# A Study on Antioxidant Activity of Bergenin and its Derivative from the Bark of *Peltophorum pterocarpum* (DC.) K. Heyne (Pan-mèzali)

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## Abstract

The present research work deals with the study of antioxidant activity of isolated bergenin from bark of *Peltophorum pterocarpum* DC. (Pan-mezali) and synthesized bergenin diethyl ether. The bark of *P. pterocarpum* was collected from Kamaryut Township, Yangon Region. Then preliminary phytochemical tests, determination of nutritional values and soluble matter contents of some organic solvents and water were also evaluated. The methanol crude extract was performed from the defatted bark sample by percolation method. Bergenin (3.26% yield, m.pt 239 – 241 °C) was isolated from methanol crude extract by column chromatographic separation technique using ethyl acetate and methanol 19:1 fraction. Then a derivative compound, bergenin diethyl ether (25% yield, m.pt 176 – 178 °C) was prepared from isolated bergenin using dry acetone and ethyl iodide by reflux method. Finally the antioxidant activity of crude extracts such as ethanol, methanol, water and isolated bergenin and also its derivative was screened by DPPH assay using UV spectrophotometer. All crude extracts and bergenin exhibited high antioxidant activity however antioxidant activity of bergenin diethyl ether was very low.

***Keywords*** **:** *Peltophorum pterocarpum* DC., phytochemical constituents, nutritional values, antioxidant activity, bergenin, bergenin diethyl ether

## Introduction

Traditional system of medicine continues to be widely practiced on many accounts. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs for infectious disease have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailment.

Medicinal plants include a various types of plants used in herbalism and some of these plants have medicinal activities. These medicinal plants are considered as a rich resourc of ingredients and which can be used in drugs development and synthesis. Besides that these plants play a critical role in the development of human cultures around the world. Moreover, some plants consider as important source of nutrition and as a result of that these plants are recommended for their therapeutic values. Medicinal plants are frequently used as a raw material for extraction of active ingredients which are used in the synthesis of different drugs.

In this study, our attention has been focused on bark of *Peltophorum pterocarpum* (DC.) K. Heyne (Syn. *Peltophorum inerme* (Roxb.) belongs to the family Caesalpiniaceae and is called Pan-mèzali (PMZL) in Myanmar (Sohail, 2007). The plant PMZL has been used for the treatments of insomnia, skin troubles, ringworm, constipation, stomatitis, dysentry, gargles and tooth powder, eye lotion and embrocation for muscular pains and sores due to its antimicrobial, antifungal, anti-inflammatory, antioxidant, antidiabetic and cardiotonic activities (Oudhia, 2003). The plant distributed in the Tropical southeastern Asia and northern Asia, Sri Lanka, Thailand, Vietnam, Indonesia, Malaysia, Popua New Guinea, Philippines and Northern Australia (Sohail, 2007). The bark contains phytoconstituents as

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stigmasterol, β-sitosterol, lupeol and lupenone (Jash *et al*., 2013). The leave and bark contains bergenin, (-) epicatechin, (+) leucocyanidin and quercetin (Sastry *et al*., 1976). Bergenin an isocoumarin was a major compound of flowers, leaves and bark of *P. pterocarpum* (Wealth of India, 1948). This plant was reported to contain phenolic compounds, tannins, flavonoids, steroids and terpenoids compounds that showed antimicrobial and antioxidant activity (Jain, 2011).

## Materials and Methods

### Sample collection

The bark of *peltophorum pterocarpum* was collected from the Yangon University Campus during the month of April 2011. The bark sample was identified at botany department of Yangon University. The sample was dried under the shade for a few days, then cut into very small pieces and were powdered.

### Preliminary Phytochemical Investigation

Phytochemical screening for bioactive principles was done by general chemical test according to the standard procedures.

### Determination of Nutritional Values

The chemical analyses for the determination of moisture content, ash content, protein content, fat content, fibre content and carbohydrate content were determined by AOAC methods.

### Determination of Soluble Matter Contents

The soluble matter contents of the sample in some organic solvents such as 95% ethanol, methanol, acetone, ethyl acetate, chloroform, petroleum ether and water were determined by the method given in "The British Pharmacopoeia", 1963.

