WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 2, 594-603. Research Article ISSN 2277– 7105

PHYTOCHEMICAL EVALUATION AND GC-MS ANALYSIS OF WHOLE PLANT EXTRACT OF *SEIDENFIA RHEEDII* (SW.) SZLACH. (ORCHIDACEAE)

Ruthisha P. K.*, Ratheesh Chandra P. and Dr. K. M. Khaleel

Department of PG Studies and Research in Botany, Sir Syed College, Taliparamba, Kannur, Kerala, India 670142.

Article Received on
05 Dec. 2016,

Revised on 25 Dec. 2016,
Accepted on 16 January 2017

DOI: 10.20959/wjpr20172-7653

***Corresponding Author**
Ruthisha P. K.
Department of PG Studies and Research in Botany, Sir Syed College, Taliparamba, Kannur, Kerala, India 670142.

ABSTRACT

The present study investigates the qualitative and quantitative evaluation of the major bioactive constituents of medicinally important plant *Seidenfia rheedii* (Sw.) Szlach. in its aqueous, ethanol, chloroform, petroleum ether extract of whole plant. Carbohydrates, proteins, aminoacids, Saponins, flavonoids,terpenoids, glycosides, tannins, alkaloids, phenols were present in the sample. Carbohydrate concentration is 82.6mg/g. Alkaloid concentration is 0 .02mg/g. Phenolics concentration is high in ethanol extract and is 109mgGAE/g. Flavonoid concentration is high in water extract and is 214mgRE/g. The percentage yield of plant extract is high in ethanol extract and is 4.02%. GC-MS analysis of chloroform extract of the plant shows 2-Chloro-5,5-dimethyl-1-phenyl-3-hexen-1-ol with Peak area 20.12 was the major compound. GC-MS analysis of Petroleum ether extract shows the major compounds Octadecane, 6-methyl- with peak area 26.52 and Oxalic acid, allyl hexyl ester with peak area 14.18.

KEYWORDS: Phytochemical evaluation, Orchidaceae, Seidenfia rheedii, GC-MS analysis.

INTRODUCTION

Rich heritage of biodiversity and traditional knowledge associated with biological resources are the treasure of India. Native people are exploiting a variety of herbs for effective curing of various ailments. The plant parts used, preparation, and administration of drugs vary from one place to another. A wide variety of chemical compounds were synthesized by plants. The chemical compounds are used to perform important biological functions. At least 12000 such compounds have been isolated so far. Chemical compounds in plants mediate their effect on

the human body through processes identical to those already well understood for the chemical compounds in conventional drugs. This enables herbal medicines to be as effective as conventional medicine.[16]

Orchidaceae is one of the largest and most diverse groups of angiosperms. It is cosmopolitan in distribution. Theophrastus, who is also called the father of Botany, gave the name 'orchid' to the group on the basis of resemblance of paired underground tubers of these plants to masculine anatomy. This resemblance was also responsible for the mistaken belief that the orchids posses aphrodisiac properties and eating of underground tubers might 'provoke Venus' and they may beget male children.[1]

Orchids are found in almost every part of the globe with the exception of poles. It is abundant in tropical and temperate regions. Orchidaceae is a monocotyledonous family and belongs to the order Asparagales. There are more than 850 genera and 25000 species.[4, 15] They occur in diverse habitat in our country with terrestrial epiphytic and saprophytic forms. The previous studies on orchids have shown them to be rich in certain biochemicals as carbohydrates, flavonoids, alkaloids, glycosides and other phytochemical contents which have great importance in medicinal field. The orchids were first put to medicinal use by the Chinese as herbal medicine.[8]

Seidenfia rheedii (Sw.) Szlach. is a member of the family orchidaceae. S.rheedii is a rare, terrestrial orchid perennial through its pseudobulb. The plant is well known for their medicinal value and commonly known as jeevak. This plant is also one of the members of ashtavarga in ayurvedic medicine and used for the preparation of chyavanaprasha. . Formely it is known as Malaxis rheedii. The plant have been claimed to possess medicinal properties in traditional system of medicine. The medicinal value of the plant was also reported in "charaka samhita" a classic ancient Indian medicinal treatise in Sanskrit.[14, 15]

MATERIALS AND METHODS

Plant collection: *Seidenfia rheedii* (Sw.) Szlach. collected from Kannur district, Kerala was taken for the study. The collected plants were washed thoroughly with tap water followed with distilled water for the removal of dust and soil particles. The plants cut into pieces were shade dried at room temperature for 15 days then coarsely powdered and used for extraction.

