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Research Article

FORMULATION AND EVALUATION OF *IN-VITRO* ANTIMICROBIAL ACTIVITY OF GEL CONTAINING ESSENTIAL OILS AND EFFECT OF POLYMER ON THEIR ANTIMICROBIAL ACTIVITY

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ABSTRACT

In the present study an attempt was made to formulate gel containing different plant extracts and essential oils and was evaluated for antimicrobial activity against different microorganism. The plants were collected and extracted with suitable solvent. Different gels were formulated by using different concentration of polymers and extracts. The evaluation was done using cup plate method for zone of inhibition and two fold dilution method for MIC (Minimum Inhibitory Concentration). Minimum Bactericidal concentration was also calculated. Sensitivity of microorganism to marketed products was also studied. MIC for antimicrobial activity of plant extracts and essential oils were studied prior to gel formulation to compare the changes in activity after incorporation in polymer gel. Propylene glycol as the co-solvent for the extract and Carbopol 940 as gelling agent showed the best results in final formulations. Gels were further subjected to evaluation of physical properties like colour, clarity, pH, consistency, Spreadability and viscosity . Zone of inhibition was between 15-23 for *S. aureus*, 18-22 for *E. coli*. 15-25 for *A. niger* and *A. varis*. For *Candida albicans*, zone of inhibition was 14-20. MIC for most of the microorganism was below 20mg/ml. Activity of plant extracts was not much affected by incorporation in gel. The gel showed promising antibacterial and antifungal activity against other strains used for the study. The gel was stable at room temperature.

Keywords: Antimicrobial activity, essential oil, gel, MIC, MBC.

INTRODUCTION

Antibiotic and multi-drug resistance is a world-wide problem in hospitals, long-stay residential centres and in the community[1]. A significant number of new therapeutics is derived from natural sources including plants, as novel drugs (systemic and topical) and antiseptics, to replace or to be used in conjunction with existing products[2,3]. Plant extracts have been developed and proposed for the same purpose in foods as natural antioxidants and/or antimicrobials. Essential oils, methanol and ethanol extracts are known to possess antimicrobial activity[4-6], *Elettaria cardamomum* as plant of Zingiberaceae family, has its seeds used commonly.

The seed and the essential oil are used as a flavouring component in a variety of foods. Seeds of *E. cardamomum* have an antibacterial activity for gram-negative bacterium (Mahady et al., 2005). Coriandrum sativum L. (Umbelliferae/Apiaceae), popularly known as coriander is medicinally believed to have several therapeutic properties: hypoglycaemic (Otoom, Al-Safi, Kerem, & Alkofani, 2006; Waheed, Miana, Ahmad, & Khan, 2006), anti-inflammatory and hypolipidaemic (Chithra & Leelamma, 1997; Chithra & Leelamma, 2000; Lal, Tkumar, & Pillai, 2004), analgesic and sedative (Chaudhry & Tariq, 2006; Emamghoreishi & Heidari-Hamedani, 2006), anxiolytic (Emamghoreishi, Khasaki, & Aazam, 2005), diuretic (Benjumea, Abdala, Hernandez-Luiz, Pérez-Paz, & Martin-Herrera, 2005; Maghrani, Zwggwagh, Haloui, & Eddouks, 2005), antioxidant (Melo, Bion, Filho, & Guerra, 2003; Ramadan, Kroh, & Morsel, 2003). Antimicrobial activity has been reported for the essential oil (EO) extracted from C. sativum seeds against different species of Candida, Gram-positive/negative bacteria, and fungi (Elgayyar, Draughon, Golden, & Mount, 2001; Hammer, Carson, & Riley, 1998; Lo Cantore et al., 2004). Cinnamomum is a genus in the Laurel family, Lauraceae, many of which are studied for their antibacterial activity by using essential oils from leaves and bark[6,7]. Cinnamon, Cinnamomum zeylanicum and cassia, Cinnamomum cassia are rich in essential oils (mainly cinnamaldehyde and eugenol) which can inhibit microbial growth[8-11]. Lavandula genus is an important member of family Lamiaceae. Lavender's essential oil is popular as a complementary medicine. These have been used for centuries as a therapeutic agent, with the more recent addition, the essential oils derived from these plants were widely used as an antibacterial in world war I (Cavanagh & Wilkinson, 2005). Transcarveol, pulegone, camphor and menthol are the main constituents of essential oil of L. Bipinnata[12]. The essential oil of L. Bipinnata showed antimicrobial activity against both bacteria and fungi, therefore it can be used as an herbal medicine[12]. Keeping in mind the activity of these commonly used essential oils, this work was conducted to develop a formulation so as to get a synergistic activity as well as easy to use formulation.

MATERIAL AND METHODS

Materials

The following chemicals were used , Methyl and Propyl paraben, Propylene glycol, PEG 300, Isopropyl alcohol, Glycerin, Triethanolamine, Methanol, Acetone, Ethanol, NaOH, Carbopol 940.

