Innovare
Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 5, 2015

Original Article

PHYTOCHEMICAL SCREENING, ANTIOXIDANT ACTIVITY OF PURE SYRINGIN IN COMPARISON TO VARIOUS SOLVENTS EXTRACTS OF *MUSA PARADISIACA* (BANANA) (FRUIT AND FLOWER) AND TOTAL PHENOLIC CONTENTS

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Received: 25 Jan 2015 Revised and Accepted: 26 Feb 2015

ABSTRACT

Objective: The study concentrated on exploring the radical scavenging activity of various banana extracts in comparison to pure syringin and the total phenolic contents of the extracts.

Methods: The banana parts were prepared and extracted by cold extraction technique and the extracts obtained were used to carry out some phytochemical screening by Trease and Evans method. The ability of the extract to scavenge free radicals was measured using 2, 2-diphenyl-1-pcrylhydrazyl radical using quercetin as a reference radical scavenger by the method of Gyamfi. Further analysis of total phenolic contents present in the extracts was carried out using Singleton and Rossi method.

Results: Tepal methanol extract was found to have the highest radical scavenging activity compared to others, such as tepal ethanol, tepal aqueous, skin methanol, flesh methanol and pure syringin. The IC_{50} value of the tepal methanol extract was found to be 22.5 µg/ml. The highest total phenolic contents (expressed as microgram of Gallic acid equivalent per gram of the extracts) were found in tepal methanol extract (8000 µg/g) and the least in Flesh methanol extract (2150 µg/g).

Conclusion: The results generated from this study showed that tepal banana extracts possess very good radical scavenging activity and as well the largest amount of phenolic contents, which could introduce phenols as the main radical scavenger in banana extracts and offering effective protection from free radicals, and the antioxidant activity of pure syringin was not obvious may be due to the presence of highly anti oxidative compounds present in banana, phenolics and flavonoids.

Keywords: Banana, Radical scavenging, Tepal, Syringin.

INTRODUCTION

Biological damage due to free radicals caused by reactive oxygen species (ROS) leads to several negative effects as they can attack lipids, proteins/enzymes, carbohydrates, and DNA in cells and tissues. They induce undesirable oxidation, causing membrane damage, protein modification, DNA damage, and cell death induced by DNA fragmentation and lipid peroxidation. This oxidative damage/stress, associated with ROS is believed to be involved not only in the toxicity of xenobiotics but also in the pathophysiological role in aging of skin and several diseases like heart disease (atherosclerosis), cataract, cognitive dysfunction, cancer (neoplastic diseases), diabetic retinopathy, critical illness such as sepsis and adult/acute respiratory distress syndrome, shock, chronic inflammatory diseases of the gastrointestinal tractt, organ dysfunction, disseminated intravascular coagulation, deep injuries, respiratory burst inactivation of the phagocytic cells of immune system, production of nitric oxide by the vascular endothelium, vascular damage caused by ischemia reperfusion known as ischemia/reperfusion injury and, release of iron and copper ions from metalloprotein [1].

All the damaging effects of ROS and reactive nitrogen species (RNS)are combated by the group of molecules called "antioxidants" which are inhibitors of the process of oxidation, even in relatively small concentration and thus have diverse physiological role in the body. Antioxidant constituents of the plant material act as radical scavengers, and help in converting the radicals to less reactive species. A variety of free radical scavenging antioxidants are found in dietary sources like fruits, vegetables and tea, etc. [2].

In vitro studies on several medicinal plants have demonstrated the antioxidant potential of phenols as direct aqueous phase radicalscavengers and as agents capable of enhancing the resistance to oxidation of low density lipoproteins implicated in the pathogenesis of coronary heart disease [3]. It is admitted that a part of the antioxidant capacity of many fruits and berries is derived from flavonoids [4, 5]. In fact, all the major polyphenol constituents of food show greater efficacy in these systems as antioxidants on a molar basis than the antioxidant nutrients vitamin C, vitamin E, and β-carotene. Differences between the antioxidant potential of selected compounds can be measured using many different techniques. Because most phytochemicals are multifunctional, a reliable antioxidant protocol requires the measurement of more than one property relevant to either foods or biological systems [6].

