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GAS CHROMATOGRAPHY-MASS SPECTROMETRY PROFILING, ANTI-INFLAMMATORY EVALUATION, AND MOLECULAR DOCKING STUDIES OF ESSENTIAL OIL AND SCENTED EXTRACT FROM THE *Porana volubilis* Burm. f. FLOWER

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ABSTRACT

Volatile components of *Porana volubilis* Burm. f. flowers from Bangkok were extracted by using steam distillation and solvent extraction prior to analysis by gas chromatography-mass spectrometry (GC-MS) and their anti-inflammatory activity was assessed *in vitro* and *in silico*. Forty-two compounds were identified with essential oil. The key volatile components were detected in the hexane-scented extract. Twenty-one compounds were detected with major volatile components of octacosanaol, dodecanoic acid, nonacosane, n-hexadecanoic acid, and tetratriacontane for ethanolic scented extract. The essential oil and extract were evaluated for their *in vitro* anti-inflammatory effect by protein denaturation assay. The essential oil showed the most potent anti-denaturation activity with IC_{50} values of 537.6 µg/mL. The primary components of essential oil were submitted ta molecular docking research based on their anti-inflammatory effect. The obtained results revealed that the main components were found as the crucial interactions for binding in the bovine serum albumin (BSA) binding site. The results obtained demonstrated the essential oil of *P. volubilis* is the potential source for the anti-inflammatory agent.

Keywords: Anti-Inflammatory, Essential Oil, Molecular Docking, *Porana volubilis*.

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INTRODUCTION

Inflammation is a physiological response defined by heat, pain, swelling, damage, infection, and physiological disruption, and it is characterized by the following responses: inactivating or destroying invasive species, removing irritants, and preparing the environment for tissue healing. This is also induced by chemical mediators released by injured tissues and migratory cells.[1] Protein denaturation is one of the inflammation mechanisms that occurs by adding external stresses or compounds such as strong acids, bases, or heat. The secondary and tertiary structures of these proteins can be altered, and their biological functions are further inhibited. To prevent the protein denaturation process, the drugs were screened by the anti-denaturation assays utilizing Bovine Serum Albumin (BSA). It was shown that numerous NSAIDs, including indomethacin, ibufenac, diclofenac sodium, aspirin at high doses, and celecoxib, could prevent BSA decomposition at pathological pH.[3] Recently, traditional medicine has been re-evaluated across the globe as a result of extensive research into numerous plant species and their beneficial medicinal principles. The enormous plant kingdom wealth can be a fresh source of novel chemicals with important anti-inflammatory properties.[4] These biological qualities are frequently attributed to essential oils (EO) found

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in plants, which are employed as herbal remedies in traditional medicine. This EO has been discovered to have a variety of characteristics, including anti-inflammatory qualities.[5] *Porana volubilis* Burm. f., is a member of the Convolvulaceae family, and is commonly known as a Snow creeper. In Thailand, it is most famously called Ladawan.[6] *P. volubilis* is a long-lived perennial vine. Research studies have demonstrated that the leaf crude extract of *P. volubilis* has anticoagulant and anti-inflammatory properties.[7] The purpose of this study was to determine the anti-inflammatory properties of *P. volubilis* essential oil and scented extract. To the best of our knowledge, this is the first systematic examination of *P. volubilis*' ethnopharmacological effects. In this study, we report the investigation of the *P. volubilis* essential oil and scented extracts, which were collected from Bangkok, Thailand. Steam distillation and solvent extractions method (hexane and ethanol) were performed in this study. GC-MS, an analytical technique, was used to isolate and analyze the chemical constituents present in essential oils, and *in vitro* anti-denaturation of the essential oil was studied against bovine BSA to know their anti-inflammatory activities' effect. Besides, the computational molecular docking study of principal volatile components was studied.

EXPERIMENTAL

Material and Methods

Plant, Chemicals and Materials

The *P. volubilis* flowers were collected from Sala Thammasop Subdistrict, Thawi Watthana District, Bangkok (13°47'38.6"N 100°22'54.8" E) between 6:00 am and 7:00 am, in December 2019. The specimen was identified by Asst.Prof.Dr. Unchalee Ninsuwan and kept in Phranakhon Rajabhat University's Department of Chemistry, Faculty of Science and Technology. Diclofenac sodium was purchased from Sigma Chemicals.

