ANTIOXIDANT ACTIVITY, CHOLESTEROL LOWERING EFFECT AND CHEMICAL INVESTIGATION OF PO-SA (STEM)

Ei Ei Sann[1], Myint Myint Soe[2], Myint Myint Khine[3]

Abstract

Po-sa (*Morus alba* L.) plant possesses many useful biological activities which has been the reason for the selection of this plant for the present study. The present work is concerned with the detection of antioxidant and cholesterol lowering activities of Po-sa (stem). Antioxidant activity of Po-sa (stem) extracts was determined by using 1,1-Diphenyl- 2-picrylhydrazyl assay method. The IC_{50} values of EtOAc, EtOH and watery extracts of stem were 2.94µg/mL, 1.21µg/mL and 1.20 µg/mL, respectively. The EtOH and watery extracts of stem showed more radical scavenging activity than EtOAc extract. Cholesterol lowering activity of crude extracts of stem was also studied by Zlatkis, Zak and Boyle method on rats model. The cholesterol levels were determined after orally administrated rats with 600 mg/kg/day of plant extracts for 10 days. It indicated that watery extract of stem has the ability to reduce bad cholesterol (Triglyceride, Total cholesterol and Very low density lipoprotein) and it also could effectively increase the good blood cholesterol (High density lipoprotein) level. By using silica gel column chromatographic separation method, umbelliferone (0.001 %) and scopoletin (0.002 %) were obtained from the EtOAc extract of the stem. The isolated compounds were identified and elucidated by melting points and by using joint application of modern spectroscopic techniques such as UV-visible, FT IR, ^{1}H NMR and mass spectrometry, compared with the reported data. Umbelliferone and scopoletin isolated from Po-sa(stem) also showed radical scavenging activity determined by using semi-quantitative DPPH staining method. The evaluation of *in-vitro* antioxidant activity of isolated compounds showed up to 6.25 µg/mL.

Keywords: Antioxidant activity, cholesterol lowering activity, Umbelliferone, Scopoletin.

Introduction

Po-sa stem is well known traditional medicinal plant and it is widely used for the treatments of Myanmar Traditional Medicinal formulation. In the

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present work, *Morus alba* L. (Po-sa) was chosen to investigate some biological activities such as antioxidant and hypocholesterolemic activities.

It belongs to one of ten species in the genus *Morus*, known as the common mulberries, which more or less juicy fruits, native to temperate Asia and North America. Leaves are known to possess triterpenes, bioflavonoid, coumarins, volatile oil, alkaloids, amino acids and other organic acids (Banskota *et al*., 2001). Leaves are a good source of ascorbic acid and rich in calcium (Wealth of India, 1962). Stem possesses antirheumatic, antispasmodic, diuretic, hypotensive and pectoral activities. They are used in the treatment of rheumatic pains and spasms, especially of the upper half of the body, high blood pressure. A tincture of the bark is used to relieve toothache. The branches are harvested in late spring or early summer and are dried for later use (Kim *et al*., 1999). A fiber is obtained from one-year old stem, it is used in weaving clothes etc. The stem bark is fibrous and is used in China and Europe for paper making.

Materials and Methods

Plant Material

Morus alba L. (Stem) was collected from Pyin Oo Lwin Township, Mandalay Region. The sample was washed with water and dried at room temperature. The dried samples were ground to get a fine powder. The drug powder were then stored in an air-tight container.

Preparation of Crude extracts

The powdered sample (800) g of Po-sa (Stem) was extracted with 80 % EtOH. The filtrate was evaporated to get 30 g of crude extract. And then partitioned between PE and EtOAc and 2 g of PE and 9 g of EtOAc crude extracts were obtained.

Screening of Biological Activities

(a) Antioxidant activity test

For the examination of *in vitro* antioxidant activity of Po-sa (stem), DPPH staining method and spectrophotometric method were used. In DPPH staining method, drop of each sample were loaded on TLC plate in order of

decreasing concentration along the row. The sheet bearing the dry spots was stained with DPPH solution. The stained of silica layer revealed a purple background with spots showed radical scavenger capacity (Soler-Rivas *et al.,* 2000). In spectrophotometric method, the sample solutions were measured by using spectrophotometer.

