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GC-MS Analysis and Molecular Docking of Bioactive Components from Leaves of *Guaiacum officinale* for Anti-inflammatory Activity

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ABSTRACT

Present study was an attempt to investigate the bioactive components present in the leaves of *Guaiacum officinale* using gas chromatography-mass spectroscopy (GC-MS) analysis and study the anti-inflammatory potential of those constituents using molecular docking studies. GC-MS analysis was done by standard protocol using the equipment JEOL GC MATE II. The identification of components was based on NIST (National Institute of Standards and Technology) Version-11 library as well as comparison of their retention indices. The molecular docking studies were done using the commercial docking software MCULE, 1-click docking. GC-MS analysis of the alcoholic extract showed the presence of ten compounds at different retention times. The phytoconstituent 8,11,14-eicosatrienoic acid was present at high concentration with % peak area of 43.3 at a retention time of 19.43 min followed by 5,7-dihydroxy-8-methoxy flavone (Wogonin) at a retention time of 17.73. All 10 compounds obtained from GC-MS analysis and diclofenac were used as the ligands in this study, with cyclooxygenase-2 (COX-2), phospholipase A2 and interleukin receptor as the molecular targets. *in silico* Docking studies revealed that the flavanoid Wogonin is having highest binding potential indicated by least docking score of -8.2, -8 and -6.9 kcal/mol on COX-2, phospholipase A2 and interleukin receptor respectively.

KEYWORDS

Guaiacum officinale, GC-MS, *in silico* Docking, Anti-inflammatory, Cyclooxygenase-2, Phospholipase A2, Interleukin-1.

INTRODUCTION

Nature remains an ever evolving source for compounds of medicinal importance. The use of medicinal plants for the treatment of parasitic diseases is well known and documented since ancient times. In traditional system of medicine, plants represent principle means of therapy for various types of ailments. In recent years, plants are being used extensively for treating different pathophysiological conditions. Pharmacological investigations of medicinal plants have provided important advances for the therapeutic approach to several pathological conditions [1]. The plant *Guaiacum officinale* has been choosen for present study which is used in traditional medicine as an

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anti-inflammatory and in treatment of various diseases associated with inflammation [2].

Guaiacum officinale (Family: Zygophyllaceae) commonly known as lignum-vitae, tree of life. A number of compounds including lignans, quinones, sesquiterpenoids and various triterpenoid saponins have also been isolated from the same source. Phytochemical screening revealed the presence of larreagenin, sitosterol and oleanolic acid [3]. The resin of *G. officinale* is used in the initial stage of angina and arthritis in homeopathy. Antibacterial activities against *Bacillus subtilis, Corynebacterium diptheriae, Enterobacter cloacae, Kelebsiella ozaenae, Proteus vulgaris, Salmonella typhi, S. pyogenes, S. lactis, S. cilreus* and *Shigella dysenteriae* were reported [4,5]. The aqueous ethanolic extracts of *G. officinale* were reported with anti-inflammatory activity [6]. The leaf, seed and twig extracts of *G. officinale* were reported for anti HIV-1 properties in primary peripheral blood mononuclear cells (PBMCs) infected with the reference HIV-1 BaL strain [7]. Although many activities were reported to this plant, till date no work was done to identify the constituents responsible for these activities. So, the objective of present study is to identify the active constituents present in this extract using GC-MS analysis and study the anti-inflammatory potential of those constituents using molecular docking studies.

Inflammation is the body defense system in response to the pathogens and injury. During the inflammation process, various inflammatory mediators are synthesized and secreted from cells and generate many cellular effects [8]. Uncontrolled inflammation lead to several chronic diseases such as cardiovascular disease, arthritis, asthma, type 2 diabetes mellitus and cancer. The mechanism of action and molecular target of various natural compounds needs to be studied for constructing a structure activity relationship [9]. Molecular docking analysis can be conducted to study the interaction of these compounds with various molecular targets of anti-inflammatory activity. Further, the structure-activity relationship can be used to develop new derivative natural compounds with higher anti-inflammatory activity. All the phytoconstituents obtained in GC-MS analysis and diclofenac were used as ligands in this study, with the cyclooxygenase-2 (COX-2), phospholipase A2, (PLA2) and interleukin receptor (IRAK) as the molecular targets.

