Evaluation of Total Phenol Content, Antimicrobial and Antioxidant Activities of Flower of *Plumeria alba* L. (Tayoksaga-hpyu)

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Abstract

This paper aims to explore the phytoconstituents, phenolic compound, total phenol content, antimicrobial and antioxidant activities of *Plumeria alba* flower which werecollected at Sagaing University of Education campus, Sagaing Region in Myanmar during June, 2017. Firstly, preliminary phytochemicals screening of selected sample was undertaken by using standard method which observed the presence of alkaloids, glycosides, flavonoids, phenolic compounds, tannins and steroids. Moreover, functional groups of phenolic compound were examined by spectroscopic method. After that, total phenol contents of ethanol, methanol, and water extracts of sample were performed by using the Folin-Ciocalteu Reagent method. In addition, antimicrobial activities of five solvent extracts were carried out using agar disc diffusion method, Finally, the antioxidant activity of ethanol, methanol, and water extracts of the sample was evaluated by 1, 1-diphenyl-2-picryl hydrazyl) DPPH Radical Scavenging Assay. The experimental results contribute that the rich phenol fractions of flowers of *Plumeriaalba* represent bioactive and a potential source of natural antioxidants.

Keywords: *Plumeria alba*, Phytoconstituents, total phenol content, Antimicrobial and Antioxidant activities, DPPH

Introduction

Medicinal plants are very important source of life saving drugs for the ever increasing world population. The developing countries greatly depend on plants, where a major role in health care is played by traditional medicine. There are over 275,000 species of flowering a plant in the world today. *Plumeria alba* is one of such plants that has been frequently used as medicine which belongs to the genus *Plumeria* and Family Apocynaceae.

Plumeria alba is commonly called White Champa, a small laticiferous tree or shrub, native of America. It is 4.5m high, occasionally grown for its ornamental and fragrant flowers.

Botanical Description of *Plumeria alba* L.

Family name	:	Apocynaceae
Botanical name	:	*Plumeria alba* L.
Myanmar name	:	Tayoksaga-hpyu
Common name	:	Pagoda tree, White champa
Part used	:	Flowers
Medicinal uses	:	Edible and eaten as fritters, ulcers, herpes and scabies

Figure1.Photographs of tree, flowersand leave of *Plumeria alba* L.

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Phytoconstituents

Phytoconstituents are biologically active; naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients. These compounds are known as secondary plant metabolites and have biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property.

Phenolic Compounds

Phenolic compounds are secondary metabolites that are derivatives of the pentose phosphate, shikimate, and phenylpropanoid pathways in plants. Natural phenolic compounds play an important role in cancer prevention and treatment.

Free Radicals

Free radicals are atoms or molecules with unpaired electron(s). Free radicals are generally highly reactive but some of them may be stable for a long time.

Reactive oxygen species

Reactive oxygen specie refers to oxygen containing free radical and non-free radical active molecules. Reactive oxygen species that are not free radicals include hydrogen peroxide, singlet oxygen and lipid hydroperoxide.

Benefits of free radicals and other reactive oxygen species

Free radicals and other reactive oxygen species are produced in all living organisms and have biological advantage. Evidences from multitude researches on free radicals and reactive oxygen species suggest that they play important roles in signal transduction, sensing of oxygen tension and regulation of functions controlled by oxygen concentration. They are essential in synthesis of energy and essential molecules. They are also involved in boosting our immune system.

Side effects of free radicals and reactive oxygen species

When free radicals and other reactive oxygen species accumulate in the body they cause damage on cells, DNA, lipid, sugar and protein. The damage caused by free radicals and reactive oxygen species, in plants and animals, could lead deterioration of foods, cell membrane dysfunction, protein modification, enzyme inactivation, break of DNA strands, brain damage and dementia.

Antioxidants

Antioxidants are chemicals that interact and neutralize with free radicals, thus preventing them from causing damage. Antioxidants are also known as "free radical scavengers". The body makes some antioxidants it uses to neutralize free radicals. These antioxidants are called endogenous antioxidants. The exogenous antioxidants are commonly called dietary antioxidants. Fruits, vegetables and grains are rich sources of dietary antioxidants.

Antioxidant activity by DPPH radical scavenging method

Antioxidants play an important role as health protecting factor which reduce the risk for chronic diseases including cancer and heart disease. A rapid simple and inexpensive method to measure antioxidant capacity of food involves the use of the free radical, 1, 1-

Diphenyl-2-picrylhydrazyl (DPPH) which is widely used to test the ability of compound to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity.

