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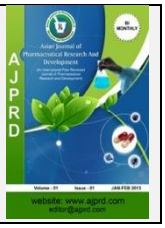

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Research Article

Formulation Development and In Vitro- Assesment of Herbal Anti-Bacterial Cream

Ratsongja Tokbi[1],Girish Kumar Vyas[2], Hariom Sharma[3], Anil Sharma[4], Manmohan Sharma[5]

[1]Research Scholar, M. Pharmacy,SPSFHS , Dr. K. N. Modi University, Newai, 304021, Rajasthan, India

[2] Associate professor, SPSFHS, Dr. K. N. Modi University, Newai, 304021, Rajasthan, India

[3] Professor, SPSFHS, Dr. K. N. Modi University, Newai, 304021, Rajasthan, India

[4] Associate professor, SPSFHS, Dr. K. N. Modi University, Newai, 304021, Rajasthan, India

[5] Associate professor, SPSFHS, Dr. K. N. Modi University, Newai, 304021, Rajasthan, India

A B S T R A C T

Objectives of the study: To evaluate various formulation factors and the topical cream formulation of Cassia Siamea flower extract for antibacterial activity. Phytochemical screening and characterization of the topical formulation (cream) made from the alcoholic extract are done after the extraction of Cassia Siamea.

Results and Discussions: Began with the extraction and was followed by confirmation of the phytochemical evaluation. According to the confirmatory study, the ethanolic extract was devoid of saponins and glycosides. Tannins, phenolics, flavonoids, and alkaloids were also discovered, but not these carbohydrates. In MIC testing, the Cassia Siamea flower extract shown good activity and effectively inhibited the growth of Propionibacterium acnes and Pseudomonas aeruginosa. The designed composition produced a very smooth cream. In accordance with I. P., evaluations were also discovered to be within the range of conventional formulas. Evaluations revealed that the overall formulation had good activity against microorganisms.

Conclusion: This study reveals that a range of phytochemicals are present in Cassia siamea flowers as an active component, producing positive findings against antibacterial activity and showing great promise as an antibacterial agent. This study also comes to the conclusion that the extraction of Cassia Siamea flowers is essential for the development of better, safer, and more affordable drugs to treat different bacterial illnesses.

Keywords: Cassia Siamea, Antibacterial, Cream, Propionibacterium acnes, Pseudomonas aeruginosa.

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*Address for Correspondence:
Ratsongja Tokbi, Research Scholar, M. Pharmacy, SPSFHS , Dr. K. N. Modi University, Newai, 304021, Rajasthan, India

INTRODUCTION:

Between 75 to 80 percent of the world's population, mostly in underdeveloped nations, still relies heavily on herbal medicine for primary healthcare [1]. This is primarily due to the widespread perception that herbal medications are cheap, readily available, and free of negative effects [2]. The World Health Organization (WHO) reports that the usage of herbal treatments is two to three times greater than that of conventional medications worldwide [3].

Hundreds of basic health centres that are meant to serve rural communities are understaffed and lack adequate medicine supplies and diagnostic tools. Traditional medical systems are mainly reliant on the rural population [4]. People tend to believe that natural plant items are healthier than synthetic medicines [5].

Extraction

The initial stage in separating the desired natural products from the base materials is extraction. According to the

extraction principle, there are several extraction procedures, including solvent extraction, distillation, pressing, and sublimation. The following steps are taken when natural product extraction advances:

- The solvent penetrates the solid matrix;
- The solute dissolves in the solvents;
- The solute is diffused out of the solid matrix;
- The extracted solutes are collected.

Types of extraction

- Maceration
- Percolation
- Decoction
- Reflux extraction
- Soxhlet extraction
- Pressurized liquid extraction (PLE)
- Supercritical fluid extraction (SFE)
- Ultrasound assisted extraction (UAE)
- Microwave assisted extraction (MAE)
- Pulsed electric field (PEF) extraction
- Enzyme assisted extraction (EAE)

Following two broad methods are detailed which are generally used for the extraction.

Maceration

Maceration is a process that is frequently used in the production of wine and is now widely used in studies involving natural products and medicinal herbs. The procedure entails soaking plant materials, often in powder or coarse form, for at least 72 hours at room temperature (25°C) while vigorously shaking the container [6, 7].

