

Chapter-33

Preliminary Phytochemical Analysis of Leaves of Plant *Cocculus Hirsutus* (L.) Diels

Dr. Bhagvan C. Kachhava

Department of Chemistry,

K.E.S.A.C.S College Kalwan (Manur) Tal- Kalwan Dist-Nashik (Maharashtra), India.

Email- bhagvankachhava@gmail.com

Abstract

Synthetic drugs produce side effects and have high risk of secondary failure. On the other hand plants are considered as an important and rich source of secondary metabolites having interesting biological activities without any adverse effect. The leaves of *Cocculus hirsutus* (L) Diels is reported in traditional system of medicine with high quality medicinal values. The present work aims to find out the various physico-chemical parameters like determination of water-soluble extractive value; alcohol soluble extractive value, loss on drying, ash values, fluorescence behavior and preliminary phytochemical analysis of leaves of *Cocculus hirsutus*. The identification of plant *Cocculus hirsutus* was done by studying macroscopical features and the final authentication was confirmed by the botanist. Preliminary phytochemical study of leaves of *Cocculus hirsutus* revealed that the presence of various phytochemicals like carbohydrates, alkaloids, phenols, flavonoids, tannins, fixed oil, fats, gums and mucilages, sterols, protein and amino acids. The physico-chemical analysis of *Cocculus hirsutus* was a useful tool to check purity as well as quality and authenticity of *Cocculus hirsutus*.

Keywords: *Cocculus hirsutus*, physico-chemical, fluorescence, ash value, Extractive value.

Introduction

In past the medicinal plant species has been widely used in traditional medicines and today also being considered as a source of development of therapeutic agents for various kinds of diseases. Consequently, it is also important to critically evaluate and understand the available scientific information about the traditional medicinal plant species and their importance. *Cocculus hirsutus* (L.) Diels is a widely distributed perennial climber mainly found in tropical and subtropical climates[1]. In Asia, it is reported from India, Myanmar, Nepal and Pakistan and southern China [2]–[4]. Traditionally the plant *Cocculus hirsutus* was preserved for its unique property of healing all types of wounds, cuts and boils in very short time with less pain. It is also used in treatment of spermatorrhoea, gonorrhoea, diarrhea, urinary troubles and hyperglycemia etc[2]. The whole aerial part of plant is used for treating eye diseases. A decoction of the leaves is taken in dysentery, eczema and urinary problem. Roots and leaves are given for Sarsaparilla, as diuretic and in gout[3]. Literature survey revealed that the leaves of the plant have been evaluated for anti-hyperglycemic[4]; antibacterial[1]; diuretic and laxative effects[5]. It is known by various names in local languages such as: Broom creeper (English); Huyer (Bengali); Vasan vel (Marathi) Farid buti, Jami ki bel (Hindi);

Kaage Mari (Kannada); Farid buti (Urdu); Paathaalagarudakkoti (Malayalam), Kaanse laharo (Nepali); Garudi, Patalagarudi (Sanskrit), Chipuru-tiga (Telegu); Kattu-k-koti (Tamil) among others [1], [6] [7].

Taxonomical Classification:[8]

Kingdom : Plantae

Phylum : Tracheophyta

Class : Magnoliopsida

Order : Ranunculanae

Family : Menispermaceae Juss

Genus : *Cocculus*

Species : *Cocculus hirsutus*.



Fig. 1: *Cocculus Hirsutus*

Materials And Method

Collection, Authentication and Processing of Plant Materials:

For present research work the plant material (leaves) get collected from Varkhede bk. (Chalisgaon) the region of North Maharashtra, India. During collection of the plant material, care was taken to identify the healthy plant leaves. It is followed by the plant material was authenticated by Dr. S.R. Kshirsagar, Taxonomist, Department of Botany, S.S.V.P.S's L. K. Dr. P.R. Ghogrey Science College, Dhule (M.S). The collected healthy leaves of plant materials were shade dried for two weeks and reduced the size by using grinding mill into coarse powder. It was stored in an air tight transparent container; it can be used for further process.

