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INVESTIGATION ON SOME PHARMACEUTICAL PROPERTIES OF THE STEM BARK EXTRACT OF *PLUMERIA ALBA* LINN.

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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 Tayok Saga - 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 stem bark of *Plumeria alba* Linn. Firstly, phytochemical screenings of the stem bark were performed. Three different solvents extracts such as ethanol, n-hexane and water of the stem bark 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 minimum inhibitory concentration (MIC) of the active extract was also determined by agar well diffusion method. The antioxidant activity of ethanol extract and aqueous extract was studied by DPPH assay. The glucose lowering activity of the ethanol and aqueous extracts of the stem bark was determined by iodometric titration. Moreover, acute toxicity of ethanol and aqueous extracts of the stem bark on the albino mice was investigated. Elemental composition of the crude sample was examined by ED-XRF (Energy Dispersive X-ray Fluorescence) studies.

KEYWORDS: Acute toxicity, Antimicrobial, Antioxidant, Anti-hyperglycemic, ED-XRF, MIC, *Plumeria alba* L.

I. 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. commonly known as Tayok Saga - aphyu in Myanmar 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 the stem bark and also the root 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] Therefore, the stem bark of Tayok Saga - aphyu was selected to investigate some pharmaceutical properties such as glucose lowering activity, antioxidant activity, antimicrobial activities and acute toxicity.

Botanical Description of Tayok Saga - aphyu[8]

Botanical name - *Plumeria alba* Linn
Family name - Apocynaceae
Myanmar name - Tayok Saga-aphyu
English name - White Champa
Part used - Stem bark

Description of Plumeria alba L.

White frangipani can grow as either a small shrub or tree ranging in height from 0.9-6.1 m with widely spaced thick succulent branches that are often covered with "knobby" protuberances. The leaves are clustered near the tips of the branches. They are large, 6-22 cm long, 2-

7 cm wide and the tip of the leaf is rounded. The leaves are dark and leathery and tend to be shiny on the upper surface with conspicuous parallel secondary veins that run from the midvein to the margins of the leaves. The flowers of this species are borne in clusters that form at the ends of the branches on a long thick stalk. Each inflorescence contains many white flowers with a small yellow center. Flowers contain five petals that are fused at the base in a short funnel-shaped tube which gradually widens as the lobes of the petals are spread out. The fruit of this species is a dry follicle which splits along one side to release the winged seeds.

***Medicinal Uses of Plumeria alba* L.**

The medicinal properties are often due to their latex which is frequently drastic and corrosive. Latex is applied to ulcers, herpes and scabies. Seeds possess haemostatic properties. Moreover its bark is bruised and applied as plaster over hard tumours. Moreover, they are used as purgative, cardiotonic, diuretic and hypotensive. This shrub has been known to possess analgesic, antitumor, antioxidant, antidiarrhoea, anticonvulsant, antimicrobial, oestrogenic and antimalarial activity. (Monika Gupta, 2016).

Figure 1: *Plumeria alba* Linn.

II. MATERIALS AND METHODS

A. Sample Collection

The stem bark of *Plumeria alba* L. was collected from Mandalay University Campus, Mandalay Region on April, 2017. They were cut into small pieces and dried in air at room temperature for four weeks and made to fine powder. Some of these were percolated with ethanol and some were used as directly.[1],[4]

B. Preliminary Phytochemical Tests for the stem bark of Plumeria alba L.

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

Test for Alkaloids

2 g of sample was boiled with 10 mL of 1% HCl for about 10 minutes and then cooled and filtered. The filtrate was treated with 5 drops of Wagner's reagents. The reddish brown precipitate was observed. It indicates the presence of alkaloids in this sample.

2 g of sample was boiled with 10 mL of 1% HCl for about 10 minutes and then cooled and filtered. The filtrate was treated with 5 drops of Dragendroff's reagents. The orange precipitate was observed. It also indicates the presence of alkaloids in this sample.

Tests for Glycoside

2 g of sample was boiled with 10 mL of distilled water for about 10 minutes and then cooled and filtered. 1 mL of distilled water and 3 drops of lead acetate solution was added to the filtrate. The formation of white precipitate indicates the presence of glycoside in this sample.

Test for Phenolic compound

2 g of sample was boiled with 10 mL of distilled water for about 10 minutes and then cooled and filtered. The filtrate was treated with 3 drops of 10% FeCl3 solution. The formation of a brown color precipitate indicates the presence of phenolic compound in this sample.

