



PHYTOCHEMICAL SCREENING, TOTAL FLAVONOID AND PHENOLIC CONTENT ASSAYS OF VARIOUS SOLVENT EXTRACTS OF TEPAL OF *Musa paradisiaca*

(Penyaringan Fitokimia, Jumlah Asai Kandungan Flavonoid dan Fenolik Pelbagai Ekstrak Pelarut Tepal *Musa paradisiaca*)

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Received: 14 April 2015; Accepted: 3 August 2016

Abstract

The objective of this research is to conduct the preliminary phytochemical screening, total flavonoid and phenolic contents assays of various solvent extracts of tepal of *Musa paradisiaca*. Phytochemical screening was carried out according to the method of Trease and Evans, total flavonoid content was measured by the aluminium chloride colorimetric assay and total phenolic content was estimated spectrophotometrically by Folin-Ciocalteu method. - Preliminary phytochemical screening reveals the presence of phenolics, flavonoids, alkaloids, tannins, terpenoids in all the three different extracts (methanolic, ethanolic and aqueous). Tepal methanolic extract has the richest content of both phenolics and flavonoids i.e. (4.27 mg GAE/g and 0.25 mg QE/g) respectively, and aqueous extract was the least i.e. (1.32 mg GAE/g and 0.164 mg QE/g). All the extracts were not significantly different with one another ($p > 0.05$). It can be hypothesised that the high contents of phenolic compounds of tepals of *Musa paradisiaca* indicated that these compounds contribute to the antioxidant activity and can be regarded as promising plant species for natural sources of radical scavenging activity with potential value for treatment of many life threatening diseases.

Keywords: phytochemical, flavonoid, phenolics, *Musa paradisiaca*

Abstrak

Objektif kajian ini adalah untuk menjalankan pemeriksaan awal ke atas ketulenan fitokimia, jumlah kandungan fenolik flavonoid dan pelbagai ekstrak pelarut tepal *Musa paradisiaca*. Penyaringan fitokimia telah dilaksanakan mengikut kaedah Trease dan Evans, dimana jumlah kandungan flavonoid diukur oleh ujian kalorimetrik aluminium klorida dan jumlah kandungan fenolik dianggarkan secara spektrofotometer dengan kaedah Folin-Ciocalteu. Penyaringan awal fitokimia menunjukkan kehadiran fenolik, flavonoid, alkaloid, tannin dan terpenoid dalam ketiga – tiga ekstrak yang berbeza (metanol, etanol dan akueus). Ekstrak metanol Tepal mempunyai kandungan yang paling tinggi di antara kedua-dua fenolik dan flavonoid iaitu sebanyak 4.27 mg GAE/g dan 0.25 mg QE/g, manakala ekstrak akueus adalah mempunyai kandungan terendah iaitu sebanyak 1.32 mg GAE/g dan 0.164 mg QE/g. Semua ekstrak tidak berbeza secara ketara antara satu sama lain ($p > 0.05$). Oleh itu, hipotesis yang boleh dibuat adalah kandungan yang tinggi sebatian fenolik daripada tepal *Musa paradisiaca* menyumbang kepada aktiviti antioksidan dan boleh dianggap sebagai spesies tumbuhan yang berpotensi untuk merawat pelbagai penyakit yang mengancam nyawa.

Kata kunci: fitokimia, flavonoid, phenolic, *Musa paradisiaca*

Introduction

Consumption of medicinal herbs protects and heals a number of ailments and they have been the principal treatment therapy in prehistoric times until the discovery of synthetic drugs in the nineteenth century [1]. This exploration of biologically active natural products has played an important role in finding new chemical entities (NCEs), for example, approximately 28% of NCEs between 1981 and 2002 were natural products or natural product-derived [2]. Health benefits have been derived from bioactive compounds that are commonly found in edible plant parts such as fruits, vegetables, flowers, leaves and have been shown to confer protection against various ailments [3]. Interestingly, many of them are known to contain large amounts of phenolic antioxidants [4]. Phytochemicals have become an intense focus of research interest because of their perceived beneficial effects for health, including anticarcinogenic, antiatherogenic, antiulcer, anti-thrombotic, anti-inflammatory, immunomodulating, antimicrobial, vasodilatory, and analgesic effects. Therefore, the search for exploitation of natural antioxidants, especially of plant origin, has greatly increased in recent years [1]. For example, chard extract (*Beta vulgaris* L. var. *cicla*) has been used as a hypoglycaemic agent by diabetic mellitus (DM) patients in Turkey [5] and it has been documented that the number of similarly various phytoconstituent such as epicatechin, rutin, quercetin, nymphayol and flavonoid extracts from *Pterocarpus marsupium* have shown to possess β -cells regeneration capacity [6].

