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Anti-bacterial activity of *Hedychium coronarium* J.Koenig's rhizome extract

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Abstract

The objectives of this independent study were to study the antibacterial activity of *Hedychium coronarium* J.Koenig's rhizome extract and its application in anti-acne gel formulation. The results of GC analysis showed that *H. coronarium* .J.Koenig's rhizome extract contains chemical compounds coronarin E, eucalyptol, (E)-labda-8, 12-diene-15, 16-dial, α-therpineol, n-hexadecanoic acid, bicyclo [3.1.0] hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1-α, 2-β., 5-α.) -e, etc. This study also found that extracts using dichloromethane and ethanol have antibacterial effects against bacteria *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Propionic acnes*, but the effects from extract using dichloromethane is better than ethanol. The minimum inhibitory concentrations (MICs) of the ethanol extract and dichloromethane extract were found to be in the range of 16.00 µg/mL – 24.41µg/mL and 6.10 µg/mL – 12.21 µg/mL respectively. Their minimum bactericidal concentrations (MBCs) of ethanol extract were shown in the range of 195.31µg/mL - 781.25 µg/mL and dichloromethane; 12.21 µg/mL – 24.41 µg/mL respectively. However, extract using ethanol was selected for formulation of acne gel due to safety reason. Besides, two formulations of acne gel were prepared, i.e., one with ethanol and another without ethanol. The formulated gels were subject to pH, viscosity and color checks; centrifugal, temperature cycling and shelf life tests. Its viscosity, pH and color had changed

slightly after the temperature cycling and shelf life tests. However, its texture, ability to spread, odor and homogeneity had not changed significantly.

Keywords: *Staphylococcus aureus/ Staphylococcus epidermidis* / *Propionic acnes*/ *H. coronarium* J.Koenig/ Rhizome

Introduction

Acne vulgarism is a skin disorder commonly found among teenagers from 15 years old until middle age. There are five types of acne: whitehead, blackhead, papules, pustules, and cyst. They often appeared on the face, neck, bust, and at the back of the body (Renu, 1989). Similar to micro-comedone it started with the accumulation of dead skin inside hair follicle. The dead skin will then discharged to the surface later under normal conditions. While the dead skin remains in the uninfected pore, the skin looks normal. Nevertheless, when the secretion of sebum by the sebaceous gland in the pore is excessive, the skin becomes oily. Oily skin stimulates the production of androgen hormone, which attracts acne-causing bacteria. These bacteria in the pore multiply causing the pore to swell. Hence, transform the affected pore into acne. This type of acne is called *Propionic* acne. Recently, there are many studies in the area of natural ingredients for acne treatments.

Butterfly lily or white ginger is the herbal plant belongs to the Zingiberaceae family. It is an herbaceous perennial plant, which can grow up to about 1.5m. It is commonly found in areas with tropical and sub-tropical climates. It can be cultivated and harvested throughout the year. Botanically, it is called *Hedychium coronarium* J.Koenig (Maha-hong). It was first discovered by J.Koenig in 1783. Sometimes, it is called *Hedychium spicatum* Lodd, (Ta-hern), *Kaempferia hedychium* Lam. (Hern-Keaw) (Pornpimon Wongsuwan, 2011). *H. coronarium* J.Koenig has been used in traditional treatments for anti-inflammation and blood pressure reduction (Lu et al., 2009). Studies have shown that rhizome extracted by methanol, ethyl acetate and dichloromethane did possess positive antibacterial effects on bacterial such as *Bacillus subtilis*, *Bacillus megaterium*, *staphylococcus aureus*, *Sarcina lutea*, *Shigella shiga*, *Pseudomonas aeruginosa* and *Salmonella typhi* (Aziz, Habib, & Karim, 2009). In another study, extract from *H. coronarium* J.Koenig's leaf and rhizome had shown

to have antioxidant and tyrosinase enzyme reduction effects (Chan et al., 2008). Another study had also shown the extreme antibacterial effects of butterfly lily extraction using dichloromethane. Butterfly lily is also commonly used in traditional medicines for anthelmintic, antifungal, analgesic and anti-inflammation purposes (Lu et al., 2009; Aziz et al., 2009; Prakash, Rajput, Kumar, & Pant, 2010). The chemical composition was identified using gas chromatography and mass spectroscopies. It contains linalool, limonene, γ-terpinene, 1, 8-ceneol, α-eudesmol, β-caryophylene, β-pinene and α-terpineol (Prakash et al., 2010; Báez, Pino, & Morales, 2011). These are the major components found in the *H. coronarium* J.Koenig rhizome (Báez et al., 2011; Santos et al., 2010). The objectives of this research are to study the antibacterial activity of *H. coronarium* J.Koenig's rhizome extract and its use as an active ingredient in anti-acne gel formulation.

