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Research Article

In vitro and *in silico* evaluation of antibacterial activities of different solvent extracts made from *Sisymbrium irio* L. seeds

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Abstract
Sisymbrium irio Linn is one of the underutilized plants, well-known for its traditional importance in Unani Medicine. The present study aimed to evaluate the antibacterial activities of different polarity-based extracts made from seeds of *S.irio* (Indian variety).The antibacterial activity of 13 different solvent extracts of *S.irio* seeds was evaluated by the Agar well-diffusion method. Among 13 different solvent seed extracts, ethanolic extract inhibited the growth of all the three bacterial strains used in the study. Further GC-MS analysis of ethanolic extract was done to reveal its phytochemical constituents. Twenty-five different compounds were identified through GC-MS analysis of ethanolic extract of *S.irio* seeds. Subsequently, for performing *in silico* antibacterial studies, the identified phytochemicals were first tested for their drug-likeability through Molinspiration software which yielded four compounds. *In silico* virtual screening via Autodock Vina was done using four phytochemicals against DNA gyrase subunit B and Dihydrofolate reductase. Out of four phytochemical studied through in silico analysis, “Benzene-1,2-dicarboxylicacid, monoamide, N-(1-cyano-1-methylethyl)” was found to inhibit DNA gyrase subunit B most effectively. The present study revealed that *Sisymbrium irio* displayed potential antibacterial activity and can be used as a good source for designing potent antibacterial agents.

Keywords: Antibacterial, Autodock Vina, GC-MS, Molecular Docking, *Sisymbrium irio*

INTRODUCTION

Most infectious diseases are not successfully treated because of the emergence of antimicrobial resistance worldwide (Mcewen and Collignon, 2018). Prescriptions of antimicrobial drugs and their continual usage in agriculture are some factors pertaining to the spread of resistance against conventional antimicrobials. There is a decline in the development of new antimicrobial compounds due to economic and scientific challenges (Chassagne, 2021). Antibiotic resistance is increasing due to the lack of new and safe antibacterial compounds; therefore, researchers focussed on developing antimicrobial compounds derived from plants. Apart from providing essential nutrients to humans, plants also give bioactive compounds that effectively treat infectious diseases (Liu, 2003). Due to the presence of phytochemicals, plants are used in various industries like food, cosmetics and, pharmaceuticals etc. The traditional medicine system is one of the healthcare systems in most developing countries. It utilizes plant and their phytochemicals to treat various diseases (Velmurugan and Anand, 2017). Medicinal plants are the reservoir of many bioactive phytochemicals with marked antioxidant, anti-cancerous, anti-inflammatory and antimicrobial activities. Medicines derived from plants are utilized to treat various infectious and chronic diseases and are usually made from crude extracts containing a combination of many phytochemicals (Sahoo and Manchikant, 2013). Though most plant species have a reservoir of secondary metabolites in them, very few of them have been explored for potential bioactive compounds (Malongane, 2017). The extracted bioactive compounds have led to the generation of potent drugs with high activity (Yadav *et al*., 2017). GC-MS is reliable among the available techniques for identifying phytochemicals such as terpeniods, aminoacids, alkaloids, flavonoids (Razack, 2018). Further-

more, the data obtained by GC-MS analysis of medicinal plant extracts can be used *in silico* analysis, thereby facilitating the discovery of potent drugs (Sliwoski *et al*., 2014). Molecular docking is a reliable and cost-effective approach for testing and making pharmaceuticals. This technique tells about the interactions between drug and receptors, which helps predict drug model's binding to the target proteins (Lee and Kim, 2019).

In this View, *Sisymbrium irio Linn (S.irio),* belonging to the family "*Brassicaceae"* is an official medicinal plant used in Unani therapy. The common name of *S.irio* is Khakshi, found in different parts of the world. *Sisymbrium* species, including North Africa, Europe, Temperate Asia, U.K., Mediterranean Islands and Pakistan, are distributed worldwide. As far as India is concerned, it can be found in Jammu and Kashmir, Punjab, Northern part of Rajasthan and some parts of Western Uttar Pradesh (Khoshoo, 1966). Aerial parts of *S.irio* are used in Unani medicine as antimicrobial in fever, expectorant, aphrodisiac, antipyretic, analgesic, gastric ulcers, cough, skin disorders, liver complaints, in the urinary tract and pulmonary infections (Haleem *et al.*, 2016). *S. irio* is found to contain many bioactive compounds like alkaloids, tannin, saponins, flavonoids, glycosides, phenolics, glucosinolates, carbohydrates, fatty acids, amino acids, proteins and steroids depicting a wide variety of pharmacological actions such as antibacterial, antifungal, anti-inflammatory, anticancer, analgesic, antipyretic, hepatoprotective and bronchoprotective (Tiwari and Bhargava, 2021). It has been reported that the aerial parts of *S.irio* have significant antibacterial activities against gram-positive and gram-negative bacterial strains (Shabnam *et al*., 2015; Al-Massarani *et al.,* 2017). Despite the proven antimicrobial activity of extracts from different parts of *S.irio*, specific phytochemicals responsible for antimicrobial action remain unexplored. Because of the importance of the plant, the present study was conducted to investigate the antibacterial activities of the different extracts from the seeds of *S.irio* (Indian variety), GC-MS of the extract giving best antibacterial activity, and further *in silico* evaluation of the antibacterial activities of the identified phytochemicals, in search of safer therapies against bacterial diseases and provide scientific proof to traditional claim of medicinal plant.

