

“Antimicrobial activity of polyherbal hair serum formulation”

Ms Sima S. Powar , Ms Shubhangi S. Badkar, Ms. Varsha Prakash Savant , Ms. Veena Vishnu Mahadik, Ms. Mohini Subhash Kamble, Mr.Yunus Salim Mullani

Sant Gajanan Maharaj College of Pharmacy, Mahagaon. Site- Chinchewadi , Tal Gadhinglaj, Dist- Kolhapur, Maharashtra-416503

Abstract:

The rise of antimicrobial resistance has prompted increased interest in alternative therapies, especially those derived from natural sources. In this context, the present study focuses on evaluating the antimicrobial activity of a polyherbal formulation consisting of

Clitoria ternatea (commonly known as butterfly pea) and *Punica granatum* (pomegranate), both of which are traditionally recognized for their medicinal and therapeutic properties.

The formulation was prepared by extracting the active constituents from the flowers of *Clitoria ternatea* and the peel of *Punica granatum* using appropriate solvent extraction methods. These plant parts are rich in bioactive compounds such as flavonoids, alkaloids, tannins, and phenolic compounds, which are known to possess antimicrobial, antioxidant, and anti-inflammatory properties. The antimicrobial activity of the prepared polyherbal extract was tested against selected bacterial and fungal strains using the agar well diffusion method. Standard microbiological procedures were followed to culture the microorganisms, and the zone of inhibition was measured to evaluate the efficacy of the formulation. The results indicated that the polyherbal extract exhibited significant antimicrobial activity, especially against Gram-positive bacteria such as *Staphylococcus aureus*, and moderate activity against fungal strains. The combination of the two plant extracts was found to be more effective than the individual component suggesting a synergistic effect

Keywords:

Clitoria ternatea, *Punica granatum*

Introduction¹:

Anti microbial activity of a substance refers to the ability of the substance to kill or inhibit the growth of microorganisms, such as bacteria, fungi, viruses, or parasites. These are called anti microbials .it can be naturally occurring, synthetic and semi-synthetic

Punica granatum* and *Clitoria ternatea both are natural occurring herbal substance and they have several uses for skin, digestive system since ancient times. They show no to minimum side effects

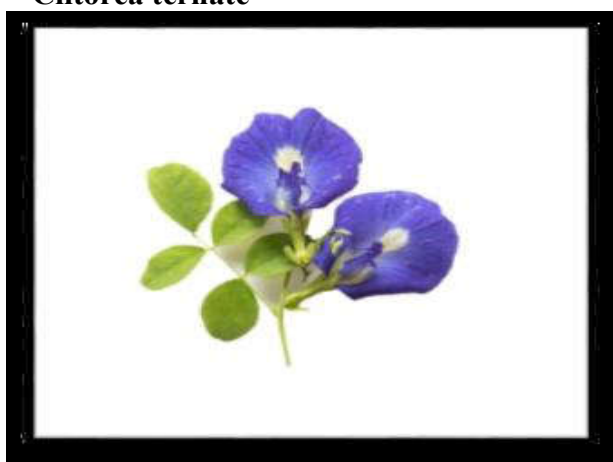
They show several properties like anti microbial, anti inflammatory, anti fungal properties

Hair growth serum is a popular cosmetic in today's time and it is therapeutic product designed to reduce hair fall, improve texture, promote hair growth, provide silky and shiny hair, and reduce frizz and protection from environmental damage

Unlike shampoo and oils, serum is light weighted liquid which forms a thin layer on hair which locks the moisture in hair, reduce frizz, and protect hair from environmental damage of which one application is enough for whole day. Herbal formulations are gaining attention due to their synergistic effect and broad spectrum biological activities. This investigation supports the growing interest in plant based medicines and potential role of polyherbal combinations against microbials.

Introduction of *clitoria ternatea* plant and pomegranate peel powder

*Clitoria ternatea*⁴



A

Img.1 *Clitoria ternatea*

Botanical Classification:

Kingdom: Plantae

Family: *Fabaceae* (Legume family)

Genus: *Clitoria*

Species: *ternatea*

Common Names: Butterfly Pea, Asian Pigeonwings, Aparajita, Neelkanthi

Description:

Type: Perennial herbaceous plant or climbing vine

Leaves: Compound with 5–7 leaflets

Flowers: Bright blue or white, often with a yellow center; single or paired

Fruit: Flat, pod-like with several seeds

Root: Taproot system

Habitat:

Native to Southeast Asia but widely cultivated in tropical and subtropical regions.

Grows well in well-drained soil with full sun to partial shade.