Physicochemical Evaluation:

The content present in leaves of *Cocculus hirsutus* in powdered form were used for determination of various physicochemical parameters such as ash values, extractive values, loss on drying, etc., according to the WHO guidelines[9].

Fluorescence Behavior:

Fluorescence analysis of crude leaves powder of *Cocculus hirsutus* was performed by treatment with different reagents as per reported procedure under (UV) light of range (264 nm and 365 nm)[10], [11]. The change in appearance of color were observed and recorded.

Method of Extraction:

The dried leaves of *Cocculus hirsutus* 2 kg was extracted by using three different solvents (Petroleum ether, Ethyl acetate and methanol) with successive hot continuous percolation method in Soxhlet apparatus. After the successful extraction, extracts were concentrated on a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.



Fig. 2: Extraction using Soxhlet Apparatus

Preliminary Phytochemical Investigation: The presence of various phytoconstituents like alkaloids, carbohydrates, glycosides, phytosterols, fixed oils, fats, saponins, phenolic compounds, triterpenes, flavonoids, proteins, amino acids, gums, mucilage, tannins and volatile oils are proven by performing preliminary phytochemical screening of extracts obtained from extraction process[12], [13].

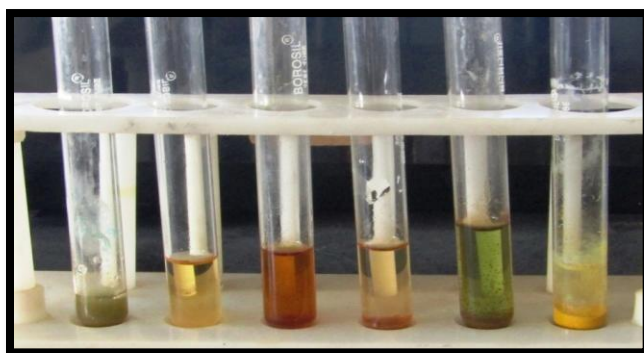


Fig.3: Preliminary Phytochemical analysis test

Test for Alkaloids: Alkaloids were tested with the help of various alkaloidal reagents such as Mayer's, Dragendorff's, reagent. E.g.: Achyranthine, Atropine, Betaine and Quinine[14].

Mayer's Reagent: It consists of two solutions'

- (i) Mercuric chloride (1.36 gm) in distilled water (60 ml)
- (ii) Potassium Iodide (5g) in distilled water (20ml). Alkaloids present in the sample give yellow (or) buff coloured (or) cream coloured precipitate.

Dragendorff's reagent: It consists of sodium Iodide (14 gm), Basic bismuth carbonate (15.2 gm) in 50 ml glacial acetic acid. These are boiled for few minutes. It was allowed to stand overnight, discarded the precipitate and took the filtrate. To every 40 ml of filtrate, 160ml of ethyl acetate and 1 ml water were added. Before conducting the test, for every 10 ml of this stock solution 20 ml of acetic acid was added and made up to 100 ml with water. Alkaloids gave orange brown precipitate.

Test for carbohydrates: -

Molish's test (General test): -

To 2-3 ml aqueous leaves extract, few drops of α -naphthol solution in alcohol was added and concentrated H_2SO_4 was added from sides of test tube which formed violet ring at the junction of two liquids.

A. For reducing sugars: -

a) Fehling's test: 1ml Fehling's A and 1 ml Fehling's B Solution were mixed and boiled for one minute. To this equal volume of test solution was added. On heating in boiling water bath for 5-10 min., initially it formed a yellow and then brick red precipitate.

b) Benedict's test: Equal volume of Benedict's reagent and test solution were mixed in test tube and heated in boiling water bath for 5 min. Solution appeared green, yellow or red color depending on the amount of reducing sugar present in test solution.

