

***IN VITRO* ANTI-ARTHRITIC ACTIVITY OF FORMULATED
HIBISCUS GEL FROM THE ETHANOLIC WHITE FLOWER
EXTRACT OF *Hibiscus rosa-sinensis* L.**

**Submitted in partial fulfillment of the requirements for the award
of BACHELOR OF PHARMACY**

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BONAFIDE CERTIFICATE

This is to certify that the thesis entitled "*IN VITRO* ANTI-ARTHRITIC ACTIVITY OF FORMULATED HIBISCUS GEL FROM THE ETHANOLIC WHITE FLOWER EXTRACT OF *Hibiscus rosa-sinensis* L.", submitted by "Karthik J (40050704), Madhavan S(40050028), Nithya Sree B(40050032), Rajalakshmi M (40050038), Vasanth E(40050054)" to the Sathyabama Institute of Science & Technology, Chennai – 600119 for the award of Bachelor of Pharmacy by research is a bonafide record of research work carried out by them under my supervision. The contents of this thesis, in full or in parts, have not been submitted to any other institute or university for the award of any degree, diploma, associate-ship, fellowship or any similar title and that it represents entirely an independent work on the part of the candidate.

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DECLARATION

We hereby declare that the work presented in the thesis entitled "**IN VITRO ANTI-ARTHRITIC ACTIVITY OF FORMULATED HIBISCUS GEL FROM THE ETHANOLIC WHITE FLOWER EXTRACT OF *Hibiscus rosa-sinensis* L.**" submitted by us to the Sathyabama Institute of Science & Technology (deemed to be University), Chennai-600119 for the award of Bachelor of Pharmacy (2020-2024) by research is an original record of research work carried out by us under the supervision of "**Mrs.G.Shanthini Nachiyar**, Assistant professor, School of Pharmacy". The contents of this dissertation, in full or in parts, have not been submitted to any other institute or university for the award of any degree, diploma, membership, fellowship, associate-ship etc.. In keeping with the general practice in reporting scientific observation, due acknowledgement has been made whenever the work described is based on the findings of other investigators.

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ABSTRACT

Arthritis, a chronic inflammatory disorder affecting joints. It impacts among the individuals of various age groups. The quest for effective remedies against arthritis has led to the exploration of natural compounds with therapeutic potential. White hibiscus (*Hibiscus rosa-sinensis* L.), a flowering plant rich in bioactive constituents, has gained attention for its purported anti-inflammatory properties. This study aims to evaluate the efficacy of an Anti-Arthritic gel formulated from the ethanolic extract of white flowers of *Hibiscus rosa-sinensis*. White hibiscus flowers are renowned for its diverse pharmacological activities, including anti-inflammatory, analgesic, and antioxidant properties, attributed with constituents such as flavonoids, phenolic compounds, and polysaccharides. Previous research suggests that these bioactive compounds may alleviate arthritis symptoms by modulating inflammatory mediators and reducing oxidative stress. The formulation of Anti-Arthritic gel offers a promising approach for localized delivery of bioactive compounds, potentially enhancing therapeutic efficacy while minimizing systemic side effects. Moreover, topical administration facilitates direct targeting of inflamed joints, providing rapid relief from pain and inflammation associated with arthritis. The findings of this study hold significant implications for the development of novel therapeutic strategies for arthritis management. If proven effective, the Anti-Arthritic gel derived from white Hibiscus flower extract could emerge as a safe and affordable alternative to conventional treatments, offering hope for individuals suffering from arthritis-induced discomfort and disability.

Keywords: *Hibiscus rosa-sinensis* white flower, Bovine serum, Gallic acid, gel formulation

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LIST OF ABBREVIATIONS

Cav	Cavity
CT	Connective Tissue
E	Epidermis
En	Endothecium
St	Stomium
VB	Vascular Bundle
IE	Inner Epidermis
Loc	Locule
Muc	Mucilage Cell
OE	Outer Epidermis
Ovl	Ovule
Plc	Placenta
VB	Vascular Bundle
IC	Inhibitory Concentration
FAA	Formalin-Aceto-Alcohol
DPX	Dibutylphthalate Polystyrene Xylene

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CHAPTER – 1

INTRODUCTION

HERBAL MEDICINES:

Herbal medicine plays a crucial role in complementary and alternative medicine. It is increasingly gaining popularity worldwide and gradually integrating into mainstream healthcare systems. Herbal medicines have been used for curing wide range of health conditions, including arthritis. Scientific evidence for the efficacy of herbal medicines in treating arthritis varies, some herbs have shown promising results in alleviating symptoms and reducing inflammation. Conventional therapy for arthritis is essential to use cautiously under the guidance of a healthcare provider. Additionally, quality control and standardized dosages are crucial when using herbal supplements to ensure safety and efficacy.

Definition and Scope:

- Herbal medicines include herbs, herbal materials, herbal preparations, and finished herbal products.
- Phytochemical constituents are derived from parts of plants or other plant materials.
- Herbal medicine is used for the prevention and treatment of diseases.

Global Acceptance:

- The use of herbal medicine transcends gender, social, and racial boundaries.
- Both developing and developed countries embrace herbal remedies.
- The annual global sale of herbal products approaches US\$62 billion due to factors like low cost, natural origin, and perceived efficacy.

Biological Complexity:

- Herbal medicines consist of biologically active natural products.
- Ingredients primarily include herbs, but some formulations may also contain fungal, minerals, ash, shells, insects, and animal parts.

- Secondary metabolites in herbs drive their pharmacological effects and potential toxicity.

Benefits and Challenges:

- Benefits:
 - **Low toxicity:** Herbal medicines are considered safe due to their natural origin.
 - **Efficacy:** Some herbs effectively address specific health conditions.
 - **Accessibility:** Herbs are readily available and easy to prepare.
- Challenges:
 - **Knowledge Gap:** Misuse and misunderstanding stem from inadequate knowledge among healthcare professionals and the public.
 - **Correct Information:** Accurate information on herbal medicines is crucial for its appropriate use.

Continued Attraction:

- The global appeal of herbal medicine will not fade.
- Comprehensive information on benefits, safety, toxicity, research, formulation, regulation, and quality control is essential.

MECHANISM OF ACTION OF PHENOLIC COMPOUNDS:

Phenolic compounds are a diverse group of chemical substances found abundantly in various fruits, vegetables, and herbs. Phenolic compounds have been recognized for their potential role in managing arthritis, particularly due to their anti-inflammatory and antioxidant properties. These compounds can modulate pro-oxidant and pro-inflammatory pathways. They help in reducing the onset and progression of arthritis disease like Rheumatoid Arthritis (RA). Phenolic compounds can act on transcription factors like nuclear factor- κ B (NF- κ B) or nuclear factor-

erythroid factor 2-related factor 2 (Nrf-2), which plays a role in regulating elements

within the antioxidant response pathways. The precise mechanisms of action may vary depending on the specific phenolic compound and the type of arthritis.

Anti-inflammatory Properties:

- Many phenolic compounds exhibit potent anti-inflammatory effects by inhibiting pro-inflammatory enzymes and signaling pathways.
- They can suppress the production of inflammatory mediators such as cytokines (e.g., Tumor necrosis factor-alpha, interleukins) and prostaglandins, thereby reducing inflammation and associated tissue damage in arthritis.
- By mitigating inflammation, phenolic compounds may help alleviate pain, swelling, and stiffness in arthritic joints.

Antioxidant Activity:

- Phenolic compounds possess strong antioxidant properties, which can counteract oxidative stress in arthritic joints.
- Oxidative stress plays a role in the pathogenesis of arthritis by promoting inflammation, cartilage degradation, and joint damage.
- By scavenging free radicals and reactive oxygen species, phenolic compounds help protect joint tissues from oxidative damage, thereby potentially slowing the progression of arthritis.

Modulation of Immune Response:

- Some phenolic compounds can modulate the immune response, which is dysregulated in autoimmune forms of arthritis such as rheumatoid arthritis.
- They may regulate the activity of immune cells, such as T cells and macrophages, and influence the production of inflammatory cytokines and autoantibodies involved in arthritis pathogenesis.
- By modulating the immune response, phenolic compounds may help attenuate autoimmunity and reduce joint inflammation and damage.

Inhibition of Cartilage Degradation:

- Certain phenolic compounds have been shown to inhibit enzymes involved in cartilage degradation, such as matrix metalloproteinases (MMPs) and aggrecanases.
- By blocking the activity of these enzymes, phenolic compounds may help preserve cartilage integrity and prevent the progression of osteoarthritis and rheumatoid arthritis.

Analgesic Effects:

- Phenolic compounds may possess analgesic properties, providing pain relief in arthritis patients.
- They can modulate pain perception pathways and inhibit nociceptive signaling, thereby reducing pain sensitivity, and improving quality of life for arthritis sufferers.

Antimicrobial Properties:

- They exhibit antimicrobial activity against a range of pathogens by disrupting microbial membranes, inhibiting biofilm formation, or interfering with microbial enzymes and DNA.

Metal Chelation:

- Some phenolic compounds have the ability to chelate metal ions, which can catalyze the generation of free radicals. By binding to these metals, phenolic compounds prevent the metals from participating in oxidative reactions.

Neuroprotective Effects:

- Some phenolic compounds have been shown to exert neuroprotective effects through their antioxidant activity, anti-inflammatory properties, and by promoting autophagy in neuronal cells.

TRADITIONAL MEDICINES USED TO CURE ARTHRITIS:

The World Health Organization (WHO) defines traditional medicine as the knowledge, skills, and practices that are based on the theories, beliefs, and experiences of different cultures. These practices are used to maintain health, and to prevent, diagnose, improve, or treat physical and mental illnesses. Traditional medicine is also known as complementary or alternative medicine. Certainly traditional systems of medicine have been practiced for centuries across various cultures.

