International Journal of GEOMATE, April, 2020, Vol.18, Issue 68, pp. 66 - 72
ISSN: 2186-2982 (P), 2186-2990 (O), Japan, DOI: https://doi.org/10.21660/2020.68.5656
Special Issue on Science, Engineering and Environment

FORMULATION, ANTIOXIDANT AND ANTIBACTERIA ACTIVITIES OF PEEL-OFF GEL MASK, ENRICHED WITH BIDARA LEAF (ZIZIPHUS SPINA-CHRISTI L.) EXTRACT

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ABSTRACT: Bidara Leaves (*Ziziphus spina -christi* L.) is the one of the herbs that already known by people to repair the broken cell and acne's problem. The bidara leaves extract can be formulated into a peel-off gel mask for facial skin. The objective of this research is to formulate the peel-off gel mask from ethanol extract of bidara leaves. The peel-off gel mask expected effective in reducing the adverse effects of free radicals and inhibits the growth of acne-causing bacteria on the face. Identification of bioactive compounds in bidara leaves extract with phytochemical test and Liquid Chromatography-tandem Mass Spectrometry (LC-MS/MS) instrument. Antioxidant activity test using *1,1 -diphenyl-2-picrylhydrazil* (DPPH) method and IC_{50} value determination. Testing of antibacterial activity of ethanol extract of bidara leaf against *Propionibacterium acne* bacteria by diffusion method. Preparation of gel mask peel-off using variation of concentration 15, 25 and 35%. Peel-off gel mask characterization by organoleptic test, physical appearance, pH value, homogeneity test, dispersion test, drying time test and microbial contamination. Ethanol extract of bidara leaves has very strong antioxidant activity with IC_{50} 23,4 ppm and antibacterial activity with inhibition of bacteria *P. acne* at a concentration $\geq$ 0.25 g/mL The results of LC-MS/MS analysis showed that there were routine compounds at retention time of 5.13 which had the potential as antioxidants and antibacterial. Peel-off gel masks obtained have antioxidant and antibacterial activity and are included in the quality requirements category of SNI 16-6070-1999 and SNI 16-4380-1996.

Keywords: Antibacterial, Antioxidant, Bidara leaves, Peel-off gel mask

1. INTRODUCTION

The utilization of natural substances as antioxidant and antibacterial agents for health product and personal care has been increasingly developed to support human basic needs. Plants as natural substances have been considerable used for health, medicine, and beauty products. In global health's context, natural source and its outcome prove that all plants contain compounds, which are clinically proven beneficial for health [1]. *Bidara* is one of the plants that is beneficial for human health. According to hadith by Bukhari and Muslim, Rasullullah presented the commendable deed to utilize *bidara* leaves in performing Islamic ritual ablution and purification on a dead body. This ritual performance gives basic knowledge to human that *bidara* leaf has beneficial effects to purify.

Bidara leaf extract has been utilized in medicines development in addition to pharmacology activities in Middle East, South East, and East Asia. *Bidara* leaf is believed to have medicinal properties to cure various diseases. *Bidara* has been used as an alternative medicine for self- purify and to cure fever, pain, dandruff, wound, inflammation, asthma, and sore eyes [2]. In traditional medicines, all parts of *bidara*, namely root, leaf, fruit, seed, and stem were used to cure [3]. *Bidara* leaf extract has been analyzed by using LC-M instrument. Soleh Putri, 2017 obtained the highest concentration of two suspected rutin compounds, which classified as one of the active flavonoid compounds [4]. Kusriani *et al.* (2015) stated that bidara leaf extract contained phenolic compounds, in total 7,192% ± 0,0198 [5]. Meanwhile, the ethanol extract of bidara leaves showed antioxidant activities with IC_{50} value 127,87 ppm [6].

