ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

In-Vitro Study of Mucolytic Activity of Extract and Essential Oil of Citrus Maxima Peel.

Nurhaidah Muctahara*, Nur Cahyani, Helni Febriani, Muhammad Isrul, and Wa Ode Yuliastri.

Pharmacy Department, STIKES Mandala Waluyakendari, South-east Sulawesi, Indonesia

ABSTRACT

Citrus maxima peel has been used empirically as mucolytic agent. The aim of this study was to examine the mucolytic activity of *Citrus maxima* peel extract obtained from maceration using methanol as solvent and essential oil extracted by distillation process. Both extracts were tested with serial concentrations i.e. 0.2%, 0.4%, 0.8%, 1.6%, and 2.4% in 80% cow's mucous. The mucolitic activity was tested using Rion® viscometer, and 0.1% acetylcysteine as positive control. The results showed that the methanol extract had mucolytic activity at the concentrations of 0.8%, 1.6%, and 2.4% while the peel essential oil did not have mucolytic activity. Phytochemical screening results showed that methanol extract contains alkaloids, terpenoids, saponins, and flavonoids. The study revealed that methanol extract of *Citrus maxima* peels have mucolytic activity and potentially to be developed as a traditional medicine for cough medicine.

Keywords: *Citrus maxima,* Mucolytic, extract, essential oil

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Cough is an important physiological reflex protecting the airways. A frequent complaint of cough is associated with virtually all pulmonary and several extrapulmonary diseases. The clearing competence of the cough reflex depends on several conditions: obstruction of the airways, bronchial collapsibility, lung volumes, respiratory muscle and laryngeal function, and the amount and viscosity of the mucus [1]. There are four distinct types of cough: dry cough, wet cough, croup cough, and whooping cough. Wet cough is caused by fluid secretions and mucus found in the lower respiratory tract (windpipe and lungs). Common causes of wet cough include infections and asthma. Coughing removes fluid from the lower respiratory tract [2].

Medicines that can be used to treat wet coughs include expectorants and mucolytics. Mucolytics are medications that change the biophysical properties of secretions by degrading the mucin polymers,DNA, fibrin, or F-actin in airway secretions, generally decreasing viscosity. Classic mucolytics depolymerize the mucin glycoprotein oligomers by hydrolyzing the disulfide bonds that link the mucin monomers [3].

Herbal products have gained increasing popularity in the last decade, and are now used by pproximately 20% of the population. Herbal products are complex mixtures of organic chemicals that may come from any raw or processed part of a plant, including leaves, stems, flowers, roots, and seeds The enduring popularity of herbal medicines may be explained by the tendency of herbs to work slowly, usually with minimal toxic side effects[4]. In Buton district South-east Sulawesi Indonesia, *Citrus maxima* peel has been used empirically as mucolytic agent.

To the best of our knowledge, There is no scientific investigation of mucolytic activity of *Citrus maxima* peel. The aim of this study was to examine the mucolytic activity of *Citrus maxima* peel extract and essential oil and phytochemical screening of the active extract. The research will reveal the potential of *Citrus maxima* peel extract as cough herbal medicine.

.

MATERIAL AND METHOD

Collection and preparation of plant extracts

Citrus maxima peels were collected from Buton District, South-east Sulawesi, Indonesia. The plant was determined by Biology Department of Haluoleo University. The peels were divided to further extraction. Fresh Peels were used in distillation, the others were dried at room temperature for 7 days. The peels were then powdered using blender.

Sample extraction

The distillation of the fresh peels carried out for 5 days produced a white thick essential oil. The dried powdered Peels were then macerated with methanol for 3 days, rotary evaporator was used to evaporate the extract to dryness at 50 °C to produce dried crude methanol extract.

Collection of Mucus

Mucus were collected from cow's intestines. The intestines were cleaned first with flowing water and cut into pieces longitudinally, then the mucosal layers were scraped. The collected mucus was stirred slowly to homogenize. The mucus were then subdivided according to the number of assay and then put into the refrigerator until evaluation was performed.