The DPPH assay method is based on the reduction of DPPH, a stable free radical with an odd electron which gives a maximum absorption at 517 nm (purple color). When antioxidants react with DPPH, which is a stable free radical becomes paired off in the presence of a hydrogen donor and is reduced to the DPPH and as consequence the absorbance decreased from the DPPH. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced from (Diphenylpicrylhydrazine; non radical) with the loss of this violet color (although there would be expected to be a residual pale yellow color from the picryl group still present).

Materials and Methods

Plant material

The flower of *Plumeria alba* was collected from Sagaing University of Education Campusduring June, 2017. The sample was cleaned by washing with distilled water and then dried at room temperature.The dried sample was grounded into purely fine powder by using an electric blender. It was stored in a well-stoppered bottle to prevent moisture changes and other contaminations and it was used throughout the experiment.

Phytoconstituents of Flower of *Plumeria alba* L.

Phytoconstituents of flower of *Plumeria alba* were examined by using standard methods to know the presence or absence of constituents such as alkaloids, glycosides, steroids, flavonoids, phenolic compound, and tannins. (Harbone, 1993)

Study on FT-IR Spectroscopy

The pure compound was isolated from 5 g of ethyl acetate extract of sample by using thin-layer and column chromatographic methods. FT-IR spectrum of pure compound was recorded in the range of 4000-450cm^{-1}in FT-IR spectroscopy.

Total Phenol Content by FCR Method

Perparation of sample solution

The sample solution was prepared by dissolving 1 mg of respective crude extract in 1 mL of distilled water.

Preparation of standard gallic acid solutions

The stock solution of standard gallic acid (1 mg/mL) was prepared by dissolving 1mg of gallic acid in 1 mL of distilled water. This stock solution was twofold diluted serially with distilled water to get the standard gallic acid solutions with the concentration of 125, 62.5, 31.25, 15.625 and 7.8125 µg/mL.

Procedure forconstruction of gallic acid standard curve

Firstly, 1 mL of different concentration of Gallic acid solution (125, 62.5, 31.25, 15.625, 7.8125 µg /mL was mixed with 5 mL of diluted F-C reagent (FCR: H2O, 1: 10) and incubated for 15 min. To each tube, 4 mL of 1 M sodium carbonate was added and the tubes were kept at room temperature for 30 minutes and the UV absorbance of reaction mixture was measured at λ_{max} 765 nm. A standard curve was prepared by plotting the absorbance against concentration of gallic acid.

Evaluation of total phenol content as gallic acid equivalent in sample

The total phenolic content (TPC) in each sample was estimated by Folin-Ciocalteu method. Each extract (1 mg) was mixed with 1mL of distilled water. To this, 5 mL of F-C reagent (1:10) was added and incubated for 15 minutes. To each tube, 4 mL of 1 M sodium carbonate solution was added and the tubes were kept at room temperature for 30 minutes and the 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 examined as μggallic acid equivalents per mg of different extract (g GAE/ mg). The TPC contents of all tested samples are described in Table 3 and Figure 3.

Antimicrobial activity

The petroleum ether, ethyl acetate, ethanol, methanol and water extracts of flower of *Plumeria alba*were investigated for antimicrobial activity against the microbial strains *Bacillus pumilus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*and *Candida albicans* using agar disc diffusion method at Development Centre for Pharmaceutical and Food Technology (DCPFT), Yangon. The antimicrobial activity was evaluated by measuring zone diameters of inhibition of microorganisms growth surrounding the sample extracts.

Screening of Antioxidant Activity of Methanol, Ethanol and Water Extracts of Flower of *Plumeriaalba*by DPPH (1, 1-diphenyl-2-picryl hydrazyl) assay

DPPH radical scavenging assay has been widely used to evaluate the free radical scavenging effectiveness of plant materials and various flavonoids and phenol compounds in food system.

Preparation of 60 M DPPH solution

DPPH (2.364 mg) was thoroughly dissolved in EtOH (100 mL). This solution was freshly prepared in the brown coloured reagent bottle and stored in the fridge for no longer than 24 hours.

Preparation of test sample solution

Sample (2 mg) and 10 mL of ethanol were thoroughly mixed by shaker. The mixture solution was filtered and the stock solution was obtained. The sample solution (20, 10, 5, 2.5, 1.25 and 0.625 μg/mL concentration) was prepared from this stock solution by dilution with appropriate amount of ethanol.

