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Research Article

Formulation of topical gel containing *Tagetes erecta* L. floral extract and its antibacterial activity

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ABSTRACT

Tagetes erecta L. (marigold) flowers were extracted to obtain crude ethanolic extract (MGE), water insoluble extract (MGP), as well as water soluble extract (MGW). All extracts were determined of total flavonoid content and antibacterial activities against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The results suggested that MGP contained the highest amount of total flavonoids (29.48%w/w quercetin equivalent) with good activity against *S. aureus* (MIC 6.25 mg/mL and MBC 25.0 mg/mL). This extract was subjected to formulate the topical gels at the concentration of 1 and 2 times of MIC. The gel formulation containing 1.25%w/w MGP with the solvent mixture of propylene glycol and ethanol (1:1) showed good physical properties. Furthermore, it exhibited the most effective antibacterial activity against *S. aureus* by agar well diffusion method with the inhibition zone of 15.15±0.43 mm. Therefore, the extract of marigold flower could be formulated as topical gel for the treatment of skin infections.

Keywords:
Tagetes erecta, Marigold, Flavonoid, Topical gel, Antibacteria, *S. aureus*

1. INTRODUCTION

Tagetes erecta L. is an annual herb belonging to family Asteraceae which is known as marigold. In Thailand, the flowers are primarily used for decoration in Buddhist festivals, weddings, and politics. The flower is plentiful of different classes of phytochemicals, such as carotenoids, phenolic acids, and flavonoids. These compounds were reported to be responsible for several pharmacological activities such as antioxidant, antimicrobial, hepatoprotective, analgesic, and wound healing activities[1]. The antibacterial activities of flavonoids have been indicated by many researchers using different assays such as agar dilution technique, paper disk diffusion assay, hole-plate diffusion method, and broth microdilution technique[2]. The flavonoids and their glycosides from marigold flower such as quercetagetin, quercetagitrin, patuletin, and patulitrin were reported to possess antibacterial activities against some Gram-positive and Gram-negative bacteria[3-5]. Consequently, the marigold flower could be a possible source of antibacterial agents.

The present study aims to formulate topical gel of *T. erecta* floral extract for treatment of skin infections. The extracts of *T. erecta* flowers were determined for their flavonoid content and antibacterial activity. The extract with the highest antibacterial activity was selected to formulate as topical gel. Physical properties and antibacterial activity of the gel were also investigated.

2. MATERIALS AND METHODS

2.1. Materials

Quercetin (HPLC grade) and resazurin were purchased from Sigma-Aldrich, Germany. Aluminium chloride hydrated was purchased from Ajax Finechem, Australia. Muller Hinton agar and Muller Hinton broth were purchased from HiMedia Laboratory Pvt. Ltd., India. Carbopol 940, propylene glycol, and glycerin were purchased from Chemipan Corporation Co., Ltd., Thailand. All solvents used were of analytical grade.

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2.2. Preparation of plant extracts

The flowers of *Tagetes erecta* L. were purchased from a farm in Plaeng Yao district, Chachoengsao province, Thailand. The flowers were washed with tap water and the florets were pulled apart. Then the florets were dried in hot air oven at 50°C before grounded into coarse powder by blender. The plant powder (800 g) was initially defatted with hexane, then the marc was extracted with absolute ethanol (8 L) using Soxhlet extractor for 24 hr. The extract was filtered and dried in vacuum rotary evaporator to obtain crude ethanolic extract (MGE). MGE (200 g) was redissolved with deionized water (1.2 L) and left in the refrigerator (15°C) overnight before filtering. The precipitate was separated and dried at 45°C to obtain water insoluble extract (MGP). While the filtrate was lyophilized to obtain water soluble extract MGW.

2.3. Determination of total flavonoid content

Total flavonoid content of the extracts was determined by aluminum chloride colorimetric method[6]. Quercetin as the reference standard was dissolved in ethanol to prepare the concentration range of 5-50 μM. Aluminium chloride solution (2.5 mL, 2%w/v) was added to 5 mL of sample or standard solutions. Then 2.5 mL of deionized water was added, and the mixture was shaken before incubation at room temperature for 10 min. The absorbance was measured at 426 nm using spectrophotometer (JASCO double-beam UV-visible spectrophotometer model V-630, Japan). The total flavonoid content of the extracts was expressed as g of quercetin equivalent per 100 g dried extract (%QE).

