Formulation of Anti Acne Herbal Gel Using Ethanolic Extract of *Tagetes Erecta* and *Hibicus Rosa-Sinensis*

[1]Rahul D. Chourasia, [2]Vaishali T. Rehapade, [3]Ruchika A. Gupta, [4]Prateema T. Harinkhede, [5]Shraddha R. Shahare

[1,2]Gondia College of Pharmacy, Gondia.

[3,4,5]Manoharbhai Patel Institute of Pharmacy (D.Pharm) Gondia.

Abstract:

Microbes in environment can cause infection on skin and it causes microbial infection. It causes mostly in warm and moist areas. Among the formulations, A1, A2 and A3 showed better release and maximum zone of inhibition against microbes. (Gel containing *Tagetes erecta*) it contains *Tagetes erecta* which are responsible for anti-microbial activity. B1, B2 and B3(gel containing *Hibiscus rosasinensis*) all these have microbial property. all these herbs have potential fight against microbes. The standard used for anti-microbial activity is Soframycin and zone of inhibition was found to be (20 mm) Thus A2, and B2, showed maximum zone of inhibition against *microbes among* them maximum zone of inhibition as compared with standard, so these have maximum antimicrobial property further the following prepared gel is used for formulation of antimicrobial gels used for microbial infection having better anti-microbial activity. 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, β-sitosterol, campesterol, stigmasterol, ergosterol, citric, tartaric and oxalic acids, cyclopropenoids and anthocyanin pigments. Polyphenol are the major plant compounds with high level of antioxidant activity.

Keywords: *Tagetes erecta, Hibiscus rosasinensi,* Acne, Soxlet extraction, Gels Preparation.

INTRODUCTION

Acne vulgaris commonly known as pimples involves many factors for its manifestation and it mainly affects the pilosebaceous follicle. It mainly indicates open and closed comedowns and inflammation causing various conditions such as papules, pustules, and nodules. The main micro- organisms responsible to cause the condition include Propionibacterium acnes, Staphylococcus aureus, and Staphylococcus epidermidis. These micro-organisms proliferate rapidly which ultimately result in development of acne. Acne is a disease characterized by inflammatory and non-inflammatory lesions.[1] Acne vulgaris is generally characterized by formation of seborrhea, come done, 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. When the chemicals produced by P. acnes destroy the cellular structure of skin cells, Staphylococcus aureus, grows causing acne lesions. [2]

Signs and Symptoms of Acne

It includes papules, nodules (large papules), seborrhea (increased oil-sebum secretion), comedones, pustules and scarring. The appearance of acne varies with skin color and it is also associated with psychological and social problems.[3]

Fig no. 1

Acne vulgaris is the most common form of acne. According to a study in The Journal of the American Academy of Dermatology, acne vulgaris usually begins during puberty, but often extends into the twenties, thirties, and beyond. It can appear all over the body, but is most common on the face, neck, chest, and back.[4] Types of lesions that are common in acne vulgaris are;

* **Papules** – Red, inflamed bumps on the skin that feel tender and have no head are called papules. Squeezing a papule will not get rid of it faster and may cause scarring.[5]
* **Whiteheads** – Whiteheads result from a pore that is blocked completely. The trapped oil, bacteria, and dead skin cells cause a white head to form on the skin's surface.[6]
* **Blackheads** – When a pore is partially blocked, blackheads often form. The trapped bacteria, oil, and dead skin slowly drains to the surface of the skin to form a blackhead.[7]
* **Pustules** – Pustules are the most common type of acne lesion. They usually appear as an inflamed red circle with a center that is white or yellow.[8]

* **Nodules** – Severe acne often causes nodules. Acne nodules are hard bumps under the skin that may be large and last for months.[9]

Herbs used as Anti-Acne: Acne is a disease characterized by inflammatory and non-inflammatory lesions. The pathogenesis includes various factors like hormonal, bacterial and immunological, which causes acne lesions.[10] This is benign condition can cause severe psychological problems. 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%. The herbal formulation for acne this will be any types of side effect properties of Tagetes erect and Hibiscus rosa sinesis for further uses of antiacne property.[11] ***Antimicrobial: -***An antimicrobial is an agent that kills microorganisms or stops their growth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against For example, antibiotics are used against bacteria and antifungals are used against fungi. They can also be classified according to their function. Agents that kill microbes are called microbicidal, while those that merely inhibit their growth are called biostatic.[12]

MATERIALS AND METHODS

Table no 1.

