ORIGINAL ARTICLE

Molecular Docking Studies of Phytochemicals from Five Medicinal Plants against Resistance genes Protein isolated from MDR *Salmonella* Typhimurium

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Abstract

Introduction: In the present study, the antibacterial activity against multidrug-resistant (MDR) *Salmonella* Typhimurium of different extracts of Five Medicinal Plants was investigated along with their phytochemical analysis by gas chromatography-mass spectrometry (GC-MS) and Molecular Docking Studies of Phytochemicals to explore antimicrobial compounds present in extracts. **Materials and Methods:** The antibacterial activities of different extracts of Five Medicinal Plants were evaluated against isolated MDR *S.* Typhimurium by disc diffusion method. Docking studies were carried out using different required docking platforms along with phytochemical analysis by GC-MS. **Results and Discussion:** The phytochemical analysis by quantitative and qualitative methods of the extract of *Allium sativum*, *Bridelia micrantha*, *Citrus lemon*, *Glycyrrhiza glabra*, and *Punica granatum* showed presence of amino acids, alkaloids, glycosides, saponins, flavonoids, steroids, and terpenoids. It was discovered that crude extracts of these plants worked well against MDR *S.* Typhimurium. The zone of inhibition values for the crude extract of *A. sativum*, *B. micrantha*, *C. lemon*, *G. glabra*, and *P. granatum* was found to be, 10.12 ± 1.71 mm, 23.50 ± 0.00 mm, 26.60 ± 0.64 mm, 29.20 ± 0.11 mm, and 19.50 ± 0.00 mm, respectively. Docking results of phyto-ligand with selected resistance genes proteins reveals that the binding energy ranges from −2.9 to −9.2 kcal/moL. *G. glabra* roots (Ligand 6-Androstanone, 3-(3, 4-dimethylphenyl)-3-methyl) showed the lowest binding energy of −9.2 kcal/moL. **Conclusion:** To produce broad-spectrum antimicrobial compounds, it is still important to assess the antibacterial potential of medicinal plants. Traditional medicine is one of the most generally available types of treatment in disadvantaged countries. According to the study's findings, phytochemicals from five medicinal plants extract have *in vitro* antibacterial activity along with phyto-ligand docking analysis providing early evidence that the plants may be used to treat MDR infections.

Key words: *Allium sativum*, *Bridelia micrantha*, *Citrus lemon*, *Glycyrrhiza glabra*, molecular docking, multidrug resistance, phyto-ligands, *Punica granatum*, *Salmonella* Typhimurium, spectral phytochemical analysis

INTRODUCTION

Salmonella is one of the notably commonly isolated food-borne infections. It is an intercontinental public health problem that causes about 94 million foodborne illnesses and 0.15 million fatalities/year. To date, more than 2500 *Salmonella* serotypes have been described and more than 60% belong to the Subsp. *Salmonella enterica*. *Enterica*, which assign to source for the number of human *Salmonella* infections.[1] *Salmonella* infections caused by invasive serotypes are very commonly fatal, necessitating prompt and effective antibiotic treatment. The advent of multidrug-resistant (MDR) *salmonella* subtypes has a significant impact on the effectiveness of the antibiotic treatment, and a growing prevalence of MDR strains may contribute to a rise in number and death rates of *Salmonella* infections.[2]

Extended spectrum beta-lactamases (ESBL) is a type of catalyst or biochemical generated by certain bacteria. Some

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antibiotics fail to treat bacterial infections because of ESBL enzymes. The existence of genes associated with tetracycline, tet (A), tet (B), and beta-lactam resistance (bla SHV, bla CMY) has been determined. ESBL-producing *Salmonella* Typhimurium strains exhibited resistance mechanisms linked to aminoglycosides, quinolones, sulfonamides, and beta-lactam resistance.[3]

Since the dawn of human civilization, several plants have been used as folk remedies. Herbal medicine continues to be the primary source of primary healthcare for 70–80% of the world's population, primarily in developing countries, due to its superior compatibility with the human body and lower risk of side effects.[4] Plants include a wide range of complex chemical elements that aid the body's natural healing processes. Plants have been shown to provide therapeutic effects for a range of diseases, including cancer and infectious diseases like malaria and tuberculosis. However, the rise in diseases that are MDR and the well-documented side effects of some common medications has brought attention to the necessity for searching for alternatives in medicine.

