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Formulation and evaluation of antimicrobial gel from methanolic leaf extract of *Clitoria ternatea L.*

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ABSTRACT

Natural medication has turned into a item of worldwide significance both therapeutic and economical. Herbal remedies are getting expanding patient compliance as they are devoid of typical side effects which are related to allopathic medicines. The main objectives of present research is to formulate and evaluate the herbal gels containing *Clitoria ternatea L*. This formulation formulated aiming less side effect and good antimicrobial effect using methanolic leaves extract of *C. ternatea L*. The plant *C. ternatea* belong to the family Fabaceae. The formulation was prepared using Carbapol 940, *Clitoria ternatea* methanolic leaf extract, propylene glycol, methyl paraben, propyl paraben, glycerine and required amount of distilled water. The prepared formulation was evaluated by different parameters like colour, homogeneity, pH, viscosity, spreadability. The gels were evaluated for antimicrobial efficiency by agar diffusion method. The herbal gels showed that formulations containing *C ternatea* methanolic leaves extract have better antimicrobial activity.

Keywords: Antimicrobial activity, *Clitoria ternatea L,* Fabaceae, spreadability, homogeneity, gel.

INTRODUCTION

Since ancient times, herbal or plant-based medicines have served as a platform for the prevention and cure of diseases and to date many more constituents of these natural sources are yet to be explored. This has enlightened scientists to find out newer compounds from the herbal source to treat many infectious diseases. Reports show that most of the medicinal plants possess antimicrobial, antioxidant, and anti-inflammatory properties, which have paved a way in the prevention of many infectious diseases, and also have potential benefits for the society. The present scenario of infectious diseases shows that there has been an alarming increase in the incidence of new and re-emerging infectious diseases. Another important concern is the development of resistance to the antibiotic in clinical use. Hence, there is a pressing need to develop a natural formulation, which can act against the microorganisms causing skin diseases(1).

A large and increasing number of patients in the world use medicinal plants and herbs for health purpose.

Therefore, scientific scrutiny of their therapeutic potential, biological properties, and safety will be useful in making wise decisions about their use. There are hundreds of significant drugs and biologically active compounds developed from the traditional medicinal plants. Plant showed wide range of pharmacological activities including antimicrobial, antioxidant, anticancer, hypolipidemic, cardiovascular, central nervous, respiratory, immunological, anti-inflammatory, analgesic antipyretic and many other pharmacological effects. The preliminary phytochemical screening showed that Clitoria ternatea contained tannins, phlobatannin, carbohydrates, saponins, triterpenoids, phenols, flavanoids, flavonol glycosides, proteins, alkaloids, antharaquinone, anthocyanins, cardiac glycosides, Stigmast-4-ene-3,6-dione, volatile oils and steroids. The plant showed many pharmacological effects including antioxidant, hypolipidemic, anticancer, anti-inflammatory, analgesic, antipyretic, antidiabetic, CNS, antimicrobial, gastro-intestinal antiparasitic, insecticidal and many other pharmacological effects(2).

Clitoria ternatea Linn. (Fabaceae) known as “Aparajita” and “Butterfly pea”, is widely used as substitute of “Shankhapushpi”. It consists of “conch” or “shankh” shaped flowers, categorized under Ayurvedic Medhya Rasayna drug that claim as brain tonic and have memory and intelligence enhancing properties. It is vigorous, strongly, persistent, herbaceous, perennial, climber, conspicuous, blue flowers legume. The plant is native to south-east Asia and distributed in tropical Asia including India. Almost all parts of the plant are reported to have medicinal properties widely used in the Ayurvedic system of medicine. Traditionally, it is recommended for the treatment of fever, rheumatism, syphilis, skin diseases, arthritis, eye and ear diseases, snake bite, scorpion sting, chronic bronchitis, indigestion, constipation in India, epilepsy, mental problems, insanity for muscular strength, etc.

Clitoria genus is inconsequential, indigenous climber and a common garden flower found throughout the tropical and subtropical regions of the world. Now the genus becomes rare in humid and sub-humid lands of Asia, America, and Africa and also in semi-arid tropical Australia. It grows from sea level to 1800 and also grown as an ornamental in the warmer parts of the world and outspread from about 20°North latitude to the Salta district in Argentina at about 24°South latitude. In Africa it grows in grasslands, often on seasonally-waterlogged black clays and in old cultivations whereas in Sudan it is grown for fodder or grazing and in Kenya it is grown in a mixture with Chloris gayana. In America, the species of this plant is spread from Florida to Texas and from New Jersey to Kentucky & Arkansas. It is commonly found in Jamaica, Puerto Rico, Turks, and Caicos Islands etc. It is found in all over India, especially in southern India up to an altitude of 1,500 m and in the Andaman Islands.

