Evaluation on pharmacognostical and phytochemical parameters of Hibiscus rosa sinensis L. – leaf

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Abstarct

The ***Hibiscus rosa sinensis*** is also known as Chinese rosa. The ***Hibiscus rosa sinesis*** plant are one of the most important sources of medicines. The medicinal plant are utilized the world in traditional system of medicine "Ayurveda". The ***Hibiscus rosa sinesis*** belong to the member of family of Malvaceae, and is one of the 300 species of the genus hibiscus. Rich source of the medicinal plant in India and is called "Botanical Garden of the World" with spacious wealth of biodiversity. In India recorded in 45,000 plant species so for of which 7,500species are used for medicinal purposes. ***Hibiscus rosa-sinensis*** are national flower of Malaysia. The genus of Hibiscus comprises about 275 species in the tropics and subtropics. It is found in China, India, and Malaysia etc. The ***Hibiscus rosa-sinensis*** are study in the various chemical constituent like: anthraquinones, phenols, alkaloids, terpenoids, cardiac glycoside, saponins, protein, flanoides, tannins, quinines, free amino acid, carbohydrate, mucilage, essential oil reducing sugar, and steroids. The plant of ***Hibiscus rosa sinensis*** are used in the various important pharmacological activity like: Hypotensive, Anti-pyritic, Anti-inflammatory, Aanti-cancer, Antioxidant, Anti-bacterial, Anti-diabetic, Wound and Healing, Anti-fungal, Antimicrobial, and, Antimicrobial, Antitumor, memory enhancement, Hepatoprotective, fibrinolytic, antitussive, Neuroprotective activity, Gastroprotective, Immune response activity, Anti-pyritic activity, Cadioprotective activity, Toxicity, Hepatoprotective effects, Antihyperlipidemic activity, Hair growth promoting activity. The review summarizes the phytochemistry, pharmacology, and flower of uses with the purpose of finding gaps demanding for future investigation and research its therapeutic therapeutic through clinical trials.

Keywords: Pharmacognosy, Phytochemistry, ***Hibiscus rosa-sinensis leaves***

Introduction:

Hibiscus rosa-sinensis is also known as Chinese rosa and belonging to Malvaceae family. The hibiscus plant are used in various medicinal purpose like as: inflammation, fever, coughs, injuries, diabetes, infection caused by fungi and bacteria, alopecia, gastric ulcers in several tropic Countres[1]. The traditionally, used of hibiscus flower has been reported of different type property antitumor properties, antipyretic property, anti-infammatory agent, have been used as analgesic [2]. Rich source of the medicinal plant in India and is called "Botanical Garden of the World" with spacious wealth of biodiversity. In India recorded in 45,000 plant

species so for [3] of which 7,500 species are used for medicinal purposes [4]. The hibiscus flower of National flower of the Malaysia [4].

Plant Profile:

Synonyms of *Hibiscus rosa-sinensis*:

Hibiscus arnottii Griff. ex Mast.; Hibiscus boryans DC.; Hibiscus cooperi auct.; hibiscus festalis Salisb., Hibiscus liliiflorus Griff. ex Mast., Hibiscus storckii Seem and Hibiscus rosiflours Stokes [5].

Scientific Classification of *Hibiscus rosa-sinensis* [6]

Kingdom	:	Plantae
Subkingdom	:	Tracheobiontas
Superdivision	:	Spermatophyte
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Subclass	:	Dilleniidae
Family	:	Malvaceae,
Genus	:	Hibiscus,
Spesies	:	Hibiscus rosa-sinensis L

Common names of *Hibiscus rosa-sinensis* [7]

Arabic : Bent EL-Kunsil, Ward EL-Jemal, Khatmah, Siniyah, Hab misk Seni, Pooq Seni
Chinese : Chinese Rosa, Fo sang, Fu sang, Zhu jin;
English : Chinese rosa, Chinese hibiscus, rose-of-China;
French : Hibiscus de chine, Hibiscus rose de Chine;
German : Chinesischer Roseneibisch;
Italian : Rose della Cina;
Japanese : Aka-bana, Fusou;
Spanish : Clavel japones;
Swedish : Hibiscus.
Hindi : Jasum, Jasson, Odhul.

