Investigation of Elemental Analysis and Antimicrobial Activity of *Ludwigia hyssopifolia* (G.Don) Exell (Taw-lay-nyin)

Shwe Ywet Wah[1], Khin Thandar Shwe[2], Nwe New Tun[3]

Abstract

In this research work, preliminary phytochemical constituents, elemental analysis and antimicrobial activity of the whole plant of *Ludwigia hyssopifolia* were carried out. It belongs to the family Onagraceae and Myanmar name is Taw-lay-nyin. It is a perennial herb. According to the preliminary phytochemical investigation; alkaloids, α-amino acids, carbohydrates, glycosides, flavonoids, phenolic compounds, reducing sugars, saponins, tannins, steroids and terpenoids were present by test tube tests. But starch, organic acids and cyanogenic glycosides were not found in the sample. From the results of elemental analysis of dried powder of *ludwigia hyssopifolia;* calcium (0.804 %), potassium (0.576 %), phosphorus (0.149 %) and sulphur (0.107 %) were relatively higher than other contents of this plant by Energy Dispersive X-Ray Fluorescence technique. Dried powder of *Ludwigia hyssopifolia* were more soluble in polar solvents; water and 95 % ethanol (16.0 % and 7.2 %) than non-polar solvents; pet-ether and ethyl acetate (2.0 % and 4.0 %). The antimicrobial activity was investigated by pet-ether, ethyl acetate, 95 % ethanol and watery extracts against six bacteria and two fungi species: *Escherichia coli*, *Bacillus subtilis*, *Bacillus pumilus*, *Pseudomonas fluorescens*, *Agrobacterium tumefaciens*, *Staphylococcus aureus*, *Candida albicans* and *Malasseiza furfur* using agar well diffusion method. The ethanol extract inhibited on all test microorganisms with inhibition zone diameters 14-26 mm. Watery extract showed a moderate antimicrobial activity with inhibition zone diameters 12-21 mm except *Staphylococcus aureus* and *Candida albicans*. Moreover, ethyl acetate extract moderately inhibited on five microorganisms with inhibition zone diameters 12-16 mm except *Pseudomonas fluorescens and Staphylococcus aureus*.

Keywords **:** *Ludwigia hyssopifolia* (G. Don) Exell, Phytochemicals, Elemental analysis, Antimicrobial activity

Introduction

The knowledge about the traditional use of medicinal plants has always explored the search for new therapeutic cures. The traditional systems of treatments with medicinal plants are often cheaper, locally available, simple medicinal preparations which bring out beneficial results. In this paper was described the elemental analysis and antimicrobial activity of the whole plants of *Ludwigia hyssopifolia* and it belongs to the family Onagraceae. It is erect aquatic or semi-aquatic annual herb, up to 2 (or) even 3 m high. English name of *Ludwigia hyssopifolia* is water primrose. It is also called Taw-lay-nyin in Myanmar. Leaves are alternate, lanceolate, up to 6.5-7.5 cm long, 1.2-2 cm wide, base decurrent to a short petiole; lateral nerves 11-17 pairs. Stem is narrowly winged, cylindric and woody below, upper stem ribbed. Flowers are solitary in leaf axils, sessile, usually four-partite, sometimes five-partite, Calyx lobes are 3-4 mm. Petals are bright yellow to orange-yellow, 2-4 mm long. Capsule pubescent is more or less cylindrical or swollen towards the apex, up to 30 mm long with many brown seeds about 0.5 mm long. In Myanmar, *Ludwigia hyssopifolia* plant is used sneeze or cough and diarrhea. This plant is used to treat fever, swelling, pain, infectious hepatitis, edema, anthelmintic,

[1] Demonstrator, Department of Chemistry, Hinthada University
[2] Professor and Head, Department of Chemistry, Taungoo University
[3] Demonstrator, Department of Chemistry, Hinthada University