Plant materials

All the plant material and fruits and seeds were obtained from local market.

Preparation of spice extracts

100 gm of *Elettaria cardamomum* and *Cinnamomum zeylanicum* was finely ground and macerated at room temperature in 100% ethanol during 5 days. The extract was subsequently filtered and concentrated to dryness. The residue was dissolved in distillate water to create a concentration of 150 mg/ml of stock solution.

Distillation of essential oil

The essential oil was obtained by the hydro distillation of fresh leaves (7.5 kg) using a Clavenger-type apparatus for 4 hr. The resulting oil/water mixture obtained was extracted using dichloromethane. The organic layer was then separated, dried over Na2SO4, filtered and the solvent removed by means of vacuum evaporation at room temperature, resulting in C. sativum essential oil. The dried leaves of *L. Bipinnata* were extracted using a soxhlet apparatus for 8 h in ether.

Preparation of extracts of camphor

The chips from the leaves and twigs were used separately to extract the essential oils and hot-water extracts by steam-distillation. The mixture of water and essential oils flowed into a collection container

where the 2 phases were separated. The anhydrous sodium sulfate was added into essential oil for absorbing moisture, and then the sodium sulphate was removed by filtration. The hot-water extracts were dried under vacuum

Microorganisms

Microorganisms used were,

Fungi: A. varis, *A. niger*, *P. notatum*,

Bacteria: *E. coli*, *S. aureus*, *P. aeruginosa*, *B. subtilis*.

Preparation of the formulations[13]

PEG 300, isopropyl alcohol and propylene glycol were used as co-solvent for dried extract and propylene glycol was chosen as the best levigator. Table I shows the constituents of the investigated preparations. Carbopol 940 was dispersed in preserved water (methyl paraben 0.18% and propyl paraben 0.02%) and glycerin overnight. The extract was dissolved in propylene glycol and was added to the polymer dispersion and stirred for 10 min, and neutralized by triethanolamine to pH 6.4 and then mixed at 300 rpm for 10 min.

Table 1: Formulation composition of gels

*Formulation	Plant extract (% (w/w))	Carbopol (% (w/w))	Propylene glycol(% (w/w))	Glycerin (% (w/w))
G1	1	0.30	20	5
G2	2	0.30	20	5
G3	1	0.40	20	5
G4	2	0.40	20	5
G5	1	0.50	20	5
G6	2	0.50	20	5
G7	1	0.75	20	5
G8	2	0.75	20	5
G9	1	1.00	20	5
G10	2	1.00	20	5

*Each formulation consists of water (propyl paraben 0.02%w/w and methyl paraben 0.18% w/w) to 100 g. All the formulations were neutralized by triethanolamine to pH=6.8.

Determination of clarity and colour

It was done with naked eyes against white background.

Determination of odour

It was done by mixing gel in water and taking the smell.

Determination of viscosity

Viscosities of the formulated gels were determined using Brookfield Viscometer. Spindle no. 7 and spindle speed 60 rpm at 25° C were used for gels, the corresponding dial reading on the viscometer was noted. Then the spindle was successively lowered .The dial reading was multiplied by the factor given in the Viscometer catalog

Determination of spreadability

Spreadability of formulations was determined by an apparatus suggested by Multimer et al. which was fabricated in laboratory and used for study. The apparatus consist of a wooden block, with a fixed glass slide and movable glass slide with one end tied to weight pan rolled on the pulley, which was in horizontal level with fixed slide.

Procedure

An excess of gel sample 2.5 g was placed between two glass slides and a 1000g weight was placed on slides for 5 minutes to compress the sample to a uniform thickness. Weight (60g) was added to the pan. The time (seconds) required to separate the two slides was taken as a measure of spreadability.

It was calculated using the formula,

$$S = m.l / t$$

Where, S - Spreadability in g.cm / sec; m - Weight tied to upper slide; l - Length of glass slide; t - Time in seconds

Length of glass slide was 11.3 cm and weight tied to upper slide was (60g) throughout the experiment

Determination of antibacterial and antifungal activity

Preparation of inoculums

For evaluation of antifungal activity, 24 hours fresh culture of fungi and bacteria were suspended in sterile water to obtain a uniform suspension of microorganism.

Determination of zone of inhibition

Antifungal and Antibacterial activity was checked by agar well diffusion method. In this method a previously liquefied medium was inoculated with 0.2 ml of Fungal and Bacterial suspension having a uniform turbidity at temperature of 40^{0}C. 20 ml of culture medium was poured into the sterile petri dish having a internal diameter of 8.5 cm. Care was taken for the uniform thickness of the layer of medium in different plates.

