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Formulation and Various Pharmacological Properties of Hibiscus Rosa Sinesis

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ABSTRACT

The natural plant products are widely use now a days because of increasing the burden of diseases. Hibiscus rosa sinesis Linn (family malvaceae), is a plant which is widely distributed throughout the world. Its leaves, barks, flowers and roots have been use in the Indian traditional system as medicine to treat various diseases. In this context a technique was employed for the formulation of gel lby using Hibiscus rosa sinesis. It mainly contains active constituent like steroids, flavonoids, tannins, mucilages, reducing sugar, riboflavin. Hibisucs rosa sinesis plants posseses Antioxidants, Antimicrobial, Antidiabetic, Antiulcer, heapatoprotective, Antifertility, Antigenotoxic and anti-inflammatory properties, which helps in the treatements of many diseases. The leaves and flowers are observed to be promoters of hair growth and aid in healing of ulcers. Flowers have been found tobe effective in the treatement of arterial hypertension and to have significant antifertility effect. Hibisucs rosa sinesis, an ornamental plant posseses different pharamacological activities. Hibiscus rosa sinesis is one of the miraculous herbal medicine that is found to have antimicrobial properties. This article compile all the information related to Hibiscus rosa sinesis Linn, and evaluation of hibiscus rosa. Hibiscus rosa sinesis commonly used for some diseases such as hypertension and as antidiabetic herbal medicine.

KEYWORDS: *Hibiscus Rosa sinesis, Antiulcer activity, China rose, Malvaceae, Tannins*

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INTRODUCTION

Hibiscus rosa-sinensis,Linn. (Malvaceae) known as China rose is an important medicinal plant. It is an evergreen woody, glabrous, showy shrub 5-8 feet in height. Leaves are bright green, short petiolated, ovate or lanceolate, more or less acuminate; irregularly and coarsely serrated towards the top, entire near base, glabrous on both sides, a few minute stellate hairs on the nerves beneath stipules, lanceolate-subulate and glabrous. Pedicels are axillary, solitary, and longer than the leaves and joined above the middle. Flowers are solitary, axillary, bell shaped, large, 4-6 inches in diameter with pistil and stamens projecting from centre. Leaves are used as emollient, anodyne, and laxative in Ayurveda. In South Asian traditional medicine, variousparts of the plant are used in the preparation of a variety of foods.[1] India is rich source of medicinal plants and is called "Botanical Garden of the World" with enormous wealth of biodiversity. There are almost 45,000 plant species recorded in India so far of which 7,500 species have been used for medicinal purposes. Hibiscus rosasinensis is a perennial ornamental plant available throughout India. It is belonging to Malvaceae family and grows as an evergreen herbaceous plant. Hibiscus rosa-sinensis, is the national flower of Malaysia. The herb Hibiscus rosa-sinensis is native to China. The genus Hibiscus comprises about 275 species in the tropics and subtropics. With attractive and colourful flowers, plants of Hibiscus are used in traditional medicine. Several articles and ancient literature have shown that the flowers of this plant possess antifertility activity, like antiimplantation, abortifacient, in rodents. Leaves are used as emollient, anodyne, and laxative in Ayurveda[2]

Hibiscus rosa sinensis Linn (Malvaceae) is an ornamental plant, native to China grown as an evergreen herbaceous plant. For the medicinal purpose, red flower variety of hibiscus is mostly preferred. It possesses various pharmacological activities such as radical scavenging, antipyretic and anti-inflammatory. Leaves and flowers of Hibiscus show potential against hair fall, and hypoglycemic activity and also having healing activity against ulcers4. The flowers also possess anti-implantation and antispermatogenic activities5,9. It is also being known to contain an anthocyanin pigment, cyanidin diglucosides, carotene, thiamine riboflavin, niacin and ascorbic acid. That is why it is traditionally used as antifertility agent from ancient time. The extracts of Hibiscus rosa sinensis have also been shown a protective effect against the tumour promotion stage of cancer development16 In this study, we evaluate the genotoxic effect of Hibiscus rosa sinensis extract in vivo, by induction of micronuclei in blood polychromatic erythrocytes of Balb/c mice.[3] The genus Hibiscus belongs to the Malvaceae family, and includes asmany as 250 annual

and perennial herbaceous species, along with a number ofwoody shrubs and small trees. Chromosome number in the species varies from n = 12 to _144, but is most commonly 14 or 18 . Gene duplication within the nuclear RNA polymerase II (rpb2) gene family and DNA sequence of the chloroplast ndhF coding region and rpl16 intron support a paraphyletic as opposed to a monophyletic origin. While most Hibiscus spp. are grown as ornamentals, H. cannabinus L., H. macrophyllus Roxb. and H. Tiliaceus L. are all cultivated as a source of fibre and others, including Hibiscus rosa-sinensis, for their pharmaceutical properties. The most highly appreciated ornamental species are H. Syriacus L. (common names Althea or Rose of Sharon), H. sinosyriacus Bailey, H. moscheutos L., H. mutabilis L. and H. rosa-sinensis, the latter showing the widest range in flower colour and shape variation.[4]