Procedure

The control solution was prepared by mixing 1.5 mL of 60 M DPPH solution and 1.5 mL of EtOH using shaker. The test sample solution was also prepared by mixing thoroughly 1.5 mL of 60 M DPPH solution and 1.5 mL of each sample solution. The mixture solutions were allowed to stand at room temperature for 30 minutes. Then, the absorbance of these solutions was measured at 517 nm by using UV-Visible spectrophotometer. Absorbance measurements were performed in triplicate for each concentration and then mean values so obtained were used to calculate percent inhibition of oxidation by the following equation. The capability to scavenge the DPPH radical was calculated by using the following equation:

$$\%\,\text{inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

This formula is the calculation of percent inhibition of (IC_{50}) value. The half maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of a substance in inhibiting a specific biological or biochemical function.

Results and Discussion

Evaluation of Preliminary Phytoconstituentsof Flower of *Plumeriaalba* L.

The preliminary phytoconstituents were performed on the crude extracts of flower of *Plumeriaalba*which show the presence of alkaloids, glycosides, flavonoids, phenolic compounds, tannins, and steroids. The results are tabulated in Table1.

Table1.Results of PhytoconstituentsofFlower of *Plumeriaalba* L.

No.	Test	Extract	Test reagents	Observation	Remark
1	Alkaloids	1% HCl	Dragendorff'sreagent	Orange ppt.	+
2	Glycosides	H_2O	10% Lead acetate	White ppt.	+
3	Flavonoids	EtOH	Mg turning, HCl	Pink color solution	+
4	Phenolic compounds	EtOH	10% $FeCl_3$	Brown color solution	+
5	Tannins	H_2O	10% $FeCl_3$+dilHCl	Yellowish brown	+
6	Steroids	C_6H_6	Acetic anhydride Conc:H_2SO_4	pptGreenish yellow color solution	+

(+) Presence, (–) Absence,ppt= precipitate

Study on FT-IR spectrumof pure compound of flower of *Plumeriaalba* L.

The FT-IR spectrum of the pure compound of the flower of *Plumeriaalba*is represented in Figure 2. Strong and broad bands absorb at 3366, and 3273 cm^{-1} represent O-H group. The symmetric and asymmetric sp^3 C-H stretching bands appeared at 2924, and 2883 cm^{-1} and the C=C stretching bands of aromatic ring observed at 1640 cm^{-1}. There is C-H in plane bending vibration of allylic group at 1457 cm^{-1}. The band at 1252 cm^{-1} shows C-C-O stretching vibration of phenol. The two bands at 1086 cm^{-1} and 945 cm^{-1}suggest C-O-C stretching vibration of ether group and C-H out of plane bending vibration of aromatic OH group whichshowed the presence of phenolic compound in sample.

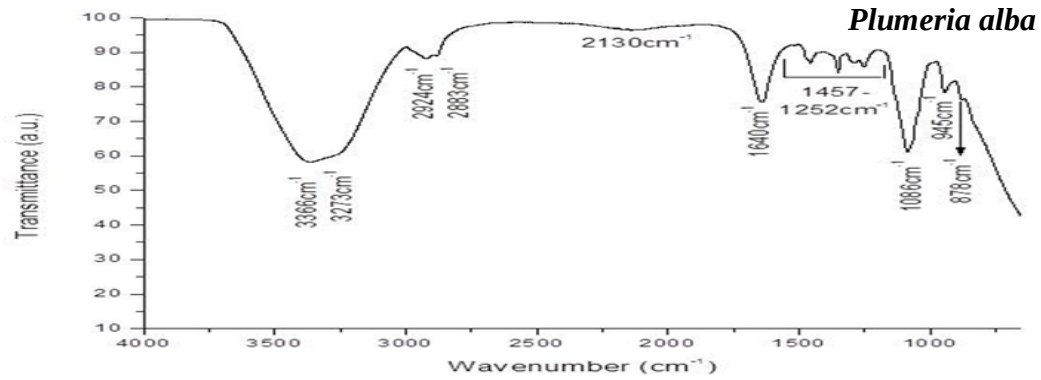

Figure 2. FT-IR spectrum of pure compound of the flower of *Plumeriaalba* L.

Total Phenol Content of Crude Extracts of Flower of *P.alba*L.

