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Research Article

Formulation and Evaluation of Anti-Bacterial Gel From Extracts of Butterfly Ash and Gold Bloom

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A B S T R A C T

The main objective of the current research study is to formulate and evaluate a topical herbal gel containing Bauhinia *variegata Linn* and Tagetes *erecta Linn* flower extracts for their anti-microbial activity against the most common pathogen Staphylococcus *aureus*.S. Aureus is most common pathogen affecting different organs of the human body and is responsible for mortalities. Flowers of Butterfly ash (Bauhinia *variegata Linn)* are traditionally used as blood cleaners, and laxatives and possess antimicrobial and wound-healing properties. Gold bloom (Tagetes *erecta Linn)* is an ornamental flowering plant. Its flower is used to treat liver problems, scabies, fever and epileptic convulsions, and skin toner. They are also known for their astringent, carminative, and stomachic properties. It is also found to be useful against microbial infections and has wound-healing properties. A total of three herbal gel formulations were prepared using 1.0% of gelling agents carbopol 934 (G1—G-3) and the formulated gels were evaluated for physical appearance, viscosity, pH, spreadability, washability, primary skin irritation tests, and antimicrobial activity by agar well diffusion technique using azithromycin as a standard. No erythema or edema was observed in the skin irritation test confirming the gel to be nontoxic and safe. An attempt has been made to formulate and evaluate a new herbal combination to treat microbial infection. Formulation G2 was found to be superior among the three formulations as it rendered very good antimicrobial activity and was found to be better in other parameters.

Keywords: Butterfly ash, Gold bloom, Flower extract, herbal gel, antibacterial

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INTRODUCTION:

Ancient medicinal systems like Ayurveda, Chinese medicine, and Unani have been using polyherbal therapy for thousands of years to treat various disorders. The usage of medicinal plants for treating various diseases has gained popularity in recent years since herbal pharmaceuticals/medicines are secure and have fewer side effects than synthetic drugs. Almost 75% of people rely on plants and plant extracts for medical purposes. It has been shown that both naturally occurring herbs and herbal components used in certain formulae can interact, which can result in mutually beneficial effects such as mutual enhancement, mutual support, mutual restraint, and mutual antagonistic effects. The major obstacles to the broad acceptance of herbal medications are their quality, safety, and lack of scientific backing for their health claims. [1]

Infectious diseases account for 50% of fatalities worldwide and are a major cause of mortality and morbidity, particularly in patients with impaired immune systems.[2]This makes it even more urgent to create new, efficient, and economical medications that can fight against pathogens without harming the host. The pathogenesis of respiratory, dermal, gastrointestinal, and urogenital tract infections is influenced by streptococcus, staphylococcus, and pseudomonas species. They also contaminate burn sites, spread nosocomial infections, and are resistant to all known treatments.[3]Antibiotics overprescribing and the inclusion of antimicrobial drugs as growth promoters in poultry and livestock production are the main causes of multi-drug resistance.[4] Plant-derived secondary metabolites are very helpful in treating infectious diseases.[5]As a result, poly-herbal/phytomedicine could be a rich source of fresh anti-microbial compounds.[6] Bauhinia *variegata Linn* (Butterfly

ash) is a medium-sized deciduous tree with numerous therapeutic uses. The Plant: Bauhinia *variegata Linn* is a herbaceous medicinal plant that grows at an altitude of 1300 meters in the Himalayan area and other parts of India. The plant, which is also known as Sigappumandarai in Tamil and Devakanchanamu in Telugu, is a member of the Caesalpiniaceae family. The plant's bark, flowers, and roots are its useful components. Bauhinia *variegata Linn's* flowers help wound healing by new skin cell formation. Anti-inflammatory and antioxidant properties are involved in wound healing. Due to its cooling and astringent properties, Bauhinia *variegata Linn*. flower powder mixed with honey helps to manage skin issues like acne and pimples.[7]

