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Antibacterial, Synergistic and β-lactamase Inhibitory Activity of *Oroxylem indicum*: An *In vitro* and Molecular Interaction Study

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Authors' contributions

This work was carried out in collaboration between all authors. Author AM designed the study, performed the experiments, wrote the protocol, and wrote the first and final draft of the manuscript. Authors SB, LS and PB performed the statistical analysis of the study and managed the literature searches. Author SD read and approved the final manuscript.

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ABSTRACT

Aims: The study was aimed to evaluate the antibacterial, synergistic and β-lactamase inhibitory potential of *O. indicum* against ampicillin resistant and Extended Spectrum β-lactamase (ESBL) producing bacterial strains.
Methods: Bacterial strains were screened for ampicillin resistance and ESBL production by disk diffusion method and modified double disc synergy test respectively. Antibacterial and synergistic activities of *O. indicum* methanol extract and ethyl acetate sub fraction of methanol extract were explored by agar well diffusion method and Checkerboard method

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respectively. Extracts were subjected to Gas chromatography and Mass spectroscopy (GC-MS) analysis to identify the bioactive compounds. Molecular docking studies were carried out to verify the β-lactamase inhibitory potential of the bioactive compounds.
Results: All bacterial strains were found to be resistant to ampicillin and only one strain was detected as ESBL positive. Ethyl acetate sub fraction exhibited strong antibacterial and synergistic activity than the methanol extract. Zone of inhibition and Minimum inhibitory concentration for ethyl acetate sub fraction was 16 mm and 15mg/ml respectively. *In vitro* interactions between plant extracts and ampicillin evaluated in terms of fractional inhibitory concentration (FIC) indices revealed synergistic effects of plant extracts. The molecular docking studies of major bioactive compounds depicted by GC-MS analysis revealed that Wogonin, a flavonoid (GLIDE Score-5.77) possessed the best inhibitory profile against β–lactamase.
Conclusion: Synergistic activity of *O. indicum* may be attributed to the β–lactamase inhibitory potential of the bioactive compounds present in the extract. The findings provide substantial basis for the future use of *O. indicum* crude extracts as potential antibacterial and antibiotic modulating agent.

Keywords: Oroxylem indicum; antibacterial; synergistic; GC-MS analysis; docking.

1. INTRODUCTION

Infectious diseases are the largest source of premature deaths, accounting for 15 million people annually [1]. The World Health Organization estimates that nearly 50,000 people die each day throughout the world from infectious diseases. It is clear that the fight against the spread of the disease is one of the most serious challenges faced and the problem is aggravated when pathogens become resistant to antimicrobial agents. The discovery of antibiotics was an essential part in combating these infectious diseases. But the non-prudent use of antibiotics led to the emergence and dissemination of antibiotic resistant strains. The bacteria silently develop several ways; like metabolic pathway alteration, target site alteration, efflux pumps and enzymatic cleavage of antibiotics to resist antibiotics. Antibiotic resistance genes database lists the existence of more than 20,000 potential resistance genes from available bacterial genome sequences [2]. These resistance genes are further transferred from one bacterial species to other; in this way burgeoning the antibiotic resistance. The emergences of antibiotic resistant strains generate the impetus for systematic global search for more effective chemotherapeutic agents.

Nature always remains as an endless source for compounds of medicinal importance. It is inferred from various combinational or synergistic studies that plant crude extracts are the blend of many bioactive compounds that act as synergistic enhancers of the antibiotics [3]. The synergistic activities of plants could be based on the antioxidant, antimicrobial, anti-inflammatory, immunomodulatory etc. Effects of the phytochemicals present in them. Phytochemicals exert their synergistic potential by enzyme inactivation/ inhibition, efflux pump inhibition, substrate deprivation, membrane disruption or intercalating into cell wall/DNA [4]. Synergy between the plant extracts and antibiotic against resistant pathogens leads to new choices for the treatment of infectious diseases. Hence, there is need for continuous exploration of multidrug resistance modulating principles from plants sources.

O. indicum, an endangered forest tree, commonly known as Sonapatha belongs to the family Bignoniaceae. The plant has a strong ethno pharmacological background, one among the

group of ten drugs named Dasamoola, used as an active ingredient of various Ayurvedic formulations. It is used to treat disease like jaundice, asthma, sore throat, laryngitis, hoarseness, diarrhoea, dysentery, infantile, erythema and measles [5].