MATERIALS AND METHODS

Plant material

Seeds of *S. irio* were purchased from the local market in Lucknow and the botanical specimen of seeds was authenticated and identified by CSIR-NISCAIR, New Delhi.

Preparation of extract

The raw material (*Sisymbrium irio* seeds) was carefully washed with distilled water to remove the pesticide spray's dirt, dust, and residues and dried under shade for 24 hours. Seeds became sticky after washing. To remove the stickiness, the seeds were dried at 40^{o}C in a hot air oven until a constant weight was obtained, and then the seeds were ground into coarse powder by an electric grinding machine. Powdered material was stored in air-tight containers/desiccators until used. The powdered seeds were extracted by Maceration using different solvents i.e water, methanol, ethanol, chloroform, ethylacetate, acetone, n-hexane, ethylacetate+methanol, ethylacetate+ethanol, ethylacetate+acetone, ethylacetate+n-hexane, chloroform+methanol, and chloroform+ethanol. A 1:1 ratio was used to make various solvent combinations as mentioned above. The extracts were made following the protocol of Shandukani *et al*., 2018 (with little modifications) and Patel *et al*., 2016 (Shandukani *et al*., 2018; Patel *et al*., 2016) .

Different solvent extracts were dissolved in dimethylsulfoxide (DMSO) and five different concentrations of 500mg/ml, 250mg/ml, 125mg/ml, 62.5mg/ml, and 31.25mg/ml, respectively, made of each extract by serial dilution for antibacterial assay.

Microorganisms and *in vitro* antibacterial activity

The antibacterial activity of each extract was determined by the Agar-well diffusion method (Naz and Bano, 2013; Prakash *et al*., 2016) against three bacterial strains, i.e. *Escherichia coli* (MTCC no: 739), *Staphylococcus aureus* (MTCC no: 96), *Pseudomonas aeruginosa (*MTCC NO: 2453). The bacterial strains were inoculated in LB broth and kept in the incubator for 24 hours at 37^{o}C. Luria Bertani medium was used for the bacterial cultures, and petriplates were prepared by pouring LB agar at 50-70^{o}C and kept for solidification under UV light for around 20 mins. After the solidification of the medium, 100µl of bacterial cultures (approximately 1x 10^{8} cfu/ml) were spread over the surface of the agar plates and the wells of 6mm diameter were punched in the Luria Bertani agar plates by using a sterile cork borer. Five different concentrations of each extract by serial dilution were made: 500mg/ml, 250mg/ml, 125mg/ml, 62.5mg/ml, and 31.25mg/ml: the volume dispensed in each well was 50µl. The amount in each well corresponded to 25mg, 12.5mg, 6.25mg, 3.125mg, and 1.5625mg, respectively. Ciprofloxacin was taken as a positive control for bacterial strains at the concentration of 2mg/ml (volume dispensed in the well is 10µl), whereas DMSO was used as a negative control. The plates were incubated at 37^{o}C for 24 hours. The zone of inhibition (diameter) was measured in mm.

Data analysis

Microsoft excel was employed to capture and enter the

data. All the collected data was then exported to SPSS for calculating p value. The data for all three bacterial species were analysed using a one-way analysis of variance (ANOVA). P value less than 0.05 was considered significant.

