# Antioxidant activity and detection of Wedelolactone in Extracts of (*Eclipta alba* L.)

**May Oo Khine[1], Myat Myat Moe[2]**

## Abstract

In this research paper, the aqueous, ethanolic and methanolic extracts of *Eclipta alba* L. were examined antioxidant activity such as DPPH radical scavenging activity, total phenolic and flavonoid contents, AChE activity and antidiabetic activity. According to this results, DPPH radical scavenging activity of aqueous extract of *Eclipta alba* L. was higher inhibition than other extracts. Maximum amount of total phenolic compound contents was observed in methanolic extract and flavonoid contents was also found in aqueous and methanolic extracts. The AChE activity of methanolic extract showed inhibition. The aqueous extract did not show antidiabetic activity. The present study reports the detection of wedelolactone in extracts of *Eclipta alba* L. by using HPLC.

***Keywords:*** antioxidant activity, DPPH, AChE, antidiabetic, Wedelolactone

## Introduction

Herbal medicine is still considered the primary form of the health care mainly in the developing countries which is about 75-80% of the world population. Plants have been in use for the treatment of various diseases and disorders since human history and form the basis of current sophisticated medicine. Many effective drugs have been originated from plant sources (Mondal, 2016).

*Eclipta alba* L. commonly known as False Daisy and is a plant belonging to the family Asteraceae. It is also named "Kyeik-hman" in Myanmar. This plant is native to the tropical and subtropical regions of the world. It is used to treat different disease in human for traditional medicine.

The plant of *Eclipta alba* L. is a widely grown plant. It has been included as a hair-growth promoter and most of its activities that include hepatoprotective, antiviral, antibacterial, spasmogenic, hypotension, homicidal, antileprotic, analgesic, antioxidant, antihaemorrhagic, anticancer and antiheptotoxic (Jadhav, 2009).

In Myanmar, a preparation obtained from the leaf juice boiled with sesame and coconut oil is used for anointing the head to render the hairs black and luxuriant. It is also used for skin disease when burn. It much more clear that this herb is more than just a hair tonic and it has wider medicinal uses. Important source of chemicals is wedelolactone, dimethyl wedelolactone exhibit antihepatotoxic activities. Thus, this study aimed to investigate antioxidant activity such as DPPH, total phenolic and flavonoids contents, AChE activity and antidiabetic activity and to detect wedelolactone in *Eclipta alba* L. using HPLC.

## Materials and Methods

### Collection and preparation of *Eclipta alba* L. Extracts

The specimens used in the research were collected from East Dagon Township, Yangon Region during May to June, 2018. The whole plants were dried in shade for two weeks when completely dried, these were pulverized by grinding machine to get the powder and stored in an airtight container for the chemical study. Firstly, dried powdered plant materials (50g) of each of which was extracted with distilled water for 1 hour(in 60°C) , macerated with 98% ethanol and 95% methanol for 12 hours with the ratio of 1: 10 (w/v). Secondly, the extracts were collected and filtered through Whatman No. 1 filter paper and then filtered with 0.45μm nylon

[1] Dr, Lecturer, Department of Botany, Dagon University
[2] Dr, Professor and Head, Department of Botany, Dagon University

membrane filter (GE Healthcare UK). Finally, the aqueous, ethanolic and methanolic extracts were yield respectively. The chemical studies were carried out at the Department of Oriental Herb Science, Jeonbuk National University, Iksan in Korea.

**Test for Antioxidant Activity**

**Preparation of DPPH (1,1-diphenyl-2-picrylhydrazyl)**

DPPH stock solution, 0.0002g of DPPH in 50ml of methanol. The solution was freshly prepared and stored on falcon tube wrapped with silver foil.

**Preparation of Test sample solution**

0.2g of test sample and 2ml of methanol were thoroughly mixed by vortex mixer. Then, the mixture solutions were placed in centrifuge. After 10 minutes, the stock solutions were obtained.

**Measurement of DPPH Radical Scavenging Activity by Spectrophotometeric method**

The control solution was prepared by mixing 200µl of methanol and 1.8µl of DPPH. Similarly, the blank solution was prepared 2µl of methanol only. The sample solution was prepared by mixing 40µl of test sample solution, 160µl of methanol and 1.8µl of DPPH solution. All solutions were kept in the dark for 30 minutes. Then, the absorbance of the solution was measured at 517nm using aUV-1601 Shimadzu Spectrophotometer. Methanol was used as standard and control. These were done in triplicate. The percentage inhibition was calculated by using the following equation: %inhibition = (1-S/C) x100, S= Absorbance of Sample, C= Absorbance of Control. The results were shown in table (1) and fig (1).