Mucolytic Evaluation

The mucus was prepared with concentration of 80% using phosphate buffer pH 7, then tested with 7 treated groups which were negative control containing only mucus and phosphate buffer, positive control containing 0.1% acetylcysteine, and 5 treatment groups administered extracts in the concentrations of 0.2% , 0.4%, 0.8%, 1.6%, and 2.4% concentration for each essential oil and methanol extract. The samples were incubated at 37 ° C for 30 minutes to allow the sample reaction conditions to conform to human physiological conditions. Then each sample viscosity was measured using Rion® viscometer.

Statistical analysis

The results were expressed as mean (triple replication) ± standard error of mean (SEM) and difference between the treatments were considered significant at $p<0.05$ using one way analysis of variance (ANOVA) and LSD post hoc test. SPSS package version 23.0 for Windows was used for the analysis.

Phytochemical Screening.

The presence of various phytochemical constituents such as alkaloids, saponins, flavonoids, sterols, and terpenoids were screened qualitatively by using standard procedures

RESULT AND DISSCUSSION

Herbal medicine had been practised for many centuries in the Indonesian community to maintain good health and to treat diseases. Although modern (conventional) medicine is becoming increasingly important in Indonesia, *Herbal medicine* is still very popular in rural as well as in urban areas [5]. The use of herbal medicine mostly is not supported by scientific studies, therefore the research is required to prove the activity of herbal medicine [6]. *Citrus maxima* is one of the plants used as traditional medicinein Indonesia. Citrus Maxima peels had been reported to have pharmacological activities such as antioxidant, antidiabetic, and antimicrobial [7-9]. The Research is one of scientific investigation of *Citrus maxima* peel pharmacological activity.

Citrus maxima peels have been used empirically as a mucolytic agent. This study aimed to examine the mucolytic activity of *Citrus maxima* peels by comparing the activity between methanol extract and essential oil distilled from the peels. Citrus peels generally contains mostly mono terpened essential oils such as α-pinene, α-terpineol, anethole, β-pinene, Camphene, camphor, citral, citronellal, citroonellol, farnesol, geraniol, myrcene, neral, terpinene [10-12]. The difference in activity between the methanol extract and essential oils needs to be determined to obtain an effective extract.

Mucus is a complex viscous adherent secretion synthesized by specialized goblet cells in the columnar epithelium that lines all of the organs that are exposed to the external environment. This includes the respiratory tract, the gastrointestinal tract, the reproductive tract, and the oculo-rhino-otolaryngeal tracts. The protective function of mucus is involved in many disease processes. Mucus is composed primarily of water (95%), but also contains salts, lipids such as fatty acids, phospholipids and cholesterol. However, the main component that is responsible for its viscous and elastic gel-like properties is the glycoprotein mucin. Mucins exhibit a tendency to aggregate and form gels which its function as the protective [13] Themucolytic action is lowers mucus viscosity by reduces the disulfide bonds in the mucins [3].

Mucus obtained was yellow brown and thick. The mucus was subdivided according to the number of planned evaluation and then located at lower the temperature to stop the activity of proteolytic enzymes that can dissolve the mucus. The mucus 80% was prepared by diluting it with a phosphate buffer solution of pH 7. The use of this buffer is to keep the mucus composition unchanged and the mucolytic activity constant at pH 7. The incubation and testing processes were carried out at 37 ° C. The temperature and pH were arranged according to the condition of human physiology. The mucolytic activity test was performed using the Rion® Viscometer because mucus had Non-Newtonian flow type [14].

The results of viscosity measurements of each treatment are shown in Table 1 and Figure 1. The increase in concentration of Methanol Extract is directly proportional to the decrease in viscosity. The ANOVA analysis show significant differences in treatment. LSD post hoc test results showed that Positive control (Acetylcystein 0.1%), methanol extract at 0.8%, 1.6%, and 2.4% concentration showed differences with negative controls. Other concentrations did not show any difference with the negative controls.