Literatiure review of *Clitoria ternatea*:

Plant profile:

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

Vernacular name:

Language	Name
Arabic -	Mazerion Hidi, Baslat el-Zuhoor
Bengali -	Aparajita
Chinese -	Die dou
English -	Blue-pea, bluebellvine, butterfly-pea, cordofan-pea, Darwin-pea
French -	Honte
German -	Blaue Klitorie
Hindi -	Aparajita
Sanskrit -	Girikarnika, Vishnukranta
Spanish -	Conchitas papito, azulejo, zapatico de la reina, zapotillo
Tamil -	Kakkanam
Telugu -	Dintena

Plant type: Perinnial herbaceous plant

Origin: Latin America or Asia

Habit: Climbing or trailing vine becoming woody with age to 10m in length, sparsely pubescent throughout.

Taxanomical classification-

- ✓ Kingdom- Plantae
- ✓ Order- Fabales
- ✓ Family- Fabaceae
- ✓ Tribe- Phaseoleae
- ✓ Subtribe- clitoriinae
- ✓ Genus-clitoria

Plant Description:

Perennial climbing or trailing herb, growing from a woody rootstock. Leaves imparipinnate with 2-4 pairs of leaflets and a terminal leaflet. Leaflets ovate to elliptic-oblong, up to 6.5 × 4 cm, mostly hairless above, pubescent below. Flowers axillary, solitary or 2 together, resupinate, large and showy, bright blue. Pod linear, oblong, 6-13 cm long, flattened, mucronate at the apex, hairless or finely pubescent(3). It is growing wild and also in gardens, bearing conspicuous blue or white flowers resembling a conch-shell. While presumably of American origin, today it is cultivated and naturalized throughout the humid tropics of the old and new world below 1600 m elevation. It

is distributed in India, the Philippines, other tropical Asian countries, South and Central America, the Caribbean and Madagascar (4).

Phytochemical review of Clitoria ternatea:

Butterfly pea yields up to 30 tons dry matter per hectare per year in favorable conditions. Plant can be exploited as a source of calcium in herbal drink due to its high calcium concentration. It contains antifungal proteins.

Leaf: The content of crude fiber and protein in the leaves were 21.5% and 21.5-29% respectively. From leaves, clitorin and kaempferol have been isolated. The leaves also contain 3-monoglucoside, 3-rutinoside, 3-neohesperidoside, 3-o-rhamnosyl-glucoside, 3-o-rhamnosylgalactoside of kaemferol, besides kaemferol-3-o-rhamnosylo- rhamnosyl glucoside. It also contains aparajitin and β- sitosterol . The flowers (blue in color) contain delphinidin-3,5- diglucoside, delphinidin-3β-glucoside, and its 3 methyl derivative, malvidin-3β-glucoside, kaemferol and cynidin chloride. A lactone- aparajitin from leaves (5).

Root: Taxaxerol and taxaxerone are present in the roots of plant. The bark of roots contains sresin, tannin, starchand flavonol glycosides. The root nodule contains glycine, alanine, valine, lecine, α-aminobutyric acid, aspartic acid, glutamic acid, arginine, ornithine, histadine, γ-aminobutyric acid(6).

Seed: The seed contains bitter acid resin as an active principle with fixed oil, tannic acid and glucose, also contains a cotyledon, which is full of granular starch and bitter in taste. There are two chemicals which are isolated from seeds viz. Sitosterol and anthoxanthin. Other than that seed-oil yields palmitic, stearic, oleic, linoleic and linolenic acids. Oils from blue and white-flower varieties have been found to have almost similar composition. Seeds also contain cinnamc acid, hexacosanol, nucleoprotein with its amino acid sequence somewhat similar to insulin(7). The seeds are very high in protein content (15-25%). The seeds contains p-hydroxycinnamic acid, flavonol-3-glycoside, ethyl-α-D-galactopyranoside, adenosine, 3,5,7,4-tetrahydroxyflavone-3-rhamnoglucoside, polypeptide, hand exacosanol. Oligosaccharides or flatulene are also present in seeds. A food dye, delphinidin 3,3',5'- triglucoside also reported in seeds. Lecin represents about 2.8% of the total extractable 11 protein from seed meal or 30 mg of lectin/30 g of C. ternatea seeds in contrast 9 mg fetuin/30 g of seeds. Tryptophan and tyrosine is also reported in the seeds.

