In silico and *in vitro* Antimicrobial Assessment of *Oroxylum indicum (L).* Kurz stem bark extract against four Bacterial and Fungal Species

Sushma[1], Mohanraj Roshni Juliet[2], Bhat Nanditha[3], Revanasiddappa Bistuvalli Chandrashekharappa[3] and Sharmila Kameyanda Poonacha[1*]

1. Central Research Laboratory, K.S Hegde Medical Academy, Nitte (Deemed to be University), Deralakatte-575018, Mangaluru, Karnataka, INDIA
2. Department of Infectious Diseases and Microbial Genomics, Nitte University Centre for Science Education and Research, Nitte (Deemed to be University), Deralakatte-575018, Mangaluru, Karnataka, INDIA
3. Department of Pharmaceutical Chemistry, NGSM Institute of Pharmaceutical Sciences, Nitte (Deemed to be University), Deralakatte-575018, Mangaluru, Karnataka, INDIA

*sharmila@nitte.edu.in

Abstract

In the present study, we used oroxylum indicum (OI) a traditional medicinal plant stem bark extract to evaluate its antimicrobial and anti-inflammatory properties. The antibacterial and antifungal properties of Oroxylum indicum stem bark extract were assessed by well diffusion assay. Further, the anti-inflammatory property of OI stem bark extract was evaluated using egg albumin denaturation and bovine serum albumin denaturation assays. The cytotoxicity was evaluated by MTT assay.

The OI extract showed antibacterial activity against Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli exhibiting the maximum zone of inhibition. From the results of antifungal activity, it was evident that the OI stem bark extract showed maximum zone of inhibition against the test organisms namely, Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus and Penicillium citricus. From our study findings, it is evident that OI stem bark extract has the potential to be used for treating bacterial and fungal infection.

Keywords: *Oroxylum indicum,* stem bark, antimicrobial.

Introduction

Worldwide plants with medicinal properties have gained a lot of attention in recent times. Medicinal plants are a treasure-house of therapeutically important compounds that are well known for possessing anti-infectious properties and are utilized for the treatment of various ailments namely digestive disorders, hysteria, respiratory and reproductive diseases, tonsilitis etc.

According to the World Health Organization (WHO), eighty percent of developing Nations, still are benefitted by utilizing traditional medicines derived from medicinal plants[9,13,25]. Out of the total estimated number of 3,74,000 plants[10], 28,187 medicinal species are used by humans. Over 20,000 species of medicinal plants are recorded by the WHO[36] which are described as one of the potential sources of novel drugs[46]. Moreover, 14–28% of higher plant species are estimated to be medicinal based on ethnomedicinal uses and 74% of bioactive plant-derived compounds were discovered from these medicinal plants[46].

In many parts of the world, there is evidence of medicinal plants having been utilized in the treatment of human ailments caused by various pathogenic microbes. Plants with known antimicrobial properties contain several bioactive constituents including alkaloids, tannins, flavonoids, saponins and glycosides which act against various microbes. These compounds may exhibit antibacterial and antifungal activities[33]. The search for novel antimicrobial compounds from medicinally important plants from many countries is an interesting and important line of research since there is an increase in the number of multidrug resistance pathogenic microbes[21].

This emerging and increasing trend is alarming and considered by the WHO to be perhaps the most important and attention seeking issue facing medical science (https://www.who.int/health-topics/antimicrobial-resistance, 2021). Therefore, there is an increasing requirement to develop new antimicrobial agents that are able to reduce the use of antibiotics and to face resistance development. This has paved the way for researchers to isolate and identify novel bioactive compounds from plants to act against microbial resistance[8,21,41,43] also in consideration of the fact that approximately 50% of current nutraceuticals and pharmaceuticals are natural products and their derivatives[15].

Nevertheless, the compounds have not yet been systematically studied and validated scientifically[5]. Antimicrobial agents of natural origin may increase antimicrobial activity against a variety of microorganisms by acting either alone or in combination with antibiotics [4,32]. Since the antimicrobial property of several medicinal plants is still unexplored, researchers are increasingly aiming the search for fast-growing novel and effective treatments[6,34].

Oroxylum indicum, (L.) Kurz is a medicinally important plant that belongs to the family Bignoniaceae. This plant is commonly known as trumpet tree (L.) and as Shyonaka in sanskrit. This plant has multiple benefits, with dual purpose as a food source mainly from fruits and seeds or as medicine with parts of the plant. The bark, leaves, fruits and seeds of

the plant are said to have a wide range of biological activities and have already been used in complementary medicine to treat human ailments. The plant cumulatively exerts antibacterial, anti-hyperglycemic, pro-neurogenesis, cardioprotective, anti-adipogenesis, anti-inflammatory, anticancer properties [35].

Material and Methods

Docking Studies: Through *in silico* computational research, the phytoconstituents' mechanism of binding with the target was examined. Using the Schrodinger 2023-1 suite device Maestro 13.5.128, an *in silico* investigation was conducted. The antibacterial properties of certain of the components in the bark extract of *Oroxylum indicum* were investigated by docking the phytoconstituents in the groove of the binding sites of DNA gyrase and Squalene epoxidase. The RCSB-PDB (https://www.rcsb.org/) library provided the 3D structure of DNA Gyrase, with PDB IDs of 2XCT. The structure of Squalene epoxidase was modeled with the Swiss Model tool, using the amino acid sequence of Squalene epoxidase from *Candida albicans* (Uniprot ID Q92206.ERG1) as the template.