Hibiscus rosa sinensis mucilage is also used as disintegrate and super disintegrate in the pharmaceutical preparations. Disintegrates are the substances which on addition to the tablets and some other encapsulated formulations in the aqueous environment, shows the disintegration of the tablet and capsule in small particles or fragments, due to which the surface area is increased and results in more rapid release of drug substance. In terms of fast dissolving tablets, disintegration has their own importance and it has received considerable attention. They work just opposite to the efficiency of binder. Their effect is enhanced when added during granulation. The tablets are breakdown into small granules and granules further disintegrate into small pieces in the solution by adding disintegrate either intragranulary or extragranulary.[5] Fast dissolving tablets required less amount of water. It dissolved into the saliva within few seconds. These tablets are beneficial for Geriatric and Pediatric patients. The herb Hibiscus rosa sinensis L. (Malvaceae) is native to China. Many species of Hibiscus are grown for their showy flowers. It is a shrub widely cultivated in the tropics as an ornamental plant and has several forms with varying colours of flowers. Hibiscus has also medicinal properties and takes part as a primary ingredient in many herbal teas. The red flowered variety is preferred in medicine. The taxonomy and the name of Hibiscus flowers in different region were depicted in Table 1. There was various studies reported that variety of Hibiscus plants have different medicinal properties. This review mainly focused on the therapeutic potential of the Hibiscus rosa sinensis plant and its applications.[6] Plants are one of the most important sources of medicines. The medicinal plants are extensively utilized throughout the world in traditional system of medicines "Ayurveda". Medicinal plant is the most exclusive source of life saving drugs for majority of the world‟s population. They continue to be an important therapeutic aid for alleviating the ailments of human kinds. The search for defence mechanism, longevity and remedies to relieve pain and discomfort drove early man to explore these immediate natural surroundings.[44]

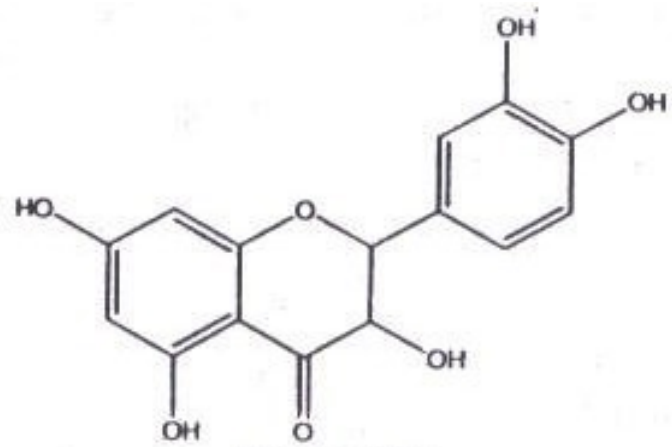
CLASSIFICATION:

Sr. No.	Kingdom	Plantae-Plants
1.	Subkingdom	Tracheobionta-Vascular plants
2.	Super division	Spermatophyta-Seed plants
3.	Division	Magnoliophyta-Flowering plants
4.	Class	Magnoliopsida-Dicotyledons
5.	Subclass	Dilleniidae
6.	Order	Malvales
7.	Family	Malvaceae-Mallow family
8.	Genus	Hibiscus L.-Rosemallow
9.	Species	Hibiscus rosa sinensis L.-Shoeblackpla.

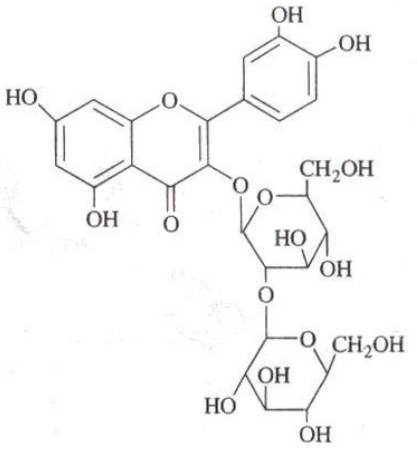
Recent years have witnessed enhanced research work reported on plants and plant products. In this regard, plants with traditional therapeutic usage are being screened more efficiently to be considered as a substitution or as a better alternative for chemical based food preservatives. Additionally, plants can be an excellent source of natural antioxidants and can be effectively used in the food industry as a source of dietary supplements or as natural antioxidants to preserve the quality and improve the shelf-life of food products.[7]

Phytochemical Analysis:

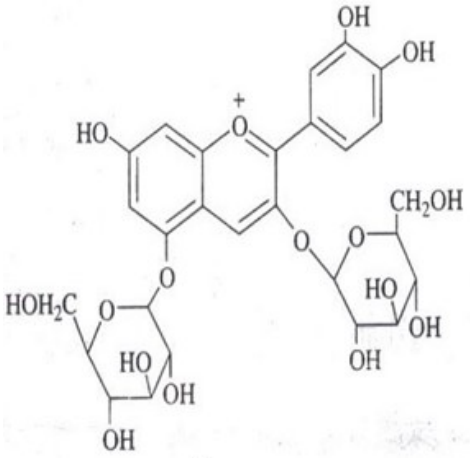
Phytochemical analysis showed that Hibiscus rosa-sinensis contained tannins, anthraquinones, quinines, phenols, flavanoides, alkaloids, terpenoids, saponins, cardiac glycosides, protein, free amino acids, carbohydrates, reducing sugars, mucilage, essential oils and steroids.[40] Hibiscus rosa-sinensis contained cyclopropanoids, methyl sterculate, methyl-2-hydroxy sterculate, 2- hydroxysterculate, malvalate and beta-sitosterol. The major anthocyanin in the flower was cyanidin 3-sophoroside. The Hibiscus rosa-sinensis revealed that amount of flavonoids was 0.171 mg/g, total phenolics 0.092 mg/g, tannins 0.073 mg/g, carbohydrates 0.356 mg/g, protein 0.247 mg/g, thiamine 0.072 mg/g, niacin 0.075 mg/g, ascorbic acid 0.0339 mg/g, riboflavin 0.087 mg/g, calcium 0.0127%, phosphorus 0.4113% and iron 0.771%[63]. The flower extract of Hibiscus rosa-sinensis (Red) contained 0.678±0.14% phenols, 0.51±0.16 % alkaloids and 7.5±0.20 % tannins. While, the flower extract of Hibiscus rosa-sinensis (White) contained 0.680±0.11% phenols, 0.50±0.18 % alkaloids and 8.9±0.21 % tannins, and the flower extract of Hibiscus rosa-sinensis (Yellow) contained 0.678±0.16% phenols, 0.48±0.16 % alkaloids and 8.5±0.20% tannins.[41,42]



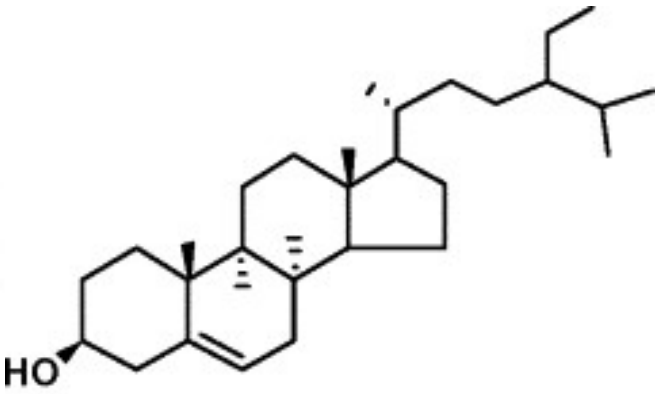
Quercetin



Quercetin-3-diglucoside



Cyanidin-3,5-



β-sitosterol

Fig.1: Habiscus rosa sinesis

Fig.2. Phenotypes of Hibiscus rosa sinensis: red (most common), pink, white, orange, and yellow[38]

MATERIALS AND METHODS:

Samples:

Fresh flowers of hibiscus and *Cassia* (*S. bicapsularis*) with no apparent physical, insect or microbial damage were collected from the University garden of Universiti Sains Malaysia, Penang. The flower petals were carefully removed and were freeze-dried (freeze dryer Model, LD53,) for 48 h at −50 °C. Samples were powdered (mesh size 30), covered with aluminum foil (to avoid exposure to light) and stored at 4 °C until analysis (performed within 24 h).

Antioxidant analysis:

Extract preparation:

Distilled water and ethanol (99.7%) were used as solvents for extraction of antioxidant compounds. A known weight of the powdered sample was mixed individually with the solvent and was shaken in an orbital shaker (Lab Companion, Model SI 600R Bench top shaker) at 160 rpm for 24 h at room temperature. Extracts were filtered through Whatman filter paper (Whatman No. 41, UK). All the filtrates were collected and transferred to screw-top glass bottles (with Teflon caps) and were covered with aluminum foil to avoid exposure to light.