B. For Monosaccharide's: -

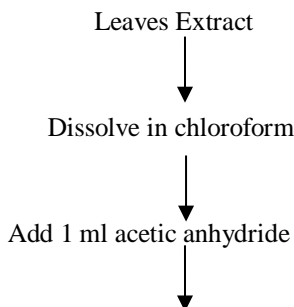
Barfoed's test: Equal volume of Barfoed's reagent and test solution were added and heated in boiling water bath and cooled. Red precipitate was observed indicating the presence of monosaccharides.

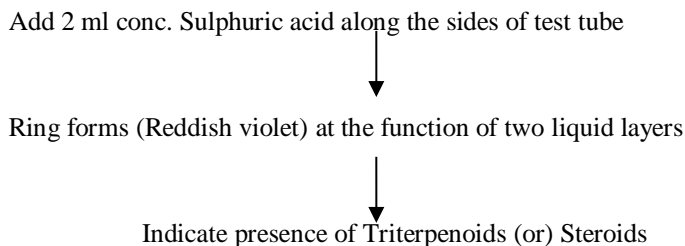
Test for Glycosides:

Small quantities of plant extracts separately are filtered. The filtrates are used for Molisch's test to detect the presence of carbohydrates [15], [16].

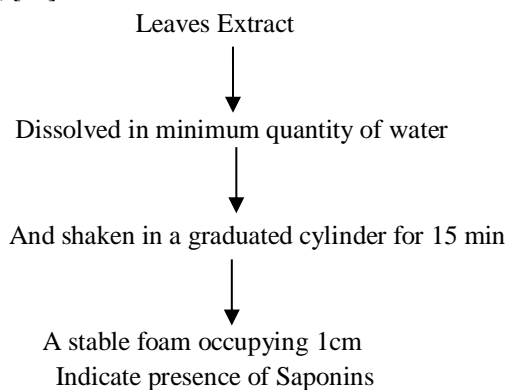
- (a) Liebermann – Burchard's test, to detect steroidal glycosides
- (b) Legal's test to detect cardiac glycosides / sterol glycosides (pink to red colour)
- (c) Borntrager's test to detect anthraquinone glycosides (Pink, red (or) violet colour).

Test for Triterpenes: Salkowski's test and Liebermann burchard's test. Dark green color in the upper layer and red color in the lower layer indicating the presence of steroids. E.g.: Sterols, Bile acids, Adrenocorticoids etc. [14] [15], [16].



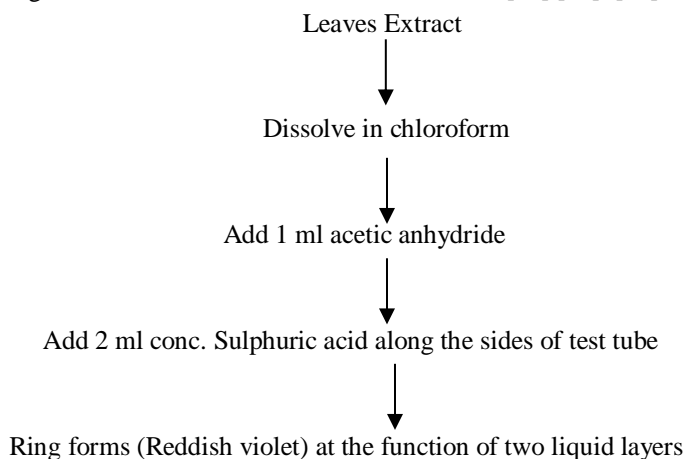


Test for Saponins: These are plant steroidal glycosides which have the property of forming foams in water (like soap in solution). They act by lowering the surface tension of water. Sample + water + shaking, Formation of honey comb like froth, Presence of froths/foams [15], [16], [17]



Test for Phenols: Phenols are the aromatic compounds in which OH (hydroxyl) group is directly attached to benzene ring depending on the attachment of OH group to benzene ring the phenols are classified. E.g.: Cresols, Catechol, etc [15], [16], [17]

Test for Triterpenes: Salkowski's test and Liebermann burchard's test. Dark green color in the upper layer and red color in the lower layer indicating the presence of steroids. E.g.: Sterols, Bile acids, Adrenocorticoids etc. [14] [15], [16].



