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Antioxidant and antibacterial response of hydroalcoholic extract of Plumeria alba leaves

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***Abstract*---**Plants are the natural treasure of antioxidants and antimicrobial agents. Present study was intended to evaluate the antioxidant and antimicrobial potential of *Plumeria alba* leaves hydroalcoholic extract (PALHE). Study involved the phytochemical screening, antioxidant, and antibacterial activity of PALHE. The PALHE was prepared using 95% ethanol as solvent through maceration method. The antioxidant activity involved determination of total phenolic content (TPC) and total flavonoid content (TFC). DPPH (1,1-Diphenyl2-picryl hydrazyl) radical scavenging assay was used for the determination of antioxidant activity of PALHE. The PALHE was investigated for its antibacterial activity against *S. aureus* and *E. coli* using well diffusion method. Various phytochemical screening tests were carried out and the results revealed the presence of carbohydrates, reducing sugar, mucilage, proteins, steroids, volatile oil, tannins, phenolic and flavonoids in the PALHE. Besides, moderate antioxidant activity was also revealed through the result of DPPH assay over the ethanolic leaves extract of the PALHE, where the IC50 was found to be 23.96 mcg/ml. Additionally, the TPC and TFC were found to be 71.04 mg (GAE/g of total phenol in terms of gallic acid equivalent) and 75.60 mg (RE/g of total flavonoid in terms of rutin equivalent) respectively. The PALHE exhibited high inhibition potential against *S. aureus* and *E. coli.* Based on the experimental results present study concludes that PALHE possess the significant antioxidant and antibacterial activity. This study also recommends that antioxidant and antibacterial potential of *Plumeria alba* leaves should be further investigated using different solvent.

***Keywords*---**Plumeria alba, total phenolic content, total flavonoid content, gallic acid, antioxidant.

Introduction

The development of microbial resistance and oxidative stress related diseases such as arthritis, diabetes, cancer, atherosclerosis, vascular diseases, metabolic syndromes and osteoporosis are becoming a major worldwide health problem. Plants are effective natural source for traditional medicines and modern medicines[1-5]. Plants are widely used in the treatment of various infectious, cellular and metabolic diseases[6-13]. Today the natural antibaceterial and antioxidant from plant source which is safe, effective and economic to replace the synthetic agents is a priority during disease treatment. Owing to the plants bearing potent biological activities several patents have been made various plant products[14-24]. Evidence suggests formulation of various polymeric, nano and other related products using plants sources[25-36]. Facts suggests that generation of free

radicals in the body under some conditions may result in cellular changes and development of various metabolic disorders such as diabetes, obesity and cancer. However, large number of evidence suggest that this can be neutralized using various plants-based antioxidants. Evidence suggests that antioxidants from plants source can effectively scavenge free radicals, thereby protect the cells, delay aging, and prevents several aging related diseases[37,38]. Plants are known to exert their wide variety of medicinal effect through their phytoconstituents that are present in different parts of the plants[39-82]. Therefore, the secondary metabolite of plants as antioxidants and antibacterials are the major targets of researchers. A few studies highlighted various solvents extracts of different parts of *Plumeria alba* (such as flowers, leaves and stems) to possess high antioxidant and antimicrobial potential[83-87]. Literary investigation suggests some leaves extracts of *Plumeria alba* as an antioxidant, but still some hydroalcoholic solvent extracts of *Plumeria alba* are not yet explored for their antioxidant and antibacterial potential. Hence present study was designed to perform the phyto chemical screening and evaluate the antioxidant potential of 95% ethanolic extract of *Plumeria alba* leaves.

Materials and Methods

Materials

The chemicals and solvents for the present study were procured from Merck and Sigma Aldrich. The *Plumeria alba* leaves were collected from the premise of AIMST University, Kedah, Malaysia. The healthy green leaves of *Plumeria alba* were chosen, stripped off, washed (to remove the impurities and dirt), air dried (in sunlight), crushed (into small pieces), homogenized (into powder), and finally stored in the airtight container for further investigations.

Preparation of *Plumeria alba* leves extract (PALHE)

Preparation of PALHE was based on the protocol of the standard research with minor modifications[83-93]. Briefly, the 100 g of *Plumeria alba* leaves powder was macerated with 300 ml of hydroalcoholic solution (ethanol and distilled water in a ratio of 95:05) in a conical flask using Bench Top Obital shaker at a speed of 81 cm/s for one week at room temperature. Next the mixture was filtered Whatman filter paper, and the filtrate was evaporated using rotavapor to yield the dark-brown colored PALHE.

