International Journal of Pharmacognosy and Pharmaceutical Sciences

ISSN Print: 2706-7009
ISSN Online: 2706-7017
IJPPS 2022; 4(1): 38-43
www.pharmacognosyjournal.net
Received: 07-04-2022
Accepted: 09-05-2022

K Devi
KGRL, College of Pharmacy, Bhimavaram, Andhra Pradesh, India

K Jacob Isaac
KGRL, College of Pharmacy, Bhimavaram, Andhra Pradesh, India

Corresponding Author:
K Devi
KGRL, College of Pharmacy, Bhimavaram, Andhra Pradesh, India

A review on *Hibiscus rosa-sinensis* leaf extraction, phytochemical screening & thin layer chromatography

K Devi and K Jacob Isaac

DOI: https://doi.org/10.33545/27067009.2022.v4.i1a.48

Abstract
The aim of the review is to give an idea about various pharmacological and pharmaceutical applications of *Hibiscus rosa-sinensis*. This review article covers selected literatures that are based on the studies conducted on *hibiscus rosa-sinensis***.** Its flowers and leaves are available all over India and well known for its Anti-diabetic property. In the present investigation aqueous extract and ethanolic extract of the flowers and leaves were made using soxhlet apparatus, water extract and maceration. The qualitative Phytochemical screening procedure was performed on each extract. Phytochemical study reveals that alkaloids, tannins, saponins. triterpenoids, coumarins, steroids, flavonoids were present in the three extracts.

Keywords: *Hibiscus rosa-sinensis,* Anti-diabetic activity, phytochemical screening

Introduction
China rose or "Queen of tropics" is often a popular name for the gorgeous flowering plant *Hibiscus rosasinensis*, as it is mainly found in south-east China and some islands in the Pacific and Indian Ocean. Dark flowers' extract is used to make eyeliners, and in shoe-blacking [1]. It was believed that the species was given the name "rosasinensis which means "Rose of China" in Latin, by the famous Swedish biologist, Carolus Linnaeus in the early 1750s. Traditionally [2, 3] Hibiscus flowers has been reported to possess antitumor properties, as well as have been used as analgesic, antipyretic, anti-asthmatic, and anti-inflammatory agents [4, 5]. Several studies have proved the presence of anti-oxidant, anti-fungal, and antimicrobial properties in flowers of *Hibiscus rosa-sinensis*. Research on extracts of stems, roots, leaves, and flowers from Hibiscus have revealed that its photochemical components contributed to beneficial findings to human's health such as antioxidant activity [6]. The flowers were also used as a contraception agent for males and females, as well as in rural regions of India. Current scientific literature suggests that more than 50% of today's clinical medications were of natural product origin. Many of them have played a significant role in pharmacological industry and in developing better therapies for various diseases [7-10]. This plant is economically very essential owing to the herbal products and medicinal uses.

Taxonomical Classification: Kingdom - Plantae,Order - Malvales, Family - Malvaceae, Genus - Hibiscus, Botanical name - *Hibiscus rosa-sinensis,* Colour of Flower - Red, White, Yellow, Pink, Orange.

Habitat & Geographical distribution [11-13]
Hibiscus rosa-sinensis was first described in 1753 by Carl Linnaeus in Species Plantarum. The specific epithet rosa-sinensis literally means "rose of China", though it is not closely related to the true roses. The exact origin of *hibiscuss rosa*-sinensis is unknown, although it has been cultivated in China, Japan and the Pacific islands for a long time. *Hibiscus rosa-sinensis* was first discovered in China or perhaps India. Botanists are not really certain, since no wild stands of *Hibiscusrosa-sinensis* have been found growing anywhere. Wherever it originated, it was brought back to Europe by explorers in the 1700s.

Morphology

Botanical description

Macroscopic

The hibiscus is an evergreen shrub, growing to a maximum of 10 m in the wild. Its bark is light-grey.(14)

Leaves: Hibiscus leaves are ovate, simple and 8 to 10.5 cm long. They are spirally arranged around a long stalk &are rich in mucilage.