Garlic is a potent antibacterial agent that inhibits the growth of both Gram-positive and Gram-negative bacteria, including *Escherichia coli*, *Salmonella*, *Streptococcus*, *Staphylococcus*, *Klebsiella*, *Proteus*, and *Helicobacter pylori*.[5,6]

The *Bridelia micrantha* stem bark's methanol extract was examined for antibacterial properties and the plant has a long history of use in ethnomedicine. The synergistic activities or the discrete actions of the phytochemicals found in the stem bark may be related to the antibacterial capabilities demonstrated by the *B. micrantha* extract. The concentration of *B. micrantha* extract affected its antibacterial action. *B. micrantha* has ability to modulate drug resistance.[7,8]

Due to the presence of Phytochemicals, citrus fruits exhibit a wide range of biological activity, including antibacterial, antifungal, antidiabetic, anticancer, and antiviral effects.[9] Different solvents, including ethanol, methanol, and acetone, are used to extract the lemon peel, and the extracts are then tested for antibacterial properties. Higher antibacterial activity is demonstrated by methanolic extract against the studied microorganisms.[10]

A well-known medicinal plant known as *Glycyrrhiza glabra* grows all over the world. One of the oldest and most popular herbs from the early practice of Ayurveda medicine, it is used both as a medicine and as a flavoring to mask the bad taste of other drugs.[11] The roots and rhizomes of *G. glabra* have been used clinically for millennia in the traditional system of medicine due to their anti-inflammatory, antiulcer, expectorant, antibacterial, and anxiolytic properties.[12]

It has been found that *Punica granatum* has anti-inflammatory,[13,14] anti-atherosclerotic,[15,16] antibacterial, and antiviral[17] characteristics. Gallocatechins, delphinidin, cyanidin, gallic acid, ellagic acid, pelargonidin, and sitosterol are among the components of *P. granatum*, and they are well known for their medicinal qualities.[18] Furthermore, it has been noted that *P. granatum* extracts have antibacterial action against *Salmonella*.[19]

The identification of secondary metabolites in medicinal plants is now primarily done via gas chromatography-mass spectrometry (GC-MS) analysis.[20] GC-MS was used to analyze and compare the components in the extracts. The chemical constituents revealed from the GC-MS analysis along with their retention time, base peak, molecular weight, molecular formula, and compound names in addition to their figures were presented according to the library used to identify compounds.[21] Plant metabolites are better prospects for use as drugs because they exhibit greater "drug-likeness and biological activity than fully synthesized molecules."[22] Drug development can be accelerated and ambiguity reduced using structure-based drug design.[23]

Computer-aided medication discovery and design can improve green technology applications in agriculture (CADD). By gathering data on genome sequences, revealing the three-dimensional structures of biomolecules, and other methods, computational tools aid in the exploration of research in molecular phytopathology and are crucial in the creation of antimicrobial agro-products against pathogens.[24]

AutoDock Vina, a new program for molecular docking and virtual screening, has been used. AutoDock Vina Vina uses a sophisticated gradient optimization method in its local optimization procedure. The rational design of medications has been proven to benefit from molecular docking.[25,26] In the field of molecular modeling, it is one of the methods that shows the preferred direction of a particle with respect to another and aids in the formation of a stable and well-built complex.[27,28]

The current study aims to determine the phytochemicals existing in the extract of *Allium sativum*, *B. micrantha*, *Citrus lemon*, *G. glabra*, *and P. granatum* by GC-MS analysis and to analyses promising prime bioactive compounds of these five medicinal plant's extracts against chosen resistance genes protein from isolated MDR *S.* Typhimurium by conducting molecular docking experiments.

MATERIALS AND METHODS

Procurement of chemicals, plant samples, bacterial samples

Nutrient agar, Nutrient Broth, and Muller Hinton Agar were purchased commercially from regional vendors as dehydrated media of Hi-Media Laboratories Limited, India, and were used as the plating medium in this experiment. In addition, chemicals and reagents of analytical quality were used.

The *A. sativum*, *B. micrantha*, *C. lemon*, *G. glabra*, *and P. granatum* plant part samples were collected from different vendors. The identification of plants components was confirmed by the Department of Pharmacognosy, PWCOP in Yavatmal. Test bacteria were isolated from samples of chicken, roadside soil, and roadside water, from five different areas of the state of Maharashtra. At PWCOP Yavatmal conducted biochemical, microbiological, MDR pattern, and molecular identification of *S.* Typhimurium analyses.[29-31]

Preparation of the extracts

Methanol was used as for the extraction process separately. The appropriate solvents were macerated with the necessary amount of plant powder. Filtration was employed to clarify the liquid, which was then concentrated and extracted using a Soxhlet system. Various solvent extracts were kept for later examination.

