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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF POLYPHENOLS EXTRACT FROM *POLYGONUM MULTIFLORUM* THUNB. ROOT

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ABSTRACT

The purpose of this research is to investigate the presence of alkaloids, saponins, flavonoids, anthraquinones and tannins compounds as the possible agent responsible for the medicinal activities, the antioxidant activities and antimicrobial activities from *Polygonum multiflorum* Thunb. root. The powdered root was analyzed positively for alkaloids, saponins, flavonoids, anthraquinones and tannins. In addition, they are also related to an antimicrobial activity and the presence of these constituents was helpful to apply in medical and food industry. The determination of antimicrobial activity of *Polygonum multiflorum* Thunb. root extracts against gram-negative *Escherichia coli* (ATCC 25922), *Salmonella enteritidis* (ATCC 13076), gram-positive: *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 11774), *Listeria monocytogenes* (CIP 74908), fungi: *Fusarium equiseti*, *Aspergillus niger* and *Trichoderma asperellum* were investigated by the paper disc diffusion method for antibiotic susceptibility testing and minimum inhibitory concentration (MIC) evaluation of dryness extract. The results showed that the dryness extract can inhibit one gram-positive bacteria (*Staphylococcus aureus*, MIC = 200 mg/mL), one gram-negative bacteria (*Salmonella enteritidis*, MIC = 400 mg/mL) and one fungus (*Trichoderma asperellum*, MIC = 100 mg/mL); it's not take effectively on *Escherichia coli*, *Bacillus subtilis*, *Listeria monocytogenes*, *Fusarium equiseti* and *Aspergillus niger*.

1. Introduction

Currently, there are a large number of herbal plants whose importance science has not been explored. All over the world, most of plants have used as the richest source of raw materials for traditional as well as modern medicine. *Polygonum multiflorum* Thunb. is one of the most popular traditional herbal plants of Vietnamese and is a main ingredient in many prescriptions during a thousand year. It was cooked with many food such as chicken, black bean (Zhou *et al*., 2010) in order to eat or use as drug to cure many diseases like tonic tension (Lim *et al*., 2014), anti-aging effects (Lin *et al*., 2008), antioxidant activity (Wang *et al*., 2008) and certain forms of cancer (Hung *et al*., 2004).

The ethnomedical uses of *Polygonum multiflorum* Thunb. that has been recorded in many provinces in Asia such as China, Korean, Japan and Vietnam. Some the scientists discovered more than 100 chemical bioactive compounds from this plant, and the major components that consisted of stilbenes,

phospholipids, quinones, flavonoids and others (Lin *et al*., 2015).

However, the content and bio-activity of these components depend on many factors such as climate, soil, harvesting season, gene, storage condition and the different extraction methods. Therefore, the determination of presence of these components is quite important in this research. Until now, many studies have demonstrated that parts of this plant contain biologically active compounds such as phenolic compounds, saponins, alkaloids, etc. They are useful in food technology or drug industry, especially root and hairy root of *Polygonum multiflorum* Thunb. Phenolic compounds in hairy root can inhibit *Staphylococcus aureus*, *Escherichia coli*, *Fusarium oxysporum* and *Aspergillus niger* (Thiruvengadam *et al.*, 2014) while phenolic compounds in root have the high antioxidant capacity (Le *and* Nguyen, 2015). There are many researches that extracted polyphenols from *Polygonum multiflorum* Thunb. root but until now there are no reports on phytochemical screening and antimicrobial activities on extract of *Polygonum multiflorum* Thunb. root. Therefore, the current research was undertaken to determine some bioactive compounds and antibacterial activities of acetone extract of *Polygonum multiflorum* Thunb. root.

2. Materials and methods

2.1. Plant collection

Polygonum multiflorum Thunb. roots were harvested from Cao Bang province (Vietnam) and the clean roots were sliced and dried at 60°C until < 12% moisture level was reached. The slices were ground into a fine powder (< 0.5 mm) and vacuum-packed.

2.2. Organisms collection

Antibacterial activity and minimum inhibitory concentration (MIC) were determined against three gram-positive bacteria as *Bacillus subtilis* (ATCC 11774), *Staphylococcus aureus* (ATCC 25923), *Listeria monocytogenes* (CIP 74908), two gram-negative bacteria as *Escherichia coli* (ATCC 25922), *Salmonella enteritidis* (ATCC 13076) and three fungi as *Fusarium equiseti*, *Aspergillus niger* and *Trichoderma asperellum* (They were kindly provided by Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh city).

