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Evaluation of Antimicrobial Activity and *In silico* DENV protease docking study of Oleoresin from wild *Piper nigrum* L.

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ABSTRACT

Wild *Piper nigrum* belongs to the family *Piperaceae* and is naturally distributed in India in the Western Ghats. The Phytochemical study has shown that the extracts of *Piper* species enhance the bioavailability of certain drugs. Work has not been carried out on antimicrobial and antiviral activity of Oleoresin extracted from root and stem of wild *Piper nigrum*. Therefore in the present study Oleoresin was extracted from the roots and stem of wild *Piper nigrum* collected from Agumbe forest of Karnataka. The extracted Oleoresin has been evaluated for its *in vitro* antimicrobial activity using the Filter paper disc method and Cut well method. Five bacteria (*Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Salmonella typhi* and *Staphylococcus aureus*) and five fungi (*Aspergillus niger, Aspergillus flavus, Penicillium spp, Drechslera spp* and *Fusarium spp*) were used as test organisms in the assay. The results revealed good antibacterial activity compared to its antifungal activity. Oleoresin showed maximum activity against *B. subtilis* and *Penicillium spp.*

Dengue is caused by an RNA virus named *Flavivirus* from *Flaviviridae*. Currently, there is no licensed drug available and to date, several vaccine candidates are still under development against the Dengue virus (DENV). So an attempt was done to test the inhibitory activity of Oleoresin against DENV protease 2VBC by *In silico* docking. This revealed potent inhibitory activity of Oleoresin against DENV protease 2VBC with the binding energy of -7.2kcal/mol. Furthermore, the study indicated that the potent inhibitor can be subjected to chemical modifications and tested for its cytotoxicity assay by *in vitro* studies. It is worth attempting to study the effects of this bioactive compound on as many bacteria and fungi as possible so that the potency of the compound could be fully understood and applied in drug development.

Keywords- *Piper nigrum* L., Oleoresin, Antimicrobial assay, DENV, Potent inhibitor.

INTRODUCTION

Genus *Piper,* the largest in the family *Piperaceae*, is distributed throughout the tropical and subtropical regions of the world. One hundred and eight species of *Piper* have been reported from the Indian subcontinent (Rahiman and Nair, 1983). Among these twenty-six are from the Western Ghats and the adjacent peninsular and coastal regions (Index Keweensis Plantarum Phanerogamarum | Nature, n.d.)(Gamble, 1915)(Rahiman, 1981)(de Candolle, 1912).

Species belonging to the genus Piper are very rich in aromatic constituents (Parmar et al., 1997). The Phytochemical study has shown that the extracts of Piper species enhance the bio-availability of certain drugs (Atal et al., 1981). The Western Ghats of the Indian peninsula are considered to be the center of origin of *Piper nigrum*, the source of medicinally and commercially very important 'Black Pepper'.

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Wild *Piper nigrum* is a climber that grows more than 10m in length adhering to the trunks of supporting trees. Fruits of *Piper nigrum* are used as a spice and also serve as remedies in Ayurveda, Siddha and Unani medicine to aid digestion, improve appetite, treat cough, cold, toothache, breathing, heart problems and skin diseases. 'Trikatu' is an ayurvedic preparation of black pepper, long pepper and ginger prescribed for a variety of diseases (Johri and Zutshi, 1992).

Studies have shown the antimutagenic properties of Bell pepper (*Capsicum annum*) and Black pepper (*Piper nigrum*) on *Drosophila melanogaster* (El Hamss et al., 2005).

Dengue virus (DENV) infection causes dengue fever (DF), dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) (Deen et al., 2006)(Dengue- The Lancet, n.d.) DENV is transmitted to humans through the Aedes mosquito and exists in four distinct serotypes. This has led to the challenge in DENV vaccine development (Monath, 1994).The study revealed the antioxidant property of Oleoresin from *Zingiber officinale* (Singh et al., 2008).

Despite the importance of *Piper nigrum* and related species in the field of pharmacology, chemistry and medicine, very little work has been reported on the organic constituents of these plants and their biological activities. Therefore the present investigation aims to extract organic constituents from wild *Piper nigrum* and to evaluate the isolated compounds for their antimicrobial assay using a broad selection of pathogenic microbes and also to study its antiviral activity against 2VBC DENV protease.

MATERIALS AND METHODS

Extraction of Oleoresin from wild *Piper nigrum*

Roots and stem of wild *Piper nigrum* were collected from Agumbe forest of Karnataka. The collected material was dried in an oven at a constant temperature of 40^0 C, powdered and used for the extraction.

