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MUCOLYTIC ACTIVITY OF ROSELLE (*Hibiscus sabdariffa* L.) CALYCES EXTRACT ON COW INTESTINAL MUCUS

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ABSTRACT

This study aimed to examine the mucolytic activity of roselle calyces extract on cow intestinal mucus in vitro. The extraction of roselle calyces was performed by the subsequence maceration method using different polarity solvent (n-hexane, ethyl acetate, and methanol). Mucolytic activity of each extract was evaluated by measuring the viscosity of mixture of cow intestinal mucus and phosphate buffer pH 7 compared to acetylcysteine as the standard mucolytic drug. The result showed that the decreasing of cow intestinal mucus and phosphate buffer pH 7 mixtures viscosity was indicated the increasing of mucolytic activity. The n-hexane, ethyl acetate, and methanol extract of roselle calyces exhibit dose-dependent mucolytic activity. Mucolytic activity of roselle calyces ethyl acetate extract with dose of 0.8% and roselle calyces methanol extract with dose of 0.6% showed no significant difference to mucolytic activity from acetylcysteine with dose of 0.1% (positive control). An increased dose of roselle calyces extract will decrease the viscosity of cow intestinal mucus and phosphate buffer pH 7 mixtures, indicating an increase in mucolytic activity.

Key words: cow intestinal, mucolytic, roselle calyces, mucus, viscosity

ABSTRAK

Penelitian ini bertujuan mengetahui aktivitas mukolitik ekstrak bunga rosela secara in vitro terhadap mukus usus sapi. Ekstraksi bunga rosela dilakukan dengan metode maserasi bertingkat dengan menggunakan pelarut yang berbeda polaritas (n-heksana, etil asetat, dan metanol). Aktivitas mukolitik masing-masing ekstrak dievaluasi dengan cara diukur viskositas campuran mukus usus sapi dan dapar fosfat pH 7 dibandingkan dengan asetilsistein sebagai obat mukolitik standar. Hasil penelitian menunjukkan bahwa penurunan viskositas campuran mukus usus sapi dan dapar fosfat pH 7 menunjukkan peningkatan aktivitas mukolitik. Ekstrak n-heksana, etil asetat, dan metanol dari bunga rosela menunjukkan aktivitas mukolitik yang bergantung terhadap dosis. Aktivitas mukolitik ekstrak etil asetat bunga rosela dengan dosis 0,8%; dan ekstrak metanol bunga rosela dengan dosis 0,6% tidak menunjukkan adanya perbedaan yang signifikan terhadap aktivitas mukolitik dari asetilsistein dengan dosis 0,1%.. Peningkatan dosis ekstrak bunga rosela akan menurunkan viskositas campuran mukus usus sapi dan dapar fosfat pH 7 yang mengindikasikan adanya peningkatan aktivitas mukolitik.

Kata kunci: usus sapi, mukolitik, bunga rosela, mukus, viskositas

INTRODUCTION

A cough can occur in a healthy or sick condition and is a normal physiological response to foreign stimulants or irritation, serving a fundamental role in the respiratory tract protection and the maintenance of airway patency. It can occur voluntarily or in response to an airway irritation and can be both reflexive and non-reflexive (behavioral) in nature. Although coughing itself reflects a coordinated effort of the respiratory muscles, the underlying control mechanisms are dependent on complex neurophysiological events. The neural pathways that control a cough have been the subject of intense investigation in recent years, and much is now known about how voluntary and evoked a cough are generated (Keller *et al*., 2017). A cough can be divided into two types, namely a productive cough that produces sputum or mucus and a non-productive cough without the presence of sputum or mucus (De Blasio *et al*., 2011).

Mucus secretion in the respiratory tract will cause blockage of the respiratory tract during respiratory process. To prevent the blockage of the respiratory tract, there is a natural mucus cleansing process. The production of over mucus will cause stimulation for the occurrence of a cough to remove excess mucus from the respiratory tract (Weinberger and Lockshin, 2017). Excessive mucus is excreted with the help of mucoactive agents. Administration of mucoactive agents aimed to increase the ability to expectorate sputum and/or decrease excessive mucus excretion. Mucoactive agents are divided into four groups: mucolytics, mucokinetics, mucoregulators, and expectorants. Mucolytics (acetyl-cysteine and bromhexine) will change the physical and chemical properties by break down the glycoprotein bonds found in mucus into smaller molecules to reduce the viscosity of mucus so it will be easily coughed out and relieve shortness of breath (Hanson, 2017).

