In-vitro Antibacterial Activity of Carbopol-Essential Oils hydrogels

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Abstract

The invention pertains to develop drug delivery systems is still challenging, and many scientists joined the race to use more effective, less toxic, low cost, and sustainable systems for drug delivery. This research was carried out to formulate mixed essential oils loaded with carbopol hydrogels, and to evaluate their antibacterial activity against selected pathogens using disk diffusion method. Screening for antibacterial activity of essential oils was done prior to the fabrication of hydrogels. Clove essential oil exhibited the strongest antibacterial activity towards all tested pathogens followed by the cinnamon essential oil. Hydrogel formulation containing mixed essential oils showed the best antibacterial results compared to single oil formulations with an inhibition zone of 18-23 mm for Staphylococcus aureus, 17-20mm for Escherichia coli, and 14-18mm for Pseudomonas aeruginosa. All hydrogel formulations were non-irritant, stable, and free of microorganisms. Minimal inhibitory concentrations of the essential oils were significantly affected by loading them in carbopol hydrogel.

Keywords: *Antimicrobial activity; essential oils; hydrogel; Carbopol Ultrez*

1. Introduction

Recently, many bacterial species are resistant to common antibiotics due to the misuse of them and naturally occurred mutations [1]; however, the inappropriate use of antibiotics accelerates the possibility of gaining antibiotic resistance mutations [2, 3]. Antibiotics and multi-drug resistance become the world-wide problem in hospitals, long-stay residential centers and in the community [4]. Due to the loss of efficacy of most common antibiotics, nowadays, the use of phytochemicals for the pharmaceutical purpose has gradually increased in many countries [5]. According to the World Health Organization (WHO), medicinal plants would be the best source to obtain a variety of drugs [6, 7]. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants [8]. Essential oils (also called volatile oils) are aromatic oily liquids obtained from plant materials (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots) [9]. They can be extracted by expression, fermentation or steam distillation which is the most commonly used for commercial production [10]. An estimated 3000 essential oils are currently known, of which only 300 are commercially important in the fragrance market [11]. Plant essential oils and extracts have been shown to possess significant activity against many types of bacteria, fungi and even viruses [12]. Some essential oils have been used in cancer treatment [13, 14], others have been used in food preservation [15], and fragrance industries [16]. Essential oils and their components are gaining increasing interest because they are readily available, non-toxic and safe. Alternative medicine is widely accepted by consumers, due to their exploitation for potential multi-purpose functional use. Carbopol is a type of synthetic polymers that offers many substantial benefits for formulators and marketers of personal care products [17]. It is exceptionally easy to use as it self-wets and disperses within minutes, it can be used in systems with moderate surfactant content, making it an ideal choice for shampoos, body washes, gels, lotions, and creams [18].

Keeping in mind the medicinal importance of essential oils and the advantages of carbopol, this work was conducted to estimate antibacterial activity of selected essential oils and fabricate carbopol

based topical hydrogel formulations, as well as to evaluate the effect of polymer on antibacterial activity of the essential oils.

2. Material and methods

2.1 Materials

Four essential oils namely; clove, cinnamon, tea tree and rosemary were obtained from Tanamera & Co (P) Ltd, Malaysia. The quality of these oils was ascertained by the company's laboratories to be more than 99 % pure. Carbopol Ultrez 21, methyl paraben, propyl paraben, glycerin, triethanolamine and ethyl acetate were obtained from Sigma-Aldrich, USA.

2.2 Methods

2.2.1. Microorganisms and preparation of inoculums

Three clinical isolates such as Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa were used in the present study, which were collected from the microbiology department, Alasmarya Islamic University. The cultures of all the microorganisms were maintained in their appropriate agar slants at 4°C throughout the study and used as stock cultures. For evaluation of antimicrobial activity, 24 hours fresh culture of bacteria were suspended in double sterile water to obtain a uniform suspension of microorganism.

