Antioxidant, Acute Toxicity Activities and Identification of Isolated Compounds from *Dichroa febrifuga* Lour.

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Abstract

From this research work, firstly, the preliminary phytochemical constituents of: carbohydrates, alkaloids, α-amino acids, phenolic compounds, steroids, saponins, terpenoids, flavonoids, glycosides, and tannins were found to be present in dry roots of *D.febrifuga*L.(Yin-pyar). According to the physiochemical analysis 5.36 % of moisture, 11.52 % of ash, 31.12 % of protein, 18.29 % of fiber, 1.41 % of fat, 27.23 % of carbohydrate and 235 kcal/100 g of energy value were found by AOAC method respectively. The collected sample was contained in Ca (126.48 ppm), Mg (121.21 ppm), Fe (17.54 ppm) determined by atomic absorption spectrophotometry method. Besides, the other toxic elements: Cd, Cu, Mn, As and Pb were not detected. The watery showed the antioxidant activity (IC_{50}= 27.18 μg/mL) higher than ethanol extract (IC_{50}= 48.26 μg/mL), determined by DPPH radical scavenging assay method. Both of watery and ethanol extracts did not show acute toxic effect up to 5000 mg/kg body weight dose on albino mice. Furthermore, from the separation of silica gel column chromatographic method, febrifugine compound: (0.05%, 138-140ºC, R_f = 0.43) was isolated from chloroform extract of *D.febrifuga* L. The isolated compound **C,** febrifugine was identified by UV visible, FTIR,[1]HNMR and [13]CNMR spectroscopy.

Keywords*: : D febrifuga*L*. , febrifugine,* antioxidant, acute toxicity

INTRODUCTION

Dichroa febrifuga **L. (Yin -pya)**

*Dichroafebrifuga*Lour.is a flowering plant in the family Hydrangeaceae. The active ingredients are febrifugine and isofebrifugine. Roots are harvested when 3-4 years old (Haruhisa, *etal.,*2006). The alkaloids febrifugine and isofebrifugine have been isolated from the leaves and roots of the plant (Kim,*etal*.,2000).

(Rasonarivo, *etal.,*2011). According to traditional medicine, these side effects, especially nausea and vomiting, may be controlled by heating the drug with a little vinegar, and by combination with other drugs as indicated in classic formulas (Ram, *et al.,*1980). In Myanmar, it is distributed especially in Pyin-Oo-Lwin area, Mandalay Region, Kachin State and Shan State.

MATERIALS AND METHODS

Sample Collection, Phytochemical Test and Nutritional Values

The young roots of *D. febrifuga*L.(Yin-pyar) samples were collected from Pyin-Oo-Lwin area, Mandalay Region and identified at the Department of Botany, University of Yangon. In order to find out types of phytoorganic constituents present in the sample, preliminary phytochemicals tests were carried out according to the appropriate reported methods (Harbone,1984).The moisture,the ash, the fat, the protein

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bymacro Kjeldahl method and the fiber content the energy value were deter-mined by AOAC method (AOAC,1990).The preparation of *D. febrifuga*sample solution was ready for analysis of mineral elements by AAS.

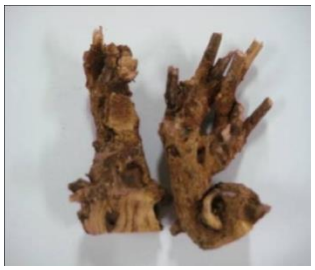


Figure 1. Photograph of root of*D. febrifuga*

Screening of Antioxidant and Acute Toxicity Activities of *D. febrifuga*

Antioxidant activity of 95 % ethanol and watery extracts of *D. febrifuga* were carried out by DPPH (1,1-Diphenyl, 2-Picryl, Hydrazyl) radical scavenging as say using UV visible spectrophotometer (Halliwell,2012). Then, IC_{50} (50 % oxidative inhibitory concentration) values were also calculated by linear regressive excel program (Kahlonene, 1999). The test sample of different concentrations of EtOH and watery extract of *D. febrifuga*were used *in vivo* by using OECD guideline 425 method. In this procedure, 18 albino rats were taken and divided into 6 groups (3 in each group) (Group I,II,III,IV,V,VI). Then, Group I,II,III were treated with watery extract as well as IV,V,VI were ethanol extract in different concentration by using a stomach tube or a suitable intubation cannula. The dose level to be used as the starting dose was selected from one of four fixed levels, 175, 550, 1750 and 5000 mg/kg body weight.