Chemical Constituents:

Anthocyanins (especially in blue flowers)

Antioxidant

Flavonoids

Triterpenoids

Saponins Alkaloid

Medicinal Uses

1. Memory booster (Medhya)
2. Anxiolytic (reduces anxiety)
3. Antioxidant
4. Antidepressant
5. Anti-inflammatory
6. Anti-diabetic
7. Antimicrobial
8. Skin and hair care – used in herbal oils and shampoos
9. Used in Ayurveda for treating:
Epilepsy (Apasmara)
Fever
Skin diseases
Urinary disorders
10. Flowers used to make herbal tea
11. Roots used as a purgative and brain tonic.
12. Leaves used in some cultures for medicinal pastes or decoctions.
13. Natural food dye (blue colour)

Punica granatum⁹**Botanical Source:****Plant:** *Punica granatum***Family:** *Lythraceae***Part Used:** Dried outer peel of pomegranate fruit**Common Names:**

Pomegranate peel powder, Anar ke chilke ka powder, डाळ िंब सालीचा चूर्ण

Img.2 Pomegranate peel powder**Pomegranate peels are rich in:**

Ellagic acid – antioxidant, anti- cancer properties
 Tannins – anti-inflammatory, astringent
 Flavonoids, Gallic acid
 Vitamin C, Polyphenols
 Alkaloids, Saponins

Medicinal & Health Benefits:

1. Digestive health
2. Antioxidant & anti-aging

3. Skin care
4. Oral health
5. Hair care
6. Immunity booster
7. Weight management

Materials & Methods^{10,11}

Selection, identification and collection, authentication of clitoria ternatea and punica granatum

Selection

The flower of clitoria ternatea and fruit of punica granatum were selected for our project

These plants were selected based on their traditional medicinal significance and reported pharmacological properties such as antioxidant, antimicrobial, and anti-inflammatory activities.

Identification and collection

Fresh flower of clitoria ternatea and fruit of punica granatum were collected from botanical garden of SGM COP, Mahagaon.

These materials were identified by the certified botanist from our college.

Authentication

The collected specimens were authenticated by comparing herbarium sheets and monographs.

Samples of the specimens were provided to institute for future reference.

Harvesting and drying

Collect the fresh materials of the selected plants i.e. fresh flowers of clitoria ternatea and fresh peel of pomegranate fruit

Remove any damaged part

- Rinsed selected parts to remove dirt and debris.
- Let the materials air dry on a clean surface
- Drying process may take several days to week to dry completely till it become crispy
- Instead we can hot air oven to dry them in minimum time
- Grinding
- Once the materials are dried, take small batches of each material separately
- Using electric grinder make fine powder of each material
- Keep these materials separately to maintain their individual properties

Sieving and storage

- Pass all the material separately through sieve no. 44 to remove any large particles obtain
- store all the powder separately in a tight glass jar

- store it in cool and dry place, keeping away from direct sunlight

Extraction

- weigh 70gm of each powdered herb
- put the powder in the cloth and keep it in the thimble of soxhlet apparatus
- connect the extractor to round bottom flask
- don't forget to add porcelain chips in the round bottom flask
- Add ethanol or methanol in the powder
- Provide heat to the apparatus
- After providing heat the mixture get separated and extract is obtained
- extraction storage
- Store it in a glass container.
- Keep it in cool and dry place.
- And away from direct sunlight.
- Glass container must sterilized.

Methodology

Formulation table:

We prepared the formulation by using following excipients

Table no.1 Formulation table

Sr. no.	Ingredients	Quantity
1	Clitorea ternatea extract	10ml
2	Punica granatum extract	10ml
3	Glycerine	1.5ml
4	Rose water	7.8ml
5	Xanthum gum	0.1g
6	Tween 80	0.3ml
7	Ethanol 20%	0.3ml

Procedure⁹

Preparation of aqueous phase

- In clean beaker, add the required amount of rose water (70% of total volume) and glycerine
- Stir continuously and add xanthum gum slowly in a required amount

- c. Continue stir about 10-15 min until the solution gets slightly thick to avoid any clumps.

Addition of active extract

- a. After preparing aqueous phase add extract of both materials
- b. Stir continuously to prepare a homogenous mixture

Emulsification

- a. Add tween 80 in a small amount in the above mixture
- b. Continue stirring to avoid clumps till the oil gets incorporated in the mixture

Addition of preservatives

- a. Add ethanol into above mixture and stir for 5-10 min.

Final mixing

- a. At last, mix all the ingredients thoroughly to ensure all excipients gets evolved equally
- b. Stir for more 5-10 min.

