DETERMINATION OF SOME CHEMICAL CONSTITUENTS AND INVESTIGATION OF SOME BIOLOGICAL ACTIVITIES OF *BRUCEA JAVANICA* LINN. (YAR-DAN-SEED)

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Abstract

This research is concerned with some phytochemical and biological investigation of crude extracts from *Brucea javanica* Linn. (Yar-dan-seed). Preliminary phytochemicals, total flavonoid content (TFC) and total phenol contents (TPC) have been carried out as chemical investigations. Preliminary phytochemical investigations done 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 (TPC) of water and ethanol extract of seeds were determined by the Folin-Ciocalteu reagent (FCR) method. TPC contents of ethanol extract (μg GAE/mg) were found to be highest content (303.24 ± 1.19). The total flavonoid contents of water and ethanol extracts of selected sample were determined by the aluminum chloride method. TFC contents of ethanol extract (mg QE/g) were found to be the highest content (286 ± 0.20). The water and ethanol extracts of the seeds were observed to possess antioxidant capacity by DPPH assay method. Among them, ethanol extract has more potent antioxidant activity (IC_{50} = 36.21 μg/mL) than other tested samples. Moreover, ethanol extract of antidiabetic activity (expressed in terms of *α*-amylase inhibitory) indicated that possessed higher anti-diabetic activity (IC_{50} = 89.12 μg/mL) than other tested samples. In the antimicrobial screening by agar well diffusion method, pet-ether and ethyl acetate extracts were found to possess high activity against all tested microorganism with the inhibition zone diameters ranging between 19 mm ~ 23 mm but other crude extracts of *B.javanica* had mild activity. According to the result, *B.javanica* (Yar-dan-seed) was found to contain highest amount of phytochemical constituents so these selected samples have more portent antioxidant, antidiabetic and antimicrobial effects.

Keywords*:* *Brucea javanica* Linn., phytochemical constituents, antioxidant, antidiabetic, antimicrobial

Introduction

Natural products such as plants have been used for the treatment of different diseases for thousands of years and they have been used as medicines in Egypt, China, India and Greece and in many countries 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 lot of research has been done and some research is still going on as it is to be mentioned that till now the best source of anticancer agents is medicinal plant. Traditional medicines from readily

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available medicinal plants offer great potential for the discovery of new anticancer drugs. Most of the 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 seed, *Brucea javanica* Linn. (Myanmar name: Yar-dan-seed) was selected. These plants have been widely used as a traditional Myanmar medicine. (Dinda *et al.*, 2015)

Family	Simaroubaceae
Botanical name	*Brucea javanica* Linn.
Myanmar name	Yar-dan-seed
Part used	Seed

Figure 1. Photographs of Brucea javanica Linn.