Preparation and Analysis of Essential Oil and Extracts

Steam Distillation

Fresh *P. volubilis* flowers (30 g) were crushed into small pieces, then, subjected to the round bottom flask (500 mL). The distilled water was poured into the flask and connected to a Clevenger apparatus. The steam distillation was carried out for 3 h, followed by a collection of the essential oil from the receiving part of the apparatus. Dichloromethane was used to separate the essential oil from the water layer. The essential oil (EO) extract obtained was dried with anhydrous sodium sulfate. After filtration, the essential oil was concentrated using a Buchi® model R-114 rotary evaporator set to 45°C for evaporating the solvent. The oil was kept in a small amber glass bottle tightly capped and stored at 4 ºC before analysis, the methods for determining traces *of* the mass of the essential oil, and the yield of oil can be calculated in percentage using the formula:

$$\text{The yield of essential oil (\%)} = \frac{\text{weight of essential oil (g)}}{\text{weight of the fresh floral sample (g)}} \times 100$$

Solvent Extraction

The fresh flowers of *P. volubilis* (100 g) were extracted with *n*-hexane and ethanol and at room temperature for 24 h in each extraction. After filtration, anhydrous Na_2SO_4 was added to eliminate residues of water. The solvent was evaporated under reduced pressure at low temperature and finally left the concrete yield. The concrete oil was weighed and recorded. Concrete oil content (%) was calculated as:

$$\text{The yield of concrete oil (\%)} = \frac{\text{weight of concrete (g)}}{\text{weight of plant sample (g)}} \times 100$$

GC-MS Analysis

Agilent 7890A/7000A GC triple quadrupole mass spectrometer (Agilent Technologies) was used as an analytical instrument equipped with DB-Wax (J&W Scientific) capillary column (30 m x 0.25 mm i.d., 0.25 µL film thickness) as separation unit. The temperature of the oven was configured as follows: start at 30 °C raised at 5 °C per min held at 240 °C. The injector port temperature and the MS transmission line have been set to 250 °C. The flow rate of carrier gas (Helium) was fixed at 1.0 mL min^{-1}. The ionization mode was selected with an electron impact at 70 eV, and the mass scan range was m/z 30-400. The

identification of oil components was accomplished based on MS scanning by the National Institute of Standards and Technology (NIST version 2011).

Inhibition of Albumin Denaturation

The anti-inflammatory activity of the essential oil and scented extracts was determined using the method of Elisha and co-workers[8] with minor modifications. The reaction was started by adding 100 µL extracts with a final concentration of 50, 100, 150, 200, 250, 500, 750, and 1000 µg/mL to 100 µL of 5 % aqueous solution of BSA. Glacial acetic acid 10 µL was used for pH adjustment. The sample reactions were incubated at 37 °C for 20 min. Afterward, the reaction mixture was heated for 10 min at 70 °C. The mixture was allowed to cool at RT for 10 min. The turbidity of the reaction was analyzed by VICTOR Nivo™ Multimode Plate Reader (PerkinElmer) at wavelength 416 nm. The blank consists of the sample and deionized water. The negative control was deionized water. Diclofenac sodium was chosen as the standard drug in this study. The experiment was done in triplicate. The inhibition percentage was obtained by using the following formula:

$$\text{The percentage of inhibition (\%)} = 100 \text{ x} \frac{(\text{Abs}_{\text{Sample}} - \text{Abs}_{\text{Blank}})}{\text{Abs}_{\text{control}}} - 1$$

The IC_{50} was calculated from a graph of inhibition against the different concentrations.

Molecular Docking Simulation

The three-dimensional (3D) structure of BSA was obtained from the Protein Data Bank PDB IDs: 4JK4. The 4JK4 structure is that of BSA co-crystallized with 3,5-diiodosalicylic acid.[9] All of the water molecules were removed from the structure of the protein. The docking of molecules was carried out using the AutoDock 4.2 software (Scripps research institute). ChemSketch version 11.0 was used to create the ligands and optimized by using Gaussian 06 package.[10] Docking was performed using the Lamarckian Genetic Algorithm following a conventional technique.[11] For each ligand, one hundred independent docking runs were performed. In addition, the Discovery Studio ver. 3.1 was used to examine the ligand's interaction with protein models. In this case, docking the native ligand again, the RMSD values were 0.84. The docked ligand was found to be superimposed with its native counterpart, which is why the RMSD values were so low. As cited in the literature[12], If the RMSD of the best-docked conformation of the native ligand from the experimental one is less than 2.0, the scoring function is appropriate. The root means square deviation (RMSD) for redocking the native 3,5-diiodosalicylic acid ligand was 0.84, indicating that the docked ligand was superimposed on the native ligand that was originally embedded.

