Phytochemical, Physico-chemical & Spectroscopic Characteristics of Ethanolic Extract of Leaf, Stem and Flower bud of *Hibiscus hispidissimus* Griffith

Thamizh Selvam N[1], Surabhi KR[2], Sanal Gopi CG[3], Neethu KR[4],
Vasanthakumar KG[5], Swamy GK[6], Acharya MV[7]

[1]Research Officer-Biochemistry,
[2]Senior Research Fellow,
[3,4] Laboratory Technicians -Biochemistry,
[5]Assistant Director (Chemistry),
[6]Assistant Director (Ayurveda),
[7]Director.
National Research Institute for Panchakarma,
(CCRAS, Ministry of AYUSH, Government of India, New Delhi.)
Cheruthruruthy, Thrissur, Kerala-679 531.
Email: nthamizhselvam@gmail.com

Abstract

The plant *Hibiscus hispidissimus* belongs to the family Malvaceae (Mallow family). The plant has wide range of medicinal uses. Considering the ethno medicinal value of *Hibiscus hispidissimus*, the present work has been taken up to document the physico-chemical composition, phytochemical details and spectrophotometric characteristics of the plant. The work has been carried out on ethanolic extract of leaf, stem and flower bud of *H. hispidissimus*. Phytochemical analysis showed the presence of saponis, tannins, glycosides, diterpenes and quinones. Spectroscopic characteristics were analyzed and found to have wide range of compounds including steroids, alkaloids, pigments like chlorophyll a and b, phenolic compounds mainly gallic acid, flavanoids like anthocyanins, flavanols, flvanones and isoflavones.

Key words: *Hibiscus hispidissimus*, phyto constituents, spectral studies, steroids, flavanoids

Introduction

The rise in population, inadequate supply of drugs, side effects of allopathic medicines, resistance to drugs and high cost treatments have made human beings to use plant as a source of medicine for a variety of diseases. Green plants which are usually the reservoir of many biochemical products can be extracted and used for various scientific experiments thus leading to the development of plant based non-toxic, non-reactive product (1). The method of using medicinal plants can be a mixture of many active components or a single active component.

The plant ***Hibiscus hispidissimus*** belongs to the family Malvaceae (Mallow family). The synonyms for the plant are *Hibiscus furcatus* DC. Non Wild and *Hibiscus aculeatus* Roxb. Non Walter. The common name of this plant is Wild hibiscus, Comfort root, Big thicket Hibiscus. The plant is a large climber having reddish stems that are covered with hooked prickles (2-6). The leaves are alternately arranged, 6-8cm, palmately 3-5 lobed, hairy and heart shaped at the base. Leaf margins are toothed, lobes are long pointed, leaf stalks 5-10cm long and prickly. Stipules are lane shaped. Yellow flowers arise singly from leaf axils which are carried on 3-5cm long prickly stalks. 8-12 bracts below the flowers with leafy appendages. Seed capsules are 1cm long, ovoid, pointed, enclosed in enlarged sepal cup. This plant is commonly found in the evergreen forests of Western Ghats. The flowering period is November-January. It is distributed throughout India. The method of propagation of the plant is by seeds (7).

The sour leaves are used as food ingredient and used in the preparation of South Indian cuisine. The leaves are the source of an ayurvedic drug Sathambasthi. This drug is one of the five acid drugs (pancamla). It is a major constituent of pancamlatailam, an oil preparation for body-anointing. The leaves are also an ingredient in Ayurvedic drug Annabhedi sinduram and Abhram. The leaves are anti inflammatory and anthelmintic (8). Tribal healers of Kerala region use this plant to treat Liver diseases. It is said to improve digestion and have anthelmintic action. The Leaves of the plant are acidic and eaten after cooking. The juice of the leaves are mixed with honey and used in treatment of eye diseases (9). In summer the roots of the plant infused in water is used as a cooling drink. Decoction of the root bark is used as remedy for poisons, swellings

and cleansing the kidneys (10). Considering the ethno medicinal value of *Hibiscus hispidissimus*, the present work has been taken up to document the phytochemial profile, physico-chemical composition, and spectrophotometric characteristics of the plant.

Materials and Methods

Plant collection and Authentication:

The plant materials were collected from Western ghat region of Kerala. The plant was authenticated by the Taxonomist, Department of Botany, St. Thomas College, Thrissur. The Specimen voucher is maintained in the Institute.