Plasma oxidative stress is significantly reduced only after a single banana meal in healthy human due to the presence of dopamine, ascorbic acid and other antioxidants present in banana [7]. Antioxidant activity was also reported with aqueous acetone extract of banana peel by β-carotene bleaching method, DPPH free radical scavenging and linoleic acid emulsion method. Glycosides and monosaccharide components are mainly responsible for the antioxidant activity [8]. Vijaya kumar [9] reported the antioxidant activity of the extracted flavonoids from *Musa paradisiaca* in rats. They found that the flavonoids from banana stimulated the activities of superoxide dismutase (SOD) and catalase (CAT) which might be responsible for the reduced level of peroxidation products such as malondialdehyde, hydroperoxides and conjugated dienes. Syringin is a phenyl propanoid glycoside compound and has been isolated from the crude extract of bark of *phellodendronchinensis*[10]. Several pharmacological actions of syringin include plasma glucose reduction, anti-oxidation, anti-cancer activity, antidepressant effect, immunomodulation etc. [11-15]. A compound isolated from *Musa paradisiaca* tepal was identified as syringin [16].

The current study aims to elucidate the radical scavenging activity of various banana extracts in comparison to pure syringin and the total phenolic contents of the extracts.

Hopefully, this study will be the first step in identifying the most suitable part of banana and solvent(s) for the isolation, identification and production of pharmacologically active drug that can be used to cure various fatal diseases like atherosclerosis, cataract, cognitive dysfunction, cancer (neoplastic diseases), and diabetic retinopathy, to mention just a few, caused by free radicals.

MATERIALS AND METHODS

Materials

The chemicals used in this study include methanol (100%) and ethanol (95%) purchased from HMB chemicals. Quercetin(98 % hplc) from Sigma Life Science, 2,2-diphenyl-1-picrylhydrazyl radical, DPPH, (95%) from Alfa Aesar, Dimethyl sulfoxide (dmso,99%) from R&M chemicals. Folin & Ciocalteu's phenol reagent from Sigma Aldrich, sodium carbonate (anhydrous) from R&M chemicals, Gallic acid from Sigma Aldrich. Syringin (10000 µg) from Sigma Aldrich. All other chemicals used were of analytical grade.

Collection and preparation plant

Musa paradisiaca, fruit and tepal, were purchased from the local market in Kuala Terengganu, Malaysia; they were identified and authenticated by Dr. Khamsah Suryati Muhammad from the faculty of Bioresources and Food industry, Universiti Sultan Zainal Abidin. The skin was peeled off and both the skin and the flesh were sliced and weighed (1.45 kg and 3.28 kg) respectively, and dried in a drier at 45 °C, their dry weights are 0.41 kg and 0.21 kg respectively. After drying, they were ground using electric blender and the weights are0.21 kg and 0.10 kg. The tepal was also prepared in a similar way and weighed (3 kg) and dried at 40 °C and then grinded (0.42 kg).

Extraction

The freshly prepared flesh, skin and tepal were extracted twice with methanol (10:2 ml/kg) by cold extraction technique and with ethanol (for the tepal). The samples were kept in the solvents for about 3 d at 25 °C with frequent shaking. Aqueous extraction of the tepal was carried out by boiling the sample with distilled water at 100 °C on a hot plate. All the extracts were vacuum filtered using Whatman filter paper and concentrated at 40 °C using a rotary evaporator while the aqueous extract was concentrated in an oven at 100 °C to obtain the crude extracts. The crude extracts were kept in fresh vials and refrigerated for further use.

Phytochemical screening of the extracts

The phytochemicals screening of the various solvents extract was carried out using standard procedures described by Trease and Evans [17]. The following tests were carried out qualitatively.

Test for glycosides

Small amount of the extracts was put in 1 ml of water in a test tube followed by the addition of 1 ml of NaOH. A yellow precipitate indicates the presence of glycosides.

Test for phenols

The extract (5000 µg) was dissolved in distilled water and 3 ml of 10% lead acetate solution was added. A bulky white precipitates indicated the presence of phenols.

