ISOLATION OF BIOACTIVE COMPOUNDS FROM BARK OF *MIMUSOPS ELENGI* ROXB. (KHA-YAY) USED IN THE TREATMENT OF HYPERGLYCEMIA

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Abstract

The present research focused on the investigation of antihyperglycemic activities of two crude extracts (70 % ethanol and water) and isolated organic compounds from bark of *Mimusops elengi* Roxb. (Kha-yay) by using adrenaline induced hyperglycemic rats. The optimum antihyperglycemic activities of two extracts showed 70 % ethanol extract (30.11 % reduction, $p < 0.01$, 1 h and 35.25 % reduction, $p < 0.01$, 2 h) and watery extract (19.94 % reduction, $p < 0.01$, 1 h and 21.61 % reduction, $p < 0.01$, 2 h) after treating with 2 g/kg body weight dose. Lupeol (0.0037 %, m.p- 215-217 °C) and spinasterol (0.0048 %, m.p- 167-169 °C) were isolated from pet-ether soluble portion of 70 % ethanol crude extract by column chromatographic method using pet-ether and ethyl acetate with increasing polarity ratio as eluent. Both compounds were identified by modern spectroscopic methods (UV, FT IR and ^{1}H NMR) and confirmed by comparison with the data reported in literature. Reduction percent of blood glucose levels of lupeol and spinasterol with a dosage of 2 mg/kg body weight showed 19.44 % ($p < 0.01$) and 22.01 % ($p < 0.01$) at 2 h respectively.

Keywords: *Mimusops elengi* Roxb., antihyperglycemic activities, lupeol, spinasterol

Introduction

Diabetes mellitus (DM) is a clinical syndrome characterized by hyperglycemia (high blood sugar level), due to deficiency or diminished effectiveness of insulin. It is the most frequently encountered metabolic disease in our societies today (Cho *et al*., 2018). Its prevalence has increased dramatically in recent years and it considered by WHO as a serious public health problem. According to IDF estimation, nearly 451 million people were suffering from diabetes in 2017 worldwide. This number is projected to reach 693 million by 2045 if current growth rate continues. It is a serious disease that can have a significant impact on the health, quality of life, and life expectancy of individuals, as well as on the health care system (Steven and Robert, 2001). The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and failure of various organs especially the eyes, kidneys, nerves, heart and blood vessels. Prevention and treatment involve maintaining a healthy diet, regular physical exercise, a normal body weight and avoiding use of tobacco. Control of blood pressure and maintaining proper food care are important for people with the disease.

Medicinal plants have been discovered and used in traditional medicine practices since prehistoric times. Plants synthesis hundreds of chemical compounds for functions including defense against insects, fungi, diseases, and herbivorous mammals (Ahn, 2017). Kha-yay is one of Myanmar medicinal plants. Botanical name is *Mimusops elengi* Roxb. (Sapotaceae family). English common names include as Spanish cherry, medlar, star flower and bullet wood. It has fragrant flowers and is planted as an ornamental and shade tree in gardens and along roads. Kha-yay contains variety of active phytoconstituents and thus possesses various kinds of biological

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and pharmacological activities. It possesses activities like antibacterial, antihemorrhoidal, antifungal, anticarciogenic, antioxidant, antineoplastic, antihyperglycemic, gastroprotective, antinociceptive , and diuretic effect, cognitive enhancing activity and cytotoxic activities (Gami, 2007). The bark can be used for cooling, cardiotonic, alexipharmic, stomachic, anthelmintic, astringent gargle and diarrhoea disease.

Materials and Methods

Sample Collection and Preparation

Bark of Kha-yay was collected from Yangon Region. The plant sample was identified at Department of Botany, Yangon University. The sample was thoroughly washed with water and cut into small parts. After being dried at room temperature for one week, the sample was made into powder and stored in air-tight container.