↓
Indicate presence of Triterpenoids (or) Steroids

Test for Flavonoids: These are also known as anthoxanthines which are yellow pigments which occur in plant kingdom 0.5 ml extract + 5-10 drops of dilute HCl + then boiled for few min. Shinoda's Test, Zn-HCl acid reduction Test. Red color Magenta color[15], [16] [18]

Test for proteins:-

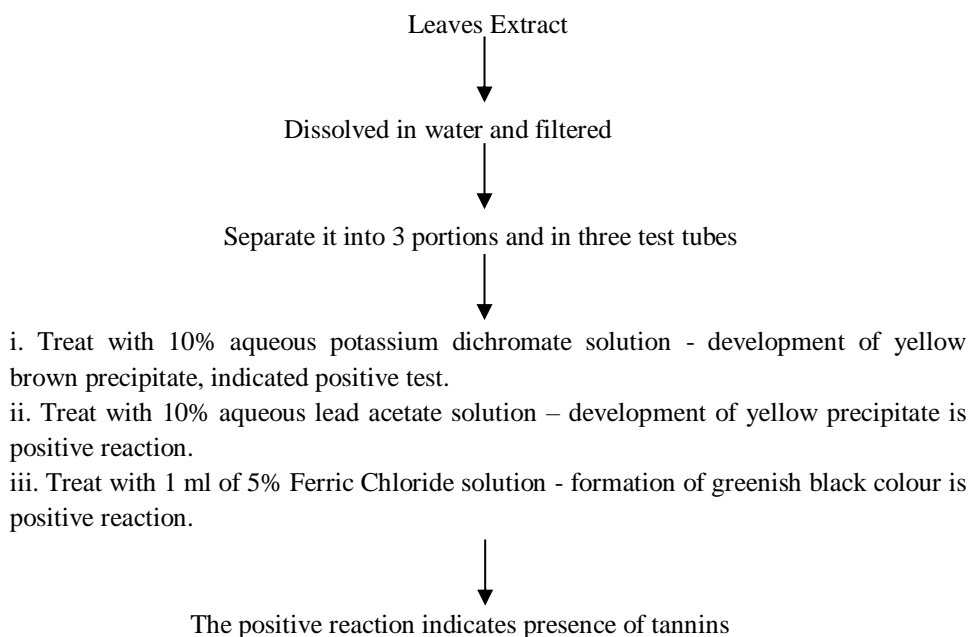
a) Biuret test (general test):

To 3 ml test solution, few drops of 4 % NaOH and few drops of 1 % CaSO₄ solution was added and observed for violet or pink color.

b) Million's test (for proteins):

3 ml of test solution was mixed with 5 ml Million's reagent to obtain white precipitate. The precipitate was further warmed which turned to brick red or precipitate was dissolved giving red color.

Test for Tannin: The tannin compounds are widely distributed in many species of plants, where they play a role in protection from predation, and perhaps also as pesticides, and in plant growth regulation[14] [16] [19]



Result And Discussion

The crude powder of *Cocculus Hirsutus leaves* showed green color in visible light, green colour under short UV (254 nm), and blue colour under long UV (365 nm). On treatment with different reagents like HNO₃, HCl, and H₂SO₄ caused noticeable color

changes in visible light and characteristic fluorescence shifts under UV light. Concentrated and diluted acids (50% HNO₃ and 50% H₂SO₄) intensified blue fluorescence, especially under long UV. These characteristic color changes indicate distinct fluorescent properties useful for identification.

