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Research Article

Formulation of Creams Containing Active Fraction of *Cassia fistula* L. Barks and its Antibacterial Activity Against *Propionibacterium acnes* and *Pseudomonas aeruginosa*

Anis Yohana Chaerunisaa[1,*], Muhaimin Muhaimin[2], Yasmiwar Susilawati[3], Tiana Milanda[3]

ABSTRACT

Background: *Cassia fistula* L. has been traditionally used to cure skin diseases. That disease can be caused by various bacteria, such as *Propionibacterium acnes* and *Pseudomonas aerugniosa*. **Objective**: the objective of this research was to formulate cream containing active fraction of *Cassia fistula* bark and to study the antibacterial activity as well as physical stability of the active substance after formulation. **Material and Methods**: The cream base were oil-in-water (O/W) and water in oil (W/O) type. Antibacterial activity test had been performed by using agar diffusion method. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were conducted by microdilution method. The active fraction was formulated into creams with concentration of 4 -6x MIC. Physical evaluation of creams including organoleptic, pH, viscosity, TLC (Thin Layer Chromatography) profiling and antibacterial activity against both tested bacteria were evaluated during 28 days of storage. **Results**: The results showed that ethyl acetate fraction was the most active, having MIC and MBC values of 175 and 350 ppm respectively against *P. acnes*, while those against *P. aeruginosa* were 400 and 800 ppm. Optimation on creams using different type of cream bases showed that either O/W or W/O creams remained stable during 28 days of storage in terms of organoleptic and pH. The viscosity increased in O/W and decreased in W/O type. Qualitative analysis by TLC profiling showed that the ethyl acetate fraction of *Cassia fistula* as chemical compounds in creams was relatively stable as the profile remained the same after 28th day of storage. Result of antibacterial activity test on cream with O/W base was unchange after 28 day, while that with W/O revealed no activity which may due to poor diffusion within the cream base as media. **Conclusion**: active fraction of *Cassia fistula* can be formulated into cream with O/W cream base system.

Key words: *Cassia fistula*, Antibacteria, Cream, *Propionibacterium acnes*, *Pseudomonas aeruginosa*.

Anis Yohana Chaerunisaa[1,*], Muhaimin Muhaimin[2], Yasmiwar Susilawati[3], Tiana Milanda[3]

[1]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Padjadjaran University, Jatinangor, Sumedang 45363, INDONESIA.
[2]Department of Chemistry Education, Faculty of Education, University of Jambi, Jambi 36361, INDONESIA.
[3]Deparment of Pharmaceutical Biology, Faculty of Pharmacy, Padjadjaran University, Jatinangor, Sumedang 45363, INDONESIA.

Correspondence

Anis Yohana Chaerunisaa

Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Padjadjaran University, Jatinangor, Sumedang 45363, INDONESIA.

E-mail: anis.yohana.chaerunisaa@unpad.ac.id

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INTRODUCTION

Skin as the tissue covering entire human body act as the physical protection from dangerous agents. Skin has the ability to protect the body from microorganisms around which will be weakened when it is been damaged thus bacterial infection will occur.[1,2]

Pseudomonas aeruginosa and *Propionibacterium acnes* are bacteria that commonly infect the human skin. *Pseudomonas aeruginosa* is a Gram-negative bacteria that can be found on the surface of medical facilities in hospitals that can cause nosocomial infections.[3] *Propionibacterium acnes* is a Gram-positive bacterium that is also part of the normal flora of the skin which also be able to cause acne.[2] Treatment for bacterial infections commonly used antibiotics. The irrational use of drugs is now causing more bacteria undergo antibiotic resistancy. *Propionibacterium acnes* had been reported to have drug resistance to erythromycin and clindamycin. All the antibiotic issues encourages the search for new drugs from natural source in this case those which have antibacterial activity.[4,5] Trengguli (*Cassia fistula* L.) is a plant which traditionally had been used to cure many diseases.[6-12] Many biological activities of this plant had been reported including antioxidant and as hepatoprotective agent, as wound healing.[10-17] According to research by Duraipandiyan and Ignacimuthu (2007), extract of trengguli have a minimum inhibitory concentration 0.156 mg / mL for *Staphylococcus aureus* and the minimum inhibitory concentration of 0.625 mg / mL for *Pseudomonas aeruginosa*. Former study from the research group reported that among the fraction of *Cassia fistula*, ethyl acetate fraction showed strongest activity against *S. aureus* and *E. Coli*.[11] Minimum Inhibitory Concentration of ethyl acetate fraction was at 0,625 % against *S. aureus* while that against *E. coli* was at 1,25%.[5] Study on antibacterial activity of the extract from *Cassia fistula* had been studied and reported in many publication. However, the application of the extract or active fraction of this plant has not been explored. In this study, the application as antibacterial agent of active fraction of *Cassia fistula* were studied as topical formulation, since the traditional use had been as topical antibacterial agent.