2.2.2. Preparation of hydrogel

The hydrogel was prepared by following the method mentioned in reference [19], with the following modifications: different amounts as mentioned in Table 1 of carbopol Ultrez 21 was dissolved in distilled water, followed by the addition of methyl paraben (0.18%), propyl paraben (0.02%) and glycerin (9.5: 0.5 w/w). Essential oils were dissolved in ethanol (10% w/w) and slowly added to the mixture and kept under magnetic stirring for 1h. The viscosity and pH were finally adjusted with triethanolamine (TEA) until the desired values were approximately reached (neutral pH and desired viscosity).

2.2.3. Evaluation of the hydrogels

The color of all the formulations was evaluated with naked eyes against a white background. The odor was determined by mixing each hydrogel in water and taking in the smell. The pH of each formulation measured by a digital pH meter (HANNA, UK). The viscosity of the microemulsion was measured by a Brookfield viscometer by using a spindle rotating at a speed of 0.5, 1, 2.5 and 5.0 rpm at 25°C. At each speed, the corresponding dial reading on the viscometer was noted. Then the spindle speed was lowered, and the corresponding dial readings were noted.

2.2.4. Skin irritation studies

Rats (180-220 g) of either sex were used for the evaluation of possible skin irritation caused by hydrogels. The animals were maintained on standard animal feed and had free access to water. They were kept under standard conditions during the study. Their fur was shaved from the back, and an area of 3 cm2 was marked on both sides, one side served as control while the other side as test. Hydrogels were applied (500 mg/animal) twice a day for 7 days and the site was observed for any sensitivity.

2.2.5. Microbial load of the essential oils loaded hydrogel

Microbial load evaluation was carried out for the optimized batches of hydrogel, after two weeks from the preparation by total plate count agar. The pour plate method was used to cultivate serially diluted portions of the hydrogel formulations samples under investigation. Enumeration was carried out on nutrient agar (NA) for bacteria, on Sabouraud agar (SA) for yeasts, and potato dextrose agar (PDA) for fungi. The NA plates were incubated at 37° C for 48 h, while SA and PDA plates were incubated at room temperature (30°± 3°C) for 48–72 h. The developed microbial colonies were counted and computed as colony-forming units per ml (cfu/ml) of hydrogel.

2.2.6. Determination of zone of inhibition

Antimicrobial screening of essential oils and hydrogel formulations was done using disk diffusion method [20-22]. It was performed by using an 18 h culture at 37°C in 10 ml of Mueller Hinton Broth. The suspensions were spread over the Mueller-Hinton agar plates using a sterile cotton swab in order to get a uniform microbial growth. The dilution of the essential oils was conducted by adding an appropriate amount of ethyl acetate. After that an empty sterilized discs (Whatman no. 3) filter paper was punched under aseptic conditions into 5 mm disc form, were impregnated with 50 µL of testing samples and placed on the inoculated agar surface. This was followed by sealing the plates with sterile laboratory para-film to avoid any eventual evaporation of the test samples, and they were left for 30 min at room temperature, to allow the diffusion of the oils and hydrogels, and incubated at a temperature of 37°C for 18 h. After the incubation period, the inhibition zone was measured with a caliper in millimeter. Studies were performed in duplicate, and mean values were calculated.

Table 1. Formulation composition of gels

*F	Carbopol % w/w	Glycerin % w/w	Ethanol % w/w	Cinnamon Oil % w/w	Clove oil %w/w	Tea tree Oil %w/w
G1	0.5	5	10	-	-	-
G2	1	5	10	-	-	-
G3	0.75	5	10	-	-	-
G4	0.75	5	10	1	-	-
G5	0.75	5	10	2	-	-
G6	0.75	5	10	3	-	-
G7	0.75	5	10	-	1	-
G8	0.75	5	10	-	2	-
G9	0.75	5	10	-	3	-
G10	0.75	5	10	-	-	1
G11	0.75	5	10	-	-	2
G12	0.75	5	10	-	-	3
G13	0.75	5	10	3	3	3

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

3. Results and discussion

3.1 Physical examination

The physical properties of hydrogel formulations are shown in Table 2. All gel formulations showed good homogeneity and spreadability. The physical appearance of all the formulations was

mostly deep white in color, and have the characteristic odor of the essential oil incorporated. The pH of the gel formulations was in the range of 6.8 to 7.2, which lies in the normal pH range of the skin and would not produce any skin irritation. It is important to say that there was no significant change in the pH values as a function of time for all prepared formulations. The formulations showed increased viscosity with the increase of polymer concentration when all other formulation additives were kept constant. Viscosity of 0.75% hydrogel was optimum and was used for formulation of the herbal hydrogels. 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.