Determination of total phenolic content, tannins, flavonoids and flavonols:

The total phenolic content of the flower extracts were determined based on the Folin–Ciocalteu (FC) method (Singleton and Rossi, 1965). In brief, 400 µL of the sample extract was mixed with 2.0 mL of FC reagent (10 times pre-diluted). Further, after incubation for 5 min at room temperature, 1.6 mL of (7.5%, w/v) sodium carbonate solution was added and the solution was mixed thoroughly and incubated for 60 min at room temperature. A suitable calibration curve was prepared using standard gallic acid solution. All the results were expressed as mg Gallic acid equivalents (GAE) per gram of sample. For total tannins, the vanillin–HCl method was. Briefly, 1 mL of the sample extracts was treated with 5 mL of reagent mixture. The color developed was read after 20 min. Suitable standard calibration curve was prepared using catechin (20–400 µg/mL) and results were expressed as mg Catechin equivalent (CE) per 100 g dry weight of the samples, respectively.

Total flavonoids in the sample extracts were determined using the aluminum chloride method as described in the report of Liu et al. (2008). In brief, for 500 mL of the sample extract solution, 2.5 mL of distilled water and sodium nitrite solution (5%, w/v, 150 mL) were added to the mixture. This mixture was maintained for 5 min., followed by addition of 300 mL of aluminum chloride (10%, w/v) and again incubated for 6 min. Followed by this, 1 mL of sodium hydroxide (1 M) was added and the mixture was diluted with 550 mL of distilled water. This solution was mixed vigorously and the absorbance of the mixture was measured immediately at 510 nm using a UV–visible spectrophotometer. Results of the total flavonoid content were expressed as mg Catechin equivalents (CE) per 100 g of dry weight of the sample.

Total flavonols in the sample extracts were evaluated based on the method described by Miliauskas et al. (2004) with slight modifications. Briefly, 1 mL of 0.15-0.05 mg/mL quercetin methanol solution with 1 mL of 2% aluminum trichloride and 3 mL of 5% sodium acetate were mixed to obtain a quercetin calibration curve. After 150 min and incubation at 20 °C, This procedure was repeated using 1 mL of the sample extract (1 mg/mL) instead of quercetin solution. Results obtained were expressed as mg Quercetin equivalent (QE) per 100 g dry weight of samples.

To determine total anthocyanins, the spectrophotometric method detailed by Abdel-Aal and Hucl (1999) was employed. Briefly, anthocyanins were extracted using acidified methanol (methanol and 1 M HCl, 85:15, v/v) with a solvent to sample extract ratio of 10:1. This was centrifuged and the absorbance was measured at 525 nm using a UV–visible spectrophotometer against a reagent blank. Cyanidin-3-glucoside (5, 10, 15, 20, and 25 mg/L, r^2 = 0.9982) was used to prepare for the standard calibration curve. Total anthocyanin contents in the flower extracts were expressed as mg cyanidin-3-glucoside equivalents (c-3-gE) per 100 g dry weight of samples.[40,9]

Plant Material:

Leaves of Hibiscus rosasinensis were collected from Gr. Noida (Uttar Pradesh, India) in the month of October-November, 2014. The plant was identified by Biotechnology Department, Gautam Buddh University, Gr. Noida and voucher specimens were deposited in that Department.

Hibiscus rosa sinensis (China rose) was procured from the local area of Greater Noida, India. Collected leaves was carefully washed and dried under shade for 24 h and then further dried in oven at 30-40°C. Size was reduced with the help of grinder. Powdered leaves were passed through sieve no. #22 and then used for further evaluation. Extraction of mucilage includes 3 steps.

Swelling Index:
It was calculated by weighing a butter paper of size 2X2 cm. then butter paper was dipped in a Petridish containing water and reweighed. After this 10 mg of the powdered sample was kept in a butter paper placing this on a Petridish containing 15 ml of water and the swelling index was calculated after 24 h and the final result was calculated using the formulae .[8]

$$\text{Swelling Index} = \frac{\text{Initial Weight} - \text{Final weigh}}{\text{Initial Weight}} \times 100 \quad (1)$$

pH of Mucilage The pH of 1% w/v solution in water was determined using digital pH meter

Short-term skin moisturizing test:
The moisturizing efficacy of 0.2% HR mucilage and 0.2% HM mucilage were examined and compared with the commercial moisturizers; 5% glycerin, 5% propylene glycol (PG), 5% butylene glycol (BG) and 0.2% hyaluronic acid (HA). The tested skins were prepared from the back of 6 months age pigs, removed the fat layer off and cut into 3 x 3 cm. The prepared skins were incubated at room temperature (25 ± 1°C) with 50-60% RH at least 30 min before use. Then, 60 µL of each test sample were applied on the skin surface. The moisture content was measured before applying and at time 10, 20 and 30 min after applying the sample using Corneometer. All of the measurements were done in triplicate. Skin moisturizing efficacy (%) was calculated as: Skin moisturizing efficacy (%) = [(At - A0)/A0] x 100 where At = skin capacitance at a specified time and A0 = skin capacitance at the base line. This method had been modified from O'Goshi et al and Leelapornpisid et al. [10]