Gas chromatography and Mass spectrometry analysis of ethanolic extract

Ethanolic extract of *S.irio* seeds, which gave comparatively better antibacterial activities against all three bacterial strains than other solvent extracts of *S.irio* seeds, was chosen for further GC-MS analysis. Ethanolic extract of *S.irio* seeds was injected in GC-MS (GCMS-QP2010 Ultra) to get the results. The sample was injected at the temperature of 260°C in split mode. The column oven temperature was 120°C. The flow rate of the column was 1.21 mL/min. Helium was used as a carrier gas. The ionization mode was used in the experiment was Electron ionization (EI) mode. Metal quadrupole mass filter was used in a scan mode to analyse the (m/z) ratio of various bioactive phytochemicals present in the sample. The phytochemicals present in the ethanolic extract of *S.irio* seeds were identified based on their retention time and by comparing their mass spectral lines with the data available in the National Institute of Standard and Technology (NIST) Library.

***In silico* studies**

GC-MS identified phytochemicals were used for further *in silico* studies.

Library preparation

All the 25 compounds identified through GC-MS were used for ligand library preparation. Molinspiration software was used to check the drug likeability of the identified compounds, which yielded 4 out of 25, following the Lipsinki rule of five. Structures of four filtered compounds were downloaded from the Pubchem database in 3D sdf format, and were finally converted into mol2 format using the Openbabel tool (O'Boyle *et al*., 2011). Autodock MGL tools was used for converting the ligands into pdbqt format required for docking through Autodock vina (Morris *et al*., 2011; Trott and Olson 2010).

Receptor preparation

The well-known antibacterial drug targets used in the study were DNA Gyrase subunit B (PDB-ID: 3TTZ) (Nosrati and Behbahani, 2020) and Dihydrofolate reductase (DHFR) (PDB-ID 3SRW) (Tiwari *et al*., 2022). DNA gyrase and DHFR are the targets of commonly used antibiotics Ciprofloxacin and Trimethoprim, respectively. The 3D crystal structure of both the target enzymes was downloaded from RCSB database. 3TTZ crystal structure is a homodimer consisting of two chains having 233 residues each and both the chains have some missing aminoacid residues. Each chain has 2 Mg^{2+} ions, unharmed during the Molecular docking. Only Chain A was used in the study to simplify the procedure, which had only three missing terminal amino acid residues. 3SRW is a crystal structure with ligand and NADP bound at its active sites. NADP was kept intact with the enzyme throughout the docking. Both the targets were converted in pdbqt format using autodock MGL tools required for docking via Autodock Vina.

Molecular docking

The molecular docking of the target enzymes with all four ligands was performed using Autodock Vina. Putative Active Site with Spheres (PASS), a computational tool, was used to predict the active sites of target enzymes used in the study (Brady and Stouten, 2000). The grid size kept was x,y,z =25 and the number of run was 10. The docked ligand-receptor complex's confirmation was visualized by Pymol (DeLano, 2002). The Ligplot+ program was used to analyse the docked ligand-receptor complex interactions (hydrophobic and H-bond interactions) (Laskowski and Swindells, 2011).

RESULTS AND DISCUSSION

Antibacterial assay

Plants are the reservoirs of various bioactive compounds that can be used for the treatment of bacterial diseases (Balandrin et al., 1985). The results of agar well diffusion are shown in Table 1, which summarizes the activity of 13 different solvent extracts made from the seeds of *S. irio* against 3 bacterial strains. Some of the extracts of *S. irio* seeds were active against bacterial strains, while some of them did not show any antibacterial activity. However, the extracts that gave negative results neither reflect the absence of active phytochemicals, nor prove the plant to be inactive. Bioactive compounds in the particular extract responsible for antibacterial properties may be present insufficiently (Taylor et al., 2001). Different polarity extracts of seeds were found to show moderate to weak antibacterial activity against the bacterial strains compared to positive control (Ciprofloxacin). Water, Acetone, Ethanol, Methanol, Ethanol+ethylacetate, and Ethylacetate+nhexane extracts were active against *E.coli.* Ethanol and Ethanol+ethylacetate extract were found to be active against *E. coli* at a minimum concentration of 6.25mg/per well. Ethanol, Ethanol+ethylacetate, Ethanol+chloroform extracts were active against *S. aureus*. Ethanol at a minimum concentration of 12.5mg/well was active against *S. aureus.* Ethanol, Methanol, Ethylacetate+nhexane, chloroform, Methanol+chloroform and ethanol+chloroform were found to be active against *P.aeruginosa.* Ethanolic

Table 1. Antibacterial activities of different solvent extracts of *Sisymbrium irio* seeds against bacterial strains

