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RESEARCH ARTICLE

Ethnobotanical value of *Withania coagulans* against carbapenem-resistant UTI pathogens

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Abstract

Antibiotic resistance has become a major global health concern due to the emergence of carbapenem-resistant bacterial species that diminish the effectiveness of conventional antibiotics. Because of this, researchers are increasingly turning to herbal products as alternative sources of antibacterial compounds. The present study aimed to evaluate the phytochemical profile and effectiveness of *Withania coagulans* Dunal activity on carbapenem-resistant pathogens. The phytochemicals were extracted by the Soxhlet extraction method with different solvents and major phytochemicals were quantified and analyzed by Gas chromatography-mass spectrometry (GC-MS). The antibacterial effect of the standard and extract was determined by the disc diffusion method as per Clinical Laboratory Standards Institute (CLSI) guidelines against drug resistant pathogens and the compound interaction was studied by the *in silico* docking method. The polar solvent showed significant yield and content of phytochemicals. The maximum yield was 3.42 %, with 11.67 mg/g of alkaloids, 12.13 mg/g of flavonoids and 6.87 mg/g of phenols, followed by ethyl acetate. Antibiotic resistance among clinical isolates was found to be 67.5 % for the resistant pattern to imipenem, followed by doripenem (62.5 %), ertapenem (60 %) and meropenem (57.5 %). The effect of extract on resistant strains was best in nonpolar extraction and less significant among polar solvents. The hexane extract and chloroform extract had the highest inhibition (26 mm), especially on *Proteus vulgaris* and *Klebsiella pneumoniae*. Molecular docking of the GC-MS-produced molecule 2,4-Dihydroxypropiophenone has shown that the molecule is a good binding molecule in the carbapenemase active site, confirming the antibacterial activity of the extract. The findings confirm that the extract of *W. coagulans* had significant antibacterial activity and could serve as a promising natural phytoremedy to overcome the emerging extended-spectrum beta-lactamase (ESBL) drug resistance infections. Formulation with nanotechnology and *in vivo* studies will be essential to validate the therapeutic potential for future clinical application.

Keywords: antibacterial; carbapenem resistance; *ESBL*; molecular docking; phytochemical analysis; withanolides

Introduction

Withania coagulans Dunal belongs to the family Solanaceae. It is a small, whitish-gray shrub that grows in Pakistan and India (1). It is often called the Indian cheesemaker or vegetable rennet because it contains withanin, an essential enzyme needed to coagulate milk into cheese. *Withania coagulans* is a well-known medicinal shrub in the Solanaceae family that has long been used in Ayurveda to treat liver issues, diabetes and inflammation (2). In accordance with biochemical studies, the plant contains abundance of steroidal lactones (withanolides), alkaloids, flavonoids and phenolic acids that support a broad spectrum of medicinal effects (3). Recent studies have shown that *W. coagulans* extracts have anti-inflammatory, antibacterial, liver-protective and anti-hyperglycemic effects in experimental animals. *Withania coagulans* has shown encouraging antibacterial activity against multidrug-resistant pathogens in recent pharmacological studies. According to a current comprehensive investigation, these extracts' antibacterial, antifungal and antiviral properties are well-established in a variety of solvent systems, underscoring the plant's extensive antimicrobial potential (4). Silver nanoparticles made from *W. coagulans* fruit extract showed notable zones of inhibition (>20 mm) against *Klebsiella pneumoniae* at low concentrations, indicating improved antimicrobial effectiveness when paired with nanotechnology especially with silver and zinc metal ions (5, 6). The emergence of carbapenem resistance among *Pseudomonas aeruginosa, Acinetobacter baumannii* and *Klebsiella pneumoniae* poses a serious threat to public health because there are only a few options to antibiotic therapy. These outcomes evidence the possibility of medicinal plants for antibiotic-resistant prophylaxis. Plant-derived phytochemicals, which provide structural diversity, multitarget modes of action and the ability to evade conventional resistance mechanisms, have drawn attention in response (7, 8). For instance, herbal remedies have been found to interfere with efflux pumps, inhibit β-lactamase activity and work in concert with current antibiotics to combat pathogens that are resistant to multiple drugs (9, 10). These results highlight the potential of botanical compounds as supplemental tactics against infections resistant to carbapenem. Therefore, the present study focused on the examination of the antibacterial role of phytochemicals extracted from *W. coagulans* medicinal plant on clinical pathogens.