Flavonoids represent the most common and widely distributed group of plant phenolics [7] and are abundant in foods; quercetin and rutin are the flavonoids most abundantly consumed [8]. *Musa paradisiaca* is one of the well-known plants of the *Musaceae* family that have been used in traditional medicine since hundred years to alleviate various diseases and health problems. Active constituent presence in the plants materials might be responsible to the beneficial of human health. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds [9]. Ethnomedicinal survey around the world revealed that the flowers of *Musa spp.* have been used to treat many illnesses. Its flowers have been traditionally used to alleviate menorrhagia, dysentery, diabetes mellitus [10], heart pain, diarrhea, stomach cramps and infantile malnutrition [11]. It was reported that the extracts of the flowers possess medicinal properties for illness such as diabetes mellitus, anaemia [12] and malaria [13]. Phenolics are the largest group of phytochemicals that account for most of the antioxidant activity in plants or plant products [14].

More than 4000 polyphenols (flavonoids, monophenols and poly phenols) are found in vascular plants. Phenolic compounds such as quercetin, rutin, naringin, catechin, caffeic acid, gallic acid and chlorogenic acid are very important plant constituents [15]. Flavonoids are the largest group of naturally occurring phenolic compounds, which occurs in different plant parts in Free State and as glycosides [16]. They are found to have many biological activities including antimicrobial, mitochondrial adhesion inhibition, antiulcer, antiarthritic, antiangiogenic, anticancer, protein kinase inhibition etc. The flavones and flavonols are the most widely distributed of all the phenolics [17]. Flavonoids are particularly beneficial, acting as antioxidants and giving protection against cardiovascular disease, certain forms of cancer and age related degeneration of cell components. Their polyphenolic nature enables them to scavenge injurious free radicals such as super oxide and hydroxyl radicals [18]. A variety of dietary plant flavonoids inhibits tumor development in experimental animal models [19]. The bi-flavonoids have the pharmacological effects like their ability to inhibit the release of histamines, the adhesion of blood platelets and the action of lens aldose reductase, to block the inflammatory effects of hepatotoxins, and to act as a heart stimulant [20]. Therefore, the objective of this paper is to carry out the phytochemical screening, total contents of both phenolics and flavonoids of various solvent extracts of *Musa paradisiaca*.

Materials and Methods

Preparation of plant extract

The tepals were selectively removed from the bracts, oven dried for one week. It was then crush grinded to fine powder using an electrical grinder. This was then divided into three portion and each portion was soaked in methanol, ethanol and distilled water for 3 days respectively. Each sample was later filtered using Whatmann's No. 1 filter paper. The filtrates were then concentrated at 42 °C to yield a dark brown semi solid using a rotary evaporator (N-1100, Shanghai, Eyela. Co. Ltd, Tokyo, China). This was repeated three times. For each extract, the

yield was expressed in percentage by dividing the quantity of dry mass obtained after extraction by the dry weight of the powder used before soaking. Dried extracts were weighed and dissolved in 10% dimethylsulphoxide (DMSO) to yield a stock solution from which lower concentrations were prepared. The filtrate was then concentrated using an electric incubator. The extract appears as semi-solid greenish brown paste. A stock solution to be used was then prepared by dissolving 100 g of the extract in 50 ml of distilled water.

Preliminary Phytochemical Screening

The phytochemical screening of the extracts was conducted using standard procedures described by Trease and Evans [21]. The following qualitative tests were carried out:

Test for saponins

One mL of the tepal extract was diluted with distilled water to 20 mL and shaken in a graduated cylinder for 15 minutes. The formation of one centimeter layer of foam indicates the presence of saponins.