Phytochemical analysis

Chemical tests for the screening and identification of bioactive chemical constituents in the medicinal plants were carried out using the standard procedures.[32,33]

Antibacterial activity

The *in vitro* antibacterial activity diameter of zone of inhibition of the plants extracts were evaluated by disc diffusion method against Isolated MDR *S.* Typhimurium.[34]

GC-MS analysis

The GC-MS analysis of selected samples was performed with Shimadzu GC-MS. The inert gas helium (99%) was used as carrier gas, at flow rate of 1.5 mL/min, Split ratio 10:1; sample size, 1 µL injected using the split less injection technique; fused capillary silica column HP-5 (30 m × 0.25 mm × 0.25 µm). Temperatures: injector: 260°C, detector: 300°C, column: 70°C, 10°C/min, 260°C (10 min). The total GC running time is at 35 min. The MS was taken at 70 eV. The MS scan parameters included a mass range of m/z 40–1000, a scan interval of 0.5 s, a scan speed of 2000 amu/s, and a detector voltage of 1.0 kV.[35]

Absorption, distribution, metabolism, and excretion (ADME) studies

The drug likeliness of various herbal compounds was screened by ADME studies. The SWISS ADME, predicts physically significant descriptors and pharmaceutically relevant properties of organic molecules. In addition to predicting molecular properties, it provides ranges for comparing a particular molecule's properties with those of known drugs. The ADME and toxicity studies of the prepared ligands were done using Swiss ADME and ProTox II tools.[36,37]

Docking analysis

Selection of target proteins

PCR and sequencing confirmation of all selected four bla_{TEM}, bla_{CTX-M}, bla_{SHV} and bla_{OXA} genes was done. Amino acid sequences selections of genes were obtained by Expasy tools. The structures of the proteins of all selected four bla_{TEM}, bla_{CTX-M}, $bla_{SHV,}$ and bla_{OXA} genes were downloaded from the SWISS model, NCBI, and RCSB protein databank.[38]

Protein preparation

Protein preparation and refinement studies were performed on Ligand structures were geometrically prepared using Pymol, PMV, and Discovery studio. The removal of water molecules, addition of non-polar hydrogen's and Charges were done. The prepared ligands can be used for further docking analysis.[39,40]

Ligand preparation

The structure of the ligands (23 Phytoconstituents and standard 7 antibiotics) was downloaded from PubChem. Ligand structures were geometrically prepared by using Pymol, PMV, and Discovery studio Visualizer. The removal of water molecules, addition of non-polar hydrogen's and Charges were done. The prepared ligands can be used for further docking analysis. The protein and ligand files in pdbqt format were prepared using MGL tools.[41]

Molecular docking using AutoDock vina

AutoDock Vina uses a hierarchical series of filters to search for possible locations of the ligand in the active site region of the receptor. The receptor grid was generated at the receptor site bound by a ligand. The ligands were then docked to the target proteins using AutoDock Vina and multiple ligands docking were done using Perl. The calculation of the gradient effectively gives the optimization algorithm from a single evaluation protocols were used for the docking. The docked protein and the ligands were viewed with Pymol, PMV and Discovery studio. Non-bonded interactions like hydrophobic was observed using Pymol and Discovery studio visualizer and these interactions can increase the binding affinity between target drug interfaces. The images of the best docked poses of the ligand and the protein were saved.[26,42,43]

RESULTS AND DISCUSSION

Phytochemical analysis

The phytochemical analysis of the extract of *A. sativum*, *B. micrantha*, *C. lemon*, *G. glabra*, *P. granatum* showed the presence of amino acids, alkaloids, glycosides, saponins, flavonoids, steroids, and terpenoids.

Antibacterial activity

Antibacterial activity of *A. sativum* bulbs, *B. micrantha* bark, *C. lemon* peels, *G. glabra* roots, *and P. granatum* peels extracts was investigated against *S.* Typhimurium isolates are studied by disk diffusion method as shown in Figure 1. Mean diameter zones of inhibition (mm) at Concentration 30 (mg/mL) of *A. sativum* bulbs, *B. micrantha* bark, *C. lemon* peels,

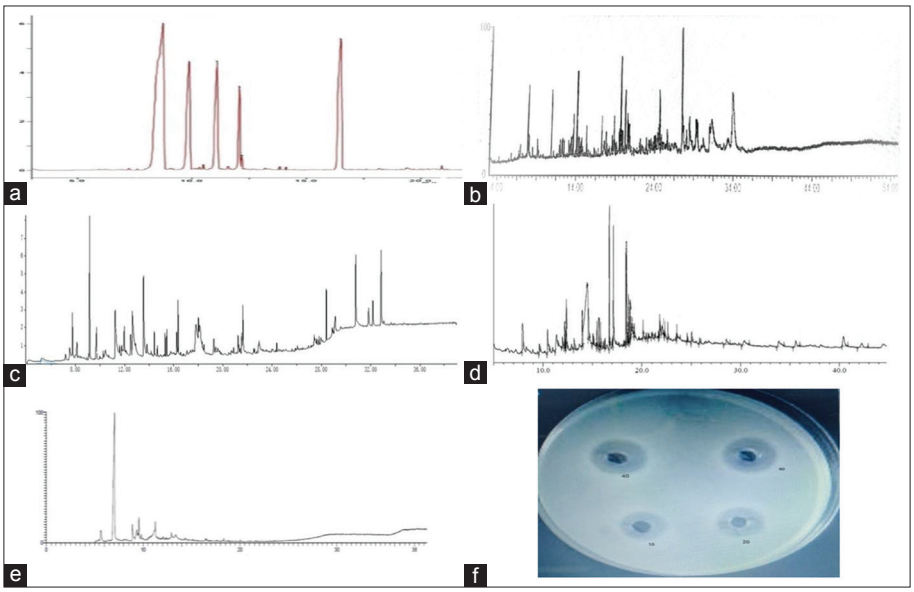

Figure 1: GC-MS Chromatogram of methanolic extract of the (a) *Alliumsativum* bulbs (b) *Bridelia micrantha* bark (c) *Citrus lemon* peels (d) *Glycyrrhiza glabra* roots, (e) *Punica granatum* peels, and (f) antibacterial activity of plants extracts

