Contents lists available at UGC-CARE

International Journal of Pharmaceutical Sciences and Drug Research

[ISSN: 0975-248X; CODEN (USA): IJPSPP]

Available online at www.ijpsdronline.com

Research Article

GC-MS Analysis and Molecular Docking of Plant-based Compounds from Medicinal Plant *Sida acuta* Burm F. for Antibacterial Potential

Adline Anita, D Selvaraj*

Department of Zoology and Research Centre, Scott Christian College (Autonomous) (affiliated to Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu, India.

ARTICLE INFO

Article history:

Received: 28 November, 2022

Revised: 14 March, 2023

Accepted: 06 April, 2023

Published: 30 May, 2023

Keywords:

Sida acuta Burm F., Molecular docking, Antimicrobial activity, Phytochemical analysis, *Escherichia coli*, GC-MS analysis.

DOI:

10.25004/IJPSDR.2023.150309

ABSTRACT

Antimicrobial-resistant bacteria cause severe public health issues and mortalities. The evolution of multi-drug resistant bacteria shifted the focus of researchers towards traditional medicine involving bioactive compounds. Plants with bioactive compounds play a pivotal role in treating human diseases. Many of the plant-based bioactive compounds were proven to have the ability to inhibit bacterial growth through different modes of action. Thus, plant-based compounds have been focused on finding potential molecules with antibacterial efficiency to overcome bacterial infection problems. So, in the present study, phytocompounds of *Sida acuta* Burm F. leaf extract were identified using GC-MS technique and phytocompounds with antibacterial potential were identified through a molecular docking study. The qualitative test carried out indicated the presence of carbohydrates, alkaloids, phenols, terpenoids, flavonoids, amino acids, steroids, glycosides, saponins, quinones and coumarins in the extract. The GC-MS analysis showed the presence of 30 phytocompounds and molecular docking studies revealed the best binding affinity of the phytocompounds Pyrido[1,2-a]pyrimidine, Acetonitrile,2-(6-phenantridinyl), 5H-Imidazo(2,1-a)isoindole,2-phenyl and Pyrido[1,2-a]pyrimidine towards *E. coli* biomolecules- 1PHO, 5I5H, 5UW2 and 6NTW respectively. The present study concludes that the phytocompounds of *S. acuta* have appreciable antibacterial efficiency.

INTRODUCTION

Public health has been threatened at the global level by number of infectious diseases. Even after the availability of many synthetic drugs for the treatment of the infection, there is still rise in the rate of infection day by day. The rise of microbial infection is due to the evolution of resistance in the microbes toward the antimicrobial drugs available.[1-3]World Health Organization reported antimicrobial resistance as one of the top ten world public health threats.[1-4] Among microbial infection, the infections caused by gram-negative bacteria have great clinical importance as they are highly resistant to antibiotics and causes high morbidity and mortality.[5,6] The microbes alter some of their mechanisms, including the drug binding site and membrane permeability, efflux pumps, enzyme degradation and the conformational alteration of the drug culminating in its activation to gain resistance against commercial antibiotics.[7-10] This has become a major problem and a great challenge for researchers to knock down the microbes' resistance and find the clinical efficient drugs to get rid of microbial infections. The researchers recommended Many strategies, in that one of the suggested strategies, was to derive plant-based compounds of antibacterial potential. As plant-based compounds belong to nature, they have numerous benefits and no side effects over synthetic drugs.[11-15]

The plant-based compounds such as alkaloids, phenols, flavonoids, tannins, steroids, fatty acids and the like, have already been proven to exhibit unique pharmacological properties of antimicrobial, anticancer, anti-inflammatory and antioxidant. Different plant species possess unique

***Corresponding Author:** Dr. D. Selvaraj

Address: Department of Zoology and Research Centre, Scott Christian College (Autonomous) (affiliated to Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu, India.

Email ✉**:** adlineanita@gmail.com

Tel.: +91 9500960228

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

activities; only a small proportion have been investigated. Still there are more new compounds to be discovered.[15-18] A suitable screening method is very important in discovering biologically active compounds. The high activity of the compounds depends upon the extraction and characterization methods used.[19,20] Generally, the GC-MS technique has been used to identify compounds and detect functional groups.[21-23] This technique is one of the best and most accurate method to detect different compounds, including alkaloids, alcohols, nitro compounds, long-chain hydrocarbons, steroids, esters, amino acids and organic acids in a small fraction of plant extract.[22] Also, computer-based tools have been used to screen medicinally active compounds, making drug discovery approaches much easier and more cost-effective.[24] Tools like molecular docking techniques give knowledge on the interaction of drug and receptors, which anticipate ligand binding to the target protein's stable binding sites.[25,26]

The medicinal plant *S. acuta* of family Malvaceae is an erect perennial shrub.[27] It is found along the roadside, fields, forests, coastal areas of India and Nepal. From ancient times, the tribal populations have been using the different parts of *S. acuta* plant as a remedy for dandruff, liver problems, kidney stones, rheumatism, nervous disorders, testicular swelling and elephantitis.[28-31] It is also used as a medication for fever, asthma, ulcers, dysentery, malaria, venereal diseases and renal inflammations.[32] Fresh leaf juices have been extensively used as anti-helminthic medicines and in treating gastric disorders.[33-35] The presence of phytochemical constituents such as saponins, tannins, alkaloids, flavonoids, phytates, phenols, oxalate, terpenoids, glycosides and steroids were reported in *S. acuta*. [36] The plant extract of *S. acuta* reported to have an antimicrobial property and several studies have proved the significant activity against the microorganism *Staphylococcus aureus, Escherichia coli, Bacillus subtilis* and *Mycobacterium phlei*.[37] It was also indicated that the property of antibacterial and antimalarial of the methanolic leaf extract of *S. acuta* are due to the presence of phytoconstituents such as alkaloids, flavonoids, terpenoids and glycosides.[38] Also, several studies have reported the use of ethanol as relatively safer solvent in the extraction process and useful in extracting wide class of compounds.[39,40] Therefore, the present study focuses on extracting compounds using ethanol and identifying the phytocomponents from ethanolic leaf extract of *S. acuta* using GC-MS techniques. Further, *in-silico* molecular docking was performed to identify the functionally active compounds with antibacterial potentials on *E. coli* protein. However, to the best our knowledge, there is no report on the molecular docking studies of bioactive compounds of *S. acuta* on *E. coli* protein.

Materials and Methods

Collection of Plant Material

The plant *S. acuta* was collected in and around Nagercoil, Kanyakumari district, Tamil Nadu, India. The plant was identified by Dr. S. Jeeva, Department of Botany, Scott Christian College (Autonomous), Nagercoil and the leaves collected were brought to Department of Zoology and Research Centre, Scott Christian College (Autonomous), Nagercoil for further analysis.