2.4. Determination of antibacterial activity

2.4.1. Bacterial strains

Two bacterial strains, *Staphylococcus aureus* TISTR 1466 (ATCC 6538) and *Pseudomonas aeruginosa* TISTR 781 (ATCC 9027) were used for determination of antibacterial activities of the plant extracts. The microorganisms were subcultured in tryptic soy agar and incubated at 35°C for 24 hr before being suspended in sterile 0.9% sodium chloride solution. The density of inoculum suspension was adjusted to the turbidity of a 10^8 colony-forming unit (CFU)/mL for agar well diffusion assay and of 10^6 CFU/mL for the minimum inhibitory concentration determination.

2.4.2. Agar well diffusion assay

The bacterial inoculum was spread onto the surface of Mueller Hinton agar plates. The concentrations of 100 and 200 mg/mL of each extract (MGE, MGP, and MGW) were prepared in dimethyl sulfoxide (DMSO). Ampicillin (Kos Introtech Co., Ltd.) at the concentration of 0.15 mg/mL was used as positive control for *S. aureus* and streptomycin (General Drug House Co., Ltd.) at the concentration of 1 mg/mL was used as positive control for *P. aeruginosa*. While DMSO was used as negative control for both strains. 50 μL of the extracts and controls were added to each well (6 mm diameter holes cut in the agar gel). The plates were incubated at 35±2°C for 18 hr. After incubation, the antibacterial activity was evaluated by measuring the inhibition zones in mm. The experiments were performed in triplicate.

2.4.3. Determination of the minimum inhibitory concentration

The minimum inhibitory concentration (MIC) against *S. aureus* was determined using broth microdilution technique. Mueller Hinton broth was used as the culture medium. The plant extracts and ampicillin (as positive control) were prepared in DMSO and DI water, respectively. The amount of inoculum (10^6 CFU/mL) added to each well was 50 μL. The concentrations obtained in the wells were between 0.05 and 100 mg/mL for the extracts and between 0.0125 and 12.5 mg/mL for ampicillin. The microplates were incubated at 37°C for 24 hr. Resazurin (20 μL, 0.4 mg/mL) was used as an indicator for visualizing bacterial growth. MIC obtains from the lowest concentration of the plant extracts or drugs that does not produce red color. The experiments were performed in triplicate.

2.4.4. Determination of the minimum bactericidal concentration

The minimum bactericidal concentration (MBC) was determined by subculturing the broths used for MIC assay onto fresh Mueller Hinton agar plates. The plates were incubated at 35±2°C for 18 hr. MBC obtains from the lowest concentration of the plant extracts or drugs that can inhibit colony growth. The experiments were performed in triplicate.

2.5. Formulation of topical gel containing marigold flower extract

Marigold flower extract that presented antibacterial activity was selected for development of topical gel with the concentration of 1 MIC or 2 MIC of the extracts. Carbopol 940 was used as gelling agent and the gel was formed by neutralization with triethanolamine. Propylene glycol, glycerin, and 95% ethanol were used as single or combined solvents for dissolving the extract. Eight formulations (F1-F8) and two gel bases (F9-F10) were prepared in this experiment as shown in Table 1. Gels were prepared by dispersing Carbopol 940 in distilled water, then neutralizing with triethanolamine. The extract

Table 1. Composition of gel formulations (%w/w).

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
MGP	0.625	0.625	0.625	1.25	0.625	0.625	1.25	1.25	-	-
Glycerin	44	-	-	-	-	-	-	-	-	-
Propylene glycol	-	30	15	30	60	30	60	30	60	30
Ethanol	-	-	15	30	-	30	-	30	-	30
Carbopol 940	0.3	0.3	0.3	0.2	0.8	0.8	0.8	0.8	0.8	0.8
Triethanolamine	0.3	0.3	0.3	0.2	0.8	0.8	0.8	0.8	0.8	0.8
Methyl paraben	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Propyl paraben	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Distilled water qs.	100	100	100	100	100	100	100	100	100	100

was separately dissolved in different solvents, then preservative was added, and this mixture was poured into the gel with homogenous mixing.

2.6. Evaluation of physical properties and antibacterial activity of topical gel

Physical appearance of gels was evaluated by visual observation. The gel formulations with stable physical appearance after kept in closed glass container at room temperature in the dark place over 2 weeks were reformulated and subjected to evaluate for pH value, viscosity, spreadability and antibacterial activity.