Data analysis

For statistical analysis, the consequences of inhibition of albumin denaturation activity had been expressed as a mean ± SD. Percentages are calculated by using the application in Microsoft Excel program (2016).

RESULTS AND DISCUSSION

Chemical Components of the Essential Oil and Scented Extract

P. volubilis EO obtained from the steam distillation process by using a Clevenger apparatus was a pale-yellow oil. The percentage yield of the obtained EO was 0.05. In the case of scented extract, via maceration technique, was performed based on two classes of solvents, which include both non-polar solvents (*n*-hexane) and polar solvents (ethanol). The corresponding yields of absolutes extraction were 0.56 and 4.38, respectively.

Table-1: Comparison of the Yield and Physical Appearances of *P. volubilis* Essential Oils Extracts

Extraction method	%yield±SD	Essential oil characteristics
Steam distillation	0.05±0.002	Clear yellow oil smells like boiled flowers.
n-Hexane	0.56±0.104	Brown sticky oil with a fragrance like fresh flowers. *n*-hexane extracts showed a more pungent scent than ethanol extracts.
Ethanol	4.38±0.016	Dark brown semi-solid with a fragrance like fresh flowers.

Note **:**Data presented as the mean ± SD (n = 3).

Analysis of EO and the scented extract was done using the gas chromatography-mass spectrometer method. The fresh flower essential oil was found to contain 42 compounds (Fig.-1A). The major compounds are

caryophyllene (1, 10.76%), *β*-eudesmene (2, 5.98%), 2-tridecanone (3, 13.39%), caryophyllene oxide (4, 8.04%), and dodecanoic acid (5, 7.02%). The For scented extract by *n*-hexane was found to contain 36 compounds (Fig.-1B). The main compounds are caryophyllene (1, 17.01%), caryophyllene oxide (4, 5.63%), dodecanoic acid (5, 8.90%), heptacosane (6, 7.78%), and triacontane (7, 8.74%). In the case of ethanolic scented extract, 21 chemical substances were recorded (Fig.-1C). The main chemical components were Octacosanal (8, 18.05%), dodecanoic acid (5, 7.88%), nonacosane (9, 8.51%), *n*-hexadecanoic acid (10, 9.00%), and tetratriacontane (11, 28.96%). The main chemical identification results of the EO and scented extract were summarized in Table-2. In particular, the major constituents observed in the essential oil and scented extract were identified in *P. volubilis*. These compounds have been found in various plants and are known to play an essential role as natural aroma compounds of bark, leaves, or flowers of some plants.[13-15] Especially, caryophyllene, caryophyllene oxide, and *β*-eudesmene are present in black pepper, black tea, cinnamon, and lavender[16], and are contributing to the unique aroma of *P. volubilis* flower. Caryophyllene and caryophyllene oxide have anticancer properties.[17] The GC-MS spectrum shows that there are a lot of different components with different retention times, as shown in Fig.-1. The mass spectrometer analyses the molecules eluted at various periods to identify their composition and structure. The large compound breaks down into smaller compounds, resulting in peaks with varying m/z ratios. These mass spectra are the fingerprint of the compound that is detectable from the data library. The GC-MS analysis of the essential oil and scented *P. volubilis* floral extract showed the existence of lots of phytochemicals that contribute to the bioactive medicinal properties of this plant.