Preparation of Plant Extract

The leaves were washed thoroughly in tap water and rinsed once with sterile water. The leaves were shade dried at room temperature for 14 days and dried leaves were powdered. Exactly 20 g of leaf powder were extracted by using 200 mL of ethanol solvent in soxhlet apparatus at a moderate temperature of 35 to 50°C. At the end of 18^{th} cycle, the extract was collected, filtered and evaporated with rotary evaporator. For further analysis, the residue was stored at 4°C in the refrigerator.

Preliminary Phytochemical Screening

The ethanolic extract of *S. acuta* was screened for phytochemicals, including carbohydrates, alkaloids, phenols, terpenoids, flavonoids, amino acids, steroids, glycosides, saponins, tannins, quinones, coumarins, anthraquinones, phlobatannins and anthocyanine by using the standard procedure.[41-49]

Gas Chromatography-Mass Spectrometry (GC–MS) Analysis

The phytoconstituents of the ethanolic leaf extract of *S. acuta* was analyzed by GC-MS analyzer of Thermo Scientific Co., Thermo GC-TRACE ultra, version 5.0, Thermo MsDsqII. About 100 µL of the extract was dissolved in 1-mL of solvent, thoroughly mixed and filtered using a membrane filter. The instrument included DB 35-MS capillary standard non-polar column with dimension, film thickness and ID of 30 m ×0.25 µm × 0.25 mm and carrier gas, helium at flow rate of 1.0 mL/min was used. The plant sample was injected in the split mode at a ratio of 1:10. The injector temperature was maintained at 240°C and the transfer line at 280°C. In the starting the instrument column was programmed at 50°C for 2 minutes, then slowly increased by 5°C/ min to 260°C and finally stabilized at 260°C for 10 minutes. The electron impact mode of the instrument was operated at 70eV. Mass spectral range was set at 42 to 350 (*m/z*). The spectrum obtained in GC-MS were matched and compared with standard spectrum available in the NIST (National Institute of Standards and Technology) library to identify the compounds.[50]

Molecular Docking

Ligand Selection and Drug Docking

About nine phytocompounds identified through the GC-MS analyzer were taken as ligand based on Lipinski rule of five parameter for the docking studies. The chemical structure of phyto-ligands were retrieved from the PubChem-NCBI database and converted into 3D structure by using online smiles translator.

Selection of Target Protein

About four biomolecules of *E. coli* were selected in order to perform protein modeling studies, 3D structure of *E. coli* biomolecules includes outer membrane porin PhoE protein (PDB-1PHO), inner membrane protein YejM protein (PDB-5I5H), intermembrane phospholipid transport system binding protein MlaD (PDB-5UW2) and probable L,D-transpeptidase YcbB protein (PDB-6NTW) were retrieved from Protein Data Bank and viewed through Discovery Studio software.

Docking Studies

Finally binding affinity for the target protein of *E. coli* and phytocompounds (ligands) of *S. acuta* were performed using Auto Dock Vina (version 4).

Results

Preliminary Phytochemical Screening

The qualitative phytochemical study revealed the presence of carbohydrates, alkaloids, phenols, terpenoids, flavonoids, amino acid, steroids, glycoside, saponins, quinones and coumarins in *S. acuta* (Table 1).

Gas Chromatography-Mass Spectrometry (GC–MS) Analysis

The GC-MS chromatogram of the ethanolic extract of *S. acuta* recorded a total of 30 peaks, each peak designated the phytocompounds corresponding to their retention time, peak area, height, molecular weight, molecular formula and mass spectral records of the known compounds recorded by the National Institute of Standards and Technology (NIST) library (Table 2) (Fig. 1).

Table 1: Preliminary phytochemical screening of ***S. acuta*** leaves

Phytochemicals	*S. acuta*
Carbohydrates	+
Alkaloids	+
Phenols	+
Terpenoids	+
Flavonoids	+
Amino acid	+
Steroids	+
Glycoside	+
Saponins	+
Tannins	-
Quinones	+
Coumarins	+
Anthraquinones	-
Phlobatannins	-
Anthocyanine	-

In-silico Molecular Docking Studies

Further, based on the Lipinski's rule of five parameters, 9 bioactive compounds were identified from the GC-MS analysis of *S. acuta* and were used for molecular docking with biomolecules 1PHO, 5I5H, 5UW2 and 6NTW of *E. coli*. The *E. coli* protein 1PHO, 5I5H, 5UW2 and 6NTW retrieved from Protein Data Bank, http://www.rcsb.org/ were shown in Fig. 4. The selected bioactive compounds 2D structure were retrieved from PubChem database. Structures of 9 bioactive compounds were shown in Fig. 2. Ciprofloxacin and Levofloxacin were used as standard controls and the chemical structure were shown in Fig. 3. The binding pattern of 1PHO, 5I5H, 5UW2 and 6 NTW protein with ligands varies according to the nature of ligand (Fig. 4). The docking score result of bioactive compounds were shown in Table 3 and the best four interaction complex for each protein target and the residues involved in the bonded interactions and non-bonded interactions are given in Table 4. 2D and 3D interaction between protein and ligand are shown in Fig. 5.

Discussion

Gram-negative bacteria are highly resistant to antibiotics due to the asymmetrical composition of lipids in cytoplasmic membrane, peptidoglycan layer and the outer membrane of the cell envelope. [51] Gram negative bacteria are known to possess high resistance against synthetic drugs and are the major causes for heavy causalities than gram-positive bacteria.[52,53] Recently, phytochemical compounds replace synthetic drugs and antibiotics in the management of gram-negative organisms that causes human diseases. The Gram-negative bacterium *E. coli* protein, which have been used in the present docking study, is a food-borne pathogen causing diarrhea, gastro-intestines, and a series of other problems.[54] A study in South Africa reported 85.7% of *E. coli* isolated from HIV-infected kids was resistant to the prophylactic drug, cotrimoxazole. [55] The resistance developed by *E. coli* and similar gram-negative bacteria against synthetic drugs made researchers to opt for alternative medicines with less side effects and

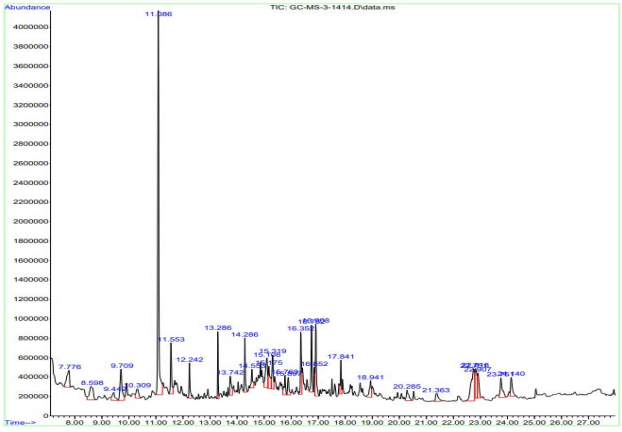


Fig. 1: GC-MS chromatogram of ethanolic leaf extract of *S. acuta*

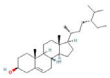
Table 2: The phytocompounds identified from GC-MS analysis of the ethanolic extract of *S. acuta* leaves

