

## “Development and Evaluation of Herbal Anti Acne Gel With Ethanolic Extract of Tagetes Erecta And Hibicus Rosa-Sinensis”

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**Abstract:** Acne is a widespread and socially distressing dermatological condition, primarily caused by *Propionibacterium acnes*, *Staphylococcus epidermidis*, and *Staphylococcus aureus*. While conventional treatment often involves the use of antibiotics, the growing concern of antibiotic resistance has prompted the exploration of safer and more sustainable alternatives, particularly those derived from natural sources. *Hibiscus rosa-sinensis*, commonly known as Hibiscus, has shown significant potential in dermatological applications due to its rich phytochemical content. It contains natural acids that function as chemical exfoliants, effectively helping to unclog skin pores. Moreover, its antibacterial and anti-inflammatory properties contribute to the prevention and treatment of acne lesions.

## INTRODUCTION

Herbal medicine also called botanical medicine or phyto medicinal refers to the use of any plant seeds barriers roots leaves bark or flower for medicinal purpose .long practiced outside of convention medicine herbalism is becoming more mainstream as up to date analysis and research shown their value in the treatment and prevention of diseases.<sup>1</sup> Ointment are greasy semisolid preparation and containing dissolved or dispersed medicament there are different type of ointment base like hydrocarbon bases usually consist of soft paraffin or its mixture with soft paraffin from a greasy film on skin inhibiting water loss thus hydrating skin because of their hydration effect on skin ointment are very effective in improving its hydration status absorption base these bases soak up water to form w/o emulsion while retaining their semisolid consistency. <sup>2</sup>The maximum amount of water that can be added to 100 gm of such a base at a given temperature is called as water number. World health organization estimated that 80 % of people worldwide rely on herbal medicines for some aspect of their primary health care for most herb contain a therapeutic effect is not known whole herb contain to produced decreased medicinal effect Along with other dosage form herbal drugs are also formulated in form of ointment an ointment is a viscous semisolid preparation used topically on a variety of body surface these include the skin and the mucus membrane of the eye vagina anus and nose.<sup>3</sup> Skin is the most vulnerable part of the body. It is a well-known fact that day to day exposure of human skin<sup>4</sup> leads to the number of problems such as acne, pimples, pigmentation and sunburn marks<sup>5</sup>. The most common skin disease now days is acne. It is a skin disorder that suppresses an individual's self-esteem concerning physical appearance and has a clinical onset during puberty and adolescence<sup>6</sup> Acne vulgaris, a multifarious chronic inflammatory state and mostly happen within a pilosebaceous unit including hair, hair follicles, sebaceous gland of the skin. Acne is categorized by non-inflammatory lesions that are whiteheads, blackheads, and inflammatory lesions that are papules, nobles, pustule, podules, and cysts.<sup>7</sup> It is characterized by several clinical conditions such as scaly red skin (Seborrhoea), erythematous papules and pustules, comedones, nodules, deep pustules, and sometimes pimples, all these symptoms are shown during the process of acne development<sup>8</sup>. Acne vulgaris is inflammation of the sebaceous (oil) gland of the skin. At first, there is excessive sebum production due to the action of androgens. These glands become blocked causing blackheads and whiteheads due to increased keratinisation of the sebaceous duct. Secondary bacterial infections occur due to Propionibacterium acnes (P. acnes) in the sebum. Lipases are produced which in turn produce free fatty acids (FFA) from

the triglycerides in the sebum.<sup>9</sup> There is a normal role of *Propionibacterium acnes* (*P. acnes*) to play in the upper layers of the skin with the resultant FFA keeping the skin pH between 5 and 6 which acts as a barrier against other bacteria and viruses. Further down in the follicle close to the dermis the free fatty acids (FFA) become pro-inflammatory, thus provoking inflammation characterised by pimples (red papules, pustules), and deeper lumps (cysts or nodules)



**Figure no.1: Acne skin**

#### **Causes of Acne vulgaris:**

##### **Infectious contribution:**

*Staphylococcus aureus* and *Propionibacterium acnes* have been recognised to *Acne vulgaris*. However, their exact contributions to the process of acne are not completely clear. So, there are many sub-strains of *P.acnes* present in regular skin and some others in long-term acne problems. Therefore, it is unclear to understand whether these strains are contributing to this condition or they are pathogenically developed. The resistance of *P. acnes* to commonly used drugs is a growing factor<sup>10</sup>. The above present strains can change, perpetuate, or familiarize to the abnormal oil production, inflammation and insufficient sloughing of acne pores. Infection with *Demodex*, which is a parasitic mite is linked with the formation of *Acne vulgaris*. Though, eradication of the mites has not improved or treat *Acne vulgaris*<sup>11</sup>.