Isolation and Characterization of Chloroform Extract by Using Silica Gel Column Chromatographic Method

The dried powdered sample of *D.febrifuga*(500g) bark was extracted with MeOH about one week three times in percolation method and filtered. Then MeOH crude extract was suspended in 0.1M HCl solution for acid hydrolysis until the pH of extract became less than 3 and then the HCl suspension was partitioned with chloroform for 5 times to remove impurities. The aqueous HCl portion was collected and adjusted to pH 9.5 with 1M NaOH solution. Then this solution was extracted successively with chloroform for 20 hours. Finally the combined chloroform extract solution was concentrated by rotary evaporator to obtain 3g of total alkaloid portion. This alkaloid portion was separated to fractions by column chromatography eluted with $CHCl_3$: MeOH in proportion of 6:1, (v/v). After checking with TLC, the fractions F_{65-93}(compound C), were successively isolated. Then the chromatogram was sprayed with 5% H_2SO_4 followed by heating, LibermannBurchard followed by heating, anisaldehyde/sulphuric acid followed by heating, 10% $FeCl_3$ and Dragendorff's reagent. The observed colouration were denoted.The isolated compound was identified by modern spectroscopic techniques such as UV-Visible ,FT-IR,^{1}HNMR and ^{13}CNMR spectroscopy (Markhan, 1982).

RESULTS AND DISCUSSION

Sample Collection,Phytochemical Test and Nutritional Values of *D. febrifuga*

According to these experiments, alkaloids, α-amino acid, carbohydrates, glycosides, flavonoids, phenolic compounds, saponins, steroids, tannins and terpenoids were found to be present but starch was not detected in collected sample, *D. febrifuga*.In this sample, the protein content (31.12%) was observed to contain the highest amount. In addition, carbohydrate (27.23%) and fiber (18.29.%) were also found to be higher than the other nutrient, moisture (5.36%) and ash (11.52%). The fat content (1.41%) was found to be the lowest amount in *D.*

febrifuga. The energy value was observed to be 235 kcal/100g. Mineral elements present in dried powder of young shoots from *D. febrifuga*were determined by Atomic Absorption Spectrometer (AAS). Ca (126.48 ppm) and Mg (121.21) were found to contain as major constituents but Fe (17.54 ppm) as trace elements.

Antioxidantand Acute Toxicity Activities *D. febrifuga*

By using DPPH free radical scavenging assay, the watery extract of *D. febrifuga* was more potent antioxidant activity than 95% ethanol extract.In acute toxicity test, there is no lethality at the dose of 5000 mg/kg b.w of the extracts and LD_{50} was supposed to be more than 5000 mg/kg b.w. It can be concluded that the ethanoic and watery extracts of *D.febrifuga*were performed practically nontoxic.

Isolation, Characterization and Identification of Isolated Compound from Chloroform Extract by Using Silica Gel ColumnChromatographic Method

The chloroform extract of (3 g) was separated by silica gel column chromatogyphy and one alkaloid and two terpenoid compounds were obtained. The fractions F_{65-93} compound C was obtained as needle shaped crystal (0.05%). The isolated compound C was recrystallized by acetone, The isolated compound **C** could be detected as a dark yellow fluorescence on TLC under UV-365 nm light and appeared as an orange zone immediately on spraying with Dragendorff reagent,the (R_f=0.43),(138-140°C). Therefore, the compound C may be alkaloid compound.The physicochemical data of isolated compound was identical with those of febrifugine in Merck index (2001). From the UV-visual spectroscopic study of isolated compound, it was observed that the maximum absorption wavelength (λ) were at 225, 264 and 310 nm. The uv spectrum of isolated compound was showed in Figure 1**.** It suggests that the compound **(C)** consists of conjugated system.

According to FT-IR spectrum in Figure 2, the presence of NH stretching vibration appeared at 3510 cm^{-1}, the alcoholic OH stretching at 3335 cm^{-1} and OH bending at 1365 cm^{-1}. The band at 2931 cm^{-1} and 2862 cm^{-1} showed the presence of asymmetric and symmetric CH stretching for CH_2 group in isolated compound C. In addition C=C stretching vibration of aromatic ring appeared at 1618cm^{-1} to 1458cm^{-1} and C-OH stretching vibration at 1272 and 1188 cm^{-1} were also present in isolated compound **(C)**. According to ^{1}H NMR spectrum, the number of protons of the active molecule could be identified. As result of the ^{1}H NMR spectrum (400 MHz) (Figure 3) and spectral data, there were nineteen protons included in isolated compound **(C)**. The one proton at 9.65 ppm attributed to hydrogen from NH group. The four protons of aromatic ring (H-5, 6, 7, 8) appeared at δ 8.27, 7.55, 7.69 and 7.65 ppm. The chemical shift value of 3.86 ppm suggested the presence of one hydrogen atom from alcoholic OH-group.According to ^{13}C NMR spectrum, the number of carbon associated in the active molecule was determined. ^{13}CNMR spectrum of isolated compound **(C)** was showed in Figure 4. Total of sixteen carbonswere observed in the spectrum. The peak at δ 161.90 ppm was due to carbonyl group which suggested the presence of 4 - quinazolone. The signal at δ 202.47 ppm was assigned as ketonic signal (carbonyl group C-2´). The signal at 144.95 ppm represents the olenfinic carbon (C-2). In addition the peaks of 124.18 and 150.20 ppm were due to two quaternary carbons (C-4a and C-8a). The benzene CH carbons (C-5, 6, 7, 8) were observed at 128.57, 127.27, 134.10 and 126.17 ppm. The remaining 7 carbon atoms were assigned as methylene (CH) and methine (CH)carbon atoms.