Chemicals and Reagents:

Chemicals and Regents of AR grade purchased from Spectrum India Ltd, Merck India Ltd, Nice India Ltd, were used.

Extraction:

The plant extraction was carried out by soxhlet extraction method using ethanol as solvent. The shade dried plant materials such as leaf, stem and flower bud were individually extracted.

Phytochemical Analysis:

The phytochemical analysis of the study material was carried out as per the standard protocols. It comprised of various tests including Salkowski test, Dragendorff's test, Keller Killani test and Ellagic acid test protocols (11-17).

Physico-chemical Analysis:

Physico-chemical analysis such as moisture content, total ash, water soluble ash, acid insoluble ash, water soluble extractive and alcohol soluble extractive were carried out as per the standard protocol mentioned below (18-22)

Loss on drying:

About 4g drug after accurately weighing was taken in a tared evaporating dish. The sample was placed in Hot air oven at 105^0C for 5hrs and weighed. The process was continued i.e. the drying and weighing at one hour interval until difference between two successive weighing correspond to not more than 0.25%. The constant weight is noted when two consecutive weighing after drying for 30min and cooling for 30min in a desiccator shown not more than 0.01g difference.

Total ash:

2-3g of the air dried drug is incinerated in a silica crucible at temperature not exceeding 450^0C until free from carbon, cool and weigh. The % of ash was calculated with reference to air dried drug.

Water soluble ash:

The ash obtained from Total ash procedure was boiled with 25ml water and insoluble matter was collected in a Gooch crucible on ashless filter paper. Then, it was washed with hot water and ignited for 15min at a temperature not exceeding 450^0C. Subtract weight of insoluble matter from weight of ash, the difference in weight represents Water soluble ash with reference to air dried drug.

Acid Insoluble ash:

The ash was boiled for 5min with 25ml dil. HCl and mix with glass rod. Filtered with Whatman paper No.1. to remove the acid and then add distilled water. For checking the acid content in the filterate, 1 drop of methyl orange was added. Appearance of yellow colour confirmed that there is no acid content. Then, Take the filter paper and fold it. Put in the crucible and incinerate up to 50^0C until filter paper burns and 100^0C up to white colour.

Water soluble extractive:

Accurately weighed sample (1g) taken in a Iodine flask and 20ml Chloroform-water mixture (0.25:100) added to the flask and kept closed for 24hrs. The next day solution was filtered and from the filtrate about 5ml was transferred to previously weighed evaporating dish and evaporated to dryness. After drying weight was noted and calculated the extractive value.

Alcohol soluble extractive:

Macerate 5g (1g) of air dried drug coarsely powdered with 100ml(20ml) of alcohol of specified strength in a closed flask for 24hrs, shaking frequently during six hours and allowing to stand for eighteen hours. Filter rapidly taking precautions against loss of solvent, evaporate 25ml of filtrate to dryness in a tared flat bottom shallow dish and dry at 105^0C to constant weight and weigh. Calculate % of Alcohol soluble extractive with reference to air dried drug.

Spectrophotometric Analysis:

Spectrophotometric characteristics were analyzed for understanding the basic chemical profiling of *H. hispidissimus* and to compare the proposition of major compounds among different parts of the plant. The ethanolic extract of different parts such as leaf, stem and flower bud of *H. hispidissmus* was taken at three different concentrations i.e. 0.5%, 0.25% and 0.125%. The extract was dissolved in ethanol for spectroscopic analysis, and pure ethanol was used as blank. The spectrum characteristics were measured after standardizing the procedures and analysis was confirmed by carrying out triplicate analysis at 200-800 nm (23-26).

Results

The phytochemical analysis showed the presence of Tannins, Saponins, Diterpenes, Quinones, Flavanoids and Phytosterols. The composition of Phytoconstituents in different parts of the plant has been mentioned in Table 1. Physico chemical parameters including Moisture content, Total ash content, Acid insoluble ash, Water soluble ash, Alcohol soluble extractive and Water soluble extractive were carried out. *H. hispidissimus* Leaf showed highest total ash (7.45gm %), followed by stem and flower bud. *H. hispidissimus* stem showed highest acid insoluble ash (0.97 gm %) followed by leaf and flower bud, mentioned in Table 2. Spectrophotometric analysis showed various prominent peaks in the spectrum of 200-800 nm representing the presence of compounds like Alkaloids, triterpenoids and steroids, flavanoids comprising anthocyanins, flavonols, flavanones, isoflavanones, glycosides, gallic acid (phenolic compounds) (Table. 3-5 and Figure. 1-3).