Flower: Two acyl moieties were determined as E-4-0-β-D-glucopyranosyl-p-coumaric acid and 6—0-malonyl-D-glucopyranose. Other six ternatins A1, A2, B1, B2, D1 and D2 in C. ternatea flower ware isolated by reverse phase HPLC. The white flower yield only kaeferol. From the petals of Clitoria ternatea L. some flavonol glycosides isolated are kaempferol 3-O-(200-O-a-rhamnosyl-600- O-malonyl)-b-glucoside; quercetin 3-O-(200-O-arhamnosyl- 600 -O- malonyl) -b- glucoside; myricetin 3 -2G -rhamnosylrutinoside; quercetin 3-2G-rhamnosylrutinoside 4. Flower also contains kaempferol 3-2G-rhamnosylrutinoside; kaempferol 3-neohesperidoside; quercetin 3-neohesperidoside; myricetin 3-neohesperidoside; kaempferol 3-rutinoside; quercetin 3-rutinoside; myricetin 3-rutinoside; kaempferol 3- glucoside; quercetin 3- glucoside; myricetin 3-glucoside. Cyanine chloride and kaempferol are isolated and identified from the flowers. Isolation of Six acylated anthocyanins A, B, C, D, E and F from blue flowers has been done with the partial characterization of kaempferol

and its 3- glucoside, robinin, quercetin and 3-glucoside and ternatins A and B (8). Blue flower of butterfly pea also contain lobelinins, which has the 3,5,3',5'-tetraglucoside substituted pattern. Deacylternatin is also reported in the blue flower petals. Various types of C. ternatea lines with different petal have been investigated for flavonoids. The newly isolated glucoside from the petals of mauve line is Delphinidin 3-O-(2″-O-α-rhamnosyl-6″-O-malonyl)-β-glucoside. Also, a group of ternatins identified from the entire blue petal lines i.e. 15 (poly) acylated delphinidin. The white petal lines do not contain anthocyanins.While ternatins are identified in all blue petal lines as 3′,5′-disubstituted polyacylanthocyanins, the mauve petal line accumulated delphinidin 3-O-(6″-O-myl)-βglucoside instead. Researchers found that the difference in flower color from blue to mauve is due to the lack of (polyacylated) glucosyl group substitutions at both the 3′- and 5′-positions of ternatins but not due to a change in the structure of an anthocyanidin from delphinidin(9).

Aim and Objective:

Aim:

- ✓ To formulate herbal antimicrobial gel using *C. ternatea* leaf extract that aiming less side effects and better antimicrobial effect.

Objective:

- ✓ Formulation of antimicrobial gel by using methanolic leaves extract.
- ✓ To evaluate effect of C. *ternatea methanolic* leaf extract on gram +ve and gram –ve bacteria.
- ✓ Evaluation of prepared antimicrobial gel for spreadability, pH, viscosity, antimicrobial activity.

Plan of work:

The research project was carried out in the following systematic scheme:

1. Collection and Authentication of plant
2. Extraction methodology: Continuous Soxhlet Extraction using ethanol as a solvent
3. Pharmacognostic evaluation:
   - 3.1. Microscopy of leaf
   - 3.2. Powder characteristics of leaf
   - 3.3. Physicochemical study
4. Phytochemical evaluation
5. Formulation and evaluation of herbal gel formulation
6. Pharmacological evaluation
   - 6.1. Antimicrobial activity

MATERIAL AND METHODS:

Collection of plant material:

The plant was collected from the locally growing area, from Akola district, Maharashtra, during the month of December. It was then botanically authenticated by Dr. Darshan Kokate, Department of Botany, K. S.K.W. College, Cidco, Nasik. The leaves were separated, dried, coarsely powdered, passed through sieve No. 40, and stored in a closed container for further use(10).