Preliminary Phytochemical Test

A few grams of dried powdered sample were subjected to the tests of steroids, terpenoids, flavonoids, glycosides, phenolic compounds, α-amino acids, carbohydrates, reducing sugars, saponins, tannins and alkaloids according to the standard procedures (Marini-Bettolo *et al*., 1981).

Preparation of Crude Extracts for Bioactivities

The dried powdered sample was percolated in 70 % ethanol and distilled water respectively. After one week, they were filtered and filtrates were concentrated to obtain 70 % ethanol and watery crude extracts.

Screening of Antihyperglycemic Activities of Crude Extracts

Healthy Wistar strain rats in male sex (250-300 g) were used in this experiment. Before the experiment, animals were kept fasting overnight for 18-20 h but were allowed free access to distilled water. The animals were divided into two groups (groups I and II) of five animals each and 70 % ethanol extract for group I and watery extract for group II were determined. All the rats were made diabetic by injecting them subcutaneously with standard dose 0.2 mg/kg body weight of adrenaline tartrate in distilled water using the method of Gupta *et al*. (1967). The blood glucose levels after injection of adrenaline were monitored in 1 h interval up to 4 h and the results were recorded. After one week, the blood glucose levels were again measured and the rats were made diabetic. Then, the blood glucose levels of each group were recorded hourly after administration of tested doses (2 g/kg body weight) from each extract by using superglucocard II and measured by blood glucose test meter (GT-1640).

The blood glucose levels were reported as mean ± Standard Error Mean (S.E.M). All results were evaluated statistically using student's t test by SPSS software. Probably level of less than 0.05 was considered significant.

Investigation of Acute Toxicity

In the present study, acute toxicity of different doses (2 g/kg, 4 g/kg and 8 g/kg body weight) of 70 % ethanol and watery extracts was evaluated by the method of Lorke (1983). The albino mice of both sexes (20-35 g) were assigned to seven groups with ten animals in each group. The animals were housed in standard cages with food and water at air conditioned room of 20 ± 5 ºC temperature with artificial light. Group I was treated with normal food and water and considered as control. Group II to IV mice were treated with 70 % ethanol extract and group V to VII mice were treated with aqueous extract orally. After oral administration of extracts, each group of mice was housed separately in a cage with free access to food and water. Observation and survivors were also observed for a total of 7 days.

Separation and Isolation of Organic Compounds

80 g of dried powdered bark was extracted with 70 % ethanol by Soxhlet extraction method for 15 h. This procedure was repeated for nine times. The combined solvents were evaporated under reduced pressure by means of a rotary evaporator and obtained ethanol extract. The ethanol extract was suspended in water and partitioned with pet-ether. Removal of the solvent from combined pet-ether layer provided pet-ether extract (6.33 g). 5 g of pet-ether extract was separated by column chromatography over silica gel (40 g) using pet-ether and ethyl acetate with increasing polarity ratio as eluent to yield 59 fractions. Each fraction obtained by column chromatography was checked by TLC. From the inspection of TLC chromatogram, the fractions of the same R_f values were combined to give 5 fractions. The fraction 2 was further purified by pet-ether and ethyl acetate (1:1 V/V) providing lupeol (26.26 mg, 0.0037 %). The fraction 5 was purified by pet-ether to provide spinasterol (34.51 mg, 0.0048 %). The structures of isolated compounds were identified by melting point determination and chemical methods such as behaviour on TLC using appropriate solvent systems and spraying reagents. In addition, spectroscopic methods such as ultraviolet, infrared and ^{1}H NMR were also employed. Furthermore, in some cases, identification was confirmed by co-TLC with authentic sample in various solvent systems and comparing with ACD Labs software.

Screening of Antihyperglycemic Activities of Isolated Compounds

Antihyperglycemic activities of isolated compounds were also investigated with a dosage of 2 mg/kg body weight according to the procedure mentioned in the previous section. Two groups (III and IV) of five animals each were divided and lupeol for group III and spinasterol for group IV were used in this experiment.