### Preparation of Methanol Crude Extract

The defatted marc (powdered sample left after extraction with pet-ether) was percolated with methanol (300 mL) for three weeks. The solvent was removed under reduced pressure in a rotary evaporator. The methanol soluble extract was obtained.

### Isolation of Bergenin from Crude Methanol

Bergenin was isolated from methanol extract by column chromatographic separation technique using ethyl acetate and methanol as eluent.

### Synthesis of Derivative from Isolated Bergenin

A derivative compound, bergenin diethyl ether was prepared from isolated bergenin using dry acetone and ethyl iodide by reflux method.

### Procedure

Bergenin (20 mg) was dissolved in 50 mL of dry acetone containing 5 g of anhydrous potassium carbonate ($K_2CO_3$) and refluxed with 3 mL of ethyl iodide for 12 h. The mixture was cooled, filtered and the acetone evaporated from the filtrate. The residue was crystallized with methanol to obtained grey needles (Tiwari and Khosa, 2009).

### Identification of Isolated Bergenin and its Derivative

The isolated bergenin and its derivative were characterized by melting point, some chemical reagents, $R_f$ value on TLC as well as UV and FT IR spectroscopic techniques.

### Investigation of antioxidant Activity by DPPH Assay method

The antioxidant activity of the three crude extracts ( MeOH and EtOH, $H_2O$), bergenin and its derivatives was examined by DPPH assay method using UV spectrophotometer (UV-7504). 60μM DPPH (1, 1-diphenyl-2-picrylhydrazyl) solution and test sample solutions (Five different Conc: 10, 5, 2.5, 1.25 and 0.625 μg/mL) were prepared. Absorbance of the solutions control solution and mixture of DPPH and different concentration of sample solutions was measured at 517 nm in triplicate for

each solution. % inhibition and standard deviation were calculated from absorbance values. Then $IC_{50}$ value was calculated by linear regressive excel program.

## Results and Discussion

### Preliminary Phytochemical Investigation

The results of phytochemical screening showed that bark of PMZL contains alkaloids, glycosides, tannins, flavonoids, steroids, terpenoids and phenolic compounds.

### Determination of Nutritional Values

Nutritional values of bark of PMZL have revealed 11.8% of moisture, 3.4% of protein, 1.5% of fat, 19% of fibre, 3% of ash, 61.3% of carbohydrate and energy values 272.3 kcal / 100 g respectively on the basis of dried sample.

### Determination of Soluble Matter Contents

The soluble matter contents of some organic solvents such as EtOH, MeOH, $CH_3COCH_3$, EtOAc, PE, $CHCl_3$ and water were 38.23%, 35.61%, 24.04%, 20.30%, 5.02%, 4.00% and 30.71% (w/w) in bark respectively.

### Preparation of Methanol Crude Extract

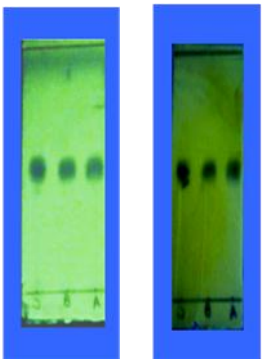
The powdered sample (100 g) bark of PMZL was successively extracted with pet-ether (60-80 °C) and methanol by percolation. Petroleum ether extract (0.24 g) and methanol crude extract (21.08 g) were obtained.

### Isolation of Bergenin from Crude Methanol

The methanol crude extract was fractionated by column chromatography using EtOAc and MeOH as eluents. Three main fractions were obtained. Yellow solid materials were observed from fraction II in the solvent system EtOAc:MeOH 19:1. The crystal was washed with PE and EtOAc and then recrystalized by acetone solvent. Colorless rhombohedron crystal (97.9 mg, 3.26 %) was obtained.

### Identification of Isolated Bergenin

Firstly, the isolated bergenin from methanol extract was characterized by determining the melting point. Its melting point was found to be 239 – 241 °C. It was soluble in chloroform, acetone, methanol and ethanol. It was UV active. Green fluorescence was observed on TLC under UV 254 nm. According to the chemical tests, bergenin also contained phenolic OH group because it gave brown coloration with 10% $FeCl_3$ and green with 5% $H_2SO_4$ followed by heating. The $R_f$ value of bergenin (0.5) was found to be identical with that of standard bergenin in EtOAc:MeOH (15:1 v/v) solvent system and they also gave the same behaviours on Co-TLC chromatogram as described in Figure 1.