2.3. Acetone extraction

The powdered root was extracted by microwave-assisted extraction (MAE) with aqueous acetone 57.35%, the ratio of materials/solvent is 1/39.98 (w/v), an extraction time of 289 seconds and microwave power of 127 W (Le *and* Nguyen, 2015). The extracts were filtered by using Whatman filter paper No. 4 and evaporated under vacuum conditions in a water bath at 45°C. After that, the residue was freeze-dried during 7 hours at -20^{o}C, < 1 mbar and dryness extract stored at 4^{o}C prior to use.

2.4. Phytochemical analysis

2.4.1. Identification of flavonoids

Ferric chloride test: Three drops of solution of 5% $FeCl_3$ was added the extract. The formation of greenish-black color indicates the presence of phenolic nucleus (Sofowora, 1993).

Sodium hydroxide test: The extract was added about 2 mL of 10% NaOH solution, yellow solution indicates the presence of flavonoids which adds on adding dilute hydrochloric acid that becomes colorless (Evans, 2002).

2.4.2. Identification of tannins

Ferric chloride test: Three drops of solution of 5% $FeCl_3$ were added the extract, production of a blue or greenish-black color that changes to olive green as more $FeCl_3$ 5% is added to indicate the presence of tannins (Evan, 2002).

Gelatin test: Few drops of 10% gelatin solution were added to the extract. Formation of a precipitate indicates the presence of tannins.

Lead sub-acetate test: Few drops of 10% lead sub acetate solution were added to the extract. Formation of a colored precipitate indicates the presence of tannins (Evan, 2002).

2.4.3. Identification of anthraquinones

Borntrager's test: The 2 mL extract was added to 5 mL chloroform in the test tube and shaken for a few minutes. The mixture was shaken with equal volume of 10% ammonia solution. After shook this mixture, there is the presence of free anthraquinones by layering such as violet, pink or red (Evan, 2002).

2.4.4. Identification of alkaloids

A small extract (2 mL) was mixed with 20 mL of 5% sulphuric acid in 50% ethanol. The mixture was cooled. Two drops of concentrated ammonia solution was added into the solution, then the equal volume of chloroform was also added and shook gently to allow the separation of the individual layers. Chloroform in the lower layer is removed. The ammoniacal layer was added drop by drop by the Dragendorff's reagent. The solution appears the reddish-brown that precipitated to indicate the presence of alkaloids (Evans, 2002).

2.4.5. Identification of saponins

Frothing test: The extract was placed in a test tube and added to 10 mL of distilled water; shook vigorously for 30 seconds then let keep for 30 minutes and observe. The formation of foam indicates the presence of saponins (Sofowora, 1993).

Haemolysis test: Few drops of an animal blood was added to the extract (prepared in normal saline) by a syringe and mixed gently by inverting the tube and allowed to keep for 15 minutes. The settling down of the red blood cells denotes the presence of saponins (Yusuf *et al.*, 2014).

2.5. Color evaluation

Color parameters were measured on extract. Values were recorded as lightness L^* (ranging from 0 to 100 corresponding to black to white), a^*: Red shade (if the value is positive), green shade (if the value is negative) and b^*: Yellow shade (if the value is positive), blue shade (if the value is negative).

2.6. Determination of total polyphenol content (TPC) and antioxidant capacity (AC) of extract

The TPC in the extract was slightly modified and determined by the Folin-Ciocalteu colorimetric method (Siddiqua *et al.*, 2010). The results were based on a standard curve obtained with gallic acid. TPC was expressed as mg of gallic acid equivalent per gram of dry weight (mg GAE/g DW).

The AC of the extract was determined by DPPH assay which was adapted from Soto *et al.* (2014) and Chmelová *et al.* (2015), it was slightly modified. Trolox was used as the standard. AC was expressed in TEAC (Trolox equivalent antioxidant capacity) determined as µmol of Trolox per gram of dry weight (µmol TE/g DW).