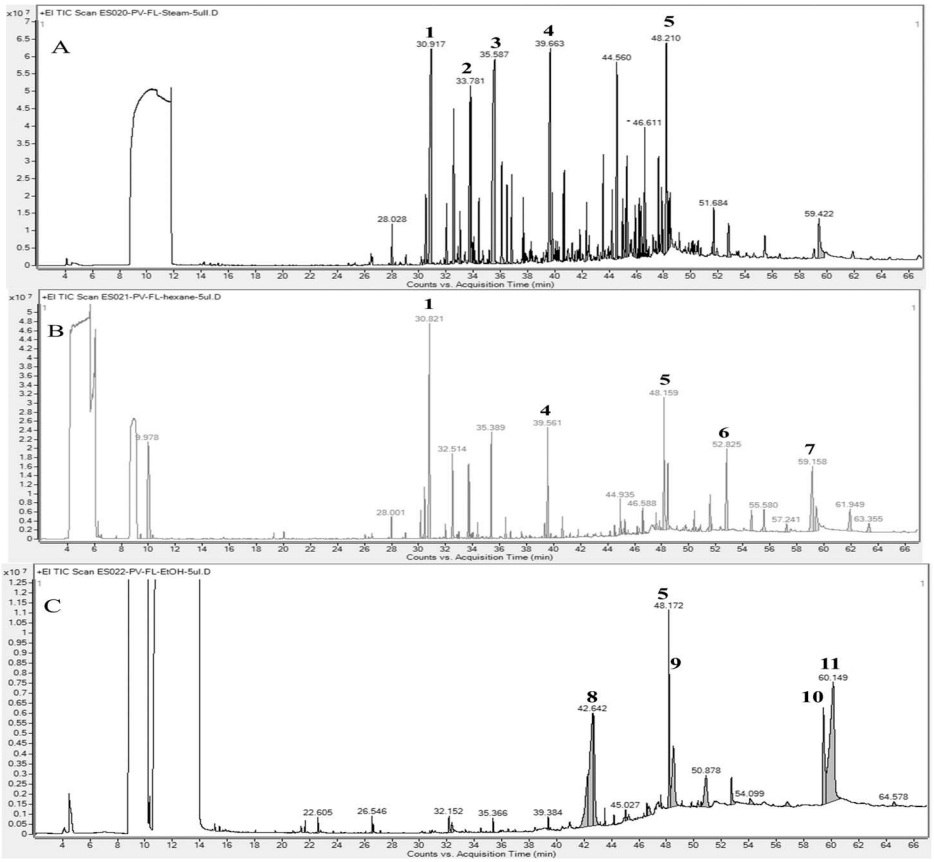


Fig.-1: GC-MS Chromatograms of *P. volubilis* Essential Oils. (A Steam Distillation Extract, B *n*-Hexane Extract, and C Ethanol Extract)

Anti-inflammatory Activity

The anti-inflammatory activity of *P. volubilis* flower extracts was measured by using anti-denaturation assays, and the results are displayed in Table-3. The IC_{50} value of diclofenac sodium was 62.8 µg/mL. The case of EO of *P. volubilis* showed a marked inhibition in protein denaturation with an IC_{50} value of 537.6 µg/mL. The IC_{50} value for the hexane-scented extract was found to be 760.5 µg/mL. The ethanolic extract

did not show activity at the highest concentration (1000 μg). The essential oil from *P. volubilis* flowers has an anti-inflammatory activity since the natural bicyclic sesquiterpene (caryophyllene (1), *β*-eudesmene (2) and caryophyllene oxide (4)) are significant components which accordance with the previous study by Chavan and co-worker who reported that caryophyllene (1), *β*-eudesmene (2) and caryophyllene oxide (4) exhibit significant analgesic and anti-inflammatory activities.[18]

Table-2: Major Chemical Compositions of *P. volubilis* Essential Oils and Extract by GC-MS Analysis

Extraction Method	RT	Area Sum %	Molecular Formula	Possible Compounds
Steam distillation	30.917	10.76	$C_{15}H_{24}$	caryophyllene (1)
	33.781	5.98	$C_{15}H_{24}$	*β*-eudesmene (2)
	35.587	13.39	$C_{13}H_{26}O$	2-tridecanone (3)
	39.663	8.04	$C_{15}H_{24}O$	caryophyllene oxide (4)
	48.210	7.02	$C_{12}H_{24}O_2$	dodecanoic acid (5)
n-Hexane	30.821	17.01	$C_{15}H_{24}$	caryophyllene (1)
	39.561	5.63	$C_{15}H_{24}O$	caryophyllene oxide (4)
	48.159	8.90	$C_{12}H_{24}O_2$	dodecanoic acid (5)
	52.825	7.78	$C_{27}H_{56}$	heptacosane (6)
	59.158	8.74	$C_{30}H_{62}$	triacontane (7)
Ethanol	42.642	18.05	$C_{28}H_{56}O$	octacosanal (8)
	48.172	7.88	$C_{12}H_{24}O_2$	dodecanoic acid (5)
	48.496	8.51	$C_{29}H_{60}$	nonacosane (9)
	59.437	9.00	$C_{16}H_{32}O_2$	*n*-hexadecanoic acid (10)
	60.149	28.96	$C_{34}H_{70}$	tetratriacontane (11)