After complete solidification of liquefied inoculated medium , the wells were made aseptically with cork borer having 6mm diameter. In each of these plate extract and gel solution was palced carefully. Plates were kept for pre diffusion for 30 mins. After it normalized to room temperature; the plates were incubated at 37ºc for 24 hrs in case of bacteria and at 27ºc for 48 hrs in case of fungi. After incubation period was over, the zone of inhibition was measured with help of Hi-media.

RESULTS AND DISCUSSION

The physiochemical property of gel containing 1% carbopol was most appropriate for the formulation of gel. The spreadability of both 0.75% and 1% carbopol was good. Gel can be used as an effective vehicle for topical administration of herbal extracts and essential oils of plants.

Clarity and colour

Colour of gels was mostly deep brown in colour. Gels were turbid except in case of *E. cardamomum* and *L. Bipinnata.*

Table 2: Clarity and colour of gels

	E. cardamomum	*C. zeylanicum*	*L. Bipinnata*	*Camphor*	*C. sativum*
Colour	Deep brown	Deep brown	Greenish	Deep brown	Deep brown
Clarity	Clear	Turbid	Clear	Turbid	Turbid

Table 3: Odour of gels

	E. cardamomum	*C. zeylanicum*	*L. Bipinnata*	*Camphor*	*C. sativum*
Colour	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic

Determination of odour

All gel formulations was having characteristic odour of extract incorporated.

Determination of viscosity

The gel formulation showed increase in viscosity with increase in concentration of polymer as all other formulation additives were kept constant. Viscosity of 1% gel was optimum and can be used for formulation of herbal gels.

Determination of Spreadability

The spreadability was optimum for formulations G7 to G10. The spreadability of both 0.75% and 1% carbopol was good.

Determination of Antibacterial and Antifungal activity:

All the extract showed activity against the microbial strains used. Lavandula extract showed highest activity against *S. aureus* among other extracts. Cinnamomum showed highest activity against *B. subtilis.* Activity of all extracts against *P. aeruginosa* was between 7-9.

Table 4: Viscosity of gels

Formulation	Viscosity
G1	1.48
G2	1.52
G3	1.90
G4	1.98
G5	2.42
G6	2.47
G7	2.98
G8	3.02
G9	3.52
G10	3.44

Table 5: Spreadability of gels

Formulation	Spreadability (gm-cm/Sec)
G1	31.22
G2	31.43
G3	29.62
G4	29.04
G5	27.82
G6	27.12
G7	24.64
G8	24.50
G9	21.46
G10	21.40

Table 6: Zone of inhibition of plant extracts.

Microbial strains	Zone of Inhibition (mm)				
	E.cardamomum	*C. zeylanicum*	*L. bipinnata*	*Camphor*	*C. sativum*
S. aureus	11	12	13	10	7
E. coli	13	12	10	12	10
B. subtilis	9	14	13	11	-
P. aeruginosa	7	7	9	5	-
A. varis	3	9	5	7	6
P. notatum	9	-	10	-	6
A. niger	-	-	-	3	-

Table 7: Zone of inhibition of individual gel formulations

Microbial strains	Zone of Inhibition (mm)				
	E.cardamomum	*C. zeylanicum*	*L. bipinnata*	*Camphor*	*C. sativum*
S. aureus	10	11	13	11	7
E. coli	11	12	9	10	10
B. subtilis	9	12	12	10	-
P. aeruginosa	6	7	9	5	-
A. varis	3	9	5	7	4
P. notatum	8	-	8	-	4
A. niger	-	-	-	3	-

Zone of inhibition of gel formulation of same plant extracts was almost similar to that of isolated plant extract activity. This shows that incorporation of extracts into polymeric gel does not decrease its activity.

MIC of most of the extracts were between 0.5-10 mg/ml. C. Sativum showed very less antimicrobial activity although Baratta et al. and Elgayyar et al. observed that essential oil of coriander inhibited microorganism.

Table 8: Table shows the MIC of plant extracts

Microbial strains	MIC (mg/ml)				
	E.cardamomum	*C. zeylanicum*	*L. bipinnata*	*Camphor*	*C. sativum*
S. aureus	10	>10	<2.5	>10	>10
E. coli	<5	<10	<2.5	>7.5	<10
B. subtilis	>10	>7.5	<2.5	<10	-
P. aeruginosa	>10	>10	<5	>7.5	-
A. varis	>10	>7.5	<5	>10	>10
P. notatum	>15	-	<7.5	-	>15
A. niger	-	-	-	>10	-

Table 9: Zone of inhibition of combined Gel formulations

Microbial strains	Zone of Inhibition (mm)
	Gel
S. aureus	13
E. coli	12
B. subtilis	13
P. aeruginosa	10
A. varis	9
P. notatum	9
A. niger	6

The gel containing different plant extracts showed synergistic effect against most of the strains. Most of the extract showed little or no activity against fungal strains but the gel showed activity against all the fungal strain used.

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