Tests for Polyphenol

2 g of sample was boiled with 10 mL of 95 % ethanol for about 10 minutes and then cooled and filtered. The filtrate was treated with 8 drops of the mixtures of 1% FeCl3 and 1% K3 [Fe (CN)6]. The greenish blue color solution was observed. It indicates the presence of polyphenol in this sample.

Test for Saponin

2 g of sample was boiled with 10 mL of distilled water for about 10 mintues and then cooled and filtered. The filtrate was shaken for 3 minutes. Formation of frothing indicates the presence of saponin in this sample.

Test for Terpene

2 g of sample was boiled with 10 mL of pet-ether for about 10 minutes and then cooled and filtered. The filtrate was treated with 2 drops of CHCl3, 2 drops of acetic anhydride and 2 drops of conc: H2SO4 were added. The yellow color solution was observed. Therefore, it indicates the presence of terpene in this sample.

Test for Flavonoid

2 g of sample was boiled with 10 mL of 95 % ethanol for about 10 minutes and then cooled and filtered. A few drop of concentrated hydrochloric acid and a few pieces of magnesium turning were added to the filtrate. Reddish brown color solution was observed. Therefore, it indicates the presence of flavonoid in this sample.

Test for Steroid

2 g of sample was boiled with 10 mL of 95% ethanol for about 10 minutes and then cooled and filtered. The filtrate was treated with 2 drops of acetic anhydride, 2 drops of CHCl3, and 2 drops of conc: H2SO4. Green yellow color solution was observed, but pink color was no observed. Therefore, it is recorded that steroid is not present in this sample.

Test for Tannin

2 g of sample was boiled with 10 mL of 95% ethanol for about 10 minutes and then cooled and filtered. The filtrate was treated with 3 drops of 10% FeCl3 and 3 drops of dil: H2SO4. The yellowish brown precipitate was observed but green color was no observed. Therefore, it is recorded that tannin is present in this sample.

Test for Reducing sugar

2 g of sample was boiled with 10 mL of distilled water for about 10 minutes and then cooled and filtered. The filtrate was treated with Benedict' solution. Reddish color solution was observed, but reddish color was no observed. Therefore, it indicates the presence of reducing sugar in this sample.

C. Preparation of Samples for Testing of Antimicrobial Activities

Dried sample powder (75 g) was extracted by n-hexane (300 ml, 60-80°C). After evaporation, 2.575 g of nonpolar fraction (3.433%) was obtained. The remaining sample powder was then extracted thoroughly with 75% ethanol to yield 20.45 g (28.23%). Water extract sample was prepared by boiling with distilled water. The resulting extracts were tested for antimicrobial activity at 500 g/ ml by Agar Well diffusion method at Development Center of Pharmaceutical and Food Research Department (PFRD), Insein Yangon. From the diameters of Inhibition zones and the minimum inhibitory concentration (MIC) of the active extract was also determined.[10]

D. Evaluation of Antioxidant Activity of Ethanol and Aqueous Extracts of Sample

In order to evaluate the antioxidant activity through free radical scavenging by the tested sample, the change in optical density of DPPH radicals is monitored in this research. The antioxidant activities of ethanol extract and aqueous extract of the stem bark of *Plumeria alba* L. was determined by DPPH (2,2-diphenyl-1-picrylhydrazyl) Radical Scavenging Assay. The results were expressed as mg ascorbic acid/ g dried sample. Each assay was carried out in triplicate. The percentage inhibition of the DPPH radical was calculated using the following formula:

$$I\% = A0 - A / A0 \times 100$$

Where I = DPPH inhibition (%)
A0 = absorbance of control sample
A = absorbance of a tested sample

The amount of sample necessary to decrease the absorbance of DPPH by 50% (IC50) was calculated graphically for the extracts in five different concentrations.[7]

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

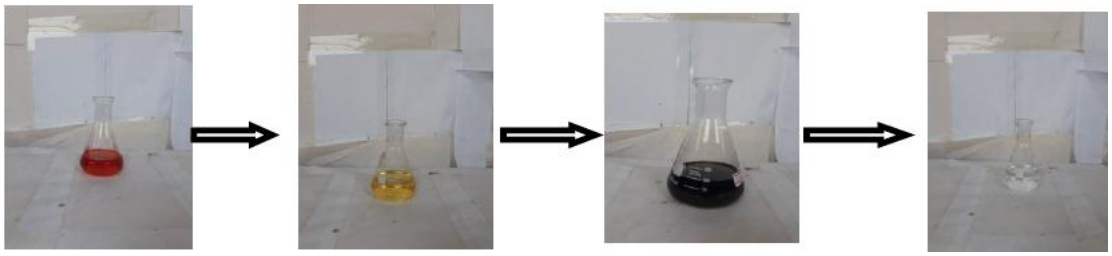
10 g of the dried powder sample was boiled with 200 mL of distilled water for 30 minutes and then cooled and filtered. 20 mL of the filtrate was mixed with 50 mL of 0.05 M glucose solution and was allowed to stand at room temperature for 15 minutes. The water extract sample containing glucose solutions were prepared for different contact times such as 30 minutes, 45 minutes and 60 minutes and 75 minutes.[9]

