Screening of Phytochemical Constituentsand Some Pharmacological Activities of *Eclipta alba* Hassk. Plant (Trailing eclipta)

Khin Nann Nyunt Swe[1], Soe Wai Mar Lwin Soe[2]

Abstract

Trailing eclipta plant is small branched annual herbaceous plant. This plant has been used as traditional medicines in many countries especially in tropical and subtropical regions. This study was subjected to screen the phytochemical constituents, elemental composition and some pharmacological activities such as antimicrobial activities, antioxidant activity and toxicity of plant sample. The phytochemical tests gave rise to positive tests for alkaloids, flavonoids, phenolic compounds, polyphenols, terpenes, steroids, glycosides and saponins, respectively. The elemental composition in this plant was detected by Energy Dispersive X-Ray Fluorescence Spectroscopy (EDXRF). In this determination, calcium was found to be the highest amount in the plant sample. Agar-well diffusion method was used to determine the antimicrobial activities of five solvent extracts on six tested microorganisms. The antioxidant activity was assessed by DPPH free radical scavenging assay. From this assessment, IC_{50} values were found to be $2.04\ \mu g/mL$ for ethanol plant extract and $1.88 \mu g/mL$ for ascorbic acid. In the toxicity tests of ethanol extract and water extract of plant sample, it was found that 2.0g/kg/day of both extracts of plant sample showed confidence dose and considered as safe.

Keywords : phytochemical, pharmacological activities, antioxidant, toxicity

Introduction

Many thousands of plants are grown throughout the world. Plants also provide food, clothes and shelter. The plants have been found useful in medicine. The medicinal effects of plants are due to metabolites especially secondary compounds by plant species. Plant`s metabolites include primary metabolites such as proteins, carbohydrate, fats, and secondary metabolites such as alkaloids, flavonoids, saponin, steroids, terpenes and tainins etc.(YusKamisha*et al*,2013).

Eclipa alba Hassk. belongs to the family of Asteraceae, is an evergreen herb of native Asia. It has been used for traditional medicines in many countries such as America, Africa, India, Thailand, China, Brazil and Myanmar. Myanmar name of this plant is commonly known as Kyeikhman. It grows well in moist places especially in paddy fields as both an erect or prostrate annual herb (Asolka, 1992).

The chemical composition of *Eclipata alba* Hassk. are steroids, alkaloids, flavonoids, triterpenes, glycosides, resin, ecliptine, nicotine, wedelic acid, apigenin and luteolin. It contain mainly coumestan i.e. wedelolactone (I) and thiophene-derivatives. It is also reported to contain many minerals that are necessary for life and bioactive phytochemicals. Many of phytochemicals such as polyphenols and flavonoids possess significant antioxidant capacities that are associated with lower occurrence and lower mortality rates of several human diseases (Jadhav, 2009).

This herb has been known for its curative properties and has been utilized as antimyotoxic, analgesic, antimicrobial, antihepatotoxic, antioxidant, antihaemorrhagic, antihyperglycemic, immunomodulatory properties and it is considered as a good rejuvenator (Jadhav, 2009).

This research was undertaken to screen the phytochemical constituents and some pharmacological activities of *Eclipta alba* Hassk.plant such as antimicrobial activities, antioxidant activity and toxicity.

[1] Dr., Lecturer, Department of Chemistry, Dagon University
[2] Demonstrator, Department of Chemistry, Sangaing University

Botanical Description

Scientific Name -- *Eclipta alba* Hassk.
Family Name -- Asteraceae
English Name -- Trailing eclipta
Myanmar Name -- Kyeikhman
Part used -- The whole plant
Medicinal Uses -- It has been used in folk medicine to treat cirrhosis, hepatitis, liver enlargement, enlarged spleen and skin disorder (Dewick,2003).

Figure (1) *Eclipta alba* Hassk.plant

Aim and Objectives

Aim

The aim of this research is to screen the phytochemical constituents and pharmacological activities of one Myanmar indigenous medicinal plant, *Eclipta alba* Hassk.

Objectives

- ❖ To collect the plant sample, *Eclipta alba* Hassk.. from Chan MyaThar Si Township, Mandalay.
- ❖ To carry out the phytochemical screening of the plant sample.
- ❖ To analyze the elemental contents of the plant sample by EDXRF spectroscopy.
- ❖ To examine the antimicrobial activities of plant sample by agar-well diffusion method.
- ❖ To investigate the antioxidant activity of ethanol extract of plant sample by DPPH scavenging assay.
- ❖ To determine the toxicity of the water extract and ethanol extract of plant sample by Litchfield and Wilcoxon's method.