2.7. Determination of antimicrobial activity and minimum inhibitory concentration (MIC) evaluation

The minimum inhibitory concentration (MIC) evaluation was determined by the paper disc diffusion method for antibiotic susceptibility testing according to Kirby-Bauer test (Bauer *et al.*, 1966). The sterile paper discs of 6 mm diameter were prepared that using various concentrations of dryness extract of powdered root (25, 50, 100, 200, 400, 800 and 1600 mg/mL); gentamicin (10 µg/disc) and ketoconazole (50 µg/disc) were used as positive controls to compare the antibacterial activity and antifungal activity, respectively; 5% dimethylsulfoxide (DMSO) was used as negative control. Firstly, 0.1 mL of bacteria suspension (0.5 McFarland standard, approximately 1.5×10^8 cfu/mL) and 0.1 mL of fungus suspension (approximately 0.4×10^4 – 5×10^4 cfu/mL) were spread on the surface of the Mueller-Hinton agar media for bacterial strains and Potato dextrose agar media for fungal strains by sterile hockey stick, respectively. Then, sterile paper discs were impregnated with 20 µL of each of extracts. The dishes were incubated during 24 hours at 37°C for bacterial strains and 72 hours at 30°C

for fungal strains. After that, the zones of inhibition were expressed in mm, as the diameters of clear zones around the discs.

2.8. Data analysis

Experimental results were analyzed by the one-way analysis of variance (ANOVA) method and significant differences among the means from triplicate analyses at ($p<0.05$) were determined by Fisher's least significant difference (LSD) procedure using Statgraphics software (Centurion XV). The values obtained were expressed in the form of a mean±standard deviation (SD).

3. Results and discussions

3.1. Identification of bioactive compounds

Phytochemical analysis of the powder of *Polygonum multiflorum* Thunb. root was successfully carried out, acetone was found to be a good solvent system for the extraction of the bioactive compounds of this plant. The powdered root was tested positive for alkaloids, tannins, anthraquinones, saponins and flavonoids (Table 1). These results agreed with the literature review on the plant which showed these compounds to be presented (Lin *et al.*, 2015).

Tannins were polyphenols which exist popularity in plant and were divided two types: condensed tannins and hydrolyzed tannins. Determining the presence of tannins in *Polygonum multiflorum* Thunb. root extract has many methods such as $FeCl_3$ test, gelatin test and lead sub-acetate in this research. Tannins play is an important role in food technology and human health because of their functions like they were antioxidant, anti-aging, anti-inflammatory, anticarcinogenic, etc. (Atanassova *and* Chiristova-Bagdassarian, 2009). Besides that, flavonoids were determined in *Polygonum multiflorum* Thunb. extract through reaction with NaOH solution to special-yellow. However, it is quite difficult to determine specific flavonoid due to various flavonoids, which can react with NaOH solution to create yellow color as flavone, isoflavone, flavanone, chalcone, etc. (Nguyễn, 2007). A group that is quite important compound is free anthraquinones which was also discovered in extract through red color in the aqueous layer above after adding chloroform and ammoniac solution. This bioactive compound is essential important ingredient and is anti-cancer, anti-bacterial, anti-inflammatory (Barnard *et al.*, 1992; Srinivas *et al.*, 2003) and enhance repairs the nucleotide in human's cells (Chang *et al.*, 1999).

Table 1. Phytochemical constituents of *Polygonum multiflorum* Thunb. root extract

No.	Phytoconstituents	*Polygonum multiflorum* Thunb. root extract
1	**Tannins**	
	a. $FeCl_3$ test	+
	b. Gelatin test	+
	c. Lead sub-acetate test	+
2	**Saponins**	
	a. Frothing test	+
	b. Haemolysis test	+
3	**Anthraquinones**	
	Borntrager's test	+
4	**Flavonoids**	
	a. $FeCl_3$ test	+
	b. NaOH test	+
5	**Alkaloids**	
	Dragendorff's test	+

+: Present

In addition, saponins also exit in *Polygonum multiflorum* Thunb. extract. This compound can be found in plant, especially medical plants such as *Paullinia pinnata* Linn (Yusuf *et al.*, 2014), *Acorus calamus* and *Lantana camara* (Mamta *and* Jyoti, 2012), etc. Saponins can dissolve easily in water and decrease the surface tension on solution to create more bubble honeycomb structure. This is a sample method to determine the presence of saponins in extract (Nguyễn, 2007). Saponins can make the settling down of the red blood cells when mixed with animal blood (Yusuf *et al.*, 2014). However, we can not determine exactly specific saponins (triterpenoid or steroid) in this case. The extract of *Polygonum multiflorum* Thunb. also has the presence of alkaloids which is bioactive and heterocyclic chemical compound. It contains nitrogen and may some pharmacological, medicinal or ecological activity in many cases (Aniszewski, 1994). Alkaloids can be found in animal and plant such as tea, coffee, pepper… These alkaloids are highly reactive substances with biological activity even in low doses (Aniszewski, 2007).