F. 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.

According to Practical Chemistry for B.Sc. I, II & III Year Students of All Indian University by O.P. Pandey, *et al*, (1997), 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.[9]

10 mL of glucose 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. In order to change, this solution was titrated with the same sodium thiosulphate solution to observe colorless solution. From the experimental data, the concentration of iodine solution can be calculated.[9]



Red color Straw color Dark blue color Colorless

Figure 2: Colour changes in iodometric titration using sodium hydroxide.

G. Determination of Glucose lowering Activity of Ethanol and Aqueous Stem Bark Extracts

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 for the prepared solution containing ethanol extract and glucose was also determined by the similar manner.[9]

H. Investigation on Acute Toxicity of Ethanol and Aqueous Extracts of the Stem Bark

The acute toxicity test of 95% ethanol and aqueous extracts of the stem bark of *Plumeria alba* L. was investigated by methods of OECD (Organization for Economic Co-operation and Development) guidelines for the testing of Chemical 423(2008). Screening of the stem bark extract was done with the dosage of 2000mg/kg, 5000mg/kg body weight in albino mice at department of Medical Research (Pyin Oo Lwin Branch).[11]

I. Determination of Elemental Compositions of the Stem Bark of Plumeria alba L.

The elemental composition of the sample was examined by EDXRF (Energy Dispersive X-ray Fluorescence) method.

III. RESULTS

A. Phytochemicals of the Stem Bark Sample

In order to estimate different types of organic compounds present in the particular sample, preliminary phytochemical tests were performed. According to the phytochemical tests, the stem bark sample contained alkaloids, flavonoid, glycoside, phenol, polyphenol, terpene, saponin, reducing sugar and tannin respectively. The results are described in table 1.

Table 1: Phytochemicals of the Stem Bark of *Plumeria alba* L.

No	Constituent	Extract	Regents Used	Observation	Results
1	Alkaloids	1%HCl	Wagner's reagents Dragendroff's reagents	Reddish brown ppt Orange ppt	+ +
2	Flavonoid	95%ethanol	conc:HCl, Mg	Reddish brown color solution	+
3	Glycoside	distilled water	10% lead acetate	White precipitate	+
4	Polyphenol	95% ethanol	1%FeCl3, 1% K3[Fe(CN)6]	Greenish blue color solution	+
5	Reducing Sugar	distilled water	Benedict's solution	Reddish color solution	+
6	Saponin	distilled water	H2O	Froth	+
7	Steroid	95%ethanol	CHCl3, acetic anhydride,conc:H2O4	yellow color solution	-
8	Tannin	95%ethanol	10%FeCl3,dilH2SO4	Yellowish brown ppt	+
9	Terpene	pet-ether	CHCl3,acetic anhydride,conc:H2So4	Yellow color solution	+

(+)= presence (-) = absence

B. Antimicrobial Activities of the Stem Bark of Plumeria alba L.

The antimicrobial activities of three different solvent extracts were determined by Agar-Well diffusion method. The ethanol extract of the plant sample responds high activities on all selected organisms such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *E. coli*. However, n-hexane and water extract of this sample have no activities on all tested organisms. The results were described in table 2.

The lowest concentration of antimicrobial agent that results in complete inhibition of microorganism

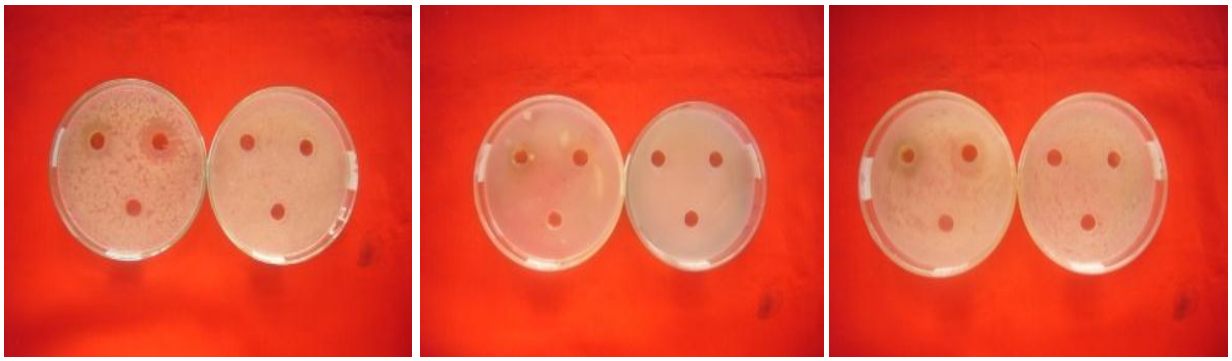
represents the MIC. The MIC values of the most active extract were calculated and the values were shown in table 3.

