JOURNAL OF PURE AND APPLIED MICROBIOLOGY, June 2015. Vol. **9**(2), p. 1371-1376

Antibacterial, Antioxidant and in *Silico* Study of *Ipomoea aquatica* Forsk

J. Helan Chandra* and M. Shamli

Department of Biotechnology, Jeppiaar Engineering College,Chennai - 600 119, India.

(Received: 13 February 2015; accepted: 30 March 2015)

Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years and in many parts of the world. Currently, thousands of plant metabolites are being successfully used in the treatment of variety of diseases. The present study deals with antibacterial and antioxidant activity of ethanol extract of leaves of plant of *Ipomoea aquatica* forsk and also to carry out *in silico* analysis of compounds against bacterial life cycle receptor. Antibacterial assay was carried out for the extract by both disc diffusion and well diffusion method. In disc diffusion method, maximum zone of inhibition (mm) was observed for *Escherichia coli* and *Bacillus cereus* when compared with S*taphylococcus aureus* and *Salmonella typhii*. In well diffusion, maximum zone of inhibition was noticed for *Escherichia coli*. Antioxidant activity of the ethanolic extracts of *Ipomoea aquatica* Forsk was performed by DPPH assay. GC-MS analysis revealed the presence of five major compounds and they were subjected for *in silico* analysis with bacterial receptor such as LuxS (1JVI), FtsZ (1S1J), FtsZ (3VOB) and LsrB (1TM2) from *Bacillus cereus, Escherichia coli,* S*taphylococcus aureus* and *Salmonella typhii* respectively by using autodock 4.2 and Cygwin. These results exhibited negative binding energy which also supports antibacterial activity of *Ipomoea aquatica* forsk.

Key words: Antibacterial activity, GC-MS analysis, Bacterial receptor, Cygwin, DPPH assay.

Plants have fed the world and cured its illness since time immemorial (Kaur and Mondal, 2014). Now-a-days, the usages of plant products are increasing in many segment of the world's population. Many plant metabolites are being successfully used in the treatment of variety of diseases. According to an estimate, 80% of the world's populations rely upon plants as the source of drugs (James *et al.*, 2009). Plant-derived products contain a great diversity of phytochemicals such as phenolic acids, flavonoids, tannins, lignin and other minor compounds. These compounds possess numerous health-related effects such as antibacterial, antimutagenic, anticarcinogenic, antioxidant, antithrombotic and vasodilatory activities (Wendakoon *et al.*, 2012).

* To whom all correspondence should be addressed.
Mob.: +91-9840406303;
E-mail: helananand@gmail.com

Ipomoea aquatica Forsk (IAF), commonly called water spinach, belongs to the family Convolvulaceae. It is cultivated commercially as an edible green leafy vegetable in Hong Kong, Taiwan and China. It is a tender, trailing or floating perennial aquatic plant found on moist soil along the margins of fresh water and in ditches, marshes and wet rice fields. It is also commonly used as a green leafy vegetable in rural India (Prasad *et al.*, 2005). It is long known as animal and aquatic organism feed, but recently, few individuals have found it a delicacy as it is being consumed as vegetable in the region. Studies with *Ipomoea aquatica* Forsk revealed that the inhibition of prostaglandin synthesis, effects on liver disease and constipation (James *et al.*, 2009).

GC-MS is extensively used for the analysis of these compounds which include esters, fatty acids, alcohols, aldehydes, terpenes etc. It is also used to detect and measure contaminants

from spoilage or adulteration which may be harmful to society. The virtual screening approach for docking small molecules into a known protein structure is a powerful tool for drug design and has become an integral part of the drug discovery process (Rizvi *et al.,* 2013). Docking algorithms fits the generated poses into the target protein under investigation, thereby helps us to develop new metabolites. Autodock module generates energetically most favorable pose are evaluated based on its complementarity to the target and found to reproduce better results compared to DOCK, Flex and GOLD (Sridhar and Helan, 2014)

In the present study, antimicrobial activity and antioxidant activity of crude leaf ethanolic extract of *Ipomoea aquatica* forsk and *in silico* study of plant compounds obtained from GC-MS with bacterial life cycle receptor such as LuxS (1JVI), FtsZ (1S1J), FtsZ (3VOB) and LsrB (1TM2) receptor molecule from *Escherichia coli, Bacillus cereus, Staphylococcus aureus* and *Salmonella typhii* respectively.