In general, *Polygonum multiflorum* Thunb. was precious herbal plant. Root had many bioactive compounds, it was very necessary in food and medical technology. These compounds are the main key in the medicinal value and the data can help us choose this valuable plant with greater quantity of medical and food industry.

3.2. Determination of total polyphenol content (TPC) and antioxidant capacity (AC) of extract

After extraction process, the TPC and AC values of extract achieve 47.53±0.79 mg GAE/g DW and 334.07±3.04 μmol TE/g DW, respectively. TPC and AC of samples from MAE method were higher than samples from China which were extracted by decoction method with deionized water as solvent (33.91±0.62 mg GAE/g DW; 257.9±3.7 μmol TE/g DW) and maceration method with 50% ethanol as solvent (40.42±0.63 mg GAE/g DW; 256.7±0.7 μmol TE/g DW) (Li *et al.*, 2007). The results show that the difference of extraction methods, land, gender, analyzation method, etc which cause the changes about TPC and AC values.

3.3. Identification of physicochemical characteristic of dryness extract

The moisture of extracts after freeze-drying was approximately 3.03±0.5% and stored at 4^{o}C. Storage conditions were quite advantageous to maintain the content and the activity of bioactive compounds such as low moisture, cold environment and less oxygen. It can be avoided the oxidization reaction and denature bioactive compounds.

The yield of dryness extract achieves 6.26±0.47%, this result is higher than recent study of Đái *et al*. (2015) (3.55%, extract from leaf and trunk of *Streptocaulon juventas* Merr.). The cause of difference was various materials, extraction method (solvent, material/solvent ratio, temperature and time extraction)… especially dewater method. Freeze-drying method can be removed completely free-water from material and a remaining part of fixed-water. Moreover, the sublimation of water does not affect significantly bioactive compounds at low temperature and low pressure (Nireesha *et al.*, 2013). This was proved that TPC and AC of dryness extract did not change significantly after freeze-drying process, TPC and AC values reached 47.15±0.88 mg GAE/g DW and 337.43±9.24 μmol TE/g DW, respectively.

Besides that, color of material also changed clearly. First, *Polygonum multiflorum* Thunb. extract has light yellow brown color. Then, the dryness extract has dark-brown color after freeze-drying. Meanwhile, L^*, a^* and b^* values also change strongly; L^* value decreases rapidly from 58.98±0.16 to 36.35±0.19; a^* value increases slowly from 8.21±0.02 to 12.77±0.1 and b^* value decrease extremely from 20.81±0.06 to 9.86±0.27. Initial color converts into dark color and turns brown-red shade. This may be explained that the loss of

water will increase the concentration of extract which lead cause to change color and the extract that was oxidized because of long freeze-drying time.

3.4. Antimicrobial activity and minimum inhibitory concentration (MIC) evaluation

Antimicrobial activity of *Polygonum multiflorum* Thunb. dryness extract was studied at various concentration (25, 50, 100, 200, 400, 800 and 1600 mg/mL) against five strains of pathogenic bacteria including two gram-negative bacteria (*Escherichia coli* – ATCC 25922, *Salmonella enteritidis* – ATCC 13076), three gram-positive bacteria (*Staphylococcus aureus* – ATCC 25923, *Bacillus subtilis* – ATCC 11774, *Listeria monocytogenes* – CIP 74908) and three fungus *Fusarium equiseti*, *Aspergillus niger* and *Trichoderma asperellum*.

DMSO is special solvent which can dissolve polar and nonpolar compounds, as the negative control that do not affect to antimicrobial result. This solvent was used widely in many studies about antibacterial method, for instance Nitiema *et al.* (2012) who used coumarin and quercetin against *E. coli* and *Salmonella*, Su *et al.* (2015) that used extract of *Polygonum cuspidatum* against *S. aureus.* In addition, DMSO is also the negative control for antifungals experiment such as *A. flavus, A. niger, C. albicans, etc.* (Usharani *et al.*, 2015) or *C. gloeosporioides* and *C. capsici* on chili (Chutrakul *et al.*, 2013).