Skin diseases require preparations that stick to the skin for a long time so that the contact time between

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active ingredient with the skin will become longer, thus provides more efficacy of drug. Skin preparation that with those property are ointments. Ointments has a very good stability due to inert base.

Cream contains more water than ointments, usually in the form of emulsion. Cream also provides a cooling effect and is easily can be cleaned with water.[12,13] However, this preparation has the disadvantage of being sticky and leaving oil stains after use. Cream preparations are alternative to ointments because they are more preferable, providing not too oily base on the skin. However, the presence of water as component in a cream formulation raises the possibility of instability of cream base which leads to the instability of the active ingredient. In this study, formulation of creams containing active fraction of trengguli were carried out. The antibacterial activity against skin microbes were studied using *Pseudomonas aeruginosa* and *Propionibacterium acnes* as model of bacterium that often infects the skin.

MATERIALS AND METHODS

Plant material

The plant material used in this study was *Cassia fistula* L. barks which collected from Manoko plantation, Lembang, Bandung, Indonesia.

Instruments used consisted of a maserator, rotary evaporator (Buchi Rotavapor R-300), a water bath (Memert), desilator toluene (Barstead), separating funnel, TLC plate, analytical balance (Mettler Toledo, AL204), oven (Memmert Memmert 200 and 400-800), spatula, incubator (Sakura, IF-4), autoclave (Hirayama), ose, volume micropipette 10-100 uL (Biohit Proline), micropipette volume 100-1000 uL (Socorex Acura 825), micropipette tip, perforator, petri dish (Pyrex), electrical heating, calipers, tweezers, incendiary spiritus, viscometer (Rion), universal pH (Merck®), and the specific pH 4.0-7.0 (Merck Milipore®) and other glassware commonly used in laboratories.

Reagents

Solvents used for extraction and fractionation were ethanol 70%, n-hexane, ethyl acetate, distilled water. Reagents used for the phytochemical screening were ammonia 10%, chloroform, hydrochloric acid 2 N, reagents Mayer, reagents Dragendorf, magnesium powder, amyl alcohol, reagent FeCl3, gelatin solution 1%, ether, reagent vanillin-sulfate (solution of vanillin 10 % in concentrated sulfuric acid), 1 N sodium hydroxide, and distilled water.

The materials used for the formulation of antibacterial cream were paraffin Liquidum (Tudapetrol (H & R). Germany), cera alba, span 80, methyl paraben, propylene glycol (Dow Chemical Pacific), stearyl alcohol, sodium lauryl sulfate, and distilled water.

Antibacterial test

Bacteria samples used in this study were *Pseudomonas aeruginosa* ATCC 27853 and *Propionibacterium acnes* clinical isolates obtained from the Laboratory of Microbiology, Faculty of Pharmacy, University of Padjadjaran. Bacterial growth medium used was Mueller-Hinton Agar with a concentration of 38 g / L (Oxoid), Mueller-Hinton Broth at a concentration of 21 g/L (Oxoid), and 0.9% the physiological NaCl (Otsuka).

Methods

Collection of samples

Simplicia used was the bark of *Cassia fistula* L. Determination of plants was conducted at the Laboratory of Plant Taxonomy, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Padjadjaran.

Extraction

The sample were ground into powder, extracted by maceration using 70% ethanol 3 times for 24 hours. Liquid extract obtained is then concentrated using a rotary evaporator and the evaporation continued using water bath until its weight was constant. Viscous extract which gained then weighed and the yield was calculated. The extract was tested organoleptic including shape, color, taste, and smell of extract.[18-20]

Phytochemical screening of extract and most active fraction

Stages in the phytochemical screening to find compounds which contained in extracts as below:

1. Alkaloids compound
2. Flavonoids compound
3. Polyphenols compound
4. Taninns compound
5. Monoterpenoid and Sesquiterpenoids compound
6. Steroids and Triterpenoid compound
7. Quinone compound
8. Saponin compound

Determination of extract parameters

Determination of the extract parameters were performed to determine the parameters of standardized extracts. The parameters tested include extract's moisture content, level of extract which water soluble, level of extract which ethanol soluble.