Preparation of extract: The powder (20 gm) was extracted with petroleum ether, chloroform and ethanol in a soxhlet apparatus (3840; Borosil Glass works Ltd., Mumbai, India) in increasing order of their polarity. Finally the dried powder was macerated using water with constant stirring for 48 hours using the orbital shaker (Rivotek; Riviera Glass Pvt.Ltd., Mumbai, India) and the extract was filtered. The extracts were concentrated, dried and stored at -20ºC in the deep freezer (RQV- 300; plus, REMI electro technik Ltd., Thane, Maharashtra, India) for further analysis.

Qualitative phytochemical screening

For preliminary phytochemical screening, plant extract of the whole plant was subjected to various qualitative chemical tests to determine the presence of various phyto-constituents like glycosides, tannins, phytosterols, proteins, amino acids, carbohydrates, flavonoids, phenolic compounds, oils and fats, and saponins.[12]

Quantitative analysis of phytochemicals

Estimation of Total carbohydrate[11]

100mg of the sample was weighed into a boiling tube and 5 ml of 2.5N Hydrochloric acid was added and hydrolyse the solution by keeping it in a boiling water bath for 3 hours. The mixture was then cool to room temperature. Then neutralise the mixture with sodium bi carbonate until the effervescence ceases. Make up the volume to 100ml and centrifuge. Then the supernatant was collected. Take 0.5ml and 1ml of the aliquots for analysis. The sample was kept in refrigerator for a few minutes. 4ml anthrone reagent was added to each test tube. The mixture is kept in a boiling water bath for 8 minutes. Cool the mixture rapidly and read the green to dark green colour at 630nm. Analysis was performed in triplicates. Glucose was used as standard.

Alkaloid determination[7]

5g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one- quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Estimation of total phenolic compounds[5]

100mg of the plant extract was dissolved in 100ml distilled water (respective solvent). Take 1 ml of the solution in a test tube. 0.5ml of 2N of Folin-ciocalteu reagent was added to the sample. Then 1.5ml of 20% Sodium carbonate solution was added. Then the volume was made up to 8ml.with distilled water. The mixture was allowed to stand for 2 hours followed by vigorous shaking. The absorbance was taken at 765nm. Analysis was performed in triplicates. Gallic acid was used as standard.

Estimation of total flavonoids[17]

0.5 Ml of sample was dissolved in 2ml of distilled water in a test tube.0.15ml of 5% sodium nitrite was added to the sample. Incubate for 6minutes at room temperature. Then 0.15ml of 10% Aluminium chloride was added. Stand for 6 minutes. 2ml of 4% sodium hydroxide solution was added. The mixture was then making up to 5 ml. with distilled water. Vortex well and incubate for 15 minutes at room temperature. Then the pink coloured solution was read at 510nm. Standard calibration curve was prepared with Rutin.

GC-MS Analysis of volatile components

GC-MS analysis of *S.rheedii* whole plant extracts were performed using Thermo Scientific Trace 1300 Gas chromatograph equipped with ISQ- QD Mass spectrometer with TG-5MS column (30m × 0.25mm ×0.25µm). Helium gas (99.999%) was used as the carrier gas at constant flow rate 1ml/minute and an injection volume of 1µl was employed. An injection port temperature of 280ºC and an ion-source temperature of 200ºC were set. The oven temperature was programmed from 60ºC for 3 minutes with an increase of 5ºC /minute to 240ºC with a hold time of 3 minutes. Then temperature was increased at a rate of 35ºC/min till 280ºC with a hold time of 5 minutes. Scan interval was programmed for 0.2 seconds with a mass range of 40-450 amu. Total GC running time was 45 minutes. The components in the extract were identified based on the mass spectra of NIST library data.

RESULTS AND DISCUSSION

The percentage yield of whole plant extracts of *Seidenfia rheedii* (Sw.) Szlach. in different solvents are presented in Table **1**. The maximum yield was obtained in ethanol extract and was 4.02% and least in petroleum ether extracts (1%). Water has an extractive yield of 3.44% and chloroform has an extractive yield of 2.92%. Preliminary phytochemical screening of whole plant extracts of Seidenfia rheedii (Sw.) Szlach. were presented in Table **2**.

