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RESEARCH ARTICLE

In Silico and *In Vitro* Approaches to Predict MepR Efflux Protein Inhibitory Activity of *Ocimum sanctum* Aqueous Leaf Extract

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Abstract

Background: *Ocimum sanctum* belonging to *Lamiaceae* family has been reported to have various medicinal values. **Aim:** The plant parts especially leaves have various pharmacological activities like antibacterial, antifungal, antioxidant, anti-inflammatory, anti-insecticidal, anti-cancer, anti-metastatic, anti-proliferative, and anti-tumour properties. **Methods**: Soxhlet extraction method was carried out to extract *O. sanctum* leaves. Then the extract was studied for its antibacterial activity against *Staphylococcus aureus* which showed a significant zone of inhibition on growth. Furthermore, through a rigorous literature survey, Oleanolic acid was found to be a predominant compound present in the aqueous leaf extract of *O. sanctum*. Then, the oleanolic acid was docked against the Multidrug Efflux Pump MepR (PDB ID: 4LLL) of *S. aureus*, which showed that the binding affinity is strong with a binding energy of -4.7 Kcal/mol. Also, oleanolic acid has passed the drug-likeness by Lipinski's rule of five and the ADME properties. **Results:** *O. sanctum* can be explored more in the future to evaluate its medicinal properties The top compound beta-sesquiphellandrene was docked against the Janus kinase 1 (PDB ID: 4I5C), Janus kinase 2 (PDB ID: 2B7A) which showed that the binding affinity is strong with binding energies -7.3 Kcal/mol and -7.4 Kcal/mol respectively. Also, the lead compound has passed the drug-likeness by Lipinski's rule of five and the ADME properties. **Conclusion:** The study shows significant cytotoxic potential in MTT assay with IC_{50} concentration of the sample nearer to 60 µg/ml. *O. sanctum* can be explored more in the future to evaluate its medicinal properties.

Major Findings: The *O. sanctum* has significant antibacterial activity against *S. aureus* and act against the lymphoma cancer cells.

Keywords: MepR, Molecular Docking, *O. sanctum,* Oleanolic Acid, *S. aureus*

1. Introduction

The promotion of traditional medicine as a means of providing healthcare has been emphasized by the World Health Organization (WHO). As a result, national research institutions are focusing on studying traditional and herbal medicinal plants. In India, sacred or holy basil, otherwise called green *tulsi* (*O. sanctum*), is a popular medicinal plant particularly in developing nations, through both independent and cooperative initiatives that thrive in homes and temples. It has long been believed to have revitalizing, tonic, and rejuvenating qualities that promote long life and good health. The leaves of *O. sanctum* are used to treat fever, pain, gastrointestinal disorders (such as dyspepsia and vomiting), worm infestations, skin conditions, and snakebite[1]. They also have expectorant, diaphoretic, antiseptic, spasmolytic, stimulant, and anticatarrhal qualities. *O. sanctum* has been shown to have significant hepatoprotective efficacy against the hepatotoxicity caused by anti-tubercular drugs, carbon tetrachloride, and paracetamol in albino rats[2]. It is thought that *O. sanctum* is the "Queen of herbs". A phytochemical examination revealed the presence of numerous substances with pharmacological significance, alkaloids, glycosides, tannins, and saponins. Numerous bio-compounds

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that were taken from *O. sanctum* have been shown to have antibacterial, anti-inflammatory, and anti-oxidant properties in a prior study[3]. Hence, in the current investigation, is to find the phytochemical in the plant leaves will have the anti-microbial and anti-cancer activity in both *in silico* and *in vitro* studies.

2. Materials and Methods

2.1 Extraction

The robust, disease-free leaves of *O. sanctum* leaves were gathered at the Thanjavur Botanical Garden in Tamil Nadu, India. We completely cleaned fresh leaves of *O. sanctum* leaves using distilled water. Then hot air oven is used to dry the leaves under 45ºC and powdered by grinding machine. using a Soxhlet extractor, the water, chloroform, and hexane extract were extracted from the powder. For later use, the dried extract was kept in a refrigerator at 4ºC in an airtight container. The extract was analyzed using conventional techniques including qualitative and quantitative analysis[4].