Fractination extract

The extract was fractionated using Liquid-Liquid Extraction (ECC). Extract as much as 20 grams dissolved in 30 mL of 70% ethanol and 170 mL of distilled water. The solution was then put into a separating funnel and added 200 mL of n-hexane as a non-polar solvent. Separating funnel was then shaken vigorously. N-hexane fraction were collected. Fraction of water was then added with 200 ml of ethyl acetate as a semi-polar solvent in separating funnel and was then shaken, left overnigh, and the ethyl acetate fraction was then tcollected. Fraction of n-hexane, ethyl acetate, and water were then concentrated using a rotary evaporator, followed by evaporation using a water bath until constant weight.[18-20]

Antibacterial activity test of extract and fraction

Antibacterial activity test of extracts and fractions were performed using agar diffusion method. 20 µL of bacterial suspension and 20 mL MHA put in a sterile petri dish and then shaken until homogeneous. Wells were developed using a perforator with diameter of 8 mm. 100 uL of sample solutios were subjected into each wells, and then incubated at 37°C for 18-24 hours. As a negative control, 50 uL solvent DMSO was used. The diameter of inhibitory zone were measured.[21]

Determination of Minimum Inhibitory Concentration (MIC) of active fraction

Determination of Minimum Inhibitory Concentration (MIC) of the most active fraction from *Cassia fistula* L. bark were performed using microdilution method with 96 wells microtiter plates. Well number one well used for negative control (100 µL MHB), number two for fraction control (MHB 100 µL and 100 µL of stock solution most active fraction). Well number three to eleven were used for sample tests containing the most active fractions of *Cassia fistula* with different concentrations and 10 µL of the bacterial suspension. Well 12 was used as positive control filled with 100 µL of MHB and 10 µL of the bacterial suspension test. Microtiter plate was closed and incubated at 37°C for 18-24 hours.

Determination of Minimum Bactericidal Concentration (MBC)

Determination of MBC value was performed by taking 10 µL solution from wells showing no bacterial growth (clear), incubated in MHA petri dish at 37°C for 18-24 hours. Petri dish containing the active fraction with the smallest concentration that showed no growth of test bacteria was determined as the MBC.

Profile Analysis Thin Layer Chromatography (TLC) of extract and the active fraction

The extracts and the most active fraction were subjected to Thin Layer Chromatography (TLC) profiling to determine the quantitative chemical compounds in the extract and fraction. TLC analysis were conducted in silica gel plates GF 254 and a mobile phase of a solvent mixture consisting toluene: ethyl acetate: formic acid at a ratio of 5: 4: 1 (Seasotiya et al., 2014). Chromatogram profile were observed in visible light, and under UV light 254 nm and 366 nm. The TLC observation also subjected to creams formulations.

Formulation of antibacterial creams

Based on the results orientation of the cream bases, formula cream base to be used in the formulation of water-in-oil base formula can be seen in Table 1. For formula oil-in-water base can be seen in Table 2.

Evaluation of dosage cream

Evaluation of dosage cream were conducted by observing the physical changes on days 1, 3, 7, 14, 21, and 28 including changes in organoleptis, vicosity pH and TLC profile.

Antibacterial activity test of creams

Antibacterial activity tof creams against *Propionibacterium acnes* and *Pseudomonas aeruginosa* were performed using agar diffusion method with perforation technique. 20 mL MHA and 20 mL bacterial suspension were put in a sterile petri dish, then shaken until homogeneous. Media was then perforated using a perforator with diameter of 8 mm. Creams were then inserted into the wells by using a spatula. The petri dishes were incubated at 37° C for 18-24 hours and then the diameter of inhibitory zone were measured.

RESULT AND DISCUSSION

Extraction of 700 grams *Cassia fistula* bark powder gave 201 gram of extract, with a rendement 28.741%. By organoleptic, a liquid extract has dark brown, distinctive odor and bitter taste. The results of phytochemical screening of extracts which were conducted according to Harborne can be seen in Table 3.

Phytochemical screening showed that the extract contains polyphenol, saponin, monoterpenes and sesquiterpenes and quinone. The result of phytochemical screening of the fraction showed that the extract contain polyphenols, saponin, flavonoids, monoterpenoid and sesquiterpenoid and quinone.

Table 1: Formula of cream with Water-in-Oil Base.

Ingredients (%)	Formula			
	FE0	FE1	FE2	FE3
Active fraction	-	4 x MIC	5 x MIC	6 x MIC
Paraffin liquidum	45	45	45	45
Cera alba	16	16	16	16
Span 80	5	5	5	5
Nipagin	0,1	0,1	0,1	0,1
Distilled water ad	100	100	100	100

Table 2: Formula of cream with Oil-in-Water Base.