Test for Flavonoids

A few drops of concentrated hydrochloric acid were added to a small amount of the extracts. Immediate development of red color indicates the presence of flavonoids.

Test for saponins

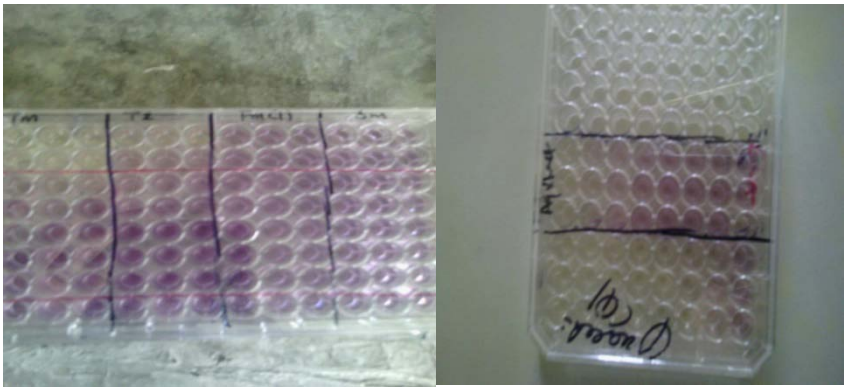
1 ml of each extract was diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 min. The formation of foam of about 0.01 m indicates the presence of saponins.

Determination of DPPH radical scavenging activity

Method of Gyamfi [18] was used with a slight modification to assay the radical scavenging capacity of the extracts. In a 96 well plate, 0.02 ml of the sample was taken from the stock and added to a total volume of 0.22 ml to achieve the required concentration (90µg/ml) and the remaining concentrations were obtained by serial dilution. 0.2 ml of DPPH dissolved in methanol was added and incubated for 30 min. The absorbance was measured at 517 nm and the ability of the sample to scavenge the radical was calculated relative to the control using the formula [19]. All measurements were performed in triplicate.

$$I(\%) = [\frac{Ac - As}{Ac}] \times 100$$

Where: I (%) = Percentage of inhibition; Ac = Absorbance of control; As = Absorbance of the sample.



Total phenolic content

Total phenolic content was determined according to the method of Singleton and Rossi [20] spectrophotometrically with some modifications. In brief, 0.25 ml of 1000 µg/ml samples was pipetted into a test tube followed by the addition of Folin-Ciocalteu's reagent (1:10 dilution) and incubated for 2-5 min. 1 ml of Sodium carbonate (7.5%) was added to the above mixture and incubated for 30 min. The absorbance of the color formed was measured at 760 nm against the blank sample. The measurements were compared to a standard curve of prepared gallic acid solutions (12.5, 6.25, 3.125 and 1.3625µg/ml). All measurements were performed in triplicate.

RESULTS AND DISCUSSION

Phytochemical screening

The various solvent extracts of banana, prepared in this work showed the presence of tested phyto chemicals like flavonoids, phenols, glycosides etc. (table 1). Several researches showed the presence of several phytochemicals in different parts of banana from different solvent extracts. Several flavonoids and related compounds (Leucocyanidin, quercetin, and its 3-O-galactoside, 3-O-glucoside and 3-O-rhamnosyl were glucoside) were isolated from the unripe pulp of plantain [21-23]. Serotonin, norepinephrine, tryptophan,

indole compounds, tannin, starch, iron, crystallisable and non-crystallisable sugars, vitamin C, B-vitamins, albuminoids, fats, mineral salts have been found in the fruit pulp of *M. Paradisiaca* and *M. Sapientum* [24]. The preliminary phytochemical screening carried out showed *M. sapientum* peels contain some secondary metabolites such as glycosides, alkaloids, saponins, volatile oil, flavonoids and tannins[25]. The preliminary phytochemical screening of ethanolic and methanolic extracts of the selected *Musa paradisiaca L. var Bontha*indicated the presence of certain secondary metabolites. Ethanolic extract was found to contain alkaloids,flavonoids, steroids, tannins, xanthoproteins and glycosides, whereas the methanolic extract revealed the presence of alkaloids, saponins, xanthoproteins and glycosides [26]. In another work, extracts of banana flower (*M. paradisiaca*) were prepared by using solvents such as chloroform,acetone, ethyl acetate, ethanol, methanol and water by maceration process. Phytochemical screening of these extracts showed the presence of various phytoconstituents like carbohydrates, steroids and triterpenoids, tannins, proteins, flavonoids, phenolic compounds, fixed oils and fats [27].

