



Anti-hyperglycemic Activity in Vitro, Acute Toxicity in Vivo and Antioxidant Activity of the Crude Extract of the Root of *Plumeria alba* L.

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Abstract

Medicinal plants are the single most productive sources for the development of drugs and play an important role in treating and preventing a variety of diseases through the world. *Plumeria alba* Linn. commonly known as Tayoke-sakar-aphyu in Myanmar is one of the medicinal plants belonging to Apocynaceae family. The pharmacological studies were carried out to investigate antimicrobial activity, antioxidant activity, anti-hyperglycemic activity in vitro and acute toxicity in vivo. The main aim of the present research is to evaluate the biological activities of the root of *Plumeria alba* L. Firstly, phytochemical screening of the collected sample was performed. Elemental composition of the crude sample was examined by EDXRF (Energy Dispersive X-ray Fluorescence) spectroscopy. Moreover three different solvents such as ethanol, ethyl acetate and n-hexane extracts of the sample were examined for their antimicrobial activities against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and *E. coli* by Agar-well diffusion method. The antioxidant activity of ethanol extract of the root of *Plumeria alba* L. was studied by DPPH (1,1-Diphenyl-2-picryl-hydryl) assay. The acute toxicity of ethanol extract of the root of *Plumeria alba* L. on *Artemia salinna* were investigated. The glucose lowering activities of the water and ethanol extracts of the sample were determined by iodometric titration.

Keywords: *Plumeria alba* L.; EDXRF; antimicrobial; antioxidant; acute toxicity.

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1. Introduction

Since ancient times, plants have been an extensive source of medicine. Vast research has been conducted in last few decades on the plants mentioned in ancient literature or used traditionally [6]. Phytochemicals, non-nutritional biologically active compounds are occurred in plant [2]. The use of plant compounds for pharmaceutical purpose has gradually increased. About 80% of individuals from developed countries use traditional medicine, which involves compounds derived from medicinal plants [6]. Herbal medicines are in great demand in the developed and developing countries for primary healthcare because of their wide biological and medicinal activities, higher safety margins and lesser costs [8]. Diabetes mellitus is a group of metabolic diseases characterized by elevated blood glucose levels (hyperglycemia) resulting from defects in insulin secretion, insulin action or both. Insulin is a hormone manufactured by the beta cells of the pancreas, which is required to utilize glucose from digested food as an energy source. Chronic hyperglycemia is associated with microvascular and macrovascular complications that can lead to visual impairment, blindness, kidney disease, nerve damage, amputations, heart disease, and stroke [3]. Diabetes mellitus is a serious metabolic disease affecting major population worldwide [5]. The plant of *Plumeria alba* (L) (Tayoke-sakar-aphyu) is mainly grown for its ornamental and fragrant flowers, is also known for its medicinal importance [8]. Despite long historical and current use of the water decoction of *Plumeria alba* (L) roots in the local management of antidiabetic, there is a dearth of scientific reports on the therapeutic potential of this plant in the management of obesity, hyperlipidemia and hyperglycemia [5]. herefore, the root of *Plumeria alba* (L) was selected to investigate phytochemicals, glucose lowering activity, elemental composition, antimicrobial activity, antioxidant activity and acute toxicity.

1.1 Botanical Description [8]

<i>Botanical name</i>	- <i>Plumeria alba</i> (L)
<i>Family name</i>	- <i>Apocynaceae</i>
<i>Myanmar name</i>	- <i>Tayoke-sakar-aphyu</i>
<i>English name</i>	- <i>White Champa</i>
<i>Part used</i>	- <i>Roots</i>



Figure 1: The *Plumeria alba* (L) plant and the root of the *Plumeria alba* (L)

2. Materials and Methods

2.1 Sample Collection

The root of *Plumeria alba* L. was collected from Mandalay University campus, Mandalay Region. They were cut into small pieces and dried in air at room temperature for three weeks. They were percolated with ethanol.

2.2 Preliminary Phytochemical Tests for the Root of *Plumeria alba* L.

All phytochemical tests of the root of *Plumeria alba* L. were performed based on J. B. Harborne (1973) [4], phytochemical methods (London: Chapman and Hall) and New Journal of Science by Chuwuma S. Ezeonu and Chigozie M.Ejikeme, 2016[1], But in this research, 2g of sample was used as the starting weight for all experiments.