Oleoresin was extracted from dried plant material by the glass column method. 50 grams of powdered material (root and stem) was taken in a glass column with glass wool at the bottom. 100ml of acetone was added to this and a contact time of 30 to 60 minutes was given by closing the column. Then the solvent was drained off and the process was repeated twice using 50 ml each of fresh acetone. The drained off extract was collected and allowed to evaporate. The thick green liquid constituted Oleoresin.

Bacterial and Fungal strains

Bacterial strains (*Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis Salmonella typhi* and *Staphylococcus aureus*) and Fungal strains (*Aspergillus niger, Aspergillus flavus, Penicillium spp, Drechslera spp* and *Fusarium spp)* were used as test organisms

Antimicrobial assay

Oleoresin isolated from *P.nigrum* root and stem was tested for its antibacterial and antifungal activity. The method adopted for this was the agar diffusion assay (Bocker, 1980).

Antibacterial assay of Oleoresin (500µg) was carried out using filter paper discs. Readymade discs of streptomycin from Himedia Private Ltd., Mumbai (10µg/disc) was used as positive control. Filter paper discs were dipped in Oleoresin, drained off and placed on seeded nutrient agar medium. After incubation at 37^0C for 24 hours, the Petri plates were screened for the presence of an inhibition zone around each disc.

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Antifungal assay of Oleoresin (500µg) was carried out using filter paper disc and cut well method in Potato Dextrose agar medium. Actidione (10µg/10ml) was used as a standard antifungal agent. About 0.1ml of standard and sample solutions were applied to different wells. The Petri plates were incubated at 28^0C for 48 hours. They were then screened for their activity.

Docking study

Molecular docking of the receptor and the ligand was carried out using AutoDock vina (Weigelt, 2010)(Kalyaanamoorthy and Chen 2011). For the docking studies crystal structure (PDB ID-2VBC) of dengue virus, NS3 protease was used. The structure was obtained from Protein Data Bank in .pdb format and subjected for optimization by removing all the heteroatoms and water molecules. This was used as a receptor in the docking study.

The grid box for Vina search space with centre x = -17.918, y =4.7193, z = 0.6507 and dimensions (A^0) with x=28.0298, y=28.8676 and z-36.8034 was chosen for the protein 2VBC on their active sites (Asp-75, Lys-104, Asn-105, Lys-117, Leu-119, Ala-125, Asp-129, Phe-130, Lys-131, Gly-133, Ser-135, Ile-139, Asn-152, Asp-160, Ser-163, Pro-174, Asp-179, Phe-183, Lys-185, Thr-200, Lys-201, Arg-202, Ile-203, Leu-212, Lys-213, Arg-215, Arg-217)

Molecular docking was carried out with the Oleoresin and the receptor (2VBC) on NS2B-NS3protease using AutoDock Vina. The best-ranked model with the lowest binding energy was analyzed further and visualized using Ligplot software.

RESULTS

Antimicrobial assay and *in silico* docking studies of Oleoresin revealed the following results.

Results of antibacterial activity

The results showed good antibacterial activity of Oleoresin in comparison with the streptomycin. *Bacillus subtilis* was found to be more sensitive to Oleoresin extract of root and stem (Plate 1 and Plate 2) followed by *Staphylococcus aureus, Salmonella typhi, Pseudomonas aerugenosa* and *E. coli* with different inhibition zone as tabulated in Table 1.

	Diameter of zone of inhibition (in mm)		
Test Organisms	Oleoresin from Root (500µg)	Oleoresin from Stem (500 µg)	Streptomycin (10µg)
Bacillus subtilis	30	29	26
Staphylococcus aureus	24	22	19
Pseudomonas aerugenosa	22	20	21
Salmonella typhi	24	20	15
E. coli	17	16	14

Table 1. Antibacterial activity of Oleoresin extract of root and stem.

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Plate 1: Antibacterial activity against B.subtilis with Oleoresin root extract.

Plate 2: Antibacterial activity against B.subtilis with Oleoresin stem extract.

Results of antifungal activity

The Antifungal activity also revealed the inhibition of *Penicillium spp* (Plate 3 and Plate 4) by the Oleoresin extract of root and stem followed by *Fusarium spp*, *Aspergillus flavus*, *Aspergillus niger* and *Drechslera spp* with different inhibition zone as tabulated in Table 2.