S. No.	*Retention Time (min)*	*Compound Name*	*Molecular Formula*	*Molecular Weight (g/mol)*	*Percent area*	*PubChem (CID)*
1	7.776	Methanaminium, 1-carboxy-N,N,N-trimethyl-, hydroxide, inner salt	$C_5H_{11}NO_2$	117.15	2.92	247
2	8.598	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	$C_6H_8O_4$	144.12	3.06	119838
3	9.442	Cyclohexanol, 3,5-dimethoxy-, stereoisomer	$C_8H_{16}O_3$	160.21	1.29	547125
4	9.709	Benzofuran, 2,3-dihydro-	C_8H_8O	120.15	3.76	10329
5	10.309	1-Buten-3-yne, 2-methyl-	C_5H_6	66.10	1.43	62323
6	11.086	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150.17	24.88	332
7	11.553	Phenol, 2,6-dimethoxy-	$C_8H_{10}O_3$	154.16	3.39	7041
8	12.242	1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydrocyclopenta[c] pyran-1-yl)ethanone	$C_{13}H_{18}O_2$	206.28	2.03	605654
9	13.742	2-Ethyl-5-propylcyclopentanone	$C_{10}H_{18}O$	154.25	1.88	574441
10	14.286	2,3,5,6-Tetrafluoroanisole	$C_7H_4F_4O$	180.1	2.60	75351
11	14.553	Tricyclo[4.2.2.0(2,5)]dec-7-ene, 7-butyl	$C_{14}H_{22}$	190.32	1.54	561940
12	15.108	d-Glycero-l-gluco-heptose	$C_7H_{14}O_7$	210.18	3.65	21139463
13	15.175	Methyl .beta.-d-galactopyranoside	$C_7H_{14}O_6$	194.18	2.33	2108
14	15.319	Methyl-(2-hydoxy-3-ethoxy-benzyl)ether	$C_{10}H_{14}O_3$	182.22	2.74	586449
15	15.897	1H-Indole, 2-(2-pyridinyl)-	$C_{13}H_{10}N_2$	194.23	1.50	270302
16	16.352	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	$C_{10}H_{12}O_3$	180.20	4.07	91753526
17	16.752	5-Ethylcyclopent-1-ene-1-carboxylic acid	$C_8H_{12}O_2$	140.18	3.89	574024
18	16.852	2-(2-Bromoethyl)-3-methyl-oxirane	C_5H_9BrO	165.03	1.27	558912
19	16.908	Guaifenesin	$C_{10}H_{14}O_4$	198.22	4.40	3516
20	18.941	3-Phenylbicyclo(3.2.2)nona-3,6-dien-2-one	$C_{15}H_{14}O$	210.27	1.86	610888
21	20.285	1H-Cyclopropa[a]naphthalene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,7,7a-t etramethyl-, [1aR-(1a.alpha.,7.alpha.,7a.alpha.,7b.alpha.)]-	$C_{15}H_{24}$	204.35	1.41	6432176
22	21.363	Methyl 2-O-benzyl-d-arabinofuranoside	$C_{13}H_{18}O_5$	254.28	1.35	22215008
23	22.751	beta.-Sitosterol	$C_{29}H_{50}O$	414.7	6.46	222284
24	22.907	7-Methoxy-1,2,3-trimethylindole	$C_{12}H_{15}NO$	189.25	2.26	605096
25	23.751	4-Pyridinamine, N-(1-naphthalenylmethylidene)-	$C_{16}H_{12}N_2$	232.28	2.56	6425626
26	23.751	5H-Imidazo(2,1-a)isoindole, 2-phenyl-	$C_{16}H_{12}N_2$	232.28	2.56	162928
27	23.751	Phenol, 3,5-bis(1-pyrrolidinyl)-	$C_{14}H_{20}N_2O$	232.32	2.56	616044
28	24.140	Acetonitrile, 2-(6-phenantridinyl)-	$C_{15}H_{10}N_2$	218.25	2.09	612758
29	24.140	Benzimidazo[2,1-a]isoquinoline	$C_{15}H_{10}N_2$	218.25	2.09	612818
30	24.140	Pyrido[1,2-a]pyrimidine	$C_{15}H_{10}N_2$	218.25	2.09	612675

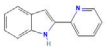
cost. Many studies on traditional medicines reported the availability of a wide spectrum of phytoconstituents as antimicrobial agents against pathogenic microbes.[56] Though the exact mechanisms of plant compounds in inhibiting the growth of microbes are undetermined, few studies have reported that the antimicrobial property of the plant compounds are due to their ability to inhibit nucleic acid synthesis, restrict the function of the cytoplasmic membrane and non-detrimental role in energy metabolism.[57] Phytochemicals are known to have a broad spectrum of antibacterial properties.[58]

The present study's qualitative phytochemical test of *S. acuta* leaves revealed the presence of carbohydrates, alkaloids, phenols, terpenoids, amino acids, steroids, glycoside, saponins, quinones, flavonoids and coumarins. Palaksha *et al.* [59] reported the presence of phytoconstituents such as alkaloids, carbohydrates, phytosterols, saponins, fixed oils, tannins, and flavonoids in the ethanolic leaf extract of *S. acuta* from East Godavari district, Andhra Pradesh, India. Tannins were found to be absent in the ethanolic leaf extract of *S. acuta*. This variation in the phytoconstituent compositions might be attributed to the difference in

5H-Imidazo(2,1-a)isoindole, 2-phenyl-

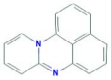


beta.-Sitosterol

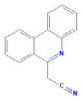


1H-Indole, 2-(2-pyridinyl)-

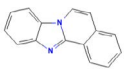
3-Phenylbicyclo(3.2.2)nona-3,6-dien-2-one



Pyrido[1,2-a]pyrimidine

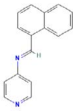


Acetonitrile, 2-(6-phenantridinyl)-



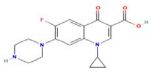
Benzimidazo[2,1-a] isoquinoline

Phenol, 3,5-bis(1-pyrrolidinyl)-

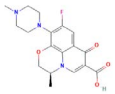


4-Pyridinamine, N-(1-naphthalenylmethylidene)-

Fig. 2: Structure of bioactive compounds identified from *S. acuta* for the analysis of molecular docking. (Photo Courtesy: http://www.pubchem.ncbi.nlm.nih.gov/)



