Essential oil was obtained by hydro distillation method in a Clevenger type apparatus. The oil was light yellow in colour (yield: 0.16%). The chemical composition of the obtained oils was determined by GC/MS. The identified compounds were presented in the table. It showed the presence of six compounds out of which two of them caryophyllene and epiglobulol are major compounds. Earlier globulol was identified from the essential oil obtained from Dehra Dun region in India. This is the first time that epiglobulol has been reported from *Vitex negundo.*

Molecular docking studies of the essential oil constituents from *Vitex negundo*

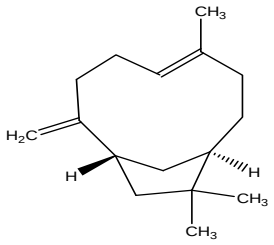
The results of the docking studies using the essential oil constituents as ligands and the bacterial proteins 1UAG, 2X5O, 3UDI and 3TYE were shown in the table. The binding scores, and the amino acids involved in the Conventional H-bond, Alkyl and pi-alkyl interactions and other forms of interactions were presented. Among the six compounds identified from the essential oil of *Vitex negundo* and which were used for docking studies, Caryophyllene and Epiglobulol have showed very good docking scores.

Table 1. The table indicates the GC MS results of essential oil composition of *vitex negundo* leaves.

Compound Name	Molecular Formula	Molecular weight	Value (iu)	Percentage
Beta-Phellandrene	$C_{10}H_{16}$	136	964	11.82%
Caryophyllene	$C_{15}H_{24}$	204	1494	21.58%
4-Terpineol	$C_{10}H_{18}O$	154	1137	5.05%
β-(Z)-farnesene	$C_{15}H_{24}$	204	1440	2.94%
Epiglobulol	$C_{15}H_{26}O$	222	1530	47.13%
5-(1-Isopropenyl-4,5-dimethylbicyclo[4.3.0]nonan-5-yl)-3-methyl-2-pentenol acetate	$C_{22}H_{36}O_2$	332	2265	11.48%

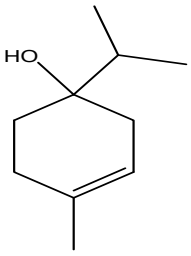


β-phellandrene

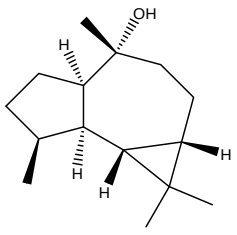


caryophyllene

3-cyclohexane-1-ol,4-ethyl-1-(1-methylethyl)-

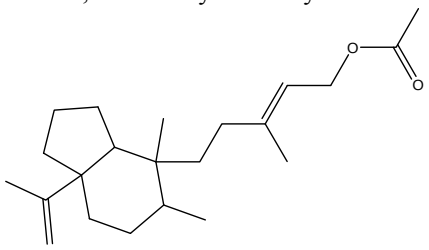


1,6,10-dodecatriene,



Epiglobulol

7,11-dimethyl-3-methylene



5-(1-isopropenyl-4,5- dimethylbicyclo[4.3.0]nonan-5-yl)-3-methyl-2-pentenol acetate

Figure 1. Structures of the compounds identified from the essential oil of *Vitex negundo.*

Caryophyllene showed docking scores of -6.3, -6.3, -6.5 and -6.8 K cal/mol with 1UAG, 2X5O, 3UDI and 3TYE proteins respectively. It showed no H-bonding and get involved only alkyl and pi-alkyl interactions with many amino acids. Similarly, epiglobulol showed docking scores of -6.5, -6.2, -6.2 and -6.3 K cal/mol with 1UAG, 2X5O, 3UDI and 3TYE proteins respectively. It showed no H-bonding except with 3UDI and get involved only alkyl and pi-alkyl interactions with many amino acids. Based on the above arguments these three compounds may be responsible for the antibacterial activity of the essential oil from *Vitex negundo*.