Test for phenols

A small amount of the ethanolic extract was taken with 1 mL of water in a test tube and 1 to 2 drops of Iron III chloride (FeCl_3) was added. A blue, green, red or purple color is a positive test.

Test for glycosides

A small amount of alcoholic extract was taken in 1 mL of water in a test tube and a few drops of aqueous NaOH were added. A yellow coloration indicates the presence glycosides.

Test for flavonoids

One to five drops of concentrated hydrochloric acid (HCl) were added to little amount of ethanolic extract of the plant material. Immediate development of a red colour indicates the presence of flavonoids.

Test for alkaloids

Two mL of extract was taken in a test tube and then 0.2 mL dilute HCl was included, followed by 1 mL of Meyer's reagent. A yellowish coloration indicates alkaloid's presence.

Test for tannins

Five mL of the tepal extract was placed in a test tube and then 2 mL of 5 % of FeCl_3 solution was added. A greenish-black precipitate indicates the presence of tannins.

Test for terpenoids

In a test tube containing 2 mL of chloroform, 0.5 mL of extract was added. This is then followed by the addition of 3 mL conc. H_2SO_4 which forms a layer. Reddish brown coloration of the interface indicates terpenoids.

Total phenolic content assay

The total phenolic content (TPC) was carried out by using the method of Folin-Ciocalteu. An aliquot (least amount) (1 mL) of extracts or standard solution of gallic acid (100, 200, 300, 400, and 500 $\mu\text{g/mL}$) was added to 25 mL of volumetric flask, containing 10 ml of decontaminated water. A blank reagent using distilled water was prepared. One mL of Folin-Ciocalteu phenol reagent was added to the mixture and shaken. After 5 mins 10 mL of 7.5% Na_2CO_3 solution was added to the mixture. The volume was then made up to the mark. Upon completion of incubation for 30-45 minutes at room temperature, the absorbance against the reagent blank was determined at 760 nm with an UV-Visible (Shimadzu Japan). TPC was expressed as mg gallic acid equivalents (GAE) [22].

Total flavonoid content assay

In this method, quercetin was used as the standard to make the calibration curve. 3.2 mg of quercetin was dissolved in 95% ethanol and then diluted to 5, 10, 20, 40, 80, 160, and 320 $\mu\text{g/mL}$. A calibration curve was made by measuring the absorbance of the dilutions at 415 nm (λ_{max} of quercetin) with a UV-1800 spectrophotometer Shimadzu Japan. Aluminium chloride (AlCl_3), 10 % and potassium acetate, 1 M solution was prepared. An amount 50 mg of each extract was accurately weighed and transferred to 10 mL volumetric flask and made up the volume

with methanol. 50 µL of each extract stock solution, 2.15 ml ethanol, 0.1 mL AlCl₃ 0.1 ml potassium acetate solution and 2.8 mL distilled water were added and mixed well. Sample blank was prepared in a similar way by replacing AlCl₃ with distilled water. Sample and sample blank of all extracts were prepared and their corresponding absorbances were measured at 415 nm. All prepared solutions were filtered through whatmann filter paper before measuring. Total flavonoid content (TFC) was expressed as mg of quercetin equivalent (QE) [23]. TFC was computed by using the following formula:

$$\text{TFC} = (R \times D \cdot F \times V \times 100) / W. \quad (1)$$

where, R - result obtained from the standard curve, D.F - dilution factor, V - volume of stock solution, 100 - For 100 g dried plant, W - weight of plants used in the experiment.

Results and Discussion

Percentage yield of extracts

The percentage yield for the various solvent extracts of tepal of *Musa paradisiaca* is shown in Table 1. Ethanolic tepal extract (ETE) has the highest percentage yield when compared to the methanolic and aqueous extract of the flower.

