

FORMULATION AND EVALUATION OF HERBAL ANTIMICROBIAL GEL FROM CLITORIS TERNATEA LINN

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ABSTRACT

Natural Imedicine has grown to be a globally important product, both medicinally and financially. Because herbal remedies don't have the common adverse effects associated with allopathic treatments, more and more patients are using them. The formulation and assessment of herbal gels are the primary goals of the current study. With Clitoria ternatea L. within The Fabaceae family includes the species C. ternatea. To create a unique topical herbal gel using flowers from the Clitoria ternatea plant and assess its antibacterial properties. Topical preparations such as gel that contain C. ternatea extract were created by combining gelling agents such Carbopol 934 P and HPMC K100M. The prepared gels under wanted Physicochemical characteristics, pH, viscosity, spreadability, homogeneity, homogenous drug content, in-vitro drug diffusion, permeability, and antibacterial activity are only a few of the evaluation factors. It was discovered that the gel worked.

Keywords: homogeneity, gel, spreadability, Fabaceae, Clitoria ternatea L, and antimicrobial activity.

1. INTRODUCTION

Herbal or plant-based remedies have been used for centuries to treat and prevent illness, and there is still much to learn about the numerous components of these natural sources. Scientists are now better equipped to treat a variety of viral diseases thanks to the discovery of novel chemicals from herbal sources. According to reports, the majority of medicinal plants include antibacterial, antioxidant, and anti-inflammatory qualities. These qualities have helped to prevent many infectious diseases and may also be advantageous to society as a whole. The current state of infectious illnesses indicates a concerning rise in the prevalence of both newly discovered and reemerging infections. The emergence of antibiotic resistance during clinical use is a significant problem. Therefore, the development of a natural formulation that can combat the bacteria causing skin illnesses is imperative (1). Medicinal plants and herbs are used by a growing number of patients worldwide for therapeutic purposes.

Known by the names "Aparajita" and "Butterfly pea," Clitoria ternatea Linn. (Fabaceae) is frequently used in place of "Shankhapushpi." It is made up of "shankh" or "conch"-shaped blossoms and is a type of Ayurvedic medicine called Medhya Rasayna. It is said to be a brain tonic and to improve memory and intelligence. It is a legume with blue flowers that is robust, persistent, herbaceous, perennial, climber, and noticeable. The plant is found throughout tropical Asia, including India, and is endemic to southeast Asia. According to reports, almost every portion of the plant has therapeutic qualities that are extensively employed in the Ayurvedic medical system.

Plant profile:

Synonym: Blue-pea, butterfly-pea, cordofan-pea, Darwin-pea

Type of plant: herbaceous perennial

Origin: Asia or Latin America

Habit: Climbing or trailing vine, sparsely pubescent throughout, becoming woody as it ages to a length of 10 meters.

Taxonomical classification-

Kingdom- Plantae

Order- Fabale

Family- Fabaceae

Tribe- Phaseoleae Subtribe- clitoriinae



Plant description :

A woody rootstock supports this perennial herb that climbs or trails. 2-4 pairs of leaflets and a terminal leaflet make up the imparipinnate leaves. Ovoid to elliptic-oblong leaflets, measuring up to 6.5×4 cm, are pubescent below and mainly hairless above. Axillary, huge and beautiful, bright blue, resupinate flowers that can be single or in pairs. Pod: hairless or faintly pubescent, linear, oblong, 6–13 cm long, flattening, mucronate at apex (3). It grows both wild and in gardens, producing striking blue or white flowers that resemble conch shells. Though it is said to have originated in America, it is now grown and naturalized below 1600 meters in elevation across the humid tropical regions of both the old and new worlds.

2. MATERIAL AND METHODS

Collection of plant material:

The plant was collected from the locally growing area, from kopargaon, Maharashtra, It was then botanically authenticate. The leaves were separated, dried, coarsely powdered, passed through sieve No. 40, and Stored in a closed container for further use(10).

Procedure for extraction:

Clitoria ternatea leaves were dried in the shade, ground into a powder, sieved through sieve No. 40, and then sealed in airtight receptacles. Soxhlet extraction was used to extract the leaves' coarse powder. To make the necessary amount of dried powder, a methanolic leaf extract of Clitoria ternatea was obtained by refluxing the powder in a flask with a circular bottom. After the resultant solvent was extracted under low pressure, a semisolid was created. This semisolid was then vacuum-dried using a rotary flash evaporator to produce a solid residue that included the C. ternatea methanolic extract. The resulting dried extract was then utilized to create an antibacterial gel (11).

