Research Article

Phytochemical Screening, Antioxidant and Alpha amylase Inhibitory Activity of *Phyllanthus acidus*

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ABSTRACT

In the present investigation, phytochemical screening, antioxidant and alpha amylase inhibitory potentials of methanolic extracts of Phyllanthus acidus fruits were investigated. The antioxidant activity was assessed through DPPH assay and reducing power assay. Phytochemical screening studies of the extract showed the presence of flavonoids, steroids, saponins and tannins. Determination of total phenolic contents revealed that the extract contains 150.3 mg/g of phenolic compounds. The free radical scavenging activity of the extract was confirmed in a DPPH assay. The extract showed the stronger radical scavenging effect with IC_{50} value of 40.5µg /ml. The reducing power of the extract increased with increasing concentration and is comparable with the standard antioxidant ascorbic acid. The present study clearly indicated that the extract exhibited good alpha amylase inhibitory activity in a dose dependent manner. The extract showed highest inhibitory activity of 72.06% with an IC_{50} value of 22.8 µg/ml.

Keywords: Antioxidant activity, Reducing power, Polyphenols, Free radicals, Phytochemicals, Alpha amylase inhibitory activity.

INTRODUCTION

In human body, the free radicals are produced as by product through frequent physiological and biochemical processes[1, 2]. Free radicals might leads to oxidative damage of biomolecules viz., lipids, proteins, DNA etc. in the body, which can initiate number of diseases like atherosclerosis, diabetes mellitus, cancer, cardiovascular diseases, neurodegenerative diseases etc. [3,4]. Plants generally contain polyphenolic compounds and these compounds protect cells against the damaging effects of reactive oxygen species such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals etc [5, 6]. Synthetic antioxidants such as butylated hydroxy toluene (BHT) and butylated hydroxy anisole (BHA) have restricted use in food industry as they are suspected to be carcinogenic [7]. Hence, the studies on natural antioxidant have gained increasingly greater importance.

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia with disturbances of carbohydrate, lipid and protein metabolism resulting from defect in insulin secretion, insulin action or both. The number of people in the world with diabetes has increased dramatically over recent years. It has predicted that by 2030, India, China and the United States will have the largest number of people with diabetes [8]. One of the effective methods to control diabetes is to inhibit the activity of alpha amylase enzyme which is responsible for the breakdown of starch to simple sugars [9]. The alpha amylase inhibitors in clinical use such as acarbose, miglitol, and voglibose produce serious side effects which includes abdominal pain, flatulence and diarrhea in the patients [10, 11]. Therefore, it is a dire need to identify and explore the amylase inhibitors from natural sources having fewer side effects. Indian traditional system of medicine practiced over thousands of years has reports of numerous antidiabetic and antioxidant plants. Nearly 200 species of plant withhypoglycemic properties have been studied [12]. Herbal remedies having high therapeutic value with minimal side effects are favoured. In this context, evaluation of the polyphenolic compounds from plants for

antioxidant and antidiabetic activity has become important tool to understand the healing property of medicinal plants.

Phyllanthus acidus commonly known as star gooseberry belonging to the family Phyllanthaceae is a common tree found in South India and Southeast Asian countries. Leaves pinnate, flowers are small and pink in colour. Fruits are drupaceous and borne in loose clusters. They are greenish yellow to creamy white, waxy, crisp, juicy, sour in taste and are a rich source of vitamin C. Medicinal properties of *Phyllanthus* species are antipyretic, analgesic, anti-inflammatory, antihepatotoxic and antiviral [13-16]. Fruits of *P. acidus* have been used for improving eyesight, memory and preventive action against Diabetes [17]. The plant is used for 28 types of remedies like cathartic, emetic, coughs, hypertension, asthma, skin diseases etc [18]. The aim of this study was to evaluate the antioxidative activity and alpha amylase inhibitory activity of *Phyllanthus acidus* using different in vitro methods.

MATERIALS AND METHODS

Chemicals

All chemicals and solvents used in the study were of analytical grade. 1, 1-diphenyl-2-picryl hydrazyl (DPPH) was purchased from Sigma Aldrich Co. St. Louis, USA. Methanol, Trichloroacetic acid, Ascorbic acid, Potassium ferric cyanide, ferric chloride, butylated hydroxyl anisole (BHA), Folin-Ciocalteau reagent, Sodium carbonate, Gallic acid, Alpha amylase, 3,5-Dinitrosalicylic acid (DNS) etc. were procured from Sd Fine chem. Ltd, India.

