Evaluation of Antibacterial and Antioxidant Activity of (Petthan) *Fernandoa adenophylla* Bark

[1]Maw Maw Than, [2]Hla Myoe Min

[1]Assistant Lecturer, [2]Associate Professor
Department of Chemistry
University of Medicine (Mandalay), Mandalay, Myanmar

***Abstract*: Firstly, (Petthan) *Fernandoa adenophylla* bark extracts were prepared using normal hexane, ethyl acetate and methanol by percolation method and antibacterial activity of these extracts were screened by agar well diffusion method using six human pathogens; *Bacillus cereus, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, Shigella boydii* and *Escherichia coli.* The result showed the significant inhibition for ethyl acetate extract and good for methanol extract. Again, antibacterial screening for ethyl acetate extract was performed by immersion bioautography method using two pathogens; *Bacillus cereus* and *Shigella boydii.* Hence, the fraction (R_f values range 0.16-0.84) of ethyl acetate extract which has broad spectrum of activity was found. Secondly, antioxidant activity of ethyl acetate and methanol extract were evaluated by DPPH assay method by comparing with that of ascorbic acid. Both extracts have good antioxidant activity. Then, TLC screening for antioxidant activity of ethyl acetate extract was done by spraying 20% solution of molybdophosphoric acid in ethanol. The R_f values given by antioxidant compounds were within the R_f values range having broad spectrum of antibacterial activity. This finding suggested that the compounds having antibacterial and antioxidant activity could be present in ethyl acetate of Petthan bark.**

***Keywords: Fernandoa adenophylla*, extract, antibacterial, human pathogen, bioautography, broad spectrum, antioxidant**

I. INTRODUCTION

The family of *Fernandoa adenophylla* (G. Don) is Bignoniaceae and is known as Petthan in Myanmar and its common name is Katsagon Tree. The Petthan plant is a deciduous small tree and the bark of Pretthan was collected from the field near Myaung-thit village, Mattayar township, Mandalay division, Myanmar. Traditionally, both of flowers and fruits of Petthan have been used for the dishes in Myanmar. In Myanmar traditional medicine, the parts of Petthan plant are found to be excluded in use of traditional medicine [1]. But it is used in folk medicine for various ailments such as night emission, amenorrhea and skin disease in Bangladesh [2]. In addition, it was very interesting news that some wild plants which were not known medicinal plant contain potent bioactive compound. For example, (Da-hat)*Tectona hamiltoniana* consists of bioactive compound, betulinic acid having high activity against HIV [3]. And it also consists of betulin and betulin aldehyde having anti-cancer activity [4]. So, the Petthan plant was interested to study the bioactivity and was evaluated for antibacterial and antioxidant activity in this study. The aim of this research is to find the common fraction of extract having antibacterial and antioxidant activity.

II. EXPERIMENT

Antibacterial Assay

The experimental work was initiated by identification of plant species, sample collection and percolation of Petthan bark. For percolation, the collected Petthan bark was cleaned, chopped and air-dried at room temperature. Analytical grade solvents were used for this percolation procedure. Three individual 100 g of the sample were percolated with each of 400 ml of n-hexane, ethyl acetate and methanol for two weeks. The obtained extracts were concentrated and dried with Rotary evaporator.

In antibacterial assay, there were two parts; screening by agar well diffusion method and immersion bioautography method [5].

Agar well diffusion method is an *in vitro* method and the three extracts and six human pathogens were used in this method. Six human pathogens were gram positive bacteria; *Bacillus cereus* and *Staphylococcus aureus*, and gram negative bacteria; *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella boydii* and *Escherichia coli*. This method was done by punching out equally spaced 8 mm-wells on a solid medium that has been heavily seeded with the individual pathogen. The wells were then filled with the solutions of extracted samples, solution of Ampicillin as a positive control and ethanol as a negative control. In this screening, 50 microliter per well of 30 mg/ml extract solution and 50 microliter per well of 10 μg/ml Ampicillin were used. These prepared plates were incubated at 37°C for 24 hours. After incubation, the inhibition zones around the well were measured by using ruler.

For immersion bioautography, ethyl acetate extract of Petthan bark was used as the test sample and *Bacillus cereus* and *Shigella boydii* were used as the test organisms according to the data which was given by agar well diffusion method. In immersion bioautography method, the first step was the preparation of the chromatogram and it was covered with a molten, seeded agar medium. After solidification and incubation, the inhibition or growth bands were visualized. To choose the best solvent system for the preparation of chromatogram, TLC were run by the following solvent systems (n-hexane : ethyl acetate = 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4). And then, the TLC plates were read at 310 nm using UV transilluminator. The R_f values of UV active spots were recorded. From these, the best solvent system (n-hexane: ethyl acetate = 2:1) was applied for bioautography. The selected ethyl acetate extract was applied to 2x7 cm TLC plate and was developed with the chosen solvent system. TLC was run in duplicate for the replicate testing of the bioautogram and dried for complete removal of solvent and covered by agar seeded medium in petri dish. After

solidification, the plates were incubated at 37°C for 24 hours. After incubation, the inhibition zones were observed as the clear zones on bioautogram.