Table 1: Phytochemicals from the various solvent extracts of *Musa paradisiaca*

Extract	Yield(%)	Glycosides	Phenols	Flavonoids	Saponins
Tepal methanol	5.87	++	++	+	+
Tepal Ethanol	5.38	++	++	+	+
Tepal Aqueous	5.09	+	+	++	-
Skin	19.49	+	+	++	-
Flesh	28.86	+	+	+	-

Key:++= present in abundance.+= present.-= Absent

DPPH radical scavenging activity

In vitro antioxidant activity of banana tepal (methanol,ethanol and aqueous),skin and flesh(methanol) and pure syringin was carried out by DPPH free radical scavenging assay method. The result showed more promising activity in tepal methanol, moderate in tepal ethanol, tepal aqueous and skin with little activity in pure syringin and flesh (fig. 1). The DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity of the different successive extracts of the plant, *Musa Paradisiaca*, showed increased activity by increasing the concentration of the sample extracts (fig. 2).

The DPPH assay method is based on the reduction of DPPH, a stable free radical. The free radical DPPH with an odd electron gives a maximum absorption at 517 nm (purple color). When antioxidants react with DPPH which is a stable free radical it becomes paired off in the presence of a hydrogen donor (e.g. a free radical-scavenging antioxidant) and is reduced to the DPPH-h and as a consequence the absorbance's decreased from the DPPH radical to the DPPH-h form results indecolorization (yellow color) with respect to the number of electrons captured [28]. More the decolorization more is the reducing ability. This test has been the most accepted model for evaluating the free radical scavenging activity of any new drug. 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 form (diphenylpicrylhydrazine; non radical) with the loss of this violet color (although there would be expected residual pale yellow color from the picryl group still present) [29]. The effective concentration of the sample required to scavenge DPPH radical by 50% (IC_{50}value) was obtained by linear regression analysis of dose-response curve plotting between percentage of inhibition and concentrations [30].

(DPPH)+h-a → DPPH-h+a

(Purple) (Yellow)

Vijaya kumar [9] reported the antioxidant activity of the extracted flavonoids from *M. Paradisiaca* in rats. They found that the flavonoids from banana stimulated the activities of superoxide dismutase (sod) and catalase, which might be responsible for the reduced level of peroxidation products such as malondialdehyde, hydroperoxides and conjugated dienes.

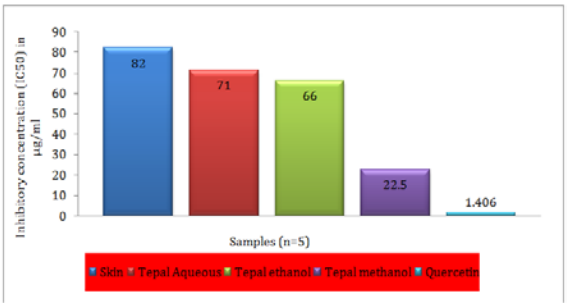


Fig. 1: Effective concentrations of sample required to scavenge Dpph radical by 50% (IC_{50})

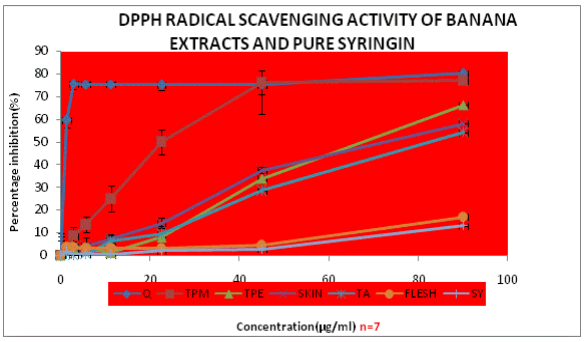


Fig. 2: DPPH Radical scavenging activity of banana extracts and pure syringin
Key: Q=Quercetin; TPM=Tepal methanol; TPE=Tepal ethanol; TA=Tepal aqueous and SY=Syringin

