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Research Article

TARGETING POLYCYSTIC OVARIAN SYNDROME INFLAMMATION: DOCKING AND PHYTOCHEMICAL PROFILING OF ANTI-INFLAMMATORY COMPOUNDS IN *LEONOTIS NEPETIFOLIA*

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ABSTRACT

Objectives: *Leonotis nepetifolia* is one of the most valuable species in folkloric treatments known to have anti-inflammatory properties devoid of studies on its interventions against polycystic ovarian syndrome (PCOS) - a chronic low-grade inflammatory disorder. This research aims to identify and classify the phytochemicals present in *L. nepetifolia* using gas chromatography-mass spectrometry (GC-MS). Molecular docking studies were then carried out to analyze their potential binding affinity and interaction with key molecular targets associated with PCOS, offering valuable insights into the fields of natural product pharmacology and women's healthcare.

Methods: The complete phytochemical profiling of methanolic extract of seeds of *L. nepetifolia* with the aid of GC-MS and the subsequent *in silico* docking of the anti-inflammatory compounds in *L. nepetifolia*, against inflammatory markers responsible for PCOS has been carried out.

Results: Cis-vaccenic acid and octadecanoic acid were the compounds identified as ligands to be docked against the inflammatory proteins responsible for PCOS. CYP19A1 and AdipoR1 were identified as hit targets with the highest binding scores of −6.4 kcaL/moL and −7.6 kcaL/moL when docked against the ligands, cis-vaccenic acid and octadecanoic acid, respectively.

Conclusion: The current study has demonstrated the potential of *L. nepetifolia* for the development of reliable and effective drugs for treating PCOS. The hit target-ligand interactions can be further investigated for its bio-activities to create new medications. To the best of the authors' knowledge, this is the first-hand report on phytochemical identification and molecular docking seeking to uncover potential compounds in *L. nepetifolia* that could alleviate PCOS.

Keywords: *Leonotis nepetifolia*, Gas chromatography-mass spectrometry, Polycystic ovarian syndrome, Inflammatory markers, Docking.

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INTRODUCTION

The remarkable evidence supporting the therapeutic properties of medicinal plants combined with long-term history in traditional medicine, has led to a growing interest in harnessing natural products. *Leonotis nepetifolia*, commonly known as Lion's ear or Klip dagga, represents a perennial herbaceous member of the *Labiatae* family, which is used widely in folkloric treatments. The *Leonotis* genus has a broad distribution across the pan tropics and consists of 12 species. In the Indian region, it is characterized by the presence of a lone species, *L. nepetifolia* [1]. The plant *L. nepetifolia* holds significance in Indian traditional medicinal systems, including Siddha, Unani, and Ayurveda, owing to its well-known medicinal properties. It has demonstrated various biological activities and is believed to have several favorable physiological effects. The *Labiatae* family, to which this plant belongs, has also been utilized in primitive cancer treatments, and every constituent of this plant possesses beneficial medicinal properties [2]. Numerous studies on *L. nepetifolia* have uncovered that it contains alkaloids, terpenoids, flavonoids, and phenols, which are likely to have anti-inflammatory, antimicrobial, antioxidant, cytotoxic, and antiviral properties [3,4].

In the 21st century, the incidence of polycystic ovarian syndrome (PCOS), an epidemic endocrine disorder, has surged. It is characterized by chronic low-grade inflammation and occurs independently of obesity [5]. PCOS patients are supposed to have risk factors such as visceral obesity, cardiometabolic risk factors, impaired glucose homeostasis, and dyslipidemia associated with inflammatory conditions. PCOS pathophysiology is driven by a destructive cycle of hyperinsulinemia, hyperandrogenism impairing folliculogenesis [6]. A combination of an anti-inflammatory diet and regular physical activity has led to moderate weight loss, improved metabolic cum hormonal balance, insulin sensitivity, and enhanced menstrual regularity in women with PCOS [7].

L. nepetifolia is known to have anti-inflammatory properties, but there were no studies on its interventions against PCOS. Hence, this research aims to identify and classify the phytochemicals present in *L. nepetifolia* using gas chromatography-mass spectrometry (GC-MS), an analytical toolgenerally utilized for its effectiveness in detecting and profiling complex mixtures of plant-based compounds. It has exceptional sensitivity in detecting compounds as minimum as 1ng, including fatty acids, volatile oils, hydrocarbons, drug metabolites, etc [8].

Following the identification of these compounds, absorption, distribution, metabolism, excretion, and toxicity (ADMET) analysis was performed to screen the compounds based on their drug likeliness before conducting molecular docking to analyze their potential binding affinity and interaction with key molecular targets associated with PCOS. Molecular docking is a key *in silico* technique that stimulates the interaction between small molecules and protein targets, providing valuable insights into their potential biological efficacy. It can provide key insights into target-ligand interactions and ligand conformations, facilitating the discovery of new drug molecules with varying affinities for targets [9,10].

