INVESTIGATION OF SOME PHYTOCHEMICAL AND BIOLOGICAL ACTIVITIES OF *OROXYLUM INDICUM* (L.) BENTH (KYAUNG-SHA) FRUITS

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Abstract

The present research is concerned with some phytochemical and biological investigation of crude extracts from *Oroxylum. Indicum* (L.) (Kyaung sha) fruits. Preliminary phytochemical investigations by test tube methods revealed the presence of alkaloids, α–amino acids, carbohydrates, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, steroids, tannins, and terpenoids, and the absence of starch in the tested samples. The total phenol contents of watery extract of fruits were determined by the Folin-Ciocalteu reagent (FCR) method. Gallic acid (3,4,5-trihydroxy benzoic acid), was used to construct a standard calibration curve for total phenol. TPC contents (μg GAE/mg) were found to be the highest content (51.28 ± 0.41). The total flavonoid contents of watery extract of fruits were determined by the aluminum chloride method. Quarcetin was used to construct a standard calibration curve for total flavonoid. TFC contents (mg QE/g) were found to be highest content (36.05 ± 1.2). The watery and ethanol extracts of the fruits were observed to possess antioxidant capacity by the DPPH assay method, with the watery extract having more potent antioxidant activity (IC_{50} = 18.28 μg/mL) than other tested samples. Moreover, the watery extract also exhibits higher antidiabetic activity, expressed in terms of α-amylase inhibitory (IC_{50} = 106.85 μg/mL than other tested samples. In the antimicrobial screening by agar well diffusion method, petroleum ether and ethyl acetate extracts were found to possess high activity against all tested microorganism with the inhibition zone diameters ranging between 18 mm ~ 23 mm but other crude extracts of fruits had mild activity. According to the result, *O. indicum* (Kyaung sha) fruits contain the highest amount of phytochemical constituents, so these selected fruits have more portent antioxidant, antidiabetic, and antimicrobial effects.

Keywords: *Oroxylum indicum* (L.) Bent, phytochemical constituents, antioxidant, antidiabetic, antimicrobial activity

Introduction

Natural products such as plants have been used for the treatment of different diseases for thousands of years. Global plants have been used as medicines in Egypt, China, India and Greece and in many countries from ancient time and an extraordinary number of modern drugs have been developed from them (Haroon, 2014). Medicinal plants remain on to be a central therapeutic assist used for alleviating ailments of human race. Over the last 2500 years, here have been very strongly built traditional systems of medicine such as Ayurvedic, and the Unani (Hong-Fang *et al.*, 2009). These plants restrain materials that can be utilized for useful purposes, of which are originators for the synthesis of drugs. Plenty of research work has been carried out on a number of medicinal herbs as well as they have been initiate to have definite action on the respiratory, nervous, circulatory, digestive and urinary organisms, sexual organs, skin, hearing, vision, and taste (Fabricant and Farnsworth, 2001). The exploration for anti-cancer means on or after plant sources started during the 1950s. Moreover, like other countries in Myanmar, there are a lot of research has been done and some research is still going on as it is to be mentioned that till

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now the best source of anticancer agents is medicinal plant. Traditional medicines from readily available medicinal plants offer great potential for the discovery of new anticancer drugs. Most of plants contain glycosides, alkaloids, terpenoids, flavonoids, etc., that are frequently implicated as having anticancer effect. In Myanmar, valuable medicinal plants are found abundantly, most of them have not been scientifically investigated yet. In this study, our attention has been focused on the Myanmar bitter medicinal fruits, *Oroxylum indicum* (L.) Bent (Myanmar name: Kyaung-sha) was selected (Figure 1). These plants have been widely used as a traditional Myanmar medicine. Some reported chemical constituents of fruits of Oroxylum indicum (L.) Bent are shown in Figure 2 (Dinda *et al.*,2015).