The modeled structure had a sequence identity of 82.42% (Ramachandran favored- 97.36%). The protein preparation tool was used to minimise energy use and optimise these protein structures after they were imported into the Maestro workspace. Oroxylum indicum bark extract contains the following compounds: Oroxylin B, Oroxylin-A-7-O-beta-d-glucuronide, Scutellarein, Baicalein, Hispidulin, Chrysin and Oroxylin A. Maestro's 2D Sketcher was used to draw the compounds' structures. The Schrodinger suite-2023-1's Ligprep module was then used to turn the 2D structures into 3D ones. With the Ligprep module, the geometry was optimised, the bond order was selected and the ligands' ionisation states were generated (via Epik).

The three-dimensional structures of the ligands were subjected to energy minimisation using the OPLS-2005 forcefield. Molecular docking analyses were then performed on the optimised ligands utilising Maestro 2023-1's Glide tool. The ligand-protein interactions and docking scores were examined to determine how well the investigated compounds bonded to the target proteins[17,30].

Plant extract preparation: The stem bark of *Oroxylum Indicum* (OI) obtained in the Mangalore region was cut into small pieces, cleaned with distilled water to remove any remaining plant debris and then dried for five days at a temperature of 27 to 30^{0}C. In this procedure, a finely ground material was put into the Soxhlet thimble chamber. In the bottom flask, the extraction solvent (99% ethanol) was heated. It then vaporised into the sample thimble, condensed in the condenser and dripped back. Once the liquid content has reached the siphon arm, the liquid contents emptied into the bottom flask again and this process was continued (72 hours). A Rotavap super fit PBU-6 rotary flash evaporator was used to dry the stem bark extract for 15 minutes at 60^{0}C. An airtight container was used to keep the dried extract at 4°C[18].

Cell suspension preparation: The density of bacterial and fungal cells in a liquid suspension was adjusted using the 0.5 McFarland standards. A measurement of the light wavelength at 600 nm was used to modify the turbidity equivalent to 1.5×10^8 cells [19].

Well diffusion assay for bacterial species: The four species of bacteria used to assess the *O. indicum* extracts' activity were as follows: *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* PA01, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 10031. Under sterilised conditions, autoclaved Petri plates were filled with Mueller Hinton agar medium. To ensure that each seedling was distributed equally, the bacterial inoculum was dispersed. The plant extracts were loaded into bored wells. Dimethyl sulfoxide (DMSO) was taken as a negative control and ciprofloxacin was utilized as a positive control. Following loading of the wells, the plates were inverted and incubated for 24 hours at 37 °C. Using a ruler, the zone of inhibition surrounding the wells was measured and reported in millimeters (mm). The experiments were performed in triplicate and averaged[7].

Quantification of the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC): The varied quantities of the plant extracts in Mueller Hinton broth (5000 ug/mL, 2500 ug/mL, 1250 ug/mL, 625 ug/mL, 312.5 ug/mL and 156.25 ug/mL) were prepared using the serial dilution method in order to ascertain the minimum inhibitory concentration (MIC). After seeding the Mueller Hinton broth with specific plant extract doses using the bacterial inoculum, it was incubated for 24 hours at 37°C. While ciprofloxacin was used to generate the positive control, the control tubes were made without the bacterial inoculation. The MIC was determined by comparing the seeded tubes' visual turbidity with that of the control tubes following a 24-hour incubation period. Following the incubation period, the MBC was recorded by incubating a loop filled with bacterial culture on the Mueller Hinton solid medium for 24 hours at 37°C[20,24].

Test to determine crude plant extract's susceptibility to antifungal agents: The agar well diffusion assay was used to assess the antifungal properties of plant extracts. Antifungal activity was employed with certain fungal strains of *Aspergillus niger*, *Aspergillus flavus, Aspergillus fumigatus* and *Penicillium citricus*. Sterilised Petri plates were filled with the potato dextrose agar medium in the germ-free Laminar air flow hood. The media was let to settle at room temperature for five to ten minutes. Using a bent rod, the spore suspension was spread out when the Petri plates were inoculated by decanting it into the middle of the medium. 50 µl of plant extracts were added to the wells after they had been punctured with a cork borer. The plant extracts were allowed to freely diffuse in the agar for five to ten

minutes. After loading the wells, plates were incubated for 48 hours at 28 °C [26].

Terbinafine served as the positive control and a well containing only DMSO was used as the negative control. The clear zone of inhibition that resulted, was measured in millimetres. The data are shown as mean ± standard deviation and were obtained from triplicate testing of the samples.