Earlier literature is rich with the reports of antimicrobial activity of *O. indicum* [6], but to the best of our knowledge, there are no reports regarding synergistic activity with β-lactam antibiotic ampicillin and β lactamase inhibitory activity of the plant against ESBL positive strains. Therefore, in consideration of the lack of literature, the present study was carried out to explore the antimicrobial and synergistic activity of the various extracts of *O. indicum* by *in vitro* analyses and β-lactamase inhibitory activity of the bioactive compounds elucidated via GC-MS analysis by *in silico* study. This study is the first integrative approach to investigate and correlate the antimicrobial, synergistic and β-lactamase inhibitory activity of *O. indicum.*

2. MATERIALS AND METHODS

2.1 Microbial Strains

Three Gram negative bacterial strains, *Escherichia coli* (MTCC 1885)*, Klebsiella pneumonia* (MTCC 4030) and *Pseudomonas aeruginosa* (MTCC 7453) were used during the study. The bacteria were procured from Institute of Microbial Technology (IMTECH), Chandigarh,

2.2 Antibiotic Discs

High potency discs of β-lactam *antibiotic, ampicillin* (10µg), amoxicillin-clavulanate (30µg), 3rd generation cephalosporins (3GC) cefotaxime (30µg), ceftriaxone (30µg), cefpodoxime (10µg) and 4th generation cephalosporins (4GC) cefepime (30µg) were purchased from Hi-media Pvt. Ltd., Bombay (India).

2.3 Collection of Plant Material

O. indicum leaves were collected from Botanical garden of Kurukshetra University, India during the period 2012-2013. Specimens were identified and authenticated by*,* Department of Botany, Kurukshetra University, Kurukshetra. The leaves were thoroughly washed with tap water followed by distilled water, dried under shade for a week and ground into fine powder. After sieving (80 mesh) they were transferred to airtight polyethylene zipper bags, labeled and stored till further use. Voucher plant specimen was deposited at the Wild Life Institute of India, Dehradun, under specimen number GS441 for future reference.

2.4 Preparation of Plant Extracts and its Fractionation

The powdered plant leaves (100g) were soaked in methanol in a clean and dry reagent bottle covered with a lid at 37ºC for overnight. The extraction was done by hot continuous soxhlet extraction method. Resulting extract was evaporated and concentrated to dryness using the rotatory evaporator at 50ºC. The aqueous solution of methanol extract was transferred into a separating funnel for partition with ethyl acetate. The sub fraction was dried in rotary evaporator. The extract and sub fraction were stored at -4ºC till further uses.

3. *In vitro* STUDIES

3.1 Antibiotic Resistance Screening Test

Resistance of test strains to ampicillin and 3rd generation cephalosporins (3GC) was determined by the disk diffusion method as described by the Clinical and Laboratory standard Institute (CLSI) standards, 2008 [7]. The test strains were first enriched in Nutrient broth for 24h at 37ºC by picking-off technique. Using sterile swab sticks, plates were seeded with 1ml of suspension of the test strains containing approximately 10^6 cells. Antibiotic discs were dispensed on the plates seeded with organisms. The plates were incubated at 37ºC for 24h and antibiotic resistance was interpreted by inhibition zones.

3.2 Modified Double Disc Synergy Test

Bacterial strains displaying diameter less than 27mm, 25mm and 17mm for cefotaxime, ceftriaxone and cefpodoxime respectively, were tested for ESBL production by modified double disc synergy test. Amoxicillin-clavulanate (20/10µg) disc was placed in the centre of the seeded plate and the discs of 3GC and 4GC were placed 15mm and 20mm apart respectively from the central disc. Any distortion in the zone of the antibiotic discs towards the disc of amoxicillin-clavulanate was considered as positive for the ESBL production [8].

3.3 Antibacterial Susceptibility Assay

Susceptibility of the ESBL positive strains to the methanol extracts and ethyl acetate sub fraction were determined using agar well diffusion method. The selected bacterial strains (10^6 CFU) were cultured on nutrient agar by using spread plate technique and wells of 8mm diameter were made for loading the extracts. Each of the bored wells was filled with 100 µl of different sample preparations. The concentration of plant extract/ sub fraction used was 30mg/ml. The plates were incubated at 37ºC for 24h and the diameter of zone of inhibition was taken as the measure of antibacterial activity of a particular extract. The negative control was 10% DMSO. Ciprofloxacin (10.0µg/ml) was used as positive control. The experiments were performed in triplicates and the mean values of the diameter of inhibition zones±standard deviations were calculated.

