IJPSR (2015), Vol. 6, Issue 3 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 20 June, 2014; received in revised form, 24 September, 2014; accepted, 16 November, 2014; published 01 March, 2015

STUDY ON PRELIMINARY PHYTOCHEMICAL AND ANTIBACTERIAL ACTIVITY AGAINST HUMAN PATHOGENS OF AN ENDANGERED ORCHID-*HABENARIA PLANTAGINEA* LINDL

M. Keerthiga [1*] and S. P. Anand [2]

Department of Biotechnology [1], Department of Botany [2], National College (Autonomous), Tiruchirappalli, India

Keywords:

Plant extract,
Antibacterial activity,
Phytochemical analysis,
Habenaria plantaginea Lindl

Correspondence to Author:
M. Keerthiga

Research scholar
Department of Biotechnology,
National College (Autonomous & CPE), Tiruchirappalli – 620 001
Tamil Nadu, India.

E-mail: keerthigamanohar@gmail.com

ABSTRACT: *Habenaria plantaginea* Lindl. (Orchidaceae) is used as a medicinal plant in several countries. In this study, ethanol, chloroform and water extracts of *Habenaria plantaginea* were evaluated for antibacterial activity against bacterial (MTCC) strains. Antibacterial activity of this plants extracts were performed using agar disc diffusion method. The antibacterial activity of ethanol extracts showed more effective followed by water and chloroform extracts against all the bacterial strains. Preliminary phytochemical studies were performed for the presence and absence of secondary metabolites in the whole plant. The Minimum Inhibitory concentration (MIC) exhibited by *H. plantaginea* extract against the bacterial strains ranged between 0.125 mg/ml to 6 mg/ml. The presence of bioactive constituents is associated with the antimicrobial activity of various solvent extracts of this plant was carried in attempt to support the use by medicinal practitioner for the treatment of various diseases. Medicinal importance of this orchid will target to meet the therapeutic demands.

INTRODUCTION: In India, orchids from 10% of the world orchid flora with Himalayas as their natural home [1] and the largest and commercially important flowering plants that over 22,500 species with 779 genera are distributed throughout the world [2]. There are 1331 species belonging to 186 genera widely distributed throughout the country [3]. The medicinal importance of orchids is known as early as 250 – 300 BC by *susruta* and *vagbhata* in ancient Sanskrit literature. Some orchid species reported to contain alkaloids, triterpenoids, flavonoids and stilbenoids.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.6(3).1100-06

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.6(3).1100-06

Ashtavarga is a group of 8 drugs in Ayurvedic formulation which are used for the preparation of tonics, such as 'Chyavanprash' which consists of Orchid species, Viz. *Habenaria intermedia* D. Don, (Riddhi), *Habenaria edgeworthi* Hook. F. (Vriddhi), *Malaxis acuminate* D. Don, (Rishbhaka). Besides these species, many Orchid species are widely used as traditional medicines by people and used in pharmaceutical industries to isolate anthocyanins, stilebnoids and triterpenoids. Some of the phytochemicals like alkaloids, anthocyanins, arundinan, bibenzyl, cypripenndin, dendrobins, gigantol, glucoside, glycosides, gymopusin, hircinol, jibantine, kinsenoside, loroglossin, nidemin and orchinol, phenanthropyran, rotundatin and moscatin are reported from Orchids [4]. In India and other parts of the world use many orchid species in their traditional system of herbal medicines. Herbal medicines have received much attention as a source of new antibacterial drugs

since they are considered as time-tested and comparatively safe both for human use and the environment [5-7].

Habenaria plantaginea Lindl. belongs to family Orchidaceae. A terrestrial Orchid. This is an endemic plant of south India. It is very common and available only from dense matter on rocks. This is native of India occurs in the forest of eastern peninsular flora from Periyakombai Hill above 450 – 650m. An ovoid – globose tuber giving rise to an erect, glabrous, bracteates stem carrying 3 to 7, sub basal, clustered, elliptic-oblong to oblong-lanceolate, sub acute to acute, subsessile, basally clasping leaves that blooms in the late summer and early fill on an erect, laxly 5 to 9 flowered, glabrous, 2 to 7 cm long inflorescence with lanceolate, acuminate, largest towards the base, setaceous margined floral bracts carrying faintly fragrant flowers.

