IN *VIVO* AND *VITRO* ANTIHYPERGLYCEMIC EFFECTS AND STRUCTURAL ELUCIDATION OF ORGANIC COMPOUNDS FROM THE BARK OF *Muntingia Calabura* L.*

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Abstract

One of the medicinal plants, namely *Muntingia calabura* L. (Hnget Thagya), belonging to the family Muntingiaceae was selected for pharmacological activity. Glucose uptake capacity by yeast cells of ethyl acetate and ethanol extracts were evaluated by using yeast cell model. Ethyl acetate extract showed the highest efficiency (84.99 ± 0.37) % and ethanol extract also showed the highest efficiency (64.95 ± 1.47) % at 25 mM of glucose solution. Thus, the ethyl acetate extract is more effective than ethanol extract. It was also found that the percent of glucose uptake by yeast cells increased at different glucose concentrations in both extracts. Furthermore, the antihyperglycemic activity of ethyl acetate extract was determined by using adrenaline-induced mice model. Ethyl acetate extract showed decrease in blood glucose levels for test animals within 90 minutes to 225 minutes. Moreover, structural elucidation of organic compound from the extract such as *β*-sitosterol, gallic acid and catechin were performed by using advanced spectroscopic methods such as FT IR, ^{1}H NMR (600 MHz),^{13}C NMR (150 MHz), DQF-COSY, HSQC and HMBC spectral data respectively.

Keywords: *Muntingia calabura* L. Antihyperglycemic, Yeast Cell, *β*-sitosterol, Gallic acid, and Catechin

Introduction

Medicinal plants are sources of important therapeutic aid for alleviating human ailments. Interest in phytomedicine started in the last 20 years and with increasing awareness of the health hazards and toxicities associated with unsystematic use of synthetic drugs and antibiotics (N.D Mahmood, *et al*., 2014). Plants are still a rich source of novel bioactive compounds with therapeutic properties like anti-inflammatory, anti-cancer, antimicrobial and so on (M. Nirmala, *et al*., 2020). The demand for plants used as traditional medicine formulations by the community is also increasing because plant-derived medicines have proven to be healthier and do not cause as many side effects as those derived from chemicals. (Arif Nur Muhammad Ansori, *et al*., 2021)

Diabetes mellitus is the collective name of metabolic abnormalities, primarily caused by the defect in secretion of insulin hormone by the pancreatic islets. It can elevate levels of blood glucose (hyperglycemia). (Gauhar Rehman, *et al*., 2017). While the type 1 is usually associated with the destruction of pancreatic β-cell by the autoimmune process, the type 2 diabetes involves a combination of insulin resistance and defective compensatory insulin secreation. (Nur Khaleeda Zulaikha Zolkeflee, *et al*., 2022). Medicinal plants offer natural antidiabetic properties with fewer side effects and the plants with analogous activity may form synergistic interactions that significantly enhance the therapeutic efficacy of treatments. (Agustingus Widodo, *et al*., 2024).

One of the indigenous plants, *Muntingia calabura* L. (Hnget Thagya) belongs to the family Muntingiaceae is a fast-growing plant that is widely found worldwide. The various part of the plant has been used to treat different types of illnesses. In Peruvian folklore medicine, the flowers and barks are used as an antiseptic, anti-diabetes, and anti-inflammatory. It possesses several medicinal values such as reducing gastric ulcer and swelling of prostate gland, and relieving headache and cold (N.D Mahmood *et al*., 2014). Moreover, leaves have been considered by the population to be a medicinal plant and have properties including anti-diabetes,

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anti-gout, anti-hypertension, laxative, antiseptic, gastro-protective, antioxidant, and anti-inflammatory activities as well as activity against productive cough, the flu, headache, and fever (Arif Nur Muhammad Ansori, *et al*., August 2021). In Colombia, the infusion of the flowers was used as a tranquillizer and tonic. In Mexico, the plant is used to treat measles, mouth pimples and stomach ache. (Sarojini, *et al*., Nov. 2018).

The primary objectives of this study were to evaluate in *vivo* and *vitro* antihyperglycemic potential of the ethyl acetate extract and to elucidate the structure of organic compounds from the bark of *Muntingia calabura* L.

Materials and Methods

Plant Material

The bark of *Muntingia calabura* L. was collected from Patheingyi Township, Mandalay Region, Myanmar.