3.4 Minimum Inhibitory Concentration

MICs of antibiotic ampicillin and methanol extracts/sub fraction were determined by micro dilution technique using 96-well microtiter plates as described by the National Committee for Clinical Laboratories standards, 2000 [9]. The bacteria inoculums were prepared in 5ml nutrient broth and incubated at 37ºC. The final inoculums were of approximately 10^6 CFU/ml. Controls with 0.5ml of culture medium without the antibiotic/extract samples and other without microorganisms were used in the tests. Tubes were incubated at 37ºC for 24h. The activity was measured as a function of turbidity at 660nm. Lack of turbidity was further confirmed by pouring suspension aliquot of 0.1ml into pre-sterilized Petri dishes with nutrient agar medium. The tests were conducted in triplicate.

3.5 Synergy Testing by Checkerboard Methods

The antibacterial effects of combining methanol extract and ethyl acetate sub fraction with antibiotic ampicillin were assessed using a checkerboard method [10]. The range of concentration of extract/ethyl acetate sub fraction and ampicillin used in the checkerboard assay was such that the dilution range encompassed the MIC for each extract/ sub fraction and ampicillin.

The fractional inhibitory concentration (FIC) was derived from the lowest concentrations of the extract or ethyl acetate sub fraction and the antibiotics in combination permitting no visible growth of the test organisms [11]. FIC indices were calculated using the formula: FIC index=(MIC of extract or sub fraction in combination/MIC of extract or sub fraction alone)+ (MIC of antibiotics in combination/MIC of antibiotics alone). In agreement with Petersen et al. [12], synergy was defined as $\Sigma FIC \leq 0.5$, additivity as $0.5 < \Sigma FIC \leq 1$, indifference as $1 < \Sigma FIC \leq 4$, and antagonism as $\Sigma FIC > 4$.

3.6 GCMS Analysis

O. indicum extracts were subjected to GC-MS analysis to identify the phytocomponents responsible for the antibacterial and synergistic activity. The tested extracts were analyzed by GC-MS using Shimadzu Mass Spectrometer-2010 series. 1µl of sample was injected in GC-MS equipped with a split injector and a PE Auto system XL gas chromatograph interfaced with a Turbo-mass spectrometric mass selective detector system. The MS was operated in the EI mode (70eV). Helium was employed as the carrier gas and its flow rate was adjusted to 1.2ml/min. The analytical column connected to the system was an Rtx-5 capillary column (length-60m×0.25mm i.d., 0.25µm film thickness). The column head pressure was adjusted to 196.6 kPa. Column temperature programmed from 100ºC (2min) to 200ºC at10ºC/min and from 200º to 300ºC at 15ºC/min withhold time 5 and 22min respectively. A solvent delay of 6 min was selected. The injector temperature was set at 260ºC. The GC-MS interface was maintained at 280ºC. The MS was operated in the ACQ mode scanning from m/z 40 to 600.0. In the full scan mode, electron ionization (EI) mass spectra in the range of 40–600(m/z) were recorded at electron energy of 70 eV. Compounds were identified by comparing mass spectra with library of the National Institute of Standard and Technology (NIST), USA/Wiley.

3.7 Docking Studies

Docking software of Schrodinger suite was used for this study. The X-ray crystal structure of the proteins β–lactamase (PDB ID: 1KE4) with refinement of 1.72Å was downloaded from the RCSB protein databank. Protein preparation and refinement studies were performed on 1KE4 using protein preparation module (Schrodinger suite, LLC). The structures of the phytochemicals revealed by GC-MS analysis and standard clauvenic acid were downloaded from Pubchem. Ligand structures were geometrically minimized using OPLS_2005 force field by Ligprep module of Maestro 9.1. The prepared ligands can be used for docking. Docking was carried out using GLIDE. GLIDE uses a hierarchical series of filters to search for possible locations of the ligand in the active site region of the receptor. The docking was done in Extra Precision mode (XP). The docked protein and the ligands were viewed with Glide Pose Viewer. Non-bonded interactions like hydrophobic was observed using LigPlot program. Pharmaceutically relevant properties of phytochemicals were predicted by QikProp

3 module. ADME and drug likeliness of the prepared ligands were studied by QikProp module of Schrodinger.

