



AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: <http://www.ajptr.com/>

Phytochemical Screening, Antioxidant and Antidiabetic Activity of Musa Acuminata, Citrus Sinensis and Phyllanthus Emblica

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ABSTRACT

The present study aims to investigate the phytochemical screening, antioxidant and anti-diabetic activity of commonly used fruits, *Musa acuminata Red Dacca* (Red Banana), *Citrus sinensis* (Orange) and *Phyllanthus emblica* (Amla). Edible part of *Phyllanthus emblica*, *Musa acuminata Red Dacca* and peel of *Citrus sinensis* are taken for the study. The phytochemical screening of ethanolic extract of *Musa acuminata* revealed strong presence of carbohydrates and glycosides and moderate presence of tannins, proteins, flavonoids, steroids and phenols. The ethanolic extract of *Citrus sinensis* showed abundance of flavonoids and least presence of carbohydrates, proteins and steroids. Strong presence of phenol was shown in *Phyllanthus emblica* and all the others were present in least amount. The antioxidant activity was determined by ferric reducing power assay (FRP) where *Phyllanthus emblica* showed maximum activity that was followed by *Citrus sinensis* peel and then *Musa acuminata*. α -Amylase inhibitory assay was performed to detect the antidiabetic activity of these fruits by comparing with commercially available drug Okamet. It was *Phyllanthus emblica* that used lowest concentration to inhibit the enzyme which was found similar to positive commercial standard Okamet that is used for reducing blood sugar level. Hence it can be concluded that *Phyllanthus emblica* has good antioxidant and antidiabetic property than *Musa acuminata* and *Citrus sinensis*.

Keywords: *Musa acuminata*, *Citrus sinensis*, *Phyllanthus emblica*, Okamet.

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Received 28 March 2015, Accepted 03 April 2015

Please cite this article as: Philip DC *et al.*, Phytochemical Screening, Antioxidant and Antidiabetic Activity of *Musa Acuminata*, *Citrus Sinensis* and *Phyllanthus Emblica*. American Journal of PharmTech Research 2015.

INTRODUCTION

Reactive oxygen species (ROS) are generated as by-products of biological processes/reactions or from exogenous factors like air pollutions, smoking, UV radiation etc¹. ROS cover a wide range of chemical components including superoxide anion, hydrogen peroxide, hydroxyl radicals, nitric oxide, and peroxy nitrite². Their overload causes generation of free radicals and lipid peroxidation inside the human body which is implicated in the etiology of many human pathogen and leads to diseases like dermatitis, melanomas or photo aging of the skin, cancer, heart disease, inflammation, arthritis, immune systems decline, brain dysfunction and cataracts etc. oxidative damages occurring in our body can be prevented by the use of antioxidants. These agents are able to remove the deleterious effects of free radicals within our body. Nowadays, considerable interest is focused on the development and evaluation of natural antioxidants and radical scavengers from plant materials which are rich in polyphenolic compounds. Anam et al (2012)³ reported that flavonoids, phenolic compounds and vitamins C are the major constituents responsible for antioxidant activities in the fresh fruits. The consumption of fruits and vegetables has been found to be associated with lowering of these diseases as they contain a large amount of phenolic compounds, antioxidants and flavonoids^{4,5}. Plant extracts derived from their parts (roots, stems, leaves, fruits) are now increasingly used in research due to their widespread, immediate availability and cheaper cost, besides having potential medicinal properties and ability to manage certain health conditions which have been growing in recognition. Globally, banana plant is one of the most widely distributed, consumed fruit in the tropical and subtropical countries⁶ and is the fourth most important agricultural product after rice, wheat and maize. The flower and stem region of banana plant are known for their anti-ulcer, diuretic, anti- diabetic and antiseptic properties^{7,8} and they have been extensively investigated. Considering the nutritional aspects, it is one of the world's leading food crops with a great source of minerals, vitamins, carbohydrates, flavonoids, phenolic compounds etc⁹. Amla which is known as *Emblica officinalis* is an Indian herb, extensively used in ayurvedic system of medicine. Amla refreshes all the organ systems of the body, provide strength, wellness and keeps us away from all the diseases by boosting our immune system. Amla fruit is a rich source of Vitamin C and tannin. Literature survey shows that it contain several alkaloids, phenolic compounds, higher concentrations of most minerals, protein and amino acids¹⁰. Several active tannoid principles (Emblicannin A, Emblicannin B, Punigluconin and Pedunculagin) have been identified which appear to account for its health benefits^{11,12,13}. *Citrus sinensis* is one of important medicinal plant in indigenous system of medicine for the treatment of