Preliminary Phytochemical Screening

Phytochemical screening of the bark of *Muntingia calabura* L. was performed by Harbone method and the results have been reported at Mandalay university research journal in 2023.

Preparation of ethyl acetate extract of the bark of *Muntingina calabura* L.

1.7 kg of air dried sample was percolated in 10.0 L of ethanol for eleven days. The ethanol extract was filtered and dried at room temperature. 80.0 g of ethanol crude extract was obtained. It was partition with ethyl acetate and the resulting extract was dried at room temperature. 18.0 g of ethyl acetate crude extract was obtained.

Determination of glucose uptake capacity by yeast cells assay

This assay was performed according to the well-defined method of Cirillo. Commercial barker's yeast was dissolved in distilled water to prepare 1 % suspension. The suspension was kept overnight at room temperature (25 ℃). On the next day, yeast cells suspension was centrifuged at 4200 rpm for 5 minutes. The processed was repeated by the addition of distilled water to the pallet until a clear supernatant was obtained. Exactly 10 parts of the clear supernatant fluids were mixed with 90 parts of distilled water to get a 10 % v/v suspension of the yeast cells.

About 5 mg w/v of plant extract was mixed with dimethyl sulfoxide (DMSO) till dissolution. The mixture was then supplemented with various concentrations (5 mM, 10 mM and 25 mM) of 0.5 mL of glucose solution and incubated for 10 min at 37 ℃. To initiate the reaction, 100 µL of yeast suspension was poured in the mixture of glucose and extract, vortexed, and incubated for another 60 minutes at 37 ℃. After incubation, the tubes were centrifuged for 5 minutes at 3800 rpm and glucose was estimated by using a spectrophotometer at 520 nm. Absorbance for the respective control was also recorded on the same wavelength. The percent increase in uptake was calculated by the formula.

$$\% \text{ increase in glucose uptake} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

A_{Sample} = Absorbance of the sample

$A_{Control}$ = Absorbance of the Control (solution having all reagents except test sample)

Metronidazole and metformin were used as standard drugs.

Study on antihyperglycemic activity of ethyl acetate extract of the bark of *Muntingia calabura* L.

The strain of *Mus musculus* was used in this study. Firstly, the mice with 40 g of body weight were selected to use and kept separately from the others. The selected mice were prepared to cause hyperglycaemic effect by using adrenaline injection shown in Figure (a). For giving adrenaline injection, the selected mice were fasted overnight for 16 hours. The animals were given intraperitoneally with adrenaline 0.2 mL/kg body weight as 6 % solution in distilled water as shown in Figure (a).

They were starved for 4 hours after injection and they were given 5 mL of 10 % glucose solution orally at hourly interval to prevent hypoglycaemic shock. They were offered unlimited amounts of standard laboratory diet food and water. After one week, the mice were used to test the antihyperglycemic activity and adrenaline was injected again to mice.

In order to determine the normal blood glucose level, the mice were fasted overnight for 16 hours before the commencement of the experiment, to ensure stable blood sugar regulation.

The selected mice were divided into three groups such as tested plant sample group, positive control group and negative control group shown in Figure (b). Each group contained five mice and given them markers by using sodium picrate solution. A total of 15 fasted mice were used to give orally for the determination of antihyperglycemic activity. The sample control group mice were administered with the plant sample extract of 1 g/kg of body weight and positive control group mice were treated with the standard drug glipizide was administered 0.5 mg/kg of body weight shown in Figure (c) and (d). Negative control group mice were administered with 0.2 mL water to mice.

During the experiment, three observations were performed at five times 225 minutes of 45 minutes interval after orally administration of the plant extract, glipizide and distilled water by using glucometer and test trips.

