

# “Formulation and Characterization of Antimicrobial Potential of Herbal Cream using *Clitoria Ternatea* Linn. Flowers (Aparajita) For the Treatment of Vaginal Infection”.

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## Abstract:

*Clitoria ternatea*, a member of the Fabaceae family, is frequently referred to as butterfly pea. It is utilised in several ayurvedic preparations and is usually referred to as "Shankpushpi" in Sanskrit. In addition to its medical applications, *C. ternatea* flowers are used as a natural food colouring and have become increasingly popular as ornamental flowers around the world. The purpose of this study was to treat vaginal infections by creating an antifungal cream with *Clitoria ternatea* Linn. (Flowers). Plant parts and flowers having antifungal, antibacterial, antioxidant, and antiaging qualities are frequently found in herbal cosmetic formulations. The safest product for everyday use that doesn't cause any negative effects is herbal cosmetics. The most prevalent gynaecological issue that women encounter during their lives is vaginal infection, or vaginitis, which is brought on by *Candida* species. The current study aims to investigate the plant's anti-candida properties. The creation of a herbal cream was also the goal. The aqueous extract of *Clitoria ternatea* Linn. flowers was used in the formulation and evaluation of the herbal cream in this study, and the results showed good results when compared to standard drugs. The herbal cream's formulation yielded highly promising and great outcomes compared to other herbal creams.

In order to uncover and demonstrate the effectiveness of a prepared dosage form, it is crucial to ensure biological activity for every formulation. In light of this, herbal creams were made and tested for anti-candida properties; the cream's anti-candida activity was assessed as an ideal preparation. The PHC (HC5) has the best anti-candida activity when compared to the conventional formulation (MCC), according to the data, and can be used to treat vaginal infections. Furthermore, thorough clinical methods must be established in order to manufacture safe and efficient medications.

**Keywords:** *Clitoria ternatea*; Anti-fungal; Vaginal infection, Anti-oxidant

## INTRODUCTION:

In primary care, vaginal *Candida* infections are the most common gynaecologic diagnosis. Up to 45% of women have had two or more vaginal *Candida* infections, and 75% are predicted to have at least one in their lifetime. Stress, poor food, poor hygiene, lack of sleep, illness, hormone imbalance, pregnancy, and antibiotic use are some of the factors that increase the risk of vaginal yeast infections in women.<sup>[1]</sup> *Clitoria ternatea* L., commonly known as Aparajita, is one of the many herbs and plants that have been used for centuries to treat a wide range of illnesses. It is mentioned in every ancient Ayurvedic source as one of the necessary herbs.<sup>[2]</sup>

The Butterfly pea, or *Clitoria ternatea*, is a perennial herbaceous plant that belongs to the Fabaceae family. The pentacyclic triterpenoids taraxerol and taraxerone, anthocyanins like ternatin and delphinidin, and flavonols like kaempferol are the primary phytoconstituents of the plant that are responsible for a variety of processes.<sup>[3]</sup> Worm infections, diabetes, pain, inflammation, vaginal irritation, and other ailments are all treated with the herb.