Phytochemical analysis:

Tests for the presence of alkaloids, tannins, glycosides, resins, steroids, saponins, flavonoids, and phenols were part of the phytochemical investigation.

Test for alkaloids:

approximately 0.5 g of methanol extract was obtained, diluted, and homogenized with 10 ml of distilled water. It was then dissolved in 20 ml of diluted HCl solution and cleared by filtering. Using Mayer's and Dragendroff's reagent, the filtrate was examined. The treated fluid was watched for any white or creamy-colored precipitation.

Tannin test:

After boiling about 2 milliliters of the extract in 1 milliliter of 1% aqueous HCl acid, the presence of tannins was determined by looking for a red precipitate.

Test for Glycosides:

0.5 g of methanol extract and 1 ml of glacial acetic acid with trace amounts of ferric chloride were combined in a test tube. One milliliter of strong sulfuric acid was added to this mixture, and it was watched to see if a reddish-brown color formed at the intersection of the two layers and if the presence of glycosides caused the upper layer to turn bluish green.

Test for Resins:

To find out if there are any resins, put 0.5 g of methanol extract in a test tube, add 5 ml of distilled water, and look for turbidity, which is a sign of resin presence.

Test for Steroids:

Put about 0.5 grams of methanol extract in a test tube, add 2 milliliters of acetic anhydride and 2 milliliters of sulfuric acid along the test tube's edges, and watch to see if the mixture turns violet or blue green.

Test for saponins:

put 0.5 g of methanol extract and 5 ml of distilled water in a test tube. We gave the mixture a good shake and looked for any lingering foam. After adding three drops of olive oil to the foam and giving it a good shake, the creation of an emulsion was checked.

Test for flavonoids:

In a test tube, roughly 0.5 g of extract was added to 10 ml of ethyl acetate, and the mixture was cooked for one minute in boiling water. After that, the mixture was filtered. After shaking 4 ml of the filtrate with 1 ml of 1% aluminum chloride solution, it was incubated for 10 minutes. When 1 milliliter of diluted ammonia solution was present, the formation of a yellow color showed the presence of flavonoids.

Test for phenol:

In a test tube, 0.5 g of extract was collected, combined with 100 ml of distilled water, and gently heated. Two milliliters of ferric chloride solution were added to this, and the creation of a green or blue color was watched(12).

Determination of physical content :

1 loss on drying:

LOD, or loss of mass, is measured as a percentage of weight per unit and can be found by following this process.

Procedure: Weighed a shallow weighing container with a glass stopper after it had been dried for thirty minutes. A sample of powdered twigs was precisely weighed and stored in a bottle. A light sideways shake was used to evenly disseminate the sample. The bottle was put in the oven. The sample underwent continuous weight drying. The bottle was placed in desiccators to bring to room temperature after drying, and then it was weighed. LOD was caused by the initial and ultimate weight differences.

2. Ash value:

Any organic material's non-volatile inorganic compound makes up its ash. Total amount of ash.

Procedure: Weigh out between two and four grams of ground air dried material precisely to the point where it turns white, signifying the absence of carbon. The residue can be cooled in a desiccator and the residue can be saturated with ammonium nitrate or roughly 2 milliliters of water. Dry in a water bath, then ignite to a steady weight on a hot plate. Weigh the residue as soon as possible after letting it cool for 30 minutes in an appropriate desiccator. Determine the dry material's total ash content in milligrams per kilogram. One gram of the sample was weighed and allowed to air dry in a tared silica dish. It was burned at a temperature of no more than 450 °C until it was carbon-free, cooled, and the ash was weighed to determine its percentage.

Water soluble ash:

Procedure: The ash was extracted using the same process as for total ash. After that, the ash was heated in 25 milliliters of water for five minutes. After filtering and gathering the insoluble material in a Grouch crucible, the mixture was heated to a maximum temperature of 450° C for 15 minutes, and it was then cleaned with hot water. The ash's weight was deducted from the weight of the insoluble substance. The water-soluble ash was represented by the weight differential. The air-dried medication was used to calculate the percentage of water soluble ash

Acid insoluble ash:

Procedure:The ash was extracted using the same process as for total ash. After boiling the ash for five minutes, 25 milliliters of 2M hydrochloric acid was added. The insoluble material was filtered, gathered in a Gooch crucible, cleaned with hot water, lit, allowed to cool in a desiccator, and then weighed. The medication that had been air-dried was used to calculate the percentage of acid insoluble ash the ash for five minutes, 25 milliliters of 2M hydrochloric acid was added.

