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Anticancer Activity of *Sida cordifolia L.*, – *Insilico* approach

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Abstract
Sida cordifolia Linn belonging to family Malvaceae is widely distributed throughout the plains of India. The various parts of *Sida cordifolia* possess different biological perspectives such as antidiabetic, anti stress, anti-inflammatory, analgesic, hepatoprotective and anticancer activity. This plant has great potential for development of ayurvedic and modern medicines. The GC-MS analysis of the ethanolic extract of *S. cordifolia* was performed and resulted in 20 major compounds. Insilico approach of anticancer activity of the compounds were studied using bioinfomatics tools against Bcl2 & VEGFR2 and it exhibited promising results.

INTRODUCTION

Medicinal plants are a good source of natural metabolites plays an important role in the health care system among the global population [1]. These natural metabolites and its derivatives have proven to be rich in medicinal plants and can be used as therapeutic agents [2]. The usage of natural products has been increased worldwide for a variety of reasons, including treatment, maintaining health and preventing diseases. Herbal medicines have been used as a remedy since ancient times to cure many diseases, including cancer [3]. The first agents to advance into clinical use were the vinca alkaloids, vinblastine and vincristine as anticancer agents [4]. Most of the plants contain antioxidant compounds which protect cells from Reactive Oxygen Species (ROS) damaging effects due to oxidative stress leading to cellular damage [5].

Cancer is a worldwide health problem due to lack of early detection methods and its increasing incidence on a global scale [6]. It is characterized by the rapid and uncontrolled growth of cells that may proliferate throughout the body [7]. According to World Cancer Report 2014, cancer is among the leading causes of mortality worldwide, with 14 million new cases and 8.2 million cancer related death and it is expected to rise by about 70% over the next 2 decades [8]. There is a high risk for cancer in people who are continually exposed to carcinogenic chemicals and harmful radiations despite their genetic disorders [9]. The main forms of treatment for cancer in humans are surgery, radiation and drugs. In recent years, a lot of anticancer drugs have been synthesized from natural sources, since there are a lot of side effects of chemotherapeutic agents even though they provide temporary relief and prolongation of life [10].

Sida cordifolia L. commonly known as "Indian Ephedra", Bala (Sanskrit) is an herb that belongs to Malvaceae family widely distributed throughout India [11]. This plant has been reported to possess analgesic, antiinflammatory, hypoglycemic and hepato-protective activities [12, 13]. Traditionally, this plant is astringent, emollient, and used in respiratory and urinary system related problems [14]. Phytochemical studies have revealed the presence of ephedrine, pseudoephedrine and vasicinone as major compounds in its leaves [15]. Bioinformatics based drug design methods have revolutionized by increasing the pace of drug discovery process [16]. Many of the naturally derived compounds are considered as biologically safe and are yet to be explored for therapeutic efficacies. Hence, in this study we intend to explore the molecular composition of *Sida cordifolia* through chromatography methods and the resultant molecules were subjected to insilico analysis towards inferring the therapeutic efficacy as anticancer drugs.

MATERIALS AND METHODS

Preparation of extract

The *Sida cordifolia* plant was collected from SASTRA University main campus, Thanjavur. It was identified and authenticated by Dr. P. Brindha, CARISM, SASTRA University. The freshly collected leaves were shade dried for two weeks and grinded into fine powder. Powdered sample (300 g) was taken with 600 ml of absolute ethanol and kept for 24 h at RT. Then, the extract was separated using filtration and the volume of the extract was measured. Then the solvent was removed by distillation and the extract was lyophilized. The extract was re-suspended in the solvent at 10 mg/ml ratio and used for further analysis.

Preliminary Phytochemical Screening

Qualitative phytochemical test for the identification of tannins, saponins, flavonoids, terpenoids, cardiac glycosides and steroids were carried out by the method described [17]. The tests were carried out to detect the bioactive components using ethanolic extract of *S. cordifolia* leaves.

Characterization by GC-MS analysis

The GC-MS analysis of the ethanolic extract of *S. cordifolia* was performed using the instrument PerkinElmer Clarus 500 system. The column employed for separation of compounds was capillary column Elite-5MS (5% diphenyl and 95% dimethyl poly siloxane). Helium at a flow rate of 1 ml/min was used as a carrier gas and the sample was injected with a volume of 1µl (split ration of 1:10). For detection, an electron ionization system was operated and mass spectra were taken at 70 eV. The injection temperature was maintained at 290˚C, the source temperature was maintained at 160˚C and the oven temperature was programmed from 60˚C (isothermal for 5 min), with an increase of 7˚C/min to 150˚C and ending at 280˚C (isothermal for 10 min). The software applied for

mass spectra and chromatograms was Turbo-Mass ver-5.2. The compounds were identified using the database of National Institute Standard and Technology (NIST). The GC-MS compounds were identified by comparing the spectrum of the unknown compounds with the spectrum of the known compounds from NIST library.