Qualitative phytochemical analysis of petroleum ether extract shows positive results For Carbohydrate, alkaloid, tannin, flavonoids, phenolic compounds. Chloroform extract shows positive results for almost all tested phytochemicals except saponin, glycosides and cardiac glycosides. Ethanol extract shows positive results for maximum number of phytochemicals tested. In ethanol extract negative result for saponin. Water extract shows positive results for phenolic compounds, flavonoids, saponins and fixed oils and fats.

The quantitative analysis of phytochemicals showed that the total alkaloid content in the 1g plant sample is 0.02 mg. The total carbohydrate content in 1g plant sample is 82.6mg. The total flavonoid and phenolic content in chloroform, petroleum ether, ethanol, and water were presented in Table **3.** Phenolic compounds obtained maximum in ethanol extract (109mgGAE/g) and least in pet ether (8.5mgGAE/g). In water and chloroform extracts phenolic compounds were 17.6mgGAE/g and 11.5mgGAE/g respectively. The flavonoid content is highest in water extract (214 mgRE/g) and least in chloroform extract (124 mgRE/g). The pet ether extract has a flavonoid content of 130mgRE/g and ethanol extract has 178mgRE/g.

GC-MS chromatogram of chloroform extract of the plant revealed 24 peaks indicating twenty four phytochemical constituents. The major compounds identified with their retention time, molecular formula, molecular weight and peak area are presented in Figure **1**,Table **4.** Among them 2-Chloro-5,5-dimethyl-1-phenyl-3-hexen-1-ol (peak area 20.12) and 2-[3-Hydroxy-4-methoxyphenyl]-semicarbazide (peak area 19.16) were major compounds. Twenty seven compounds were identified in the petroleum ether extract of the plant. Among them Tetradecane, 1-iodo- (peak area 10.52), Octadecane, 6-methyl- (26.52), Oxalic acid, allyl hexyl ester (14.18) were the major compounds. Figure **2**, Table **5** shows the GC-MS chromatogram and components of petroleum ether extract.

Renjini Haridas et al identified 42 compounds in methanol extract of Malaxis rheedei and they concluded that the compounds identified have anticarcinogenic, antibiabetic, antimicrobial and antioxidant properties.[13] The compound 2-Piperidinone, N-[4-bromo- n-butyl]- is an alkaloid and has Antimicrobial, Antioxidant, Anti-inflammatory functions.[10] Hexadecanoic acid, ethyl ester is reported to be a property of Antioxidant, Hypocholesterolemic, Nematicide, Pesticide activity[3] (E)-9-Octadeconoic acid ethyl ester is reported to have Anti-inflammatory, Anticancer, hypocholesterolemic activity.[9] Literature shows other compounds also have properties in medical field.

Table 1: Extractive yield of whole plant extracts of S.rheedii in different solvents

Sl.No	Solvent	Yield (%)
1	Petroleum Ether	1
2	Chloroform	2.92
3	Ethanol	**4.02**
4	Water	3.44

Table 2: phytochemical screening tests for various phytochemicals

Sl.No	Phytochemicals	Pet.ether	Chloroform	Ethanol	Water
1	Carbohydrate	+	+	+	+
2	Protein	-	+	+	-
3	Amino acid	-	+	+	-
4	Alkaloid	+	+	+	-
5	Flavonoid	+	+	+	+
6	Tannin	+	+	+	-
7	Terpenoid	-	+	+	-
8	Saponin	-	-	-	+
9	Phenol	+	+	+	+
10	Glycosides	-	-	+	-
11	Cardiac Glycosides	-	-	+	-
12	Fixed oils and Fats	-	+	+	+

+indicates presence of phytochemicals and – indicates absence of phytochemicals.