Ciprofloxacin

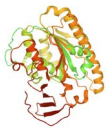


Levofloxacin

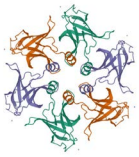
Fig. 3: Structure of Ciprofloxacin and Levofloxacin used for the analysis of molecular docking. (Photo Courtesy: http://www.pubchem.ncbi.nlm.nih.gov/)

1PHO

5I5H



5UW2



6NTW

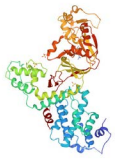


Fig. 4: The target Protein 1PHO, 5I5H, 5UW2 and 6NTW (Photo Courtesy: Protein Data Bank, http://www.rcsb.org/)

climatic conditions under which the plant grew or due to the stage of the plant. [60,61] Multiple factors are involved in the extraction of bioactive compounds from plants such as solvent selection, phytoconstituents chemistry, extraction technique, time and temperature.[62] Major phytoconstituents, such as flavonoids, possess antioxidant, antibacterial, antifungal, antiviral, antiallergic, antitumor and antithrombotic properties, whereas the phenols were observed to have potential antioxidant and free radical scavenging activities. The alkaloids act as analgesic and antimicrobial agents.[63,64] Saponins contribute to antibacterial, antidiabetic, anti-inflammatory and anticancer activities.[65] Tannins are reported to have inhibitory action on bacterial growth of *E. coli, Vibrio cholera, S. aureus*, *Bacillus cereus* and *Streptococcus faecalis*.[66-68] The presence of these phytoconstituents in the extracts of *S. acuta* might be responsible for contributing therapeutic value to the plant.

The GC-MS analysis of the ethanolic leaf extract of *S. acuta* led to the identification of 30 components, many of which with therapeutic properties. Based on Dr. Duke's Phytochemical and Ethnobotanical Databases, Methanaminium, 1-carboxy-N,N,N-trimethyl-, hydroxide, inner salt, a nitrogen compound have been reported to possess antimicrobial property, 1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydrocyclopenta[c]pyran-1-yl)ethenone has antihistamine activity, 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol, a phenolic compound, possesses antimicrobial, antioxidant, anti-inflammatory and antifungal activities. Similarly, beta-sitosterol possesses antimicrobial, anti-inflammatory, anticancer, antiasthma, diuretic and hepatoprotective activities. A study reported that beta-sitosterol with hypoglycemic activity promotes the synthesis of serum insulin with an inhibitory effect on the α-amylase enzyme.[69,70] The phenolic compound, phenol,2,6-dimethoxy isolated from *Rhizophora apiculate* is reported to have significant antioxidant activity.[71] A previous study suggested that the antibacterial and antioxidant effect of *Litchi chinensis,* wood vinegar is due to phenol,2,6-dimethoxy.[72] Through the suppression of NF-κB and MAPK activation, and histone acetylation proved that 2-methoxy-4-vinylphenol isolated from pine needles have anti-inflammatory activity.[73] A study reported the antineoplastic activity associated with ellipticine retained with heterocyclic compound, Benzimidazo[2,1-a]isoquinoline. Pyrido[1,2-a]pyrimidine with insecticidal activity against *Aphis craccivora* and *Sogatella furcifera*.[74]

The identification of bioactive compounds with antimicrobial properties and less toxicity is an elaborated study that would consume more time. Hence, utilizing *in-silico* computational approaches such as molecular docking, chemo informatics and artificial intelligence has

Table 3: Molecular docking score for bioactive compounds of S. acuta with 1PHO, 5I5H, 5UW2 and 6NTW

S.No.	*Compound Name*	*Pubchem ID*	*1PHO*	*5I5H*	*5UW2*	*6NTW*
			Docking score in Kcal/mol			
1	5H-Imidazo(2,1-a)isoindole, 2-phenyl-	162928	-7.6	-7.2	-6.4	-7.1w
2	beta.-Sitosterol	222284	-7.8	-7.3	-5.8	-7.2
3	1H-Indole, 2-(2-pyridinyl)-	270302	-6.9	-7.3	-6.2	-6.7
4	3-Phenylbicyclo(3.2.2)nona-3,6-dien-2-one	610888	-6.6	-7.9	-5.9	-6.6
5	Pyrido[1,2-a]pyrimidine	612675	-8.1	-7.6	-6.1	-7.5
6	Acetonitrile, 2-(6-phenantridinyl)-	612758	-7.5	-8	-6.1	-6.5
7	Benzimidazo[2,1-a]isoquinoline	612818	-7.8	-7.6	-6.3	-7.4
8	Phenol, 3,5-bis(1-pyrrolidinyl)-	616044	-6.3	-6.6	-5.7	-6.2
9	4-Pyridinamine, N-(1-naphthalenylmethylidene)-	6425626	-7.9	-7.3	-6.2	-7.4
Standard Drugs						
10	Ciprofloxacin	2764	-7.6	-7.2	-6.1	-7.5
11	Levofloxacin	149096	-7.6	-7.9	-7.1	-7.6

Table 4: The best four interaction complex for each protein with residues involved in bonded and non-bonded interactions

S.No	*Complex*	*Docking Score (-kcal/mol)*	*No of H Bonds*	*No of Alkyl Bonds*	*Residues involved in Bonded Interactions*	*Residues involved in Non-Bonded Interactions*
1	1PHO-612675	-8.1	-	3	-	Lys46, Tyr58, Lys89
2	1PHO -6425626	-7.9	1	4	Lys10	Lys46, Glu48, Tyr58, Lys89
3	1PHO-222284	-7.8	-	4	-	Lys46, Tyr58, Arg60, Phe85
4	1PHO-612818	-7.8	-	2	-	Tyr58, Asp97
5	5I5H-612758	-8.0	1	2	Asp406	Phe412, Ile453
6	5I5H-610888	-7.9	-	2		Phe412, Ile453
7	5I5H-612675	-7.6	1	3	Pro454	Asp406, Phe412, Ile453
8	5I5H- 612818	-7.6	1	1	Asp406	Phe412
9	5UW2 - 162928	-6.4	1	3	Ile101	Arg102, Ala140, Met141
10	5UW2-612818	-6.3	1	4	Asn91	Tyr40, Gln88, Ile127, Lys129
11	5UW2- 270302	-6.2	2	4	Asn91, Leu128	Tyr40, Gln88, Ile127, Lys129
12	5UW2- 6425626	-6.2	1	3	Gln88	Tyr40, Gln88, Lys129, Asp130
13	6NTW- 612675	-7.5	1	3	Asp230	Ala226, Leu227, Pro352
14	6NTW- 612818	-7.4	1	4	Asp230	Ala226, Leu227, Trp234, Pro352
15	6NTW-6425626	-7.4	-	4	-	Ala226, Leu227, Pro352, Arg586
16	6NTW-222284	-7.2		3	-	Ala343, Trp347, Pro599
17	1PHO- 2764	-7.6	2	4	Ser95, Arg140	Lys46, Glu48, Tyr58, Phe85
18	5I5H- 2764	-7.2	2	3	Ser407, Arg451	Asp406, Phe412, Pro454
19	5UW2- 2764	-6.1	3	-	Gly51, Gln110, Met141	-
20	6NTW- 2764	-7.5	2	3	Tyr446, Arg490	Asp435, Pro512
21	1PHO- 149096	-7.6	5	1	Lys46, Tyr58, Ser95, Asp97, Arg140	Glu48
22	5I5H- 149096	-7.9	1	5	Arg451	Ile404, Asp406, Phe412, Gly452, Ile453
23	5UW2- 149096	-7.1	1	4	Gly51	Ile101, Thr103, Ala140, Gln110
24	6NTW- 149096	-7.6	-	1	-	Pro512