Phytochemical screening of PALHE

The prepared PALHE was subjected to phytochemical screening as per the experimental protocol mentioned in the standard literature with slight modification[91-93].

Estimation of Total Phenolic Content (TPC) of PALHE

The PALHE was subjected to TPC determination using Folin–Ciocalteu method, using standard experimental protocol with slight modification[88-93]. Briefly, 1 mL of gallic acid (10–100 µg/mL) solution was mixed with 1 mL of 10% (w/v) Folin–

Ciocalteu reagent. After 5 min, 2.0 mL of Na_2CO_3 (2.5%) was subsequently added to the mixture and incubated at room temperature for 2 hours. Similarly, PALHE solution was prepared. Next, the absorbance for standard and PALHE was measured using UV Spectrophotometer (Shimazu, UV-1800) at 750 nm. The resultant experimental data was expressed as mg/g of gallic acid equivalent in mg per gram (mg GAE/g) of dry extract.

Estimation of Total Flavonoid Content (TFC) of PALHE

The PALHE was subjected to TFC determination uisng standard protocol with slight modification[91-93]. Briefly, 1 mL of rutin solution (10–100 µg/mL) was mixed with 0.1 ml of 10 % aluminium chloride, 0.1 ml of 1 M potassium acetate solution and 2.8 ml of distilled water. Similarly, PALHE solution was prepared. The standard and PALHE mixtures were incubated for 1 hour at room temperature followed by recording of absorbance at 415 nm against blank. The resultant experimental data was expressed as mg/g of rutin equivalent in mg per gram (mg RE/g) of dry extract.

DPPH Radical Scavenging Assay

The PALHE was further subjected to DPPH (1,1-Diphenyl2-picryl hydrazyl) assay following the standard experimental protocol with minor modification[91-93]. The radical scavenging activity of PALHE was done to measure its antioxidant potential using DPPH method. Briefly, 2.5 mL of extract solution (10–100 µg/mL) in ethanol was added to 1 ml of 0.3 mM of alcoholic solution of DPPH. Similarly, standard solution of ascorbic acid was prepared. The mixtures were kept aside in a dark for 30 minutes and absorbance was recorded 518 nm. The percentage of DPPH• scavenging was estimated using following expression:

$$\% \text{ scavenging of DPPH}\bullet = [(A_0 - A_1)/A_0] \times 100 \quad \ldots.(1)$$

where A_0 = absorbance of the control and A_1 = absorbance of the test extracts.

Preliminary Screening for Antibacterial activity

Preparation of bacterial culture

Bacterial strains of *E. coli* were used to evaluate the inhibitory potential of the PALHE. The preparation of bacterial culture was done as per the standard protocol given in the literature[94-103]. The prepared stock culture of *E. coli* was maintained at 4°C. Subcultures were prepared by transferring loopful of microorganism colonies from stock culture into the nutrient broth and incubated for 24 hours at 37 ℃ in the incubator. The broth turbidity indicated the microbial growth.

Well Diffusion Method

The inhibitory potential of PALHE against *E. coli* was based on well diffusion method using standard protocol with slight modification[103]. Briefly, 20 µl of nutrient broth containing broth organism was poured into Muller Hinton agar

plate. Three wells were made on the agar medium with cork borer, in one of the well 1 mg/ml of PALHE was added, whereas in other two standard (ciprofloxacin 1 µg/ml) and blank were added. The plate was incubated for 24 hours at 37ºC. The diameter of zone of inhibition around wells was measured in milliliters (mm) in triplicate and average values were calculated.

Results and Discussion

Phytochemical Screening

The observations and results of phytochemical screening of PALHE are presented in the table 1. The results of phytochemical screening of PALHE, revealed the presence of various primary and secondary metabolites. In summary, the PALHE is present with carbohydrates, mucilage, fats and oils, volatile oils, anthraquinone glycosides, flavonoids, alkaloids, tannins and phenolic compounds. The flavonoids and phenolic compounds are vital for the antioxidant activity. However, alkaloids are important in antimicrobial activity. Besides that, flavonoids and phenolic compounds exhibits anti-inflammatory and anti-cancer activities. Study reports that *Plumeria alba* possess many other medicinal properties[83-87].