Extraction procedure:

The leaves of *Clitoria ternatea* were dried under shade, powdered and sieved through sieve No.40 and stored in air tight containers. The coarse powder of the leaves was extracted by a soxhlet extraction method. The required quantity of dried powder was taken in a round-bottomed flask and refluxed with methanol to obtain methanolic leaf extract of Clitoria ternatea. The resulting solvent was then removed under reduced pressure, and thus formed a semisolid, which was vacuum dried using a rotary flash evaporator, to get a solid residue which was the methanolic extract of *C. ternatea*. The dried extract thus obtained was used for the formulation of antimicrobial gel(11).

Phytochemical study:

It comprised of tests for the presence of Alkaloids, Tannins, Glycosides, Resins, Steroids, Saponins, Flavonoids and Phenols.

Test for Alkaloids

About 0.5 gm of methanol extract was taken in a test tube and was diluted and homogenized with 10 ml distilled water, dissolved in 20 ml dilute HCl solution and clarified by filtration. The filtrate was tested with Dragendroff's and Mayer's reagent. The treated solution was observed for precipitation of white or creamy colour.

Test for Tannins:

About 2 ml of extract was boiled with 1 ml of 1% aqueous HCl acid was taken and observed for the red precipitation which showed that presence of tannins.

Test for Glycosides:

About 0.5 gm of methanol extract was taken in a test tube and 1 ml glacial acetic acid containing traces of ferric chloride was added to it. To this solution, 1 ml concentrated sulphuric acid was added and observed for the formation of reddish brown colour at the junction of the two layers and the upper layer turned bluish green in the presence of glycosides.

Test for Resins:

For the tests concerning the presence of Resins, 0.5 gm of methanol extract was taken in a test tube and 5 ml of distilled water was added to it and observed for turbidity which indicates the presence of Resins.

Test for Steroids:

About 0.5 gm of methanol extract was taken in a test tube and 2 ml of acetic anhydride was added to it and 2 ml of sulphuric acid was added by the sides of the test tube and observed for the colour change to violet or blue green.

Test for Saponins:

About 0.5 gm of methanol extract was taken in a test tube and 5 ml distilled water was added to it. The solution was shaken vigorously and observed for persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Test for Flavonoids:

About 0.5 gm of extract was introduced into 10 ml of ethyl acetate in a test tube and heated in boiling water for 1 min. The mixture was then filtered. About 4 ml of the filtrate was shaken with 1 ml 1% aluminium chloride solution and incubated for 10 min. Formation of yellow colour in the presence of 1 ml dilute ammonia solution indicated the presence of flavonoids.

Test for Phenols:

About 0.5 gm of extract was taken in a test tube, mixed with 100ml distilled water and heated gently. To this, 2 ml of ferric chloride solution was added and observed for the formation of green or blue colour(12).

DETERMINATION OF PHYSICAL CONTENT

1. Loss on Drying:

LOD is the loss of mass expressed as percentage w/w and can be determine by following procedure.

Procedure-

Weighted a glass stopper, shallow weighing bottle that has been dried for 30 min. powdered twigs sample was kept in the bottle and weighted accurately. Sample was distributed evenly by gentle sidewise shaking. Placed the bottle into the oven. Sample was dried to constant weight. After drying is completed, the bottle was allowed to come to room temperature in desiccators before weighing. Difference in initial weight and final weight gave LOD.

2. Ash value:

The ash of any organic material is composed of their non-volatile inorganic compound.

Total ash:

Procedure-

Place about 2-4 gm of ground air dried material accurately weighed in a °C until it is white indicating the absence of carbon. Cool in a desiccator and the residue with about 2ml of water or a saturated solution of ammonium nitrate. Dry on a water bath then on hot plate and ignite to a constant weight. Allow the residue to cool in suitable desiccators for 30 min, and then weigh without delay. Calculate the content of total ash in mg/g of the dried material.

A sample of 1gm was weighted and air dried in a tared silica dish. It was incinerated at a temperature not exceeding 450 °C until it was free from carbon, cooled and the ash was weighted and percentage of ash was calculated.

Water soluble ash:

Procedure-

The ash was obtained as per the method described above for total ash. The ash obtained was boiled for 5 minutes with 25 ml of water. Filtered and the insoluble matter was collected in a Grouch crucible, washed with hot water and ignited for 15 minutes at a temperature not exceeding 450° C. The weight of the insoluble matter was subtracted from the weight of the ash. The difference in weight represented the water- soluble ash. Percentage of water soluble ash was calculated with the air-dried drug.