Flower: The Flowers are bisexual, large and showy, grow up to 25 cm wide, stalked and arising singly from the upper leaf axils. The five free petals joined at the base may be white, yellow or red colour. Sepals are joined in a five-lobed cup with an epical, five to seven lobes. The superior ovary has five stigmas with a long style. The plant flowers perennially. (15-20)

Fruit: The ovoid fruit has up to 20 seeds, is beaked and splits into five parts.

Microscopic

Hibiscus rosa-sinensis is a perennial shrub, evergreen shrub with tap root growing 2.5–5 m (8–16 ft) tall and 1.5–3 m (5–10 ft) wide, with glossy leaves and solitary, brilliant red flowers in summer and autumn. (21-23) The five-petaled flowers are 10 cm (4 in) in diameter, with prominent orange-tipped red anthers. Cultivars and hybrids have flowers in a variety of colours as well as red: white, pink, orange, peach, yellow and purple. Some have double colour flowers.

At the bottom of every *hibiscus* bud is the calyx which is green in colour. The pointed ends of the calyx are the sepals. (24-27) When the hibiscus begins to bloom, the petals begin to grow which contains multiple petals and multiple colours. The ovary and other female parts of the flower lie in the main structure of the *hibiscus*, the pistil, which is long and tubular. The *hibiscus* has both male and female parts on the same flower. The five hairy red spots on the top of the flower is the stigma (female part) of the flower. The stigma is located at the end of the style branch. At the top of the pistil is known as the stigma, where pollen is collected, and in the middle is the style, which is the section that the pollen travels down to the ovary. The ovary lies at the bottom of the blossom and the *hibiscus* has only one ovary which is superior. (28-31)

The male part (stamen) of the flower consists of stem-like filaments and each filament ends with the pollen-producing anther. The anthers, which release the pollen, sits on the filament and these two organs make up the stamen, the male part of the flower. Overall, the *hibiscus* is a dicot, with solitary (axillary), complete, perfect flowers, which have a superior ovary, regular symmetry, and axile placentation. They have five carpels, five locules, five sepals, and the number of stamens may vary. (31-35)

The root is a branched tap root. The stem is aerial, erect, green, cylindrical and branched. The leaf is simple, with alternate phyllotaxy and is petiolate. The leaf shape is ovate, the tip is acute and margin is serrated. Venation is unicostate reticulate. (Venation is branched or divergent.) Free lateral stipules are present. (36-40)

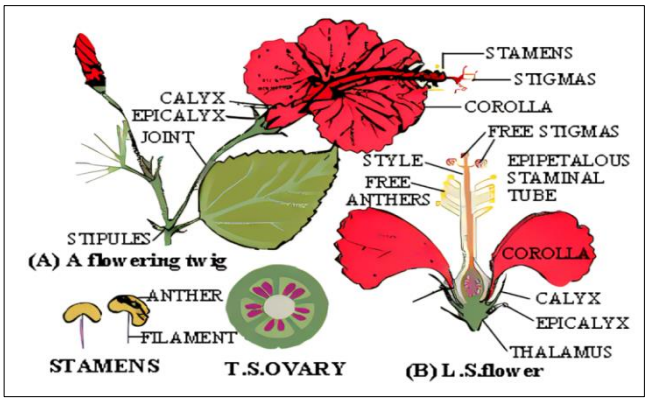

Fig 1: *Hibiscus rosa sinensis*

Fig 2: Flowers of *Hibiscus rosa-sinensis*

Vernacular names: Hindi - Jasum, Gulhar, Bengali - Jaba, English - Hibiscus, Malayalam - Bunga Raya, Tamil – Sembaruthi, Telugu – Mandara, Tribal Name – Raktajaba (Chakma).

Chemical constituents [41-45]

The phytochemical analysis as shows that for *Hibiscusrosa-sinensis* contain tannins, anthraquinones, phenols, flavanoides, alkaloids, saponins, cardiac glycosides, protein,

free amino acids, carbohydrates, reducing sugars, mucilage, essential oils and steroids.