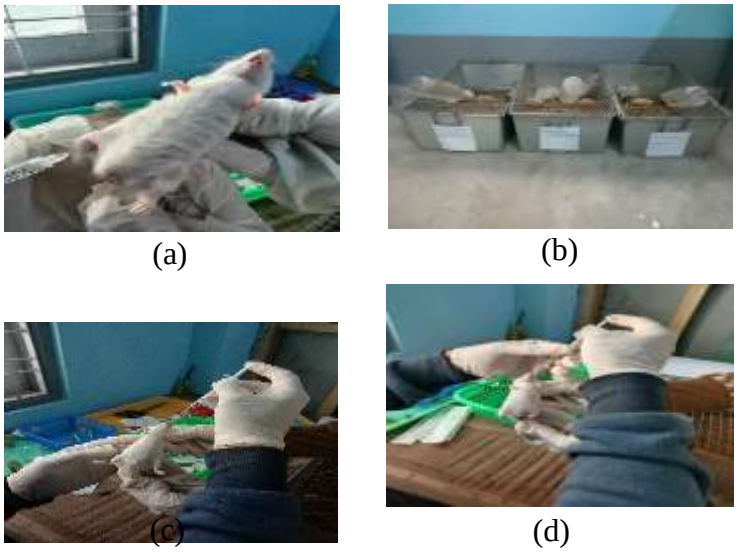


Figure 1. (a) Induction of diabetes to mouse
(b) Division of mice into three groups
(c) Treatment with the selected plant extracts
(d) Treatment with the selected standard drug

Isolation of organic compounds from the bark of *Muntingia calabura* L.

8.0 g of ethyl acetate extract was fractionated on a silica gel column by using *n*-hexane and ethyl acetate as eluents. Each fraction obtained by column chromatography was checked by TLC, iodine and anizaldehyde dipping reagent. From the inspection of TLC chromatogram, the fractions of the same R_f values were combined and 12 combined fractions were obtained. The fraction 7 was re-chromatographed for three times by using *n*-hexane and ethyl acetate as eluents to get *β*-sitosterol compound. Moreover, the combined fraction 12 was also re-chromatographed by using *n*-hexane and acetone as eluents and it provide to get the gallic acid, and catechin compounds. The spectra of isolated organic compounds such as ^{1}H NMR, ^{13}C NMR, HSQC, HMBC and DQF-COSY spectra were measured at Meijo University, Nagoya, Japan.

Results and Discussion

Effect of ethanol and ethyl acetate extracts on glucose uptake capacity by yeast cells

Effect of ethanol and ethyl acetate extract on glucose uptake capacity by yeast cells was shown in Table 1. According to the experimental observation, both extracts promoted the uptake of glucose across the plasma membrane of yeast cells. The glucose uptake capacity of ethyl acetate and ethanol extracts can comparable to that of known drug metronidazole and metformin. However, the effect of metformin on glucose uptake by yeast cells at 5 mM and 10 mM glucose concentration was a bit higher as compared to ethanol extract.

Table 1 Glucose Uptake Capacity by Yeast Cells

Sample Name	Concentrations	% Increased in Glucose Uptake (Mean ± SEM)			Method
		5 mM Glucose	10 mM Glucose	25 mM Glucose	
Muntingina calabura L. (Ethyl Acetate Extract)	5.0 mg/mL	81.48 ± 0.47	82.70 ± 0.37	84.99 ± 0.32	Cirillo VP (Glucose Uptake Capacity by Yeast Cells)
Muntingina calabura L. (Ethanol Extract)	5.0 mg/mL	62.02 ± 0.89	62.86 ± 0.32	64.95 ± 1.47	
Metronidazole	5.0 mg/mL	26.60 ± 1.33	42.01 ± 2.38	31.93 ± 3.42	
Metformin	5.0 mg/mL	72.00 ± 1.02	72.81 ± 1.40	64.52 ± 2.34	

Effect of ethyl acetate extract of *Muntingia calabura* L. on blood sugar level

Effect of ethyl acetate extract of *Muntingia calabura* L. on blood sugar level was shown in Table 2. According to observation, ethyl acetate extract showed decrease in blood glucose levels for test animals within 90 minutes to 225 minutes.

Table 2 Effect of ethyl acetate extract of *Muntingia calabura* L. on blood sugar level

Groups	Dose	Blood Glucose Level Mean (mg/dL) ± SD					
		0 min	45 min	90 min	135 min	180 min	225 min
Diabetic (negative control)	0.2 mL/kg	108.6 ± 7.64	185 ± 6.52	165.6 ± 13.85	150.8 ± 25.0	132.6 ± 33.7	117 ± 21.20
Muntingina Calabura L. (EtOAc Extract)	1.0 g/kg	104.8 ± 6.69	132.4 ± 6.84	126 ± 9.38	109.8 ± 12.60	104 ± 14.78	85.8 ± 11.19
Positive Control (Glipizide)	0.5 mg/kg	117 ± 10.8	152.6 ± 9.66	121.8 ± 17.38	106 ± 15.15	104.2 ± 8.35	90.6 ± 9.37