Table 1. Percentage yield of various solvent extracts of tepal of *Musa paradisiaca*

Extract	Initial weight	Final weight	% Yield
Tepal (methanol)	508.9	7.53	1.50
Tepal (ethanol)	508.9	7.54	1.51
Tepal (aqueous)	508.9	7.13	1.40

Preliminary phytochemical screening

Preliminary phytochemical screening shows the presence of phenols, glycosides, flavonoids, alkaloids, tannins and terpenoids in all three extracts i.e. methanol, ethanol and aqueous. MTE has the highest contents of phenols, followed by the ETE and aqueous tepal extract (ATE) the least. Nonetheless, the aqueous extract possesses flavonoids in abundance. Glycosides, alkaloids, tannins and terpenoids were all found to be present. All extracts were tested negative for saponins.

Total phenolic content (TPC)

The TPC of the extracts was determined by extrapolation from the calibration curve ($Y = 0.18x + 0.1039$; $R^2 = 0.994$) prepared from the gallic acid concentrations (Figure 1) and expressed in mg of gallic acid equivalence (GAE) per gram. The amount of phenolic compounds in the various extracts was obtained from regression equation and the values were expressed in gallic acid equivalence (Figure 2). There was no significant difference ($p > 0.05$) in all the three different extracts.

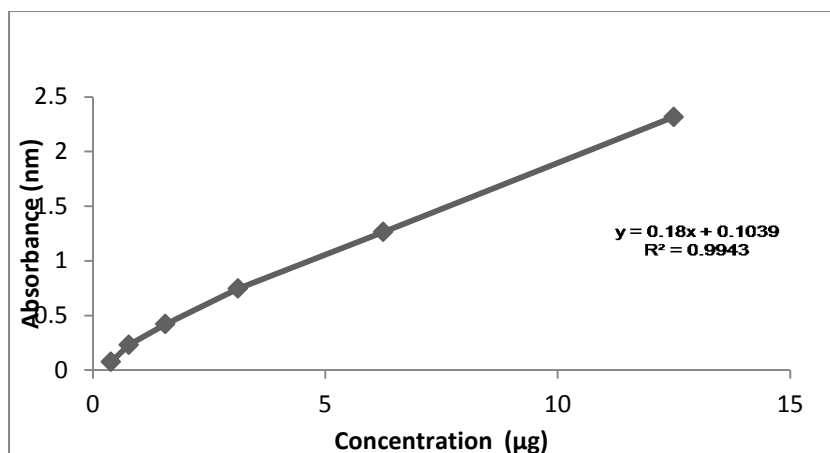


Figure 1. Gallic acid calibration curve

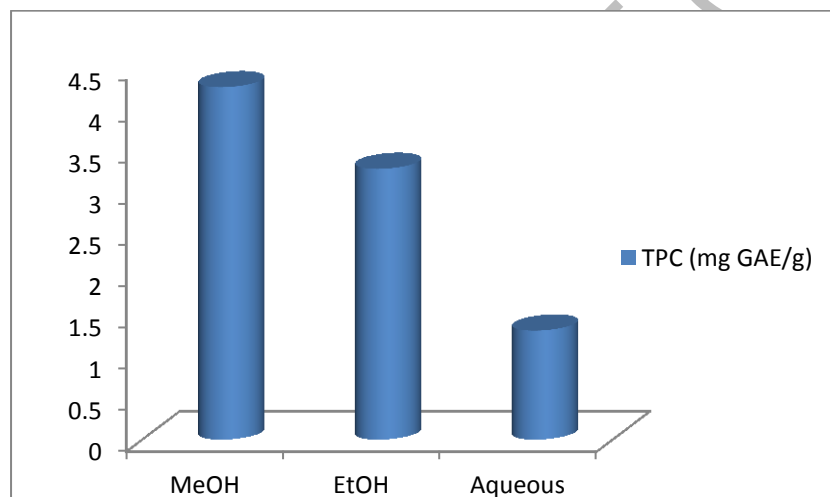


Figure 2. Total phenolic content of various solvent extracts of tepal of *M. paradisiaca*

Total flavonoid content (TFC)

The TFC content of the tepal extracts was also determined by extrapolation from the calibration curve ($Y = 0.4638x + 0.0778$; $R^2 = 0.992$) prepared from the quercetin concentrations (Figure 3) and expressed in mg of quercetin equivalence (QE) per gram. The amounts of flavanoid compounds in the various extracts were obtained from regression equation and the values were expressed in quercetin equivalence (figure 4). The values from all the extracts were not significantly different ($p > 0.05$).