Siddha:

- The Siddha system of medicine is one of the traditional medicinal systems of India that originated in South India.
- It is based on ancient principles and practices that have been passed down through generations.
- In Siddha medicine, arthritis is known as "Vatha Kuzhanthai" or "Sandhigata vatham".
- It is believed that arthritis results from an imbalance of the three doshas (humor) – vata, pitta, and kapha.
- Siddha practitioners use a holistic approach to diagnose and treat arthritis.

Ayurveda:

- Ayurveda, the traditional system of medicine in India, utilizes a variety of plants for the treatment of arthritis.
- In Ayurveda, arthritis is known as "Amavata" or "Sandhigata Vata,"
- it is believed that arthritis results from an imbalance of the doshas (Vata, Pitta, and Kapha), leading to the accumulation of toxins (ama) in the joints.
- Ayurvedic treatment for arthritis focuses on balancing the doshas, removing toxins from the body, reducing inflammation, and strengthening the joints and tissues.
- Ayurvedic treatments for arthritis are individualized based on the patient's constitution (prakriti), dosha imbalance, and specific symptoms.

Unani:

- Unani medicine, a traditional system of medicine practiced widely in South Asia, employs various plants with medicinal properties to treat conditions like arthritis.
- The Unani system of medicine, also known as Greco-Arabic medicine.
- It is based on the principles of Hippocratic-Galenic medicine and was developed by Greek and Arab physicians.
- In Unani medicine, arthritis is known as "Waja'ul-Mafasil" or "Wurma."
- It is believed that arthritis results from an imbalance of the four humors—blood, phlegm, yellow bile, and black bile and the presence of excess waste products (mawad-e-ghaleezah) in the body.
- Unani treatment for arthritis aims to restore the balance of humors, eliminate waste products, reduce inflammation, and strengthen the joints and muscles.

Homeopathy:

- Homeopathy is a holistic system of medicine founded by **Samuel Hahnemann** in the late 18th century.
- It operates on the principle of “**like cures like**”, where highly diluted substances that produce symptoms in healthy individuals are used to treat similar symptoms in sick patients.
- The highly diluted substances stimulate the body's natural healing processes.
- Some of the homeopathic remedies include Rhus Toxicodendron, Bryonia alba, Arnica montana, Calcarea carbonica, Ruta graveolens etc.
- Homeopathy is a highly individualized form of medicine, and remedies are prescribed based on the unique symptoms and constitution of each person.

Naturopathy:

- Naturopathy is a system of healthcare that emphasizes the body's innate ability to heal itself.
- Naturopathy seeks to address the root causes of illness rather than merely treating symptoms.
- It is based on the principles of natural healing, holistic health, and prevention,

drawing on a combination of traditional healing practices, modern scientific knowledge, and individualized patient care.x

- Naturopathic treatments may include a wide range of therapies, including clinical nutrition, herbal medicine, homeopathy, hydrotherapy, physical medicine, acupuncture, lifestyle counselling, and mind-body approaches such as meditation and stress management techniques.
- It aims to restore the balance and promote vitality in the body, mind, and spirit.

HERBAL MARKETED PRODUCT FOR ARTHRITIS:

Simhanada Guggulu:

- Simhanada Guggulu is an Ayurvedic formulation renowned for its therapeutic effects in managing arthritis. It is imbued with potent analgesic, anti-inflammatory, and pain-relieving properties, which are attributed to the bioactive components within the preparation.
- This formulation is traditionally made by combining several herbal ingredients known for their medicinal properties.
- The primary ingredients include Triphala (a combination of Haritaki, Bibhitaki, and Amla), purified Gandhaka (sulfur), and Guggulu (Commiphora mukul), with the addition of Castor oil. These components work synergistically to address the symptoms of arthritis.

Maha Rasnadi Kada:

- Maha Rasnadi Kada, also known as Maharasnadi Kwath, is a classical Ayurvedic formulation widely used for its therapeutic benefits in managing arthritis and other musculoskeletal disorders.
- It is a polyherbal decoction that acts primarily on bones, joints, muscles, and nerves, providing relief from pain, swelling, inflammation, and stiffness associated with arthritis.
- The formulation contains a blend of several herbs, including Rasna (Pluchea lanceolata), Eranda (Castor Plant), Devdaru (Cedrus Deodara), and many others, which are known for their anti-inflammatory and analgesic properties.

- Maharasnadi Kada is recommended for a variety of conditions such as rheumatoid arthritis, osteoarthritis, gout, sciatica, backache, and even paralytic disorders.

Mahanarayan Tailam:

- Mahanarayan Tailam, also known as Mahanarayana Thailam, is a classical Ayurvedic oil renowned for its effectiveness in treating various ailments, particularly arthritis.
- This medicated oil is formulated from a mixture of potent herbs and is used for external application, oral consumption, and in several Ayurvedic therapies.
- Mahanarayan Tailam contains a variety of herbs such as Bilva (Bael root), Ashwagandha (Indian Ginseng), Brihati (Indian Nightshade), and many others, which are known for their healing properties.
- These ingredients are processed in a base of sesame oil, along with other substances like goatmilk and herbal juices, to enhance the oil's therapeutic effects.

Biofreeze Gel:

- Biofreeze gel is a topical analgesic designed to provide temporary relief from arthritis pain.
- The active ingredient in Biofreeze gel are Menthol USP, Ilex Paraguariensis Leaf Extract, Arnica Montana Flower Extract, Calendula Officinalis Extract, Aloe Barbadensis Leaf Extract, Boswellia Carterii Resin Extract, Camellia Sinensis Leaf Extract, Arctium Lappa Root (Burdock) Extract, Melissa Officinalis (Lemon Balm) Leaf Extract.

Arnicare Gel:

- Arnicare Gel is a homeopathic product that is used for pain relief.
- It's made from Arnica montana, a type of mountain daisy. The gel is applied topically and is known for its anti-inflammatory and pain-relieving properties.
- It's commonly used to treat sore muscles, bruises, and joint pain associated with arthritis.

CHAPTER – 2

LITERATUREREVIEW

1. **Yolandasalinasetal.,2023**Reportedtheanti-microbialactivityandphenolic compositionofvarietiesof*Hibiscussabdariffa*Linnwithredandwhitecalyces.
2. **Dianyangwangetal.,2022**Identifiedthepotentialof*Hibiscussabdariffa* LinnandHibiscusacidtoreverseskin aging.
3. **Nohamalmoraieetal.,2022**Reportedtheeffectof*Hibiscusroselle*juiceon Mood improvement in healthy adults.
4. **Mengje Xiao et al.,2022** Studied the anti-influenza properties of tiliroside isolated from *Hibiscus mutabilis* Linn.
5. **Akshada et al.,2022** Reported anti-inflammatory, anti-convulsant, anti-pyretic, anti-bacterial, anti-tussive, anti-parasitic, dermatological, neuro-protective, anti-oxidant, and anti-diabetic effect in *hibiscus rosa-sinesis*.
6. **Priyanka v et al.,2022** Showed the results obtained from red flowers of *Hibiscus rosa-sinensis* proves a marked effect on dermatological and cardioprotective action.
7. **Amol g jadhao et al.,2021** Explored the anti-fertility activity in rodents by using red flowers of *Hibiscus rosa-sinensis*.
8. **Dr.Aliesmailetal.,2021** Explainedtheredflowersof*Hibiscusrosa-sinensis* whichhavevarioushealthbenefits,includingmenstrualcycleregulation,liver disorders,highbloodpressure,stomachpain,eyeproblems,andaphrodisiac effect, anti-inflammatory, anti-microbial effect.

9. **LTM Ngan et al.,2021** Anti-bacterial activity in variants of *Hibiscus rosa-sinensis* with red and white flower against antibiotic-resistant strain of *Helicobacter Pylori* and identification of flower constituents.

10. **Tiadjefferyetal.,2021**Reviewedtheeffectivenessof*hibiscusrosa-sinensis* fortreatmentofmetabolicsyndrome.

11. **Hieu tran trung et al.,2020** Identified the potential effects of *Hibiscus rosa-sinensis* on skin aging.

12. **Christopher nguyen et al.,2019** Proved that *Hibiscus rosa-sinensis* flower extract selectively induced apoptosis in breast cancer cells and positively interacts with common chemotherapeutics.

13. **M Madhu babu et al.,2018** Evaluated the Hepato-protective activity of ethanolic extract of whole plant of *Hibiscus plantifolius Linn* in Rifampicin induced hepatotoxicity in Wister Albino rats.

14. **Mortho madhu babu et al.,2018** Evaluated anti-diarrheal activity of ethanol extractof*Hibiscusplatanifolius*incastoroilinduceddiarrheainWisterAlbino rats.

15. **Ghazala riza et al.,2018** Conducted review on phytochemistry and therapeuticuses of *Hibiscus sabdariffa Linn*.

16. **Mukhopadhyaygoutametal.,2018**Haveexploredthepotentialactivityof *Hibiscusrosa-sinensis*flowersintreatingepilepsy,leprosy.

17. **Ali esmail et al.,2018** Reported *Hibiscus rosa-sinensis* have various pharmacological effects which include reproductive, anti-diabetic, and anti-inflammatory properties.

18. **Asmaamissoumetal.,2018** Proved that the flower of *Hibiscus rosa-sinensis* have been used for analgesic, anti-pyretic, anti-asthmatic, and anti-inflammatory purpose as well as being claimed to have anti-cancer activity.

19. **Ishrat mahmood et al.,2017** Proved the *Hibiscus rosa-sinensis* explore its pharmacological application towards anti-fertility, anti-inflammatory, anti-microbial, anti-diabetic, and anti-pyretic activity.

