Antioxidant Activity, Antimicrobial Activity and Functional Group Identification of Isolated Compound from the Stem Bark of *Millingtonia hortensis* L.f.

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Abstract

In the present study, the stem bark of *Millingtoniahortensis* L.f. was collected from Mandalay University Campus, MahaAungMyay Township, Mandalay Region. Firstly, phytochemical screening of the selected sample was performed. Elemental composition of the crude sample was examined by EDXRF (Energy Dispersive X-ray Fluorescence) spectroscopy. The antimicrobial activities of three different solvents such as ethanol, ethyl acetate and n-hexane extracts of the sample were tested by Agar- well diffusion method. The antioxidant activity of ethanol extract of the stem bark of *Millingtoniahortensis* L.f. was studied by DPPH (1,1-Diphenyl-2-picryl-hydrayl) assay. Moreover pure bioactive organic compound was isolated from ethanol extract of the sample by Thin- Layer and Column Chromatography. The functional groups of pure isolated compound from the stem bark of *Millingtonia hortensis* L.f. were identified by FT-IR (Fourier-transform infrared) spectroscopy.

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Key words; *Millingtonia hortensis* L., phytochemical, EDXR, DPPH,

Introduction

All over the world, diseases attack rich and poor alike. There are many different types of diseases, but the most common worldwide are infection diseases. Scientists interest the natural herbal medicine which is useful for the treatment of various diseases and to investigate the relevant phytochemical constituents.Traditional medicines are madefrom plants, animal's products and minerals. Up to eighty percent of people in developed countries, medicinal plants are used in the name of herbal drugs to treat various chronic diseases because of its fewer side effects. In most of the countries, roots, leaves, barks, seeds, fruits and flowers are used in traditional medicine. These plants may save many lives if they are used correctly. Therefore, this research was carried out in order to know the antioxidant activity, mineral compositions and caffeine content in dried green tea leaves collected from YwanganTownship, Southern Shan State.In the field of natural products, the indigenous medicinal plants are generally considered to be the source of new products and compounds. The medicinal effects of plants are due to metabolites especially secondary compounds produced by plants.

Plant metabolites include primary metabolite such as carbohydrates, fat, nucleic acid and secondary metabolites such as alkaloids, flavonoids, saponin, steroids, terpenes, tannin, polyphenols etc.

Hence, the plants are needed to analyze whether they have the characteristics medicinal uses or not. This sense, *Millingtonia hortensis* L.f. was selected for chemical analysis.The stem bark of *Millingtonia hortensis* L.f. is used traditionally as mainly lung tonic, anti asthmatic and antimicrobial. The scientific activities reported so far from the plants are antifungal, larvicidal, antioxidant and antiproliferative activities. The tree is favorite garden and avenue tree. The tree is considered ornamental and the pleasant fragrance of the flowers renders it ideal as a garden tree. The wood is also used as timber and the bark is as an inferior substitute for cork. The leaves are also used as a cheap substitute for tobacco in cigarettes.In this research work, the preliminary phytochemical screening was performed. The antimicrobial activities of the stem

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bark of *Millingtonia hortensis* L.f. was tested with three solvent systems by using Agar well diffusion method in DCPT (Development Center of Pharmaceutical Technology), Insein, Yangon. The antioxidant activity of ethanol extract of stem bark of *Millingtonia hortensis* L.f. was determined by using DPPH.In addition, the pure bioactive compound was isolated by utilizing Thin Layer and Column Chromatographic methods and the isolated compound was identified by Fourier Transform Infrared Spectroscopic methods.

Botanical Description of tea

Botanical Name	:	*Millingtonia hortensis* L.f.
Family Name	:	Bignoniaceae
English Name	:	Tree Jasmine
Myanmar name	:	Eigarit
Habit	:	Tree
Part Uses	:	Bark

Figure : Plant, Flowers, Leaves and Bark of *Millingtonia hortensis* L.f.

Experimental

Sample Collection

The stem bark of *Millingtonia hortensis* L.f. was collected from Mandalay University Campus, Mandalay Region, Myanmar to investigate antioxidant activity, antimicrobial activity, and mineral composition and to isolate pure organic compound. The sample was cut into small pieces and allowed to dry in air. The air driedsamplewas made to powder and was used throughout the experiment.

Screening of Phytochemical Constituents of *Millingtonia hortensis* L.f.