Table 2. Molecular docking of the constituents identified from the essential oil of *vitex negundo*

LIGANDS	DOCKING DETAILS	1UAG	2X5O	3UDI	3TYE
5-(1-Isopropenyl-4,5-dimethylbicyclo[4.3.0]nonan-5-yl)-3-methyl-2-pentenol acetate	Binding score(K cal/mol)	-6.8	-6.0	-6.3	-6.7
	Conventional H-bond	ASN:211	-	ASN:402	ASN:196, SER:221, PHE:222
	Alkyl and pi-alkyl	ALA:328	PHE:422, ALA:417, HIS:183, LEU:416	ALA:34	LYS:220, ARG:68, 219 PRO:69, VAL:231 HIS:256
	Others	-	-	-	-
β-(Z)-farnesene	Binding score	-5.1	-3.2	-5.5	-5.6
	Conventional H-bond	-	-	-	ALA:190
	Alkyl and pi-alkyl	VAL:335, LEU:330,339	TYR:3, AGR:27	ARG:481, ILE:645, VAL:649	PHE:71
	Others	LEU:333 (Unfavorable acceptor-acceptor)	-	ARG:482 (C-H Bond) TYR:485 (Pi-Sigma)	PHE:71 (Pi-Sigma)
β-Phellandrene	Binding score	-5.1	-4.9	-6.1	-5.4
	Conventional H-bond	-	-	-	-
	Alkyl and pi-alkyl	VAL:335, LEU:330,333,339	LEU:333, HIS:267, PHE:303	ARG:481,482, TYR:485, VAL:649, ILE:645	PHE:71, TRP:123, PHE:189
	Others	-	-	-	-
Caryophyllene	Binding score	-6.3	-6.3	-6.5	-6.8
	Conventional H-bond	-	-	-	-
	Alkyl and pi-alkyl	VAL:232, ALA:328, HIS:267, PHE:230	ALA:414 LEU:416 PHE:422	ILE:558, 555, PHE:257,554 LEU:253, HIS:256	VAL:226, LEU:197,244 ALA:240, ILE:223, PHE:222, MET:200
	Others	-	-	TYR:567 (Pi-Sigma)	-
4-Terpineol	Binding score	-4.8	-4.1	-4.6	-5.8
	Conventional H-bond	-	-	-	-
	Alkyl and pi-alkyl	VAL:364, LEU:339, TYR:358	ALA:26, MET:145	ALA:261, TYR:266	PHE:71, PRO:69, TYR:130, TRP:123
	Others	-	-	TYR:567 (Pi-Sigma)	-
Epiglobulol	Binding score	-6.5	-6.2	-6.3	-6.3
	Conventional H-bond	-	-	GLN:285	-
	Alkyl and pi-alkyl	TYR:358, LEU:330,333,339, VAL:335	ALA:414, LEU:416, PHE:422	TYR:418,244, PRO:243	LYS:2,274 ILE:272
	Others	-	-	-	-