20. **Maria herranz et al.,2017** Reported multi target effect of Hibiscus sabdariffa polyphenols, an opportunity for a global approach to obesity.

21. **Sherif t s hassan et al.,2016** Revealed the presence of phytochemical compounds for the action anti-microbial, anti-parasitic and Anticancer activity in the flower of *Hibiscus sabdariffa*.

22. **Zulfiqar ali khan et al.,2014** Proved anti-oxidant and anti-bacterial activities of hibiscus Rosa-Sinensis Linn flower extracts.

23. **Zoriah aziz et al.,2013** Gave a systematic review in decreasing effect of serum lipid in Hibiscus sabdariffa Linn.

24. **Radwar et al.,2013** Showed an anti-inflammatory effect on ethanolic extract containing red flowers of *Hibiscus rosa-sinensis*.

25. **P Ruban et al.,2012** Showed in-vitro anti-bacterial activity of Hibiscus rosa-Sinensis flower extract against human pathogens.

26. **Vishnukumaretal.,2012** Gave a detailed report on roots of *Hibiscus rosasinensis* in treating anti-dyslipidemic and anti-oxidant activity.

CHAPTER–3

AIM&SCOPE OFWORK

AIM:

To formulate Anti-Arthritic gel using ethanolic white flower extract of *Hibiscus rosa-sinensis* L. and evaluate its *in-vitro* Anti-Arthritic activity.

SCOPE OF WORK:

From the literature survey it was found that polyphenolic derivatives such as flavonoids & phenol have Anti-Arthritic activity. Flavonoids and phenols extracted from various plants were used in the treatment of arthritis related disease like inflammation and gout. It was also revealed from the literature survey that the anti-arthritic activity was not reported by using the extract of *Hibiscus rosa-sinensis* white flower.

- To collect *Hibiscus rosa-sinensis* (white flower) & authentication of the flower's reproductive part.
- To prepare Petroleum ether, Ethyl acetate, Ethanol, and Aqueous extract of *Hibiscus rosa-sinensis* (white flower).
- To perform phytochemical screening using flower extract to identify the presence of phenolic compounds.
- To perform Thin Layer Chromatography to identify the active component present in the ethanolic white flower extract of *Hibiscus rosa-sinensis* L.
- To perform anatomical characterization of the reproductive part of *Hibiscus rosa-sinensis* (white flower).
- To estimate the total phenolic content present in the ethanolic white flower extract of *Hibiscus rosa-sinensis* L.
- To formulate Anti-Arthritic gel using *Hibiscus rosa-sinensis* L. (white flowers).
- To evaluate the physical parameters of the formulated Anti-Arthritic gel.
- To demonstrate the *in-vitro* activity of the formulated gel by Bovine Serum Albumin [BSA] denaturation inhibition assay.

CHAPTER – 4

MATERIALS&METHODS

PLANT PROFILE OF WHITE FLOWER OF *Hibiscus rosa-sinensis* L.:

Hibiscus-rosa-sinensis L., commonly known as Chinese hibiscus, China rose, or Hawaiian Hibiscus is a tropical flowering plant belonging to the family Malvaceae.



Figure 4.1: White Flowers of *Hibiscus rosa-sinensis* L.

Geographical Source:

- Although it is widely cultivated as an ornamental plant in tropical and subtropical regions, its native range is Vanuatu.
- This archipelago in the South Pacific Ocean is where *Hibiscus rosa-sinensis* L. naturally occurs.

Cultivation:

- Over time, it has been cultivated in various other places, including China, Japan, and the Pacific islands.
- Cultivating *Hibiscus rosa-sinensis* is an art steeped in tradition, yet continually evolving with advancements in horticultural science.
- The plant is adaptable to various growing conditions, including pots, containers, open fields, greenhouses, and polyhouses.
- General cultivation requirements for *Hibiscus rosa-sinensis* are climate, sunlight, soil, watering, fertilization, pruning, pest and disease control and protection from frost.

- *Hibiscus Rosa-Sinensis* can reach up to 15 feet in height and 6 feet in width, with flowers that can be up to 6 inches in diameter and occur in a variety of colors.

Colour variants of Hibiscus white flowers:

- The plant produces striking flowers with five petals, typically red, and prominent orange-tipped red anthers.
- hybrids have expanded the color palette to include white, pink, orange, peach, yellow, blue, and purple blooms. Some varieties even have double flowers.

Morphological characters of Hibiscus white flower:

- *Hibiscus rosa-sinensis* is a bushy, evergreen shrub or small tree, growing up to 2.5–5 meters (8–16 feet) tall and 1.5–3 meters (5–10 feet) wide.
- Its glossy leaves are simple, ovate, and serrated, while its flowers bloom during summer and autumn.

Table 4.1: Taxonomical classification

Kingdom	Plantae
Subkingdom	Tracheobionta
Super Division	Magnoliophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Dilleniidae
Order	Malvales
Family	Malvaceae
Genus	<i>Hibiscus</i>
Species	<i>rosa-sinensis</i>
Botanical name	<i>Hibiscus rosa-sinensis</i>

Nutrients and Chemical Components:

Anthocyanins:

- Cyanidin-3-sophoroside is a major anthocyanin found in *Hibiscus rosa-sinensis* L. flowers.
- Anthocyanins are powerful antioxidants that help reduce oxidative stress and inflammation in the body.

Flavonoids:

- Quercetin-3-diglucoside, kaempferol-3-xylosylglucoside, and cyanidin-3,5-diglucoside are flavonoids present in the flower.
- Flavonoids have anti-inflammatory properties and may contribute to joint health.

Other Compounds:

- Tannins, phenols, and saponins are also found in *Hibiscus rosa-sinensis*.
- These compounds play various roles, including antioxidant and anti-inflammatory effects.

Pharmacological Properties:

This plant exhibits several pharmacological activities:

- Antioxidant
- Anticancer
- Anti-inflammatory
- Antidiabetic
- Antibacterial

Traditional Uses:

All parts of *Hibiscus rosa-sinensis* are used in traditional medicine:

- Treating fever
- Addressing inflammation
- Combating bacterial infections

- Acting as a contraceptive agent

Advantage:

- The plant's comparatively low toxicity levels position it as a viable therapeutic agent for potential use in medical applications.

COLLECTION OF FLOWERS SAMPLE:

The white flowers of the *Hibiscus rosa-sinensis* plant were collected from a local area in Chennai, Tamil Nadu, India and authenticated in Siddha Research Institute, Arumbakkam, Chennai-600106. (Form No: PCOG002-ACF)

PREPARATION OF EXTRACTS:

Collected white flowers of *Hibiscus rosa-sinensis* were converted into moderately coarse powder and extracted with various solvents like petroleum ether, ethyl acetate, ethanol and aqueous extract for 3 consecutive days by cold maceration. The solvent was removed under reduced pressure and the yield of extract was calculated.



Figure 4.2: Preparation of coarse powder

ANATOMICAL CHARACTERIZATION OF THE REPRODUCTIVE PART OF *Hibiscus rosa-sinensis* L. (WHITE FLOWER)

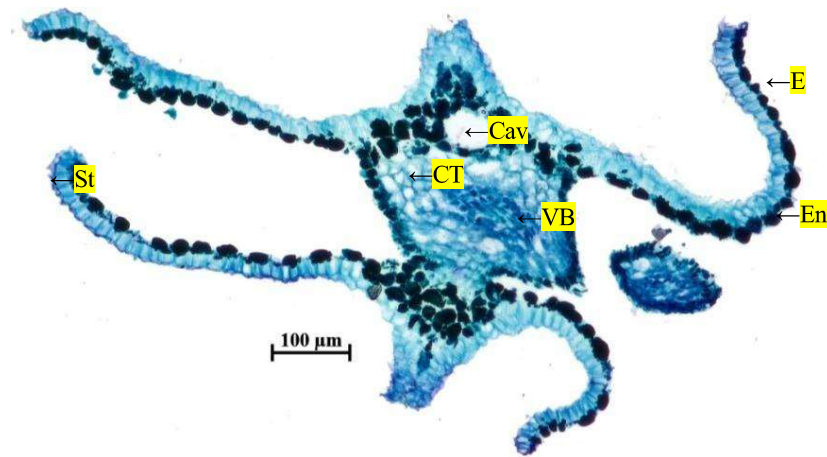
The Anatomical characterization of nonreproductive parts of the white flower of *Hibiscus rosa-sinensis* using microtome. A microtome is a cutting tool used to produce extremely thin slices of material known as sections, with the process being termed micro sectioning. Important in science, microtomes are used in microscopy for the preparation of samples for observation under transmitted light or electron radiation.

Methodology:

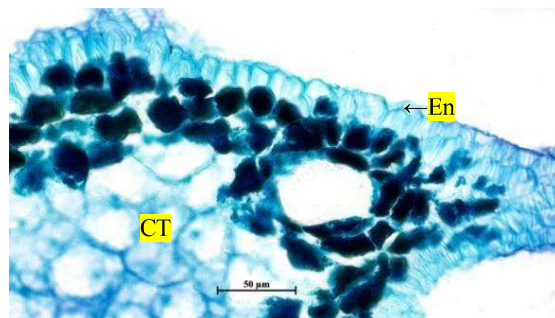
The sample was fixed in Formalin-Aceto-Alcohol (FAA). Dehydration was carried out using the Xylol:Alcohol series. Paraffin wax was melted and poured into a paper boat and with the help of forceps, the specimens were transferred to the molten wax. Gently placed the boat in cold water for 20 to 30 min followed by peeling off the paper boat. With the help of a sharp blade made an incision on the wax block and trimmed the upper lower and lateral sides of the block straight and parallel leaving the specimen surrounded by 1 mm of wax. Fix the cut piece of block on the wooden holder with the help of a hot spatula. Lock the hand wheel at the position at which the block holder is in the highest position. After setting the thickness scale at 10 μ m sectioning is done. Once the ribbon is about 5 inches detach from the microtome blade and mount on slide previously smeared with adhesive. After the dewaxing with Xylol, the sections were stained with 1 % toluidine blue and finally mounted with Dibutylphthalate Polystyrene Xylene (DPX). These sections were taken with the help of a Leica rotary microtome.