##### **Dietary Contribution:**

The two factors that are diet and acne but the relationship between these two factors is unclear and there is no good quality evidence for dietary combination. However, a high level of glycaemic diet is associated with the worsening of *Acne vulgaris*. A positive connection between the use of milk, chocolates or salt, and an increase in the severity of *Acne vulgaris* has also been recommended. However, the contribution of chocolates is uncertain, as they can be made with different quantities of sugar, with or without milk<sup>12</sup>.

##### **Genetic contribution:**

For specific subjects, the predisposition to Acne vulgaris might be clarified by a genetic component. This recommendation has been supported by some studies that have estimated the rate of acne among first degree relatives, along with twin studies. There are varieties of genetic factors, which have been attributed to acne, such as polymorphisms in IL-1 $\alpha$ , TNF- $\alpha$ , and CYP1A1 amongst others<sup>13</sup>.

### **Hormonal changes:**

Hormonal changes, such as puberty and menstrual cycles in the human body, seem to contribute to the development of Acne vulgaris. An increase form of some sex hormones, especially the hormone that are changed during puberty and pregnancy, cause the follicular glands to develop more sebum.<sup>14</sup> The use of anabolic steroids typically has similar effects. Hormones, which have been recognized and present in the formation of acne so, Vulgaris, they consist of testosterone, dehydroepiandrosterone and, dihydrotestosterone, as well as insulin-like growth factor 1 linked with the formation of acne. The formation of Acne vulgaris in adult women might be due to a fundamental condition such as Cushing syndrome, polycystic ovary syndrome or hirsutism. An ointment may or may not be medicated ointment contain medicament dissolved suspended or emulsified in the base Ointment are used topically for several purpose e.g. as protectants antiseptic emollient Ointment base are almost always anhydrous and generally contain one or more medicaments in suspension or solution or dispersion ointment bases may be hydrocarbon oleaginous etc.<sup>15</sup> In an earlier study medicinal plants has been reported to be very beneficial in wound care and promoting the rate of wound healing with minimal path discomfort and sharing to the patient.<sup>16</sup> Acne vulgaris commonly known as pimples involves many factors for its manifestation and it mainly affects the pilosebaceous follicle.<sup>17</sup> It mainly indicates open and closed comedowns and inflammation causing various conditions such as papules, pustules, and nodules.<sup>1</sup> The main micro-organisms responsible to cause the condition include Propionibacterium acnes, Staphylococcus aureus, and Staphylococcus epidermidis.<sup>2</sup> These micro-organisms proliferate rapidly which ultimately result in development of acne. Acne is a disease characterized by inflammatory and non-inflammatory lesions.<sup>18</sup> The pathogenesis includes various factors like hormonal, bacterial and immunological, which causes acne lesions. Propionibacterium acnes and Staphylococcus epidermidis have been recognized as pus-forming bacteria triggering an inflammation in acne.<sup>19</sup> The present research work deals with formulation and evaluation of herbal cream against this etiologic agent of acne vulgaris.<sup>20</sup> The pathogenesis includes various factors like hormonal, bacterial and immunological, which causes acne lesions.<sup>21</sup> The term acne is obtained from Greek language “acme” which means prime of life.