EXPERIMENTAL

Collection, identification and extraction of plant material: The leaves of *G. officinale* (GO) were collected at the medicinal garden of Chebrolu Hanumaiah Institute of Pharmaceutical Sciences. The plant was authenticated by the Department of Botany, Acharya Nagarjuna University, Guntur and voucher specimen was preserved. The leaves were separated, dried, powdered and then extracted with alcohol as solvent by using soxhelation for 4 cycles. Then the extracted drug was further evaporated using simple distillation apparatus to obtain the concentrate [10].

GC-MS analysis of alcoholic extract: GC-MS analysis was performed using the equipment JEOL GC MATE II. The equipment has a DB 35-MS Capillary Standard non-polar column with dimensions of 30 mm × 0.25 mm ID × 0.25 µm. The carrier gas used is helium with at rate of 1.0 mL/min. The injector was operated at 250 °C and the oven temperature was programmed as follows: 110 °C hold for 3.50 min, up to 200 °C at the rate of 10 °C/min-No hold, up to 280 °C at the rate of 5 °C/min-12 min hold and total GC running time is 40 min. The identification of components was based on NIST (National Institute of Standards and Technology) Version-11 library as well as comparison of their retention indices [11].

Docking studies: Selection of molecular targets for anti-inflammatory activity:

a) Cyclooxygenase-2 (COX-2): The COX enzymes (COX-1 and COX-2) catalyze the biosynthesis of prostaglandins, prostacylins and thromboxanes, from arachidonic acid. COX-1 is constitutively expressed in most tissues, while COX-2 is expressed in specific tissues and is induced by cytokine and growth hormones. COX-1 possesses regulatory effects on platelet aggregation and gastric mucous biosynthesis, while COX-2 is involved in pathological conditions such as inflammation, pain and fever [12].

b) Phospholipase A2 (PLA2): Phopholipase A2 (PLA2) enzymes are required to increase the level of arachidonic acid for metabolism and biosynthesis of eicosanoid under physiological condition as well as in inflammatory cell activation [13].

c) Interleukin-1: Contribution of interleukin-1 (IL-1), a proinflammatory spectra, in the inflammation network is important. It propagates and amplifies signals; furthermore, the signaling pathways mediated by IL-1 and other cytokines receptors may communicate in various cross-talk mechanisms. Therefore, inhibition of IL-1 receptor would have profound effects on overall inflammatory responses. Interleukin-1 receptor-associated kinase 4 (IRAK-4) plays a pivotal role in signaling cascades associated with the immune and inflammatory diseases and may be an effective therapeutic target for various diseases associated with deregulated inflammation [14].

Preparation of target proteins: The compounds identified in GC-MS and diclofenac were docked to the selected 3 target proteins from Homo sapiens. The structures of COX-2 (SC-PDB ID: 4COX), PLA2 (SC-PDB ID: 1DB4) and IRAK-4 ((SC-PDB ID 2NRU) were obtained from database available in 1-click docking of MCULE software.

Preparation of ligands: The structures of native ligands from each target macromolecules were prepared to separate from the protein, water and miscellaneous substances. The structures of the 11 ligands were sketched in MCULE software.

Docking method validation: To ensure that the docking studies were valid and represented the reasonable potential binding model, the docking methods and parameters used were validated by redocking experiment. Each copy of native ligand was docked into the native protein to determine the ability of the program to reproduce the orientation and position of the ligand observed in the crystal structure.

