Formulation And Evaluation of Herbal Hand Sanitizer by Using Ethanolic Flower Extract of *Clitoria Ternatea*

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***Abstract* - Hand hygiene is essential for preventing the spread of infectious microorganisms. Conventional hand sanitizers mainly contain alcohol and synthetic antimicrobial agents, which may cause skin irritation with repeated use. Herbal formulations are considered safer alternatives due to their natural bioactive compounds. The present study aimed to formulate and evaluate a herbal hand sanitizer using ethanolic flower extract of *Clitoria ternatea*. The plant extract was prepared using Soxhlet extraction with ethanol. The sanitizer was formulated using ethanol, glycerine, perfume, distilled water, and the plant extract. Preliminary phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins, and steroids. Antimicrobial activity was evaluated using agar well diffusion method and minimum inhibitory concentration against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. The formulation was also evaluated for organoleptic properties, pH, viscosity, and skin irritation. Results showed good antimicrobial activity and acceptable physicochemical properties. The herbal sanitizer was safe, stable and effective for microbial control.**

Keywords: **Antimicrobial activity, *Clitoria ternatea*, Ethanolic extract, Herbal hand sanitizer, Time kill assay.**

I. INTRODUCTION

Hand hygiene plays an important role in preventing infections caused by microorganisms. Microbes present on the hands can easily spread diseases through direct or indirect contact. Hand sanitizers are widely used as disinfecting agents because they reduce microbial contamination quickly and effectively. Most commercial sanitizers contain chemials like hydrogen peroxide which may cause dryness and irritation on the skin when used frequently[1,2].

Herbal products have gained attention because they are safer and contain natural bioactive compounds with antimicrobial activity. *Clitoria ternatea*, commonly known as butterfly pea, is a medicinal plant belonging to the Fabaceae family. The plant contains several phytochemicals such as flavonoids, alkaloids, tannins, anthocyanins and phenolic compounds. These compounds exhibit antimicrobial, antioxidant, anti-inflammator and therapeutic properties.

The flower extract of *Clitoria ternatea* has been reported to possess significant antimicrobial activity against various bacterial and fungal strains. Due to these properties, the plant can be used as a natural antimicrobial agent in disinfectant formulations[3].

The present study focuses on the formulation and evaluation of an alcohol-based herbal hand sanitizer containing ethanolic flower extract of *Clitoria ternatea*. The objective of the study is to develop an effective and safe herbal disinfectant with antimicrobial activity against pathogenic microorganisms.

II. MATERIALS AND METHODS

Extraction of Plant Material

The ethanolic extract of *Clitoria ternatea* flowers was obtained using the Soxhlet extraction method.

Procedure:

Fresh or dried flowers were crushed using a mortar and pestle to increase surface area. Approximately 14 g of the powdered sample was placed in a cellulose extraction thimble. The thimble was inserted into the Soxhlet extractor. Ethanol was added to a round-bottom flask attached to the Soxhlet apparatus. The solvent was heated using an isomantle to allow evaporation.Vapours passed through the condenser and condensed solvent collected in the extraction chamber.The solvent siphoned back into the flask once the chamber was filled, repeating the extraction cycle.The extraction process was carried out for approximately 4 hours.The obtained extract was collected and concentrated for further analysis.

Preliminary Phytochemical Screening
The ethanolic extract was subjected to qualitative phytochemical tests to identify major secondary metabolites[4].
Antimicrobial Activity
Agar Well Diffusion Method
Principle:
The antimicrobial agents present in the sample diffuse into agar medium inoculated with microorganisms. The inhibition of microbial growth produces a clear zone around the well.
Agar-well diffusion method procedure:

a. Nutrient Agar Medium
The medium was prepared by dissolving 2.8 g of the commercially available Nutrient Agar Medium (HiMedia) in 100ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petri plates (25-30ml/plate) while still molten.

b. Nutrient broth
Nutrient broth was prepared by dissolving 2.8 g of commercially available nutrient medium (HiMedia) in 100ml distilled water and boiling to dissolve the medium completely. The medium was dispensed as desired and sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 minutes.

Procedure:
Petriplates containing 20ml nutrient agar medium were seeded with 24hr culture of bacterial strains were adjusted to 0.5 Optical Density value according to McFarland standard. Wells were cut and concentration of test sample CT (500, 250, 100 and 50 µg/ml) was added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the wells. Gentamicin was used as a positive control[5,6].

Antifungal activity:
Principle:
The anti-fungal agent present in the given sample was allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular, as there will be a confluent lawn of growth. The diameter of the zone of inhibition can be measured in millimetres.

