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Evaluation and Formulation of Anti-Microbial Gel Using Lavender Oil and Rosemary Oil

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ABSTRACT

Introduction: Salvia Rosemary is a medicinal herb of great importance for traditional applications against several kinds of disease. Because of the range of bioactive compounds, the genus of Salvia Rosemary is regarded as one of the most significant genus utilised in many medical systems. This plant was chemically investigated for its bioactive compounds in the current analysis.

Materials and Methods: The essential oil of Rosemary extract by using clevenger apparatus with the help of distillation method.

Result: The anti-microbial gel was evaluated for appearance and homogeneity, grittiness, pH, viscosity, particle size etc. The anti-microbial gel was white in colour which feels smooth on application and have aromatic odour. The antimicrobial activity of herbal gel was compared with standard and it's found to be less effective.

Conclusion: It was inferred from the result that anti-microbial gel was good in appearance, homogeniety and easily spreadable. The anti-microbial gel was prepared using gelling agent like carbopol 934 along with polyethylene glycol and the formulation were subjected to various evaluation parameters. The pH of all three formulations were in the range of the pH of the skin. The Antimicrobial gel was less effective then standard gel as they have rosemary oil and lavender oil as their key ingredients because of their anti-microbial property.

Keywords- Salvia Rosemary, Antimicrobial gel.

I. INTRODUCTION

Rosemary (Rosmarinus officinalis L.) is an evergreen brush belonging to the Lamiaceae family. In natural conditions, it can reach from approx 1 m to even 2.5 m in height (1). Which is widely distributed in various regions of the world like in America, Europe, Asia (2). Two distinct ecotypes of *Rosmarinus officinalis*, namely Cevoli and Lunigiana, were cultivated in the littoral area near Pisa, in northern Tuscany, Italy (3).

This species is a widely grown plant that is valued for its nutritional content and pharmacological qualities, which have made it well-known in regional and traditional medicine (4). Because of its antibacterial and antioxidant properties, it is employed as a food flavouring and preservative in the food industry. Additionally, R. officinalis is utilised in cosmetics (5).

The two ecotypes were distinguished by their distinct yield and essential oil composition of leaves, flowers, and stems, which were obtained from different positions of the plants. Compared to the Lunigiana ecotype, the Cevoli ecotype plant yielded the largest amount of dry matter (6).

Typically, Soxhlet extraction or traditional maceration were used to create extracts with non-volatile chemicals (7). They are made up of several chemical substances, including ketones, alcohols, hydrocarbons,

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phenols, aldehydes, and esters. Furthermore, the phytochemical content and advantageous qualities of the extract are influenced by the parameters and extraction technique selected (8).

The present study examined the chemical profile of eighteen wild populations of rosemary spread throughout the Tyrrenian Sea and its environs (9). In order to reduce the possible impact of geographic surroundings on volatile composition (phenotype), which might obscure the real genotypic differences, the populations were first farmed for two years in a homogeneous environment (10)

One of these plants is rosemary (*Rosmarinus officinalis*), a plant with medicinal properties [**8**], of which extracts appear in the composition of hundreds of cosmetics. A Google search of the words "*Rosmarinus*" and "cosmetics" returns approximately 2,390,000 results (as of August 2020). In this search, it was observed that derivatives of rosemary are formulated in essential oils for massages and aromatherapy, rosemary alcohol, gels, shampoos, soaps, rosemary water, cleansing milk, deodorant, anti-wrinkle cream, aftershave lotion, hydrating facial cream, cream for the eye contour area, etc.

Since 2000, an average of 120 papers have been published per year dealing with various aspects of rosemary [**9**]. The latest reviews demonstrate its phytochemical, biological, and nutritional properties [**9**,**10**]; its anti-inflammatory power [**11**]; its power as a therapeutic and prophylactic agent [**12**]; and its topical applications [**13**].

Gels are semisolid formulations meant to be applied to the skin or to mucous membranes that are accessible, such as those in the mouth (11). Gels are made up of two interpenetrating systems in which the colloidal particles also referred to as the gelator or gellant—are evenly dispersed throughout a solvent or dispersion media to produce a three-dimensional matrix called the gel (12).

