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Original Article

NADES EXTRACT OF GEDONG MANGO LEAVES AND MULBERRY LEAVES IN SPRAY GEL AS A SUNSCREEN

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ABSTRACT

Objective: This study's purpose was to formulate a spray gel containing the combination extract of gedong mango leaves and mulberry leaves as a sunscreen preparation.

Methods: Each leaf was extracted by Natural Deep Eutectic Solvent-Maceration Assisted Extraction (NADES-MAE) method. The extract (gedong mango and mulberry leaves) formulated in Carbopol Spray Gel/GM-CarSG) in a ratio of GL and ML were 3:0.5 (GM-CarSG 1); 3:3 (GM-CarSG 2); and 3:6 (GM-CarSG 3). Furthermore, the spray gel was evaluated, and the SPF value was determined.

Results: The physical appearance, spreadability, pH, and viscosity of GM-CarSG 1, GM-CarSG 2, and GM-CarSG 3 meet the requirements. In addition, the three preparation have a sunscreen activity, with the SPF value of GM-CarSG 1, GM-CarSG 2, and GM-CarSG 3 being 2.08+0.03; 2.78+0.05; and 3.53+0.04, respectively.

Conclusion: The results showed that the combination of GL and ML extract is potentially used as a sunscreen in spray gel preparation. The best formulation is GM-CarSG 3, which has the highest SPF value among them.

Keywords: Gedong mango leaves, Mulberry leaves, Spray gel, SPF, Sunscreen

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INTRODUCTION

Indonesia is a tropical country where the influence of sunlight is powerful on the skin. Excessive exposure to sunlight, both UV A, UV B, and UV C, can interfere with skin health, such as erythema, skin cancer, and loss of skin elasticity. Sunscreen is a cosmetic preparation used to protect the skin from sun exposure so that cosmetics can prevent health problems on the skin [1]. Cosmetic preparation is available in creams, lotions, powders, soaps, masks, and scrubs. Spray gel preparations are commonly used as antiseptics. Spray gel is developed with a viscosity of 500-5000 cPs and can come out through the hole in a spray bottle. Spray gel preparations also have advantages compared to other topical preparations because this preparation is safer, easy to wash, and use [2].

Gedong mango (*Mangifera indica* L.) is a plant often found in Indonesia. Mango contains mangiferin with several pharmacology activities: analgesic, antidiabetic, antimicrobial, antiviral, anti-allergic, and UV protection Fields [3]. Nurdianti and Rahmiyani (2016) found that cream containing extract of mango leaves 1% has potent antioxidant activity (IC_{50}) of 50.54 ppm [4]. The other plant with antioxidant activity is mulberry (*Morus alba* L.). *Morus alba* L. is one of the TOGA (*Tanaman Obat Keluarga*/family medicinal plant plants) used as herbal medicine. Mulberry leaves contain alkaloids, flavonoids, polyphenols, and terpenoids that play roles as antioxidants [5]. The study of 0.75%, 3.75%, and 6.75% mulberry leaf extract in cream preparations showed intense antioxidant activity with IC_{50} values of less than 50 ppm [5]. On the other hand, Wimpy *et al.* (2020) suggested a positive relationship between antioxidant activity and sunscreen. The antioxidant activity correlated with the SPF value. The increase in SPF value results in the growth of antioxidant activity.

MATERIALS AND METHODS

Materials

Gedong mango leaves samples were obtained from Dawuan Village, Tengah Tani, and Cirebon, while the mulberry leaves were obtained from Sampiran Village, Talun, and Cirebon. Sodium acetate and lactic acid, triethanolamine, and sodium ideas were received from PT. Merck. Choline chloride, glycerol, Carbopol 940, propylene glycol, propylparaben, and methylparaben were obtained from PT. Global, sodium metabisulfite, ethanol 96%, and distilled water were obtained from Bratachem. All the materials were used without further purification.

The tools used in this study were oven (Memmert-UN 55), microwave (Krisbow), digital ultrasonic bath (Oxides Xten), homogenizer (IKA RW-20), analytical balance (Ohaus), viscometer (Brookfield-LV), and spectrophotometer (Shimadzu UV Mini-1240). This study was done in the Sekolah Tinggi Farmasi, Muhammadiyah Cirebon.