**Spray with 10% $FeCl_3$**

A = isolated bergenin (m.pt = 239-241 °C)
B = mixture (isolated and std bergenin)
C = standard bergenin
Solvent system = EtOAc:MeOH (15:1)
$R_f$ = 0.5
Adsorbent = Silica gel 60 $F_{254}$ (Aluminium Sheet)

Figure 1 Co TLC chromatograms of isolated and standard bergenin

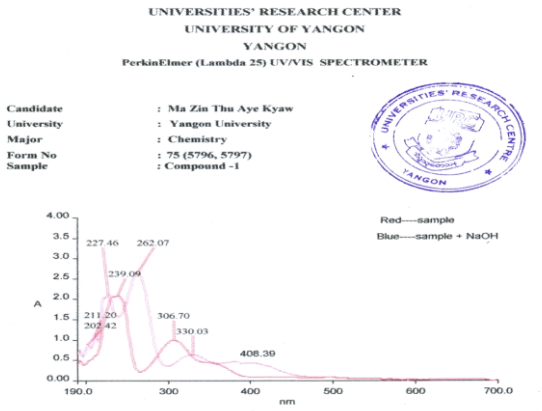



**Figure 2 UV spectra of isolated bergenin in MeOH and NaOH**

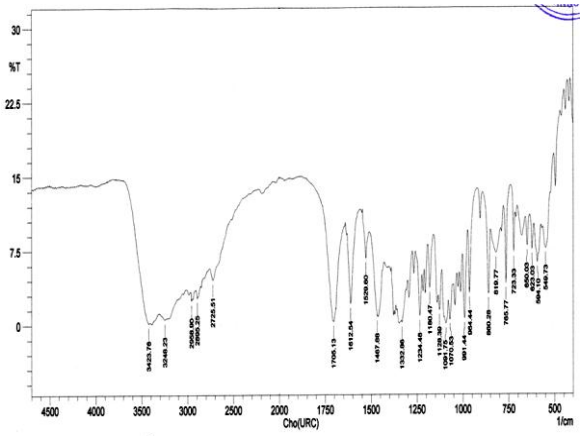



**Figure 3 FT IR spectrum of isolated bergenin (KBr)**

The UV spectra of isolated bergenin (Figure 2) showed the maximum absorption wavelength $\lambda_{max}$ at 227 and 306 (sh) nm in MeOH indicating the presence of conjugated double bond due to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions. When a small amount of 10% NaOH solution was added into the sample solution $\lambda_{max}$ shifted to 239 and 330 (sh) nm indicating that red shift (longer wavelength) due to phenolic OH groups. The absorption spectrum may be sensitive to control of pH. Chromophores involving an acidic or basic group will be affected by pH, e.g. the bathochromic shift (to longer wavelength) and hyperchromic peak (greater intensity) of phenates compared to their parent phenol. This is a useful test for a phenolic system.

The FT IR spectrum of isolated bergenin (Figure 3) showed that a broad strong band absorbed at 3423 and 3248 $cm^{-1}$ due to O-H stretching vibration of and phenolic and alcoholic OH groups. The symmetric and asymmetric aliphatic C-H stretching bands appeared at 2958 and 2895 $cm^{-1}$. The band at 1705 $cm^{-1}$ indicated the presence of α, β – unsaturated lactone group. The bands at 1612 and 1529 $cm^{-1}$ that corresponded to C=C stretching revealed the presence of aromatic ring in this compound. In addition, C-H bending of $CH_2$ group appeared at 1467 $cm^{-1}$. Absorption band at 1332 $cm^{-1}$ indicated the O-H bending of phenol group. The band at 1234, 1091, and 1070 $cm^{-1}$ can be attributed to C-O-C stretching vibration of cyclic ring. The absorption band at 1180 $cm^{-1}$ showed to C- O stretching vibration of phenolic OH group. Weak intensity band at 860 and 765 $cm^{-1}$ was attributed to C-H bending vibration of aromatic ring. So, the isolated compound was assigned as bergenin. Consequently, it chemical structure is shown in Figure 4.