2.2 Qualitative Screening of Phytochemicals

The following techniques were performed to analyse the phytochemicals present in the *O. sanctum* leaves extracts Wagner's test, sodium hydroxide test, copper acetate test, Salkowski Test, foam test, ferric chloride test, lead acetate test, Borntrager's test, fluorescence test, Kellar Killani's test, Spot test[5].

2.3 Total Phyto-constituents Quantification

The phyto-constituents of the glycine max seed extracts were measured for total alkaloid, total flavonoid, total phenolic, total saponin, and total tannin content as per the stated[6].

2.4 GC-MS Quantification of Phytochemicals

The GCMS-QP2010 SE was used to quantify the phytoconstituents in the glycine max seed extracts. An RTX-5 MS capillary column of 0.25m in diameter and 25m in length was used to detect and quantify chemical components. The GC operated within a temperature range of 45°C to 290°C, with a 5°C rise every minute. Oven and injection port temperatures were set to 115°C and 295°C, respectively. The flow rate was 1.5 mL/min, and he was used as a mobile phase carrier gas. The interface temperature was 290°C, and the ionizer temperature was 235°C. The Wiley library was used to compare the chemicals, and the detector voltage was adjusted to zero.

2.5 DPPH Antioxidant Activity

Using the DPPH test, the extract of *O. sanctum* leaves radical-scavenging ability was determined. Different concentrations of the plant extract were made as the stock solution. Each concentration of the extract was incubated for 30 minutes in a dark environment after 1 ml of DPPH was added. At 520 nm, the plant extract's absorbance was finally computed. The standard solution utilized was ascorbic acid[7]. The formula was used to determine the proportion of scavenging activity.

$$\text{Percentage inhibition} = \frac{(\text{Absorbance of control Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

2.6 Antibacterial Activity

The paper disc method is used to determine the antibacterial activity of the plant extract. The respective nutrient agar medium was prepared and autoclaved at 121°C for 30-45 min to sterilize the contaminating microorganisms[8]. Then it was allowed to cool down at room temperature and when it reached nearly 50°C, the media was poured into the culture plates and allowed for further solidification. Then the overnight grown *S. aureus* culture was added to the plates by pour plate method and incubated at 50°C for 12-24 hrs. The extract along with the standard and control were tested in the plate and zone of inhibition was measured[9].

2.7 Molecular Modelling

The three-dimensional structures of MepR (PDB ID: 4LLL). This got back from the protein Data bank (PDB) and the excess chains, bound molecules, and ions were removed and the protein was prepared for docking. The three-dimensional structures of oleanolic acid (PubChem ID: 10494) were retrieved from PubChem and the energy minimization of the ligand was performed and the ligand was prepared for the docking[10].

2.8 Drug Likeness and ADME Evaluation

The drug-likeness properties by Lipinski's /Pfizer's rule of five, ADME properties of the oleanolic acid were evaluated using Molinspiration. Lipinski's rule states that the H-bond donor and H-bond acceptor should

be less than 5 and 10 respectively. Also, the molecular weight and partition coefficient (log P) should be less than 500 Da and 5 respectively. ADME properties including hydrophilicity (High solubility: -3 to -1), lipophilicity (high solubility: 0 to 5), and GI absorption and bioavailability (0.55- 10% oral availability in animal models) of the drugs in the body were also evaluated[9].

2.9 MTT Assay

Assemble cells and test chemicals onto 96-well plates with a final capacity of 100 µl per well. For the intended length of exposure, incubate. To reach a final concentration of 0.45 mg/ml, add 10 µl of MTT solution each well. Keep the incubation temperature at 37°C for one to four hours. To dissolve formazan crystals, add 100 µl of Solubility solution to each well. To guarantee total solubilisation, mix. Note the absorbance at 570 nm.

3. Results and Discussion

3.1 Qualitative Determination *O. sanctum* Leaves

The chemical components of an extract of *O. sanctum* leaves in several solvents (hexane, chloroform, and aqueous) are shown in Table 1. Numerous phytoconstituents, including alkaloids, terpenoids, phenol, tannin, anthraquinone, steroids, proteins, and amino acids, were found in the result. In addition, compared to other extracts, the aqueous extract displayed a large amount of secondary metabolites.