3.8 Statistics

All experimental results are expressed as mean±standard deviation (SD) of three determinations. One-way analysis of variance was performed on the means to determine whether they differed significantly, with $P<0.05$ being regarded as significant.

4. RESULTS AND DISCUSSION

4.1 Disc Diffusion Assay

Resistance profiling of the microbial strains was done by using β-lactam antibiotic ampicillin (Table 1). All three bacterial strains were found to be resistant to ampicillin. Results were interpreted according to the CLSI zone diameter interpretative standards, 2008. This result is in accordance with the previous work [13] which also reported about prevalence of resistance to β–lactams antibiotics in *P. aeruginosa* and *E. coli*.

Table 1. Antibiogram of the bacterial strains

Antibacterial agent	Disc content	Zone of inhibition (mm)		
		E.C	K.P	P.A
Ampicillin	10µg	R	R	R

E.C: E. coli, K.P: K. pneumoniae, P.A: P. aeruginosa. R: resistant

4.2 Modified Double Disc Synergy Test

Results were interpreted in accordance with Clinical and Laboratory standard Institute (CLSI) susceptibility guidelines. All the tested strains displayed diameter less than 27mm, 25mm and 17mm for cefotaxime, ceftriaxone and cefpodoxime respectively, so all the three strains were tested for ESBL production by modified double disc synergy test. *P. aeruginosa* was detected as ESBL producer as it showed a clear extension of the edge of inhibition towards the amoxicillin-clavulanate disk. Thus, further study was carried out by using the ESBL positive *P. aeruginosa* strain. Our results are in agreement with earlier reports [14] which also reported ESBL production in *P. aeruginosa*. However, there are some reports [15] which reported the β lactamase production in *E. coli* also, which is contradictory to the present study. Differences among the results could be related to the use of strains with different resistance profiles and different MTCC numbers.

4.3 Antibacterial Susceptibility Testing

Antibacterial activity was exhibited by both the methanol extract as well as sub fraction. The zone of inhibition for methanol extract was 12mm (Table 2). Similarly ethyl acetate sub fraction of *O. indicum* (ZOI 16mm) possessed significant inhibitory activity. Antibacterial activity of *O. indicum* was also reported by earlier reports [16].

Table 2. Zone of inhibitionin millimeter (mm) produced by methanol extract/ethyl acetate sub fraction (30mg/ml) against ESBL positive *P. aeruginosa*

Microorganism	M.E	E.F	CIP
P. aeruginosa	12.00±0.00	16.00±1.00	23.00±0.00

M.E: Methanol extract, E.F: Ethyl acetate sub fraction, CIP: Ciprofloxacin

4.4 Minimum Inhibitory Concentration (MIC)

MIC is considered as the gold standard for determining the susceptibility of organism to antimicrobials. MIC for ampicillin was 50μg/mL, 30mg/ml for methanol extract and 15 mg/ml for ethyl acetate sub fraction (Table 3). The present findings revealed that same bacterial strain exhibited different levels of susceptibility to the different extracts. The differences in bacterial susceptibility to the extracts may be due to the presence of different bioactive compounds in different extracts.

Table 3. Minimum Inhibitory concentrations of methanol extract/ethyl acetate sub fraction (30mg/ml) and the ampicillin (10µg/ml) alone and in combination with ampicillin

Microorganism	M.E	E.F	AMP	M.E+AMP	E.F+AMP
P. aeruginosa	30	15	50	15,5	1.87,2.5

M.E: Methanol Extract, E.F: Ethyl Acetate sub fraction, AMP: Ampicillin

4.5 Synergy Testing by Checkerboard Methods

Combination studies revealed that *significant reduction was achieved in the MIC of the ampicillin in combination with plant extract (*Table 3). *Pseudomonas aeruginosa* strain resistant to ampicillin become sensitized in combination with extracts. The *in vitro* interaction between methanol extract/ sub fraction and ampicillin was evaluated in terms of fractional inhibitory concentration (FIC) indices (Table 4). The FIC indices revealed that ethyl acetate sub fraction of the methanol extract exhibited strong synergistic activity than the methanol extract. Ethyl acetate sub fraction-ampicillin combinations showed synergistic interaction while methanol extracts showed additive interaction. The exact mechanism for the reduction of β-lactam (ampicillin) resistance by the natural antimicrobials is unknown but it may be due to the enzymatic inhibition by the bioactive compounds present in the extract. As the tested strain was β-lactamase producer which confer resistance to the β-lactam antibiotics by catalyzing the cleavage of the β-lactam ring, so the synergistic potential may be due to the β-lactamase inhibition. *In silico* docking studies were performed to validate the enzymatic inhibition by plant extract.