Results of Antibacterial Assay

The antibacterial activities for the three extracts of Petthan bark were listed as in following table-1. Although ethyl acetate and methanol extract showed high activity against all six pathogens, n-hexane extract showed moderate activity for five pathogens without *Escherichia coli*.

Table-1

Antibacterial Activity of Extracts of Petthan Bark

Micro organisms	Inhibition Zone(mm) in diameter of extracts and Ampicillin			
	normal Hexane	*Ethyl acetate*	*Methanol*	*Ampicillin*
B. cereus	6	14	12	7
S. aureus	3	15	9	26
P. aeruginosa	8	16	12	7
S. typhi	3	16	19	4
S. boydii	7	12	7	-
E. coli	-	15	12	6

The inhibition zones of *Bacillus cereus* and that of *Shigella boydii* which were observed on bioautogram were described by comparing with the duplicate chromatogram which was under UV as in figure-1 and figure-2 respectively. The R_f values range of clear zone on bioautogram for anti-*Bacillus cereus* and anti-*Shigella boydii* activity were 0.06-0.84 and 0.16-0.95 respectively.

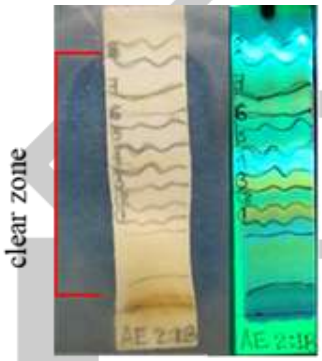

Figure-1 Bioautogram for *B. cereus*

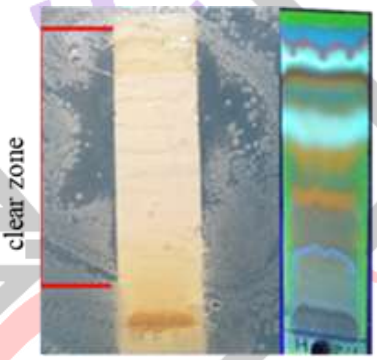

Figure-2 Bioautogram for *S. boydii*

Assay of Antioxidant Activity

In this assay, there were also two parts. The first method, DPPH assay method, was performed to evaluate antioxidant activity of the extracts [6]. In TLC screening method, spray reagent was 20% solution of molybdophosphoric acid in ethanol [7]. Ethyl acetate extract, methanol extract and standard ascorbic acid were samples for DPPH assay method and only ethyl acetate extract was sample of TLC screening method.

In DPPH assay, 2 ml of different concentrations (0.9766, 1.9532, 3.9063, 7.8125 and 15.625 µg/ml) of each sample extract solution was mixed with 2 ml of 0.4mM DPPH solution in methanol and kept for 30min in the dark at room temperature. After that, 2 ml of varying concentrations (0.0625, 0.125, 0.25, 0.5 and 1 µg/ml) of aqueous solution of ascorbic acid was also mixed with 2 ml of the above DPPH solution and also kept for 30 min in the dark at room temperature. The absorbance of each mixture and blank DPPH solution were determined at 517 nm using Cary 60 UV-Visible Spectrophotometer and ascorbic acid was served as a positive control. The % scavenging activity was calculated using the equation as in Eq.1.

$$\text{Scavenging activity (\%)} = (A\text{-}B/A) \times 100 \qquad (1)$$

Where A was the absorbance of control (DPPH solution without the sample), B was the absorbance of mixture. Then % scavenging activity was plotted against concentration and IC_{50} value was calculated by linear regression analysis.

In TLC screening method, ethyl acetate extract was loaded to 1x7 cm TLC plate and was developed with n-hexane :ethyl acetate(2:1) solvent system and dried for complete removal of solvent. After spraying 20% molybdophosphoric acid solution to chromatogram, the chromatogram was heated about 120°C. Blue color bands were appeared as the proof for antioxidant activity and the R_f values were calculated.

Results of Antioxidant Assay

The % scavenging activity of the two extracts and standard ascorbic acid were assessed by the DPPH assay as shown in Table-2, Table-3 and Table-4. Both extracts exhibited very-closed DPPH free radical scavenging effects results to each other. The figure showing % scavenging activity of the two extracts was also shown in figure-3. IC_{50} value of ascorbic acid and that of ethyl acetate and methanol extract were found to be 0.2447 µg/ml, 1.1807 µg/ml and 1.3415 µg/ml respectively.