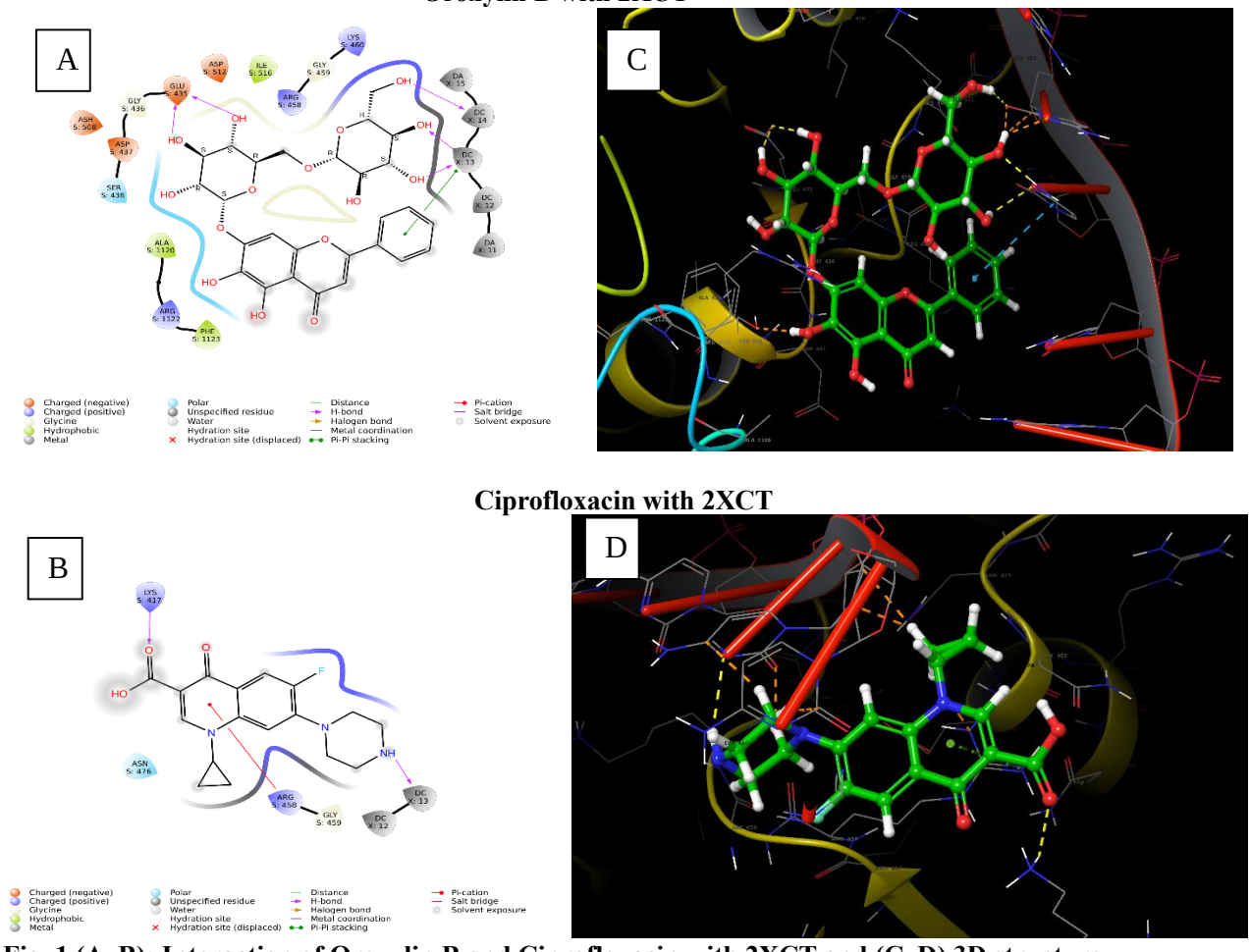
Quantification of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC): The serial dilution approach was used to prepare the various concentrations of plant extracts in potato dextrose broth (5000 ug/mL, 2500 ug/mL, 1250 ug/mL, 625 ug/mL, 312.5 ug/mL and 156.25 ug/mL) in order to determine the minimum inhibitory concentration (MIC). The potato dextrose broth was seeded with particular plant extract doses using a fungal inoculum and it was then incubated at 28 °C for 48 hours. Although the positive control was created using Terbinafine, the control tubes were not infused with fungi. After a 48-hour incubation period, the MIC was ascertained by comparing the optical turbidity of the seeded tubes with that of the control tubes. After the incubation period, a loop containing bacterial culture was incubated on the potato dextrose solid medium for 48 hours at 28 °C in order to record the MFC [1].

Data Analysis: All of the test data were saved in a Microsoft Excel spreadsheet. Prism software 8.0 and Microsoft Excel were used as statistical tools to evaluate the data. A graphical representation of the results was used with Mean ± SD. The threshold for determining statistical significance was $p<0.05$.

Results

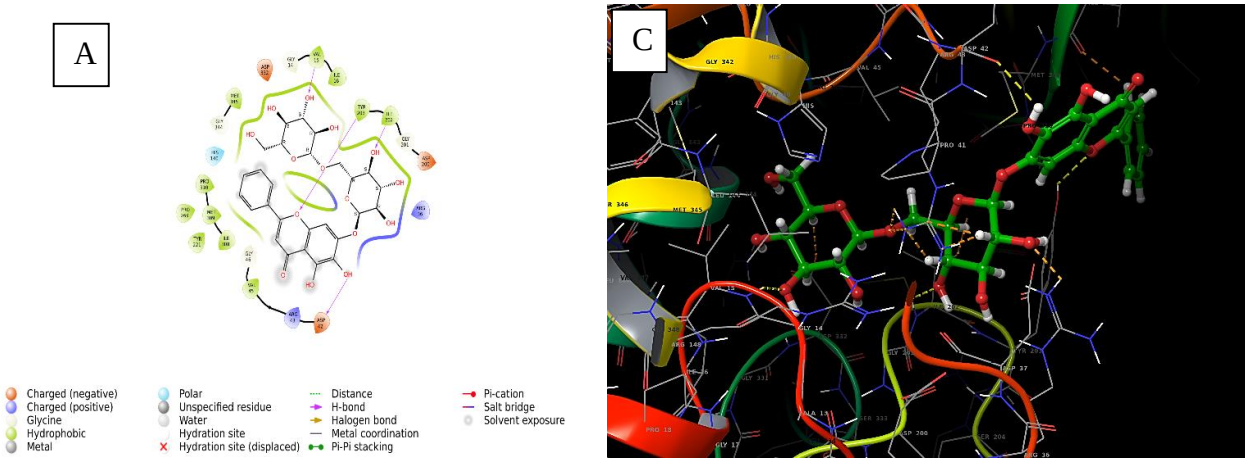
Molecular Docking: Oroxylin B, Oroxylin-A-7-O-beta-d-glucuronide, Scutellarein, Baicalein, Hispidulin, Chrysin and Oroxylin A, found in the bark extract of *Oroxylum indicum*, were successfully docked against the proteins DNA gyrase, Squalene epoxidase. Oroxylin B indicated the potential triggering of antimicrobial property through *in silico* experiments. Molecular docking analyses have been conducted in the target binding site grooves of DNA gyrase (PDB ID: 2XCT), Squalene epoxidase. With binding scores of Oroxylin B being -9.398, -7.619 and the standard drugs ciprofloxacin -3.051, terbinafine -2.761 (Table 1-2) respectively, Oroxylin B has demonstrated the best binding energies with DNA gyrase, Squalene epoxidase.

