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Alpha-amylase and alpha-glucosidase inhibitory property and antioxidant potential of *Polyalthia longifolia* bark extracts

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ABSTRACT

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and it is one of the free radical mediated ailments. Among the various anti-diabetic therapeutic approaches one major strategy is reducing gastrointestinal absorption of glucose by inhibition of carbohydrate metabolizing enzymes alpha-amylase and alpha-glucosidase In the present study the aqueous extracts of *Polyalthia longifolia* bark has been assessed for the inhibition of alpha-amylase and alpha-glucosidase and total antioxidant potential (FRAP assay). *In vitro* antioxidant activity of the ethanol extract (polar solvent) and hexane extract (non-polar solvent) has been assessed using various antioxidant model systems, *viz* DPPH, ABTS, NO, OH, SO and inhibition of *in vitro* lipid peroxidation. The aqueous extract of the bark exhibits highest alpha-amylase and alpha-glucosidase inhibition. Ethanol extract of *Polyalthia longifolia* is found to possess highest DPPH, ABTS, nitric oxide, hydroxyl, superoxide scavenging activity at lower concentration compared to the hexane extract. Also the ethanolic extract of *Polyalthia longifolia* is found to be good inhibitor of *in vitro* lipid peroxidation. Saturation in the enzyme inhibition and free radical scavenging activity has been attained in a concentration dependent manner. This study indicates significant alpha-amylase and alpha-glucosidase inhibition, free radical scavenging potential and lipid peroxidation inhibition of *Polyalthia longifolia* bark which can be exploited for the treatment of various free radical mediated diseases like diabetes mellitus. Studies are being done on the various solvent extracts of the bark for enzyme inhibition to isolate the active compounds with potent inhibition for alpha-amylase, alpha-glucosidase and antioxidant potential.

Key words: *Polyalthia longifolia,* Alpha-Amylase, Alpha-Glucosidase, Antioxidant Activity, Ethanol, Hexane, Bark.

1. INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder of multiple etiologies in which chronic hyperglycemia is caused by defects or alterations in either the secretion or action of insulin. This results in derangement of carbohydrate, fat and protein metabolism. Type 2 diabetes is caused either predominantly by insulin resistance with a relative deficiency of insulin or by impaired insulin secretion that may or may not be accompanied by insulin resistance and accounts for about 90%-95% of all diagnosed cases of diabetes in adults [1].

Oxidative stress is known to play a significant role in the development and progression of DM [2]. Excessive generation of free radicals and depleted levels of free radical scavenging enzymes have been demonstrated in animal models of diabetes [3-4]. Free radicals are formed disproportionately in diabetes by glucose oxidation, nonenzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins. Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes and also increase lipid peroxidation and beta-cell apoptotic pathways activation [5]. The primary oxygen derived free radicals are superoxide anion, hydroxyl, hydroperoxyl, peroxyl and alkoxyl radicals and non free radicals are hydrogen peroxide, hypochlorous acid, and ozone and singlet oxygen. Antioxidants are compounds which even at a low concentration are able to offer protection against the oxidative stress by scavenging the free radicals, inhibiting the lipid peroxidation and by other mechanisms thereby reducing the damage by neutralizing the free radicals and thus prevent disease [6-7].

Recent reports suggest that a major strategy in antidiabetic therapeutic approach is to decrease post-prandial hyperglycaemia. This can

be achieved through the inhibition of carbohydrate hydrolyzing enzymes such as alpha-amylase and alpha-glucosidase [8]. Alpha amylase and glucosidase inhibitors are used for developing new target drugs for the treatment of diabetes, obesity and hyperlipidaemia [9]. Phytoantioxidants from food and medicinal sources are gaining importance because of their safety and availability. Plants have long been used for the treatment of diabetes, particularly in developing countries where most people have limited resources and do not have access to modern treatment. Due to the possible importance of the amylase and glucosidase inhibitors in animal and human nutrition, extensive research has been conducted on their properties and biological effects [10].

Polyalthia is a large genus of shrubs and trees found in tropic and sub-tropic regions. It belongs to the family of Annonaceae. *Polyalthia longifolia* is commonly known as the mast tree [11]. It is a small medium-sized tree with linear-lanceolate leaves, 1 to 1.5 cm broad, occurring in Sri Lanka and now grown in tropical parts of India on road side and garden for their beautiful appearance. It is found to have a unique place in the traditional medicinal practice of Indian ayurvedic system and has been proved to be successful in the cure of various diseases like cancer, ulcer, inflammation, and hepatotoxicity. Researchers have reported that it possesses flavonoids, alkaloids, sesquiterpenes, diterpenes, saponins, quercetin, bulbocapnin, a-sitosterol, stigmasterol campest, enihalimane diterpenes, and sesquiterpenoid [12]. Similarly, the ethanolic extract of *Polyalthia longifolia* leaves have been shown to possess potent nitric oxide radical scavenging activity [13]. The diterpenes, alkaloids, steroid and miscellaneous lactones were isolated from its bark. The stem bark extracts and isolated compounds were studied for various biological activities like antibacterial, cytotoxicity and antifungal activity [13-14].