Acne is not a serious type of disease but it impacts the life quality of the patient<sup>22</sup> This is benign condition can cause severe psychological problems.<sup>23</sup> It can show its symptoms and come out in any stage of life but mostly it affects at age 12-24, the estimated population is around 85%.<sup>24</sup> Acne vulgaris is generally characterized by formation of seborrhea, comedones, inflammatory lesions and presence of bacteria *Propionibacterium acnes*, *Staphylococcus epidermidis* and *Staphylococcus aureus* in the follicular canal and sebum production. *P. acnes* have been described as an obligate anaerobic microorganism. It is implicated in the development of inflammatory acne by its capability to activate complements and by its ability to metabolize sebaceous triglycerides into fatty acids, which chemotactically attract neutrophils.<sup>25</sup> On the contrary, *epidermidis*, an aerobic organism, usually involves in superficial infections within the sebaceous unit.<sup>26</sup> When the chemicals produced by *P. acnes* destroy the cellular structure of skin cells, *Staphylococcus aureus*, grows causing acne lesions. These factors provide a potential target for treatment. *P. acnes*, *S. epidermidis* and *S. aureus* are the target sites of anti-acne drugs.<sup>27</sup>

## **MATERIALS AND METHODS**

### **SAMPLE COLLECTION AND AUTHENTICATION**

The Flower of *Tagetes erecta* species (*Erecta*), *Hibiscus rosa sinensis* species (*Rosa sinensis*), were collected from the local area of Balaghat M.P (India) in the month of November 2024. The plant material were identified and authenticated by the Department of Botany. the collected flower were shade dried under normal environmental condition.

### **EXTRACTION:**

Extraction were done by soxhlation process. The Soxhlet extraction process heats the solvent (ethanol) to boiling temperature (>78°C). The evaporated ethanol is contained within the apparatus by the condenser unit; however, the apparatus should be placed under a fume hood in case of escape.

- By simple maceration
- Using mortar pestle
- Addition of substrates in a series and calculated quantity

### **Procedure of Extraction**

1. Herbal material is placed in SOXHLATE thimble. Solvent is heated under reflux
2. Condensation and extraction with “fresh” solvent. Solutes are transferred from the extraction chamber into the reservoir.

3. Continuous repetition of the extraction.

4. Exhaustive extraction gets complete.



**Fig.no.2 Extractions**

### **PHYSICOCHEMICAL PARAMETER**

#### **Determination of ash values**

Used to determine quality and purity of a crude drug and to establish the identity of it. Ash contains inorganic radicals like phosphates, carbonates, and silicates of sodium, potassium, magnesium, calcium etc. These are present in definite amount in a particular crude drug.

**Used to determine foreign inorganic matter present as an impurity.**

#### **Determination of total ash value: -**

1. Total ash value of the sample =  $100(x-z)/y$  %
2. Weigh the empty silica crucible.
3. Weigh about 2gm of the powdered drug into silica crucible.
4. Then it was placed into muffle furnace until the temperature reach up to 350°C and the temperature was maintained at 100°C.
5. Then take out the silica crucible from the muffle furnace when all carbon was burned off from the crude drug. And cooled in a desiccator
6. The ash was weighed and the percentage of total ash with reference to the airdried sample of crude drug was calculated.

**Determination of Acid insoluble ash value**

- 1.The ash from the dish used for total ash was washed by using 25 ml of diluted hydrochloric acid into a 100ml beaker.
- 2.Boiled it for five minutes in a Bunsen burner. A mere gauze was placed over the Bunsen burner.
- 3.Filtered it through an ash less filter paper and the residue were washed twice with hot water. and ignite a crucible in a flame, cool and weight.
- 4.The filter paper and residue put into the crucible together, gently heated until vapors cease to be evolved and then more strongly until all carbon was removed. Cool in a desiccator.
- 5.The residue was weighted and acid insoluble ash of crude drug with reference to the air-dried sample of the crude drug was calculated.

**Determination of Loss on Drying**

- 1.About 1.5g of the powdered drug was weighted into a weighted flat and thin porcelain dish.
- 2.Dried it in the oven at 100°C-105°C, until two consecutive weighing do not differ by more than 0.5 mg.
- 3.Cool in a desiccator and weigh. The loss in weight was recorded as moisture.

**PHYTOCHEMICAL SCREENING**

- 1.Detection of alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered.
  - a.Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). The yellow-colored precipitate was indicated the presence of alkaloids.
- 2.Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered it. The filtrates sample was used to test for the presence of carbohydrates.