Plant material collection and Preparation of extract

The plant material consisting of mature fruits of *Phyllanthus acidus* (L.) Skeels was collected from local market, Mysore, Karnataka, India. The materials were identified and authenticated by Department of Studies in Botany, University of Mysore. The fruits were cleaned and washed under running tap water then dried at 40º C in an oven for 3 days. The dried fruits were powdered using a grinder. The crude methanolic extract was obtained by extracting 100 grams of dried fruit powder in 500ml of methanol on a water shaker for 72 hrs. Extract was further concentrated using rotary vacuum evaporator at 45-50 °C and stored at 4°C.

Phytochemical Screening

The extract was analyzed for the active phyto-constituents such as phenols, flavonoids, alkaloids, tannins, saponins, terpenoids etc according to the standard protocol [19].

Determination of the total phenolic content

The amount of total soluble phenolic content present in the extract was evaluated according to Folin-Ciocalteu method [20]. Briefly, the extract (1mg/ml) was mixed with 20 µl of Folin-Ciocalteau reagent (1:10) and 50 µl of aqueous 2.5% Na_2CO_3. The mixtures were allowed to stand for one hour at room temperature. Absorbance was measured at 765 nm using spectrophotometer. The standard graph was plotted using different concentrations of gallic acid. Total phenolic content was expressed as mg gallic acid equivalent/gram of dry weight of extract.

DPPH radical scavenging assay

DPPH radical scavenging activity was measured using the method described by Oktay et al [21]. The reaction mixture contained 0.1 ml of fruit extract at different concentrations and 5 mL of 0.004% solution of DPPH in methanol was incubated for 30 minutes in dark. After incubation, discoloration was measured at 517 nm. Ascorbic acid was used as a positive control. The percentage inhibition was calculated using the following formula,

$$\textbf{\% inhibition} = [(A_0 - A_1)/A_0] \times 100$$

Where, A_0 is the absorbance of the control. A_1 is the absorbance of the extract.

Reducing Power assay

The reducing power of the extract was determined according to the method of Oyaizu [22]. Different concentrations of plant extract and standard BHA solutions were mixed with 2.5 ml of 0.2M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was then incubated at 50°C for 20 min. Following incubation, 2.5 ml of 10% trichloro acetic acid was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. After centrifugation, 2.5ml upper layer solution was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1 % $FeCl_3$ solution. The absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated the increased reducing power.

Alpha amylase inhibitory assay

The α-amylase inhibitory activity of the extract was evaluated using the method of Bernfeld [23]. Briefly, 1mL of different concentrations of the extract (100-500µg/ml) was pre-incubated with α-amylase 1U/mL for 30 min and there after 1 mL of starch solution was added. The mixture was further incubated at 37°C for 10 min. Then the reaction was stopped by adding 1 mL Dinitrosalicylic acid reagent and the contents were heated in a boiling water bath for 5 min. The absorbance of the mixture was measured at 540 nm. A control was prepared without plant extract. The reducing sugar released from starch was measured as maltose equivalent from a standard graph. Acarbose was used as positive control. Anti-diabetic activity was expressed as percentage of inhibition and was calculated using the following formula,

$$\% \text{ inhibition} = [(Ac - Ae)/Ac] \times 100$$

Where Ac is the absorbance of the control and Ae is the absorbance of the extract.

Statistical analysis

All the analyses were carried out in triplicate and the results were expressed in mean± SD.

RESULTS AND DISCUSSION

Phytochemical Screening and Determination of the total phenolic content

The performed qualitative phytochemical studies of the extract showed the presence of flavonoids, steroids , saponins and tannins (Table 1). Determination of total phenolic contents revealed that the extract showed 150.3 mg/g of phenolic compounds. The phenolic concentration of the extract was expressed as milligram of gallic acid equivalents per gram of extract. Phenols are very important plant constituents because of their free radical scavenging ability due to their hydroxyl groups [24]. It has been reported that phenolic compounds are associated with antioxidant activity and play a crucial role in stabilizing lipid peroxidation [25]. Consumption of polyphenolic compounds up to 1g daily from diet has remarkable inhibitory effects on mutagenesis and carcinogenesis in humans [26]. The result of the present work strongly suggests that phenolic compounds are important components of this plant and some of their pharmacological effects could be attributed to the presence of these valuable constituents.

