Section A-Research paper
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DEVELOPMENT AND PHARMACOLOGICAL ASSESSMENT OF HERBAL FORMULATION FOR SKIN CARE

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OBJECTIVE

The aim of present research work was development and pharmacological assessment of herbal formulation for skin care like acne acne disease. Acne is the most common skin problem which can occur due to a bacterial infection

MATERIALS AND METHOD

The herbal gel was prepared by using Cascabela thevetia extract, Carbopol-940, Tamarind gum, HPMC, Poloxamer 407, Lecithin, Isopropyl myristate, Triethanolamine, Methyl paraben and distilled water. The prepared herbal gel was evaluated by using a number of parameters. The anti-inflammatory and antiacne activity was assessed with paw edema and invitro antimicrobial activity.

RESULTS AND DISCUSSION

Optimized formulation F7 of CTEE containing combination 1% carbopol 934 and 1% HPMC K4M was found to be excellent for skin application for the treatment of anti-inflammatory. Poly phenolic compounds CTEE presents in ethanol extract of *Cascabela thevetia* leaves. It is key components for producing anti-inflammatory activity. It may act as antioxidant and potential inhibitors of cyclo-oxygenase, lipoxygenase and nitric oxide synthase. This gel can be effective for various skin diseases such as abscesses, boil, burns and eczema. Consequently, CTEE bioactive compounds containing is appropriate for topical application. It can offer multiple benefits for skin diseases along with treating inflammation

CONCLUSION

The prepared gel formulation can be used for the treatment and management of acne diseases.

Keywords: *Cascabela thevetia*, Nanogel, Acne, *Propionibacterium acnes*

INTRODUCTION

Herbal medicine (also known as herbalism) is the study of pharmacognosy and the use of therapeutic herbs, which form the foundation of traditional medicine. There is minimal scientific evidence for the safety and efficacy of plants used in 21st century herbalism, which does not typically establish purity or dose criteria. Herbal medicine frequently incorporates fungal and bee products, as well as minerals, shells, and animal parts. Phytomedicine or phytotherapy are additional terms for herbal medicine [1-2].

Alternative and pseudoscientific techniques of employing unprocessed plant or animal extracts as untested medications or health-promoting substances are referred to as paraherbalism. Paraherbalism is based on the assumption that keeping multiple compounds from a specific source with less processing is safer or more effective than produced goods, a view for which no proof exists [3-4].

Cascabela thevetia, commonly known as Yellow Oleander or Lucky Nut, is an evergreen shrub or small tree belonging to the Apocynaceae family. It is native to tropical regions of Central America, the Caribbean, and parts of South America. *Cascabela thevetia* is widely cultivated for its attractive yellow flowers and ornamental value, although it is important to note that all parts of the plant, especially the seeds, are highly toxic.

Cascabela thevetia, commonly known as the yellow oleander or lucky nut tree, is a flowering plant belonging to the family Apocynaceae. It is native to tropical regions of North and South America, but it is also cultivated in many other parts of the world for its ornamental value. The plant contains a variety of chemical constituents, including cardiac glycosides, alkaloids, flavonoids, and phenolic compounds. These constituents contribute to both the plant's medicinal properties and its toxicity. *Cascabela thevetia* is primarily grown for ornamental purposes, it also has a long history of use in traditional medicine. However, due to its toxicity, its medicinal use should be strictly avoided without professional guidance. The plant's extract has been used in some cultures for treating certain ailments, including skin conditions, ringworm, and even as an

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abortifacient. However, it is crucial to note that self-medication or ingestion of any part of the plant can be extremely dangerous and potentially lethal [5].

MATERIALS AND METHOD

Collection of Plants

The plants were collected from the Garden of College of pharmacy, dried, pulverized and stored until further use.

Chemicals and Reagents

Emulsifying wax, white soft paraffin, liquid paraffin, methanol, agar which were obtained from SD Fine Chemicals, 315 - 317, T V Industrial Estate, 248, Worli Road, Mumbai, Maharashtra-400030.

Preparation of the Extracts [6-12]

To prepare the sample, one kilogram of *Cascabela thevetia* leaves were crushed into coarse powder and defatted using Soxhlet's extractor with petroleum ether (65°-85°C). The residue obtained was then extracted with ethanol. The collected liquid ethanol extract was further evaporated by using rotator evaporator. The ethanol extract was storied in desiccators for further analysis. The phytochemical tests were done

Qualitative Phytochemical Analysis [6-12]

The dried ethanolic extracts were subjected to various color reactions to identify the nature of the phytoconstituents.

Test for Alkaloids

a. Hager’s test: Extract was treated with a few drops of Hager’s reagent (saturated solution of picric acid) formation of yellow precipitate indicates the presence of alkaloids.

b. Mayer’s test: Extract was treated with Mayer’s reagent (potassium mercuric iodide solution) – formation of cream precipitate shows the presence of alkaloids.

c. Dragendorff’s test: Extract was treated with Dragendorff’s reagent (potassium bismuth iodide solution) – orange precipitate shows the presence of alkaloids.

d. Wagner’s test: Extract treated with Wagner’s reagent (iodine, potassium – iodide solution) appearance of reddish brown precipitate indicates alkaloids.

Test for Steroids

a. Liebermann – Burchard test: 10 mg of extract was dissolved in 1 ml of chloroform. 1 ml of acetic anhydride was added following the addition of 2 ml of concentrated sulphuric acid. Formation of reddish violet color precipitate at the junction indicates the presence of steroids.

b. Salkowski test: 1 ml of concentrated sulphuric acid was added to 10 mg of extract dissolved in 1 ml chloroform. A reddish brown layer exhibited by chloroform layer and green fluorescence by the acid layer suggests the presence of steroids.

Test for Flavonoids

a. Ferric chloride test: To the extract few drops of neutral ferric chloride solution was added. Blackish red color formation shows the presence of flavonoids.

b. Lead acetate test: To the extract lead acetate solution was added. Presence of flavonoid is indicated by formation of yellow precipitate.

c. Magnesium ribbon test: Few fragments of magnesium ribbon were added and concentrated hydrochloric acid was added along the sides of the test tube. Magenta color formation indicates the presence of flavonoids.

d. Zinc-hydrochloride test: To the extract, a pinch of zinc dust was added followed by addition of concentrated hydrochloric acid along the sides of the tube. Magenta color indicates presence of flavonoids.

Test for Saponins

a. 1 ml of extract was diluted with 10 ml of distilled water and shaken in a graduated cylinder for 15 min. Formation of 1 cm layer of foam indicates the presence of saponins.

b. Haemolysis test: 2 ml of 1.8% sodium chloride solution was taken in two test tubes. To one test tube 2 ml of distilled water was added and to another tube 2 ml of extract was added. Blood was obtained by pricking the thumb and 5 drops of blood was added to each tube. The contents were gently mixed and observed under the microscope. Haemolysis indicates the presence of saponins.

Test for Proteins and Amino Acids

a. Millon's test: Extract was treated with Millon's reagent (mercuric nitrate in nitric acid). Red color formation indicates the presence of proteins and amino acids.

b. Biuret test: Extract was treated with sodium hydroxide and copper sulphate solution drop wise. Violet color shows the presence of proteins and amino acids.

c. Ninhydrin test: Extract treated with Ninhydrin reagent, ammonia and heated. Violet color is formed indicating the presence of proteins and amino acids.

Test for Glycosides and Sugars

a. Molisch's Test: 2 ml of concentrated sulphuric acid was added to 2 ml of extract solution. Then it was treated with Molisch's reagent with 15% ethanolic α-naphthol. Formation of a reddish violet ring indicates the presence of glycosides and sugars.

b. Fehling's Test: 5 ml of extract solution was mixed with 5 ml of Fehling's solution and boiled for 5 min. Formation of brick red precipitate demonstrated the presence of glycosides and sugars. [Fehling's solution A: 34.64 g of copper sulphate solution was dissolved in a mixture of 0.5 ml of sulphuric acid and sufficient water to produce 500 ml.

Fehling's solution B: 176 g of sodium potassium tartrate and 77 g of sodium hydroxide are dissolved in sufficient water to produce 500 ml. Equal volumes of A and B solution are mixed at time of use]

c. Benedicts Test: - To 5 ml of extract solution, 5 ml of Benedicts solution (1.73 g of cupric sulphate, 1.73 g of sodium citrate and 10 g of anhydrous sodium carbonate were dissolved in water and the volume is made up to 100 ml with water) was added in a test tube and boiled for a few min. Development of brick red precipitate confirmed the presence of glycosides and sugars.

d. Barfoed's Test: Extract was treated with Barfoed's reagent (copper acetate in water and glacial acetic acid). Appearance of red color is a positive test for presence of glycosides and sugars.

e. Legal test: Extract was dissolved in pyridine. Sodium nitroprusside solution was added to it. Pink red or red color produced indicates presence of glycosides and sugars.

f. Borntrager's test: Few ml of dilute sulphuric acid was added to the test solution. It was then boiled, filtered and the filtrate was extracted with ether or chloroform. The organic layer was then separated and ammonia was added. Pink, red or violet color was produced in the organic layer to indicate the presence of glycosides and sugars.

Test for Phenolic Compounds & Tannins

a. Ferric chloride: test 5 ml of extract was allowed to react with 1 ml of 5% Ferric chloride solution. Bluish black coloration indicated the presence of phenolic compounds and Tannins.

b. Gelatin test: When extract was treated with gelatin solution, white precipitate formed indicates the presence of phenolic compounds and Tannins.

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c. Lead acetate test: 5 ml of extract was treated with 1 ml of 10% lead acetate solution in water. Yellow color precipitate shows the presence of phenolic compounds and Tannins.

In-vitro antimicrobial activity of *Cascabela thevetia*[13-15]

Agar well diffusion method

Culture of test microbe

For the cultivation of the bacterial strains, Nutrient broth medium (NBM) was prepared using 8% nutrient broth in double distilled water and agar-agar. It was subjected to autoclaving at 15 Ibs psi for 25–30 min s. Agar test plates were prepared by pouring 15 ml of NBM into petri dishes under aseptic condition and allowed to stand for room temperature for stabilization. Bacterial cell cultures were maintained in peptone saline solution by regular subculturing and were incubated at 37OC for 24 h.

Development of topical formulation

Method of preparation

Measured quantity of *Cascabela thevetia* ethanol extract, Methyl Paraben, Glycerin and weighed quantity of Polyethylene glycol will dissolved in about little amount of distilled water in a beaker and stirred at high speed using mechanical stirrer or (sonicator). Then Carbopol-940 added slowly to the beaker containing above liquid while stirring. Neutralized the solution by slowly adding triethanolamine solution with constant stirring until the gel is formed. Carbopol 940- Gelling Polymer, Triethanolamine- gelling agent, pH Adjusting agent, Neutralizer Methyl Paraben- Preservative, Distilled Water, Glycerin and Polyethylene Glycol- solvents some other agents will used for different development of gel that are mentioned in below table 1.

Table 5.1: Formulation composition of the topical preparation of CTEE

Ingredients %	F1	F2	F3	F4	F5	F6	F7
Cascabela thevetia extract	1	1	1	1	1	1	1
Carbopol-940	2	1					1
Tamarind gum			2	1			
HPMC					2	1	1
Poloxamer 407	35	35	35	35	35	35	35
Lecithin	10	10	10	10	10	10	10
Isopropyl myristate	10	10	10	10	10	10	10

Triethanolamine	1.3	1.2	1.3	1.2	1.3	1.2	1.2
Methyl paraben	qs	qs	qs	qs	qs	qs	qs
Distilled water	qs	qs	qs	qs	qs	qs	qs

Evaluation of topical formulation

Prepared formulations were evaluated for various physicochemical parameters such as colour, homogeneity, pH, spreadability, viscosity and drug content (total phenolic content) (Dev et al 2019).

Appearance and consistency:

The physical appearance visually checked for the texture of flavonoid rich fraction extract gel based formulations and observations may be like stated in Table.

Wash-ability

Formulations applied on the skin and then ease and extent of washing with water were checked manually and observations may be like stated in table.

Extrude-ability determination of formulations [15]

The extrusion of the gel from the tube is an important during its application and in patient acceptance. Gel with high consistency may not extrude from tube, whereas low viscous gels may flow quickly, and hence, suitable consistency is required to extrude the gel from the tube. Extrudability of gel formulation was found to be good.

Measurement of pH

5 gm of gel formulation was dispersed separately in 45 ml of water, and the pH of the suspension was determined using digital pH meter (digital pH meter, 335, systronics, Noroda, Ahmedabad). Measurements of pH of all formulations were carried out in triplicate and the averages of three readings were noted.

Homogeneity

Formulations were tested for homogeneity by visual inspection after the formulations have been set in the container. They were tested for their appearance and presence of any aggregates.

Measurement of viscosity

The viscosity of gel was determined by using a Brookfield viscometer DVII model with a T-Bar spindle in combination with a helipath stand. 50 g of gel was filled in a 100 ml beaker. T-bar

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spindle (T95) was used for the measurement of viscosity of all the gels. The helipath T-bar spindle was moved up and down and viscosity was measured using spindle 7.

Determination of Spread ability

Principle:

An important criterion for hair gels is that it must possess good spread ability. The spread ability is a term expressed to denote the extent of area to which the gel readily spreads on application to hairs. The therapeutic efficacy of a formulation also depends on its spreading value. A special apparatus has been designed to study the spread ability of the formulations. Spread ability is expressed in terms of time in seconds taken by two slides to slip off from formulation, placed between, under the application of a certain load. Lesser the time taken for the separation of two slides, better the spread ability.

Method:

Spread ability is expressed in terms of time in seconds taken by two slides to slip off from the gel when placed in between the slides under the direction of a certain load. The excess amount of sample was placed between the two glass slides and a definite amount of weight was placed on these glass slides to compress the glass slides of uniform thickness. A weight of 70 g was added and the time required to separate the two slides was noted. Spread ability was calculated using the formula

$S = M.L / T$

Where, M = wt tied to upper slide, L = length of glass slides, T = time taken to separate the slides.

Drug content determination

Drug content was determined by dissolving accurately weighed 1 g of gel in phosphate buffer of pH 6.8. After suitable dilution, total phenolic content were determined spectrophotometrically following Folin-Ciocalteau method described previously with minor modification. Absorbance was recorded by using UV-visible spectrophotometer at 765 nm and the concentration is determined for estimating drug content.

***In-Vitro* Skin Permeation**

Diffusion studies of the all the formulations were carried out in Franz diffusion cell through a sigma membrane. In diffusion cell, sample (0.1 g) was applied on dialysis membrane in donor compartment. The entire surface of membrane kept in contact with the receptor compartment

containing phosphate buffer (pH 6.8) as the dissolution medium. Magnetic stirrer was used for stirring the receptor compartment. The temperature maintained was 37±1 °C. The study was carried out for 8 h with samples removed at 0.5, 1, 2, 4, 6, 8 h. The sample was withdrawn at a predetermined period of time and the same volume was replaced with fresh phosphate buffer (pH 6.8). Samples were analyzed for total phenolic content according to the Folin-Ciocalteu method and absorbance values were measured at 765 nm using UV/Visible spectrophotometer .

Stability

The stability studies will be carried out for all the formulations. The formulations were kept at two different temperatures 4 ± 2°C and 30 ± 2°C, 65 RH, for three months. The pH and the viscosity of the formulations, which were determined after three months, were compared with the initial pH and Statistical analysis. All experimental measurements will be carried out in triplicate and expressed as an average of three analyses ± standard deviation. Statistical analyzes performed by the t-test .

Acute Toxicity Studies

The Institutional Animals Ethics Committee approved the use of animals for the present study (Ethical Clearance number: P.Col/48/2020/IAEC/VMPC) and the acute toxicity will be carried out as per the OECD 423 guidelines. Twelve female Swiss Albino Mice weighing between 20 and 25 g and between age eight and twelve weeks procured for the experimental trial. The animals maintained under controlled environmental conditions (30-70% humidity and temperature – 22 ± 3°C) and exposed to a photoperiod of 12 hours of daylight and 12 hours of night, in an animal house, approved by the committee for the purpose of control and supervision of experiments on animals. The selected animals fed with standard feed and drinking water and monitored on a regular basis.

The animals selected randomly and grouped three animals per group. They kept fasting four hours prior to the treatment and the test substance administered in a single dose by the oral route.

Subsequently, the dose is gradually increased with each step, starting from 5 mg / kg then to 50, 300, 2000 mg / kg. After the substance had been administered, the food could be withheld for a further one to- two hours, in mice.

The animals observed for changes in skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic, and central nervous systems, and somatic motor activity and behavior pattern. They are noted individually after dosing, at least once during the first 30 minutes,

periodically during the first 24 hours, with special attention given during the first four hours, daily, and thereafter for a total of 14 days.

In-vivo anti-inflammatory using carrageenan of CTEE

Induced Paw Edema method

The Intraplanter injection of carrageenan to hind paw in rats induced an increasing in the paw thickness. This edema had a rapid onset and reached a peak at 5 h after the challenge. Pretreatment with plant extract based hydrogel, standard Piroxicam gel resulted in % inhibition in paw edema respectively at 5 h as compared to plain carbopol gel show significantly suppressed the increase in paw edema after carrageenan injection. It is evident that plant extract based Hydrogel on pretreatment showed significant ($p<0.05$) percentage inhibition of edema as compared to plain carbopol gel which could be due to enhanced permeation of bioactive constituents through skin. Hence prepared plant extracts based hydrogel containing bioactive constituents produced significant anti-inflammatory effect [16-17].

Determination of In-vitro Anti-Inflammatory Activity [16-17]

The in vitro anti-inflammatory activity of the extracts was established by the assessment of inhibition of albumin denaturation, membrane stabilization, and antiproteinase activity, as described by the previous studies with slight modifications. The freeze-dried extract obtained from each solvent was serially diluted in dimethyl sulfoxide (DMSO) from 25 to 500 µg/mL. Aspirin (100 µg/mL, Sigma-Aldrich, Singapore), a standard anti-inflammatory drug, was used as a positive control, whereas DMSO was used as a negative control.

Anti-acne activity of developed topical formulation [16-17]

The antibacterial activities of optimized topical formulation were measured using modified agar well diffusion method. In this method, nutrient agar plates were seeded with 0.2 ml of 24 h broth culture of *Acne vulgarism*. The agar plates were allowed to solidify and a sterile 8 mm borer was used to cut wells of equidistance in each of the plates. 0.5 ml of formulations and marketed clindamycin gel were introduced into the wells. The plates were incubated at 37°C for 24 hours. The antibacterial activities were evaluated by measuring the zones of inhibition (in mm) (Sahu et al., 2019).

RESULTS AND DISCUSSION

Extraction of *Cascabela thevetia* leaves

Extraction Yield Three solvents with different polarities were chosen for extraction of the *Cascabela thevetia leaves* part.

Table 6.1: Extraction and percent yield of the *Cascabela thevetia* leaves extracts

Solvent Used	Percentage Yield	Colour of Extracts
60% ethanol extract	17.8%	Dark brownish

Each value is expressed as means ± SD of three independent extractions (n = 3) within a column are significantly different ($P < 0.05$).

Evaluation of topical formulation

Cascabela thevetia (CTEE) loaded gel topical formulations were prepared using various different polymers such as carbopol934, Tamarind seed gum and HPMC K4M as gelling agent. Triethanolamine was used in the formulations for neutralize the pH. Methyl paraben; propyl paraben were used as preservatives for stabilization of gel. All the prepared gel formulations showed good organoleptic properties such as green color, aromatic odor, and were found good homogeneity and spread ability. All the prepared gel formulations showed good pH properties and were found in the range of 6.9-7.0. These ranges of pH showed that lies in the normal pH range of the skin. Prepared formulation F7 viscosity of gel formulation containing carbopol-934 and HPMC K4M as gelling agent was found to be excellent with good spread ability than gels than other formulations. Drug content of all the formulation was found to be more than 97.6%

Wash-ability and skin irritation property

Wash-ability and skin irritation property of all prepared formulation were performed and found that prepared gels were not produce any type of skin irritation, redness, or edema on application and free from dermatological reaction.

Extrude-ability determination of formulations

The optimized topical gel formulation showed good extrude-ability determination of formulations

Table 6.2: Physicochemical evaluations of gel formulations of *Cascabela thevetia* ethanol extract (MAEE)

Formulation code	color	Homogeneity	pH	Spread ability	Drug content %
F1	Slight yellow	Good	6.8±0.03	26.30±1.12	93.7±0.4
F2	Slight yellow	Good	6.6±0.02	34.30±1.21	91.4±0.3
F3	Slight yellow	Good	6.5±0.09	34.30±1.15	94.2±0.5
F4	Slight yellow	Good	6.7±0.25	35.30±1.34	92.9±0.6
F5	Slight yellow	Good	7.1±0.15	34.30±1.18	95.8±0.5
F6	Slight yellow	Good	6.7±0.22	37.30±1.16	94.7±0.2
F7	Slight yellow	Good	6.8±0.12	35.30±1.13	98.6±0.3

All values are expressed as mean ± SD, n=3

Viscosity

Viscosities of all prepared formulations were estimated by using spindle 7. The results are shown in the table. The formulation F7 showed optimum viscosity 4621±.02 centipoises.

Table 6.3: Measurement of viscosity of gel formulations of MAEE

Formulation code	Spindle 7 (Centipoises)
F1	4521±.04
F2	4533±.02
F3	4523±.04
F4	4531±.02
F5	4518±.05
F6	4517±.03
F7	4621±.03

In Vitro Skin Permeation

The optimized topical gel formulations were showed good skin permeation property

Acute Toxicity Studies

The optimized topical gel formulation showed no significant skin irritation on skin surface. The application of topical gel after no edema formation was seen. These results represented or indicating skin acceptability of these formulations for topical application.

***In-vitro* drug release study**

In vitro bioactive compound CTEE drug release of topical gel formulation F7 gel was shown that the drug release profile was controlled. Formulation F7 was establish to be optimal since it presented greater controlled release (98.6%) in 8hrs as opposed to previous formulations as shown in fig. In first 2hrs more than 20-31% drug discharge was observed, the in-vitro drug release rate are shown in Table.

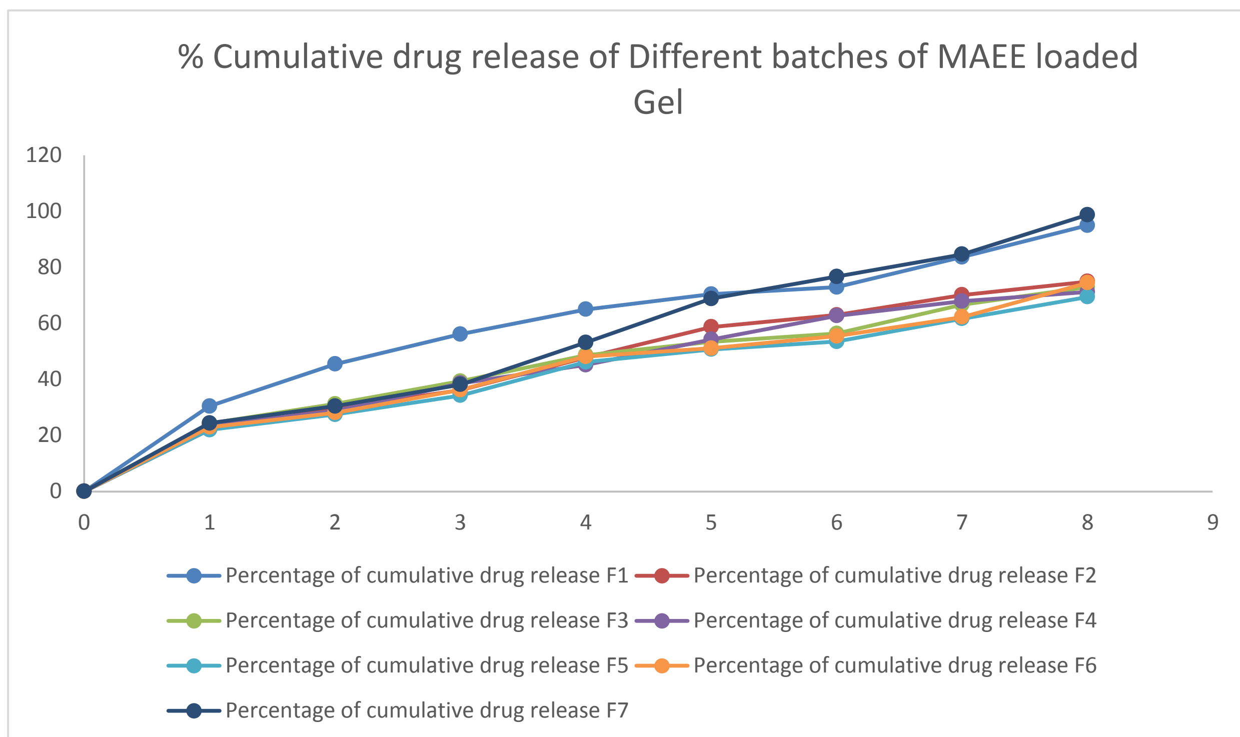

Fig. 6.1: % Cumulative drug release profiles of prepared topical gel

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Table 6.4: % Cumulative drug release through Different batches of CTEE loaded Gel

Time	**Percentage of cumulative drug release**						
Hrs	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**
0	0	0	0	0	0	0	0
1	30.41	22.02	24.30	23.53	21.98	22.91	24.33
2	45.437	29.62	31.22	28.71	27.42	28.05	30.42
3	56.067	36.13	39.28	38.57	34.21	36.20	38.12
4	64.925	47.81	48.61	45.15	46.18	48.10	53.14
5	70.353	58.67	53.43	54.22	50.66	51.09	68.77
6	72.892	62.92	56.32	62.64	53.47	55.41	76.63
7	83.631	70.09	66.62	67.85	61.65	62.17	84.58
8	94.890	74.88	72.88	71.16	69.37	74.42	98.69

Pharmacokinetic model

Drug release data obtained from the optimal batch was subjected to release pattern analysis, which showed that the formulation F7 followed Higuchi model as the regression coefficient value of 0.988 was very close to 1 as shown in Table and fig. to.

Table 6.5: Pharmacokinetic modelling of Optimized batch of MAEE (F7)

Models	**R^2= Regression coefficient**
Zero order	0.891
First order	0.985
Higuchi model	0.988
Korsmayer papas	0.663

Zero order

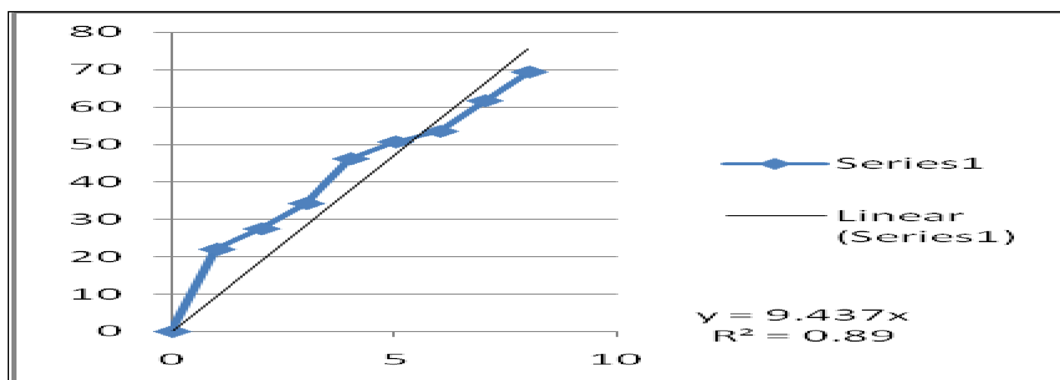

Fig 6.2: Zero order plot for optimized microsponges batch F-7

First order

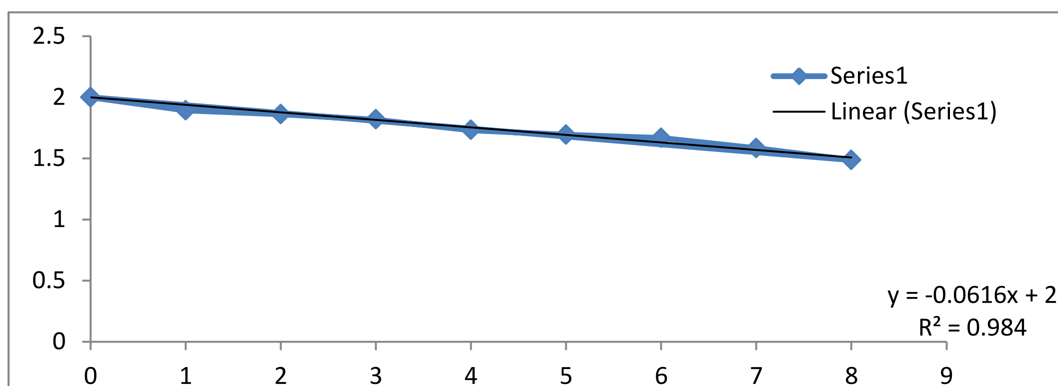

Fig 6.3: First order plot for optimized microsponges batch F-7

Higuchi

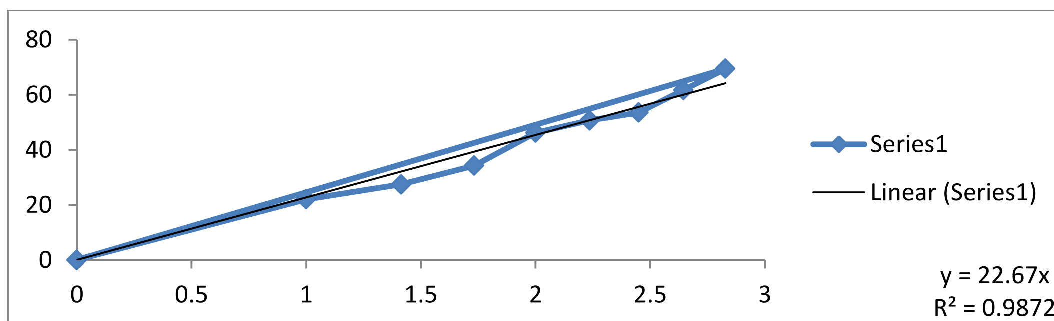

Fig 6.4: Higuchi model for optimized microsponges batch F-7

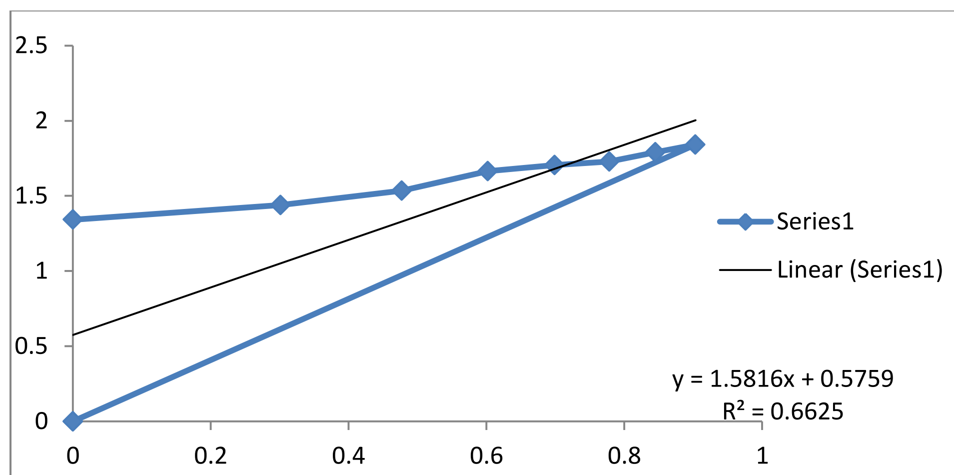

Fig 6.5: Korsmayer papas plot for optimized microsponges batch F-7

In-vivo anti-inflammatory using carrageenan

The anti-inflammatory study suggests that formulation F7 was superior to that of all formulation gel. Therefore it was proved that sample (F7) is more effective in the treatment of inflammation.

Table 6.6: Measurement of carrageenan-induced paw edema volume in rats treated with topical formulations

Treatment	Paw volume(ml)a (Percentage inhibition of edema)			
	1h	2h	3h	4h
Control (gel base)	1.21±0.03	1.3±0.18	1.23±0.02	1.09±0.03
Diclofenac gel	0.71±0.08** (43.09)	0.54±0.03** (56.17)	0.38±0.03** (70.15)	0.27±0.08** (76.02)
Optimized-F7 formulation	0.92±0.04** (26.70)	0.75±0.04** (37.60)	0.53±0.05** (59.79)	0.62±0.03** (47.35)

Values are expressed as mean ± SEM (Number of animals, n=6); one-way analysis of variance (Anova) followed by Dunnett's test. Probability values of 0.05 ($p<0.05$) or less were considered statistically significant; p**<0.01 p***<0.001 Vs control.

Treatment with formulation **F7** showed 58.79% reduction of paw edema when compared to the control group at 3hrs after from carrageenan injection. Optimized formulation F7 of CTEE significantly reduced the paw oedema during period of experiment in comparison to control group ($p<0.01$).

Optimized formulation F7 of CTEE containing combination 1% carbopol 934 and 1% HPMC K4M was found to be excellent for skin application for the treatment of anti-inflammatory. Poly phenolic compounds CTEE presents in ethanol extract of *Cascabela thevetia* leaves. It is key components for producing anti-inflammatory activity. It may act as antioxidant and potential inhibitors of cyclo-oxygenase, lipoxygenase and nitric oxide synthase. This gel can be effective for various skin diseases such as abscesses, boil, burns and eczema. Consequently, CTEE bioactive compounds containing is appropriate for topical application. It can offer multiple benefits for skin diseases along with treating inflammation.

Stability

The stability studies were carried out for formulation F7 only. The formulation F7 were kept at two different temperatures 4 ± 2°C and 30 ± 2°C, 65 RH, for three months. Stability studies results showed that there is no significant change in viscosity and drug content. It represented that prepared gel formulation of CTEE were found to hold good stability on storage up to 3 month.

Antiacne activity of developed topical formulation

The antibacterial activities of optimized topical formulation were measured using modified agar well diffusion method. In this method, nutrient agar plates were seeded with 0.2 ml of 24 h broth culture of *Acne vulgarism*. The agar plates were allowed to solidify and a sterile 8 mm borer was used to cut wells of equidistance in each of the plates. 0.5 ml of formulations and marketed clindamycin gel were introduced into the wells. The plates were incubated at 37°C for 24 hours. The antibacterial activities were evaluated by measuring the zones of inhibition was found to be C (-ve control) 12±0.32 mm, Optimized topical formulation 23±0.72mm and Marketed product26±0.22mm respectively (Sahu et al., 2019). The results of antibacterial evaluation are shown in Table.

Table 6.7: Observations of *in vitro* anti acne activity

Sample code	Zone of inhibition diameter (mm) ± SD
C (-ve control)	12±0.32

Optimized topical formulation	23±0.72
Marketed product	26±0.22

Standard deviation means n =3.

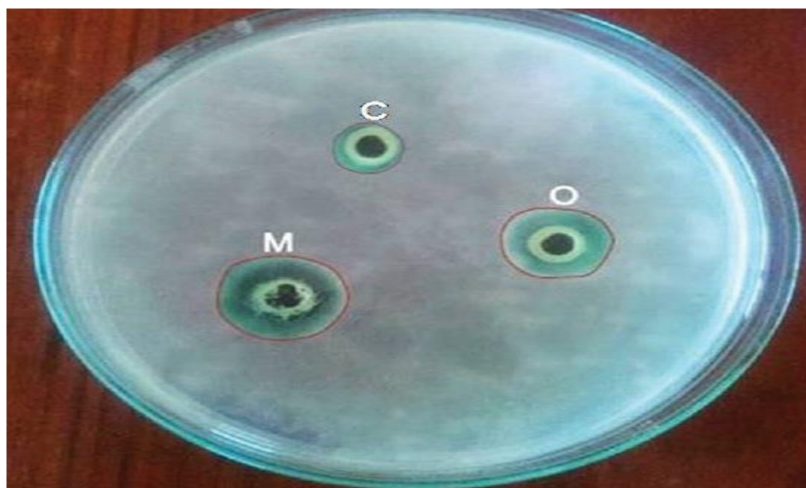

Fig 6.6: In vitro anti-acne activity against *Acne vulgarise*

The formulation F7 was showed satisfactory physical properties and drug release profile. The drug release pattern was comparable to marketed gel formulation. The optimized formulation F7 showed good anti-inflammatory and antiacne property which indicate that CTEE bioactive compounds can be used for other skin disease. Researchers need to do more research to find out other application of its compounds. This topical formulation can be the best option for the marketed gel containing NSAID's or SAID's

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CONCLUSION

The ethanolic extract of *Cascabela thevetia leaves* part yield was found to be 17.8%. Prepared formulation F7 viscosity of gel formulation containing carbopol-934 and HPMC K4M as gelling agent was found to be excellent with good spread ability than gels than other formulations. Drug content of all the formulation was found to be more than 97.6%. All the prepared optimized

formulation showed good physical properties such as wash-ability and skin irritation property, extrude-ability determination of formulations, color, homogeneity, Ph, spread ability and drug content %. The physical properties of prepared herbal topical formulation physical properties were found to be batter F7 than the other prepared formulation. Therefore F7 was used further research work study. Viscosities of all prepared formulations were estimated by using spindle 7. The results are shown in the table. The formulation F7 showed optimum viscosity 4621±.02 centipoises. In vitro bioactive compound CTEE drug release of topical gel formulation F7 gel was shown that the drug release profile was controlled. Formulation F7 was established to be optimal since it presented greater controlled release (98.6%) in 8hrs as opposed to previous formulations. Drug release data obtained from the optimal batch was subjected to release pattern analysis, which showed that the formulation F7 followed Higuchi model as the regression coefficient value of 0.988. Optimized formulation F7 of CTEE containing combination 1% carbopol 934 and 1% HPMC K4M was found to be excellent for skin application for the treatment of anti-inflammatory. Poly phenolic compounds CTEE presents in ethanol extract of *Cascabela thevetia* leaves extract. The formulation F7 was showed satisfactory physical properties and drug release profile. The drug release pattern was comparable to marketed gel formulation. The optimized formulation F7 showed good anti-inflammatory and antiacne property which indicate that CTEE bioactive compounds can be used for other skin disease. Researchers need to do more research to find out other application of its compounds. This topical formulation can be the best option for the marketed gel containing NSAID's or SAID's

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