Low (20-70 mg/dL)
Normal (72-126 mg/dL)

Determination of the structure of *β*-sitosterol

β-sitosterol ($C_{29}H_{50}O$): white crystal, melting point – 138-142 ℃ (R_f = 0.56, *n*-hexane: ethyl acetate, 8:2 v/v). It was UV inactive. FT IR (KBr): ν(cm^{-1}) 3426 (-OH), 3028 (=CH), 2961, 2938 (sat-C-H), 1452, 1443, 1383, 1368 (C-OH), 1055 (C-C-O): ^{1}H NMR (600 MHz, $CDCl_3$) (δ_H /ppm): 0.71-2.32 (47 H, m, CH_3, CH_2, CH), 3.57 (1H, m, CH-OH), 5.38 (1H, m): ^{13}C NMR (150 MHz, $CDCl_3$) (δ_C /ppm): 26 sp^3 carbons 11.9 – 56.8, one sp^3 oxygen bearing methine carbon, 71.8, one sp^2 quaternary carbon 121.7 and one sp^2 methine carbon 140.7.

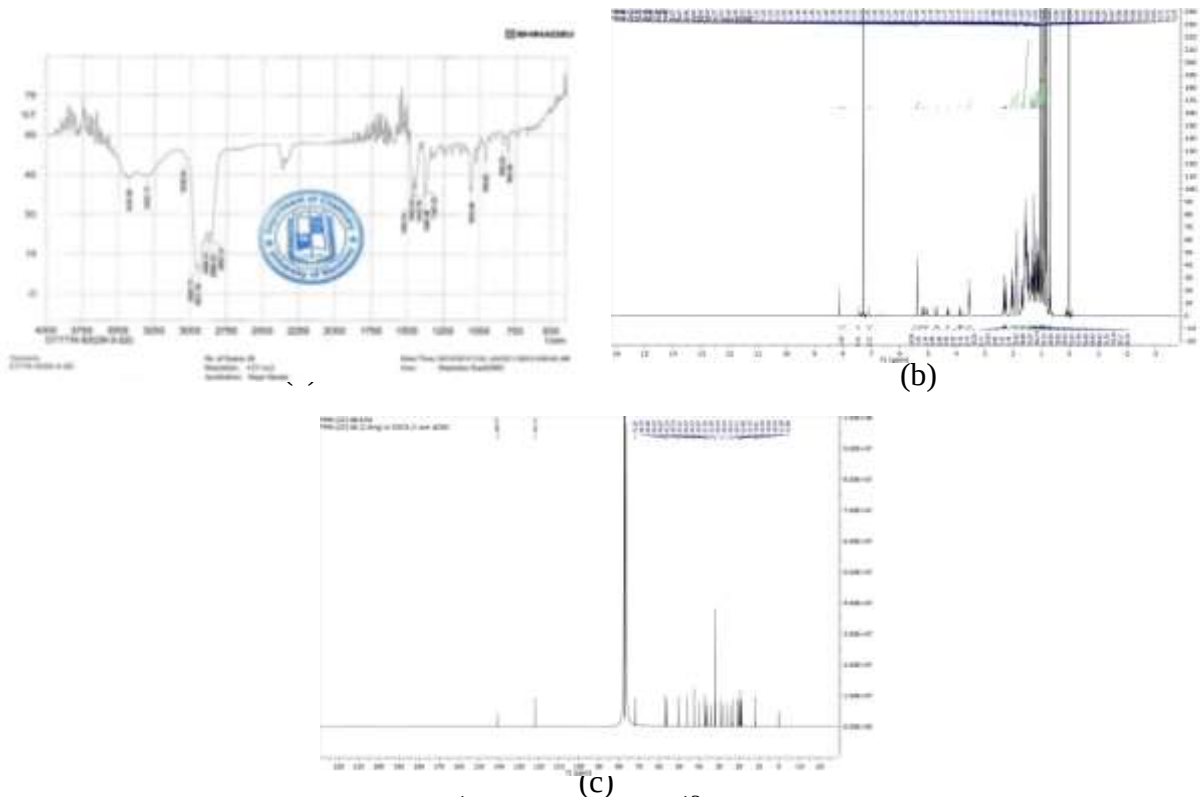
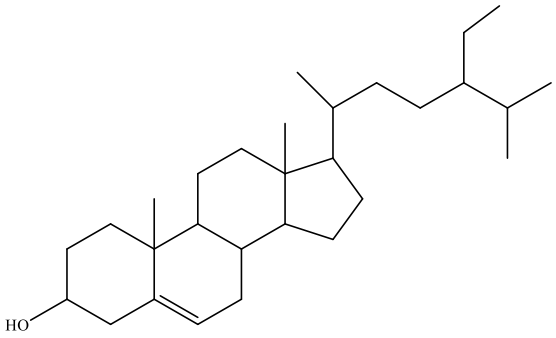