Fig. 1. Study plant sample collection site.

Materials and Methods

Extraction of phytochemical

The dried leaf powder of *W. coagulans* procured from Thiruvarangam (Fig. 1) was successively extracted by the hot continuous extraction method using a Soxhlet apparatus. The Soxhlet thimble compartment was filled with 50 g of leaf powder that had been packaged with non-absorbent cotton. The material was extracted successively at 50–60 °C using 250 mL of different solvents in the sequence of increasing polarity: hexane, chloroform, ethyl acetate and methanol for 8 cycles at 6 hr. The extract was collected and condensed under a vacuum evaporator. The solvent of the extract was reduced under room temperature and the extracted samples were refrigerated at 4 °C. The yield of each extract was recorded as follows:

$$Y\,(\%) = W_e/W_s \times 100$$

Where,

Y= Percentage of yield

W_e = Weight of extract

W_s= Weight of sample taken.

Quantitative phytochemical study

Estimation of total phenolic content

Total phenol content was estimated by the FC method. The reaction mixture consists of 0.5 mL extract and 0.1 mL of 0.5 N Folin -Ciocalteu reagent (FC); it was incubated at 37 °C for 5 min. After incubation, 2.5 mL of saturated sodium carbonate was added and it was further incubated for 30 min. After incubation the colour developed was read at 760 nm using a spectrophotometer. Gallic acid was used as the standard.

Estimation of flavonoids

About 0.5 mL of the extract, 0.5 mL of aluminium chloride and 0.5 mL of potassium acetate were placed into a test tube (120 mL). The tube was allowed to stand at room temperature for half an hour. Using a spectrophotometer, the colour developed was measured at 417 nm. Quercetin served as the standard, while distilled water served as the blank.

Estimation of alkaloid

Five milligrams of each *W. coagulans* extract were dissolved in 10 mL dimethyl sulfoxide (DMSO). After filtering, 1 mL of 2 N hydrochloric acid was added and filtered. About 0.5 mL of phosphate buffer solution and 5 mL of bromocresol green reagent were added to the filtered sample. The mixture was vigorously agitated with 5 mL of chloroform, collected in a 10 mL volumetric flask and diluted with chloroform to the 10 ml volume. In a spectrophotometer, the absorbance of the test was measured at 470 nm in relation to the reagent blank. The total alkaloid content was expressed as mg of AE/g of extract.

Isolation of clinical pathogens

Clinical pathogens were collected at Helikem Diagnostics and Research Laboratory, Tiruchirappalli, Tamil Nadu and subcultured on MacConkey agar and characterized by biochemical characters such as Gram stain, IMViC, catalase and oxidase.

Carbapenem-resistance screening

In compliance with the guidelines of the Clinical Laboratory Standards Institute (CLSI), further antimicrobial susceptibility testing was carried out on clinically isolated organisms that were demonstrating resistance to imipenem and meropenem. The suspension of 0.5 McFarland standard turbidity in saline was prepared and swabbed over the surface of a Mueller-Hinton agar (MHA) plate. The antibiotic discs were placed on the agar and incubated for 24 hr at 37 °C. The zone of inhibition was measured using a zone scale.

Modified Hodge test (MHT)

A 0.5 McFarland culture of ATCC *Escherichia coli* 25922 was diluted 1:10 in sterile saline. It was inoculated on a Mueller-Hinton agar plate and a disc containing 10 µg of imipenem was placed in the middle of the plate after it had dried for 5 min. Test organisms were chosen, inoculated in a straight line at least 20 mm from the disc's edge and incubated overnight. They were checked for enhanced growth in the vicinity of the test organism. The production of extended-spectrum beta-lactamase (ESBL) was indicated by a cloverleaf zone or distortion of inhibition surrounding the imipenem disc.

Imipenem-EDTA combined disc test

Ten microliters of 0.5 M EDTA solution were added to 10 µg imipenem discs, which were mounted on a plate that had already been seeded with the test pathogens. The zone of inhibition between the imipenem and EDTA-amended discs was recorded. A zone greater than 7 mm variation was considered positive for metallo-beta-lactamase (MBL) production.