Lipinski's analysis: Lipinski's rule says that to evaluate drug likeness and determine the pharmacological activity. The Lipinski's properties like molecular weight, log p, number of hydrogen bond donors and acceptors. Lipinski's parameters to satisfy the retrieved phytocompounds of *Guaiacum officinale* were analyzed, using PubChem tool [15].

RESULTS AND DISCUSSION

In the present study, alcoholic extract of the leaves of *Guaiacum officinale* was subjected to GC-MS analysis to identify the potential phytocompounds present in it. In this study, the GC-MS analysis clearly showed the presence of 10 compounds in Table-1 and its chromatogram is shown in Fig. 1. Out of these compounds 8,11,14-eicosatrienoic acid was present at high concentration with % peak area of 43.3 at a retention time of 19.43 min, followed by 5,7-dihydroxy-8-methoxy flavone (Wogonin) at a retention time of 17.73 min. All these phytoconstituents and standard anti-inflammatory drug diclofenac were subjected to predict the anti-inflammatory potential through *in silico* docking analysis by using the commercial docking software MCULE, 1-click docking. All the compounds were docked against 3 major inflammatory targets cyclooxygenase-2, phospholipase-A2 and interleukin-1.

The goal of ligand and protein docking is to predict the predominant binding model of a ligand with a protein of known three dimensional structures [16]. To study the binding modes of bioactive compounds in the binding site of target proteins**,** intermolecular flexible docking simulations were performed and energy values were calculated from the docked conformations of the receptor ligand complexes [17]. Lipinski's rule five is important for drug development where a pharmacologically active phytocompounds should have not more than 5 hydrogen bond donors, not more than 10 hydrogen bond acceptors, molecular weight under 500 dalton, Partition coefficient A log P is less than 5. All the constituents except nonanedioic acid dioctyl ester satisfied the Lipinski's properties.

The phytocompounds had the potential to dock with the target proteins and their interaction details are listed in Table-2. The identified phytocompouds exhibited the docking score between -4.9 to -8.2 kcal/mol against all the three targets. Of

TABLE-1
GC-MS OF ALCOHOLIC EXTRACT OF *G. officinale*

Compound	Rt	Peak area (%)	m.w.	m.f.
Cyclopentane undecanoic acid methyl ester	17.05	0.68	268	$C_{17}H_{32}O_2$
5,7 dihydroxy, 8-methoxy flavone (WOGONIN)	17.73	27.5	269	$C_{16}H_{12}O_5$
Oleic acid	18.8	2.75	282.05	$C_{18}H_{34}O_2$
8,11,14 Eicosa trienoic acid	19.43	43.3	306.76	$C_{20}H_{34}O_2$
5-methyl-Z-5 docosene	20.08	4.8	322	$C_{23}H_{46}$
Docosanoic acid (BEHENIC ACID)	20.28	6.8	340	$C_{22}H_{44}O_2$
Octanoic acid pentadecyl ester	21.18	8.25	353	$C_{23}H_{46}O_2$
Benzene, 1-(2-butenyl)oxy)-4-(4'-butyl-(1,1'-bicyclohexyl)-4yl	22.35	2.06	354	$C_{25}H_{34}O$
Eicosapentaneoic acid, 2,6, 11, 15, 19 -pentamethyl-ethyl ester	23.95	2.06	400	$C_{27}H_{48}O_2$
Nonanedioic acid dioctyl ester	26.18	1.5	412	$C_{25}H_{48}O_4$

