



PHYTOCHEMICAL ESSENCES OF FOUR HYBRID FLOWER *PLUMERIA ACUMINATA* IN POLAR AND NON-POLAR SOLVENTS EXTRACT

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ABSTRACT

Plumeria sp. is traditionally used by Malaysian for various folk treatments. In this study *Plumeria acuminata* plant with different colour of flowers such as pink, light red, red and white were extracted using petroleum ether, chloroform, methanol and cold water. The extracts were screened for the presences of phytochemicals essences. In common, the extracts of *Plumeria acuminata* shows the presences of reducing sugars, saponins, flavonoids, tannins, anthraquinone and alkaloids. Prominent phytochemicals substances were presence in cold water extracts followed by methanol, chloroform and petroleum ether. Quantitative studies can be further done on these extracts to quantify the bioactive substances.

Keywords: *Plumeria acuminata*, Various extraction solvents, Phytochemical assay

INTRODUCTION

Medicinal plants play an important role in the health benefits of individuals and communities at large. Before the time of modern technology, traditional methods were used in investigating indigenous medicinal plant properties without any quality control measures. Medicinal herbs were the target of big drug corporates and researchers in the hunt for novel drug discovery. Huge investment is being pumped into probing the magical properties of certain plants that are recorded in traditional medicine and also in exploring their yet unknown characteristics. Traditional medicine is practiced in almost all culture. In all traditional medicines, plants play a major and important role as therapeutic agents. *Plumeria acuminata* belonging to the family Apocynaceae is commonly known as Frangipani, 'perungalli' in Tamil and 'bunga kemboja' in Malay [1]. In Malaysia, only three species are popularly cultivated, *Plumeria obtusa* Linn., *P. acuminata* Ait. and *P. rubra* Linn [2]. It is widely distributed throughout South East Asia. 5 The flowers are generally large, waxy and very fragrant, in terminal or lateral stalked clusters. The colour of the blooms can vary from white to red and most have a delightful aroma that is most intense during the early part of the day [1].

There are four hybrids flower of *Plumeria sp.* that is being studied from which each has different colours of flowers; white, pink, light red and red. In the traditional medicinal system, different parts of the plant have been mentioned to be useful in a variety of diseases. The plant material is widely used as a purgative, remedy for diarrhea and to cure for itch [3]. The milky juice is employed for the treatment of inflammation and rheumatism. However,

there is no scientific report or verification of the use of this plant in the treatment of these conditions. There are experimental trials on animal models, conducted by different laboratories and authors in various places. The methanol extract of *Plumeria acuminata* from India exhibited significant anti-inflammatory activity on the tested experimental rat models [4]. As per Gupta, 2007, the methanolic leaves extract possesses potent antipyretic and antinociceptive properties which are mediated via peripheral and central inhibitory mechanism [5]. Besides the methanolic leaves extract exhibits potent antioxidant, free radical scavenging properties [6], Gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus* and *Micrococcus luteus*) more susceptible to the methanolic extracts than the Gram-negative bacteria [7].

Since phytochemicals from plant source are mostly safe and easily available, potential *Plumeria rubra* Linn. with phytochemical constituents was subjected to test. This would be the first scientific study to evaluate the phytochemical activity of *Plumeria acuminata* hybrid flowers.

MATERIALS AND METHODS

Plant materials:

Samples of *Plumeria acuminata* were collected in the surrounding areas of AIMST University, Kedah in Jun 2010. The flowers of *Plumeria acuminata* was collected from 4 different hybrids which are white, pink, light red and red. The plant materials were air-dried first and then oven-dried at 50 °C for 2 days and were all ground to fine powder using a heavy duty blender. The powder was then used for solvent extraction [8].

Samples preparation:

The powdered plant material was soaked in four different solvent which spans from a highly non-polar to polar solvents which are petroleum ether, chloroform, methanol and cold water for 7 days. The suspension was stirred from time to time and then the extract was filtered and the solvent was completely removed by a rotary evaporator according to solvents boiling point. The extract was dried in the oven at 40°C for 1 day to obtain a thick paste. The

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four solvents extract (petroleum ether, chloroform, methanol and cold water) were then wrapped in aluminium foil and stored at 4°C.