Numerous herbal treatments practiced may be individual drugs or a combination of different drugs to treat various ailments in different systems of medicine. Distinct traditional medical systems have acknowledged Tagetes erecta *Linn* (Gold bloom), marigold, as having a medicinal benefit for treating heterogeneous human disorders. There are 50 species of annual or perennial herbaceous plants in the genus Tagetes, which belong to the family Compositae/Asteraceae. Locals call the plant Tagetes erecta Linn. Genda Phool (Marigold). These plants bloom yearly that grow, with heights varying from dwarfs of 6-8 inches to medium and taller plants that reach heights of 10 to 3 feet. They yield huge pompon-like double flowers (up to 5 inches across) and have a brief flowering season from midsummer to frost. This flowering plant is one of the most chosen plants for gardens and produces a highly scented essential oil (Tagetes oil). Tagetes oil is fashioned into expensive perfumes. This plant's many parts, especially the flowers, are used in folk medicine to treat varied kinds of illnesses. The leaves are used as an antiseptic in kidney problems, muscle pain, and piles. As per Ayurvedic literature, the flower is helpful for liver problems, scabies, astringent, carminative, stomachic, fevers, and epileptic fits.[8]The numerous topical formulations include liposomes, polar gel formulations, creams, ointments, and hydrocarbon-based formulations. These topical preparations can transform the skin's barrier function. Gels are semisolid solutions that may include a suspension of big organic molecules or small inorganic particles. Semisolid dosage forms are designed to induce the desired therapeutic activity at specific locations in the epidermal tissue during dermatological medication therapy.[9]

The skin's epidermis protects against environmental microorganisms and acts as an infection barrier.[10] Numerous bacteria, fungi, and viruses are responsible for many skin ailments. When applied to the skin or other accessible mucous membranes, semisolid dosage forms have the potential to either treat or alleviate a pathological condition and help against a harmful environment.[11]

Objectives:

The current study intended to formulate and evaluate a polyherbal antibacterial gel containing alcoholic extracts of Bauhinia *variegata Linn* and Tagetes *erecta Linn* flowers at different concentrations. From the literature, it has been observed that newer antibacterials are to be investigated to meet the challenges of resistance to the existing antimicrobials/antibacterials in the market. The formulated gels were assessed for stability, pH, viscosity, spreadability, washability, and antibacterial efficacy. It was attempted to formulate an alternative andcost-effective formulation with easily available herbs to already available anti-bacterial gel in the market

MATERIALS AND METHODS:

Collection of butterfly ash (Bauhinia *variegata Linn)* and Gold Bloom (Tagetes *erecta Linn)*Flowers:

The flowers of Bauhinia *variegata Linn* were collected from the college campus of Kamla Nehru College of Pharmacy, Butiburi, Nagpur, Maharashtra, India. The flowers of Tagetes *erecta Linn* were purchased from the local market and were authenticated by Dr. Jagdish Baheti (Pharmacognosist). The collected materials were cleaned and dried and were taken care that there should not be any microbial growth. The dried flowers were then reduced to coarse powder with the help of a mixer- grinder, and stored in an airtight container for further process of extraction.[7], [13]

Preparation of Butterfly Ash, Bauhinia Variegata Linn Flowers Extract:

The fresh flowers of Bauhinia *variegata Linn* were collected and dried in the shade. Then the dried flowers were powdered to get a coarse powder. Used, 50 ml petroleum ether to do defatting of Bauhinia *variegata Linn* flower powder (25g). The residue was collected,andair dried, and then the defatted plant material was individually extracted in 50 ml of different solvents (for at least 24hr/solvent) with increasing polarity *viz*. ethyl acetate, chloroform, methanol, and ethanol respectively. Each extract was preserved separately. The marc left after ethanolic extract was collected and dried under the shade to get a dry mass. [7, 13,14]

Preparation of Tagetes *erecta Linn* (Marigold) Flowers Extract:

The fresh flowers of Tagetes *erecta Linn* were collected and dried in the shade. Then the dried flower was powdered to get a coarse powder. About 40 g of the drug was mixed with 99% methanol (as solvent) to about 3/4th of the vessel for 72 hours (cold maceration process). The methanolic extract was prepared and obtained a dark orange color residue. The solvent was then Slowly evaporated at 40°C using a rotary machine to get a concentrated extract. [7,15]