Existing research provided the evidence of anti-inflammatory properties in *L. nepetifolia* extracts, while some studies underlined the impact of inflammation on PCOS patients. The integrated approach of phytochemical identification and molecular docking seeks to uncover potential lead compounds in *L. nepetifolia* that could alleviate PCOS symptoms, offering valuable insights into the fields of natural product pharmacology and women's healthcare.

METHODS

Plant authentication

Healthy seeds from flowers of *Leonotis nepetifolia* were procured from Kalvarayan hills of Kallakurichi district at Tamil Nadu, located at an altitude of 609.6–914.4 m m.s.l. The specimen as shown in (Fig. 1a) exhibited a tall, erect frame with a quadrangular, slightly pubescent stem (Fig. 1b) typical of the *Lamiaceae* family. Leaves were opposite, ovate to lanceolate, with serrated margins and long petioles, showing prominent reticulate venation as seen in (Fig. 1b and c). Distinctive spherical verticillaster inflorescences were observed at the leaf axils (Fig. 1d), each comprising bright orange, bilabiate, tubular flowers emerging from spiny, subulate bracts—key diagnostic features of the species. These morphological traits, particularly the characteristic flower whorls and stem-leaf architecture, conclusively confirm the plant's identity as *L. nepetifolia*.

Extraction from seeds

The seeds of *L. nepetifolia* were thoroughly washed multiple times, shade-dried, ground, and extracted with 250 mL of 95% of methanol solvent for 24 h. Whatmann No. 1 filter paper was utilized to filter the extract, and the resulting powder was redissolved in the same solvent used for extraction to prepare a 100 mg/mL solution. These seed extracts were subsequently utilized for GC-MS analysis.

Qualitative phytochemical analysis

The qualitative phytochemical study of methanolic extracts of seeds of *L. nepetifolia* was carried out with standard tests and techniques to ascertain the presence of various groups of phytoconstituents.

GC-MS analysis

The Shimadzu 2010 Plus model, equipped with an AOC-20i auto-sampler, was used to perform GC-MS qualitative analysis. The resulting chromatogram was analyzed using a tagged mass spectrophotometer. The GC-MS was operated using a standard column (RTX 5Ms) with a diameter of 0.32 mm, length of 30 m, and thickness of 0.50 µm. The instrument functioned in electron impact mode at 70 eV, using 99.9% helium gas as the mobile phase at a constant flow rate of 1.73 mL/min. A sample loading volume of 0.5 µL (split ratio of 10:0) was used, with an injecting temperature of 250°C and an ion source temperature of 200°C. To obtain the chromatograph, incubation of the sample was performed at an isothermal temperature of 50°C for 2 min, followed by an increase in temperature to 280°C at a rate of 8°C/min, and ending with an isothermal period of 20 min at 280°C. Spectrums were recorded using a scan period of 0.30 s, and molecule segments were recorded from 40 to 450 Da. The complete chromatograph was prepared in a total time of 40.33 min. The comparative percentage of each molecule was computed by comparing the total peak area and the average peak area. The conversion of analytical data to digital data was achieved using Turbo Mass Ver-5.2.0.

Component identification

The data obtained from the GC-MS analysis of the methanol extract were interpreted using the standard database of the National Institute of Standards and Technology. As a result, the names, molecular weights, and structures of the separated components were generated accordingly.

ADMET analysis for drug-likeliness

Lipinski's rule of five aids us in screening for materials with potential drug-likeliness. SwissADME was used to perform the ADMET analysis and to determine the compounds satisfying Lipinski's rule.

Molecular docking

The three-dimensional (3D) X-ray crystallographic structure of various proteins responsible for PCOS were retrieved from the Protein Data Bank database, and the structures of phytocompounds isolated from *L. nepetifolia* seeds that were reported to have anti-inflammatory properties were obtained from the PubChem database. ADMET analysis was performed. The aforementioned phytochemical ligands were docked against the set of proteins responsible for PCOS using PyRx-virtual screening tool. Each protein was docked with a ligand 10 times to validate the binding energies obtained. Protein-ligand interactions were analyzed based on binding free energy scores and molecular interaction profiles, and the compounds exhibiting strong binding affinities and significant molecular interactions were identified. Phytocompounds that exhibit a high binding affinity to target proteins were termed hit compounds due to their elevated affinity scores. These hit compounds and the associated proteins are considered druggable and can be further investigated in subsequent studies.