Family	Bignoniaceae
Botanical name	Oroxylum indicum (L.) Benth
English name	midnight horror, Indian trumpet flower
Myanmar name	Kyaung sha
Part used	Fruits

Figure 1. Photographs of *Oroxylum indicum* (L.) Benth (Kyaung-sha) Fruits

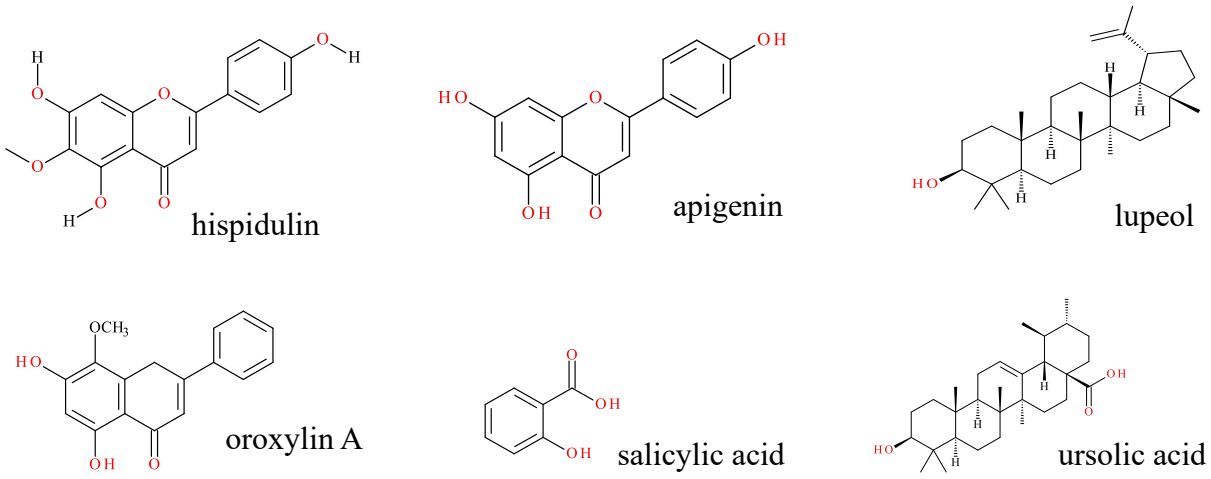


Figure 2. Some reported chemical constituents of *Oroxylum indicum* (L.) Benth (Kyaung-sha) Fruits

The main aim of this research was to investigate some phytochemical constituents from the fruits of *Oroxylum indicum* (L.) Benth (Kyaung-sha) and to evaluate some biological activities such as antimicrobial activity, antioxidant activity and antidiabetic activity.

Materials and Methods

Sample Collection

The fruits sample of *Oroxylum indicum* (L.) Benth (Kyaung-sha) was collected from Pathein Township, Ayeyawady Region in October, 2020. After being collected, the scientific name of the sample was identified by authorized botanists at Botany Department, Pathein University.

Sample Preparation

The fresh sample was cleaned and washed with water and then air-dried. The dried sample was ground using grinding machine. And then this powdered sample was kept in the sealed air-tight container to prevent moisture changes and other contamination. It was then used without further purification or refining.

Preparation of crude extracts by direct extraction methods for screening of some biological activities

Each dried powdered sample (2 g) was extracted with 50 mL of petroleum ether (60-80 °C) for 6 h by using a Soxhlet extractor. The filtrate was concentrated by removal of the solvent under reduced pressure to give the respective petroleum ether crude extract. The ethyl acetate, 95% ethanol, and watery extracts were prepared by similar manner mentioned in the above procedure. Each extract was dried at normal pressure in a water bath and stored in the refrigerator for screening some biological activities.

Preliminary Phytochemical Test

A few gram of dried powder of selected sample was subjected to the tests of alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, starch, tannins, steroids, terpenoids according to the standard procedures (Harborne, 1984).

Determination of Chemical Constituents of the Watery Extract of *Oroxylum indicum* (L.) Benth (Kyaung-sha) Fruits

(a) Determination of total phenol content by Folin-Ciocalteu Reagent (FCR) method

The total phenol content (TPC) of watery extract of *Oroxylum indicum* (L.) Benth (Kyaung-sha) fruits were estimated by Folin-Ciocalteu (FC) method according to the procedure described by Song *et al.*, (2010). The extract solution (1000 µg/mL) was mixed with 5 mL of F-C reagent (1:10) in a test tube and incubated for about 5 min. To each test tube, 4 mL of 1 M sodium carbonate was added and the test tubes were kept at room temperature for 15 min and UV absorbance of reaction mixture was measured at λ_{max} 765 nm. The blank solution was prepared as the above procedure by using distilled water instead of sample solution. Total phenol content was estimated as milligram gallic acid equivalent per gram (mg GAE/g) of extract.