Table 3: Total phenolic and flavonoid content in the whole plant extract of S.rheedii

Sl.no	Solvent	Phenolics(mgGAE/g)	Flavonoids(mgRE/g)
1	Petroleum Ether	8.5	130
2	Chloroform	11.5	124
3	Ethanol	**109**	178
4	Water	17.6	**214**

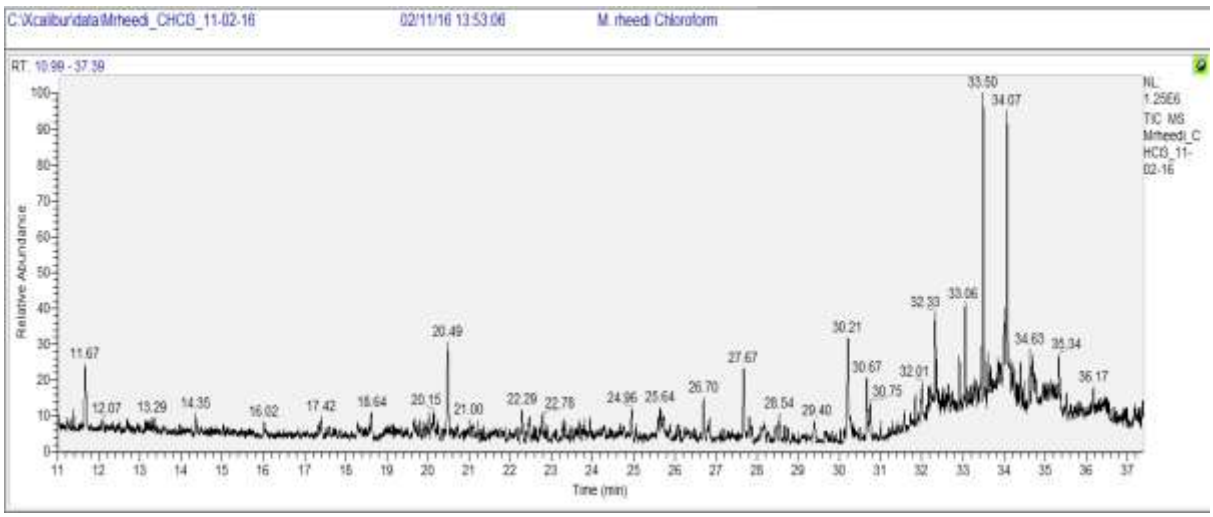

Figure 1: GC-MS chromatogram of chloroform extract of S.rheedii(Sw.) Szlach.

Table 4: Components identified in the chloroform extract of Seidenfia rheedii (Sw.) Szlach. Whole plant sample

RT	Compound name	Peak area	Mole. Formula	Mole.wt
11.67	2-Buten-1-ol, 2-methyl-	4.09	C5H10O	86
20.49	phenol, 4-(1,1-dimethylethyl)-2(1,1dimethylpropyl)-	8.26	C15H24O	206
22.29	4-Dodecene, (E)-	0.46	C12H24	168
24.96	Tetradecane, 1-iodo-	0.20	C14H29I	324
26.70	6-Tridecene	2.13	C13H26	182
27.67	1-Hexadecyne	5.68	C6H30	222
30.21	n-Hexadecanoic acid	8.37	C16H32O2	256
30.67	Silane, trichlorodocosyl-	2.71	C22H45Cl3Si	442
30.75	3,6-Octadecadiynoic acid, methyl ester	0.44	C19H30O2	290
31.85	2,4,6,8-Tetramethyl -1 -undecane	0.30	C15H30	210
32.01	4-Cyclopropylcarbonyloxytridecane	1.14	C17H32O2	268
32.33	10-Heptadecen-8-ynoic acid, methyl ester, (E)-	6.41	C18H30O2	278
32.91	1-iodo-2-methylundecane	1.60	C12H25I	296
33.06	2H-Tetrazole, 5-(4-methoxybenzyl)-	4.71	C9H10N4O	190
33.50	**2-Chloro-5,5-dimethyl-1-phenyl-3-hexen-1-ol**	**20.12**	**C14H17ClO**	**236**
33.61	2-phenyl-2,5-cyclohexadiene-1,4-dione,4-oxime	2.29	C12H9NO2	199
33.68	2-chloro-5,5-dimethyl-1-phenyl-3-hexen-1-ol	0.24	C14H17ClO	236
34.01	2-piperidinone,N-[4-bromo-n-butyl]-	3.86	C9H16BrNO	233
34.07	**2-[3-Hydroxy-4-methoxyphenyl]-semicarbazide**	**19.16**	**C8H11N3O3**	**197**
34.15	2-Dodecen-1-yl(-)succinic anhydride	0.18	C16H26O3	266
34.23	7-decen-1-ol acetate	0.23	C13H22O2	210
34.40	Formic acid,neopentyl ester	1.44	C6H12O2	116
34.63	Oxalic acid, allyl nonyl ester	2.03	C14H24O4	256
35.34	Octane, 2,7-dimethyl-	2.52	C10H22	142