Table 1: List of phytoconstituents selected for docking analysis

Medicinal plant	Code	Phytochemicals
Allium sativum	Ligand 3	9-Octadecenoic acid (Z)-, methyl ester
	Ligand 1	1,2,3-propanetriyl ester
	Ligand 20	Squalene
	Ligand 5	Allicin
	Ligand 8	Diallyl disulfide
	Ligand 19	Pyrogallol
	Ligand 4	Ajoene
Bridelia micrantha	Ligand 15	L-Ascorbic acid, 6-octadecanoate
	Ligand 12	Gallic acid
	Ligand 10	Ellagic acid
	Ligand 23	Vitamin A Aldehyde
Citrus lemon	Ligand 7	Campesterol
	Ligand 22	Stigmasterol
	Ligand 16	limonene
	Ligand 14	Gamma-Sitosterol
Glycyrrhiza glabra	Ligand 11	Ethyl pipecolin
	Ligand 9	Eicosane
	Ligand 2	5-oxo-pyrrolidine-2-carboxylic acid methyl
	Ligand 17	Phenol, 2-[3,4-dihydro-8-methyl-8-(4-methyl-3-pentenyl
	Ligand 21	Stigmasta-5,22-dien-3-ol, (3.beta.,22E)-
	Ligand 13	Gamma.-Sitosterol monohydrate
	Ligand 6	Androstanone, 3-(3,4-dimethylphenyl)-3-methyl
Punica granatum	Ligand 18	Punicalagin

G. glabra roots, *and P. granatum* peels extracts were found as 10.12 ± 1.71 mm, 23.50 ± 0.00 mm, 26.60 ± 0.64 mm, 29.20 ± 0.11 mm, and 19.50 ± 0.00 mm, respectively, which were considerable to different standard antibiotics used as positive control [Figure 1e].

The polymerase chain reactions for ESBL genes that are bla_{CTX-M}, bla_{OXA}, $bla_{TEM,}$ and bla_{SHV} were performed for MDR *S.* Typhimurium isolates and desired amplifications bands were obtained on 1% agarose gel as 867 bp, 1510 bp, 656 bp, and 1100 bp, respectively.[31]

GC-MS analysis

Total 128 phytochemicals identified in the methanolic extracts of the *A. sativum* bulbs, *B. micrantha bark*, *C. lemon* peels, *G. glabra* roots, and *P. granatum* peels by GC-MS, out

Table 2: Phytoconstituents ADMET analysis

Code	Pubchem ID	Drug likeness	Bioavailability	Molecular weight	Toxicity
Ligand 3	44151529	0.67	0.85	647.07	N
Ligand 1	22833298	0.22	0.56	1059.54	Y
Ligand 20	638072	−0.9	0.55	410.72	N
Ligand 5	65036	−0.84	0.55	162.27	N
Ligand 8	16590	−1.06	0.55	146.27	Y
Ligand 19	1057	−1.36	0.55	126.11	Y
Ligand 4	5386591	−1.01	0.55	234.4	N
Ligand 15	54725318	0.4	0.56	442.59	N
Ligand 12	370	−0.22	0.56	170.12	Y
Ligand 10	5281855	−1.11	0.55	302.19	Y
Ligand 23	638015	0.5	0.55	284.44	N
Ligand 7	173183	0.59	0.55	400.68	N
Ligand 22	5280794	0.62	0.55	412.69	N
Ligand 16	22311	−1.54	0.55	136.23	N
Ligand 14	457801	0.78	0.55	414.71	N
Ligand 11	5745779	1.62	0.55	466.63	N
Ligand 9	8222	−1.03	0.55	282.55	N
Ligand 2	384213	0.34	0.56	355.38	N
Ligand 17	629585	0.92	0.55	406.51	N
Ligand 21	6432745	0.62	0.55	412.69	N
Ligand 13	1.33E+08	0.78	0.55	432.72	N
Ligand 6	633770	0.33	0.55	392.62	N
Ligand 18	44584733	−0.29	0.17	1084.72	N