**Total Phenolic Compound Content (TPC)**

Total phenolic compound content of each extract was determined by Folin-Ciocalteu,s reagent. The sample 100µl was prepared by mixing 2ml of 2%$Na_2Co_3$. The mixture was left at room temperature for 3 minutes. Then, 100µl of 50% Folin-Ciocalteu,s reagent was added to the mixture and left for 30 minutes. The absorbance of the solution was determined at 700nm using a UV-1601 Shimadzu spectrophotometer. These were done in triplicate. The equivalent values of the extract were calculated using the following equation: Sample (Abs) = 0.1523/0.8965. The results were shown in Table (2).

**Total Flavonoid Content (TFC)**

Total flavonoid content of each extract was determined by 10% $AlCl_3.6H_2O$. The sample 250µl, 1ml of distilled water and 75µl of 5% $NaNo_2$ were mixed and incubated for 5 mins. Then, 150µl of 10% $AlCl_3.6H_2O$ was added into the mixture. After 6 minutes of incubation, 500µl of 1M NaOH was added into mixture and left for 11 minutes. The absorbance of the solution was determined at 500nm using a UV-1601 Shimadzu spectrophotometer. These were done in triplicate. The equivalent values of the extract were calculated using the following equation: Sample (Abs) 0.0848/0.0002. The results were shown in Table (3).

**Test for AChE acitivity (acetylcholinesterase)**

**Measurement of AChE activity by Spectrophotometeric Method**

The control solution was prepared by mixing with 150 µl methanol, 1.5 ml of buffer (Tris), 150 µl of AChE solvent. The sample solution was prepared by mixing with 150 µl of test sample solution, 1.5 ml of buffer (Tris) and 150 µl of AChE

solvent. All solution were shaked at room temperature for 10 minutes. And then adding by 150 µl of DTNB solvent and 75 µl of acetylthiocholine. Then, the absorbance of the solution was measured at 405 nm using a UV-1601 Shimadzu Spectrophotometer. These were prepared in triplicate. The percentage inhibition was calculated using the following equation: % inhibition = $(1-\frac{S}{C})\times 100$, S = Absorbance of sample, C = Absorbance of control. The result were shown in Table (4).

**Test for Antidiabetic activity (α-glucosidase)**

**Measurement of Antidiabetic activity by Spectrophotometric Method**

The control solution was prepared by mixing with 200 µl α-glucosidase and 100 µl of Distilled water, methanol and ethanol respectively.

The sample solution was prepared by mixing with 200 µl α-glucosidase, 100 µl of test sample solution. All solution were shaked at incubator, 37°C for 10 minutes. And then adding with 100 µl of ρ-nitrophenyl-α, D-glycopyra-noside (ρNPG) were shaked at incubator for 20 minutes. Then adding with 2 ml of sodium phosphate buffer. ($Na_2PO_3$). the absorbance of the solution was measured at 405 nm using a UV-1601 Shimadzu Spectrophotometer. These were prepared in triplicate. The percentage inhibition was calculated using the following equation: % inhibition = $(1-\frac{S}{C})\times 100$, S = Absorbance of sample, C = Absorb`ance of control. The result were shown in Table (5).

**High Performance Liquid Chromatography (HPLC)**

The aqueous, ethanolic and methanolic extracts of *Eclipta alba* L. were analysed for the presence of wedelolactone using HPLC. The column used was $C_{18}$ Silica. Wavelength was 270nm and the flow rate was 1.0 ml / min. 0.5 % Fomic acid: Acetonitrile (70:30) was used as mobile phase.

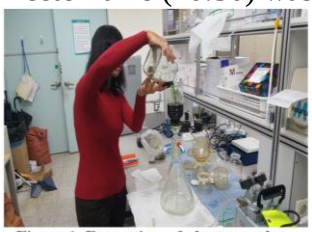

Figure 1. Extraction of plant sample


Figure 2. UV-visible spectrophotometer

Figure 3. Experiment of the test

Figure 4. HPLC analysis

## Result and Discussion

**Outstanding Characters of *Eclipta alba* L.**

Perennial herb; Leaves Opposite lance-like with a toothed edge and hairy; Flower White, small and arranged in small clusters; Fruit Achene; Seed Dark brown and non-endospermic.