Research conducted by Haeria *et al.* (2016) suggested that the ethanol extract of bidara leaves contained high concentration of antioxidant, with flavonoid compounds 1,5312% in total and IC_{50} value 90,9584 ppm [7]. Ali *et al.* (2015) revealed that *bidara* leaf extract shows antibacterial activities to prevent pathogen-bacteria growth, i.e. *Proteus vulgaris*, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* that can be potentially added into the active substances in facial-cosmetic product [8].

Facial cosmetic can be produced in various forms. One of those forms is peel-off mask, which is practically used and easy to clean [6]. Ariani and

Wigati (2019) used the formulation of peel-off mask as acne cream with Polyvinyl alcohol (PVA) as the main formula of gelling agent; hydroxypropyl methylcellulose (HPMC) as viscosity-increasing agents; and propylene glycol (PG) as humectant agent [9]. The formulation of peel-off gel mask with selected basis substances, i.e. PVA with higher concentration than HPMC, did produce the most excellent characteristic of peel- off gel mask [10]. Therefore, a research on peel-off gel mask, enriched with ethanol extract of *bidara* leaf (*Z. spina-christi* L.) with various concentrations, is supposed to conduct. This new formula is expected to increase antioxidant and antibacterial activities of peel-off gel mask.

2. RESEARCH METHOD

2.1 Material And Equipments

Bidara leaves used in this research (approximately 1 kg), were obtained from *bidara* farm in Sumenep, East Java and identified in Herbarium Bogoriense, LIPI Bogor. A solvent used was 96% technical grade ethanol. Bacterial isolate of *P. acne* was obtained from Laboratorium Biologi, Universitas Indonesia (Biology Laboratory, University of Indonesia). Tryptic Soy Agar was used as a medium to culture antibacterial compounds. DPPH was used to analyze antioxidant activities. PVA, HPMC, propylene glycol, and aquades, as well as commercial product to compare, were used to form peel-off gel mask. The equipments used in this research were Spectrophotometer UV Vis, LC-MS/MS, analytical weighing balance, laboratory glassware, pH meter, oven, vacuum rotary evaporator, incubator, and autoclave.

2.2 Extraction and Phytochemical Analysis of Bidara Leaves

Bidara leaves (Z. spina -christi L.) were grinded, weighed and put into a container to be macerated. Afterwards, the macerated bidara leaves were added with 96% ethanol until the leaves were completely soaked. After the soaking procedure, the soaked bidara leaves were stirred for 30 min. The container was covered and kept for 24 h at room temperature. After the maceration process, the yields were filtered to separate the filtrates and the residue. The filtration of bidara leaf extract was collected and condensed in a vacuum rotary evaporator. The solvent was further evaporated to obtain thick-ethanol extract of bidara leaves. Afterwards, the ethanol extract of bidara leaves were ready to use as the test samples. Phytochemical analysis on bidara leaf extract to examine steroid/triterpenoid, flavonoid, alkaloid, phenolat, tannin, and saponin compounds, was conducted by using proper reagents.

2.3 Identification of Active Compound of Ethanol Extract of Bidara Leaves using LCMS/MS Method

Ethanol extract of *bidara* leaves (1 mg) was weighed and diluted in methanol. Leaf samples (10 µL) were taken and injected to LCMS/MS through column C-18 (2 x 150 mm) with the flow rate 0,2 mL/min. Chromatography analysis and mass spectrophotometry were observed by using a software program, Mass Lynx (Version 4.1), to identify the suspected chemical structures.

2.4 Formulation of Peel-off Gel Mask

Polyvinyl alcohol (PVA) was added with aquades four times and heated until the PVA was transparent and homogenous. Hydroxypropyl methylcellulose (HPMC) was grown with aquades for 30 min. PVA and HPMC were mixed in a mortar and grinded until the mixture was homogenous. The mixture was added with proplylene glycol, gradually diluted with ethanol and odour, and then homogenized. The peel-off gel mask was formulated with varied concentration of bidara leaf extract, 25, 30, and 35% (b/v).