Literature review:

Morphology *Hibiscus rosa-sinensis*:

Hibiscus rosa-sinensis are belonging the member of phylum 'Magnoliophyta', hibiscus is a flowering plant that has true leaves, root and stems, as well as carpels enclosing ovules, and to the 'class' Magnolipsida,[8]. Generally iIt was found in tropical and sub tropical regions. Hibiscus rosa-sinensis grow like small trees are called 'shrubs'. The plants are 4 meters tall, evergreen, and has ovate branches with stalks that are 10cm wide and 15cm long. Flower are found on long stalks, measure about 20cm wide. The central column includes a style that has 5 lobes at the tip, and its covers with numerous yellow anthers [9'10].

(Figure-1: Whole Plant of *Hibiscus rosa-sinensis*)

Traditional use:

- The hibiscus rosa-sinensis are used in Siddha and traditional Tamil System in South India.
- Hibiscus is used in the medication of herbal teas. It is antioxidant and most effective in the reduction of cholesterol situation [11].
- Hibiscus plants are used in the traditional drug for treating snap, diseases of respiratory tract and loss of appetite, and respiratory tract disease [12].
- In India, Hibiscus plants are leaves and flowers have been used for the anti-fertility, contraceptive, abortion, Demulcent, cough.
- The Hibiscus flower is used in the perfumes and sachcets [13].
- In Nigeria, Hibiscus has been used to treatment of constipation [14].
- The flower and leaves are used in this factory play major part in hair treatment [15].
- The hibiscus leaves are used in the treatment of burning sensations, skin disease, and constipation [16].

Chemical constituent:

The ***Hibiscus rosa-sinensis*** found to be anthraquinones, phenols, alkaloids, terpenoids, cardiac glycoside, saponins, protein, flanoides, tannins, quinines, free amino acid, carbohydrate, mucilage, essential oil reducing suger, and steroids [17].

The eatable protein of flower (61.6%) reported to the following nutrient composition (per 100g): moisture 89.8%, fat 0.36, nitrogen 0.064%, calcium 4.04 mg, crude fiber 1.56%, iron 1.69 mg,thiamine 0.031 mg, ascorbic acid 4.16 mg, riboflavin 0.048 mg and niacin 0.61 mg. The ***Hibiscus rosa-sinesis*** are reported to contain quercetin-3-di-O-B-D-glucoside; quercetin-3-7-di-O-B-D-glucoside; quercetin-3-O-B-D-sophorotriside; and Kaempferol and Kaempferol-3-O-B-xylosylglucoside [18].

Quantitative of phytochemical evaluated of the flower in ***Hibiscus rosa-sinensis*** are the amount of present of total flavonoids was 0.171mg/g, phenolics 0.092 mg/g, tannins 0.0735 mg/g, thiamine 0.072 mg/g, calcium 0.0127mg/g, protein 0.247mg/g, carbohydrates 0.356mg/g, niacin 0.075mg/g, ascorbic acid 0.0339mg/g, riboflavin 0.087mg/g, iron 0.771% and phosphorus 0.4113% [19].

The fresh flower in ***Hibiscus rosa sinesis*** gave 0.30-0.50 v/w% of essential oil. The different type of constituents are identified in the essential oil included: 50.568%: 1-idooundecane, 7.641%: neopentane, 11.056%: 1, 2-benezenedicarboxylicacid isodecyl octyl ester, 1.556%: 2, 2, 4-trimethyl 3-pentanone, 2.404%: 2-cyclopentylethanol, 1.543%: 2-propenamide, 1.543%: 1-4 butanedily ester, 3.993%: 1-tetrazole-2-ylethanone, 1.480%: 4-tirfluoroacetoxyoctane and 3.993% amylnitrite [20].

Pharmacological Activity:

Hibiscus rosa sinesis plants shows different pharmacological activities like: antioxidant, antifungal, antibacterial, dermatological effect, effect on hair growth, neuroprotective activity.

Antioxident Effect:

The radical scavenging extraction of the flower excerpts of ***Hibiscus rosa-sinensis*** where are determined on the percent inhibition of DPPH and ferric reducing antioxidant power assays. The result show the flower excerpt contained high quantum of antioxidant conditioning, depended on birth detergents. Water less excerpt of hibiscus is high tannin and anthocyanin contents, and showed high ferric reducing antioxidant power [21].