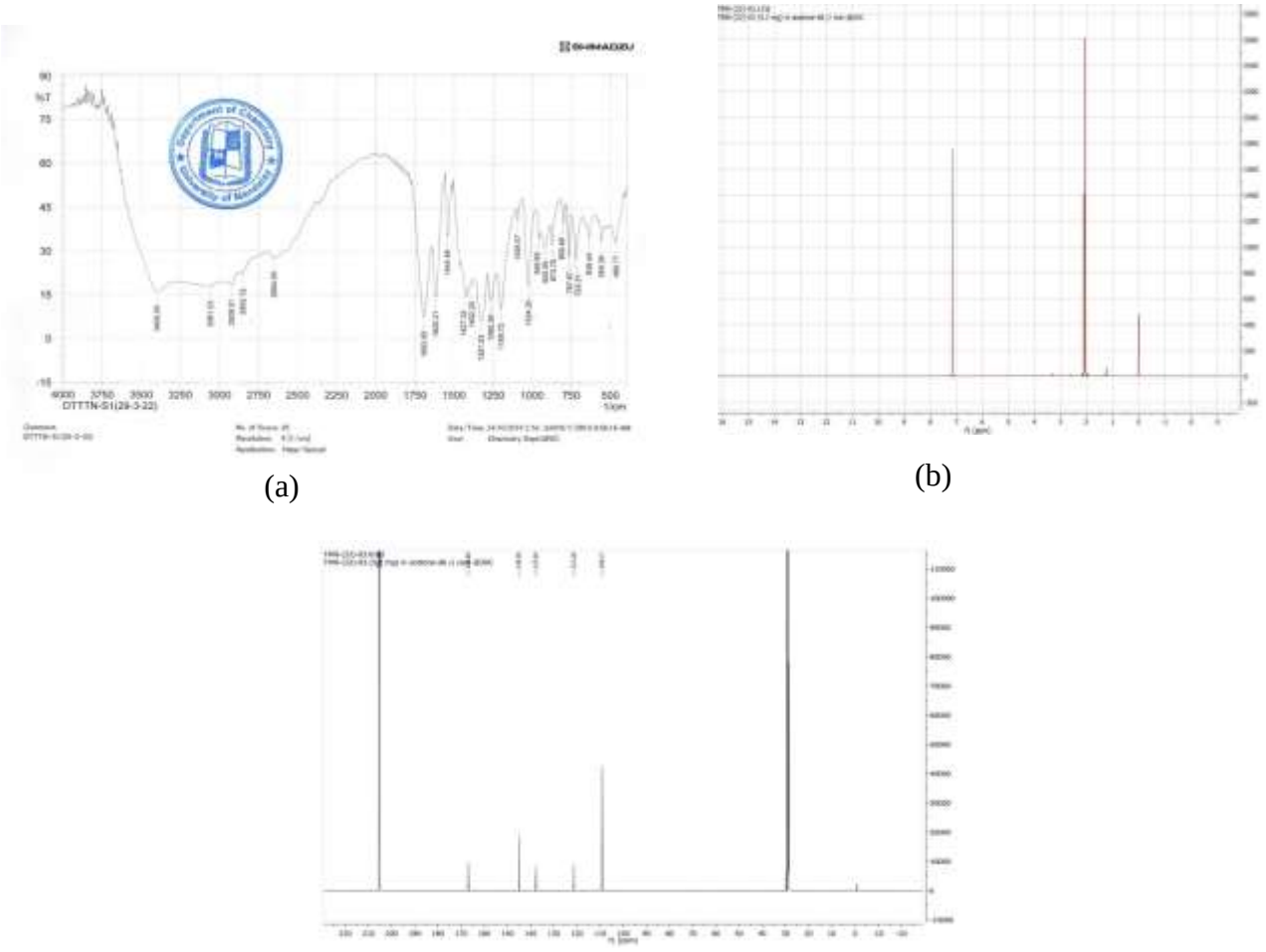
Figure 2. (a) FT IR (b) ^{1}H NMR and (c) ^{13}C NMR spectra of *β*-sitosterol

According to these spectral data, the structure of *β*-sitosterol could be assigned as shown below.