Synergy testing

Using the gold standard method, all the isolates were subjected to *in vitro* synergy testing for the antibiotics, such as imipenem+tigecycline and meropenem+colistin. The plates were incubated at 37 °C for 18 hr. The zone sizes surrounding each disc have been determined following incubation. A valid phenotypic test to confirm ESBL production was defined as a difference of > 5 mm between the zone diameters of either the imipenem disc and

the corresponding tigecycline (10 µg) or Colistin (10 µg) discs.

Molecular characterization by sequencing

Antibiotic-resistant strains were characterized by 16S rDNA analysis. Using the spin-win DNA extraction kit (Himedia, Mumbai), DNA was extracted from a 24 hr old culture. Primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) were used to amplify the 16S gene. PCR was performed in a 25 µL volume that contained 50 ng DNA template with 0.5 µL (3 U/µL) Taq polymerase, 2.5 µL of 10X reaction buffer with $MgCl_2$ (1.5 mM), 0.5 µL (200 µM) dNTPs and 0.5 µL of primer (10 pm/µL). Preliminary denaturation at 94 °C for 5 min, denaturation at 94 °C for 30 sec, annealing at 55 °C for 4 sec, extension at 72 °C for 1 min 45 sec and post-elongation at 72 °C for 10 min were the temperature parameters under which the amplification process was conducted over 30 cycles. Sanger sequencing was performed on the PCR-amplified products. After Sanger sequence analysis, the phylogenetic tree was constructed using MEGA BLAST.

Antibacterial effect of extract

Different concentrations of extract (50, 100, 250 and 500 µg/mL) were made from a stock of 5 mg/mL and loaded onto sterile discs. The preloaded discs were placed on each plate seeded with isolated ESBL strain at 0.5 McFarland as described by the standard Kirby-Bauer method. A disc loaded with methanol served as the negative control, while AMC served as the positive control. All the plates were incubated for 48 hr at 37 °C. The development of the zone of inhibition around the microorganisms was determined.

Gas chromatography mass spectrum (GC-MS) analysis

The methanol extract at 500 µg/mL was subjected to GC-MS analysis using Thermo MS DSQ II system. The equipment includes a DB 35-MS capillary standard non-polar column with dimensions of 30 m × 0.25 mm ID × 0.25 µm film thickness. At an average velocity of 1.0 mL/min, helium was used as the transport gas. The electron energy was set at 70 eV and the oven was preheated to 250 °C. The temperature was gradually increased to 280 °C for 3 min after maintaining 15 min at 60 °C. Injector temperature was 250–300 °C. The components have been determined and the results were documented after evaluating them with those from NIST and Willey libraries linked to the GC-MS device.

In silico docking of identified ligand

The crystal structures of *E. coli* proteins (pdb:3DW0) were obtained as 3D structures in PDB format from the AlphaFold Protein Structure Database. Docking tests were conducted using the dock module of MOE (Molecular Operating Environment), version MOE 2019.0102.2. After removing all water molecules from the target protein receptors, hydrogen atoms were incorporated into the protein structure using 3D protonation with standard geometry. The program's default parameters were used to minimize energy and ligand preparation. The ligand was docked against the protein's rigid interaction pocket. The force field-based scoring function was used to determine the free energy of ligand binding from a specific position.

Results and Discussion

Extraction and phytochemical study

The study plant (certificate number: HGB/PA/018-25) was authenticated by the Department of Plant Taxonomy, HeteroGene Biotech Pvt Ltd., Chennai, Tamil Nadu, India. The best solvent extraction was observed with methanol (5.14 g; 3.42 %), which was followed by ethyl acetate (3.93 g; 2.62 %) and chloroform (3.77 g; 2.51 %). Water had the lowest extraction efficiency at 1.27 g (0.84 %), whereas hexane produced 2.87 g (1.91%). This hierarchy (methanol > ethyl acetate > chloroform > hexane > water) demonstrates that solvent polarity affects yield of extraction (Table 1). These results are in line with prior studies that showed polar solvents extract a broad range of hydrophilic bioactive compounds from medicinal plants than non-polar solvents (11, 12). On the other hand, non-polar solvents, such as hexane extracts, typically include lipophilic components, which reduced yields but enhance terpenoids recovery (13, 14). Despite being highly polar water frequently has low recovery rates due to its restricted matrix permeability and inability to separate less polar plant compounds.