Antioxident eventuality of different solvent excerpts of ***Hibiscus rosa-sinenis*** was estimated by estimation of total flllavonoids contents, total phenolic contents, DPPH free revolutionary scavenging exertion and chance inhibition of linoleic acid oxidation capacity [22].

Dermetological effects:

The effect of china rose (HRSF), Calotropisgigantea (CGF) and Polyherbal formulation. (HCF) was examined in stimulating hair growth in stress induced alopecia animal model in comparision with minoxidil. On comparision HRSF, CGF, HCF and minoxidil, it's been observed that HRSF also as HCF herbal formulation application showed better growth that the patch with minoxidil [23].

Effect on hair growth:

The hair growth potential of aqueous flower extract was evaluated in ***Hbiscus rosa-sinensis***. In-vivo and In-vitro after 30dayes, 2% of extract resulted in a mean of 18.68= 0.3 mm of hair length, compared to 19.24= 0.4 mm with 2% of minoxidil in Wister rats. Invcrease in hair follicle length was observed In-vitro as 1.73=0.18 mm, in comparision to 1.95= 0.14mm by positive control, after 72 hours of incubation [24].

The leaf petroleum ether extract are effect was also investigated in albino of male rats. The 1% extract Resulted in 65% anagen, 2% catagen and 33% telogen in hair follicle population, compared to anagen 64%, catagen 1%, and 35% telogen in minoxidil treated group. Leaf

ethanolic extract contributed to 17= 1.2 mm hair length, compared to 19.36= 0.4 mm with minoxidil after 30 days [25].

Neuroprotective activity:

The methanolic extract of ***Hibisus rosa-sinensis*** roots are beneficial effects on the central nervous system in Swiss albino mice and Wistar rats. Using acetic acid to induce writhing, 200mg/kg i.p of extract resulted in an analegesic activity as inhibited the pain sensation by 78.5%, compared to 81.0% in 30 Mg/kg Diclofenac treated group [26].

The anxiolytic effect of roots extract was also demonstrated, as in elevated plus maze, mice spent more time in open arms. In addition, the extract's phytochemical were tannins, flavonoids, saponins, and glycosides [26].

Antimicrobial effect:

Antimicrobial activity of a 70 percent methanolic extract of ***Hibiscus rosa-sinensis*** prtals against Staphylococcus aureus, E. Coli, Bacillus subtilis, Pseudomonas aeruginosa, and Proteus vulgaris was investigated. Hibiscus rosa-sinensis showed antimicrobial activity against E. Coli and P.vulgaris, with zones of inhibition of 17.06 and 18.96mm, respectively [27].

Cytotoxic effect:

The genotoxic effect of extract was studied using a dose of 250mg/kg orally in mice (dose repeated every 24 hr for 7 day. In additional, a group of mice was used for subacute study). The extract rendered significant protection cyclophosphamide induced genotoxicity. Indicating significant anti-genotoxic effects [28].

The result of acute toxicity test revealed that maximum toxic dose was above 5 g/kg in mice, which show that plant extract was relatively safe [29].

Anti-bacterial activity:

The methanol extracts prepared from plant of ***Hibiscus rosa-seinensis*** leaves were shown to have antimicrobial activity against pseudomonas aeruginosa, Enterobacter aerogenes, Esherichia coli, and streptococcus pyogenes. These micro organism were obtained from infected skins, and the chemical compounds responsible for the antibacterial activity may be due to flavonoids, tannins, terpenoids, saponins, or alkaloids identified inthe study [30].

Antibacterial activity has been also observed using disc diffusion method against E.coli and S. Aureua at different concentration of methanolic flower and leaves extract varying from 31.25 to 500 mg/ml. These were compared with positive control gentamicin (1 mg/ml) and methanol is negative control [31].

Antifungal activity:

The previous studies, the methanol extracts prepared from the leaves of the ***Hibiscus rosa-sinensis*** were shown to have antimicrobial activity against Aspergillus niger, Candida albicans, Trichophyton rubrum and Candida parapsilosis. These fungi werevobtained from infected skins, and the chemical compounds responsible for the antifungal activity may be due to flavonoids, tannins, terpenoids, saponins, or alkaloids identified in the study [32].