Estimation of chlorophyll and carotene:
1 g of leaf sample was weighed and was ground in pestle and mortar with 5 ml distilled water to a paste. The contents were transferred to a centrifuge tube and the total volume was made up to 10 ml with distilled water. 0.5 ml from the tube was transferred to a tube containing 4.5 ml of 80% acetone. The contents were centrifuged at 4000 rpm for 15 min. The absorbance of the supernatant was measured at the following wavelengths-645, 663, 490, 638 nm and the content of chlorophyll was calculated.[11]

PROPERTIES OF HIBISCUS ROSA SINESIS:
Antioxidant activity:
Ethanolic (95.0%) extract of flowers strongly scavenged hydrogen peroxide for 96 ± 2.35 % inhibition with a concentration of 50 µg/ml while the standard antioxidant, ascorbic acid produced 76.33 ± 1.25 % radical scavenging activity at 100 µg/ml concentration. It was reported that molecules identified by GC-MS analysis mainly belonged to classes of alkaloids, tannins, steroids, glycosides, and flavonoids, and may be also the reason behind the high radical scavenging activity. Free radicals such as those generated from hydrogen peroxide play a crucial role in the progress of tissue damage. Any substances which have the ability to remove these, such as H. rosa sinensis phytochemicals, will protect the cell system and components from cytotoxic damage. DPPH radical scavenging activity was observed using 80% methanol flower extract as 75.46 ± 4.67 %, and 80% ethanol flower extract as 64.98 ± 2.11%, compared to 77.54 ± 4.77% for BHT as a positive control. The scavenging of DPPH free radicals was measured at 515 nm using a UV visible spectrophotometer. Moreover, the total phenolic contents of the methanolic and ethanolic extracts were 61.45 ± 3.23 and 59.31 ± 4.31 mg/100g dry extract, and total flavonoid contents were 53.28 ± 1.93 and 32.25 ± 1.21 mg/100g dry extract respectively. Because it was observed that methanolic extract had higher amount of phenolics and flavonoids as well as contributed to higher scavanging activity than ethanolic extract, this strongly suggest that they are responsible for the anti-oxidant activity. According to another study, aqueous stem extracts resulted in 15.1 ± 4.5 scavenging activity against DPPH radicals compared to methanolic extracts which exhibited only 9.75 ± 1.15 scavenging activity. [38] The antioxidant activity of the plant extracts was examined on the basis of the scavenging effect on the stable DPPH free radical (Brand-Williams *et al.,*1995). DPPH (0.002%) was freshly prepared in methanol and kept in the dark for 30 minutes.[24]

Genotoxicity Test:
Genotoxicity was induced by the administration of cyclophosphamide in six group of animal (each group contained 5 mice individually). To investigate the protective effect of Hibiscus rosa sinensis (HRS) against the Genotoxicity induced byCP, group 1 mice received drinking water (1 ml per day by gavage) for 15 consecutive days and were treated intraperitoneally (i.p.) on day 15 with 0.9% NaCl. Group 2received drinking water (1 ml per day by gavage) for 15 days, and mice were treated with CP (20mgkg_1 body weight) on day15. Groups 5 and 6 received extract of Hibiscus rosa sinensis (group 4, 200mg/kg body weight; group 5, 400mg/kg body weight) by gavage for 15 days before treatment with CP on day 15.[3]

Estrogenic and Antiestrogenic Activity:
Ethanol extract at 400 mg/kg was found to be active amongst the two treatments in post-coital antifertility testing. Hence, it was subjected to a detailed investigation for potential estrogenic and antiestrogenic activity.[20]

Ethanol extract at 400 mg/kg was found to be active amongst the two treatments in post-coital antifertility testing. Hence, it was subjected to a detailed investigation for potential estrogenic and antiestrogenic activity. The uterine weight and vaginal cornification method was employed for this assay (17). Colony-bred immature ovariectimized female albino rats, 21–23 days old, weighing between 35 and 45 g, were used (18–22). They were divided into four groups, consisting of six rats each. The first group served as a control and received vehicle only The uterine weight and vaginal cornification method was employed for this assay (17). Colony-bred immature ovariectimized female albino rats, 21–23 days old, weighing between 35 and 45 g, were used (18–22). They were divided into four groups, consisting of six rats each. The first group served as a control and received vehicle only (acacia 1%). The second group received ethinyl estradiol in olive oil, 1 µg/rat per day, subcutaneously. The third group received the ethanol extract at a dose of 400 mg/kg body weight. The fourth group received, in addition to ethinyl estradiol, a test dose of the ethanol extract at 400 mg/kg body weight. [46]