Acid insoluble ash:

Procedure-

The ash was obtained as per the method described above for total ash. The ash obtained was boiled with 25 ml 2M hydrochloric acid for 5 minutes. Filtered and the insoluble matter was collected in Gooch crucible, washed with hot water, ignited, cooled, in a desiccator and weighed. The percentage of acid insoluble ash was calculated with reference to the air-dried drug.

3. Extractive value determination

This method determines the number of active constituents in given amount of medicinal plant material when extract with solvents. The extraction of any crude drug with a particular solvent yields a solution containing different phytoconstituents. The composition of these Phytoconstituents in that particular solvent yields a solution containing different phytoconstituents. The composition of these Phytoconstituents in that particular solvent depends upon the nature of the nature of the drug and solvent used.

Alcohol soluble extractive value-

Accurately weight 5gm of air-dried crude drug was taken in closed flask and macerated with 100ml of 95% ethanol for 24hrs. It was shaken frequently during the first 6hrs and allowed to stand for 18hrs and filter rapidly taking precaution against loss of ethanol. 25ml of the filtrate was taken and evaporate to dryness in tarred flat bottom shallow dish dry 105C and weighed. The percentage of ethanol soluble extractive value was calculated with reference to air dried drug.

Water soluble extractive value-

Accurately weight 5gm of air-dried crude drug was taken in closed flask and macerated with 100ml of distilled water for 24 hrs. It was shaken frequently during the first 6 hrs and allowed to stand for 18 hrs and filter rapidly taking precaution against loss of distilled water. 25ml of the filtrate was taken and evaporate to dryness in tarred flat bottom shallow dish dry 105 °C and weighed. The percentage of water soluble extractive value was calculated with reference to air dried drug(13).

4. Determination of tapped density (ρT):

This parameter is useful to calculate the compressibility index and Hausner Ratio of powder. It is determined by placing a graduated cylinder containing a known mass of drug or formulation on a mechanical tapper apparatus,

which is operated for fixed number of taps (~1000) until the powder bed volume has reached a minimum. It is denoted as g/ml.

Tapped density (ρT) = Mass of powder (M)/ Tapped volume (V_t)

5. Determination of bulk density (ρB):

This parameter gives an understanding of powder flowability and compressibility. It is determined by pouring presieved (40 mesh) bulk into a graduated cylinder via a large funnel and measuring the volume and weight(15).

Bulk density (ρB) = Mass of powder (M)/ Bulk volume (V_b)

6. Determination of angle of repose (θ):

A funnel is secured with its tip at a given height H, above graph paper that is placed on a flat horizontal surface. Powder or granulation is carefully poured through the funnel until the apex of the conical pile just touches the top of the funnel. The diameter of the base of conical pile is then determined to calculate the angle of repose(16).

Angle of repose (θ) = $\tan^{-1}$ (h/r)

7. Hausner ratio:

Depending on the material, the compressibility index can be determined using V_{10} instead of V_0. If V_{10} is used, it is clearly stated in the results(17).

Hausner ratio = Tapped density/ Bulk density

8. Carr's index:

The Carr's index (Carr's index or Carr's Compressibility Index) is an indication of the compressibility of a powder. It is named after the scientist Ralph J. Carr, Jr. The Carr index is calculated by the formula,

Carr's index =100[ρT – ρB/ρB]

Where ρB is the freely settled bulk density of the powder, and ρT is the tapped bulk density of the powder after "tapping down".

Formulation of Herbal gel formulations

For the preparation of gel formulation, firstly take carbopol 940 which was then dispersed in distilled water then methyl paraben, propyl paraben and glycerine were added and kept for overnight. Take the leaf extract of *Clitoris ternatea* in propylene glycol which was then added in polymer dispersion. Remaining quantity of water was then added and neutralized to pH 7 with triethanolamine by constant stirring(18).

Table 1: **Formulation of gel base**

Ingredients	Quantity
Carbopol 934 (%)	1
Extract (%w/w)	2.5
Methyl paraben (0.5%) (ml)	0.2
Propyl paraben (0.2%)(ml)	5
Triethanolamine (ml)	0.5
Ethanol (ml)	3
Purified water	Q.S

Evaluation of Herbal gels:

Physical evaluation:

The herbal gel was evaluated for colour, consistency and odour. They appear light greenish yellow and opaque, free from grittiness and homogenously dispersed

pH:

The pH of all the formulated herbal gels was measured by using pH paper.

Viscosity:

Viscosity of herbal gels was determined by using Brookfield rotational viscometer at 100 rpm using spindle no.64.