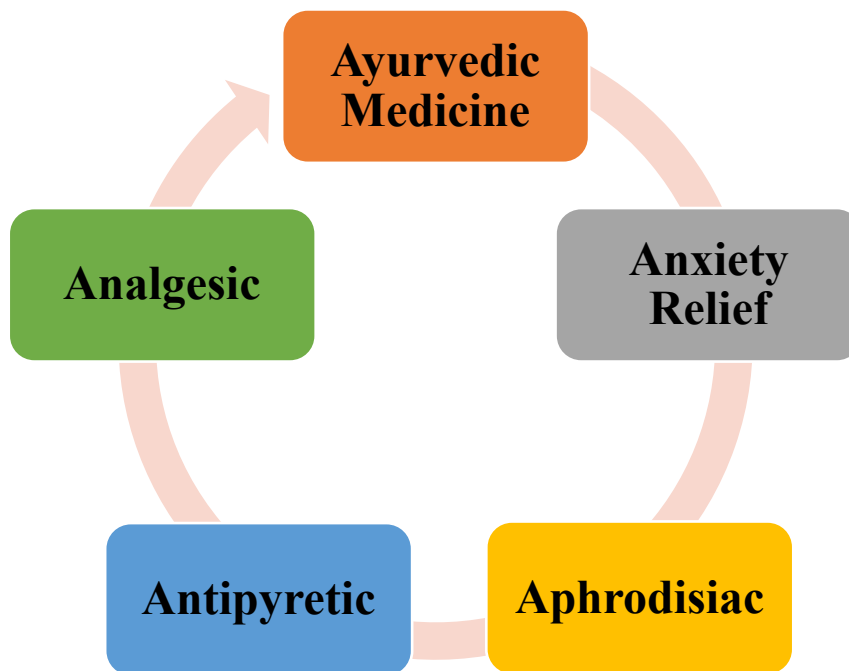


**Fig1.1: Clitoria Ternatea Linn**

**(Aparajita)**

#### **BENEFITS OF CREAM USING CLITORIA TERNATEA LINN. (APARJITA) FLOWER:-**

1. **Hydration and Rejuvenation:** Vitamins A and C found in abundance in aparajita flowers can help revitalise and support a glowing complexion.
2. **Antioxidant Properties:** The flower has antioxidants that can help shield the skin from free radical damage, which may lessen ageing symptoms and encourage healthy skin.
3. **Soothing and Calming:** calming and soothing qualities, aparajita is appropriate for a variety of skin types, including sensitive ones.
4. **Anti-Inflammatory:** Anti-inflammatory natural substances found in butterfly pea blossoms can calm sensitive skin and lessen redness.
5. **Antifungal properties:** Clitoria ternatea exhibits antifungal activity against various fungi, including *Candida hoffmannii*.
6. **Other uses:**



### AIM & OBJECTIVE:

**Aim:-** Preparation of herbal cream containing aqueous extract of *Clitoria Ternatea* Linn. (flowers) for the treatment of vaginal infection.

**Objective:-**

- The main objective was to prepare herbal cream. In this formulation of herbal cream we use Aparajita flower and shatavari as an active ingredient for the treatment of vaginal infection and various other like bees wax, propyl paraben, borax, and coconut oil, rose water.
- Treating vaginal infections: includes itching, burning, discharge and pain.
- Different evaluation tests are carried out for the prepared herbal cream such as appearance, spreadability, pH, Viscosity, phytochemical analysis. Prepared formulation passes all the given evaluation tests.
- Thus, the prepared formulation of herbal cream was effective and safe for vaginal infection .

### LITERATURE REVIEW:

1) **Kumar. A et.al (2016) :-** This research article reviewed about “**Extraction, Phytochemical Screening, Separation and Characterisation of Bioactive Compounds from leaves Extracts of *Clitoria Ternatea* Linn. (Aparjita)**”. The main objective of research study was to extract, separate and characterize the bioactive compounds by using different phytochemical screening, thin layer chromatography and spectroscopy techniques. The extraction was performed by successive hot continuous Soxhlet extraction in order to the polarity of the solvents i.e, Petroleum Ether. As per the phytochemical investigation carbohydrates, glycosides, flavonoids, tannins, saponins etc was found to be present in the various extracts of leaves of *Clitoria Ternatea* Linn.<sup>[4]</sup>

2) **Sharma. P et.al (2019):-** This research article reviewed about “**Butterfly pea (*Clitoria ternatea*) Flower Extract (BPFE) and It's use as a pH-Dependent Natural colorant**”. It includes color- changing Anthocyanin, Extraction using solvent alcohol and maintaining temperature for extraction (40°C-80°C) and evaluation parameters like pH. Storage conditions and processing their effects on color and quality of BPFE. It has also mentioned the potential protective properties of sucrose.<sup>[5]</sup>

3) **Sharma. u & Dwivedi. S et.al (2021):-**This research article reviewed about “**Develpoment and Characterization of Herbal Formulation Containing Aqueous Extract of Clitoria Ternatea Linn.(Flowers) for the treatement of Vaginal Infection**”. This includes selection of plant material , collection of authentication of plant, preparation and characterization of extracts, their Anti-candida activity, Screening of antifungal activity by disc diffusion method, preparation of cultural plate and lastly Formulation of herbal cream and evaluating evaluation paramters like pH, Viscosity, Spreadability etc. The results of formulation with code HC5 were found to be most promising and excellent among all herbal creams formulated when compared with standard formulation.<sup>[6]</sup>