Antiulcer Activity:
All the extracts of H. rosa sinensis roots were screened for antiulcer activity by pyloric ligation method [16,17] in rats. The animals were divided into eight groups (n=6) and made to fast for 18 hours. Group I served as control and received vehicle. Group II-VII received petroleum ether, alcohol and aqueous extract (250 and 500 mg/kg/p.o.) respectively. Group VIII received the standard drug, lansoperazole (8 mg/

kg/p.o.). Four hours after the pyloric ligation, the animals were sacrificed by decapitation. Then the stomach was cut open along the greater curvature and the inner mucosal membrane was examined for ulcer lesions, ulcer score and the parameters like gastric volume, pH, free acidity and total acidity were determined and compared with control.[12]

Anti- anxiety activity:

Discussed about the anxiety induce exploratory and locomotar activity performed by Hibiscus rosa sinensis. It is a medicinal plant which has a refrigerant and calming effect. According to this study, for determining the exploratory behaviour, alcoholic and chloroform extracts of Hibiscus rosa sinensis were used. From the result the difference is obtained that the ethanolic extract showed better performance in comparison to chloroform extract. There is no effect of the both extract on the urination and defecation of animals. According to the result Hibiscus rosa sinensis showed anti anxiety activity.[39]

Antibacterial assay:

Disc Diffusion: In this method aqueous and organic flower extract were introduced into a disc 0.5 mm (himedia) and then allowed to dry. The disc was completely saturated with the test compound at concentration of 40 mg/ml. Then this disc was placed directly on the surface of Muller Hinton agar plates swapped with the test organism and the plates were incubated at 34°C for 24h. Screening of antibacterial activity of the plant extract was performed by disc diffusion technique which is highly effective for rapidly growing microorganisms. All the microorganisms used in this study were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka, Bangladesh. The sample solution of the material to be tested was prepared by dissolving a definite amount of material in respective solvent (1ml) to attain a concentration of 500µg/disc.[19]

Anticonvulsant activity:

The ethanolic extracts of flower of Hibiscus rosa sinensis which is used to exhibit anticonvulsant activity. The anticonvulsant activity lied in the acetone soluble part of ethanolic extract of Hibiscus rosa sinensis flower according to bioassay guided fractionation. This fraction saves the animals from lithium-pilocarpine, electro shock and kindling, and pentylene tetrazole which are responsible for inducing convulsion in mice. It was proved that the ethanolic extract of Hibiscus rosa sinensis showed significant anticonvulsive activity. [39]

Anti-microbial study:

Antibacterial activity was investigated using disc method. With the help of sterile wire loop, the test was inoculated into a test tube containing Mueller Hinton broth. As per the composition, Mueller Hinton Agar was prepared by using sterile distilled water and was sterilized at 121oC at 15lb pressure for 15 min in an autoclave. The medium was cooled at room temperature and poured in sterile petri plates and were allowed to solidify. Bacterial inoculums was swabbed over the medium using sterile cotton swab. Sterile disc was placed on medium, on which 20 µl of complex suspension was added. Zone of inhibition were observed and measured after incubation at 30oC for 18-20 hrs.[43]

Antidaibetic Activity:

Top three countries to have highest number of people with diabetes are India (31.7 and 79.4), China (20.8 and 42.3) and U.S. (17.7 and 30.3) in millions respectively.[1] Diabetes is classified mainly as Type 1 diabetes or insulin dependent diabetes mellitus (IDDM), Type 2 diabetes or non-insulin dependent diabetes mellitus (NIDDM) and gestational diabetes mellitus (GDM). Type-1diabetes that affects more than 5.3 million people worldwide[2]. Approaches to the control of diabetes mellitus are aimed at prevention of hyperglycemia involving dietary manipulation and use of plant therapies. More than 1200 plants are used worldwide in traditional medicine for their alleged hypoglycemic activity[4]. Few reviews are published on medicinal plants useful for treatment of diabetes. Isolation of plant extracts for antiviral, antidiabetic and antioxidant properties have been reported.[45]

Wound healing activity:

Wistar albino rats (150–250 g body weight) of either sex were selected. They were acclimatized for a period of 7 days to the laboratory environment. They were housed individually in polypropylene cages at 23°C ± 1°C in 12:12 h dark: light cycle, with free access to standard pellet feed and water. [47]

A Extraction of components:

Aqueous Extraction: Air dried powder of flower Hibiscus rosa-sinensis is taken and weighed 4 g of powder was boiled in 100 ml distilled water and filtered with Whatman filter paper no. 1. The filtrate was collected and then stored at 5°C.