Disc diffusion method:
Potato Dextrose Agar Medium. The potato dextrose agar medium was prepared by dissolving 20 gm of potato infusion, 2 gm of dextrose and 1.5 gm of agar in 100ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto100mm petriplates (25-30 ml/plate) while still molten.

Procedure:
Petri plates containing 20ml potato dextrose agar medium were seeded with 72 hr culture of fungal strain (*Candida albicans*), with different concentrations of sample CT (500, 250, 100 and 50 µg/ml)was added. The plates were then incubated at 28°C for 72 hours. The anti-fungal activity was assayed by measuring the diameter of the inhibition zone formed around the wells. Amphotericin B was used as a positive control[7].

Minimum Inhibitory Concentration:
MIC values are used to determine susceptibilities of bacteria to drugs and also to evaluate the activity of new antimicrobial agent.

Procedure:
The broth microdilution assay method was used to determine the MICs for the susceptibility of *Escherichia coli* in sterile disposable flat-bottomed 96-well microtiter plates. Briefly, the 0.5 McFarland inoculum suspensions were further diluted 1:100 in nutrient broth before inoculation. 50µL of the bacterial suspension was seeded into a 96- well plate containing 50 µL of test sample with serial concentrations. The final inoculum of the bacteria was approximately5×105CFU/mL. The final concentrations of CT were 500, 250, 125, 62.5, 31.25, 15.6, 7.8, 3.9, 1.9 and 0.9 µg/mL. The plates were then incubated at 37 °C for 18–24 h. The results were read at 600 nm using a microplate reader. Changes of colour were observed and recorded. All the tests were done in triplicates[8,9].

Formulation of disinfectant:
Measure ethanol using a small graduated cylinder and pour it into a clean 100 mL glass beaker. Add 5ml of *Clitoria ternatea* flower extract to the ethanol using a measuring cylinder or micropipette (depending on volume). Add glycerol using a measuring cylinder. Since glycerol is thick and sticky, rinse the cylinder with a small amount of

sterile distilled water and add the rinsing back into the beaker to ensure the full amount is transferred.Add sterile distilled water to the mixture until the total volume reaches 100mL. Cover the beaker immediately with a lid to prevent ethanol evaporation. Mix thoroughly using a sterile glass rod to ensure uniform blending. Transfer the final 100 mL solution into a clean, labelled storage bottle (e.g., a 100 mL plastic or glass container) and close it tightly.

Ingredients of hand sanitizer:

Table 1: Ingredients of hand sanitizer

S.NO.	Materials Required	For 100ml
1	Ethanol	72.9 ml
2	*Clitoria ternatea* flower extract	1 ml
3	Glycerine	1.5 ml
4	Perfume	1 ml
5	Distilled water	q.s

Evaluation of hand sanitizer:

1) *Organoleptic Evaluation*

Appearance:

The sanitizer formulation was examined to determine whether it was clear, free from sediment or cloudiness, and possessed a uniform texture (gel or liquid form).

Odour:

The fragrance of the sanitizer was evaluated to ensure that it was pleasant and not overpowering. The formulation was also checked to confirm the absence of any unpleasant or strong chemical odours.

2) Evaluation of pH

pH paper was taken.The pH paper was dipped into the sanitizer solution.The colour change of the pH paper was observed.The resulting colour was compared with the standard colour chart.The corresponding pH value was recorded.

3) Viscosity

The Ostwald viscometer was cleaned using warm chromic acid solution. The viscometer was mounted vertically on a suitable stand.Distilled water was filled into the dry viscometer up to mark G.The time required for water to flow from mark A to mark B was recorded in seconds. The measurement was repeated at least three times to obtain an accurate reading. The viscometer was rinsed with the test liquid and then filled up to mark A. The time required for the test liquid to flow between the marks was recorded. The density of the liquid was determined as mentioned in the experimental procedure.

4) Skin Irritation Test

A 2 × 2 cm area was marked on the inner forearm or behind the ear.A small quantity of the sanitizer was applied evenly over the marked area. The formulation was allowed to remain on the skin for 24 hours. The test area was not washed or rubbed during the observation period. The site was then examined for any signs of irritation such as redness, itching, burning sensation, swelling, or rash.

5) Visual Test

During visual inspection, the ingredients and the final product were carefully examined to ensure purity and acceptable appearance. The formulation was evaluated for clarity, uniformity and overall aesthetic quality. Since patient adherence and compliance are important, the product was also assessed to ensure that it exhibited good visual appeal and elegance in appearance[10].

6) Time kill Assay

Time Kill Assay is carried out to evaluate an antimicrobial test material and assess the *Invitro* reduction of a microbial population of test organisms after exposure to a test material.