Materials and Methods

Sample collection and preparation

Eclipta alba Hassk. (Trailing eclipta) plants were collected from Chan MyaThar Si Township, Mandalay, Myanmar for chemical analysis.

After sample collection, the plant materials were cut into small pieces and dried in air. Then, these dried samples were ground into powder by grinderand stored in air-tight container to prevent moisture changes and contamination.

Phytochemical screening

The various solvents extracts of plant sample were prepared to analyze the presence of certain phytochemicals. Analysis was done for alkaloids, flavonoids, phenolic compounds,

polyphenols, terpenes, steroids, glycosides, saponins, lipophilic compounds and cyanogenic glycosides.

Analysis on elemental contents

The elemental contents of plant sample were analyzed by Energy Dispersive X-Ray Fluorescence (EDXRF) spectrophotometer.

Determination of some pharmacological activities of extracts of plant sample

The five solvents such as n-hexane, pet-ether, chloroform, ethyl acetate and ethanol were used to analyze the antimicrobial activities of plant sample. The antimicrobial activities of plant sample were analyzed by agar-well diffusion method on six microorganisms such as *Bacillus substilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumalis, Candida albicans and Escherichia coli.*

DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical scavenging assay was chosen to assess the antioxidant activity of plant sample. In this study, the ethanol extract of plant sample was used. TheDPPH radical-scavenging assay measures the ability of the compound to donate hydrogen to DPPH radical. The DPPH radical-scavenging activity of plant sample was also determined based on median inhibition concentration (IC_{50}). In this experiment, ascorbic acid was used as standard antioxidant.

The water extract and ethanol extract of plant sample were used to determine the toxicity of plant sample. The animal models (Mus. Musculus) were used in this study. The mice with 30 g of body weight and age of 4-6 weeks were selected to use and kept separately.

Table (1) Determination of some pharmacological activities of crude extract of plant sample

No.	Activities	Method
1.	Antibacterial Activities	Agar-well Diffusion method
2.	Antioxidant Activity	DPPH Radical Scavenging Assay
3.	Toxicity	Litchfield and Wilcoxon'smethod

Results and Discussion

Phytochemical constituents of plant sample

Preliminary phytochemical analysis was performed in order to know different types of organic compounds present in the plant sample. Analysis on the extracts of plant sample revealed the presence of phytochemicals such as alkaloids, flavonoids, glycosides, phenolic compounds, polyphenols, saponins, steroids, and terpenes, respectively. These phytochemicals are known to exhibit medicinal as well as physiological activities. The results are shown in Table (2).

Table (2) Results of phytochemical constituents of plant sample

No.	Constituents	Extract	Reagents	Observation	Result
1.	Alkaloids	1% HCl	Dragendroff's reagent	Orange ppt.	+
2.	Flavonoids	95% ethanol	Conc: HCl, Mg	Pink color	+
3.	Glycosides	Water	10% lead acetate	White ppt.	+
4.	Phenolic compounds	Water	1% $FeCl_3$	Purplish color	+
5.	Polyphenols	95% ethanol	1% $FeCl_3$ + 1% $K_3[Fe(CN)_6]$	Green blue color	+
6.	Saponins	Water	-	Froth	+
7.	Steroids	95% ethanol	$CHCl_3$, Acetic anhydride, H_2SO_4	Green color	+
8.	Terpene	Pet-ether	$CHCl_3$, Acetic anhydride, H_2SO_4	Pink color	+
9.	Lipophilic compounds	Water	0.5M KOH	No deeper green color	-
10.	Cyanogenic glycoside	Water	Con: H_2SO_4, sodium picrate paper	No brick-red color	-

(+) = Presence , (-) = Absence

According to the Table (2), plant sample gave the positive tests for alkaloids, flavonoids, phenolic compounds, polyphenols, terpenes, steroids, glycosides and saponins respectively. Lipophilic compounds were not present. Cyanogenic glycosides which generally possess toxic property was found to be absent in the plant sample.

Elemental contents of plant sample by EDXRF Spectroscopy

The plant sample contain minerals .It displays many beneficial properties. Minerals are important for our body to stay healthy. The elemental composition in plant sample was examined by EDXRF spectroscopy. The results are shown in Table (3).