4) **Siddham. P et.al (2023):-** This research article reviewed about “ **Phytochemical Analysis and Antimicrobial Screening of Clitoria Ternatea. Linn**”. study includes various preliminary test and screening of phytochemicals and microbial screening. It concludes that the study of phytochemical screening for leaves of Clitoria Ternatea showed the presence of active component like saponins, tanins, flavonoids, amino acids, alkaloids, proteins and other various constituents. Antimicrobial investigation reveals that the current plant extract is found to be useful against S. typhi and S. aureus basically ethanol are effective against s.typhi and s. aureus.<sup>[7]</sup>

5) **Paymalle.L et.al (2024):-** This paper reviewed about “**A systematic review on exploration of therapeutic potential of Aparajita (Clitoria Ternatea Linn)**”. Study includes Analgesic effect of Clitoria Ternatea Linn , Hepatoprotective and Antioxidant Activity of Clitoria Ternatea Linn, Hypoglycemic effect , Diuretic and Antibacterial effect of Clitoria Ternatea Linn. For evaluating the antibacterial properties of Clitoria Ternatea Linn. For this, they used organic solvent (Petroleum ether, Ethyl acetate, and methanol) extracts from the leaves. The extract was tested against Bacillus cereus, Staphylococcus aureus, and Salmonella typhi by agar disc and well diffusion methods. The results of this study were that methanolic extract affected the activity of Bacillus cereus to a greater extent followed, Proteus vulgaris, and Salmonella typhi. Petroleum ether extract affected the activity of salmonella typhi to a great extent followed by Proteus vulgaris, Bacillus cereus.<sup>[8]</sup>

### Plant profile: *Clitoria Ternatea Linn.*

#### Botanical source

- **Scientific Name:** *Clitoria Ternatea Linn.*
- **Family:** Fabaceae
- **Common Names:** Butterfly pea, Asian pigeonwings, Blue bellvine, Blue pea, Cordofan pea or Darwin pea,



**Fig: 1.2: *Clitoria Ternatea Linn.* plant**

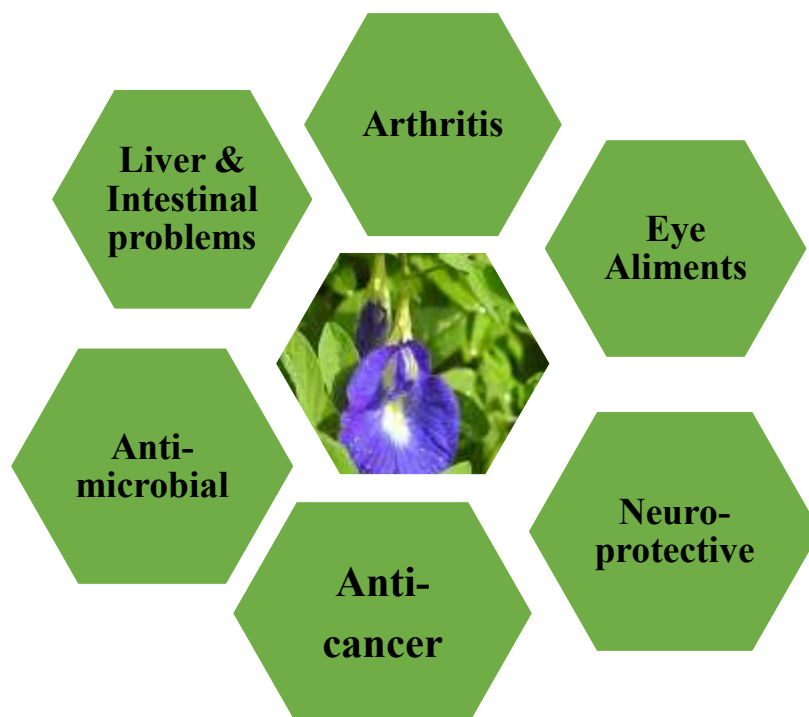
*Clitoria ternatea* Linn is a member of the fabaceae family. It has elliptic, obtuse leaves and is a perennial herbaceous plant. It thrives in damp, neutral soil and grows as a vine or creeper. Its flowers, which are solitary and have faint yellow patterns, are its most remarkable feature. They are a beautiful deep blue colour. They measure roughly 3 cm (1+1/4 in) in width and 4 cm (1+1/2 in) in length. Some types produce pink and white blooms. Several triterpenoids, flavonol glycosides,