**Fehling's Test:**

Filtrates samples were hydrolyzed with the dilute HCl, and neutralized with alkali then heated with Fehling's A and B solutions. The red colored precipitate indicated the presence of reducing sugars.

- 1.Detection of glycosides: Sample extracts were hydrolyzed with the dilute HCl, and then subjected to test for glycosides.
- 2.Detection of steroids and terpenoids: - In 1 ml of ethanol plant extract, 1ml of chloroform was added and 2-3 ml of acetic anhydride was mixed. Then 1-2 drops of concentrated H<sub>2</sub>SO<sub>4</sub> were added. Dark green coloration of the solution indicated the presence of steroids and pink or red coloration of the solution indicated the presence of terpenoid.

**Detection of saponins**

Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes only. Then formation of 1 cm layer of foam indicated that presence of saponins.

**Detection of phenols**

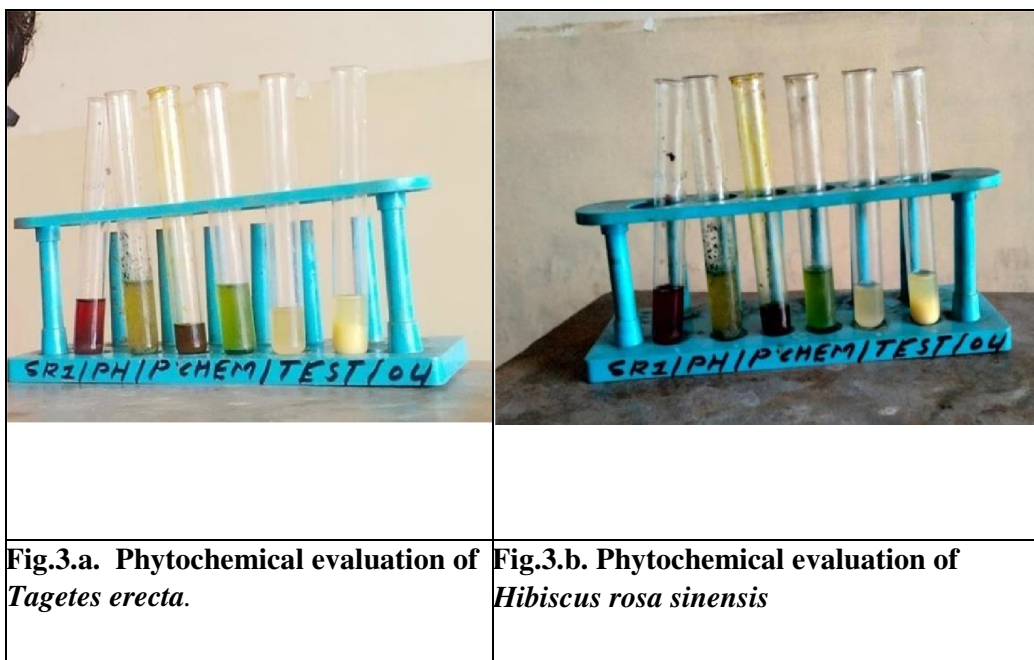
Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. The bluish black color was indicated that presence of phenols.

**Detection of proteins and amino acids**

Xanthoproteic Test: The extracts were treated with few drops of concentrated nitric acid. Then the formation of yellow color indicated that presence of proteins.

**Detection of flavonoids: -**

In ethanol extract 10% NaOH was added and dilute HCl was added to that solution. The change of color from yellow to colorless provides the positive result

**HIBISCUS ROSA SINENSIS****PARAMETER OF ANTIOXIDANT ACTIVITY****A.  $\alpha$ ,  $\alpha$ -diphenyl- $\beta$ -picryl-hydra Zyl (DPPH) radical scavenging assay**

The free radical scavenging activity was measured by using 2, 2-diphenyl-1-picrylhydrazyl or 1, 1-diphenyl-2- picrylhydrazyl by the method of McCune and Johns.[12] The reaction mixture consisted of 1.0 ml of DPPH in methanol (0.3 mM) and 1.0 ml of the extract. After

incubation for 10 min in dark, the absorbance was measured at 517 nm. DPPH scavenging activity was expressed in terms ascorbic acid equivalent (mg/g).[8]