carminative, diuretic, flatulence, leucorrhoea, spitting of blood and glands in the neck. A decoction is also used as a vermifuge and purgative. The plant exhibits a variety of biological activities including anticancer, antibacterial, antidiarrheal and anti-ulcer activities. This plant can be extracted black dye from the whole plant. *Ludwigia hyssopifolia* is widely distributed in wet places and rice growing areas of the country up to foothill areas to throughout of Asia, south Asia including Myanmar, Australia, and Pacific Islands (Barua, 2010). Das, *et al*., 2007 were reported that the presence of chemical constituents; piperine, palmitic acid, isovanillin, β-sitosterol, stigmasterol-3-O-β-D-glucopyranoside, gallic acid, ethyl gallate, oleanolic acid, 2,4,6-trihydroxybenzoic acid, ursolic acid, kaemferol, ginsenoside Rb_1, 6β, 24-hydroxy tormnetic acid, xanthyletin (+) trans-decursidinol, β-sitosterol-β-D-glucopyranoside, 6β, 23-hydroxy tormnetic acid in that plant. The aim of this research work is to study the elemental analysis and antimicrobial activity of *Ludwigia hyssopifolia* (G. Don) Exell (Taw-lay-nyin).

Figure 1 Photograph of the whole plant of *Ludwigia hyssopifolia* (G. Don) Exell (Taw-lay-nyin)

Calcium

Calcium is the most abundant mineral in the human body. Calcium has several main functions in the body such as several of the clotting factors in the blood plasma, which are functionless in the absence of calcium ions.

Potassium

Potassium is an essential mineral that has many roles in your body. It helps regulate muscle contractions, maintain healthy nerve function and regulate fluid balance. A low-potassium diet is rarely the cause of potassium deficiency, or hypokalemia.

Sulphur

Sulphur is the third most abundant mineral based on percentage of total body weight. It is essential for life. It is required for vital amino acids and to create protein for cells and tissues and for hormone, enzymes and antibodies.

Antimicrobial Activity

An antimicrobial is an agent that kills microorganisms or inhibits their growth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. Antibacterials are used to treat bacterial infections. The toxicity of humans and other animals from antibacterial is generally considered low. An antibiotic is a drug that kills or slows the growth of bacteria. Antibiotics are one class of antimicrobials, a large group which also includes anti-viral, anti-fungal and anti-parasitic drugs.

Materials and Methods

Sample Collection and Preparation

The whole plants of *Ludwigia hyssopifolia* were was collected from Pauk-Kone Village, Zalun Township, Ayeyarwady Region in December 2019. It was confirmed at the Department of Botany, Hinthada University. The whole plants of Taw-lay-nyin were washed with water and dried at room temperature. The collected

dried samples were ground into powder by grinder. Then, the dried powdered samples were stored in the air-tight containers to prevent the moisture and other contaminations.

Chemicals

Petroleum ether, ethyl acetate, 95 % ethanol, glucose, yeast , peptone, concentrated hydrochloric acid, concentrated sulphuric acid, sodium hydroxide, distilled water, Dragendorff's, Wagner's, Mayer's, sodium picrate, α-naphthol, Bromocresol green, lead acetate, potassium ferricyanide, Benedict's reagent, iodine, acetic anhydride and gelatin

Apparatus

Energy dispersive X-ray Fluorescence Spectrometer **(**Shimadzu's EDX-7000/8000), conical flasks, test tubes, beakers, measuring cylinder, funnels, glass tube, glass rod, electric balance, thermometer, watch clock, filter paper, water bath, porcelain basin, autocleaved, petridishes, cork borer and digital caliper.

Procedure

Preliminary Phytochemical of *Ludwigia hyssopifolia*

In order to know the preliminary phytochemical constituents such as alkaloids, α-amino acids, carbohydrates, cyanogenic glycosides, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, starch, steroids, organic acids, tannins and terpenoids present in the whole plant of *Ludwigia hyssopifolia* were carried out according to the test tube methods.

Quantitatively Elemental Analysis of *Ludwigia hyssopifolia* by Energy Dispersive X-ray Florescence (EDXRF) Spectrometry

For this analysis, pellets of the dried powder sample were first made and measured by Energy Dispersive X-ray Fluorescence (EDXRF) Spectrometer (Shimadzu's EDX-7000/8000) at the Universities Research Centre (URC), Monywa University, Sagaing Region. EDXRF Spectrometer can analyze the elements from Na to U under vacuum condition. X-ray fluorescence uses X-rays to excite an unknown sample. The individual elements in each sample are detected by using semiconductor (Si-Li) that permits multi-elements, simultaneous analysis.