The indigenous people especially in hilly regions take immense pride in treasuring this plant because of its high utility in traditional healing and cure floriculture trade. *Habenaria plantaginea* tubers used as folk medicine to treat cough, asthma, helminthiasis, insanity and snake bite [8]. The plant has been used as medicine for the treatment of tuberculosis and paralysis [9].

MATERIAL AND METHODS:

Collection of Plants:

The whole plant of *Habenaria plantagniea* was collected from the Periyakombai Hill, Namakkal district, Tamil Nadu. The specimen was identified with the help of regional floras and the voucher specimen was deposited at St. Joseph's college (Autonomous), Tiruchirappalli, Tamil Nadu, India.

Preparation of extracts:

Aqueous extraction:

100 grams of dried powder were extracted in distilled water for 6 hrs at slow heat. Every 2 hrs it was filtered through 8hrs layers of muslin cloth and centrifuged at 5000 g for 15 min. The supernatant was collected. This procedure was repeated twice and after 6 hrs the supernatant was concentrated to make the final volume one-fifth of the original volume.

Solvent extraction:

100 grams of dried leaves were extracted with 200 ml of two different solvents such as ethanol and chloroform kept on a rotary shaker for 24 h. Thereafter, it was filtered and centrifuged at 5000 g for 15 min. The supernatant was collected and the solvent was evaporated to make the final volume one-fifth of the original volume. It was stored at 4^{o}C in airtight bottles for further studies.

Percent extractive values were calculated by the following formula.

$$\text{Percent Extract} = \frac{\text{Weight of dried extract}}{\text{Weight of dried plant material}}$$

Microorganisms:

In vitro antimicrobial activity was examined for various extracts such as water, ethanol and chloroform of the species, *Habenaria plantaginea* against eight bacterial species which include the gram positive strains viz., *Staphylococcus aureus* (MTCC 3160), *Bacillus subtilis* (MTCC 441), and *Streptococcus pyogenes* (MTCC 442) and gram negative strains viz., *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 424), and *Klebsiella pneumoniae* (MTCC 3384), All the bacterial strains were maintained at 4°C on nutrient agar slants until further use.

Antimicrobial assay:

The plant extracts were tested for their effect against the growth of pathogenic bacteria by disc diffusion method. The various solvent extract of *Habenaria plantaginea* at different concentrations was employed for antimicrobial activity. The antibiotic discs, Streptomycin (30µg) served as positive control for bacteria. The bacteria tested were inoculated into nutrient agar. After the incubation period of 24 hours at a temperature of 35°C, three or four colonies isolated from these media were inoculated on 4ml of nutrient broth and incubated for 2 hrs at 35°C.

The cultures were adjusted with sterile saline solution to obtain turbidity. Petri dishes containing Muller-Hinton agar medium were streaked separately with these microbial suspensions of bacteria and fungi. Sterile filter paper discs impregnated with 25, 50, 75 and 100mg/ml extracts and control discs were applied over the culture plates. After equilibrium at 4°C, the plates were

incubated overnight at 37°C and the diameter of any resulting zones of inhibition was measured. Triplicates were maintained for all these experiments. Based on the diameter of the zone of inhibition, antibacterial susceptibility was ranked[10]. Inhibition zones were measured and compared with the standard reference antibiotics. Activity index for each extracts was calculated.

$$\text{Activity Index (AI)} = \frac{\text{Inhibtion zone of the sample}}{\text{Inhibition zone of the standard}}$$

Minimum Inhibitory Concentration (MIC):
Minimum Inhibitory Concentration (MIC) was determined by Micro dilution method using serially diluted plant extracts. The extracts were diluted into different concentrations of 0.125 - 8mg/ml respectively with DMSO. Then each tubes was filled with 1ml of sterile nutrient broth and inoculated with 0.1ml of broth culture of the test organism (inoculums contains $1\text{-}2\times10^{-7}$ CFU/ml). The tubes were incubated aerobically at 37ºC for 18-24hrs. The control tubes were maintained for each test tube. Inhibition of growth observed in those test tubes (No turbidity) which has lowest or minimum concentration of extract. This lowest or minimum concentration was considered as Minimum Inhibitory Concentration (MIC) [11]