#### **B. Nitric oxide (NO) radical scavenging assay**

3.0 ml of sodium nitroprusside in phosphate buffer (10 mM) was added to 2.0 ml of extract (1:200 dilutions). The resulting solution was then incubated at 25°C for 60 min. To 5.0 ml of the incubated sample, 5.0 ml of Griess reagent (1% sulphanilamide, 0.1% naphthyethylene diamine dihydrochloride in 2% H<sub>3</sub>PO<sub>3</sub>) was added and absorbance of the chromophore formed was measured at 540 nm. [8,13] NO radical scavenging activity was expressed in terms of ascorbic acid equivalent (mg/g).

#### **C. Ferric reducing antioxidant power (FRAP) assay**

0.2 ml of the extract (1:20 dilution) was added to 3.8 ml of FRAP reagent (10 parts of 300 mM sodium acetate buffer at pH 3.6, 1 part of 10.0 mM TPTZ solution and 1 part of 20 mM FeCl<sub>3</sub>. 6H<sub>2</sub>O solution) and the reaction mixture was incubated at 37°C for 30 min and the increase in absorbance at 593 nm was measured.[8] The antioxidant capacity based on the ability to reduce ferric ions of sample was expressed in terms of ascorbic acid equivalent.

#### **PREPARATION OF GELS**

Various gel formulations were prepared using Carbopol 940, HPMC, sodium alginate, sodium CMC as gelling agents. Required quantity of gelling agent was weighted and dispersed in a small quantity of distilled water to form a homogeneous dispersion. The extracts were dissolved in suitable solvent (propylene glycol) and added to the above solution. Other excipients (methyl paraben and propyl paraben) were also added with continuous stirring. The final weight of the gel was adjusted to 5g with distilled water. The gels were stored in wide mouthed bottles. Entrapped air bubbles were removed by keeping the gels in vacuum oven for 2hrs.the Prepared gels are inspected by vision in case of any microbial growth the sample is rejected.

**Table no.1:Formulation of gels of extracts Gela:**

| <b>S.N.</b> | <b>EXTRACT</b>           | <b>GRAM</b> |
|-------------|--------------------------|-------------|
| 1.          | Tagets erecta            | 1.5 g       |
| 2.          | Hibiscus<br>rosasinensis | 2g          |

**Table no.2:Gel B:**

| S.No. | EXTRACT               | GRAM |
|-------|-----------------------|------|
| 1.    | Tagets erecta         | 2g   |
| 2.    | Hibiscus rosasinensis | 1.5g |

Storage of gels: the prepared gels are kept in air tight container for 7 days at different locations, after 7 days one form each location is selected and antimicrobial activity was checked.

|       |    |                                 |
|-------|----|---------------------------------|
| GEL A | A1 | Kept in Room temperature at Lab |
|       | A2 | Kept in refrigerator            |
|       | A3 | Kept in Incubator               |
| GEL B | B1 | Kept in Room temperature at Lab |
|       | B2 | Kept in refrigerator            |
|       | B3 | Kept in Incubator               |

## ANTIMICROBIAL ACTIVITY

### Agar well diffusion method

#### 1. Nutrient Agar Medium (1 L)

The medium was prepared by dissolving 1gm. beef extract, 1gm peptone and 0.5gm sodium chloride in 1000ml of distilled water and boiled to dissolve the medium completely and then add agar media by continuous stirring. The dissolved medium was autoclaved at 15 lbs. pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm Petri plates (25-30ml/plate) while still molten.

#### 2. Nutrient broth (1L)

One liter of nutrient broth was prepared by dissolving 1gm. beef extract, 1gm peptone and 0.5gm sodium chloride in 1000ml distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by autoclaving at 15 lbs. pressure (121°C) for 15 minutes.