Table 2. Physical properties of the hydrogels

F	Physical Appearance	homogeneity	Spreadability (gmcm/Sec)	Viscosity (cps)	pH
G1	Colorless, Transparent	Homogeneous	27.12	109480	7.2
G2	Colorless, Transparent	Homogeneous	21.40	198577	7.1
G3	Colorless, Transparent	Homogeneous	24.64	159480	7.2
G4	Deep white	Homogeneous	25.73	159678	6.9
G5	Deep white	Homogeneous	25.87	159322	6.8
G6	Deep white	Homogeneous	27.65	159655	6.9
G7	Deep white	Homogeneous	25.58	159499	7.0
G8	Deep white	Homogeneous	26.23	159122	6.9
G9	Deep white	Homogeneous	27.84	159566	6.9
G10	Deep white	Homogeneous	24.97	159419	7.1
G11	Deep white	Homogeneous	25.11	157486	6.9
G12	Deep white	Homogeneous	26.21	159444	6.8
G13	Deep white	Homogeneous	27.66	162496	6.8

3.2. Antibacterial activities of the essential oils and their hydrogels

The activity of two essential oils, namely; clove and cinnamon was relatively similar, compared to rosemary and tea tree. However, most of the concentration of the essential oil and the incorporated essential oils showed activity against the microbial used. Cinnamon oil showed the highest activity against *S. aureus* among other essential oils. Clove oil showed the highest activity against both *E. coli,* and *P. aeruginosa*. Activity of formulation G13 was the highest among all the other formulations and the oils concentrations.

Table 4 a. Zone of inhibition of essential oils								
	Zone of Inhibition (mm)							
	Cinnamon oil				**Clove oil**			
Concentration	100%	75%	50%	25%	100%	75%	50%	25%
S. aureus	26	24	16	13	22	22	14	10
E. coli	12	12	9	9	18	21	14	11
P. aeruginosa	12	11	9	10	27	23	20	13

Table 4 b. Zone of inhibition of essential oils								
	Zone of Inhibition (mm)							
	Rosemary oil				**Tea tree oil**			
Concentration	100%	75%	50%	25%	100%	75%	50%	25%
S. aureus	16	13	7	6	15	15	11	10
E. coli	6	6	7	6	6	7	6	8
P. aeruginosa	6	6	7	8	7	6	6	8

The antibacterial activity of all prepared formulations is shown in Table 5. The formulations that contained different essential oils (G13) showed synergistic effect against all pathogens. Most of the single essential oil loaded with hydrogels showed little or no activity against bacterial species as Figure 1 presents. It can be clearly observed that there is significant difference in the antibacterial activity between the single and mixed essential oils loaded with hydrogels. The skin irritation studies show that all the tested animals tolerated the applications of hydrogels and no signs of irritation were noticed during the whole period of study.

Table 5. Zone of inhibition of hydrogel formulations			
	Microbial strains		
	S. aureus	***E. coli***	***P. aeruginosa***
G1	-	-	-
G2	-	-	-
G3	-	-	-
G4	-	-	-
G5	-	7	7
G6	10	10	8
G7	-	6	-
G8	8	9	9
G9	12	11	10
G10	-	6	-
G11	6	8	-
G12	7	9	-
G13	20	18	16