Studying the plants and plant parts for alpha-glucosidase and alpha-amylase inhibition as well as antioxidant potential becomes important in choosing good candidature plants for preclinical studies on antidiabetic property. Hence the present study was considered to evaluate the extent of alpha-amylase and alpha-glucosidase inhibition and *in vitro* antioxidant property of *Polyalthia longifolia* bark for its potential use in prevention and treatment of diabetes mellitus using various *in vitro* assays.

2. MATERIALS AND METHODS

2.1 Plant Collection

The bark of *Polyalthia longifolia* was collected from Institute of Forest Genetics and Tree Breeding, Coimbatore and identified by the botanist from Botanical Survey of India, Tamil Nadu Agricultural University campus, Coimbatore, Tamil Nadu, India. The Voucher specimen is preserved in Department of Biochemistry, Avinasilingam University, Coimbatore for future reference.

2.2. Chemicals

Alpha-amylase, Alpha-glucosidase, p-nitro phenyl alpha-D glucopyranoside, DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS (2,2-azinobis(3-ethyl benzothiazoline-6-sulfonic acid) were purchased from Sigma Aldrich company Ltd. The standards curcumin and ascorbic acid were procured from High media. All other chemicals and solvents used in this study are of analytical grade.

2.3. Technological processes

2.3.1. Preparation of plant extracts

The bark of *Polyalthia longifolia* was shade dried, ground to a fine powder and stored at room temperature. The dried bark was crushed into a coarse powder using a mechanical grinder. The bark powder was extracted in various solvents, *viz* aqueous, ethanol and hexane.

2.3.1.1. Aqueous extract

One part of the powdered bark was macerated in five parts of distilled water, kept in a boiling water bath for half an hour. The extract was centrifuged at 10,000 rpm for ten minutes and the supernatant was used for analysis (PLA).

2.3.1.1. Ethanol extract

One part of the powdered bark was macerated in five parts of ethanol, kept in shaker for 48 hours at 40°C. Filtered and collected the solvent. The solvent was evaporated to obtain the ethanol extract of *Polyalthia longifolia* (PLE).

2.3.1.2. Hexane extract

One part of the powdered bark was macerated in five parts of hexane, kept in shaker for 48 hours at 40°C. Filtered and collected the solvent. The solvent was evaporated to obtain the hexane extract of *Polyalthia longifolia* (PLH)

2.4. Analytical methods

2.4.1. Alpha-amylase inhibition assay

α-amylase inhibitory property of the aqueous extracts were determined by the method of Bernfield [15] with slight modifications as described below. In brief 100 µl of the extract was allowed to react with 200 µl of porcine pancreatic α-amylase enzyme (Sigma-Aldrich 3176) and

100 µl of 200 mM phosphate buffer (pH-6.9). After 20 min of incubation 100 µl of 1% starch was added. The same was performed for the control where 200 µl of enzyme was replaced by the buffer. The enzyme standard was prepared by dissolving 1 mg of porcine pancreatic α-amylase enzyme in 10 ml of phosphate buffer (pH 6.9). After incubation for 5 minutes 500 µl of DNS was added to both the control and test. The tubes were kept in a boiling water bath for 10 minutes. The absorbance was recorded at 540 nm using a spectrophotometer and the percentage of α-amylase inhibition was calculated using the formula

Inhibition (%) = 100 (Absorbance $_{Control}$ - Absorbance $_{Test}$ / Absorbance $_{Control}$)

Suitable reagent blank and inhibitor controls were also carried out and subtracted. Dose dependent variation in the α-amylase inhibition was measured using 25 µl to 200 µl of the aqueous extracts.

2.4.2 Alpha-glucosidase inhibition assay

The α-glucosidase inhibitory activity was determined by measuring the release of 4-nitrophenol from paranitrophenyl α-D glucopyranoside [16]. The assay mixtures for these experiments contained 0.3 ml of 10mM para-nitrophenyl α-D-glucopyranoside, 1.0 ml of potassium phosphate (0.1M, pH: 6.8), 0.2 ml of enzyme solution and 0.2 ml of inhibitor extract , all in a final volume of 1.7 ml. Following an incubation time of 30 min at 37°C, the reaction was terminated by the addition of 2.0 ml of 100 mM sodium carbonate. The liberated p-nitrophenol was determined at 400 nm using spectrophotometer. The % inhibition rates were calculated using the formula, Inhibition (%) = 100 (Absorbance $_{Control}$ - Absorbance $_{Test}$ / Absorbance $_{Control}$). Suitable reagent blank and inhibitor controls were also carried out and subtracted. Dose dependent variation in the α-glucosidase inhibition was measured using 25 µl to 200 µl of the aqueous extracts.

2.4.3 Total antioxidant activity

The total antioxidant activity of the extracts of *Polyalthia longifolia* viz PLE and PLH was assayed by FRAP method (Ferric Reduction Antioxidant Power) [17]. The reaction mixture containing different volumes of extracts made up to a final volume of 1.5 ml to which 1.5 ml of FRAP reagent was added and the absorbance measured at 593 nm in 1cm light path at 37°C. A standard solution of ascorbic acid was tested in parallel.