2.6.1. pH measurement

The pH of gel was measured using pH meter (Mettler Toledo S20 SevenEasy™) at room temperature.

2.6.2. Viscosity measurement

The viscosity of gel was measured using a Brookfield viscometer, model LVDV-II+ with T-bar spindle No. 95, 0.5 rpm at 26.8°C.

2.6.3. Spreadability

Spreadability of gel was determined by parallel plate method[7-8]. Gel (1 g) was spread between two horizontal plates (20×20 cm). The standardized weight (100 g) was placed on the upper plate for 1 min and diameter (∅) of the gel was measured. In this experiment, the measured diameters of the gel were characterized as: very stiff gel ∅≤40 mm, stiff gel 47≥∅> 40 mm, semi-stiff gel 55≥ ∅>47 mm, semifluid gel 70≥∅>55 mm, and fluid gel ∅>70 mm.

2.6.4. In vitro antibacterial activity

The gel formulations with stable physical appearance over 2 weeks were subjected to evaluate for antibacterial activity against *S. aureus* using agar well diffusion assay as described above. Gentamicin cream (0.1%w/w) from Seng Thai Co., Ltd. was used as positive control. The amount of selected gels and controls (0.1 g each) were directly applied into the wells.

3. RESULTS AND DISCUSSION

3.1. Total flavonoid content of marigold flower extracts

According to previous research, several flavonoids in marigold flower were extracted using ethanol or methanol as solvents[9-10]. In our study, ethanol was selected as a solvent in extraction process due to it is generally recognized as bio-solvent and safe substance in foods, drugs, and cosmetics[11-12]. The dried powder of marigold flowers (800 g) was extracted with absolute ethanol to obtain the crude ethanolic extract (MGE) with the percentage yield of 32.55%. MGE was redissolved with deionized water and the precipitate (MGP), as well as the lyophilized aqueous phase (MGW) were separated to yield 4.41% and 20.72%, respectively. By aluminum chloride colorimetric method, the total flavonoid contents in these extracts were calculated from the regression equation based on calibration curve of quercetin, y= 63.83x-0.0053, R^2=0.9997. The highest amount of flavonoid content equivalent to quercetin was found in MGP (29.48% QE) followed by MGW (3.17% QE), and MGE (1.59% QE).

3.2. Antibacterial activity of marigold flower extracts

The results from agar well diffusion assay of three extracts from marigold flower revealed that among them, MGP exhibited the highest antibacterial activities against *S. aureus* and *P. aeruginosa*. The inhibition zone diameters of all extracts and controls are shown in Table 2. At both concentrations (100 and 200 mg/mL), MGP and MGW were able to inhibit *S. aureus* whereas MGE showed inhibitory activity only at the concentration of 200 mg/mL. Additionally, MGP showed antibacterial activity against *P. aeruginosa* with 9.13±0.24 mm inhibition zones at the concentration of 200 mg/mL, while MGE and MGW had no activity. Moreover, the MIC and MBC of MGP against *S. aureus* were determined as 6.25 and 25.0 mg/mL, respectively (Table 3). Many researchers reported that flavonoids possessed antibacterial activities. Flavonoids such as quercetagetin and its

Table 2. The inhibition zone diameters of marigold flower extracts.

Test samples	Concentrations (mg/mL)	Inhibition zone (mm±SD)	
		S. aureus	***P. aeruginosa***
MGE	100	ND	ND
MGW		9.33±0.62	ND
MGP		14.93±1.99	ND
MGE	200	8.32±0.06	ND
MGW		11.55±0.84	ND
MGP		15.57±0.66	9.13±0.24
Streptomycin	1	NA	19.98±0.20
Ampicillin	0.15	45.70±0.70	NA
DMSO	-	ND	ND

NA: not applicable, ND: not detected

Table 3. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of marigold flower extracts against *S. aureus*.

Test samples	MIC (mg/mL)	MBC (mg/mL)
MGW	50.00	ND
MGP	6.25	25.00
Ampicillin	0.10	0.20

ND: not detected

derivatives (quercetagetin 6,3′-dimethyl ether, quercetagetin 7-methyl ether, and quercetagetin 6-O-β-D-glucopyranoside, etc.) were isolated from flowers of *T. erecta*[3]. Patuletin which is quercetagetin 6-methyl ether showed potent antibacterial activities against *Corynebacterium* spp., *Staphylococcus* spp., *Streptococcus* spp., and *Micrococcus luteus*[4]. Furthermore, patulitrin which is 7-O-glucoside of patuletin isolated from *T. erecta* flower, also showed activities against *Alcaligens faecalis*, *Bacillus cereus*, *Campylobacter coli*, *Escherchia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Streptococcus mutans* and *Streptococcus pyogenes*[5].