RESULTS AND DISCUSSION

GC-MS analysis

The GC-MS analysis of the ethanolic extract of *S. cordifolia* was performed using the instrument PerkinElmer Clarus 500 system and the results are as follows (Fig1 & Table 1):

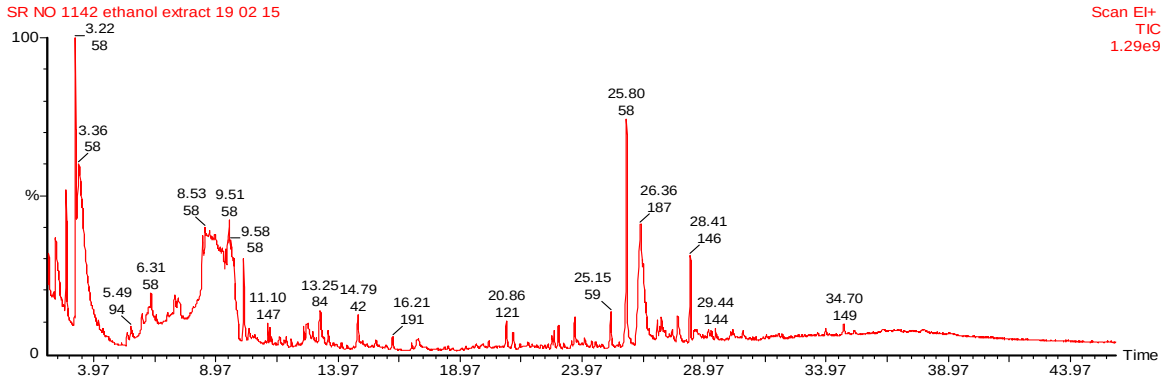

Fig 1: GC-MS analysis of the ethanolic extract of *S. cordifolia*

Ligand No	Compound name
1	methyl 2-hydroxybenzoate
2	4-methylbenzaldehyde
3	(5R)-5-methylimidazolidine-2,4-dione
4	methyl (2S)-5-oxopyrrolidine-2-carboxylate
5	1,3-dimethyl-1,2,3,4-tetrahydropyrimidine-2,4-dione (or) 1,3-dimethylpyrimidine-2,4-dione
6	1-methyl-1,2,3,4-tetrahydropyrimidine-2,4-dione (or) 1-methyl-3H-pyrimidine-2,4-dione
7	3-hydroxybenzohydrazide
8	2,4-di-tert-butylphenol
9	2-(3,4-dimethoxyphenyl)ethan-1-ol (or) 2-(3,4-dimethoxyphenyl)ethanol
10	(2R,6S)-2,6-dimethylcyclohexan-1-one
11	(2E)-N-[1-(dimethylcarbamoyl)propyl]-N-propylbut-2-enamide
12	4-(2,6,6-trimethylcyclohex-1-en-1-yl)butan-2-one
13	(5R)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)decan-3-one
14	3-(pyrrolidin-2-yl)propanoic acid
15	3-[(3E,7E)-3,7-dimethyl-9-(phenylsulfanyl)nona-3,7-dien-1-yl]-2,2-dimethyloxirane
16	(3S)-3-acetyl-6-methyl-3,4-dihydro-2H-pyran-2,4-dione
17	3,5-diazatricyclo[7.4.0.0^{2,6}]trideca- 1(13),2(6),3,7,9,11-hexaene
18	4-[(1E)-3-hydroxyprop-1-en-1-yl]-2-methoxyphenol
19	1-ethyl-N,2-dimethyl-N-phenyl-1H-1,3-benzodiazole-5-carmoxamide
20	1,7-dimethyl-6,7-dihydro-1H-purin-6-one (or) 1,7-dimethylpurin-6-one

Table 1: List of compounds from GC-MS analysis of the ethanolic extract of *S. cordifolia*

Insilico analysis

Structure:

Crystal structure was available for the BCL2 and VEGFR2 available in PDB ID 4LXD (co-crystallized with SORAFENIB) and 4SAD (co-crystallized with Navitoclax) respectively.

Preparation and docking:

Protein preparation wizard in maestro was used to prepare the structures by fixing adding hydrogen atoms; assigning bond orders; incomplete side chains and the water beyond 5 amstrong distance from the ligand are deleted. The protonation states were retained as it was in the crystal structure. Further the structure was minimized using restrained minimization with OPLS force field. The ligands were prepared using ligprep module of Schrödinger by minimizing the ligands with OPLS with unaltered ionization states and one low energy conformation of the each ligand is saved [18,19,20].