Solvent Extraction: 4g air dried powder of flower of Hibiscus rosa-sinensis was placed in 100 ml of organic solvent Hexane in plugged conical flask. After that it was kept in a rotary shaker at 190-220 rpm for 24 hrs. Then filtered and centrifuged it at 10000 rpm for 5 min. The filtrate was collected and the solvent was evaporated by solvent distillation apparatus. It was then stored at 40°C in air tight bottles for further study.[13]

Drying method: Hibiscus flowers were cut and air dried at room temperature 3-5 days. The dried flowers were ground in an electric grinder to fine powder and sieved with a 2mm sieve. The dried powder was kept in air tight containers at room temperature for incorporation in sample.[14]

Sample preparation: Ingredient required for biscuit are given in. Refined wheat flour was replaced with hibiscus powder at the levels of 5%, 10% and 15%.

Test materials: Hibiscus flowers and Lettuce were purchased from commercially available herbalist and markets. Extraction of Hibiscus flowers and Lettuce Decoctions were prepared from 15 g of dry and milled Hibiscus flowers and Lettuce by boiling for 15 min. in 1000 ml distilled water. After boiling it has been cooled at room temperature and filtered through a filter paper. The applied concentrations (5 g/l and 10 g/l) were prepared by dilution of these stock solutions. Antimitotic activity Allium cepa has been used for evaluating cytotoxic properties since the early 1920's (GRANT 1982). Small onion bulbs are carefully unscaled and cultivated on top of test tubes filled with different concentrations from decoction of flowers. Tap water and Lettuce has been used as control groups. The test tubes were kept in an incubator at 24±2°C. After 72 h the roots were counted and their lengths were measured for each onion. The emerged roots has been fi xed with glacial acetic acid/absolute alcohol (1/3 v/v).[15] H. rosa sinensis flowers were collected from Tiruchirappalli district, Tamil Nadu, India. The plant was authenticated by Dr. S. Kalavathy, Associate Professor, Department of Botany, Bishop Heber

College, Trichy, and molecular taxonomy of the plant was done by sequencing the 18SrDNA of the plant. H. Rosa sinensis L., flowers were shade-dried at room temperature, pulverized by a mechanical grinder, sieved through 40-size sieve mesh. 500 g of fine flower powder was suspended in 1500 ml of ethanol for 24 h at room temperature. The mixture was filtered using a fine muslin cloth followed by filter paper (Whatmann No: 1). The filtrate was placed in a water bath to dry at 40°C and the final ethanol-free clear residue was used for the study.[16]

Macroscopic study:

The fresh plant was taken for various macroscopic organoleptic evaluation like colour, odour, size, shape, taste, appearance, texture, fracture etc.

Microscopy Study:

Qualitative microscopic evaluation was carried out by taking transverse sections of fresh root of Hibiscus rosa sinesis. The thinnest section was selected and cleared by boiling with chloral hydrate solution for 20mins and then carefully stained with phloroglucinol and HCl (1:1). Then mounted on a slide and a cover slip was placed over it and observed the different histological characters.[17] The plant samples were collected from Shahid Chamran University farmlands in Khuzestan Province, Iran IN June, 2009. The taxonomic identification of these plants was done comparing with existing herbarium in Biology Department of Shahid Chamran University.[18]

Abscisic Acid Determination:

Petals (80-100 mg FW) were extracted with distilled water (water:tissue ratio 10:1v/w) for 16 h at 4°C in the dark. Quantitative analysis was performed on crude aqueous extracts using a solid-phase radioimmunoassay based on a monoclonal antibody (DBPA1) raised against free (*S*)-ABA, as described previously (Vernieri et al., 1991).[21]

Evaluation on floating tablets Angle of repose:

The frictional force in a loose powder was measured by conventional funnel method. The powder was allowed to flow through the funnel fixed to a stand at definite height. The angle of repose was then calculated by measuring the height and radius of the heap of powder formed using the formula 1.

$\tan \theta = h/r$

$\theta = \tan (h/r)$ ……(1)

Where,

θ = angle of repose, h = height, r = radius[22]

Histopathology and Light microscopy:

The testis were fixed in 10% formalin and embedded in paraffin. Five-micron thick sections were prepared and stained with Hematoxylin and Eosin (H&E). The specimens were examined under Olympus/3H light microscope-Japan.[23]

Preliminary chemical:

analysis was performed to identify the bioactive components. Petroleum ether (PEHRS) and methanol extracts (MEHRS) of Hibiscus rosa sinensis at different doses of 200 and 400mg/kg was suspended in distilled water and administered orally.[25]

Test for Flavonoids:

Crude extract (1 g) was added with 5 ml ethanol, boiled and filtered. A few drops of concentrated HCl and magnesium tape ribbon (1-2 cm) were added. Colours ranging from orange to red indicated flavones, red to crimson indicated flavonols and crimson to magenta indicated flavonones.[26]

Acute toxicity studies (LD50):