Table 1. Phytochemical characteristics of ethanolic extract of *H. hispidissimus*

S. No	Phytochemical test details	Leaf	Stem	Flower bud
1	Tannins (Lead sub-acetate test)	+++	++	+
2	Saponins (Foam test)	+++	-	-
3	Alkaloids Dragendorff's test Hager's test	+ +	- -	- -
4	Terpenoids (Salkowsky's test)	-	++	+
5	Cardiac glycosides (Keller-killani)	++	+++	+
6	Flavanoids (Alkaline reagent test)	-	++	+
7	Glycosides (Fehling's test)	++	+++	+
8	Anthraquinones (Modified Borntrager's test)	-	-	+
9	Coumarins	+	-	-
10	Reducing compounds (Benedict's test)	+++	+++	-
11	Phlobatannins	-	-	-
12	carotenoids	-	-	-
13	Lignins	-	-	-
14	Resins	-	-	-
15	Diterpenes	+++	+++	+
16	Quinones	+	-	-
17	Phytosterols and sterols	-	++	+
18	Triterpenoids	-	++	+

+++ Highly Present ++ Medium + Trace - Not present

Table 2. The physicochemical analysis of Leaf, Stem and Flower Bud of *H. hispidissimus*

S. No.	Physico-chemical parameters	Leaf (gm %)	Stem (gm %)	Flower bud (gm %)
1	Moisture content	11.630	11.875	11.515
2	Total ash	7.450	5.495	4.690
3	Acid Insoluble ash	0.440	0.970	0.070
4	Water soluble ash	3.560	3.600	2.510
5	Alcohol soluble extractive	20.750	-	-
6	Water soluble extractive	34.910	-	-

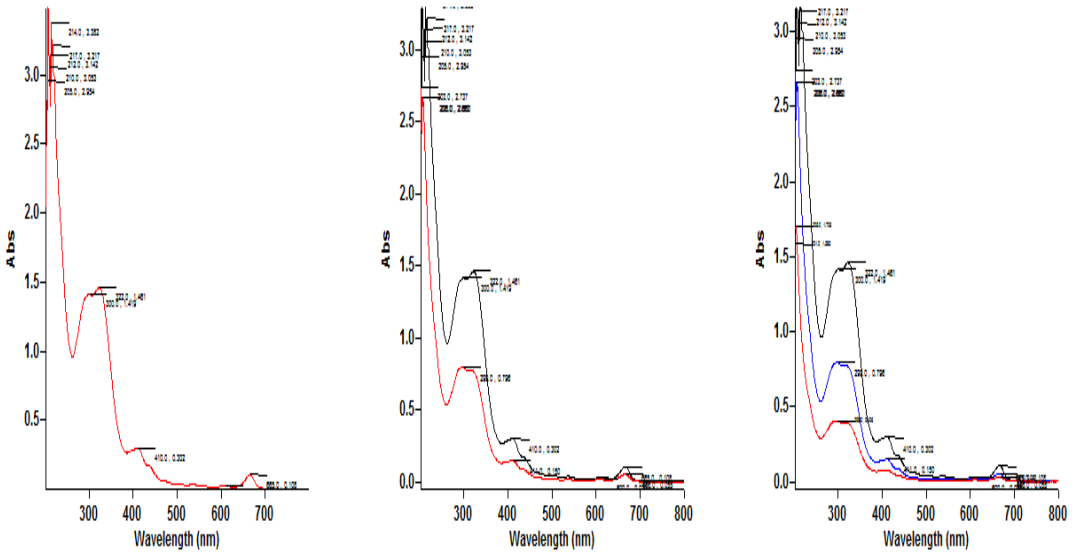

Figure 1. Spectroscopic analysis of ethanolic extract of *H. hispidissimus* Stem at different concentrations.

Table 3. Comparative data of spectroscopic analysis of ethanolic extract of *H. hispidissimus* Stem at different concentrations.