The goal of the current study was to create an anti-microbial gel composition using *rosemary* and lavender oils (13). Escherichia coli and Enterobacter cloacae are two examples of foodborne bacteria that are susceptible to the potent antibacterial properties of linalyl acetate and linalol, the active ingredients in lavender oil (14). Other chemicals found in essential oils, such limonene, αpinene, and β-pinene, have the ability to fight many pathogenic germs that affect humans by having antibacterial properties (15).

Rosemary oil demonstrated the lowest inhibitory concentration against Escherichia coli. The antibacterial properties of *rosemary* oil have been demonstrated in several investigations against Salmonella choleraesuis, Clostridium perfringens, Bacillus cereus, E. coli, and Staphylococcus aureus (16,17).

II. MATERIAL AND METHOD

Authentication:

Plant of *Rosemary* were gathered from krishna nursery, balupur near flyover (Dehradun Uttarakhand, india) and certified by a botanist called botanical survey of india by northern regional centre,192, kaulagarh road P.O, -KDMIPE Dehradun -248195, Uttarakhand, india.

Extraction:

To extract the essential oil by clevenger apparatus was placed in a round bottom flask column, of which the lower and higher parts were connected to a water flask and a condenser, respectively. The water vapour produced in the flask crosses the plant, charged with essential oil then to the condenser, where it is condensed. After condensation, the oil is separated from water by decantation.

Figure: 1 Extraction of the essential oil

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Procedures for antimicrobial gel preparation

The antimicrobial gel is prepared After being continuously stirred throughout the dispersion of carbopol 934 in 05 ml of distilled water, the mixture was allowed to solidify overnight. To the aforesaid combination, add additional lavender oil, rosemary oil, ethanol, and PEG 400. Then, add the remaining distilled water to bring the amount up to 10 millilitres. A smooth anti-microbial gel was formed by thoroughly mixing all of the ingredients with carbapol 934. Lastly, several formulations are created to alter the needed pH to between 4.5 and 5.5 in order to create the necessary consistency in the gel. Several assessment criteria were applied to the antimicrobial gel that had been manufactured.

Formulation of Anti-Microbial Gel

Table 1: Depicting formulation of antimicrobial gel

S. No.	Ingredients	F1	F2	F3
1	Lavender oil	2ml	2.5ml	3.5ml
2	Rosemary oil	1ml	1.5ml	2ml
3	Carbopol 934	0.2gm	0.21gm	0.25gm
4	Ethanol	0.5ml	0.8ml	0.9ml
5	PEG 400	0.5ml	0.8ml	0.9ml
6	Distilled water	10ml	12ml	15ml

Formulation:1

Formulation: 2

Formulation: 3

Figure 2: Formulation of antimicrobial gel

Evaluation of anti-microbial gel

Physical Evaluation: The prepared antimicrobial gel was examined for colour, homogeneity, consistency, and olfactory appearance. It was also examined for tactile (feel) and tactile appearance (smelling) (18).

pH: By using a pH metre, the pH was found (19).

Viscosity: By using a Brookfield viscometer, the viscosity of antimicrobial gel is determined (20).

Spreadability: The spreadability of a material is determined by measuring the time it takes for two slides to separate from the gel and be placed in between each other under a specific load. The shorter time it takes for two slides to separate, the more spreadable the material. The following formula is used to compute it:

$$S = M. L / T$$

Where, M = weight tied to upper slide
L = length of glass slides
T = time taken to separate the slide (21).

Stability Studies: A three-month duration of varying temperatures was used to conduct stability tests on anti-microbial gel with regards to pH and physical stability.

TEM (Transmission Electron Microscopy): It is use to determine a morphology of anti-microbial gel formulation by the help of TEM. It is done from NIPER, Mohali.

Antimicrobial study

Test organism

Gram positive bacteria: Staphylococcus aureus

Gram negative bacteria: *E.coli* Standard: Povidone iodine.

III. RESULT AND DISCUSSION

Physical evaluation: All the prepared gels using different polymers in different concentrations were light yellowish in colour which feels smooth on application. The prepared gel have an aromatic odour.

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Table 2: Physical evaluation results of formulation F1, F2, F3

Formulation Code	Appearance	Feel on application	Odour
F1	Light yellowish	Smooth	Aromatic
F2	Light yellowish	Smooth	Aromatic
F3	Light yellowish	Smooth	Aromatic

pH: All four of the formulations had pH values between 4 and 5.5, which are in the range of the skin's pH. Range displayed in the table.