(b) Determination of total flavonoid content by aluminium chloride method

The total flavonoid content (TFC) of watery extract of *Oroxylum indicum* (L.) Benth (Kyaung-sha) fruits was estimated by Aluminium Chloride method according to the procedure described by Song *et al.* (2010). The extract solution (1000 µg/mL) was mixed with 1.5 mL of methanol, 0.2 mL of 1% $AlCl_3$ solution and 2.8 mL of distilled water. The absorbance of reaction mixture was measured at λ_{max} 415 nm. The blank solution was prepared as the above procedure by using distilled water instead of sample solution. Total flavonoid content was estimated as milligram quercetin equivalent per gram (mg QE/g) of extract.

Investigation of Some Biological Activities of Crude Extracts of *Oroxylum indicum* (L.) Benth (Kyaung-sha) Fruits

(a) Determination of antioxidant activity of crude extracts of *Oroxylum indicum* (L.) Benth (Kyaung-sha) fruits

In this experiment, DPPH (2 mg) was thoroughly dissolved in ethanol (100 mL). This solution was freshly prepared in the brown coloured reagent bottle and stored in the fridge for no longer than 24 h. The crude extracts of *O. indicum* (2 mg) and 10 mL of ethanol were thoroughly mixed by shaker. The mixture solution was filtered and the stock solution was obtained. By adding with ethanol, the sample solutions in different concentrations of 200, 100, 50, 25, 12.5, 6.25 and 3.125 μg/mL were prepared from the stock solution. The effect on DPPH radical was determined by using the method of Marinova and Batchvarov (2011). The control solution was prepared by mixing 1.5 mL of 50 μM DPPH solution and 1.5 mL of ethanol using shaker. The test sample solution was also prepared by mixing thoroughly 1.5 mL of 50 μM DPPH solutions and 1.5 mL of each sample solution. The mixture solutions were allowed to stand at room temperature for 30 min. Then, the absorbance of each solution was measured at 517 nm by using UV-1650 spectrophotometer. Absorbance measurements were done in triplicate for each concentration and then mean values so obtained were used to calculate percent inhibition of oxidation. The capability to scavenge the DPPH radical was calculated by using the following equation:

$$\% \text{ RSA} = \frac{A_{control} - (A_{sample} - A_{blank})}{A_{control} \times 100}$$

Where, %RSA = Percent of Radical Scavenging Activity

$A_{control}$ = absorbance of the control (DPPH only) solution

A_{blank} = absorbance of the blank (EtOH + Test sample solution) solution

A_{sample} = absorbance of the test sample solution

(b) Determination of antidiabetic activity of crude extracts of *Oroxylum indicum* (L.) Benth (Kyaung-sha) fruits

Determination of α-amylase inhibitory activity

In alpha amylase assay, the starch-iodine was used. First 2 mL of (0.5%) substrate starch solution and 1 mL of tested solution (Acarbose standard drug and crude) of seven different concentrations such as 200, 100, 50, 25, 12.5, 6.25, and 3.125 μg/mL were added in a bottle and this mixture was incubated for 3 min at room temperature. To start the reaction, 1 mL of α-amylase was added to the above solution followed by incubated for 15 min at room temperature. To stop the reaction, 4 mL of 0.1M HCl was added to this mixture, and to detect the reaction, 1 mL of iodine-iodide indicator (1 mM) was added to the mixture. Absorbance was read at 650 nm by UV spectrophotometer in the visible region. The control solution was prepared as the above procedure by using phosphate buffer (0.02M, pH 6.5) instead of drug solution.

All the experiments were done in triplicate. Percent inhibition of each sample solution was calculated by using the following formula. Standard deviation (SD) and 50% inhibition concentration (IC_{50}) value in μg/mL were calculated by computer excel program.

$$\% \text{ Inhibition} = \frac{A_{Sample} - A_{Control}}{A_{Sample}} \times 100$$

Where,

$A_{control}$ = the absorbance of the control solution

A_{sample} = the absorbance of sample solution

(c) Determination of antimicrobial activity of crude extracts of *Oroxylum indicum* (L.) Benth (Kyaung-sha) fruits