2.3 Determination of Elemental Composition of the Root of *Plumeria alba* L.

The elemental composition of the root of *Plumeria alba* L. was examined by EDXRF (Energy Dispersive X-ray Fluorescence) method.

2.4 Determination of Antimicrobial Activities of the Root of *Plumeria alba* L.

Asian Journal of Plant Science and Research by Thamaraiselvi, P.Lallitha* and P.Jayanthi (2012), [11] described the studies on antimicrobial activities by agar well diffusion method. So, the antimicrobial activities of the three solvent extracts such as n-hexane, ethanol and ethyl acetate extracts were determined by Agar Well diffusion method at Development Center of Pharmaceutical and Food Research Department (PFRD), Insein Yangon. The antimicrobial activities of three different solvent extracts were measured from the diameters of Inhibition zones as shown in figures, 2.

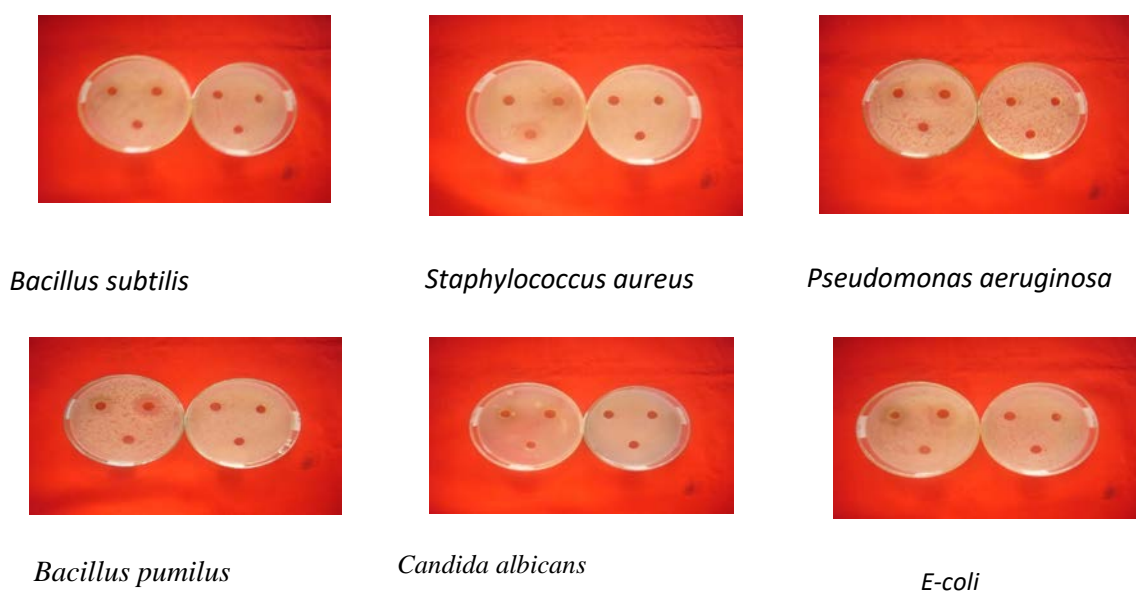


Figure 2: Antimicrobial activities of three solvents extracts of the sample

2.5 Determination of Antioxidant Activity of Ethanol Extract of *Plumeria alba* L.

Saudi Pharmaceutical Journal by Md Nur Alam And his colleagues 2013 [7], reported that the evaluation of antioxidant activity by using DPPH assay. In order to examine the antioxidant activity through free radical scavenging by the test sample, the change in optical density of DPPH radicals is monitored in this research. The antioxidant activity of ethanol extract of the root of *Plumeria alba* L. was determined by DPPH (1,1-Diphenyl-2-picryl-hydrazyl) Radical Scavenging Assay in Department of Biotechnology, Mandalay Technological University.

2.6 Determination of Glucose Lowering Activity of the Root of *Plumeria alba* L.

According to Practical Chemistry for B.Sc.I, II & III Year Students of All Indian University by O.P. Pandey And his colleagues (1997)[9], the glucose lowering activity of water extract and ethanol extract of the sample was determined by iodometric titration. In order to carry out iodometric titration, the concentration of iodine solution was determined by titrating with 0.05 M of standard glucose solution.