Name of the extract	Concentration mg/ml	Escherichia coli (zone of inhibition in mm)	Staphylococcus aureus (zone of inhibition in mm)	Pseudomonas aeruginosa (zone of inhibition in mm)
Water extract	31.25	0	0	0
	62.5	0	0	0
	125	0	0	0
	250	0	0	0
	500	10	0	0
Acetone	31.25	0	0	0
	62.5	0	0	0
	125	0	0	0
	250	0	0	0
	500	10	0	0
Ethanol	31.25	0	0	0
	62.5	0	0	0
	125	10	0	12.67+_0.57
	250	11	10	14.67+_0.57
	500	12.33+_0.57	11	18
Methanol	31.25	0	0	0
	62.5	0	0	0
	125	0	0	11
	250	0	0	12.67+_0.57
	500	10	0	15.33+_0.57
Ethyl acetate	31.25	0	0	0
	62.5	0	0	0
	125	0	0	0
	250	0	0	0
	500	0	0	10
Ethanol+ethyl acetate	31.25	0	0	0
	62.5	0	0	0
	125	6	10	0
	250	10.67+_0.57	11	0
	500	11.67+_0.57	12	0
Ethyl acetate+n-Hexane	31.25	0	0	0
	62.5	0	0	0
	125	0	0	10
	250	10	0	11
	500	12	0	12
Methanol+Ethyl acetate	31.25	0	0	0
	62.5	0	0	0
	125	0	0	0
	250	0	0	0
	500	0	0	0
Acetone+ethylacetate	31.25	0	0	0
	62.5	0	0	0
	125	0	0	0
	250	0	0	0
	500	0	0	0
Chloroform	31.25	0	0	0
	62.5	0	0	0
	125	0	0	0
	250	0	0	10
	500	0	0	11

Contd……...

Table 1. Contd…….

Methanol+Chloroform	31.25	0	0	0
	62.5	0	0	0
	125	0	0	0
	250	0	0	10
	500	0	0	11
Ethanol+Chloroform	31.25	0	0	0
	62.5	0	0	0
	125	0	0	0
	250	0	0	10
	500	0	10	11
n-hexane	31.25	0	0	0
	62.5	0	0	0
	125	0	0	0
	250	0	0	0
	500	10	0	0
Ciprofloxacin(+control)(2mg/ml)		36	33.67+_0.57	35.67+-0.57
DMSO(-control)		0	0	0

extract was active against *P.aeruginosa* at the minimum concentration of 6.25mg/well. The ethanolic extract of *S.irio* seeds was found to be active against all the bacterial strains as compared to other solvent extracts. A study conducted by Vohora *et al*. 1980, showed that the ethanolic extracts of *S.irio* seeds inhibit the growth of gram-positive and gram-negative bacterial strains (Vohora *et al*., 1980). In another study conducted by Shabnam *et al*. (2015) on the Pakistan variety of *S.irio* seeds, showed that n-hexane extract of *S.irio* seeds inhibited the growth of *P. aeruginosa*, Ethanolic and water extract of the seeds inhibited the majority of gram-positive and gram-negative bacterial strains used in the study (Shabnam *et al*., 2015). In the present study nhexane extract did not show any activity against test bacterial strains, while water extract inhibited the growth of *E.coli* only, and ethanolic extracts inhibited the growth of all three strains. In the present study, the crude seeds extracts were a mixture of many compounds. The phytochemical showing antimicrobial activities might be present in very low amount in crude extracts, resulting in lower antimicrobial activities compared to positive control ciprofloxacin (which is a pure compound). Ethanolic crude seed extract of *S. irio* had shown significant antimicrobial activity amongst 13 different solvent extracts. Thus ethanolic extract were selected for further phytochemical investigation through GC-MS to determine the composition of bioactive compounds.