Extraction of gedong mango (GL) and mulberry leaves (ML) by NADES-MAE method

Gedong mango (GL) and mulberry leaves (ML) were extracted by the Microwave Assisted Extraction (MAE) method using NADES solvent. A total of 1 g powder dried materials were soaked in 20 ml of Natural Deep Eutectic Solvent (NADES). GL was extracted for 19 min, while ML was removed for 18 min. The NADES solvent used for GL was sodium acetate and lactic acid (1:3 in distilled water), and the MAE extraction was done for 1 h at 70 °C. Mulberry leaves were extracted using choline chloride and glycerol (1:2). The extraction was carried out for about 1 h at 80 °C. Then the extract was filtered and evaporated to remove the solvent [6, 7].

Phytochemical screening

Alkaloids

The small amount of the sample was diluted with 2 ml of distilled water. Two milliliters solution of 2% HCl was added, heated for 5 min, and filtered. The filtrate is reacted with three drops of Mayer reagent. Positive alkaloids with white precipitate or yellow precipitate formed [8, 9].

Flavonoids

The sample was diluted with 5 ml of distilled water, then five drops of magnesium powder and concentrated HCl were added, respectively. The formation of red or orange indicates flavonoid presence [8, 9].

Saponins

A total of 2 ml of the extract was diluted with 5 ml of aquadest, then ten drops of KOH were added, heated for 5 min, and shaken for 15 min. Positive saponins formed with the high of 1 ml and were stable for 15 min [8, 9].

Steroids dan triterpenoids

A piece of extract reacted with 1 ml of Liberman-Burchard reagent. The appearance of green indicates steroids, and the red or purple color indicates the presence of triterpenoids [8, 9].

Tannins

A piece of extract reacted with 2 ml of $FeCl_3$. Tannins and polyphenolic compounds are indicated with a blackish green or dark blue [8].

Formulation of spray gel (CarSG)

The spray gel preparation used the combination of Gedong mango leaves and mulberry leaves in the ratio of 3: 0.5; 3: 3; and 3:6. Carbopol 940 was poured into distilled water (70 °C), then stirred using a magnetic stirrer. Triethanolamine (TEA) was added and started until homogenous. Methyl paraben and propyl paraben were dissolved in propylene glycol. Sodium-EDTA and sodium metabisulfite were dissolved with distilled water separately, then mixed. This mixture was added to the base and stirred until homogenous [10–12]. The extract was combined with propylene glycol and added to the ground. Distilled water was added until the final volume with continuous stirring (table 1). CarSG contains the spray gel base without GL dan ML extracts.

Table 1: Preparation of GM-CarSGEvaluation of spray gel (CarSG)

CarSG	Carbopol (g)	GL Extract (g)	ML extract (g)	Propylene glycol (ml)	Methyl paraben (mg)	Propyl paraben (mg)	Sodium metabisulfite (mg)	Sodium-EDTA (mg)	TEA (ml)	Distilled Water (ml)
CarSG	0.8	No extract		10	180	20	100	100	1	87.8
GM-CarSG 1	0.8	3	0.5	10	180	20	100	100	1	84.3
GM-CarSG 2	0.8	3	3	10	180	20	100	100	1	81.8
GM-CarSG 3	0.8	3	6	10	180	20	100	100	1	78.8

Evaluation of spray gel (CarSG)

Organoleptic

An organoleptic test was carried out by observing the preparation's color, smell, and texture. The organoleptic test aims to see the physical practice. The results of the spray gel preparation are expected not to be cloudy and clear, and this is no air bubbles [10, 11, 13].

Homogeneity

The homogeneity test was carried out by applying 3 g of the preparation on a glass slide, then pasting another glass slide on it, pressing until the entire surface was evenly distributed, and observing. Homogeneity testing aims to see whether there are particles that have not been mixed evenly or homogeneously. The results of the spray gel preparation are expected to have no lumps and no solid particles [10, 11, 13].

pH measurement

The pH test is carried out using a pH meter (Mettler Toledo). The electrode was dipped into the spray gel sample and recorded the measurement. The pH of the preparation should be in the range of 4.5-6.5 [10, 11, 13].