Table 1: Summary of Phytochemical Screening Test

Phytochemical test	PALHE
Carbohydrates	+
Gums	-
Mucilage	+
Proteins	+
Amino Acids	+
Fats and Oils	+
Steroids	+
Volatile Oils	+
Cardiac Glycosides	-
Anthraquinone Glycosides	+
Saponin Glycosides	-
Cyanogenetic Glycosides	-
Coumarin Glycosides	-
Flavonoids	+
Alkaloids	+
Tannins and Phenolic Compounds	+

Where, (+) positive represent presence, and (-) negative represent absence

Total Phenolic Content

It has been documented that phenolic compound are important in antioxidants and also highly distributed in various plant species[83-93]. The Folin-Ciocalteu (F-C) method was used for determination of the total phenolic content of the Plumeria alba leaves extract which gallic acid was used as standard. A calibration curve was constructed by using the absorbance values obtained at various

concentration of gallic acid. The F-C method showed a blue colour complex which resulted from the transfer of electrons in alkaline medium from phenolic compounds to phosphomolybedic phosphotungstic acid complexes. The table 2, showed the contents of total phenols which were determined by using FollinCiocalteu reagent in term of gallic acid equivalent (standard curve equation: y = 0.0098x + 0.0168, R2 = 0.9993). The concentration used to determine the total phenolic content was 10.0 mcg/ml. UV spectrophotometer was used to detect the absorbance at 750nm. From the gallic acid standard curve produced, gallic acid equivalent of Plumeria alba was calculated to be 71.04 mg GAE/g

Table 2: UV-absorbance value for gallic acid

No.	Concentration (µg/ml)	Absorbance of standard at 750nm			
		First Trial	Second Trial	Third Trial	Mean
1	10	0.128	0.133	0.123	0.128 ± 0.005
2	20	0.195	0.222	0.190	0.202 ± 0.017
3	40	0.480	0.307	0.410	0.399 ± 0.087
4	60	0.621	0.621	0.583	0.608 ± 0.022
5	80	0.823	0.809	0.817	0.802 ± 0.007
6	100	0.998	1.062	0.928	0.996 ± 0.067

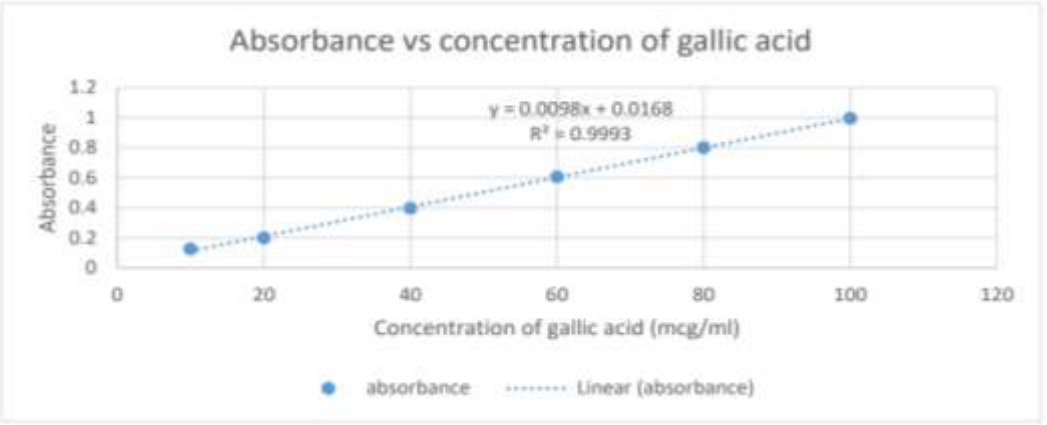

Figure 1: Standard curve of Gallic acid for total phenolic content

Table 3: UV-absorbance value for PALHE

No.	Concentration (mcg/ml)	Absorbance of sample at 750nm				Equivalent gallic acid (mg GAE/g)
		First Trial	Second Trial	Third Trial	Mean	
1	10	0.749	0.763	0.626	0.713 ± 0.075	71.04