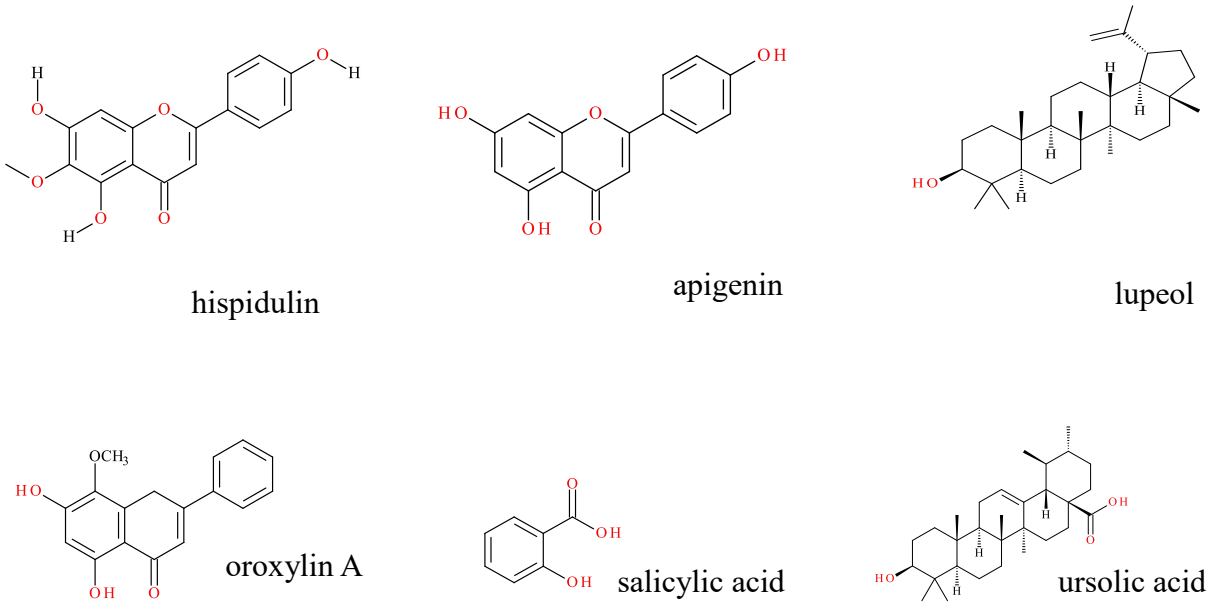


Figure 2. Some reported chemical constituents of *Brucea javanica Linn.*

The main aim of this research was to investigate some phytochemical constituents from the seeds of *Brucea javanica* Linn. (Yar-dan-seed) and to evaluate some biological activities such as antimicrobial activity, antioxidant activity and antidiabetic activity.

Materials and Methods

Sample Collection

The seeds sample of *Brucea javanica* Linn. (Yar-dan-seed) was collected from Yangon Region in October, 2020. After being collected, the scientific name of the sample was identified by authorized botanists at Botany Department, Yangon University.

Sample Preparation

The fresh sample was cleaned by washing with water and 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 pet-ether (60-80 °C) for 6 h by using soxhlet extractor. The filtrate was concentrated by removal of the solvent under reduced pressure to give the respective pet-ether crude extract. Preparation of ethyl acetate extract, 95% ethanol and water extracts were prepared by similar manner mentioned in above procedure. Each extract was dried at normal pressure on a water bath and stored under 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 Water and Ethanol Extracts of *Brucea javanica* Linn. (Yar-dan-seed)

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

The total phenol content (TPC) of watery and ethanol extracts of selected sample was 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 read 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 and ethanol extracts of selected sample 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 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 *Brucea javanica* Linn. (Yar-dan-seed)

(a) Investigation of antioxidant activity of Crude Extracts of *Brucea javanica* Linn. (Yar-dan-seed)

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 *Brucea javanica* Linn. (Yar-dan-seed)

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 concentration 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 in above solution followed by incubated for 15 min at room temperature. To stop the reaction, 4 mL of 0.1M HCl was added in this mixture and to detect the reaction, 1 mL of Iodine-iodide indicator (1 mM) was added in the mixture. Absorbance was read at 650 nm by UV spectrophotometer in the visible region. The control solution was prepared as above procedure by using phosphate buffer (0.02 M, 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 *Brucea javanica* Linn. (Yar-dan-seed)

The antimicrobial activity of crude extracts *Brucea javanica* Linn. (Yar-dan-seed) 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 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 at 7.2 with 0.1 M sodium hydroxide solution and 1.5 g of agar was added. Nutrient agar 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 and plugged with cotton wool and autoclaved at 121 ºC for 15 minutes. Then the tubes were cooled down to 60 ºC and poured into sterilized petri-dish and 0.1 mL of spore suspension was also added into the dishes. The agar was allowed to set for 30 minutes after which 10 mm plate agar well was made with the help of sterilized cork border. After that, about 0.1 mL each of the prepared extract solution was introduced into the agar well and incubated at 37 ºC for 24 hours. 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 zone of inhibition diameter.