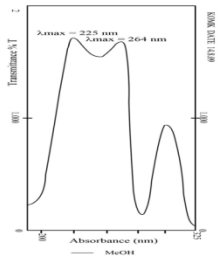
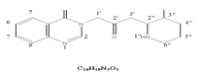


Figure1 Ultraviolet spectrum of isolated active compound C from *D. febrifuga*



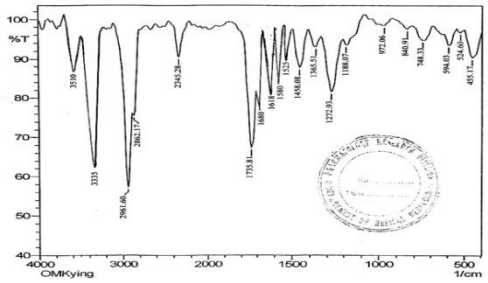


Figure 2FT IR spectrum of isolated active compound C from root of *D febrifuga*

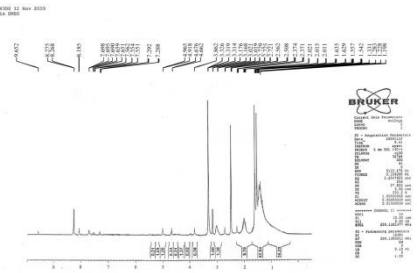


Figure 3 ^{1}H NMR spectrum of isolated active compound C from root of *D febrifuga*

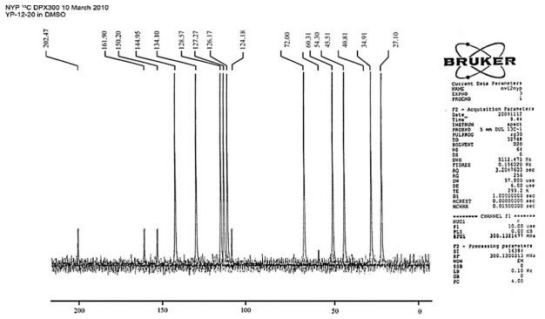


Figure 4 ^{13}C NMR spectrum of isolated active compound, C from root of*D febrifuga* L.

CONCLUSION

From this study ,the phytochemical investigation of selected sample was found to contain α-amino acid, carbohydrate, flavonoids, glycosides, phenolic compounds, alkaloids, reducing sugar, saponins, steroids and tannins. Starch was not observed in it. The identification of active principal compound **(C)** was obtained from the data of UV, IR, ^{1}H NMR and ^{13}C NMR spectroscopic studies. The maximum absorption at the wavelength (λ_{max}) 225, 264 and 310 nm were observed from the UV-visual spectroscopic study of isolated compound (C). It suggests that the compound (C) consists of conjugated system. According to the FT-IR spectrum in the presence of NH stretching vibration , the alcoholic OH stretching and OH bending, the presence of C=O group, C=C stretching vibration of aromatic ring and C-OH stretching vibration were denoted in isolated compound **(C)**. In this study by using NMR spectroscopy number of protons and chemical structure of active compound were determined. As result of the ^{1}H NMR spectrum (600 MHz) and spectral data, there were nineteen protons included in isolated compound. The one proton at 9.65 ppm attributed to hydrogen from NH group. The four protons of aromatic ring (H-5, 6, 7, 8) appeared at δ 8.27, 7.55, 7.69 and 7.68 ppm. The chemical shift value of 3.86 ppm suggested the presence of one hydrogen atom from alcoholic OH-group. Moreover, ^{13}CNMR spectrum described a total of 16 carbons which consist of carbonyl groups (C-H), ketonic carbon (C-2´), olefinic carbon (C-2), two quaternary carbons

(C- 4a and C- 8a), methylene (CH) and methine (CH) carbon atoms. Characterization of active principal by UV spectra, FT IR spectrum, ^{1}HNMR and ^{13}C NMR spectrum in addition to physical properties exhibited that the isolated active principal was identical with febrifugine, a known compound of *D.febrifuga* L.

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