RJC

RASĀYAN ***J. Chem.***
Vol. 12 | No. 2 |1002 - 1010| April - June | 2019
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

ANALYSIS CHEMICAL COMPOUNDS AND ANTIMICROBIAL ACTIVITY OF RED ONION (*Allium cepa* L.) BULB SKIN EXTRACT

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ABSTRACT

Recently, drug resistance because of widespread abuse and uncontrol use of antibiotics has become an increasingly difficult problem, with the result that making the development of alternative antimicrobial very urgent handling. Aim of this study was to investigate chemical compounds and antimicrobial activity of outer layers (skin) of red onion bulbs from various extracts using different solvents according to their polarity. In this study, outer layers (skin) of onion (*Allium cepa* L.) bulbs extract by directly maceration method with methanol as solvent and stratified maceration method based on solvent polarity (hexane, ethyl acetate and methanol). Analysis of chemical compound of hexane, ethyl acetate and methanol extract carried by thin layer chromatography. Phytochemical screening carried out on outer layers of onion (*Allium cepa* L.) bulbs in simplicia powder. An antimicrobial activity using agar well diffusion method. The culture of microorganism used were *Staphylococcus aureus* ATCC 29737, *Escherichia coli* ATCC 10536 and *Candida albicans* ATCC 10231. Phytochemistry screening positive results of simplicia were showed against the class secondary metabolites of chemical compounds, namely alkaloids, flavonoids, glycosides, saponins, triterpenoids and tannins but not contained anthraquinone glycosides and cyanogenic glycosides. Analysis of chemical compounds of hexane, ethyl acetate and methanol extract produced different chemical compounds, namely triterpenoids and steroids (for hexane extract), polyphenols and alkaloids (for ethyl acetate extract), triterpenoids, polyphenols and alkaloids (for methanol extract). Data showed that extract from stratified maceration method using methanol provided the most extensive inhibitory diameter. Extract of directly maceration method using methanol and extract of stratified maceration method using ethyl acetate showed not so different antimicrobial activity, whereas for an extract from stratified maceration method using hexane was not provide antimicrobial activity at all.

Keywords: Chemical Compounds, Secondary Metabolites, Antimicrobial, *Allium cepa* L., Phytochemical Screening, Thin Layer Chromatography, Extract

INTRODUCTION

Development and improvement of the resistance of microorganism for human infection, then needed new antimicrobial discoveries and developments[1]. Plants produce chemical compounds derived from plant secondary metabolite compounds, which can have an effect as antimicrobial[2]. A number of studies have been carried out in various parts of the world by extracting plants to searching for antimicrobial activity[3]. Main groups of plant compounds with antimicrobial activity had been reported, such as phenolics (simple phenols, phenolic acid, flavonoids, flavones, flavonols, tannins, coumarins and quinones); terpenoids; essential oils; and also alkaloids[4]. Allium is spread over almost 750 species throughout the hemisphere. Allium is not only known as a vegetable that has a taste and use as a spice, but also as a plant that has

http://dx.doi.org/10.31788/RJC.2019.1225153

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medicinal properties. Among other types of Allium, *Allium cepa* L. (red onion) is one of the oldest plants in the world. Many epidemiological studies confirm that consuming foods containing bulbs is associated with a reduced risk of developing various forms of cancer, cardiovascular and neurodegenerative diseases[5]. This effect is caused because red onion contains a number of secondary metabolites that are high and biologically active, namely phenolic compounds[5,6,7], especially flavonoids and some organic sulfur compounds[5,6,8]. The most common flavonoids found in red onion are quercetin[5,7] and anthocyanin[5]. It has been reported previously, that red onion extract which is rich in phenol compounds shows antiproliferative activity, antimutagenic, anticancer[6], antiulcer, antispasmodic and antidiarrhoeal properties[9]. Bulbs found in the onion extract has also been reported to be antidiabetic, antithrombotic, antiasthmatic, antioxidant, antimicrobial, antiaging, hypoglycaemic and platelet antiaggreration[6]. Up to now in the literature were reported data about antimicrobial action of the mature onion bulbs mostly but not for outer layers (skin) of red onion (*Allium cepa* L.) bulbs. Red onion bulbs can be stored for a long time in a dry state if without peeling, this showed that the onion skin possessed an active compound that protects the bulbs. This onion skin is widely used in making pindang eggs (food traditional in Java and Sumatera, Indonesia), it aims to increase the storage time of boiled eggs. Aim of this study was to investigate chemical compounds (secondary metabolites) and antimicrobial activity of outer layers (skin) of red onion bulbs from various extracts using different solvents according to their polarity.