Table(3) Results of elemental contents of plant sample

No	Elements	Symbols	Results (%)
1	Calcium	Ca	40.025
2	Potassium	K	29.650
3	Chlorine	Cl	18.438
4	Sulphur	S	5.482
5	Iron	Fe	3.888
6	Strontium	Sr	0.747
7	Manganese	Mn	0.675
8	Zinc	Zn	0.484
9	Copper	Cu	0.355
10	Bromine	Br	0.257

According to experimental results, calcium was found to be the highest in the plant sample. It can be seemed that plant sample is a rich source of minerals that are necessary for healthy life.

Antimicrobial activities of plant sample

The study of antimicrobial activities of plant sample was performed by agar-well diffusion method on six microorganisms. The results are tabulated in Table (4) and Figure (2).

Table (4) Results of antimicrobial activities of plant sample

Sample	Solvents	Organisms					
		I	II	III	IV	V	VI
Trailing eclipta plant	EtOH	15mm	15mm	15mm	15mm	13mm	15mm
	EtOAc	18mm	24mm	24mm	22mm	20mm	25mm
	$CHCl_3$	13mm	14mm	13mm	14mm	0	14mm
	Pet-ether	-	-	-	-	-	-
	n-hexane	-	-	-	-	-	-

Clear zone diameter
Well diameter = 10 mm, 10-14 mm = (+), 15-19 mm = (++),
20 mm and above = (+++)

Organisms
I = *Bacillus subtilis*II = *Staphylococcus aureus*
III = *Pseudomonas aeruginosa* IV = *Bacillus pumalis*
V = *Candida albicans*VI = *Escheric*

Antioxidant activity of ethanol extract of plant sample

The antioxidant activity was studied on the ethanol extract of sample by DPPH assay. In this experiment, ascorbic acid was used as a standard antioxidant. On the basis of absorbance values, percent inhibition of ethanol extract in different concentrations was calculated. According to results, it can be seen that as the concentration was increased, the percent of inhibition of oxidation was also increased. The IC_{50} values were determined by using linear regressive excel program. IC_{50} value for extracted sample (IC_{50} = 2.04 µg/ml) was comparable to that of standard ascorbic acid (IC_{50} = 1.88µg/ml).Therefore, ethanol extract of plant sample showed significant antioxidant activity.

Table (5) % Inhibition and IC_{50} Values of Ethanol Extract of plant extract

Extract	**Concentration** (µg/mL)	**Mean Absorbance**	**Mean % Inhibition**	**IC_{50}**(µg/mL)
EtOHextract (Sample)	1.25	0.182	36.806	2.04
	2.5	0.122	57.639	
	5	0.102	64.583	
	10	0.093	67.708	
	20	0.065	77.431	
Ascorbic acid	1.25	0.085	33.20	1.88
	2.5	0.037	66.61	
	5	0.034	69.59	
	10	0.029	77.13	
	20	0.023	84.63	

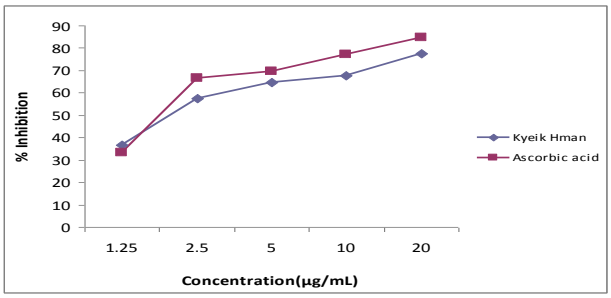

Figure (3) % Inhibition Vs Concentration (µg/mL) of ethanol extract of plant sample and standard ascorbic acid

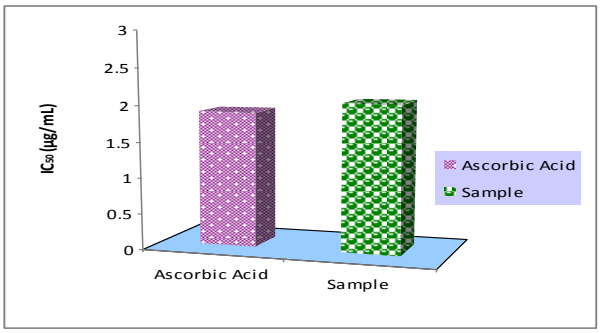

Figure (4) Bar-graph of IC_{50} values for standard ascorbic acid and plant sample

Toxicity test for water extract and ethanol extract of plant sample

500mg/kg/day,1000mg/kg/day,1500mg/kg/day, and 2000mg/kg/day does were used to examine the toxicity test for both extracts such as ,water extract and ethanol extract of plant sample. 25 mice were administrated orally. Both extracts of plant sample gave the normal condition of their health. They were survived for 10 days after administration. No mortalities were observed among the mice tested with both extracts of plant.