Kingdom	**Plantae**
Order	Asterales
Family	Asteraceae
Genus	*Tagetes*
Species	*erecta*

Fig.2 ***Tagetes erecta(marigold)***

- **Synonyms:** Chinese hibiscus, China rose, Hawaiian hibiscus, rose mallow
- **Biological Source:** In the Bengal, Indonesia tropical Asia China, Japan
- **Chemical constituent:** Hibiscus rosa-sinensis contained cyclopropanoids, methyl sterculate, methyl-2-hydroxy sterculate, 2- hydroxysterculate, malvalate and beta-sitosterol. The major anthocyanin in the flower was cyanidin 3- sophoroside, d tannins, anthraquinones, quinines, phenols, flavonoids', alkaloids, terpenoids, saponins, cardiac glycosides, protein, free amino acids, carbohydrates, reducing sugars, mucilage, essential oils and steroid[13]
- **Family:** Malvaceae
- **Uses:** Anti-inflammatory, antipyretic, Antiparasitic, Antimicrobial, Antidiabetic and hypolipidemic and analgesic effects[14]

*2.**Hibiscus rosa-sinensis (Gudhal)***

Table no 2

Kingdom	**Plantae**
Order	Malvales
Family	Malvaceae
Genus	*Hibiscus*
Species	*rosa sinensis*

Fig. 3 Gudhal

- **Synonyms:** Aztec marigold, Mexican marigold, big marigold

- **Biological Source:** tropical deciduous forests, thorny forests, cloud forests and pine-oak forests. it can be found in China, India, Zambia, Zimbabwe, South Africa and Australia[15]
- **Chemical constituent**: The plant T. erecta has been shown to contain quercetagetin, a glucoside of quercetagetin, phenolics, syringic acid, methyl-3, 5dihydroxy-4- methoxy benzoate, quercetin, vinyl and ethyl gallate. Lutein is an ox carotenoid, or xanthophyll, containing 2 cyclic end groups (one beta and one alphaionone ring) and the basic C-40 isoprenoid structure common to all carotenoids.[16]
- **Family:** Asteraceae
- **Uses:** antimicrobial, anti-inflammatory, hepatoprotective, wound healing, insecticidal, analgesic activity.

SAMPLE COLLECTION AND AUTHENTICATION

The Flower of *Tagetes erecta* species (***Erecta),*** *Hibiscus rosa sinensis* species ***(Rosa sinensis)*****,** were collected from the local area of Gondia (India) in the month of November 2024.

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.

1. By simple maceration
2. Using mortar pestle
3. Addition of substrates in a series and calculated quantity

Procedure of Extraction [17]

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.4. Extractions

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[18]

Formulation of gels of extracts Gel A Table.3

S.N.	EXTRACT	GRAM
1.	*Tagets erecta*	1.5 g
2.	*Hibiscus rosasinensis*	2g

Gel B: Table 4.

S.N.	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. **Table 5.**

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. [19]

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 (121ºC) 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 µl 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**.** [20]

RESULTS

Total Ash value Table 6.

S.N.	Plants (Flower)	Ash Value
1.	***Tagetes erecta***	4.95% w/w
2.	***Hibiscus rosasinensis***	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

Acid insoluble ash Table 7.

S.N.	Plants (Flower)	Acid insoluble ash value
1.	***Tagetes erecta***	0.2% w/w
2.	***Hibiscus rosasinensis***	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.

Loss On drying Table 8.

S.N.	Plants (Flower)	Loss on drying (LOD)
1.	***Tagetes erecta***	7.46 % w/w
2.	***Hibiscus rosasinensis***	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 [21]

Table Parameters of antioxidant activity Table 9

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:

Fig.5 Gel of *Tagets erecta* (Gel A)

Fig.6 Gel of *Hibiscus rosasinensis* (Gel B)