various ailments. Studies shows that *Citrus* has been used for ailments like diabetics, infections, hypertension, oxidative damages etc.¹⁴. The leaves and the peel of the fruit have been used to kill mosquito larvae and mites¹⁵. There are strong evidences that essential oil of *C. sinensis* showed larvicidal, repellent and fumigant activities against *Aedes aegypti* L. mosquitoes¹⁶. Folk medicine uses the leaf extracts of *C.sinensis* to treat neurological disorders and to help the digestion of food¹⁷. Although several compounds obtained from *C. sinensis* have been reported, flavonoids exhibit a broad spectrum of pharmacological properties¹⁸. The most prevalent flavanones like hesperidin, naringin, tangeretin and nobiletin are shown to present in *C. sinensis*. Hesperidin was shown to have anti- inflammatory, antihypertensive, diuretic, analgesic and hypolipidemic properties. It also has antioxidant, anti-allergic, vasoprotective and anti-carcinogenic actions¹⁹.

MATERIAL AND METHODS

Selection and procurement of plants

Healthy and disease free, easily available three different fresh fruits *Musa acuminata* Red Dacca (Red banana), *Citrus sinensis* (orange) and *Phyllathus emblica* (amla) were collected from local market of Avadi. The plants were authenticated at the Department of Botany, Government Arts and Science College, Nandanam, Chennai, (India).

Preparation of extracts

The fruits were washed with distilled water. Crushed and grinded fruits were dipped separately in ethanol medium. The solution were covered with aluminum foil and kept at 4°C in a refrigerator for overnight. Thereafter it was subjected to centrifugation at 8000 rpm for 10 min which is then filtered using Whatmann No.1 filter paper. The resultant supernatant was subsequently oven dried at a temperature of 45°C to form a powdery residue. This powder was used for further studies²⁰.

Phytochemical Screening

The phytochemical screening of *Musa acuminata* Red Dacca, peel of *Citrus sinensis* and *Phyllathus emblica* were carried out as described by Nweze et al (2004)²¹ and Senthilkumar and Reetha (2009)²².

Antioxidant Activity

Antioxidant potential of ethanolic extracts of *Musa acuminata* Red Dacca, *Citrus sinensis* and *Phyllathus emblica* were determined by Ferric reducing power assay (FRP)²³. All the assays were carried out in triplicate.

Ferric reducing power assay (FRP)

The reducing power of *Musa acuminata* Red Dacca, *Citrus sinensis* and *Phyllathus emblica*

extracts was quantified by the method described previously by Shafiqu *et al* (2013) with minor modification. Red banana, Orange peel and Amla were taken as test solutions in the concentration range of (0.5, 1.0, 1.5) mg in different test tubes. 1 ml of ethanol (50%) was added and mixed with 5 ml phosphate buffer (2 M, pH 6.6) and 5 ml potassium ferricyanide (1%). These mixtures were incubated at 50°C for 20 min. 5 ml of trichloroacetic acid (10%) was added and the mixtures were centrifuged at 3000 rpm for 10 min. The upper layer of the solution (5 ml) was mixed with 5 ml of distilled water and 1 ml of ferric chloride (0.1%). Ascorbic acid was used as standard. Control sample was prepared without any extract or ascorbic acid. The absorbances of the pink colour mixture were measured spectrophotometrically at 700 nm. Increased absorbance of the mixture indicates increased reducing power.

Antidiabetic Activity

Antidiabetic activity of ethanolic extracts of *Musa acuminata* Red Dacca, peel of *Citrus sinensis* and *Phyllanthus emblica* were determined by α -Amylase inhibitory assay. All the assays were carried out in triplicate.