Procedure:

The time-kill assay method was used to assess the bactericidal activity at varying concentrations over time. Nutrient broth, along with 10ml (*Escherichia coli)* of the test organism suspension, was introduced into a 15ml tube, and the IC50 Concentration of the test CT was added. For the negative control, 9 ml of sterile distilled water and 1ml of the test organism were added. The plates were then incubated at 37 °C for 24 h. The results were observed every 0, 1, 6, 12 and 24 hrs. Then the results were read at 600 nm using a Calorimeter. Changes of colour were observed and recorded. The reduction of microbial population and percentage of inhibition was calculated[11].

III. RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

Table 2: Preliminary Phytochemical Screening

S. No.	Extract	Observations
1	Alkaloids	+
2	Amino acids	+
3	Anthraquinone glycoside	-
4	Cardiac glycoside	+
5	Flavonoid	+

6	Saponin	-
7	Steroids	+
8	Tannins	+

Antimicrobial activity

Table 3: Means ± Standard Deviation of zone of inhibition obtained by sample *Clitoria ternatea* against and *Escherichia coli*

S. No	Name of the test organism	Name of the test sample	Zone of inhibition(mm) Mean±SD				
			500 µg/ml	250 µg/ml	100 µg/ml	50 µg/ml	PC
1.	*Staphylococcus aureus*	*Clitoria ternatea*	13.15±0.35	7.85±0.07	0	0	23.8±0.28
2.	*Escherichia coli*		14.9±0.42	13.55±0.49	0	0	22.65±0.35

Antifungal activity

Table 4: Means ± Standard Deviation of zone of inhibition obtained by sample *Clitoria ternatea* against *Candida albicans.*

S.NO	Name of the test organism	Name of the test sample	Zone of inhibition(mm) Standard Deviation ± Mean				
			500 µg/ml	250 µg/ml	100 µg/ml	50 µg/ml	PC
1.	*Candida albicans*	*Clitoria ternatea*	14±0.56	0	0	0	23.45±0.63

Minimum Inhibitory Concentration

Table 5: Minimum Inhibitory Concentration

S. No.	Name of the test sample	Name of the Microorganisms	MIC	MIC- 50
1.	*Clitoria ternatea*	*Staphylococcus aureus*	250 µg/ml	125 µg/ml
2.	*Clitoria ternatea*	*Escherichia coli*	250 µg/ml	125 µg/ml
3.	*Clitoria ternatea*	*Candida albicans*	500 µg/ml	250 µg/ml