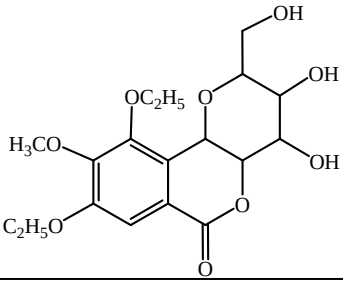



**Figure 5 Structure of prepared bergenin diethyl ether**

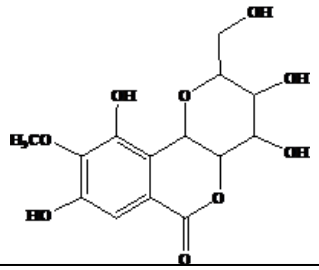



**Figure 4 Structure of isolatred bergenin**

**Synthesis of Derivative from Isolated Bergenin**

A derivative compound, bergenin diethyl ether was prepared by refluxed method using dry acetone, anhydrous $K_2CO_3$ and ethyl iodide. The yellow needles material was obtained which was washed with PE and EtOAc and then recrystallized by methanol solvent. The grey needle (5 mg, 25% yield) was crystallized out.

There are five –OH groups in bergenin compound, two are attached to the benzene ring and the others are in glycoside residue. In the preparation of bergenin diethyl ether the two active hydrogens in phenolic-OH group are substituted by ethyl group. Therefore phenolic character is absent in a derivative compound. Complete ethylation in the more active hydrogen atoms of two phenolic OH groups was confirmed by Co-TLC chromatograms (Figure 6).

**Identification of Prepared Bergenin Diethyl Ether**

Bergenin diethyl ether was prepared as a gray needle from isolated bergenin and it was UV active. Its melting point was found to be 176 – 178 °C. It was soluble in chloroform, acetone, methanol and ethanol. Its $R_f$ value was found to be 0.54 with EtOAc:MeOH (15:1 v/v) solvent system. According to the chemical tests, bergenin diethyl ether did not contain phenolic OH group because it did not give brown coloration with 10 % $FeCl_3$.

The structure of bergenin diethyl ether was also studied by UV and FT IR spectral data. The UV spectra of bergenin diethyl ether (Figure 7) showed the maximum absorptions wavelength $\lambda_{max}$ at 248 and 298 (sh) nm in MeOH. When a small amount of 10% NaOH was added to sample solution neither red nor blue shift was observed. It can be concluded that ethyl group are substituted completely in only phenolic OH group.

The FT IR spectrum of bergenin diethyl ether (Figure 8) showed that the broad absorption band which occurs at 3308 $cm^{-1}$ was due to the O-H stretching vibration band. The symmetric and asymmetric aliphatic C-H stretching band of $CH_2$ and $CH_3$ groups appeared at 2977, 2932 and 2877 $cm^{-1}$. The band at 1734 and 1705 $cm^{-1}$ indicated the C=O stretching vibration of α, β – unsaturated lactone group. The band appeared at 1596 $cm^{-1}$ indicated the presence of aromatic CH stretching. The band at 1478 and 1372 $cm^{-1}$ showed to C-H bending of $CH_2$ and $CH_3$ groups. The band at 1328 $cm^{-1}$ referred to OH bending alcoholic OH group. In addition, the absorption bands at 1240 and 1040 $cm^{-1}$ were appeared due to C-O-C stretching vibration of cyclic ring and that at 1127 and 1105 $cm^{-1}$ indicated the presence of C-O stretching band of alcohol group. The peak due to C-H in out of plane bending vibration of aromatic ring occurred at 853 and 763 $cm^{-1}$. The observed FT IR spectral data of bergenin diethyl ether were consisted with those of reported bergenin diethyl ether Consequently, prepared derivative compound was identified as bergenin diethyl ether and its structure is shown in Figure 5.