Measurement of viscosity

The viscosity of each preparation was examined by using a Brookfield LV Viscometer. About 150 grams of the trial was put into the sample holder, selecting the appropriate spindle and speed. The spindle was dipped into the practice, and the dial reading was noted. The following equation calculated viscosity:

$$\text{Viscosity } (\eta) = (\text{dial reading}) \times \text{Factor}. \quad (1)$$

The viscosity of the preparation should be in the range of 500-5000 cPs [13].

Spreadability

Spreadability was measured by spraying the preparation on a mica plastic at a distance of 3, 5, 10, 15, and 20 cm. The spray pattern, diameter, and weight (g) were observed. The spray pattern was examined to evaluate the quality of the spray applicator used in the [14].

Determination of SPF value

The SPF value was measured using a UV-Vis spectrophotometer at 290-360 nm at an interval of 2.5 nm, three times for each sample. The blank used was 70% ethanol. The absorption of each wavelength was calculated using the modified A. J Petro formula [10, 15].

$$\text{Ar} = \frac{[\,1.25\,(\text{A290}+\text{A360})+2.5\,(\text{A292.5}+\cdots+\text{A357.50})\,]}{70} \quad \ldots\ldots (2)$$

$$\text{As} = \frac{125}{\text{M}} \times \text{Ar} \ldots\ldots (3)$$

M is the concentration of sample solution (mg/l), and SPF value wa calculated by the following equation:

$$\text{SPF Value} = \text{antilog}\,(2 \times \text{As}) \ldots\ldots (4)$$

The effectiveness of sunscreen was classified into 5five types according to the SPF value (table 2).

Table 2: Classification of sunscreen based on SPF value [16, 17]

SPF value	Effectiveness of sunscreen
2-4	Minimum protection
4-6	Intermediate protection
6-8	Extra protection
8-15	Maximum protection
≥ 15	Ultra protection

Data analysis

The data from the results were presented in the form of mean±standard error of the mean (SEM). The statistical comparison was performed using the Scheffe test [18]. The results are considered significant when $P<0.05$ [19].

RESULTS

Gedong mango leaves (GL) and mulberry leaves (ML) were dried in the oven at 40^0C to prevent the reduction of the phytochemical compound in this herbal. This process was carried out to obtain dried materials that will not be easily damaged by reducing the leaves' water content. Then the dry leaves were ground to get the dry powder because the powder provided a larger area of the simplicia that easily penetrated with the solvent in the extraction [3, 8].

In this study, the GL extract was made using NADES-MAE Method and evaporation using rotary evaporation to obtain the thicker extract. The use of NADES solvent because it is “green-solvent,” non-toxic, non-flammable, and non-explosive [20]. The extraction

process is carried out using the MAE (Microwave Assisted Extraction) method because the amount of solvent required is small and the time required is short [21]. NADES provides tremendous antioxidant activity for some plants, such as wild thyme Fields[Fields22]. The other study proposed that the combination of NADES-MAE will be an efficient and sustainable method in extracting antioxidants in some medicinal plants Fields[23]. Various solvent and extraction methods suggested increased antioxidant activity in GL and ML extracts.

Table 3: Phytochemical screening of ethanol extract GL and ML leaves

Secondary metabolites	Extract	
	GL	ML
Alkaloids	+	-
Flavonoids	+	+
Saponins	+	+
Steroids and Triterpenoids	-	-
Tannins	+	+

Note: (+) = indicates present, (-) = indicates absent/not detected

Phytochemical screening was examined to determine the secondary metabolite contained in extracts. These compounds are identified by reagents that provide the characteristics of each secondary metabolite [24]. The tests included alkaloids, flavonoids, saponins, steroids, triterpenoids, and tannins. The results of identifying secondary metabolites of gedong mango leaf extract and mulberry extract can be seen in table 3. Ethanol extract of GL contains alkaloids, flavonoids, and saponins, but ML didn't have an alkaloid [24].

