STUDIES ON PHYTOCHEMICALS SCREENING, ANTIMICROBIAL ACTIVITIES AND ANTIOXIDANT ACTIVITIES FROM THE ROOTS OF *GIRARDINIA HETEROPHYLLA* DENE*

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ABSTRACT

The roots of *Girardinia heterophylla* Dene. were selected for this research. Phytochemical screening was done by means of standard method. Antimicrobial activities of the crude extracts of the sample were tested by Agar-well diffusion method on six selected organisms. The antioxidant activity of ethanol extract of the roots of *Girardinia heterophylla* Dene. was determined by using DPPH assay. From the phytochemical tests of the crude extracts, the sample contains many chemical constituents such as alkaloid, flavonoid, terpenoid, free sugar, polyphenol and glycoside respectively. The antimicrobial activities of the crude extracts of the sample were tested by Agar well diffusion method on six selected microorganisms such as *Bacillus subtilis, Staphylococcus aureus, Pseudomas aeruginosa, Bacillus pumilus, Candida albicans* and *E.coli*. The results of the antimicrobial activity showed that all the crude extracts of the plant possesses antibacterial activity.

Keywords : *Girardinia heterophylla*, roots extract, antibacterial activity, antifungal activity.

1. INTRODUCTION

Herbal medicine is the oldest form of healthcare known to medicine. The plants provided food, clothing, shelter and medicine. Much of the medicinal use of plants seems to have been developed through observations of wild animals. Plants and plant extracts have been used as a major source of medicines since ancient time and that is why a large number of drugs are de-rived from different kinds of plants in the world.[1] Many drugs commonly used today are of herbal origin. About 25 percent of the prescription drugs dispensed in the United States contain at least one active ingredient derived from plant material. Some are made from plant extracts; others are synthesized to mimic a natural plant compound.

Research on locally-derived natural products such as medicinal remedies and essential oils has great potential to contribute to substainable livelihoods by providing new opportunities for income and ensuring access to affordable and effective medicines for common ailments.[4,5] The World Health Organization (WHO) has compiled a list of more than 21,000 plant species supposedly used in south-east Asia. Meanwhile WHO estimated that about 74 percent of the 121 bio-active plant-derived drugs are presently in use world wide. [6] Many herbs are known to possess antimicrobial properties. The plant "*Girardinia"* is used to treat many diseases. Traditional treatments may provide valuable clues for the development of new oral hypo-glycemic agents and simple dietary adjuncts, as one of the most important plant *Girardinia heterophylla*, Dene. Many patients have been benefited by this herb. Hence the antimicrobial property of the medicine was investigated.

Antioxidants are widely use as ingredients in dietary supplements in the hope of maintaining health and preventing diseases such as cancer and coronary heart disease. In addition, to these uses in medicine, antioxidants have many industrial uses such as preservatives in food and cosmetics and preventing the degradation of rubber and gasoline.[7] These observations suggested that antioxdiants might help prevent these conditions. There is some evidence that antioxdiants might help prevent diseases such macular degeneration,[11] suppressed immunity due to poor nutrition[12] and neurodegeneration.[13] Other substances in fruits and vegetables (possibly flavonoids) or a complex mixture of substances may contribute to the better cardiovascular health of those who consume more fruit and vegetables.[14,15] Although some levels of antioxidant vitamins and minerals in the diet are required for good health, there is considerable doubt as to whether antioxidant supplementation is beneficial and if so, which antioxidant(s) are beneficial and in what amounts.[9,10] In this research involves the

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preliminary phytochemical screening, antimicrobial activity, and finally the measurement of the antioxidant activity of one Myanmar indigenous medicinal plant, (Phetya).

2. MATERIALS AND METHODS

2.1 Plant Materials

The sample *Girardinia heterophylla* Dene. was collected from Pyin Oo Lwin Township, Mandalay Division, Myanmar. The plant was screened and identified by authorized botanist from Botany Department, Mandalay University, Myanmar. The roots were washed, cut into pieces and allowed to air dried well. These pieces were ground to powder, stored in well-stoppered bottle and used throughout the experiment. Commercial grade reagents and solvents were used without further purification. UV-VIS Spectrometer (Shimadzu, Japan) was used for the determination of antioxidant activity.