The structures (4LXD and 4ASD) taken for the docking was already co-crystallized with its respective inhibitors (SORAFENIB and Navitoclax analogue for 4LXD and 4ASD respectively). Hence the grid points were set by selecting ligand binding sites of co-crystallized ligand. Glide XP (extra precision) was used to dock all the prepared ligands. The scaling factor of vdw radii was set to 0.8 and partial charges were set to standard cutt off of 0.15. Ligand sampling was set to flexible and one best pose per ligand is saved.

Compound Name	Glide gscore kcal/mol
3-[(3E,7E)-3,7-dimethyl-9-(phenylsulfanyl)nona-3,7-dien-1-yl]-2,2-dimethyloxirane	-6.146
1-ethyl-N,2-dimethyl-N-phenyl-1H-1,3-benzodiazole-5-carmoxamide	-5.768
(5R)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)decan-3-one	-5.728
4-(2,6,6-trimethylcyclohex-1-en-1-yl)butan-2-one	-4.989
methyl 2-hydroxybenzoate	-4.623
4-[(1E)-3-hydroxyprop-1-en-1-yl]-2-methoxyphenol	-4.604
2,4-di-tert-butylphenol	-4.56
3,5-diazatricyclo[7.4.0.0^{2,6}]trideca- 1(13),2(6),3,7,9,11-hexaene	-4.785
2-(3,4-dimethoxyphenyl)ethan-1-ol (or) 2-(3,4-dimethoxyphenyl)ethanol	-4.236
1-ethyl-N,2-dimethyl-N-phenyl-1H-1,3-benzodiazole-5-carmoxamide	-5.878
(2E)-N-[1-(dimethylcarbamoyl)propyl]-N-propylbut-2-enamide	-4.017
4-methylbenzaldehyde	-3.927
3-hydroxybenzohydrazide	-3.908
3,5-diazatricyclo[7.4.0.0^{2,6}]trideca- 1(13),2(6),3,7,9,11-hexaene	-4.123
methyl (2S)-5-oxopyrrolidine-2-carboxylate	-3.65
(2R,6S)-2,6-dimethylcyclohexan-1-one	-3.585
1,7-dimethyl-6,7-dihydro-1H-purin-6-one (or) 1,7-dimethylpurin-6-one	-3.522
(5R)-5-methylimidazolidine-2,4-dione	-3.411
1-methyl-1,2,3,4-tetrahydropyrimidine-2,4-dione (or) 1-methyl-3H-pyrimidine-2,4-dione	-3.386
(3S)-3-acetyl-6-methyl-3,4-dihydro-2H-pyran-2,4-dione	-3.234
1,3-dimethyl-1,2,3,4-tetrahydropyrimidine-2,4-dione (or) 1,3-dimethylpyrimidine-2,4-dione	-3.104
3,5-diazatricyclo[7.4.0.0^{2,6}]trideca- 1(13),2(6),3,7,9,11-hexaene	-4.809
3-(pyrrolidin-2-yl)propanoic acid	-2.56
methyl 2-hydroxybenzoate	-4.105

Table 2: BCL2- ligand docking scores

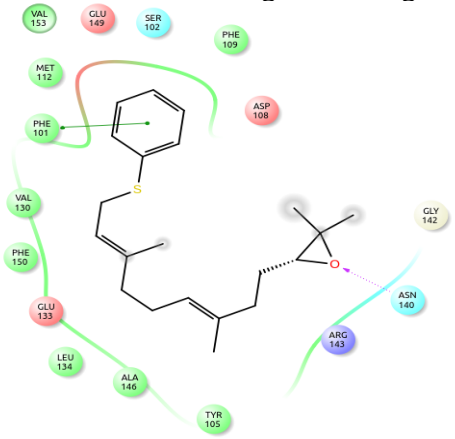

Fig 2: 2D interaction map of Ligand 15 [3-[(3E,7E)-3,7-dimethyl-9-(phenylsulfanyl)nona-3,7-dien-1-yl]-2,2-dimethyloxirane] with Bcl2

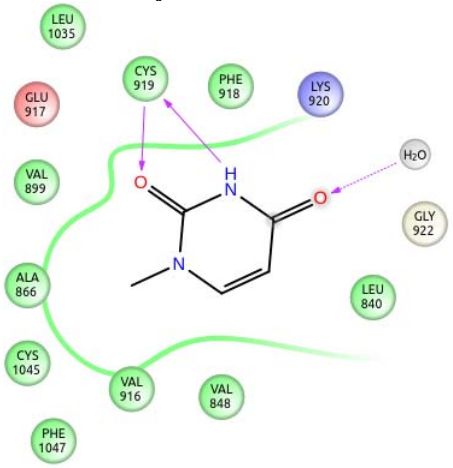

Fig 3: 2D interaction map of ligand 6 [1-methyl-1,2,3,4-tetrahydropyrimidine-2,4-dione (or) 1-methyl-3H-pyrimidine-2,4-dione] with VEGFR2.