Results and Discussion

The phytochemical investigation showed the presence of steroids, terpenoids, flavonoids, glycosides, phenolic compounds, α-amino acids, carbohydrates, reducing sugars, saponins, tannins and alkaloids in bark of plant sample.

For the study on antihyperglycemic activities, 70 % ethanol crude extract produced its antihyperglycemic effect at 1 h (30.11 %, $p < 0.01$) and 2 h (35.25 %, $p < 0.01$) (Table 1 and Figure 1 a). The percent inhibitions of blood glucose levels were 30.11 %, 35.25 %, 6.69 % and 4.84 % at one hour interval. The blood glucose levels of watery crude extract were significantly decreased at 1 h (19.94 %, $p < 0.01$) and 2 h (21.61 %, $p < 0.01$) compared with that of control

rats (Table 2 and Figure 1 b). The percent inhibitions of blood glucose levels were 19.94 %, 21.61 %, 15.30 % and 6.02 %. From these results, it may be deduced that 70 % ethanol extract exhibits more antihyperglycemic effect than watery extract. In the case of isolated compounds, the blood glucose levels were significantly reduced after 2 h oral administration of each compound where 19.44 % reduction ($P < 0.01$) for lupeol and 22.01 % reduction ($P < 0.01$) for spinasterol (Tables 3, 4 and Figures 2 a, 2 b). The percent reductions of blood glucose levels were 16.18 %, 19.44 %, 14.16 % and 11.74 % for lupeol and 13.45 %, 22.01 %, 18.52 % and 10.22 % for spinasterol. From this data, it was found that the isolated compounds from bark of selected plant possessed the antihyperglycemic potency.

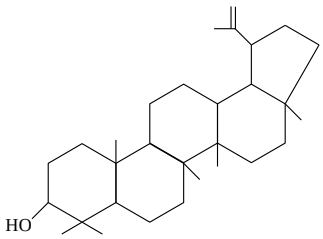
The acute toxicity screening for plant crude extracts (70 % ethanol and water) was done with the dosage of 2 g, 4 g and 8 g/kg body weight in mice. The condition of mice was denoted after one week treatment. All the animals remained alive and did not show any visible symptoms of toxicity like restlessness, respiratory disorders, convulsion, aggressive activities, coma and death at the tested dosage. So, the median lethal dose (LD_{50}) was more than 8 g/kg body weight. From these results, it was found that both plant extracts were free from acute toxic or harmful effects under experimental condition (Table 5).

Identification of Isolated Organic Compounds

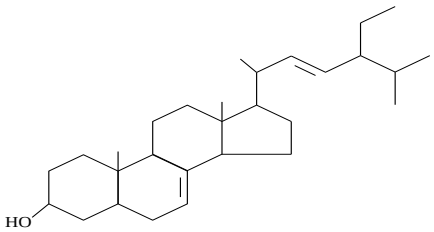
Lupeol ($C_{30}H_{50}O$) : colourless needle shape crystals, (R_f = 0.47, PE : EtOAC – 9:1 V/V), m.p. 215-217 °C (Lit. - 217°C, Merck Index, 2001). It was UV inactive. FT IR (KBr) ν_{max} (cm^{-1}): 3454 (O-H), 3070 (C=CH_2),2936, 2869 (C-H),1642, 1633 (C=C), 1461 (C-H), 1382 (CH_3), 1037 (CH-OH), 998 (C-H) : ^{1}H NMR (400 MHz,$CDCl_3$) (δ_H/ppm): 0.75-2.2 (47H,m,CH_3, CH_2, CH), 3.21 (1H,dd,-CH-OH), 5.11 (1H,d,=CH_2),5.51(1H,d,=CH_2) (Figures 3 and 4)