TS of Anther:

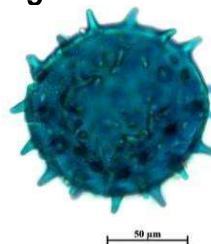
TS of the mature ruptured anther is irregular in outline; it shows an outer epidermal layer followed by endothelial cells formed of compactly arranged cylindrical cells; all other contents and pollen grains are dehiscent leaving four empty lacunae; two anther lobes are connected by parenchymatous connective tissue; a small vascular strand can be seen at the center of connective tissue; some secretion cavities can be seen near the connective tissue (Fig 5.1).



TSofHibiscusrosa-sinensisL.anther



Enlargedviewofanther

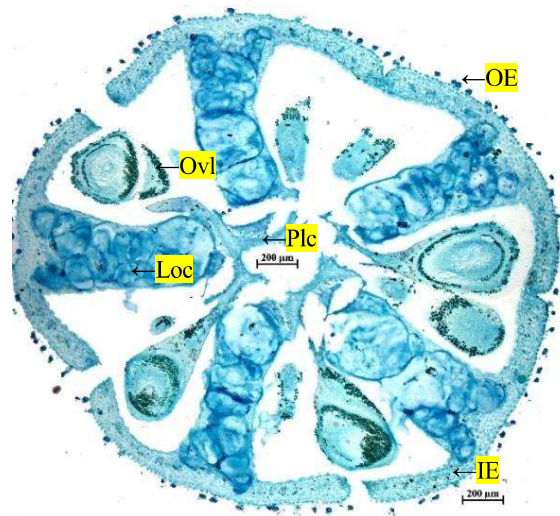


SpinyPollen grains

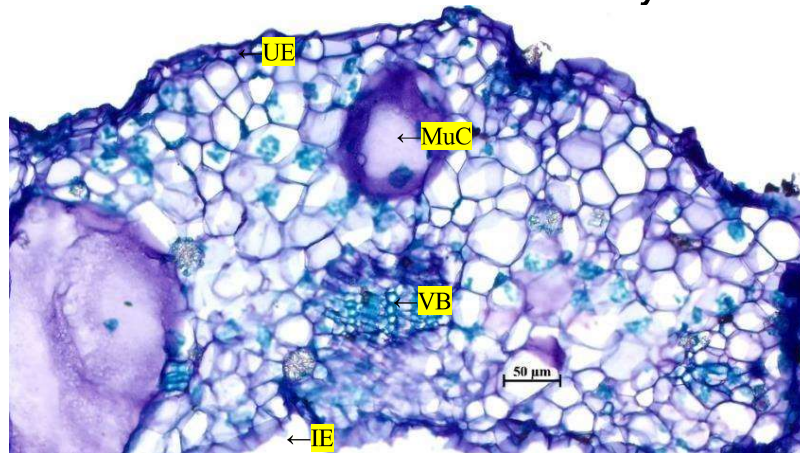
Figure4.3T.SofReproductivePartofHibiscusrosa-sinensisL.(WhiteFlower)

TSofOvary:

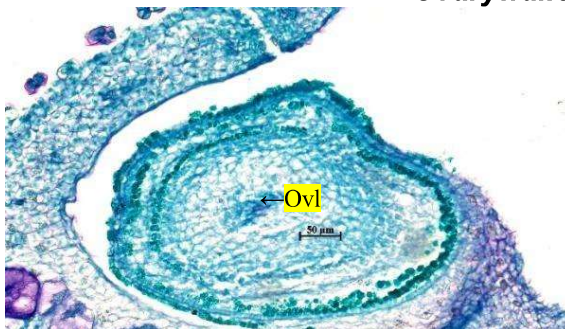
A transverse section of the pentacarpellary ovary is almost rounded in outline showing five locules, each locule containing two ovules. The ovary shows an outer and inner epidermis enclosing a parenchymatous mesophyll in between. The mesophyll is formed of rounded, moderately thin-walled parenchyma with small intercellular spaces. It is traversed by five vascular strands and mucilage cavities. There are five locules with thick walls and numerous mucilage cells; attached to the inner locular wall ovules are seen attached to the placenta (Fig 5.4 – 5.7)



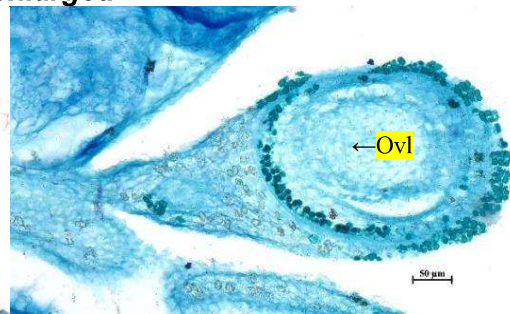
TS of Hibiscus rosa-sinensis L. ovary



Ovary wall enlarged



Developing ovule



Mature ovule

Figure 4.4: T.S of the Ovary of Hibiscus rosa-sinensis L. (White Flower)

**PRELIMINARY PHYTOCHEMICAL SCREENING FOR THE VARIOUS
EXTRACTS OF *Hibiscus rosa-sinensis* L. (WHITE FLOWER):**

Organoleptic Evaluation:

Nature	-	Coarse powder
Colour	-	Greenish white
Odour	-	Characteristic odour
Taste	-	Mild astringent

4.5.2. Test for alkaloids:

About 2ml of the Hibiscus flower extract was mixed with 1gm of calcium hydroxide and add 5ml of water to make it into a smooth paste and set aside for 5 minutes. It was then evaporated to dryness in a porcelain dish on a water bath. To this 200ml of chloroform was added, mixed well, and refluxed for half an hour on a water bath. Then it was filtered and the chloroform was evaporated. To this 5ml of dilute hydrochloric acid was added followed by 2ml of each of the following reagents.

Mayer's Reagent -white precipitate

Dragendorff's reagent -orange precipitate

Test for flavonoids:

Shinoda test:

A little of the Hibiscus extract was heated with alcohol and filtered. To the test solution magnesium turnings and few drops of concentrated hydrochloric acid were added and boiled for five minutes. Red or magenta color was obtained indicating the presence of flavonoids.

Alkali Test:

To the small quantity of Hibiscus extract is added with 10% aqueous sodium hydroxide solution. Yellow orange color was produced indicating the presence of flavonoids.

Acid Test:

To the small quantity of Hibiscus extract is added in test tube with few drops of concentrated Sulphuric acid were added. Yellow to crimson color was obtained indicates the presence of flavonoids.

Test for phenols:***Ferric chloride test:***

To 2ml of hibiscus flower extract is added in a test tube with 5% ferric chloride, blue or violet color was obtained indicating the presence of phenols.

Test for terpenoids:

To 2ml of hibiscus flower extract was warmed gently with tin and thionyl chloride the color of the solution turned to pink indicating the presence of terpenoids.

Test for tannins:

In 10 ml of distilled water 0.5 g of the powdered flower material was mixed and boiled in a test tube and then filtered. A few drops of 0.1% ferric chloride were added and appearance of blue-black or brownish green color indicates the presence of tannins.

Test for Phlobatannins:

A 2 ml of flower extracts were boiled with 1% aqueous Hydrochloric acid. Formation of red precipitate indicates the presence of Phlobatannins.

Test for Saponin:

2 ml of flower extract was shaken vigorously with 5 ml distilled water to obtain stable persistent foam. The formation of emulsion indicates the presence of saponins.

Test for Cardiac glycosides:

5 ml of aqueous flower extracts was treated with 2 ml of glacial acetic acid containing one drop of Ferric chloride solution and then 1 ml of concentrated sulphuric acid were carefully added. Formation of brown ring at the interface

indicates the presence of a deoxysugar which is characteristics of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just spreading gradually throughout the layer.

Test for Carbohydrates:

Few drops of Benedict's reagent was added in test solution (alkaline solution containing cupric citrate complex) and boiled in water bath, formation of reddish brown precipitate indicates the presence of carbohydrate.

Table 4.2: Preliminary phytochemical screening for the various extracts of flower of *Hibiscus rosa-sinensis* L.

Tests	Pet. ether Extract	Ethyl acetate Extract	Ethanol extract	Aqueous extract
1. Test for alkaloids				
a. Mayer's reagent	-	-	+	+
b. Dragendorff's reagent	-	-	+	+
2. Test for flavonoids				
a. Acid test	-	+	+	+
b. Alkali test	-	-	+	+
c. Shinoda test	+	-	+	+
3. Test for phenols				
a. Ferric chloride test	+	+	+	+
4. Test for terpenoids	+	+	+	+
5. Test for Tannins	-	-	-	-
6. Test for Saponin	-	-	-	-
7. Test for Cardiac glycosides	-	-	-	-
8. Test for Carbohydrates	-	-	+	+

ESTIMATION OF TOTAL PHENOLIC CONTENT OF ETHANOLIC EXTRACTS OF *Hibiscus rosa-sinensis* L. (WHITE FLOWER):

Principle:

The total phenolic content of the *Hibiscus rosa-sinensis* white flower extract was determined by **Folin – Ciocalteu** Reagent method. This reagent consists of a mixture of phosphotungstate and phosphomolybdate which is reduced, during oxidation of the phenolic substances, into a mixture of blue molybdenum and tungsten oxides. The intensity of color is proportional to the amount of oxidized phenolic compounds and it can be estimated as gallic acid equivalents at 765nm.