2.4.1 Antioxidant activity testing

A 2 mL sample was put into a culture tube, then added with 2 mL of DPPH 0,002%. The mixture was homogenized then incubated in a dark room, at room temperature for 30 min. The absorbance was measured by using UV-Vis Spectrophotometry and obtained wavelength 516 nm. A percentage inhibition was calculated and equated into a linear regression analysis to obtain IC_{50} value. The absorbance value of DPPH, before and after added with samples, was further measured. Furthermore, the outcome was denoted by linear regression analysis with extract concentration (ppm) as abscissa on x-axis and inhibition percentage value, i.e. antioxidant as ordinate on y-axis, and IC_{50} value at inhibition percentage 50% $y = ax+b$.

2.4.2 Antibacterial activity testing

Antibacterial activity testing of bidara leaves was conducted by performing preliminary test on ethanol extract of bidara leaves, with the following steps: (i) TSA medium (25 mL) was inserted into petri dish; (ii) added with bacterial suspension (0,25 mL); (iii) homogenized until the medium was solid and firm. Furthermore, holes were made in medium and applied with 100 µL extract, then incubated at 37 ºC for 24 h. Ethanol extract testing

of bidara leaves was conducted on thick extract, with extract concentration 1; 0,5 ;0,25; 0,125 g/mL. Inhibition zone formed was observed; diameter of inhibition zone was measured.

2.4.3 Characterization of peel-off gel mask

The analysis of formulation result from ethanol extract of peel- off gel mask of *bidara* leaves includes: organoleptic test or testing on panellist's preference, physical appearance, pH value, dispersion test, drying-time test, viscosity test, and microbial pollution test to identify the gel mask quality.

3. RESULT AND DISCUSSION

3.1 Extraction and Phytochemical Analysis on *Bidara* Leaves

Bidara leaves were grinded and extracted by using maceration method with 96% ethanol, resulted thick and dark green extract; yield percentage 19%. Phytochemical screening test of ethanol extract of *bidara* leaves showed positive results on the six reagents with colour change when added with reagents, proved that bidara leaf extract contains steroid, flavonoid, alkaloid, tannin, phenolat, and saponin compounds (Table 1). Ethanol extract of *bidara* leaves was expected to have potential antioxidant and antibacterial activities.

Table 1 Phytochemical screening test

Class of Compounds	Reagent	Colour Change	Conclusion
Steroid	Lieberman Burchard	Green	+
Flavonoid	Mg+HCl	Orange	+
Alkaloid	Wagner	Precipitated Brown	+
Phenolat	Aquades + $FeCl_3$ 1%	Black	+
Tannin	$FeCl_3$ 1%	Blackish Green	+
Saponin	Aquades	Foamed	+

3.2 Identification of Active Compounds in Ethanol Extract of *Bidara* Leaves using LCMS/MS Method

The analysis of the suspected compounds was carried out by comparing the similarity of mass spectra samples and mass spectra compounds, using online database Mass Bank, The Human Metabolome Database, and Mass Bank of North America. Chromatogram in fig. 2 illustrated some peaks of ethanol extract compounds from *bidara* leaves. The m/z value 611,1687 was fragmented to be m/z 303,0536 at retention time 5,15. Ionization used in this research was $[M+H]^+$ to determine the real m/z value 610,1687. The prediction of compound molecular formula was $C_{27}H_{30}O_{16}$. Compared with online database Mass Bank, the findings showed some similarities of mass spectra as a result from rutin mass spectra measurement, i.e. m/z 611; 465; 303. Phytochemical screening test showed flavonoid compounds. Thus, the compound appeared at retention time 5,15 was suspected as flavonoid compound, namely rutin.