Both GL and ML can be formulated into spray gel with Carbopol 940 as a base (fig. 1). GM-CarSG. The spray gel of GM-CarSG 1, GM-CarSG 2, and GM-CarSG 3 was transparent, yellowish, and had a characteristic odor. A gel containing the lower concentration has a light color and becomes dark color depending on the concentration of extract in the preparation [25, 26]

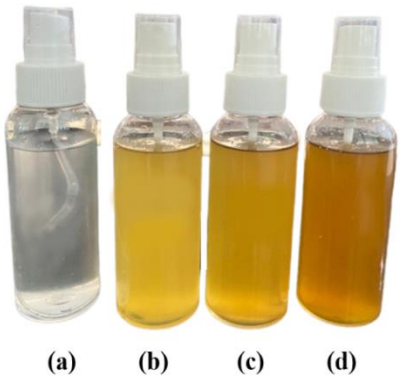

Fig. 1: Spray gel of CarSG (a), GM-CarSG 1 (b), GM-CarSG 2 (c), and GM-CarSG 3 (d)

The homogeneity of GM-CarSG 1, GM-CarSG 2, and GM-CarSG 3 was examined to observe the distribution of excipients and the extracts. All the preparation are homogenous because Carbopol 940 was completely swelled in water resulting in the transparent gel. The extract distribution in GM-CarSG 1, GM-CarSG 2, and GM-CarSG 3 were homogeneous because the extract dissolved in the gel base [10, 11].

The pH of GM-CarSG 1, GM-CarSG 2, and GM-CarSG were 4.52+0.03; 4.52+0.03; and 4.52+0.03, respectively (table 4). The increased concentration of extracts raises the pH. GL extracts are more acidic than ML. GL extract has pH 3,86 dan ML extracts is 5,81. The presence of extract decreases the pH of the gel base (CarSG) but is still in the range of the requirements of skin pH (4,5-6,5) because if the pH under 4,5 will cause irritation, and if the pH above 6.5 will cause the scaly skin [13].

Table 4: Physical characteristics of CarSG, GM-CarSG 1, GM-CarSG 2, and GM-CarSG 3

CarSG	Color	Homogeneity	pH
CarSG	-	Homogeneous	6.04+0.02
GM-CarSG 1	+	Homogeneous	4.52+0.03†
GM-CarSG 2	++	Homogeneous	4.73+0.04†
GM-CarSG 3	+++	Homogeneous	4.90+0,06†

Note: -= colorless,+= light yellow,++=yellow,+++: dark yellow. The results were expressed as the mean±SD (n = 3). †p<0.0001, compared to CarSG

The added extract decreases the pH of CarSG (table 4). The addition of a section reduced the viscosity of CarSG but was still in the range of spray gel (500-5000 cPs). The gel should have enough consistency to quickly come out from the spray hole [9, 17, 18]. The diameter of the spray is affected by the viscosity of the spray gel. The increase in density leads to a decrease in the spray's diameter because the gel is more viscous, limiting the spray field area [28].

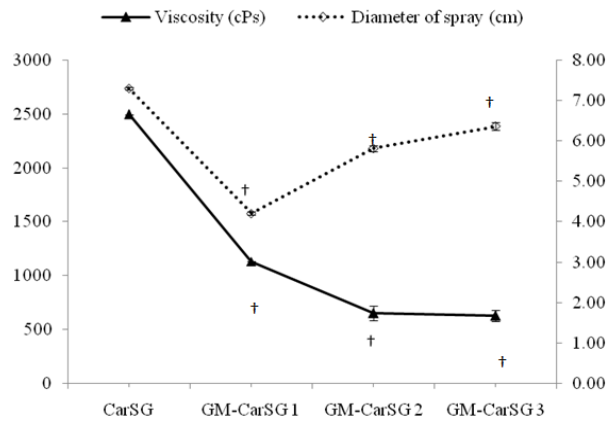

Fig. 2: Viscosity and diameter spray of CarSG, GM-CarSG 1, GM-CarSG 2, and GM-CarSG 3. The results are expressed as the mean±SD (n = 3). The viscosity and diameter of spread of GM-CarSG 1, GM-CarSG 2, and GM-CarSG 3 have a significant difference with CarSG (†p<0.0001)

Determination of SPF was done to evaluate the sunscreen effectivity. With the increase of SPF value, the capability of UV protection will increase [16, 29]. The SPF value was determined using a UV-vis spectrophotometer at 290-320 nm and interval 2,5 nm [30]. The SPF value of GM-CarSG 1, GM-CarSG 2, and GM-CarSG slightly increase but still in minimum protection (table 5).