All phytochemical tests of the sample were carried out based upon J. B. Harborne (1973), phytochemical methods (London: Chapman and Hall) and New Journal of Science by Chuwuma S. Ezeonu and ChigozieM.Ejikeme, 2016. But in this research, 2g of sample was used as the starting weight for all experiments. The results are described in table (1).

Determination of Elemental Composition of *Millingtonia hortensis* L.f. by EDXRF

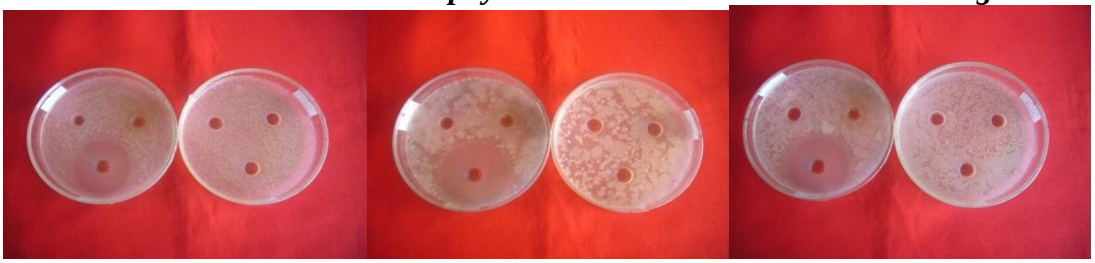
The mineral contents in ***Millingtonia hortensis*** **L.f.** were examined by EDXRF spectroscopy at Department of Physics, University of Mandalay. The results are tabulated in table (2).

Determination of Antimicrobial Activities of *Millingtonia hortensis* L.f.

Asian Journal of Plant Science and Research by Thamaraiselvi, P.Lallitha* and P.Jayanthi (2012), described the studies on antimicrobial activities by agar well diffusion method. So, the antimicrobial activities of the three solvent extracts such as n-hexane, ethanol and ethyl acetate extracts were determined by Agar Well diffusion method at Development Center of Pharmaceutical and Food Research Department (PFRD), Insein Yangon. The antimicrobial activities of three different solvent extracts were measured from the diameters of inhibition zones, shown in Figures. The results are shown in Table (3).

***Determination of Antimicrobial ofMillingtonia hortensis* L.f.**

Bacillus subtilis ***Staphylococcus aureus*** ***Pseudomonas aeruginosa***



Bacillus pumilus ***Candida albicans*** ***E. coli***

Figure : Antimicrobial Activities of Stem Bark of Millingtonia hortensis L.f.

Determination of Antioxidant Activity of *Millingtonia hortensis* L.f. by DPPH Assay

Saudi Pharmaceutical Journal by MdNurAlam*, *et al*, 2013, reported that the evaluation of antioxidant activity by using DPPH assay.In order to examine the antioxidant activity through free radical scavenging by the test sample, the change in optical density of DPPH radicals is monitored in this research. The antioxidant activity of *Millingtonia hortensis* L.f. was determined by DPPH (1, 1-Diphenyl-2-picryl-hydrazyl) Radical Scavenging Assay in Department of Biotechnology, Mandalay Technological University. The observed data are tabulated in table (4).

Isolation ofPure Organic Compounds from Ethanol Crude Extract by Colum Chromatography

Procedure

3 g of the crude extract obtained from the stem bark of ***Millingtonia hortensis*** **L.f.** was separated by column chromatography. The column was vertically clamped and filled with a small amount of n-hexane solvent. A small piece of cotton wool was inserted and tamped at

the bottom of the column. The gel slurry was prepared by mixing about 30 g of gel and 120 ml of n-hexane. It was introduced immediately into the column and the external wall of the column was tapped with a small rubber tube to affect the removal of air bubbles. The height of the adsorbent was about 12 cm. Care must be taken not to dry the adsorbent while the experiment was carried out. The top was closed as soon as the solvent level in the column reaches the adsorbent layer. Then the concentrated crude extract was dissolved in a minimum amount of EtOAc and introduced into the column along the wall by the use of a micro pipette.When the sample had just reached the adsorbent, pure sand was added on the solute to obtain a sand layer of 1 cm in thickness. The eluting solvent (n-hexane) was poured into the column, the tap was opened and adjusted the flow rate. As the layer began to separate and move down the column, a small dry bottle was placed under the tap of the column to collect the eluent. About 2 ml of each fraction was collected in small bottles by using the eluting simultaneously in the order as mentioned above. 311 fractions were collected. Each and every fraction was checked by TLC. Fraction V and VI showed one spot on TLC and UV active.