To release the soluble bioactive compounds, the method is designed to weaken and rupture the plant's cell wall. The entire mixture is pressed and samples were filtered over the course of three days using Whatman no. 1 filter paper.

The type of phytochemical recovered from the samples depends on the solvent used in maceration, where heat is transported by convection and conduction [8].

Soxhlet extraction

The crude extract is finely pulverised and then placed in a porous bag or "thimble" composed of sturdy filter paper, which is then inserted in the Soxhlet apparatus' chamber. In flask A, the extracting solvent is heated, causing its vapour to condense.

The crude drug extract in the thimble is extracted by active contact as the condensed extractant drips into it. The liquid inside the chamber syphons into the flask when the level reaches the syphon tube's top.

This operation is carried out continuously until a solvent drop from the syphon tube cannot evaporate without leaving behind residue.

The benefit of this method over other extraction techniques is the ability to extract significant amounts of bioactive metabolites with a very tiny amount of solvent, which saves time, energy, and money.

When transformed into a continuous extraction technique on a medium or large size, it can become considerably more cost-effective and viable [9]. On a small scale, it is typically employed as a batch process.

Topical Drug Delivery System

Topical drug administration is a localized drug delivery system anywhere in the body through ophthalmic, rectal, vaginal and skin as topical routes. Skin is one of the most readily accessible organs on human body for topical administration and is main route of topical drug delivery system.

Challenges of developing topical drug delivery system

- Container Selection and Product Stability
- Skin Penetration
- Cosmetic Acceptability
- Topical Route of Drug Administration

Purpose of Topical Preparation

This is directly concerned with the site of action and the desired effect of the preparation. Topical preparations may be used for

- Surface effects
- Appendage effects
- Stratum corneum effects
- Viable epidermal and dermal effects

Creams

Creams are semisolid emulsion systems with opaque appearances as contrasted with translucent ointments. Their consistency and rheologic character depend on whether the emulsion is water in oil or oil-in-water type and/or the nature of the solids in the internal phase. Oil-in-water emulsions are most useful as water-washable bases whereas water-in-oil emulsions are emollient and cleansing.

Types of creams

1. When an emulsion in which the oil is dispersed as droplets throughout the aqueous phase is termed an oil-in-water (O/W) emulsion.
2. When water is the dispersed phase and an oil the dispersion medium, the emulsion is of the water-in-oil (W/O) type.

Contents:

- *Thickeners and Emulsifying agents*
- Preservatives and Antioxidants
- Buffer agents

Plant Description and details

Senna siamea, also known as Siamese cassia, kassod tree, cassod tree and cassia tree, is a legume in the subfamily Caesalpinioideae. It is native to South and Southeast Asia, although its exact origin is unknown.

Figure 1: Cassia Siamea Tree

Botanical Name: Cassia siamea

Common Name: Kassod tree

AIM AND OBJECTIVES

Aim

The basic aim of this research work is to evaluate different formulation parameters and antibacterial activity of Cassia Siamea flower extract in form of topical cream.

Objectives

In order to achieve this goal, the following major objectives were set

- Extraction of Cassia Siamea with Soxhlet Apparatus.
- Phytochemical screening of Cassia Siamea flower alcoholic extraction.
- To identify and characterize active phytoconstituents from the plant extract.
- To prepare and characterize topical formulation (cream) from the alcoholic extract.
- To determine antibacterial activity by in vitro model.

MATERIALS AND METHODS

My work was started from plant identification, collection, and authentication. After this, process moves up and wentfor extraction then chemical analysis was completed according to my prior set objectives of this research work. Then, formulation development and evaluation of the preparation was processed.

Plant Collection and authentication

Cassia Siamea flowers were collected from Dr. K. N. Modi University in Newai, Tonk, Rajasthan. It was authentified by herbarium department of Rajasthan University. The plant authentication no. is RUBL21359 on Receipt no. 34415 issued by botany department, RU, Jaipur for Cassia Siamea.