Extraction of Dried Powder of *Ludwigia hyssopifolia*

To determine the amount of soluble organic constituents in various solvents; petroleum ether, ethyl acetate, 95 % ethanol and water were carried out by direct extraction method**.** In that experiment, 25 g of dried powdered sample was separately extracted with 75 mL of each of solvents for 6 hours and then the filtrates were filtered. The filtrate was placed in a weighed porcelain basin and evaporated to dryness on a water-bath until it was completely dried. After dried, the extract with the basin were weighed. Percentages of extracted materials were calculated by getting the amount of dried extract by abstracting weight.

Screening of Antimicrobial Activity of Various Crude Extracts of *Ludwigia hyssopifolia*

Antimicrobial activity of crude extracts; petroleum ether, ethyl acetate, ethanol and watery extracts from the whole plant of *Ludwigia hyssopifolia* were determined by using Agar-well diffusion method. Test microorganisms: *Escherichia coli* (AHU 5436), *Bacillus subtilis* (IFO 90571), *Bacillus pumilus* (IFO 12092), *Pseudomonas fluorescens* (IFO 94307), *Agrobacterium tumefaciens* (NITE 09678), *Staphylococcus aureus* (AHU 8465), *Candida albicans* (NITE 09542) and *Malassezia furfur* (AUV 0255) which are supported from Microbiology Laboratory Department of Biotechnology Development Center, Pathein University. This experiment was conducted at the Department of Chemistry, Hinthada University.

In this experiment, Glucose (0.5 g), peptone (0.3 g) and yeast extract (0.3 g) were mixed with distilled water and the solution was made up to 100 mL with distilled water. The pH of this solution was adjusted at 6.5 with 0.1 M HCl and 0.1 M NaOH solution and then 1.5 g of agar was added. The nutrient agar medium was put into sterilized conical flask and plugged with cotton wool and then autocleaved at 121 ^{o}C for 15 minutes. After cool down to 40 ^{o}C, 3 drops of suspended strain was inoculated to the nutrient agar medium was poured into the sterilized petridishes and left 10-15 mins in order to set the agar. After that the agar wells were made with a 8 mm sterilized cork borer and the wells filled with 0.2 mL of each extract sample to be tested. Finally, the plates were incubated at 27 ^{o}C for 24-48 hours in incubator. After incubation, the diameters of inhibition zone (ID.) including 8 mm wells were measured with digital caliper.

Results and Discussion

Preliminary Phytochemical Investigation of *Ludwigia hyssopifolia* (Taw-lay-nyin)

The results of the preliminary phytochemical constituents were presented in Table 1, it was observed that alkaloids, α-amino acids, carbohydrates, glycosides, reducing sugars, saponins, phenolic compounds, flavonoids, tannins, steroids and terpenoids were present in the whole plant of *Ludwigia hyssopifolia*. But starch, organic acids and cyanogenic glycosides were not detected by test tube test. According to the results data, the mostly important phytochemical constituents were present in that whole plant. That is consistently with biological activities of that plant.

Table 1 Results of Preliminary Phytochemical Test of *Ludwigia hyssopifolia*

No.	Types ofcompounds	Extracts	Reagent	*Ludwigia hyssopifolia* Observation	Remarks
			Dragendorff's	Orange ppt	+
			Wagner's	Reddish Brown ppt	+
1.	Alkaloids	1 % HCl	Sodium picrate	Yellow ppt	+
			Mayer's	White ppt	+
2.	α-amino acids	H_2O	Ninhydrin	Purple colour	+
3.	Carbohydrates	H_2O	10 % α-naphthol conc: H_2SO_4	Red ring	+
4.	Cyanogenic Glycosides	H_2O	Sodium picrate solution	No brick-red colour	-
5.	Flavonids	95 % EtOH	dil: NaOH & dil: HCl	Yellow colour	+
6.	Glycosides	H_2O	10% lead acetate	White ppt	+
7.	Organic acids	H_2O	Bromocresol green Indicator	No green colour	-
8.	Phenolic Compounds	H_2O	5 % $FeCl_3$ and 1 % potassium ferricyanide	Dark blue colour	+
9.	Reducing Sugars	H_2O	Benedict's reagent	Green colour	+
10.	Saponins	H_2O	Distilled water	Marked Frothing	+
11.	Starch	H_2O	Iodine solution	No blue colour	-
12.	Steroids	PE	Acetic anhydride and conc: H_2SO_4	Green colour	+
13.	Tannins	95 % EtOH	1 % Gelatine and 5 % $FeCl_3$	Green colour	+
14.	Terpenoids	$CHCl_3$	Acetic anhydride and conc: H_2SO_4	Red colour	+