## Antioxidant activity of Eclipta alba L.

### DPPH – radical scavenging assay

DPPH is stable nitrogen free radicals and has been widely used to determine the free radical scavenging ability of various samples (Pukumpuang, 2014). The aqueous and methanolic extracts of *Eclipta alba* L. showed strong DPPH radical scavenging activities than ethanolic extract. The extracts showed a better scavenging activity than the control. The results indicated that the extracts of *Eclipta alba* L. contain compounds responsible for scavenging the radical. Phenol, flavonoid, tannin, alkaloid and glycoside are good antioxidant substance and prevent or control oxidative stress related disorder. According to the results, the aqueous extract of *Eclipta alba* L. had high antioxidant activity. The percentage inhibition of DPPH radical scavenging activities of extracts of this plant were presented in Table. 1, Figure 6.

**Table.1 2,2-Diphenyl 1-picryhydrazyl (DPPH) Radical scavenging activity**

| Sl. No. | Control (abs) | Concentration (µl) | Sample (abs) | % inhibition 0f free radical DPPH= ( 1- sample/control) x 100% |
|---|---|---|---|---|
| **1.** | **1.077** | **40** | **DW- 0.272**<br>**0.468**<br>**0.393** | **75**<br>**57**<br>**64** |
| 2. | 1.091 | 40 | EtOH- 0.746<br>0.782<br>0.788 | 16<br>23<br>29 |
| 3 | 1.068 | 40 | MeOH- 0.676<br>0.273<br>0.468 | 37<br>75<br>56 |

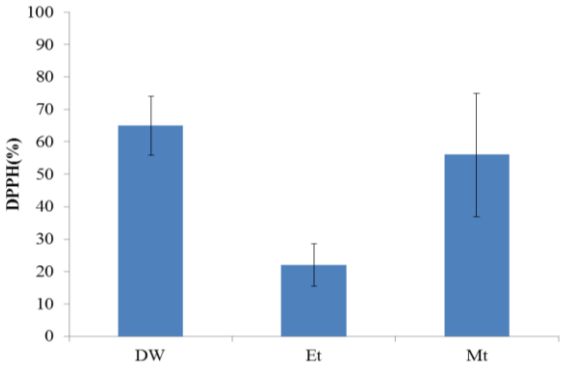


**Figure.6 2,2-Diphenyl 1-picryhydrazyl Radical scavenging activity (DPPH)**

## Total Phenolic Compound Content (TPC)

Phenolic compounds such as phenolic acids, tannins and flavonoids are widely found from plant. Phenolic acids are one of the major groups acting antioxidant activity by donating hydrogen or electron. Phenols present in the plants are strong antioxidants and have an important role in the health care system (Thenomozhi,2011). They have been shown to possess various biological activities including anti-inflammatory, anticarcinogenic, anti- atherosclerotic activities and they might be related to antioxidant activity (Pukumpuang, 2014). The results from Table 2. showed that methanolic extract of *Eclipta alba* L. had total phenolic content higher than aqueous and ethanolic extracts .

**Table.2 Total Phenolic Compound Content (TPC)**

| Sl. No. | Control (abs) | Concentration (µl) | Sample (abs) | Total phenolic content (mg) = $\frac{\text{Sample} - 0.1523}{0.5963}$ |
|---|---|---|---|---|
| 1. | Gallic acid | 100 | DW- 1.235<br>1.062<br>1.222 | 1.815<br>1.525<br>1.793 |
| 2. | Gallic acid | 100 | EtOH- 0.746<br>0.782<br>0.788 | 0.996<br>1.055<br>1.066 |
| **3** | **Gallic acid** | **100** | **MeOH- 1.715<br>1.869<br>1.777** | **2.619<br>2.878<br>2.724** |

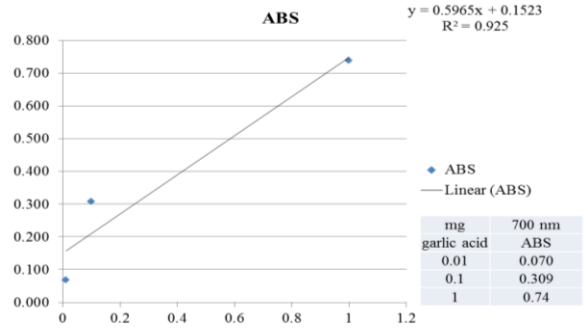


**Figure.7 Standard of Phenol**

## Total Flavonoid Content (TFC)

It was found that flavonoids were the largest class of phenolic in this study. Flavonoids in plant are mainly present as glycosides and some are as aglycones. They have shown to possess biological activities including antioxidant activity, hepatoprotective, anti-inflammatory and anticancer activities. Maximum amount of flavonoids content was observed in aqueous extract but minimum in ethanolic and methanolic extracts of *Eclipta alba* L. The results shown in Table. 3.