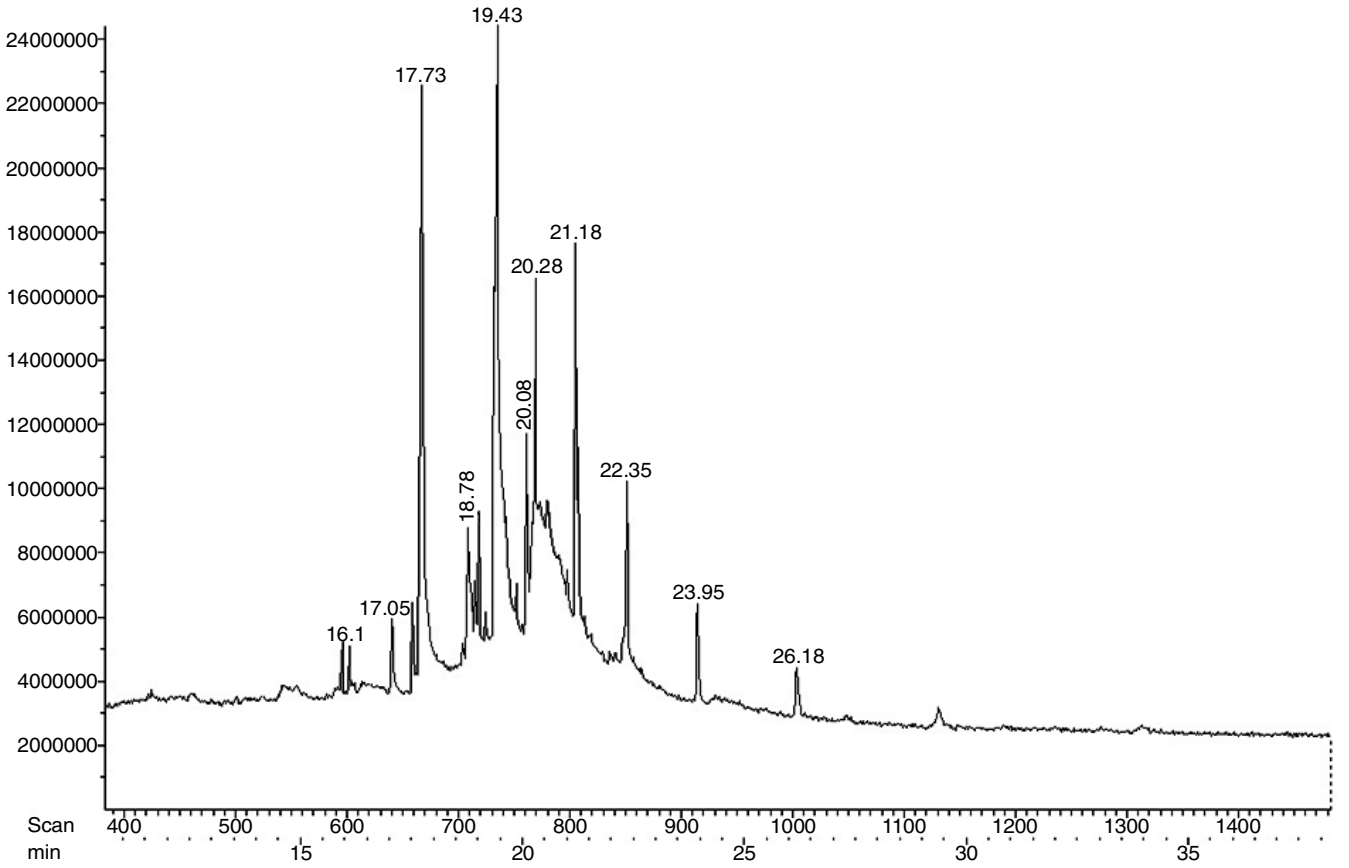

Fig. 1. GC-MS chromatogram of alcoholic extract of *G. officinale*

TABLE-2
DOCKING SCORES ON DIFFERENT PROTEINS

Compound	COX-2	PLA2	IL-1
Cyclopentane undecanoic acid methyl ester	-6.8	-5.6	-4.9
5,7 dihydroxy, 8-methoxy flavone (Wogonin)	-8.2	-8	-6.9
Oleic acid	-6.5	-5.7	-5.1
8,11,14 Eicosa trienoic acid	-7.5	-6.2	-5.7
5-methyl-Z-5 docosene	-7.4	-5.5	-5.1
Docosanoic acid (Behenic acid)	-7.1	-5.4	-5.2
Octaneoic acid pentadecyl ester	-6.8	-5.4	-4.5
Benzene, 1-(2-butenyl)oxy)-4-(4'-butyl-(1,1'-bicyclohexyl)-4-yl	-7.8	-8.4	-7.1
Eicosapentaneoic acid, 2,6, 11, 15, 19 -pentamethyl-ethyl ester	-6.6	-5.8	-5.4
Nonanedioic acid dioctyl ester	NA	NA	NA
Diclofenac	-8.1	-7.6	-6.4

which, the maximum binding energy was found in Wogonin (-8.2 kcal/mol) with COX-2 (Fig. 2).

Reports unveil that flavanoids have anti-inflammatory properties and increased intake of flavanoids reduced inflammatory symptoms [18]. Furthermore, oleic acid is one another indigenous compound which has an antioxidant property and acts as a 5-α-reductase inhibitor [19]. Present results are consistent with the previous reports stating that a diverse range of flavonoids occur in traditional medicines that exert potent anti-inflammatory activity. Least docking score shows good binding energy and hence more efficient in blocking the activity of the particular protein. Analysis of ligand binding interaction with