Table 1: Plant description

S. No.	Name	Local Name	Family	Part used
1	Cassia siamea	Cassod tree, assod tree	Fabaceae	Flower

Preparation of extract

Soxhlation:

Dried flowers (weight-50g) were ground to obtain a powder using pestle mortar. This was then taken in a muslin bag and subjected to Soxhlet extraction using ethanol (90%) as solvent maintained at 60^{o}C for 24 hours. The Ethanolic Extract of Cassia Siameaflowers were made free from the solvent by using rotary evaporator with a standard temperature of 45^{o} C [10].

Maceration:

Crude samples were extracted by maceration process using ethanol (60% v/v) and distilled water as solvent in the ratio of 1:6. The maceration was carried out at room temperature for 24 hours and the extract was filtered using Whattman no.1 filter paper. The residue left was again subjected to second maceration under similar conditions as in first maceration.

Then, the filtrate obtained from first and second maceration of all the plant extracts were concentrated in rotary evaporator at 175 mmHg pressure at 40 rpm and 5 °C chilling temperature. Further drying was performed in vacuum desiccator at pressure of 60 mbar until dry. Thus, obtained dried ethanolic extract was stored at 4 °C in refrigerator for further use. Stock solution of concentration 1 mg/ml was prepared in ethanol [11].

Phytochemical analysis

The presence of phytochemical constituents in the test sample was determined using qualitative phytochemical tests.

The phytochemical screening to find compounds which contained in extracts as below by [12]

- Alkaloids compound
- Flavonoids compound
- Polyphenols compound
- Tannins compound
- Monoterpenoid and Sesquiterpenoids compound
- Steroids and Triterpenoid compound
- Quinone compound 8. Saponin compound

Thin Layer Chromatography (TLC) of Cassia Siamea flower extract

The extracts and the most active fraction were subjected to Thin Layer Chromatography (TLC) profiling to determine the quantitative chemical compounds in the extract and fraction.

TLC analyses were conducted in silica gel plates GF 254 and a mobile phase of a solvent mixture consisting toluene: ethyl acetate: formic acid at a ratio of 5: 4: 1.

This phase was selected according to an article written and published by Seasotiya et al., in 2014. Chromatogram profile was observed in visible light, and under UV light 254 nm and 366 nm. The TLC observation also subjected to creams formulations [13].

Figure 2: TLC preparation and chamber saturation process

Microbiological activity determination:

Antibacterial activity test of extract and fraction

Agar diffusion was used to investigate the antibacterial activity of extracts and fractions. A sterile petri dish was filled with 20 mL MHA and 20 L bacterial solution, and the mixture was then agitated until homogenous. A 8 mm-diameter perforator was used to create wells. Each well received 100 uL of the sample solutions, which were subsequently incubated for 18 to 24 hours at 37 °C. DMSO, a solvent, in 50 uL was employed as a negative control. The inhibitory zone's diameter was measured [14].

Determination of Minimum Inhibitory Concentration (MIC) of active fraction

Using the microdilution method and 96 well microtiter plates, the minimum inhibitory concentration (MIC) of the most active fraction from Cassia siamea flowers was determined. The first well was used as a negative control (100 l of MHB), while the second was utilised as a fraction control (100 l of MHB and 100 l of the stock solution's highest active fraction). The most active portions of Cassia fistula at varying concentrations and 10 L of the bacterial suspension were tested in samples from wells three to eleven. 100 l of MHB and 10 l of the bacterial suspension test were employed in Well 12 as the positive control. Microtiter plate was sealed and incubated for 18–24 hours at 37° C[14].

Cream formulation and evaluations:

Formulation

The cream base was melted in mortar and pestle by heating it in water bath, and the calculated amount of extract (3 %, 5% and 10% w/w) was added in the molten base. The mixture was triturated until the homogeneous mixture was formed. The cream was stored for further evaluation [15].