2.6.1 Preparation of Sample Solution Containing Water Extract and Glucose at Different Contact Times

10 g of the dried powder sample was boiled with 200 mL of distilled water for 30 minutes and then cooled and filtered. 20 mL of the filtrate was mixed with 50 mL of 0.05 M glucose solution and was allowed to stand at room temperature for 15 minutes. The sample and glucose solutions were prepared for different contact times such as 30 minutes, 45 minutes and 60 minutes and 75 minutes.

2.6.2 Preparation of Sample Solution Containing Ethanol Extract and Glucose at Different Contact Times

Dried powdered sample (about 150 g) was percolated with 600 mL of 95 % ethanol for four months. 0.25 g of ethanol extract was mixed with 50 mL of 0.05 M glucose solution and was allowed to stand at room temperature for 15 minutes. The same mixture solutions were prepared for different contact times such as 30 minutes, 45 minutes, 60 minutes and 75 minutes.

2.6.3 Determination of Glucose Lowering Activity of the Extract by Iodometric Titration

10 mL of the prepared water extract sample solution was taken in a conical flask and 20 mL of 0.05 M iodine solution and 45 mL of 0.1 M sodium hydroxide solution were added into the flask. This flask was closed and was kept in the dark for 15 minutes. When 6 mL of 1 M hydrochloric acid was added into the flask free from the dark, red color solution was obtained. This red color solution was titrated with 0.05 M sodium thiosulphate solution until straw color solution was obtained. Then, 1 mL of starch indicator solution was added into the straw color solution. Then dark blue color was obtained. This solution was titrated with the same sodium thiosulphate solution to observe colorless solution. From the experimental data, the decrease in amount of glucose for water extract of the sample solution can be calculated. Similarly, the amount of decreased glucose amount for the prepared solution containing ethanol extract and glucose was also determined by the similar manner.

2.7 Determination of Acute Toxicity of Ethanol Extract of *Plumeria alba* L.

J.Myan.Acad.Tech.1 by SabaiAnd his colleagues 2001[10], informed about the toxicity test for some Myanmar Medicinal Plants Using Brine Shrimp (*Artemia salina*). Therefore in this research the acute toxicity of the selected medicinal plant was examined with *Artemia salina*.

In this research, selected *Artemia salina* were divided into six groups. Each group contains 20 brine shrimp larvae of *Artemia salina*. A total of 20 *Artemia salina* were used to give orally the different concentrations of sample extracts. After administering the extract orally, the *Artemia salina* were watched for 6 hours for their health and behavior.



Figure 3: Brine Shrimp Larvae and extract orally

3. Results and Discussion

3.1 Results of Phytochemicals in the Root of *Plumeria alba* L.

In order to estimate different types of organic compounds present in the root of *Plumeria alba* L., preliminary phytochemical tests were performed. According to the results of phytochemical tests, the root of *Plumeria alba* L. contained alkaloids, flavonoid, glycoside, phenol, polyphenol, terpene, saponin, reducing sugar and tannin respectively.

3.2 Antimicrobial Activities of the Root of *Plumeria alba* L.

The antimicrobial activities of three different solvent extracts were determined by Agar-Well diffusion method at Development Center of Pharmaceutical and Food Research Department (PFRD), Insein Yangon. The results are described in table 1.

As describe in table, ethanol extract of the sample responds the high activities on three tested organisms such as *Staphylococcus aureus*, *Bacillus pumilus* and *Candida albican* but medium activity on the remaining tested organisms. N-hexane extract of sample responds the medium activities on three tested organisms such as *Staphylococcus aureus*, *Bacillus pumilus* and *Candida albican* but low activity on the remaining tested organisms. Ethyl acetate extract of sample responds the low activities on two tested organisms such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* but no activities on the remaining tested organisms. Therefore, the ethanol extract of the sample is the most effective on all selected organisms.

Table 1: Results of Antimicrobial Activities of the Root of *Plumeria alba* L.