Mucus is a complex liquid in the form of mucoproteins and mucopolysaccharides. Respiratory tract mucus has a composition of 97%-98% waters and 2%-3% solids (mucin proteins, non-mucin proteins, salts, lipids, cellular debris) (Jacquot *et al*., 1992; Fahy and Dickey, 2010). Gastrointestinal mucus has a composition of 98% water and 2% solids (Pelaseyed *et al*., 2014). Due to the similarity of gastrointestinal mucus composition to respiratory tract mucus composition, then gastrointestinal mucus may serve as an in vitro model to replace respiratory tract mucus for mucolytic activity test.

Herbal products have shown several activities for the treatment of respiratory disorders, such as mucolytic, expectorant, antitussive, nasal-decongestant, and antibacterial. The many advantages of herbal medicine cause increased use of herbal medicines. Several studies have shown an association between phytochemical compounds contained in plants to the mucolytic effects of plants. The phytochemical compound contained in plants such as alkaloids, flavonoids, tannins, saponins, glycosides, steroids, and terpenoids are reported to have several synergistic effects for the treatment of a cough, such as mucolytics, mucokinetics, mucoregulators, expectorants, and antibacterial (Adesina *et al*., 2017).

Roselle calyces has many pharmacological effects that have been studied in silico, in vitro, in situ and/or in vivo, including: immunomodulation (Kadri *et al*., 2013), hepatoprotective (Ezzat *et al*., 2016), cardioprotective (Obouayeba *et al*., 2015), antidiabetic (Rosemary *et al*., 2014), antibacterial (Lusida *et al*., 2017), anticancer (Prabhakaran *et al*., 2017), antihypertensive (Abubakar *et al*., 2015), antimalarial (Nerdy, 2017), antinociceptive (Ali *et al*., 2011), anti-inflammatory (Ali *et al*., 2011; Obouayeba *et al*., 2015), and antidiarrheal (Ali *et al*., 2011). However, the mucolytic activity of roselle calyces has not been reported yet. Thus, in this study, the researchers were interested to examine the mucolytic effect of the roselle calyces extract by in vitro on cow intestinal mucus.

MATERIALS AND METHODS

Preparation of Extract

This research used roselle calyces samples obtained from Wonosari, Tanjung Morawa, Deli Serdang, North Sumatera, Indonesia. The criteria of roselle calyces samples was fresh and dark red color. Samples were sorted, washed, drained, cut, dried, powdered, and sieved. Extract was prepared using stratified maceration with different polarity solvent, start with non-polar solvent (n-hexane), followed by semi-polar solvent (ethyl acetate), and ended with the polar solvent (methanol). Each solvent was macerated 2 times, the first maceration was carried out using 10 L solvent, stirred daily for 5 days (5x24 hours), and the second maceration was done using 5 L solvent, stirred daily for 3 days (3x24 hours). Each aqueous extract was evaporated with a rotary evaporator until a viscous extract was obtained. The extracts obtained were tested for phytochemical content of alkaloids, flavonoids, tannins, saponins, glycosides, steroids, and terpenoids.

Preparation of Cow Intestinal Mucus and Phosphate Buffer pH 7 Mixtures

The cow intestinal is washed with running water, cut longitudinally, scraped mucous layer (not to the capillary blood vessels and fat), collected in beaker glass, stirred until homogeneous until the color turn to white yellowish and/or white brownish. Phosphate buffer pH 7 was prepared by mixed 125 mL of 0.2 M potassium dihydrogen phosphate with 72.75 mL of 0.2 N sodium hydroxide solution and diluted up to 500 mL with CO_2 free water. Mixture of cow intestinal mucus and phosphate buffer pH 7 was prepared by mixed 300 g of cow intestinal mucus with 700 g of phosphate buffer pH 7 and stirred until homogeneous.