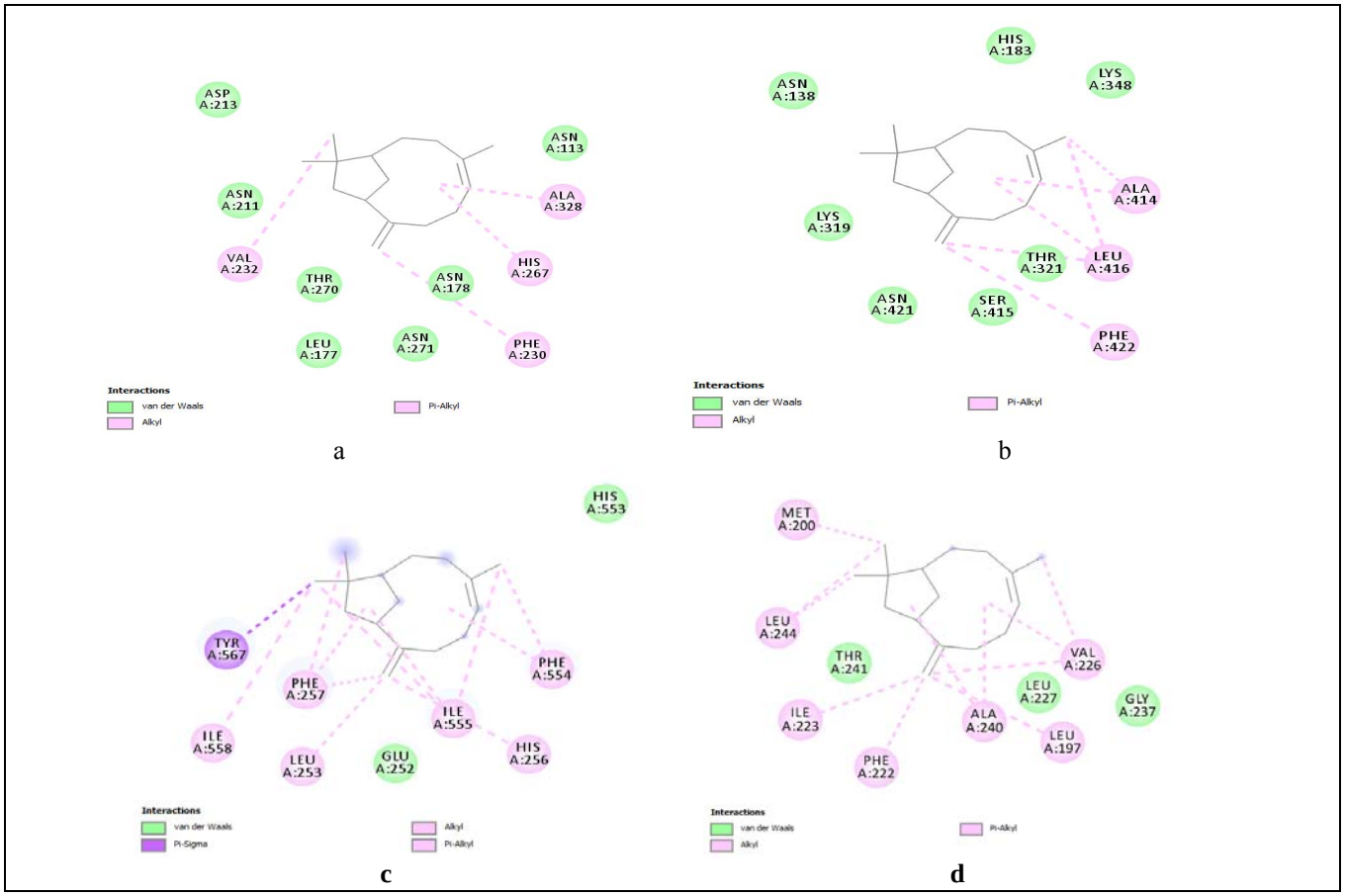


Figure 2. Molecular docking of Caryophyllene against a. 1UAG, b.2X5O, c. 3UDI, d. 3TYE