The total phenolic contents of ethanol and water extracts of sample were examined using the diluted Folin-Ciocalteu Reagent (FCR). Gallic acid standard curve is required to evaluate the total phenol content of the selected sample. Because gallic acid is 3, 4, 5-trihydroxy benzoic acid and it is a kind of total phenol. Gallic acid standard curve is shown in Figure 3.

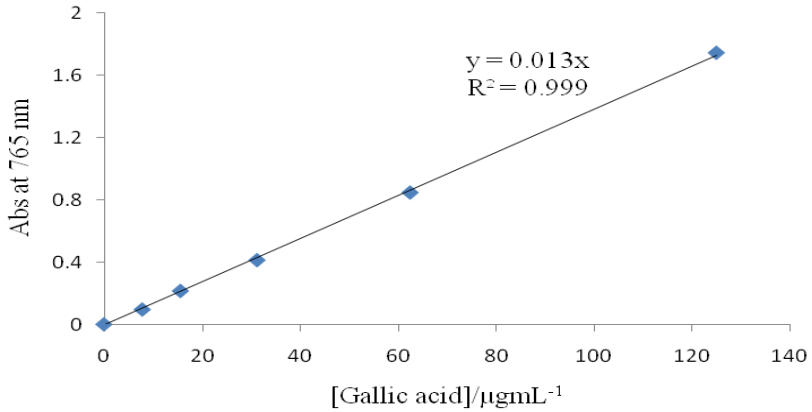

Figure 3. Gallic acidstandard graph

The results of total phenol contents for ethanol, methanol and water extracts are described in Table2.

Table 2.Total Phenol Content of Ethanol, Methanol and WaterExtracts of Flower of *P. alba*byFolin-Ciocalteu Method

Sample	TPC (□ g GAE □ SD) / mg	
	EtOHMethanol	Water
Flower	42.23□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□	36.13□□□□□□

(Data expressed as (□g Gallic Acid Equivalents GAE, (mean SD) in 1 mg of sampleextract)

According to experimental data, methanol extract (67.73µg/mg) of selected sample was observed to be more total phenol content than ethanol extract (42.23µg/mg) and water extract (36.13µg/mg) of sample.

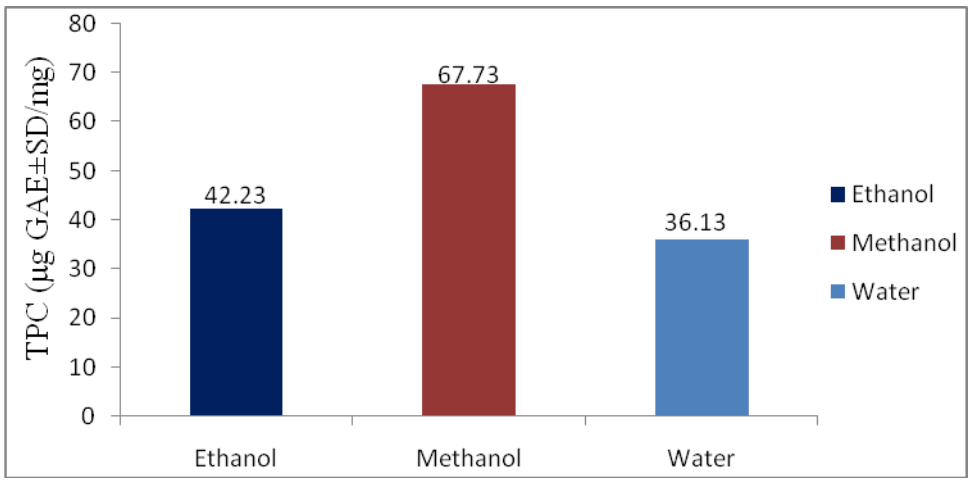

Figure 3.Bar graph for total phenol content of ethanol, methanol andwater extracts of flowerof *P. alba* L.

Antimicrobial activity

Antimicrobial activity of petroleum ether, ethyl acetate, ethanol, methanol, and aqueous extracts of flower of *P. alba* was screened against *Bacillus pumilus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus*and *Candida albicans*. The results of the zone of inhibition are summarized in Table 3 and Figure 4.

Table 3.The results of antimicrobial activities of flower of *P. alba* L.