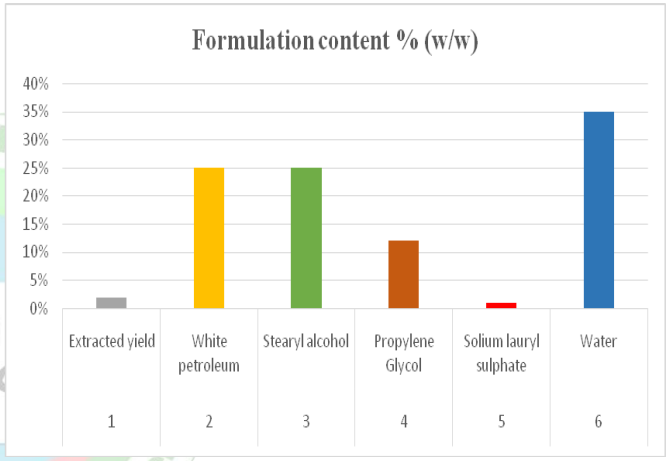

Chart 1: Formula Chart presentation for cream preparation

Table 2: Formula of Cream preparation

Sr. No.	Component	Function	F 1 Content % (w/w)	F 2 Content % (w/w)	F 3 Content % (w/w)
1	Extracted yield	Antibacterial	3	5	10
2	White petroleum	Oil base of o/w emulsion	27	25	20
3	Cetyl Alcohol	Thickener	5	5	5
4	Propylene glycol	Humectant	4	4	4
5	Solium lauryl sulphate	Surfactant/emulsifier	1	1	1
6	Methyl paraben	Preservative	0.2	0.2	0.2
7	Water	Aqueous base of o/w emulsion	Q. S. (upto 100 gram)	Q. S. (upto 100 gram)	Q. S. (upto 100 gram)

Evaluations

Evaluation parameters of the ointment are given below

A. Organoleptic Evaluation

Various organoleptic characters as colour, grittiness, odour, homogeneity, and physical stability were determined as per the method given by Fatima et al. 2017 [16].

B. PH

pH of Cream The pH was measured using a calibrated pH meter at 4 and 7 pH. 5 gm of cream was weighed and dispersed in 45 ml of water to determine the pH of suspension at room temperature [17].

C. Spreadability

The spreadability was determined by placing excess of sample in between two slides which was compressed to uniform thickness by placing a definite weight for definite time. The time required to separate the two slides was

measured as spreadability. Lesser the time taken for separation of two slides results better spreadability [17].

Spreadability was calculated by following formula

S=M×L/T
Here,
S= Spreadability
M= Weight tide to the upper slide
L= Length of glass slide
T= Time taken to separate the slides

A. Diffusion studies

The diffusion study of formulations was carried out by preparing agar nutrient medium. A hole board at the centre of medium and formulations were by placed in it. The time taken by formulations to get diffused through was noted. (After 60 minutes) [17].

B. Loss On Drying

Percentage residue indicates the water content present in formulation. It was determined as per the method given by Fatima et al. 2017 [18] . 5 grams of cream was weighted in a cleaned and tarred Petri dish. Then, it was kept in oven at 105°C until constant weight was obtained. Then, it was cooled in desiccator and weighted. Percentage residue was calculated by using following equation:

C. % Residue = $\frac{\text{D. Initial weight of the cream}}{\text{F. Final weight of the cream}}$ E. X 100

G. Solubility

Soluble in boiling water, miscible with alcohol, ether, chloroform is observed.

H. Washability

Formulations were applied on the skin at 2 x 2 cm^2 area and then ease extend of washing with water was checked.

I. Irritancy Test

Mark an area (1sq.cm) on the left-hand dorsal surface. The cream was applied to the specified area and time was noted. Irritancy, erythema, edema, was checked if any for regular intervals up to 24 hours and reported.

J. Stability study

Physical stability test of the formulation was carried out for four weeks at various temperature conditions like 2°C, 25°C and 37°C. The formulations were found to be physically stable at different temperature i.e., 2°C, 25°C and 37°C within four weeks [17].

RESULTS AND DISCUSSIONS

As the objective of the present study was to formulate and evaluate formulations of cream from ethanolic extract of *Cassia Siamea* flowers and evaluate the efficacy with different quality determination tests.

Physicochemical parameter's evaluations of Cassia Siamea *flowers*

Ash value determination is known as the physicochemical parameter's evaluation of *Cassia Siamea* flowers. This determination was done with the help of article written by Rao, Y. et. al., in 2009 [12, 19].