Chemical tests of extracts^{5,6}

Tests for clitoria ternatea

A. Tests for Alkaloids

For this test, mix the residue with dilute HCl

Table no. 2 tests for alkaloids

Tests	Results
1. dragondroff's test 2-3ml filtrate + dragondroff's reagent	Positive
2. mayer's test 2-3 ml filtrate + mayer's reagent	Positive
3. hager's test 2-3 ml filtrate + hager's reagent	Positive
4. wagner's test 2-3 ml filtrate + wagner's reagent	Positive



Img 6. Test for alkaloids

B. Tests for Tannin and phenolic content

Table no. 3 tests for tannin and phenolic content

Tests	Results
1. 5% FeCl_3 solution	Negative
2. lead acetate solution	Positive
3. gelatine solution	Negative
4. bromine water	Positive
5. acetic acid	Positive
6. dil. Potassium permanganate solution	Positive
7. potassium chromate	Positive
8. dilute HNO_3	Positive
9. Dilute iodine solution	Positive

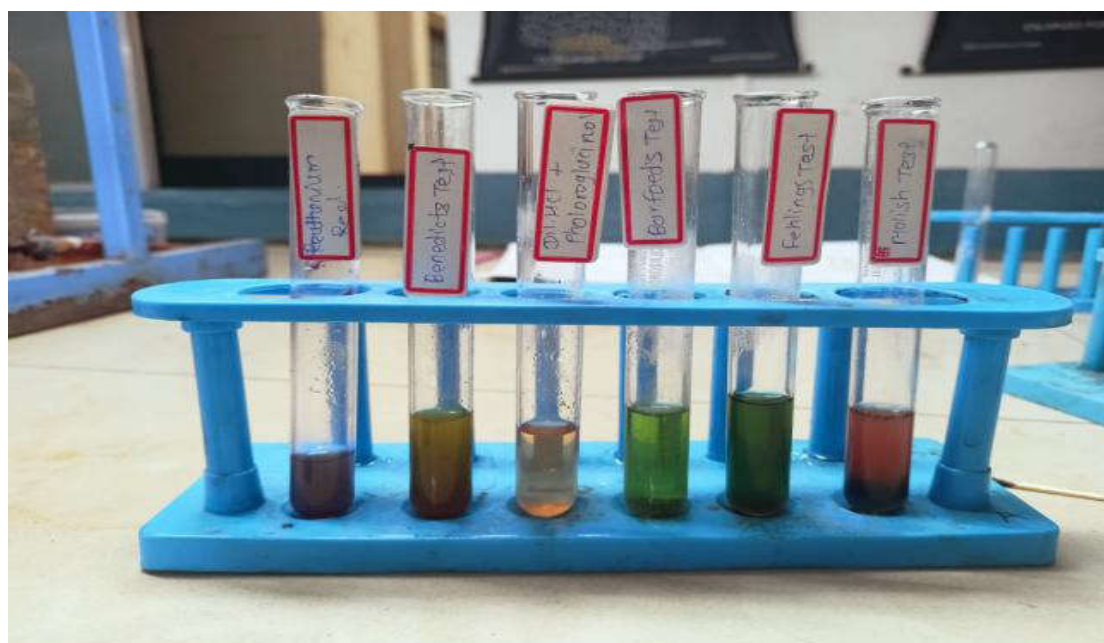


Img 7. Tests for tannins and phenolic content

C. Tests for Carbohydrates

Table no. 4 tests for carbohydrates

Tests	Results
1. molish test To 2-3 ml of aqueous extract+ few drop of alpha naphthol solution in alcohol,shaken and added conc.	Negative
2. fehling's test 1 ml fehling's solution A + 1ml fehling's solution B was mixed and boiled for 1 min. added equal volume of test solution. Heated in boiling water for 5-10 min.	Positive
3. benedict's test Equal volume of benedict's reagent and test solution in test tube were mixed.heated in boiling water bath for 5 min.	Positive
4. barfoed's test Equal volume of barfoed's reagent and test solution were added. Heated for 1-2 min, in boiling water bath and cooled	Positive
5.tollen's phloroglucinol test Mix 2.5 ml conc. Hcl and 4 ml 0.5 % phloroglucinol. Add 1-2 ml test solution,heat.	Positive
6.iodine test Mix 3 ml test solution and 2 drop of dilute iodine solution.	Positive
7. ruthenium red test In powdered drug add few drops of ruthenium red reagent.	Positive