Materials and Methods

Obtained material: Butterfly Lili or *H. coronarium* J.Koenig's rhizomes were from Mukdaharn province of Thailand. There were collect in February 2019.

Maceration extraction: Flesh *H. coronarium* J. Koenig's rhizome (10 kg) was cut into slices. The rhizome slices were then dried and pulverized into 2.4 kg of rhizome powder. *H. coronarium* J. Koenig's rhizome was extracted by maceration method using absolute ethanol and dichloromethane. The powder to solvent mass ratio was 1:3. Two types of rhizome extracts were obtained: ethanol extract and dichloromethane extract.

Gas chromatography: The extracts were analyzed by GC/MS (Shimadzu, QP2010 Ultra) using GC mode. The column of GC unit selected was DB5. The injection mode selected was "split mode". Injected temperature was set at 230°C. The mode of flow selected was linear injecting at 16.1 kPa. The flow rate was set at 1.5 mL/min; purged flow rate set at 3.0 mL/min; oven temperature set at 50°C and heat up for 5 min. Then diluted crude, volume up to marking six in the column, was injected. Then oven temperature was increased to 140°C and hold for 10 min. After that all balance crude was injected. Oven temperature was then increased to 230 °C and hold for 10 min. After injection, carrier gas helium was selected; interface temperature was set as 250°C. At interface temperature, 50% (minimum scanned area) of the area in the gas

chamber was scanned. The results were then recorded and printed (Santos et al., 2010; Gershon, Shugar, & Ballinger, 1996).

Antibacterial activity: Antibacterial activity of *H.coronarium* J.Koenig rhizome extract was determined by broth well dilution method with slight modifications (Stephen et al., 2005; Clinical and Laboratory Standard Institute, 2018). The samples were tested using three cultured bacteria: *Staphylococcus aureus* ATCC6538, *Staphylococcus epidermidis* ATCC1228 and *Propionic acne* ATCC6919. The tests were conducted by the Centre of Analysis Product Quality (MU-CAPQ) of Mahidol University, Thailand. Two stocks were prepared for inhibition zone tests. One stock was prepared using 200mg of crude mixing with 1.0 mL (1000 µL) of DMSO (dimethyl sulfonate). Another stock was prepared using 200 mg of crude mixing with 1.0 mL (1000 µL) of ethanol.

Minimum inhibitory concentration (MICs): The ethanol stocks were prepared using 0.1mg of crude diluted with 500 µL of ethanol. For dichloromethane stock, it was prepared using 0.1mg diluted with 500 µL of dichloromethane. Two set of broths well were used. Each set of broth contained 20 wells. To determine the MIC of test stock, 250 µl of test stock prepared was dropped into the first well. Progressively, the volume dropped into the subsequent well was 50% of that of previous well i.e. second well volume was 125 µl, third well volume was 112.5 µl until all the 20 wells in the set were filled.

Minimum bactericidal concentration (MBCs): The procedures to determine MBC was similar to inhibition zone test using disk diffusion. The difference was in the type of stock used. In the case of MBC tests, the stocks used were obtained from the first broth well to the well having the clear zone in MIC test.

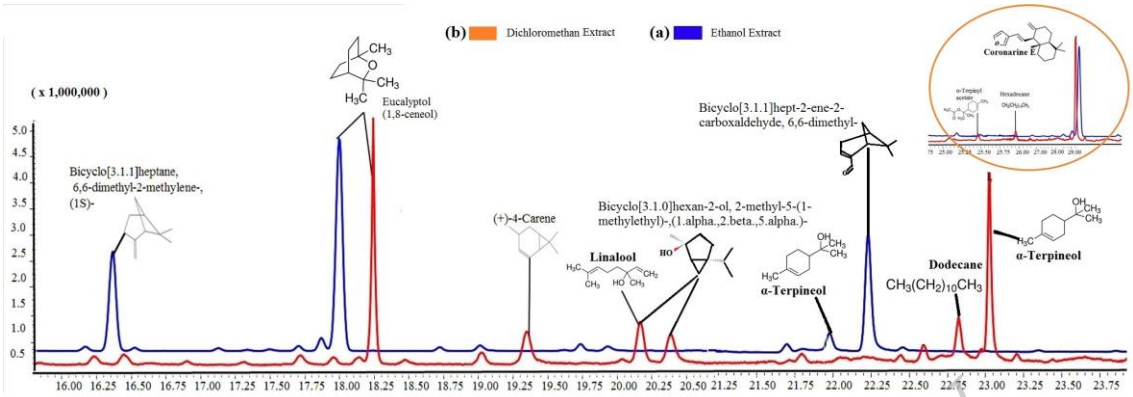
Formulation of acne gel: The mixing order used for base gel formulation with some modifications (Flick, 1992). The base gel was prepared using gelling agent carbopol®934 mixing with distilled water. Then triethanolamine (TEA) was added while stirring. Ethanol was subsequently added to form a homogeneous base gel. *H.coronarium* J.Koenig's rhizome extract was added later to form acne gel. Gels prepared were then subject to various physical properties checks and stability tests.