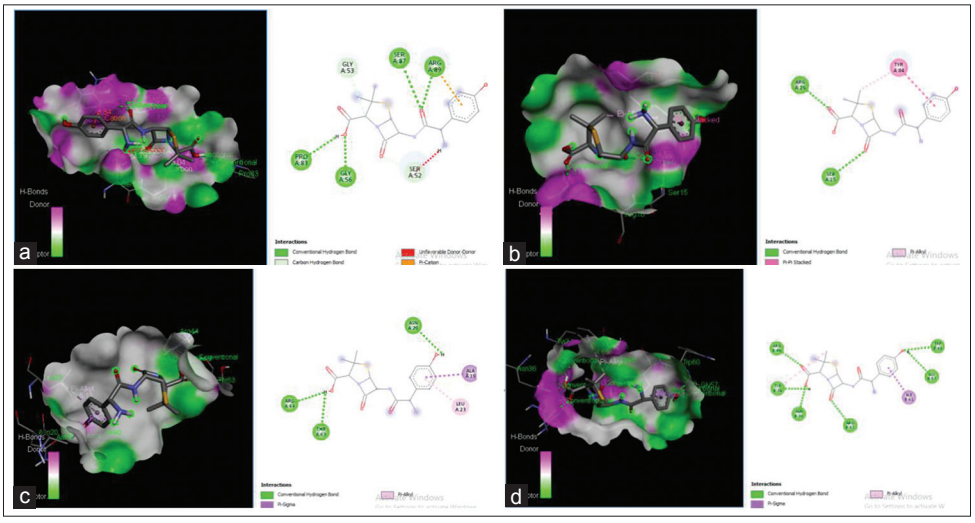

Figure 2: Docking results analysis of Amoxicillin with (a) CTX-Protein 1 (b) TEM-Protein 2 (c) SHV- Protein 3 (d) OXA- Protein 4

of which 23 phytochemicals were selected for the docking analysis with all four resistant gene proteins on the basis of their ADME, bioavailability, toxicity analysis listed in [Tables 1 and 2, Figure 1a-d] along with details about plant, code, and phytochemicals name.

Docking analysis

We discovered that all 23 phytochemicals interact with bla_{CTX-M}, bla_{OXA}, $bla_{TEM}$and bla_{SHV} genes proteins in some way after looking at molecular interaction findings from docking experiments with 7 standard antibiotics. Selected 7 antibiotics shows binding energy ranges from −4.7 to −7.7 kcal/moL. Among which amoxicillin shows lowest when docked with all four proteins that are 7.7 kcal/moL 3D and 2D interactions for amoxicillin with these four proteins are shown in [Figure 2 and Table 3]. Docking results of phyto-ligand with selected resistance genes proteins reveal that the binding energy ranges from −2.9 to −9.2 kcal/moL. As compared to standard antibiotics the phyto-ligands seems to be prominent option for combating the MDR *S.* Typhimurium. Among all phyto-ligands screened the ligand 6 shows lowest docking energy that is −9.2 kcal/moL. This *in silico* study suggest Phytoconstituents from methanolic extract of these plants are *A. sativum* bulbs (Ligand19-Pyrogallol), *B. micrantha* bark (Ligand10-Ellagic acid), *C. lemon* (Ligan7-Campesterol), *G. glabra* roots (Ligand6-Androstanone, 3-(3, 4-dimethylphenyl)-3-methyl), *and P. granatum* peels (Ligand18-Punicalagin) in consideration to antimicrobial

Table 3: Antibiotics with protein docking score

Code (For Phyto-ligands)	CTX (Protein 1)	TEM (Protein 2)	SHV (Protein 3)	OXA (Protein 4)
Amoxicillin	−5.7	−6.2	−5.8	−7.7
Cefotaxime	−5.5	−5.9	−5.6	−6.8
Chloramphenicol	−4.7	−5.1	−5.1	−5.8
Enrofloxacin	−6.0	−6.3	−6.7	−7.4
Gentamicin	−5.7	−5.2	−5.7	−7.2
Nalidixic acid	−5.6	−5.6	−5.6	−6.4
Tetracycline	−6.2	−7.0	−6.7	−7.4