Img 8. Test for carbohydrates

D. Test for Protein

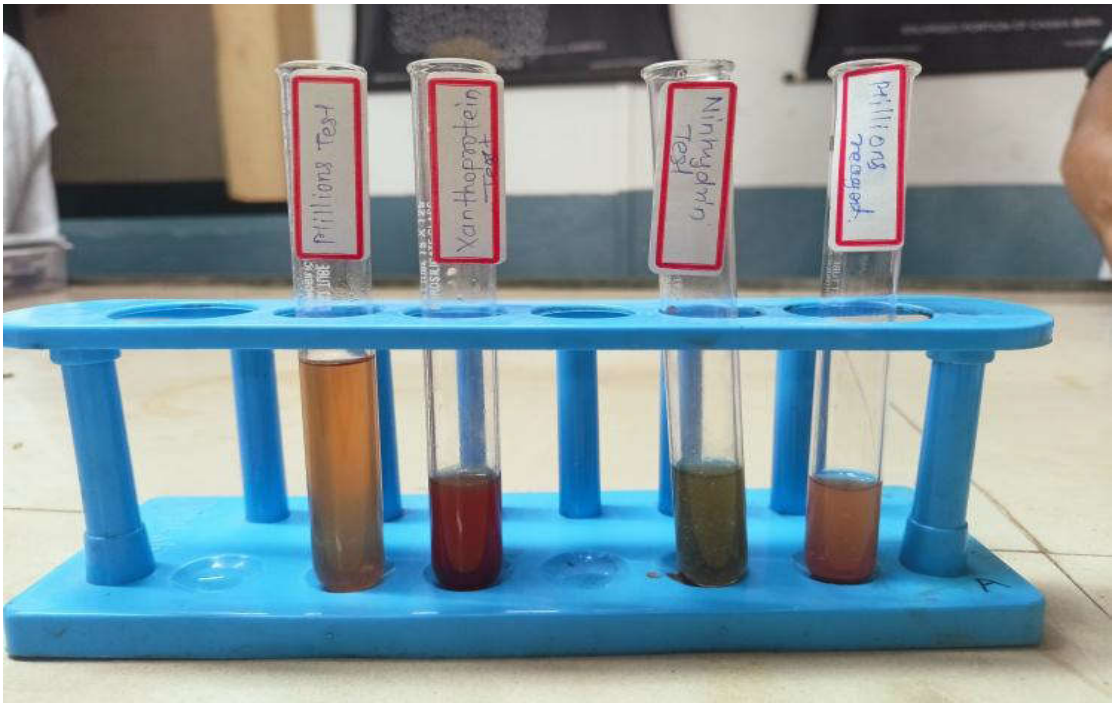
Table no. 5 tests for protein

Test	Result
1. Million's test 3 ml test solution with 5 ml millon's reagent	Positive
2. xanthoprotein test Mixed 3 ml test solution with 1 ml conc. H2SO4	Negative

E. Test for amino acids

Table no. 6 tests for amino acids

Test	Results
1. ninhydrin test (general test) Heat 3ml test solution and 3 drops 5% ninhydrin solution in boiling water bath for 10 min.	Positive
2.million's test Heat 3 ml test solution with 3 drops of millon's reagent	Positive



Img.9 Tests for protein and amino acids

F. Test for saponin

Table no. 7 tests for saponins

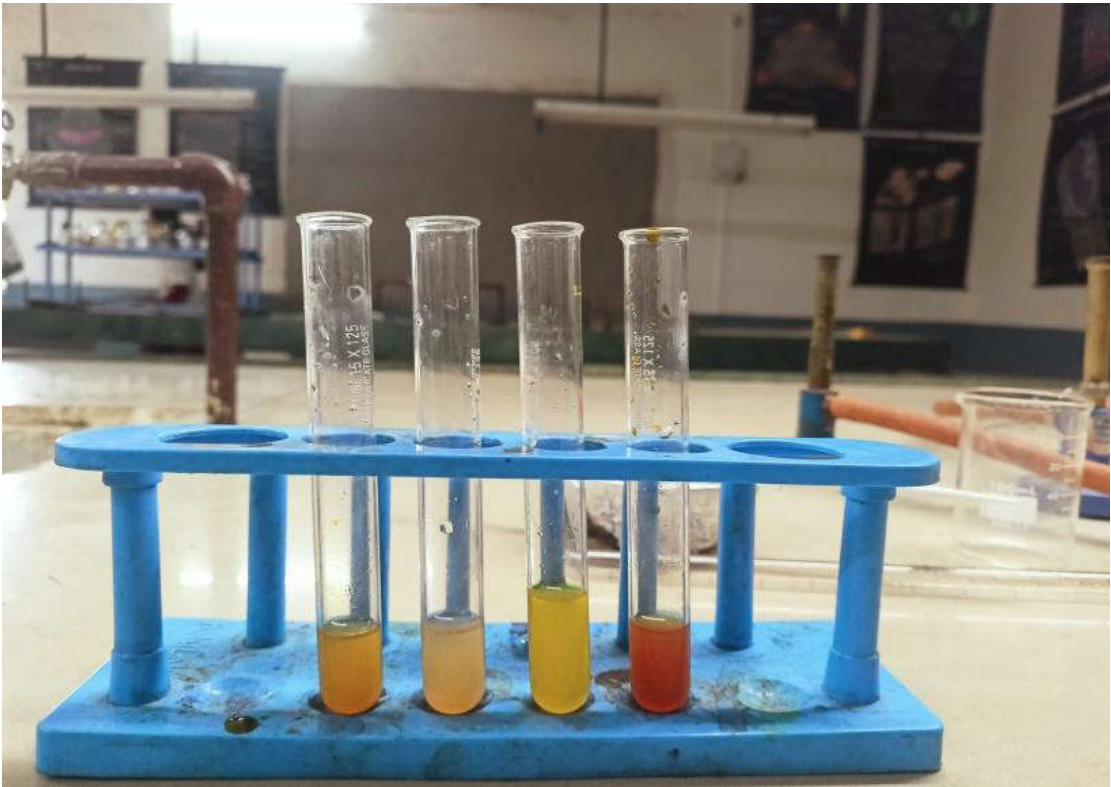
Test	Result
1. take small amount of extract and mix this in water and shake	Positive