Quantitative analysis of the primary phytochemical categories in *W. coagulans* samples revealed a clear dependence on solvent polarity (Table 2). The hexane extract contained 1.67 ± 0.05 mg/g of alkaloids, while the methanol extract contained 11.67 ± 0.34 mg/g. The flavonoid contents showed similar trends between hexane (8.15 ± 0.25 mg/g) and methanol (12.13 ± 0.15 mg/g). The water extract exhibited the lowest phenolic content (8.14 ± 0.21 mg/g), whereas the methanol extract had the highest (16.87 ± 0.33 mg/g). Several studies have reported that, because of its strong polarity and protic nature, methanol diffuses inside cells, increasing the extraction of phytochemicals such as flavonoids, phenolics and alkaloids (15, 16). Additionally, several studies have demonstrated that phytochemical content and extraction yield rise with solvent polarity, with methanol frequently outperforming less polar solvents (17-19). On the other hand, only lipophilic components can be removed by non-polar solvents like hexane, resulting in substantially lower amounts of phytochemicals. Although water is the most polar solvent, its efficiency may be restricted by insufficient extraction of specific additional metabolites and low volume transit across plant tissues (20).

Table 1. Percentage of yield of extraction among different solvent

S. No	Solvent	Weight (g)	Percentage
1	Hexane	2.87	1.91
2	Chloroform	3.77	2.51
3	Ethyl acetate	3.93	2.62
4	Water	1.27	0.84
5	Methanol	5.14	3.42

Table 2. Quantitative phytochemical analysis of major compounds estimated (mg/g)

Phytochemical	Hexane	Chloroform	Ethyl acetate	Methanol	Water
Alkaloids	1.67 ± 0.05	2.56 ± 0.11	3.12 ± 0.21	11.67 ± 0.34	4.12 ± 0.15
Flavonoids (mg/g)	8.15 ± 0.25	8.87 ± 0.09	11.01 ± 0.04	12.13 ± 0.15	5.73 ± 0.435
Phenolics (mg/g)	10.56 ± 0.65	9.67 ± 0.12	12.14 ± 0.08	16.87 ± 0.33	8.14 ± 0.21

Carbapenem resistance among pathogens

Four carbapenem antibiotics were tested on 40 clinical isolates and results on resistance (R) and sensitivity (S) were given in Table 3. Among the isolates, meropenem resistance was 57.5 %, doripenem 62.5 %, ertapenem 60 % and imipenem 67.5 %. About 32.5%–42.5 % of isolates were still completely sensitive to the carbapenem during the investigation (Fig. 2). Imipenem showed the greatest degree of resistance, followed by doripenem, ertapenem and meropenem. Given that carbapenems are frequently regarded as last-line treatments for multi-drug-resistant Gram-negative infections, a 65 % imipenem-resistant incidence is very concerning (Table 3). This study shows a disturbing level of carbapenem-resistance among Gram-negative isolates associated with clinical pathogens; more than 50 % with substantial imipenem-resistance (65 %) is a significant finding and an important concern, because of carbapenems antibiotic failure to treat. This enhanced resistance greatly limits therapeutic alternatives and increases the chance of treatment failure in clinical settings. Numerous investigations have shown carbapenemase-producing organisms in Gram-negative UTIs, including bla NDM, bla IMP and bla VIM gene carriers (21). The result indicates greater carbapenems as compared to Indian literature. It has been discovered that between 20 and 35 percent of Indian UTI bacteria are resistant to carbapenem (22, 23). For example, urine specimens from hospitalized patients exhibited higher carbapenem- esistance for *E. coli* and less for *K. pneumoniae,* as reported earlier (24, 25). The examined pathogens varied in their resistance to imipenem (65 %). The confirmation assays (Table 4) demonstrated that six isolates (R38, R36, R32, R37, R21 and R25) expressed carbapenemase inhibited by EDTA action. The antibiogram test revealed that *Citrobacter koseri* (R39) was highly sensitive to all tested antibiotics. Phylogenetic analysis confirmed the presence of various pathogens, with their GenBank accession numbers as follows: *C. koseri* (PX112644), *K. pneumoniae* (PX112645, PX112647), *P. vulgaris* (PX112646, PX112651), *P. aeruginosa* (PX112648, PX112652), and *Streptococcus pneumoniae* (PX112653)

