Phytochemical and Antimicrobial Studies of *Ludwigia hyssopifolia* (G.Don) E*x*ell

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Abstract

Ludwigia hyssopifolia (G.Don) E*x*ell. is annual herb belongs to the family Onagraceae in the order Myrtales. It is locally known as Taw-lay-hnyin. This plant was collected from Hinthada University Campus, Hinthada Township, Ayeyarwaddy Region. The morphological characters of vegetative and reproductive parts of the fresh specimens were investigated with the help of available literatures. The preliminary phytochemical tests were conducted from the powdered sample of the whole plant. The presence of alkaloid, phenolic compound, flavonoid, terpenoid, starch, reducing sugar, glycoside, saponin, tannin, α-amino acid and carbohydrate were found in the e*x*amination. Elemental analysis of powdered sample was examined by using EDXRF spectrometer. Percentage of calcium was higher than the other elements.Antimicrobial activities of various crude extracts were carried out by using paper disc diffusion assay with four test organisms. The methanolic extracts e*x*hibited the highest against on different pathogenic microorganisms. These results showed that *Ludwigia hyssopifolia* (G.Don) E*x*ell is endowed with many active constituents, minerals and antimicrobial properties that can be found useful in medicinal activities as well as possible application in nutrition and pharmacology.

Key words : *Ludwigia hyssopifolia* (G.Don) E*x*ell, phytochemical tests, antimicrobial activity

Introduction

Ludwigia hyssopifolia (G.Don) E*x*ell. is commonly known as Taw-lay-hnyin in Myanmar and water primrose in English name. It is one of the traditionally used medicinal plants in Myanmar. It is distributed all over the world, particularly in both temperate and tropical regions of the world (Valkenburg, 2002).

The plant is useful in carminative, diuretic, anthelmintic, diarrhoea and dysentery. A poultice of the plant is used to treat pimples, boils and other infections. The leaves are used in a febrifuge decoction. A cold infusion of the roots is used to treat syphilis (Sujanapal, 2016).The leaf sap is also taken orally to stave off threatened abortion, flatulence and constipation (Kirtikar and Basu, 1987).

The objectives of this study are to identify and confirm the morphological characters, to e*x*amine the phytochemical properties, to determine the elemental analysis and to evaluate the antimicrobial activity of *Ludwigia hyssopifolia* (G.Don) E*x*ell.

Materials and Methods

Collection and identification

The specimens of *Ludwigia hyssopifolia* (G.Don) E*x*ell. were collected from Hinthada University Campus, Hinthada Township, Ayeyarwaddy Region in Myanmar, especially during the flowering and fruiting periods from June, 2017 to February, 2018. After the collection, the plants were identified with the help of available literatures Backer, 1963; Hooker, 1973; Dassanayake, 1995; Hong Kong Herbarium, 2008 and Sujanapal, 2016.

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Preliminary phytochemical investigation of *Ludwigia hyssopifoli*a (G.Don) E*x*ell.

The preliminary phytochemical investigation on powdered sample were carried out to determine the presence or absence of the chemical constituents such as alkaloid, phenolic compound, flavonoid, terpenoid, starch, reducing sugar, glycoside, saponin, tannin, α - amino acid and carbohydrate according to the method of British Pharmacopoeia, 1968; Marini Bettolo, 1981; Central Council for Research in Unani Medicine, 1987; Trease and Evans, 2002 and Harborne, 2005, at Department of Botany, Hinthada University.

Elemental Analysis of *Ludwigia hyssopifolia* (G.Don) E*x*ell

The relative concentration of elements in powdered sample of *Ludwigia hyssopifolia* (G.Don) E*x*ell. was analyzed by using Energy Dispersive X-Ray Fluorescence (EDXRF) spectrometer (EDXRF 700, Shimadzu) technique at the Universities Research Center, Yangon.

Antimicrobial activity of various solvent e*x*tracts from *Ludwigia hyssopifolia* (G.Don) E*x*ell.

Various crude extracts of the plant such as acetone, chloroform, ethyl acetate, ethanol, methanol, petroleum ether and distilled water extracts were used for antimicrobial study. Screening of antimicrobial activity was done by paper disc diffusion technique according to Madigan and Martinko (2005), at Department of Botany, Hinthada University. The four test organisms (three bacterial strains and one fungal strain) were utilized for antimicrobial activity.