(b) Screening of hypocholesterolemic activity

The examination of *in vivo* hypocholesterolemic activity of crude extracts was done by Zlatkis *et al.* method (1953) in rat model. There were nine rats which were divided into three groups, each contained three animals. After ten days duration, the blood was collected from the tail of rats to determine the serum (Triglyceride, Total cholesterol and Low density lipoprotein) cholesterol levels.

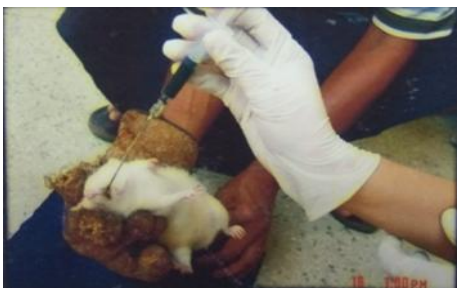

Figure 1: Photograph of administration of samples to male winstar strain rat by oral route

Isolation of compounds from Po-sa (Stem)

The ethyl acetate extract (9 g) was chromatographed on a silica gel column using PE: EA (2:1v/v) solvent system and three fractions were collected by checking TLC behavior. Compound 1 was obtained from column chromatograph of fraction I with solvent system CH_2Cl_2: EA (65:1v/v). Compound 2 was obtained from fraction II eluting with PE : CH_2Cl_2 : EA solvent system (1:20:0.5v/v). The isolated compounds were characterized by melting point determination and TLC, UV-vis, FT IR, ^{1}HNMR and mass spectroscopic methods.

Results and Discussion

DPPH radical scavenging activity by spectrophotometric method

The antioxidant activity of EtOAc, 80 % EtOH and H_2O extracts was tested according to DPPH radical scavenging activity assay by using spectrophotometric method. The H_2O extract was found to be more potent than the other extracts compared with standard BHT (Table 1). From these results, increase in concentration showed increase in percent inhibition, i.e. increase free radical scavenging activity. The lower IC_{50} value indicates the greater antioxidant activity.

Table 1: Oxidative Inhibition % in Various Concentrations and IC_{50} Values of Extracts of Po-sa (Stem)

Extracts	% Inhibition in various concentrations (µg/mL)					IC_{50} (µg/mL)
	0.625	1.25	2.5	5	10	
Po-sa -EtOH (stem)	40.61	50.64	60.85	69.27	84.00	1.21
Po-sa-H_2O (stem)	28.01	51.97	62.48	70.06	78.61	1.20
Po-sa-EtOAc (stem)	28.18	37.52	48.06	59.00	77.33	2.94
BHT	14.04	54.82	74.22	77.13	87.40	1.17

BHT = Butylated Hydroxy Toluene

Hypocholesterolemic activity

In cholesterol lowering activity, 80 % EtOH and watery extract of Po-sa (stem) could respectively reduce 8.84 % and 9.27 % of total cholesterol after ten days duration when treated with 600 mg/kg/body weight/day dose. Both of the 80 % EtOH and watery extracts did not only decrease the serum total cholesterol, triglyceride, very low density lipoprotein and low density lipoprotein cholesterol levels, but also they could raise the good cholesterol, high density lipoprotein levels (Table 2). Watery extract was the most effective in lowering triglyceride as well as very low density lipoprotein and the higher ability to increase high density lipoprotein cholesterol. The 80 % EtOH extract was the highest potency to reduce low density lipoprotein cholesterol level.