Results and Discussion

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 selected sample. The total phenol content of the watery and ethanol extracts of *Brucea javanica* Linn. (Yar-dan-seed) was determined with spectrophotometric method by using Folin-Ciocalteu reagent. The total

phenol content of the ethanol extract of *B. javanica* was 303.24 ± 1.19 mg GAE/g. The total flavonoid content of sample was determined with spectrophotometric method by aluminium chloride reagent and ethanol extract of selected sample was found to be 286.01 ± 0.20 mg QE/g. The results are shown in Table1

Table 1. Total Phenol and Total Flavonoid Contents of Water and Ethanol Extract of *Brucea javanica Linn*. (Yar-dan-seed)

Extracts	Total Phenol Content (mg GAE ± SD)/g of extract	Total Flavonoid Content (mg QE ± SD)/g of extract
Water	189.52 ± 0.66	171.55 ± 0.11
Ethanol	303.24 ± 1.19	286.01±0.20

According to the experimental results, phenol and flavonoid compounds were detected in water and ethanol extracts 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 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.

The antioxidant activity was measured in terms of hydrogen donating or radical scavenging ability of the water and ethanol extracts of selected sample by using the stable radical DPPH. The results are shown in Table 2. The ethanol extract of selected samples was found to be the low (IC_{50}) value 36.21 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 *Brucea javanica* Linn. (Yar-dan-seed) 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	
Water	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	41.55
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	36.21

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 *Brucea javanica* Linn. (Yar-dan-seed) was investigated. The inhibitory effect of watery and ethanol extracts were analyzed. The percentage inhibition of *α*-amylase by water and ethanol extracts were 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 water and ethanol extracts were calculated and the results are respectively tabulated in Table (3). The water and ethanol extracts of bitter selected plant was also explored for the *in vitro α*-amylase inhibition and their activity was compared with standard anti-diabetic drug, acarbose. The 50% a–amylase inhibition potency (IC_{50}) of water and ethanol extracts of the selected sample was ranging between 89.12-153.98 μg/mL, indicating that crude extracts possessed potent *α*-amylase inhibition activity but these extracts low inhibition activity than standard acarbose (IC_{50} 20.92 μg/mL).

Table 3. IC_{50} Values of Water and Ethanol Extracts of *Brucea javanica Linn*. (Yar-dan-seed) by *α*-Amylase Inhibition Assay

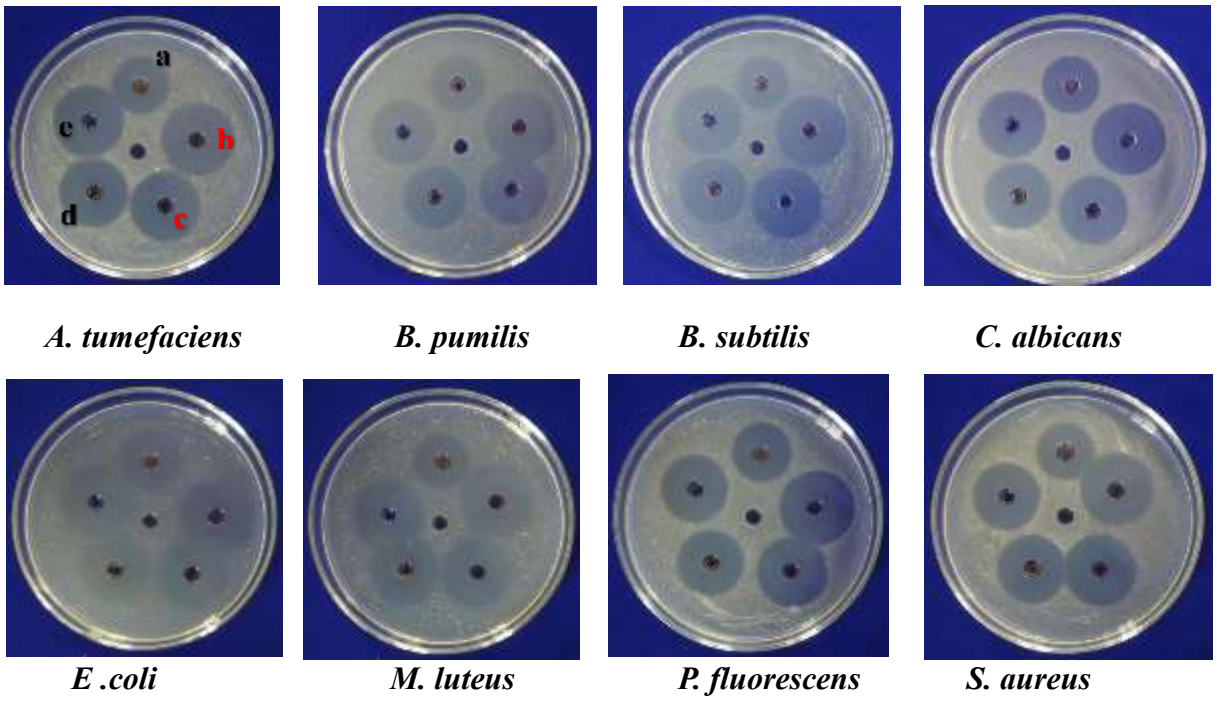
Samples	**% Inhibition in different concentrations (μg/mL)**							**IC_{50} (μg/mL)**
(Extracts)	**3.125**	**6.25**	**12.5**	**25**	**50**	**100**	**200**	
Water	4.07 ± 0.20	7.29 ± 0.32	10.10 ± 0.30	23.13 ± 0.34	46.35 ± 0.16	49.43 ± 0.10	57.75 ± 0.07	153.98
Ethanol	7.29 ± 0.32	16.04 ± 0.26	20.77 ± 0.24	37.62 ± 0.15	45.29 ± 0.11	48.32 ± 0.57	65.99 ± 0.04	89.12