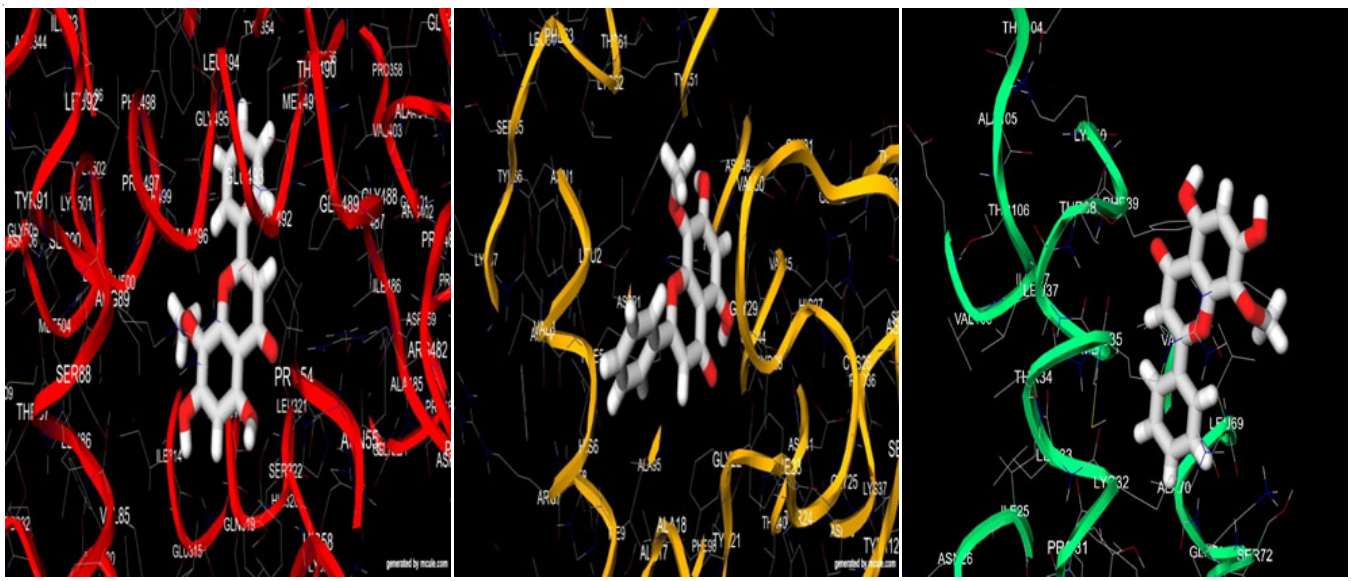

Fig. 2. Images of docking of Wogonin on Cox-2, PLA-2, Interleukin-1

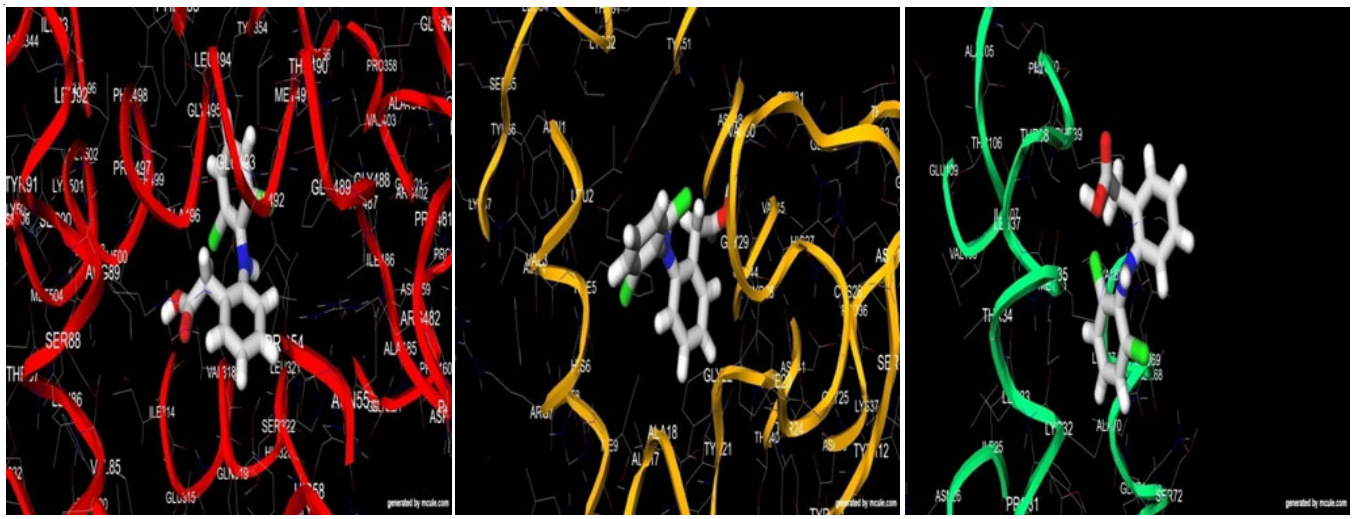

Fig. 3. Images of docking of diclofenac on Cox-2, PLA-2, Interleukin-1

the target proteins can be useful for invention of preventive drug for inflammation and associated disorders from natural sources.

The target proteins COX-2, PLA2, IL-1 when counteracted with the standard anti-inflammatory drug, diclofenac exhibited a least score of -8.1, -7.6 and -6.4 kcal/mol, respectively (Fig. 3). The phytoconstituent 5,7-dihydroxy-8-methoxy flavone (commonly termed as Wogonin) obtained from GC-MS of *G. offinale* as second major constituent exhibited least docking score with energy values of -8.2, -8.0 and -6.9 kcal/mol against COX-2, PLA2, IL-1 respectively. The results indicate that Wogonin is having highest binding affinity than that of diclofenac.

The results obtained from this study would be useful in both understanding the inhibitory mode as well as in rapidly and accurately predicting the activities of new inhibitors on the basis of docking scores.

Conclusion

The present study provides an evidence for the isolated compounds from the leaves of *G. offinale* as new potent anti-inflammatory compounds. The results suggested that the compound Wogonin, as potential lead molecules for developing novel anti-inflammatory drug which can confer better acceptability. The *in silico* assay endorses the reference compound diclofenac as an established anti-inflammatory drug. The efficacy of these potent phytocompounds can be further elucidated with *in vivo* studies.

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