Sample Name: *H. hispidissimus* Stem Ethanol **0.5%** =200-800 nm		Sample Name: *H. hispidissimus* Stem Ethanol **0.25%** =200-800 nm		Sample Name: *H. hispidissimus* Stem Ethanol **0.125%** =200-800 nm	
Wavelength	Absorbance	Wavelength	Absorbance	Wavelength	Absorbance
665.0	0.108	666.0	0.055	667.0	0.030
609.0	0.029	411.0	0.150	298.0	0.406
410.0	0.302	299.0	0.796	205.0	1.706
322.0	1.461	208.0	2.660	201.0	1.585
217.0	3.217	206.0	2.662	-	-

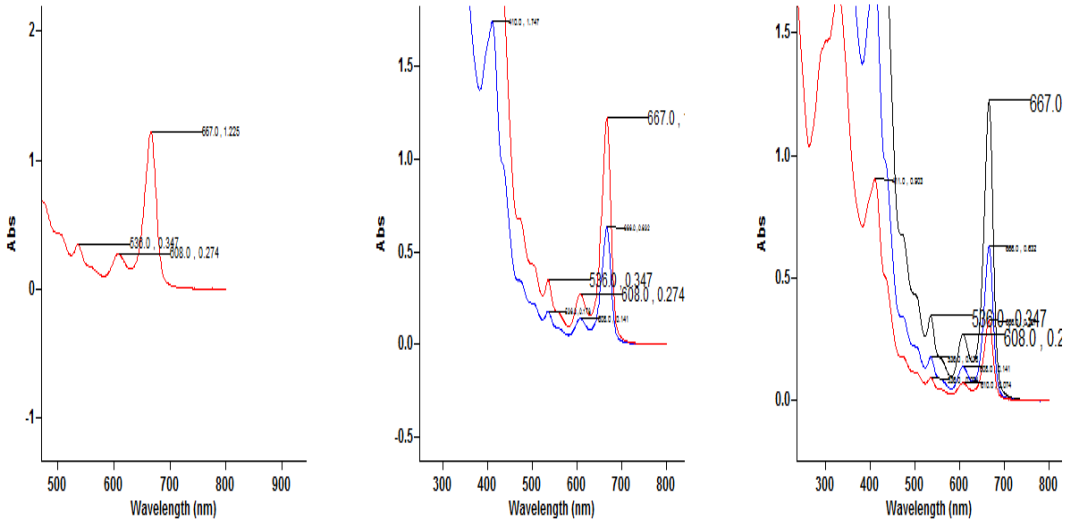

Figure 2. Spectroscopic analysis of ethanolic extract of *H. hispidissimus* Leaf at different concentrations.

Table 4. Comparative data of spectroscopic analysis of ethanolic extract of *H. hispidissimus* Leaf at different concentrations.

Sample Name: *H. hispidissimus* Leaf Ethanol **0.5%** =200-800 nm		Sample Name: *H. hispidissimus* Leaf Ethanol **0.25%** =200-800 nm		Sample Name: *H. hispidissimus* Leaf Ethanol **0.125%** =200-800 nm	
Wavelength	Absorbance	Wavelength	Absorbance	Wavelength	Absorbance
667.0	1.225	666.0	0.632	666.0	0.327
608.0	0.274	608.0	0.141	610.0	0.074
536.0	0.347	536.0	0.178	536.0	0.094
416.0	3.428	410.0	1.747	411.0	0.903
370.0	2.988	348.0	2.544	330.0	1.671

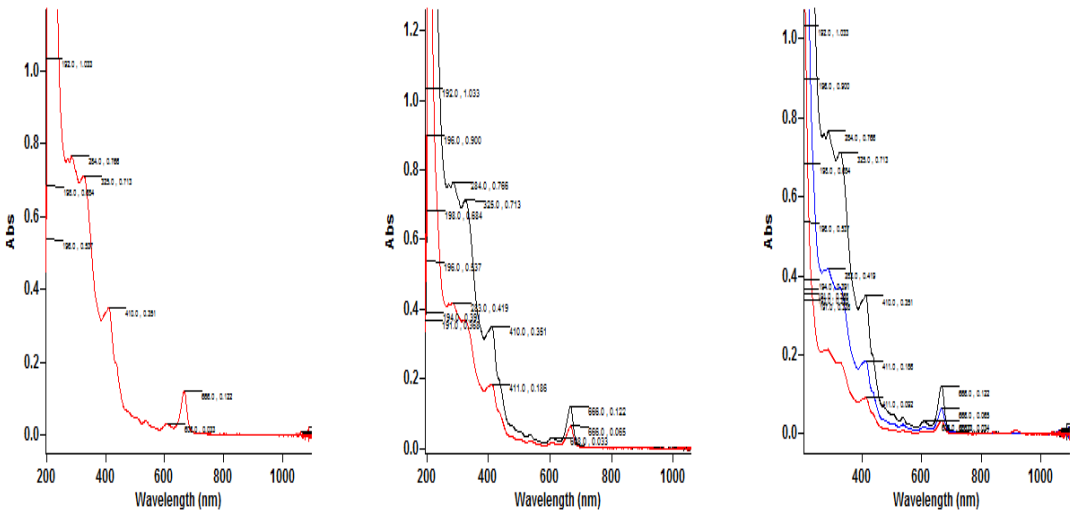

Figure 3. Spectroscopic analysis of ethanolic extract of *H. hispidissimus* Flower bud at different concentrations.