**Table. 3 Total Flavonoid Content (TFC)**

| Sl. No. | Control (abs) | Concentration (µl) | Sample (abs) | Total flavonoid content(mg) = $\frac{\text{Sample} - 0.1523}{0.5963}$ |
|---|---|---|---|---|
| **1.** | **Catechin** | **250** | **DW-1.512<br>1.286<br>1.393** | **7136<br>6006<br>6541** |
| 2. | Catechin | 250 | EtOH-0.452<br>0.644<br>0.597 | 1836<br>2796<br>1561 |
| 3. | Catechin | 250 | MeOH- 1.323<br>1.301<br>1.446 | 6171<br>6081<br>6806 |

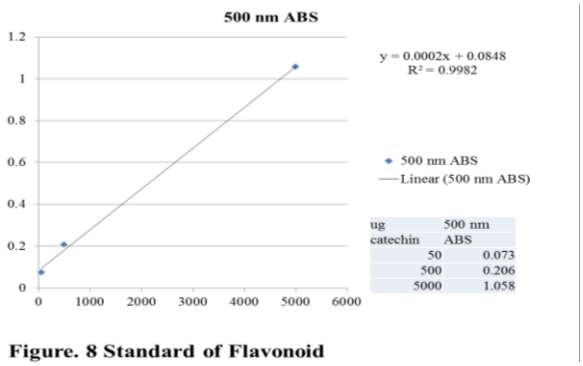


**Figure. 8 Standard of Flavonoid**

## AChE activity (acetylcholinesterase)

AChE tends to become deposited within the neurofibrillary tangles and amyloid plaques associated with Alzheimer's disease ( Dhanasekaran,2015). The aqueous and ethanolic extracts were not showed AChE activity. The methanolic extract showed inhibition activity. The AChE inhibition activities of extracts from *Eclipta alba* L. are presented in Table. 4, Figure 9.

**Table.4 AChE activity of different extracts of *Eclipta alba* L.**

| Sl. No. | Control (abs) | Concentration (µl) | Sample (abs) | % inhibition of AChE activity = ( 1- sample/control) x 100% |
|---|---|---|---|---|
| 1. | 2.751 | 150 | DW- 2.767<br>2.799<br>3.068 | No detect |
| 2. | 2.767 | 150 | EtOH- 2.767<br>2.799<br>2.767 | No detect |
| **3** | **2.960** | **150** | **MeOH- 2.101<br>2.011<br>3.215** | **37<br>32<br>error** |

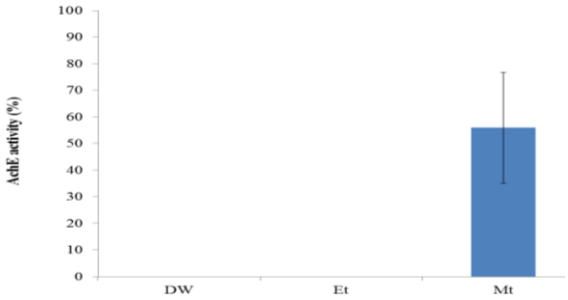


**Figure.9 AchE activity (acetylcholinesterase)**

## Antidiabetic activity

There was inhibitory activity against α-glucosidase enzyme. The ethanolic and mehanolic extracts of *Eclipta alba* L. show good inhibitory percentage of antidiabetic activity. The aqueous extract of *E. alba* L. did not show activity. A comparative α-glucosidase percentage inhibition are shown in Table. 5, Figure 10.