EXPERIMENTAL

The study was conducted at the Phytochemistry Laboratory, Microbiology Laboratory and Research Laboratory, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, North Sumatera. Plant material used in this study was outer layers of onion (*Allium cepa* L.) bulbs extract, plant material obtained from Jamin Ginting 10, Tanah Seribu, South Binjai, Medan, North Sumatera, Indonesia. Plant material was done purposively without comparing with similar plants in other areas. Identification of plants was done in "Herbarium MEDANENSE" Botany Research Center of Biology, University of Sumatera Utara. Phytochemical screening carried out on outer layers of onion (*Allium cepa* L.) bulbs in simplicia powder form for detecting chemical compounds alkaloids, anthraquinone glycosides, flavonoids, saponins[10,] glycosides[11], cyanogenic glycosides[12], triterpenoids, steroids and tannins[10]. Sample extraction was done by directly maceration method and stratified maceration method[11]. The solvent of directly maceration method using methanol and stratified maceration method using hexane, ethyl acetate and methanol, respectively. Analysis of chemical compound of hexane, ethyl acetate and methanol extract carried by thin layer chromatography (TLC). Stationary phase using aluminium plate of TLC silica gel 60 F_{254} (Merck®) and mobile phase was used hexane : ethyl acetate (7:3 and 3:7) (pro analysis, Merck®) with visualization 50% sulfuric acid acid in methanol[13], Liebermann-Bourchard[14], Dragendorff and 5% iron (III) chloride[13]. An antibacterial activity using Mueller Hinton Agar[15] (Merck®) for *Staphylococcus aureus* ATCC 29737 and *Escherichia coli* ATCC 10536 and also antifungal was determined on Sabourauds Dextrose Agar (Merck®) using *Candida albicans* ATCC 10231[16]. A suspension of each microorganism (10^6 cfu/mL) was obtained by measuring the absorbance of the solution at wavelength 620 nm using an ultraviolet spectrometer[1]. Antimicrobial activity was determined using agar well diffusion method with slightly modification[17,18,19,20]. The first layer of the media was made by pouring 10 mL of Mueller hinton agar (MHA) into a sterile petri dish, then left to solidify. After solidification, on the surface of the first layer, poured 0.1 mL of a suspension of test bacterial inoculum and 20 mL of MHA as the second layer, then homogenized. Steril cork borers were immediately placed and arranged on the second layer media, so that the observation area did not overlap. Furthermore, the steril cork borers were lifted slowly from the surface of the agar media, so that wells was formed. Solution of the test extract with various concentrations and blanks (mixtures of dimethylsulfoxide and ethanol) was included in the wells as much as 0.1 mL. After incubation at 37 °C for 24 h[21] for bacteria and at 25°C for 48 h for fungi, the inhibition diameter were observed, measured and recorded. All tests were performed in triplicate.

RESULTS AND DISCUSSION

Result of Onion (*Allium cepa* L.) identification conducted in "Herbarium MEDANENSE" Botany Research Center of Biology, Universitas Sumatera Utara, indicated that the plants used in this study was

the plant of outer layers of onion (*Allium cepa* L.) bulbs and included Alliaceae family. As much as 5 kg fresh outer layers of onion bulbs (*Allium cepa* L.) was obtained 3.23 kg simplicia. Result of direct maceration from 400 g simplicia was produced 22.2599 g extract. Results of stratified maceration from 600 g simplicia was respectively obtained 2.8599 g of hexane extract, 15.1504 g of ethyl acetate extract and 25.7558 g of methanol extract.

Result of Secondary Metabolites Analysis as Chemical Compound by Phytochemistry Screening Method

Phytochemistry screening results of outer layers (skin) simplicia of onion (*Allium cepa* L.) bulbs were showed positive results against the class of chemical compounds alkaloids, flavonoids, glycosides, saponins, triterpenoids and tannins but not contained anthraquinone glycosides and cyanogenic glycosides. Results of phytochemistry screening of simplicity can be presented in the data below (Tabel-1).