Spinasterol ($C_{29}H_{48}O$) : white amorphous powder (R_f = 0.46, PE : EtOAC – 2:1 V/V), m.p. 167-169°C (Lit. - 168-169°C, Merck Index, 2001). It was UV inactive. FT IR (KBr) ν_{max} (cm^{-1}): 3447 (O-H), 2940, 2867 (C-H), 1686, 1638 (C=C), 1451 (C-H), 1376 (CH_3), 1042 (C-OH), 882 (C-H) : ^{1}H NMR (400 MHz,$CDCl_3$) (δ_H/ppm): 0.65-2.5 (44H, m, CH_3, CH_2, CH), 3.20 (1H, m, CH-OH), 4.51 (1H, m, C=CH), 4.74 (1H, m, C=CH), 5.30 (1H, m, C=CH)

^{1}H NMR (ACD Labs software) (δ_H/ppm): 0.66-2.7 (44H, m, CH_3, CH_2, CH), 3.40 (1H, m, CH-OH), 5.01 (1H, m, C=CH), 5.17 (1H, m, C=CH), 5.48 (1H, m, C=CH) (Figures 5 and 6)



Lupeol



Spinasterol

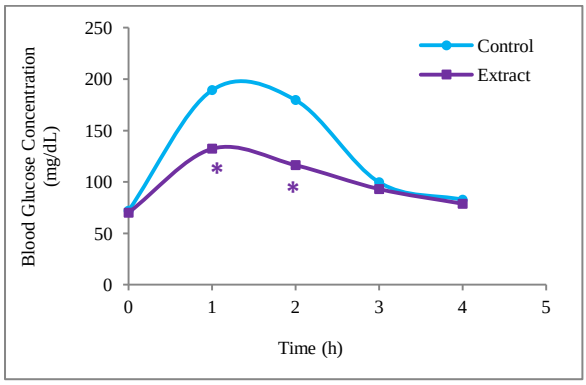
Table 1 Mean Blood Glucose Levels (± SEM) in Adrenaline Induced Hyperglycemic Rats with 70 % Ethanol Extract (2 g/kg Body Weight Dose)

Test Group	Blood Glucose Concentration (mg/dL)				
	0 h	**1 h**	**2 h**	**3 h**	**4 h**
Group I (Control) (n=5)	72.0 ± 1.0	189.33 ± 12.6	179.67 ± 0.6	99.67 ± 5.5	82.67 ± 14.6
Group I (Extract) (n=5)	70.0 ± 2.0	132.33 ± 22.7 (30.11 %R)*	116.33 ± 13.7 (35.25 %R)*	93.00 ± 7.2 (6.69 %R)	78.67 ± 12.2 (4.84 %R)

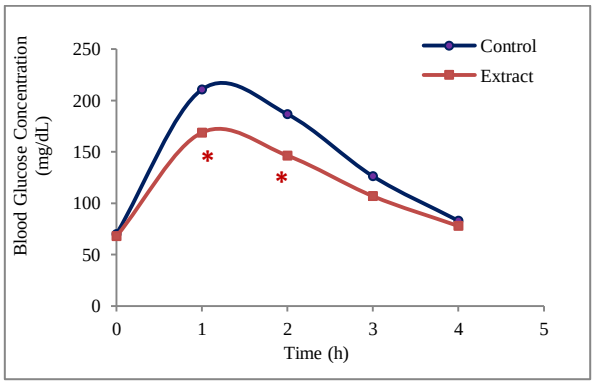
* = $p < 0.01$ R = Reduction in hyperglycemia

Table 2 Mean Blood Glucose Levels (±SEM) in Adrenaline Induced Hyperglycemic Rats with Watery Extract (2 g/kg Body Weight Dose)

Test Group	Blood Glucose Concentration (mg/dL)				
	0 h	**1 h**	**2 h**	**3 h**	**4 h**
Group II (Control) (n=5)	70.0 ± 2.0	210.67 ± 18.5	186.67 ± 11.7	126.33± 4.9	83.0 ± 13.9
Group II (Extract) (n=5)	68.0 ± 2.6	168.67 ± 6.0 (19.94 %R)*	146.33 ± 4.7 (21.61 %R)*	107.0 ± 10.6 (15.30 %R)	78.0 ± 10.1 (6.02 %R)



* = $p < 0.01$ (a)