Table 5. Comparative data of spectroscopic analysis of ethanolic extract of *H. hispidissimus* Flower bud at different concentrations.

Sample Name: *H. hispidissimus* Flower Bud Ethanol **0.5%** =200-800 nm		Sample Name: *H. hispidissimus* Flower Bud Ethanol **0.25%** =200-800 nm		Sample Name: *H. hispidissimus* Flower Bud Ethanol **0.125%** =200-800 nm	
Wavelength	Absorbance	Wavelength	Absorbance	Wavelength	Absorbance
666.0	0.122	666.0	0.065	666.0	0.034
608.0	0.033	411.0	0.186	411.0	0.092
410.0	0.351	283.0	0.419	203.0	1.251
325.0	0.713	205.0	2.206	197.0	0.338
284.0	0.766	196.0	0.900	193.0	0.355

Discussion and Conclusion

The plant *Hibiscus hispidissimus* is widely used by the traditional healers for various medical ailments. The present study has been taken up to evaluate the phytochemical, physico-chemical and spectroscopic analysis of *H. hispidissimus*. The phytochemical studies revealed the presence of phenolic compounds, steroids, alkaloids, saponins and glycosides. The semi quantitative test showed the levels of phyto-constitutents is varying among plant parts i.e. stem, leaf and flower bud. The physico-chemical proximate composition studies exhibited the composition of water soluble and acid soluble ash contents and extraction percentage.

Spectroscopic analysis showed the highly presence of photosynthetic pigments like chlorophylls, phycobilins and cytochromes, saponins, terpenoids, alakaloids and glycosides in the ethanolic extract of leaf. The compounds such as flavanoids, tannins, triterpenoids and pigments like anthocyanins and cytochromes were highly present in stem part of *H. hispidissimus*. The ethanolic extract of flower bud of *H. hispidissmus* exhibited high composition of anthroquinones, phycobilins, flavanoids and higher class of phenolic compounds. These reports were in close to the report presented by the Thamizhselvam *et al.,* (2015) and Krishnakumar *et al.*,(2008) (27,28). The further research on isolation and characterization of individual compounds from different extracts may contribute value addition to its ethnomedicinal value of the plant.

Acknowledgement

Authors are thankful to the Director General, CCRAS, New Delhi for his enormous support and encouragement. The authors are also expressing their gratitude to staff of NRIP, Cheruthuruthy for their extended help and co-operation.

Conflict of Interest: Nil

References

[1] A Textbook of Pharmacognosy and Phytochemistry, Kumar GS and Jayaveera KN. S. Chand & Company Pvt. Ltd.2014
[2] https://www.flickr.com/photos/dinesh_valke/with/8290396443
[3] Flowering Plants of Kerala, Publisher-KFRI, Peechi Contents: 5094 plants, 880 exotics, 100 more multiple keys for finding plants, 20,000 more plant images, 6,800 local/trade/common names.2011
[4] Indian Medicinal Plants, a compendium of 500 species, Volume 3 , Editors : Warrier PK, Nambiar VPK and Ramankutty C, Orient Longman Publications Pg 146-149
[5] Bindu TK, Saidutty A, Alkhathilan HZ, Shafi PM. Chemical investigation on the leaves of Hibiscus furcatus. Asian Journal chem.1997; 9: 734
[6] Barve VH, Hiremath SN, Pattan SR and Pal SC. Phytochemical and Pharmacological Evaluation of Hibiscus mutabilis leaves. Journal of chemical and pharmaceutical Research. 2010; 2(1): 300-309
[7] www.toxicologycentre.com/English/plants/Botanical/njaranpuli.html
[8] Malvaceae of Southern Peninsular India. A Taxonomic Monograph. Sivarajan VV, Pradeep AK. Daya publishing House, Delhi.1996; 107-108
[9] Salem MZM, Perez JO, Salem AZM. Studies on biological activities and Phytochemical composition of Hibiscus species- A Review, Life Science journal 2014; 11(5): 1-8
[10] Herbal plants. Brief explanation about herbal plants and their medicinal uses, Senthil Kumar, Oct-2014
[11] Reddy MN and Mishra GJ. Preliminary Phytochemical screening and Antibacterial analysis of the leaf extracts of Launaea procumbens Roxb. International Journal of Phytopharmacology. 2012; 3(2): 147-151.
[12] Kavit M, Patel BN and Jain BK. Phytochemical analysis of leaf extract of Phyllanthus fraternus. Research Journal of Recent Sciences. 2013;Vol 2: 12-15
[13] Vinoth B and Manivasagaperumal R. Phytochemical analysis and antibacterial activity of Cardiospermum halicacabum Linn. International Journal of Current Science and Technology.2013; Vol2: 009-012
[14] Phytochemical methods, A guide to modern techniques of plant analysis. Harborne JB, Springer publications, 2015. London.
[15] Medicinal plants & Traditional medicine in Africa; Sofowara A, John Wiley & Sons; 1982
[16] Moses A, Bernard S, Oriko OR, Edward A. Preliminary Qualitative Analysis of Phytochemical Constituents of the Endemic Aloe tororoana Raynolds in Tororo, Eastern Uganda. Global Advanced Research Journal of Agricultural Science. 2014; Vol. 3(3) pp: 096-099