Table 2: Effect of Po-sa (Stem) Crude Extracts on Blood Cholesterol in Rat Models

Samples	TC (Decreasing %)	TG (Decreasing %)	VLDL-C (Decreasing %)	LDL-C (Decreasing %)	HDL-C (Increasing %)
80% EtOH extract PSS	8.84	5.1	5.56	19.62	2.56
H_2O extract PSS	9.27	15.63	14.29	12.93	9.09
Distilled water	-	-	-	-	-

PSS = Po-sa stem
TC = Total Cholesterol
TG = Triglyceride
VLDL = Very low density lipoprotein
LDL = Low density lipoprotein
HDL = High density lipoprotein

Identification of the Isolated Compounds

Compounds 1 (6 mg, 0.001 %) and compound 2 (13.8 mg, 0.002 %) were characterized by physical and chemical methods and then identified by spectroscopic techniques.

Compound 1 (Umbelliferone): MS m/z; 161 (M-H), 133.1, 92.9. UV λ_{max}^{MeOH}nm; 220, 329; UV $\lambda_{max}^{MeOH+NaOH}$nm : 221, 363. FT IR υ_{max}^{KBr} cm^{-1}: 3398 (υ_{O-H}), 1705 ($\upsilon_{C=O}$), 1616-1562 ($\upsilon_{C=C}$), 1234-1053 ($\upsilon_{asy-C-O}$), 840 ($\delta_{oop-C-H}$). ^1H NMR δ (ppm) 6.2 (3H, d, J=9 Hz), 8.0 (4H, d, J=9Hz), 7.3 (5H, d, J=8Hz), 6.9 (6H, dd, J=8 Hz), 6.8 (8H, d, J=1 Hz). All of these chemical shifts are very similar to those in the spectrum of umbelliferone (Harborne, 1941; Mya Thandar Aung, 2007).

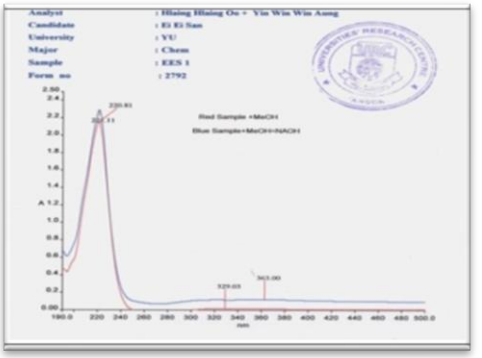

Figure 2: UV spectrum of the isolated compound 1
Solvent: (a) MeOH
(b) MeOH+NaOH

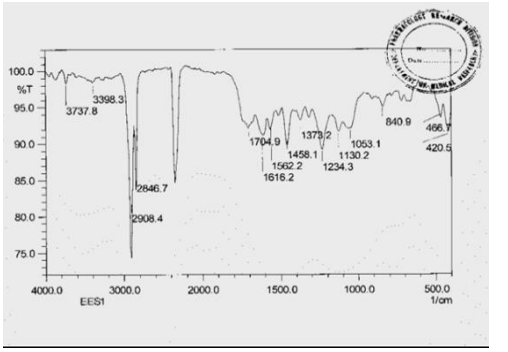

Figure 3: FT IR spectrum of isolated the compound 1(KBr)