Pyrido[1,2-a]pyrimidine with 1PHO (- 8.1 Kcal/mol)

4-Pyridinamine, N-(1-naphthalenylmethylidene) with 1PHO (- 7.9 Kcal/mol)z

beta.-Sitosterol with 1PHO (- 7.8 Kcal/mol)v

Benzimidazo[2,1-a]isoquinoline with 1PHO (- 7.8 Kcal/mol)

Acetonitrile, 2-(6-phenantridinyl) with 5I5H (- 8.0 Kcal/mol)

3-Phenylbicyclo(3.2.2)nona-3,6-dien-2-one with 5I5H (- 7.9 Kcal/mol)

Pyrido[1,2-a]pyrimidine with 5I5H (- 7.6 Kcal/mol)

Benzimidazo[2,1-a]isoquinoline with 5I5H (- 7.6 Kcal/mol)

5H-Imidazo(2,1-a)isoindole, 2-phenyl with 5UW2-162928 (- 6.4 Kcal/mol)

greatly increased during the last decade in the sector of design, development, and discovery of medicine.[75,76] The molecular docking studies makes it easier and provides

Benzimidazo[2,1-a]isoquinoline with 5UW2 (- 6.3 Kcal/mol)

1H-Indole, 2-(2-pyridinyl) with 5UW2 (- 6.2 Kcal/mol)

4-Pyridinamine, N-(1-naphthalenylmethylidene) with 5UW2- (- 6.2 Kcal/mol)

Pyrido[1,2-a]pyrimidine with 6NTW (- 7.5 Kcal/mol)

Benzimidazo[2,1-a]isoquinoline with 6NTW (- 7.4 Kcal/mol)

4-Pyridinamine, N-(1-naphthalenylmethylidene) with 6NTW (-7.4 Kcal/mol)

beta.-Sitosterol with 6NTW (-7.2 Kcal/mol)

Fig. 5: The 2D interaction and 3D docked complex of the ligands with *E. coli* proteins

in depth understanding about the molecular interactions along with docking score to know the affinity of the ligands with proteins.[77,78] Several molecular docking approaches have enabled virtual screening of protein and ligand interactions which would help discover suitable pharmaceutical agents with high output, within a short duration and in a cost-effective manner.[75,79] In the present investigation, 9 phytocompounds from *S. acuta* have been docked against four *E. coli* protein- 1PHO, 5I5H, 5UW2, 6NTW to find out potential phytocompounds with good

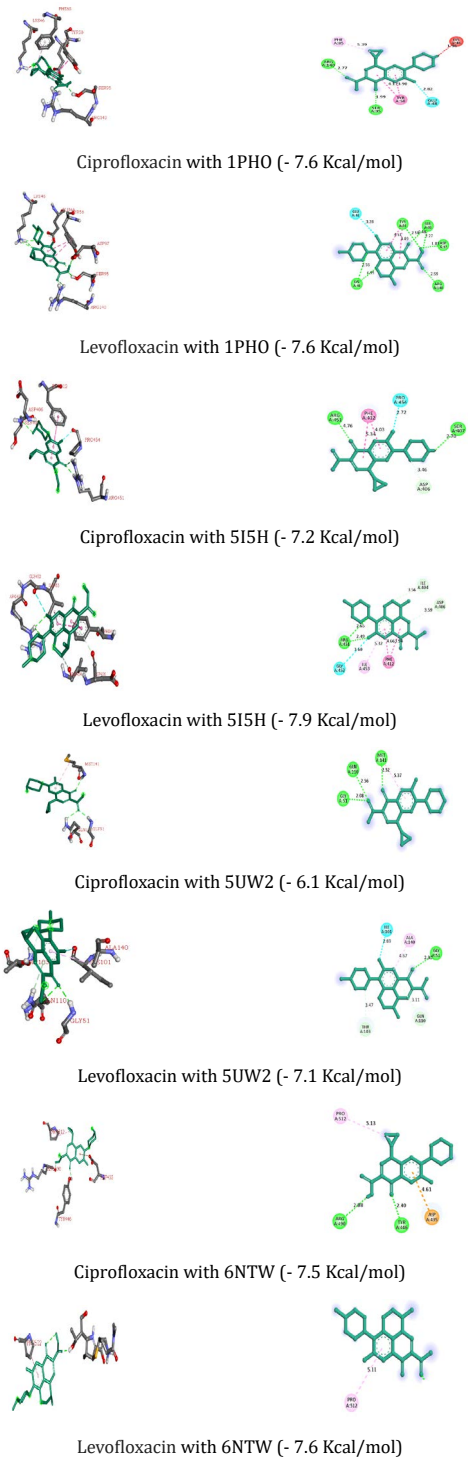


Fig. 6: The 2D interaction and 3D docked complex of the ligands (Standard drugs Ciprofloxacin and Levofloxacin) with *E. coli* proteins