(+) = presence, (-) = absence, ppt = precipitate

Qualitatively Elemental Analysis of the Whole Plant of *Ludwigia hyssopifolia*

In this work, relative abundance of elements present in the whole plant of were *Ludwigia hyssopifolia* determined by EDXRF spectrometer. According to EDXRF spectrum of the dried powder of *Ludwigia hyssopifolia*, it was showed that the relative abundance of calcium (0.804 %), potassium (0.576 %), phosphorus (0.149 %) and sulphur (0.107 %) were more higher than other respective elements see in Figure 2 and Table 2. A trace amount of silicon (0.085 %), manganese (0.070 %), iron (0.018 %), titanium (0.002 %), zinc (0.002 %), copper (0.002 %) and strontium (0.001 %) were present in this sample.

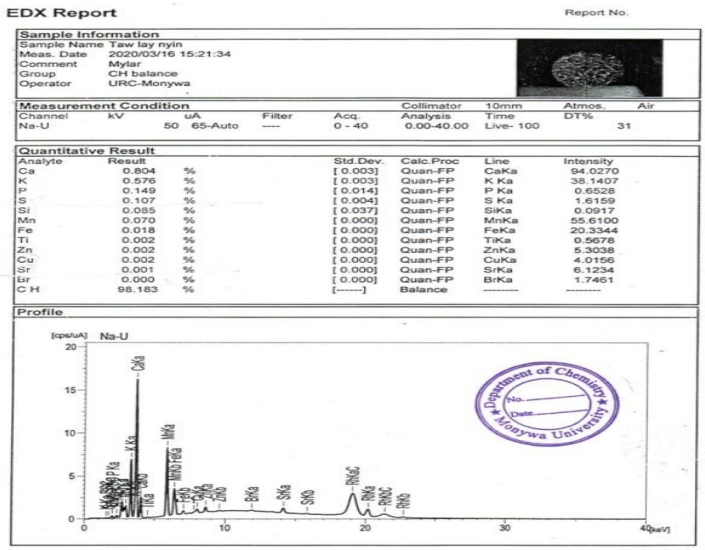

Figure 2 EDXRF spectrum of the whole plant of *Ludwigia hyssopifolia*

Table 2 Relative Abundance of Some Elements in the Whole Plant of *Ludwigia hyssopifolia*

No.	Elements	Relative Abundance (%)
1.	Calcium (Ca)	0.804
2.	Potassium (K)	0.576
3.	Phosphorous (P)	0.149
4.	Sulphur (S)	0.107
5.	Silicon (Si)	0.085
6.	Manganese (Mn)	0.070
7.	Iron (Fe)	0.018
8.	Titanium (Ti)	0.002
9.	Zinc (Zn)	0.002
10.	Copper (Cu)	0.002
11.	Strontium (Sr)	0.001
12.	Hydrocarbon (CH)	98.183

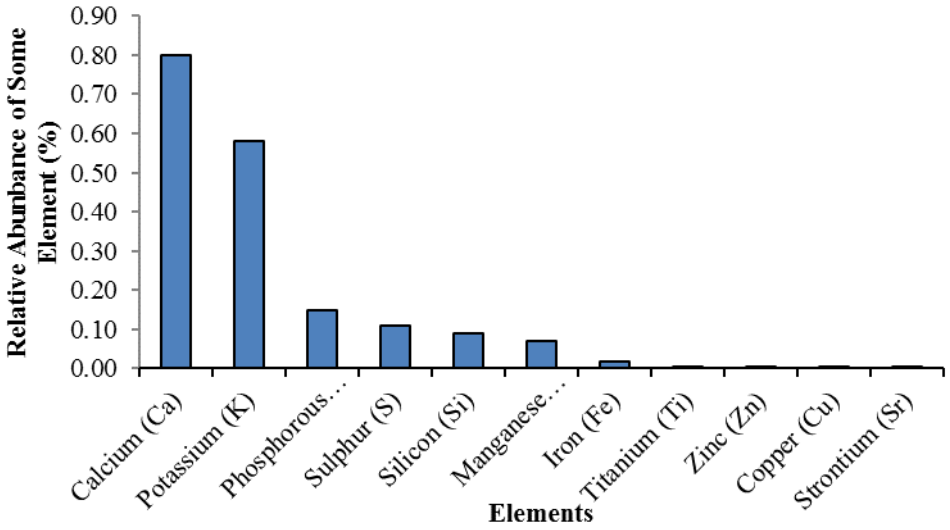

Figure 3 Histogram of relative abundance of some elements in the whole plant of *Ludwigia hyssopifolia*