3.3. Physical properties and antibacterial activity of topical gel

The extract with the highest antibacterial activity against *S. aureus*, MGP, was selected for development of topical gel. Ten formulations were prepared as a preliminary. Different solvents (glycerin, propylene glycol, and ethanol) were used for dissolving the extract. The formulations containing 0.625%w/w of the extract (F1, F2, F3, F5, and F6) were homogeneous transparent yellow gels, while other three formulations (F4, F7, and F8) containing 1.25%w/w of the extract were dark yellow gels. Two gel bases, F9 and F10, were transparent colorless gels. Carbopol 940, a gelling agent, was used in the range of 0.2-0.8%w/w. Among all formulations, gel formation was not observed in F4 due to an insufficient amount of gelling agent (0.2%w/w). In addition, the pH values of all formulations using a pH test strip were 5 except for F4 were 4. After 2-week storage at room temperature, the pH values of F1 and F3 decreased from 5 to 4 while those of other formulations remained constant. Since, swelling of Carbopol is caused by an increase in solubility with increasing pH, a decrease in pH could affect the ability of Carbopol to form gel. The decreasing of viscosity was observed in F1-F3 (0.3%w/w of Carbopol 940) but it was not observed in F5-F10 (0.8%w/w of Carbopol 940). This could be owing to the pH of gel declining and the amount of Carbopol 940 used in F1-F3 was insufficient to stabilize the gel. Besides, the amounts of solvent in formulation also affected the physical stability of gel. The solvent to gelling agent ratios were 146:1 in F1 and 100:1 in F2 and F3 that were higher than the ratio in F5-F10 (75:1). As the result, F5-F10 were selected to reformulate and subject to further studies. The pH values and viscosity of F5-F10 were shown in Table 4. The pH and viscosity of gel were impacted by the extract concentration. The pH and viscosity decreased as the concentration increased. However, the pH values were in the range of 4.82-5.55 which were suitable for skin application[13].

Spreadability of semi-solid preparation is inversely

Table 4. The pH and viscosity of F5-F10.

Formulations	pH values	Viscosity (cps)
F5	5.46	715320
F6	5.10	815600
F7	5.17	685400
F8	4.82	741200
F9	5.55	817500
F10	5.25	888700