Table 3: Physicochemical parameter's determination

S. No.	Physicochemical parameters	Observation
1	Loss of drying	7.00%
2	Total Ash value	2.60%
3	Water soluble ash	0.85%
4	Acid insoluble ash	1.3%
5	Foreign matter	Nil

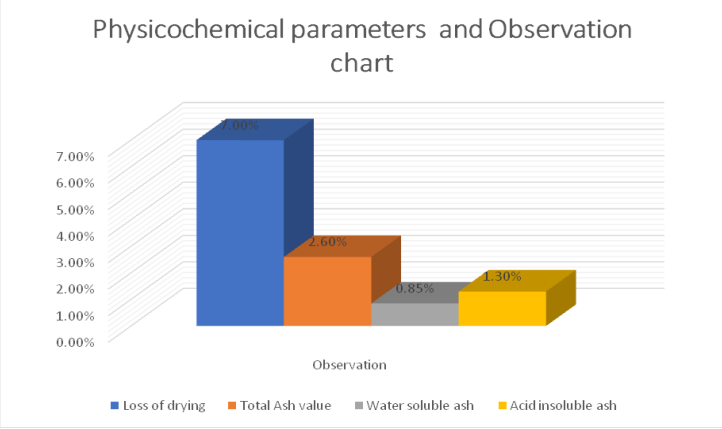

Chart 2: Physicochemical parameters and observation

Preparation of yield:

Dried flowers 50 grams extracted in Soxhlet extraction using ethanol (90%) and the yield obtained was 4% (w/w), 2 grams.

Figure 3: Dried extract analysis

The Soxhlet extraction was done and the liquid yield was collected. Found yield is mentioned in table below:

Table 4: Extraction yield obtained from *cassia siamea* flowers

S. No.	Crude drug Taken quantity	Yield Obtained
1.	50 Grams	(2 grams) or 4%

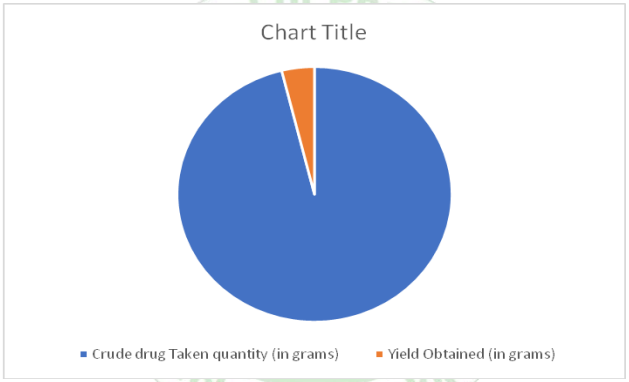

Chart 3: Extraction yield with comparison to total quantity taken for extraction

Phytochemical analysis:

The presence of phytochemical constituents in the test sample was determined using qualitative phytochemical tests.

The preliminary phytochemical analysis of the plant extract of *Cassia Siamea* flowers showed the presence of carbohydrates, alkaloids, glycoside, phenolic compound, and tannins, saponins, flavonoids, fixed oils, and fat test [12,20].

Table 5: Phytochemical evaluation from ethanolic extract of *Cassia Siamea* flowers

Phytochemical tests			
S. No.	**Phytochemical constituents**	**Test**	**Results**
1	Carbohydrate	Fehling's test	**Positive**
2	Alkaloids	Mayer's test, Wagner's test,	**Positive**
3	Glycoside	Molish' test,	**Negative**
		Keller-Killani test Positive with con.	**Negative**
4	Phenolic compound and Tannins	With ferrous sulphate and sodium potassium Tartrate, lead acetate test, ferric chloride test	**Positive**
5	Saponins	Foam test	**Negative**
6	Flavonoids	With NaOH, with lead acetate test	**Positive**
		With H2So4, zinc test	**Positive**
7	fixed oils and fat test	Spot test	**Positive**

Figure 4: Phytochemical evaluations of cassia siamea flower extract

Phenolic content and Flavonoids Analysis

Some phenolic and flavonoid contents were found present in the testing. Both are presented hereunder [20].

Figure 5: Phenolic content and Flavonoids

Table 6: Phenolic and Flavonoids determination

S. No.	Phytochemical constituents	Results
1	Phenolic	**Present**
2	Flavonoids	**Present**

TLC Determination

TLC analysis was done to investigate the compounds in the extract and fractions qualitatively. The findings indicated that the most active fraction of Cassia fistula, the extract, has the same TLC profile as the most active fraction, indicating that these spots are those that are responsible for the antibacterial activity[13, 21]. The results of TLC profile can be seen in following table and Figure.

Table 7: TLC and Rf value determination

Thin Layer Chromatogram profile of Ethanolic Extract of *Cassia Siamea* flower					
Sample	Spot No.	Rf value	Visible light	UV light	
				254nm	366 nm
Extract	1	0.37	-	-	Blue
	2	0.47	-	-	Blue
	1	0.58	-	-	Red
	2	0.73	-	-	Red
Ethyl Acetate fraction	1	0.51	-	-	Blue
	2	0.67	-	-	Blue
	1	0.7	-	-	Red
	2	0.92	-	-	Red

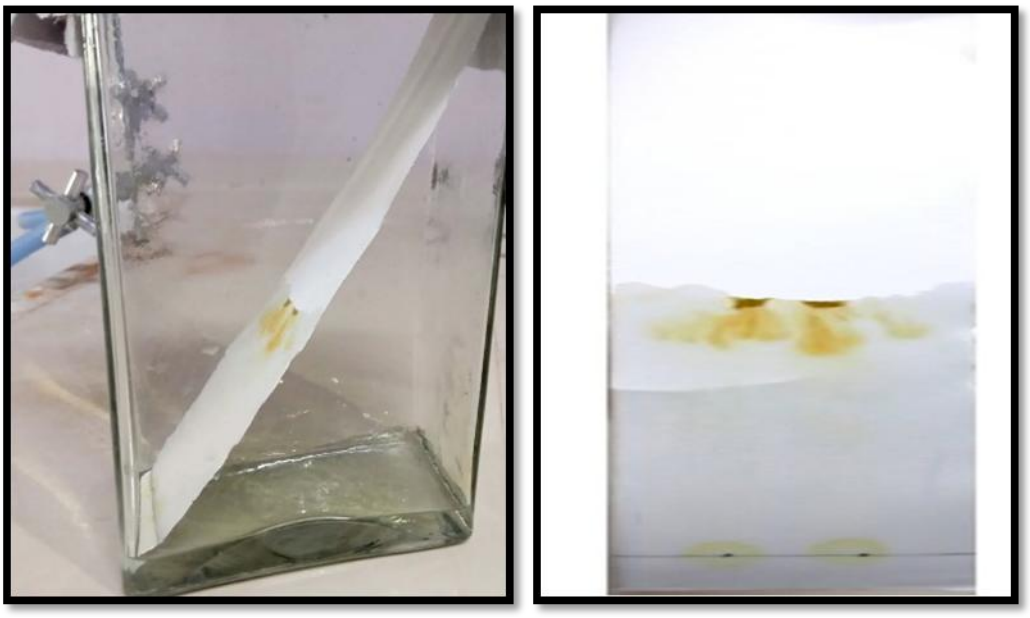

Figure 6: TLC preparation, determination and Rf value determination

These characteristics are divided in different categories for results of cream evaluation and better understanding as follows.

Microbiological Determination

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of active fraction Results of the determination of the most active fraction MIC *Cassia Siamea* flowers can be seen in Table no. . The lowest concentration which inhibited the visual growth was recorded as MIC. Positive results were decided when the well showing no growth of bacteria (visually observed as clear solution), and it was determined as the MIC. Based on Table 8, it can be concluded that MIC of the active fraction of *Cassia Siamea* flowers against *Propionibacterium acnes* was 175 ppm, while that against*Pseudomonas aeruginosa* was 400 ppm [14].

The swab test to examine MCB value against *Propionibacterium acnes* were performed on media agar from the wells with concentration of 175, 200, 300 and 350 ppm. Determination of MCB against *Pseudomonas aeruginosa*, were performed on agar medium of the wells with concentration of 400, 600, 700 and 800 ppm. After they were plated into media agar and incubated for at 37^{o} C for 18-24 hours, it was found that the value of MCB for *Propionibacterium acnes* was 350 ppm, while that *Pseudomonas aeruginosa* was 800 ppm [14].

Table 8: Minimum Inhibitory Concentration of ethanolic extract

Minimum Inhibitory Concentration of extract *Cassia Siamea* flowers against selected bacteria			
Concentration of the sample (most active fraction) of Cassia fistula (ppm)		Bacterial Growth	
		P. acnes	*P. aeruginosa*
1	800	-	-
2	700	-	-
3	600	-	-
4	400	-	-
5	350	-	+
6	300	-	+
7	200	-	+
8	175	-	+
9	150	+	+
10	100	+	+
11	87.5	+	+
12	75	+	+
13	50	+	+

Evaluation of Cream

The present study was done for preparation and evaluation of cream formulations. For this the herbal extracts were prepared by using Soxhlet process were used for all three formulations.

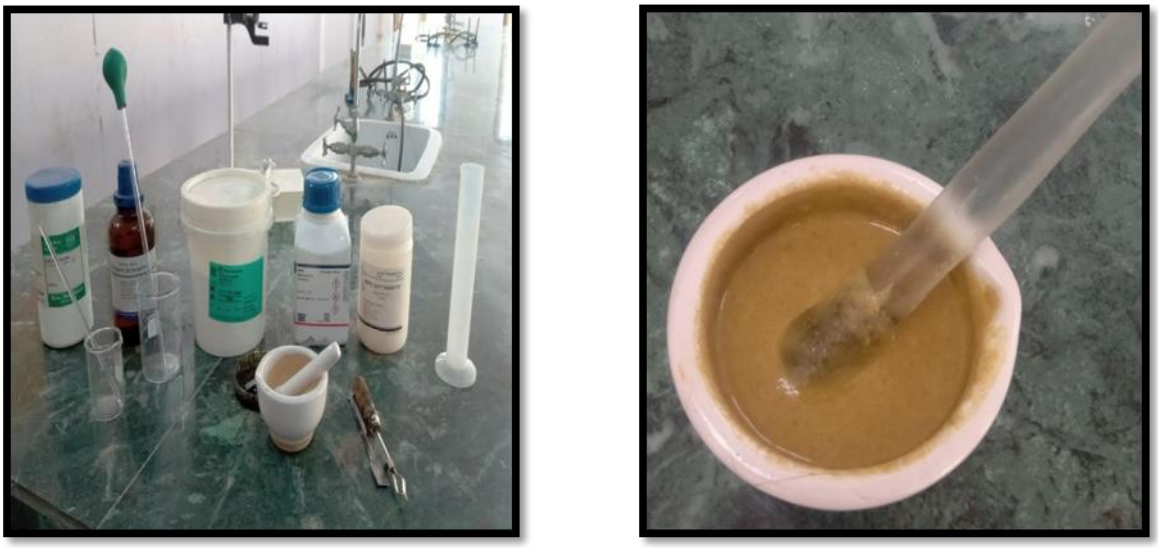

Figure 7: Cream formulation excipients and extract

The concentration was decided by MIC determination, The physicochemical properties were studied which shows satisfactory results for spreadability, washability, solubility, loss on drying and others [16,17]. Which are elaborated hereunder:

Table 9: The physicochemical properties of Cream

S. No.	Characteristics	Formulation 1	Formulation 2	Formulation 3
1	Colour	Light Brown	Light Brown	Light Brown
2	Odour	Pleasant	Pleasant	Pleasant
3	Consistency	Smooth and homogenous	Smooth and homogenous	Smooth and homogenous
4	P^H	6.1	6.4	6.3
5	Spreadability(seconds)	7	8	7.5
6	Diffusion study (after 60 min)	0.7 gm	0.6 gm	0.8 gm
7	Loss on drying	0.2%	0.3%	0.2%
8	Solubility	Soluble in boiling water, miscible with alcohol, ether, chloroform	Soluble in boiling water, miscible with alcohol, ether, chloroform	Soluble in boiling water, miscible with alcohol, ether, chloroform
9	Washability	Good	Good	Good
11	Non irritancy	Non irritant	Non irritant	Non irritant
12	Stability study	Stable	Stable	Stable

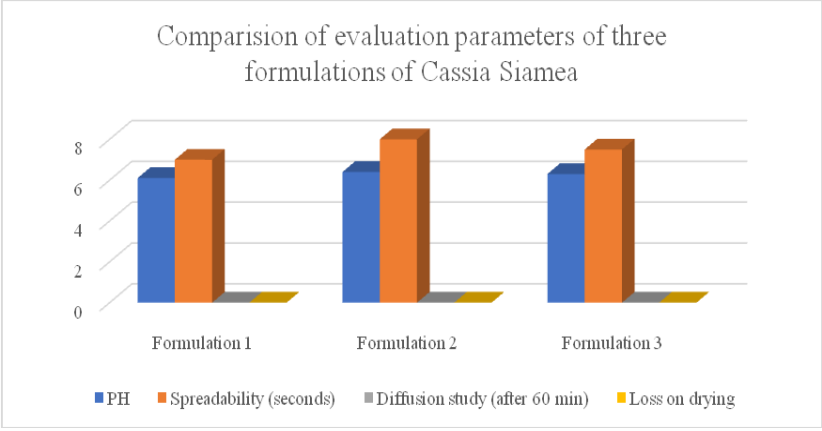

Chart 4: physicochemical parameters of prepared cream

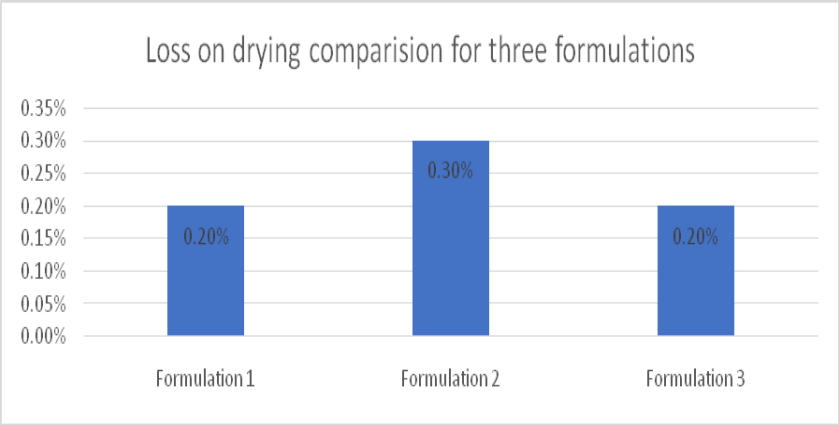

Chart 5: Comparison of loss on drying for all three formulations of Cassia Siamea

Stability study of cream: The stability test was carried out for 30 days at room temperature. Color change and phase separation of cream was not observed.

The formulations were placed for a stability study at different temperature conditions like 2°C, 25°C and 37°C more than four weeks (about 30 days). There were no changes observed in Color change, phase separation spreading ability,and diffusion study as well as irritant effect [16, 17].

Table 10: Stability Test of Cream

Sr. No.	No. of Days	Colour	Phase Separation
1	5	No Change (Light Brown)	No Phase separation observed
2	10	No Change (Light Brown)	No Phase separation observed
3	15	No Change (Light Brown)	No Phase separation observed
4	20	No Change (Light Brown)	No Phase separation observed
5	25	No Change (Light Brown)	No Phase separation observed
6	30	No Change (Light Brown)	No Phase separation observed

So, here some experimental results are shown for herbal plant *Cassia Siamea* and a preparation of cream and its evaluations were done. The overall results found satisfactory. In this research work I found formulation 2 and formulation 3 more effective as compared to formulation 1. So out of these two I selected formulation 2 for the evaluation and found all results with in good efficacy.

CONCLUSION

The current study concludes that Cassia Siamea flowers extraction is necessary in order to develop better, safer, and more cost-effective medications for treating various bacterial infections. This study demonstrates that Cassia Siamea flowers has a variety of phytochemicals as an active ingredient, which produce good results against antibacterial activity and have a high potential as an antibacterial agent. The anti-bacterial properties of Cassia Siamea herbal cream. The finished product spread easily on the skin's surface, had no irritating effect, diffused effectively, and found stable at various temperatures.

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