Phytochemical Analyses

This is qualitative analyses which are used to detect the presence of flavanoids, reducing sugar, saponin, tannins, anthraquinone and alkaloid [9]. Petroleum ether, chloroform, methanol, and cold water extracts were subjected to the qualitative phytochemical analysis.

Qualitative Phytochemical screening

Benedict Test

Benedict test was used to detect the presence of reducing sugar. 2ml of various solvent's crude extract was added with 1ml of prepared Benedict solution in a test tube. The mixture was shaken vigorously. Then it was heated in a water bath at 45°C for 10 minutes. After 10 minutes the test tube was taken from the water bath to check any colour changes. A brick red precipitate indicates the presence of reducing sugars.

Frothing Test

Frothing test was conducted to detect the presence of saponins. 2 ml of crude various solvent's extract was pipetted to the test tube and shook vigorously. Then it was left to stand for 10 minutes. A thick persistent froth indicates the presence of saponins.

Borntrager's Test

To detect the presence of anthraquinone derivatives, Borntrager test was conducted. Firstly 10% of diluted ammonia is prepared; the crude of various solvent's extract was added with 1ml of dilute ammonia (10%) and shaken vigorously. The colour change was recorded. A pink-red colour in the ammonia layer shows anthraquinone derivatives.

Flavonoids Test

To test the presence of flavanoids, 2 ml of crude methanol extract was added in a test tube. Diluted 0.5ml sodium hydroxide (NaOH) and diluted 1ml hydrogen chloride (HCl) was first prepared before adding to the crude. It was prepared by preparing the diluted NaOH (1g of NAOH diluted in 100ml distilled water) and preparing diluted HCL (1ml of HCL diluted in 100ml distilled water). 1 ml of diluted NaOH was added to 1ml extract and continued by adding 1ml of diluted HCL. The solubility and colour change indicates the presence of flavonoids.

Tannins Test

Ferric chloride solution was used to test for tannins. 15% of ferric chloride was prepared by diluting 15g of ferric chloride in 100ml distilled water. 2ml of 15% ferric chloride was added to 1ml of crude methanol extract. A blue or green colour indicates the presence of tannins.

Alkaloid Test

To test the presence of alkaloid, ammonical chloroform, 10% sulphuric acid, and Mayer's reagent was prepared. Ammonical chloroform was prepared by adding 50ml of ammonia and 50 ml of chloroform together. 10% of sulphuric acid was prepared by adding 10.5ml of 95% sulphuric acid to 89.5ml of distilled water. Mayer's

reagent was prepared by adding 1.36g of mercury chloride together with 5g of potassium iodine and topping up with distilled water to 100ml. Test was done by adding 2 ml of extract with 2 ml of ammonical chloroform, 10 drops of 10% sulphuric acid and 0.5ml of Mayer's reagent.

RESULTS

The petroleum ether, chloroform, methanol and cold water crude flower extracts of *Plumeria acuminata* (white, pink, light red and red) exhibits presences of phytochemical constitutes. Frothing test ascertains the presence of saponins by the thick persistent froth that was formed. These positive results can be seen on all the cold water extracts of different cultivars and on chloroform extract of the white and pink cultivar. The Borntrager's test gave a positive result where there was formation of pink-red colour in the ammonical layer. This shows the presence of anthraquinone derivatives. Positive results for Borntrager's test can be seen in all cold water and methanol extracts of the different cultivars. A cream coloured precipitation produced during alkaloid test by using Meyer's reagent indicates the presence of alkaloids. These positive results can be seen in all petroleum ether and methanol extracts of the different cultivars, on the light red cultivar of the chloroform extract and on all the cold water extracts except for the red cultivar. Presence of flavonoids was determined by performing sodium hydroxide flavonoid test. Upon addition of sodium hydroxide to the plant extract, the solution turned to yellow colour. Discolouration of the solution after adding dilute hydrochloric acid attests the presence of flavonoids. Positive results for flavonoid test can be seen in all the cold water and methanol extracts of the different cultivars.