Determination of the structure of gallic acid

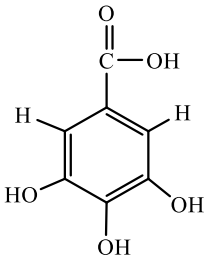
Gallic acid ($C_7H_6O_5$): pale-fawn crystal, melting point- 258-265 ℃, (R_f = 0.25, *n*-hexane: Acetone – 1:2 v/v). It was UV active. FT IR (KBr): ν (cm^{-1}) 3401 (-OH), 3061 (=CH), 2926, 2853 (sat-C-H), 1694 (C=O),1620 (C=C), 1427, 1402, 1327 (C-OH), 1265 (C-C-O), 1199, 1096, 1024 (C-O-C): ^{1}H NMR (600 MHz, acetone) (δ_H /ppm): 7.16 (2H, s, =CH): ^{13}C NMR (150 MHz, Acetone) (δ_C /ppm) two sp^2 methine carbons 109.2, three oxygen bearing quaternary carbons 145.0, 137.6, one sp^2 quaternary carbon 121.2 and one carbonyl carbon 166.8.



(a) (b)

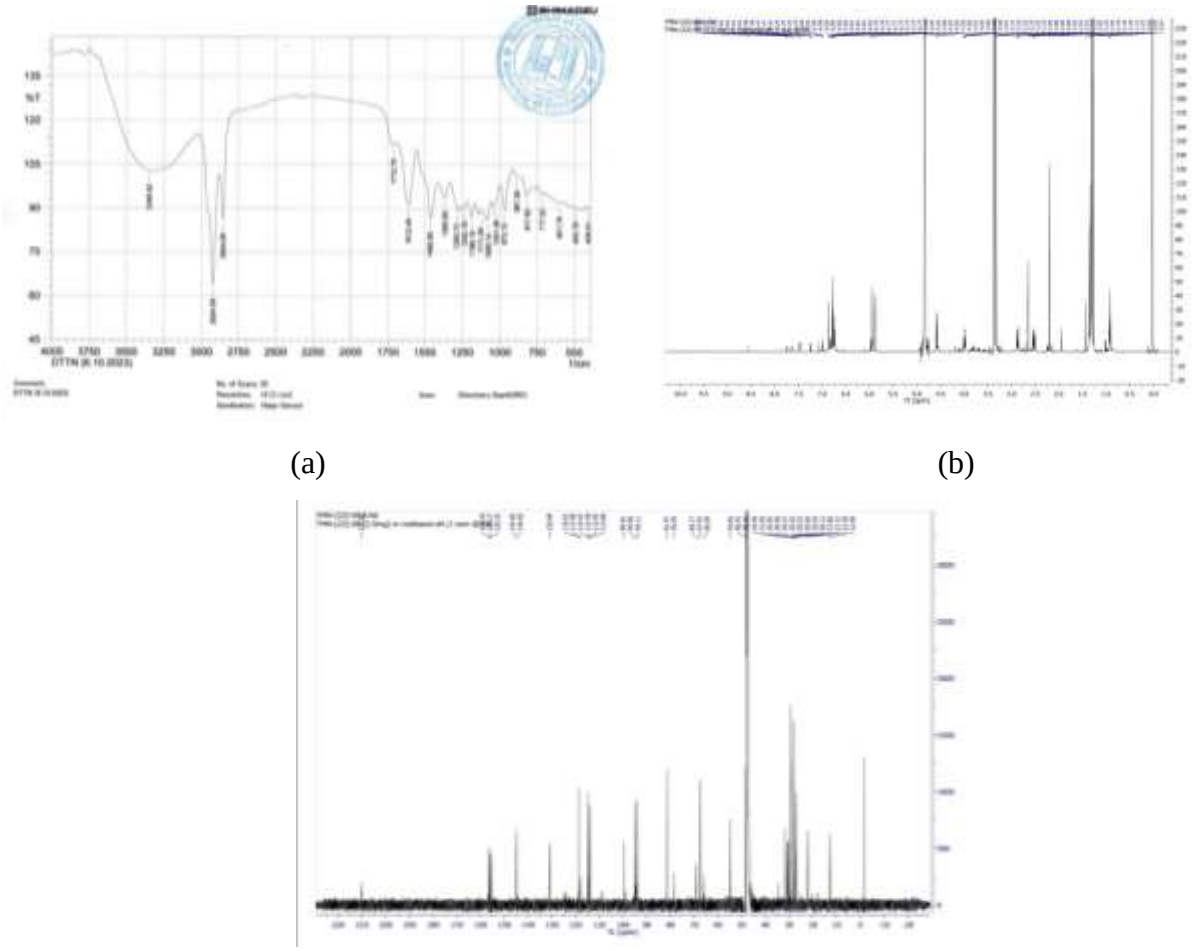
Figure 3. (a) FT IR (b) ^{1}H NMR and (c) ^{13}C NMR spectra of gallic acid