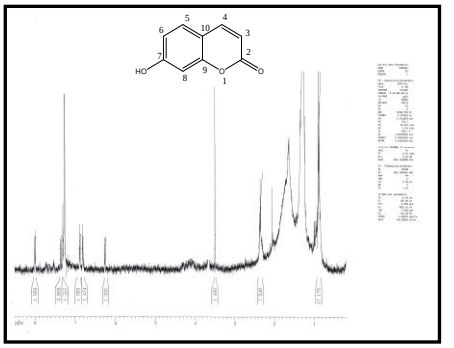

Figure 4: ^{1}H NMR spectrum of the isolated compound 1

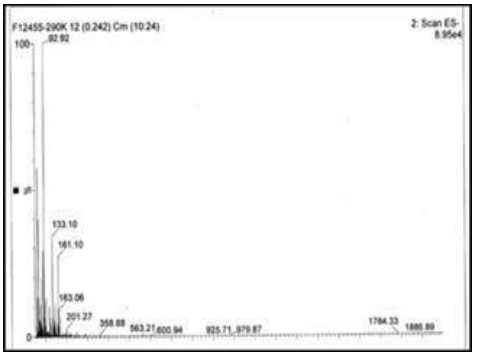

Figure 5: ESI-mass spectrum of the isolated compound 1

Compound 2 (Scopoletin): Colourless crystal, m.p. 201 °C. UV λ_{max}^{MeOH}nm: 215, 344; UV $\lambda_{max}^{MeOH+NaOH}$ nm: 218, 390. FT IR υ_{max}^{KBr}cm^{-1}: 3325 (υ_{O-H}), 3055 (υ_{C-H} of C=CH), 2920 (υ_{C-H} of –CH_3, CH_2), 2850 (υ_{C-H} of –OCH_3), 1705 ($\upsilon_{C=O}$) of δ lactone, 1604($\upsilon_{C=C}$ of aromatic ring). ^{1}H NMR δ(ppm): 6.2 (3H, d, J = 9 Hz), 7.6 (4H, d, J= 9Hz), 6.8 (5H, S), 6.9 (8H, S), 3.7 (6H-OMe, S). All these spectral data of compound 2 were agree with the reported data of scopoletin (Merck index, 2001), (Harbone, 1993), (Khin Tar Yar Myint, 2010).

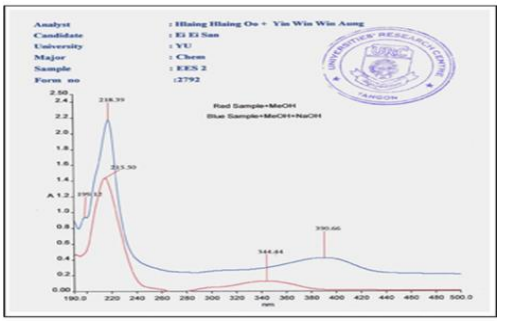

Figure 6: UV spectrum of isolated the compound 2 Solvent: (a)MeOH (b) MeOH+NaOH

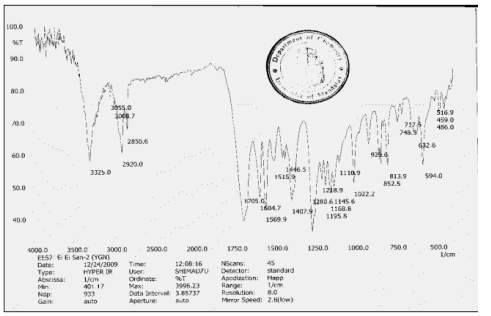

Figure 7: FT IR spectrum of the isolated compound 2(KBr)

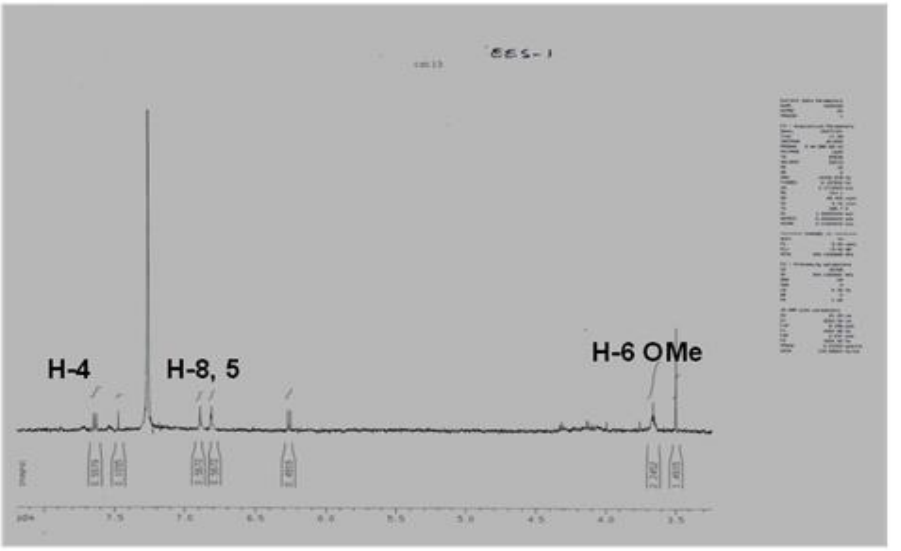

Figure 8: ^{1}H NMR spectrum of isolated compound 2

Rapid screening of antioxidant activity of two isolated compounds by DPPH staining method