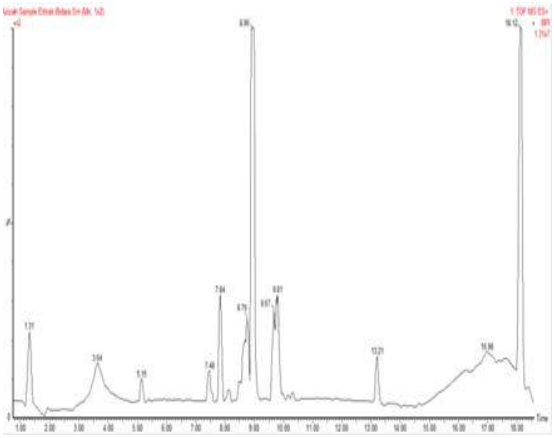

Fig 1 Chromatogram of ethanol

3.3 Extract of *Bidara* Leaves

Rutin is a derivative compound from flavonoid, i.e. flavonol. Rutin is a condensation result between quercetin and glycone rutinoside. Quercetin-3-O-rutinoside and quercetin have antioxidant activities [11]. Flavonoid prevents free radicals through free radical scavenger process by donating hydrogen atom to alter radicals being inactive [12]. According to Pelczar dan Chan (2006), quercetin may also act as antibacterial because its phenolic group performs protein denaturation and damage microbial cell membranes [13]. Quercetine and its derivative compounds were first reported to have antivirus activities [14].

3.4 Antioxidant Activity of Ethanol on Bidara Leaves

Ascorbic acid was used to observe antioxidant activities. Research found that ascorbic acid showed strong antioxidant activity, with IC_{50} value 3,606 ppm. Antioxidant activity of bidara leaf extract (*Z. spina-christi* L.) also classified into highly-strong

antioxidant activity, with IC_{50} value 23,4. However, antioxidant activity of ethanol extract of *bidara* leaves is lower than ascorbic acid.

Table 2 Antioxidant activity test on ethanol extract of *bidara* leaves

Conc. of bidara (ppm)	Ads	% Inhibition	Linear Reg.	IC_{50} (ppm)
0	0,264	0		
2,5	0,185	29,92 ± 0,0042		
5	0,172	35,04 ± 0,0007	y= 0,7921 x 31,479	
10	0,156	40,90 ± 0,0014		23,4
20	0,134	49,43 ± 0,0007	R^2 = 0,9911	
40	0,093	64,96 ± 0,0007		
80	0,018	93,37 ± 0,0007		

Note: Means ± Standard Deviation (SD)

3.5 Antibacterial Activity of Ethanol Extract of *Bidara* Leaves

Antibacterial test was conducted on thick extract without dilution process and some varied concentrations, i.e. concentration 1; 0,5; 0,25, and 0,125g/mL, respectively. Positive control was antibiotic, generated inhibition zone 37,80 mm. Negative control was sterilized aquades, generated inhibition zone 8 mm. Results of antibacterial test of *bidara* leaf extract at concentration 0,125 g/mL showed the same inhibition zone with negative control; proved that leaf extract at that concentration did not show inhibition activities against *P. acne* bacteria. Concentration extract of 0,25 g/mL showed diameter of clear zone 14,40 mm; Concentration 0,5 g/mL resulted inhibition diameter 15,90 mm; Concentration 1 g/mL resulted inhibition diameter 17,50 mm and thick extract without dilution process 18,85 mm. *Bidara* leaf extract (*Z. spina-christi* L.) showed inhibition activities against *P. acne* at concentration ≥ 0,25 g/mL. Results obtained from responses of substances in diffusion holes, marked by the area clearness or diameter of inhibition zone at each concentration.