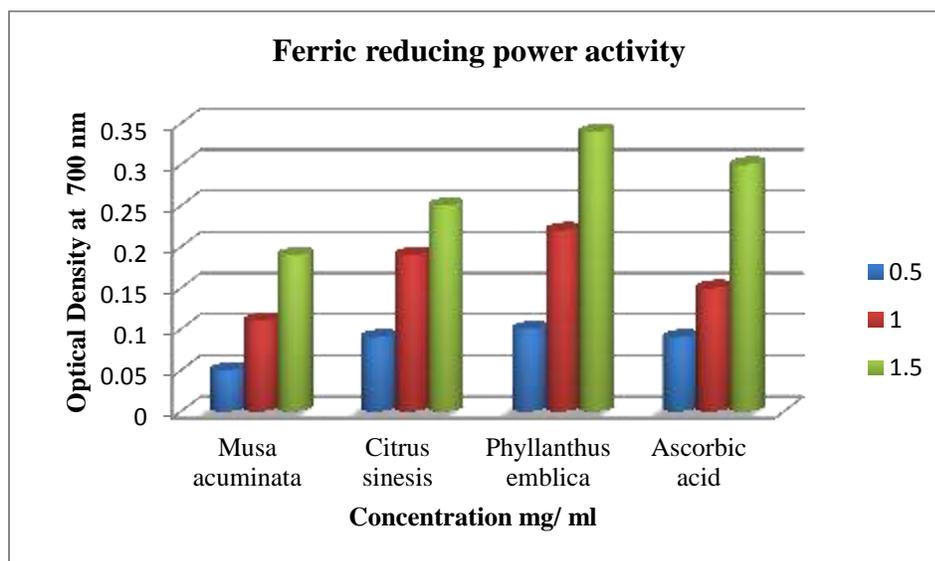
α -Amylase inhibition assay

α -Amylase inhibitory ability of *Musa acuminata* Red Dacca, peel of *Citrus sinensis* and *Phyllanthus emblica* was analyzed as described by Miller (1959)²⁴ with a modified dinitrosalicylic acid (DNS) method²⁵. The substrate starch and the enzyme was prepared from potato and saliva respectively. Test samples and standard drug (Okamet) were prepared using DMSO in different concentration. The reaction was arrested by adding 3, 5 dinitro salicylic acid (DNS). Three sets of experiments were conducted; test, blank and control. One ml of each test and enzyme mixture was conducted at 25 °C for half an hour. From this 1 ml of sample mixture was taken in another test tube and added 1 ml of starch solution (0.5%) and incubated for 3 min at 25 °C again. Finally 1 ml of DNS reagent was added and heated the mixture in water bath at 85 °C for 15 min. The mixture solution was cooled by adding 9 ml of distilled water and observed the readings at 540 nm. In case of blank solution, the DNS solution was added prior to the starch solution while the rest of the experimental procedure remains same. For control solution instead of fruit extract 1 ml of DMSO was added. Remaining procedure was the same. Control represents 100% enzyme activity. Inhibition of starch hydrolysis by α -amylase inhibitor results in a diminished absorbance at 540 nm in comparison with the control.

Calculation of IC₅₀

The concentration of the plant extracts required to inhibit 50% of the enzyme (IC₅₀) was calculated by using the percentage inhibition activities (I %) at different concentrations of the extracts.

of *Citrus sinensis* have shown profound antioxidant activity. The reducing power of *Phyllanthus emblica* was shown maximum that was followed by *Citrus sinensis* peel and then *Musa acuminata* (Figure 1).



Values are gives as Mean \pm SD of three replicates

Figure 1: Ferric reducing power activity of *Musa acuminata* Red Dacca, *Citrus sinensis* peel and *Phyllanthus emblica*

Reducing power of the extracts was increased with quantity of sample. The reducing power of the extracts were comparable to the standard ascorbic acid. The reported antioxidant activity is due to the presence of phytochemicals like phenols, tannins, flavonoids, glycosides and saponins³¹ in these plants. The result of α - amylase inhibitory activities are shown in (Figure 2). The inhibitory activity of *Phyllanthus emblica* was found similar to positive commercial standard drug Okamet that is used for reducing blood sugar level. Concentration of a sample at which the inhibition percentage reaches 50% is the IC_{50} . Lower the IC_{50} higher the antidiabetic activity . After the drug (0.44 \pm 0.17 mg/ml) it was *Phyllanthus emblica* that used lowest concentration to inhibit the enzyme (0.45 \pm 0.56 mg/ml). That is highest antidiabetic activity is shown by *Phyllanthus emblica* followed by *Citrus sinensis* peel and then *Musa acuminata* Red Dacca .The drug and *Phyllanthus emblica* showed almost similar (IC_{50}) value (Table 2).