anthocyanins, and steroids are among the chemical substances that have been extracted from *C. ternatea*. The heat-stable portion of the *C. ternatea* extract has yielded cyclic peptides called cliotides.<sup>[9]</sup> Several anthocyanins, most notably ternatins, which are polyacylated derivatives of delphinidin 3,3',5'-triglucoside (Da-T), give *C. ternatea* its blue hue.<sup>[10]</sup> It has delicate, twining stems that are 0.5–3 m long. The pinnate leaves have five to seven elliptic to lanceolate leaflets that are three to five centimetres long and hairy beneath. Solitary flowers range in colour from deep blue to blue mauve or occasionally white; the fig is 4–5 cm long and has a very short pedicellate. Up to ten seeds are contained in flat, linear, beaked pods that are 6–12 cm long, 0.7–1.2 mm diameter, and somewhat pubescent.

The seeds are 4.57 mm long, 3–4 mm diameter, olive, brown or black, and frequently mottled. Originally from humid and sub humid tropical lowlands, butterfly peas can withstand drought in the seasonally dry tropics (500–900 mm of rainfall) and have withstood moderate frost damage in the subtropics (26°S).<sup>[11]</sup>

Throughout its natural range, it can be found in grassland, open woodland, shrub, riverine vegetation, and disturbed areas. Full sun is ideal for butterfly pea growth. Ascetics, visceral expansion of the abdomen, sore throats, and skin conditions are all treated with root. Asthma and burning sensation can also be relieved by the roots. The entire plant is used to treat jaundice, migraine, throat and eye infections, skin conditions, asthma, swollen joints, ear pain, eruptions, fever, UTIs, constipation, snakebite, indigestion, leprosy, and disorders of the central nervous system. For the treatment of snakebite and scorpion sting, the root, stem, and flower are advised. Root is often given to kids as a general tonic with honey and ghee to help with mental and physical strength. Many people utilise seeds and leaves as a brain tonic to improve their memory and intelligence. The antidote for snake bites was made of juice and flowers. Antioxidants such as p-coumaric acid, delphinidin-3,5-glucoside, and kaempferol are said to be present in the plant. Additionally, blue peas are high in vitamins A, C, and E.<sup>[12]</sup> A drink, cake, ice cream, candy, and other traditional foods and sweets frequently contain this plant's colourant in varying amounts.

#### Traditional uses of *Clitoria ternatea* linn:



#### Plant profile: *Asparagus racemosus*

##### Botanical source:

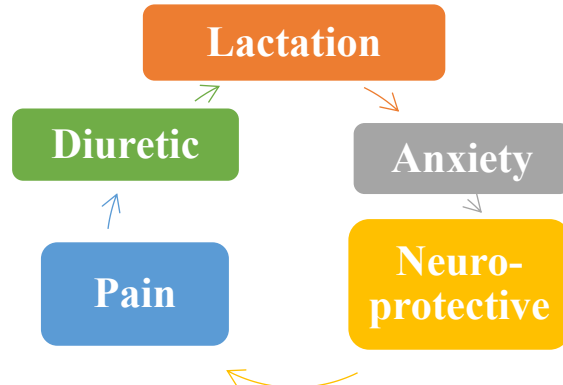
- **Scientific Name:** *Asparagus racemosus*
- **Family:** Asparagaceae
- **Common Names:** Shatavari, Satawar, Satamuli



**Fig 1.3: *Asparagus racemosus***

*Asparagus racemosus* (*A. racemosus*), a member of the Liliaceae family, is found at low elevations all over India and is also referred to as Satawar, Satamuli, and Satavari. The name Shatawari means “curer of a hundred diseases”. The plant's dried roots are made into a medication.<sup>[12]</sup> When it comes to issues with the female reproductive system, this plant works wonders. The two primary works on Ayurvedic remedies, Charak Samhita (authored by Charak) and Ashtang Hridayam (written by Vagbhata), both include *Asparagus racemosus* (*A. racemosus*) in their lists of formulae for treating women's health issues.<sup>[13-16]</sup> *A. racemosus* is a well-known Ayurvedic rasayana that helps with diseases of the neurological system, dyspepsia, tumours, inflammation, neuropathy, and hepatopathy. It also prolongs life, boosts immunity, improves mental function, vigour, and adds vitality to the body. Steroid saponins (Shatavarins I–IV) found in the roots of *A. racemosus* are the main active ingredients. In India, Sri Lanka, and the Himalayas, *A. racemosus* is widespread. It is 1-2 meters tall and grows best in rocky, gravelly soils at elevations of 1300-1400 meters in the piedmont plains.