Gas chromatography and Mass spectroscopic analysis

GC-MS analysis of Ethanolic extracts from seeds of *S.irio* revealed the presence of 25 constituents as shown in Table 2. The main phytochemicals present are as follows: 4-(Dimethoxymethyl)-1,2-Dimethylbenzene), Phenol, 2,4-bis(1,1-dimethylethyl)-, Benzene-1,2-dicarboxylic acid, monoamide, N-(1-cyano-1-methylethyl), Pentadecanoic acid, 14-methyl-, methyl ester, n-Hexadecanoic acid, Docosanoic acid, docosyl ester, 9,12-Octadecadienoic acid (Z,Z), 9- Octadeacenoic acid(Z), methyl ester, Tetradecanoic acid, methyl ester, Oleic acid, 9-Octadecenoic acid (Z), 2-Cyclohexen-1-one, 3,5,5-trimethyl-2-(2-propenyl), 7-(Bromomethyl)-7- Pentadecene, Octadecanoic acid, 2-propenyl ester, Hexadecanoic acid, 2-Hydroxy-1,3-Propanediyl ester, Palmitoyl chloride, 1,2-Benzenedicarboxylic acid, 9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester, Octadecanoic acid, 2,3-dihydroxypropyl ester, Cholest-5-ene, 3-methoxy-,(3.Beta.), Cholesta-3,5-diene, Ergost-5-en-3-ol,(3.Beta.), (3S,8S,9S,10R,13R,14S,17R)-17-((2R,5R)-5-Ethyl-6-methylheptan-2-yl)-3-methoxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthrene, Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1), 5,11,17,23- Tetratert-butylpentacyclo[19.3.1.1~3,7~.1~9,13~.1~15,19~]Octacosa-1(25),3(28),4,6,9(27),10,12,15(26),16,18,21,23Dodecaene-25,26,27,28-tetrol. The extract contained a high percentage of oleic acid and palmitic acid as shown in Table 2. Thus, phytochemical investigation of ethanolic extracts (giving the best antimicrobial activity amongst all the polarity-based extracts) of *S.irio* seeds showed the presence of phytochemicals as mentioned above. These phytochemicals may be responsible for the different pharmacological activities of the plant, like antioxidant, wound healing, antipyretic, and antimicrobial activities (Hailu *et al*., 2021). Further investigation of antimicrobial activities of identified phytochemicals and their mechanism of antibacterial action was done with

Table 2. GC-MS analysis of bioactive compounds from ethanolic seeds extract of *Sisymbrium irio L*

Name of the compounds	R.Time	Area%	Mol. formula	M. wt (in Daltons)
4-(Dimethoxymethyl)-1,2-dimethylbenzene	6.699	0.45	$C_{11}H_{16}O_2$	180.25
Phenol, 2,4-bis(1,1-dimethylethyl)	9.010	1.11	$C_{14}H_{22}O$	206
Benzene-1,2-dicarboxylic acid, monoamide, N-(1-cyano-1-methylethyl)	9.989	0.52	$C_{12}H_{12}N_2O_3$	232.24
Pentadecanoic acid, 14-methyl-, methyl ester	13.498	0.51	$C_{17}H_{34}O_2$	270.46
n-Hexadecanoic acid	14.011	26.94	$C_{16}H_{32}O_2$	256.43
Docosanoic acid, docosyl ester	14.961	0.59	$C_{44}H_{88}O_2$	649.19
9,12-Octadecadienoic acid (Z,Z)	15.135	0.62	$C_{18}H_{32}O_2$	280.45
9- Octadecenoic acid (Z), methyl ester	15.189	1.26	$C_{19}H_{36}O_2$	296.48
Tetradecanoic acid, methyl ester	15.422	0.24	$C_{15}H_{30}O_2$	242.40
Oleic Acid	15.685	40.02	$C_{18}H_{34}O_2$	282.47
9-Octadecenoic acid (Z)-	15.888	5.55	$C_{36}H_{68}O_4$	564.9
2-Cyclohexen-1-one, 3,5,5-trimethyl-2-(2-propenyl)	16.483	0.22	$C_{12}H_{18}O$	178.28
7-(Bromomethyl)-7-pentadecene	16.536	0.68	$C_{16}H_{31}Br$	303.33
Octadecanoic acid, 2-propenyl ester	16.761	0.37	$C_{21}H_{40}O_2$	324.55
Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester	16.963	0.21	$C_{35}H_{68}O_5$	568.92
Palmitoyl chloride	18.624	1.08	$C_{16}H_{31}ClO$	274.88
1,2-Benzenedicarboxylic acid	18.901	0.52	$C_8H_6O_4$	166.13
9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	20.023	1.44	$C_{21}H_{40}O_4$	356.55
Octadecanoic acid, 2,3-dihydroxypropyl ester	20.204	0.79	$C_{21}H_{42}O_4$	358.56
Cholest-5-ene, 3-methoxy-, (3.Beta.)	21.721	0.43	$C_{28}H_{48}O$	400.69
Cholesta-3,5-diene	22.077	5.13	$C_{27}H_{44}$	368.65
Ergost-5-en-3-ol, (3.Beta.)	23.266	0.34	$C_{30}H_{50}O_2$	400.69
:(3S,8S,9S,10R,13R,14S,17R)-17-((2R,5R)-5-Ethyl-6-methylheptan-2-yl)-3-methoxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17 -tetradecahydro-1H-cyclopenta[a]phenanthrene	24.423	3.67	$C_{30}H_{52}O$	428.75
Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)	30.126	3.19	$C_{42}H_{63}O_3P$	646.94
5,11,17,23-Tetratert-butylpentacyclo [19.3.1.1~3,7~.1~9,13~.1~15,19~]octacosa-1(25),3(28),4,6,9(27),10,12,15(26),16,18,21,23-Dodecaene-25,26,27,28-tetrol	35.939	5.12	$C_{44}H_{56}O_4$	648.93

the help of virtual screening.