Samples	solvent	Inhibition zone					
		1	2	3	4	5	6
<i>Plumeria alba</i> (L)	n-hexane	12mm (+)	15mm (++)	13mm (+)	15mm (++)	15mm (++)	-
	EtOAc	-	11mm (+)	11mm (+)	-	-	-
	EtOH	18mm (++)	23mm (+++)	18mm (++)	20mm (+++)	20mm (+++)	-

Agar well -10 mm (-) = no activity

10mm-14mm (+) = low activity

15mm-19mm (++) = medium activity

20mm above (+++) = high activity

Organisms

(1) *Bacillus subtilis*

(2) *Staphylococcus aureus*

(3) *Pseudomonas aeruginosa*

(4) *Bacillus pumilus*

(5) *Candida albicans*

(6) *E-coli*

3.3 Antioxidant Activity of Ethanol Extract of *Plumeria alba* L.

Table 2: % Inhibition of Various Concentrations of Ethanol Extract of the Sample

Concentration (µg/mL)	Mean Absorbance	%DPPH Radical Scavenging	IC ₅₀ (µg/mL)
7.8	1.8785	47.62382865	36.144
15.0	1.8685	47.88877733	
31.0	1.8213	49.20515395	
62.5	1.6671	53.50568942	
125.0	1.5517	56.7241187	

The results of antioxidant activity of ethanol extract of the root of *Plumeria alba* L. by using DPPH assay are shown in table 2.

IC₅₀ value was calculated by using linear regressive equation

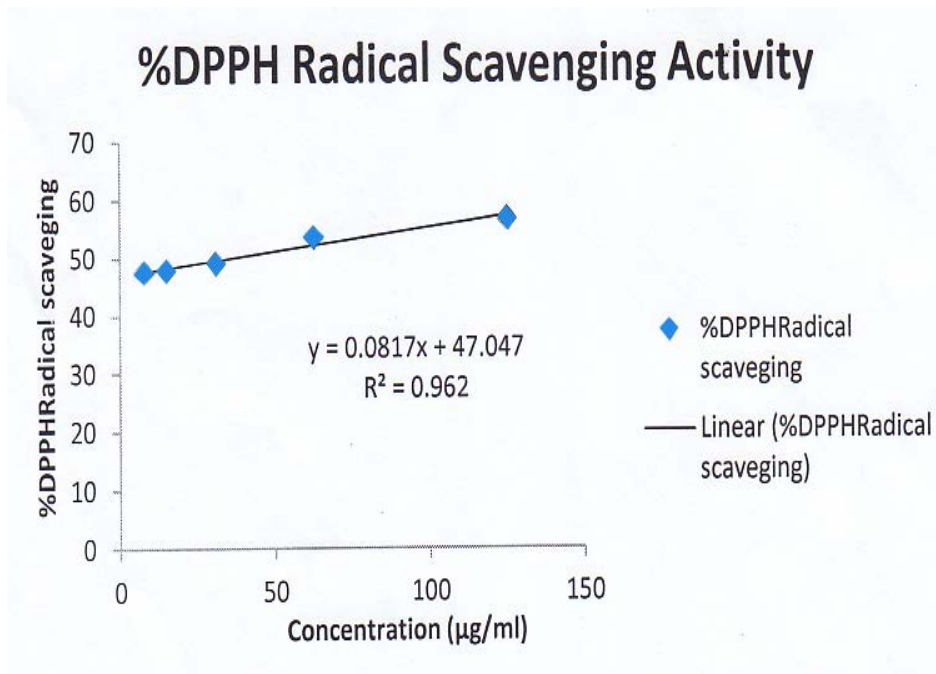


Figure 4: %Inhibition of Different Concentrations of Ethanol Extract

According to table 2 and figure 4, the IC₅₀ value of the ethanol extract of the root of *Plumeria alba* L. was 36.144 µg/mL and that of the standard ascorbic acid was 0.02195µg/mL. The ethanol extract of the sample showed the antioxidant activity but it was observed that the antioxidant activity of ethanol extract of the root of *Plumeria alba* L. was found to be lower than that of standard ascorbic acid.

3.4 Elemental Compositions of the Root of *Plumeria alba* L.

From the information of EDXRF report, it was recorded that the root of *Plumeria alba* L. was a rich source of minerals for health benefit especially calcium and potassium. Meanwhile, it was also known that the toxic metal such as lead, mercury were not present in this sample.