Total Activity (TA) determination:
Total activity is the volume at which the test extract can be diluted with the ability to kill microorganisms. It is calculated by dividing the amount of extract from 1gm plant material by the MIC of the same extract or compound isolated and is expressed in ml/g [12]

$$\text{Total Activity (TA)} = \frac{\text{Extract per gram dried plant part}}{\text{MIC of extract}}$$

Identification Tests for Phytochemical Constituents:
The tests were performed to find out the presence of active chemical constituents such as alkaloids, terpenes, flavones, flavonoids, steroids, reducing sugars, carbohydrates, tannins, anthraquinones, glycosides, cardiac glycosides by the following procedure. Phytochemical analysis was carried out for all the extracts using standard methods [13, 14]

Statistical Analysis:
Mean value and Standard deviation were calculated for each test bacteria. Data were analysed by one – way ANOVA and p values were considered significant at $p > 0.005$ [15]

RESULTS AND DISCUSSION: The Whole plant powder of *Habenaria plantaginea* Lindl. was subjected to successive solvent extraction. Percentage yield of the selected successive extract were recorded in **Table 1** and **Figure 1**.

TABLE 1: SUCCESSIVE EXTRACTION OF THE WHOLE PLANT OF *HABENARIA PLANTAGINEA* LINDL.

Parameter	Values % (w/w)
Water Extract	22.98
Ethanol Extract	12.49
Chloroform Extract	5.698

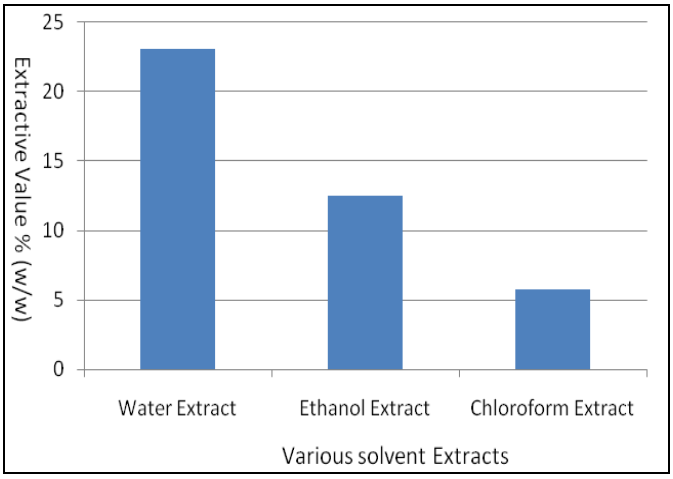

FIGURE 1: SUCCESSIVE EXTRACTION OF THE WHOLE PLANT OF *HABENERIA PLANTAGINEA* LINDL.

The antibacterial activity of the plant extracts from *Habenaria plantaginea* Lindl.was studied against Bacterial MTCC strains *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus pyogenes*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* using agar disc diffusion method. The result revealed that inhibitory effects of test samples was dose dependent as the concentration increased the zone of inhibition was also increased. The water extracts of this plant showed maximum activity against *Bacillus subtilis*, followed by *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Escherichia coli*.

The ethanol extracts of this plant showed maximum activity against *Bacillus subtilis* followed by *Escherichia coli, Streptococcus pyogenes, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Staphylococcus aureus*. Chloroform extracts of this plant (leaves) showed maximum activity against *Streptococcus pyogenes, Klebsiella pneumoniae* followed by *Escherichia coli, Bacillus subtilis, Staphylococcus aureus* and *Pseudomonas aeruginosa.* The antibacterial activity of ethanol extracts showed more effective followed by chloroform and water extracts against all the bacterial strains. The results were recorded and tabulated **(Table 2)**. The study was reporting that ethanolic extract of *H.plantaginea* whole plant showed greater antibacterial activity against some species of gram positive and gram negative pathogenic bacteria than the water extracts.

