

**ANATOMICAL, PHYTOCHEMICAL AND *In vitro* ANTI-ARTHRITIC  
SCREENING FROM WHITE FLOWERS OF *Hibiscus rosa-sinensis* L.  
GEL.**

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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 Flowers, Bovine serum, Gallic acid, Carbopol-94.

## INTRODUCTION

Arthritis, a chronic and debilitating condition, affects millions worldwide, causing pain, stiffness, and reduced mobility. While conventional treatments offer relief, herbal medicines have emerged as a complementary approach, leveraging the therapeutic potential of plants. Phenolic compounds, a diverse group of bioactive molecules, have shown promise in alleviating arthritis symptoms. This article explores the benefits, challenges, and mechanisms of action of herbal medicines and phenolic compounds in arthritis management. Herbal medicines have been used for centuries to treat various health conditions, including arthritis. These natural remedies have gained popularity due to their perceived safety, efficacy, and low cost. Herbal medicines encompass herbs, herbal materials, herbal preparations, and finished herbal products, containing phytochemical constituents derived from plants. Phenolic compounds, abundant in fruits, vegetables, and herbs, have been recognized for their anti-inflammatory, antioxidant, and immunomodulatory properties. These compounds have shown potential in managing arthritis by, Reducing inflammation and oxidative stress, Modulating the immune response, Inhibiting cartilage degradation, Exhibiting analgesic and antimicrobial effects. Phenolic compounds exert their therapeutic effects through various mechanisms, including, Anti-inflammatory activity: inhibiting pro-inflammatory enzymes and signaling pathways, Antioxidant activity: scavenging free radicals and reactive oxygen species, Immune response modulation: regulating immune cell activity and cytokine production, Cartilage protection: inhibiting enzymes involved in cartilage degradation. Herbal medicines and phenolic compounds offer several benefits, including: Low toxicity and natural origin, Efficacy in alleviating arthritis symptoms, Accessibility and ease of preparation. However, challenges persist, such as: Knowledge gaps among healthcare professionals and the public, Need for accurate information on herbal medicines, Quality control and standardized dosages. Herbal medicines and phenolic compounds hold promise in arthritis management, offering a complementary approach to conventional treatments.<sup>[1-10]</sup> Further research is necessary to fully understand their mechanisms of action, optimize their therapeutic potential, and address the challenges associated with their use. By exploring the benefits and challenges of herbal medicines and phenolic compounds, can unlock their potential in improving the quality of life for arthritis sufferers.



**Figure 1: White flowers of hibiscus rosa-sinensia.**

## MATERIALS AND METHODS

*Hibiscus-rosa-sinensis* L., commonly known as Chinese hibiscus, china rose, or Hawaiian hibiscus is a tropical flowering plant belonging to the family malvaceae. The white flowers of the *Hibiscus -rosa-sinensis* plant were collected from a local area in Chennai, tamilnadu, india and authenticated in siddha research institute, arubakkam, Chennai-600106.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 seven consecutive days by cold macerartion.<sup>[11-17]</sup>

The solvent was removed under reduced pressure and yield of extract was calculated.



**Figure 2: Coarse powder of White flowers of Hibiscus rosa-sinensis.**

The Anatomical characterization on reproductive 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

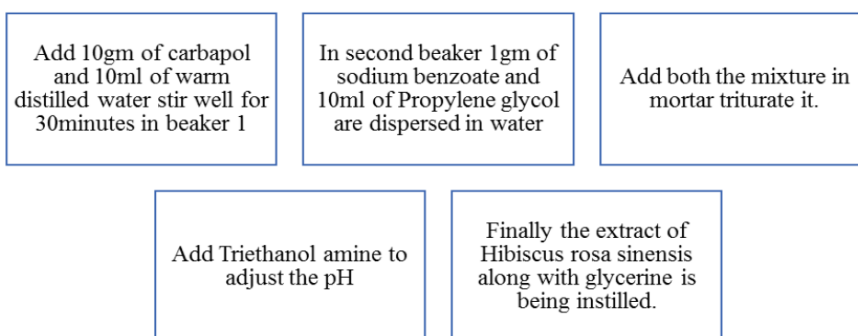
science, microtomes are used in microscopy for the preparation of samples for observation under transmitted light or electron radiation.