binding affinities. The result has been compared with antibiotic compounds, ciprofloxacin and levofloxacin. 1PHO shows higher binding affinity of -8.1 Kcal/mol with a ligand, pyrido[1,2-a]pyrimidine by non-bonded interaction with amino acid residues Lys46, Tyr58 and Lys89. Ciprofloxacin showed binding affinity of -7.6 Kcal/mol with 1PHO by hydrogen bonds with amino acid residues Ser95 and Arg140, and four non-bonded interaction with amino acid residues Lys46, Glu48, Tyr58 and Phe85. Another antibiotic compound, levofloxacin showed docking score of – 7.6 Kcal/mol when interacting with 1PHO. The interaction led to the formation of five bonded interactions with Lys46, Tyr58, Ser95, Asp97 and Arg140, and one non-bonded interaction with Glu48. 5I5H showed higher binding affinity of -8.0Kcal/mol with ligand acetonitrile, 2-(6-phenantridinyl)- (Fig. 3) than the control which showed -7.2Kcal/mol. 5I5H formed a hydrogen bond interaction with the amino acid residue Asp406 and non-bonded interaction with amino acid residues Phe412 and Ile453. Ciprofloxacin interacting with 5I5H, showed two hydrogen bonds with amino acid residues Ser95 and Arg140, and three alkyl bonds with amino acid residues such as Asp406, Phe412 and Pro454, the docking score was -7.2 kcal/mol but levofloxacin showed docking score of -7.9 with bonded interaction with amino residue Arg451 and non-bonded interaction with amino residues Ile404, Asp406, Phe412, Gly452 and Ile453. One hydrogen bond was formed with Ile101 and three alkyl bonds were formed with amino acid residues such as Arg102, Ala140, Met141 when 5UW2 interacted with 5H-Imidazo(2,1-a)isoindole, 2-phenyl- (Fig. 4). The docking score was -6.4 Kcal/mol. The antibiotic compound, Ciprofloxacin interacted with amino acid residues such as Gly51, Gln110 and Met141 by forming three hydrogen bonds but Levofloxacin showed non-bonded interaction with Ile101, Thr103, Ala140 and Gln110, and hydrogen bond interaction with amino acid Gly51. The protein of *E. coli*, 6NTW interacted with pyrido[1,2-a]pyrimidine by forming one hydrogen bond and three alkyl bond with amino acid residues Asp230 and Ala226, Leu227, Pro352, respectively, the docking score was -7.5Kcal/mol, where the interaction of ciprofloxacin with 6NTW also showed the same docking score (Fig. 5). Ciprofloxacin showed bonded interaction with two amino acid residues (Tyr446 and Arg490) and two non-bonded interactions with residues Asp435 and Pro512. Levofloxacin showed affinity of -7.6 Kcal/mol by the formation of an alkyl bond with Pro512. The phytocompounds of *S. acuta* showed better affinity towards the *E. coli* protein than the antibiotic compounds which was similar to the molecular docking study reported on the phytocompounds of *Lantana camara* towards *E. coli* protein 1PHO, 5I5H, 5UW2 and 6NTW.[80]

Conclusion

The present investigation clearly revealed that phytochemical of ethanolic leaf extract of *S. acuta* has considerable antimicrobial activity. The GC-MS result of the *ethanolic leaf extract of S. acuta* provided tentative identification of antibacterial compounds such as methanaminium, 1-carboxy-N,N,N-trimethyl-,

hydroxide, inner salt; 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol; beta.-Sitosterol; phenol, 2,6-dimethoxy and other potential biologically active secondary metabolites. The molecular docking showed that the phytoconstituents of this plant has antibacterial potential. The phytochemicals pyrido[1,2-a]pyrimidine possessed highest binding affinity with 1PHO, acetonitrile, 2-(6-phenantridinyl) showed best binding affinity with 5I5H, 5H-Imidazo(2,1-a)isoindole, 2-phenyl has still better affinity with 5UW2 and Pyrido[1,2-a]pyrimidine showed good binding affinity with 6NTW. Thus, our study suggests that ethanolic leaf extract of *S. acuta* contains many phytochemical compounds with antibacterial property and may be utilized as a therapeutical source for developing beneficial drugs. However, further research on *in-vitro* screening of pharmacological activities, fractionation, isolation of pure compounds from the extract of *S. acuta* is in progress.