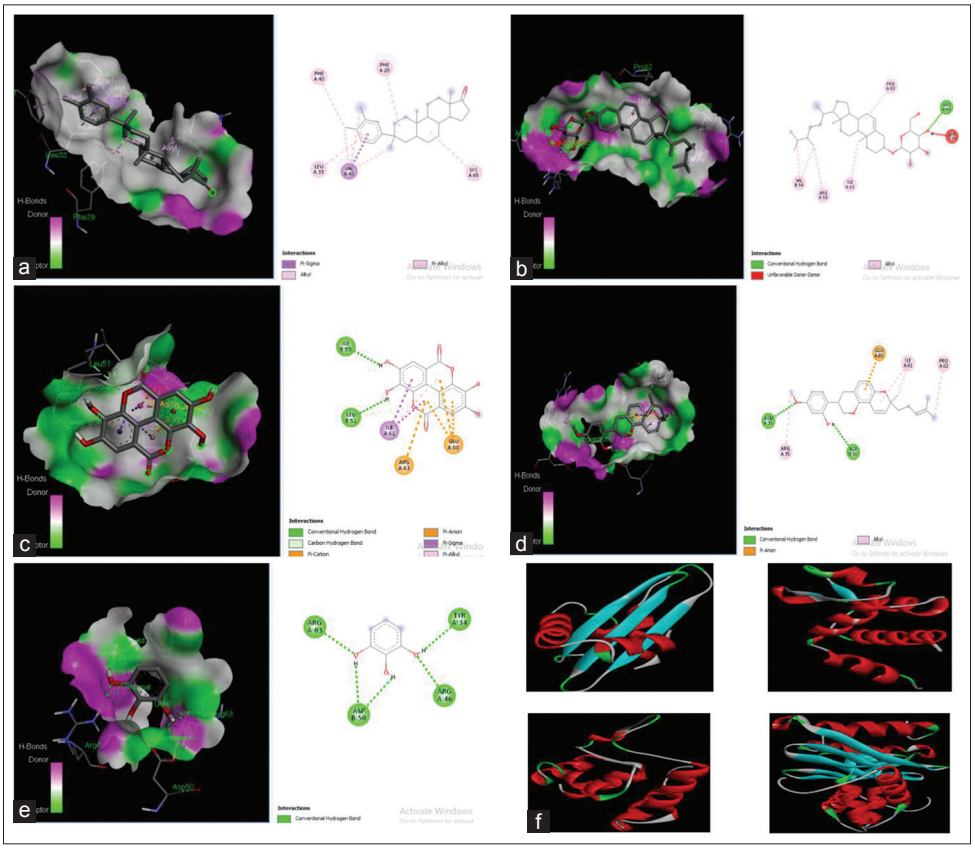

Figure 3: Docking results analysis of Phyto-ligands with OXA-Protein 4 (a) Ligand 6, (b) Ligand 7, (c) Ligand10, (d) Ligand 18, (e) Ligand 19, and (f) Protein structures

Table 4: Phyto-ligand with protein docking score

Code (For Phyto-ligands)	CTX (Protein 1)	TEM (Protein 2)	SHV (Protein 3)	OXA (Protein 4)
Ligand 3	−4.1	−4.8	−5.3	−5.0
Ligand 01	−4.8	−4.7	−4.6	−5.2
Ligand 20	−5.1	−4.7	−6.2	−6.4
Ligand 5	−3.1	−3.5	−3.4	−4.1
Ligand 8	−2.9	−3.2	−3.1	−3.1
Ligand 19	−4.2	−4.5	−4.6	−5.2
Ligand 4	−3.3	−3.6	−3.7	−4.1
Ligand 15	−4.6	−4.8	−4.5	−5.6
Ligand 12	−4.7	−5.1	−4.9	−5.5
Ligand 10	−6.5	−6.5	−6.8	−7.9
Ligand 23	−5.7	−5.6	−6.1	−5.9
Ligand 7	−7.0	−7.2	−8.0	−8.3
Ligand 22	−6.6	−7.6	−7.9	−7.0
Ligand 16	−4.3	−5.1	−4.8	−5.1
Ligand 14	−6.7	−7.1	−7.4	−7.0
Ligand 11	−6.3	−7.2	−7.2	−7.6
Ligand 9	−6.4	−6.9	−7.1	−7.7
Ligand 2	−6.3	−6.3	−5.8	−6.6
Ligand 17	−7.3	−7.3	−7.6	−7.7
Ligand 21	−6.9	−7.1	−7.8	−7.1
Ligand 13	−6.5	−7.3	−7.6	−6.5
Ligand 6	−8.0	−8.8	−9.2	−8.9
Ligand 18	−7.2	−7.2	−7.6	−7.7

effect on MDR *S.* Typhimurium. The final intermolecular energy, inhibition constants, and hydrogen bond formation during the interaction of phyto-ligands and resistance genes proteins could all be used to evaluate molecular docking data [Figure 3 and Table 4].

The lowest binding energy was found in *G. glabra* roots (Ligand6-Androstanone, 3-(3, 4-dimethylphenyl)-3-methyl), which was −9.2 kcal/moL. However, the binding energy represented in the table is primarily the result of the chemical interactions shown in Figure 3. Covalent bonds, alkyl bonds, pi bonds, and hydrogen bonds were primarily produced as a result of chemical interaction, which accounts for the protein-ligand binding stability.

CONCLUSION

In this study, we have indicated the antimicrobial activity *A. sativum* bulbs, *B. micrantha* bark, *C. lemon* peels, *G. glabra* roots, *and P. granatum* peels among all of these of *G. glabra* roots (Ligand6- Androstanone, 3-(3, 4-dimethylphenyl)-3-methyl) highest in this categories against MDR *S.* Typhimurium.

DATA AVAILABILITY STATEMENT

This article contains all of the data generated or analyzed during this investigation.