Preparation of antimicrobial activity test

Paper disc diffusion method was used according to the method described by Madigan and Martinko, 2005. The assay medium (agar 1.8 g, sucrose 1.0g, NaCl 0.1 g, yeast e*x*tract 0.3g, distilled water 100 ml, pH 7.0) was utilized for these test organisms. Test organisms (50µl) was added to 100 ml assay medium, and then poured into plates. After solidification, about 0.2ml of crude e*x*tracts was impregnated onto the paper disc with the size of 6mm diameter and 0.8mm thickness on the test agar plates and these plates were incubated for 24-48 hours at 30°C. After 24-48 hours, clear zones (inhibitory zones) surrounding the test discs were measured. These zones indicate the presence of the bioactive compounds which inhibit the growth of test organisms.

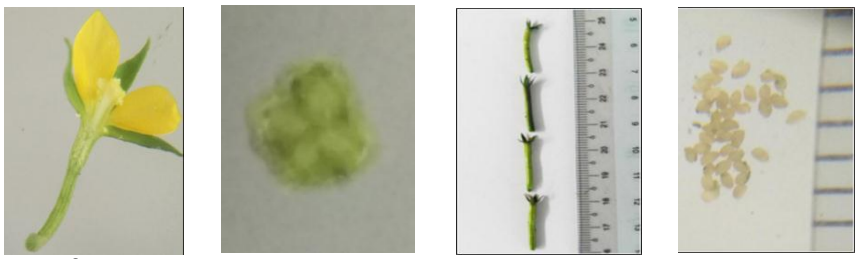
Results

Morphological characters

Annual herbs, about 5-300cm high, erect, much branched, stem upper portion quadrangular. Leaves simple, alternate, elliptic-lanceolate, 1.0-10.0cm long and 0.5-3.0cm wide, the bases attenuate, the margins entire, the tips acuminate, stipulate, petioles short, 3.0mm long. Inflorescences are a*x*illary and solitary cymes. Flowers are yellow, bracteate, bracteolate, subsessile, complete, bise*x*ual, actinomorphic, tetra merous, cyclic, epigynous. Caly*x* lobe 4 (2-4mm long) aposepalous, valvate, sepaloid, persistent, calyx tube not produced above the ovary. Petals 4, apopetalous, valvate, petaloid (yellow) 3-5 mm long, 2mm wide, ovate-elliptic, stamens 4+4, biseriate, apostemonous, filaments unequal, anther dithecous,

dorsifixed, longitudinal dehiscence. Ovary(4), syncarpous, tetralocular, axile placentation, one ovule in the locule, style terminal stout (1-1.5mm), stigma capitate. Capsule cylindrical terete (1.5-3cm long), subsessile. Seeds obovoid, numerous.

Inflorescence



L.S of Flower T.S of Ovary

Fig.(1) Morphological characters of *Ludwigia hyssopifolia* (G.Don) E*x*ell.

Preliminary phytochemical investigation of *Ludwigia hyssopifolia* (G.Don) E*x*ell.

The preliminary phytochemical test of powdered sample of *Ludwigia hyssopifolia* (G.Don) E*x*ell. indicated that the presence of alkaloid, phenolic compound, flavonoid, terpenoid, starch, reducing sugar, glycoside, saponin, tannin, α–amino acid and carbohydrates. The results were shown in Table (1).

Table 1. Preliminary phytochemical investigation of *Ludwigia hyssopifolia* (G.Don) E*x*ell.

No	Tests	Extract	Test Reagents	Observation	Results
1	Alkaloid	1% HCL	1.Dragendroff's reagent 2.Mayer's reagent 3.Wagner's reagent	Black ppt White ppt Black ppt	+ + +
2	Phenolic compound	1% HCL	3% Ferric Chloride	Brown	+
3	Flavonoid	EtOH	Conc: HCL & Mg	Greenish brown	+
4	Terpenoid	MeOH	H_2SO_4	Bluish green	+
5	Starch	H_2O	Iodine solution	Bluish black	+
6	Reducing sugar	H_2O	Benedict's solution	Brick red	+
7	Glycoside	H_2O	10%leadacetate solution	White ppt	+
8	Saponin	H_2O	Distilled water	Frothing	+
9	Tannin	H_2O	3% Ferric chloride	Black	+

			solution		
10	α-amino acid	H_2O	Ninhydrin reagent	Purple	+
11	Carbohydrate	H_2O	10% α napthol and conc: H_2SO_4	Red ring	+