The antifungal activity of root, leaves and flower ethyl alcohol extracts from ***H. rosa sinenis*** was also investigated. It was reported thet using disc diffusion method, the growth of both Candidaparapsilosis and Aspergillus niger for 1.5 vm were inhibited by flower extracts as highest zones of inhibition at concentration 10 microgram/ml. In addition, Candida parapsilosis was inhibited optimally for 2.2 cm by leaves extracts and for 1.5 cm by roots extracts at 10 microgram/ml and 7.5microgram/ml concentration respectively [33].

Material Methods:

Collection and Authentication of plant:

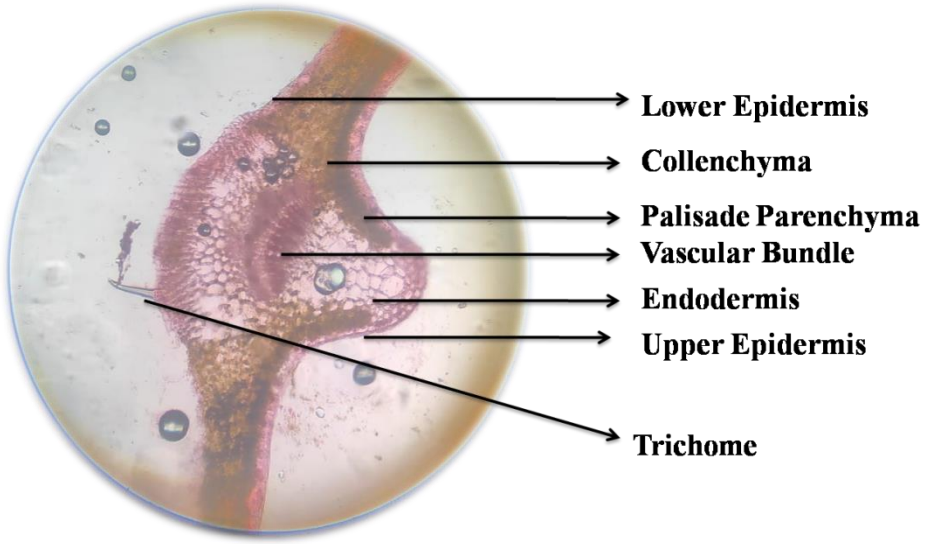
Fresh, mature, ***Hibiscus rosa-seinensis*** were are collected from the medicinal Garden of Pharmacy College, Itaura, Azamgarh, Uttar-Pradesh, India. The plant was further identified and authenticated from the department of botany, Banaras Hindu University, Banaras, India by a Voucher Specimen no. Malva. 2023/01.

Preparation of Plant Material:

The fresh leaves of hibiscus plant are collect from Pharmacy College, Azamgarh. Then wash the leaves with water, dry at room temperature for 1 to 2 weeks. The dry leaves are grinding by using mortar – pestle and leaves for convert in to powder sample.

Microscopy *Hibiscus rosa-seinensis* leaves:

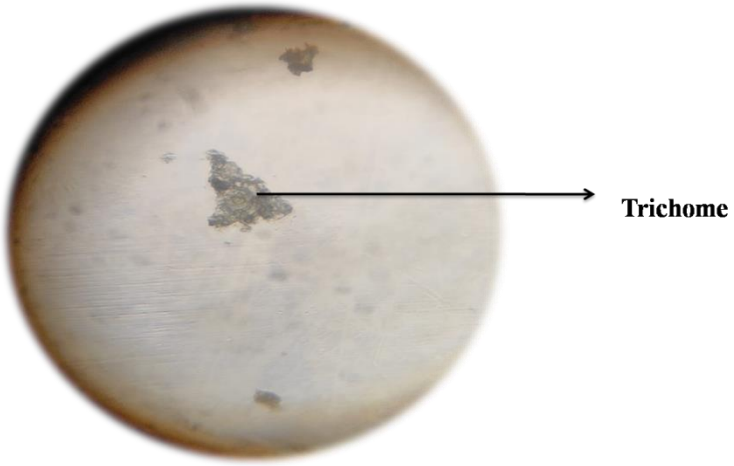
The hibiscus leaves were soaked in water to obtain sufficient moistening for section cutting. The free hand thin transverse section of leaves were prepared and collected in a borosilicate Petridis plate filled with water. The best section were preferred, mounted on glass slide using glycerine, covered slip and observed under light microscope. The presence of some anatomical characters and features were noted and photographed [34].