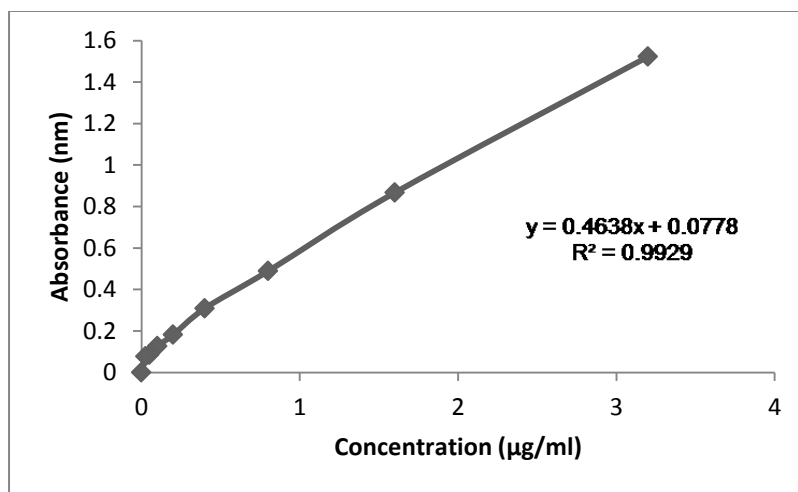


Figure 3. Quercetin calibration curve.

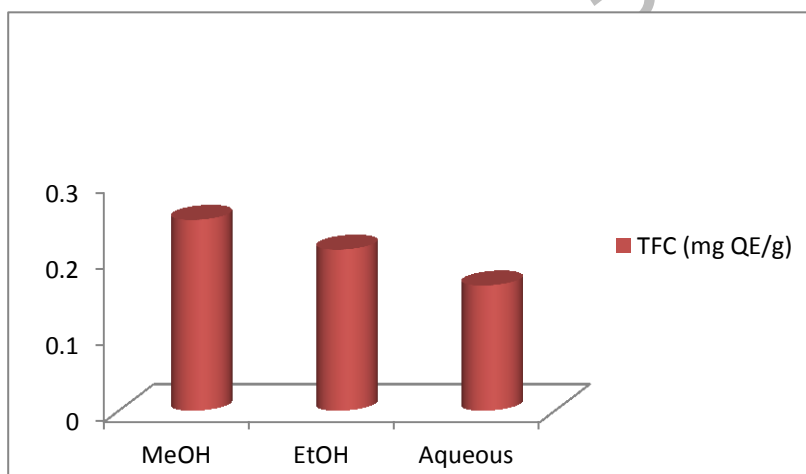


Figure 4. Total flavonoid content of various solvent extracts of tepal of *M. paradisiaca*

Preliminary phytochemical screening reveals the presence of phenols, flavonoids, glycosides, alkaloids, tannins and terpenoids. Saponins were tested negative in all the three different extracts (Table 2). The total phenolic content (TPC) of the tepal (methanolic, ethanolic and aqueous) extracts of *Musa paradisiaca* was determined using the Folin-Ciocalteu method. This method allows the estimation of all the flavonoids, anthocyanins and non-flavonoid phenolic compounds of all the phenolics available in the samples [24]. The results for total phenolic content in the studied plant extracts are presented in the graphs (Figure 2). The results show that MTE has the richest source of phenolics, total phenolic content is 4.272 mg GAE/g while on the contrary, ATE possesses the lowest amount of phenolics i.e. 1.32mg GAE/g. The total phenolic contents did not vary significantly ($p > 0.05$) in different solvent extracts. This is in agreement with the researchers [25,26], who found that yield of total phenolic was dependent on the method and choice of solvent. The recovery of polyphenols from plant materials is influenced by the solubility of the phenolic compounds in the solvent used in the extraction process. Furthermore, solvent polarity will play a key role in increasing phenolic solubility [27]. Thus, it is difficult to produce a standard extraction procedure suitable for phenol extraction of all the plant samples. As the results indicated, it is apparent that the recovery of