RESULTS AND DISCUSSION

Preliminary phytochemical screening using GCMS

The seed extract of *L. nepetifolia* was carried out to analyze the different phytocompounds using preliminary phytochemical screening. A total

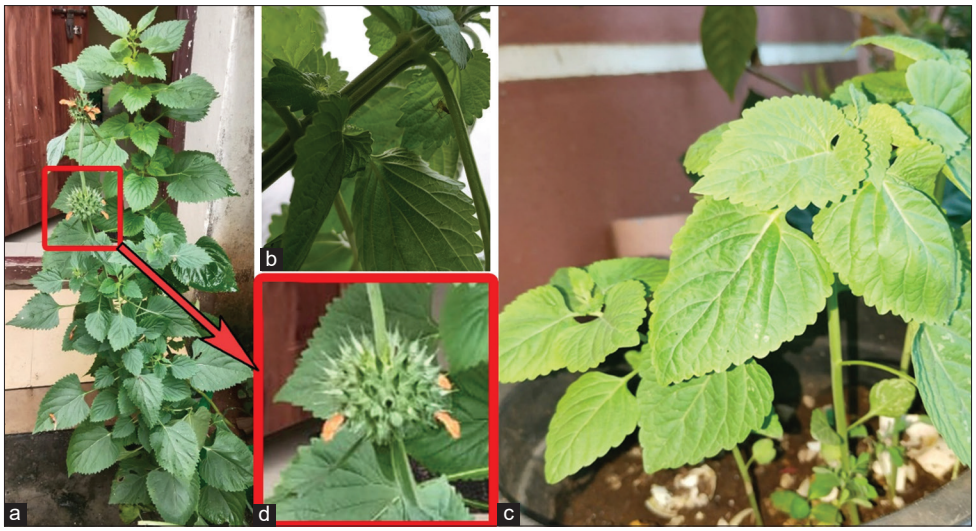


Fig. 1: Morphology of *Leonotis nepetifolia* (a) Plant (b) Stem (c) Leaves (d) Flower

of 30 phytochemicals were identified and pooled into various groups. Preliminary phytochemicals tests for all the screened compounds with their respective chemical groups are itemized in Table 1.

Phytochemical analyses of *L. nepetifolia* have shown that the plant has a diverse range of bioactive compounds across various parts. Earlier studies on its aerial parts identified tannins, terpenes, sterols and fats, flavonoids, and iridoidsas noted by Sobolewska *et al*. [11]. Additional research has found alkaloids, saponins, and steroids in methanol extracts of *L. nepetifolia* leaves [12]. Some reports have also identified specific compounds such as allenic acid, iridoids, glycosides, and terpenoids [13], underscoring the plant's richness in bio-active compounds with promising medicinal properties. Furthermore, Da Silva Almeida *et al*. reviewed the presence of flavonoids, diterpenes, fatty acids, iridoids, and coumarins, consolidating the understanding of this species' phytochemical diversity [14].

The research findings align with these prior studies, particularly in the identification of alkaloids, terpenoids, steroids, and fatty acids. However, the methanolic seed extract of *L. nepetifolia* in this study also revealed unique phytochemical groups that have not been reported in earlier research, such as carboxylic acids and esters, dicarboxylic acids and phthalates, cyclic ketones and aldehydes, triazines, spiro compounds, lactones, and organosilicon compounds. The presence of these novel phytocompounds can be attributed to environmental influences, geographic distribution, and genetic diversity among the plant populations, which collectively impact the genetic and metabolic composition of phytochemicals. These groups exhibit diverse chemical properties, suggesting their potential in various biological activities such as antioxidant, cytotoxic, anti-cancer, antimicrobial, antidiabetic, anti-inflammatory, anti-plasmodial, and anti-helminthic activities [14].

Table 1: Preliminary phytochemical analysis to detect active constituents in *Leonotis nepetifolia*

Group	Methanol extract of *Leonotis nepetifolia*
Alkaloids	+
Carboxylic acids and esters	+
Dicarboxylic acids and phthalates	+
Flavonoids	-
Cyclic ketone and aldehydes	+
Phenolics	-
Terpenoids and steroids	+
Triazines	+
Saponins	-
Resins	-
Spiro compounds	+
Fatty acid derivatives	+
Volatile oils	-
Fixed oils	-
Lactones and organosilicon compounds	+

Phytochemical analysis of seed extracts using GC-MS

The chromatographs of methanolic extracts of seeds of *L. nepetifolia* are illustrated in Fig. 2. Thirty secondary metabolites in the methanolic extract of *L. nepetifolia* seeds, along with their peak area percentage and molecular weight, are itemized in Table 2. The choice of solvent is critical for the efficient extraction of bioactive compounds, with methanol frequently favored for its ability to extract a broad spectrum of phytochemicals. Methanol extracts typically yield higher concentrations of phenolic compounds, flavonoids, and alkaloids than the other solvents [15]. Methanol's polarity facilitates the extraction of both polar and semi-polar compounds, enhancing bioactive molecule yield and enabling the identification of unique compounds that may contribute to the therapeutic properties [16,17]. This study broadens the phytochemical profile of *L. nepetifolia*, laying the foundation for future research into its bioactive properties and potential therapeutic applications.