Sr. No.	Testing	Visible Light	Short UV (254nm)	Long UV (365 nm)
1	Powder	Dark Green	Green	Light Blue
2	Powder + 1 N NaOH in ethanol	Green	Dark Green	Blue
3	Powder + HNO ₃ (1:1)	Dark brown	Light Green	Dark blue
4	Powder + 1 N HCL	Light Brown	Dark green	Violet
5	Powder + H ₂ SO ₄ (1:1)	Green	Green	Dark green
6	Powder+ 50% H ₂ SO ₄	Light Green	Light Green	Blue
7	Powder + 50% HNO ₃	Blue	Light Blue	Dark blue

Table No. 1 Fluorescence behavior of the *Cocculus Hirsutus* leaves powder with different chemical reagents

Ash Value[20] [21]:

The determination of ash value of crude sample is very important and it is useful in analyzing the quality and purity of crude drug sample. It usually shows the presence of naturally inorganic salts and impurity adhering to it. The main intention of ash value detection is to remove all traces of organic matter which may be interfering in analytical determination. Procedure used for determination of ash values such as total ash, acid insoluble ash, and water-soluble ash as per I.P.

Determination of total ash value:

About 3.5gm of powdered drug was weighed accurately into a tarred silica crucible and incinerated gradually up to dull red hot until free from carbon. The crucible was cooled and weighed, repeated for constant value.

Percentage of total ash was calculated with reference to air-dried substance it found to be 5.10 ± 0.02 . (See Table No. 2)

Determination of acid insoluble ash value:

Ash obtained from the total ash was boiled with 25ml of 2N HCl for few minutes. The insoluble matter was collected on an ashless filter paper, washed with hot water. The filter paper was transferred into a tarred silica crucible and ignited up to dull red hot until free from carbon and weighed. The percentage of acid insoluble ash was found to be 3.34 ± 0.03 (Table No. 2).

Determination of acid soluble ash value: The ash obtained from total ash was boiled with 25ml of water for 5 minutes. The insoluble matter was collected on an ashless filter paper, washed with hot water and ignited for 15 minutes at a temperature above 250^oC. The weight of insoluble matter was subtracted from the weight of total ash. The difference in weight represents the water soluble ash value. The percentage of water-soluble ash was found to be 2.00 ± 0.02 .

Sr. No.	Analyzed parameters	Observation in (% w/w)
1	Total ash value	5.10 ± 0.02
2	Acid insoluble ash value	3.34 ± 0.03
3	Acid soluble ash value	2.00 ± 0.02

Table 2: Percentage of ash values of *Cocculus Hirsutus* leaves powder Determination of extractive values[21]:

Extractive value is useful for determining the nature of chemical constituent present as well as quality of drug. Hence it is important to use the suitable solvent which can able to dissolve appropriate quantity of desired substance.

Determination of Alcohol Soluble Extractive Value:

About 15gm of the air-dried coarse powder of *Cocculus Hirsutus* leaves was macerated with 100ml of 90% ethanol in a closed flask for 24 hours shaking frequently during the first 6 hours and allowed to stand for 18 hours. Thereafter, it was filtered rapidly. 25ml of the filtrate was evaporated to dryness in a tarred flat bottom shallow dish, dried at 105⁰C and weighed. The percentage of ethanol soluble extractive value was found to be 5.90 ± 0.19 (See Table No. 3)

Determination of Water-Soluble Extractive Value:

Powdered *Cocculus Hirsutus* leaves (15gm) were weighed accurately and macerated with 100ml of water in a closed flask for 24 hours. It was shaken frequently during the first 6 hours and allowed to stand. After 18 hours it was filtered rapidly. Then 25ml of the filtrate was evaporated to dryness in a tarred flat-bottom shallow dish, dried at 105⁰C and weighed. The percentage of water-soluble extractive was 6.60 ± 1.00.