phenolic compounds was dependent on the solvents used and their polarity. This may be attributed to the fact that wide range of phenols can dissolve in aqueous methanol mixtures. On the other hand, methanol is a good solvent system for the extraction of polar antioxidants [28]. There is a strong link between phenolic content and free radical scavenging activity [29,30]. Phenolics possess a broad spectrum of biochemical activities such as antioxidant, antimutagenic, anti-carcinogenic and even the ability to modify the gene expression [31]. Polyphenols are the largest group of phytochemicals that account for most of the therapeutic activity in plants or plant products [32]. This plant is generally reported to contain substantial amounts of polyphenols with pharmacological properties which includes flavonoids and tannins [33]. The results obtained in the present study revealed that the level of phenolic compounds in these extracts was considerable and falls within the values of commonly consumed green vegetables. Hence consumption of these plants will enhance the health benefits by absorbing and neutralizing free radicals, quenching singlet oxygen, or decomposing peroxides [3].

Table 2. Preliminary phytochemical screening of various solvent extracts of *Musa paradisiaca*

S.N.	Test	T (MeOH)	T (EtOH)	T (aqueous)
1	Saponin	—	—	—
2	Phenols	+++	++	+
3	Glycosides	++	++	+
4	Flavonoids	+	+	++
5	Alkaloids	+	+	+
6	Tannins	++	+	+
7	Terpenoids	+	+	+

plus (+) indicates the presence and minus (—) signifies absence.

Total flavonoid content (TFC) of different extracts of tepals of *Musa paradisiaca* was determined by $AlCl_3$ method. Determining the total flavonoids by using $AlCl_3$ is based upon the formation of stable complex between $AlCl_3$, keto and hydroxyl groups of flavones and flavonoids. The results for total flavonoid content in the studied plant extracts are presented in the graph (Figure 4). The results show that MTE has the richest source of flavonoids, i.e. total content is 0.25 mg QE/g while on the contrary, ATE possess the lowest amount of flavonoid i.e. 0.164 mg QE/g. this is contrary to the findings of [34] where it was found that ethanolic extract has the highest flavonoid content. Flavonoids display a crucial role in scavenging the free radicals and these are the phyto constituents that should be focused on for investigation of many biological activities. Phytochemicals especially polyphenols constitute a major group of compounds that act as primary antioxidants [15].

More than 4000 polyphenols (flavonoids, monophenols and poly phenols) are found in vascular plants. Phenolic compounds such as quercetin, rutin, narigin, catechin, caffeic acid, gallic acid and chlorogenic acid are very important plant constituents [15]. Medicinal plants are known to produce diverse substances possessing antioxidant properties having ability to protect the human body against cellular oxidation. Anti-oxidation are vital substances which possess the ability to protect the body from damage caused by free radicals inducing oxidative stress [35]. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide of lipid hydroxyl and therefore halt the oxidative mechanism that leads to degenerative diseases [36]. Flavonoids are proactive polyphenolic compounds found in most plants and cannot be synthesized or produced by the humans [37]. It was found to be effective in controlling various biological activities and is anti-inflammatory, anti-angiotic, antimicrobial, antioxidant, reduced hypertension, and has anti-cholesterol properties [38, 39]. In recent times, research interest has been paid to polyphenols and flavonoids as a result of their antioxidant capacity which is principally based on the redox properties of their hydroxyl groups and the structural relationship between different functional groups in their structure which enable them to actively serve as free radical scavengers, reducing agents, singlet oxygen quenchers metal chelators, and hydrogen donors [40].

Conclusion

It can be postulated that the contents of phenolic and flavonoid compounds of tepal of *Musa paradisiaca* contribute to the radical scavenging activity and can be regarded as promising plant species for natural sources of antioxidant with potential value for treatment of many life threatening diseases. The process of extraction and identification of active principles responsible for the free radical scavenging property of tepal extract of *Musa paradisiaca* through bioactivity guided fraction is under progress in order to understand the possible mechanism of action of tepal of *Musa paradisiaca*. Utilization of this tepal will be of advantage to mankind and increased in its consumption will help in prevention of chronic life style diseases.

Acknowledgement

The authors would like to acknowledge the support of Faculty of Medicine, Universiti Sultan Zainal Abidin (UniSZA), for providing the necessary fund on this research work.

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