Withania coagulans extract activity on Carbapenem-resistant strains

Promising antibacterial activity was recorded for hexane (HE) and chloroform (CE) extraction over *P. vulgaris* and *K. pneumoniae* (26 mm). Similarly, *S. pneumoniae* (25 mm) and *C. koseri* (24 mm) were highly sensitive to the ethyl acetate-extract, suggesting that *W. coagulans has* potential as a source of nonpolar antibacterial therapeutic agent (Table 5). All the tested pathogens were found to be resistant to water extract but moderately sensitive to methanol extract, demonstrating a 13–22 mm zone of inhibition. The pathogen *P. aeruginosa* was the least susceptible organism, especially to polar extracts. The observed pattern of activity is in line with studies showing that non-polar extractions yield more recoverable bioactive molecules (26). The activity of ethyl acetate and chloroform extracts against *P. aeruginosa* and *K. pneumoniae*

Table 3. Number of antibiotic-resistant isolates registered among 40 tested pathogens

Isolate	MRP	DOR	IMP	ETP	Isolate	MRP	DOR	IMP	ETP
R1	26	R	R	R	R21	R	R	R	R
R2	R	R	R	R	R22	35	R	R	R
R3	R	R	R	R	R23	R	R	R	R
R4	23	26	24	20	R24	R	R	R	R
R5	I10	I12	I 8	I 10	R25	R	R	R	R
R6	18	20	22	25	R26	32	32	-36	34
R7	29	R	R	R	R27	28	30	-32	28
R8	R	R	R	R	R28	28	29	-30	32
R9	R	R	R	I 8	R29	I-7	R	R	I-8
R10	I 15	I17	I 23	I 25	R30	R	R	R	R
R11	32	34	34	36	R31	- 30	-30	R-31	32
R12	33	34	36	32	R32	R	R	R	R
R13	R	R	R	R	R33	-29	-30	-31	32
R14	R	R	R	R	R34	I-6	R	R	I-7
R15	32	36	R	34	R35	28	30	32	31
R16	32	34	32	34	R36	R	R	R	R
R17	I 17	R	R	I 16	R37	R	R	R	R
R18	32	34	32	36	R38	R	R	R	R
R19	34	32	R	34	R39	R	R	R	R
R20	R	R	R	R	R40	R	R	R	R

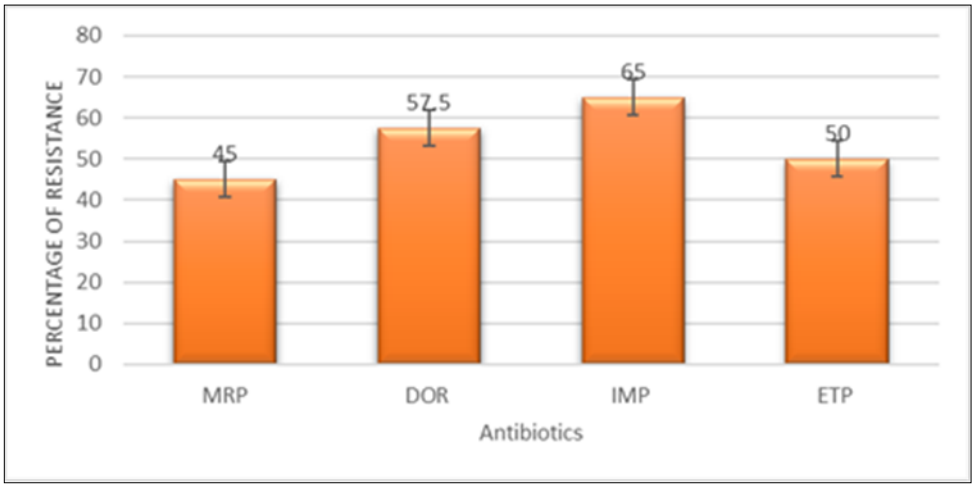

Fig. 2. Percentage of resistance among carbapenem antibiotics [MRP: meropenem; DOR: Doripenem; IMP: Imipenem; ETP: Ertapenem].