Materials Required:

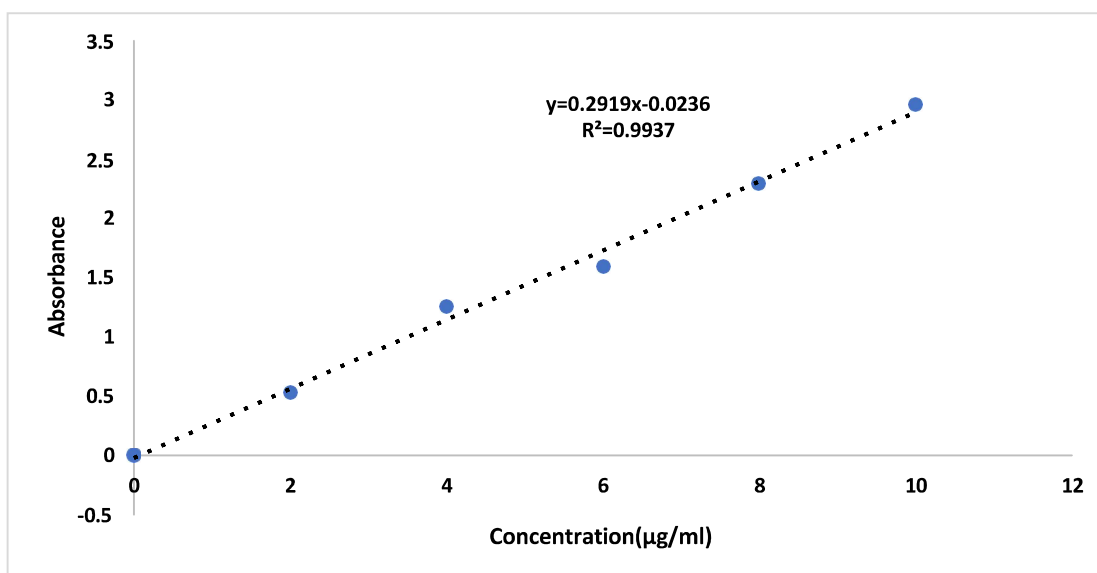
1. Ethanolic white flower extract of *Hibiscus rosa-sinensis* L.
2. Gallic acid
3. 10% sodium carbonate solution
4. Folin–Ciocalteu reagent

Procedure:

The total phenol content of various flower extracts of *Hibiscus rosa-sinensis* white flower was estimated using **Folin–Ciocalteu** reagent method. The 1ml of ethanol extract from 1mg/ml (1000µg/ml) was transferred into separate test tubes. To this solution, Folin–Ciocalteu Reagent 0.5ml and 1ml of sodium carbonate were added and final volume was made up to 10ml with distilled water. The mixture was allowed to stand for 1 hour with intermittent shaking. The absorbance of the reaction mixture was measured at wavelength 765nm. A calibration curve was generated using absorbance reading of gallic acid at different concentrations (2, 4, 6, 8, 10µg/ml). The reaction mixture without sample was used as the blank. The total phenolic content in the various extracts were expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE/g).

Table4.3:Concentrationvsabsorbanceofgallicacid

Concentration($\mu\text{g/ml}$)	Absorbance
0	0
2	0.523
4	1.253
6	1.587
8	2.295
102	2.956

**Figure4.5CalibrationcurveofGallicacid****Table4.4:Concentrationvsabsorbanceoftestsample**

Sample solution ($\mu\text{g/ml}$)	Weight of flower extract per ml(mg)	Absorbance	mg of GAE equivalent/gm of extract*
100	1	1.115	386.182 $\pm$ 2.76
100	1	1.084	
100	1	1.112	

THINLAYERCHROMATOGRAPHY:

- ThinlayerchromatographyofEthanolicextractcontainingwhiteflowerof *Hibiscusrosa-sinensis* L.
- Asmall spotofsample was placed onThin layer chromatographyplate bya capillary tube, then it was introduced into the beaker containing the solvent.
- The Thin Layer Chromatography was performed to analyze both Flavonoid and Phenol compounds in the sample.
- The experiment successfully separated the compounds based on their differingaffinitiesforthestationaryphaseandthemobilephase,allowingfor individual identification and characterization. The R_f values calculated and compared.

Table4.5:Thinlayerchromatographyofflavonoid

SolventSystem (MobilePhase)	Ratio	Detector
Toluene	4	UVchamber254nm
Ethylacetate	2	
Formicacid	1	

RetentionFactor(R_f)ofFlavonoid =0.81



Figure4.6:Thinlayerchromatographyofflavonoid

Table 4.6: Thin layer chromatography of phenol

Mobile Phase	Ratio	Detecting Agent	Standard
Butanol	4	Folin phenolic reagent	Gallic acid
Acetic Acid	3		
Water	2		

Retention Factor (R_f) of Phenol = 0.45

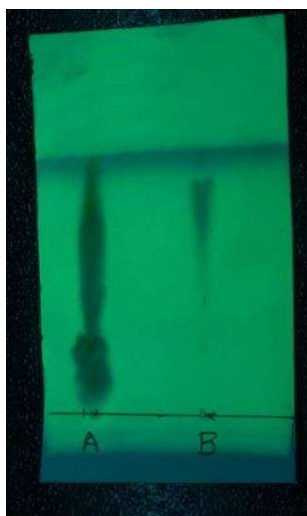


Figure 4.7: Thin layer chromatography of phenol

FORMULATION&EVALUATIONOF GEL:

FormulationofWhiteFlowerHibiscusGel:

Table4.7:FormulationofwhiteflowerHibiscus gel

S.No	Ingredients		FormulationCode		
			F1	F2	F3
1	Ethanoliceextractof <i>Hibiscus rosa-sinensis</i> L. (white flower)	Active Ingredient	10ml	20ml	30ml
2	PropyleneGlycol	Humectant	10ml	10ml	10ml
3	SodiumBenzoate	Preservative	10g	10g	10g
4	Glycerine	Emollient	1drop	2drops	3drops
5	Carbapol940	Gelling polymer	10g	10g	10g
6	Triethanolamine	p ^H adjuster	q.s	q.s	q.s
7	PurifiedWater	Vehicle	q.s	q.s	q.s

Methodology:

- AccuratelyweighedCarbopol940wastakeninabeaker(1)anddispersed with 10ml of distilled water at 40° with constant stirring for 30 mins.
- Inanotherbeaker(2)1gofsodiumbenzoateand10mlofpropyleneglycol was taken and dispersed in water.
- Beaker(2&1)wastrituratedin mortar.
- TriethanolaminewasaddedtoadjustthepH.
- ThenethanolicflowerextractofHibiscusrosasinenisalongwithglycerine was added.

Evaluationof WhiteFlowerHibiscusGel:

Appearance:

Thepreparedgelswereinspectedvisuallyforclarity,colourandpresenceof any particle.

Clarity:

The clarity of various formulations was determined by visual inspection and it was graded as follows; turbid: +, clear: ++, very clear (glassy): +++.

p^H:

The p^H of gel was determined using digital p^H meter. 2 gm White Hibiscus gel was stirred in distilled water till a uniform suspension is formed. The volume was made up to 40 ml and pH of the solution was measured.

Homogeneity:

All developed gels were tested for homogeneity by visual inspection after the gels have been set in the container for their appearance and presence of any aggregate.

Spreadability:

It was determined by wooden block and glass slide apparatus for the determination of spreadability, excess of sample was applied in between two glass slides and they were compressed to form a uniformly thick layer of gel. Initially 10 gm weight was tied to the thread and left for 5 minutes, and then the weight was increased by 1 gm at every step. The time required to separate the two slides, i.e. the time in which the upper glass slide moves over the lower plate was taken as measure of Spreadability.

IN-VITRO ANTI-ARTHRITIC ACTIVITY BY BOVINE SERUM ALBUMIN (BSA) DENATURATION INHIBITION ASSAY

Rheumatoid arthritis is an autoimmune disorder. One among the cause for the disease is due to the denaturation of the protein. Anti-Arthritic activity was studied by inhibition of protein denaturation method.

Methodology:

- About 100, 200, 300, 400, 500 µg/ml of sample was taken in series of test tubes.
- To each test tube 1% bovine serum albumin was added.
- This mixture was pre-incubated for 10-15 minutes, followed by heating for 20 minutes.
- The resulting solution was cooled down to room temperature and absorbance was recorded at 660 nm.
- Acetylsalicylic acid was taken instead of test item as a positive control.