Acknowledgment

This research has no acknowledgment

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Bandow JE, Brötz H, Leichert LI, Labischinski H, Hecker M. Proteomic approach to understanding antibiotic action. Antimicrobial Agents and Chemotherapy. 2003;47(3):948–955. Available from: doi.org/10.1128/aac.47.3.948-955.2003
2. Baym M, Stone LK, Kishony R. Multidrug evolutionary strategies to reverse antibiotic resistance. Science. 2016;351(6268):1-21. Available from: doi.org/10.1126/science.aad3292
3. Gupta M, Sharma R, Kumar A. Comparative potential of simvastatin, Rosuvastatin and Fluvastatin against bacterial infection: An *in silico* and *in vitro* study. Oriental Pharmacy and Experimental Medicine. 2019;19:1–17. Available from: doi.org/10.1007/s13596-019-00359-z
4. WHO, 2023. Available from: www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance
5. Oznur Ak, Batirel A, Ozer S, Çolakoğlu S. Nosocomial infections and risk factors in the intensive care unit of a teaching and research hospital: A prospective cohort study. Medical Science Monitor. 2011;17(5):29-34. Available from: doi.org/10.12659/msm.881750
6. Hormozi SF, Vasei N, Aminianfar M, Darvishi M, Saeedi AA. Antibiotic resistance in patients suffering from nosocomial infections in Besat Hospital. European Journal of Translational Myology. 2018;28(3):304-308. Available from: doi.org/10.4081%2Fejtm.2018.7594
7. Ruppé É, Woerther PL, Barbier F. Mechanisms of antimicrobial resistance in Gram-negative bacilli. Annals of Intensive Care. 2015;5(21):1-15. Available from: doi.org/10.1186%2Fs13613-015-0061-0
8. Gilbert P, Allison DG, McBain AJ. Biofilms *in vitro* and *in vivo*: do singular mechanisms imply cross-resistance. Journal of Applied Microbiology. 2002;92:98S–110S. Available from: pubmed.ncbi.nlm.nih.gov/12000619/
9. Li XZ, Plésiat P, Nikaido H. The challenge of efflux-mediated antibiotic resistance in Gram-negative bacteria. Clinical Microbiology Reviews. 2015;28(2):337-418. Available from: doi.org/10.1128/cmr.00117-14
10. Naas T, Dortet L, Iorga BI. Structural and Functional Aspects of Class A Carbapenemases. Current Drug Targets. 2016;17(9):1006-1028. Available from: doi.org/10.2174/1389450117666160310144501
11. Cunha MDBA. Antibiotic side effects. Medical Clinics of North America. 2001;85(1):149–85. Available from: doi.org/10.1016/s0025-7125(05)70309-6
12. George P. Concerns regarding the safety and toxicity of medicinal plants—an overview. Journal of Applied Pharmaceutical Science. 2011;1(06):40–44. Available from: japsonline.com/abstract.php?article_id=121&sts=2
13. Mann A, Amupitan JO, Oyewale AO, Okogun JI, Ibrahim K, Oladosu P, Lawson L, Olajide I, Nnamdi A. Evaluation of *in vitro* antimycobacterial activity of Nigerian plants used for treatment of respiratory diseases. African Journal of Biotechnology. 2008;7(11):1630–1636. Available from: doi.org/10.5897/AJB08.438
14. Sahoo N, Manchikant P. Herbal drug regulation and commercialization: An Indian industry perspective. Journal of Alternative and Complementary Medicine. 2013;19(12):957–963. Available from: doi.org/10.1089%2Facm.2012.0275
15. Yuan H, Ma Q, Ye L, Piao G. The traditional medicine and modern medicine from natural products. Molecules. 2016;21(5):559. Available from: doi.org/10.3390%2Fmolecules21050559
16. Anand U, Jacobo-Herrera N, Altemimi A, Lakhssassi N. A comprehensive review on medicinal plants as antimicrobial therapeutics: potential avenues of biocompatible drug discovery. Metabolites. 2019;9(11):258. Available from: doi.org/10.3390/metabo9110258
17. Semwal DK, Chauhan A, Kumar A, Aswal S, Semwal RB, Kumar A. Status of Indian medicinal plants in the International Union for Conservation of Nature and the future of Ayurvedic drugs: Shouldn't think about Ayurvedic fundamentals. Journal of Integrative Medicine. 2019;17(4):238–243. Available from: doi.org/10.1016/j.joim.2019.04.008
18. Malongane F, McGaw L J, Mudau FN. The synergistic potential of various teas, herbs and therapeutic drugs in health improvement: A review. Journal of the Science of Food and Agriculture. 2017;97(14):4679–4689. Available from: doi.org/10.1002/jsfa.8472
19. Keskes H, Belhadji S, Jlail L, Feki AE, Damak M, Sayadi S, Allouche N. LC-MS–MS and GC-MS analyses of biologically active extracts and fractions from *Tunisian Juniperus* phoenice leaves. Pharmaceutical Biology. 2017;55(1):88–95. Available from: doi.org/10.1080/13880209.2016.1230139
20. Yadav R, Khare RK, Singhal A. Qualitative phytochemical screening of some selected medicinal plants of Shivpuri District (MP). International Journal of Life Sciences Scientific Research. 2017;3(1):844–847. Available from: dx.doi.org/10.21276/ijlssr.2016.3.1.16
21. Fan S, Chan J, Zong Y, Hu G, Jia J. GC-MS analysis of the composition of the essential oil from *Dendranthema indicum* Var. Aromaticum using three extraction methods and two columns. Molecules. 2018;23(3):1-11. Available from: doi.org/10.3390%2Fmolecules23030576
22. Razack S, Kumar KH, Nallamuthu I, Naika M, Khanum F. Antioxidant, biomolecule oxidation protective activities of *Nardostachys jatamansi* DC and its phytochemical anlaysis by RP-HPLC and GC-MS. Antioxidants. 2015;4(1):185–203. Available from: doi.org/10.3390%2Fantiox4010185
23. Satapute P, Murali KP, Kurjogi M, Jogaiah S. Physiological adaptation and spectral annotation of Arsenic and Cadmium heavy metal-resistant and susceptible strain *Pseudomonas taiwanensis*. Environmental Pollution. 2019;251:555–563. Available from: doi.org/10.1016/j.envpol.2019.05.054
24. Sliwoski G, Kothiwale S, Meiler J, Lowe EW. Computational methods in drug discovery. Pharmacological Reviews. 2014;66(1):334–395. Available from: doi.org/10.1124%2Fpr.112.007336
25. Lee K, Kim D. In silico molecular binding prediction for human drug targets using deep neural multi-task learning. Genes. 2019;10(11):1-16. Available from: doi.org/10.3390/genes10110906
26. Bharathi A. Roopan SM, Vasavi CS, Munusami P, Gayathri GA, Gayathri M. In silico molecular docking and in vitro antidiabetic studies of dihydropyrimido (4,5-a] acridin-2-amines). BioMed Research International. 2014;1:1–10. Available from: doi.org/10.1155/2014/971569

27. Karou D, Dicko MH, Sanon S, Simpore J, Traore AS. Antimalarial activity of *Sida acuta* Burm. F. (Malvaceae) and *Pterocarpus erinaceus* Poir. (Fabaceae). Journal of Ethnopharmacology. 2003;89(2):291-294. Available from: doi.org/10.1016/j.jep.2003.09.010
28. Tayade SK, Patil DA. Ethnomedicinal wisdom of tribals of Nandurbar district (Maharashtra). Natural Product Radiance. 2006;5:64–69. Available from: www.semanticscholar.org/paper/Ethnomedicinal-wisdom-of-tribals-of-Nandurbar-Tayade-Patil/152ed52fb3c4860aa395484ee7a194bee5a1fb22
29. Mishra SB, Dwivedi S, Shashi A, Prajapati K. Ethnomedicinal uses of some plant species by ethnic and rural peoples of the Salem District of Tamil Nadu with special reference to the conservation of vanishing species. Ethnobotanical Leaflets. 2008;12:873–887. Available from: opensiuc.lib.siu.edu/cgi/viewcontent.cgi?article=1154&context=ebl
30. Silja VP, Varma KS, Mohanan KV. Ethnomedicinal plant knowledge of the Mullu kuruma tribe of Wayanad district, Kerala. Indian Journal of Traditional Knowledge. 2008; 7(4): 604–612. Available from: nopr.niscpr.res.in/bitstream/123456789/2389/1/IJTK%207%284%29%20604-612.pdf
31. Gairola S, Sharma J, Gaur RD, Siddiqi TO, Painuli RM. 2013. Plants used for treatment of dysentery and diarrhoea by the Bhoxa community of district Dehradun, Uttarakhand, India. Journal of Ethnopharmacology. 2013;150(3):989–1006. Available from: dx.doi.org/10.1016/j.jep.2013.10.007
32. Dinda B, Das N, Dinda S, Dinda M. The genus Sida L.—A traditional medicine: Its ethnopharmacological, phytochemical and pharmacological data for commercial exploitation in herbal drugs industry. Journal of Ethnopharmacology. 2015;176:135–176. Available from: doi.org/10.1016/j.jep.2015.10.027
33. Ramachandran VS, Nair NC. Ethnobotanical observations on irulars of Tamil Nadu (India). Journal of Economic and Taxonomic Botany. 1981;2:183-190. Available from: eurekamag.com/research/005/391/005391883.php
34. Akilandeswari S, Senthamarai R, Valarmathi R, Prema S. Screening of Gastric Antiulcer Activity of *Sida acuta* Burm F. International Journal of PharmTech Research. 2010;2(2):1644-1648. Available from: www.semanticscholar.org/paper/Screening-of-Gastric-Antiulcer-Activity-of-Sida-Akilandeswari-Senthamarai/99f2c20c83b46ba1827d1dcf857d661ec7c83094
35. Naik MR, Venugopalan V, Kumaravelayutham P. Ethnoveterinary uses of medicinal plants among the Lambani community in Chitradurga district, Karnataka, India. Asian Pacific Journal of Tropical Biomedicine. 2012;2(2): S470–S476. Available from: doi.org/10.1016/S2221-1691(12)60256-1
36. Shittu, Alagbe JO. Phyto-Nutritional Profiles of Broom Weed (*Sida acuta*) Leaf Extract. Annals of Clinical Toxicology. 2020;3(2):1032. Available from: www.remedypublications.com/annals-of-clinical-toxicology-abstract.php?aid=6545
37. Anani K, Hudson JB, De Souza C, Akpkagana K, Tower GHN, Amason JT, Gbeassor M Investigation of medicinal plants of Togo for antiviral and antimicrobial activities. Pharmaceutical Biology. 2000;38(1):40-45. Available from: doi.org/10.1076/1388-0209(200001)3811-bft040
38. Richa SS, Sharma ML. Phytochemical investigations and anatomical study of three species of *Sida acuta*. Biolife. 2014;2(2):622-9. Available from: doi.org/10.5281/zenodo.7214403
39. Sultana B, Anwar F, Ashraf M. Effect of Extraction Solvent/Technique on the Antioxidant Activity of Selected Medicinal Plant Extracts. Molecules. 2009;14(6):2167–2180. Available from: doi.org/10.3390/molecules14062167
40. Abarca-Vargas R, Malacara CFP, Petricevich VL. (2016). Characterization of chemical compounds with antioxidant and cytotoxic activities in bougainvillea x buttiana holttum and standl, (Var. rose) extracts. Antioxidants. 2016;5(4): 45. Available from: doi.org/10.3390/antiox5040045
41. Raaman N. Phytochemical Techniques. New India Publishing Agency, New Delhi, 2006,19-24. Available from: www.researchgate.net/publication/292756650_Phytochemical_techniques
42. Deshpande PK, Gothalwal R, Pathak AK. Phytochemical analysis and evaluation of antimalarial activity of *Azadirachta indica*. The Pharma Innovation Journal. 2014;3(9):12-16. Available from: www.researchgate.net/publication/306117945_Phytochemical_analysis_and_evaluation_of_antimalarial_activity_of_Azadirachta_indica
43. Singh V, Kumar R. Study of Phytochemical Analysis and Antioxidant Activity of *Allium sativum* of Bundelkhand Region. International Journal of Life Sciences Scientific Research. 2017;3(6):1451-1458. Available from: dx.doi.org/10.21276/ijlssr.2017.3.6.4
44. Harborne JB. Phytochemical Methods. London Chapman and Hall, Ltd. 1973; 49-188. Available from: www.researchgate.net/profile/Br-Rajeswara-Rao/post/What-is-the-most-efficient-method-for-extraction-of-phytochemicals-from-plants/attachment/59d6460ec49f478072eae359/AS%3A273831246139392%401442297864226/download/Phytochemical+methods-Harborne.pdf
45. Silva GO, Abeysundara AT, Aponso MM. Extraction methods, qualitative and quantitative techniques for screening of phytochemicals from plants. American Journal of Essential Oils and Natural Products. 2017;5(2):29-32. Available from: www.essencejournal.com/pdf/2017/vol5issue2/PartA/5-1-31-491.pdf
46. Ray S, Chatterjee S, Chakrabarti CS. Antiproliferative Activity of Allelo chemicals present in aqueous extract of *Synedrella nodiflora* (L.) Gaertn. In Apical Meristems and Wistar Rat Bone Marrow Cells. Iosr Journal of Pharmacy. 2013;3(2):1-10. Available from: dx.doi.org/10.9790/3013-3220110
47. Trease G E, Evans WC. 1989. A Textbook of Pharmacognosy. Bacilliere tinal Ltd., London, 13th edition. Available from: dl.konkur.in/post/Book/MedicalScience/Trease-and-Evans-Pharmacognosy-16th-Edition-%5Bkonkur.in%5D.pdf
48. Njoku OV, Obi C Onyema OM. Phytochemical constituents of some selected medicinal plants. African Journal of Pure and Applied Chemistry. 2011;10(66):228-233. Available from: doi.org/10.5897/AJB11.1948
49. Obouayeba AP, Diarrassouba M, Soumahin EF, Kouakou TH. Phytochemical analysis, purification and identification of *Hibiscus Anthocyanins*. Journal of Pharmaceutical, Chemical and Biological Sciences. 2015;3(2):156-168. Available from: www.jpcbs.info/2015_3_2_5_Obouayeba.pdf
50. NIST 23 Gas Chromatography library with search software 2023/2020/2017, Wiley Specialty libraries. Available from: www.sisweb.com/software/nist-gc-library.htm
51. Lugtenberg B, Alphen LV. Molecular architecture and functioning of the outer membrane of *Escherichia coli* and other gram-negative bacteria. Biochimica et Biophysica Acta- Reviews on Biomembranes. 1983;737(1):51–115. Available from: doi.org/10.1016/0304-4157(83)90014-x
52. Villegas MV, Quinn JP. An update on antibiotic-resistant gram-negative bacteria. Infections in Medicine. 2004;21(12):595–599. Available from: www.researchgate.net/publication/291842550_An_update_on_antibiotic-resistant_gram-negative_bacteria
53. Canli K, Altuner EM, Akata I. Antimicrobial screening of *Mnium stellare*. Bangladesh Journal of Pharmacology. 2015;10(2):321–325. Available from: doi.org/10.3329/bjp.v10i2.22463
54. Miri ST, Dashti A, Mostaan S, Kazemi F, Bouzari S. Identification of different *Escherichia coli* pathotypes in north and north-west provinces of Iran. Iranian Journal of Microbiology. 2017;9(1):33–37. Available from: www.ncbi.nlm.nih.gov/pmc/articles/pmc5534002/
55. Madhi SA, Petersen K, Madhi A, Khoosal M, Klugman KP. Increased disease burden and antibiotic resistance of Bacteria causing severe community-acquired lower respiratory tract infections in human immunodeficiency virus type 1-infected children. Clinical Infectious Diseases. 2000;31(1):170–176. Available from: doi.org/10.1086/313925
56. Prasad MA, Zolnik CP, Molina J. Leveraging phytochemicals: the plant phylogeny predicts sources of novel antibacterial compounds. Future Science OA. 2019;5(7):FSO407. Available from: doi.org/10.2144/fsoa-2018-0124
57. Lee KA, Kim KT, Nah SY, Chung MS, Cho S, Paik HD. Antimicrobial and antioxidative effects of onion peel extracted by the subcritical water. Food Science and Biotechnology. 2011;20:543–548. Available from: doi.org/10.1007/s10068-011-0076-8
58. Aziz M, Karboune S. Natural antimicrobial/antioxidant agents