Oroxylin B with 2XCT



Ciprofloxacin with 2XCT

Fig. 1 (A, B): Interaction of Oroxylin B and Ciprofloxacin with 2XCT and (C, D) 3D structure. Oroxylin B with Squalene epoxidase



Terbinafine with Squalene epoxidase

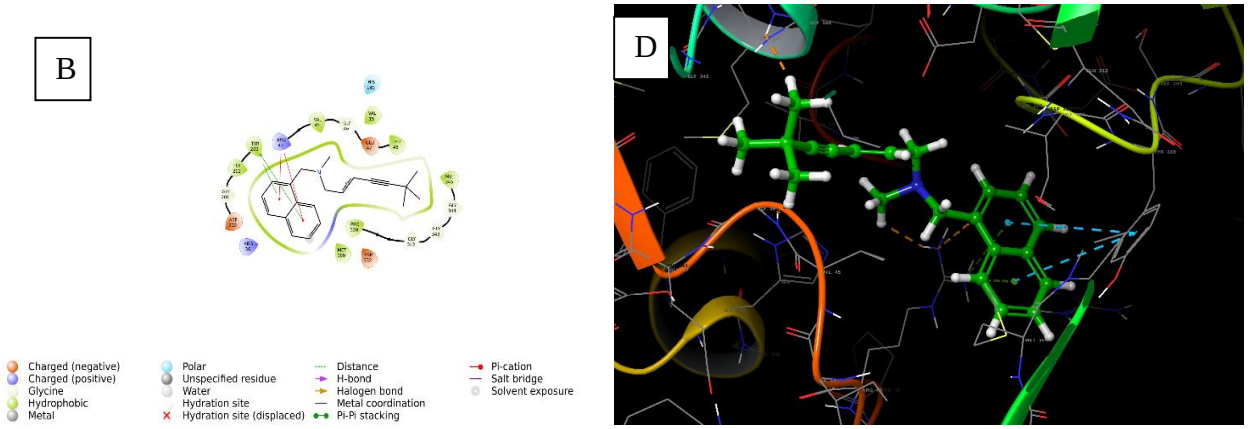


Fig. 2(A, B): Interaction of Oroxylin B and Terbinafine with Squalene epoxidase and (C, D)3D structure.

Fig. 3: *Oroxylum indicum* tree and stem bark

Table 1
Target DNA gyrase
PDB ID: 2XCT

COMPOUNDS	DOCK SCORES
OROXYLIN B	-9.398
OROXYLIN A-7-o-BETA-d-GLUCURONIDE	-5.278
SCUTELLAREIN	-4.666
BAICALEIN	-4.445
HISPIDULIN	-4.441
OROXYLIN A	-4.349
CHRYSIN	-4.062
CIPROFLOXACIN	-3.051

Table 2
Target Squalene epoxidase (Monooxygenase)

COMPOUNDS	DOCK SCORES
OROXYLIN B	-7.619
OROXYLIN A-7-o-BETA-d-GLUCURONIDE	-7.162
SCUTELLAREIN	-5.649
BAICALEIN	-5.493
CHRYSIN	-4.658
OROXYLIN A	-4.415
HISPIDULIN	-4.411
TERBINAFINE	-2.761

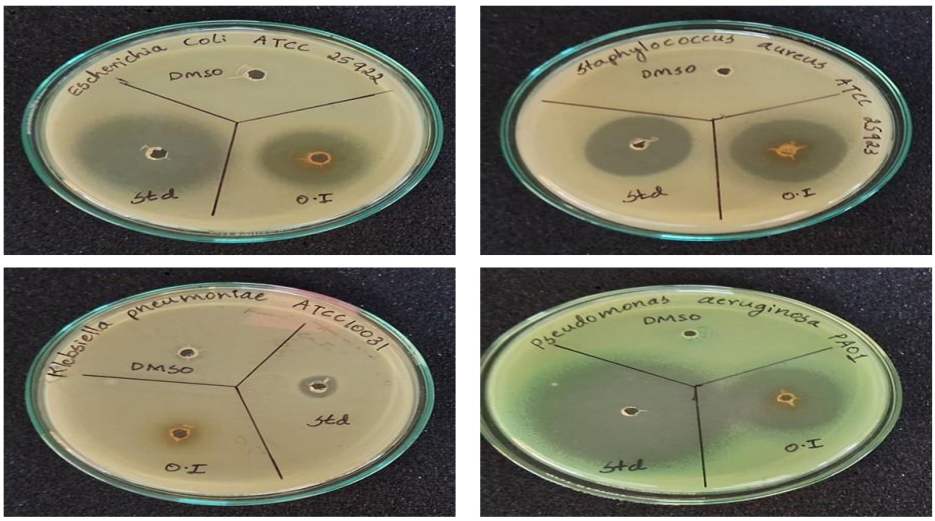


Fig. 4: Antibacterial activity of *Oroxylum indicum* stem bark extract. The drug ciprofloxacin was used as positive control.

Antibacterial activity: The antibacterial activity of ethanolic extracts of stem bark was carried out with *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* PA01, *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 10031 (Table 3, figure 4). From the results, it is evident that the ethanolic extract of stem bark of *Oroxylum indicum* showed maximum zone of inhibition against test bacteria. The activity was observed against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* with a zone of inhibitions at 10.33±0.57 mm, 13.33±0.57mm and 10.66±0.57 mm respectively when used at 10 mg/mL (in DMSO) 50 µL per well. Extract did not show activity against *Klebsiella pneumoniae*.