The percent inhibition for protein denaturation was calculated using:

$$\text{Percentage inhibition} = 100 - \frac{\text{Absorbance of Sample} - \text{Absorbance of control}}{\text{Absorbance of control}}$$

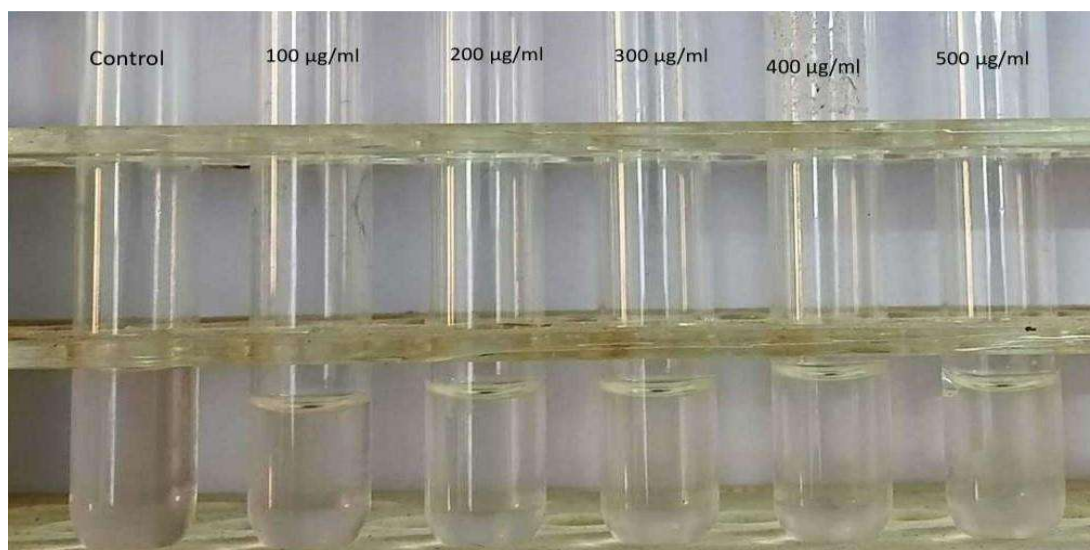


Figure 4.8: Dilution of control and test sample

Table 4.8: Inhibition of Bovine Serum Albumin denaturation assay

Concentrations ($\mu\text{g/mL}$)	Absorbance			Average	Inhibition%
	I	II	III		
Control	0.137	0.140	0.134	0.137	0
100	0.065	0.065	0.058	0.063	54.258 $\pm$ 0.0023
200	0.042	0.044	0.039	0.042	69.586 $\pm$ 0.0015
300	0.031	0.029	0.031	0.030	77.859 $\pm$ 0.0007
400	0.021	0.020	0.019	0.020	85.401 $\pm$ 0.0006
500	0.013	0.012	0.011	0.012	91.241 $\pm$ 0.0006

*mean of three readings $\pm$ SEM

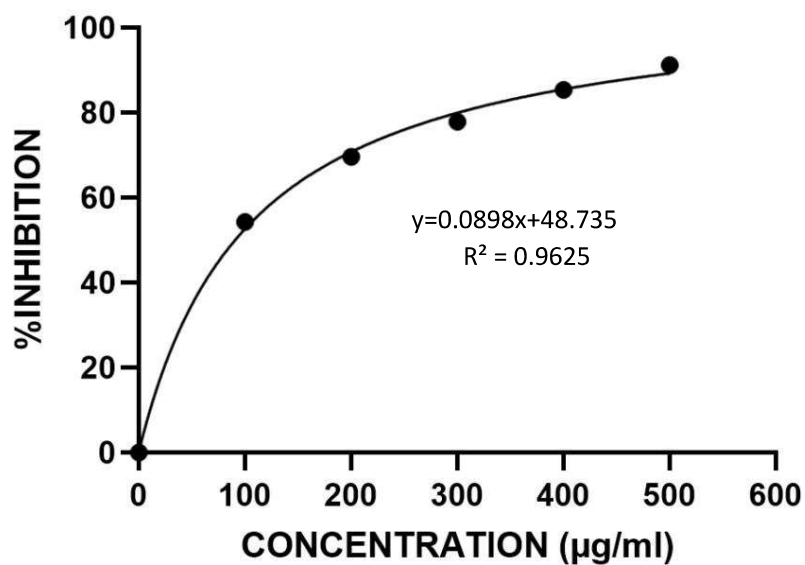


Figure 4.9: Calibration Curve of BSA denaturation assay

Statistical analysis:

All data were denoted as mean $\pm$ standard deviation (SD). Results were obtained from at least three independent experiments, each performed in singlet, duplicate, and triplicate. Statistical analysis was determined using one-way analysis of variance (ANOVA) by Graphpad prism 6.0 software. *p* values less than or equal to 0.05 were considered significant.

CHAPTER – 5

RESULT&DISCUSSION

RESULTFORANATOMICALCHARACTERIZATION

Anatomical characterization of nonreproductive part of *Hibiscus rosa-sinensis* L. (white flower) describes the anatomical features of mature ruptured anther with typical characteristics associated with pollen release and reproduction function on flowering plant. It also describes about the pentacarpellary ovary having typical traits linked to flowering plant's growth and seed production.

RESULTFORPRELIMINARYPHYTOCHEMICALSCREENING:

Preliminary phytochemical screening of *Hibiscus rosa-sinensis* L. (white flower) using various extracts like Petroleum ether, Ethyl acetate, Ethanol, Aqueous extract reveals the presence of Alkaloids, Flavonoids, Phenols, Terpenoids, and Carbohydrate only in the Ethanol & Aqueous extract. So we have chosen ethanol as a solvent for extraction because it has demonstrated superior capacity for extracting a diverse range of phytochemical constituents, including alkaloids, flavonoids, phenols, terpenoids, and carbohydrates, from *Hibiscus rosa-sinensis* L. (white flower).

RESULTFORESTIMATIONOFTOTALPHENOLICCONTENT

Estimation of total phenolic content in the ethanolic extract of *Hibiscus rosa-sinensis* L. (white flower) results in 386.182 ± 2.76 mg of GAE equivalent/gm of extract.

RESULTFORTHINLAYERCHROMATOGRAPHY:

Thin Layer Chromatography was performed by using suitable solvent system and it indicates that the R_f value correlates with Rutin and Gallic acid.

RESULTFORPHYSICALEVALUATIONOF GEL:

The physical evaluation of anti-arthritic gel formulations F1, F2, F3 include appearance, colour, clarity, p^H, homogeneity and spreadability were measured. For F1, the appearance was homogenous, the colour was light green, the clarity was ++, p^H was 5.93 ± 0.18 , the homogeneity was good, the spreadability was 4.85. For F2, the appearance was homogenous, the colour was light green, the clarity was

++, p^H was 6.66 ± 0.13 , the homogeneity was good, the spreadability was 5.02. For F3, the appearance was homogenous, the colour was light green, the clarity was +++ , p^H was 6.90 ± 0.09 , the homogeneity was good, the spreadability was 6.27. From this F3 have the best Formulation which correlates with Range of pH, Spreadability, Homogeneity.

Table 5.1: Physical evaluation of white flower Hibiscus Gel

S.No	FORMULATIONS	F1	F2	F3
1.	Appearance	Homogenous	Homogenous	Homogenous
2.	Colour	Light Green	Light Green	Light Green
3.	Clarity(+, ++, +++)	++	++	+++
4.	p^H	5.93 ± 0.18	6.66 ± 0.13	6.90 ± 0.09
5.	Homogeneity(Good\Bad)	Good	Good	Good
6.	Spreadability gm.cm/sec	4.85	5.02	6.27

RESULT FOR IN-VITRO ANTI-ARTHRITIC ACTIVITY:

The F3 Hibiscus Gel sample demonstrated significant Anti-Arthritic activity, exhibiting 91.451% inhibition at a concentration of 500 $\mu\text{g/ml}$ in the Bovine Serum Albumin (BSA) denaturation inhibition assay. Additionally, the IC_{50} value for the hibiscus gel sample was determined to be 14.087 $\mu\text{g/ml}$, indicating its potency in inhibiting protein denaturation associated with arthritis.

CHAPTER–6

SUMMARY,CONCLUSION&FUTURESCOPE

SUMMARY:

CHAPTER 1- Describes about the general introduction of herbal medicines, biological complexity and its scope. Mechanism of action of phenolic compounds such as Anti-Oxidant Activity, Anti-Arthritic Activity, Anti-Microbial, Analgesic effects, Immune response and Neuroprotective activity etc., Traditional medicines used to cure arthritis and herbal market product for arthritis.

CHAPTER 2 - Talks about the evaluation of previous studies that have been published on the creation and description of anti-arthritis gel. Additionally, the literature on *Hibiscus rosa-sinensis* L. was reviewed with particular attention.

CHAPTER 3 – Explains about the aim of formulating gel using white flowers of *Hibiscus rosa-sinensis* and evaluating its physical parameters. It also gives an idea about the future perspective of the formulated gel.

CHAPTER 4 - Describes the Anatomical characterization was done for the reproductive part of the flower. It also includes the geographical source, collection of flower, collection of plant sample, preparation of flower extracts, phytochemical screening of the flower extracts such as alkaloids, flavonoids, phenols and terpenoid. Estimation of phenolic compound was performed. Thin layer chromatography was performed for phenols and flavonoids. It also describes about the formulation of herbal gel and its evaluation, physical evaluation such as measurement of pH, appearance, spreadability, clarity, and homogeneity, etc., were studied. *In-vitro* anti-arthritic study was estimated using Bovine Serum Albumin Denaturation Inhibition Assay method and also a comparative study with acetylsalicylic acid was performed.

CHAPTER 5 – Provides an insight into the result obtained from phytochemical screening, anatomical characterization, thin layer chromatography, estimation of

total phenolic compounds, and Bovine Serum Albumin [BSA] denaturation inhibition assay using the white flowers of *Hibiscus rosa-sinensis*.

CHAPTER 6 - Describes the summary, conclusion and future perspective of research work of formulation and evaluation of Anti-Arthritic gel.