The insoluble material was filtered, gathered in a Gooch crucible, cleaned with hot water, lit, allowed to cool in a desiccator, and then weighed. The medication that had been air-dried was used to calculate the percentage of acid insoluble ash.

3. Determining the extractive value:

This technique counts the active ingredients in a certain volume of medicinal plant material by extracting it using solvents. Any crude medication that is extracted using a specific solvent produces a solution that has several phytoconstituents. A solution with a variety of phytoconstituents is produced by the combination of these phytoconstituents in that specific solvent. The nature of the medication and solvent utilized will determine how these phytoconstituents are composed in that specific solvent

Alcohol soluble extractive value:

Five grams of air-dried crude medication, precisely weighed, were macerated for twenty-four hours in a closed flask with one hundred milliliters of 95% ethanol. In order to prevent ethanol loss, it was shook often for the first six hours, let it stand for eighteen hours, and then swiftly filtered. 25 milliliters of the filtrate were taken, dried at 105 degrees Celsius in a tarred, shallow dish, and then weighed. With reference to air-dried medication, the percentage of ethanol soluble extractive value was computed.

Water soluble extractive value:

Five grams of air-dried crude medicine, precisely weighed, were macerated for twenty-four hours in a closed flask with one hundred milliliters of distilled water. In order to prevent losing any of the distilled water, it was shaken often for the first six hours, then left to stand for eighteen hours and filter quickly. A quarter of a liter of the filtrate was removed, dried at 105°C in a shallow dish with a tarred bottom, and weighed. Using the medication air dried as a reference, the percentage of water soluble extractive value was computed (13).

4. Determining the tapped density (ρ_T):

This quantity is helpful in determining the powder's Hausner Ratio and compressibility index. A graduated cylinder with a known mass of medication or formulation is placed on a mechanical tapper device to determine it. which is run for a predetermined amount of time (around 1000 taps) or until the volume of the powder bed is at its lowest.

It is expressed in g/ml. Mass of powder (M) / Tapped volume (V_t) equals tapped density (ρ_T).

5. Determination of bulk density (ρ_B):

This parameter provides information on compressibility and powder flowability. Using a large funnel to pour presieved (40 mesh) bulk into a graduated cylinder, the volume and weight are measured (15). Mass of powder (M) / Bulk volume (V_b) equals bulk density .

6. Determination of angle of repose:

A funnel is fastened above graph paper that is laid out horizontally and level, with its tip at a specified height H. The funnel is gradually filled with powder or granulation until the top of the funnel is barely touched by the apex of the conical pile. The angle of repose is then calculated using the diameter of the conical pile's base (16). Repose angle $(\theta) = \tan^{-1} (h/r)$.

7. Hausner ratio:

Rather than using V₀ to calculate the compressibility index, V₁₀ can be used, depending on the material. The results make it obvious whether or not V₁₀ is used (17). Tapped density / Bulk density is the Hausner ratio.).

8. Carr's index:

Also known as the Carr's Compressibility Index, the Carr's index provides information on how compressible a powder is. Ralph J. Carr, Jr., a scientist, is honored by the name. Carr's index = $100[\rho_T - \rho_B/\rho_B]$ is the formula used to calculate the Carr index. Where the powder's freely settled bulk density (ρ_B) and its tapped bulk density (ρ_T) upon "tapping down" are given

Formulation of herbal gel:

To prepare the gel formulation, dissolve carbopol 940 in distilled water first. Then, add methyl paraben, propyl paraben, and glycerine, and let it sit overnight. Consider the Clitoris alternata leaf extract in propylene glycol, to which polymer dispersion was subsequently added. After that, the remaining water was added, and triethanolamine was added while swirling constantly to bring the pH down to 7 (18).

Sr.no.	Ingredients	Quantity
1	Carbapol 934(%)	1gm
2	Extract (%w/w)	2.5ml
3	Methyl paraben (0.5%)	0.2ml
4	Propyl paraben (0.2%)	5ml
5	Triethanolamine	0.5ml
6	HPMC	0.6gm
7	Glycerine	5ml
8	Purified water	Q.S

Clitoris Ternatea gel



Evaluation of herbal gel :

Physical assessment: The color, consistency, and smell of the herbal gel were assessed. They have an opaque, light greenish yellow appearance, no grittiness, and a uniformly distributed .

pH: Using pH paper, the pH of each of the herbal gel formulations was determined.