Thin Layer Chromatograms of Pure Compound I and II

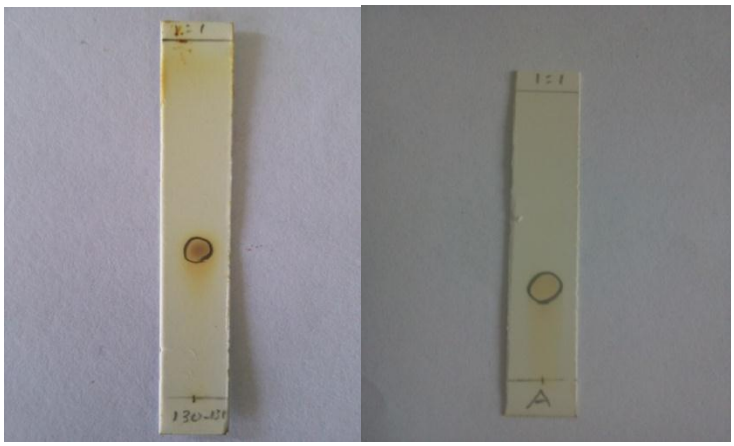


Table :R_f Values of Compound I and II

Compound	R_f	Solvent system(n-hexane : EtOAc)
Compound I	0.4	1: 1
Compound II	0.32	2:3

In this study, only pure isolated compound I was study to identify functional groups, to measure melting point and to perform additional test such as polyphenol test.

Identification of Functional Groups of Isolated Pure Compound I by FT-IR Spectroscopy

The isolated pure compound I was identified by FTIR spectroscopic studies. The FT-IR spectral data are tabulated in table (5).

Determination of Melting Point of the Isolated Compound I

The melting point of the isolated compound I was determined by melting point apparatus.The melting point value is described in table (6).

Polyphenol Test for Isolated Pure Compound I

Isolated pure compound was dissolved in 5 m of EtOH in a test tube. When each of 5 drops of 1% $FeCl_3$ and 1% $K_3[Fe(CN)_6]$ solution was added, green color solution was obtained.

Results and Discussion

According to phytochemical tests, the chemical constituents of *M. hortensis* L.f. are described in table 1.

Table (1) Results of the chemical constituents of *Millingtonia hortensis* L.f.

No	Test	Reagent	Observation	Inference
1	Alkaloid	Wagner's solution	Redddish brown ppt	+
2	Glycoside	10 % lead acetate	White ppt	+
3	Phenolic	10 % $FeCL_3$	Blue black color solution	+
4	Reducing sugar	Benedict's solution	No red ppt	-
5	Tannins	10 % $FeCL_3$dilH_2SO_4	Yellowish brown ppt	+
6	Saponin	$NaHCO_3$	Froth like comb	+
7	Lipophenol	0.5 KOH, 4drops of NaOH	Deep color solution	+
8	Flavonoid	conc: HcL, Mg turning	Pink color ppt	+
9	Steroid	Acetic anhydride, conc; H_2SO_4	Green color solution	+
10	Terpene	Acetic anhydride conc; $H_2SO_4CHCL_3$	Geenish color solution	+
11	Polyphenol	1 % $FeCL_3$ and 1% K_3 {Fe (CN_6)}	Greenish color solution	+

(+) = presence (-) = absence

From the observation of phytochemical tests, it can be seen that *Millingtonia hortensis* L.f**.** contain several chemical constituents as shown in above table.
The mineral compositions of the sample are recorded in table (2).

From the determination of elemental compositions, the results of metal compositions are tabulated in table (2).

Table (2) The Elemental Compositions of Crude Sample by EDXRF

Symbol	Element	Concentration (%)
Ca	Calcium	1.429
K	Potassium	1.083
Si	Silicon	0.7560
Al	Aluminium	0.2761
Fe	Iron	0.2010
Cl	Chlorine	0.1429
P	Phosphorus	0.0839
S	Sulfur	0.02592
Ti	Titanium	0.01605
Ba	Barium	0.01250
Sr	Strontium	0.01539
Cu	Copper	0.00671
Rb	Rubidium	0.00296
Zn	Zinc	0.00244

From theEDXRF report, it can be recorded that the*Millingtonia hortensis* L**.**f. is rich source of minerals for health benefit especially potassium and calcium. Meanwhile, it is also known that the toxic metals such as lead, mercury are not present in this sample.

The results of antioxidant activity of the stem bark of the sample and that of ascorbic acid are described in table (3).

Table (3) Antioxidant Activities on the Stem Bark of *Millingtonia hortensis* L.f.