Table 4. Confirmatory test on antibiotic resistance

Isolate	Organism	EDTA -Synergy	EBL Production	Modified Hodge Tet	GenBank Accession Number
R39	*Citrobacter koei*	–	–	–	PX112644
R38	*Klebsiella pneumoniae*	+	+	+	PX112645
R36	*Proteus vulgaris*	+	+	+	PX112646
R32	*Klebsiella pneumoniae*	+	+	+	PX112647
R37	*Pseudomonas aeruginosa*	+	+	+	PX112648
R21	*Proteus vulgaris*	+	+	+	PX112651
R25	*Pseudomonas aeruginosa*	+	+	+	PX112652
R40	*S. pneumoniae*	–	–	–	PX112653

Table 5. Antibacterial activity of *W. coagulans* extract at 500 µg/mL

Pathogen	IMP	ME	CE	HE	EA	AE
C. koei	5	18	18	11	24	0
K. pneumoniae	7	22	24	26	23	0
P. vulgaris	7	15	26	24	22	0
K. pneumoniae	8	19	26	26	24	0
P. aeruginosa	5	16	9	22	-	0
P. vulgaris	5	20	12	24	12	0
P.aeruginosa	8	13	-	-	10	0
S. pneumoniae	10	22	21	20	25	0

reached significant zones of inhibition, indicating that non-polar fractions with a complex fatty acid composition may have potent antibacterial qualities. Previous studies have shown that *W. coagulans* fruits and leaf extracts demonstrated broad-spectrum antimicrobial activity against many pathogens (27-29).

All the pathogens responded to the methanolic extract and its chromatogram confirms multiple peaks containing many fatty acid derivatives (Fig. 3). The GC–MS profile shows Cyclotetrasiloxane (octamethyl) as the leading compound, followed by ethylphosphonic acid derivatives and aromatic ethyl naphthyl compounds, which make up most of the sample composition. The analysis indicates that the sample contains mainly silicone-based substances and aromatic organic compounds. The main compounds identified in the sample included Cyclotetrasiloxane, octamethyl (29.294 min/9.25), Cyclotetrasiloxane, octamethyl (37.592 min/7.38), Ethylphosphonic acid bis(2-hydroxyethyl) ester (32.824 min/7.35), Cyclotetrasiloxane, octamethyl (16.917 min/6.94), Ethyl [5-hydroxy -1-(6-methoxy-2-naphthyl)] (41.282 min/6.24), Ethyl [5-hydroxy-1-(6-methoxy-2-naphthyl)] (42.236 min/5.13) and Ethyl [5-hydroxy-1-(6-methoxy-2-naphthyl)] (43.493 min/4.15). The researchers chose 2,4-dihydroxypropiophenone as their docking target because this phenolic ketone compound appears in aromatic metabolite studies. Previous research on *W. coagulans* discovered multiple bioactive fatty acids, which included both saturated and unsaturated types that enhance therapeutic effects (30) (Table 6).

The binding affinity score of -5.9 kcal/mol around the cavity volume of 286 Å^3 by 2,4-dihydroxypropiophenone demonstrated inhibition of the carbapenemase enzyme functional cavity (Fig. 4). The compound formed hydrophobic and hydrogen contacts with important active-site residues while firmly positioned within the catalytic pocket (Table 7). According to hydrogen bond studies, the ligand formed two hydrogen bonds with asparagine residues (Asn1 and Asn167) and three hydrogen bonds with serine residues (Ser70, Ser237 and Ser238). These polar interactions suggest that the hydroxyl groups of 2,4-dihydroxypropiophenone play a crucial role in anchoring the molecule within the enzyme's binding pocket. Hydrophobic interactions were observed with Trp105, Leu167 and Phe166, which provide additional stabilization through van der Waals and π–π stacking effects. The pharmacodynamic perspective of *W. coagulans* phytochemicals reveals presence of many polyunsaturated fatty acids and