Table-1: Results of Phytochemistry Screening of Outer Layers Simplicia of Onion (*Allium cepa* L.) Bulbs

No.	Chemical Compound	Reagent or Treatment	Results (color / precipitate)	Conclusion
1.	Alkaloids	Dragendorff Bouchard at Mayer	(+) orange-brown (+) yellow-brown (+) turbidity and a white precipitate	(+)
2.	Anthraquinone glycosides	NaOH	not occurred intensively redness color on NaOH layer	(-)
3.	Flavonoids	Zn add concentrated hydrochloric acid Mg add concentrated hydrochloric acid	(+) red	(+)
4.	Glycosides	Molisch Fehling Liebermann-Burchard	(+) purple rings (+) orange-red precipitate (+) red	(+)
5.	Saponins	Hot water and shaken	(+) stability foam	(+)
6.	Cyanogenic glycosides	Pikrat sodium	not occurred orange color in Whatman paper	(-)
7.	Tanins	$FeCl_3$ 1%	(+) green-black	(+)
8.	Triterpenoids / Steroid	Liebermann-Burchard	(+) old purple	(+) triterpenoids

Note: (+) = positive compound, (-) = negative compound

Results of simplicia phytochemistry screening on outer layers of onion (*Allium cepa* L.) bulbs indicated the existence of chemical compounds in methanol extract from directly maceration using methanol, this showed methanol as a solvent that can dissolve various compounds.

Result of Chemical Compound Analysis by Thin Layer Chromatography

Results of chemical compounds analysis of hexane extract, ethyl acetate extract and methanol extract from stratified maceration method yield carried out thin layer chromatography (TLC). Stationary phase used aluminum plate of TLC silica gel 60 F_{254} (Merck®). Mobile phase used hexane:ethyl acetate (7:3 for hexane extract and 3:7 for ethyl acetate and also methanol extract) (pro analysis, Merck®) with visualization agent was 50% sulfuric acid in methanol to display the entire class of chemical compounds contained in each extract, Liebermann-Bourchard to detect the presence of free compounds of triterpenes and steroids, Dragendorff to detect alkaloid class of compounds and 5% iron (III) chloride to detect a class of polyphenolic compounds. Results variety chromatograms and Rf value for each extract can be seen in Table-2, 3 and 4 below.

Chromatograms result of hexane extract with using hexane:ethyl acetate (7:3) as mobile phase and 50% sulfuric acid in methanol as visualization was provided 13 spots that showed the presence of at least 13 compounds. Chromatogram results using Liebermann-Bourchard as visualization was obtained 7 spots (6

of them was purple and 1 green) which mean there was triterpenoids compound (purple) and steroids compound (green) whereas visually, $FeCl_3$ 5% and Dragendorff was not visible spots, it means that there were not alkaloids compounds detected by Dragendorff and no polyphenol compounds detected by $FeCl_3$ 5%.

Table-2: Results of Chromatogram and Rf Value of Hexane Extract of Outer Layers of Onion (*Allium cepa* L.) Bulbs

Chromatograms result with using mobile phase hexane:ethyl acetate (7:3)				
visually	H_2SO_4 50% reagent	Liebermann-Burchard reagent	$FeCl_3$ 5% reagent	Dragendorff reagent
not visible	0,90(p)	0,76(p)	not visible	not visible
	0,80(p)	0,67(p)		
	0,76(p)	0,55(g)		
	0,70(g)	0,48(p)		
	0,67(v)	0,38(p)		
	0,55(y)	0,31(p)		
	0,51(p)	0,20(p)		
	0,48(y)			
	0,38(v)			
	0,36(g)			
	0,31(y)			
	0,20(b)			
	0,15(v)			

Note: b = brown, g = green, p = purple, v = violet, y = yellow

Table-3: Results of Chromatogram and Rf Value of Ethyl Acetate Extract of Outer Layers of Onion (*Allium cepa* L.) Bulbs

Chromatograms result with using mobile phase hexane:ethyl acetate (3:7)				
visually	H_2SO_4 50% reagent	Liebermann-Burchard reagent	$FeCl_3$ 5% reagent	Dragendorff reagent
not visible	0,90(br)	not visible	0,90(g)	0,59(y)
	0,88(p)		0,80(g)	0,44(y)
	0,82(p)		0,64(yg)	0,29(b)
	0,80(br)		0,46(yg)	
	0,64(br)		0,38(yg)	
	0,61(p)		0,35(bl)	
	0,59(b)			
	0,46(br)			
	0,44(b)			
	0,38(br)			
	0,35(g)			
	0,29(y)			