#### USES:-



#### MATERIALS AND METHODOLOGY :-

##### ▪ Selection of plant material

1. The herb *Clitoria ternatea* Linn. has long been used by people to treat gynaecological disorders, particularly vaginal infections. However, no systematic study has been done to date to create an effective herbal formulation that uses the flower alone or in combination to treat gynaecological disorders (vaginitis); for this reason, this herb was chosen for it.<sup>[17-22]</sup>
2. For issues pertaining to the female reproductive system, this herb is quite beneficial. Vagbhata's Ashtang Hridayam and Charak's Charak Samhita, the two primary texts on Ayurvedic remedies, include *Asparagus racemosus* (*A. racemosus*) in their formulations for treating women's health disorders. According to reports, the pharmacological properties of the root extract of *A. racemosus* include immunomodulatory, antidiabetic, antioxidant, antiulcer, and antidiarrheal properties.<sup>[23]</sup>



Fig 1.4 : Dried *Clitoria Ternatea* Linn.



Fig 1.5 : *Asparagus racemosus*

▪ **Preparation and characterization of extracts**

- **Preparation of *Clitoria ternatea* linn (Aparjita) extract:-** Using maceration process in which whole parts of flower was selected and was kept in contact with a specific solvent (ethanol) covered with cotton ball or foil paper for 2-3 days at room temperature and filter it out.



Fig 1.6 : Aqueous *Clitoria Ternatea* Linn. Extract

- **Preparation of *Asparagus racemosus* (Shatavari) extract:-** Using maceration process in which whole plant was selected and was kept in contact with a specific solvent (ethanol) covered with paper for 2-3 days at room temperature and filter it out.



**Fig 1.7: *Asparagus racemosus* Extract**

- **Characterization of extract:-** The aqueous extract of dried plant material of *Clitoria ternatea* Linn. (flowers) and *Asparagus racemosus* (Shatavari) CTF were characterized for their color, odor, and pH and were recorded in present investigation.
- **Preparation of culture plate:-** The nutrient agar media were prepared by dissolving media in 1000ml of distilled water and sterilized by autoclaving at 121°C for 1 hour. The media were cooled and poured in sterilized Petri plates to solidify at room temperature.<sup>[24]</sup>
- **Evaluation of zone of inhibition:-** The re-cultured fungal strains were used for antifungal evaluation. The strains were streak on the nutrient agar media and the drug entrapped discs were placed. For negative control, disc of distilled water and for positive control ciprofloxacin (10 µg/ml) were used. The disc of *Clitoria ternatea* Linn. Cream was mixed in 10 ml distilled water and 1ml concentration was added into agar plate. The petri plates were kept in incubator for 24 hrs. After 24 hrs, the petri-plates were checked for zone of inhibition. The zone of inhibition diameter was recorded. The zone of inhibition was calculated by subtracting diameter of sample or standard or control by diameter of disc. The more the zone of inhibition the more will be antifungal activity.

#### INGREDIENTS AND THEIR USES:

SR.NO	INGREDIENTS	QUANTITY	CATEGORY
1	Aqueous Extract of <i>Clitoria ternatea</i> (AECT)	4 ml	Antifungal activity
2	Aqueous Extract of <i>Asparagus racemosus</i>	3 ml	Antioxidant, antibacterial , improves skin elasticity.