Ingredients (%)	Formula			
	FF0	FF1	FF2	FF3
Active fraction	-	4 x MIC	5 x MIC	6 x MIC
Propylene glycol	50	50	50	50
Stearyl alcohol	5	5	5	5
Sodium lauryl sulfate	1	1	1	1
Distilled water ad	100	100	100	100

Table 3: Phytochemical Screening results of *Cassia fistula* extract.

Class of Chemical Compounds	Result
Alkaloids	-
Tannins	-
Polyphenols	+
Saponin	+
Flavonoids	+
Monoterpenoid and sesquiterpenoid	+
Steroids	-
Triterpenoid	-
Quinone	+

Description: (+): detected; (-): not detected

Examination on parameter of extract showed that the moisture content of the extract was 9.95%, which fulfill requirements. From 2.0293 gram of extract sample, water soluble component was 1.5578 grams giving the rendement of 76.76%. From 2.043 gram of extract, alcohol soluble component was 1.6199 grams giving the rendement of 80.82%.

Fractination extract

Fractionation were conducted using n-hexane, ethyl acetate and water, solvent with different polarity. The fraction then were evaporated, and the weight rendement were calculated to give the result as can be seen in Table 4.

Antibacterial activity of extract and fraction

As preliminary study, antibacterial activity of the extract and fractions of *Cassia fistula*'s bark were conducted against both tested bacteria by performing antibacterial activity test using agar diffusion method. The results can be seen in Table 5. In can be concluded that ethyl acetat fraction is the most active fraction of *Cassia fistula* having potent antibacterial activity. Non-polar fraction obtained from n-hecxane solvent was concluded to be non pontential fraction since it showed no inhibition growth during the antibacterial test. Accordingly, further research on cream formulation was conducted by using ethyl acetate fraction as active compound.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of active fraction

Results of the determination of the most active fraction MIC *Cassia fistula* bark can be seen in Table 6. The lowest concentration which inhibited the visual growth was recorded as MIC. Positive results were dicided when the well showing no growth of bacteria (visually observed as clear solution), and it was determined as the MIC.[21]

Based on Table 6, it can be concluded that MIC of the active fraction of *Cassia fistula*'s bark against *Propionibacterium acnes* was 175 ppm, while that against *Pseudomonas aeruginosa* was 400 ppm.

The swab test to examine MCB value against *Propionibacterium acnes* were performed on media agar from the wells with concentration

Table 4: The Fractionation result from *Cassia fistula* Ethanolic Extract.

Fraction	Weight Fraction (g)	Rendement (%)
n-Hexane	0,44	0,724
Ethyl Acetate	19,37	31,86
Water	8,48	13,95

Table 5: Antibacterial Activity of Extract and Fractions of *Cassia fistula's* Bark against *Propionibacterium acnes* and *Pseudomonas aeruginosa* at concentration 50000 ppm.

Sample	Inhibition Zone Diameter (mm)	
	Propionibacterium acnes	Pseudomonas aeruginosa
Extract	186	164
n-hexane fraction	135	-
Ethyl acetate fraction	212	196
Water fraction	172	155

*diameter well 8 mm

Table 6: Minimum Inhibitory Concentration of Active Fraction from *Cassia fistula's* Bark against *Propionibacterium acnes* and *Pseudomonas aeruginosa*.

Concetration of the sample (most active fraction) of Cassia fistula (ppm)	Bacteria Growth	
	P. acnes	P. aeruginosa
1600	-	-
1400	-	-
1200	-	-
800	-	-
700	-	-
600	-	-
400	-	-
350	-	+
300	-	+
200	-	+
175	-	+
150	+	+
100	+	+
87.5	+	+
75	+	+
50	+	+

Description:
(+) : Bacteria growth was found
(-) : Bacteria growth was not found

of 175, 200, 300 and 350 ppm. Determination of MCB against *Pseudomonas aeruginosa*, were performed on agar medium of the wells with concentration of 400, 600, 700 and 800 ppm. After they were plated into media agar and incubated for at 37°C for 18-24 hours, it was found that the value of MCB for *Propionibacterium acnes* was 350 ppm, while that *Pseudomonas aeruginosa* was 800 ppm.

Profile of Thin Layer Chromatography (TLC)

TLC analysis were performed to study qualitatively on the chemicals consisting in the extract and fractions. The result showed that extract and ethyl acetate fraction as the most active fraction of *Cassia fistula* have the same TLC profile suggesting that these spot are the one which responsible for the antibacterial activity. The results of TLC profile can be seen in Table 7 and Figure 1.