3.2 Quantitative Determination *O. sanctum* Leaves

The above-mentioned Table 2 represents the Quantitative determination of chemical constituents (%) of hexane, chloroform and aqueous extracts of *O. sanctum* leaves respectively. In general, the higher composition of alkaloids, flavonoids, and phenolics presence in the plant extracts corresponds to significant pharmacological properties while the saponins and tannins presence involved in lower pharmacological activities. From the above tables 1 and 2, it is evident that the aqueous extracts of *O. sanctum* show a higher presence of the phytoconstituents, and these extracts were chosen for further studies.

3.3 GCMS Analysis

The water leaf extract of *O. sanctum* was subject to the GCMS study and the phytoconstituents in this study we

Table 1. Initial phytochemical screening for different *O. sanctum* leaf extracts

S. No.	Chemical constituent	Hexane extract	Chloroform extract	Aqueous extract
1	Alkaloids	positive	positive	Highly positive
2	Anthraquinones	Negative	Negative	positive
3	Flavonoids	positive	positive	Highly positive
4	Glycoside	Negative	positive	Negative
5	Protein and Free amino acid	Negative	Negative	positive
6	Phenol	Negative	Negative	Highly positive
7	Quinones	Negative	Negative	Negative
8	Reducing sugar	Negative	positive	Negative
9	Saponins	positive	positive	Negative
10	Steroids	Negative	Negative	positive
11	Terpenoids	positive	Negative	Highly positive
12	Tannins	Negative	positive	positive

Table 2. Quantitative determination of phytoconstituents of various extracts of *O. sanctum* leaves

S. No.	Extracts	Quantitative determination of chemical constituents (%)				
		Alkaloids	Flavonoids	Saponins	Tannins	Phenols
1	**Aqueous extract**	28.80±0.75	21.50±0.25	2.25±2.25	4.15±1.45	16.50±0.85
2	**Chloroform extract**	20.45±1.5	14.60±1.15	2.65±1.755	2.02±1.55	10.20±1.05
3	**Hexane extract**	19.65±0.95	17.75±1.50	1.85±1.85	3.95±1.35	7.80±0.50

found a number of phytoconstituents in that oleanolic acid shows higher concentration and has several pharmaceutical activities in the yearly study shown in this study we take oleanolic acid for molecule docking.

3.4 DPPH Radical Scavenging Activity

The findings showed that at higher extract concentrations, *O. sanctum* leaves had a significant capacity to scavenge radicals, specifically 2,2-diphenyl-1-picrylhydrazyl. Using ascorbic acid as a reference, the absorbances were measured at 570 nm (Table 3).

3.5 Antibacterial Activity

The antibacterial activity of the aqueous leaf extract of *O. sanctum* against *S. aureus* was performed by paper disc method of disc size 5 mm. The antibacterial activity and the zone of inhibition produced by the sample along with the control and standard were given in respectively. The zone of inhibition created by the sample was about 11 mm and it was close and even higher to the standard ampicillin used in the test (Table 4).

3.6 Molecular Modelling Studies

Oleanolic acid and the Efflux pump MepR were set up and docked using Autodock 4.2 mode. Table 5 provides the binding energies in Kcal/mol as well as the interacting hydrophobic residues located around 4 Å from the protein's ligand. For the drug-likeness determined by Lipinski's Rule of Five and the oleanolic Acid's ADME characteristics predicted by molinspiration. Molecular docking, drug-likeness, and ADME properties evaluation were used to assess the binding affinities of the protein-ligand complex, physiochemical properties such as molecular weight and formula, Lipinski's filters such as H-bond donor and H-bond acceptor, Molecular mass (Da), liphophilicity (LogP), and ADME properties (Tables 6 and 7). The protein-ligand interaction surface models and cartoon models are displayed in Figures 1 and 2 respectively.