Table 3 Antibacterial activity test on ethanol extract of bidara *leaves*

Concentration of Bidara (g/mL)	Inhibition Diameter (mm)
0,125	8 ± 0
0,250	14,40 ± 1,56
0,500	15,90 ± 1,98
0,100	17,50 ± 1,70
Pekat	18,85 ± 2,76
Kontrol (+)	37,80 ± 1,56
Kontrol (-)	8 ± 0

Note: Means of inhibition diameter ± Standard Deviation; Control (+) is tetracycline and Control (–) is sterilized aquades

3.6 Formulation of Peel-Off Gel Mask of Ethanol Extract of *Bidara* Leaves

The homogenized substances of peel-off gel mask was added with extract concentration, 0,25, 30, and 35%, respectively. The concentration was taken from the results of antibacterial activity test on *bidara* leaf extract, which showed inhibition power at concentration ≥ 0,25 g/mL.

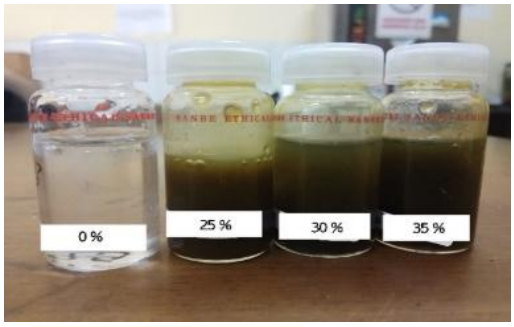

Fig.2 Formulation of peel-off gel mask

The peel-off gel mask obtained was F0 with extract concentration 0%, resulted transparent or clear cream; F1 with 25% extract concentration in faded green colour; F2 with 30% extract concentration in light green colour; F3 with 35% extract concentration in dark green colour (Figure 3). The green colour in peel-off gel mask was resulted from *bidara* leaf extract. The thickness of green colour in peel-off gel mask increased equally with the adding of *bidara* leaf extract.

3.7 Antioxidant Activity of Peel-off Gel Mask of *Bidara* Leaf Ethanol Extract

Based on the results of antioxidant activity test on peel-off gel mask, the higher amount ethanol extract of *bidara* leaves (*Z. spina-christi* L.) added into the peel-off gel mask, the higher antioxidant activities performed. It was in accordance with the increasing inhibition percentage value of peel-off gel mask and the increasing ethanol extract concentration of *bidara* leaves. The inhibition percentage was lower compared with the ethanol extract of *bidara* leaves. The smaller amount of extract concentration than the amount of peel-off gel mask used in this research led to low percentage of inhibition. The decrease of inhibition percentage might be caused by the amount of ethanol extract of *bidara* leaves that was not sufficient or less, hence the active compounds did not optimally work. However, the peel-off gel mask showed high antioxidant potentials, compared with commercial product with the lowest inhibition value.

Table 4 Antioxidant activity test of peel-off gel mask

Sample	Inhibition percentage (%)
Commercial (F0)	18,389 ± 0,737
(Sample without additional extract)	3,163 ± 0,847
(Sample added with 25% extract) F2	38,638 ± 3,876
(Sample added with 30% extract) F3	50,862 ± 1,219
(Sample added with 35% extract) Thick extract of *bidara* leaves	65,743 ± 3,227
Ascorbic acid	80,823 ± 3,823
	94,568 ± 0,415

Note: Means of inhibition percentage ± standard deviation

3.8 Antibacterial Activity of Peel-off Gel Mask of Ethanol Extract of *Bidara* Leaves

Antibacterial activity test on peel-off gel mask of ethanol extract of *bidara* leaves showed results that the higher concentration the ethanol extract of *bidara* leaves added into the peel-off gel mask, the larger the inhibition activities against *P. Acne* performed. Results were obtained from responses of substances in diffusion holes, marked by the area clearness or diameter of inhibition zone. The difference of extract concentration added to each samples led to different clear zones. The higher extract concentration of *bidara* leaves added, the larger amount antibacterial compounds contained. The larger amount antibacterial compounds obtained from *bidara* leaf extract, the larger amount *P. acne* bacteria damaged, either the structure or metabolic system. Research found bacteria interrupted by antibacterial compounds were killed or inhibited. Findings showed that formulation with the largest width of inhibition zone was peel-off gel mask of *bidara* leaf extract, formula F3 added with 35% extract, which showed inhibition zone 9,10 mm. The sample with the smallest width of inhibition zone was commercial peel-off gel mask, with inhibition zone 6 mm, same with peel-off gel mask without additional extract as the negative control. Antibacterial activity test showed that peel-off gel mask formula added with ethanol extract of *bidara* leaves performed antibacterial activities against *P. acne*. The result was relatively small compared with the inhibition zone of ethanol extract of *bidara* leaves before formulated in peel-off gel mask. The low inhibition zone in peel-off gel mask was found to be caused by high viscosity, led to become lower antibacterial activities. The bond between gel bases and high viscosity was tighter, hence the active compounds were more difficult to diffuse [15].