Note: b = brown, bl = blue, br = brownish red, g = green, p = purple, y = yellow, yg = yellowish green

Chromatograms result of ethyl acetate extract (in table 3) with using hexane:ethyl acetate (3:7) as mobile phase and 50% sulfuric acid in methanol as visualization was provided 12 spots that showed the presence of at least 12 compounds. Chromatogram results using $FeCl_3$ 5% as visualization was obtained 6 spots (5 of them was green and yellowish green and 1 blue) which means there was polyphenols compound. Chromatogram results using Dragendorff has obtained 1 spot (brown) that indicated alkaloids compounds, whereas visually and Liebermann-Bourchard was not visible spots, it means that there were not triterpenes and steroids compounds detected.

Chromatograms result of methanol extract (in table 4) with using hexane:ethyl acetate (3:7) as mobile phase and 50% sulfuric acid in methanol as visualization was provided 16 spots that showed the presence of at least 16 compounds. Chromatogram results using Liebermann-Bourchard as visualization was obtained 3 spots which means there was triterpenoids compound. Chromatogram results using $FeCl_3$ 5% as visualization was obtained 8 spots (7 of them was variation green and 1 blue) which mean there was

polyphenols compound. Chromatogram results using Dragendorff has obtained 1 spot (brownish yellow) that indicated alkaloids compounds.

Table-4: Results of Chromatogram and Rf Value of Methanol Extract of Outer Layers of Onion (*Allium cepa* L.) Bulbs

Chromatograms result with using mobile phase hexane:ethyl acetate (3:7)				
visually	H_2SO_4 50% reagent	Liebermann-Burchard reagent	$FeCl_3$ 5% reagent	Dragendorff reagent
not visible	0,92(b)	0,61(p)	0,92(yg)	0,46(o)
	0,87(b)	0,31(p)	0,81(yg)	0,22(o)
	0,81(b)	0,17(p)	0,77(g)	0,11(by)
	0,76(o)		0,71(g)	
	0,72(b)		0,68(bl)	
	0,67(b)		0,56(g)	
	0,60(o)		0,52(g)	
	0,56(b)		0,37(g)	
	0,52(o)			
	0,50(b)			
	0,46(o)			
	0,37(b)			
	0,30(b)			
	0,22(y)			
	0,17(y)			
	0,11(b)			

Note: b = brown, bl = blue, by = brownish yellow, g = green, o = orange, p = purple, y = yellow, yg = yellowish green

Results of Antimicrobial Activity

Antibacterial Activity of Extract of Outer Layers of Onion Bulbs (*Allium cepa* L.) for *Staphylococcus aureus* (SA) ATCC 29737 can be seen in Fig.-1. Antibacterial activity against SA showed increasing inhibitory diameter when the extract concentration was increased. However, for extracts, SMH was not showed antibacterial activity against SA in various extract concentrations. Based on the data presented of the identification of the component chemical compounds on the hexane extract containing dominant of triterpenoid (Table-2). This data showed that hexane extract from outer layers of onion bulbs (*Allium cepa* L.) produced from maceration extraction using hexane solvents contains triterpenoid compounds that were not had antibacterial activity against SA. Antibacterial activity against SA showed that extraction by stratified maceration method using methanol solvent (SMM) at a concentration of 150 mg / ml provided the greatest antibacterial activity with the highest inhibitory diameter values (d = 12.00 mm). The antibacterial activity SMM also has a wider inhibitory diameter compared to the methanol extract produced from directly maceration method (DMM). The results of the analysis of chemical compounds for DMM extracts (Table-1) and SMM (Table-4) also showed the components of each chemical compound. DMM extract has a variety of plant chemical compounds (secondary metabolites), such as the class of chemical compounds alkaloids, anthraquinone glycosides, flavonoids, glycosides, saponins, tanins and triterpenoids caused by using of methanol solvents by directly maceration method. The SMM extract used methanol solvents but extraction had been done before using hexane and ethyl acetate solvents in a row, this causes the number of chemical compound components in the SMM extract to decrease because previously the other chemical compounds had been drawn by solvents hexane and ethyl acetate. The SMM extract had triterpenoid and phenol chemical compounds (Table-4). These results provided the possibility that extracts had fewer chemical compounds so gave greater antibacterial activity. The results obtained also caused to the presence of triterpenoid and phenol compounds from the SMM extract having greater antibacterial activity against SA than these compounds in the DMM extract which had many other chemical compounds.

Antibacterial Activity of Extract of Outer Layers of Onion Bulbs (*Allium cepa* L.) for *Escherichia coli* (EC) ATCC 10536 can be seen in Fig.-2. Antibacterial activity against EC showed increasing inhibitory diameter when the extract concentration was increased. However, for extracts SMH was not showed antibacterial activity against EC in various extract concentrations. This result was the same as the

antibacterial activity against SA. Antibacterial activity against EC showed that extraction by stratified maceration method using methanol as solvent (SMM) at a concentration of 150 mg / ml provided the greatest antibacterial activity (d = 11.16 mm) then followed by SME (d = 10.50 mm) and DMM (d = 9.60 mm). SME extract provided stronger antibacterial activity against EC than DMM extract. The analysis of the components of the chemical compounds of the SME extract showed that the SME extract containing phenol compounds (Table 3) which were indicated stronger in inhibiting EC growth compared with DMM extracts. DMM extract has a variety of plant chemical compounds (secondary metabolites), such as the class of chemical compounds alkaloids, anthraquinone glycosides, flavonoids, glycosides, saponins, tanins and triterpenoids caused by the use of methanol solvents by directly maceration method. The SME extract used ethyl acetate solvents, but previously extracted using hexane solvents, this resulted indicated was not detection of triterpenoid and steroid compounds because the compounds such as free triterpenoids and free steroids were withdrawn by hexane solvents. These results provided the possibility that extracts had a component of chemical compounds in the phenol group provided stronger antibacterial activity against EC than these phenol compounds combined with other chemical compounds such as those found in DMM extracts.

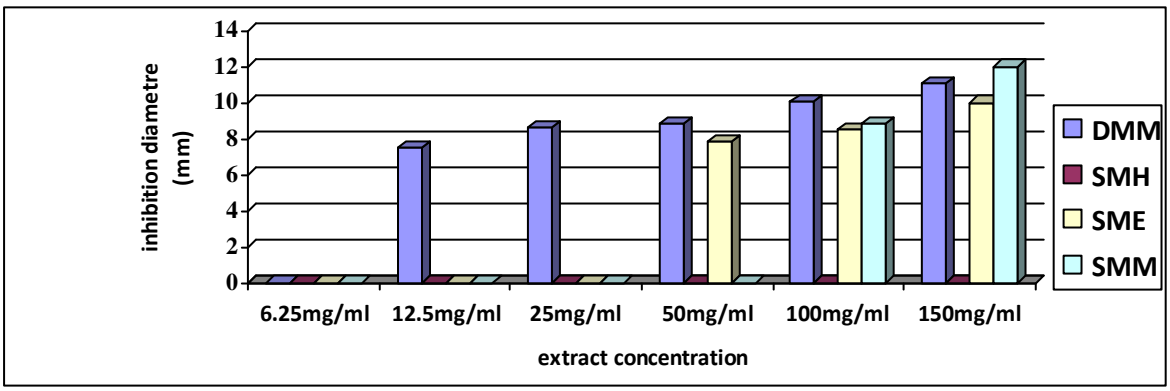

Fig.-1: Antibacterial Activity of Extract of Outer Layers of Onion Bulbs (*Allium cepa* L.) for *Staphylococcus aureus* ATCC 29737

Note: DMM= extract of directly maceration method using methanol, SMH= extract of stratified maceration method using hexane, SME= extract of stratified maceration method using ethyl acetate, SMM= extract of stratified maceration method using methanol.