Total Flavonoid Content

Total flavonoid content assay is a method which is used to determine the flavonoid content in extracts. The flavonoids are polyphenolic compounds which are water soluble and widely distributed in plant as their glycoside. It is also documented that flavonoids show antioxidant activity and have significant effect on human nutrition and health[83-93]. Scavenging and chelating are the process of the mechanism of action of the flavonoids. From the result obtained from the table, it was clearly showed that the increases in concentration of rutin will result in increases of the absorbance. By using the graph which constructed from the absorbance values of rutin at various concentration, the flavonoid contents of the extracts in terms of rutin equivalent (the standard curve equation: y = 0.0037x + 0.0402, R 2 = 0.9931) was calculated to be 75.60mg GAE/g. However, that the solvent used for the extraction may affect the result of the total flavonoids detected in the plant. It was found out that there the total flavonoids content detected was higher in methanolic extract rather than No. Concentration (mcg/ml) Absorbance of sample at 415nm Value Mean ± SD Equivalent rutin (mg RE/g) 1 10 0.381 0.275 0.304 0.320 ± 0.055 75.60 64 ethanolic as methanol greater cell wall penetration than ethanol (Hafizul Rahman et al 2014). Besides, a study by Dawood et al based on the literature review has shown that the flower part of the plant has higher total flavonoid content compared to leaves part. As a result, it can be said that the total flavonoids content may differ significantly among different parts of the plant.

Table 4: UV-Absorbance value for Rutin

No.	Concentration (µg/ml)	Absorbance of standard at 415nm			
		First Trial	Second Trial	Third Trial	Mean
1	10	0.082	0.089	0.085	0.085 ± 0.004
2	20	0.113	0.125	0.118	0.119 ± 0.006
3	40	0.157	0.175	0.168	0.167 ± 0.009
4	60	0.251	0.275	0.267	0.264 ± 0.012
5	80	0.328	0.356	0.346	0.343 ± 0.014
6	100	0.403	0.418	0.412	0.411 ± 0.008

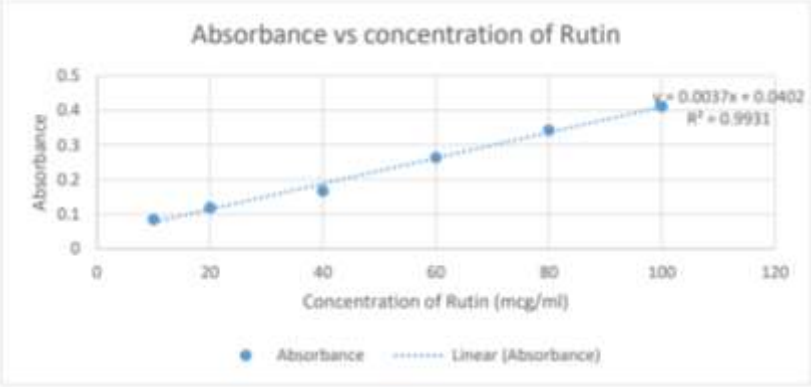

Figure 2: Standard curve of rutin for total flavonoid content

Table 5: UV-absorbance value for PALHE

No.	Concentration (mcg/ml)	Absorbance of sample at 750nm				Equivalent gallic acid (mg GAE/g)
		First Trial	Second Trial	Third Trial	Mean	
1	1	0.381	0.275	0.304	0.320 ± 0.055	75.60

DPPH Scavenging Assay

The DPPH is an organic free radical which is stable. However, at the spectrum of 515-528 nm, DPPH accepts a free radical species or an electron as they lose their absorption spectrum. DPPH assay is the most common and familiar technique for determine and evaluate the antioxidant free radical scavenging activity[104]. This is because it is simple and easy to carry out. Table 6 presents the summary of DPPH scavenging by standard ascorbic acid. The antioxidant activity of the leaves of Plumeria alba ethanolic extract was assessed by % DPPH (1, 1-diphenyl-2picrylhydrazyl) scavenging method. The DPPH antioxidant assay was conducted according to the procedure described. Ascorbic acid was served as a reference standard for this experiment. The result obtained are given in the following tables and figure. These days, it is important to discover new classes of compound with antioxidant ability to overcome various diseases such as cardiovascular disease, atherosclerosis, lung diseases and cancer as these diseases are resulted by the oxidative damage caused by the free radical to numerous biological substances, including DNA, protein, and lipid membranes. Some synthetic antioxidant such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertbutylated hydroxyquinone (TBHQ) have been added to foodstuff. However, there are several studies proved that these synthetic antioxidant may possess harm to human body. As a result, it is essential to produce antioxidant which is natural based to overcome such problem and protect human body from reactive oxygen species. DPPH is a stable nitrogen cantered free radical which can be effectively scavenged by antioxidants and shows strong absorbance at 518nm. The change in absorbance of DPPH radicals caused by the extracts was due to the reaction between antioxidant molecules and the extracts, which resulted in the scavenging of the radical by the hydrogen donation. Extend of DPPH radical scavenged determined by the decreases in the intensity of violet colour in the form of IC50. In the present study, it was used to evaluate the radical-scavenging effects of Plumeria alba leaves powder extract. The absorption decreases as antioxidants donate protons to this radical. It was observed that increases in the concentration of the extract will result in higher percentage of antioxidant activity. However, the ethanolic extract of the Plumeria alba leaves has shown lower antioxidant activity as compared to standard used in the assay which is ascorbic acid. The IC50 values observed for standard and extract was 3.89 mcg/ml and 23.96 mcg/ml.