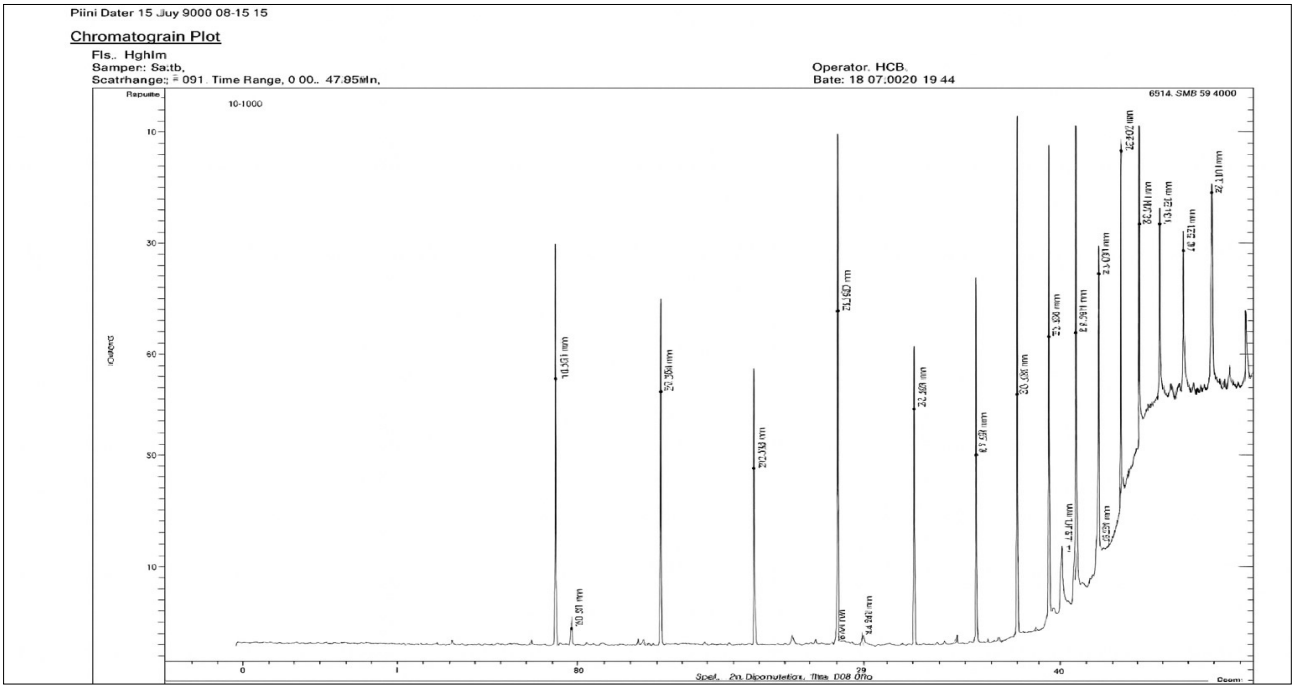

Fig. 3. GC-MS spectrum of methanol extract of *W. coagulans.*

Table 6. NIST library matched peaks and compound names

Peak No	RT (min)	Compound name	Area	percentage
1	16.917	2,2,4,4,6,6,8,8-Octamethyl-1,3,5,7-tetraoxa-2,4,6,8-tetrasilacyclooctane	13256	6.94
2	17.611	1-(1,1-Dimethylethyl)-3,5-dimethyl-2,4-dinitrobenzene	1378	0.721
3	21.58	Hexadecamethylcyclooctasiloxane	12554	6.572
4	25.659	Octadeamethyl-cyclononasiloxane	9800	5.131
5	29.098	3-nitropropylbenzene	195	0.102
6	29.294	octamethylcyclotetrasiloxane	17674	9.252
7	30.484	Acetic acid, 4-(dihydroxy-9	848	0.444
8	32.824	Phosphonic acid, ethyl-, bis(trimethylsilyl) ester	14030	7.345
9	35.74	Phosphonic acid, ethyl-, bis(trimethylsilyl) ester	10928	5.721
10	37.592	Octadeamethyl-cyclononasiloxane	14096	7.379
11	39.028	Ethyl 5-hydroxy-1-(6-methoxyphenyl)-1H-imidazole-4-carboxylate	13580	7.11
12	39.6	2-Thiophenecarboxylic acid	1990	1.042
13	39.712	2-(3-Thenoylamino)prop-2-enamide	4102	2.147
14	40.232	Ethyl 5-hydroxy-1-(6-methoxyphenyl)-1H-imidazole-4-carboxylate	12386	6.484
15	40.404	Ethyl 1-(4-hydroxy-3-isopropylphenyl)-1H-imidazole-4-carboxylate	3515	1.84
16	41.282	Ethyl 5-hydroxy-1-(6-methoxyphenyl)-1H-imidazole-4-carboxylate	11918	6.239
17	42.236	Ethyl 5-hydroxy-1-(6-methoxyphenyl)-1H-imidazole-4-carboxylate	9817	5.14
18	43.083	Ethyl 5-hydroxy-1-(6-methoxyphenyl)-1H-imidazole-4-carboxylate	7917	4.145
19	43.966	octamethylcyclotetrasiloxane	6657	3.485
20	44.99	octamethylcyclotetrasiloxane	5702	2.985
21	46.239	1,4-Naphthalenedicarboxylic acid	13204	6.912
22	47.011	(5β)-16-(Hydroxyimino) androstane-3,17-dione	1473	0.771
23	47.72	1-(2,4-Dihydroxyphenyl)propan-1-one	3998	2.093