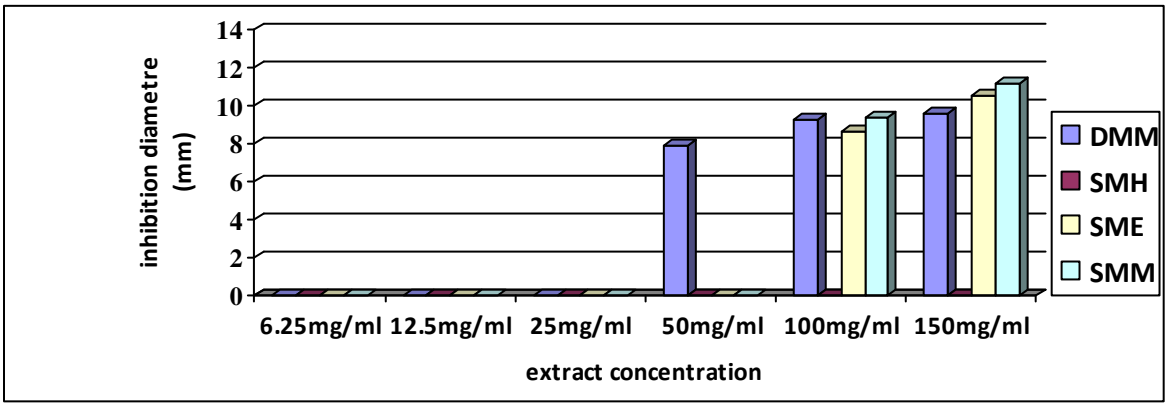

Fig.-2: Antibacterial Activity of Extract of Outer Layers of Onion Bulbs (*Allium cepa* L.) for *Escherichia coli* ATCC 10536

Note: DMM= extract of directly maceration method using methanol, SMH= extract of stratified maceration method using hexane, SME= extract of stratified maceration method using ethyl acetate, SMM= extract of stratified maceration method using methanol.

Chemical compounds found in plants have the advantage of being able to tolerate well in the body and have low side effects compared to most chemotherapy drugs. Such data has been appointed on previous research there were synergistic effects between plant chemical compounds if they are in an extract, these

compounds include triterpenes, saponins, polyphenols, pectin, steroids and other active chemical compounds[22]. Various plant chemical compounds such as alkaloids, terpenoids, tannins[2,23], flavonoids[2,23,24], saponins[2,25], glycosides, anthraquinone[2], phenolic, flavonons and derivatives terpenes[26] became modern plant chemical compounds that can attract various types of microorganisms. It was also reported that phenol as the chemical compound can inhibit the growth of *Salmonella thypi* and *Escherichia coli*[23,25]. Cell membrane damage has been reported to have occurred as a result of interacting with plant chemical compounds, for example in phenol compounds have several working mechanisms, including membrane disruption, protein binding, inhibiting protein synthesis, inhibiting enzymes, cell wall destruction, disulfide bridge formation and intercalation with the cell wall and/or DNA[27,28]. Alkaloids have intercalation with the cell wall and/or DNA working mechanism[27]. The triterpenoid compound has a membrane disruption mechanism[27,29].

Antifungal Activity of Extract of Outer Layers of Onion Bulbs (*Allium cepa* L.) for *Candida albicans* (CA) ATCC 10231 can be seen in Figure 3. Antifungal activity against CA showed increasing inhibitory diameter when the extract concentration was increased. However, for extracts, SMH was not indicated antifungal activity against CA at various extract concentrations. Based on the data of chemical compounds analysis on the hexane extract contained dominant triterpenoid (table 2). This data indicated that hexane extract from maceration extraction using hexane solvents contain a class of triterpenoid compounds that did not have antifungal activity against CA. Antifungal activity against CA showed that extraction with stratified maceration methods using methanol as solvent (SMM) at a concentration of 150 mg / ml provided the greatest antifungal activity with the most extensive inhibitory diameter values (d = 10.66 mm). The antifungal activity of methanol extract (SMM) also has a wider inhibitory diameter compared to the methanol extract produced from the direct maceration method (DMM). DMM extract has various plant chemical compounds (secondary metabolites), such as alkaloids, anthraquinone glycosides, flavonoids, glycosides, saponins, tanins and triterpenoids (Table-1) caused using methanol as solvent by directly maceration method. The SMM extract has triterpenoid and phenol chemical compounds (Table-4). These results provided the possibility that extracts that had fewer chemical compounds giving greater antifungal activity. The results obtained also caused to the presence of triterpenoid and phenol compounds from the SMM extract having greater antifungal activity against CA than these compounds in the DMM extract which had many other chemical compounds.