The antimicrobial activity of crude extracts of *Oroxylum indicum* (L.) Benth (Kyaung-sha) fruits was determined against eight strains of microorganisms such as *Agro tumefaciens* (NITE 09678), *Bacillus pumilus* (IFO 12092), *Bacillus subtilis* (IFO 90517), *Candida albicans (*NITE 09542), *Escherichia coli* (AHU 5436), *Micro luteus* (NITE 83297), *Pseudomonas fluoresens* (IFO 94307) and *Staphylococus aureus* (AHU 8465) by employing the agar well diffusion method. To prepare the agar plate, firstly, peptone (0.5 g) and sodium chloride (0.25 g) were mixed in distilled water and made up to 100 mL with distilled water. The pH of this solution was adjusted to 7.2 with a 0.1 M sodium hydroxide solution, and 1.5 g of agar was added. Nutrient agar medium was prepared according to method described by Cruick (1975). Briefly, nutrient agar was boiled, and 20-25 mL of the medium was poured into a test tube, plugged with cotton wool, and autoclaved at 121 ºC for 15 min. Then the tubes were cooled down to 60 ºC and poured into sterilized petri dishes, and 0.1 mL of spore suspension was also added to the dishes. The agar was allowed to set for 30 min, after which a 10 mm plate agar well was made with the help of a sterilised cork borer. After that, about 0.1 mL of each of the prepared extract solutions was introduced into the agar well and incubated at 37 ºC for 24 h. The inhibition zone (clear zone) appeared around the agar well, indicating the presence of antimicrobial activity. The extent of antimicrobial activity was measured from the inhibition zone diameter of. The measurements were conducted at Botany Department, Pathein University.

Results and Discussion

Phytochemical Constituents

The phytochemical tests revealed that alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, steroids, tannins and terpenoids were found to be present but starch was absent in fruits of *Oroxylum indicum* (L.) Benth (Kyaung-sha).

Total Phenol and Total Flavonoid Contents of Watery Extract of *Oroxylum indicum* Fruits

The total phenol content of the watery extract of *Oroxylum indicum* (L.) Benth (Kyaung-sha) fruits was determined with spectrophotometric method by using Folin-Ciocalteu reagent. The total phenol content of the watery extract of *Oroxylum indicum* (L.) Benth (Kyaung-sha) fruits was 51.28 ± 0.41 mg GAE/g. The total flavonoid content of sample was determined with spectrophotometric method by aluminium chloride reagent and was found to be 36.05 ± 1.2 mg QE/g. The results are shown in Table1.

Table 1. Total Phenol and Total Flavonoid Contents of Watery Extract of *Oroxylum indicum* (L.) Benth (Kyaung-sha) Fruits

Chemical Constituents	Contents
Total Phenol Content (mg GAE ± SD)/mg of extract	51.28 ± 0.41
Total Flavonoid Content (mg QE ± SD)/g of extract	36.05 ± 1.2

According to the experimental results, phenol and flavonoid compounds were detected in watery extract of selected sample. Besides their established antioxidant activity, many phenolic

compounds may exhibit significant antimicrobial activity. Since many plant extracts are rich in phenolic compounds, this is of particular interest for the development of natural alternatives to synthetic preservatives in food and cosmetic applications. Flavonoids are also present as a potent water-soluble antioxidant and free radical scavengers, which prevent from the oxidative cell damage and also have strong anticancer activity. It also helps in managing diabetes induced oxidative stress.

Antioxidant Activity of Crude Extracts of *Oroxylum indicum* (L.) Fruits by DPPH Assay

The antioxidant activity was measured in terms of hydrogen donating or radical scavenging ability of the watery and ethanol extracts of selected sample by using the stable radical DPPH. The results are shown in Table 2. The watery extract of selected fruit was found to be the low (IC_{50}) value 18.28 μg/mL than the ethanol extract of selected fruit, low IC_{50} value indicate the more potent antioxidant activity. However, the watery and ethanol extracts of selected sample were weaker activity than the standard ascorbic acid (IC_{50} = 1.08 μg/mL).

Table 2. Antioxidant Activity of Watery and Ethanol Extracts of *Oroxylum indicum* (L.) Benth (Kyaung-sha) Fruits by DPPH Assay

Samples (Extracts)	% RSA (mean ± SD) in different concentrations (μg/mL)							IC_{50} (μg/mL)
	3.125	6.25	12.5	25	50	100	200	
Watery	31.78 ± 0.54	34.63 ± 0.54	45.25 ± 0.83	55.53 ± 0.15	69.43 ± 0.26	88.43 ± 0.40	97.93 ± 0.26	18.28
Ethanol	34.37 ± 0.54	39.64 ± 0.26	42.57 ± 0.54	47.15 ± 0.26	58.89 ± 0.54	65.54 ± 0.26	73.32 ± 0.26	31.07

Andiabetic (α-Amylase Inhibitory) Activity of Crude Extracts of *Oroxylum indicum* Fruits