CONCLUSION:

Rheumatoid arthritis is an autoimmune inflammatory disease and it is primarily characterized by synovitis (inflammation in joints). Hibiscus flowers had been traditionally used for curing various disease like Anti-Inflammation, Anti-Arthritis, Alopecia etc. It is also used as immunomodulators. Anatomical characterization of the reproductive part of *Hibiscus rosa-sinensis* white flower was performed. Anatomical characterization of the reproductive part of *Hibiscus rosa-sinensis* white flower provides insights into its morphological features and structural composition. Phytochemical screening of various extract of white flower revealed the presence of alkaloid, flavonoids, phenols and terpenoids. Among these compounds, flavonoids are polyphenolic compounds that have been found to play a major role in reducing inflammation. To further examine, Thin layer chromatography was performed using the ethanolic extract of *Hibiscus rosa-sinensis* white flower which proves the presence of phenolic compounds. Phenolic compounds are known for their antioxidant and anti-inflammatory effects. They reduce the onset and progression of arthritis disease like Rheumatoid Arthritis. Phenolic compounds influence the synthesis of inflammatory cytokines and autoantibodies that are involved in the inflammatory process of arthritis. The extract's flavonoid and phenolic content may help reduce inflammation in the synovial membrane, thereby alleviating the symptoms of rheumatoid arthritis. Based on the phytochemical screening the antiarthritic activity was proven in the ethanolic extract of *Hibiscus rosa-sinensis*. Arthritis can be cured using various traditional system of medicines like siddha, ayurveda, unani, homeopathy and naturopathy etc. Siddha system of medicines focuses on improving digestion, detoxification, and strengthening the body's natural healing mechanisms. Ayurvedic treatment system for arthritis focuses on balancing the doshas-Vata, Pitta, and Kapha. Unani treatment for arthritis aims to restore the balance of humors, eliminate waste products, reduce inflammation, and strengthen

the joints and muscles. Homeopathic treatment includes conventional medications, physical therapy, lifestyle modifications, and other complementary therapies. Naturopathy aims to restore the balance and promote vitality in the body, mind, and spirit. Herbal marketed formulations include Simhanada Guggulu, Maha Rasnadi Kada, Mahanarayan Tailam, Biofreeze gel, Arnicare Gel etc.

FUTURE PERSPECTIVES:

- To evaluate in future by using biological methods such as in-vivo study in wistar rat with the formulated Anti-Arthritic gel.
- It will also be a source for isolating polyphenolic phytoconstituents.
- It can also be incorporated with other Malvaceae species for preparing polyherbal formulations.
- The scope of white flowers helps the future researcher to formulate Nano formulation with other hibiscus species.
- Researcher's curiosity as increased their finding with herbal therapies in curing various diseases.
- White flowers of hibiscus play a vital role as Anti-Oxidant, Anti-inflammatory and immunomodulator.
- To perform stability studies for the formulated anti-arthritic gel.
- In future the formulated gel can be customized to meet individual patient needs based on genetic makeup, disease phenotype, and other clinical parameters.
- Formulated gel can be developed into implantable gels for controlled drug release.
- Conducting clinical trials to evaluate the safety, efficacy, and tolerability of the formulated Anti-Arthritic gel in human subjects would be a crucial next step towards its clinical application and regulatory approval.
- Optimization of the formulation parameters, such as gel composition, concentration of active ingredients, and delivery system, could enhance the stability, bioavailability, and therapeutic effects of the Anti-Arthritic gel.

REFERENCES

1. Kumar A, Singh A. Review on Hibiscus rosa-sinensis. International Journal of Research in Pharmaceutical and Biomedical Sciences 2012; DOI:10.4236/ijbise.2014.714107
2. Jesica J Mejia, Jose G Ceballos. Antioxidant Capacity and Flavonoid Composition in Hibiscus rosa-sinensis Cultivars; 2023; DOI:10.3390/molecules28041779
3. Braz J Med Biol Res. Antibacterial activity of Hibiscus rosa-sinensis L. red flower against antibiotic-resistant strains of Helicobacter pylori and identification of the flower constituents. 2021; DOI:10.1590/1414-431X2020e10889.
4. 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; DOI:10.21276/ijprhs.2016.05.02
5. Dr. Suchandragoswami, Shaliniyadav, Pushpendrakumar. A Review on the Pharmacological Activities of Hibiscus Rosa Sinensis. 2023 ISSN: 2249-7781
6. Agarwal S, Prakash R, Srivastava A, Mathur RM. Quantitative and qualitative analysis of phytochemicals, present in flower extract of Hibiscus rosa-sinensis. Int J Scientific Res. 2016; DOI:10.36106/ijsr
7. Smruti Sohani, Ravindra Chouhan. Phytochemistry & nutritional composition of Hibiscus rosa sinensis. ISSN: 2455-541X
8. Mohammed Ali, Prawez Alam, Vijender Singh, Jameel. Phytochemical investigations of the leaves and flowers of Hibiscus rosa-sinensis L. DOI:10.53879/id.54.10.11189
9. Shilpi Patel and Madhavi Adhav. Comparative Phytochemical Screening and Thin Layer Chromatographic Analysis of Ethanolic Leaf Extracts of Morphotypes of Hibiscus Rosa-Sinensis Linn. ISSN: 2277-7695 TPI 2016
10. Sumathy Rengarajan, Vijayalakshmi Melanathur. A comparative study of in vitro antimicrobial activity and TLC studies of petals of selected Indian medicinal plants. DOI:10.22159/ajpcr.2016.v9i5.13476

11. K.N.V.Rao, Geetha K., Alagar Raja. M, David Banji. Quality control study and standardization of *Hibiscus rosasinensis* L. flowers and leaves as per WHO guidelines. E-ISSN: 2278-4136 P-ISSN: 2349-8234 JPP 2014; 3(4): 29-37
<https://www.phytojournal.com/archives?year=2014&vol=3&issue=4&part=A&ArticleId=409>
12. Asmaa Missoum. An update review on *Hibiscus rosasinensis* phytochemistry and medicinal uses. 2018 ISSN: 2454-5023
13. Raduan SZ, Hakim MN. Anti-inflammatory effects of *Hibiscus rosa-sinensis* L. and *Hibiscus rosasinensis* var. *alba* ethanol extracts. Int J Pharm Pharm Sci. 2013; ISSN- 0975-1491 <https://ir.unimas.my/id/eprint/11330/1/7844.pdf>
14. Anusha Bhaskar, Nithya, and Vidhya, V.G. Phytochemical screening and in vitro antioxidant activities of the ethanolic extract of *Hibiscus rosasinensis* L. 2011 ISSN 0976-1233 CODEN(USA): ABRNBW
15. Shilpi Patel and Madhavi Adhav. Comparative phytochemical screening of ethanolic extracts (flower and leaf) of morphotypes of *Hibiscus Rosa-sinensis* Linn. 2016 E-ISSN: 2278-4136 P-ISSN: 2349-8234
16. Sana Wahid , Samiyah Tasleem and Sajid Jahangir. PHYTOCHEMICAL PROFILING OF ETHANOLIC FLOWER EXTRACT OF *HIBISCUS ROSA-SINENSIS* AND EVALUATION OF ITS ANTIOXIDANT POTENTIAL . 2019 ISSN 2277– 7105.
17. M. J. Divya, C. Sowmia, K. P. Dhanya, K. Joona. Screening of antioxidant, anticancer activity and phytochemicals in methanolic extract of *Hibiscus rosa-sinensis* leaf extract. 2013 ISSN: 0975-8585. URL: [http://rjpbcs.com/pdf/2013_4\(2\)/\[142\].pdf](http://rjpbcs.com/pdf/2013_4(2)/[142].pdf)
18. Poornima, M (2012) *Phytochemical Screening, Elemental Analysis and Antimicrobial Activity on Flower Part of Three Hibiscus Species.* <http://repository-tnmgrmu.ac.in/id/eprint/2654>
19. Mukhopadhyay Goutam, Dutta Kr Prabir. Authentication and Photochemical Screening of *Hibiscus Rosa Sinensis*. 2018 E ISSN 2348 –1269, P ISSN 2349-5138
20. Josline Y Salib, Enas N Daniel, Sally M Abdel. Polyphenolic compounds from flowers of *Hibiscus rosa-sinensis* Linn. and their inhibitory effect on alkaline phosphatase enzyme activity in vitro. DOI: [10.1515/znc-2011-9-1003](https://doi.org/10.1515/znc-2011-9-1003)

21. Smail Amtaghri, Adil Qabouche, Miloudia Slaouil. A Comprehensive Overview of *Hibiscus rosa-sinensis* L.: Its Ethnobotanical Uses, Phytochemistry, Therapeutic Uses, Pharmacological Activities, and Toxicology. DOI: [10.2174/1871530323666230522113405](https://doi.org/10.2174/1871530323666230522113405)
22. Hui-Min Shen, Chun Chen, Bing Wang, Wen-Feng Cai. The N-butyl alcohol extract from *Hibiscus rosa-sinensis* L. flowers enhances healing potential on rat excisional wounds. 2017 DOI: [10.1016/j.jep.2017.01.016](https://doi.org/10.1016/j.jep.2017.01.016)
23. Jamadar MJ, Shaikh RH. Preparation and evaluation of herbal gel formulation. *Journal of Pharmaceutical Research and Education*. 2017;1(2):201-4.
24. Saidaiyah Pidigam, Amarapalli Geetha, Someshwar Rao Pandravada, Suhail Khan, Vishnukiran Thuraga, Natarajan Sivaraj and Kattula Nagaraju. Series: Medicinal and Aromatic Plants of the World, Year: 2023, Volume 9, Page 279 DOI: [10.1007/978-3-031-44914-7_14](https://doi.org/10.1007/978-3-031-44914-7_14)
25. Saptarini NM, Pratiwi R, Maisyarah IT. Colorimetric method for total phenolic and flavonoid content determination of fig (*Ficus carica* L.) leaves extract from West Java, Indonesia. *RASAYAN J. Chem.*, Vol 15, NO.1, 2022
26. Wahid S, Tasleem S, Jahangir S. Phytochemical profiling of ethanolic flower extract of *Hibiscus rosa-sinensis* and evaluation of its antioxidant potential. *World J. Pharm. Res.* 2019;
27. Al-Snafi AE. Chemical constituents, pharmacological effects and therapeutic importance of *Hibiscus rosa-sinensis* - A review. *IOSR PHR J Pharm.* 2018;
28. Vastrad JV, Byadgi SA. Phytochemical screening and antibacterial activity of *Hibiscus rosa-sinensis* leaf extracts. *International Journal of Current Microbiology and Applied Sciences*. 2018;
29. Johnson S, Abdul Razack S, Sellaperumal P, Ramakrishnan G, Jayakrishnan P. Antioxidant activity of iron isolated from petals of *Hibiscus rosa sinensis*. *EC Microbiology*. 2017;
30. Ghosh A, Dutta A. GC-MS analysis and study of potential antioxidant activity of the crude ethanolic flower extract of *Hibiscus rosa sinensis* L. (wild variety) by hydrogen peroxide scavenging assay. *International Journal of Current Trends in Science and Technology*. 2017;