Table 2. Antibacterial activities of extract of *Polygonum multiflorum* Thunb. root

Bacterial strains	Zone of inhibition (mm)							
	DMSO (5%)	Gentamycin (10 µg/disc)	Concentration of dryness extract (mg/mL)					
			50	100	200	400	800	1600
E. coli	-	19.67 ± 0.58^{d}	-	-	-	-	-	-
S. enteritidis	-	14 ± 1.00^{Bb}	-	-	-	8.67 ± 0.58^{Aa}	10 ± 1.00^{Aa}	12.67 ± 0.58^{B}
S. aureus	-	15.67 ± 1.15^{Cc}	-	-	8.33 ± 1.53^{A}	10.33 ± 1.15^{ABa}	11.67 ± 0.58^{Ba}	NT
B. subtilis	-	15.33 ± 0.58^{bc}	-	-	-	-	-	-
L. monocytogenes	-	11.33 ± 0.58^{a}	-	-	-	-	-	-

-: not detect, NT: not tested.
Various lowercase letters in the same column denote significant difference ($p<0.05$).
Various uppercase letters in the same row denote significant difference ($p<0.05$).

Table 3. Antifungal activities of extract of *Polygonum multiflorum* Thunb. root

Fungal strains	Zone of inhibition (mm)								
	DMSO (5%)	Ketoconazole (50 µg/disc)	Concentration of dryness extract (mg/mL)						
			25	50	100	200	400	800	1600
A. niger	-	14 ± 1^{a}	NT	NT	-	-	-	-	-
F. equiseti	-	25 ± 1^{b}	NT	NT	-	-	-	-	-
T. asperellum	-	26.67 ± 2.08^{Cb}	-	-	8.33 ± 0.58^{A}	$8.67\pm0,58^{A}$	11 ± 1^{B}	NT	NT

-: not detect, NT: not tested.
Various lowercase letters in the same column denote significant difference ($p<0.05$).
Various uppercase letters in the same row denote significant difference ($p<0.05$).

Besides, gentamicin used also as positive control and had clearly effective in five of bacteria. Concentration of gentamicin was quite low (10 µg/disc) but antibacterial capacity for each bacteria strain was very different. Inhibitor zone of positive control listed in susceptible order: *L. monocytogenes* < *S. enteritidis* < *B. subtilis* < *S. aureus* < *E. coli* (Figure 1). Gentamicin is antibiotic which belongs to aminoglycosides group, can prohibit protein synthesis and destroys bacteria cell membrane system. Gentamicin diffuse inside the periplasmic space and the transport across the cytoplasmic membrane requires metabolic energy from the electron transport system in an oxygen-dependent process. Then, gentamicin binds quickly bacteria ribosome and inhibitory protein synthesis process. It decreases the

exactly of information RNA that results in the wrong combination of amino acid in polypeptide chain of bacteria (Zembower *et al.*, 1998).

The antifungal mechanisms of ketoconazole that cause increased membrane permeability, inhibition of uptake of precursors of RNA, DNA and synthesis of peroxidative and oxidative enzymes. In addition, ketoconazole derivatives inhibit the biosynthesis of ergosterol, the main sterol in the membranes of fungi. The demethylation from lanosterol to ergosterol was blocked by ketoconazole. This lead to be leaky membranes, permeability changes and fungi were easily inhibited (Van-Tyle, 1984). The results show that ketoconazole (50 μg/disc) inhibited these fungi and the inhibitor zone of positive control listed in susceptible order: *A. niger* < *F. equiseti* < *T. asperellum* (Figure 2).

Table 2 and 3 show that dryness extract of *Polygonum multiflorum* Thunb. has antimicrobial activity against a gram-positive bacteria (*S. aureus*, MIC of 200 mg/mL and inhibition zone of 8.3±1.53 mm), a gram-negative bacteria (*S. enteritidis*, MIC of 400 mg/mL and inhibition zone 8.67±0.58 mm) and a fungus (*T. asperellum*, MIC of 100 mg/mL and inhibition zone 8.33±0.58 mm). Inhibition zones of *S. enteritidis*, *S. aureus* and *T. asperellum* were "sensitive" (inhibition zone from 8 to 14 mm), this results were evaluated similar with antimicrobial level of some essential oils (Ponce *et al.*, 2003). Antimicrobial effect increases with the increase of concentration of dryness extract and depends on many different factors, for instance the presence of flavonoids in plant is advantageous against bacterial pathogen and fungi because flavonoids can destroy the cell membrane (Ikigai *et al.*, 1993), reduce permeability of membrane (Tsuchiya *and* Iinuma, 2000) and inhibit the nucleic acid synthesis (Mori *et al.*, 1987). Each type of flavonoids can inhibit microorganism by many different pathways.