Table-2

Antioxidant Activity of Ethyl Acetate Extract

conc. (µg/ml) of extract	Absorbance		% scavenging activity	IC_{50} (µg/ml)
	DPPH	*mixture*		
0.9766	2.5783	1.3118	49.1213	1.1807
1.9532		1.2824	50.2621	
3.9063		1.2384	51.9678	
7.8125		1.1970	53.5741	
15.625		1.1293	56.1999	

Table-3

Antioxidant Activity of Methanol Extract

conc. (µg/ml) of extract	Absorbance		% scavenging activity	IC_{50} (µg/ml)
	DPPH	*mixture*		
0.9766	2.5783	1.2931	49.8279	1.3415
1.9532		1.2749	50.5508	
3.9063		1.2535	51.3795	
7.8125		1.2195	52.6998	
15.625		1.0970	57.4526	

Table-4

Antioxidant Activity of Ascorbic Acid

conc. (µg/ml) of Ascorbic acid	Absorbance		% scavenging activity	IC_{50} (µg/ml)
	DPPH	*mixture*		
0.0625	2.5783	1.5000	41.8221	0.2447
0.1250		1.2914	49.9127	
0.2500		1.1920	53.7680	
0.5000		1.1380	55.8624	
1.0000		1.0706	58.4765	

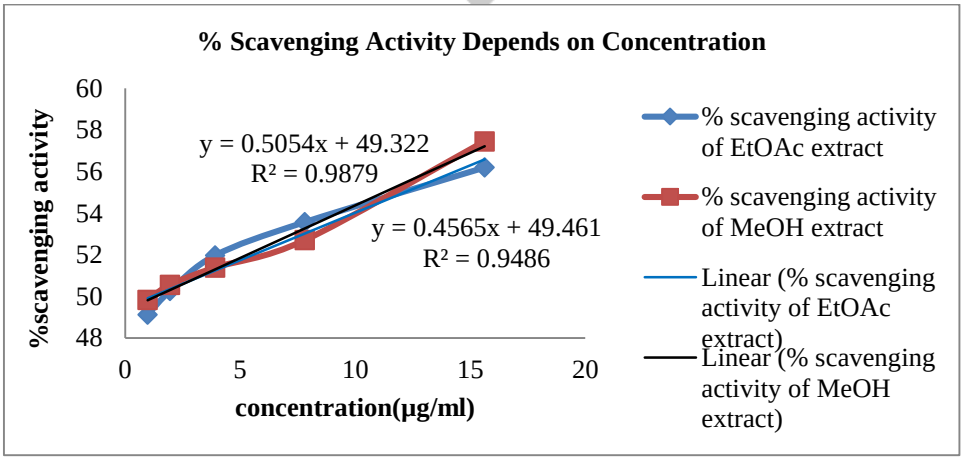

Figure -3 % Scavenging Activity depends on concentration of the two Extracts

The figure of chromatogram which was compared with the two bioautograms was shown in figure-4. The R_f values of the bands having antioxidant activity were A=0.26, B=0.32, C=0.48 and D=0.73 respectively.

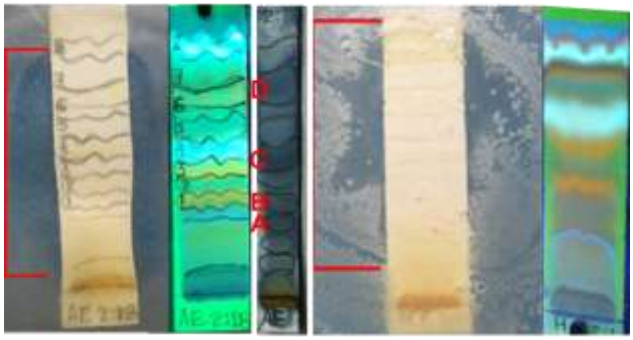

Figure-4 comparison of R_f values for antioxidant and antibacterial activity of ethyl acetate extract

III. ANALYSIS

In agar well diffusion method, ethyl acetate extract showed significant inhibition zones and methanol extract showed good inhibition zones for all six pathogens. So, these extracts could have broad spectrum of antibacterial activity. According to the bioautography result, the R_f values range 0.16-0.84 was the common fraction of anti-*Bacillus cereus* and anti-*Shigella boydii* due to the overlapping of inhibition zone for *Bacillus cereus* and *Shigella boydii.* This revealed that the common fraction could have broad spectrum of activity.

In antioxidant activity, IC_{50} value of methanol extract was comparable with that of ethyl acetate extract. The comparison of IC_{50} value of standard ascorbic acid with IC_{50} values of the two extracts suggested that antioxidant activity of the two extracts will be lower than that of ascorbic acid. And the R_f values having the antioxidant activity were compared with the R_f values range of the common fraction of antibacterial activity. According to this, some antioxidant compounds of ethyl acetate extract (R_f =0.26, 0.32, 0.48 and 0.73) could have the activity of anti-*Bacillus cereus* and anti-*Shigella boydii.*

IV. CONCLUSION

In conclusion, both ethyl acetate extract and methanol extract of Petthan bark could be the source of potent antibacterial medicine in the treatment of bacterial diseases. In addition, these extracts could aslo have antioxidant potential. Especially, the compounds which have broad spectrum of antibacterial and antioxidant activity could be present in ethyl acetate extract.

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