Molecular docking

Molecular docking is a reliable and widely used computational method that helps to predict chemical ligands' binding affinities against particular receptors. The compounds filtered through Molinspiration software following Lipinski rule of five with their acceptable values of molecular weight, hydrogen bond acceptor, hydrogen bonds donor and miLogP are tabulated in Table 3.

The Autodock vina score of selected four phytochemicals and the positive control against DNA gyrase subunit B are represented in Table 4. Selected phytochemicals "Benzene-1,2-dicarboxylicacid, monoamide, N-(1-cyano-1-methylethyl)", "1,2-Benzenedicarboxylic acid", "4-(Dimethoxymethyl)-1,2-dimethylbenzene" and "2-Cyclohexen-1-one, 3,5,5-trimethyl-2-(2-propenyl)" had the docking score of -7.3, -6.4, -5.8, and -5.4 kcal/mol respectively. The docking score of positive control Novobiocin and Ciprofloxacin are -7.4 and -7.3 kcal/mol respectively.

The docking score of the phytochemical "Benzene-1,2-dicarboxylicacid, monoamide, N-(1-cyano-1-methylethyl)" found to be equivalent to the positive controls. Compound "Benzene-1,2-dicarboxylicacid, monoamide, N-(1-cyano-1-methylethyl)" makes 2 H bonds

and 9 hydrophobic interactions with the active sites aminoacids residues of the receptor and corresponds to lowest binding score of 7.3 kcal/mol. Positive control Novobiocin makes 2 H bonds and 12 hydrophobic interactions with active site residues whereas other positive control Ciprofloxacin makes 3 H bonds and 7 hydrophobic interactions with active site aminoacid residues of the receptor. Thus Phytochemicals" Benzene-1,2-dicarboxylicacid, monoamide, N-(1-cyano-1-methylethyl) PubChem id 5" identified through GCMS of ethanolic extract of *S.irio* seeds found to be the better inhibitor of bacterial DNA Gyrase subunit B in comparison to other three phytochemicals, also found to have almost equal binding free energy to the positive control. Thus it can be further optimized and can be tested for its in vitro efficacy. The two-dimensional representation of best-docked molecule (PubChem id 590819) and Positive controls (ciprofloxacin and novobiocin) with DNA gyrase subunit B receptor is shown via Ligplot^{+} in Fig.1.

Similarly, the above four phytochemicals were docked against antibacterial drug targets DHFR. Table 5: represents the Autodock vina score of selected four phytochemicals along with the positive control against antibacterial drug target DHFR. Phytochemicals 2-Cyclohexen-1-one, 3,5,5-trimethyl-2-(2-propenyl), Benzene-1,2-dicarboxylicacid, monoamide, N-(1-cyano-1-methylethyl), 1,2-Benzenedicarboxylic acid and 4-(Dimethoxymethyl)-1,2-dimethylbenzene has the docking score of -6, -5.9, -5.7 and -5.6 respectively. The docking score of positive control Trimethoprim was -7. For phytochemicals, the binding score is mostly contributed to by lipophilic interactions. Out of all four phytochemicals only "1,2-Benzenedicarboxylic acid" found to have one H bond with aminoacid residues present in the active cleft of the receptors. All three phytochemicals only have hydrophobic interactions with the active site residues, whereas positive control makes 3 H bonds and 5 hydrophobic interactions with the active site residues. Thus, Present study could conclude that the phytochemical "2-Cyclohexen-1-one,3,5,5- trimethyl-2-(2-propenyl)" with Pubchem id: 104531 found to be the better binder (Vina score = -6) of DHFR in comparison to other three phytochemicals used in the analysis, but in comparison to positive control trimethoprim, the binding free energy or vina score of "2-Cyclohexen-1-one,3,5,5-trimethyl-2-(2-propenyl) Pubchem Id : 104531" is little more. This phytochemical can be hybridized with other