Formulation of antibacterial creams

In development of formula of cream base, six different cream bases containing different type of emulsion system had been formulated. Some formulation showed changes in color after a few hours of product preparation due to the flavonoids content in active fraction which probably interact with triethanolamine in the formulation which has strong alkaline property. The phenolic and flavonoids can interact with alkaline derivate[22]. It is concluded that formulas with triethanolamine were not possible to be used as cream base.

The results of antibacterial cream dosage formulations water-in-oil base and oil-in-water base with various concentrations of the most active fractions can be seen in Table 8. Physical evaluation on creams containing active fraction of *Cassia fistula* were conducted by observing physical changes during 28 days of storage. By organoleptic observation, cream with water-in-oil base and oil-in-water base were unchanged either in consistency, smell, color, and homogenity. It can be concluded that the addition of *Cassia fistula* fraction as active substance in cream formulations did not affect the stability.

During the 28 days of storage, the pH of either cream with water-in-oil or oil-in-water base did not change and remained at pH 5. This pH also fulfilled the requirements pH for topical preparation (4.0 - 7.0). It can be concluded that the addition of active fraction of *Cassia fistula* did not affect the pH when cream was used as topical preparation, either with water-in-oil or oil-in-water base.

The results of observation on viscosity of cream with water-in-oil base containing different concentrations of *Cassia fistula* active fraction can be seen in Figure 2.

Table 7: Thin Layer Chromatogram profile of Ethanolic Extract and Most Active Fraction of *Cassia fistula*'s Bark.

Sample	Spot No.	Rf	Visible light	UV (color)	
				254 nm	366 nm
Extract	1	0.39	-	-	Blue
	2	0.49	-	-	Blue
	3	0.55	-	-	Red
	4	0.76	-	-	Red
Ethyl Acetate fraction	1	0.50	-	-	Blue
	2	0.64	-	-	Blue
	3	0.69	-	-	Red
	4	0.90	-	-	Red

(-) = undetected

Table 8: Formulation of Cream with Various Concentrations of *Cassia fistula*'s ethyl acetate fraction.

Base type	Formula	Consistency	Color	Odor	Homogenity
Water-in-oil	FE0	Thick Cream	White	Odorless	Homogen
	FE1	Thick Cream	Brown	Odorless	Homogen
	FE2	Thick Cream	Brown	Odorless	Homogen
	FE3	Thick Cream	Brown	Odorless	Homogen
Oil-in-water	FF0	Thick Cream	White	Distinctive odor	Homogen
	FF1	Thick Cream	Mocca brown	Distinctive odor	Homogen
	FF2	Thick Cream	Mocca brown	Distinctive odor	Homogen
	FF3	Thick Cream	Mocca brown	Distinctive odor	Homogen

Description:
FE0 = Water-in-oil base cream without ethyl acetate fraction
FE1 = Water-in-oil base cream with ethyl acetate fraction 4 x MIC
FE2 = Water-in-oil base cream with ethyl acetate fraction 5 x MIC
FE3 = Water-in-oil base cream with th ethyl acetate fraction 6 x MIC
FF0 = Oil-in-water base cream without ethyl acetate fraction
FF1 = Oil-in-water base cream with ethyl acetate fraction 4 x MIC
FF2 = Oil-in-water base cream with ethyl acetate fraction 5 x MIC
FF3 = Oil-in-water base cream with ethyl acetate fraction 6 x MIC

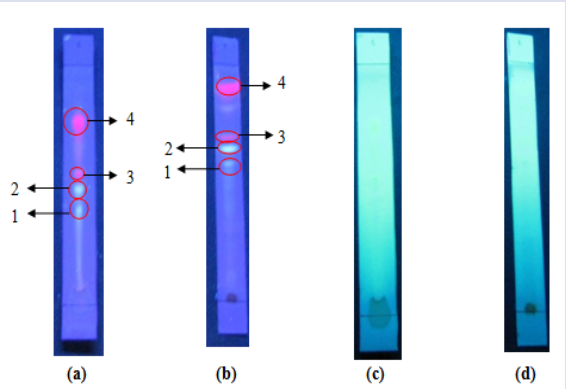

Figure 1: TLC profile of extract and active fraction of *Cassia fistula*'s bark (a) Extract at 366 nm, (b) Ethyl acetate fraction at 366 nm, (c) Extract at 254 nm and (d) Ethyl acetate fraction at 366 nm.