Hyperglycemia has been a classical risk in the development of diabetes and the complications associated with diabetes. Therefore, control of blood glucose levels is critical in the early treatment of diabetes mellitus and reduction of macro and microvascular complications. One therapeutic approach is the prevention of carbohydrate absorption after food intake, which is facilitated by inhibition of enteric enzymes including *α*-glucosidase and *α*-amylase present in the brush borders of intestine. In this study, the *α*-amylase inhibitory activity of *Oroxylum indicum* (L.) Benth (Kyaung-sha) fruits was investigated. The inhibitory effects of watery and ethanol extracts were analyzed. The percentage inhibition of *α*-amylase by watery and ethanol extracts was studied in a concentration range of 3.125-200 μg/mL. The percentage inhibition of the sample on *α*-amylase enzyme activity increased with increasing the concentrations. From the percentage inhibition, the respective IC_{50} values for the watery and ethanol extracts were calculated and the results are respectively tabulated in Table 3. The watery and ethanol extracts of bitter selected plant were also explored for *in vitro α*-amylase inhibition and their activity was compared with that standard of the anti-diabetic drug, acarbose. The 50% α–amylase inhibition potency (IC_{50}) of watery and ethanol extracts of the selected sample ranged between 106.85-153.98 μg/mL, indicating that crude extracts possessed potent *α*-amylase inhibition activity but these extracts has a lower inhibition activity than standard acarbose (IC_{50} =20.92 μg/mL).

Table 3. IC_{50} Values of Watery and Ethanol Extracts of *Oroxylum indicum* (L.) Benth (Kyaung-sha) Fruits by α-Amylase Inhibition Assay

Samples (Extracts)	% Inhibition in different concentrations (µg/mL)							IC_{50} (µg/mL)
	3.125	6.25	12.5	25	50	100	200	
Watery	4.07 ± 0.20	7.29 ± 0.32	10.10 ± 0.30	23.13 ± 0.34	46.35 ± 0.16	49.43 ± 0.10	57.75 ± 0.07	106.85
Ethanol	7.29 ± 0.32	16.04 ± 0.26	20.77 ± 0.24	37.62 ± 0.15	45.29 ± 0.11	48.32 ± 0.57	65.99 ± 0.04	153.98

Screening of Antimicrobial Activity of Various Crude Extracts of *Oroxylum indicum* Fruits

Screening of the antimicrobial activity of various crude extracts such as petroleum ether, ethyl acetate, 80 % ethanol, and watery extracts of *Oroxylum indicum* (L.) Benth (Kyaung-sha) fruits was done by employing the agar well diffusion method (Table 4 and Figure 3). In this study, the samples were tested on eight pathogenic microorganisms, such as *Agro tumefaciens, Bacillus pumilus, Bacillus subtilis, Candida albicans, Escherichia coli, Micro luteus, Pseudomonas fluoresens* and *Staphylococus aureus.* From these results, it was found that the watery extract of the selected sample did not exhibit any antimicrobial activity against all tested microorganisms, whereas the petroleum ether, ethyl acetate, and ethanol extracts of the selected sample exhibited inhibition zone diameters ranging from 13 ~ 22 mm, respectively, against all tested microorganisms. The selected sample of ethanol extract showed less activity, while petroleum ether and ethyl acetate extracts were observed to be most effective in antimicrobial activity. Therefore, all the crude extracts of the selected sample, except the watery extract of *Oroxylum indicum* (L.) Benth (Kyaung-sha) fruits, exhibited antimicrobial activity against all microorganisms tested. Among the crude extracts, petroleum ether and ethyl acetate extracts from the *Oroxylum indicum* fruits showed the most pronounced antimicrobial activity against all microorganisms tested. However, the selected sample possesses smaller inhibition zone diameters than the standard antimicrobial drug chloramphenicol (29 ~ 30 mm).