It was inferred from the results that use of plant extracts and antibiotics could inhibit the growth of antibiotic resistant bacteria better than the use of plant extracts/ antibiotics alone. The plant extracts thus stand out as genuine source of potential resistance modifying agents. Our results are in agreement with various reports which showed that plant extracts/ phytochemicals increase the activity of various antibiotics with significant reduction in the MICs of the antibiotics [17,18].

Table 4. Effects of combining methanol extracts/ethyl acetate sub fractions with ampicillin against ESBL positive *P. aeruginosa*

Microorganism	FIC A (M.E)	FIC B (AMP)	FIC	Remarks	FIC A (E.F)	FIC B (AMP)	FIC	Remarks
P. aeruginosa	0.50	0.10	0.60	Additive	0.12	0.05	0.17	Synergistic

M.E: Methanol Extract, E.F: Ethyl Acetate sub fraction, AMP: Ampicillin, FIC: Fractional Inhibitory Concentration, FIC A: MIC of plant extract tested in combination/MIC of plant extract tested alone, FIC B: MIC of ampicillin tested in combination/MIC of ampicillin tested alone, FICI=FIC A+FIC B

4.6 GCMS Analysis

GCMS of methanol extract and ethyl acetate sub fraction was conducted to determine the nature of phytochemicals which were responsible for antibacterial and synergistic activity. GC-MS chromatogram of methanol and ethyl acetate sub fraction of *O. indicum* are shown in (Figs. 1 and 2). Palmitic acid and citronellol were found to be most prevalent in *O. indicum* ethyl acetate sub fraction and methanol extract. Citronellol is strongly effective against several species of microbes [19], so it may be responsible for antibacterial activity of plant extracts. Other significant compounds revealed by GC-MS analysis were 5- Hydroxy methyl furfural, linoleic acid, oleyl alcohol, phthalic acid dibutyl ester, stigmastenol, stigmasterol, squalene, vitamin E and wogonin; having substantial medicinal properties.