3	Bees Wax	3.5 g	Emolient to soften and soothe skin, help to lock in moisture for hydrated skin
4	Borax	0.4 g	Emulsifying agent, Stabilizer, Buffering agent.
5	Propyl Paraben	0.4 g	Preservative
6	Coconut Oil	4 ml	Reduce inflammation, support healing, moisturizes skin.
7	Distilled Water	Q.S	Solvent
8	Rose Water	Q.S	Fragrance/ perfume

**Table1.1: Ingredients and their uses****Preparation of herbal vaginal cream :**

**Cream:-** It is defined as semi-solid emulsions which could be oil in water(o/w) and water in oil (w/o) type.

**PROCEDURE:-**

- 1) Take one beaker and melt the bees wax and coconut oil together at 75-80°C, (oil phase) heat it and mix well.
- 2) In another beaker dissolve borax, propyl paraben , aqueous clitoria ternatea extract and aqueous Asparagus racemosus extract in rose water (aqueous phase) and heat to same temperature to get a clear solution.
- 3) Slowly add aqueous phase to oil phase with constant stirring until a uniform mixture is obtained.
- 4) Keep stirring mixture continuously while it cools to room temperature.
- 5) Transfer cream into air tight container and store it.

**Antimicrobial Activity of Cream Formulations:**

The antibacterial activity of cream formulations was perform by standard agar well diffusion method against staphylococcus aureus, E. coli and bacillus. Nutrient broth/agar was used to cultivate bacteria. In order to recover the lyophilized culture, the desire amount of culture was aseptically transferred in nutrient broth and maintained in an incubator at 37 °C for 3 hrs. to form inoculums. The media was poured in petri plates aseptically and kept for 30 minutes for solidification. After 30 minutes, the fresh inoculums of different culture were spread on to solidified nutrient agar plates. Wells were made using sterile small test tubes of 5mm diameter on petri plates at proper positions. The cream was added in to agar wells, aseptically. Then the agar plates were incubated at 37°C for 24 hrs. After 24 hrs. of incubation, the zone of inhibition was investigated.<sup>[25]</sup>

**Nutrient medium:**

Beef extract- 1gm

Peptone- 1gm

NaCl- 0.5gm

Distilled water - 100ml

Agar- 3gm



Fig 1.8 : Ecoli



Fig 1.9: S. Aureus

## RESULT & DISCUSSION:

### Evaluation parameter for cream-

#### 1. Organoleptic properties:-

##### ➤ Colour-

The colour of the formulation was checked manually and observed as light blue .

##### ➤ Odour-

Odour was found to be characteristic.

##### ➤ Consistency-

Consistency was found to be smooth with visual observation.

➤ **Phase separation-** The cream is kept in a closed container at 25-30°C not exposed to light. Phase separation was observed regularly for 30 days, no any kind of phase separation is observed.

#### 2. pH-

0.5 g cream was taken and dispersed in 50 ml distilled water and then pH was measured by using digital pH meter.



**Fig 1.10: Digital pH meter**

**3. Spreadability-** It is used to identify the extend of spreadability by cream on the skin. A small quantity of sample was placed on glass slide and another slide was placed above them;100g of weight was placed on the slide. The time taken for the cream to get spread on the slide was noted and measured which was found to be 10 cm in 60 sec.

$$S = m \times l/t$$

S= Spreadability

m=Weight placed on slide

l= Length of the glass slide

t=Time taken in seconds



**Fig 1.11: Spreadability of Cream**

**4. Homogeneity-**

The formulation was tested for the homogeneity by visual appearance and by touch.

### 5. Stability-

The study was carried out for 2 months and no change was noticed. The stability studies were carried out by storing at different temperature conditions like 20°C, 25°C , 40°C for 2 month.

### 6. Viscosity:

Viscosity of the formulation was determined by Brookfield viscometer at 100rpm and the result was found to be 4480Cp (27.0C).



Fig 1.12: Viscosity of cream

### 7. Irritability-

Small amount of cream was applied on skin and kept for few minutes and found to be non irritable.

SR.NO	Parameters	Result
1.	Colour	Light blue
2.	Odour	Characteristic
3.	pH	7
4.	Viscosity	4480cP (27.0 C)
5.	Irritability	Non-irritant
6.	Spreadability	10g.cm /sec

7.	Consistency	Consistent
8.	Stability	Observed for 30 days (Stable)
9.	Homogeneity	Homogenous
10.	Texture	Smooth
11.	Phase separation	No phase separation

**Table 1.2: Parameters & Result**

#### 8. ZONE OF INHIBITION:

It was determined by minimum inhibitory concentration using agar well diffusion method. Agar well diffusion method was used to evaluate antibacterial activity of *Clitoria ternatea* flower extract. To prepare nutrient agar about 5 gm nutrient agar was added to 100 ml of distilled water, pH was adjusted at 7.0 and was autoclaved. It was allowed to cool upto 45°C. About 60 ml of seeded nutrient agar seeded with microorganism was poured in each of two Petri plates and allowed to solidify. Wells were bored into the agar using a sterile 6mm diameter cork borer. *Cleome viscosa* linn extract at different concentration were added into the well, allowed to stand at room temperature for about 2 hour and incubated at 37°C. Standard were set in parallel. Zones of inhibition was determined after 24 hours. The effect were compared with that of standard.