Therefore, it was found that 2000mg/kg/day of both extracts of plant sample showed confidence dose and considered as safe. The following Table (6) showed the results for toxicity test.

Table (6) Results of toxicity of water extract and ethanol extractof plant sample

No.	Dose	Tested Mice	Dead	Alive
1	500 mg/kg/day	5	-	5
2	1000 mg/kg/day	5	-	5
3	1500 mg/kg/day	5	-	5
4	2000 mg/kg/day	5	-	5

Conclusion

In this research, *Eclipta alba* Hassk.. (Trailing eclipta) plant was selected for chemical analysis because it is one of the well-known Myanmar medicinal plants. An attempt has been made to investigate the phytochemical constituents, minerals and antimicrobial activity, as well as anitoxidant activity of *Eclipta alba* Hassk.. ethanol extract. The plant sample was found to be contained alkaloids, flavonoids, phenolic compounds, polyphenols, terpenes, steroids, glycosides and saponins. These phytochemicals confer antimicrobial activity on the plant extract. From the experimental results, $CHCl_3$, EtOH and EtOAc extracts were found to be remarkable antimicrobial activity. Therefore, these extracts can be used in medicinal formulation to treat the diseases related to tested microorganisms.

According to the elemental analysis, the plant contains macro (Ca, K, Cl, S) and micro (Mn, Zn, Cu) elements that are necessary for health benefits. Moreover, antioxidant properties are very important due to the harmful role of free radicals in food and biological systems. The antioxidant activity of the plant extract was evaluated by DPPH method. In the DPPH assay, IC_{50} value of *Eclipa alba* Hassk. was found to be 2.04µg/mL and that of ascorbic acid was 1.88 µg/mL.These IC_{50}values were comparable. Thus, the ethanol extract of the plant showed the significant antioxidant activity.

From the toxicity test, it can be observed that 2.0 g/kg/day dose of both ethanol and water extracts showed confidence dose and considered as safe. It showed no toxicity.

Therefore, the selected plant could be considered as a source of valuable phytochemicals, minerals, antimicrobial and antioxidants for medicinal purposes. There can be strong scientific evidence to use this plant in Myanmar traditional medicine.

Finally, more detailed research for this plant sample should be performed from the pharmacology point of view.

Acknowledgement

We would like to express our heartfelt gratitude to Rector, Dr.WinNaing, Dagon University for his interest and encouragement on this research paper. We would like to thank to Dr. Nu Nu Yi, Pro-rector, Dagon University for her encouragement and interest on this research. We also convey special gratitude to Dr. Nay ThweKyi, Pro-rector, Dagon University for her patient guidance and invaluable advice for research. We deeply thank to Dr. Cho Cho Win, Professor and Head, Department of Chemistry, Dagon University for her invaluable advice and encouragement for the research work. We would like to express our sincere thanks to Dr.Ahmar Yi, Professor and Head, Department of Chemistry, Taunggyi University for her invaluable guidance and encouragement for this research work.

References

Asolkar, A.V., (1992), "Glossary of Indian Medicinal Plants with active Principles." Publication and information directorate (CSIR), New Delhi.

Chakrabory, P., (1998), "A Text Book of Microbiology", New Central Book Agency Ltd, 423.

Dewick, P.M.,(2003). "Medicinal Natural Products," "A Biosynthetic Approach," Second Edition, JOHN WILEY & SONS LTD.

Fiabane, A.M., Williams D.R., (1977), "The Principles of Bio-inorganic Chemistry", The Chemical Society, London, P-15.

Harbone, J.B.(1984). *Phytochemical Methods: A Guide to ModernTechniques of Plant Analysis*. 2nd edit, Charpman and Hall Ltd., U.S, A.

Halliwell, B., (2008). "Are polyphenols antioxidants or pro-oxidant? What do we learn from cell culture and in vivo studies?"

Jadhav,V.M, *etal.*,(2009).*Journal of Pharmacy Research* 2[8], 1229-1231.

Kadam V.J. ,Ghosh R., (2009). *International Journal of Pharmtech Research*. 1[4], 1480-1484.

Kritikar, K.R., Basu, BD. (1975). Chroeinica Botanical Indian Medicinal plants. New Delhi.

YusKamisha, Faizah Othman, HjMohdSaadQodriyah, and KamsiahJarrin , 2013.
"Parkiaspeciosa Hass: A potential phytomedicine".