Table 6: DPPH Radical Scavenging Assay value for Ascorbic acid

No.	Concentration (µg/ml)	Percentage of Antioxidant Scavenging Activity			
		First Trial	Second Trial	Third Trial	Mean±SD

1	1	0.6283	0.5375	0.5829	0.5829 ± 0.0454
2	2	0.4614	0.4581	0.4598	0.4597 ± 0.0016
3	4	0.3850	0.2966	0.3408	0.3408 ± 0.0442
4	6	0.0932	0.1188	0.1060	0.1060 ± 0.0128
5	8	0.0133	0.0154	0.0144	0.0144 ± 0.0011
6	10	0.0144	0.0080	0.0097	0.0097 ± 0.0017

Table 7: DPPH Radical Scavenging Assay value for PALHE

No.	Concentration (µg/ml)	Percentage of Antioxidant Scavenging Activity			
		First Trial	Second Trial	Third Trial	Mean
1	1	0.8681	0.8096	0.7633	0.8137 ± 0.053
2	2	0.8047	0.7442	0.7720	0.7736 ± 0.0302
3	4	0.7549	0.7417	0.7590	0.7519 ± 0.0090
4	6	0.7484	0.7048	0.7477	0.7336 ± 0.0250
5	8	0.6804	0.6921	0.7151	0.6959 ± 0.0177
6	10	0.6769	0.6413	0.6899	0.6694 ± 0.0252

Preliminary antibacterial Screening

In present study, the PALHE was evaluated for its antibacterial response using well diffusion method[105]. The results so obtained are given in table 8. A The incidences of bacterial resistance towards conventional antibiotics raises the demand for evaluation of alternative antimicrobials[103]. As per the literature available over different parts of Plumeria alba plant and very less literature was available over antimicrobial potential of Plumeria alba plant leaves. Hence, investigators of present study planned to evaluate the in-vitro inhibition potential of PALHE *S. aureus* and *E. coli* using well diffusion method. The PALHE was prepared using hydroalcoholic solvent. The PALHE was investigated for inhibitory potential (using well diffusion method) and phytochemical screening. The PALHE was investigated for its zone of inhibition against *S. aureus* and *E. coli.* The PALHE showed good inhibitory effect over the growth of *S. aureus* and *E. coli.*

Table 8: Zone of inhibition of PALHE

	Zone of inhibition (in mm)	
	S. aureus	*E. coli*

PALHE	22	20
Ciprofloxacin	25	25
Control	--	--

Conclusion

Present study was intended to study the primary and secondary metabolites, total phenolic content, total flavonoid content, antioxidant and antibacterial activity of PALHE. The present study establishes that PALHE possess carbohydrates, mucilage, fats and oils, volatile oils, anthraquinone glycosides, flavonoids, alkaloids, tannins and phenolic compounds. These compounds may allow PALHE with potential antioxidant, antimicrobial, anti-inflammatory and anti-cancer activity. The total phenolic content and total flavonoid content in the present study support this statement. Present study concludes that hydroalcoholic extract of *Plumeria alba* leaves possess significant antioxidant and antibacterial potential. Present study recommends that in future further isolation and metabolic studies should be performed to establish the antioxidant mechanism and antibacterial potential of PALHE.

Conflict of Interest

The authors have no conflicts of interest regarding this investigation**.**

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