The standard antibiotic ciprofloxacin showed the zone of inhibition at 17.66 ± 0.57 mm,11.66±0.57mm,3.66±0.57mm and 23.66±0.57mm against *Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia* and *Pseudomonas aeruginosa* when used at 1mg/ mL (in DMSO) 10 µL per well.

Antifungal activity: The antifungal activity of ethanolic extract of stem bark against *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus* and *Penicillium citricus* is shown in table 4 and figure 5. From the results, it is evident that the ethanolic stem bark extract of *Oroxylum indicum* showed maximum zone of inhibition against test organisms. The activity was observed against *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus* and *Penicillium citricus* with a zone of inhibitions at 3.33±0.57 mm, 10.33±0.57 mm, 3.66±0.57 mm and 12.33±0.57 mm respectively when used at 10 mg/mL (in DMSO) 50 µL per well.

The standard antifungal drug, terbinafine showed the zone of inhibitions at 13.66 ± 0.57mm, 28.33 ± 0.57 mm,20.33 ± 0.57mm and 23.33 ± 0.57mm against *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus* and *Penicillium citricus* respectively when used at 64 µg/ mL (in DMSO).

Minimum inhibitory and bactericidal concentration of *O. indicum* extract: The minimum inhibitory concentration for the bacteria ranged from 5 mg/ml, 2.5mg/ml, 0.312mg/ml and 5mg/ml against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae* respectively. The minimum bactericidal concentration (MBC) was determined as there was no visible growth observed at the lowest concentration of extracts. The MBC *of O. indicum* extract was not detected in any of the bacterial strains used in the study (Table 5).

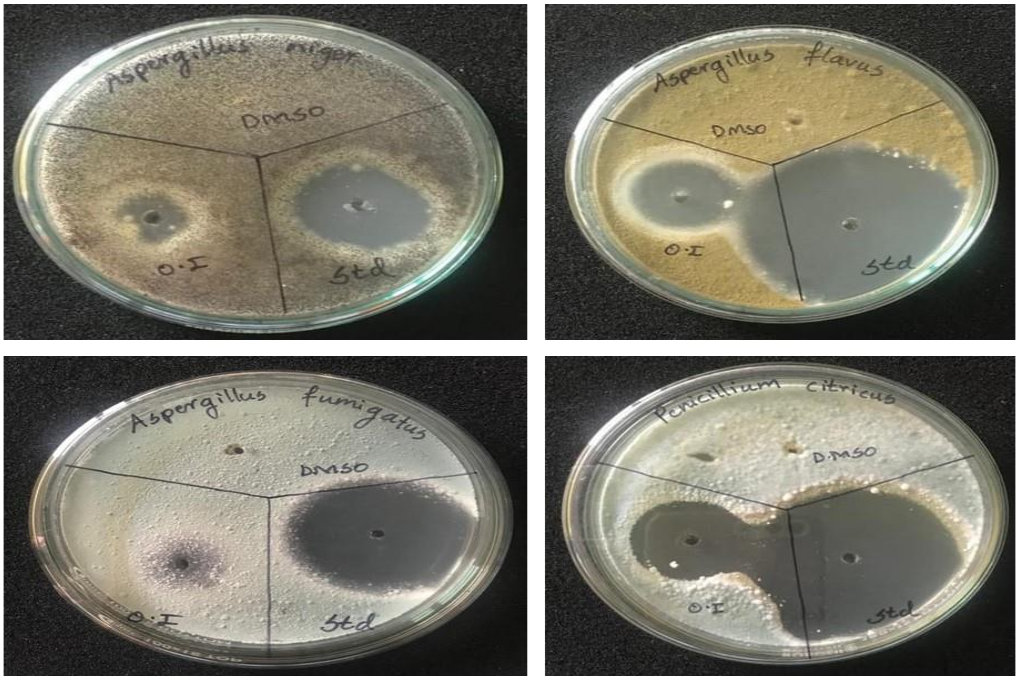


Fig. 5: Antifungal activity of *Oroxylum indicum* stem bark extract. The drug Terbinafine was used as positive control.

Table 3
Antibacterial activity Disc diffusion method - Inhibition zone in mm

Test organisms	***Escherichia coli***	***Staphylococcus aureus***	***Klebsiella pneumonia***	***Pseudomonas aeruginosa***
O.indicum extract	10.666±0.577	10.333±0.577	0.0±0.0	13.333±0.577
Ciprofloxacin	17.666±0.577	11.666±0.577	3.666±1.154	23.666±0.577

Table 4
Antifungal activity Disc diffusion method - Inhibition zone in mm

Test organisms	***Aspergillus niger***	***Aspergillus flavus***	***Aspergillus fumigatus***	***Penicillium citricus***
O.indicum extract	3.333±0.577	10.333±0.577	3.666±0.577	12.333±0.577
Terbinafine	13.666±0.577	28.333±0.577	20.333±0.577	23.333±0.577

Table 5
MIC and MBC of *O.indicum* extract against Bacteria

Test extract	**Activities**	***Escherichia coli* (mg/ml)**	***Staphylococcus aureus* (mg/ml)**	***Klebsiella pneumonia* (mg/ml)**	***Pseudomonas aeruginosa* (mg/ml)**
O.indicum extract	MIC_{50}	0.312	5	5	2.5
	MBC	ND	ND	ND	ND

ND = Not detected

Table 6
MIC and MFC of *O. indicum* extract against Fungi

Test extract	**Activities**	***Aspergillus niger* (mg/ml)**	***Aspergillus flavus* (mg/ml)**	***Aspergillus fumigatus* (mg/ml)**	***Penicillium citricus* (mg/ml)**
O.indicum extract	MIC_{50}	2.5	0.312	1.25	0.625
	MFC	ND	5	ND	5

ND = Not detected.