The differences between bergenin and bergenin diethyl ether were checked by Co TLC. $R_f$ values of bergenin was found to be 0.5 and 0.54 for bergenin diethyl ether with EtOAc: MeOH (15: 1 v/v) solvent system. On TLC chromatogram, both compounds gave dark green and pale green with 5% $H_2SO_4$ followed by heating. Bergenin gave brown with 10% $FeCl_3$ solution indicating the presence of phenolic OH group. Bergenin diethyl ether did not give any color with 10% $FeCl_3$ solution due to the absence of phenolic OH group.

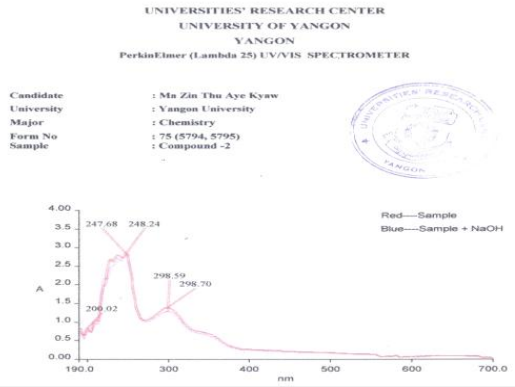



**Figure 7 UV spectra of bergenin diethyl ether in MeOH and NaOH**

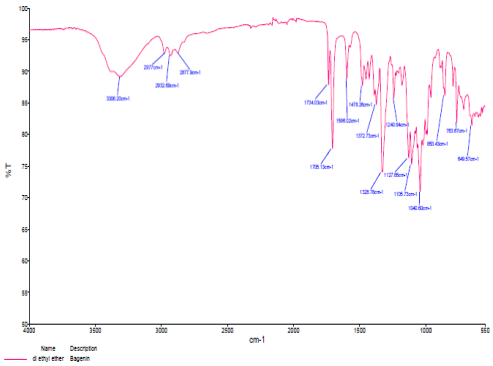
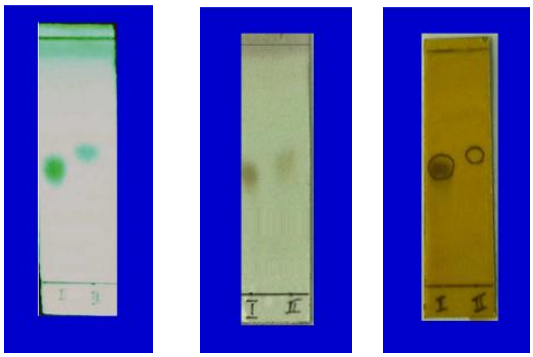


**Figure 8 FT IR spectrum of prepared bergenin diethyl ether (KBr)**



**Under UV-254** | **Spray with 5% $H_2SO_4$** | **Spray with 10% $FeCl_3$**

**I = bergenin**

**II = bergenin diethylether**

**Solvent system = EtOAc :MeOH (15:1)**

**$R_f$ = 0.5 (Compound I)**

**$R_f$ = 0.54 (Compound II)**

**Adsorbent = Silica gel 60 $F_{254}$ (Aluminium Sheet)**

**Figure 6 Co-TLC chromatograms of bergenin and bergenin diethyl ether**

**Antioxidant Activity of Plant Extracts, Bergenin and Bergenin Diethyl Ether**

In *in vitro* antioxidant activity study, the radical scavenging activity of crude extracts, bergenin and standard vitamin C were expressed in term of % inhibition and $IC_{50}$ (50% inhibition concentration) values. The results are described in Table 1 and Figures 9.

Among the extracts, EtOH extract showed the more antioxidant power than the other extracts and isolated bergenin was found to be more effective than the all extracts of bark sample in free radical scavenging activity. The isolated bergenin ($IC_{50}$ = 0.65 µg/mL) and EtOH extract ($IC_{50}$ = 0.96 µg/mL) showed stronger radical scavenging activity than the standard vitamin C ($IC_{50}$ = 1.17 µg/mL). But, the $IC_{50}$ value of prepared bergenin diethyl ether was not detected until 400 µg/mL, therefore the bergenin diethyl ether exhibited very low antioxidant activity because of polyphenolic character was lost in its chemical structure.