Ferric chloride test showed a bluish green colour change indicating the presence of tannins. Positive results for tannin test can be seen in all petroleum ether and cold water extracts of different cultivars. Benedict's test shows the presence of reducing sugars by forming a brick red precipitation. Positive results for reducing sugar test can be seen on all cold water and methanol extracts of different cultivars and also on chloroform extracts of white and pink cultivars.

DISCUSSION

Plants are able to produce a large number of diverse bioactive compounds. Medicinal plants are currently in considerable significance view due to their special attributes as a large source of therapeutic phytochemicals that may lead to the development of novel drugs [10]. Awareness of pharmaceutical industries in formulating skin products from natural sources contribute to increasing interest in research and industrial application of medicinal plants [11]. Most of the phytochemicals from plant sources such as phenolics and flavonoids have been reported to have a positive impact on health and cancer prevention [12]. With regards to the beneficial phytochemicals in medicinal plants and the shift towards natural products in pharmaceuticals and cosmeceuticals

industry qualitative and quantitative analysis of phytochemical constituents in medicinal plants become significant investigations [13]. Every part of *Plumeria acuminata* flower can be used medicinally [14]. Chemical compounds that can be found in the crude extract of *Plumeria acuminata* by performing various phytochemical tests are shown in Table 1. The studies have indicated that different extraction solvents such as petroleum ether, chloroform, methanol and cold water used for extraction harvested different compounds from the dried flowers. The selection of solvents plays an import part in the extractions of the phytochemical constituents. Absences of aromatic organic compounds in the extract may very less likely be due to the type of solvent used in the extraction because solvents used ranges from highly polar to non-polar. An ideal solvent dissolves the desired compound and leaves the other

constituents. For extracting non-polar compounds like fats, oils, and lipids non-polar solvents are used. The extraction of fixed oil, chlorophyll, steroids, terpenoids and aglycones can be affected by the use of hexane. Extraction of highly polar compounds like glycosides, sugars, amino acids, proteins, and polysaccharides can be done with polar solvents such as ethanol and water. In the case of flavonoids, less polar ones such as isoflavones, flavanones, methylated flavones, and flavonols are extracted with low polar solvents such as chloroform, dichloromethane, diethyl ether or ethyl acetate and the polar flavonoids and flavonoid glycosides are extracted with alcohols or aqueous alcohol mixtures. Besides this, for volatile compounds, the solvent should be of a low boiling point so that it can be easily removed, without denaturing the compounds extracted at high temperatures.

Table 1: phytochemical tests of *Plumeria acuminata*

<i>Plumeria acuminata</i> flowers	Extracts																							
	Petroleum ether						Chloroform						Methanol						Cold water					
Tests	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
White			+		+					+		+		+	+			+	+	+	+	+	+	+
Pink			+		+		+			+		+		+	+			+	+	+	+	+	+	+
Light red			+		+				+	+				+	+			+	+	+	+	+	+	+
Red			+		+		+			+				+	+			+	+	+	+		+	+

Note: 1: Saponins; 2: Anthraquinones; 3: Alkaloids; 4: Flavonoids; 5: Tannin; 6: Reducing sugar

Petroleum ether is and highly non-polar solvent where contributes for the extractions of fats, oil and lipid non-polar compounds. Thus, this solvent only demonstrated presences of tannins and alkaloids for all hybrids flower. Water has high polarity compared to all other solvents, consequently the four hybrids flower shows high phytochemical constituents in cold water extracts. Therefore, the cold water extract is suitable for the evaluation of pharmacological activities such as anti-inflammatory, antinociceptive, antimicrobial and also antioxidant properties.

CONCLUSION

The water crude extracts of four hybrid flowers *Plumeria acuminata* has shown the capability of extracting saponins, flavanoids, tannins, reducing sugars and alkaloids in the phytochemical analysis. High content of phytochemical constituents in all four hybrid flowers gives an insight that, the hybridization of the flower colour doesn't effects the phytochemical constituents in water extracts.

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