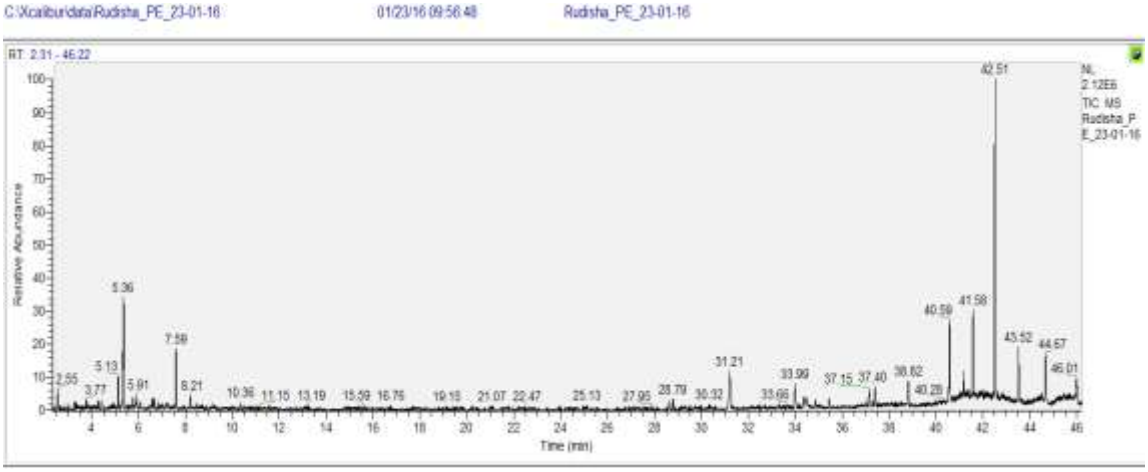

Figure 2: GC-MS chromatogram of pet. Ether extract of Seidenfia rheedii (Sw.) Szlach.

Table 5: Components identified in the pet ether extract of Seidenfia rheedii (Sw.) Szlach. whole plant sample

RT	Compound name	Peak Area	Mol.For.	Mol.wt
2.13	Ethanamine,2-methoxy	1.25	C3H9NO	75
2.27	Oxetane,2,4-dimethyl-trans-	0.09	C5H10O	86
2.55	Oxirane, (propoxymethyl)-	1.24	C6H12O2	116
3.28	Isopropylsulfonyl chloride	0.09	C3H7ClO2S	142
5.13	3,6-octadecadiynoic acid,methyl ester	2.18	C19H30O2	290
5.36	**Oxalic acid, allyl hexyl ester**	**14.18**	**C11H18O4**	**214**
5.75	Acetic Anhydride	0.54	C4H6O3	102
5.91	Nitroxide,bis(1,1-dimethylethyl)	0.58	C8H18NO	144
6.59	Pentane,2-bromo-	0.32	C5H11Br	150
7.59	Hydroperoxide, pentyl	5.15	C5H12O2	104
8.21	Amyl nitrite	0.82	C5H11NO2	117
28.64	Hydroxylamine,O-(2-methylpropyl)-	0.12	C4H11NO	89
28.79	Oxalic acid,ethyl propyl ester	0.38	C7H12O4	160
31.21	n-Butyl nitrite	5.04	C4H9NO2	103
33.99	2-Piperidinone, N-[4-bromo-n-butyl]-	2.37	C9H16BrNO	233
34.38	Hexanoic acid, 6-bromo-	0.09	C6H11BrO2	194
37.15	Octane, 2,7-dimethyl-	1.55	C10H22	142
37.40	Phenylacetic acid, 4-hydrazino-, ethyl ester	1.59	C10H14N2O2	194
38.82	1-Iodo-2-methylnonane	2.42	C10H21I	268
40.59	**Tetradecane, 1-iodo-**	**10.52**	**C14H29I**	**324**
41.16	Bis-(3,5,5-trimethylhexyl) phthalate	1.67	C26H42O4	418
41.58	Oxalic acid, allyl nonyl ester	6.69	C14H24O4	256
41.90	9-Octadecenoic acid(Z)-,phenylmethyl ester	0.05	C25H40O2	372
42.51	**Octadecane, 6-methyl-**	**26.52**	**C19H40**	**268**
43.52	1-Iodo-2-methylundecane	4.93	C12H25I	296
44.67	Heptadecane, 2,6-dimethyl-	5.31	C19H40	268
46.01	Decane, 2,9-dimethyl-	2.94	C12H26	170