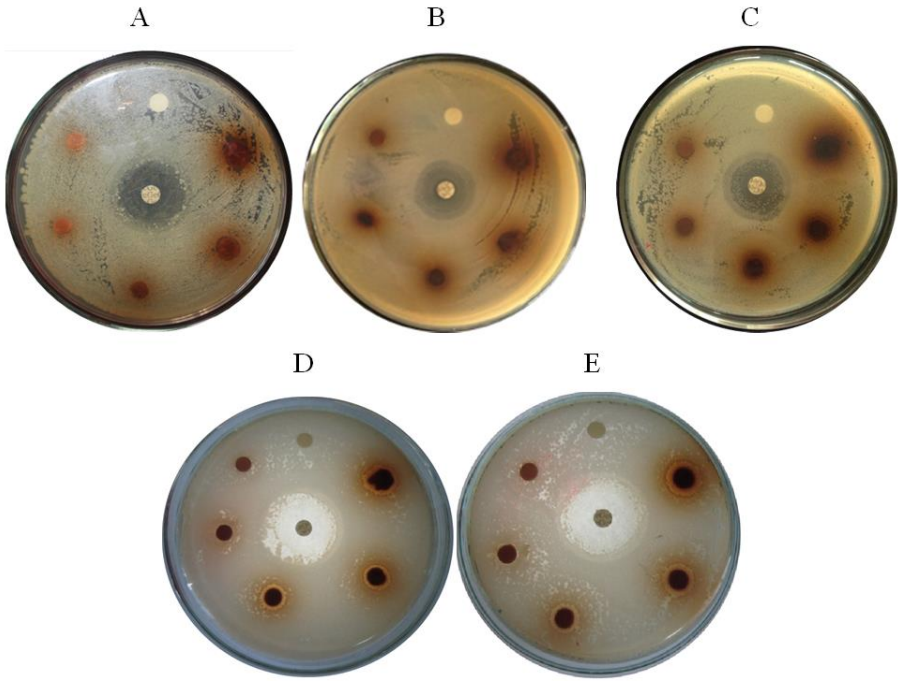

Figure 1. Inhibition zones for *E. coli* (A), *B. subtilis* (B), *L. monocytogenes* (C), *S. enteritidis* (D) and *S. aureus* (E).

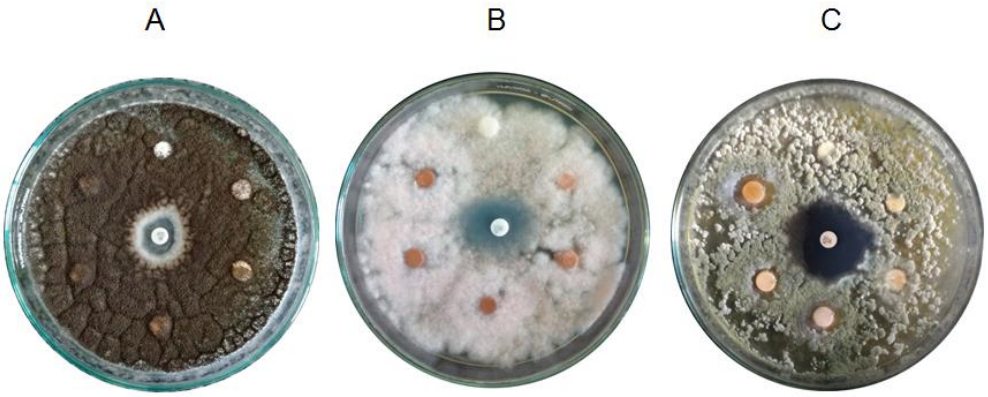

Figure 2. Inhibition zones for *A. niger* (A), *F. equiseti* (B), *T. asperellum* (C)