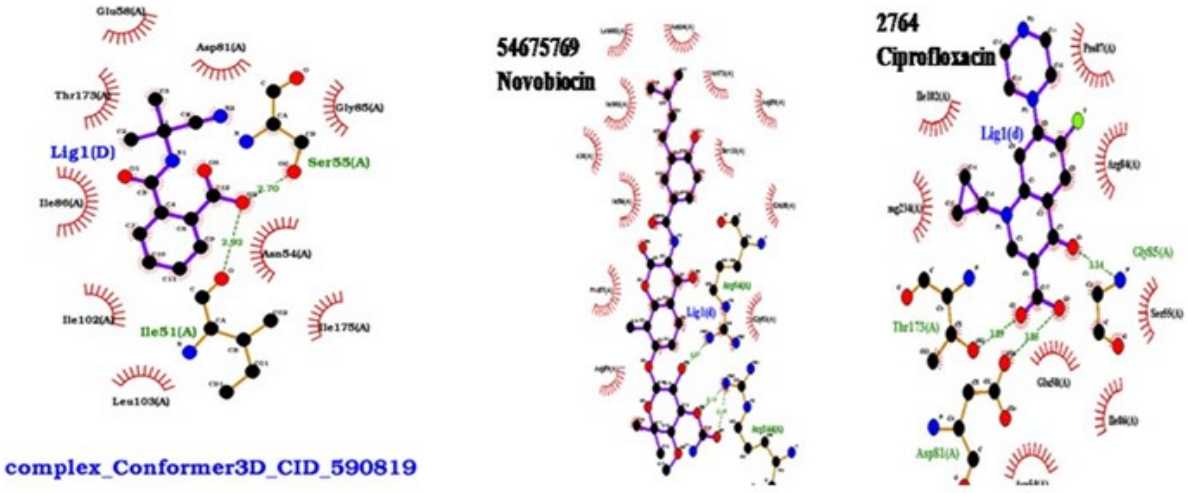

Fig.1. *Showing the 2D representation of the interactions of best-docked phytochemical and positive controls with DNA Gyrase B receptor via Ligplot*$^{+}$

Table 3. Representing the filtered phytochemicals following Lipinski rule of five

Phytochemicals	Pubchem CID	Molecular wt. (in daltons)	nON	nOHNH	mi LogP
4-(Dimethoxymethyl)-1,2-dimethylbenzene	14636865	180.25	2	0	2.67
Benzene-1,2-dicarboxylic acid, monoamide, N-(1-cyano-1-methylethyl)	590819	232.24	5	2	1.25
2-Cyclohexen-1-one, 3,5,5-trimethyl-2-(2-propenyl)	104531	178.28	1	0	2.96
1,2-Benzenedicarboxylic acid	1017	166.13	4	2	1.03

*Note: nON= no of Hydrogen bonds acceptor, nOHNH= no of Hydrogen bonds donor

Table 4. Representing phytochemicals and the positive controls docked to DNA Gyrase subunit B receptor along with their AutoDockVina score, number of interactions and residues interacting with the compounds.

Phytochemicals with their Pubchem CID	Autodock vina score	No. of H bonds and residues involved	No of Hydrophobic interactions and residues involved
Benzene-1,2-dicarboxylicacid, monoamide, N-(1-cyano-1-methylethyl): 590819	-7.3	2; Ile51, Ser55	9; Glu58, Asp81, Gly85, Asn54, Ile175, Leu103, Ile102, Ile84, Thr173
1,2-Benzenedicarboxylic acid: 1017	-6.4	4; Thr173, Asp81, Ser55, Ile51	4; Ile175, Ile86, Leu103, Asn54
4-(Dimethoxymethyl)-1,2-dimethylbenzene: 14636865	-5.8	1; Thr173	8; Ile102, Ile175, Ile51, Asn54, Ile86, Glu58, Asp81, Gly85.
2-Cyclohexen-1-one, 3,5,5-trimethyl-2-(2-propenyl): 104531	-5.4	-	7; Ile86, Pro87, Thr173, Glu58, Asn54, Asp81, Gly85
Novobiocin:	-7.4	2; Arg84, Arg144	12; Ile51, Asn54, Ser55, Glu58, Asp81, Gly85, Ile86, Pro87, Ile102, Leu103, Ser139, Ile175
Ciprofloxacin	-7.3	3; Asp81, Gly85, Thr173	7; Asn54, Ser55, Glu58, Arg84, Ile86, Pro87, Ile102

Table 5. Representing phytochemicals and the positive control docked to DHFR receptor along with their AutoDockVina score, number of interactions and residues interacting with the compounds.