Tests for punica granatum

A. Test for alkaloid

Table no. 8 tests for alkaloid

Tests	Results
1. dragondroff's test 2-3ml filtrate + dragondroff's reagent	Positive
2. mayer's test 2-3 ml filtrate + mayer's reagent	Positive
3. hager's test 2-3 ml filtrate + hager's reagent	Positive
4. wagner's test 2-3 ml filtrate + wagner's reagent	Positive



Img.10 Tests for alkaloids

B. Tests for Tannin and phenolic content

Table no. 9 tests for tannin and phenolic content

Tests	Results
1. 5% FeCl_3 solution	Positive
2. lead acetate solution	Positive
3. gelatine solution	Negative
4. bromine water	Positive
5. acetic acid	Negative
6. dil. Potassium permanganate solution	Positive
7. potassium chromate	Positive
8. dilute HNO_3	Positive
9. Dilute iodine solution	Positive

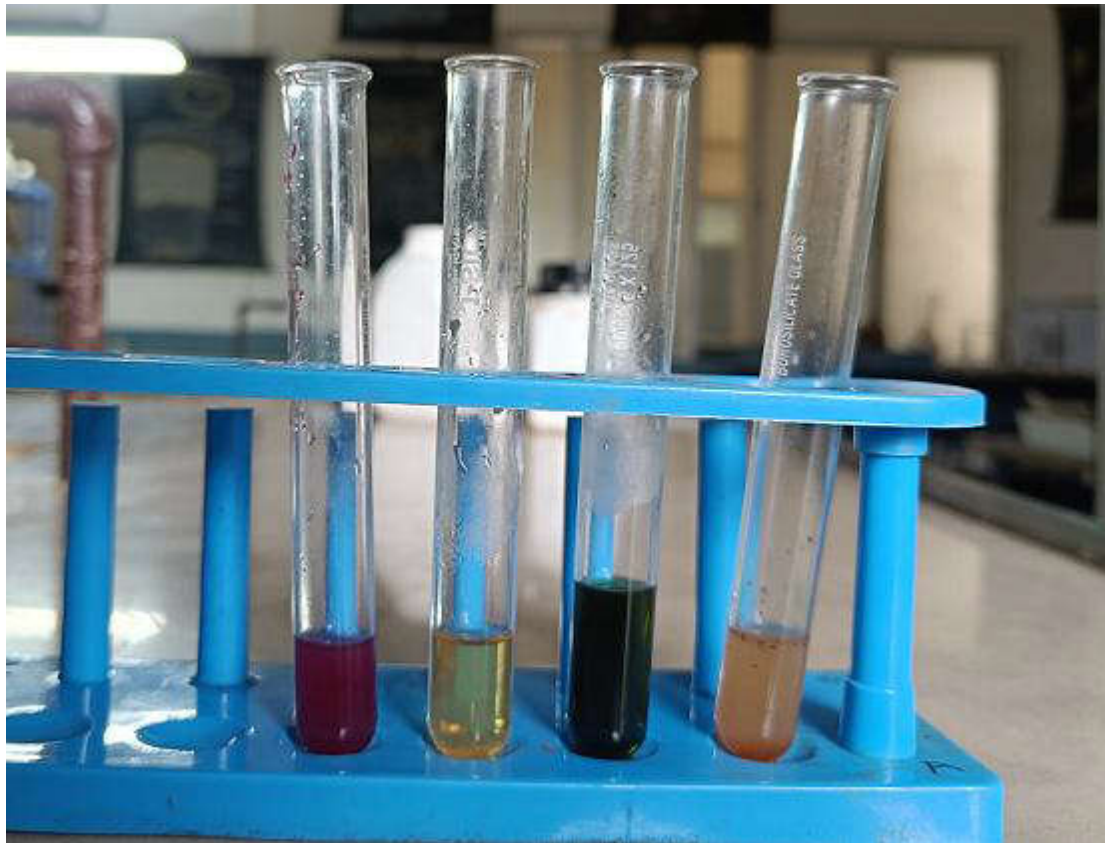


Img.11 Tests for tannins and phenolic contents

C. Tests for Carbohydrates

Table no. 10 tests for carbohydrates

Tests	Results
1. Molish test To 2-3 ml of aqueous extract+ few drop of alpha naphthol solution in alcohol,shaken and added conc. H ₂ so ₄ from side of the test tube	Positive