Compound name	Glide gscore kcal/mol
1-methyl-1,2,3,4-tetrahydropyrimidine-2,4-dione (or) 1-methyl-3H-pyrimidine-2,4-dione	-7.731
3-[(3E,7E)-3,7-dimethyl-9-(phenylsulfanyl)nona-3,7-dien-1-yl]-2,2-dimethyloxirane	-7.591
(5R)-5-methylimidazolidine-2,4-dione	-7.572
1-ethyl-N,2-dimethyl-N-phenyl-1H-1,3-benzodiazole-5-carmoxamide	-7.257
4-methylbenzaldehyde	-7.195
4-[(1E)-3-hydroxyprop-1-en-1-yl]-2-methoxyphenol	-7.174
3-hydroxybenzohydrazide	-7.132
methyl (2S)-5-oxopyrrolidine-2-carboxylate	-6.932
3,5-diazatricyclo[7.4.0.0^{2,6}]trideca- 1(13),2(6),3,7,9,11-hexaene	-7.306
1,3-dimethyl-1,2,3,4-tetrahydropyrimidine-2,4-dione (or) 1,3-dimethylpyrimidine-2,4-dione	-6.839
(3S)-3-acetyl-6-methyl-3,4-dihydro-2H-pyran-2,4-dione	-6.835
methyl 2-hydroxybenzoate	-6.797
(5R)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)decan-3-one	-6.757
4-(2,6,6-trimethylcyclohex-1-en-1-yl)butan-2-one	-6.338
2-(3,4-dimethoxyphenyl)ethan-1-ol (or) 2-(3,4-dimethoxyphenyl)ethanol	-6.117
3,5-diazatricyclo[7.4.0.0^{2,6}]trideca- 1(13),2(6),3,7,9,11-hexaene	-6.54
1,7-dimethyl-6,7-dihydro-1H-purin-6-one (or) 1,7-dimethylpurin-6-one	-6.043
(2R,6S)-2,6-dimethylcyclohexan-1-one	-5.796
2,4-di-tert-butylphenol	-5.559
1-ethyl-N,2-dimethyl-N-phenyl-1H-1,3-benzodiazole-5-carmoxamide	-7.271
3-(pyrrolidin-2-yl)propanoic acid	-5.491
3,5-diazatricyclo[7.4.0.0^{2,6}]trideca- 1(13),2(6),3,7,9,11-hexaene	-7.258
methyl 2-hydroxybenzoate	-6.992
(2E)-N-[1-(dimethylcarbamoyl)propyl]-N-propylbut-2-enamide	-4.205

Table 3: Vegfr2-ligand docking scores

Among all the compounds studied 3-[(3E,7E)-3,7-dimethyl-9-(phenylsulfanyl)nona-3,7-dien-1-yl]-2,2-dimethyloxirane was found be a potential inhibitor of Bcl2, as it showed significant binding affinity with a glide score of -6.146 Kcal/mol (Table.2, Fig 2) and also showed pi-pi stacking interactions with Phe101 and hydrogen bonding with side chain of Asn140. In case of Vegfr2, 6 [1-methyl-1,2,3,4-tetrahydropyrimidine-2,4-dione (or) 1-methyl-3H-pyrimidine-2,4-dione] showed two hydrogen bonded interactions with the peptide backbone of Cys919 stabilized by a water mediated hydrogen bonding (Table 3, Fig 3). Hence, these compounds shall prove as potential inhibitors for the discussed targets, which corroborates the earlier reports of anticancer activity of *Sida cordifolia* [21,22].

CONCLUSION

GC-MS analysis of the ethanolic extract of *S. cordifolia* resulted in 20 major compounds. Glide scoring function approximates the ligand binding free energy and it is used widely for screening compounds from virtual screening. From the glide scoring, it can be observed that the ligand 15 [3-[(3E,7E)-3,7-dimethyl-9-(phenylsulfanyl)nona-3,7-dien-1-yl]-2,2-dimethyloxirane] was found to be efficient in binding to BCL2. Similarly, ligand 6 [1-methyl-1,2,3,4-tetrahydropyrimidine-2,4-dione (or) 1-methyl-3H-pyrimidine-2,4-dione] was efficient in binding with VEGFR2.

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