Hibiscusrosa-sinensis contained cyclopropanoids, methyl sterculate, methyl-2-hydroxy sterculate, 2-hydroxysterculate, malvalate and beta-sitosterol. The major anthocyanin in the flower was cyanidin 3-sophoroside. Quantitative phytochemical evaluation of the flowers of *Hibiscus rosa-sinensis* revealed that the amount of ascorbic acid 0.0339 mg/g, riboflavin 0.087 mg/g, calcium 0.0127%, phosphorus 0.4113% and iron 0.771%.

The flowers contained four types of flavonoids, rutin, quercetin and myricetin. The flowers also contained substantial quantities of proanthocyanidins and anthocyanins. Other compounds were isolated from the flowers included cyclopeptide alkaloids, vitamins, thiamine, riboflavin, niacin and ascorbic acid. Crushed red varities yield dark-purplish dye, anthocyanin pigment and cyandindiglucoside.

Table 1: Quantitative phytochemical analysis of *Hibiscus rosa-sinensis*

Phytoconstituents	mg/100 g dry weight
Carbohydrates	0.356
Flavonoids	0.171
Phenol	0.092
Protein	0.247
Tannins	0.073

Uses [46-49]

- Flowers and leaves were taken orally for constipation and painful bowel motion.
- The flowers and leaves were churned into a mucilaginous juice with water and filtered. About half a cup of the filtrate was taken by mouth every day before going to bed act as good laxative.
- A decoction of root is used for venereal diseases and fevers.
- Diuretic used for Kidney trouble.
- Buds are used in treatment of vaginal and uterine discharges.
- Leaves and flowers are good for healing ulcers and for promoting growth and colour of hair.
- Hot water extract of flowers is taken orally for menorrhagia, bronchitis, as an emnenagogue for treatment of menarche Flower decoction alone with "jaggary" is drunk, and as a contraceptive in Ayurvedic medicine

Materials & Methods [50-51]

Columns of different types, beakers, test tubes, conical flasks, rotary evaporator, weighing balance, filter paper, Merc Millipore TLC silica gel 60 F_{254} plate, 10% H_2SO_4 in methanol visualizing solution, TLC chamber, Hot plate. All solvents used were analytical grade and are distilled before use.

Instruments Used [52-56]

Table 2: Instruments – Model

Instruments	Make/Model
TLC glass plates silica gel 60 F254	Merck, Germany.
Silica gel (60-120, 100-200 and 230-400) mesh	Qingdao Marine Chemical, China.

Drying

The whole plant material was shade dried to avoid decomposition of bioactive constituents. The dried whole plant was allowed to mechanical grinding.

Extraction [57]

The dried whole plant material of *Hibiscus rosa-sinensis* was extracted using hexane, chloroform, ethyl acetate, methanol and aqueous solvents by successive solvent extraction. The extracts are filtered and concentrated by using Rotary Vaccum evaporator.

The procedure adopted and amount of the extract obtained was given in figure.

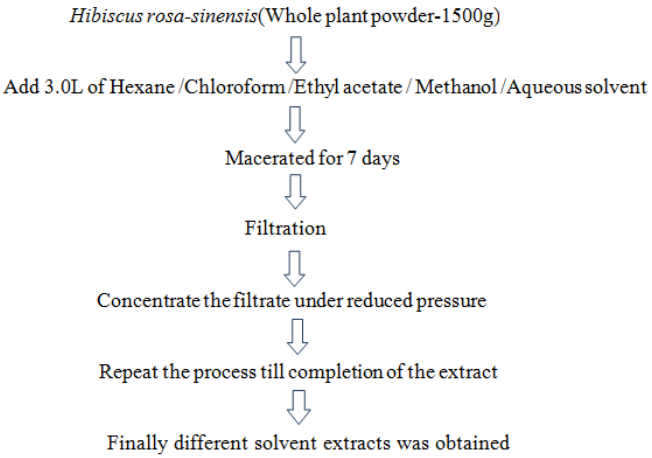

Fig 3: Scheme of solvent extraction of whole plant of *Hibiscus rosa-sinensis*by different solvent polarity

Qualitative screening of phytochemicals [58-64]

The various solvent extracts of *Hibiscus rosa-sinensis* were analysed for its Phytoconstituents like alkaloids, phytosterols, triterpenoids, flavonoids, phenolic compounds, tannins, carbohydrates and proteins.