(+) = Present

Elemental Analysis of *Ludwigia hyssopifolia* (G.Don) Exell

The content element in powdered sample of the plant was quantitatively determined by using EDXRF (Energy Dispersive X-ray Fluorescence spectrometer). It was found that Calcium (Ca), Potassium (K), Chloride (Cl), Iron (Fe), Manganese (Mn) and Sulphur (S) were found as mineral macronutrients. Zinc (Zn), Titanium (Ti), Strontium (Sr) and Copper (Cu) were found as micronutrients. Among these elements Calcium and Potassium were abundant but Zinc, Titanium, Strontium and Copper were small amount in this plant. The results were shown in Table (2), Figure (2) and (3).

Table 2. Relative concentration of elements from powdered sample of *Ludwigia hyssopifolia* (G.Don) Exell.

No.	Elements	Content in %
1	Calcium (Ca)	40.230
2	Potassium (K)	34.128
3	Chloride (Cl)	11.417
4	Iron (Fe)	6.603
5	Manganese (Mn)	4.403
6	Sulphur (S)	1.782
7	Zinc (Zn)	0.450
8	Titanium (Ti)	0.439
9	Strontium (Sr)	0.350
10	Copper (Cu)	0.198

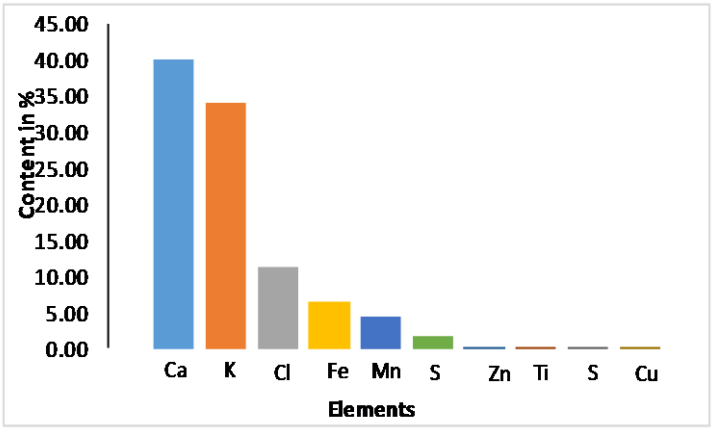


Fig. (2) Relative concentration of elements from powdered sample of *Ludwigia hyssopifolia* (G.Don) E*x*ell

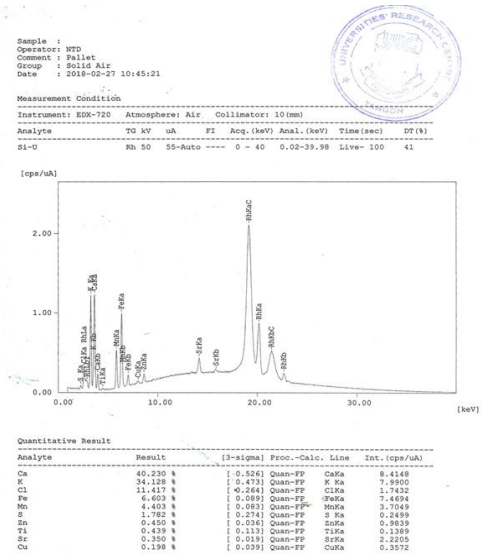


Fig. (3) Relative concentration of elements from powdered sample of *Ludwigia hyssopifolia* (G.Don) E*x*ell.

Antimicrobial activity of *Ludwigia hyssopifolia* (G.Don) E*x*ell.

In this investigation, acetone, chloroform, ethanol, methanol and distilled water extracts observed against on *Aspergillus flavus, Bacillus subtilis, Escherichia coli and Pseudomonas fluorescens*. Ethyl acetate and petroleum ether extract did not show antimicrobial activity on all tested organisms. The results were shown in Table (3) and Figure (4).

Table 3. Antimicrobial activity of different solvent e*x*tracts from *Ludwigia hyssopifolia* (G.Don) E*x*ell.