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  $\mu$ 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). The sections were taken with the help of a Leica rotary microtome.<sup>[18-26]</sup>

### Thin layer chromatography

Thin layer chromatography of Ethanolic extract containing white flower of *Hibiscus rosa-sinensis* L. A small spot of sample was placed on Thin layer chromatography plate by a 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 differing affinities for the stationary phase and the mobile phase, allowing for individual identification and characterization. The Rf values calculated and compared.<sup>[27-36]</sup>

### Formulation of gel



### Appearance

The prepared gels were inspected visually for clarity, colour and presence of any particle.<sup>[36-46]</sup>

### Clarity

The clarity of various formulations was determined by visual inspection and it was graded as follows; turbid: +, clear: ++, very clear (glassy): +++. 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, the results were shown in Table 6&7.

### *In-Vitro* Anti-Arthritic activity by Bovine serum Albumin 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.

About 100, 200, 300, 400, 500  $\mu\text{g/ml}$  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. Acetyl salicylic acid was taken instead of test item as a positive control. The percent inhibition for protein denaturation was calculated using and results in Table 8.

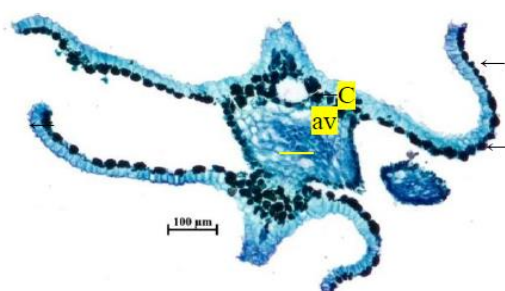
Percentage inhibition =  $100 - \frac{\text{Absorbance of Sample} - \text{Absorbance of control}}{\text{Absorbance of control}} \times 100$  <sup>[47-59]</sup>

## RESULT AND DISCUSSION

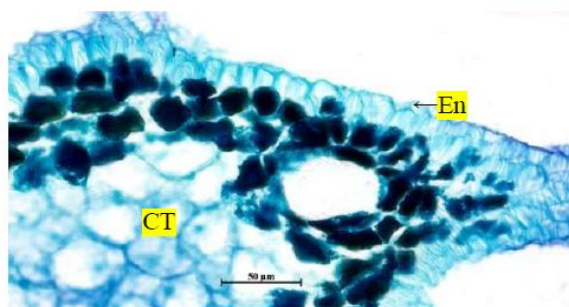


**TS of anther**

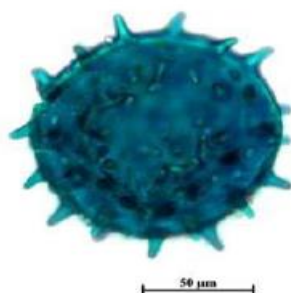
TS of the mature ruptured anther is irregular in outline; it shows an outer epidermal layer followed by endothelial cells formed of compactly arranged cylindrical cells; all other contents and pollen grains are dehiscid leaving four empty lacunae; two anther lobes are connected by parenchymatous connective tissue; a small vascular strand can be seen at the center of connective tissue; some secretion cavities can be seen near the connective tissue shown in Figure 3.



**TS of *Hibiscus rosa-sinensis* L. anther**



**Enlarged view of anther**

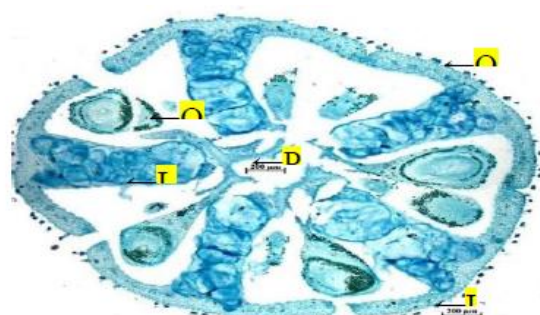


**Spiny Pollen grains**

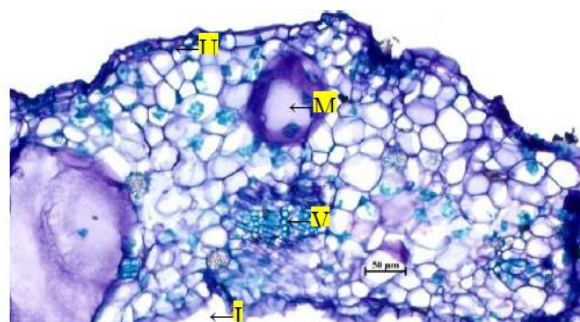
**Figure 3: T. S. of Reproductive Part of *Hibiscus rosa-sinensis* L. (White Flower).**