The results of viscosity measurements of each treatment are shown in Table 1 and Figure 1. The increase in concentration of Methanol Extract is directly proportional to the decrease in viscosity. The ANOVA analysis show significant differences in treatment. The results showed methanol extract showed mucolytic activity at 0.8, 1.6, and 2.4% concentration. LSD post hoc test results showed that Positive control

ISSN: 0975-8585

(Acetylcystein 0.1%), methanol extract at 0.8%, 1.6%, and 2.4% concentration showed differences with negative controls. The 2.4% of methanol extract concentration showed the greatest viscosity decrease but the decrease was not as effective as acetylcysteine 0.1%. The results shows the essential oils do not have mukolytic activity. All concentrations did not show significant differences with negative controls.

Table 1: Viscosity measurements of each Treatment

Treatment	Concentration	Viscosity (dPa·s)
Negative control		2.47±0.12
Positive control (Asetylcystein)	0.1 %	0.90
Methanol Extract	0.2 %	2.53±0.12
	0.4 %	2.43±0.06
	0.8 %	2.10±0.17
	1.6 %	1.87±0.12
	2.4 %	1.37±0.06
Essential Oil	0.2 %	2.43±0.06
	0.4 %	2.43±0.06
	0.8 %	2.43±0.06
	1.6 %	2.40
	2.4 %	2.43±0.06

Fig 1: viscosity measurements of each Treatment

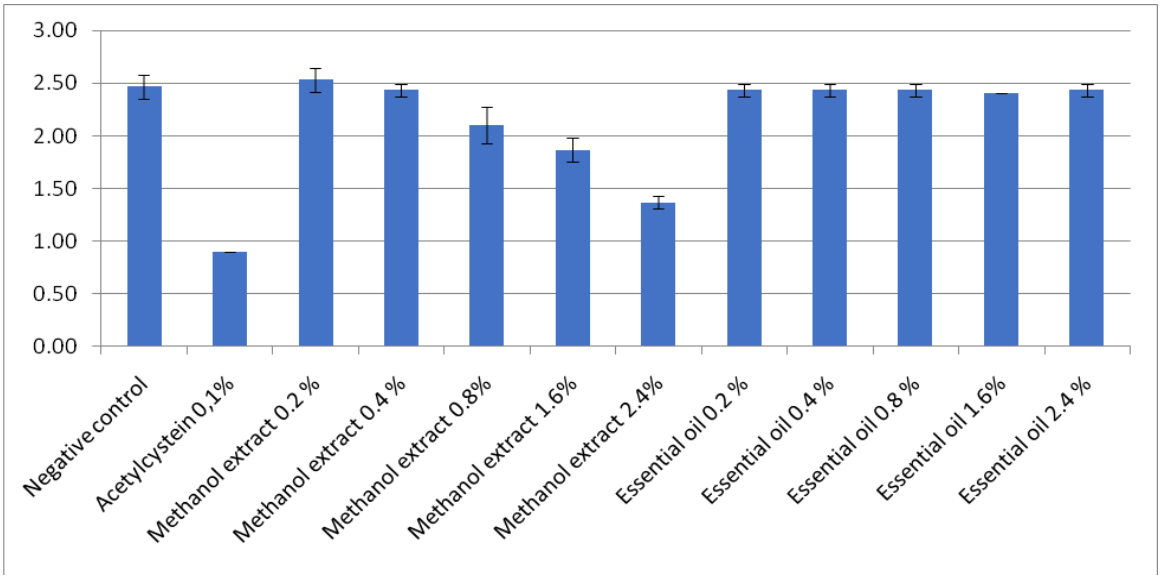

Table 2: Phytochemical Screening of Methanol Extract

Compound	Result
Alkaloids	(+)
Sterols	(-)
Terpenoids	(+)
Saponins	(+)
Flavanoids	(+)
Triterpenoids	**(-)**