* = $p < 0.01$ (b)

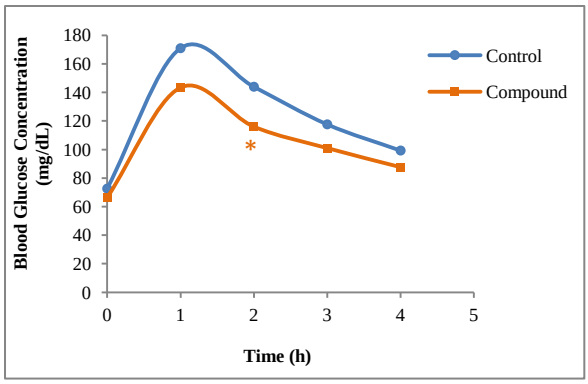
Figure 1 Time course effect of (a) 70 % ethanol extract and (b) watery extract on adrenaline induced hyperglycemic rats

Table 3 Mean Blood Glucose Levels (± SEM) in Adrenaline Induced Hyperglycemic Rats with Isolated Compound (Lupeol) (2 mg/kg Body Weight Dose)

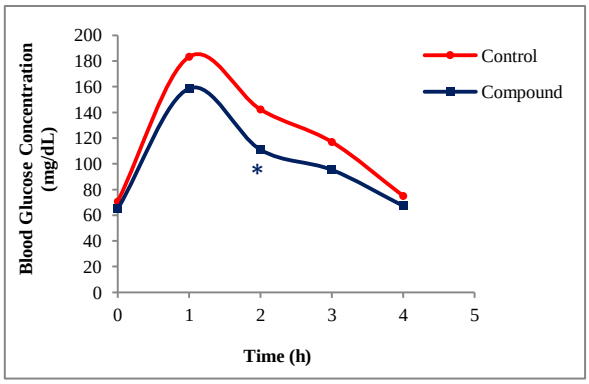
Test Group	Blood Glucose Concentration (mg/dL)				
	0 h	**1 h**	**2 h**	**3 h**	**4 h**
Group III (Control) (n=5)	72.67 ± 6.5	171.00 ± 22.6	144.00 ± 14.1	117.67 ± 3.5	99.33 ± 4.2
Group III (Lupeol) (n=5)	66.33 ± 5.1	143.33 ± 9.1 (16.18 %R)	116.00 ± 6.2 (19.44 %R)*	101.00 ± 4.4 (14.16 %R)	87.67 ± 4.2 (11.74 %R)

Table 4 Mean Blood Glucose Levels (± SEM) in Adrenaline Induced Hyperglycemic Rats with Isolated Compound (Spinasterol) (2 mg/kg Body Weight Dose)

Test Group	Blood Glucose Concentration (mg/dL)				
	0 h	1 h	2 h	3 h	4 h
Group IV (Control) (n=5)	70.67 ± 2.5	183.33 ± 12.5	142.33 ± 16.3	117.00 ± 13.7	75.00 ± 15.4
Group IV (Spinasterol) (n=5)	65.00 ± 3.0	158.67 ± 19.5 (13.45 %R)	111.00 ± 4.0 (22.01 % R)*	95.33 ± 4.2 (18.52 %R)	67.33 ± 3.1 (10.22 %R)



(a)



(b)

* = p < 0.01

* = p < 0.01

Figure 2 Time course effect of (a) isolated compound (Lupeol) and (b) isolated compound (Spinasterol) on adrenaline induced hyperglycemic rats

Table 5 Results of Acute Toxicity Test of 70 % Ethanol and Watery Extracts

Group No.	Doses (g/kg)	Ratio of Dead & Tested Mice No.	Observed % Dead	Expected % Dead	Observed-Expected
I (Control)	0	0:10	0	0	0
II & V (Ethanol and Water)	2	0 :10	0	0	0
III & VI (Ethanol and Water)	4	0 :10	0	0	0
IV & VII (Ethanol and Water)	8	0 :10	0	0	0