Extraction of Crude *Ludwigia hyssopifolia* Extracts

In this research work, the various crude extracts; pet-ether, ethyl acetate, 95 % ethanol and water extracts of dried powder of *Ludwigia hyssopifolia* were done by direct solvent extraction. From the results, it was observed that the amounts of polar phytochemical constituents; ethanol and water extracts were higher than nonpolar constituents; petroleum ether and ethyl acetate extracts in *Ludwigia hyssopifolia* see in Table 3. From these result data, this plant may be concluded that the high polarity phytochemical constituents are more soluble than nonpolar phytochemical constituents.

Table 3 Yield Percent of Extracted Amount of *Ludwigia hyssopifolia*

No	Solvent used in Extraction	Weight of powdered sample (g)	Weight of extract (g)	Yield (%)
1	Petroleum ether (PE)	25	0.5	2.0
2	Ethyl acetate (EtOAc)	25	1.0	4.0
3	Ethanol (EtOH)	25	1.8	7.2
4	Water (H_2O)	25	4.0	16.0

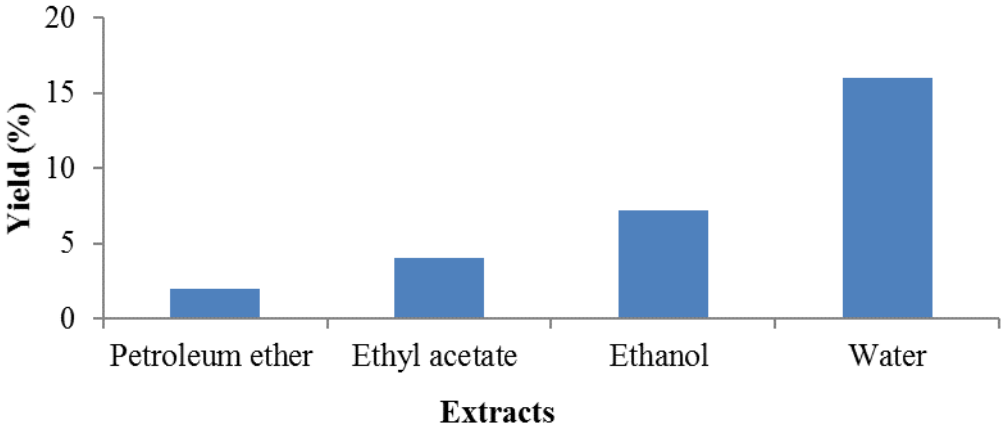

Figure 4 A bargraph of relative abundance of elements in the whole plant of *Ludwigia hyssopifolia*

Antimicrobial Activity of *Ludwigia hyssopifolia*

In the screening of antimicrobial activity by agar well diffusion method, petroleum ether extract of *Ludwigia hyssopifolia* was not inhibited on all tested microorganisms. Ethyl acetate extract of *Ludwigia hyssopifolia* showed a moderate antimicrobial activity against six microorganisms with inhibition zone diameter ranged in 12 mm to 16 mm except *Pseudomonas fluoresens* and *Staphylococcus aureus*. Ethanol extract of *Ludwigia hyssopifolia* with inhibition zone 23 mm to 26 mm is the most significant against growth of *Escherichia coli*, *Bacillus subtilis*, *Agrobacterium tumefaciens* and *Malassezia furfur* compare with standard control chloramphenicol with inhibition zone 22 mm to 24 mm. Besides, this extract exhibited moderate against on growth of *Bacillus pumilus, Pseudomonas fluorescens, Staphylococcus aureus* and *Candida abicans* with inhibition zone diameter ranged in 14 mm to 26 mm. In addition, watery extract of *Ludwigia hyssopifolia* showed a moderate antimicrobial activity against *Escherichia coli, Bacillus subtilis, Bacillus pumilus, Pseudomonas fluorescens, Agrobacterium tumefaciens* and *Malassezia furfur* with inhibition zone diameter ranged in 12 mm to 21 mm and there was no inhibition zone for *Staphylococcus aureus* and *Candida albicans see* in Table 4 and Figure 5. From the results data, ethanol and watery extracts may be used for the treatment of those microorganisms related diseases.