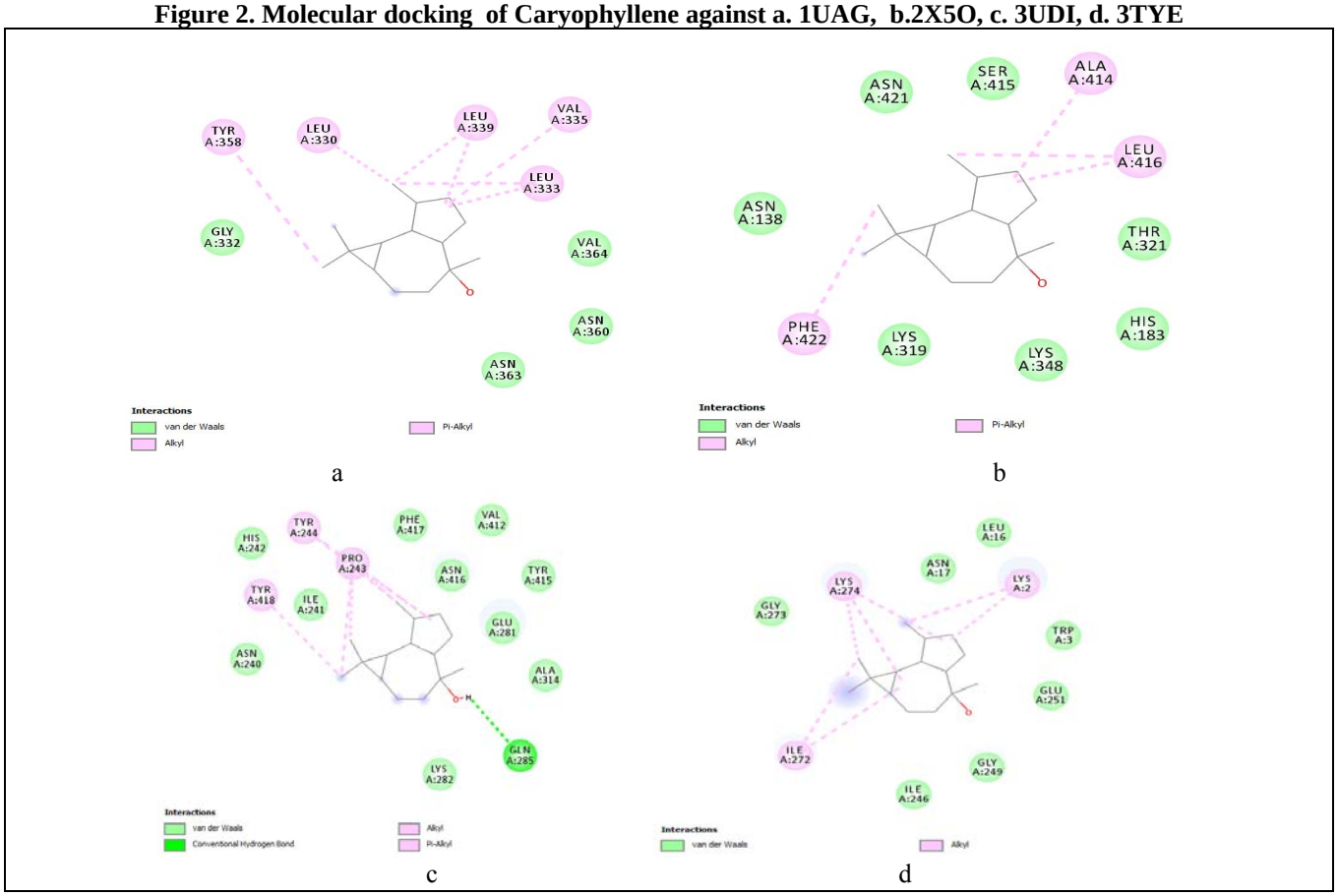


Figure 3. Molecular docking of epiglobulol against a. 1UAG, b. 2X5O, c. 3UDI, d. 3TYE

Acetylcholine esterase (AChE) inhibition activity

Eventhough, *Vitex negundo* exhibited a large number of biological activities, the acetylcholine esterase (AChE) inhibition activity was not yet explored. In the present work we carried out the acetylcholine esterase (AChE) inhibition activity of the essential oil obtained from *V. negundo*. The results are shown in the figure 3 and the IC_{50} value for the acetylcholine esterase (AChE) inhibition activity is found to be 13µg/ml.

It showed a dose dependent AChE inhibition activity. At a concentration of 50 µg/ml of the essential oil it shows 89.7% activity. This indicates that the essential oil from *Vitex negundo* exhibits a strong AChE inhibition activity.