Gel formulation from Bauhinia *variegata Linn* and Tagetes *erecta Linn* Flower Extracts

Dispersed 1gm of carbopol 934 in 25 ml of distilled water and kept the beaker aside to swell the carbopol 934 for half an hour. Then, the above mixture was stirred to a gel from carbopol 934. The necessary amounts of methylparaben and propylparaben were dissolved in hot water (5 ml) followed by the addition of propylene glycol 400 to the cold mixture. Further, both the extracts in different concentrations were mixed with the above mixture, and the final volume of 100 ml was made up by adding the remaining amount of distilled water. Triethanolamine was added dropwise to the carbopol 934 gel after the combined materials had been well mixed with constant stirring to achieve the desired consistency and to adjust pH. [17,18]

Table:1 Composition of Antimicrobial Gels from Butterfly Ash (Bauhinia *variegata Linn)* flower extract and Gold Bloom (Tagetes *erecta Linn) flower extract*

Sr. No.	Ingredients	Control	G1	G2	G3
1.	Carbopol 934 PNF (g)	1	1	1	1
2.	Bauhinia *variegata Linn.* flower extract (BAE- %)	-	1	1	0.5
3.	Tagetes *erecta Linn.*flower extract (TEE- %)	-	0.5	1	1
4.	Glycerine (ml)	5	5	5	5
5.	Triethanolamine	q.s.	q.s.	q.s.	q.s.
6.	Methylparaben (g)	0.2	0.2	0.2	0.2
7.	Propylparaben (g)	0.1	0.1	0.1	0.1
8.	Distilled water	q.s.	q.s.	q.s.	q.s.

Figure 1: Anti-microbial gel from Butterfly Ash (Bauhinia *variegata Linn)* flower extract and Gold Bloom (Tagetes *erecta Linn) flower extract*s

Evaluation of Herbal Gel:

The formulations were evaluated for their appearance, color, and odor. pH of the formulations was determined using a digital pH meter KNCP PH (ELICO-LI-120).

Spreadability Test:

Generally, steel blocks are employed to measure the spreadability of the gel formulations. Steel blocks were applied to measure the spreadability of the gels, which is grounded on the slip, and the drug characteristics of the gel put on the ground slide and the excess gel (approximately 2 g) under analysis. Placed the gel between the two slides followed by 200 g weight on the top of both slides for 5 minutes to expel air and to provide a uniform gel film between the slides. The edges were cleaned by scraping. The time (in seconds) required to cover a distance of 7.5 cm by the top slide was noted.The following formula was used to determine the spreadability efficacy of the prepared gels. [17,18]

S= M.L/ T

Where,

M = Weight tied to upper slide L = Length of glass slide

T = Time duration of separating the slide

Viscosity:

Utilizing spindle no. 64 of a Brookfield viscometer, Brookfield Rv DH^{+} Pro.The viscosity of the herbal gel was measured at 100 revolutions per minute. [17,18,19]

Skin Irritation Test:

Ten healthy participants, both male and female, under went the test. Each volunteer's hand received about 1g of the gel preparation for a predetermined period. After 2 hours, the test area was observed for any visible signs that might result from skin irritation.[17,19]

Washability:

Formulations were applied on the skin and the extent of water washing was manually assessed. [19]

Anti-microbial Activity, MIC Determination Study:

It was determined by minimum inhibitory concentration using the agar well diffusion method. The agar well diffusion method was used to evaluate the antibacterial activity of the gel. Nutrient agar about 5g was added to 100ml of distilled water, pH was adjusted at 7.0, and was then autoclaved. It was allowed to cool up to 45 degrees Celsius. About 60 ml of nutrient agar was seeded with microorganisms, poured into each of the two petri plates, and allowed to solidify. Wells were bored into the agar using a sterile 6mm diameter cork borer. Gels of different concentrations were added into the well, allowed to stand at room temperature for about 2 hours, and incubated at 37^{0}C. Standards were set in parallel. A zone of inhibition was found after 24 hours. The effects were compared with that of the standard.[17,18,19]

RESULTS AND DISCUSSION

Percentage extraction was found to be 5% and 4.5% for Bauhinia *variegata Linn.* flower extract and Tagete s*erecta Linn flower* respectively. Flower extracts of both plants were formulated into a gel and were evaluated for physicochemical characteristics and their antibacterial effectiveness. A 1% concentration of each extract in the formulation G2 rendered good antimicrobial activity.The formulations were yellowish-green in color with a characteristic odorand smooth texture.