Table 3. DPPH radical scavenging activity of distilled water extract of *O. sanctum*

S. No.	Concentration(µg\ ml)	Absorbance at 570nm	
		Ascorbic Acid	*Ocimum sanctum*
1	Blank	0.0	0.0
2	0.2	82.90	35.4
3	0.4	86.78	38.78
4	0.6	87.65	51.66
5	0.8	91.57	58.34
6	1.0	93.45	62.49

3.7 MTT Assay

The MTT assay was performed to determine the cytotoxic potential of the compound/extract on the U937 lymphoma cell line. The different concentrations (20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml, and 100µg/ml) along with the control were estimated for their cytotoxicity on the lymphoma cancer cells. The results showed that the IC_{50} concentration of the sample was nearer to the 60µg/ml of the sample treated on the lymphoma cancer cells. The cell viability and the cytotoxic profiles of the sample were given in respectively (Table 8 and Figure 3).

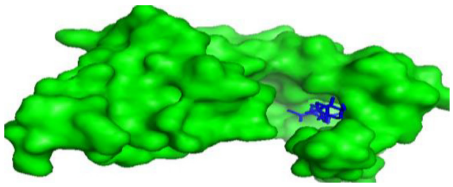


Figure 1. Surface model of MepR protein-oleanolic acid.

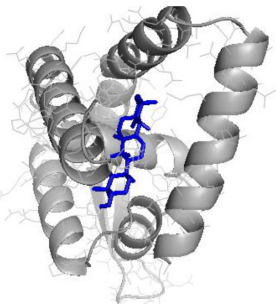


Figure 2. Cartoon model of MepR protein -oleanolic acid.

Table 4. Zone of inhibition of aqueous leaf extract of *O. sanctum*

S. No.	Microorganism Name	Extract	Zone of inhibition in mm		
			Standard (mm)	Control	Sample (mm)
1	*S. aureus*	*O. sanctum* aqueous leaf extract	11	Nil	9

Table 5. Binding energy and interactions of protein-ligand complex

Compound name	Protein name	Binding energy (Kcal/mol)	Interacting Hydrophobic residues around 4 Å
Oleanolic acid	MepR	-4.7	PHE9, ILE12, SER13, MET16, LEU115, MET127

Table 6. Physiochemical and drug likeness properties of oleanolic acid

Properties	Values
Molecular formula	$C_{30}H_{48}O_3$
Molecular weight (g/mol)	456.7 g/mol
H-bond donor	2
H-bond acceptor	3
Lipophilicity (LogP)	6.06

Table 7. ADME properties of oleanolic acid

Properties	Values
Lipophilicity (LogP)	6.06
Hydrophilicity (LogS)	-7.32
Topological polar surface area	57.53 Å^2
Gastrointestinal absorption	Low
Blood-Brain Barrier permeability	No
Bioavailability	0.85
Synthetic accessibility	6.08

Table 8. MTT cytotoxicity assay

S. No.	Tested sample concentration (µg/ml)	Mean value of live viability (%)
1	Control	100±0.00
2	100 µg/ml	28.65±1.68
3	80 µg/ml	40.25±2.15
4	60 µg/ml	49.65±2.36
5	40 µg/ml	59.35±1.54
6	20 µg/ml	71.85±1.98

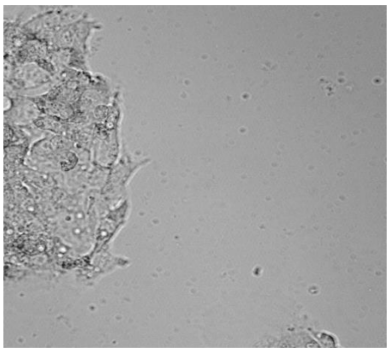


Figure 3. MTT assay of the sample at 60 µg/ml (Nearest value to IC_{50}).

4. Conclusion

The aqueous leaf extract of *O. sanctum* was extracted using Soxhlet's apparatus. The clear zone of inhibition of the extract clearly shows its antibacterial activity against *S. aureus.* The predominant compound oleanolic acid was docked against the MepR (PDB ID: 4LLL) of *S. aureus* which showed that the binding affinity is strong with binding energies -4.7 Kcal/mol. Also, it has passed Lipinski's rule of five and the ADME properties. We conclude that the *O. sanctum* has significant antibacterial activity against *S. aureus* and it can act against the lymphoma cancer cells.

5. Author contribution

R. Sumathi did the entire work of this manuscript. Author has read and agreed to the published version of the manuscript.

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