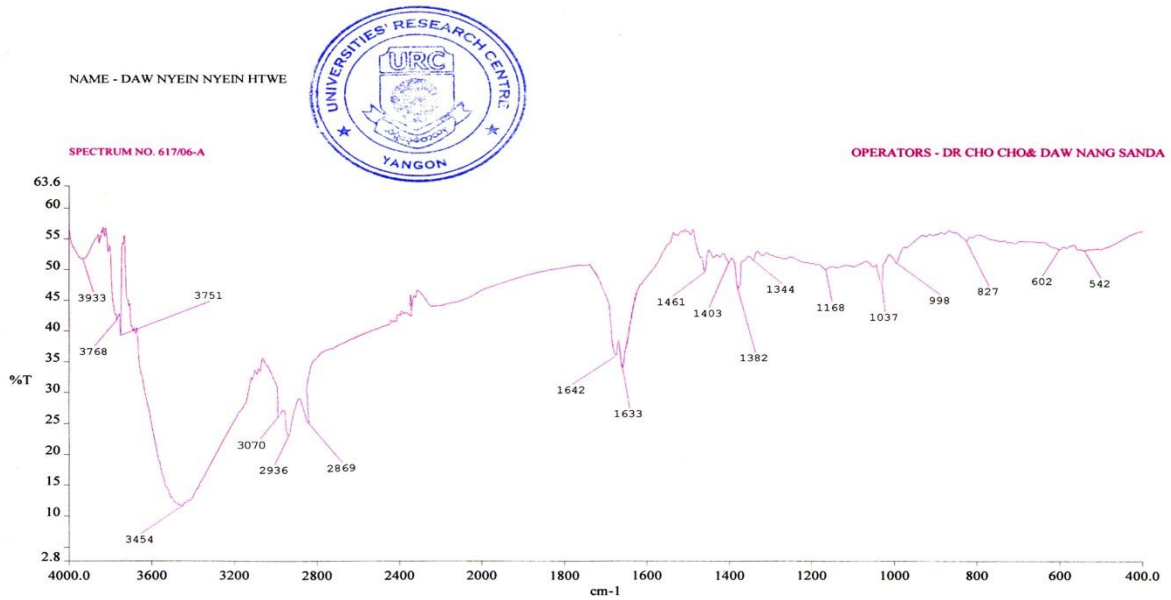


Figure 3 FT IR spectrum of isolated compound (Lupeol) (KBr)