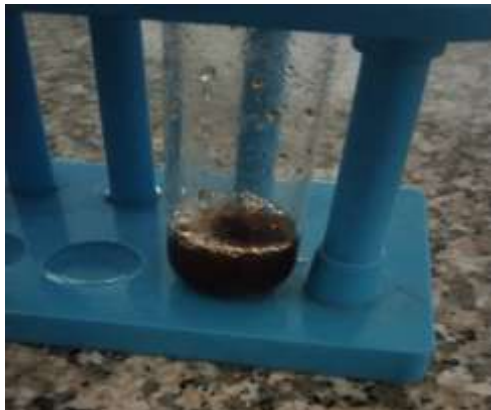





**Fig1.13: Zone of Inhibition of S. aureus and E. coli**


Compound	S.Aureus	E.coli
10µg/ml	2mm	01mm
20µg/ml	5mm	04mm
40µg/ml	6mm	05mm
80µg/ml	8mm	09mm
Ciprofloxacin	28mm	22mm

**Table 1.3: Zone of Inhibition of S.aureus and E.coli**

### 9. Phytochemical Analysis of Clitoria Ternatea Linn. :-

SR.NO	PHYTOCHEMICAL TEST	OBSERVATION	RESULT
1.	<b>Alkaloids:</b> Few ml extract +add a drop of Mayer's reagent by side of test tube.	White creamy precipitate	
2.	<b>Amino acid:</b> 1ml of extract + few drops of Ninhydrin reagent.	Purple colour	
3.	<b>Steroids:</b> 1ml extract+ dissolve 10ml of chloroform + 10ml conc. sulphuric acid.	Yellow + green fluorescene	No change
4.	<b>Cardiac glycosides:</b> Extract in glacial acetic acid + add few drops of Fecl3 + conc. Sulphuric acid.	Brown colour	

5.	<b>Flavonoids:</b> 1ml extract + few drops of dil.NaOH + add dilute acid.	Yellow to colourless	No change
6.	<b>Tanin:</b> 5ml extract + few drops of 1% lead acetate	Yellow precipitate	
7.	<b>Phenol:</b> 2ml extract + 2ml of Fecl3	Green colour	
8.	<b>Terpenoids:</b> 5ml extract + 2ml chloroform + 3ml conc. Sulphuric acid	Reddish brown colour	

9.	<b>Reducing sugar:</b> 1ml extract + 5-8 drop of fehling's reagent and boiled it.	Brick red colour precipitate	
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## DISCUSSION:

The herbal cream was prepared and evaluated. the herbal vaginal cream was formulated in the laboratory and found to be compared with various parameters such as appearance, pH, and viscosity, spreadability, irritability and found to be satisfied with all required characterization. Thus, the developed formulation can be used as an effective cream for using it to treat the vaginal infection. It contained aqueous extract of *Clitoria Ternatea* Linn which shows antifungal activity, aqueous extract of *Asparagus racemosus* showing antioxidant, antibacterial activity and improves skin elasticity. Other ingredients like Bees wax (emollient), Borax as an emulsifying agent and stabilizer , propyl paraben as a preservative , coconut oil which reduces inflammation and moisturizes skin.

After using cream skin was smooth with no irritancy. Herbal cosmetics showed lesser or no side effects, hence use of herbal cosmetic is increased.

## CONCLUSION:

Extract of *Clitoria Ternatea* Linn. (Aparajita Flowers) and *Asparagus racemosus* (Shatavari) was choosen for this study based on various journals and reports indicating the antimicrobial potential which is very important for treating Vaginal infection. The pH of the prepared herbal cream was nearer to the skin pH, Also the cream was safe in respect to the skin irritation and allergic sensitization. After application of cream, skin becomes soft and is non-irritant.

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