Minimum inhibitory and fungicidal concentration of *O. indicum* extract: The minimum fungistatic concentration of extracts ranged from 2.5mg/ml, 0.312mg/ml, 1.25mg/ml and 0.625mg/ml against *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus* and *Penicillium citricus* respectively. The minimum fungicidal concentration (MFC) of the *O. indicum* extract ranged from 5mg/ml against *Aspergillus flavus* and *Penicillium citricus.* However, the minimum fungicidal concentrations of the *O.indicum* extract were not detected against *Aspergillus niger* and *Aspergillus fumigatus* (Table 6).

Discussion

The emergence of resistant bacterial and fungal strains to traditional antimicrobial medications, together with the unfavourable consequences of anti - biotherapy, has led to a heightened quest for natural products as substitutes for these pathogens. Medicinal plants have been used since the dawn of civilization to treat a wide range of health issues including bacterial and fungal diseases[42]. In addition to being useful in treating infectious disorders, plant extracts and natural chemicals also mitigate many of the side effects of conventional antimicrobials[16]. This study evaluated the stem bark extract from *Oroxylum indicum* for its antibacterial and anti-inflammatory properties.

Antibiotics derived from extracted plants are risk-free, efficient and rarely cause adverse effects[31]. The biological activities of active phytochemicals, such as their antibacterial properties against pathogens, aid in the development of novel antibiotic medications[2,14]. The antibacterial and antifungal properties of the medicinal herb *Oroxylum indicum* were examined in this study.

Numerous β-lactamase producers including *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas Aeruginosa*, have emerged as a significant clinical concern. A greater degree of thought has been on the use of natural antibacterial agents, particularly those derived from plants, because most of these species are regarded as universally acknowledged to be safe and effective. Natural goods are in fact widely utilized as nutraceuticals, food preservatives and may be as pharmaceuticals for the treatment and prevention of a wide range of illnesses and ailments including as cancer, cardiovascular disease, aging and many more. For these reasons, there has been a recent global education campaign focused on the identification, application and extraction of biologically and pharmacologically active chemicals derived from plants [39].

In this study, the stem bark of *Oroxylum indicum (Sonapatha)* is used. Locally, the stem bark of Sonapatha is used to kill maggots on cattle wounds[22,23,27,45]. Since ancient times, sonapatha has been utilized to cure a variety of human illnesses. Sonapatha is used in Ayurveda and traditional medicine for a variety of ailments; it is one of the main ingredients in Brahma Rasayana, Dashmularishta, Dhanawantara Ghrita, Amritarishta, Narayana Taila, Dantyadyarista and Chyavanprasha[3,11]. The antibacterial report of the medicinal plant *Oroxylum indicum* extracts is summarized in table 1. The *Oroxylum indicum* extract showed dose-dependent potential activity and affected the tested pathogens. The crude ethanol extract is more potent against bacterial strains *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

As per our previously published study[35], the ethanol stem bark extract of *Oroxylum indicum* revealed the presence of glycosides, alkaloids, flavonoids and tannins. The majority of these phytochemicals form the basis for the therapeutic efficacy of plants and are currently used as starting materials to produce novel medications. This investigation revealed that the OI stem bark extract exhibited efficacy against the pathogens employed, underscoring the potential of herbal remedies and their prospective application in local medicine. Tannins and flavonoids function similarly, giving rise to a stable free radical and forming complexes with nucleophilic amino acids in proteins that cause the protein to become inactive and lose its function. They also exhibit potential antimicrobial effect because they may target microbial cells that have surface-exposed adhesins, cell wall polypeptides and membrane-bound enzymes[37].

Gycosides and alkaloids exhibit antibacterial property by inhibiting the formation of bacterial cell walls, altering the permeability of cell membranes, preventing bacterial metabolism and inhibiting the synthesis of proteins and nucleic acids[47]. In this study, the more susceptible test organisms to the ethanol stem bark extract were *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. *O. indicum stem* bark extract exhibited antibacterial activity against both Gram positive bacterial strains such as *S. albus, S. aureus* and Gram-negative bacterial strains such as *B. subtilis* and *B. cereus* mediating the presence of a broad spectrum of antibacterial compounds in the plant.

In the present study, *O. indicum* stem bark ethanol extract was found to exhibit antifungal activity against *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus* and *Penicillium citricus.* The phytochemicals found in the ethanol extract of *O. indicum* could be the reason for its antifungal activity. Novel antifungal medicines and new therapeutic targets are very much needed, especially in light of the growing antifungal resistance. More knowledge about the cellular architecture of fungi and associated processes has recently led to the development of promising antifungal medications that may not target human cells and have a broad therapeutic index. In order to target drug-resistant representatives, novel plant derived drugs with a broad spectrum of antifungal action may be able to circumvent the absence of sensitive diagnostic tools[44].

Conclusion

We conclude that the *Oroxylum indicum* stem bark extract has the potential to be used for treating microbial (fungus

and bacterial strain) infection. The extract of the medicinal plant, *O.indicum* showed both antibacterial and antifungal biological activities in a wide range. The variations in concentration, purification and isolation of bioactive compounds in the extract can provide us more sustainable results.

Thus, *Oroxylum indicum* is a useful medicinal plant and its further assessment is important, which can provide help in the discovery of new antimicrobials and antimicrobial resistance modifiers.

Acknowledgement

The authors acknowledge the support from Nitte (Deemed to be University).