Results and Discussion

The ethanol (ETOH) extract was brownish in color while the dichloromethane (DCM) extract looked dark brown. Both extracts possess strong herbal odor. Both extracts looked thick but DCM extract seems to be thicker than ETOH extract. The yield of ETOH extract and DCM extract were 29.33 ± 0.05 % and 7.00 ± 0.02 % respectively. The difference in yields is due to ethanol molecules being more polarized than that of DCM, hence producing a higher yield (Sigma-Aldrich Corporation, 2014). The physical color and appearances of ethanol extract and dichloromethane extract are shown in Figure 1.

Figure 1 Ethanol extract (a) and dichloromethane extract (b)

The chemical compositions of the extracts were found to contain more than 30 components. The major compounds identified in ethanol extract were Coronarin E (47.88%), eucalyptol (31.00%), (E)-Labda-8(17), 12-diene-15, 16-dial (13.24%) and α-Terpineol (11.91%). The dichloromethane extract contained Coronarin E (36.15%), Eucalyptol (27.57%), (E)-Labda-8(17), 12-diene-15, 16-dial (13.24%) and Bicycloheptane [3.1.1] 6, 6-dimethyl-2-methylene-(1S)-(13.03%).



Note. Ethanol (a), Dichloromethane (b)

Figure 2 Chromatogram of ethanol extract (a) and Dichloromethane extract (b)

Both ethanol and dichloromethane extracts had demonstrated positive inhibitory effects against bacteria *S.aureous*, *S.epidermidis* and *P.acne*. The inhibition zones is showed in Table 1. Inhibition zones of ethanol extract compared with positive control by used EtOH solution, these bacteria were found to be 10 mm for *S.aureous*, 12.67 mm for *S.epidermidis* and 16 mm for *P.acne*. And inhibition zone of dichloromethane were found 0 mm for *S. aureous* after compared with positive control by used DMSO (Dimethyl sulfoxide), 15.67 mm for *S. epidermidis* and, 5 mm. for *P. acne*. Because of dichloromethane volatility and chlorinated solvent miserable in the water, were then not occurred the inhibition zone for *S. aureous*, one possibility it's has resistant and/or bacteria immune (Ankita Sharma, & Kanika Sharma, 2011; Lulitanond, 2018).

Table 1 Inhibition zone of *H.coronarium* J.Koenig's rhizome extracts

Microorganism	Inhibition Zone Diameter (mm.)			
	Ethanol extract	Control ETOH	Dichloromethane extract	Control DMSO
Staphyrococcus aureus	10.00	0.00	0.00	14.00
Staphyrococcus epidermidis	12.67	0.00	15.67	0.00
Propionibacterium acnes	16.00	0.00	5.00	15.00

Minimum inhibitory concentrations (MICs) and Minimum bactericidal concentrations (MBCs) of *H. coronarium* J.Koenig's rhizome extracts are shown in Table 2. The MICs of ethanol extract against bacteria *S.aureous, S.epidermidis* and *P.acne* were found to be 24.41 µg/mL, 12.67 µg/mL and 16.00 µg/mL respectively. The MBCs were 781.25 µg/mL, 195.31 µg/mL and 390.63 µg/mL respectively. For dichloromethane extract, the MICs against bacteria *S.aureous, S.epidermidis* and *P.acne* were found to be 12.21 µg/mL, 12.21 µg/mL and 6.10 µg/mL respectively; The MBCs were 24.41 µg/mL, 48.83 µg/mL and 12.21 µg/mL respectively.