FUNDING

For the submission this study, it does not include any research funding.

COMPETING INTERESTS

There are no competing interests in this study, according to the authors.

ETHICAL APPROVAL

Because in present study animals were not used as result of this, ethical approval was not required.

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REFERENCES

1. Crump JA, Sjölund-Karlsson M, Gordon MA, Parry CM. Epidemiology, clinical presentation, laboratory diagnosis, antimicrobial resistance, and antimicrobial management of invasive *Salmonella* infections. Clin Microbiol Rev 2015;28:901-7.
2. Eng SK, Pusparajah P, Mutalib NS, Ser HL, Chan KG, Lee LH. *Salmonella*: A review on pathogenesis, epidemiology and antibiotic resistance. Front Life Sci 2015;8:284-93.
3. Bouchrif B, Hello SL, Pardos M, Karraouan B, Perrier-Gros-Claude JD, Ennaji M, *et al*. Ceftazidime-resistant *Salmonella enterica,* Morocco. Emerg Infect Dis 2009;15:1693-5.
4. Avato P, Tursil E, Vitali C, Miccolis V, Candido V. Allylsulfide constituents of garlic volatile oil as antimicrobial agents. Phytomedicine 2000;7:239-43.
5. Reuter HD, Koch HP, Lawson DL. Therapeutic effects and applications of garlic and its preparations. In. Koch HP, Lawson LD, editors. Garlic: The Science and Therapeutic Applications of *Allium sativum* L and Related Species. 2nd ed. Baltimore, MD: William and Wilkins; 1996. p. 135-212.
6. Biswas T, Dwivedi UN. Plant triterpenoid saponins: Biosynthesis, *in vitro* production, and pharmacological relevance. Protoplasma 2019;256:1463-86.
7. Douglas K, Gitonga A. Antimicrobial activity of *Bridelia micrantha* and *Grewia plagiophylla* leaf extracts. Br J Pharm Res 2016;12:1-7.
8. Ochwang"i DO, Kimwele CN, Oduma JA, Gathumbi PK, Kiama SG, Efferth T. Cytotoxic activity of medicinal plants of the Kakamega County (Kenya) against drug-sensitive and multidrug-resistant cancer cells. J Ethnopharmacol 2018;215:233-40.
9. Akhilesh K, Raghvendra P, Vikas S, Madhulika G. Antimicrobial property of lemon peel extract. Natl J Universal Pharm Life Sci 2012;52:382-6.
10. Amit P, Arti K, Sudeep KT. Evaluation of antimicrobial activity and phytochemical analysis of *Citrus limon*. J Pharm Biomed Sci 2011;13:101-7.
11. Singh R, Chandrqa R, Mridula B, Luthra PM. Antibactreial activity of *Glycyrrhiza Glabra* L. extract on pathogenic bacteria. Curr Sci 2002;83:737.
12. Pillai TV. Dictionary of Medicine, Chemistry, Botany and Allied science. Vol. 3. p. 212-17.
13. Ricci D, Giamperi L, Bucchini A, Fraternale D. Antioxidant activity of *Punica granatum* fruits. Fitoterapia 2006;77:310-12.
14. Anchez-Lamar AS, Fonseca G, Fuentes JL, Cozzi R, Cundari E, Fiore M, *et al*. Assessment of the genotoxic risk of *Punica granatum* L. (Punicaceae) whole fruit extracts. J Ethnopharmacol 2007;115:416-2.
15. Aviram M, Rosenblat M, Gaitini D, Nitecki S, Hoffman A, Dornfeld L *et al*. Pomegranatem juice consumption for 3 years by patients with carotid artery stenosis reduces common carotid intima-media thickness, blood pressure and LDL oxidation. Clin Nutr 2004;23:423-3.
16. Parmar HS, Kar A. Protective role of *Citrus sinensis*, *Musa paradisiaca,* and *Punica granatum* peels against diet-induced atherosclerosis and thyroid dysfunctions in rats. Nutr Res 2007;27:710-8.
17. Zhang J, Zhan B, Yao X, Gao Y, Shong J. Antiviral activity of tannin from the pericarp of *Punica granatum* L. against genital Herpes virus *in vitro*. Zhongguo Zhong Yao Za Zhi 1995;20:556-76.
18. Lansky EP, Newman RA. *Punica granatum* (pomegranate) and its potential for prevention and treatment of inflammation and cancer. J Ethnopharmacol 2007;109:177-206.
19. Naz S, Siddiqi R, Ahmad S, Rasool SA, Sayeed SA. Antibacterial activity directed isolation of compounds from *Punica granatum*. J Food Sci 2007;72:M341-5.
20. Kumari R, Mishra RC, Yadav A, Yadav JP. Screening of traditionally used medicinal plants for their antimicrobial efficacy against oral pathogens and GC-MS analysis of *Acacia nilotica* extract. Indian J Tradit Knowl 2019;18:162-8.
21. Park NH, Jang HR, Lee SJ, Boby N, Park SC. Chromatographic mass spectrometric analysis, antimicrobial and antioxidant effects of ethanolic garlic extract. Int J Gas Chromatogr Antioxidant Phytomed 2017;9:324-1.
22. George TK, Tomy A, Jisha MS. Molecular docking study of bioactive compounds of *Withania somnifera* extract against topoisomerase IV type B. Proc Natl Acad Sci India Sec B Biol Sci 2019;90:381-90.
23. Umesh HR, Ramesh KV, Devaraju KS. Molecular docking studies of phytochemicals against trehalose-6-phosphate phosphatases of pathogenic microbes. Beni-Seuf Univ J Basic Appl Sci 2020;9:5.
24. Stumpfe D, Ahmed HE, Vogt I, Bajorath J. Methods for computer-aided chemical biology: Design of a benchmark system for the evaluation of compound selectivity. Chem Biol Drug Des 2007;70:182-94.
25. Eberhardt J, Santos-Martins D, Tillack AF, Forli S. AutoDock Vina 1.2.0: New docking methods, expanded force field, and python bindings. J Chem Inf Model 2021;61:3891-8.
26. Trott O, Olson AJ. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. J Comput Chem 2010;31:455-61.
27. Ganugapati J, Mukkavalli S, Atimamul S, Siva SD, Sahithi A. *In silico* modeling and docking studies of New Delhi metallo-beta-lactamase-1 (super bug). Int J Eng Sci Technol 2011;3:2427-34.
28. Lakshmi P, Radhika S, Annamalai A. Molecular