According to these spectral data, the structure of gallic acid could be assigned as shown below.



Determination of the structure of catechin

Catechin ($C_{15}H_{14}O_6$): orange crystal, melting point- 175-177 ℃ (R_f = 0.38, *n*-hexane: Acetone – 1:1 v/v). It was UV active. FT IR (KBr): ν (cm^{-1}) 3348 (-OH), 2924, 2855 (sat-C-H), 1613 (C=C), 1466, 1366 (C-OH), 1281, 1242 (C-C-O): 1188, 1111, 1080, 1042 (C-O-C): ^{1}H NMR (600 MHz, methanol) (δ_H /ppm): 6.86 (1H, d, *J*=2.0 Hz), 6.77 (1H, d, *J*=8.1 Hz), 6.73 (1H, dd, *J*=8.1,2.0 Hz), 5.95 (1H, d, *J*=2.3 Hz), 5.87 (1H, d, *J*=2.3 Hz), 4.58 (1H, d, *J*=7.5 Hz), 3.99 (1H, td, *J*=7.5, 5.4 Hz), 2.87 (1H, dd, *J*=16.1, 5.4 Hz), 2.52 (1H, dd, *J*=16.1,5.4 Hz): ^{13}C NMR (150 MHz, methanol) (δ_C /ppm) five sp^2 methine carbons 118.6, 114.6, 113.8, 94.1 and 94.9, five sp^2 oxygen bearing quaternary carbons 156.5, 156.17, 155.5, 144.85, 144.82, two sp^2 quaternary carbon 130.8, 99.4, two sp^3 oxygen bearing carbons 81.47, 67.4 and one sp^3 methylene carbon 27.11.

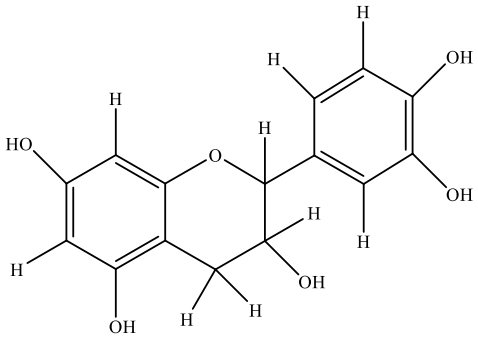


(a) (b)

(c)

Figure 4 (a) FT IR and (b) ^{1}H NMR and (c) ^{13}C NMR spectra of catechin

According to these spectral data, the structure of catechin could be assigned as showed below.



Conclusion

In this present research work, glucose uptake capacity by yeast cells of ethanol and ethyl acetate extracts was evaluated by Cirillo method. The ethyl acetate extract had greater efficiency in increasing the glucose uptake by yeast cells as compared to standard drugs metronidazole and metformin whereas the ethanol extract had greater efficiency as compared to standard drugs metronidazole and lower efficiency than metformin. In addition, it was found that the percentage of glucose uptake by yeast cell increased at different glucose concentrations (5 mM, 10 mM and 25 mM) in both extracts. The antihyperglycemic activity of ethyl acetate extract was determined by using adrenaline-induced mice model. After administration, the blood glucose levels reduced to normal range within 90 to 225 minutes. The ethyl acetate extract of the bark of *Muntingia calabur*a L. showed a significant reduction in blood glucose levels for tested mice. Moreover, the bark of *Muntingia calabura* L. contains bioactive compounds such as *β*-sitosterol, gallic acid and catechin. These compounds have antioxidant activity and other medicinal properties. Thus, the selected plants possessed antihyperglycemic activity in *vitro* and *vivo* tests.

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