Phytochemical screening results show that methanol extract contains Alkaloids, terpenoids, saponins, and flavonoids(Table 2). Several studies have reported these compounds as mucolytic agent. Maretta proved that ethanol extract of *Piper Miniatum* induces mucolytic activity in cow mucus in vitro and contains flavonoid group compounds. Prastiyomentioned that *Hibiscus* leaf extract had mucolytic activity and contained saponins and flavonoids. In addition, Wijayanti proved that *Momordicacharantia* leaves prepared as infusa had mucolytic activity and they containedsaponin, flavonoids, and polyphenols [14].

CONCLUSION

Methanol extract has mucolytic activity and essential oil doesnot have the mucolytic activity. Methanol extract at the concentration of 2.4% showed the greatest mucolytic activity but the activity was not as effective as acetylcysteine 0.1%. Phytochemical screening results show that methanol extract contains Alkaloids, terpenoids, saponins, and flavonoids. The study reveal that methanol extract of Citrus maxima peels have mucolytuc activity and potentially to be developed as a traditional medicine for cough medicine.

ACKNOWLEDGEMENTS

The authors wish to express gratitude to PKM Kemenristek DIKTI as financial support for implementation of this study.

REFERENCES

[1] Kardos P. Management of cough in adults. Breathe. 2010;7(2):122-33.
[2] Parents.2017. Cough Symptoms and Treatment. Available at http://www.parents.com/health/cough/cough/ [Accessed 27 July 2017].
[3] Rubin BK. Mucolytics, expectorants, and mucokinetic medications. Respiratory Care. 2007;52(7):859-65.
[4] Bent S. Herbal medicine in the United States: review of efficacy, safety, and regulation. Journal of general internal medicine. 2008;23(6):854-9.
[5] Woerdenbag HJ, Kayser O. Jamu: Indonesian traditional herbal medicine towards rational phytopharmacological use. Journal of Herbal Medicine. 2014;4(2):51-73.
[6] Firenzuoli F, Gori L. Herbal medicine today: clinical and research issues. Evidence-Based Complementary and Alternative Medicine. 2007;4(S1):37-40.
[7] Xu G, Ye X, Chen J, Liu D. Effect of heat treatment on the phenolic compounds and antioxidant capacity of citrus peel extract. Journal of Agricultural and Food chemistry. 2007;55(2):330-5.
[8] Mokbel MS, Hashinaga F. Evaluation of the Antimicrobial Activity of Extract from limitan (Citrus grandisOsbeck) Fruit Peel. Pakistan Journal of Biological Sciences. 2005;8(8):1090-5.
[9] Kim GN, Shin JG, Jang HD. Antioxidant and antidiabetic activity of Dangyuja (Citrus grandisOsbeck) extract treated with Aspergillussaitoi. Food Chemistry. 2009;117(1):35-41.
[10] Yang X, Zhang G, Cui P. The GC/MS analysis of the chemical constituents of pomelo peel volatile oil [J]. Journal of Wuhan Institute of Chemical Technology. 2001;2:003.
[11] Zhou Y, Qiao H, Wang L, Liu X. GC-MS analysis of essential oil from pomelo peel obtained in rong country. Journal of Guangxi University (Natural Science Edition). 2003;29(1):70-2.
[12] Sawamura M, Shichiri KI, Ootani Y, Zheng XH. Volatile constituents of several varieties of pummelos and characteristics among citrus species. Agricultural and biological chemistry. 1991;55(10):2571-8.
[13] Bansil R, Turner BS. Mucin structure, aggregation, physiological functions and biomedical applications. Current opinion in colloid & interface science. 2006;11(2):164-70.
[14] Wulandari, R.L., Windriyati, Y.N. and Budiarti, A. Mucolytic Activity of Methanol Fraction from Ethanolic Extract Piper crocotum Ruiz and Pav.e-PublikasiFakultasFarmasi. 2011;64-70.