Total phenolic content

Total phenolic contents (in µg/g of extract), as affected by the extracting solvents were ordered from high to low: tepal methanol extract>tepal ethanol extract > skin methanol extract>aqueous extract>Flesh methanol (table 2). The total phenolic contents were found remarkable in methanol soluble fraction to be8000 µgGallic acid equivalent/g extract. Gallic acid was used as a standard compound, the standard curve (fig. 3) and the total phenols were expressed as µg/g Gallic acid equivalent using the standard curve equation: y = 0.0952x+0.1009, where y is absorbance at 760 nm and x is total phenolic content in extracts banana expressed in µg/g. The phenolic content of plants may contribute directly to their antioxidant action [31]. The extract with highest phenolic content showed the highest antioxidant activity.

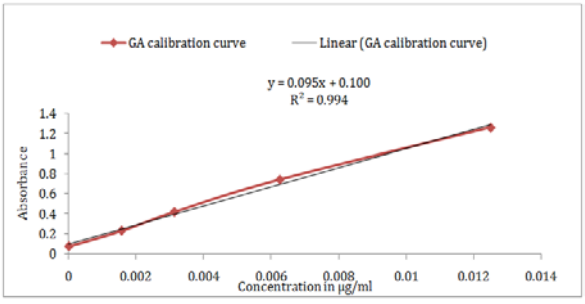


Fig. 3: Standard curve for gallic acid used as a standard

Phenolic compounds are a class of antioxidant agents which act as free radical terminators and their bioactivities may be related to their abilities to chelate metals, inhibit lipoxygenase and scavenge free radicals [32]. They are very important in chelating redox-active metal ions, inactivating lipid free radical chains, and preventing

hydro-peroxideconversions into reactive oxy-radicals as they have been generally recognized.

The anti-oxidative effect is mainly due to phenolic components, such as phenolic acids, and Phenolic diterpenes [33]. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [34].

Table 2: Total phenols present in each extract (gae µg/g of extract)

Sample	Absorbance (mean±Sd)	gae µg/g extract
Tepal Methanol	0.873±0.037	8000
Tepal Ethanol	0.695±0.092	6240
Tepal aqueous	0.342±0.006	2530
Skin	0.429±0.015	3450
Flesh	0.306±0.061	2150

For the measurements of the reducing ability, it has been found that the Fe^{3+}–Fe^{2+}transformation occurred in the presence of extract samples which were postulated previously by [35]. Tanaka M, *et al.,*[36]has observed a direct correlation between antioxidant activity and total phenolic contents of certain plant extracts. Methanol extract had the highest total phenolic recovery, as well as the highest antioxidant activity when determined by the DPPH assay. Thus, this result indicates that the selective extraction of natural materials, by an appropriate solvent, is important for obtaining fractions with high antioxidant activity. The results also showed that different solvent extracts contained different antioxidant capacities in terms of reducing and radical scavenging power.

CONCLUSION

Musa paradisiaca, a common and highly valued plant use as food and medicine have been undergoing several pharmacological investigations and been proved to be very effective in curing various diseases through its medicinally active secondary metabolites. This study is in line with various literatures in confirming the anti-oxidative effect of various extracts of banana and the direct relation between the antioxidant activities with the total phenolic contents present.

ACKNOWLEDGEMENT

The authors express gratitude to Vice Chancellor, Universiti Sultan Zainal Abidin, Malaysia,and Eng. Dr. Rabiu Musa kwankwaso, Kano state governor, Nigeria for their enormous contribution, and Suleiman Danladi and Miera for their tireless effort in many aspects of the work. May Almighty Allah reward them all.

CONFLICT OF INTERESTS

No conflict of interest so far

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