Screening of antimicrobial activity of various crude extracts such as pet-ether, ethyl acetate, 80 % ethanol and water extracts of *B. javanica* were done by employing 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 water extract of selected sample did not exhibit any antimicrobial activity against all tested microorganisms whereas pet-ether and ethyl acetate and ethanol extracts from selected sample exhibited inhibition zone diameters ranged between in 13.34 ~ 22.53 mm respectively against all tested microorganisms. The selected sample of ethanol extract showed less activity and pet-ether and ethyl acetate extracts were observed to be most effective in antimicrobial activity. Therefore, all the crude extracts of selected sample, except water extract of *B. javanica* exhibited antimicrobial activity against all microorganisms tested. Among the crude extract, pet-ether and ethyl acetate extracts of selected sample showed the most pronounced antimicrobial activity against all microorganisms tested. However, the selected sample low inhibition zone diameters than standard antimicrobial drug chloramphenicol (28.33 ~ 31.31 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	-	14.10	20.24	20.83	29.29
B. pumilis	-	14.10	18.25	19.53	29.48
B. subtilis	-	14.62	19.66	22.53	28.89
C. albicans	-	13.54	19.04	21.28	30.34
E .coli	-	14.10	20.24	20.83	29.29
M. luteus	-	14.10	18.25	19.53	29.48
P. fluorescens	-	14.62	19.66	22.53	28.89
S. aureus	-	13.54	19.04	21.28	30.34

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= EA, 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 of the fruits of *B. javanica.* In the preliminary phytochemical results alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, steroid, tannins and terpenoids were found to be present except starch in selected sample. According to the chemical investigation the water and ethanol extract of selected sample contains high total phenolic and total flavonoid contents. This showed high antimicrobial activity (20 ~ 40 mm) 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 water and ethanol extracts of selected sample also showed DPPH free radical scavenging activity assay (IC_{50} = 41.55 mg/mL and IC_{50} = 36.21 μg/mL respectively) has antioxidant activity. The water and ethanol extracts of selected sample possessed the antidiabetic activity due to its *α*-amylase inhibitory effect (IC_{50}= 153.98 μg/mL and IC_{50}= 89.12 μg/mL respectively). The result obtained from this research strongly indicated that tested crude extracts of *Brucea javanica* Linn. (Yar-dan-seed) may play an important role in medicinal properties used *in vitro* and may be effective.

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