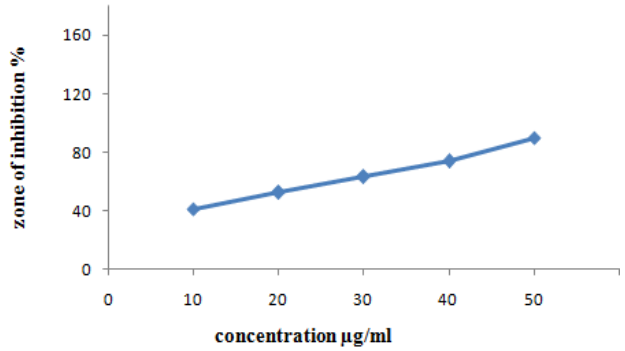


Figure 4. The acetylcholine esterase inhibition activity of the essential oil obtained from *V. negundo*.

CONCLUSION

The essential oil from *Vitex negundo* was obtained by hydro distillation method using Clevenger type apparatus and the chemical composition determined. It showed the presence of six compounds out of which two of them caryophyllene and epiglobulol are major compounds.

The molecular docking studies were carried out for the essential oil constituents against the bacterial proteins 1UAG, 2X5O, 3UDI and 3TYE. The binding scores, and the amino acids involved in the Conventional H-bond, Alkyl and pi-alkyl interactions and other forms of interactions were presented. Among the six compounds identified from the essential oil of *Vitex negundo* and which were used for docking studies Caryophyllene and Epiglobulol have showed very good docking scores.

We tested the acetylcholine esterase (AChE) inhibition activity of the essential oil obtained from *Vitex negundo*. The Acetylcholine esterase activity of the essential oil from *Vitex negundo* shows that the IC_{50} value of 13µg/ml. This indicates that the essential oil from *Vitex negundo* exhibits a strong AChE inhibition activity.

REFERENCES

1. Khare CP. Encyclopedia of India Medicinal plants. Spinger Verlange Berline Heidelberg. New York 2004, pp. 474-476.
2. Mahalakshmi R, Rajash P, Ramesh N, Balasubramanion V, Kanan VR. Hepatoprotective activity on *Vitex negundo Linn.* (verbanaceae) by using wister albino rats in ibuprofen indused model. International journal of pharmacology 2010; 1-6.
3. Gautam LN, Shrestha SL, Wagle P, Tamrakar BM. Chemical constituents from *Vitex negundo (linn.)* of nepalese origin Scientific World 2008; 6: 6.
4. Rastogi T, Bhutada V, Moon K, Aswar PB, Khadabadi SS. Comparative studies on anthelmintic activity of Moringa olifera and *Vitex*. Asian J Research Che 2009; 2: 122-127.
5. Sharma MM, Khanna P, Saini R, Batra A. Potential plant source of a promising drug for cancer chemotherapy: *Vitex negundo L*. Journal of Economic and Taxonomic Botany 2006; 30: 269-273.
6. Tandon V, Gupta RK. Histomorphological changes induced by *Vitex negundo* in albinorats. Indian Journal of Pharmacology 2004; 36: 176-177.
7. Ziegler K, Diener A, Herpin C, Richter R, Deutzmann R, Lockau W. Molecular characterization of cyanophycin synthetase, the enzyme catalyzing the biosynthesis of the cyanobacterial reserve material multi-L-arginyl-poly-L-aspartate (cyanophycin). Eur J Biochem 1998; 254(1): 154–9.
8. Perdih A, Kotnik M, Hodoscek M, Solmajer T. Targeted molecular dynamics simulation studies of binding and conformational changes in E. coli MurD Proteins 2007; 68(1): 243–54.
9. Meng EC, Shoichet BK, Kuntz ID. Automated docking with grid-based energy evaluation. Journal of Computational Chemistry 2004; 13(4): 505-524.