Homogeneity:

Inhabitancy of any aggregates in the gel formulations was visually inspected and the homogeneity was approved.

Spreadability:

The spreadability of gel formulations was determined by measuring the spreading diameter of 1g of gel between two horizontal plates(11).

Grittiness:

All the formulations were evaluated microscopically for the presence of any appreciable particulate matter which was seen under light microscope. Hence, obviously the gel preparation fulfils the requirement of freedom from particulate matter and form grittiness as desired for any topical preparation.

Antibacterial activity:

The antibacterial screening of herbal gels was done by disc diffusion method. The gels were tested against bacterial agents namely S. aureus and E. coli. Nutrient agar media was sterilized and poured into petri plates. After solidification, 0.1ml of the inoculum was spread over the agar evenly using a rod. 6mm diameter cavity was prepared and formulated gel is placed in the cavity. A standard antibiotic was used as the control. The inoculated plates are incubated for 24 hours. Later, the zone of inhibition around the disc was measured and recorded(19).

Results and discussion:

Physicochemical study

The physicochemical properties such as ash values, extractive values, loss on drying, tapped density, bulk density, etc were determined and given in Table

Table 1: Physicochemical study

Sr. No.	Parameter	Value
1	Total ash	16%
2	Acid in soluble ash	1.5%
3	Water soluble ash	5%
4	Loss on drying	9%
5	Alcohol Soluble extractive value	8.9%
6	Water soluble extractive value	25.6%
7	Tapped density	0.26 g/ml
8	Bulk density	0.18 g/ml
9	Angle of Repose	17.59°

10	Hausner ratio	1.44
11	Carr's index	30.76 %

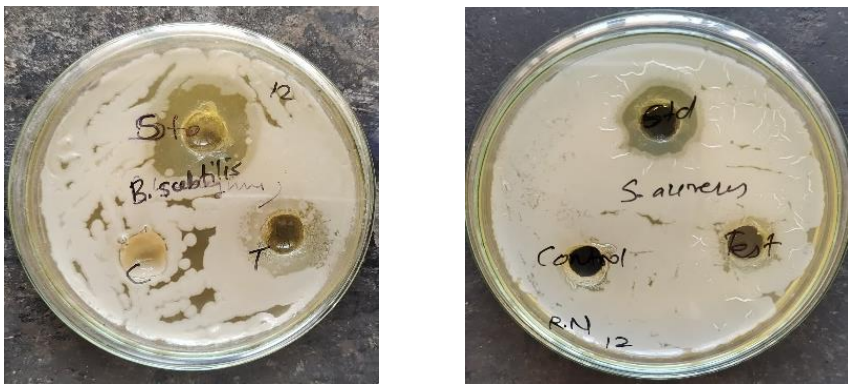
Preliminary Phytochemical Study

The phytochemical analysis of the methanolic extract was performed and the results have been given in (Table 2). The preliminary phytochemical screening of extracts revealed the presence of phytoconstituents like alkaloid, tannins, steroids, flavonoids, carbohydrates, reducing sugars, monosaccharide, cardiac glycosides, anthraquinone glycosides, etc.

Sr. No	Extract	Methanol
1	Alkaloids	+
2	Flavonoids	+
3	Saponins	+
4	Steroids	+
5.	Glycosides	+
6	Terpenoids	+
7	Tannins	+
8	Phenols	+
9	Resins	+
10	Protein	+

1.1 Pharmacological study:

1.1.1 Antimicrobial activity



On *B. subtilis* On *S. aureus*

Figure 6: Antimicrobial zone of inhibition by Agar Diffusion method

Table 3: Antimicrobial activity of *Trachyspermum ammi* leaves

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

Conclusion:

The qualitative analysis of Clitoria ternatea shows the presence of bioactive compounds such as Alkaloids, Tannins, Glycosides, Resins, Steroids, Saponins, Flavonoids and Phenols. From the present investigation, it has been revealed that herbal gels of plant *Clitoria ternatea* leaves extract can be formulated using carbopol 940 as polymer with other ingredients and the evaluation of physical parameters shown satisfactory results. From the antibacterial activity it was found that prepared herbal gels of *Clitoria ternatea* leaves extract were significantly active against tested pathogens which was comparable with standard antibiotic. Hence, from the overall results, finally it was concluded that the formulated herbal gels have significant antimicrobial properties and hence will be better, safe and effective than allopathic medications

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Annexure:

Errata: