Formulation of Topical Gel Containing *Cymbopogon Citratus* Oil & Evaluation of its Antimicrobial Activity

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Abstract **- Since ancient era, herbal- or plant-based medicines has been used for the prevention, cure & mitigation of diseases. The volatile oil obtained from the fresh leaves of the plant is one of the most important essential oils in the food, cosmetics, and pharmaceutical industries. Considering these advantages, herbal gel containing *Cymbopogon citratus* oil was prepared. Solvent extraction method was used for extraction of essential oils. After extraction, some of physicochemical properties of the extract were determined .Then topical gel was prepared by taking carbopol 934 ,triethanolamine methyl and propyl paraben ,propylene glycol and three different measured amount of *Cymbopogon citratus* oil was incorporated to gel and labeled as F1,F2 and F3 and evaluated for physical parameters and *in-vitro* antimicrobial activity against *E.coli*. The formulation F3 shows best formulation for antimicrobial activity than F1 and F2.**

Index Terms **- *Cymbopogon citratus* oil, antimicrobial activity, carbopol 934.**

I.INTRODUCTION

Since ancient era, herbal- or plant-based medicines has been used for the prevention, cure & mitigation of diseases and time to time more and more herbal constituents of these natural sources are get enhanced. In research scientist have been proved newer compounds from the herbal to treat many infectious diseases. Reports shows that most of the medicinal plants possess antimicrobial, antioxidant, and anti-inflammatory properties, which has been used in the prevention of many infectious diseases, and also and thus beneficial for the society[1] The current scenario of infectious diseases emphasis that there has been an lighten increase in emerging infectious diseases.[2]The main task toward scientist is the development of resistance to the antibiotic in clinical use. Therefore, development a natural formulation is best option to act against the microorganisms develops skin diseases. Although a lot of pharmacological investigations have been showed that lemongrass oil *Cymbopogon citratus* belonging to family has /anti-amebic[3], antibacterial activity[4], ant-filarial Activity and antifungal Activity [5]antimycobacterial Activity[6] Antinociceptive Effect[7]. The volatile oil obtained from the fresh leaves of the *Cymbopogon citratus* is one of the most important essential oils in the food, cosmetics, and pharmaceutical industries. Gels are defined as substantially dilute cross linked system, which exhibits no flow when in the Steady state. Topical gel preparation has remained one of the most popular and important pharmaceutical dosage form. As a result the therapeutic effects can be avoided or minimized. Example of drug commonly prepared in topical gel from includes gastrointestinal Irritating non-steroidal anti-inflammatory drugs and antibacterial, Antifungal. For topical treatment of dermatological disease as well as skin care, a wide variety of vehicles ranging from solids to semisolids and liquid Preparations is available to clinicians and patients. Within major group of semisolid preparations, the use or transparent gels has expanded both in cosmetics and in pharmaceuticals preparations. Therefore, the present study was aimed to develop and evaluate topical Gel containing *Cymbopogon citratus* oil & Evaluation of its antimicrobial activity.

II. CHEMICALS AND REAGENTS

Carbapol 934, triethanolamine, propylene glycol, were obtained from Loba Chemie Pvt. Ltd, Nagpur, India. Methyl Paraben, Propyl Paraben were obtained from Samar Chemical Pvt. Ltd, Nagpur, India. Glycerin was obtained from Apurva Chemicals, Gondia, India.

III. SAMPLE COLLECTION

The lemongrass (*Cymbopogon citratus*) leaves was collected from Bajiroji Karanjekar agriculture campass & its authenticity was done by Dr. A. A, Jagia, Professor and head of the department of botany, Sakoli. Fresh lemongrass (*Cymbopogon citratus*) leaves were cut with 6 cm height from the root in the morning due to the volatility of the aromatic compound in the lemongrass leaves. After collecting, the plant material, it was partially dried at room temperature for maximum of 4 days, then kept in a seal plastic bag at ambient temperature and protected from sun light. The lemongrass leaves (*Cymbopogon citratus*) were reduced in size by using a knife and crushed using mortar for size reduction since extraction yield increases as the particle size decreased.[8]

IV. MOISTURE CONTENT DETERMINATION

The moisture content of lemongrass was determined from 5g fresh sample which was dried at 100-105 ^{0}C in oven for four hours till the sample becomes gray in color. Then it was cooled in dessicator and measured in a digital balance.

Moisture content of the lemongrasses sample was measured in the following equation. [9]

$$Moisture\ \% = \frac{W1-W2}{W1} \times 100$$

Where: W1 is the original weight of the sample before drying and W2 is weight of the sample after drying

V. PROCEDURE FOR SOLVENT EXTRACTION OF ESSENTIAL OILS

500 g of lemongrass (*Cymbopogon citratus*) crush powder was weighted and was placed in a clean round bottomed flask and about 700ml of N-hexane solvent was poured into the flask. Then it was allowed to stand for 48hours with continuous shaking with electrical shaker. After the limited time was completed, the extract containing the solvent was decanted into another beaker. This includes highly volatile aroma molecules as well as non-aroma waxes and other lipids. The solvent was removed from the extract using rotary evaporator at 40 ^{0}C. The waxy mass that remains is known as the concrete. The concentrate is further processed to remove the waxy materials which dilute the pure essential oil. To prepare the essential oil the waxy concentrated extract was stirred with alcohol (ethanol). During the stirring process only essential oils were dissolved in ethanol but not the waxy substances. As a result two layers were formed and separated easily using separatory funnel. Ethanol was then evaporated at 78^{0}C and the yield of oil was determined as the different between the final weight of the beaker with extract and the initial weight of the empty beaker.

VI. DETERMINATION OF THE YIELD OF LEMONGRASS OIL

The yield of extracted essential oil was calculated using the following equation.

$$\text{Percentage yield} = \frac{Weight\ of\ oil}{weight\ of\ lemon\ grass} \times 100\ \%$$

VII. PHYSICOCHEMICAL PARAMETERS OF LEMONGRASS OIL

Physicochemical characterization determines the physical and chemical properties of the extracted oil like pH, boiling temperature, solubility, acid value, iodine value, saponification value etc.[10].

A] Determination of pH

4g of the lemongrass oil was poured into a clean dry 50ml beaker; and then, 25ml of distilled water was added into the beaker and heated on hot plate till boiling with slow stirring and left to cool down. Then it was filtered into 25ml volumetric flask and filled with distilled water to the mark and was determined by using a calibrated pHmeter.[11]

B] Boiling temperature

25ml of lemongrass oil was placed into borosilicate glass and a thermometer was inserted and placed on the heating mantle, and the oil in the borosilicate was started to be circulated which leads the boiling of oil and the temperature on the thermometer was recorded.

C] Solubility

The solubility of lemongrass oil was analyzed by adding 1g of lemongrass oil sample into 10 ml of 70% ethanol and water respectively and the solubility was observed.

D] Saponification value

1g of lemongrass oil was accurately weighed and dissolved in 50ml of 2.5 N of potassium

Hydroxide solution. This procedure was performed together with blank experiment which was without the oil. The mixture was refluxed for two hours and cooled. The unreacted KOH was titrated with standard 0.5 N of oxalic acid by adding 2-3 drops of phenolphthalein indicator until it became colorless. After that, the saponification value was determined using the following equation [12].

Saponification value = $\frac{56\,(V1-V2)}{2XW}$

Where: W is the weight of oil, V1 is the volume of 0.5 N of oxalic acid for blank. V2 is the volume of 0.5 N of the oxalic acid for sample.

E] Acid value

50ml of neutral ethyl alcohol was heated with 5g of oil sample in a 250ml beaker until the mixture began to boil. The heat was removed and was titrated with 0.1M KOH solution, using two drops of phenolphthalein indicator with continuous shaking and finally a permanent pink colour was obtained at the end point.

Acid value = $\frac{56.1\,(Number\ of\ ml\ consumed}{W} \times 0.1\ M\ NaOH$

Where: 56.1 is molecular weight of KOH, M is Morality of KOH and V is volume of KOH used and W is weight of the sample

VIII. PREPARATION OF GEL

Carbopol 934 gels were prepared by adding carbopol 934 in water and by neutralizing with triethanolamine to pH 6.4. Weighed amount of methyl and propyl paraben were added to the water prior to the addition of carbopol 934. In another beaker, the required quantity of propylene glycol was taken in another test tube to which accurately measured the amount of Lemon grass oil corresponding to its MIC was incorporated and finally this mixture was added to the beaker containing carbopol with stirring. The glycerin was also added to the polymer dispersion and stirred continuously till it forms a homogenous product. The volume was made up with distilled water and stirring was done vigorously. [13] The same method was adopted to formulate three different formulations by taking 0.8, 0.9 and 1 ml of lemongrass oil and labeled as F1, F2 and F3.All the prepared gels were then subjected to evaluation tests. The composition of different gel formulations is listed in Table.2.