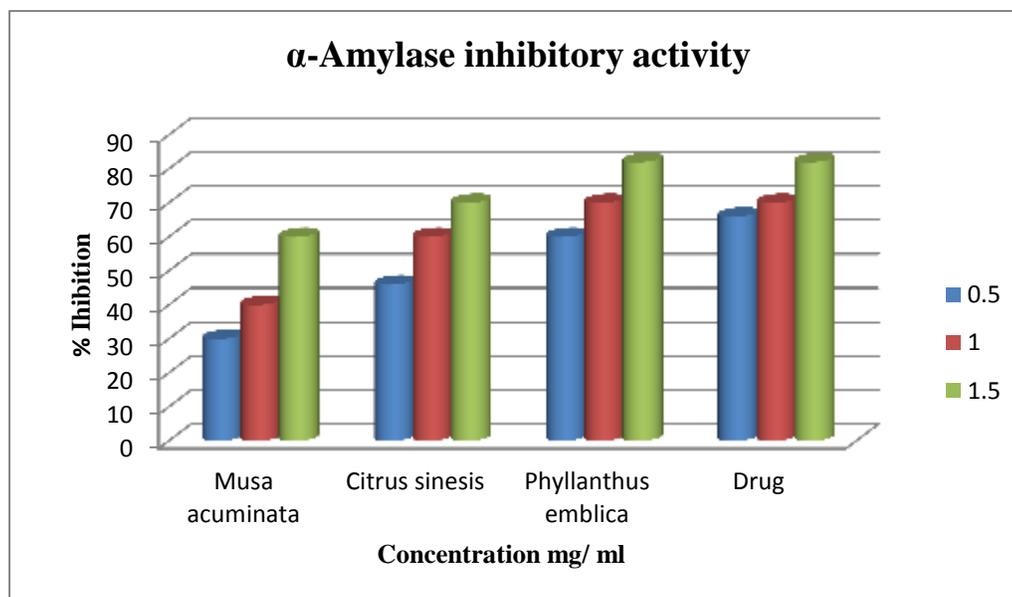
Antidiabetic activity of Orange may be due to Bioflavonoid such as hesperidin present in citrus fruit peels which play antidiabetic role by the regulation of glucose regulatory enzyme that decrease the activity of glucose -6-phosphatase and phosphoenol pyruvate³². In *Phyllathus emblica*, antidiabetic effect may be by inhibiting glycogenolysis and hepatic glucogenesis and glucose absorption from intestine or by increasing glucose absorption in cells of peripheral tissues

and hepatic glycogenesis, and by stimulation of insulin secretion³³.

Table 2: α -Amylase inhibitory activity (IC₅₀) of *Musa acuminata* Red Dacca, *Citrus sinensis* peel, *Phyllanthus emblica* and Drug

Extract / Standard	(IC ₅₀) mg/ml
<i>Musa acuminata</i> Red Dacca	1.25±0.82
<i>Citrus sinensis</i> peel	0.78±0.19
<i>Phyllanthus emblica</i>	0.45±0.56
Drug Okamet	0.44±0.17

Values are given as Mean \pm SD of three replicates



Values are gives as Mean \pm SD of three replicates

Figure 2: α -Amylase inhibitory activity of *Musa acuminata* Red Dacca, *Citrus sinensis* peel, *Phyllanthus emblica* and Drug.

CONCLUSION

Herbal molecules are safe and can overcome the resistance produced by the pathogens. Almost all the parts of the plant, namely leaves, flowers, fruits, bark, roots, stem and seeds are known to have various medicinal properties. Fruits used in this study such as red bananas, oranges and amla showed varied phytochemical, antioxidant and antidiabetic properties that offer great medical benefits. Therefore the use of traditional herbals such as *Citrus sinensis* peel and *Phyllanthus emblica* as alpha amylase inhibitors can be used for the prevention and cure of diabetes that will not have any side effects.

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