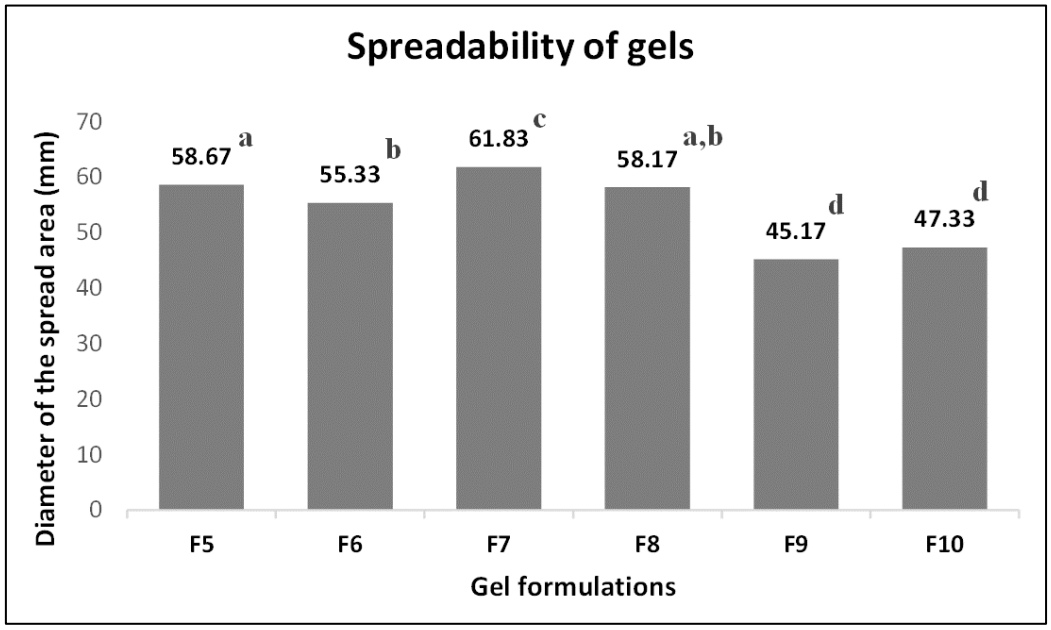

Figure 1. Results of spreadability of gel given by the diameter of the spread area in mm (characterized as: very stiff gel ∅≤40 mm, stiff gel 47≥∅> 40 mm, semi-stiff gel 55≥∅>47 mm, semifluid gel 70≥∅>55 mm, and fluid gel ∅>70 mm), different letters (a-d) show statistically significant differences at 95% confidence interval (p<0.05).

proportional to viscosity. The higher the viscosity, the lower the spreadability which is produced. The results of spreadability test in the term of spread area diameter (∅) of the gel formulations (F5-F10) were shown in Figure 1. There were 4 formulations, F5-F8, which were characterized as semifluid gels. F9 was characterized as stiff gel while F10 as semi-stiff gel. As indicated earlier, the viscosity of gel were impacted by the extract concentration. The spread area diameters of gel bases (F9 and F10) were less than that of the gels loaded with marigold flower extract (F5-F8). Different solvents were used in the gel bases, propylene glycol was used in F9 whereas a mixture of propylene glycol and ethanol in the ratio of 1:1 was used in F10. Polarity of solvent is a factor which influenced on viscosity and consistency of gel preparation. Viscosity and consistency of gel decrease if polarity of solvent decreases[14]. Propylene glycol which contains 2 hydroxyl groups in the molecule, possesses more polar than ethanol. In compared with F9, F10 showed higher degree of spreadability but there was no statistically significant difference. Propylene glycol was also used in F5 and F7 as a solvent for dissolving the extract, MGP. The spread area diameters of F7 and F5 which were greater than that of F9 revealed that the extract altered the viscosity of gel formulation. In addition, the increasing of the extract concentration in gel formulation, the increasing of spreadability of gel was produced. This was also occurred in the formulations using the mixture of propylene glycol and ethanol (1:1) as solvent. The spreadability of F8 was higher than that of F6 but not statistically different, as well as the spreadability of F10 was the least. In contrast, when considered the formulations with the same concentration of MGP, the spreadability of the formulations using propylene glycol as solvent was greater than that of the formulations using the solvent mixture.

Antibacterial activity against *S. aureus* of the topical gel formulations was determined using agar well diffusion assay and the results were shown in Table 5. The growth inhibition zone was not observed in gel bases (F9 and F10) and gel bases without preservative (F9a and F10a). The activity of gel formulations was the result of

Table 5. The inhibition zone diameters of topical gel formulations against *S. aureus*.

Formulations	Inhibition zone (mm±SD)
F5	13.50±0.41[a]
F6	14.12±0.06[a,b]
F7	14.58±0.23[b,c]
F8	15.15±0.43[c]
F9 (gel base)	ND
F10 (gel base)	ND
F9a (gel base without preservative)	ND
F10a (gel base without preservative)	ND
Gentamicin cream (0.1%w/w)	26.94±0.80[d]

NA: not applicable, ND: not detected, different letters (a-d) show 5 statistically significant differences at 95% confidence interval (p<0.05).

the activity of extract. When the concentration of the extract increased from 0.625% to 1.25%, the inhibition zone increased but the difference was not significant. While altering solvent in the formulation showed a greater impact on its activity. When compared between the formulations with the same amount of MPG, F7 exhibited a significantly larger inhibition zone than F5, and this was also observed in F6 and F8. Interestingly, F8, which contained 1.25%w/w MGP and the solvent mixture exhibited the most effective antibacterial activity. Previous study suggested that co-solvents such as ethanol and propylene glycol could be used to increase the solvent's hydrophobicity which improved the solubility of antibacterial agents and increased their activity[15].

4. CONCLUSION

The extract of marigold flower with the highest amount of flavonoid content as well as the highest antibacterial activity against *S. aureus*, MGP, was selected for development of topical gel. The gel formulation containing 1.25%w/w MGP with the solvent mixture of propylene glycol and ethanol (1:1) had good physical properties with the most effective antibacterial activity. Therefore, the extract of marigold flower could be formulated as topical gel for the treatment of skin infections.

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