Table 1: Composition of different gel formulation.

Ingredients	F1(quantity in ml)	F2(quantity in ml)	F3(quantity in ml)
Lemon grass oil	1	1.5	2
Carbapol 934	0.5	0.6	0.8
Poly ethylene glycol	15	15	15
Glycerin	5	5	5
Methyl paraben	0.18	0.18	0.18
Propyl paraben	0.02	0.02	0.02
Distilled water	q.s	q.s	q.s

IX. EVALUATION OF GEL FORMULATION[14,15]

A] Physical appearance

Color and odor was evaluated visually.

B] Determination of pH

It was evaluated using a digital pH meter. The pH of the gel was measured by dropping the glass electrode into the formulation.

C] Determination of viscosity

The viscosity of the prepared gels was determined using Brookfield viscometer by using spindle no. 64 at 10 rpm and temperature of 25ºC.

D] Determination of spreadability

It was evaluated via using a glass slide and wooden block apparatus. The gel formulation (1 g) was kept on a preset glass slide and another movable glass slide was placed over the first glass slide and 50g weight was added to the slide for 5 minutes. The time used for the separation of slides was noted. It can be measured by using the given formula:

S = M× $\frac{L}{T}$

Where, S = spreadability in g.cm/s

M = mass in grams

T = time in seconds.

E] Determination of homogeneity

The homogeneity of formulated gels was evaluated visually. The formulations were evaluated for appearance and existence of aggregates.

F] Determination of antimicrobial activity

Agar cup plate method was used for screening of antimicrobial activity of gel containing lemon grass oil. All formulations of gel were placed aseptically in cups of agar plate which was previously inoculated with culture. The plates were left at ambient temperature for 30 mins prior to incubation at 37◦C for

24 hrs. The broad-spectrum antibiotic i.e., tetracycline was used as positive control for obtaining comparative results. Plates were observed after 24-48 hrs incubation for the appearance of the zone of inhibition. Antimicrobial activity was evaluated by measuring the diameter of zones of inhibition (millimeters) of microbial growth.

X. RESULT & DISCUSSION

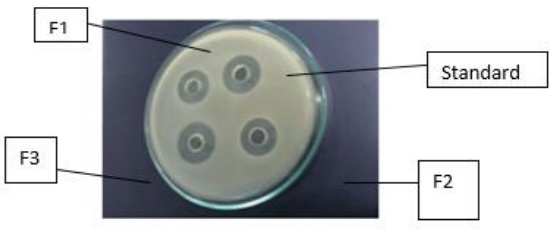
Physicochemical analysis for plant extracts is very essential to decide the validity and way of using extract in appropriate manner. According to this logic, the n-hexane extract of lemongrass essential oil has characterized with different physicochemical parameters. The result shows that moisture content of lemongrass leaves was 20.7%.The result for phycochemical parameter for lemon grass oil is shown in following table no. 2

PH	BOILING TEMPERATURE	SOLUBILITY	ACID VALUE	SAPONIFICATION VALUE ETC
5.95	215°c	dissolved in 70 % of ethanol but not in water	2.103	138.2

Physicochemical characteristics for the formulation (F1, F2 and F3) of gels was examined and results are shown below Table no.3.

Evolution parameter	Formulation (F1)	Formulation (F2)	Formulation (F3)
Colour	Pale yellow	Pale yellow	Pale yellow
Odour	Aromatic	Aromatic	Aromatic
PH	5.5	5.4	5.4
Viscosity	36548	34569	32856
Spreadability (g cm/s)	44.8	28	25.5
Homogeneity	good	good	good

Formulation F1, F2 and F3 are evaluated for its in-vitro antimicrobial activity by using standard tetracycline .The results are shown in following figure no, 1.



XI. CONCLUSION

Accords to literature survey and current investigation we found that the oil obtained from leaves of plant lemongrass *Cymbopogon citrates* possess potential antimicrobial activity. Out of the formulated gel preparation F3 gel was less good in appearance and showed better antimicrobial effect than other gel formulations. Therefore our study will definitely open up a scope for future utilization of lemongrass oil for therapeutic purposes and can be further used commercially to develop topical gels after conducting clinical trials on human beings.

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