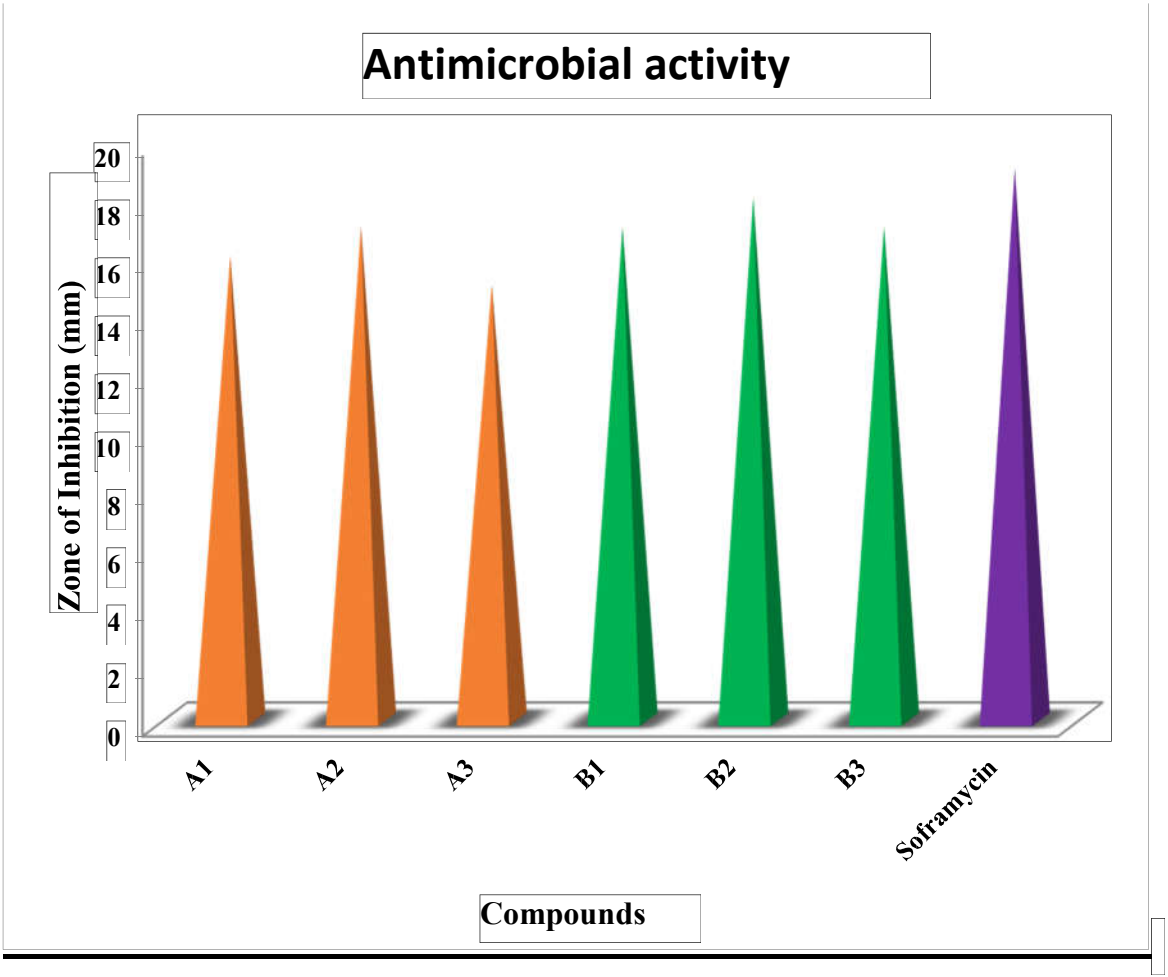
GEL A

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	
3	B 3	

Graf for zone of inhibition



Results:

Results of antimicrobial activity reveled 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.

RESULT AND 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, β-sitosterol, campesterol, stigmasterol, ergosterol, citric, tartaric and oxalic acids, cyclopropenoids and

anthocyanin pigments. Polyphenol are the major plant compounds with high level of antioxidant activity.