CONCLUSION

The medicinal value of plants lies in some chemical substances that have a definite physiological action on the human body. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. The present study identifies that the selected plant S.rheedii (Sw.) Szlach**.** contains various bioactive constituents and their presence made the plant highly medicinal. The major compounds identified through GC_MS analysis have specific application in medicine. So the study revealed the presence of medicinally important constituents in the studied species. Based on the reported phyto constituents some more pharmacological as well as clinical

studies may be carried out for producing a proper validation of the medicinal orchid S.rheedii (Sw.) Szlach**.**

ACKNOWLEDGEMENT

We are thankful to the faculties and Head of the department of PG studies and research centre in botany Sir Syed College, Taliparamba, Kannur, Kerala for providing necessary laboratory facilities, and also thankful to the Kannur University for the financial assistance to carry out this research. We acknowledge the research scholars of the Sir Syed college, Taliparamba for giving us support.

REFERENCES

1. Amritpal Singh, Sanjiv Duggal.*(2009)* Medicinal Orchids - An Overview Ethnobotanical Leaflets *13: 399-412.*
2. Bulpitt J, Li Y, Bulpitt PF and J Wang, (2007). The Use Of Orchids In Chinese Medicine. JRSM, 100(12): 558-563.
3. Farina Mujeeb, Preeti Bajpai and Neelam Pathak. (2014), Phytochemical Evaluation, Antimicrobial Activity, and Determination of Bioactive Components from Leaves of Aegle marmelos. Bio Med Research International; 1-11.
4. Gutierrez, R M P (2010). Orchids: A review of uses in traditional medicine, its phytochemistry and pharmacology. Journal of Medicinal Plant Research 4(8): 592-638.
5. Hagerman A, Muller I and Makkar H. 2000. Quantification of tannins in tree foliage. A laboratory manual, Vienna: FAO/IAEA.4-7.
6. Hanna B Margonska, Dariusz L Szlachetko;(2001), Materials to the revision of the genus Seidenfia(orhidaceae, malaxidinae),with a description of a new species. Polish botanicl journal, 46(1): 47-62.
7. Harborne J. 1973. Phytochemical methods. Chapman and Hill, Ltd London: 49-188.
8. Jeewan Singh Jalal, Pankaj Kumar and Y.P.S Pangtey, (2008), Ethnomedicinal Orchids of Uttarakhand, Western Himalaya. Ethnobotanical Leaflets, 12: 1227-30.
9. Manonmani R and Catharin Sara S. (2015), GC-MS Analysis of Bioactive Components of an Important Medicinal Fern Actiniopteris radiata (Swartz) Link. World Journal of Pharmaceutical Research, 4(4): 1860-69.

10. Meenakshi VK, Gomathy S, Senthamarai S, Paripooranaselvi M and Chamundeswari KP.(2012) GC-MS Determination of the Bioactive Components of Microcosmus exasperatus Heller, 1878. J. Curr. Chem. Pharm. Sc; 2(4): 271-276.
11. Morris. D. L. (1948), Quantitative determination of carbohydrates with Dreywoods Anthrone reagent, Science, 107: 254-255.
12. Raaman, N., (2006): *Phytochemical Techniques*. New India Publishing Agency, New Delhi India. pp: 19-24.
13. Renjini Haridas, Manorama S, Sindhu S, Binu Thomas.,(2016), International journal of Experimental Pharmacology; 6(1): 11-15.
14. Sharma Pushpa, Mahajan Nipun, Garg Pankaj. Singh Gurkirpal, Dadhwal Sumit, Sharma Sakshi; 2011, Malaxis acuminate a review, IJRAP; 2(2): 422-425.
15. Stewart, J & M., Griffith (1995). Manual of Orchids.Timber Press Portland, Oregon.
16. Vasishta P C (1992). Taxonomy of Angiosperms. R Chand & Co publishers- New Delhi-2.
17. Zhishen J, Mengecheng Taxonomy, Jianming W. 1999. The determination of flavonoid content on mulberry and their scavenging effects on superoxide radical. Food Chem. 64: 555-559.