Table 5 Antibacterial test on peel-off gel mask

Formulation	Inhibition Diameter (mm)
Commercial	6 ± 0
F1	7,65 ± 0,49
F2	8,50 ± 1,13
F3	9,10 ± 0,85
Control (+)	32,30 ± 0,42
Control (–)	6 ± 0

Note: Means of inhibition diameter ± standard deviation; Control (+) is t tetracycline and Control negative (–) is *peel-off* gel mask without additional extract.

3.9 Characterization of Gel Mask and Hedonic Test on Panellists

Characterization on formula of peel-off gel mask of ethanol extract of *bidara* leaves includes physical appearance, pH value, viscosity test, dispersion test, drying -time test, and microbial pollution test to study the characteristic of each peel-off gel mask sample. Characterization test

showed that all samples have fulfilled the requirements of SNI 16-6070-1999, concerning facial-mask quality and SNI 16-4380-1996, concerning facial-cleanser product.

Table 6 Activity and characteristic of peel-off gel mask

Charac	Com	F0	F1	F2	F3
Phy	Hmg steril	Hmg steril	Hmg steril	Hmg steril	Hmg steril
pH Value	6,56	6,62	5,62	5,53	5,43
Disp (cm)	5,3	5,5	5,25	4,765	4,53
Dry (min)	25	29	26	24	22,5
Visc (cP)	6020	8141	12039	13367	16653
Micr (coloni/ gram)	N/A	N/A	N/A	N/A	N/A

Notes:
Charac= Characteristic; Com= Commercial;
Disp= Dispersion; Dry= Drying time;
Hmg= Homogenous Micr= Microbial Pollution; Phy = Physical appearance; Steril = Sterilized; Visc= Viscosity.

Furthermore, statistical hedonic test on panellist preference showed peel-off gel mask formula F1 indicated the highest level of preference, with the average value 3,87. Commercial peel-off gel mask showed the same subset-class, with the preference level 3,84. Peel-off gel mask formula F0 showed preference value 3,78 on second subset-class. Peel-off gel mask formula F2 and F3 were on first sub-set with preference value 3,02 and 2,64. These values were significantly different with second subset-class, suspected it was due to the colour change, odour, and texture [16]. The panellists preferred white colour and fresh odour. However, moderate addition of extract in formula F1 (25%) still could be accepted by the panellists because the green colour was not very thick; the odour was not too sour; and the thickness texture was sufficient. Therefore, based on organoleptic test, formula F1 was chosen as the best formulation.

4. CONCLUSION

Ethanol extract of *bidara* leaves was expected to contain (i) rutin compounds, (ii) IC_{50} value 23,4 ppm, and (iii) antibacterial activity of *P. acne* against ethanol extract of *bidara* leaves (*Z. spina-christi* L.) at concentration ≥ 0,25 g/mL. Based on organoleptic test, the best formulation of peel-off gel mask of ethanol extract of *bidara* leaves was formula F1 with 25% extract addition. The formulation, which showed antioxidant activity 38,638% and inhibition zone against *P. acne* 7,65 mm, has fulfilled the quality requirement of SNI 16-6070-1999 and SNI 16-4380-1996.

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