2.2 Preliminary Phytochemical Screening

The preliminary phytochemical screening of the roots of *Girardinia heterophylla* Dene. were carried out to know the different kinds of phytochemical constituents at Department of Chemistry, University of Mandalay, Myanmar to detect the different kinds of chemical constituents in the sample [7] [8] [9] [10].

2.3 Determination of Antimicrobial Activity on Crude Extracts

The antimicrobial activities of the crude extracts from the root*s* of *Girardinia heterophylla* Dene. were tested by Agar-well diffusion method on six selected microorganisms such as *Bacillus subtilis, Staphylococcus aureus, Pseudomas aeruginosa, Bacillus pumilus, Candida albicans* and *E.coli* in PRD (Pharmaceutical Research Department), in Yangon, Myanmar [11].

2.4 Determination of Antioxidant Activity by DPPH Assay

In this experiment, 1, 1-diphenyl-2-picryl hydrazyl (DPPH) powder was used as stable free radical according to Manzocco and *et al*., 1998. (Manzocco, 1998) Ascorbic acid was used as standard antioxidant. Ethanol (Analar grade) was also used as solvent. The absorbance was determined at 517 nm wavelength by using UV- VIS spectrophotometer [12] [13].

3. RESULTS AND DISCUSSION

3.1 Preliminary Phytochemical Screening of Plant Sample

The results obtained for the phytochemical screening from the roots of *Girardinia heterophylla* Dene. were showed the presence of alkaloid, flavonoid, terpenoid, free sugar, polyphenol and glycoside. respectively as shown in the table (1).

Table (1) Results of Phytochemical Test of *Girardinia heterophylla* Dene.

No.	Constituents	Extract	Reagents	Observation	Results
1	Alkaloid	Water	Dragendraff's	Orange	+
			Mayer's Solution	Cream color ppt	+
			Wagner's Solution	Reddish brown ppt	+
2	Flavonoid	Ethanol	EtOH, Mg coil, HCl	Pink color	+
3	Glycoside	Water	10% lead acetate	White ppt	+
4	Polyphenol	Ethanol	10% $FeCl_3$+1%$K_3[Fe(CN)_6]$	Green -blue	+
5	Steroid	Ethanol	Pet ether, conc. H_2SO_4	No reddish brown	-
6	Terpene	Ethanol	$CHCl_3$,H_2SO_4, Acetic anhydride	Two layer Red ppt	+
7	Phenol	Water	1% $FeCl_3$	Puplish	+
8	Sugar	Water	Fehling's solution	Yellow ppt	+
9	Saponin	Water	$NaHCO_3$	forth	+
10	Lipophilic	Water	0.5N KOH	Deep color	+

(+) = presence of constituents (-) = absence of constituents

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3.2 Antimicrobial Activities of Crude Extracts from the roots of *Girardinia heterophylla* Dene.

The results of the antimicrobial activities of the crude extract of the sample are given in the table (2) and figure (1).

Table (2) Antimicrobial Activities of Crude Extracts from the roots of *Girardinia heterophylla* Dene.

Sample	Solvent Used for Extraction	Diameter of Inhibition Zone (mm)					
		I	II	III	IV	V	VI
Crude Extract	EtOAc	11(+)	11(+)	16(++)	15(++)	15 (++)	11(++)

Microorganisms
I = *Bacillus subtilis* II = *Staphylococcus aureus* III = *Pseudomonas aeruginosa*
IV = *Bacillus pumilus* V = *Candida albicans* VI = *E. coli*

Agar well- 10 mm, 7mm~11mm (+); 12mm~16mm(++); 17mm above (+++)

Bacillus substilis *Staphylococcus aureus* *Pseudomonas aeruginosa*

Bacillus pumalis *Candida albican* *E-coli*

Figure (1). Antimicrobial activities of the crude extract of the sample

According to this table, ethyl acetate extracts of the sample show low activities on *Bacillus subtilis*, *Staphylococcus aureus* and *E. coli* and medium activities on *Pseudomonas aeruginosa*, *Bacillus pumilus* and *Candida albicans*.