Image 1: Minimum Inhibitory Concentration of *Staphylococcus aureus*

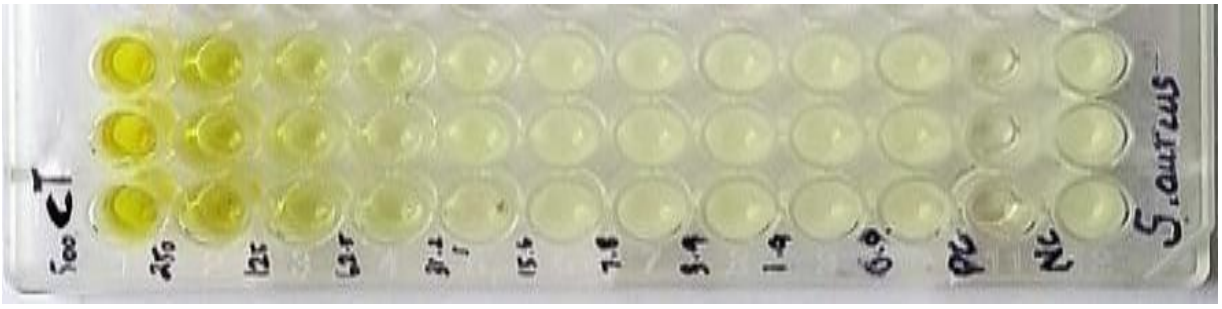


Image 2: Minimum Inhibitory Concentration of *Escherichia coli*

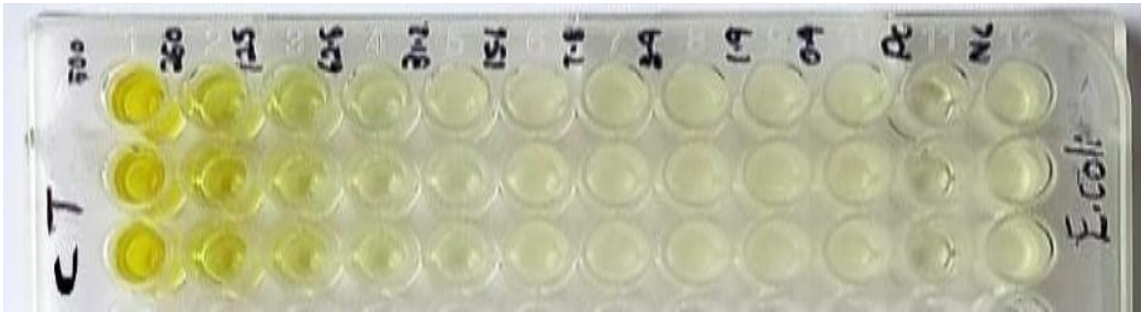
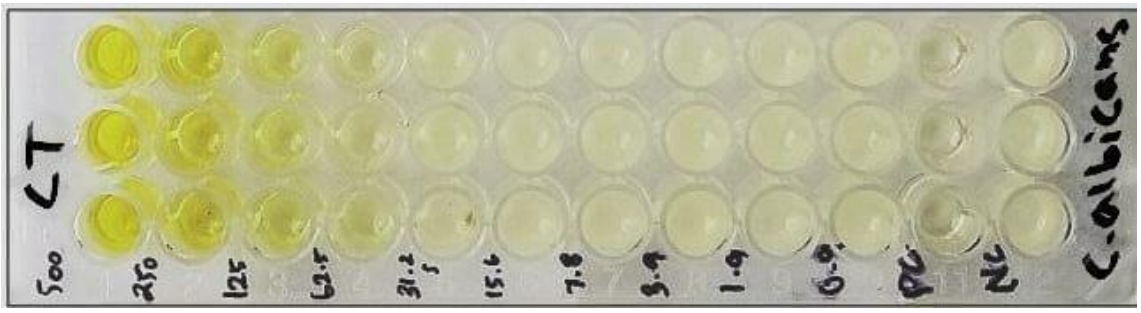


Image 3: Minimum Inhibitory Concentration of *Candida albicans*



Formulation of hand sanitizer

Image 4: Hand sanitizer

Hand sanitizer was prepared by following the procedure, packed and labeled neatly.

Evaluation of disinfectant

1) Organoleptic Evaluation:

Appearance: Clear green colour

Odour: Pleasant

2) Evaluationof pH: 5.5

3) Viscosity: 0.6786cp

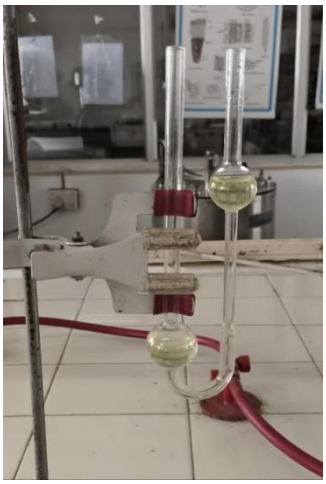


Image 5: Ostwald viscometer

4) Skin irritation Test: No irritation

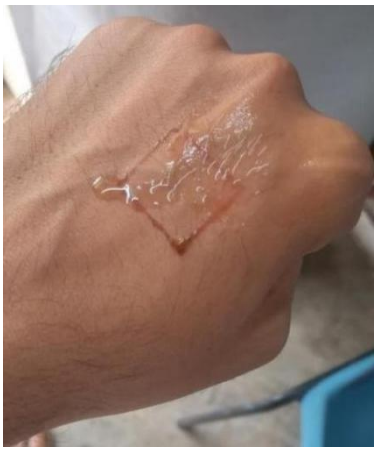


Image 6: Skin irritation test

5) Visual Test: No gritty particles were found

6) Time kill assay:

Percentage of inhibition:

Table 6: Percentage of inhibition

S. No	Time in Hours	Percentage of inhibition(in triplicates)			Mean value %
1	1 hour	93.87	94.32	93.55	93.91
2	6 hours	97.68	98.12	97.34	97.71
3	12 hours	98.10	98.42	97.75	98.16
4	24 hours	99.21	99.45	99.08	99.25

The results indicate that the herbal sanitizer containing *Clitoria ternatea* extract provides antimicrobial activity along with acceptable formulation characteristics.

IV. CONCLUSION

The present study successfully formulated and evaluated a herbal hand sanitizer using ethanolic flower extract of *Clitoria ternatea*. The formulation showed good antimicrobial activity against bacterial and fungal strains. The sanitizer also exhibited acceptable physicochemical properties and did not cause skin irritation. The results suggest that *Clitoria ternatea* extract can be used as a natural antimicrobial agent in hand sanitizer formulations. The developed herbal sanitizer may serve as a safe and effective alternative to conventional chemical disinfectant.

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