Solvents / Test organism	Acetone	$CHCl_3$	EtOAc	EtOH	MeOH	PE	DW
Aspergillusflavus	8mm	11mm	-	19mm	21mm	-	18mm
Bacillus subtilis	8mm	11mm	-	23mm	24mm	-	18mm
Escherichia coli	8mm	11mm	-	18mm	20mm	-	18mm
Pseudomonas fluorescens	8mm	11mm	-	21mm	24mm	-	20mm

Paper disc size 6mm

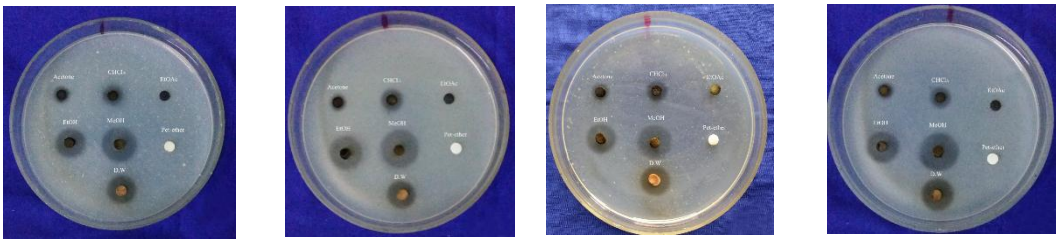


Fig. (4) Antimicrobial activity of various solvent extracts from *Ludwigiahyssopifolia* (G.Don)E*x*ell

Discussion and Conclusion

In this research, the morphological characters, preliminary phytochemical tests,elemental analysis and antimicrobial activities on the powdered sample of the plant *Ludwigia hyssopifolia* (G.Don) E*x*ell. had been undertaken.

In morphological study, the plants are annual herb, stem upper portion quadrangular. The leaves are simple, alternate and lamina elliptic-lanceolate. The inflorescences are a*x*illary and solitary cymes. The flowers are yellow, caly*x* lobe 4 and caly*x* tube not produced above the ovary. Stamens 4+4, biseriate and unequal. Ovary inferior and four locular. The fruits are Capsule and terete. The seeds are many, obovoid, dimorphic, free above and embedded in corky endocarp below. These characters are in agreement with those mentioned by Backer, 1963; Hooker, 1973; Dassanayake, 1995; Hong Kong herbarium, 2008 and Sujanapal, 2016.

The preliminary phytochemical screening of the plant *Ludwigia hyssopifolia* (G.Don) E*x*ell. revealed the presence of alkaloid, phenolic compound, flavonoid, terpenoid, starch, reducing sugar, glycoside, saponin, tannin, α-amino acid and carbohydrate. The active principles could be responsible for the antimicrobial properties.

In the elemental analysis, calcium, potassium, chloride, iron, manganese and sulphur were found as mineral macronutrients, whereas zinc, titanium, strontium and copper were found as micronutrietns. Calcium was most abundant in powdered sample. According to the literature, calcium is the main constituent for bones and teeth and it has keys metabolic functions. It also assists the functions of nervous system and muscles. The health benefit of calcium is prevention colon cancer and reduction of obesity.

The antimicrobial activities of various crude e*x*tracts (acetone, chloroform, ethyl acetate, ethanol, methanol, petroleum ether and distilled water) were determined by using paper disc diffusion method with four test microorganisms. These test organisms were *Aspergillus flavus, Bacillus subtilis, Escherichia coli* and *Pseudomonas fluorescens*. In this result, acetone, chloroform, ethanol, methanol and distilled water e*x*tracts showed antimicrobial activity against on all test organisms. The ethyl acetate and petroleum ether extracts did not actively inhibit the growth of the test organisms. The methanolic e*x*tract showed the strongest antimicrobial activity among the seven e*x*tracts against the three bacterial strains and one fungal strain. This indicates that active principles which inhibits the growth of microorganisms tested may dissolve better in methanol than the other solvents. This is also suggests that methanol crude extract possesses the strongest antimicrobial property that could be used in the formulation of medicinal treatment of related diseases. The results of this study indicated *Ludwigia hyssopifolia* (G.Don) E*x*ell. to have potential antimicrobial activities that could be used as a scientific evidence to support traditional usage of this plant.

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