MATERIALS AND METHODS

Materials

All chemicals and reagents were of analytical grade and purchased from Hi-media Laboratories Pvt. Ltd., Mumbai, India and Sd-fine Chemicals. Ltd., Mumbai, India

Plant Materials

The leaves of water spinach (*Ipomoea aquatica* Forsk) were obtained from Potheri Lake in Kancheepuram District, Tamil Nadu. After harvesting, the leaves were separated and washed under tap water. Leaves were dried in the dark room for 5 days. The samples were ground in grinding mill for 10 seconds to produce a powder with an approximate size of 0.525mm.

Extraction of Plant

Ten grams (10g) of dried and grounded leaves were placed in a soxhlet apparatus. Extraction was performed with 100 ml of an organic solvent (ethanol) for 6h. Extract is poured in Petri plate in order to remove solvent (Bimakr *et al.,* 2011).

Anti Bacterial Assay

Disc Diffusion Method

Dried and sterilized filter paper discs (6 mm diameter) containing the test samples from the stock of 0.1g/ml are placed on Muller Hinton (MH) agar medium uniformly seeded with the test microorganisms such as Gram negative bacteria (*Escherichia coli* and *Salmonella typhii*) and *Gram* positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*) by spread plate method. Standard antibiotic (tetracycline t30) discs and blank (sterile water) discs were used as positive and negative control respectively. These plates were kept at low temperature (4°C) for 1 hour to allow maximum diffusion of the test materials to the surrounding media. The plates were then inverted and incubated at 37°C for 24 hours for optimum growth of the organisms. The antimicrobial activity of the test agent was then determined by measuring the diameter of zone of inhibition expressed in millimeter (Benkeblia, 2004).

Well Diffusion Method

Antimicrobial susceptibility testing was done using the well diffusion method to detect the presence of anti-bacterial activities of the plant samples (Kaur and Mondal, 2014). Petri plates containing 20 ml Mueller Hinton medium were seeded with the bacterial strains such as Gram negative bacteria (*Escherichia coli* and *Salmonella typhii*) and *Gram* positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*) by using spread plate method. Wells were punchered and stock was prepared 0.1g/ml of extracts. From the stock, dilutions were made to obtain 250µg/100µl, 500µg/100µl, 750µg/100µl and 1000µg/100µl of plant extracts and poured into wells respectively. The plates were then incubated at 37°C for 24 hours. Erythromycin (0.05%) was used as positive control and analysis was done in triplicates. The diameter of zone of inhibition can be measured in millimeters.

Phytochemical Analysis

Phyto-chemical analyses were carried out according to the Yadav and Agarwala, (2011)

Antioxidant Activity by DPPH Assay

The electron donating ability of the obtained extracts was measured by bleaching a purple solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (Blois,1958). Percentage of free radical scavenging activity was expressed in percent inhibition from the given formula:

$$\text{Inhibition \%} = \frac{(\text{absorbance of control} - \text{absorbance of sample}) * 100}{\text{Absorbance of control}}$$

GC-MS Analysis

GC-MS analysis was carried out in Indian Institute of Technology, Madras using the instrument JEOL - JMS gc mat ell with GCMS solution version 2.53 software. The sample volume was 1.0 µl. The sample of ethanolic extract was run for 30 minutes.

Docking Study

Docking studies were carried out for the compounds of the *Ipomoea aquatica* Forsk identified in GC-MS analysis against receptors which were selected based on their role in bacterial life cycle. Ligands chosen were n-hexadecanoic acid, 12-octadecenoic acid, methyl ester, 9,12,15 octadecatrienoic acid, ethyl ester [Z,Z,Z], pentadecanoic acid,14 methyl-methyl ester and decanoic acid,10-[2-hexacyclopropyl]. These structures were downloaded from PubChem in SDF format and converted into MOL format using Chemsketch 12.01 (freeware version, ACD Labs). The receptor such as Quorum sensing protein LuxS(1JVI), FtsZ(1S1J), FtsZ(3VOB) and LsrB(1TM2) Were obtained from *Escherichia coli, Bacillus cereus, Staphylococcus aureus* and *Salmonella typhii* respectively (Saravanakumar and Helan, 2013).

By using AutoDock 4.2, the target and ligand files can be converted into PDBQT format files (Target. pdbqt , Ligand. pdbqt) and these files were subjected to undergo Grid and Docking Parameter (a.gpf and a.dpf). Molecular docking was performed using Cygwin and finally the results are analyzed (Rizvi *et al.,* 2013).

RESULTS AND DISCUSSION

Antibacterial Assay

In disc diffusion method, the ethanol extracts exhibited maximum activity (Figure-1) in the *Escherichia coli* and *Bacillus cereus* when compared with other microorganism. In well diffusion, ethanol extract exhibited the maximum zone of inhibition (Figure-2) for *E.coli* at the maximum concentration (1000µg/100µl). It clearly indicates that the ethanol extracts of *Ipomoea aquatica* forsk were more potent against the gram negative bacteria than the gram positive bacteria. The potential for developing antimicrobials from higher plants appears rewarding as it would lead to the development of a phytomedicine to act against microbes (Parekh and Chanda, 2006).

Phytochemical Analysis

Qualitative analysis for phytochemical constituents revealed the presence of Proteins, Carbohydrates, Steroids, Tannins, Glycosides and Phenols which are medically active. Saponins, and Terpenoids were absent in ethanol extract of the plant. Phytochemical compounds derived from plant products serve as a prototype to develop less toxic and more effective medicines in controlling the growth of microorganism (Sen and Batra, 2012). The results obtained from this work revealed that the plants contained medically active compounds which are connected with antimicrobial properties in plants (Arora *et al.,* 2013).

Antioxidant Activity by DPPH Assay

Figure-3 shows that the ethanol extracts of *Ipomoea aquatica* Forsk in the concentration of 50µg (19.49 ± 0.70) and 100µg (24.48 ± 0.41) have the high antioxidant activity when compared with concentration of 25µg (14.95 ± 1.44). It might be due to the presence phenolic compounds from ethanol extracts of *Ipomoea aquatica* Forsk which were more efficient antioxidants than Butylated hydroxytoluene (Yadav and Agarwala, 2011).

The antioxidant activity of Ethanolic plant extract from *Ipomoea aquatica* Forsk was determined using a methanol solution of DPPH reagent. Unlike *in vitro* generated free radicals such as the hydroxyl radical and superoxide anion, DPPH has the advantage of being unaffected by certain side reactions, such as metal ion chelation and enzyme inhibition. Radical scavenging activities are important due to the deleterious role of free radicals in foods and also in biological systems. DPPH assay evaluated the ability of antioxidants to scavenge free radicals (Mundhe *et al.,* 2011).

GC-MS Analysis

GC-MS analysis for the ethanolic extract of *Ipomoea aquatica* Forsk (IAF) revealed the presence of 5 compounds. The Chromatogram (Figure-4) shows 5 prominent peaks in the retention time range 17.17 – 19.55. The compounds identified from the peak of chromatogram are n-hexadecanoic acid, 12-octadecenoic acid,methyl ester, 9,12,15

Table 1. Compound present in GC-MS analysis

S.No	Rt(min)	Compound name	Structure	Molecular formula	Molecular weight
17.9	n-hexadecanoic acid			$C_{16}H_{32}O_2$	256.4241
18.9	12-octadecenoic acid,methyl ester			$C_{19}H_{36}O_2$	296.4879
19.55	9,12,15 octadecatrienoic acid,ethyl ester [Z,Z,Z],			$C_{20}H_{34}O_2$	306.4828
17.17	pentadecanoic acid,14 methyl-methyl ester			$C_{17}H_{34}O_2$	270.4507
19.08	decanoic acid,10-[2-hexacyclopropy			$C_{10}H_{20}O_2$	172.2646

Table 2. Docking analysis of ligands against the bacterial life cycle receptor

PDB Id	Ligands	Binding Energy (Kcal/Mol)	No.of Hydrogen Bond	Amino Acid
1JVI	n-hexadecanoic acid	-4.71	2	Lys 35
	12-octadecenoic acid,methyl ester	-4.95	5	Lys 35, Pro 50, Glu 83, Ser 6
	9,12,15 octadecatrienoic acid,ethyl ester [Z,Z,Z],	-5.22	4	Lys 35, Pro 50, Tyr 89, Ser 6
	pentadecanoic acid,14 methyl-methyl ester	-4.38	5	Tyr 85, Glu 83
	decanoic acid,10-[2-hexacyclopropyl]	-4.91	5	Lys 35, Glu 83, Ser 6
1TM2	n-hexadecanoic acid	-6.42	4	Gly 130, lys 120, Ser 117
	methyl-methyl ester12-octadecenoic acid,methyl ester	-4.81	8	Phe 340, gly 40, asp116-3, asn129, Tyr 127.
	9,12,15 octadecatrienoic acid,ethyl ester [Z,Z,Z],	-5.94	3	Asp 116, asp 118, pro 220
	pentadecanoic acid,14	-7.59	6	Asp 116,asn 129, gln 169
	decanoic acid,10-[2 hexacyclopropyl]	-7.81	6	Lys 35,Tyr 312, lys 120,gln 130

octadecatrienoic acid,ethyl ester [Z,Z,Z], Pentadecanoic acid,14methyl-methyl ester and Decanoic acid,10-[2-hexacyclopropyl] according to NIST library.

Docking Study

Docking studies showed the formation of hydrogen bond interactions between the sterols and ester compounds with the active sites of bacterial life cycle receptors. In the present study, formation of Hydrogen bond was observed with LuxS (1JVI) and LsrB (1TM2) receptor and no hydrogen bond formation with FtsZ (3VOB) and FtsZ (1S1J) receptor. An observed Negative binding energy value explains about the maximum antibacterial activity as well as the interaction between ligand and receptor during the hydrogen bond formation. Bioinformatics approach could be used to mimic, investigate the insights of molecular interactions due to their inexpensiveness, less time consuming, higher reproducibility with low compound synthesis requirements and have the potential of reduced utilization of animals, thereby assisting us by providing mechanistic information ligand-protein interactions (Sridhar and Helan, 2014)

Plant-based antimicrobial studies have enormous therapeutic potential as they could serve

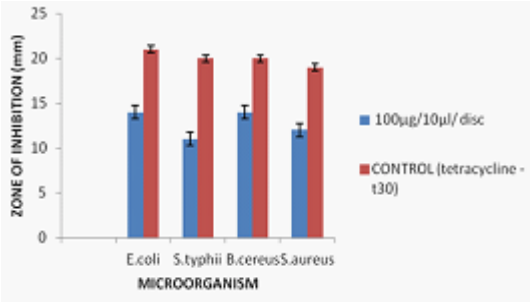

Fig. 1. Antibacterial activity against ethanol extract of leaves of plant *Ipomoea aquatica* forsk by Disc Diffusion Method

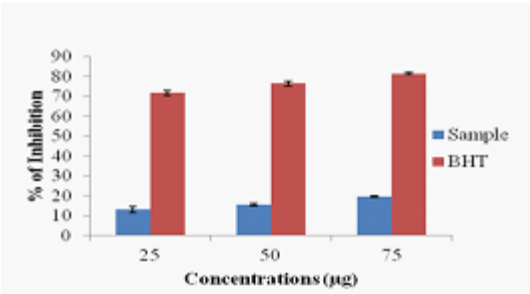

Fig. 3. Antioxidant activity of ethanolic extract of *Ipomoea aquatica* Forsk by DPPH assay

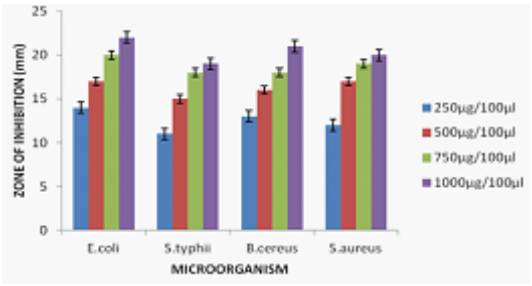

Fig. 2. Antibacterial activity against Ethanol extract of leaves of plant *Ipomoea aquatica* forsk by Well Diffusion Method

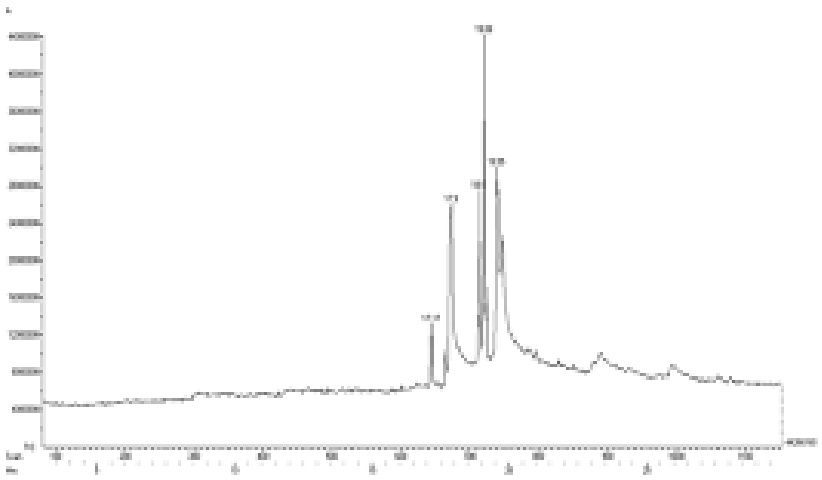

Fig. 4. GC-MS result for Ethanolic plant extracts

the purpose with lesser side effects that are often associated with synthetic antimicrobial agents. Preliminary investigation clearly showed that the phytochemicals derived from plant products serve as a prototype to develop less toxic and more effective medicines in controlling the growth of microorganism. The ethanolic extracts of leaves of *Ipomoea aquatica* forsk showed the maximum antioxidant activity based upon the different concentration of extracts. *In silico* study with the negative binding energy of the above sterols and ester compounds supported *in vivo* antibacterial activity and motivates the new area in the development of antibiotics.

ACKNOWLEDGEMENTS

Authors are thankful to Staff, Department of Biotechnology and Management, Jeppiaar Engineering College, Chennai for providing the necessary facilities.

REFERENCES

1. Kaur, S., Mondal, P., "Study of Total Phenolic and Flavonoid Content, Antioxidant Activity and Antimicrobial Properties of Medicinal Plants". *J Microbiol. Exp*, 2014; **1**: 1-6.
2. James, O., Nnacheta, O.P., Wara, H.S Aliyu, U.R., "*In vitro* and *In vivo* studies on the antioxidative activities, membrane stabilization and cytotoxicity of water spinach (*Ipomoea aquatica forsk*) from ibaji ponds, Nigeria". *Int.J. PharmTech Res*, 2009; **1**(3):474-482.
3. Wendakoon, C., Calderon, P., Gagnon,D., "Evaluation of Selected Medicinal Plants Extracted in Different Ethanol Concentrations for Antibacterial Activity against Human Pathogens". *Journal of Medicinally Active Plants.*, 2012; **1**(2): 60-68.
4. Prasad N.K., Divakar S., Shivamurthy R.G., Aradhya S.M., "Isolation of a free radical-scavenging antioxidant from water spinach (*Ipomoea aquatica Forsk*)". *J Sci Food Agric*, 2005; **85**: 1461-1468.
5. Rizvi, S.M.D., Shakil, S., Haneef, M., "A Simple click by click protocol to perform docking: Autodock 4.2 made easy for non-bioinformaticians". *EXCLI Journal*, 2013; **12**: 831-857.
6. Sridhar, S., Helan, J.C., "Involvement of Computational tools towards In Silico remediation - Synthetic textile dyes interacting with Azoreductase". *International Journal of ChemTech Research*, 2014; **6**(9):4412-4416.
7. Bimakr, M., Rahman, R.A., Taip, F.S., Ganjloo, A., Salleh, L.M., Jinap, S.J., Hamid, A., Zaidul, I.S.M., "Comparison of different extraction methods for the extraction of major bioactive flavonoid compounds from spearmint (*Mentha spicata* L.) Leaves". *Food and bioproducts processing*,2011; **8**(9):67-72.
8. Benkeblia, N., "Antimicrobial activityof essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium sativum*)". *Lebensm.-Wiss. U.-Technol.*, 2004; **37**:263-268.
9. Yadav, R.N.S., Agarwala, M., "Phytochemical analysis of some medicinal plants". *Journal of Phytology*, 2011; **3**(12):10-14.
10. Blois, M.S., "Antioxidant determination by the use of stable free radicals". *Nature*, 1958; **181**:1199-2000.
11. Saravanakumar,S., Helan, J.C., "*In silico* analysis of compounds characterized from *Caesalpinia sappan* L with Bacterial life cycle receptors". *Int J. of Appl.Biol.*, 2013; **1**:72-75.
12. Parekh, J., Chanda, V.S., "In vitro Antimicrobial Activity and Phytochemical Analysis of Some Indian Medicinal Plants", *Turk J Biol*, 2007; **31**: 53-58.
13. Sen, A., Batra, A., "Evaluation of Antimicrobial activity of different solvent extracts of medicinal plant: *Melia azedarach* l." *Int J Curr Pharm Res*, 2012; **4**(2):67-73.
14. Arora, S., Kumar, D., Shiba., "Phytochemical, Antimicrobial and Antioxidant Activities of Methanol extract of leaves and flowers of *Ipomoea cairica*" . *Int J Pharm Pharm Sci*, 2013; **5**(1): 198-202.
15. Mundhe, S.K., Kale, A.A., Gaikwad, A.S., Deshpande, R.N., Kashalkar, V.R., "Evaluation of phenol, flavonoid contents and antioxidant activity of *Polyalthia longifolia*". *J. Chem. Pharm. Res.*, 2011; **3**(1):764-769.