Table 2 Minimum inhibitory concentrations (MIC) and Minimum bactericidal concentrations (MBC) of *H. coronarium* J.Koenig's rhizome extracts

Microorganisms	Ethanol extract		Dichloromethane extract	
	MIC(µg/ml)	MBC(µg/ml)	MIC(µg/ml)	MBC(µg/ml)
Staphyrococcus aureus	24.41	781.25	12.21	24.41
Staphyrococcus epidermidis	12.67	195.31	12.21	48.83
Propionibacterium acnes	16.00	390.63	6.10	12.21

Acne gel was successfully formulated using gelling agent Carbopol®934 and *H. coronarium* J.Koenig rhizome extract were specific and interpolated by refer to MBCs result of *P.acne* 390.60 µg/mL of ethanol extract. Two formulations of acne gel were prepared, i.e., one with ethanol and another without ethanol as shown in Table 3 and Figure. 3. The pH values of acne gel with ethanol were found to be in the range of 7.10–7.96 and 5.83-5.89 for acne gel without ethanol. The pH values of both gels are within the acceptable range of 4.5-8.0 (Barel, Paye, & Maibach, 2010). The gels appeared translucent and yellowish brown in color.

Table 3 Order of mixing for acne gel with ethanol and without ethanol formulation.

Ingredients	INCI Name	w/w (g)			
		C	D	E	F
Part A					
Water	Distillation water (DI)/Aqua	63.60	93.60	63.10	93.10
Carbopol® 934 NF (Cosmetic grade)	acrylic acid and C10-C30 alkyl acrylate cross polymer	0.50	0.50	0.50	0.50
Triethanolamine	Triethanolamine	0.60	0.60	0.60	0.60
Part B					
Ethanol	Denature Ethyl alcohol 95%	30.00	-	30.00	-
H.Coronarium J.Koenigs rhizome Extract.	*H.Coronarium* J.Koenigs rhizome Extract.	-	-	0.50	0.50
PolyPropylene glycol (USP/EP;Phamasueti cal grade)	Propylene glycol	5.00	5.00	5.00	5.00
Part C					
Phenochem NIB (Propyl paraben)	Paraben in 2-phenoxyethanol	0.30	0.30	0.30	0.30
Total		100.00	100.00	100.00	100.00

Figure 3 Anti-acne gel with ethanol (E), and without ethanol (F)

They have mild turmeric smell. Their textures are soft, smooth and spread well when applied on backhand and forehead. The viscosity of the formulated acne gels range 8,690 cP – 30,720 cP. It was higher than the minimum viscosity of 2,050 cP. When subject to centrifugal tests at 3,800 RPM for 30 min. and 5,000 RPM for 10

min., the gel remained homogeneous with no separation. After the temperature cycle test cycling between 4°C and 45°C there is a slight change in color to more brownish. This is common among herbal based products. Besides, the viscosity and pH of gels were reduced marginally after the temperature cycle test. The formulated acne gel has also passed the 30 days shelf life test at 27° C and 45° C with a slight change in color to more brownish. The physical appearance after the self life test is shown in Figure 4.

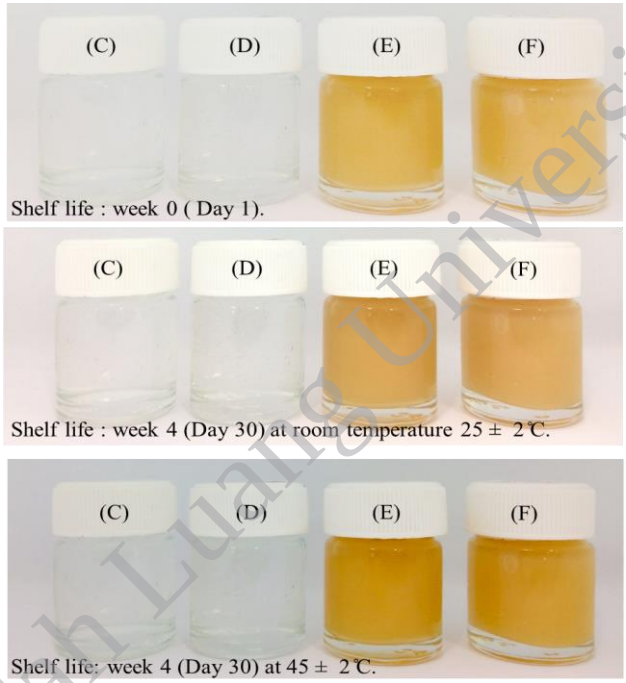


Figure 4 Physical appearance of gel after 30 days kept on the shelf.

Conclusions

H. coronarium J.Koenig's rhizome extract has again shown its positive antibacterial activity effects. In this study, a stable acne gel was successfully formed using the extract as an active ingredient. Potentially, it can be further developed into an effective herbal based acne gel in the future.

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