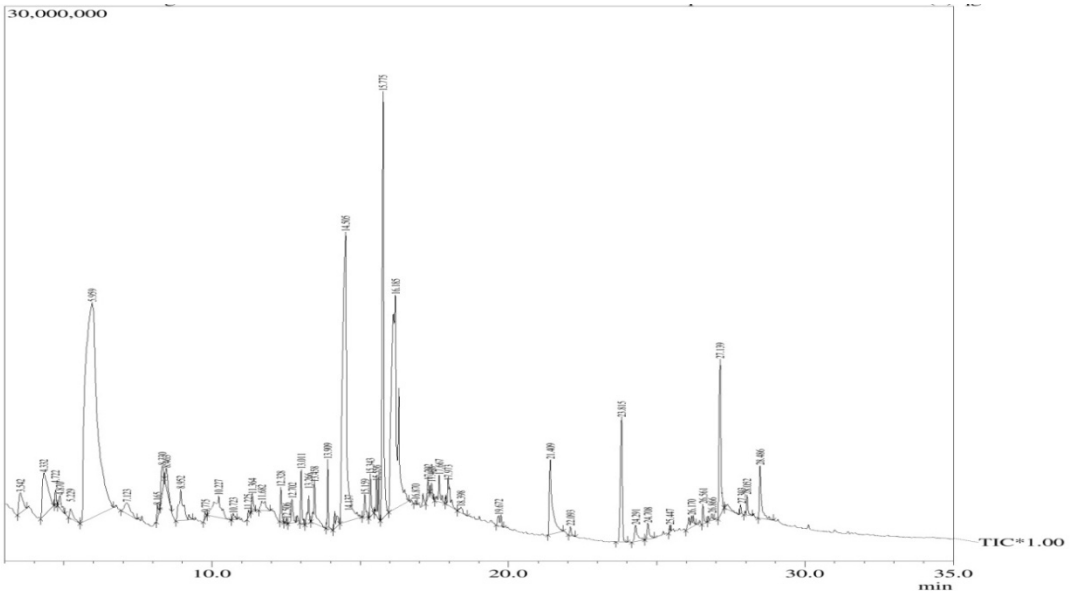

Fig. 1. GC-MS chromatogram of *O. indicum* methanol extract

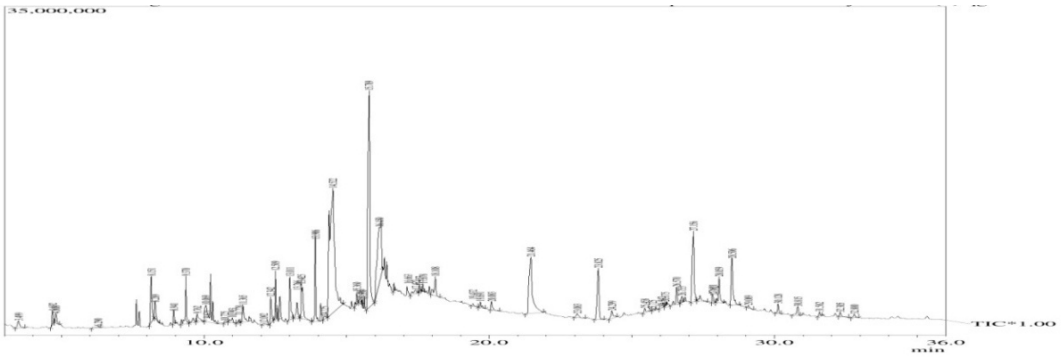

Fig. 2. GC-MS chromatogram of *O. indicum* ethyl acetate sub fraction

4.7 Docking Studies

Synergistic activity of *O. indicum*, as evidenced earlier by *in vitro* experiments may be due to the inhibition of β lactamase. The *in silico* docking studies were carried out to screen phytochemicals regarding their potential to inhibit β-lactamase. The GC-MS reports revealed high percentage of citronellol, 5-Hydroxy methyl furfural, linoleic acid, oleyl alcohol, palmitic acid, phthalic acid dibutyl ester, stigmastenol, stigmasterol, squalene, vitamin E and wogonin presence in methanol extract and ethyl acetate sub fraction. All compounds were subjected to ADME and *in silico* studies. Clauvenic acid as inhibitor of β-lactamase was also docked with the β –lactamase and used as reference in the study. The ADME profile showed that citronellol, stigmastenol, stigmasterol and wogonin exhibited good bioavailability (Table 5.1). Further all ligands were subjected to *in silico* studies to analyze their efficacy against the target proteins β–lactamase. The structures of the target proteins β–lactamase (*Escherichia coli*) was obtained from the Protein Data Bank and refined using the protein preparation wizard. The *in silico* study was done under the assumption that a high docking score will indicate strong inhibition of the enzyme The study revealed that wogonin, a flavonoid was the most active compound followed by stigmasterol, stigmastenol and citronellol with GLIDE Score of -5.77, -4.50, -4.36 and -3.79 respectively (Table 5.2).

Thus, Wogonin offered profound promise regarding inhibition of β–lactamase. The following amino acids: Glu 272, Leu274, Lys 67, Ser 64, Trp 271, Tyr 150, and Val65were found to be the key residues playing important role in stabilizing the complex (Figs. 3 & 4). The *in vitro* antimicrobial activity of flavonoids against Extended Spectrum β-Lactamase producing bacterial strains is also reported earlier [20] and this is further confirmed by our *in silico* studies that flavonoids have ability to reverse β–lactam resistance, thus can be co-administered as β-lactamase inhibitors to restore the activity of β-lactam antibiotics. The docking studies thus provide strong evidence that the molecular base of the synergistic activity is probably the presence of β–lactamase inhibitors in the extract.