3.3 Determination of Antioxidant Activity of Ethanol Extract of the Sample by DPPH Assay

The antioxidant activity of the ethanol extract of the roots of *Girardinia heterophylla* Dene. was determined in DPPH free radical scavenging assay. From this assay, IC50 value of the roots of *Girardinia heterophylla* Dene. was found to be 3.211124 µg/ml and showed low activity than standard ascorbic acid compared with its IC50 (1.724812 µg/ml) value. The antioxidant activity results of the ethanol extract from the roots of *Girardinia heterophylla* Dene using DPPH assay were shown in the table (3).

Table(3) Radical Scavenging Activity and IC_{50}

Sample	% RSA (mean ±SD) in different concentration (µg/mL)					IC_{50} (µg/mL)
	0.625	1.25	2.5	5	10	
Ascorbic acid	41.6	53.6	62	85.4	92.2	1.724812
Ethanol Extract of crude sample	33.12	48.7	51.6	54.2	56.8	3.211124

IC50 value of the ethanol extract of the sample was calculated by using linear regressive equation.

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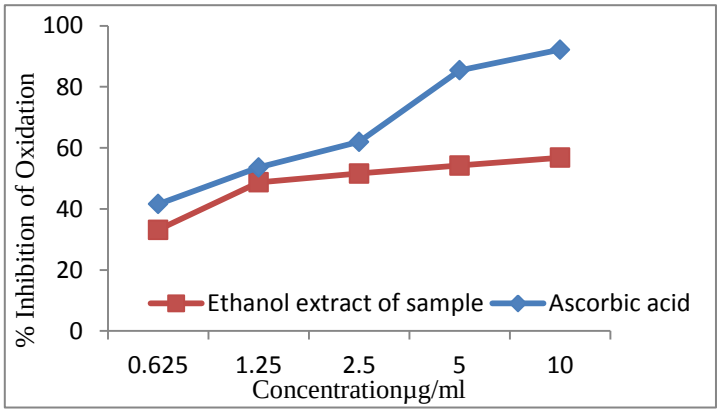

Figure (2). Plot of % inhibition vs. concentration of crude extract and Standard Ascorbic acid

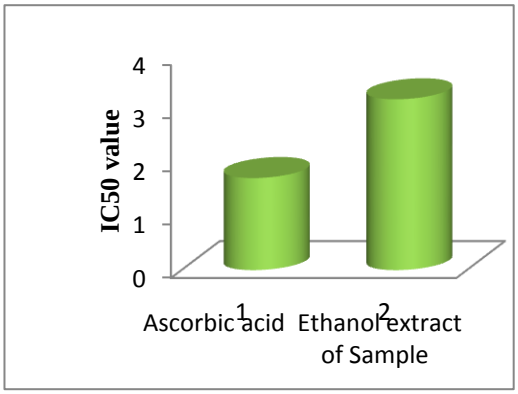

Figure (3). Plot of % inhibition vs. concentration of crude extract and Standard Ascorbic acid

CONCLUSION

In this research work, firstly Myanmar indigenous medicinal plant *Girardinia heterophylla* Dene was selected for phytochemical screening and antimicrobial activities. The crude extract of Phetya consists of alkaloid, flavonoid, terpenoid, free sugar, polyphenol and glycoside. According to figure (2) and (3) the absorbance values of *Girardinia heterophylla* Dene. and ascorbic acid are not considerably different at lower concentrations. But at higher concentration levels (5.0 μg / ml-10 μg / ml) the differences of absorbance between *Girardinia heterophylla* Dene. and standard ascorbic acid became much larger. Consequently at lower concentration levels, *Girardinia heterophylla* Dene. responds high antioxidant activity. In addition, the radical scavenging activity (IC_{50} values) of the ethanol extract of the sample was found to be 1.25 μg/ml.

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