According to Scalbert (1991) and Rebecca *et al.* (2009), there are three hypothesis that might explain the antimicrobial mechanism of tannins on microorganism: inhibition of enzyme activity by complexing with substrates of bacteria and fungi; direct action of tannins on the microorganism metabolism, through the inhibition of oxidative phosphorylation; a mechanism involving the complexation of tannins with metabolic ions, decreasing the availability of essential ions to the metabolism of the microorganisms. Besides, tannins in extract also can inhibit gram-negative and gram-positive bacterial, especially condensed tannins (Zarin *et al.*, 2016). However, hydrolyzed tannins can inhibit some specific bacteria such as *S. aureus* (Lim *et al.*, 2006). In addition, some studies show that these compounds almost don't affect resistance of yeast and fungi because they have thick walled structure and high chitin content (Madigan *and* Martinko, 2006) but this results show that *T. asperellum* was inhibited by the present of tannins in extract. Besides, tannins in *Stryphnodendron adstringens* extract also inhibited *Candida albicans* (Santos *et al.*, 2009).

According to Lin *et al.* (2015), anthraquinones in *Polygonum multiflorum* Thunb. root include rhein, emodin, aloe-emodin, physcion, chrysophanol, etc. After entering the cell membrane, emodin will bind to DNA and destroy bacteria (Lu *et al.*, 2011). Furthermore, rhein, emodin and aloe-emodin also inhibit respiration of some bacteria, especially *S. aureus* (Chen *et al.*, 1963) and interfere in redox of enzyme NADH dehydrogenase in bacteria (Zhang *and* Chen, 1986). The present of anthraquinones in extract could be due to the leaks in the cell wall or perhaps some alteration in the membrane permeability, resulting in the loss of the cytoplasm and fungus were inhibited (Phongpaichit *et al.*, 2004).

Some bioactive compounds except polyphenols can inhibit microorganism such as saponins and alkaloids. These compounds also present in *Polygonum multiflorum* Thunb. root extract. Alkaloids can inhibit the synthesis of DNA, RNA and cellular respiration of microorganism (Aniszewski, 2007). There are many studies show that the bacteria which was inhibited by alkaloids in plant, for instance like *L. monocytogenes* and *S. typhimurium* that were inhibited by alkaloid extract of *Pangium edule* (Chye *and* Sim, 2009); *E. coli, S. aureus, B. cereus, S. carmonum,* etc. were sensitive with alkaloids extract of *Sida acuta* (Karou *et al.*, 2005). Alkaloids from *Lupinus luteus* L. extract can inhibit many fungi such as *Rhizoctonia solani, Phoma exigua,* etc. (Sas-Piotrowska *et al.*, 1996).

The presence of saponins also contributed significantly to the antibacterial activity of the extract. However, saponins affect strongly gram-positive bacteria more than gram-negative bacteria and fungi (Soetan *et al.*, 2006). Antimicrobial activity depends on structure of aglycon of saponins. The possible antimicrobial mechanism of saponins was due

to the reduced glucose utilization efficiency in microorganisms, then affecting their growth and proliferation, reducing the activity of key enzymes in physiological metabolism and suppressing the synthesis of relevant proteins, and finally executing the antibacterial effect (Yu *et al.*, 2013). Some bacteria was inhibited by saponins extract from plant, for instance *E. coli, S. aureus, K. pneumonia, B. subtilis* and *P. aeruginosa* were inhibited by saponins extract from *Anabasis articulate* (Maatalah *et al.*, 2012). Saponins from *Gymnema sylvestre* and *Eclipta prostrata* leaves extract inhibited *A. flavus, A. niger, A. fumigatus* (Gopiesh-Khanna *and* Kannabiran, 2008).

The obtained result shows that there are many bioactive compounds in the dryness extract. They can inhibit microorganism by many different mechanisms. Hence, combination of these compounds has the anti-microorganism effect better than single compound.

4. Conclusions

The presence of bioactive compounds was determined. The powdered root had many bioactive compounds such as alkaloids, saponins, tannins, flavonoids and anthraquinones. These components were quite precious compound to apply to medical technology and food industry. In addition, acetone dryness extract from *Polygonum multiflorum* Thunb. root proved to be effective against both one gram-positive bacteria (*Staphylococcus aureus*) at MIC of 200 mg/mL, one gram-negative bacteria (*Salmonella enteritidis*) at MIC of 400 mg/mL and a fungus (*Trichoderma asperellum*) at MIC of 100 mg/mL. Therefore, the result shows that this plant had antioxidant and antimicrobial potentials. It may be used as alternative natural sources applicable to medicine, agriculture and food products.

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