TABLE 2: ANTIMICROBIAL ACTIVITY OF VARIOUS EXTRACTS OF *HABENARIA PLANTAGINEA* AGAINST HUMAN PATHOGENS

Test microorganisms	Values	Solvents											
		Water				Ethanol				Chloroform			
		25mg	50mg	75mg	100mg	25mg	50mg	75mg	100mg	25mg	50mg	75mg	100mg
Bacillus subtilis	IZ±S.D	-	-	-	10±0.26	10±0.33	14±0.23	21±0.56	28±0.34	10±0.24	12±0.39	13±0.23	15±0.34
	AI	-	-	-	0.909	0.909	1.272	1.909	2.545	0.909	1.090	1.181	1.363
Staphylococcus aureus	IZ±S.D	-	-	-	13±0.12	10±0.24	11±0.24	12±0.27	18±0.24	15±0.27	17±0.06	18±0.48	19±0.24
	AI	-	-	-	0.866	0.666	0.733	0.8	1.2	1.003	1.133	1.2	1.266
Streptococcus pyogenes	IZ±S.D	-	-	10±0.26	12±0.43	10±0.29	13±0.23	17±0.42	20±0.25	14±0.29	17±0.07	19±0.36	22±0.18
	AI	-	-	0.476	0.571	0.476	0.619	0.809	0.952	0.666	0.809	0.904	1.047
Escherichia coli	IZ±S.D	-	10±0.26	11±0.13	14±0.24	10±0.33	15±0.27	20±0.10	22±0.24	16±0.09	17±0.23	19±0.25	20±0.23
	AI	-	0.625	0.6875	0.875	0.625	0.9375	1.25	1.375	1.009	1.0625	1.187	1.25
Pseudomonas aerginosa	IZ±S.D	-	-	10±0.23	11±0.27	10±0.13	11±0.19	15±0.08	19±0.23	13±0.12	14±0.25	15±0.34	17±0.14
	AI	-	-	0.416	0.458	0.416	0.458	0.625	0.791	0.541	0.583	0.625	0.708
Klebseilla pnemoniea	IZ±S.D	-	10±0.21	12±0.14	13±0.17	10±0.23	12±0.25	17±0.28	19±0.34	14±0.25	17±0.24	19±0.65	22±0.09
	AI	-	0.714	0.857	0.928	0.714	0.857	1.214	1.357	1.025	1.214	1.357	1.571

IZ – Inhibition zones in mm, S.D – Standard Deviation, AI – Activity Index, - - No zone formation.

The results of the Minimum Inhibitory concentrations (MICs) of extracts from different solvents such as water, ethanol and chloroform of *Habenaria plantaginea* determined against *Escherichia coli, Klebsiella pneumoniae, Bacillus subtilis, Pseudomonas aeruginosa, Streptococcus pyogenes* and *Staphylococcus aureus* were presented in **Table 3** and **Figure 2.**

TABLE 3: SCREENING MIC (MG/ML) PERFORMANCE OF DIFFERENT EXTRACTS OF *HABENARIA PLANTAGINEA* LINDL. AGAINST PATHOGENIC ORGANISMS.

Test microorganisms	Water Extract	Ethanol Extract	Chloroform Extract
Bacillus subtilis	-	0.250	0.500
Staphylococcus aureus	-	0.500	2.500
Streptococcus pyogenes	-	1.25	0.125
Escherichia coli	6.000	3.500	1.000
Pseudomonas aeruginosa	-	5.000	4.000
Klebseilla pneumoniae	5.000	2.000	0.250

MICs of ethanol and chloroform extracts of *H. plantaginea* Linn against *Escherichia coli, Klebsiella pneumoniae, Bacillus subtilis, Pseudomonas aeruginosa, Streptococcus pyogenes* and *Staphylococcus aureus* were 0.250 – 5 mg/ml. MICs value of petroleum ether extract of *H. plantaginea* Linn against *Escherichia coli,* and *Klebsiella pneumoniae* were 5 – 6 mg/ml and there was no activity against *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa* and *Streptococcus pyogenes.*

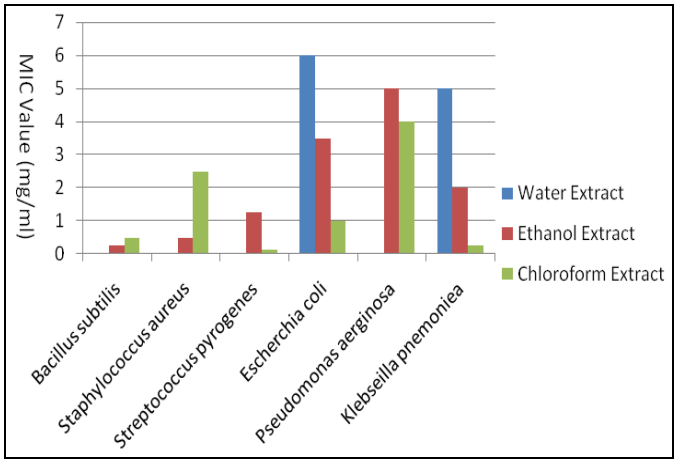

FIGURE 2: SCREENING MIC (µG/ML) PERFORMANCE OF DIFFERENT EXTRACTS OF *HABENERIA PLANTAGINEA* LINDL. AGAINST PATHOGENIC ORGANISMS.