(Figure-2: T. S. of *Hibiscus rosa senisis* leaf)

Powder Microscopy:

The ***Hibiscus rosa senisis*** leaf powder was prepared by grinding by using mortar – pestle. Then the powder was placed on a glass slide, mounted in glycerine and observed under the microscope for the examination of microscopic features [34].



(Figure-3: Powder Microscopy of *Hibiscus rosa senisis* leaf powder**)**

Physiochemical Character:

The physic-chemical parameters of ***Hibiscus rosa senisis*** like as: a total ash, moisture content, swelling index, foaming index, water soluble extractive value, alcohol soluble extractive value was performed according to the standard methods [34'35'36].

Determination of Ash value:

Total Ash value:

Place about 2-4g of the ground air-dried material, accurately weighed, in a previously ignited and tared crucible (usually of platinum or silica). Spread the material in an even layer and ignite it by gradually increasing the heat to 500- 600°C until it is white, indicating the absence of carbon. Cool in a desiccator and weigh. If carbon-free ash cannot be obtained in this manner, cool the crucible and moisten the residue with about 2 ml of water or a saturated solution of ammonium nitrate R. Dry on a water-bath, then on a hot-plate and ignite to constant weight. Allow the residue to cool in a suitable desiccator for 30 minutes, then weigh without delay. Calculate the content of total ash in mg per g of air-dried material.

Determination of Foaming Index:

Reduce about 1 g of the plant material to a coarse powder (sieve size no. 1250), weigh accurately and transfer to a 500-ml conical flask containing 100ml of boiling water. Maintain at moderate boiling for 30 minutes. Cool and filter into a 100-ml volumetric flask and add sufficient water through the filter to dilute to volume.

Pour the decoction into 10 stoppered test-tubes (height 16cm, diameter 16mm) in successive portions of 1 ml, 2 ml, 3 ml, etc. up to 10 ml, and adjust the volume of the liquid in each tube with water to 10ml. Stopper the tubes and shake them in a lengthwise motion for 15 seconds, two shakes per second. Allow to stand for 15 minutes and measure the height of the foam.

Determination of Swelling Index:

Carry out simultaneously no fewer than three determinations for any given material. Introduce the specified quantity of the plant material concerned, previously reduced to the required fineness and accurately weighed, into a 25- ml glass-stoppered measuring cylinder. The internal diameter of the cylinder should be about 16mm, the length of the graduated portion about 125 mm, marked in 0.2-ml divisions from 0 to 25 ml in an upwards direction. Unless otherwise indicated in the test procedure, add 25 ml of water and shake the mixture thoroughly every 10 minutes for 1 hour. Allow to stand for 3 hours at room temperature, or as specified. Measure the volume in ml occupied by the plant material, including any sticky mucilage. Calculate the mean value of the individual determinations, related to 1 g of plant material.

Determination of Moisture content:

About 1.50 gm. of powdered drug was weighed accurately in a tared porcelain dish, which was previously dried at 105C in hot air oven to constant weight and then weighed. From the difference in weight, the percentage loss of drying with reference to the air-dried substance was calculated and recorded.

% Moisture content = Total moisture content / Total wt. of powder X 100

Determination of Extractive value:

Determination of alcohol soluble extractive value:

The maceration of powdered seed material was carried out with 30ml methanol in a closed conical flask for 24 h. The solution thus, obtained was filtered through filter paper and filtrate was evaporated to dryness. The residue was dried at 105 ºC, weighed and extractive value was determined with respect to air dried sample.

Determination of Water soluble extractive value:

The seed powder sample was macerated with 50ml distilled water in a closed conical flask for 24 h. After maceration, the filtrate was allowed to dry on a water bath and then at 105 ºC to obtain a constant weight. The percentage of water soluble extractive value was determined with reference to air dried seed material.