Sr. No.	Analyzed parameters	Observation in (% w/w)
1	Alcohol soluble extractive value	5.90 ± 0.19
2	Water Soluble Extractive Value	6.60 ± 1.00
3	Loss on drying	3.35 ± 0.90

Table 3: percentage of extractive values of *Cocculus Hirsutus* leaves powder

Phytoconstituents	Petroleum Ether Extract	Ethyl Acetate Extract	Methanol Extract
Alkaloids	– (Absent)	+ (Present)	+ (Present)
Carbohydrates	–	–	+
Glycosides	–	+	+

Phytosterols	+	+	-
Saponins	-	+	+
Phenolic Compounds	-	+	+
Triterpenes	+	+	+
Flavonoids	-	+	+
Proteins	-	-	+
Tannins	-	+	+

Table 4: Preliminary Phytochemical analysis of the leaves of *Cocculus hirsutus*

Conclusion

In the above study, the fluorescence behaviour of the plant material treated with reagents like HNO₃, HCl, and H₂SO₄ was examined under visible light, short-wave UV (254 nm), and long-wave UV (365 nm). The change in colour of powder helped in detecting the possible presence of phytoconstituents and consider as an important parameter for purity and quality investigation. The good quality of the plant material was proved by the distinct colour changes of the crude powder under different light sources. It indicates the good quality and purity of drug for further analytical and pharmacological investigations.

Physicochemical parameters such as extractive values, loss on drying, water- and alcohol-soluble ash values were quantitatively determined. It was also observed that the water-soluble extractive value was higher than the alcohol-soluble extractive value, indicating a predominance of polar constituents.

The preliminary phytochemical screening shows that the nature of the solvent notably influences the extraction of phytochemicals. Non-polar petroleum ether extract mainly yielded phytosterols and triterpenes, on the other hand the moderately polar ethyl acetate extract contained a broader range of secondary metabolites. The methanol extract showed the maximum number of phytoconstituents, particularly polar compounds, demonstrating that methanol is the most efficient solvent for extracting bioactive constituents. This indicates that the plant possesses diverse phytochemicals with potential therapeutic value and supports further pharmacological and phytochemical investigations using methanolic extracts.

Reference

[1] B. R. Panda, S. R. Mohanta, U. S. Mishra, S. Kar, B. K. Panda, and P. Chakraborty, "Antibacterial activity of the leaves of *Cocculus hirsutus*," INDIAN DRUGS-BOMBAY-, vol. 44, no. 2, p. 108, 2007.