Seperation based on method of TLC chromatography [64-68]

The crude methanolic extract was initially monitored on TLC using different solvent systems with Ethyl acetate-Hexane, Methanol-Ethyl acetate, to check the maximum number of spots appearing and identified.

Discussion

Hibiscus rosa-sinensis belongs to the family Malvaceae, flowers and leaves of this plant traditionally used as anti-diabetic activity and cosmetics. The macro & micro scopical characters along with physicochemical and fluorescence characters of flower and leaf powder and sections of *Hibiscus rosa sinensis* Linn. is used to establish the pharmacognostical standards and qualitative parameters as per pharmacopoeia and WHO guidelines. The in vitro Anti-diabetic screening a-glucoamylase enzyme is dose dependent. Maximum inhibition was observed in flower alcoholic extract prepared by Maceration.

The TLC study of flower extracts confirmed the presence of various phytochemical constituents such as Flavonoids, glycosides, tannin, glycosides. Further the thin layer chromatography profile may serve as characteristic fingerprint for flower extracts of morphotypes it could be concluded that morphotypes of *Hibiscus rosa-sinensis* Linn.

Contained rich verity of similar type of phytocostituents which can be used for drug.

Conclusion

Hibiscus rosa-sinensis Linn plant powder was collected. The Phytochemical analysis of various solvent extracts found that it is rich in flavonoids and glycosides.

Future work:

Further purification and characterization & mechanism of action, by in-vivo and in-vitro, acute toxicity studies, In silico studies and finger printing analysis are to be studied in future.

.