Fluorescence characteristics

Table 2 present the fluorescence characteristics of the leaves sample. The sample was green in colour, while their solvent extracts fluorescence differently in normal and UV lights. The shade dried, powdered sample of ***Hibiscus rosa senisis*** exhibited distinct fluorescence characteristics as could be seen in the data provided in the **Table 2.**

Preliminary Phytochemical Analysis: [37]

Test for Carbohydrate:

Molish's Test:

To 2-3 ml. aqueous extract, add few drop of alpha-naphthol solution in alcohol, shake and conc. H_2SO_4 from side of the test tube, Violet ring is formed at the junction of two acid.

Test for Amino Acid:

Ninhydrin Test:

Heat 3 ml test solution and 3 drops 5% Ninhydrin solution in boiling water bath 10 min. Purple or bluish color appears.

Test for Flavonoids:

Shinoda Test:

To dry powder or extract, add 5ml. 95% ethanol, few drop conc. HCL and 0.5 g magnesium turning, Pink color observed.

Test for Alkaloids:

Dragendorff's test:

To 2-3ml. filtrate, add few drops Dragendorff's reagent. Orange brown ppt is formed.

Test for Steroids:

Salkowski reaction:

To 2ml of extract, add 2ml. chloroform and 2ml. conc. H_2SO_4, Shake well Chloroform layer appears red and acid layer show greenish yellow fluorescence.

Test for tannins:

To 2-3ml. of aqueous or alcoholic extract, add few drops of 5% $FeCl_3$ deep blue-black color.

Test for glycoside:

Test for cardiac glycoside:

Legal's test (Test for cardenoloids):

To aqueous or alcoholic extract, add 1 ml. pyridine and 1ml nitroprusside. Pink to red color appears.

RESULT:

Table 1: Physicochemical parameter of *Hibiscus rosa senisis* leaves:

S. No.	Physico-chemical Parameter	Result
1.	Total Ash	16.33%
2.	Foaming Index	Nill
3.	Swelling Index	0.7cm
4.	Moisture Content	10%
5.	Alcohol Soluble Extractive Value	16%
6.	Water Soluble Extractive Value	9%

Table 2: Fluorescence characteristics of *Hibiscus rosa senisis in different solvents**

S.NO.	Solvent	Day Light	UV Light (254nm)
1	Hexane	Green	Pale green
2	Benzene	Dark green	Green
3	Chloroform	Yellowish green	Yellowish green
4	Ethyl acetate	Green	GREEN
5	Petroleum ether	Light green	Green
6	Acetone	Green	Dark green
7	Ethanol	Pale green	Green
8	Water	Green	Yellowish green
9	Acid (50% H_2SO_4)	Greenish brown	Greenish black
10	Methanol	Green	Dark green
11	Leaves powder as such	Pale brown	Pale brown

***Shade dried powdered material**

Table 3: Preliminary phytochemical investigation of *Hibiscus rosa senisis* leaves:

S. No.	Test	Aqueous Extract	Methanol Extract
1.	Alkaloids	Present (+)	Present (+)
2.	Glycosides	Absent (+)	Present (+)
3.	Carbohydrates	Present (+)	Present (+)
4.	Tannins	Present (+)	Present (+)
5.	Flavonoids	Present (+)	Present (+)
6.	Amino Acid	Absent (-)	Absent (-)
7.	Steroids	Absent (-)	Absent (-)

Discussions:

Phytochemical analyses of ***Hibiscus rosa senisis*** leaf was performed. The result of phytochemical analysis of this extract revealed the presence of tannins, alkaloids, glycosides, flavonoids and carbohydrates.

The observations made in the present study have clearly showed the bioactive potential of the plant ***Hibiscus rosa senisis*** in medicine. The leaves sample exhibited fluorescence

characteristics, ash values, swelling index, foaming index, moisture contents and exhaustive extractive values. Leaves sample exhibited qualitative phytochemical properties.

ACKNOWLEDGEMENT

Authors express sincerely thanks to Mr. Bajrang Tripathi, Manager of Pharmacy College, Azamgarh for providing research facilities.

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