	The diameter of zone of inhibition (in mm)		
Test Organisms	Oleoresin from Root (500µg)	Oleoresin from Stem (500 µg)	Actidione (10µg)
Penicillium spp	24	16	21.5
Fusarium spp	19	11	11
Aspergillus flavus	16	10	20.3
Aspergillus niger	14	9	19.5

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Drechslera spp	11	8	16

Table 2. Antifungal activity of Oleoresin extract of root and stem.

Plate 3: Antifungal activity against Penicillium spp with Oleoresin root extract.

Plate 4: Antifungal activity against Penicillium spp with Oleoresin stem extract.

Results of docking study

The ligplot software analysis of Oleoresin showed the hydrophobic interactions- *Glu-230, Glu-66, Glu-233, Arg-418, Trp-402, Lys-201, Lys-357, Gly-420, Glu-468, Tyr-354, Glu-333, Trp-350* and hydrophilic interactions- *Lys-199, Asn-416, Asn-464* against DENV protease, 2VBC with the binding energy of -7.2kcal/mol as shown in Fig 1.

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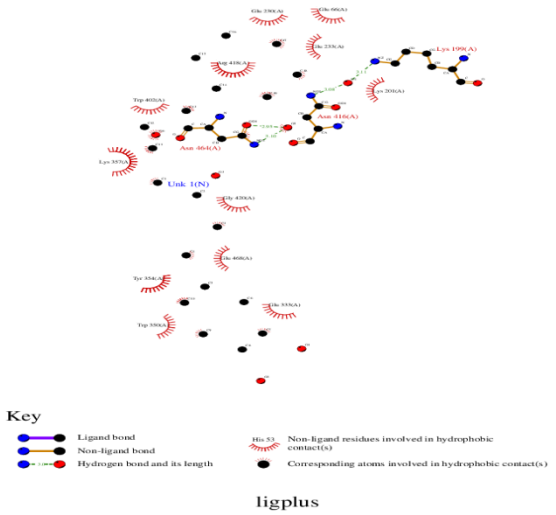

Fig 1. Ligplot of Oleoresin with 2VBC.

Hydrophobic interactions- Glu-230, Glu-66, Glu-233, Arg-418, Trp-402, Lys-201, Lys-357, Gly-420, Glu-468, Tyr-354, Glu-333, Trp-350.

Hydrophilic interactions- Lys-199, Asn-416, Asn-464.

DISCUSSION

Although several antibiotics are widely used in medicine, the search for antimicrobial substances from plants will continue as better and safer drugs to combat bacterial and fungal infections because of their biodegradable nature and being relatively safer for human beings. The results of the antimicrobial activity indicate that Oleoresin is a good antibacterial agent but not so effective antifungal agent against the organisms tested. The extract of Oleoresin from both root and stem showed almost similar activity.

Among the five bacteria screened for their response to Oleoresin, all were found to be sensitive. Out of the five sensitive bacteria, *Bacillus subtilis* (Gram- positive) showed maximum sensitivity followed by *Staphylococcus aureus* (Gram-positive), *Pseudomonas aeruginosa* (Gram-negative), *Salmonella typhi* (Gram-negative) and *E. coli* (Gram-negative). Oleoresin is found to be more effective against *Bacillus subtilis* and slightly less effective against *E. coli*. Gram-positive bacteria showed better inhibition than Gram-negative bacteria. Significant antibacterial activity was observed in all bacteria.

A study of antifungal activity of Oleoresin showed maximum inhibitIon with Penicillium spp. Oleoresin exhibited poor inhibition in the case of *Fusarium spp, Aspergillus flavus, Aspergillus niger and Drechslera spp.* In comparison to the antibacterial activity, Oleoresin was not much effective as an antifungal agent.

The results indicated potent inhibitory activity of Oleoresin with DENV protease, 2VBC with the binding affinity of -7.2kcal/mole (Hydrophobic interactions- *Glu-230, Glu-66, Glu-233, Arg-*

418, *Trp-402*, *Lys-201*, *Lys-357*, *Gly-420*, *Glu-468*, *Tyr-354*, *Glu-333*, *Trp-350;* Hydrophilic interactions- *Lys-199*, *Asn-416*, *Asn-464*).
Thus it can be concluded that Oleoresin is more potential to be used as an antibacterial agent, antifungal agent and also as an inhibitor of DENV protease. Furthermore, studies can also be designed to synthesize these compounds or the chemically modified compounds of these molecules and could be used for medical purposes.

Conflict of interests

There is no conflict of interest in the present study.

Ethical considerations

The present study didn't involve animals hence did not require approval by the ethical committee.

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