#### 3. Soframycin (standard antibacterial agent)

**PROCEDURE**

Petri plates containing 20ml nutrient Agar medium were seeded with 24hr culture of bacterial strains. Wells were cut and 20  $\mu$ l of the plant extracts (namely ethanolic and hydroalcoholic extracts) were added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993). soframycin was used as a positive control.

**RESULT AND DISCUSSION****Table no.3:Total Ash value**

| S.N. | Plants (Flower)              | Ash Value |
|------|------------------------------|-----------|
| 1.   | <i>Tagetes erecta</i>        | 4.95% w/w |
| 2.   | <i>Hibiscus rosasinensis</i> | 5.5% w/w  |

**Result:** Total ash value gives an estimation about purity and quality of drug. Water soluble ash value gives an estimation of inorganic contents. According to the results obtained of both plants indicated that they do not contain any impurities and no adulteration was found in samples

**Table no.4: Acid insoluble ash**

| S.N. | Plants (Flower)              | Acid insoluble ash value |
|------|------------------------------|--------------------------|
| 1.   | <i>Tagetes erecta</i>        | 0.2% w/w                 |
| 2.   | <i>Hibiscus rosasinensis</i> | 2 % w/w                  |

**Result:** The residue remaining after incineration is ash content of drug. Acid insoluble ash values represented detecting presence of silica and oxalates in drug.

**Table no.5: Loss On drying**

| S.N | Plants (Flower)              | Loss on drying (LOD) |
|-----|------------------------------|----------------------|
| 1.  | <i>Tagetes erecta</i>        | 7.46 % w/w           |
| 2.  | <i>Hibiscus rosasinensis</i> | 78 % w/w             |

**Result:** Loss on drying is an unspecific analytical technique removing not only water but all other impurities like alcohol etc. According to result obtained the sample doesn't contain any impurities

**Table no.6: Result of Phytochemical screening - *Tagets erecta* (Marigold)**

| S.N. | PHYTO CHEMICALS | TEST                  | EXTRACT OF <i>TAGETS ERECTA</i> |
|------|-----------------|-----------------------|---------------------------------|
| 1.   | Alkaloids       | Wagner test           | +ve                             |
|      |                 | Hager test            | +ve                             |
|      |                 | Dragendroff's test    | +ve                             |
| 2.   | Carbohydrate    | Fehling's test        | -ve                             |
|      |                 | Barfoed's test        | -ve                             |
|      |                 | Molisch test          | -ve                             |
| 3.   | Steroids        | Liebermann test       | -ve                             |
| 4.   | Phenols         | 10% FeCl <sub>3</sub> | -ve                             |
|      |                 | Dil. HNO <sub>3</sub> | -ve                             |
| 5.   | Glycoside       | Keller kiliani test   | -ve                             |
|      |                 | Anthraquinone test    | -ve                             |
| 6.   | Proteins        | Biuret test           | -ve                             |
|      |                 | Million's test        | -ve                             |
|      |                 | Xanthoprotein test    | -ve                             |
| 7.   | Saponins        | Frothing test         | +ve                             |
| 8.   | Amino Acid      | Ninhydrin             | -ve                             |
| 9.   | Tannins         | 5% FeCl <sub>3</sub>  | +ve                             |

**Where:** - Positive(+ve) and Negative(-ve)

**Result:** The sample of *Tagets erecta* shows presence of above (+ve).

**Table no.7: Result of Phytochemical screening *Hibiscus rosasinensis* (Gudhal)**

| S.N. | Phytochemicals | Test                  | Extract Of <i>Hibiscus rosasinensis</i> |
|------|----------------|-----------------------|---|
| 1.   | Alkaloids      | Wagner test           | +ve                                     |
|      |                | Hager test            | +ve                                     |
|      |                | Dragendroff's test    | +ve                                     |
| 2.   | Carbohydrate   | Fehling's test        | -ve                                     |
|      |                | Barfoed's test        | -ve                                     |
|      |                | Molisch test          | -ve                                     |
| 3.   | Steroids       | Liebermann test       | -ve                                     |
| 4.   | Phenols        | 10% FeCl <sub>3</sub> | -ve                                     |
|      |                | Dil. HNO <sub>3</sub> | -ve                                     |
| 5.   | Glycoside      | Keller kiliani test   | -ve                                     |
|      |                | Anthraquinone test    | -ve                                     |
| 6.   | Proteins       | Biuret test           | -ve                                     |
|      |                | Million's test        | -ve                                     |
|      |                | Xanthoprotein test    | -ve                                     |
| 7.   | Saponins       | Frothing test         | +ve                                     |
| 8.   | Amino Acid     | Ninhydrin             | -ve                                     |
| 9.   | Tannins        | 5% FeCl <sub>3</sub>  | +ve                                     |