References

1. Braglia L, Bruna S, Lanteri S, Mercuri A, Portis E. An AFLP-based assessment of the genetic diversity within *Hibiscus rosa-sinensis* and its place within the Hibiscus genus complex. Scientia Horticulturae. 2010;123:372-378.
2. Melzer MJ, Simbajon N, Carillo J, Borth WB, Freitas-Astúa J, Kitajima EW, *et al*. A cilevirus infects ornamental hibiscus in Hawail. Archives of Virology. 2013;158:2421-2424.
3. Reddy UK, Rajesh S, Sindhu G, Aruna B. Herbs used in formulating poly herbal hair oila review. Indo American Journal of Pharmaceutical Research. 2017;4:1527-1539.
4. Kumari OS, Rao NB, Reddy VK. Phyto-chemical analysis and antimicrobial activity of *Hibiscus rosa-sinensis* World Journal of Pharmacy and Pharmaceutical Sciences. 2015;4:766-771.
5. Gomare KS, Mishra DN. FTIR spectroscopic analysis of phytochemical extracts from *Hibiscus rosa-sinensis* L. used for hair disorder.International Journal of Recent Trends in Science and Technology. 2018, 70-75.
6. Vastrad JV, Byadgi SA. Phytochemical screening and antibacterial activity of *Hibiscus rosa-sinensis* leaf extracts. International Journal of Current Microbiology and Applied Sciences. 2018;7:3329-3337.
7. Bhakta S, Das SK. A review on *Hibiscus rosa* sinensis: a rural traditional medicine for contraception. International Journal of Engineering and Information Systems. 2017;1:77-79.
8. Udo J, Ben MG, Etuk CU, Tiomthy Al. Phytochemical, proximate and antibacterial properties of *Hibiscus rosa-sinensis* L. Leaf. Journal of Medicinal Plants Studies. 2016;4:193-195.
9. Tokusoglų O, Yildirim Z. Effects of cooking methods on the anthocyanin levels and antioxidant activity of a local turkish sweet potato [*Ipomoea batatas* (1) lam] cultivar hataykirmizi: boiling. steaming and frying effects. Turkish Journal of Field Crops. 2012;17:89.
10. Uddin B, Hossan T, Paul S, Ahmed T, Nahar T, Ahmed S, *et al*. Antibacterial activity of the ethanol extracts of *Hibiscus rosasinensis* leaves and flowers against clinical isolates of bacteria. Bangladesh Journal of Life Sciences. 2010;22:65-73.
11. Wee YC. Tropical trees and shrubs: A selection for urban planting. Singapore: Sun Tree Publishing, 2003, p. 65
12. Lohar L. The Ayurvedic Pharmacopoeia of India. 2007, 108-109.
13. Kumari OS, Rao NB. Reddy VK Phyto chemical analysis and antimicrobial activity of *Hibiscus rusă* sinensis. World Journal of Pharmacy and Pharmaceutical Sciences. 2015;4:766-771.
14. Kaur S, Lakshmanan P. *Hibiscus rosa* siness leaves polysaccharide as solubility enhancing spent for poorly soluble drug. Pharm Pharmacol Int J. 2015;2:40.
15. Prasad MP. In vitru Phytochemical analysis and antioxidant studies of Hibiscus species. Int J Pure AppBiosci. 2014;2:83-88.
16. Trwar U, Yadav P, Nigam D. Study on Phytochemical screening and antibacterial potential of methanolic flower and leaf extracts of *Hibiscus rosa-sinensis* International Journal of Innovative and Applied Research. 2015;3:9-14.
17. Divya MJ, Sowmia C, Dhanya KP, Joona K. Screening of antioxidant, anticancer activity and phytochemicals in methanolic extract of *Hibiscus rosa-sinensis* leaf extract Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2013;4:1308-1316.
18. Khire CP. Indian medicinal plants An illustrated dictionary. Springer Science, BusinessMedia, LLC. 2007, 310.
19. Sugumaran M, Poornima M, Sethuvani S. Phytochemical and trace element analysis of *Hibiscus rosa sinensis* Linn and Hibiscus syriacus Linn flowers. NPAIJ. 2012;8:341-345.
20. Agarwal S, Prakash R, Srivastava A, Malur RM. Quantitative and qualitative analysis of phytochemicals, present in flower extract of *Hibiscus rosa-sinensis*. Int Scientific Res. 2016;5:78-79.
21. Wong SK, Lim YY, Chan EWC Antioxidant properties of Hibiscus species variation attitudinal. change coastal influence and floral colour change Journal of Tropical Forest Science. 2009;21:307-315.
22. Purushothaman A, Meenatchi P, Saravanan S, Sundaram R, Saravanan N. Quantification of total phenolic content, HPLC analysis of flavonoids and assessment of antioxidant and anti-haemolytic activities of *Hibiscus rosa-sinensis* L. flowers in vitro. Int. J Pharma Res Health Sci. 2016;4:1342-1350.
23. Nakamura Y, Hidaka M, Masaki H, Seti H, Unzumi T. Major anthocyanin of the flowers of Hibiscus (*Hibiscus rosa-sinensis* L.), AgricBiol Chem. 1990;54:3345-3345.
24. Intergan C, Concepcion Alejo LG, Carpus VK, Salud RD, Rosariol D, Gamez R. Composition Philippine foods IV. Philippine J Sc. 1955;84:343-364.
25. Khokhar I, Ahmad A. Studies in medicinal plants of Pakistan: New cyclopeptidealkoloids from the flowers of *Hibiscus rosa-sinensis*. Sci. Int. (Lahore). 1992;4:147-150.
26. Wealth of India, Raw Materials. Vol. VI H-KNISC, CSIR. New Delhi. 1997, 91-92.
27. Subramanium SS, Nair AGR. Flavonoids of four Malvaceous plants. Phytochemistry. 1972;11:1518-1519.
28. Agarwal S, Prakashi R. Essential oil composition of solvent extract of Hibiscus rusa-sinensis flower. Orient J Chem. 2013;29:813-814.
29. Khan ZA, Naqvi SA, Mukhtar A, Hussain Z, Shahzad SA, Mansha A. Antioxidant and antibacterial activines of *Hibiscus rosasinensis* Linn lower extracts: Pak J Pharm Sci. 2014;27:469-474.