Sample	Inhibition zone diameters of various solvent extracts against organisms						
	Solvents	I	II	III	IV	V	VI
Flower	Methanol	-	+	-	-	+	-
	Ethyl acetate	+++	+++	+++	+++	+++	+++
	Ethanol	+	+	++	-	+	+
	Aqueous	++	-	++	-	+	+
	Petroleumether	-	-	-	-	-	-

Agar-well ~ 10 mm

10 mm ~ 14 mm	(+)	I	=	*Bacillussubtilis*
15 mm ~ 19 mm	(++)	II	=	*Staphylococcus aureus*
20 mm above	(+++)	III	=	*Pseudomonas aeruginosa*
(+) = low activity		IV	=	*Bacillus pumilus*
(++) = medium activity		V	=	*Candida albicans*
(+++) = high activity		VI	=	*Escherichia coli,*
(–) = absent				

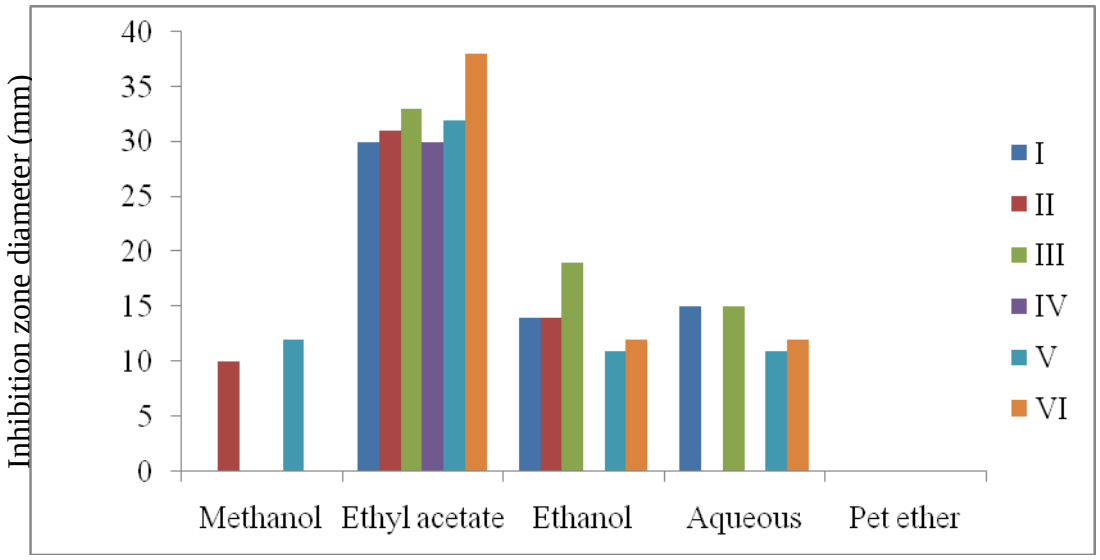

Figure 4.Bar graph for antimicrobial activity of flower of *P.alba*L.

According to these results, ethyl acetate extracts of sample show high activity on all tested organisms such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans,* and *Escherichia coli*. Petroleum ether extracts sample show no activity on six selected organisms and methanol, ethanol and aqueous extracts exhibit

low antimicrobial activity showed the presence of phenolic compound. Therefore, the flower of *P.alba* consists of bioactive compounds.

Antioxidant Activity of Ethanol, Methanol and Water Crude Extracts of Flower of *P.alba*by DPPH Radical Scavenging Assay

The extracts or their constituents when mixed with DPPH decolorized due to hydrogen donating ability. The radical scavenging activity of crude extracts was described by % RSA and IC_{50} (50% inhibitory concentration). These results are shown in Table 4 and Figures 5.

Table 4. Radical Activity (IC50) of EtOH, Methanol, and Water Crude Extracts of Flower of *P. alba* and Ascorbic Acid

Extracts	% RSA (mean ±SD) in different concentration (µg/mL)						IC_{50} (µg/mL)
	0.625	**1.25**	**2.5**	**5**	**10**	**20**	
Ethanol	35.19	50.89	60.45	72.82	80.12	81.99	0.99
	□□□□□	□□□□□	□□□□□	□□□□□	□□□□□	□□□□□	
Methanol	36.19	55.89	66.45	75.82	87.12	91.99	0.87
	□1.01	□□□□□	□□□□□	□□□□□	□ 0.51	□ 0.30	
Water	19.98	29.52	41.3	57.76	71.62	76.81	3.61
	□ 0.88	± 0.6	± 1.97	± 1.37	± 0.46	± 0.4	
Ascorbic acid	25.2	53.58	65.53	74.82	83.32	91.21	1.17
	± 1.4	± 0.88	± 1.13	± 0.59	± 0.78	± 0.48	

According to above result, when the concentration of sample was increased, the % RSA was also increased.Methanol extract was found to be the higher antioxidant activitythan ethanol and water extracts of sample. Hence, the antioxidant property of methanol extract was evaluated to be effectiveness whencompared with the property of standard ascorbic acid (IC_{50}=1.17 (□g/mL).