References

1. Aidoo D.B., Konja D., Henneh I.T. and Ekor M., Protective effect of bergapten against human erythrocyte hemolysis and protein denaturation *in vitro*, *International Journal of Inflammation*, **2**, 1-7 **(2021)**

2. Alghazeer R., El-Saltani H., Saleh N., Al-Najjar A. and Hebail F., Antioxidant and antimicrobial properties of five medicinal Libyan plants extracts, *Natural Science (Irvine)*, **4(5)**, 324–35 **(2012)**

3. Balkrishna A., Ayurved Jadi-Booti Rahasya, Divyaprakasan, Divya Yog Mandir Trust: Haridwar, India **(2005)**

4. Bazzaz F., Khameneh B.S., Zahedianostad B. and Hosseinzadeh M.R., *In vitro* evaluation of antibacterial activity of verbascoside, *lemon verbena* extract and caffeine in combination with gentamicin against drug-resistant *Staphylococcus aureus* and *Escherichia coli* clinical isolates, *Avicenna Journal of Phytomedicine*, **8(3)**, 246-253 **(2018)**

5. Bazzaz F., Sarabandi B., Khameneh S. and Hosseinzadeh B., Effect of Catechins, Green Tea Extract and Methylxanthines in Combination with Gentamicin against *Staphylococcus aureus* and *Pseudomonas aeruginosa*-Combination Therapy Against Resistant Bacteria, *Journal of Pharmacopuncture*, **19(4)**, 312-318 **(2016)**

6. Begum M.M. et al, Ethnopharmacological inspections of organic extract of *Oroxylum indicum* in rat models: A promising natural gift, *Evidence Based Complementary and Alternative Medicine*, **2019**, 1-13 **(2019)**

7. Bhuyan D.J., Vuong Q.V., Chalmers A.C., van Altena I.A. Bowyer M.C. and Scarlett C.J., Phytochemical, antibacterial and antifungal properties of an aqueous extract of *Eucalyptus microcorys* leaves, *South African Journal of Botany*, **112**, 180-185 **(2017)**

8. Chavan S.S., Damale M.G., Shinde D.B. and Sangshetti J.N., Antibacterial and Antifungal Drugs from Natural Source: A Review of Clinical Development. In: Natural Products in Clinical Trials, Benthan Science Books, Sharjah, United Arab Emirates, 114-164 **(2018)**

9. Chin Y.W., Balunas M.J., Chai H.B. and Kinghorn A.D., Drug discovery from natural sources, *An Official Journal of the American Association of Pharmaceutical Scientists*, **8(2)**, 239-53 **(2006)**

10. Christenhusz M. and Byng J., The Number of Known Plants Species in the World and its Annual Increase, *Phytotaxa*, **261**, 201-217 **(2016)**

11. Dinda B., Sil Sarma I., Dinda M. and Rudrapaul P., *Oroxylum indicum* (L.) Kurz, an important Asian traditional medicine: from traditional uses to scientific data for its commercial exploitation, *Journal of Ethnopharmaco*logy, **161**, 255-78 **(2015)**

12. Duganath N., Kumar S., Kumanan R. and Jayaveera K.N., Activity of traditionally used medicinal plants, *International Journal of Pharma Bio Sciences*, **1**, 1-7 **(2010)**

13. Duraipandiyan V., Ayyanar M. and Ignacimuthu S., Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India, *BMC Complementary and Alternative Medicine*, **6(1)**, 35 **(2006)**

14. Edziri H., Mastouri M., Cheraif I. and Aouni M., Chemical composition and antibacterial, antifungal and antioxidant activities of the flower oil of *Retama raetam* (Forssk.) Webb from Tunisia, *Natural Product Research*, **24(9)**, 789–96 **(2010)**

15. Emma N.Q., Antonio R.S. and Marta A.V., Screening Antifungal Activities of Selected Medicinal Plants, *Journal of Ethnopharmacology*, **74(1)**, 89-96 **(2001)**

16. Iwu M., Duncan A.R. and Okunji C.O., New Antimicrobials of Plant Origin, In Janick J., ed., Perspectives on new crops and new uses, Alexandria, ASHS Press **(1999)**

17. Jadhav N.C., Pahelkar A.R., Desai N.V. and Telvekar V.N., Design, synthesis and molecular docking study of novel pyrrole-based α-amylase and α-glucosidase inhibitors, *Medicinal Chemistry Research*, **26(10)**, 2675–91 **(2017)**

18. Jalalvand A.R. et al, Chemical characterization and antioxidant, cytotoxic, antibacterial and antifungal properties of ethanolic extract of *Allium Saralicum* R.M. Fritsch leaves rich in linolenic acid, methyl ester, *J of Photochemistry and Photobiology*, **192**, 103-112 **(2019)**

19. Javed B., Nawaz K. and Munazir M., Phytochemical analysis and antibacterial activity of tannins extracted from *Salix alba* L. against different gram-positive and gram-negative bacterial strains, *Iranian Journal of Science and Technology Transaction A-Science*, **44(5)**, 1303–14 **(2020)**

20. Javed B., Farooqc F., Ibrahimd M., Abbase H.A.B., Jawwadf H., Zehrag S.S., Ahmadh H.M., Sarweri A., Malikj K. and Nawaza K., Antibacterial and antifungal activity of methanolic extracts of *Salix alba* L. against various disease causing pathogens, *Brazilian Journal of Biology*, **83**, e 243332 **(2023)**

21. Khameneh B., Diab R., Ghazvini K. and Fazly Bazzaz B.S., Breakthroughs in bacterial resistance mechanisms and the potential ways to combat them, *Microbial Pathogenesis*, **95**, 32-42 **(2016)**

22. Lalrinzuali K., Vabeiryureilai M. and Chandra G., Phytochemical and TLC Profiling of *Oroxylum indicum* and *Milletia pachycarpa*, *Journal of Plant Biochemistry and Physiology*, **3**, 1-8 **(2015)**