SampleConcentration(mg/ml)	Absorbance	% inhibition	IC_{50} mg/ml
50	**0.168**	**82.80**	**8.23**
25	**0.279**	**71.44**	
12.5	**0.400**	**59.06**	
6.25	**0.506**	**48.21**	
3.125	**0.608**	**37.77**	

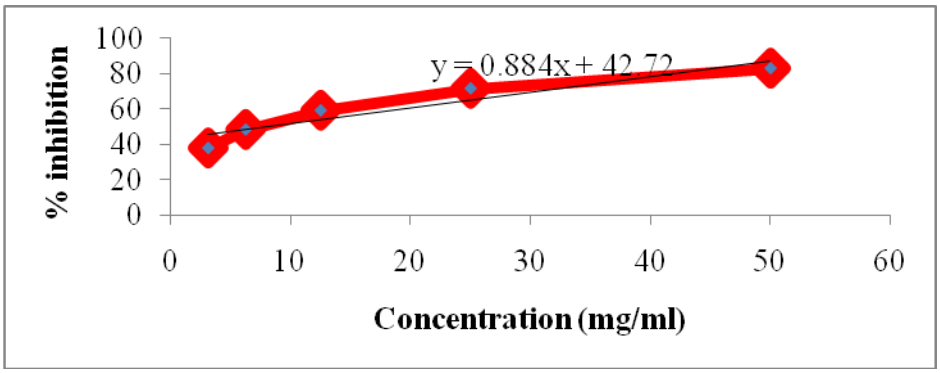


Figure : Plot of % Inhibition Vs Concentration of Standard Ascorbic Acid

Table (4) % Inhibition of various concentration of sample

Concentration (mg/ml)	Mean Absorbance	Mean % inhibiton	IC_{50}
400	0.445	52.35	342.98
300	0.472	49.46	
200	0.586	37.96	
100	0.781	16.38	

According to the observation of IC_{50} value of the sample, the tested sample shows antioxidant activity but its activity is not as good as standard ascorbic acid having IC_{50}value 8.23 µg/mL.

The FTIR spectrum of isolated pure compound is as shown in the following.