- [2] K. R. Kirtikar, "Basu., BD Indian Medicinal Plants," Int. Book Distrib., pp. 1038–1063, 1918.
- [3] K. M. Nadkarani 3rd, "Indian Materia Medica, 3rd Edn. popular prakashan," 1982, Bombay.
- [4] S. Badole, N. Patel, S. Bodhankar, B. Jain, and S. Bhardwaj, "Antihyperglycemic activity of aqueous extract of leaves of *Cocculus hirsutus* (L.) Diels in alloxan-induced diabetic mice.," Indian J. Pharmacol., vol. 38, no. 1, p. 49, 2006.
- [5] S. Ganapaty, G. K. Dash, T. Subburaju, and P. Suresh, "Diuretic, laxative and toxicity studies of *Cocculus hirsutus* aerial parts," Fitoterapia, vol. 73, no. 1, pp. 28–31, 2002.
- [6] K. K. Shrestha, S. Bhattarai, and P. Bhandari, Handbook of Flowering Plants of Nepal (Vol. 1 Gymnosperms and Angiosperms: Cycadaceae-Betulaceae). Scientific Publishers, 2018.
- [7] R. Logesh, N. Das, A. Adhikari-Devkota, and H. P. Devkota, "*Cocculus hirsutus* (L.) W.Theob.(Menispermaceae): A Review on Traditional Uses, Phytochemistry and Pharmacological Activities," Medicines, vol. 7, no. 11, p. 69, 2020.
- [8] "*Cocculus hirsutus* (L.) Diels." Accessed: Sept. 06, 2021. [Online]. Available: <https://www.gbif.org/en/species/7930800>
- [9] "World Health Organization. Quality control methods... - Google Scholar." Accessed: Sept. 06, 2021. [Online]. Available:
- [10] "Shrestha S, Kaushik VS, Eshwarappa RSB, Subaramaihha SR, Ramanna LM, Lakkappa DB. Pharmacognostic studies of insect gall of *Quercus infectoria* Olivier (Fagaceae). Asian Pac J Trop Biomed. 2014; 4(1): 35-39." Accessed: Sept. 06, 2021. [Online]. Available: <https://www.sciencedirect.com/science/article/abs/pii/S2221169114602057>
- [11] Ghasemi Dehkordi N, Sajadi SE, Ghannadi AR, Amanzade Y, Azadbakht M, Asghari GHR, Amin GHR, Hajiakhondi A, Taleb AM. Iranian herbal pharmacopoeia. Tehran: Ministry of Health and Medical Education, 2002.
- [12] "Kumar VR and Venkatachalam VV: Physicochemical evaluation and phytochemical investigation of the leaves of *Grewia tiliaefolia* vahl. Der Pharmacia Lettre 2016; 8(20): 52-56."
- [13] "Rathi V, Rathi JC, Pande MS and Tamizharasi S: Pharmacognostic evaluation and phytochemical studies on leaves of *Albizia lebbek* Benth. PHCOG J 2009; 1(1): 30-32."
- [14] "Vaishali Rai M, Vinitha Ramanath Pai, Pratapchandra Kedilaya H, Smitha Hegde (2013). Preliminary Phytochemical Screening of Members of Lamiaceae Family: *Leucas linifolia*, *Coleus aromaticus* and *Pogestemon patchouli*int. J. Pharm. Sci. Rev. Res., 21(1) 22, 131-137."
- [15] "Dharmendra Singh, Poonam Singh, Abhishek Gupta, Shikha Solank, Ekta sharma, Rajeev Nem (2012). Qualitative Estimation of the Presence of Bioactive Compound in *Centella asiatica*: An Important Medicinal Plant, International Journal of Life Science and Medical Science, Vol. 2 Issus. 1, 5-7".

- [16]“Yusuf, A. Z., Zakir, A., Shemau, Z., Abdullahi, M. and Halima, S. A. (2014). Phytochemical analysis of the methanol leaves extract of *Paullinia pinnata* linn, *Journal of Pharmacognosy and Phytotherapy*, Vol. 6(2), 10-16”.
- [17]“Kangogo K. Geoffrey, Kagira M. John, Maina Naomi and Karanja M. Simon (2014). Qualitative Phytochemical Screening of *Camellia sinensis* and *Psidium guajava* Leave Extracts from Kericho and Baringo Counties, *International Journal of Advanced Biotechnology and Research(IJBR)*, 5, 3, 506-512”.
- [18]“Krishnaiah D, Sarbatly R, Bono A (2007). Phytochemical antioxidants for health and medicine – A move towards nature. *Biotechnol. Mol. Biol, Rev.*, 1(4): 097-104.”.
- [19]“Mohammad Abdul Motalib Momin, Sm Faysal Bellah, Sarder Mohammad Raussel Rahman, Ahmed Ayedur Rahman, Gazi Mohammad Monjur Murshid, Sarder Mohammad Saker Billah, Talha Bin Emran (2014)., *Phytopharmacological evaluation of ethanol extract of Sida cordifolia L. Roots*. *Asian Pac J Trop Biomed*; 4(1): 18-24.”.
- [20]Government of India ministry of Health and Family welfare. *Indian Pharmacopeia*, vol.II New Delhi, Controller of publication. 1996, A-52-A-54.
- [21]“Dr.A. P. Rajput¹ Bhagvan .C. Kachhava² PHYSICOCHEMICAL STUDIES ON LEAVES OF *SOYMIDA FEBRIFUGA* ADR. JUSS. FAMILY : MELIACEAE *Science, Technology and Development* Volume IX Issue IX SEPTEMBER 2020 ISSN : 0950-0707 Page No : 659”.