31. Sobhy EA, AbdElaleem KG, AbdElaleem HG. Potential antibacterial activity of *Hibiscus rosa-sinensis* flower extracts. *International Journal of Current Microbiology and Applied Sciences*. 2017;
32. Zahid H, Rizwani GH, Khalid L, Shareef H. Comparative profile of *Hibiscus schizopetalus* (Mast) hook and *Hibiscus rosa-sinensis* L. (Malvaceae). *Journal of Pharmacognosy and Phytochemistry*. 2016;5(1):131-6.
33. 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-hemolytic activities of *Hibiscus rosa-sinensis* L. flowers in vitro. *Int J Pharma Res Health Sci*. 2016;
34. Agarwal S, Prakash R, Srivastava A, Mathur RM. Quantitative and qualitative analysis of phytochemicals, present in flower extract of *Hibiscus rosa-sinensis*. *Int J Scientific Res*. 2016;
35. Goldberg KH, Yin AC, Mupparapu A, Retzbach EP, Goldberg GS, Yang CF. Components in aqueous *Hibiscus rosa-sinensis* flower extract inhibit in vitro melanoma cell growth. *J Tradit Complement Med*. 2016;
36. Udo IJ, Ben MG, Etuk CU, Tiomthy AI. Phytochemical, proximate and antibacterial properties of *Hibiscus rosa-Sinensis* L. Leaf. *Journal of Medicinal Plants Studies*. 2016;
37. Nayak D, Ashe S, Rauta PR, Nayak B. Biosynthesis, characterisation and antimicrobial activity of silver nanoparticles using *Hibiscus rosa-sinensis* petals extracts. *IET Nanobiotechnol*. 2015;
38. Tiwari 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;
39. Kaur S, Lakshmanan P. *Hibiscus rosa-sinensis* leaves polysaccharide as solubility enhancing agent for poorly soluble drug. *Pharm Pharmacol Int J*. 2015;
40. Kumari OS, Rao NB, Reddy VK. Phyto-chemical analysis and anti-microbial activity of *Hibiscus rosa-sinensis*. *World Journal of Pharmacy and Pharmaceutical Sciences* 2015;
41. Salem M., Olivares J., Salem A. Studies on biological activities and phytochemicals composition of *Hibiscus* species. A review. *Life Sci*. 2014;

42. Khalid L, Rizwani GH, Sultana V, Zahid H, Khursheed R, Shareef H. Antidepressant activity of ethanolic extract of *Hibiscus rosa Sinensis* Linn. *Pak J Pharm Sci.* 2014;
43. Rao K., Geetha K., Raja A., Banji D. Quality control study and standardization of *Hibiscus rosa-sinensis* L. flowers and leaves as per WHO guidelines. *J. Pharm. Phytochem.* 2014;
44. Agarwal S, Prakash R. Evaluation of antibacterial activity of *Hibiscus rosa-sinensis* flower extract against *E. coli* and *B. subtilis*. *Biological Forum –An International Journal* 2014;
45. Prasad MP. In vitro Phytochemical Analysis and Antioxidant Studies of *Hibiscus* Species. *International Journal of Pure & Applied Bioscience.* 2014;
46. Khan ZA, Naqvi SA, Mukhtar A, Hussain Z, Shahzad SA, Mansha A, Ahmad M, Zahoor AF, Bukhari IH, Ashraf-Janjua MR, Mahmood N, Yar M. Antioxidant and antibacterial activities of *Hibiscus rosa-sinensis* Linn flower extracts. *Pak J Pharm Sci.* 2014;
47. Raduan SZ, Hakim MN. Anti-inflammatory effects of *Hibiscus rosa-sinensis* L. and *Hibiscus rosasinensis* var. *alba* ethanol extracts. *Int J Pharm Pharm Sci.* 2013;
48. Pekamwar SS, Kalyankar TM, Jadhav AC. *Hibiscus rosa-sinensis*: A review on ornamental plant. *World Journal of Pharmacy and Pharmaceutical Sciences* 2013;
49. 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;
50. Sheth F., De S. Evaluation of comparative antioxidant potential of four cultivars of *Hibiscus rosa-sinensis* L. by HPLC-DPPH method. *Free Radic. Antioxid.* 2012
51. Garg D., Shaikh A., Muley A., Marar T. In vitro antioxidant activity and phytochemical analysis in extracts of *Hibiscus rosa-sinensis* stem and leaves. *Free Rad. Antiox.* 2012;
52. Patel R., Patel A., Desai A., Nagee A. Study of secondary metabolites and antioxidant properties of leaves, stem and root among *Hibiscus rosa-sinensis* cultivars. *Asian J. Exp. Biol. Sci.* 2012;

53. Bhaskar A, Nithya V. Evaluation of the wound-healing activity of *Hibiscus rosasinensis* L. (Malvaceae) in Wistar albino rats. *Indian J Pharmacol.* 2012;
54. Ruban P, Gajalakshmi K. In vitro antibacterial activity of *Hibiscus rosasinensis* flower extract against human pathogens. *Asian Pac J Trop Biomed.* 2012;
55. Sugumaran M, Poornima M, Sethuvani S. Phytochemical and trace element analysis of *Hibiscus rosasinensis* Linn and *Hibiscus syriacus* Linn flowers. *NPAIJ.* 2012;
56. Kumar A, Singh A. Review on *Hibiscus rosa-sinensis*. *International Journal of Research in Pharmaceutical and Biomedical Sciences* 2012;
57. Upadhyay SM, Upadhyay P, Ghosh AK, Singh V, Dixit VK. Effect of ethanolic extract of *Hibiscus rosa-sinensis* L., flowers on hair growth in female Wistar rats. *Der Pharmacia Lettre* 2011;
58. Soni D, Gupta A, Solanki R, Jana GK. Pharmacognostical, phytochemical, and physiochemical findings over the root extract of *Hibiscus rosa sinensis* [Malvaceae]. *J Nat Prod Plant Resour.* 2011;
59. Moqbels FS, Naik PR, Najma HM, Selvaraj S. Antidiabetic properties of *Hibiscus rosa sinensis* L. leaf extract fractions on nonobese diabetic (NOD) mouse. *Indian J Exp Biol.* 2011;
60. Bhaskar A, Nithya V, Vidhya VG. Phytochemical screening and in vitro antioxidant activities of the ethanolic extract of *Hibiscus rosa-sinensis*. *Ann Biol Res.* 2011;
61. Uddin B, Hossan T, Paul S, Ahmed T, Nahar T, Ahmed S. Antibacterial activity of the ethanol extracts of *Hibiscus rosa-sinensis* leaves and flowers against clinical isolates of bacteria. *Bangladesh J Life Sci.* 2010;
62. Jadhav VM, Thorat RM, Kadam VJ, Sathe NS. Traditional medicinal uses of *Hibiscus rosa-sinensis*. *Journal of Pharmacy Research* 2009;
63. Vankar P., Srivastava J. Comparative study of total phenol, flavonoid contents and antioxidant activity in *Canna indica* and *Hibiscus rosa sinensis*: Prospective natural food dyes. *Int. J. Food Eng.* 2008;
64. Khandelwal K.R. Practical pharmacognosy, 19th edition, Nirali Prakashan; 2008.

65. NayakBS, RajuSS, OretteFA, RaoAVC. Effectsof *Hibiscus rosasinensis*l (Malvaceae) on wound healing activity: a preclinical study in a sprague Dawley rat. *The International Journal of Lower Extremity Wounds*. 2007;
66. ShivanandaNayakB, SivachandraRajuS, OretteFA, ChalapathiRao AV. Effects of *Hibiscus rosa sinensis*L (Malvaceae) on wound healing activity: a preclinical study in a Sprague Dawley rat. *Int J Low Extrem Wounds*. 2007;
67. GilaniA., BashirS., JanbazK., ShahA. Presenceofcholinergicandcalcium channel blocking activities explains the traditional use of *Hibiscus rosa-sinensis* in constipation and diarrhea. *J. Ethnopharmacol*. 2005;
68. Ragunathan, V., Sulochana, N., A new flavonol bioside from the flowers of *Hibiscus vitifolius* Linn. And its Hypoglycemic activity. *Journal of Indian Chemical Society* 1994;
69. V., Sulochana, N., A new flavonol bioside from the flowers of *Hibiscus vitifolius* Linn. And its Hypoglycemic activity. *Journal of Indian Chemical Society* 1994;
70. Khokhar I, Ahmad A. Studies in medicinal plants of Pakistan: New cyclopeptide alkaloids from the flowers of *Hibiscus rosa-sinensis*. *Sci Int. (Lahore)* 1992;
71. Nakatani M, FukunagaY, HaseT. Aliphatic compounds from *Hibiscus rosa-sinensis*. *Phytochemistry* 1986;
72. FahnA. *Plant anatomy*. Third Edition Pergamon Press, Oxford; 1980.
73. Dwivedi R., Pandey S., Tripathi V. Role of japapushpa (*Hibiscus rosa-sinensis*) in the treatment of arterial hypertension. A trial study. *J. Res. Indian Med*. 1977;
74. WallisTE. *Analytical Microscopy - Its aims and methods in relation to foods, water, spices and drugs*. Third Edition. Boston: Little, Brown and Company; 1965.