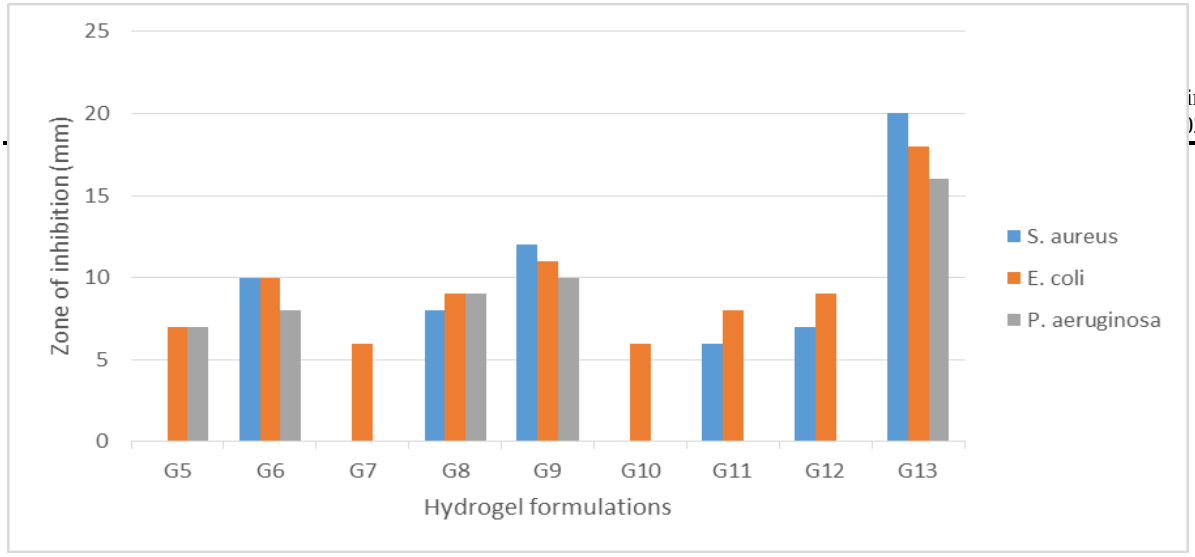

Figure 1. Inhibition of *S. aureus*, *E. coli*, and *P. aeruginosa* with prepared Carbopol hydrogel formulations (G3 to G13) by disc diffusion method.

Antibacterial activity of rosemary and tea tree oil in this study did not concur with the findings in many previous studies. In the study of [23], they evaluated antibacterial activity of seven types of rosemary, and most of them showed strong antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. In our study, only strong concentrations of rosemary worked with *S. aureus* but not with the other two pathogenesis, thus it was excluded from hydrogel preparations. The same conclusion can be obtained when we compared the results of tea tree oil in our study with [24] and [25]. Tea tree essential oil was not eliminated in our study because it has wound healing activity and it was used to enhance the properties of hydrogels [26]. In contrast, cinnamon and clove essential oils showed excellent activity even in weak concentration, which concurred with many studies, including [27-29]. In the hydrogel, the antibacterial compounds in the essential oils cross-linked and immobilized within the network, which was well suspended in the network. This explains the low and effective concentrations in the formulation 13. From this study, it can be concluded that all the tested essential oils (cinnamon, clove, rosemary, and tea tree) possess different antibacterial activities. Clove oil has the most potential antimicrobial properties against *S. aureus*, *E. coli* and *P. aeruginosa*, followed by cinnamon essential oil with slightly lower effect, compared to the other tested essential oils (clove > cinnamon > tea tree EO > rosemary). Although all the pathogens were affected, *S. aureus* was more sensitive to clove and cinnamon oils, than *E. coli* and *P. aeruginosa*. Hydrogel formulations showed better antimicrobial activity, even in low concentration of oil, which confirms the ability of Carbopol polymer as a drug delivery system.

4. Conclusion

Based on the results obtained from this study, it can be concluded that the antibacterial activity of essential oils can be positively affected and enhanced with the integration of carbopol. All hydrogel formulations were non-irritable, stable, and free of microorganisms. Minimal inhibitory concentrations of the essential oils were significantly affected by loading them in carbopol hydrogel. Essential oils loaded with hydrogels can be used as an alternative and amenable solution to traditional antibiotic treatments.

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