Table 3: pH results of formulation F1, F2, F3

S. No.	F1	F2	F3
Ph	4.5	4.7	4.8

Viscosity: Viscosities of the gels were measured by the Brookfield viscometer in centipoises. The viscosity of different formulations at different rpm are given below:

Table 4: Viscosity results of formulation F1, F2, F3 at different RPM

S. No.	F1	F2	F3
RPM	2.8	7	12
VISCOSITY	860	350	210

Spreadability: All the prepared gels using different polymers in different concentrations were spreadable. The formulation F-1 showed the maximum Spreadability followed by second and third.

Anti-microbial study: As the zone of inhibition of the herbal gel was found to be better than against both the bacteria S. aureus and E. coli, the antibacterial activity of the gel was found to be superior to that of ordinary Povidone iodine. The values are listed in Table No. 02. Comparing the F-3 gel formulation with the commercial formulation, it was discovered to have the best antibacterial action.

Table 5: Zone of inhibition of different gel formulation

Test organism	Standard	F1	F2	F3
S.aureus	2.7cm	2.5cm	2.6cm	3cm
E.coli	2.5cm	3.4cm	2.5cm	3.4cm

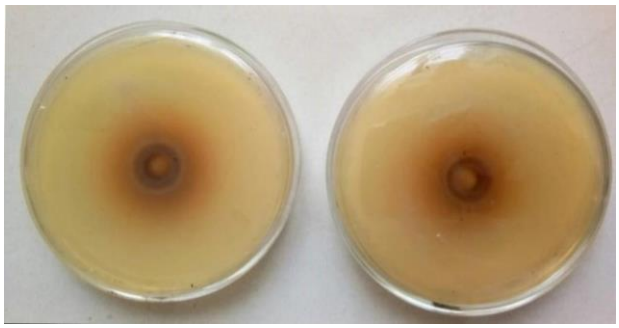

Figure 3: Zone of inhibition of standard (Povidone iodine) for S.aureus and E.coli

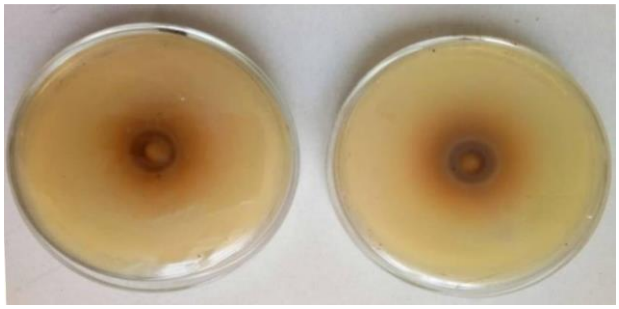

Figure 4: Zone of inhibition of F-1 for E.coli and S.aureus

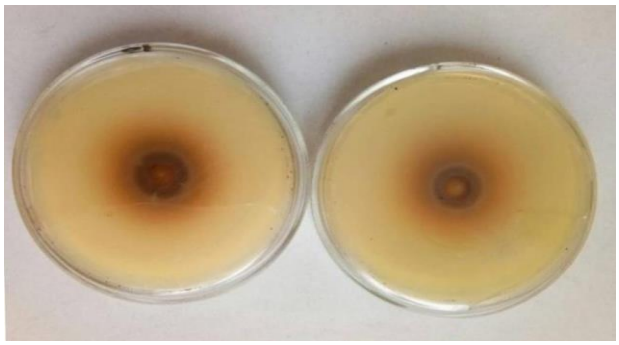

Figure 5: Zone of inhibition of F-2 for E.coli and S.aureus

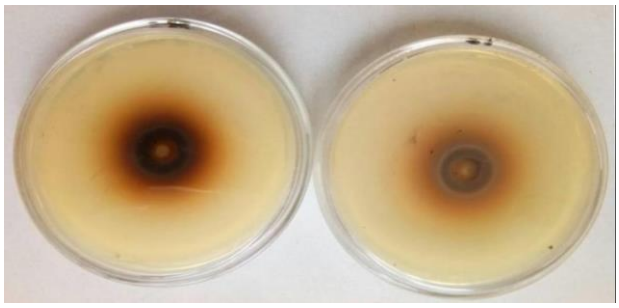

Figure 6 Zone of inhibition of F-3 for E.coli and S.aureus

Transmission Electron Microscopy

The morphology of hydrogel was analysed by the help of transmission electron microscope. The result are shown in figure given below. Most of the particles were spherical with only few irregular shaped particles.