Among the identified compounds in seeds, it was uncovered that, n-Hexadecanoic acid, cis-Vaccenic acid, and octadecanoic acid were the most prevalent components, accounting for 64.95% of the total peak area. In addition, the extract contained other compounds such as 3-piperidinemethanol, hexadecanoic acid, methyl ester, and docosanoic acid, methyl ester, in mere amounts. Although <1% in peak area, palmitic acid vinyl ester has some exclusive properties such as anti-tumor, nematicidal properties, lubricant anti-androgenic properties exerting a crucial influence on protein palmitoylation [18].

The potential biological activities of 15 compounds, represented in Table 3, were organized based on peak area percentage hierarchy. The GC-MS analysis of methanolic extracts of *L. nepetifolia* seeds has shown the therapeutically important compounds in the group of alkaloids, antioxidants, fatty acids, terpenes, and so on. The most prominent compound identified in seed extracts is n-Hexadecanoic acid, which demonstrated antioxidant, antibacterial, and anti-tumor properties [19-21] as well as a wide spectrum of other phytochemical activities, including anti-tumor, and immunostimulant properties [20,22]. This is followed by cis-Vaccenic acid, which has been reported to display antibacterial activity [23,24] Anti-inflammatory and antioxidant properties [25]. Another prevalent compound is

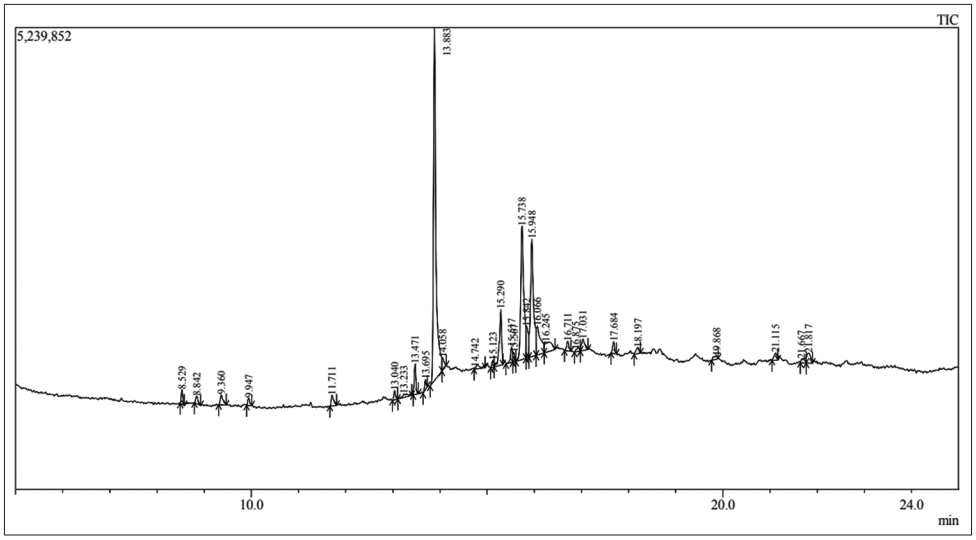


Fig. 2: Gas chromatography-mass spectrometry chromatogram of methanol extract of the seeds of *Leonotis nepetifolia*

Table 2: Phytochemicals identified in the methanolic extract from the seeds of *Leonotis nepetifolia*