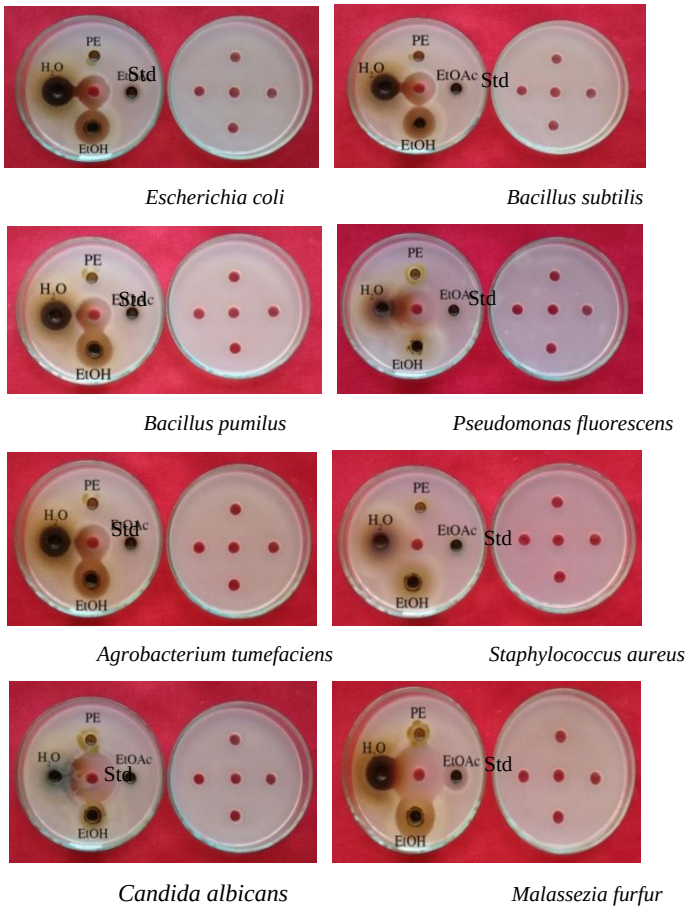

Figure 5 The images of inhibition zones of various crude extracts against eight microorganisms

Std - Standard (Chloramphenicol) H_2O - watery extract
PE - petroleum ether extract EtOH - ethanol extract
EtOAc - ethyl acetate extract

Table 4 Inhibition Zone Diameters of Various Crude Extracts of *Ludwigia hyssopifolia* (Taw-lay-nyin) Against Eight Microorganisms in Agar Well Diffusion Method

No.	Microorganisms	Inhibition zone diameter (mm)				
		PE extract	EtOAc extract	EtOH extract	H_2O extract	Standard of Chloramphenicol
1.	*Escherichia coli*	-	12	23	20	22
2.	*Bacillus subtilis*	-	12	24	20	22
3.	*Bacillus pumilus*	-	12	24	20	37
4.	*Pseudomonas fluorescens*	-	-	19	12	23
5.	*Agrobacterium tumefaciens*	-	12	25	21	23
6.	*Staphylococcus aureus*	-	-	14	-	-
7.	*Candida albicans*	-	13	18	-	30
8.	*Malassezia furfur*	-	16	26	21	24