23. Lalrinzuali K., Vabeiryureilai M. and Jagetia G.C., Topical application of stem bark ethanol extract of Sonapatha, *Oroxylum indicum* (L.) Kurz accelerates healing of deep dermal excision wound in Swiss albino mice, *Journal of Ethnopharmacology*, **227**, 290-299 **(2018)**

24. Mativandlela S.P.N., Lall N. and Meyer J.J.M., Antibacterial, antifungal and antitubercular activity of (the roots of) *Pelargonium reniforme* (CURT) and *Pelargonium sidoides* (DC) (Geraniaceae) root extracts, *South African Journal of Botany*, **72(2)**, 232–7 **(2006)**

25. Mishra A., Sharma A.K., Kumar S., Saxena A.K. and Pandey A.K., *Bauhinia variegata* leaf extracts exhibit considerable antibacterial, antioxidant and anticancer activities, *Biomed Research International*, **6**, 1-10 **(2013)**

26. Mizushima Y. and Kobayashi M., Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins, *Journal of Pharmacy and Pharmacology*, **20(3)**, 169–73 **(1968)**

27. Nakahara K., Trakoontivakorn G., Alzoreky N.S., Ono H., Onishi-Kameyama M. and Yoshida M., Antimutagenicity of some edible Thai plants and a bioactive carbazole alkaloid, mahanine, isolated from *Micromelum minutum*, *Journal of Agricultural and Food Chemistry*, **50(17)**, 4796–802 **(2023)**

28. Pandeya K. and Kumar S., Perspective on Plant Products as Antimicrobials Agents. A Review, *Pharmacologia*, **4**, 469-480 **(2013)**

29. Penesyan A., Kjelleberg S. and Egan S., Development of novel drugs from marine surface associated microorganisms, *Marine Drugs*, **8(3)**, 438–59 **(2010)**

30. Ramachandran G.N., Ramakrishnan C. and Sasisekharan V., Stereochemistry of polypeptide chain configurations, *Journal of Molecular Biology*, **7**, 95-9 **(1963)**

31. Rehman A., Rehman A. and Ahmad I., Antibacterial, antifungal and insecticidal potentials of *Oxalis corniculata* and its isolated compounds, *International Journal of Analytical Chemistry*, **16**, 1-5 **(2015)**

32. Ríos J.L. and Recio M.C., Medicinal plants and antimicrobial activity, *Journal of Ethnopharmacolgy*, **100**, 80-4 **(2005)**

33. Rojas R., Bustamante B., Bauer J., Fernández I., Albán J. and Lock O., Antimicrobial activity of selected Peruvian medicinal plants, *Journal of Ethnopharmacolgy*, **88**, 199–204 **(2003)**

34. Savoia D., Plant-derived antimicrobial compounds alternatives to antibiotics, *Future Microbiology*, **7(8)**, 979-90 **(2012)**

35. Sharmila K.P., Kumar Bhandary S. and Kumari S., Phytochemical profile and in-vitro antioxidant potencies of *oroxylum indicum* stem bark extract, *International Journal of Pharmaceutical Sciences and Research*, **12(7)**, 3791-98 **(2021)**

36. Srinivasan D., Nathan S., Suresh T. and Lakshmana P.P., Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine, *Journal of Ethnopharmacolgy*, **74(3)**, 217–20 **(2001)**

37. Stern J.L., Hagerman A.E., Steinberg P.D. and Mason P.K., Phlorotannin-protein interactions, *Journal of Chemical Ecology*, **22(10)**, 1877–99 **(1996)**

38. Sun Y., Yang A.W.H. and Lenon G.B., Phytochemistry, Ethnopharmacology, Pharmacokinetics and Toxicology of *Cnidium monnieri* (L.) Cusson, *International Journal of Molecular Sciences*, **21(3)**, 1006 **(2020)**

39. Suurbaar J., Mosobil R. and Donkor A.M., Antibacterial and antifungal activities and phytochemical profile of leaf extract from different extractants of *Ricinus communis* against selected pathogens, *BMC Research Notes*, **10(1)**, 660 **(2017)**

40. Talari S., Antibacterial activity of stem bark extracts of *Oroxylum indicum* an endangered ethnomedicinal forest tree, *IOSR Journal of Pharmacy and Biological Sciences*, **7(1)**, 24–8 **(2013)**

41. Talib W.H., Anticancer and Antimicrobial Potential of Plant-Derived Natural Products, Phytochemicals-Bioactivities and Impact on Health, Rasooli Iraj, ed., InTech **(2011)**

42. Tamokou J.D.D., Kuiate J.R. and Gatsing D., Antidermatophytic and toxicological evaluations of dichloro methane methanol extract, fractions and compounds isolated from *Coula edulis*, *Iranian Journal of Medical Sciences*, **36(2)**, 111-2 **(2011)**

43. Tortorella E. et al, Antibiotics from deep-sea microorganisms: Current discoveries and perspectives, *Marine Drugs*, **16(10)**, 355 **(2018)**

44. Vahedi-Shahandashti R. and Lass-Flörl C., Novel antifungal agents and their activity against Aspergillus species, *Journal of Fungi*, **6(4)**, 213 **(2020)**

45. Warrier P., Nambiar V. and Ramankutty C., Indian Medicinal Plants-A Compendium of 500 Species, Orient Longman Ltd. **(1995)**

46. Yadav R. and Agarwala M., Phytochemical Analysis of some Medicinal Plants, *Journal of Phytology*, **3**, 10-14 **(2011)**

47. Yan Y., Li X., Zhang C., Lv L., Gao B. and Li M., Research progress on antibacterial activities and mechanisms of natural alkaloids: A review, *Antibiotics*, **10(3)**, 318 **(2021)**.

(Received 05th November 2024, accepted 08th January 2025)