2. fehling's test 1 ml fehling's solution A + 1ml fehling's solution B was mixed and boiled for 1 min. added equal volume of test solution. Heated in boiling water for 5-10 min.	Positive
3.tollen's phloroglucinol test Mix 2.5 ml conc. Hcl and 4 ml 0.5 % phloroglucinol. Add 1-2 ml test solution,heat.	Positive
4. ruthenium red test In powdered drug add few drops of ruthenium red reagent.	Positive



Img.12 Tests for carbohydrates

D. Test for Protein

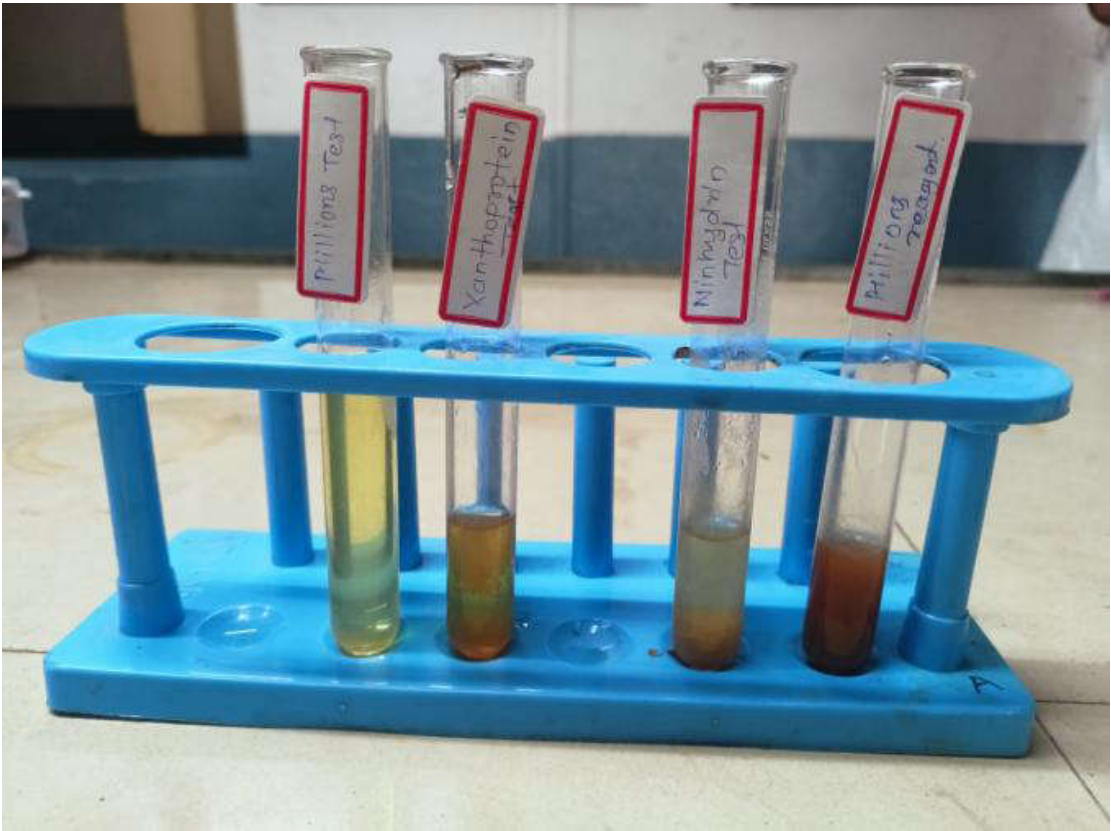
Table no. 11 tests for protein

Test	Result
1. Million's test 3 ml test solution with 5 ml millon's reagent	Positive
2. xanthoprotein test Mixed 3 ml test solution with 1 ml conc. H2SO4	Negative

E. Test for amino acids

Table no. 12 tests for amino acids

Test	Results
1. ninhydrin test (general test) Heat 3ml test solution and 3 drops 5% ninhydrin solution in boiling water bath for 10 min.	Positive
2.million's test Heat 3 ml test solution with 3 drops of millon's reagent	Positive