Agar well - 8 mm

9 mm ~ 14 mm (+) low activity

15 mm ~ 19 mm (++) moderate activity

20 mm above (+++) highest activity

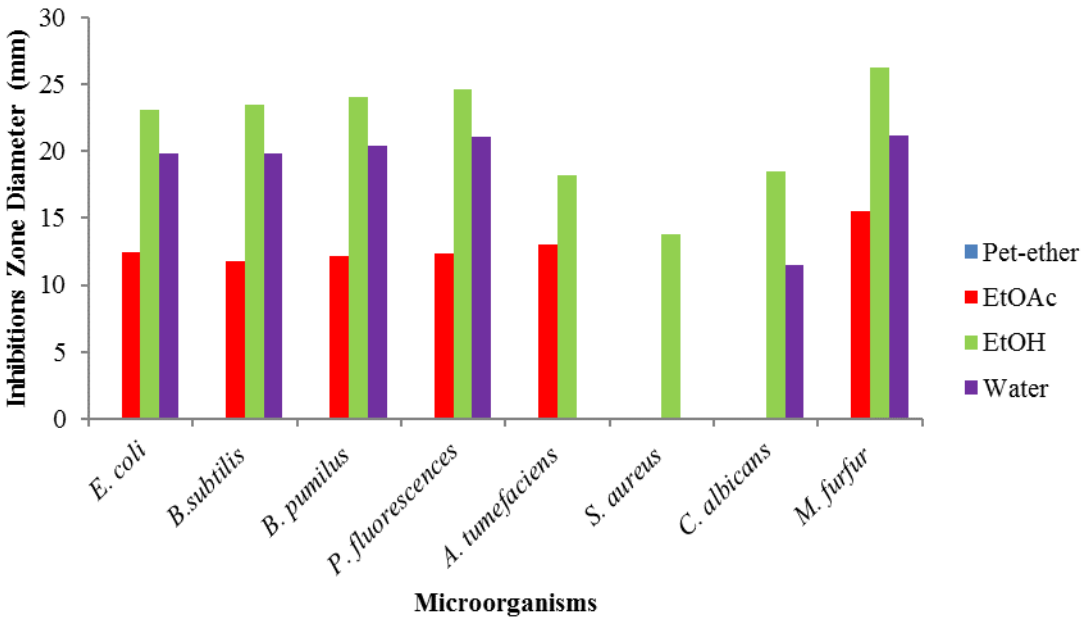

Figure 6 Comparison of inhibition zone diameters of various crude extracts of *Ludwigia hyssopifolia* against eight microorganisms by agar well diffusion methods

Conclusion

The preliminary phytochemicals in *Ludwigia hyssopifolia*; alkaloids, α-amino acids, carbohydrates, glycosides, flavonoids, phenolic compounds, reducing sugars, saponins, tannins, steroids and terpenoids were found out. But starch, organic acids and cyanogenic glycosides were not detected by test tube test. According to the EDXRF elemental analysis, the percent of relative abundance of calcium (0.804 %), potassium (0.576 %), phosphorus (0.149 %) and sulphur (0.107 %) were higher than other related element of that plant. *Ludwigia hyssopifolia* plant may be observed that the high polarity phytochemical constituents are more soluble than nonpolar phytochemical constituents. The antimicrobial activity of various extracts; ethanol extract is significantly inhibited against on all test microorganisms (ID.14-26 mm), watery extracts is inhibited on six microorganisms (ID. 12-21 mm) exception of *Staphylococcus aureus* and *Candida albicans*, and ethyl acetate extract showed a moderate antimicrobial activity against six microorganisms (ID. 12-16 mm) exception of *Pseudomonas fluorescens* and *Staphylococcus aureus* were investigated. Especially, since the all three extracts were inhibited on *Malassezia furfur* (ID. 16-26 mm), this may be used for the treatment of related that fungi diseases such as dermatitis, danfruff, fungemia and pneumonia.

Acknowledgements

The authors would like to express their profound gratitude to Rector, Dr. Theingi Shwe and Pro-Rectors Dr. Yee Yee Than and Dr. Aye Lwin, Hinthada University for their permission. And also express appreciation to Professor and Head, Dr. Tin Htwe Mu and Professors Dr. Moe Ohmmar and Dr. Yin Yin Myint, Department of Chemistry, Hinthada University for encouragement to do this research work.

References

Achenabch, H. and W.J.Fietz. (1986). "Investigation of the Constituents of Piper Amalago-30 Neco Amids of the Piperine-type". *Planta Med*, **1**, 12-18

Ball, G.steven and K.M.Matthew. (2003). "From Bacterial Glycogen to Starch, Understanding the Biologeneis of the Plant Starch Granule*". Annual Review of Plant Biology*, **54**, 207-233

Barua, I. (2010). "The Genus *Ludwigia* (Onagraceae) in India". *Rheedea*, **20**(1), 59-70

Collin, C.H. (2004). *Microbiology Methods*. London: 8th Ed. Butter worth and Co., Publisher Ltd.,

Cruickshank, R., J.P. Duguid., B.P. Marminor and R.H.A. Swain. (1995). *Medicinal Microbiology*. Lodon, 12th Ed, Churchill Living Stone Ltd., **11**

Das, B., J.Kundu., S.C. Bachar., M.A.Uddin and J.K.Kundu. (2007). "Antitumor and Antibacterial Activity of Ethylacetate Extract of *Ludwigia hyssopifolia Linn* and Its Active Principle Piperine". *Pak.J.Pharm. Sci.*, **20** (2), 128-131.