3.5 Glucose Lowering Activity of Water Extract of *Plumeria alba* L.

The glucose lowering activity of the root of water extract of the sample solution was determined by titration method. The results are shown in table 3.

As shown in table, the more contact between the water extract of the sample solution and glucose solution the greater the glucose lowering activity. It can be recorded that the percent of glucose lowering activity is the maximum at the contact time 60 minutes.

Table 3: The Percent of Decreased Amount of Glucose in Water Extract Sample

No	contact time (min)	Initial amount of glucose (mmol)	Left amount of glucose (mmol)	Decreased amount of glucose (mmol)	decreased amount of glucose(%)
1	15	2.5	2.4290	0.0710	2.84
2	30	2.5	2.3065	0.1935	7.74
3	45	2.5	2.2715	0.2285	9.14
4	60	2.5	2.2540	0.2460	9.84
5	75	2.5	2.2540	0.2460	9.84

3.6 Glucose Lowering Activity of Ethanol Extract of *Plumeria alba L.*

The glucose lowering activity in the root of ethanol extract of the sample solution was also determined by titration method. The results are shown in tables 4. By observing the experimental results, the less contact between the ethanol extract of the sample solution and glucose solution the more the glucose lowering activity. It can be recorded that the percent of glucose lowering activity is the maximum at the contact 15 minutes.

Table 4: The Percent of Decreased Amount of Glucose in Ethanol Extract

No	contact time (min)	Initial amount of glucose (mmol)	Left amount of glucose (mmol)	Decrease amount of glucose (mmol)	% of Decrease amount of glucose
1	15	2.5	2.1100	0.3900	15.6
2	30	2.5	2.1600	0.3400	13.6
3	45	2.5	2.2350	0.2650	10.6
4	60	2.5	2.2725	0.2275	9.1
5	75	2.5	2.3475	0.1525	6.1

3.7 Acute Toxicity of Ethanol Extract of the Root of *Plumeria alba L.*

The toxicity of ethanol extract of the root of *Plumeria alba L.* was determined by using selected *Artemia*

salina. The results are recorded in table 5.

Table 5: Mortality for Acute Toxicity of the Root of *Plumeria alba* L.

Dosage (ppm)	log Dosage	Alive	Dead	Accumulated alive	Accumulated dead	Ratio Dead: Total	Mortality
4000	3.60	0	20	0	72	72: 72	1.00
2000	3.30	4	16	4	52	52: 56	0.93
1000	3.00	6	14	10	46	46: 56	0.82
500	2.69	7	13	17	32	32: 39	0.82
250	2.39	9	11	26	19	19: 42	0.45
125	2.09	12	8	38	8	8 43	0.19

Number of brine shrimp larvae =20

By studying the recorded data, when the *Artemia salina* were treated with different dosage of ethanol extract of the sample for six hours, it was found that 12 *Artemia salina* were alive per 20 at the sample concentrations about 125ppm.

4. Conclusion

According to the phytochemical tests, the test sample, *Plumeria alba* L. consists of many d phytochemical compounds. From the EDXRF information, some minerals such as calcium, potassium and silicon are present as significant amount in the sample. However, it can be clearly known that the toxic metal such as lead and mercury are not present in this sample. In addition, from the comparison of the results of antimicrobial activities of three different extracts, it can be seen that the ethanol extract of the sample is more effective than that of the other extracts on all tested organisms. By the antioxidant activity determination, it was observed that the ethanol extract of the sample showed antioxidant activity. However, its antioxidant activity was found to be very low compared with standard ascorbic acid. Moreover, both water extracts and ethanol extracts of the sample can reduce glucose content. In comparing the glucose lowering activity, it can be seen that the more contact between the water extracts of the sample solution and glucose solution the greater the glucose lowering activity. But the less contact between the ethanol extracts of the sample solution and glucose solution the more the glucose lowering activity. By examining the toxicity of the ethanol extract of sample with *Artemia salina*, it was recorded that concentrations of the sample, until 125 ppm is safe to the tested organisms. Therefore it can be recommended that the root of *Plumeria alba* L. can be used to lower the glucose level.

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