Img.13 Tests for protein and amino acids

F. Test for saponin
Table no. 13 tests for saponins

Test	Result
1. take small amount of extract and mix this in water and shake	Positive

Antimicrobial test of extract⁸

Anti microbial activity was performed by following method

1. Formulation of Polyherbal Extract

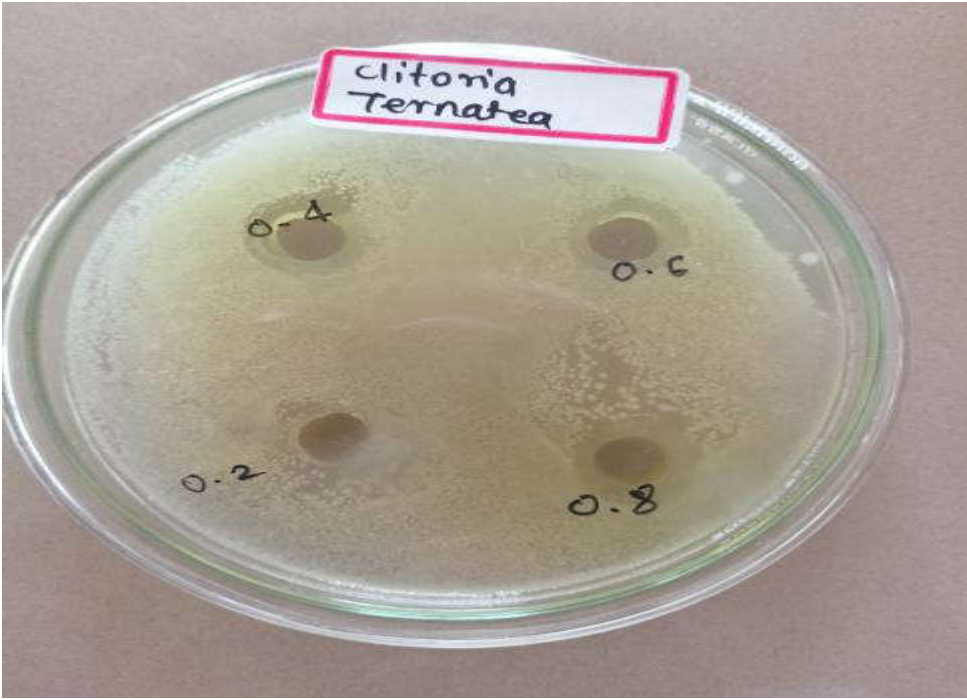
Equal portions of *Clitoria ternatea* and *Punica granatum* extracts were combined in various concentrations (0.2, 0.4, 0.6, and 0.8) to prepare polyherbal formulations.

2. Microorganisms Used

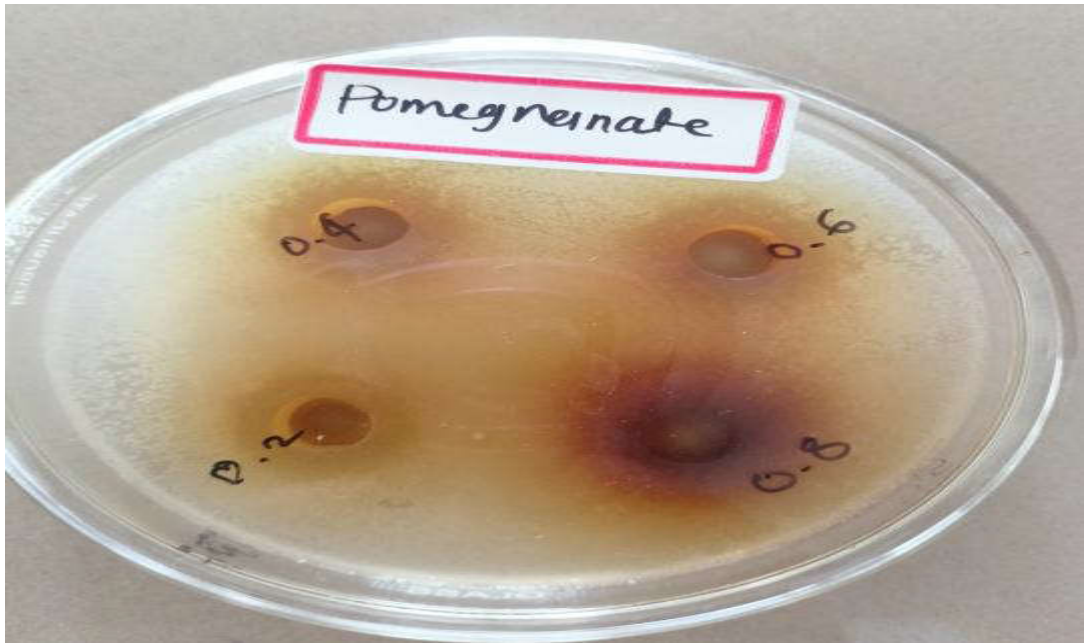
The antimicrobial activity was tested against common bacterial strain *Staphylococcus aureus* which were obtained from a microbiology lab culture bank.