**Table. 5 Antidiabetic activity of different extracts of *Eclipta alba* L.**

| Sl. No. | Control (abs) | Concentration (µl) | Sample (abs) | % inhibition of Antidiabetic activity = ( 1-sample/control)x100% |
|---|---|---|---|---|
| 1. | 1.030 | 100 | DW- 1.057<br>1.060<br>1.055 | No detect |
| 2. | o.794 | 100 | EtOH- 0.401<br>0.284<br>0.569 | 50<br>64<br>30 |
| 3 | 1.078 | 100 | MeOH- 0.655<br>0.678<br>0.732 | 39<br>37<br>32 |

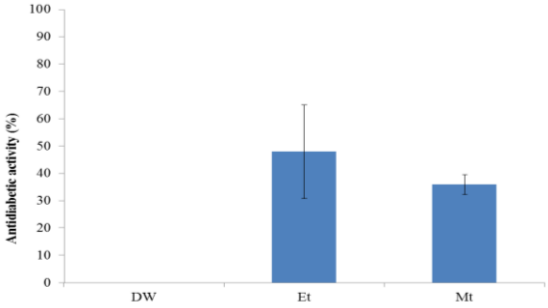


**Figure.10 Antidiabetic activity**

## High Performance Liquid Chromatography (HPLC)

The aqueous, ethanolic and metholic extracts of *Eclipta alba* L. were analysed for the presence of wedelolactone using HPLC. The aqueous extract showed three peaks at Rt 2.52, 3.63 and 4.44 min Figure 11. The area of the peak at Rt 3.6min was found to increase whereas the other peaks showed a decrease. So, the peak at 3.6 min assigned to wedelolactone. The ethanolic extract showed 7 peaks at Rt 2.94, 4.18, 5.39, 6.97, 10.33, 11.33 and 19.06 respectively Figure 12. The area of the peak at Rt 2.94 min was found to increase whereas the other peaks showed a decrease. So, the peak at Rt 2.94 min was assigned to wedelolactone. The methanolic extract showed 6 peaks Rt 2.59, 4.00, 4.77, 6.48, 10.55 and 17.50 respectively Fig 13. The area of the peak at Rt 4.00 was found to increase whereas the peaks showed a decrease . So, the peak at Rt 4.00 min was assigned to wedelolactone.

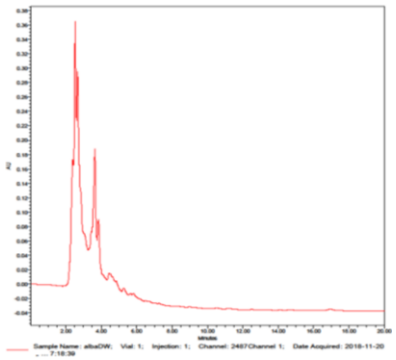

Figure 11. HPLC chromatogram of the aqueous extract of *Eclipta alba* L. at 270nm

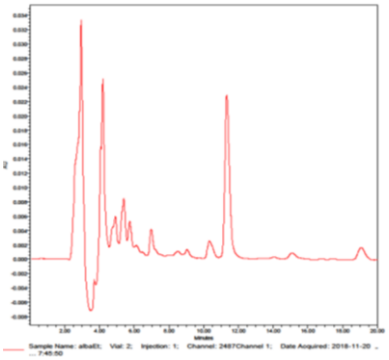

Figure 12. HPLC chromatogram of the ethanolic extract of *Eclipta alba* L. at 270nm

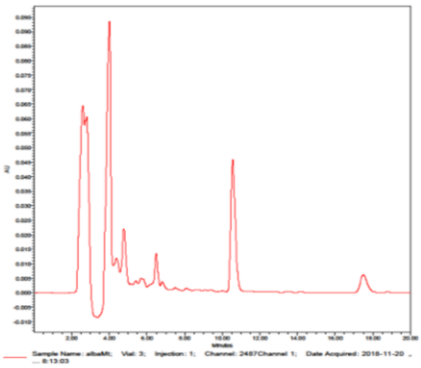

Figure 13. HPLC chromatogram of the methanolic extract of *Eclipta alba* L. at 270nm

## Conclusion

Aqueous, ethanolic and methanolic extracts were investigated for antifree radical activity by DPPH assay, total phenolic and flavonoid contents, AChE activity and Antidiabetic activity. It should be considered for the antioxidant properties and also beneficial role in their prevention of human disease. The method established using HPLC techniques facilities the convenient and rapid quality control of traditional medicines and their pharmaceutical preparation. Hence, sensitive and comprehensive analytical techniques are needed to acquire a better understanding of the pharmacological basis of the herbs and to enhance the product quality control.

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