DPPH radical scavenging assay

It is a dire need to search effective antioxidants from natural sources as alternatives to synthetic antioxidant in order to prevent the free radicals implicated diseases which can have serious effects on the cardiovascular system [27, 28]. In the present investigation, the strong free radical scavenging activity of the extract was confirmed in a DPPH assay. DPPH is relatively stable nitrogen centered free radical that easily accepts an electron or hydrogen radical to become a stable diamagnetic molecule [29]. The methanolic extract showed the stronger scavenging effect with IC_{50} value of 40.5µg /ml which is comparable to standard antioxidant ascorbic acid. The ascorbic acid showed IC_{50} value of 12µg/ml. The free radical scavenging activity was found to increase with increasing concentration of the extract (Table 2). Hydrogen-donating ability of the antioxidant molecule contributes to its free radical scavenging nature [30].

Reducing power assay

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity [31]. The present study clearly showed the reductive capabilities of the methanolic fruit extract (Table 3). The reducing power of the extract increased with increasing concentration and is comparable with the standard antioxidant butylated hydroxyl anisole (BHA). The reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action through breaking the free radical chain by donating a hydrogen atom [32].

Alpha amylase inhibitory assay

Oxidative stress plays a critical role in the development of diabetes complications. Free radicals are formed disproportionately during diabetes due to glucose oxidation and the subsequent oxidative degradation of glycated proteins [33]. Plants have long been used to treat diabetes, as their principal bioactive components showed good anti-diabetic and anti-oxidant properties [34]. The present study clearly indicated that the extract exhibited good alpha amylase inhibitory activity in a dose dependent manner which is comparable to a standard drug acarbose. Extract showed highest inhibitory activity of 72.06% with an IC_{50} value of 22.8 µg/ml. The standard drug acarbose showed IC_{50} value of 12.7 µg/ml (Table 4).

In conclusion, the results of the present study clearly indicated that methanolic fruit extract of *Phyllanthus acidus* showed good antioxidant and alpha amylase inhibitory activity. The extract can be used as easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry. The therapeutic potentiality of the fruit could be exploited in the management of post prandial hyperglycemia in treatment of Type 2 diabetes mellitus.

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Table 1: Showing phytochemical constituents of the methanolic fruit extract of *Phyllanthus acidus*

Carbohydrates	Terpenoids	Saponins	Steroids	Alkaloids	Glycosides	Tanins	Flavanoids
--	--	+	+	--	--	+	+

+ indicates presence of constituents
-- indicates absence of constituents

Table 2: DPPH radical scavenging activity of the methanolic fruit extract of *Phyllanthus acidus* and Ascorbic acid

Concentration of extract and ascorbic acid (µg)	% inhibition of methanolic extract	% inhibition of ascorbic acid
20	31 ± 1.2	85.4 ± 1.2
40	42 ± 2.6	93.0 ± 1.2
60	61 ± 0.9	97.0 ± 0.9
80	72 ± 1.6	98.4 ± 1.5
100	86 ± 1.2	98.8 ± 1.7

Values are shown in mean ± SE

Table 3: Reducing power activity of the methanolic fruit extract of *Phyllanthus acidus* and BHA

Concentration of extract and BHA (µg)	Reducing property (absorbance) of methanolic extract	Reducing property (absorbance) of ascorbic acid
20	0.22 ± 0.04	0.52 ± 0.03
40	0.51 ± 0.06	0.86 ± 0.05
60	0.87 ± 0.03	1.17 ± 0.08
80	0.96 ± 0.2	1.52 ± 0.3
100	1.06 ± 0.08	1.72 ± 0.2

Values are shown in mean ± SE

Table 4: Alpha amylase inhibitory activity of *Phyllanthus acidus* methanolic fruit extract and acarbose

S. No.	Concentration of extract and acarbose(µg)	% of inhibition of extract	% of inhibition of acarbose
1	20	42.70 ± 0.32	63.6 ± 0.51
2	40	51.60 ± 0.62	71.7 ± 0.62
3	60	60.51 ± 0.42	79.4 ± 0.6
4	80	66.12 ± 0.17	81.4 ± 0.41
5	100	72.06 ± 0.08	92.31 ± 0.72

Values are shown in mean ± SE

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