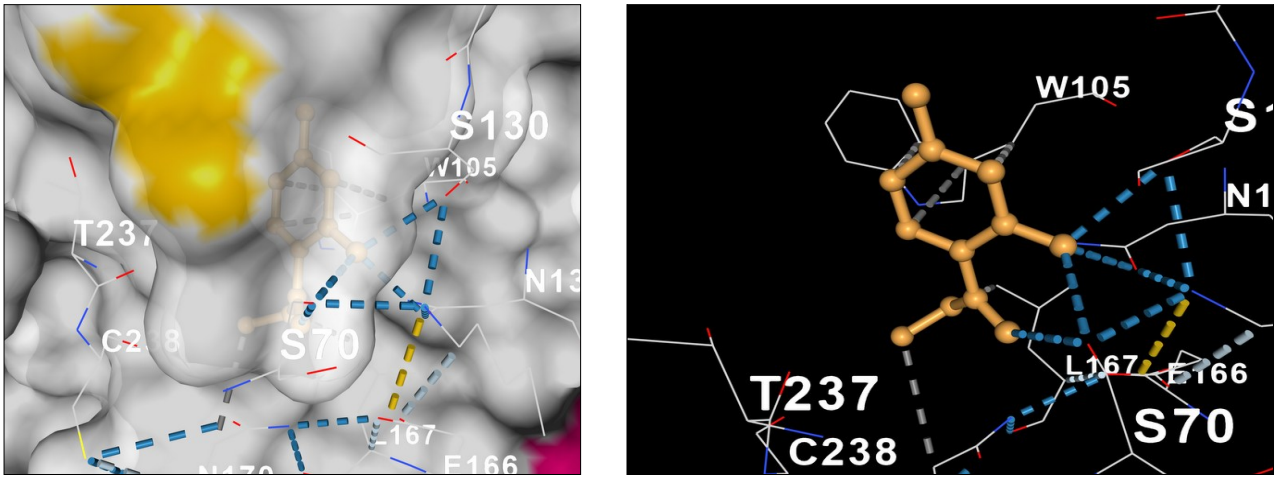

Fig. 4. Interaction of 2,4-dihydroxypropiophenone over carbapenemase.

Table 7. Binding affinity of 2,4-dihydroxypropiophenone on carbapenemase

Cavity size(Å³)	Score kcal/mol	Hydrogen bond	Hydrophobic
286	-5.9	3 Ser, 2 Asn	3 Trp, 1 Leu, 1 Asn

alkaloids capable to inhibiting many receptors have been reported earlier (30). To the best of our knowledge, this is the first publication that describes how bioactive chemicals from *W. coagulans* interact with carbapenem-degrading enzymes using molecular docking.

Conclusion

The current study unequivocally demonstrates that the antimicrobial activity of *W. coagulans* and the recovery of bioactive compounds are significantly impacted by the non-polar fractions, supporting that lipophilic phytochemicals play a major role in antibacterial activity. Among the identified compounds, 2,4-Dihydroxypropiophenone was selected for molecular docking because it is a phenolic ketone with known biological activity. Docking studies found hydrophobic interactions and stable hydrogen bonding involving key catalytic residues, as well as a strong binding affinity for the carbapenemase enzyme active site. The results of the study show that *W. coagulans* has a great deal of potential as a source of bioactive substances that successfully fight carbapenem-resistant pathogens. These findings suggest that *W. coagulans* is a novel plant-based antimicrobial drug and emphasize the urgent need for alternate approaches to treatment.

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Authors' contributions

SD conceptualized and supervised the overall research work. She provided guidance in experimental design, data interpretation and critical revision of the manuscript. IJ conducted

experimental studies, including extraction, antimicrobial assays and data analysis. She also contributed to the preparation of figures, tables and the initial drafting of the manuscript. All authors read and approve the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process: During the preparation of this work, the authors used "Grammarly" solely for grammar, spelling and punctuation correction at the final editing stage. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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