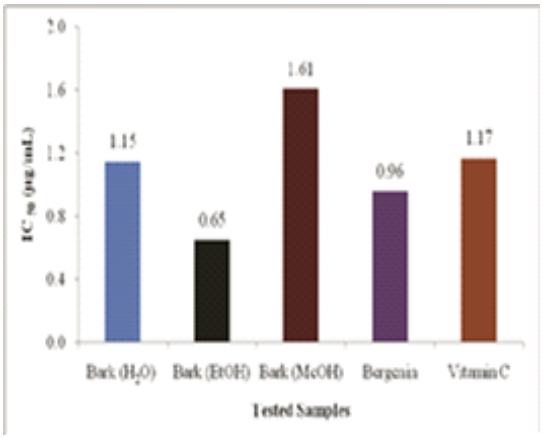



**Figure 9 Bar graph of $IC_{50}$ values of some crude extracts from bark of PMZL, bergenin and vitamin C**

The plant exhibited potent antioxidant activity. Because of flavonoids and tannins in the bark sample is responsible for the free radical scavenging effects observed. Flavonoids and tannins are phenolic compounds which are a major group of compounds in the bark of PMZL. These compounds act as primary antioxidants or free radical scavengers.

**Table 1 Percent (%) Oxidative Inhibition and $IC_{50}$ Values of some crude Extracts from Bark of Pan-mèzali, Bergenin and Standard Vitamin C**

| Tested samples | % RSA (mean ± SD) in different concentration (µg/mL) | | | | | $IC_{50}$ (µg/mL) |
|---|---|---|---|---|---|---|
| | 0.625 | 1.25 | 2.5 | 5 | 10 | |
| Bark ($H_2O$) | 35.99 ± 1.44 | 52.62 ± 2.49 | 80.88 ± 3.81 | 89.44 ± 0.38 | 91.69 ± 0.80 | 1.15 |
| Bark (EtOH) | 49.29 ± 6.28 | 72.57 ± 2.49 | 87.45 ± 2.50 | 91.02 ± 0.66 | 92.27 ± 0.25 | 0.65 |
| Bark (MeOH) | 19.52 ± 0.88 | 39.04 ± 1.54 | 77.44 ± 1.10 | 89.48 ± 0.44 | 90.75 ± 0.88 | 1.61 |
| Bergenin | 44.31 ± 6.28 | 55.11 ± 2.49 | 59.02 ± 3.10 | 65.92 ± 3.81 | 72.57 ± 2.49 | 0.96 |
| Vitamin C | 25.2 ± 1.40 | 53.58 ± 0.88 | 65.53 ± 1.13 | 74.82 ± 0.59 | 83.32 ± 0.78 | 1.17 |

The isolated bergenin and prepared bergenin diethyl ether had been unambiguously characterized by Co TLC and UV spectra. Test with DPPH showed that bergenin is a good free radical scavenger and presents excellent antioxidant activity. Because the presence of polyphenolic portion in the chemical structure is responsible for the antioxidant activity of bergenin.

## Conclusion

Bergenin (3.26% yield, m.pt 239 – 241 °C) was isolated from methanol crude extract by column chromatographic separation technique. Then a derivative compound, bergenin diethyl ether (25% yield, m.pt 176 – 178 °C) was prepared from isolated bergeninin. In the antioxidant activity of crude extracts and isolated bergenin and also its derivative, ethanol, methanol and water crude extracts and isolated bergenin exhibited high antioxidant activity however antioxidant activity of bergenin diethyl ether was very low. Therefore bergenin compound having polyphenolic group is fundamental importance for its antioxidant activity and modification of its structure

can be envisaged to reduce its radical scavenging property. In this study, the presences of tannins and phenolic compounds in bark showed antioxidant activity.

## Acknowledgements

The authors acknowledge the Department of Higher Education (Lower Myanmar), Ministry of Education, Yangon, Myanmar, for allowing us to carry out this research programme and Pro-Rector Dr Daw Nu Nu Yee and Dr Daw Nay Thwe Kyi, Dagon University and Professor Dr Daw Cho Cho Win, Head of Department of Chemistry and Professor Dr Daw khin Than Win, Department of Chemistry, Dagon University for their kind provision of the opportunity to submit this research paper.

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