Table 2: Antimicrobial Activities of the Stem Bark of *Plumeria alba* L.

		Inhibition zone					
Samples	**Extract**	**1**	**2**	**3**	**4**	**5**	**6**
Plumeria alba (L)	water	12mm(+)	15mm (++)	13mm(+)	15mm(++)	15mm(++)	-
	n-hexane	-	-	-	-	-	-
	EtOH	18mm(+++)	23mm(+++)	18mm(++)	20mm(+++)	20mm(+++)	-

Agar well -10 mm (-) = no activity
10mm-14mm (+) = low activity
15mm-19mm (++) = medium activity
20mm above (+++) = high activity
1 = *Bacillus subtilis*
2 = *Staphylococcus aureus*
3 = *Pseudomonas aeruginosa*
4 = *Bacillus pumilus*
5 = *Candida albicans*
6 = *E-coli*

Bacillus subtilis sublilis ***Staphylococcus aureus*** ***Pseudomonas aeruginosa***



Bacillus pumilus ***Candida albicans*** ***E-coli***

Figure 3: Antimicrobial activities of different solvents extracts.

Table 3: MIC Values of Ethanol Extract.

No	Organisms	MIC(g/ml)
1	*Bacillus subtilis*	10.5
2	*Staphylococcus aureus*	50.0
3	*Pseudomonas aeruginosa*	15.5
4	*Bacillus pumilus*	20.0
5	*Candida albicans*	50.0
6	*E-coli*	5.25

C. Antioxidant Activity of Ethanol and Aqueous Stem Bark Extracts

Table 4: % Inhibition of various concentration of Ethanol Extract.

Sample Concentration (□g/ml)	Mean Absorbance	Mean % inhibition	IC_{50} MIC (µg/ml)
50	0.297	68.50	17.99
25	0.350	61.61	
12.5	0.483	48.78	
6.25	0.562	40.41	
3.125	0.608	35.52	

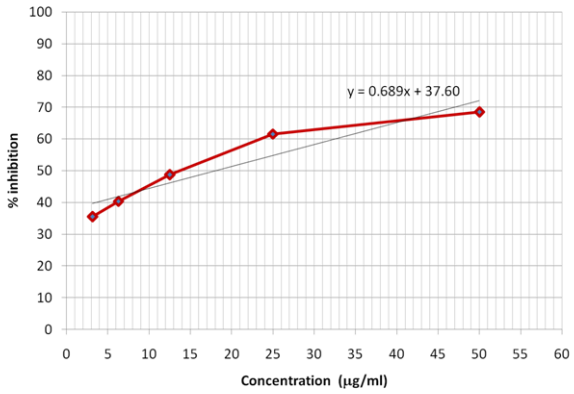


Figure 4: Plot of % Inhibition Vs Concentration of Ethanol Extract.

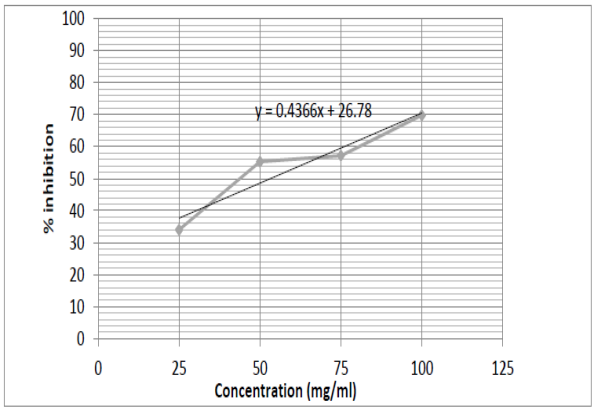


Figure 5. Plot of % Inhibition Vs Concentration of Stem Bark of Plumeria alba L.

Table 5: % Inhibition of various concentration of water Extract.