Table 5: SPF value formula spray gel

Sampel	SPF	Category
Positive Control (PC)	4.11+0.18	Intermediate protection
GM-CarSG 1	2.09+0.03†	Minimum protection
GM-CarSG 2	2.78+0.05†	Minimum protection
GM-CarSG 3	3.53+0.04*	Minimum protection

The results are expressed as the mean±SD (n = 3). SPF value of GM-CarSG 1 and GM-CarSG 2 has a significant difference from PC (†p<0.0001), while GM-CarSG 3 has a significantly different from PC (*p<0.05)

The SPF value of GM-CarSG 3 is higher than GM-CarSG 1 dan GM-CarSG 2. The addition of extract increases SPF value because the concentration of metabolites rises [31]. The sunscreen activity of GM-CarSG 1 and GM-CarSG 2 were significantly different compared to CarSG (†p<0.0001), while GM-CarSG 3 was a significant difference compared to CarSG (*p<0.05). So that all preparation be able to use as sunscreen.

DISCUSSION

In this study, the GL extract was made by using NADES. NADES is a liquid made u of significant metabolites (e. g., sugars, sugar alcohols, organic acids, amino acids, and amines) held together by strong intermolecular forces, especially hydrogen bonds [32]. NADES solvent is cheaper, environmentally friendly, non-volatile, made using simple preparations, and safe for consumption because it is food grade and has low toxicity [20].

When carrying out extraction with solvents, the solvent should fulfill the following criteria: high selectivity (polarity), safety (low toxicity, nonexplosive, and nonflammable), neutral, easy to separate from target compounds, low viscosity (allow ease of transfer mass), low boiling temperature (prevent degradation of combinations), and economical (as cheap as possible). To increase polarity, some of the conventional volatile organic solvents used in plant extraction include petroleum ether, n-hexane, toluene, diethyl ether, chloroform, dichloromethane, ethyl acetate, acetone, n-butanol, ethanol, and methanol [2]. Generally, these solvents are used in large quantities, mainly when maceration or percolation is used, causing the high cost of extraction. Organic solvents are relatively toxic due to the possibility of residual solvents in the extracts produced.

Green solvents are alternative solvents meeting “green” criteria, so they are environmentally friendly and relatively safe [3]. Several advantages of green solvents include that they are easy to prepare (synthesized with ecologically friendly materials and procedures), less hazardous, low energy, recyclable, biodegradable, etc. [4]. The extraction process is carried out using the MAE (Microwave Assisted Extraction) method because the amount of solvent required is small and the time required is short. The microwave energy can penetrate the deeper dried materials [21]. Screening of phytochemical compounds found that both the extract GL and ML contain polyphenol. Polyphenol is one of the plant's primary compounds with antioxidant activity. The NADES-MAE method increased the polyphenol compound and the antioxidant activity of turmeric and thyme [22, 23].

GL and ML extract were used in spray gel as sunscreen. The spray gel was made by Carbopol 940 as a gelling agent, sodium metabisulfite, methyl paraben, propyl paraben as a preservative, and sodium-EDTA as a chelating agent. Propylene glycol was used as a humectant and triethanolamine (TEA) to adjust the pH of the preparation [10, 11]. Both extracts have a sunscreen activity based on the SPF value because the capability of UV protection will increase [33]. Mango and Mulberry leave in CarSG can be used as UV protection if the SPF value is a minimum of two and the UV protection is high if SPF is more than 15 [34].

CONCLUSION

The study suggested that using gedong mango leaves and mulberry extract in spray gel preparation has a sunscreen activity; the combination of gedong mango leaves extract. Mulberry leaves extract is well formulated in spray gel preparation with Carbopol 940 as a gel base because the physical characteristic fulfills the requirements of organoleptic, homogeneity, pH, viscosity, and diameter of spread. Spray gel containing extract of GL: ML (3:6) has the highest SPF value. These preparations have minimal sunscreen activity because they have an SPF value of 2-4. Both mangoes leave extract and mulberry extract have the potential to use in topical preparation as a sunscreen that helps protect the skin from UV light and prevents the skin from erythema, irritation, and other skin diseases because of UV radiation.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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