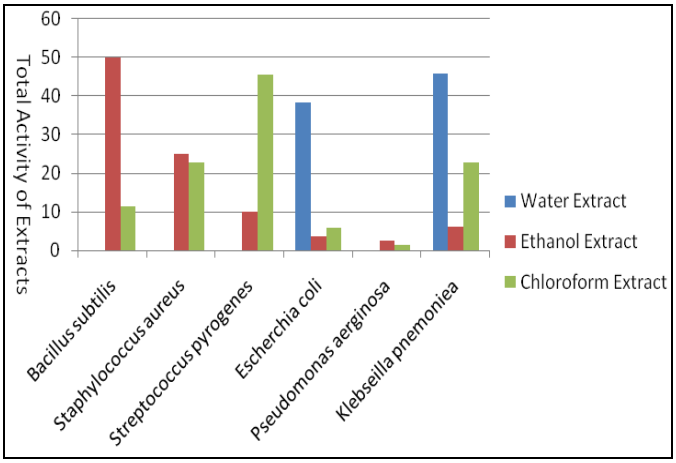

FIGURE 3: SCREENING TOTAL ACTIVITY PERFORMANCE OF DIFFERENT EXTRACTS OF *HABENERIA PLANTAGINEA* LINDL. *AGAINST PATHOGENIC ORGANISMS.*

Total Activity:

Total activity indicates the volume at which extracts can be diluted with still having ability to kill microorganism **(Table 4** and **Figure 3)**. Mostly ethanolic extracts showed high value of total activity (49.96 & 24.98) against *Bacillus subtilis* and *Staphylococcus aureus* respectively, which proves the potential of extracts to inhibit growth of the test microorganisms, even at low concentration. Total activity values were calculated in ethanolic solvent extracts (6.245) followed by water extract (45.96) & chloroform extract (22.792) against *Klebseilla pneumoniae*.

The findings revealed that ethanolic extracts from *Habenaria plant*aginea contain phytochemicals wtich offer an enormous potential as bio control of these pathogens and source of antimicrobial agents of therapeutic importance.

TABLE 4: SCREENING TOTAL ACTIVITY PERFORMANCE OF DIFFERENT EXTRACTS OF *HABENARIA PLANTAGINEA* LINDL. AGAINST PATHOGENIC ORGANISMS

Test microorganisms	Water Extract	Ethanol Extract	Chloroform Extract
Bacillus subtilis	-	49.96	11.396
Staphylococcus aureus	-	24.98	22.792
Streptococcus pyogenes	-	9.992	45.584
Escherichia coli	38.3	3.568	5.698
Pseudomonas aeruginosa	-	2.498	1.424
Klebseilla pneumoniae	45.96	6.245	22.792

Phytochemical studies:

All the test phytochemicals as alkaloids, flavanoids, tannins and Phenolic compounds, Phytosterols, saponins, and glycosides were detected in different solvent extracts, but carbohydrates, Proteins and amino acids were absent in all three solvent extracts **(Table 5).** Many compounds belonging to these secondary metabolite groups have been reported to their antimicrobial activity. The results showed that the antibacterial activity of the plant were comparable with the results of previous researches using extracts of other orchid species of the genus *Bulbophyllum kaitense* [16] and *Cymbidium aloifolium* [17]

TABLE 5: PRELIMINARY PHYTOCHEMICAL SCREENING OF *HABENARIA PLANTAGINEA* LINDL

Phytochemical Test	Plant Extracts		
	Aqueous Extract	Ethanol Extract	Chloroform Extract
Alkaloids	+	+	+
Flavonoids	+	+	-
Steroids	+	-	-
Cardiac Glycosides	-	+	-
Terpenoids	-	+	+
Triterpenoids and Steroids	-	-	-
Phenol	-	+	-
Tannins	+	+	+
Sopanins	-	-	-
Phlobatannins	-	-	-
Reducing Sugar	+	+	+
Anthroquinones	-	-	-
Gum and Mucilages	-	-	-

CONCLUSION: The development of resistance in common human pathogens and emergence of new infectious pathogens intrinsically resistant to the currently available antibiotics demonstrates the urgent importance of identifying novel natural antimicrobial agents. There will be an increasing need for microbial inhibiting substances from plants. The traditional medicinal plants represent a reservoir of antimicrobial agent. Present study shows, *Habenaria plantaginea* extract shows the most potent antimicrobial activity against five standard species microorganisms. Therefore, *Habenaria plantaginea* extract and its compounds might be potentially valuable as a natural food preservative.