S. no.	R. time	Peak area%	Name of the compound
1	8.529	0.83	Naphthalene, 1, 2, 3, 4, 4a, 5, 6, 8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1alpha.,4 alpha., 2 8.842 271741 0.64 87835 0.82 3.09 Undecanoic acid, 10-methyl-, methyl ester
2	8.842	0.64	Undecanoic acid, 10-methyl-, methyl ester
3	9.36	1.06	Dodecanoic acid
4	9.947	0.61	1,2-Benzenedicarboxylic acid, diethyl ester (CAS) Ethyl phthalate
5	11.711	1.03	Tetradecanoic acid
6	13.04	0.62	Phthalic acid, butyl undecyl ester
7	13.233	0.77	Cyclododecanone (CAS) CYCLODODECANON
8	13.471	1.98	Hexadecanoic acid, methyl ester
9	13.695	0.67	7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione
10	13.883	34.16	n-Hexadecanoic acid
11	14.058	0.93	1,3,5-Triazine, 2,4,6-tris (cyanomethoxy)-
12	14.742	0.65	Cholestan-6-one, 3-chloro-, (3 alpha., 5 alpha.)- (CAS) 5 alpha-Cholestan-6-one, 3 alpha-chloro-
13	15.123	0.51	Cyclopentane, heneicosyl-
14	15.29	5.05	10-Octadecenoic acid, methyl ester
15	15.517	1.13	Heneicosanoic acid, methyl ester
16	15.567	0.53	6-Octadecynoic acid, methyl ester
17	15.738	16.68	cis-Vaccenic acid
18	15.842	2.59	3-Piperidinemethanol
19	15.948	14.11	Octadecanoic acid
20	16.066	4.58	Bicyclo [3.2.1] octan-3-one, 6-hydroxy-, exo-(.+-.)-
21	16.245	2.65	Cyclohexane, 1,2,4,5-tetraethyl- (CAS)
22	16.711	0.87	Palmitic acid vinyl ester
23	16.875	0.55	Propanal, 2,2-dimethyl-, oxime (CAS) Pivalaldehyde, oxime
24	17.031	1.46	1,1,1-Trifluoroheptadecen-2-one
25	17.684	0.91	Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester (CAS) Glycerol 1,3-dihexadecanoate
26	18.197	0.73	(E)-13-Docosenoic acid
27	19.868	0.49	2-Nonadecanone 2,4-D.N.P.H.
28	21.115	0.88	3-Dimethylsilyloxypentadecane
29	21.667	0.77	Dihydro-3-methylene-5-methyl-2-furanone
30	21.817	1.53	Docosanoic acid, methyl ester

octadecanoic acid which showed anti-inflammatory, antibacterial, and antioxidant properties in its extracts [26]. Acetylcholinesterase inhibitors, present in phthalic acid and butyl undecyl ester, have curative properties for the symptomatic treatment of Alzheimer's disease and other dementias, which was found to be present only in the seed extract. These two compounds were also reported in n-hexane extracts of *L. nepetifolia* [27].

Molecular docking

Target proteins associated with PCOS and selected ligands

PCOS is characterized by chronic low-grade inflammation, reflected in elevated levels of inflammatory markers such as interleukin-6 (IL-6), C-reactive protein (CRP), and tumor necrosis factor-α (TNF-α) [18,39,40]. Anti-inflammatory strategies have proven effective in managing PCOS symptom; lifestyle intervention combining an anti-inflammatory diet with exercise led to moderate weight reduction, enhanced metabolic and hormonal health profiles, and improved menstrual cyclicity in overweight and obese women with PCOS [7]. The polygenic nature of PCOS involves multiple genes and biochemical pathways that interfere with ovulation and androgen metabolism. The genes CYP17A1, CYP11A1, and CYP19A1 which influence notable pathways involved in steroidogenesis, are among the key contributors of PCOS. Hyperandrogenism, a defining characteristic of PCOS, is driven by androgen receptor (AR)-mediated mechanisms that are central to its pathogenesis [41]. Adiponectin, along with its receptors AdipoR1 and AdipoR2, contributes significantly to the pathogenesis of PCOS. Hyperandrogenism and hyperinsulinemia, prevalent in PCOS, lead to aberrant expression of AR, which may adversely affect implantation [42].

The phytocompounds with anti-inflammatory activity in *L. nepetifolia* that have the potential for being a drug have been docked against inflammatory markers along with various proteins associated with PCOS. The ADMET analysis was performed with the aid of SwissADME, identifying Cis-vaccenic acid and octadecanoic acid as potential anti-inflammatory agents in *L. nepetifolia* [43]. The Lipinski's rule of five was used to estimate the drug-likeness of the selected compounds, and the results of SwissADME analysis on Lipinsk's rule are tabulated in Table 4. A total of 12 proteins associated with PCOS were docked against the identified anti-inflammatory agents using PyRx – virtual screening tool.

Analysis of docking score

The drug development process has been profoundly influenced and enhanced by innovations in computational technology. Molecular docking is a technique used to identify potential ligands for protein structures, serving a vital role in structure-based drug design and nutraceutical research [44,45].

The best protein interactions with Cis - vaccenic acid and octadecanoic acid are tabulated in Tables 5 and 6, respectively. After analyzing the active proteins by binding free energy score and molecular interaction profile, all the inflammatory markers (IL-6, CRP, and TNF-α) have exhibited binding affinities ranging from −5.7 kcaL/moL to −6.4 kcaL/moL when docked with cis-vaccenic acid (Table 5). Of interest CYP19A1 protein, which is responsible for steroidogenesis [41] showed the best binding score of −6.4 kcaL/moL against cis-vaccenic acid (Fig. 3a). (Fig. 3a-f) illustrate the two-dimensional and three-dimensional binding interactions of various proteins with cis-vaccenic acid.