Table 5.1. ADME profile of various phytochemicals

Standard/ herbal active compound	Lipinski's rule	Lead like rule	Human oral absorption	Caco2 cell permeability	MDCK cell permeability	Skin permeability	Blood brain barrier penetration
Clauvenic acid	Suitable	Suitable	Moderately absorbed	Low	Low	High	High
Citronellol	Suitable	Suitable	Well absorbed	High	High	High	High
5- Hydroxy methyl furfural	Suitable	Suitable	Moderately absorbed	High	Middle	High	High
Linoleic acid	Suitable	Suitable	Well absorbed	Middle	Middle	High	High
Oleyl alcohol	Suitable	Suitable	Well absorbed	High	High	Low	High
Palmitic acid	Suitable	Suitable	Well absorbed	Middle	Middle	High	High
Phthalic acid dibutyl ester	Suitable	Suitable	Well absorbed	Middle	Middle	High	High
Stigmastenol	Suitable	Suitable	Well absorbed	High	High	High	High
Stigmasterol	Suitable	Suitable	Well absorbed	High	High	High	High
Squalene	Suitable	Suitable	Well absorbed	High	High	Low	Low
Vitamin E	Suitable	Suitable	Well absorbed	High	High	Low	High
Wogonin	Suitable	Suitable	Well absorbed	High	High	High	High

Table 5.2. Molecular docking results of β–lactamase with different phytochemicals

Standard/ herbal active compound	Compound ID	Molecular weight	XP glide score with β –lactamase	Number of hydrogen bond	Interacting residues
Clauvenic acid	CID:5280980	199.16	-2.16	2	Arg148, Glu272, Ser64, Thr68, Tyr112, Trp271 and Val65.
Citronellol	CID:8842	156.26	-3.79	1	Glu272, Gln256, Leu238, Met273, Trp271 and Val234
5- Hydroxy methyl furfural	CID:237332	126.11	-3.76	3	Glu272, Tyr112 and Ser64
Linoleic acid	CID:5280450	280.24	-2.33	1	Ala31, Ala231, Glu272, Gln256, Ile33, Ile227 , Met230, Met273,Val30, Val65 and Val234
Oleyl alcohol	CID:5284499	268.27	-2.04	1	Ala31, Ala231, Glu272, Gln256, Ile33, Ile227, Val65 and Val234.
Palmitic acid	CID:985	256.24	-2.20	2	Arg258, Asn237, Glu272, Gln256, Ile33, Leu238, Leu274 and Val234.
Phthalic acid dibutyl ester	CID:20393	278.34	-2.75	2	Arg258, Glu272, Ile283, Leu274, Met273, Trp276 and Val278.
Stigmastenol	CID:3080632	414.71	-4.36	1	Ala29, Ala31, Gly270, Leu62, Leu238, Met230, Thr68, Trp271, Val30, Val65 and Val234.
Stigmasterol	CID:5280794	412.69	-4.50	1	Ala29, Ala31, Ala 231, Gly27, Leu62, Leu238, Met28, Met230, Thr68, Trp271, Val30, Val65 and Val234.
Squalene	CID:638072	410.72	-5.80	2	Ala29, Ala31, Ala 231, Gly27, Leu62, Leu238, Met28, Met230, Thr68, Trp271, Val30, Val65 and Val234.
Vitamin E	CID:14985	430.71	-4.63	1	Ala29, Ala31, Ala 231, Glu272, Gln256, Leu62, Leu274, Met28, Met273, Thr68, Trp271, Val30, Val65 and Val234.
Wogonin	CID:5281703	284.26	-5.77	2	Glu 272, Leu274, Lys 67, Ser 64, Trp 271, Tyr 150, and Val65.