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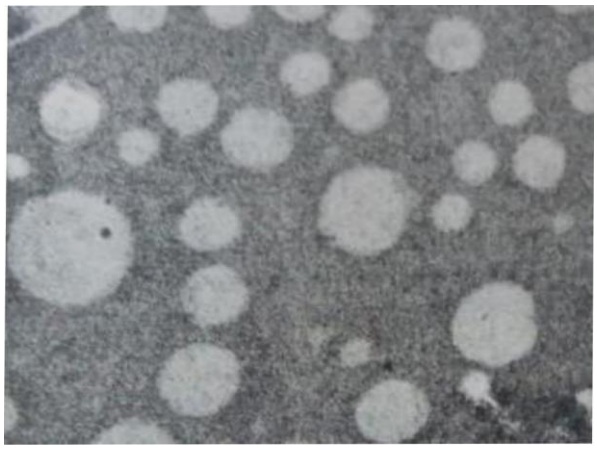

Figure 7: TEM image of formulation F1

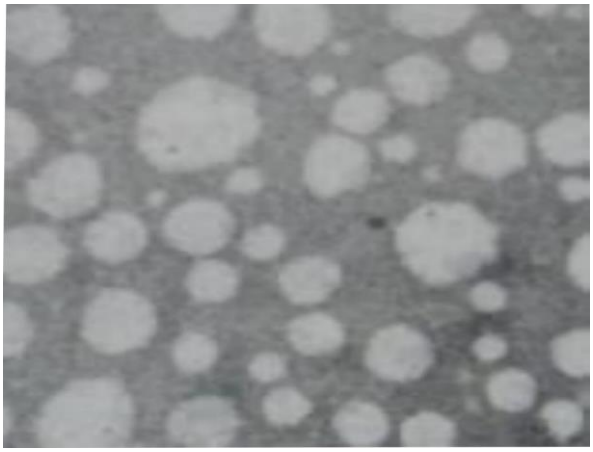

Figure 8: TEM image of formulation F2

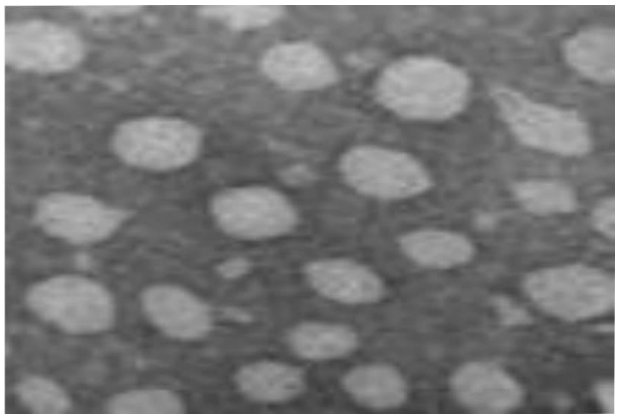

Figure 9: TEM image of formulation F3

Stability study: The created gel compositions' appearances have not changed after one month of storage at room temperature, in a humidity chamber, and at a refrigerated temperature.

The colour of F-3 gel does not fade in a refrigerator after 30 to 90 days under various storage settings, unlike other formulations that were unstable in their state and caused the pH to drop. In comparison to previous formulations, formulation F-3 was more stable and the colour did not shift.

Table no. 06: Effect of temperature on (pH) stability of Anti-microbial gel

S.No.	Storage conditions	C-1	F-1	F-2	F-3
1	Room Temperature	4.05	4.07	4.14	5.05
2	Humidity Control	3.95	4.00	4.08	4.15
3	Refrigration Temperature	4.05	4.10	4.12	4.22

IV. CONCLUSION

Based on the results, it was concluded that the anti-microbial gel had an excellent look, homogeneity, and absorbency. The formulation was evaluated according to a number of criteria after the anti-microbial gel was created using polyethylene glycol and a gelling agent such as carbopol 934. With a Brookfield viscometer, the viscosity of the antimicrobial gel was determined. Each of the three formulations had a pH between 4 and5.5, which is the range of the skin. Since rosemary and lavender oils are main constituents in the anti-microbial gel due to its anti-microbial properties, the antimicrobial gel worked better than the commercial gel.

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