3. Antimicrobial Activity Testing

The agar well diffusion method was used to evaluate antimicrobial activity. Agar plates were inoculated with test microorganisms, and wells were filled with different concentrations of the polyherbal formulation (e.g. 0.2, 0.4, 0.6, and 0.8). Zones of inhibition were measured after 24 hours of incubation at 37°C. Maximum effects were shown at 0.8 concentrations



Img.14 Antimicrobial activity of Img. *Clitoria ternatea*



Img.15 Antimicrobial activity of punica granatum

9 Evaluation tests of formulation

1. Physical characteristics

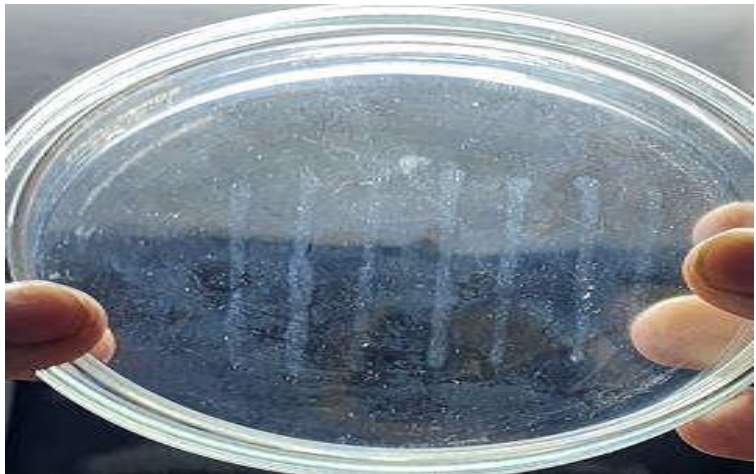
Table no. 14 physical characteristics

Parameter	Result
Colour	Woody brown
Odour	Pleasant
State	Slightly viscous

2. Homogeneity test

Table no.15 homogeneity test

Experiment	Result
Clarity test	Absence of white and black spots



Img. 16 Test of homogeneity

3. Determination of pH
Table no. 16 determination of pH

Experiment	Result
Digital pH meter	4.7



Img.17 pH determination

4. Viscosity study of Serum Formulation
Table no. 17 test for viscosity

Sr.no.	Formulation	Viscosity (cp) 10rpm	Viscosity (cp) 20rpm	Viscosity (cp) 50rpm	Viscosity (cp) 100rpm
1.	F1	600.0	200.0	80.00	40.00
2.	F2	800.0	200.0	240.0	120.0

5. Spreadability test
The formulation shows optimum spreadability



Img. 25 Spreadability test

10 Conclusion

Staphylococcus aureus (Staph) infection, especially folliculitis, can affect hair, leading to symptoms like small, painful pimples or boils at the base of hair follicles. The successful formulation and evaluation of polyherbal hair serum utilizing *clitoria ternatea* and *punica granatum* have yielded promising result. This natural hair serum offers an effective and environmental friendly solution to combat hair issues. The antimicrobial activity of *clitoria ternatea* and *punica granatum* shows effective results which makes the formulation an alternative use to synthetic products.

11 Reference

1. www.researchgate.net :
2. <https://www.who.int>
3. <https://www.nlm.nih.gov>
4. www.ijprajournal.com :-
Mrs. Neethu Vijayan, Ansiya A., Fatima Hussainar, Fatima K K, Hadiya C. Kabeer, Rumaiza Ansar.
Formulation and evaluation of hair serum using *clitoria ternatea* and *cyathium cinereum* for dandruff.
International journal of pharmaceutical research and applications
volume 10, Issue 1 Jan-Feb 2025, pp: 541-548
5. https://pmc.ncbi.nlm.nih.gov/articles/PMC8076379/pdf/13197_2020_Article_4745.pdf
6. <https://ndl.iitkgp.ac.in/>
7. <https://www.embrapa.br/>
8. www.elsevier.com/locate/ijfoodmicro
9. N.S. Al-Zoreky
Antimicrobial activity of pomegranate (*Punica granatum* L.) fruit peels
International Journal of Food Microbiology
10. <https://link.springer.com/>
11. <https://doi.org/10.1007/s13197-020-04745-3>