Acute toxicity studies of the plants extracts were conducted for doses of the plant extracts administered to animals in this study. Oral LD50 determination was done by the method of lorke (1983) using rats. No mortality in the rats was observed up to 3000 mg/kg.[27]

Extraction of mucilage:

The fresh Hibiscus rosa-sinensis leaves were collected and washed with water. The leaves were crushed and soaked in water for 5–6 h, boiled for 30 min and left to stand for 1 h to allow complete release of the mucilage into the water. The mucilage was extracted using a multi layer muslin cloth bag to remove the marc from the solution. Acetone (in the quantity of three times the volume of filtrate) was added to precipitate the mucilage. The mucilage was separated, dried in an oven at 35°C, collected, grounded, passed through a # 80 sieve and stored in a desiccator at 30 °C & 45% relative humidity till use.[28]

Statistical analysis:

All values were expressed as Mean ± SE. (n = 10 in each groups). One way ANOVA was applied to test for significance of biochemical data of the different groups. Significance is set at $p < 0.001$.[29]

Hibiscus Leaf Spot:

Hibiscus leaves with small sunken light brown to brown spots (1-2 mm diameter) having halo-like yellowing around the lesions were collected in the germplasm collection and breeding nurseries of IPB. Infected leaf samples were washed with distilled water and incubated overnight in Petri dishes lined with moist sterile filter paper to allow growth of the causal organism.[30]

Formulation and evaluation of hydro gel from hibiscus rosa sinesis:

Evaluation of Gel: The prepared gel was evaluated on the basis of following parameters like : Physical evaluation, measurement of pH, spreadibility, viscosity, skin irritation, extrudability etc.

1. Physical evaluation - Physical parameters such as color and appearance will be checked.
2. Measurement of pH – pH of gel was measured with the help of pH meter.
3. Spreadibility – Spreadibility was determined by the apparatus which consist of wooden block, which was provided by the pulley at one end. By this method spreadibility was measured on the basis of slip and gel characteristics of gel. An excess of gel (2 gm) was placed on the on glass slide. [31]

RESULTS AND DISCUSSION:

The physical parameters of formulation on the basis of viscosity, color, pH, skin irritation. The antibacterial activity of Hibiscus rosa sinesis cultivars leaf extract of solvent acetate, hexane bacillus substillis and gram negative bacteria. Hibiscus rosa sinesis methanol medicinal plant extract and tested bacterial strains are presented as MIC and

MBC value. Hibiscus rosa sinesis are to be good sources of antimicrobial activity.[34]

Hibiscus rosa sinesis are reported that, the phytochemical analysis showed present of alkaloids, proteins, steroids, and carbohydrates in Hibiscus rosa sinesis flower extract .Hibiscus rosa sinesis extract reported the presented of different medicinal valuable constituent include Alkaloids, glycosides, phytosteols, phenolic compounds, saponins, flavonoids and proteins and amino acid .[35] Hibiscus rosa sinensis is an ornamental plant available throughout India. Hibiscus rosa sinensis plant has been said to be useful against several diseases, including diabetes, inflammation and hepatic disease. leaf and stem extract showed strong antioxidant potential revealed that reduces the risk of various diseases. The control of microorganisms, intermittent occurrences of epidemics due to drug resistant microorganisms and previously unknown disease causing microbes pose a huge risk to public health. Hibiscus flower and leaf extract showed an antimicrobial effect against various pathogenic bacteria, hence reduces the risk of various infectious diseases.[37]

CONCLUSION:

Hibiscus rosa sinensis possess many properties and this plant may procured at large scale for providing herbal alternative to many diseases. This study shows that on biochemical basis plant used in the treatment and prevention of various diseases and disorders. The phytochemical screening on qualitative analysis shows that the plant is rich in alkaloids, terpenoids, flavonoids, glycosides, reducing sugar, Fatty materials, saponins, gums and mucilage.[32]According to the data obtained it is concluded of Hibiscus rosa sinesis habe pharmaceutical and pharmacological activities .this plant is effective for herbal to many diseases. Such as antiulcer, antibacterial, anticonvulsant, antipyretic etc. This extracted widely used for the medicinal purposes. The phytochemical screening on qualitative analysis shows that the plant is rich in alkaloids, terpenoids, glycosides, reducing sugar, fatty materials, saponins, gums and mucilage.[33] The current review discussed the chemical constituents, pharmacological effects and therapeutic important of Hibiscus rosa sinesis as a promising medicinal plant with wide range of pharmacological activity which could be utilized in several medical application because of its effectiveness and safety.[36] The antioxidant properties of the Hibiscus rosa sinensis plants are particular interest in view of the oxidative modification. The diabetes related complications like hyperglycimia, hypercholesteromia, hyperlipidemia also controlled by the Hibiscus rosa sinensis plants in animals.[37]

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