It has been observed that AdipoR1 receptor, which is said to exert the anti-inflammatory effect of adiponectin, was typically reduced in individuals with PCOS [46]. ThisAdipoR1 when docked against octadecanoic acid, showed the best binding affinity of −7.6 kcaL/moL (Fig. 4a). Enhanced adiponectin signaling reduces inflammation by inhibiting TNF-α as well as improves insulin sensitivity [47,48]. In addition, other inflammatory markers such as IL-18, IL-6, CRP, TNF-α along with AR showed reasonable binding affinities (−4.5−−5.9 kcaL/moL) while docked with octadecanoic acid (Table 6). (Fig. 4a-f) illustrate the two-dimensional and three-dimensional binding interactions of various proteins with octadecanoic acid.

Table 3: Biological activity of the most prevalent phytochemicals in the methanol extract of seeds of *Leonotis nepetifolia*

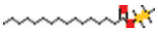
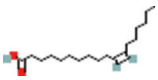
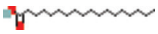
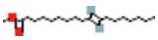
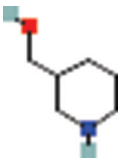
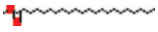
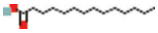
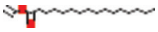
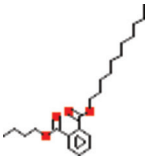
S. no.	Name of compound	Molecular formula	Molecular wt. (g/moL)	Peak area %	Molecular structure	Phytochemical activity
1	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	34.16		Antioxidant and antibacterial activities [19,20]. nematicide, hypocholesterolemic, pesticide, antitumor, immunostimulant, chemopreventive, cancer preventive, flavor, lubricant, haemolytic, 5-a reductase inhibitor, antiandrogenic, and lipoxygenase inhibitor [22,28]
2	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282.46	16.68		Antibacterial activity [23,24] anti-inflammatory and antioxidant compounds [25]
3	Octadecanoic acid	$C_{18}H_{36}O_2$	284.48	14.11		Positive impacts on thrombogenic and atherogenic risk factors among males and Anti-inflammatory [26]
4	10-octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.49	5.05		Antibacterial, anti – inflammatory antioxidant, antifungal, decrease blood cholesterol [29]
5	3-Piperidinemethanol	$C_6H_{13}NO$	115.17	2.59		Psychoactive compound [30]
6	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45	1.98		Antimicrobial effect[31] anti-inflammatory and anti-fibrotic effect[32] Anticancer[33] Antioxidant, antibacterial and antifungal [34]
7	Docosanoic acid, methyl ester	$C_{23}H_{46}O_2$	354.61	1.53		Elucidate the structure and function of p53[35] Anti-peptic ulcer agent, Antioxidants [36]
8	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	1.03		Nematicide, Cancer-Preventive [25]
9	Palmitic acid vinyl ester	$C_{18}H_{34}O_2$	282.25	0.87		Pesticide, Nematicide, Lubricant, Flavor, Antiandrogenic, Hemolytic 5-Alpha reductase inhibitor, Antitumour, Antimicrobial, Antifungal, Antibacterial [37]
10	Phthalic acid, butyl undecyl ester	$C_{23}H_{36}O_4$	376.5	0.62		Selective acetylcholinesterase and butyryl cholinesterase inhibitors [38]

Table 4: Drug-likeness of ligands with anti-inflammatory properties

Compound name	Lipinski's rule of five				
	MoL. weight (<500 Da)	LogP (<4.15)	Hydrogen acceptor (<10)	Hydrogen donor (<5)	Drug-likeness
Octadecanoic acid	284.48	4.67	2	1	Yes
Cis-vaccenic acid	282.46	4.57	2	1	Yes

Table 5: Binding affinities of various proteins responsible for PCOS when docked against cis–vaccenic acid

S. no.	Proteins	Binding affinities (kcaL/moL)**	No. of hydrogen bonds
1	CYP19A1	−6.4±0.24	5
2	Interleukin-18	−6.2±0.35	2
3	Androgen Receptor	−5.9±0.67	1
4	TNF-α*	−5.9±0.51	-
5	CRP*	−5.8±1.02	1
6	CYP17A1	−5.7±0.51	2

**Mean of 10 docking trials; *CRP: C-reactive protein, TNF-α: Tumor necrosis factor alpha, PCOS: polycystic ovarian syndrome

Table 6: Binding affinities of various proteins responsible for PCOS when docked against octadecanoic acid

S. no.	Proteins	Binding affinities (kcaL/moL)**	No. of hydrogen bonds
1	AdipoR1	−7.6±0.72	1
2	Interleukin-18	−5.9±0.67	3
3	Androgen receptor	−5.8±0.62	2
4	TNF-α*	−5.6±0.78	-
5	CRP*	−5.3±1.29	1
6	Interleukin-6	−4.5±1.12	1

**Mean of 10 docking trials; *CRP: C-reactive protein, TNF-α: Tumor necrosis factor alpha, PCOS: Polycystic ovarian syndrome

It is evident from the aforementioned results that bioactive compounds such as Cis-vaccenic acid and Octadecanoic acid from *L. nepetifolia* can efficiently bind with the inflammatory markers such as IL-18, IL-6, CRP, and TNF-α. Hence the present study offers valuable insights for further research in drug design and formulation to treat women with PCOS. To the best of the author's knowledge, this is the first report on bioactive compounds from underutilized, ethnobotanically important *L. nepetifolia* that are subjected to molecular docking for screening pharmacological potential against PCOS.