Viscosity: Using a Brookfield rotational viscometer with spindle number 64, the viscosity of the herbal gels was measured at 100 rpm.

Homogeneity: The homogeneity of the gel compositions was confirmed by visually inspecting the presence of any aggregates.

Antibacterial activity:

The disc diffusion method was used to screen herbal gels for microorganisms. *S. aureus* and *E. coli* were the two bacterial agents that the gels were tested against. After sterilizing, nutrient agar medium was added to petri dishes. Following solidification, a rod was used to equally distribute 0.1 ml of the inoculum over the agar. A cavity with a 6 mm diameter was made, and the formed gel was inserted into it. The control was an antibiotic that is often used. For a whole day, the infected plates are incubated. Later, measurements and records were made of the inhibitory zone surrounding the disk (19).

3. RESULT AND DISCUSSION

Sr.no.	Parameters	Value
1	Total ash	17%
2	Acid in soluble ash	1.5%
3	Water soluble ash	5%
4	Loss on drying	9%
6	Alcohol soluble extractive Value	8.8%
7	Water soluble extractive Value	24.6g/ml
8	Tapped density	0.25g/ml
9	Bulk density	0.17g/ml
10	Angle of repose	16.58
11	Hausner ratio	1.40
12	Carr's index	30.60

investigation of physicochemistry The physical-chemical characteristics, include bulk, tapped density, extractive values, loss upon drying, and ash valuesbulk density, tapped density, drying loss, and other factors were calculated and are given in table.

Preliminary Phytochemical analysis:

The methanolic extract underwent phytochemical investigation, the findings of which are presented in (Table 2). Anthraquinone glycosides, alkaloids, tannins, steroids, flavonoids, carbohydrates, reducing sugars, monosaccharides, cardiac glycosides, and other phytoconstituents were found in the extracts during the initial phytochemical screening process.

Sr.no.	Extract	Methanol
1	Alkaloids	+
2	Steroids	+
3	Saponin	+
4	Flavonoids	+
5	Glycosides	+
6	Terpenoid	+
7	Phenol	+
8	Tanins	+
9	Resins	+
10	Protein	+

Pharmacological study:

Antimicrobial activity –



On *B. subtilis*



On *S. aureus*

Sr. no.	Micro-organisms	Zone of Inhibition		Percentage Inhibition (%)
		Methanolic extract (60µg/mL)	Streptomycin (Standard drug) (60µg/mL)	
1.	<i>Bacillus subtilis</i>	23	30	76
2.	<i>Staphylococcus aureus</i>	13	20	65

4. CONCLUSION

Clitoria ternatea has been shown to contain bioactive substances, including alkaloids, tannins, glycosides, resins, steroids, saponins, flavonoids, and phenols. The results of this experiment indicate that carbopol 940 can be used as a polymer in the formulation of herbal gels including plant extract from *Clitoria ternatea* leaves, together with other substances. The evaluation of physical parameters also yielded excellent results. The antibacterial activity of *Clitoria ternatea* leaf extract in produced herbal gels was found to be very active against tested pathogens, comparable to that of conventional antibiotics. Thus, based on the total data, it was ultimately determined that the herbal gel formulations possess strong antibacterial qualities, making them superior to allopathic medicines in terms of safety and efficacy.