Moreover, it is interesting to note that the anti-BSA denaturation activity of *P. volubilis* has not been previously reported in the literature. To further investigate the effects of anti-BSA denaturation, the five main components of steam distillation extract, including caryophyllene (1), *β*-eudesmene (2), 2-tridecanone (3), caryophyllene oxide (4) and dodecanoic acid (5) were selected for further molecular docking study due to its potential anti-denaturation activity.

Table-3: Results of Anti-BSA Denaturation Activity of *P. volubilis* Extracts

Sample	IC_{50} value ± SD (μg/mL)
Steam distillation extract	537.6±23.4
n-Hexane extract	760.5±16.8
Ethanol extract	inactive
Sodium diclofenac*	62.8±2.6

Note **:**Data are expressed as mean ± standard deviation)n = 3(*standard drug)

Docking Study of *P. volubilis* EO Main Chemical Constitutions

Molecular docking was used to obtain the functional and structural characteristics of the primary components within the BSA's binding site in order to examine the binding affinities that were related to the mechanism of inhibition. BSA is a monomeric protein comprised of 583 amino acid residues. The structure of BSA was built by three homologous helical domains. The domain names are classified as I, II, and III. Domain I compose of amino acids from 1 to 195, while domains II and III have amino acid sequences from 196–383 and 384–583, respectively. Each domain is composed of two subdomains (A and B). Two major drug binding sites are found in sub-domains IIA (Sudlow site I) and IIIA (Sudlow site II), as shown in Fig.-2.[19] The BSA protein (4JK4) is considered for this study as a target to bind with the ligands identified in GC-MS, which include caryophyllene (1), *β*-eudesmene (2), 2-tridecanone (3), caryophyllene oxide (4) and dodecanoic acid (5). The detailed interactions of selected compounds (compounds 1, 2, 3, 4, and 5) at the binding site of the BSA are given in Table-4 and Fig.-3. The order of the stability of binding free energies is 5 > 3 > 1 > 2 > 4 which exhibited the ΔG values of -5.56, -5.95, -7.02, -7.11, and -7.64 kcal.mol^{-1}, respectively. In the case of diclofenac sodium showed better binding to BSA protein with an estimated free energy of binding of -7.92 kcal.mol^{-1}.

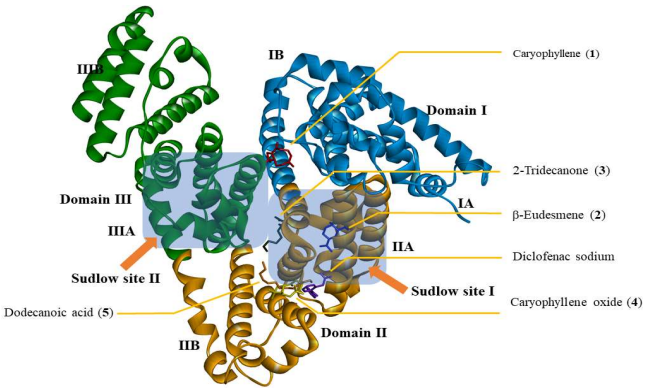


Fig.-2: The BSA Monomer Structure Obtained from Homology Modeling Indicating the Domains and the Binding Sites