Table 4. Inhibition Zone Diameters of Crude Extracts by Agar Well Diffusion Method

Microorganism	Inhibition zone diameters (mm)				
	H_2O	EtOH	EtOAc	PE	STD
A. tumefaciens	-	14	20	21	29
B. pumilis	-	14	18	20	29
B. subtilis	-	15	20	23	29
C. albicans	-	13	19	21	30
E. coli	-	14	20	21	29
M. luteus	-	14	18	20	29
P. fluorescens	-	15	20	22	29
S. aureus	-	14	19	21	30

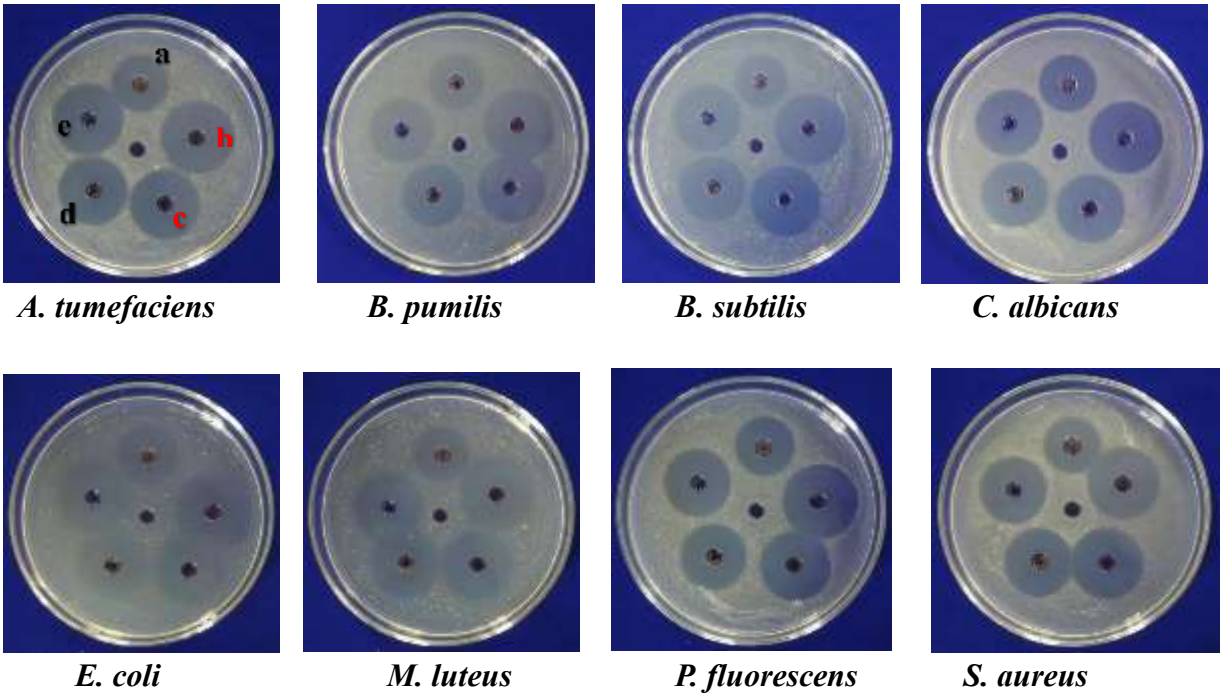
Agar well diameter (8 mm) No activity (-)

10 mm – 14 mm = weak activity (+)

15 mm – 19 mm = moderate activity (++)

20 mm and above = potent activity (+++)

STD = chloramphenicol



a= H_2O, b= EtOH, c= EtOAc, d= PE & e= Standard

Figure 3. Screening of antimicrobial activity of the crude extracts by agar well diffusion method

Conclusion

The following inferences could be deduced from the overall assessment of the chemical and biological investigation on the fruits of *Oroxylum indicum* (L.) Benth (Kyaung-sha). In the preliminary phytochemical results, alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, steroids, tannins, and terpenoids were found to be present, but starch was absent in the selected sample. According to the chemical investigation, the watery extract of the selected sample contains significant amounts of TPC (51.28 ± 0.41 mg GAE/mg) and TFC (36 ± 1.4 mg QE/g). The extracts showed the high antimicrobial activity (13 mm ~ 22mm) against *Agro tumefaciens, Bacillus pumilus, Bacillus subtilis, Candida albicans, Escherichia coli, Micro luteus, Pseudomonas fluoresens* and *Staphylococus aureus* due to the presence of flavonoids and phenols. The watery and ethanol extracts of the selected sample also showed DPPH free radical scavenging activity IC_{50} = 18.28 mg/mL and IC_{50} = 31.07 μg/mL respectively, as antioxidant activity. The watery and ethanol extracts of the selected sample possessed the antidiabetic activity due to its α-amylase inhibitory effects (IC_{50}= 106.85 μg/mL and IC_{50}= 153.98 μg/mL respectively). The result obtained from this research indicated that the tested crude extracts of *Oroxylum indicum* (L.) Benth (Kyaung-sha) fruit may play an important role in medicinal properties used *in vitro* and may be effective.

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