5. REFERENCE

- [1] Viswanad V, Aleykutty N, Jayakar B, Zacharia S, Thomas L. Development and evaluation of antimicrobial Herbal formulations containing the methanolic extract of *Samadera indica* for skin diseases. *J Adv Pharm Technol Res*. 2012;3(2):106–11.
- [2] Kshetrimayum B. Medicinal Plants and Its Therapeutic Uses. *Med Plants Its Ther Uses*. 2017;(January).
- [3] Kirtikar, K.R. and Basu BD. *Indian Medicinal Plants*, Vol. II. Allahabad: Lalit Mohan Publication; 1935. 1120–1122 p.
- [4] Mukherjee PK, Kumar V, Kumar NS, Heinrich M. The Ayurvedic medicine *Clitoria ternatea*-From Traditional use to scientific assessment. *J Ethnopharmacol*. 2008;120(3):291–301.
- [5] Michael Gomez S., Kalamani A. Butterfly Pea (*Clitoria ternatea*): A Nutritive Multipurpose Forage Legume for the Tropics – An Overview. Vol. 2, *Pakistan Journal of Nutrition*. 2003. P. 374–9.
- [6] KM N. *Indian Materia Medica*, Volume 1. First. Revised & Enlarged by Nadkarni, AK. Bombay Popular Prakashan; 2005. 354–355 p.
- [7] Chopra RN, Nayar SL CI. *Glossary of Indian Medicinal Plants*. 7th ed. New Delhi: NISCAIR Press, CSIR, Dr. K. S. Krishnan. Marg; 2006. 71 p.
- [8] Terahara N, Saito N, Honda T, Toki K, Osajima Y. Structure of ternatin D1, an acylated anthocyanin From *Clitoria ternatea* flowers. *Tetrahedron Lett*. 1989;30(39):5305–8.
- [9] Kazuma K, Noda N, Suzuki M. Flavonoid composition related to petal color in different lines of *Clitoria Ternatea*. *Phytochemistry*. 2003;64(6):1133–9.
- [10] Kamilla L, Mnsor SM, Ramanathan S, Sasidharan S. Antimicrobial activity of *Clitoria ternatea* (L.) Extracts. *Pharmacologyonline*. 2009;1:731–8.
- [11] Pingili D, Amminbavi D, Awasthi A, Khan FMS. Formulation, Evaluation and in Vitro Antibacterial Screening of Herbal Gel Containing *Manilkara Hexandra* (Roxb.) Dubard Leaf Extract. *Int J Pharm Sci Res [Internet]*. 2018;9(2):702–7. Available from: [http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L620516902%0Ahttp://dx.doi.org/10.13040/IJPSR.0975-8232.9\(2\).702-07](http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L620516902%0Ahttp://dx.doi.org/10.13040/IJPSR.0975-8232.9(2).702-07)
- [12] Manjula P, Mohan CH, Sreekanth D, Keerthi B, Devi BP. PHYTOCHEMICAL ANALYSIS OF LITORIA TERNATEA LINN ., A VALUABLE PHYTOCHEMICAL ANALYSIS OF CLITORIA TERNATEA LINN ., A VALUABLE MEDICINAL PLANT. 2015;(June).
- [13] Singh MPVV, Prajapati VK. Pharmacognostical Screening of Blue Flowered Variety of *Clitoria ternatea* Linn. – A Phytocentric Overview. *Int J Res Rev*. 2022;9(5):168–81.
- [14] Lachman L, Lieberman H, Kanig J. *The Theory and Practice if Industrial Pharmacy*. 1991. 66–86 p.
- [15] Honarpour M, Koederitz L, Harvey a H, Pop I, Pop I, Ingham D, et al. Bulk Density and Tapped Density Of Powders. *World Heal Organ [Internet]*. 2012;XXXIII(2):81–7. Available from: <http://www.tandfonline.com/doi/abs/10.1080/19942060.2014.11015516%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/15003161%5Cnhttp://cid.oxfordjournals.org/lookup/doi/10.1093/cid/cir991%5Cnhttp://www.scielo.cl/pdf/udecada/v15n26/art06.pdf%5Cnhttp://www.scopus.com/i>
- [16] Beakawi Al-Hashemi HM, Baghabra Al-Amoudi OS. A review on the angle of repose of granular Materials. *Powder Technol [Internet]*. 2018;330(May):397–417. Available from: <https://doi.org/10.1016/j.powtec.2018.02.003>
- [17] Kaleem MA, Alam MZ, Khan M, Jaffery SHI, Rashid B. An experimental investigation on accuracy of Hausner Ratio and Carr Index of powders in additive manufacturing processes. *Met Powder Rep [Internet]*. 2021;76(xx):S50–4. Available from: <https://doi.org/10.1016/j.mprp.2020.06.061>
- [18] Sandeep DS, Nayak P, Jose J, Rishal Relita M, Sumana DR. Formulation and evaluation of antibacterial Herbal gels of *Murraya koenigii* leaves extract. *Res J Pharm Technol*. 2017;10(6):1798–801.
- [19] Arumugam M, Panneerselvam R. In vitro propagation and antibacterial activity of *Clitoria ternatea* Linn. *Asian Pac J Trop Biomed [Internet]*. 2012;2(2 SUPPL.):S870–5. Available from: [http://dx.doi.org/10.1016/S2221-1691\(12\)60326-8](http://dx.doi.org/10.1016/S2221-1691(12)60326-8)