Evaluation of Mucolytic Activity

Negative control was prepared by mix 0.45 g tween 80 with 29.55 g cow intestinal mucus and phosphate buffer pH 7 mixtures in a beaker glass, and stirred until homogenous. Positive control was prepared by adding 0.03 g acetylcysteine and 0.42 g tween 80 into beaker glass. The mixture was stirred until homogenous, added with 29.55 g cow intestinal mucus and phosphate buffer pH 7 mixtures, and then stirred until homogenous. The final concentration of acetylcysteine in the mixture was 0.1%. Test sample prepared by adding 0.06 g, 0.12 g, 0.18 g, 0.24 g, and 0.30 g of each extract into 0.39 g, 0.33 g, 0.27 g, 0.18 g, and 0.15 g tween 80, respectively. The mixture was stirred until homogeneous, added with 29.55 g cow intestinal mucus and phosphate buffer pH 7 mixtures, and re-stirred until homogeneous. The final concentration of extract in the mixture was 0.2%, 0.4%, 0.6%, 0.8%, and 1.0%. Composition of the mixture for negative control, positive control, and test sample are shown in Table 1.

The sample of each treatment (negative control, positive control, and test sample) was incubated for 30 min at 37° C. Each treatment performed in six repetitions. The viscosity of each treatment was measured by using viscometer and density measurements were made using pycnometer. Viscosity was calculated by multiplying the flow time and the density. The viscosity data were analyzed statistically with one-way analysis of variance (ANOVA) with 95% confidence level and least significant difference (LSD) technique.

RESULTS AND DISCUSSION

The extraction of roselle calyces using stratified maceration with different polarity of solvent (n-hexane, ethyl acetate, and methanol) to separate the chemical constituents contained in roselle calyces based on the solubility. Extraction of 1000 g of roselle calyces dried powder produced 31.8 g n-hexane extract (dark green), 15.4 g of ethyl acetate extract (green), and 38.9 g of methanol extract (dark red). Phytochemical constituents

Table 1. Composition of the mixture for negative control, positive control, and test sample

No	Item	Control		Test sample				
		(–)	(+)	0.2%	0.4%	0.6%	0.8%	1.0%
1.	Tween 80	0.45	0.42	0.39	0.33	0.27	0.21	0.15
2.	Acetylcysteine	–	0.03	–	–	–	–	–
3.	Extract	–	–	0.06	0.12	0.18	0.24	0.30
4.	Mucus	29.55	29.55	29.55	29.55	29.55	29.55	29.55
∑	Total	30.00	30.00	30.00	30.00	30.00	30.00	30.00

contained in roselle calyces extract (n-hexane, ethyl acetate, and methanol) are shown in Table 2.

The phytochemical constituents contained in roselle calyces n-hexane extract are different and less than the ethyl acetate extract and the methanol extract of roselle calyces. The content in roselle calyces n-hexane extract differs from roselle calyces ethyl acetate extract and roselle calyces methanol extract, this is because the extraction by stratified maceration using different polarity of solvent principles is dissolves like. Compounds having similar polarity properties will be extracted in the same solvent polarity. Thus, in stratified maceration, different polarity of solvent will be extracted the different polarity compound (Altemimi, 2017).

Further evaluation was carried out on mucolytic activity to cow intestinal mucus and phosphate buffer pH 7 mixtures with viscosity measurement of various treatments (negative control, positive control, and test sample) in vitro. Data of viscosity measurements of various treatments (negative control, positive control, and test sample) were shown in Table 3.

The result data showed that roselle calyces extract had mucolytic activity shown by different viscosity between roselle calyces extract and negative control. The increasing of roselle calyces extract dose will cause decreasing of viscosity and indicating an increase of mucolytic activity, therefore, the mucolytic activity of roselle calyces extract was dose dependent. This is in accordance with previous studies of roselle calyces, which provide a various increasing activity with the increase of concentrations or doses, such as immunomodulation (Kadri *et al*., 2013), hepatoprotective (Ezzat *et al*., 2016), cardioprotective (Obouayeba *et al*., 2015), antidiabetic (Rosemary *et al*., 2014), antibacterial (Lusida *et al*., 2017), anticancer (Prabhakaran *et al*., 2017), antihypertensive (Abubakar *et al*., 2015), antinociceptive (Ali *et al*., 2011), anti-inflammatory (Ali *et al*., 2011; Obouayeba *et al*., 2015), and antidiarrheal (Ali *et al*., 2011).