30. Ameena K, Dilip C, Saraswathi R, Krishnan PN. Sankar, Simi SP. Isolation of the mucilages from *Hibiscus rosa-sinensts* Linn and Olra (*Abelmoschus esculentus* linn.) and studies of the binding effects of the mucilages Asian Pacific Journal of Tropical Medicine, 2010, 539-543.
31. The Wealth of India. First Supplement Series mw materials. National Institute of Science and Communication. CSIR, New Delhi, India. 2002;3:306-387.
32. Shal V, Patul R. Studies on mucilage from *Hibiscus rosa sinensis* Linn, us oral disintegrant, International Journal of Applied Phormaceutics. 2010, 18-21.
33. Sanito SB, Kazi T, Jatni WB, Makhija PM, Shar GQ, Mahar KP. Determination and evaluation of mineral constituents of medicinal plants used for the treatment of asthma and other ailments by alomic absorption spectrophotometry. Pak J Aral Environ Chem. 2013;14:61-67.
34. Nakatani M, Fulkumaga Y, Hase T. Aliphatic compounds from *Hibiscus rosa-sinensis* Phytochemistry. 1986;25:449-452.
35. Aggrawal SK, Rastoga PR. Triterpenoids of *Hibiscus rosa-sinensis* Linn. Indian J Pham. 1971;33:41-42.
36. Nandan Kumar J, Pandey LK. *Hibiscus rosa-sinensis*: China rose, Phytophamm. 2008, 4-5.
37. Rao KNV, Geetha K, Alagar Raja M, David B. Journal of Pharmacognosy and Phytochemistry. 30.
38. Kholkute SD, Mudgal V, Udupa KN. Studies on antifertility potentiality of *Hibiscus rosa sinensis*. Parts of medicinal value, selection of species and season alvariations, Planta Medica. 1977;31:35-39.
39. Jadhav VM, Thorat RM, Kadam VJ, Sathe NS. *Hibiscus rosa sinensis* Linn, A Review, Journal of Pharmacy Research. 2009;2:1168-1173.
40. Kasture VS, Chopde CT and Deshmukh VK. Anticonvulsive activity of Albizzialebbeck, *Hibiscus rosasinensis* and Butea monosperma in experimental animals, Journal of Ethnopharmacol. 2000;71:65-67.
41. Prakash A0. *Hibiscus rosasinensis* Linn extracts. 1979, 501-503.
42. Murthy DR, Reddy CM and Patil SB. Effect of benzene extract of *Hibiscus rosasinensis* on the estrous cycle and ovarian activity in albino mice, Biological and Pharmaceutical bulletin. 1997;20:756-758.
43. Adhirajan N, Kumar TR, Shanmugasundaram N, Babu M. In vivo and in vitro evaluation of hair growth potential of *Hibiscus rosa sinensis* Linn, Journal of Ethnopharmacology. 2003;88:235-239.
44. Venkatesh S, Thilagavathi J, ShyamSundar D. Anti-diabetic activity of flowers of *Hibiscus rosa* sinensis, Fitoterapia, 2008;79:79-81.
45. Yamasaki H, Uefuji H, Sakihama Y. Bleaching of the red anthocyanin induced by superoxide radical, Archives of Biochemistry and Biophysics. 1996;332:183-186.
46. Gauthaman KK, Saleem MT, Thanislas PT, Prabhu VV, Krishnamoorthy KK, Devaraj NS. Cardioprotective effect of the *Hibiscus rosa sinensis* flower in an oxidative stress model of myocardial ischemic reperfusion injury in rat, BMC Complementary and Alternative Medicine. 2006;20:32.
47. Ozmen, Ali. Cytotoxicity of *Hibiscus rosa sinensis* flower extract, Caryologia. 2010;63:157-161.
48. Singh MP, Singh RH, Udupa KN. Anti-fertility activity of a benzene extract of *Hibiscus rosa sinensis* flower on female albino rats, Planta Medica. 1982;5:91-94.
49. Siddiqui AA, Wani SM, Rajesh R, Alagarsamy V. Phytochemical and pharmacological investigation of flowers of *Hibiscus rosa sinensis* Linn, Indian Journal of Pharmaceutical Sciences. 2006;68:127-130.