**TS of ovary**

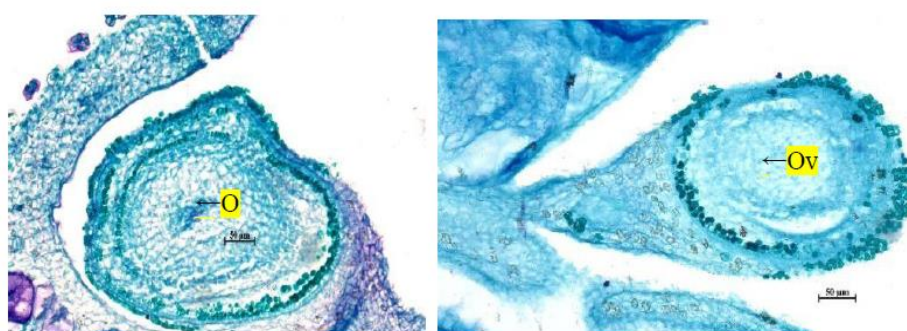
A transverse section of the pentacarpellary ovary is almost rounded in outline showing five locules, each locule containing two ovules. The ovary shows an outer and inner epidermis enclosing a parenchymatous mesophyll in between. The mesophyll is formed of rounded, moderately thin-walled parenchyma with 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 at below Figure 4.



*TS of Hibiscus rosa-sinensis L. Ovary*



**Ovary wall enlarged**



*Developing ovule and Mature ovule*

**Figure 4: T. S. of the Ovary of Hibiscus rosa-sinensis L. (White Flower).**

**Table 1: Preliminary phytochemical screening for the various extracts of flower of *hibiscus rosa sinensis L.***

Tests	Pet ether extract	Ethyl acetate extracts	Ethanol extract	aqueous extract
<b>Test for alkaloids</b>				
a. Mayers Reagent	-	-	+	+
b. Dragendroffs Reagent	-	-	+	+
<b>Test for flavonoids</b>				
Acid test	-	+	+	+
Alkali Test	-	-	+	+
Shinoda Test	+	-	+	+
<b>Test for phenols</b>				
Ferric chloride test	+	+	+	+
<b>Test for terpenoids</b>	+	+	+	+
<b>Test for tannins</b>	-	-	-	-
<b>Test for saponins</b>	-	-	-	-
<b>Test for cardiac glycosides</b>	-	-	-	-
<b>Test for carbohydrates</b>	-	-	+	+

#### Estimation of total phenolic content of ethanolic extract of *hibiscus rosa-sinensis* L. (white flower)

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.

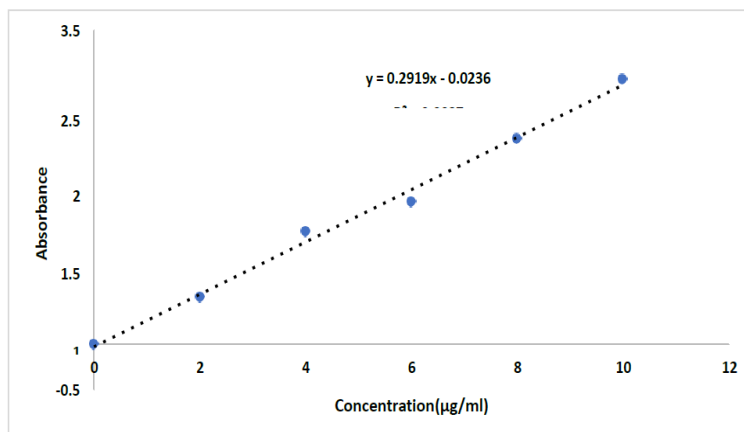
The total phenol content of various flower extracts of *Hibiscus rosa-sinensis* white flower was estimated using **Folin–Ciocalteu** reagent method. The 1ml of ethanol extract from 1mg/ml(1000µg/ml) was transferred into separate test tubes. To this solution, Folin–Ciocalteu Reagent 0.5ml and 1ml of sodium 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) and the results are tabulated in Table 2.

**Table 2: Concentration vs absorbance of gallic acid.**

Concentration (µg/ml)	Absorbance
0	0
2	0.523



4	1.253
6	1.587
8	2.295
102	2.956



**Figure 5: Calibration curve of gallic acid.**

**Table 3: Concentration vs absorbance of test sample.**

Sample solution (µg/ml)	Weight of flower extract per ml (mg)	Absorbance	mg of GAE equivalent/gm of extract*
100	1	1.115	386.182 ± 2.76
100	1	1.084	
100	1	1.112	

**Table 4: Thin layer chromatography of flavonoid.**

Solvent system (Mobile phase)	Ratio	Detector
Toluene	4	UV chamber 254nm
Ethyl acetate	2	
Formic acid	1	