Concentration (µ gg/ml)	Mean Absorbance	Mean % inhibition	IC^{50} (µg/ml)
100	0.318	69.77	53.236
75	0.453	57.18	
50	0.473	55.29	
25	0.698	34.02	

The IC50 value of ethanol extract of the sample was found to be 17.99 where as that of water extract was 53.236.

D. Glucose Lowering Activity of Water Extract of Plumeria alba L

The glucose lowering activity of the stem bark water extract was determined by titration method. The results are shown in table 6.

Table 6: The Percent of Decreased Amount of Glucose in Water 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	2.325	0.1750	7.00
2	30	2.5	2.2065	0.2935	11.74
3	45	2.5	2.2015	0.2985	11.94
4	60	2.5	2.1950	0.3050	12.20
5	75	2.5	2.1950	0.3050	12.20

E. Glucose Lowering Activity of Ethanol Extract of the Stem Bark

The glucose lowering activity of the stem bark ethanol extract was also determined by titration method.
The results are shown in table 7.

Table 7: Percent of Decreased Amount of Glucose in Ethanol Extract.

No	contact time (min)	Initial amount of glucose (mmol)	2.0100	Decrease amount of glucose (mmol)	% of Decrease amount of glucose
1	15	2.5	2.0600	0.4900	19.60
2	30	2.5	2.1350	0.4400	17.60
3	45	2.5		0.3650	14.60
4	60	2.5	2.1725	0.3275	13.10
5	75	2.5	2.2495	0.2505	10.02

F. Acute Toxicity of Ethanol and Aqueous Extracts of the Stem Bark

The LD50 (Lethal Dose) of the two tested sample extracts were more than 5000 mg/kg.

G. Elemental compositions of the Stem Bark of Plumeria alba L.

The mineral composition of the crude sample powder was shown in table 8.

Table 8: Elemental Compositions of *Plumeria alba* L.

No	Element	Symbol	Relative abundant (%)
1	Calcium	Ca	0.551
2	Potassium	K	0.317
3	Silicon	Si	0.314
4	Aluminium	Al	0.081
5	Sulfur	S	0.072
6	Iron	Fe	0.033
7	Titanium	Ti	0.004
8	Phosphorus	P	0.003
9	Manganese	Mn	0.002
10	Stronium	Sr	0.002
11	Copper	Cu	0.002
12	Zinc	Zn	0.001

IV. DISCUSSION

According to phytochemical tests, the stem bark of *Plumeria alba* L. collected from Mandalay University Campus was rich in phytochemicals. From the information of antimicrobial activity evaluation, it could be observed that the ethanol extract showed positive activity in preliminary screening while the nonpolar solvent extract was not active. The finding results showed that the ethanol extract has broad spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria, yeast such as *Candida albicans*. Among Gram-positive and Gram-negative bacteria, activity was in the range of 50 to 5.25 g/mL. In general activity against Gram-negative bacteria is greater than Gram-positive bacteria. The most sensitive Gram-positive bacteria is *Bacillus subtilis* having 10.5 g/mL MIC value while *Bacillus pumilus* has MIC value of 20 g/mL. The most sensitive Gram-negative bacteria are E.coli and *Pseudomonas aeruginosa* having 5.25 and 15.5 g/mL MIC values respectively. The MIC values of *Staphylococcus aureus* and *Candida albicans* are 50 g/ml.

As the IC50 values and the antioxidant capacity are inversely proportional values. So, by comparing IC50 values of ethanol and aqueous extracts, ethanol extract showed high activity than that of aqueous extract.

As shown in table 6, 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.

By observing the experimental results in table 7, 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.

The LD 50 of the two tested sample extracts was more than 5000 mg/kg. So, acute toxicity study revealed that there was no toxic sign and lethality at the dose of 5000 mg/kg. From the EDXRF information, it was recorded that the stem bark of sample powder was a rich source of minerals for health benefit especially calcium and potassium. Meanwhile, the toxic metals such as lead, mercury were not present in this stem bark sample.

V.CONCLUSION

Based on experimental data, it can be concluded that the ethanol extract of the *Plumeria alba* L. stem bark has good antimicrobial activity. The active principle is polar in nature as it extractable with 75% ethanol. The active fraction has a broad spectrum of activity against bacteria and fungi. Moreover the active ethanol extract has high antioxidant capacity. 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. In addition, the present study proves that both ethanol and aqueous extracts have no serious acute toxic effects and the crude material has no harmful toxic heavy metals. The collected the stem bark of *Plumeria alba* L. is rich in antimicrobial compounds and can reduce the glucose content. Therefore it can be recommended that the stem bark of *Plumeria alba* L. is an important phytopharmaceutical material. Further bioassay –guided purification for the isolation of active principle is in progress.

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