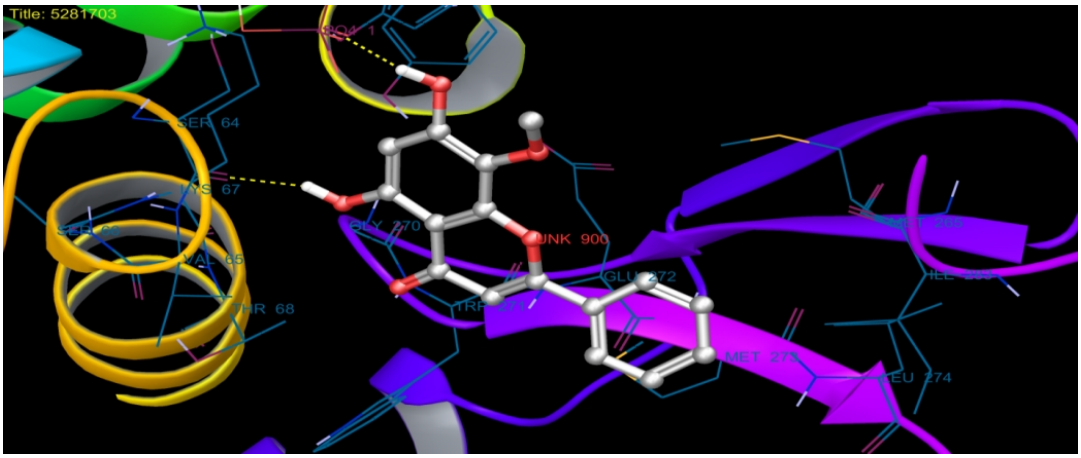

Fig. 3. Docking of β–lactamase with Wogonin

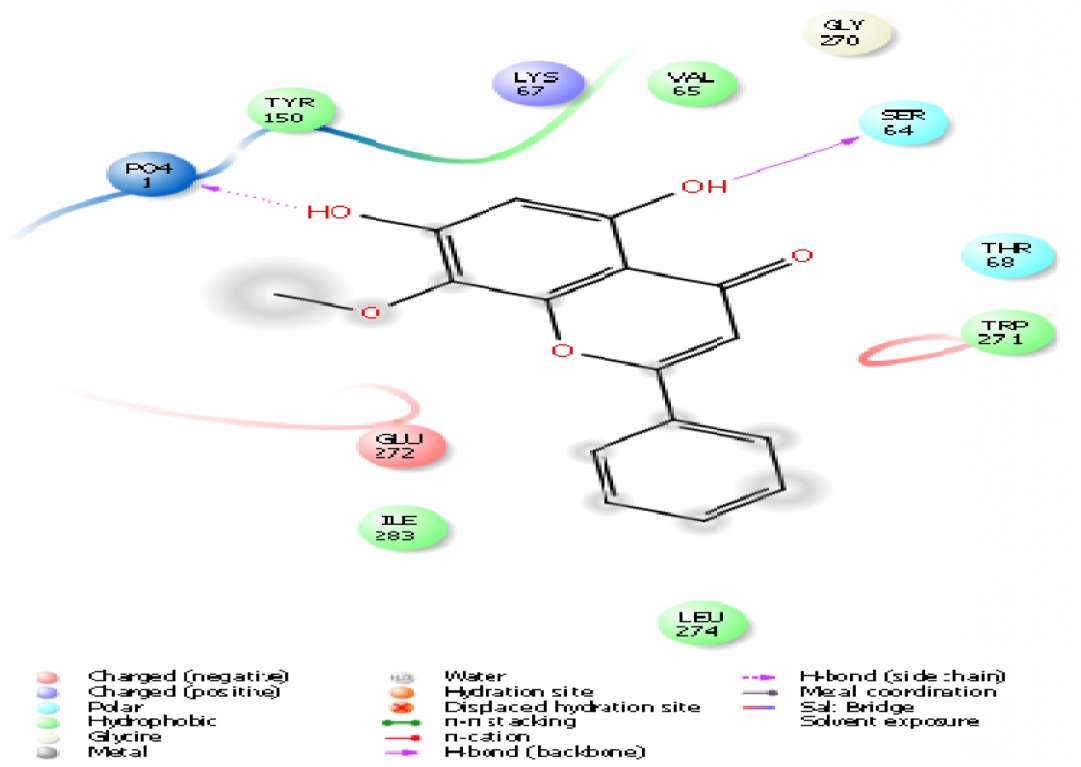

Fig. 4. Ligplot image of interaction between β–lactamase with Wogonin

5. CONCLUSION

The presence of medicinally significant phytocomponents in the *Oroxylem indicum* extract implies the pharmaceutical importance of the plant. The results demonstrated a significant antibacterial and synergistic activity of *O. indicum* ethyl acetate sub fraction and methanol

extract against ESBL positive *P. aeruginosa* strain. The docking studies confirmed the β–lactamase inhibitory potential of the bioactive compounds revealed via GC-MS analysis and suggested that the synergistic effect may be due to β–lactamase inhibitory property of bioactive compounds present in the plant. Thus the findings, for the first time, investigate and correlate the antimicrobial, synergistic and β-lactamase inhibitory activity of *O. indicum* and provide substantial basis for the future use of *O. indicum* crude extracts as potential antibacterial and antibiotic modulating agent. Further studies are needed on animal model to evaluate the bioactivity and to ascertain the pharmacological activity of the phytochemicals present in the plant.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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