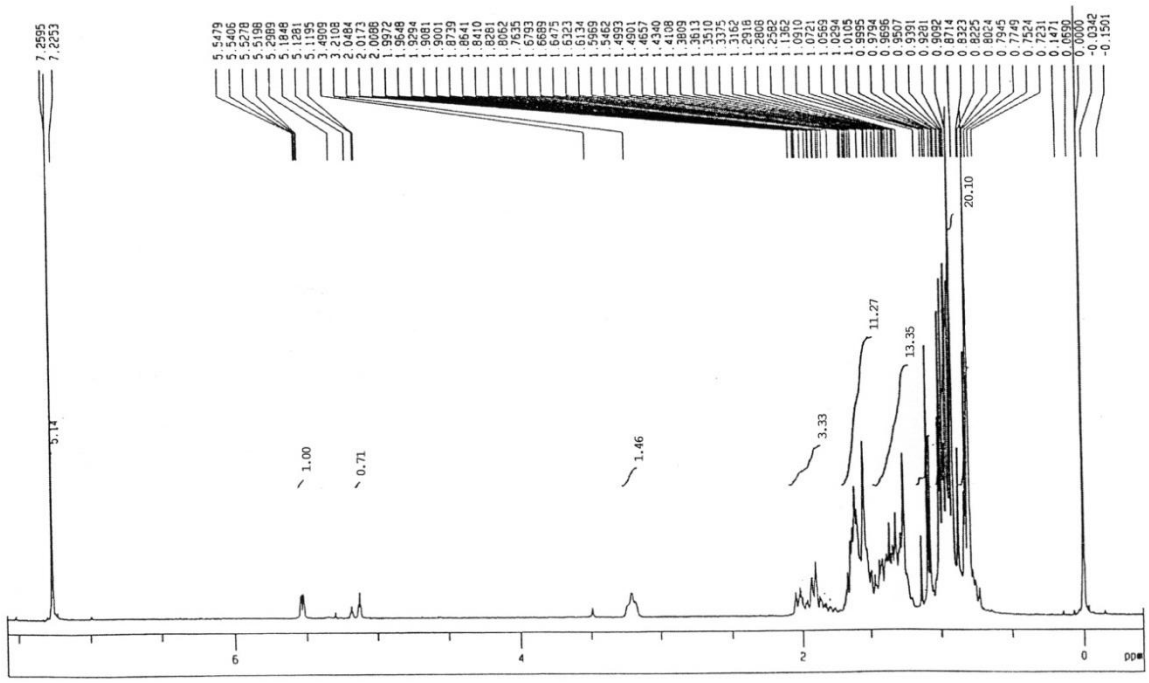


Figure 4 ^{1}H NMR (400 MHz in $CDCl_3$) spectrum of isolated compound (Lupeol)

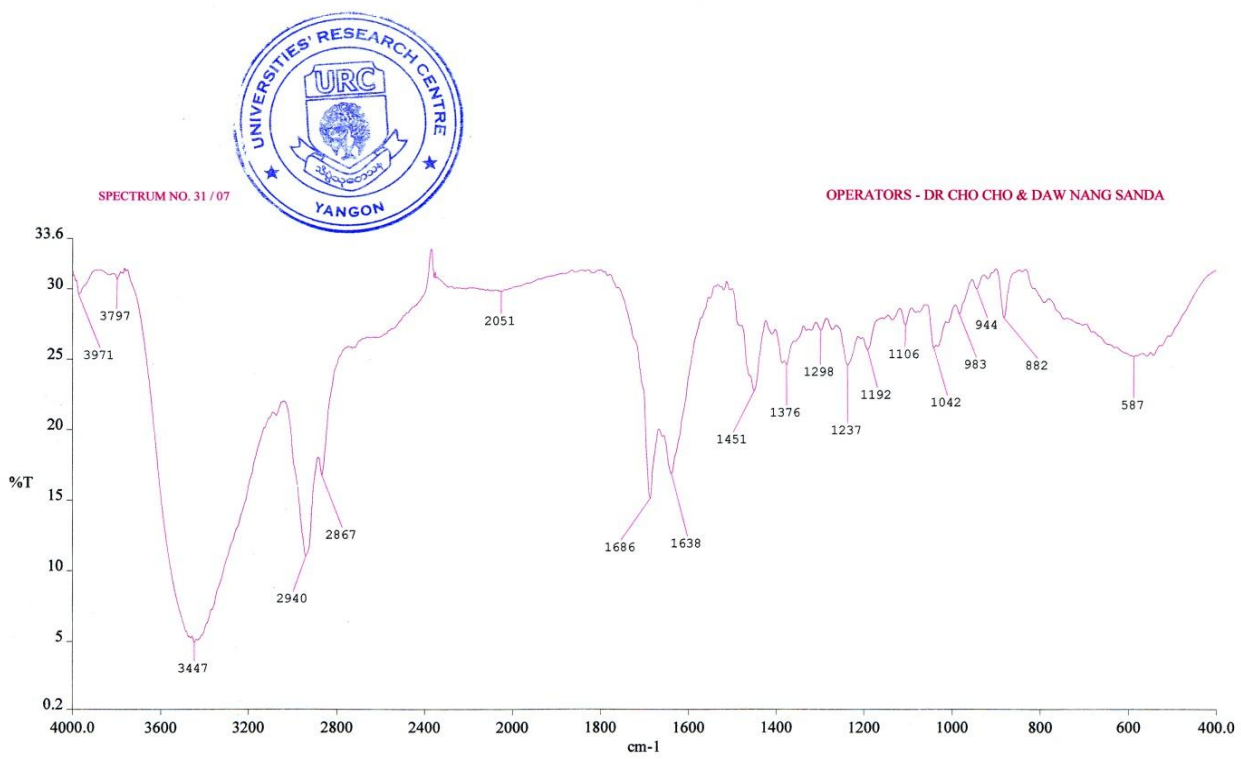


Figure 5 FT IR spectrum of isolated compound (Spinasterol) (KBr)