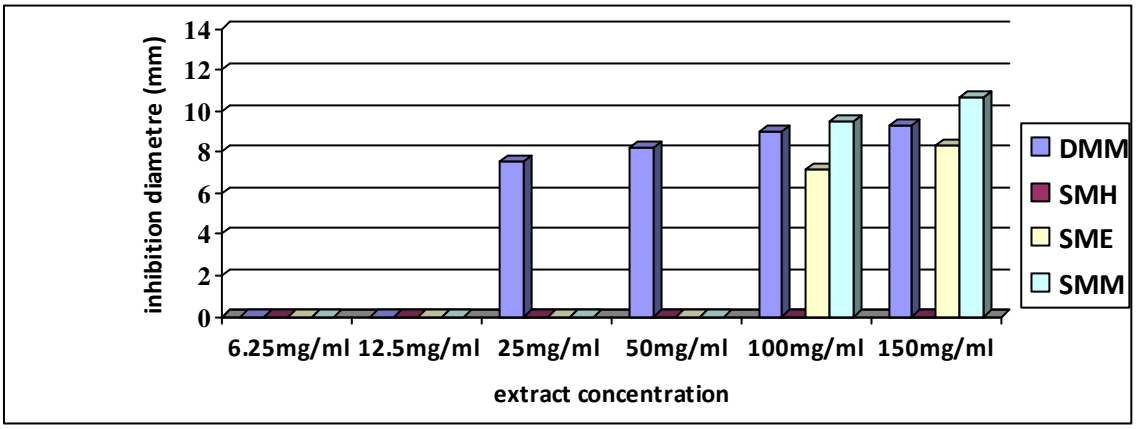

Fig.-3: Antifungal Activity of Extract of Outer Layers of Onion Bulbs (*Allium cepa* L.) for *Candida albicans* ATCC 10231

Note: DMM= extract of directly maceration method using methanol, SMH= extract of stratified maceration method using hexane, SME= extract of stratified maceration method using ethyl acetate, SMM= extract of stratified maceration method using methanol.

The antimicrobial activity of the natural product, especially plants, was not only determined by the class of chemical compounds, but also determined by the relationship between the effects of chemical compounds of plants and the cells of microorganisms that they affect. This can be seen from the results that had been obtained, where DMM extract was stronger antibacterial activity against SA than SME, but the antibacterial activity of SME extracts against EC was stronger than compared to DMM extracts. In

terms of solvent polarity, it was showed that more nonpolar of plant chemical compounds affected bacterial cells which tend to be nonpolar as well. Research on the relevance of these plant chemical compounds to microorganism cells can be done for further studies.

Infection caused by species of Candida is the main cause of opportunistic fungal infections throughout in the world. Candidemia and other forms of invasive candidiasis are the causes of increased rates of morbitity and mortality. *Candida albicans* is a type of fungus that grows by causing infections in the mucosa and blood flow system[30]. Components of chemical compounds such as flavonoids contained in extracts have a working mechanism in the cytoplasmic membrane area or on the cell wall which caused damage to the function and structure of cells. Some previous researchers stated that antimicrobial activity was more strongly related to the synergistic effects of flavonoids and other components of phenolic compounds compared to their single compounds[31]. In previous studies it has been reported that the skin of the onion extract contains much higher levels of flavonoids than its commonly edible bulbs. In this study it was found that crude extract obtained from acetone, ethanol and its mixture with water contained higher levels of phenolic and quercetin compounds, antioxidant and antimicrobial activity against *Escherichia coli*, *Pseudomonas fluorescens*, *Bacillus cereus*, *Aspergillus niger*, *Trichoderma viridae* and *Penicillium cyclopium* which is stronger than the onion bulbs that are commonly edible[9].

CONCLUSION

Phytochemistry screening results of simplicia were showed positive results against the class of chemical compounds alkaloids, flavonoids, glycosides, saponins, triterpenoids and tannins but not contained anthraquinone glycosides and cyanogenic glycosides. Analysis of chemical compounds of hexane, ethyl acetate and methanol extract produced different chemical compounds, namely triterpenoids and steroids (for hexane extract), polyphenols and alkaloids (for ethyl acetate extract), triterpenoids, polyphenols and alkaloids (for methanol extract). The antimicrobial activity of extracts indicated a variety of different results. Data showed that extract from stratified maceration method using methanol as solvent (SMM) provided the most extensive inhibitory diameter. Extract from directly maceration method using methanol as solvent (DMM) and extract from stratified maceration method using ethyl acetate as solvent (SME) showed not so different antimicrobial activity, whereas for an extract from stratified maceration method using hexane as solvent (SMH) was not provide antimicrobial activity at all.

ACKNOWLEDGMENT

All the authors are indebted to the Universitas Sumatera Utara for allowing and facilitating the publication of this work. This research is funded by Universitas Sumatera Utara through non PNBP funding in accordance with TALENTA's research in contract 2017.

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