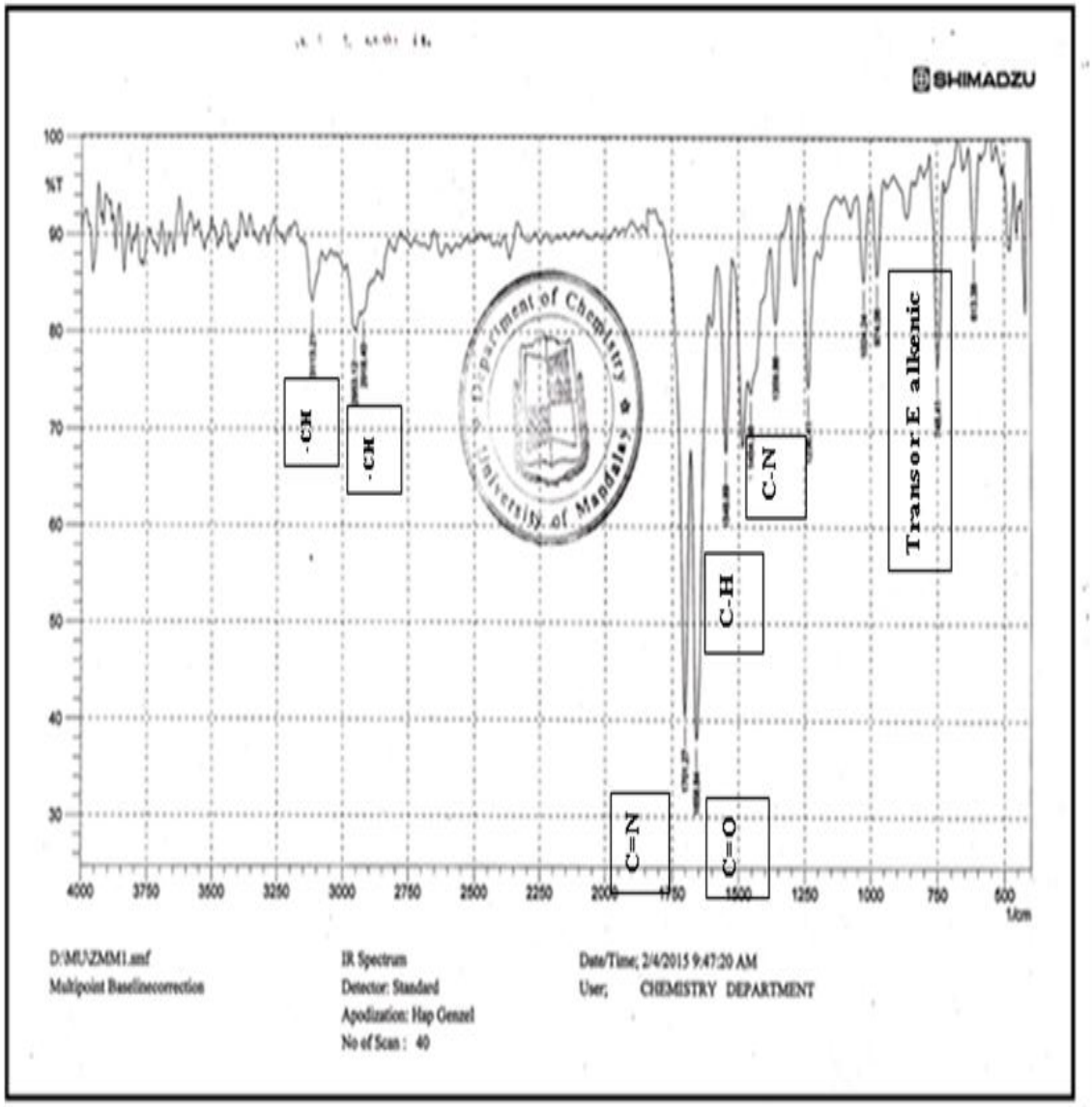


Figure (3) FTIR spectrum of isolated pure compound

The observed FTIR spectral data are tabulated in the following table (5).

Table (5) Comparison of FT-IRspectral dataof Isolated Pure Compound

No	**Assignments of Isolated Pure Compound**	Frequency (cm^{-1})
1	O – H stretching vibration of alcohol	3412.56
2	C – H stretching vibration of sp^2 hydrocarbons	3014.84
3	asymmetrical and symmetrical C – H stretching vibration of sp^3 hydrocarbons	2928.04, 2862.46
4	C=O stretching vibration of carbonyl group	1683.91
5	C = C ring skeletal stretching vibration of aromatic benzene ring	1602.90, 1518.03
6	OH in plane bending vibration of phenol group	1384.94
7	C – C – O stretching vibration of alcohol groups	1284.63, 1238.34
8	C – O – C stretching vibration of ether group	1111.03, 1028.09
9	C – H out of plane bending vibration of trans or E and cis or Z alkenic group	912.34, 765.77

FTIR spectral data of isolated compound I from the stem bark of *Millingtoniahortensi*s L.are nearly identical to that of PureHispidulin.

Confirmation of Pure Compound I by Melting Point Determination

The isolated pure compound can be identified by melting point determination. The observed melting point is compared with that of the reference compound in table (6).

Table (6) Comparison of melting Point of Isolated compound I and reference compound(Hispidulin)

Sample	**Melting Point (°C)**
Isolated compound	205**°C** - 206**°C**
Reference compound(Hispidulin)	207**°C**

Melting point of isolated compound was found to be (205ºC – 206 ºC). It agrees with reference compound Hispidulin.

Confirmation of Pure Compound I byColor Reaction Test for Polyphenol

The isolated compound could be confirmed by polyphenol test.

Figure : Color Reaction Test for Polyphenol

Conclusion

In this research work, Myanmar indigenous medicinal plant *Millingtonia hortensis*L.f. (Eigarit) was chemically investigated by solvent extraction and Thin Layer Chromatographic methods. According to preliminary phytochemical screening of selected sample, the stem bark of *Millingtonia hortensis* L.f. is rich in many chemical constituents.From the antimicrobial activity examination by Agar well diffusion method, the ethyl acetate extract of stem bark of *Millingtonia hortensis* L.f.responds the highest activities on all selected organisms such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans*and *E. coli*. But the n-hexane and ethanol extract of this plant sample have no activity on all selected organisms.According to EDXRF results, it can be known that the tested sample is composed by so many minerals.According to results of measuring antioxidant activity, it can be known that antioxidant activity of the sample (IC_{50}342.98 μg/ml) is notas good as standard ascorbic acid (IC_{50} 8.23 μg/ml). In addition, pure organic compound (12.5 mg, 0.42 %) could be isolated by Thin Layer and Column Chromatographic methods.The isolated pure organic compound was assumed to be Hispidulin according to polyphenol test, melting point determination and FTIR identification.

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