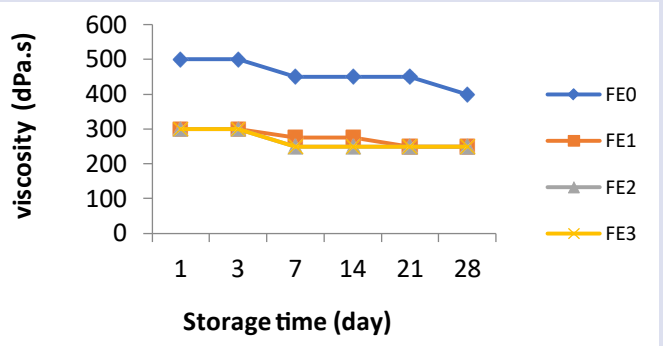

Figure 2: Viscosity of cream containing different concentration of *Cassia fistula* active fraction in water-in-oil base during 28 days of storage.
FE0=Water-in-oil base cream without ethyl acetate fraction
FE=Water-in-oil base cream with ethyl acetate fraction 4 x MIC
FE2=Water-in-oil base cream with ethyl acetate fraction 5 x MIC
FE3=Water-in-oil base cream with ethyl acetate fraction 6 x MIC

It can be seen that the viscosity decreased during 28 days of storage. Statistical analysis using ANOVA One Way Design Block Complete Random (DBLA) (α = 0,05), gave conclusion that the addition of active fraction of *Cassia fistula*'s bark effected the viscosity of the cream with water-in-oil base. The observation on changes in viscosity of creams with oil-in-water base containing different concentrations of active fraction of *Cassia fist*ula can be seen in Figure 3.

The viscosity of oil-in-water base cream with different concentration of *Cassia fistula* active fraction increased during 28 days of storage. Statistical analysis using ANOVA One Way Design Block Complete Random (DBLA) (α = 0,05), showed o that the addition of t active fraction of *Cassia fistula* bark affected the viscosity of the cream with oil-in-water base.

Profile of Thin Layer Chromatography

Analysis of thin layer chromatography (TLC) from creams with various concentration of active fraction of *Cassia fistula* was carried out confirm that the compound of the fraction remains stable after formulation into

cream as well as after 28 days of storage. The eluen used for analysis were toluene: ethyl acetate: formic acid (5: 4: 1). The results showed that the spots on TLC profile of creams resembled the spot of ethyl acetate fraction of *Cassia fistula's* bark as standard. Creams with water-in-oil base (Fig.a and b), revelaed blue spot at 254 nm which suspected as spot from base of the cream. However, after 28th day, although the profile remain the same, the retardation factor (Rf) were different from that of the ethyl acetate fraction. It may due interaction between cream base with component on the fraction as active ingredient the during storage. The results of the profile TLC analysis of creams can be seen in Figure 4.

Antibacterial activity of creams

Antibacterial activity test were performed on first day and 28th day of storage. When using water-in-oil base, creams containing active fraction did not show antibacterial activity which was observed as clear inhibition zone on *Propionibacterium acnes* and *Pseudomonas aeruginosa* agar media. This phenomena may due to poor diffusion of active ingredient on the medium since the cream base was lipophilic. Therefore, active fraction as active ingredient could not inhibit bacterial growth. In contrast, cream with oil-in-water base showed antibacterial activity against both tested bacteria, which can be seen in Table 9.

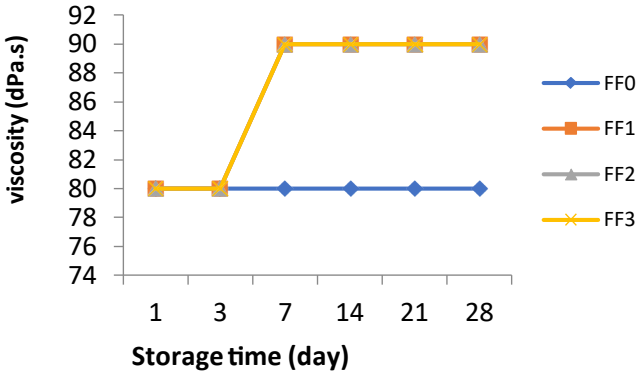

Figure 3: Viscosity of cream containing different concentration of *Cassia fistula* active fraction in oil-in-water base during 28 days of storage.
FE0=oil-in-water base cream without ethyl acetate fraction
FE=oil-in-water base cream with ethyl acetate fraction 4 x MIC
FE2=oil-in-water base cream with ethyl acetate fraction 5 x MIC
FE3=oil-in-water base cream with ethyl acetate fraction 6 x MIC