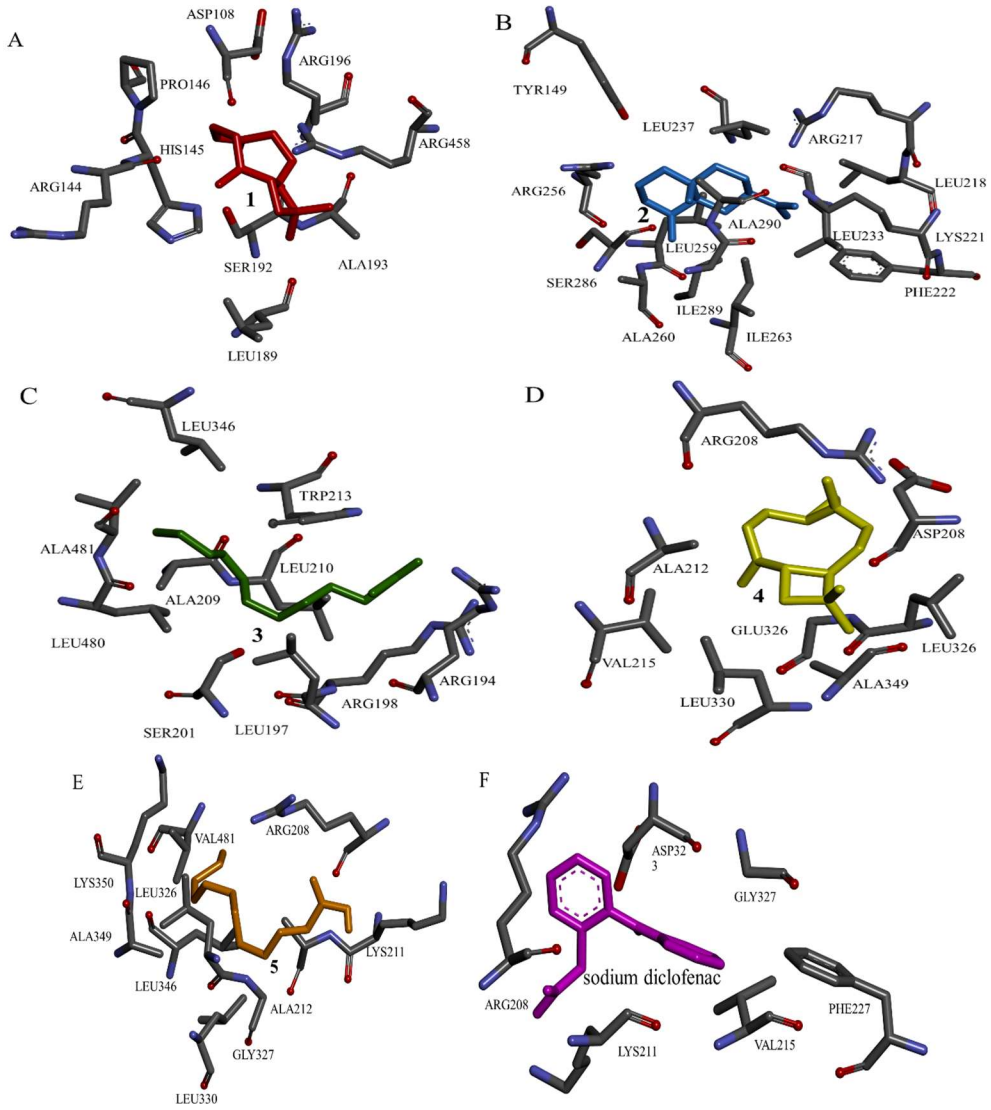


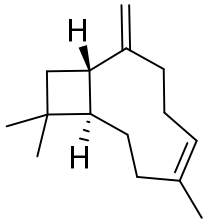
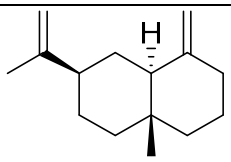
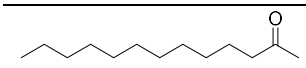
Fig.-3: Molecular Docking Results of BSA Complexed with (A) Caryophyllene (1), (B) *β*-eudesmene (2), (C) 2-Tridecanone (3), (D) Caryophyllene Oxide (4), (E) Dodecanoic Acid (5) and (F) Diclofenac Sodium

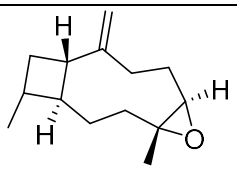
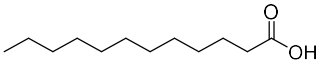
The diclofenac sodium showed interactions at domain II with amino acid residues Arg208, Lys211, Phe227, Asp323, and Leu326. Notably, caryophyllene (1) was surrounded by various residues at domain I, including

Asp108, Arg144, His145, Pro146, Leu189, Ser192, and Ala193. *β*-Eudesmene (2) was also adjacent to many residues at domains I and II, including Tyr149, Arg217, Leu218, Lys221, Phe222, Leu233, Leu237, Arg256, Leu259, Ala260, Ile263, Ile289, Ser286, and Ala290Compoundnd 3 was observed to establish the interactions with binding site residues in domain II, including Arg194, Leu197, Arg198, Ser201, Leu210, Ala209, Trp213, Leu346, Leu480, and Val481.