docking analysis of phyto-ligands with multi drug resistant β-lactamase of *Staphyloccus aureus*. Trends Bioinformatics 2011;4:23-34.
29. Gaikwad RP, Channawar MA, Mohale D, Kochar NI, Anil V, Chandewar MA, *et al.* Evaluation of the antimicrobial activity of the stem bark extracts of *Bridelia micrantha* against MDR *salmonella typhimurium*. Neuro Quantol 2022;20:872-6.
30. Gaikwad RP, Channawar MA, Mohale D, Kochar NI, Chandewar AV, *et al*. Evaluation of the antimicrobial activity of the *Punica granatum* Peel extract against isolated MDR *salmonella typhimurium*. Acta Sci Pharm Sci 2023;7:2-7.
31. Gaikwad RP, Channawar MA, Mohale DS, Kochar NI, Chandewar AV. Genotypic and phenotypic characterization of MDR *Salmonella typhimurium* isolated from various sources in Maharashtra state. Biol Forum Int J 2023;15:66-72.
32. Varsha S, Agrawal RC, Sonam P. Phytochemical screening and determination of anti-bacterial and anti-oxidant potential of *Glycyrrhiza glabra* root extracts. J Environ Res Dev 2013;7:1552-8.
33. Velvizhi S, Annapurani S. Estimation of total flavonoid, phenolic content, and free radical scavenging potential of *Glycyrrhiza glabra*. Asian J Pharm Clin Res 2018;11:231-5.
34. Ates DA, Erdourul ZT. Antimicrobial activities of various medicinal and commercial plant extracts. Turk J Biol 2003;27:157-62.
35. Kumar A, Vijayalakshmi K. GC-MS analysis of phytochemical constituents in ethanolic extract of *Punica granatum* peel and *Vitis vinifera* seeds. Int J Pharm Bio Sci 2011;2:461-8.
36. Banerjee P, Eckert AO, Schrey AK, Preissner R. ProTox-II: A webserver for the prediction of toxicity of chemicals. Nucleic Acids Res 2018;46:W257-63.
37. Bienert S, Waterhouse A, De Beer TA, Tauriello G, Studer G, Bordoli, *et al*. The SWISS-MODEL Repository-new features and functionality. Nucleic Acids Res 2017;45:D313-9.
38. Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R, *et al.* SWISS-MODEL: Homology modelling of protein structures and complexes. Nucleic Acids Res 2018;46:W296-303.
39. The PyMOL Molecular Graphics System. Version 1.3. Austria: Schrödinger LLC.
40. Coon SI, Sanner MF, Olson AO. Re-Usable Components for Structural Bioinformatics. New Jersey, USA: Citeseer; 2001.
41. BIOVIA, Dassault Systèmes, BIOVIA Workbook, Release 2020; BIOVIA Pipeline Pilot, Release 2020. San Diego: Dassault Systèmes; 2020.
42. Guex N, Peitsch MC, Schwede T. Automated comparative protein structure modeling with SWISS-MODEL and Swiss-PdbViewer: A historical perspective. Electrophoresis 2009;30:S162-73.
43. Birhanu BT, Lee EB, Lee SJ, Park SC. Targeting *Salmonella typhimurium* invasion and intracellular survival using pyrogallol. Front Microbiol

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