It was observed that compounds 1 and 2 from *Morus alba* Linn. (stem) showed antioxidant activity on the TLC plates. After staining, white spots with strong intensity was found by staining with the amount of 12.5 µg of dry matter for compounds (Figure1). The intensity of white colour depends upon the amount and nature of radical scavenger present in the sample.

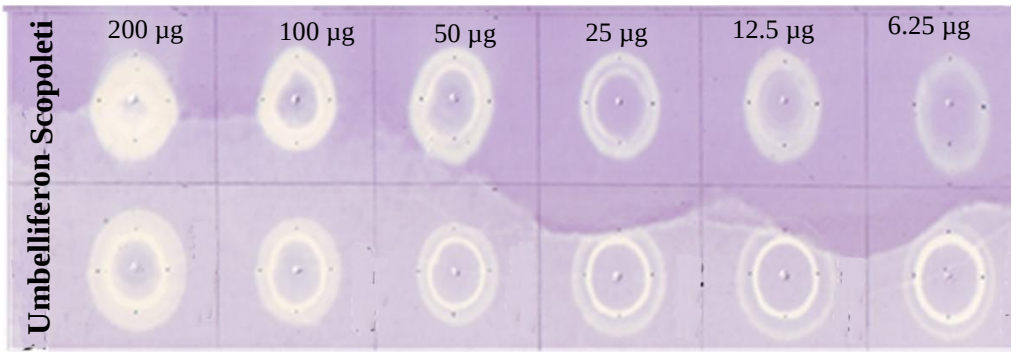

Figure 9: Screening of antioxidant activity of the isolated compounds from Po-sa (stem) by DPPH Dot-Blot assay

Conclusion

The results of this study, Po-sa (stem) was found to possess many pharmacological activities such as antioxidant, antidiabetes, antimicrobial and hypocholesterolemic activity. From the experimental results, the isolated compounds umbelliferone and scopoletin showed the radical scavenging activity by using DPPH staining method. EtOH, watery and EtOAc extracts showed antioxidant activity with IC_{50} values (1.20-2.94 µg/mL). The radical scavenging activity of watery extracts is more potent than EtOH, and EtOAc extracts. The hypocholesterolemic activity, the watery extract of Po-sa (stem) has the ability to reduce bad cholesterol (Total cholesterol, Triglyceride and Very low density lipoprotein) and it also could effectively increase the good blood cholesterol (High density lipoprotein) level. Umbelliferone (0.001 %) and scopoletin (0.002 %) were obtained from ethyl acetate extract of Po-sa (stem) by column chromatography on silica gel using PE:EA (2:1). The isolated compounds containing the coumarin skeleton showed the radical scavenging activity. The isolated compounds were identified by TLC, UV, FT IR, ^{1}HNMR and mass spectroscopic methods. They are also confirmed by melting point determination and compounds with reported data.

Acknowledgements

The authors would like to thank the Department of Higher Education, Ministry of Education, Yangon, Myanmar, for the permission of doing this research. We would like to express our deepest gratitude to Dr Tin Tun, Rector and Dr Mar Lar, Pro-rector, Taungoo University, for their permission to do this research. Special thanks are extended to Professor and Head, Dr Mi Mi Kyaing and Professor Dr Nay Mar Soe, Department of Chemistry, Taungoo University, for their valuable advices and kind encouragement and for providing research facilities.

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