**Where:** - Positive(+ve) and Negative(-ve)

**Result:** The sample of *Hibiscus rosa sinensis* shows presence of above (+ve).

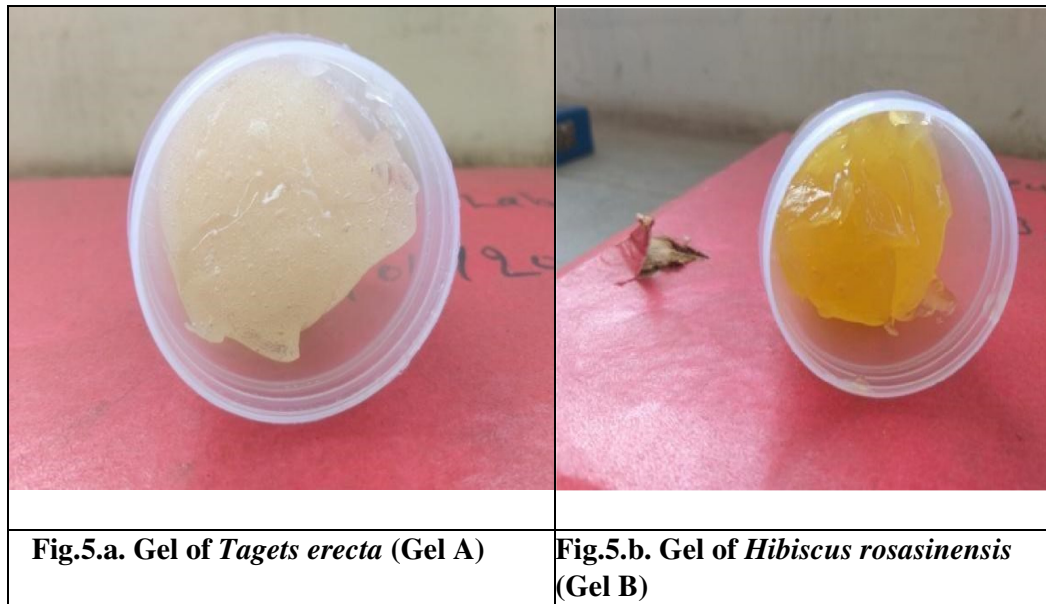
**Table no.8: Parameters of antioxidant activity**

| S.N. | Antioxidant activity                       | Tagetes erecta | Hibiscus rosasinensis |
|------|--|----------------|-----------------------|
| 1    | DPPH scavenging activity                   | 9.75 ± 1.15    | 15.1 ± 4.5            |
| 2    | Nitric oxide (NO) radical scavenging assay | 7.2 ± 0.15     | 6.05 ± 0.42           |

|   |  |             |            |
|---|--|-------------|------------|
| 3 | Ferric reducing antioxidant power (FRAP) assay | 11.0 ± 0.31 | 10.9 ± 1.7 |
|---|--|-------------|------------|

**Result:** Antioxidant potential indicates the rate of neutralizing of free radicals, both the samples showed the optimum anti-oxidant potential.