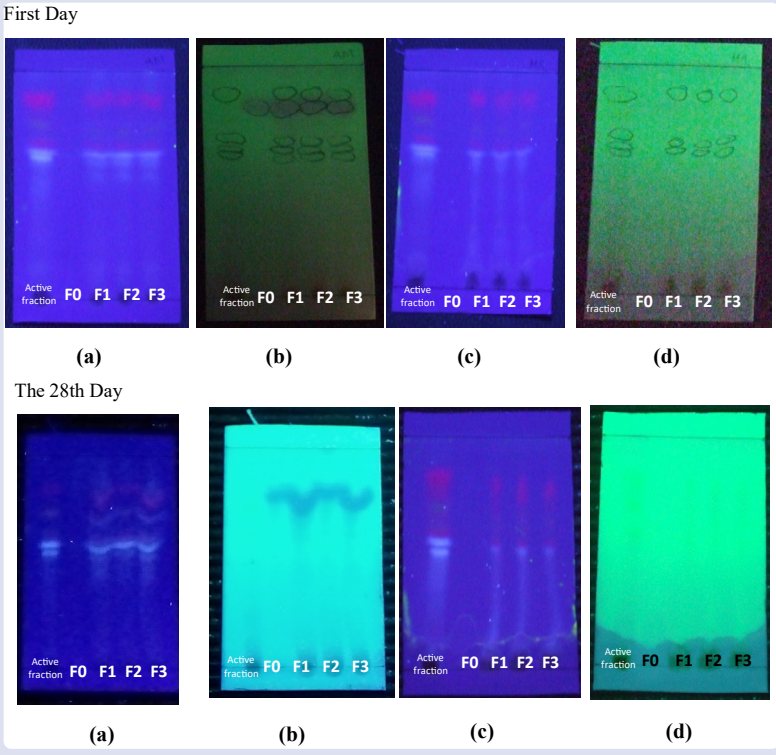

Figure 4: TLC profile of sample of ethyl acetate fraction (Active fraction), F0: cream without ethyl acetate fraction, and creams containing active fraction of *Cassia fistula* in F1: cream water-in-oil base (4-6 x MIC) at 366 nm, F2: cream water-in-oil base (4-6 x MIC) at 254 nm, F3: cream oil-in-water base (4-6 x MIC) at 366 nm, and F3: cream oil-in-water base (4-6 x MIC) at 254 nm.

Table 9: Antibacterial Activity of Cream with Oil-in-Water Base containing Various Concentrations of Active Fraction of *Cassia fistula*.

Formula	Inhibition Zone Diameter (mm)			
	P. acnes		P. aeruginosa	
	Day 1	Day 28	Day 1	Day 28
FF0	10.2	10.1	-	-
FF1	10.5	11	11	-
FF2	10.9	11	11	-
FF3	11.6	10.1	12.45	-

diameter hole = 8 mm
FF0=Oil-in-water base cream without ethyl acetate fraction
FF1=Oil-in-water base cream with the concetration of ethyl acetate fraction 4 x MIC
FF2=Oil-in-water base cream with the concetration of ethyl acetate fraction 5 x MIC
FF3=Oil-in-water base cream with the concetration of ethyl acetate fraction 6 x MIC

CONCLUSION

Ethanolic extract and ethyl acetate fractions of *Cassia fistula* L. Barks have activity against bacteria *Propionibacterium acnes* clinical isolates and *Pseudomonas aeruginosa* ATCC 27853. The value of MIC of ethyl acetate fraction was 175 ppm against *P. acnes* and 400 ppm against *P. aeruginosa*. Ethyl acetate fraction can be formulated into a cream preparation with limitation of component with strong alkaline properties, due to possible incompatibility with flavonoids. Phytochemical screening of *Cassia fistula* extract which showed positive result of flavonoids could be the explanation for this limitation. The creams containing various concentrations of ethyl acetate fraction were physically stable in terms of organoleptic and pH during 28 days of storage. The viscosity changed during 28 days of the preparation. Thin Layer Chromatography profile as qualitative analysis for chemical stability revealed the same spot between creams of oil in water with ethyl acetate fraction as standars which can be assumed that the formulation were chemically stable during 28 days of storage. Additionally, feasible cream base for topical preparation was oil-in-water type since they provided activity against tested bacteria compared to that with water-in-oil cream base due to more diffusional base as media.

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CONFLICTS OF INTEREST

The authors declare none.