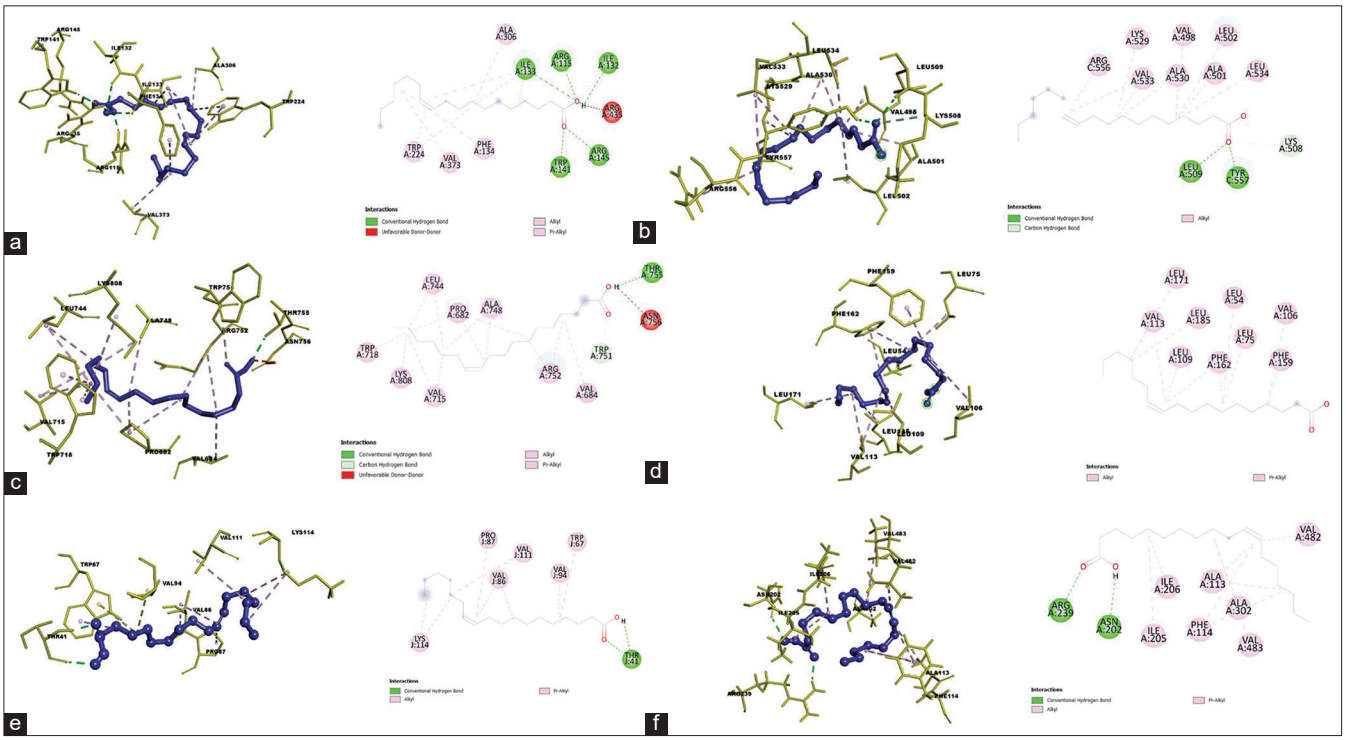


Fig. 3: 3-dimensional and 2-dimensional binding mode of cis-vaccenic acid against (a) CYP19A1 (b) Interleukin -18 (c) Androgen receptor (d) tumor necrosis factor-α (e) C- reactive protein (f) CYP17A1