**Preparation of gels**





**Result of Antimicrobial Activity:**

**Table no.9:GEL A**

| Table S.N. | Gel | Antimicrobial activity |
|------------|-----|------------------------|
| 1          | A 1 |                        |

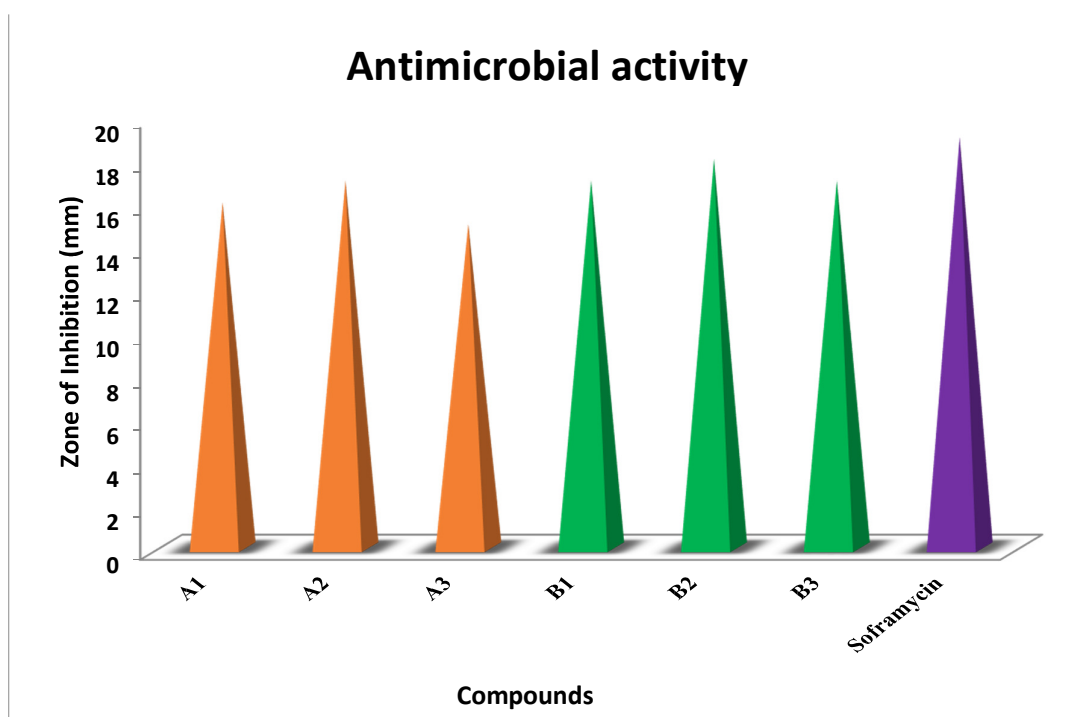
|   |     |  |
|---|-----|--|
| 2 | A 2 |  |
| 3 | A 3 |  |

GEL B

| S.N. | Gel | Antimicrobial activity   |
|------|-----|--|
| 1    | B 1 |  |
| 2    | B 2 |  |



### Graf for zone of inhibition



### Results:

Results of antimicrobial activity revealed that all samples shown the appreciable zone of inhibition as compared to standard soframycin. Sample A2 and B2 shown most prominent antimicrobial activity as compared to other formulation samples.

### **DISCUSSION**

Our preliminary phytochemical analysis for *Tagetes erecta* and *Hibiscus rosasinensis* flowers revealed the presence of high content of phenolics, carbohydrates, flavonoids, and tannins in aqueous and methanolic extracts. Phytochemical studies have revealed the presence of several chemicals, including flavonoids, flavonoid glycosides, hibiscetin, cyanidine, cyanidin

glucosides, taraxeryl acetate,  $\beta$ -sitosterol, campesterol, stigmasterol, ergosterol, citric, tartaric and oxalic acids, cyclopropenoids and anthocyanin pigments.<sup>[18]</sup> Polyphenol are the major plant compounds with high level of antioxidant activity. This activity is due to their ability to adsorb, neutralize and to quench free radicals.<sup>[19]</sup> Their ability as free radical scavenger could also be attributed to their redox properties, presence of conjugated ring structures and carboxylic group which have been reported to inhibit lipid per oxidation.<sup>[20]</sup> Total phenolics, flavonoids and tannins in methanolic extract of leaves were highest among all extracts. Since phenolics and flavonoids are responsible for the antioxidant activity, and high amount present in the extract indicates good antioxidant activity. Flavonoids like kaempferol and quercetin have been shown to exhibit the action through effects on membrane permeability, and by inhibition of membrane-bound enzymes such as the ATPase and phospholipase A2.

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