50. Shivananda Nayak B, Sivachandra Raju, Orette FA, Chalapathi Rao AV. Effect of *Hibiscus rosa sinensis* Linn (Malvaceae) on wound healing activity: A preclinical study in a Sprague Dawley rat, International Journal of Lower Extremity wounds. 2007;6:78-81.
51. Vimalin Hena J. Antibacterial potentiality of *Hibiscus rosa-sinensis* solvent extract and aqueous extracts against some pathogenic Bacteria, Journal of Herbal Science and Technology. 2010;4:21-23.
52. Singh N, Nath R, Kohli RP. A Pharmacological investigation of some indigenous drugs of plant origin for evaluation of their antipyretic, analgesic and anti-inflammatory activities, Journal of Research and Education in Indian Medicine. 1978;132:58-62.
53. Bhakuni OS, Dhar ML, Dhar MM, Dhawan BN, Mehrotra BN. Screening of Indian plants for biological activity, Indian Journal of Experimental Biology. 1969;7:250-262.
54. Renu. Fungitoxicity of leaf extracts of some higher plants against Rhizoctoniasolani Kuehn, National Academy of Sciences. 1983;6:245-246.
55. Van Den Berghe DA, Levan M, Mertens F, Vlietinck AJ, Lammens E. Screening of higher plants for biological activities, Journal of Natural Products. 1978;41:463-467.
56. Prakash AO. Acid and alkaline phosphatase activity in the uterus of rat treated with *Hibiscus rosa sinensis* Linn extracts, Current Science. 1979;48:501-503.
57. Sharma S, Sultana S. Effect of *Hibiscus rosa sinensis* extract on hyperproliferation and oxidative damage caused by benzoyl peroxide and ultra violetradiations in mouse skin, Basic and Clinical Pharmacology and Toxicology. 2004;95:220-225.
58. Olagbende Dada SO, Ezeobika EN, Duru FI. Anabolic effect of *Hibiscus rosa sinensis* Linn leaf extracts in immature albino male rats, Nigerian Quarterly Journal of Hospital Medicine. 2007;17:5-7.
59. Sangeetha A, Zubaidah Z, Dayang F. Preliminary Screening of Antibacterial Activity Using Crude Extracts of *Hibiscus rosa sinensis*, Tropical Life Sciences Research. 2009;20:109-118.
60. https://www.researchgate.net/publication/328103257. phytochemical and physicochemical properties of *Hibiscus rosa sinensis* leaves extract a comparison between conventional and microwave assisted extraction.
61. Iqbal Hussian, Riaz Ullah, Muhammad Khurram, Naseem Ullah, Mohammad Zahoor, Jehangir K. Phytochemical analysis of selected medicinal plants, African Journal of Biotechnology. 2011;10:7487-7492.
62. Obadoni BO, Ochuko PO. Phytochemical studies and Comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria, Global Journal of Pure and Applied Sciences. 2001;8:203-208.
63. Boham AB, Kocipai AC. Flavonoid and condensed tannins from Leaves of *Hawaiian vaccininum*

vaticulum and vicalycinium, Pacific Science. 1994;48:458-463.
64. Sadasivam S and Manickam A. Biochemical methods for agricultural sciences, Wiley Eastern Limited Publications. 1992;2:59-63.
65. Obadoni BO, Ochuko PO. Phytochemical studies and Comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria, Global Journal of Pure and Applied Sciences. 2001;8:203-208.
66. Shanthi P, Amudha P. Evaluation of the phytochemical constituents of acmellacalva, International Journal of Pharma and Bio Sciences. 2010;1:309-311.
67. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent, Journal of Biological Chemistry. 1951;193:256-275.
68. Deepak Koche. Trace element analysis and vitamins from an Indian medicinal plant *Nepeta hindostana* (roth) haines, International Journal of Pharmacy and Pharmaceutical Sciences. 2011;3:53-54.