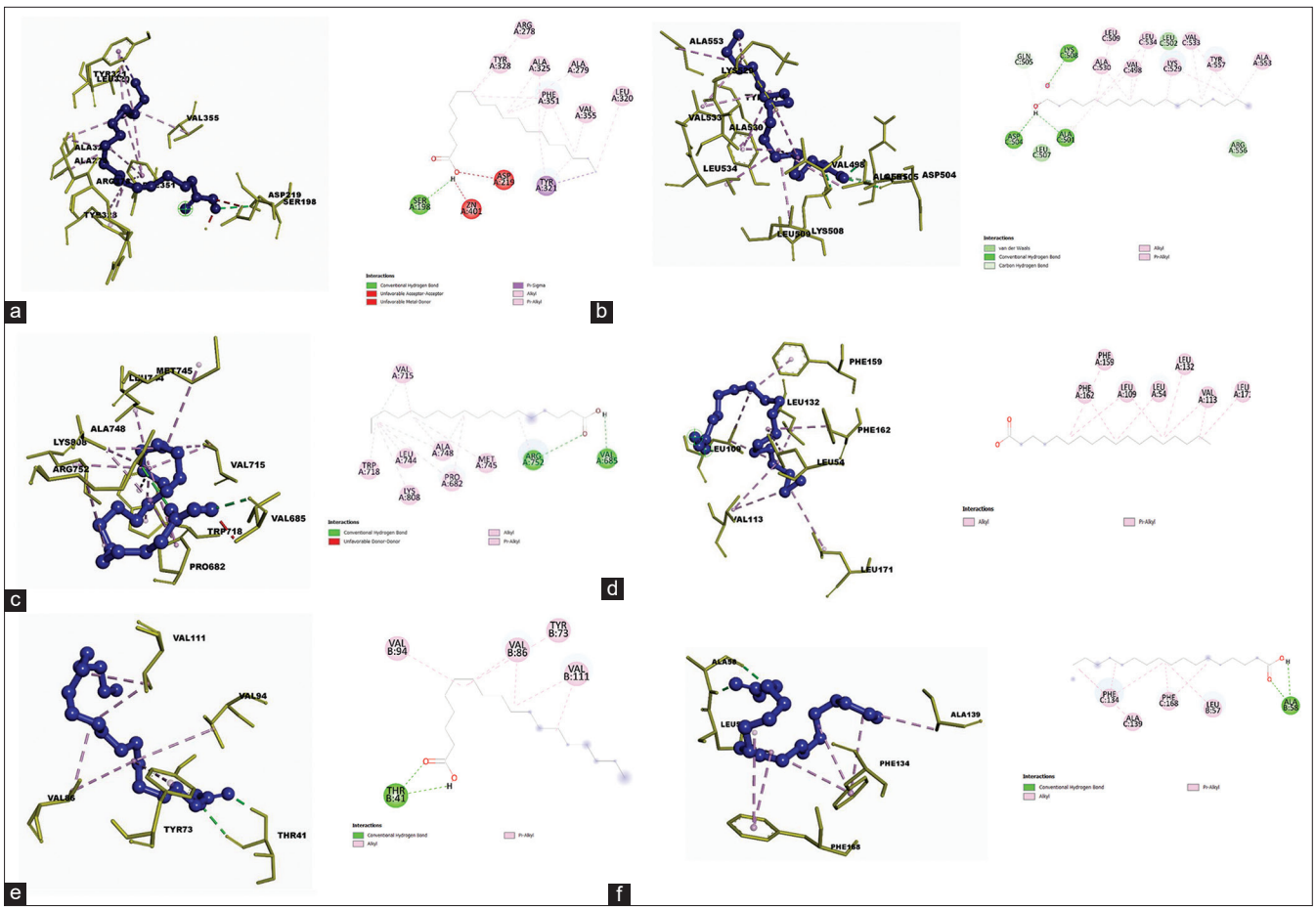


Fig. 4: 3-dimensional and 2-dimensional binding mode of cis-vaccenic acid against (a) AdipoR1 (b) Interleukin-18 (c) Androgen receptor (d) tumor necrosis factor-α (e) C-reactive protein (f) Interleukin-6

CONCLUSION

The present study focused on the exploration of certain bioactive compounds from the seeds of *L. nepetifolia* and identifying its therapeutic potential for PCOS. Methanolic extract of seeds of *L. nepetifolia* uncover two lead compounds with anti-inflammatory properties. Identifying and docking those compounds, i.e., Cis-vaccenic acid and Octadecanoic acid with various inflammatory markers (IL-6, IL-18, CRP, TNF-α, AR, AdipoR1 and AdipoR2) responsible for PCOS revealed favorable binding affinities. *In silico* docking scores revealed that CYP19A1 protein, which is responsible for steroidogenesis, showed the best binding score of −6.4 kcaL/moL against cis-vaccenic acid, while AdipoR1 had a maximum binding affinity of −7.6 kcaL/moL against octadecanoic acid. These results suggest that *L. nepetifolia* holds the potential for the development of reliable and effective drugs for treating PCOS. However, further research, including bioactivity assessments and clinical trials, is essential for the formulation of new drug therapies.

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AUTHOR'S CONTRIBUTIONS

M. Merlin Monisha: Laboratory work, Material preparation, Data collection and analysis, Manuscript preparation.

Dr. M. Prakash: Data analysis, Manuscript preparation and revision.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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