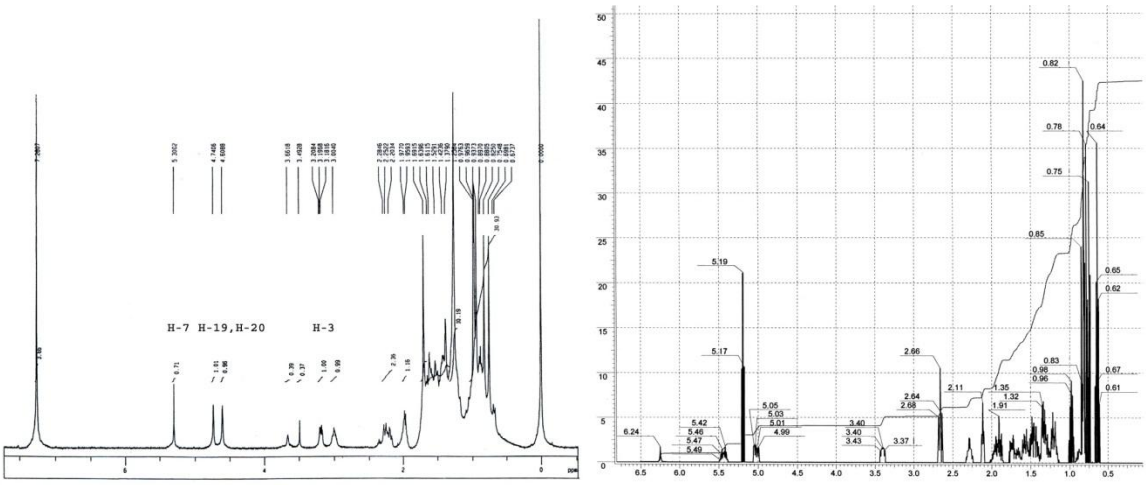


Figure 6 ^{1}H NMR (400 MHz in $CDCl_3$) spectrum of Spinasterol compared with predicted spectrum of ACD Labs software

Conclusion

Two crude extracts and two isolated compounds from the bark of Kha-yay were investigated the antihyperglycemic activities by using adrenaline induced hyperglycemic rats. The effective doses were observed 2 g/kg body weight for each extract and 2 mg/kg body weight for isolated compounds. 70 % ethanol extract showed the optimum antihyperglycemic effect at 1 h (30.11 % reduction, $p < 0.01$) and 2 h (35.25 % reduction, $p < 0.01$), and watery extract decreased blood glucose level at 1 h (19.94 % reduction, $p < 0.01$) and 2 h (21.61 % reduction, $p < 0.01$) compared with control group. From these results, 70 % ethanol extract was found to be more potent than watery extract in antihyperglycemic activity. The effective reduction percent of blood glucose levels of isolated compounds after 2 h showed 19.44 % ($p < 0.01$) for lupeol and 22.01 % ($p < 0.01$) for spinasterol. So, Antihyperglycemic activities of two isolated compounds are not quite different.

In acute toxicity test, the maximum dose for tested crude extracts (70 % ethanol and water) was found to be 8 g/kg body weight. From the result, LD_{50} was more than 8 g/kg body weight and it was inferred that both extracts were free from acute toxic or harmful effects.

According to this scientific investigation, it may be inferred that two crude extracts (70 % ethanol and water) and two isolated compounds (lupeol and spinasterol) of Kha-yay bark possessed antihyperglycemic activities.

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