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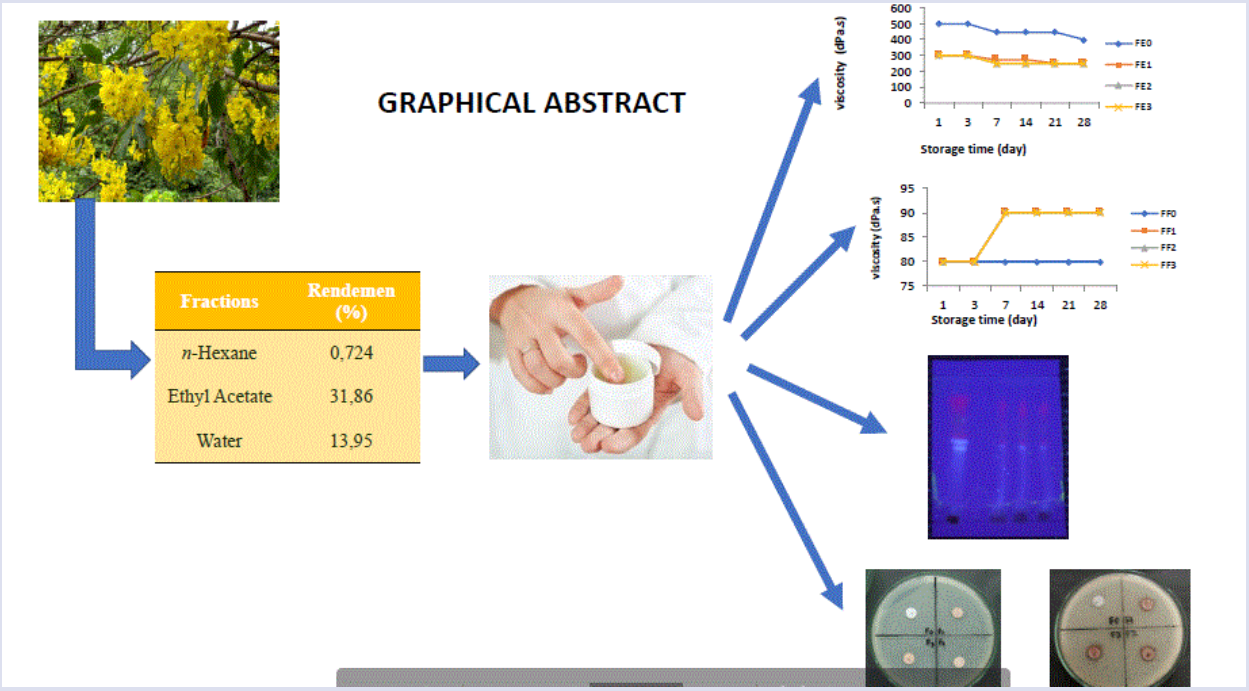
GRAPHICAL ABSTRACT



ABOUT AUTHORS

Anis Yohana Chaerunisaa: Associate Professor at Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Padjadjaran University, Jatinangor, Indonesia. She currently work on many research in the area of Pharmaceutical Technology and drug delivery including solid, liquid and semisolid dosage forms as well as developing polymer and other excipients for pharmaceutical dosage forms. She wrote dissertation on release adjustment of drug combined in one dosage form. She also has many experiences on drug discovery from herbals either as active compound or alternative for adjuvant therapy. She wrote many publications about her research on pharmaceutical dosage forms in many journals, as well as some books and book chapter. She is also pursuing interests on development of nanoparticles from synthesis and herbal active compounds.

Muhaimin Muhaimin: Associate Professor at Department of Chemistry Education, Faculty of Education, University of Jambi, Jambi, Indonesia. He has experience in the area of Organic Chemistry, Pharmaceutical Technology, and Pharmacognosy, working in drug delivery, drugs discovery of herbal plants, antimalaria and infectious disease. His research also focus on polymer and organic chemistry from mechanistic studies to synthetic methodology and the synthesis of natural products as well as of structurally intriguing heterocyclic compounds. The central focus of the research is the design of new methods for the synthesis of bioactive and synthetically versatile compounds. Other his research focus on innovative drug delivery systems with controlled drug release.

Yasmiwar Susilawati: Associate Professor at Department of Pharmaceutical Biology, Faculty of Pharmacy, Padjadjaran University, Jatinangor, Indonesia. She has been working on many research in the area of Pharmacognosy, phytochesmistry and etnopharmacognosy. Her dissertation was about finding new antidiabetic compounds from herb *Peperomia pellucida*. Many researches on pharmaceutical active compound has been her current interest. Some of her project has been developed into many pharmaceutical dosage forms and this has been her focus of interest during the last few years.

Tiana Milanda: Associate Professor at Department of Pharmaceutical Biology, Faculty of Pharmacy, Padjadjaran University, Jatinangor, Indonesia. Her research interest is about microbiology and biotechnology. She worked on molecular and genetic engineering for therapeutic purposes, and currently has been focused on finding the antimicrobial agent for resistant bacteria. She also doing the research on the development of antivirus from some herbals with the activity in inhibiting Neuraminidase receptors.

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