[17] Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and Extraction: A Review. Internationale Pharmaceutica Sciencia. Jan-Mar 2011; Vol I: 8-106.
[18] Suhas SN, Vilas DV. Pharmacognostic evaluation of dried leaf powder of Cardiospermum halicacabum Linn and dried seed hull powder of Hydnocarpus petandra (Buch.ham) Oken. International Journal of Res Ayurveda Pharm. 2014; 5(4): 502-507
[19] Pratap GP, Sudarsanam G, Jyothi B, Prasad GP and Rasheed NMA. Pharmacognostical and analytical studies of leaves of Cardiospermum canescens wall. International Journal of phytomedicine. 2012;4: 386-394
[20] Rao KNV, Geetha K, Alagar RM, Banji D. Quality control study and standardization of Hibiscus rosa-sinensis flowers and leaves as per WHO guidelines. Journal of Pharmacognosy and Phytochemistry. 2014; 3(4): 29-37
[21] Soni D, Gupta A, Solanki R, Jana GK. Pharmacognostical, phytochemical and physiochemical findings over the root extract of Hibiscus rosa-sinesis [Malvacae]. J. Nat. Prod. Plant Resour. 2011, 1 (4): 73-79
[22] Protocol for testing Ayurvedic, Siddha and Unani medicines, Dr. D.R. Lohar. Govt of India, Dept of Ayush, Pharmacopoeial Laboratory of Indian Medicines, 2007
[23] Anand T, Gokulakrishnan K. Phytochemical Analysis of Hybanthus enneaspermus using UV, FTIR and GC- MS. IOSR Journal of Pharmacy May-June, 2012;Vol. 2, Issue 3: PP:520-524
[24] Santhanakrishnan D, Shankar SN and Chandrasekaran B. Studies on the phytochemistry, spectroscopic characterization and antibacterial efficacy of salicornia brachiata. International journal of pharmacy and pharmaceutical sciences. 2014; Vol 6:430-432
[25] Janakiraman N, Sathish S, Johnson M. UV-Vis and FTIR Spectroscopic studies on Peristrophe bicalyculata (Retz) nees. Asian Journal of Pharmaceutical and Clinical Research. 2011; Vol 4, Issue 4: 125-129
[26] Sathish S , Janakiraman N , Johnson M. Phytochemical Analysis of Vitex altissima L. using UV-VIS, FTIR and GC-MS. International Journal of Pharmaceutical Sciences and Drug Research. 2012; 4(1): 56-62
[27] Thamizh Selvam N, Surabhi K R, Swami GK. Ethno Medicinal value of Hibiscus hispidissimus: A Review. International Journal of Current Science and Technology. June 2015; 3: 28-31
[28] Krishnakumar NM, Latha PG, Suja SR, Shine VJ, Shyamal S, Anuja GI, Pradeep S, Shikha P, Somasekharan Unni PK, Rajasekharan S. Hepatoprotective effect of Hibiscus hispidissimus Griffith, ethanolic extract in paracetamol and CCl_4 induced hepatotoxicity in Wistar rats. Indian Journal of Experimental Biology. September 2008; 46: 653-659.