Roselle calyces n-hexane extract in various doses of administration did not show a similar or stronger mucolytic activity to the positive control, proven by the higher viscosity in the administration of roselle calyces n-hexane extract than the viscosity in the administration 0.1% acetylcysteine. Roselle calyces ethyl acetate extract with dose 0.8% and roselle calyces methanol extract with dose 0.6% show a similar mucolytic activity to the positive control, demonstrated by the similar viscosity in the administration of roselle calyces ethyl acetate extract and roselle calyces methanol extract to the viscosity in the administration 0.1% acetylcysteine. Roselle calyces ethyl acetate extract with dose 1.0% and roselle calyces methanol extract with dose 0.8% and 1.0% show a stronger mucolytic activity to the positive control, demonstrated by the lower viscosity in the administration of roselle calyces ethyl acetate extract and roselle calyces methanol extract than the viscosity in the administration 0.1% acetylcysteine.

The administration of roselle calyces extract in various polarities and concentrations has mucolytic activity exhibited by significantly different viscosities

Table 2. Phytochemical constituents contained in roselle calyces extract (n-hexane, ethyl acetate, and methanol)

Number	Compound	Extract		
		N-Hexane	Ethyl acetate	Methanol
1	Alkaloids	–	+	+
2	Flavonoids	–	+	+
3	Saponins	–	+	+
4	Glycosides	–	+	+
5	Tannins	+	+	+
6	Steroids	+	–	–
7	Terpenoids	+	–	–

Table 3. Data of viscosity measurements of various treatments (negative control, positive control, and test sample) and subset results of one-way analysis of variance (ANOVA) with 95% confidence level and least significant difference (LSD)

Number	Treatment	Viscosity (cP) Average±standard deviation	Subset (Group)
1	Negative control	7.2073±0.0055	A
2	Positive control	7.1264±0.0035	I
3	N-Hexane 0.2%	7.1938±0.0040	B
4	N-Hexane 0.4%	7.1861±0.0036	C
5	N-Hexane 0.6%	7.1759±0.0025	E
6	N-Hexane 0.8%	7.1663±0.0024	F
7	N-Hexane 1.0%	7.1575±0.0018	G
8	Ethyl acetate 0.2%	7.1860±0.0023	C
9	Ethyl acetate 0.4%	7.1656±0.0034	F
10	Ethyl acetate 0.6%	7.1455±0.0029	H
11	Ethyl acetate 0.8%	7.1239±0.0020	I
12	Ethyl acetate 1.0%	7.1088±0.0039	J
13	Methanol 0.2%	7.1811±0.0037	D
14	Methanol 0.4%	7.1555±0.0032	G
15	Methanol 0.6%	7.1280±0.0035	I
16	Methanol 0.8%	7.1012±0.0029	K
17	Methanol 1.0%	7.0753±0.0031	L

between roselle calyces extract and negative control. Not all polarity extracts and extract concentrations have a similar mucolytic activity to the positive control activity, but there are certain extract polarities and certain extracts concentrations that have a similar mucolytic activity to the positive control activity, and even certain extract polarities and certain extract concentrations are better than positive controls. The data show that the administration of roselle calyces ethyl acetate extract with dose of 0.8% and roselle calyces methanol extract with dose of 0.6% showed activity did not differ significantly with positive control. This may be due to phytochemical constituents contained in the extract (Muctahara *et al*., 2017). Flavonoids contained in roselle calyces ethyl acetate extract and roselle calyces methanol extract (but not contained in roselle calyces n-hexane extract) are predicted to be responsible for better mucolytic effects than other compounds (Zhang and Zhou, 2014).

CONCLUSION

An increased dose of roselle calyces extract will decrease the viscosity of cow intestinal mucus and phosphate buffer pH 7 mixtures, indicating an increase in mucolytic activity.

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