Caryophyllene oxide (4) forms hydrogen bonds with Asp323, and compound 4 was encircled by a variety of hydrophobic, polar, and charged residues such as Arg208, Val215, Leu326, Gly327, and Leu330. In the case of compound 5 bound to BSA with residues, including Arg208, Lys211, Ala212, Leu326, Gly327, Leu330, Leu346, Lys350, Ala349, and Val481 (Fig.-3). The above molecular docking study depicted that five EO main chemical constitutions fit into the hydrophobic pocket close to Sudlow site I. The Sudlow site I is the main anti-inflammatory drug-bindinsitees of BSA.[20] Based on this docking information and an evaluation of anti-inflammatory efficacy, the inhibitory activity of five major compounds, and the overall activity of steam distillation extract of *P. volubilis* flower in anti-BSA denaturation was confirmed.

Table-4: The Results of Molecular Docking of *P. volubilis* EO Main Chemical Component and Diclofenac Sodium

Ligand	Estimated free energy of binding (kcal.mol^{-1})	Interacting residues	Interaction distance (Å)
 Caryophyllene (**1**)	-7.02	Asp108	2.744
		Arg144	3.137
		His145	3.255
		Pro146	3.161
		Leu189	2.922
		Ser192	2.953
		Ala193	3.381
		Leu189	2.922
		Ser192	2.953
		Ala193	3.381
 *β*-Eudesmene (**2**)	-7.11	Tyr149	3.559
		Arg217	3.674
		Leu218	3.249
		Lys221	3.406
		Phe222	3.492
		Leu233	3.802
		Leu237	3.655
		Arg256	2.172
		Leu259	3.585
		Ala260	3.302
		Ile263	3.453
		Ile289	3.608
		Ser286	2.914
		Ala290	3.480
 2-Tridecanone (**3**)	-5.95	Arg194	2.920
		Leu197	3.946
		Arg198	3.613
		Ser201	3.352
		Leu210	3.740
		Ala209	3.094
		Trp213	3.450
		Leu346	3.578
		Leu480	3.329
		Val481	3.901
	-7.64	Arg208	2.663
		Val215	3.938

Ligand	Estimated free energy of binding (kcal.mol^{-1})	Interacting residues	Interaction distance (Å)
 Caryophyllene oxide (**4**)		Asp323	3.237
		Leu326	3.757
		Gly327	3.873
		Leu330	3.597
 Dodecanoic acid (**5**)	-5.56	Arg208	3.076
		Lys211	3.746
		Ala212	3.999
		Leu326	3.966
		Gly327	3.951
		Leu330	3.890
		Leu346	3.135
		Lys350	3.491
		Ala349	3.888
		Val481	3.549
Diclofenac sodium	-7.92	Arg208	3.746
		Lys211	2.916
		Phe227	3.140
		Asp323	3.913
		Leu326	3.503

CONCLUSION

In conclusion, the preliminary examination for the volatile oil components from the *P. volubilis* flower was performed using GC-MS. The bioactive compounds of the essential oil and scented extracts were identified by retention time and mass spectrum in comparison with a mass spectral library collection. The result of GC-MS analysis showed that *P. volubilis* essential oil consists of 42 components. In the case of scented extracts, 36 compounds were recorded in *n*-hexane extract, and 21 components in ethanol extract. The essential oil and *n*-hexane extracts displayed mild *in vitro* anti-denaturation with IC_{50} values of 537.6, and 760.5 mg/mL, respectively. Furthermore, molecular docking studies of the main components of essential oil indicated the tested, which can bind to BSA and act as an anti-denaturing agent for BSA protein. It may be concluded that *P. volubilis* flower essential oil possesses significant *in vitro* and *in silico* anti-inflammatory activities.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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