Phytochemicals	Autodock vina score	No. of H bonds and residues involved	No. of Hydrophobic interactions and residues involved
2-Cyclohexen-1-one, 3,5,5-trimethyl-2-(2-propenyl)	-6	-	7; Leu55, Leu21, Val32, Ile51, Thr47, Leu29, Phe93
Benzene-1,2-dicarboxylicacid, monoamide, N-(1-cyano-1-methylethyl)	-5.9	-	8; Ile51, Leu21, Phe93, Asp28, Leu55, Val32, Leu29, Ala8.
1,2-Benzenedicarboxylic acid	-5.7	1; Gln20	4; Thr47, Ser50. Ile51, Leu21
4-(Dimethoxymethyl)-1,2-dimethylbenzene	-5.6	-	9; Leu21, Ile51, Val32, Thr47, Leu6, Phe93, Ala8, Val7, Asp28.
Trimethoprim	-7	3; Leu6, Asp28, Phe93	5; Val7, Ala8, Leu21, Leu29, Val32

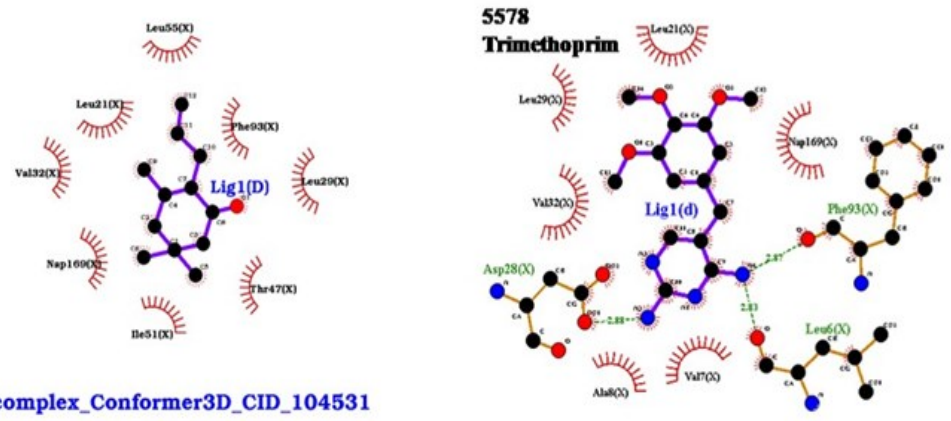

Fig. 2. *Showing the 2D representation of the interactions of best docked phytochemical and positive control with DHFR receptor via Ligplot*[+]

strong binder of DFR further enhancing the inhibition of bacterial DHFR, and help tackle the problem of antibiotic resistance. The two dimensional representation of best dockedmolecule (pubchem id: 104531) and Positive control (Trimethprim) with DHFR receptor is shown via Ligplot^{+} in Fig. 2.

In case of DNA Gyrase subunit B phytochemical "Benzene-1,2-dicarboxylicacid, monoamide, N-(1-cyano-1-methylethyl)" with Pubchem CID "590819" has shown almost equal binding score with positive controls along with equivalent number of H bonds and Hydrophobic interactions with the active site residues of the receptor. The phytochemical "Benzene-1,2-dicarboxylic acid, monoamide, N-(1-cyano-1-methylethyl)" gave the best result against DNA gyrase subunit B receptor *in silico* studies. Therefore, further optimization of the above-mentioned phytochemical is required for its *in vitro* efficacy as it showed the best results *in silico* investigation.

Conclusion

The present research confirmed the ethanolic extracts of the Indian variety of *S.irio* seeds showed significant antimicrobial activities comparable to other solvent extracts. A GC-MS analysis of ethanolic seed extract was done to determine its phytochemical constituents. Twenty-five active phytochemicals were identified through GC-MS analysis. For *In silico* analysis of antibacterial activities, the drug-likeability of 25 identified compounds was tested through Molinspiration software. Finally, four compounds out of 25 followed Lipinski's Rule of five. *In silico*, antibacterial activities of four resultant phytochemicals were evaluated against DNA gyrase subunit B and DHFR. "Benzene-1,2-dicarboxylicacid, monoamide, N-(1-cyano-1-methylethyl)" Pubchem CID "590819" found to inhibit DNA gyrase subunit B as it has a comparable docking score with its positive control. Thus it can be further optimized and tested for its *in vitro* efficacy against DNA gyrase subunit B. In the case of DHFR, phytochemical "2-Cyclohexen-1-one,3,5,5- trimethyl-2-(2-propenyl)" extracted from *S. irio* seeds can further be hybridized with other DHFR inhibitors or antibiotics to achieve potency against multiple drug- resistant bacteria, can be optimized and tested for its in vitro efficacy. Thus, it can be concluded that *S. irio* is a viable candidate for finding bioactive phytochemicals that serve as antimicrobial agents and may potentially contribute to developing new pharmaceuticals. This study provides additional scientific support for the plant's traditional claims.

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Conflict of interest

The authors declare that they have no conflict of interest.

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