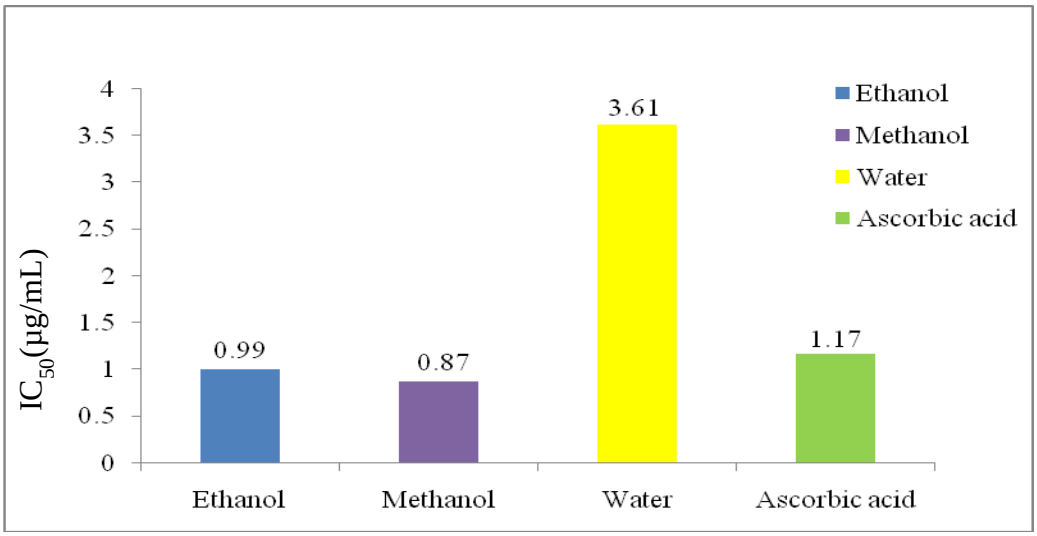

Figure 5.A bar graph of IC_{50} ((µg/mL) of ethanol, methanol and water crude extracts of flower of*P.alba*and ascorbic acid

Antioxidant activity was found to be highest in methanol extract followed by ethanol and water extracts. Therefore, flower of *Plumeriaalba* are suitable not only to eat but also to use as medicine.

Conclusion

The preliminary phytoconstituents screening showed the presence of alkaloids, glycosides, flavonoids, phenolic compounds, tannins, and steroids in the crude extracts of flower of *P.alba*. Hence,it canuse medicinally for health benefits.

FT-IR spectrum of pure compound isolated from the sample represented the presence of phenolic compound.One of the antioxidant factors, total phenol content (TPC) was measured by spectrophotometer using the Folin-Ciocalteu method. Total phenol content of methanol extract (67.73µg/mg) of selected sample was observed to be more total phenol content than ethanol extract (42.23µg/mg) and water extract (36.13µg/mg) of sample.

In addition, regarding the scabies and laxative, antimicrobial activities of five solvent extracts wereinvestigated by agar disc diffusion method.Among them, ethyl acetate extract indicated high activities on all tested organisms.

Furthermore, the ethanol, methanol and water extracts of flower of *P.alba* were scavenged by DPPH using spectrophotometer. The methanol extracts (IC_{50}=0.87□g/mL) revealed the strongest antioxidant capacity using DPPH radical scavenging method when compared with ethanol (IC_{50}=0.99□g/mL) and water extracts (IC_{50}=3.61□g/mL) of sample. Therefore, the rich phenol fractions of flower of *P.alba* show a potential source of natural antioxidants.

Due to the presence of active phytoconstituents, antimicrobial and antioxidant activities, *Plumeriaalba*flowers are suitable to eat and can be used as medicine for human health.The present study provided important information for the further application of *Plumeriaalba* flowers.

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