**Retention Factor ( $R_f$ ) of Flavonoid = 0.81**

**Table 5: 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**

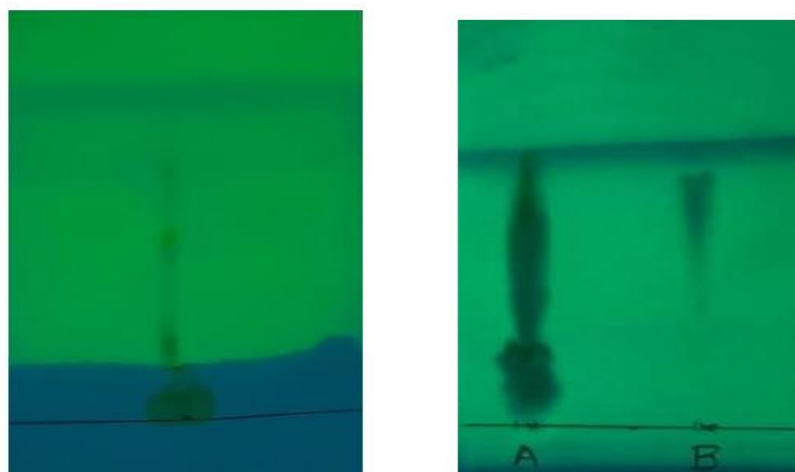


Figure 6: Thin layer chromatography of flavonoids.

Table 6: Formulation of Gel with White Flowers of Hibiscus rosa sinensis.

Sl. No	Ingredients	F1	F2	F3
1	Ethanol extract of Hibiscus rosa-sinensis L.(White flowers)	10ml	20ml	30ml
2	Propylene glycol	10ml	10ml	10ml
3	Sodium Benzoate	10gm	10gm	10gm
4	Glycerine	1ml	2ml	3ml
5	Carbapol 940	10gm	10gm	10gm
6	Triethanol amine	q.s	q.s	q.s
7	Purified water	q.s	q.s	q.s

## Evaluation of white flower hibiscus gel

Table 7: 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.	pH	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

Table 8: Inhibition of bovine serum albumin denaturation assay.

Concentrations (µg/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±0.0023
200	0.042	0.044	0.039	0.042	69.586±0.0015
300	0.031	0.029	0.031	0.030	77.859±0.0007
400	0.021	0.020	0.019	0.020	85.401±0.0006
500	0.013	0.012	0.011	0.012	91.241±0.0006

\*mean of three readings ± SEM

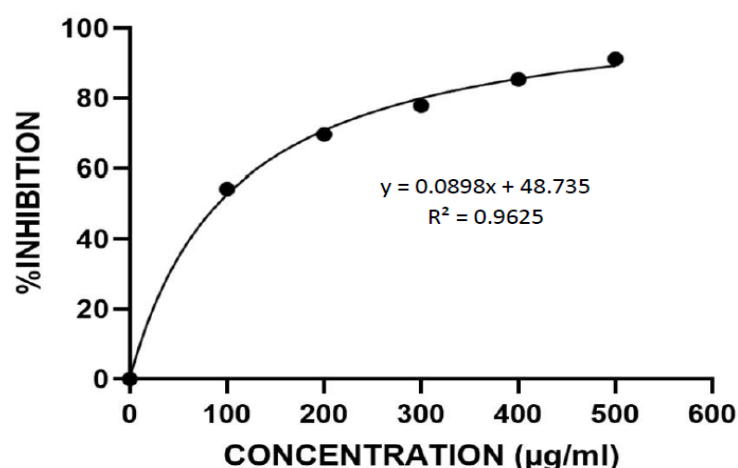


Figure 7: Calibration Curve of BSA denaturation assay.

### 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 Graph pad prism 6.0 software. *p* values less than or equal to 0.05 were considered significant.

### DISCUSSION

Anatomical characterization on reproductive 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. 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 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). Estimation of total phenolic content in the ethanolic extract of *Hibiscus rosa-sinensis* L. (white flower) results in  $386.182 \pm 2.76$  mg of GAE equivalent/gm of extract. Thin Layer Chromatography was performed by using suitable solvent system and it indicates that the *R<sub>f</sub>* value correlates with Rutin and Gallic acid. The physical evaluations of anti-arthritic gel formulations F1, F2, F3 include appearance, colour, clarity, *p<sup>H</sup>*, homogeneity and spreadability were measured. For F1, the appearance was homogenous, the

colour was light green, the clarity was ++,  $p^H$  was  $5.93 \pm 0.18$ , 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  $6.66 \pm 0.13$ , 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  $6.90 \pm 0.09$ , the homogeneity was good, the spreadability was 6.27. From this F3 have the best Formulation which correlates with Range of pH, Spreadability, Homogeneity. 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 IC50 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.

## 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.

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