REFERENCES:

1. Medhi R.P. and Chakrabarti, S: Traditional knowledge of NE people on conservation of wild orchids. Indian J. Tradn. Knowl.2009; 8(1): 11-16.
2. Mabberley D.J: Mabberley's plant book: A portable dictionary of plants, their classification and uses. Cambridge University Press, Cambridge, 2008; 3:606.
3. Chowdhery H.J: Orchid Diversity in North – Eastern states of India. J. Orchid Soc. India. 2009; 23(1 & 2): 19 – 42
4. Singh A and Duggal Sanjiv: Medicinal Orchids – An Overview. Ethnobotanical Leaflets 2009; 13: 399 – 412.
5. Umamaheswari A, Nanu A and Shreevidya R: Evalution of antibacterial activity of *Boerhaavia diffusa* L. leaves. Int J Green Pharm 2010; 4:235-41.
6. Khan MA, Islam MT and Sadhu SK: Evaluation of phytochemical and antimicrobial properties of *Commelina diffusa* Burm. f. Int J Green Pharm 2011;11:235-41.
7. Nayak BS, Ellaiah P and Dinda SC: Antibacterial, antioxidant and antidiabetic activities of *Gmelina arborea* roxb fruit extracts. Int J Green Pharm 2012; 6:224-30.
8. Maridass M, Zahir Hussain MI, Raju G: Phytochemical Survey of Orchids in the Tirunelveli Hills of South India. Ethnobotanical Leaflets 2008; 12: 705 – 712.
9. Mohammad HM: Therapeutic Orchids: Traditional uses and recent advances – An Overview. Fitoterapia 2011; 82: 102 – 140.
10. Periyasamy Ashokkumar, Rajkumar and Mahalingam Kanimozhi: Phytochemical Screening and Antimicrobial Actiivity from Five Indian Medicinal Plants against Human Pathogens. Middle-East Journal of Scientific Research. 2010; 5(6): 477-482.
11. Ammara Hassan, salma Rahman, Farah Deepa and Shahid Mahmud: Antimicrobial activity of some plant extracts having hepatoprotective effects. Journal of Medicinal Plants Research 2009; 3(1): 20-23.
12. Eloff JN: Quantifying the bioactivity of the plant extracts during Screening and bioassay – guided fractionation. Phytomedicine 2004; 11(4): 370 – 371.
13. Harborne JB: Phytochemical methods: A guide to modern techniques of plant analysis. Champman and Hall, London. 1973; 279.
14. Raaman N: Phytochemical Techniques, New India Publishing Agency, New Delhi. 2006; 19.
15. Jain T, Sharma K: Assay of antibacterial activity of *Polyalthia longifolia* (Benth. & Hook.) Leaf extracts. J of Cell & Tissue Res. 2009; 9(2): 1817 – 20.
16. Kalaiyarasan A, Ahmed john S, Edward A: Antimicrobial Activity of Orchid. Root Eastern Peninsular Flora in India. Nature and Science 2012; 10(11):63 – 67.
17. Radhika B, Murthy J.V.V.S.N and Nirmala Grace D Nirmala Grace: Preliminary phytochemical analysis & antibacterial activity against clinical pathogens of medicinally important orchid *Cymbidium aloifolium* (l.) Sw. International Journal of Pharmaceutical Sciences and Research. 2013; 4(10): 3925-3931.

How to cite this article:
Keerthiga M and Anand SP: Study on Preliminary Phytochemical and Antibacterial Activity against Human Pathogens of an Endangered Orchid-*Habenaria Plantaginea* Lindl. Int J Pharm Sci Res 2015; 6(3): 1100-06.doi: 10.13040/IJPSR.0975-8232.6 (3).1100-06.