Table 2: Extraction yield of flowers of Bauhinia *variegata Linn* and Tagetes *erecta Linn*

Sr. No.	Plant	Solvent	Extraction Process	% Yield
1.	*Bauhinia variegate Linn.*	Ethanol	Maceration	5%
2	*Tageteserecta Linn.*	Methanol	Maceration	4.5%

Table 3: Physicochemical Evaluation of Gels from flower extracts of Bauhinia *variegata Linn* and Tagetes erecta *Linn*

Sr. No.	**Characteristics**	**G1**	**G2**	**G3**
1.	Color	Reddish	Yellowish green	Brownish
2.	Odor	Characteristic	Characteristic	Characteristic
3.	Consistency	Smooth	Smooth	Smooth
4.	pH	5.20	5.26	5.27
5.	Spreadability	7.0 cm	7.1cm	7.0cm
6.	Viscosity	4400cP	4478cP	4528cP
7.	SkinIrritation	Non irritant	Non irritant	Non irritant
8.	Washability	Washable	Washable	Washable

Table 4: Result of Agar Well Diffusion Testfor MIC

Test Organism	Zone of inhibition (mm)		
	G1	G2	G3
Staphylococcus Aureus	5	8	7
Standard Azithromycin	10		

DISCUSSION:

An attempt was made to formulate a gel to explore the natural extracts *Bauhinia variegata Linn. And Tagetes erecta Linn* to explore their effects as a topical agent.The pH was between 5.26-6.0 and is suitable for topical formulation and the formulations spread easily after the application on the skin. All the formulations were tested for skin irritation, and no signs of irritation, flare, or redness were observed after applying the gels. The formulated gel (G2) was found to be yellowish-green in color with a characteristic odor. The Observations confirmed that the formulation (G2) is within acceptable limits and that all parameters, including pH, viscosity, and spreadability, are suitable for topical preparation. The formulation was free of microorganisms because it did not produce any microbial growth when introduced in agar media.

CONCLUSION:

Many plants have been investigated for compounds with medicinal activities. Plants include a wide variety of medications. Many studies have reported that the activity of an individual plant can be increased when given in combination with others with the same activity. There fore, from the literature survey, no studies are reported for the combination of Bauhinia *variegata Linn* and Tagetes *erecta Linn*. extract. As these plants show good antimicrobial activity. A significant effort has been made to combine the active ingredients of both extracts to create an effective polyherbal antibacterial gel. Bauhinia *variegata Linn* and Tagetes *erecta Linn* extracts were utilized in this study because of their reported antibacterial properties. The zone of inhibition results indicated that this herbal gel was effective as an antibacterial agent. As a result, they can be used in the treatment of skin diseases and bacterial infections.The current study has a significant impact on the development of a cost-effective herbal antibacterial gel for low-income areas. The present herbal formulation can be useful in curing skin diseases due to S. *aureus*. The natural flower extracts included in this composition can further be evaluated against different Gram-positive and Gram-negative bacteria to treat skin diseases. The different secondary metabolites present in the flowers might be responsible for the antibacterial activity and they can also be isolated and studied individually in the future.

Abbreviations:

BAE-Bauhinia *variegata Linn* (Butterfly Ash) Extract

TEE-Tagetes *erecta Linn (*Gold Bloom)Extract

MIC- Minimum Inhibitory concentration

S. *aureus*-Staphylococcus *aureus*

G1, G2, G3- Antibacterial Gel Formulations from flower extracts of Butterfly Ash (BAE) and Gold Bloom (TEE)

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