REFFERENC

1. Apriani EF, Rosana Y, Iskandarsyah I. Formulation, characterization, and in vitro testing of azelaic acid ethosome-based cream against Propionibacterium acnes for the treatment of acne. J Adv Pharm Technol Res 2019; 10:75-80.
2. Aswal A, Kalra M, Rout A. Preparation and evaluation of polyherbal cosmetic cream. Der Pharmacia Lettre. 2013; 5(1):83-8.
3. Das Kuntal, Dang Raman, Machale U Manjunath. Formulation and evaluation of a novel herbal gel of Stevia extract. Iranian J Dermatol. 12:2009; 117-122.
4. Daud FS, Wankede S, Joshi M, Pande G. Development of herbal anti-acne gel and its evaluation against acne causing bacteria Propianibacterium acne and Staphylococcus epidermidis. Int J Res Ayurveda Pharm. 2013; 4(5):781-6.
5. Esra Maltaş, Ahmet Uysal, Salih Yıldız and Yusuf Dura. (Evaluation of Antioxidant and Antimicrobial Activity Of Vitex Agnus Castus L). Fresenius Environmental Bulletin, 2010; 19(12): 3094-3099.
6. Hamnerius N: Acne-aetiology and pathogenesis. Treatment of Acne. 1996; 32: 29-38.
7. Jadoon, Saima; Karim, Sabiha; Asad, Muhammad Hassham Hassan Bin; Akram, Muhammad Rouf; Kalsoom Khan, Abida; Malik, Arif; Chen, Chunye; Murtaza, Ghulam (2015). Anti-Aging Potential of Phytoextract Loaded-Pharmaceutical Creams for Human Skin Cell Longetivity. Oxidative Medicine and Cellular Longevity, 2015.
8. Kim JY Song JY Lee EJ and Park SK: Rheological properties and microstructure of carbopol gel network system. Colloid polymer Science. 2003; 281: 614-623.
9. Lee I. S., Lee A. R., Lee H., et al. Psychological distress and attentional bias toward acne lesions in patients with acne. Psychology, Health & Medicine . 2014;19(6):680–686. doi: 10.1080/13548506.2014.880493
10. Leyden JJ: Current issues in antimicrobial therapy for the treatment of acne. J Eur Dermatol Venereol. 2001; 15(3): 51-55.
11. Malhotra, Swati. “Antibacterial Activity of Leaf Extract of Mexican Marigold (Tagetes Erecta) Against Different Gram Positive and Gram-Negative Bacterial Strains.” Journal of Pharmacy Research (2012): n. page. Print.

12. Mekjaruskul C, Kumkarnjana S, Fangkrathok N, Kengkittipat W, Sirichat N.Potential Cosmeceutical Applications and Evaluation of Human Skin Irritation of Tagetes erecta L. Flower Extract. Pharmacog Res. 2021;13(4):199-207.
13. Milani M. Acne, anti-acne therapies and epidermal barrier functions: the role of adjuvant treatments. OA Dermatology. 2013; 1(1):4.
14. Patil A. C., Patil A. R., Patil A. A. C., et al. Formulation and evaluation of polyherbal anti-acne gel. Research Journal of Topical and Cosmetic Sciences . 2017;8(2):p. 61. doi: 10.5958/2321-5844.2017.00007.3
15. Prod J. N., Resour P., Zohra S. F., Meriem B., Samira S., Muneer M. A. Phytochemical screening and identification of some compounds from Mallow. Journal of Natural Product and Plant Resources . 2012;2(4):512–516
16. Satpute and Kalyankar Tm. "Development and evaluation of herbal cream for the treatment of acne." Journal of Pharmacognosy and Phytochemistry 8 (2019): 26182624.
17. Sawarkar A H, Khadabadi SS, Mankar M.D. Development and biological evaluation of herbal anti acne gel. Int. J PharmTech Res. 2:2010; 2028-2031.
18. Shahtalebi MA, Asghari GR, Rahmani F, Shafiee F, Jahanian-Najafabadi A. Formulation of Herbal Gel of Antirrhinum majus Extract and Evaluation of its Anti-Propionibacterium acne Effects. Adv Biomed Res 2018; 7:53.
19. Shukla KV, Choudhary N, Pathak R, Formulation and Evaluation of Topical Polyherbal Antiacne Gels Containing Luffa Acutangula, Amaranthus Spinosus and Morus Alba, Journal of Drug Delivery and Therapeutics. 2019; 9(4-s):439-444.
20. Sinha, Priyam; Srivastava, Shruti; Mishra, Nidhi; Yadav, Narayan Prasad (2014). New Perspectives on Antiacne Plant Drugs: Contribution to Modern Therapeutics. BioMed Research International, 2014, 1–19.
21. supriya agnihotri; Sharad wakode, Anoop agnihotri. formulation and evaluation of herbal antiacne gel of myrica esculenta Asian J Pharm Clin Res. 2016; 9(4):358-361.