2.4.4 Total phenol content

The total phenol content was determined with Folin-Ciocalteu reagent using pyrocatechol as the standard. To 0.1 ml of ethanolic extract of *Polyalthia longifolia* added 0.5 ml of diluted Folin Ciocalteau reagent followed by 2.0 ml of 20% Na_2CO_3 solution after 30 min and mixed thoroughly. The tubes were placed in a boiling water bath for exactly 1 minute. Cooled and measured the absorbance at 650 nm. From the standard graph calculated the amount of polyphenols and expressed as mg of phenols per g of the sample [18].

2.4.5 Free radical scavenging activity

A stock solution of 1mg/ml of *Polyalthia longifolia* ethanol extract (PLE), hexane extract (PLH) was prepared. This was diluted to get various concentrations (20-100 µg/ml) in the final volume of reaction mixture. PLE was dissolved in ethanol, while PLH was dissolved in Dimethyl Sulfoxide (DMSO). The free radical scavenging activity of the extracts were analyzed by following the various standard *in vitro* radical generating model systems *viz.*,1,1-diphenyl-2-picryl hydrazyl (DPPH), 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), superoxide anion (SO), hydroxyl (OH) and nitric oxide (NO). Inhibition of *in vitro* lipid peroxidation was studied using goat liver as a model system. In all the experiments deionized water served as blank and reaction mixtures without extracts served as control samples. The percentage scavenging (or) inhibition was calculated according to the formula

Percentage scavenging (or) Inhibition = (C-T/C) × 100

Where C is the absorbance of control and T is the absorbance of test. All the experiments were performed in triplicates and the mean values were taken for results.

2.4.5.1. DPPH scavenging activity

The effect of PLE and PLH on DPPH scavenging activity was estimated as per the following method [19]. Aliquots containing different concentrations (20-100 µg) of ethanol extract (PLE); hexane extract (PLH) were made up to 1 ml. To this 2 ml of DPPH (0.1 mM) was added. In the control, 2 ml of DPPH and 1 ml of distilled water was added. All the tubes were incubated at 37°C for 20 min. Absorbance of reaction mixtures was recorded at 517 nm.

2.4.5.2. ABTS radical decolourization assay

The ABTS stock reagent mixture was prepared by mixing 88µl of 140 mM potassium persulphate with 5 ml of 7 mM ABTS stock solution (pH 7.4). The working ABTS reagent was prepared by diluting the stock solution with ethanol to give an absorbance of 0.7 ± 0.5 at

734 nm. Aliquots containing different concentrations (20-100 µg) of ethanol extract (PLE), hexane extract were taken separately and the final volume was made up to 1ml with distilled water. One ml of ABTS cation working solution was added to the tubes and ABTS solution with equal amount of distilled water serves as control. The reaction mixtures were incubated at 28ºC for 30 min. Absorbance was measured at 734 nm. The effect of PLE and PLH on ABTS scavenging activity was determined [20].

2.4.5.3. Nitric oxide scavenging activity

Aliquots containing different concentrations (20-100 µg) of PLE and PLH were taken in different tubes. One ml of sodium nitroprusside (10 mM) was added to various tubes containing different extracts and the volume was made up to 500 µl using distilled water. Tubes were incubated at room temperature for 2.5 hrs. To the reaction mixture, 1 ml of greiss reagent (prepared by mixing an equal volume of 1% sulphanilamide in 2% orthophosphoric acid with 0.1% N-(napthyl) ethylene diamine hydrochloride in water) was added. One ml sodium nitroprusside and 500 µl of distilled water without the extracts served as control. Absorbance was recorded at 546 nm. Curcumin was used as a reference compound. The nitric oxide scavenging potential of the PLE and PLH extracts was assayed [21].

2.4.5.6. Super oxide anion scavenging activity

Aliquots containing different concentrations (20-100 µg) of PLE and PLH were taken in different tubes. To the extracts added 1 ml of nitroblue tetrazolium solution (156 µM in 100 mM phosphate buffer pH 7.4) and 1 ml of NADH solution (468 µM in 100 mM phosphate buffer pH 7.4). The volume was made up with distilled water and reaction started by adding 100µl of phenazine methosulphate solution (60 µM in 100 mM phosphate buffer pH 7.4). The reaction mixture was incubated at 25ºC for 5 min. Curcumin was used as a reference compound. Absorbance was measured at 560 nm and the superoxide anion scavenging activity of PLE and PLH extracts was calculated [22].

2.4.5.7 Hydroxyl radical scavenging activity

Aliquots containing different concentrations (20-100 µg) of PLE and PLH were taken in different tubes. The reaction mixture finally contains 1 ml of phosphate buffer, 100 µl of 1mM EDTA, 100 µl of 20 mM hydrogen peroxide, 100 µl of 2-deoxyribose (30 mM), 100 µl of 1mM ferric chloride and 100 µl ascorbic acid (1mM). The tubes were incubated at 37ºC for 30 min. Added 1 ml of 2.8% trichloroacetic acid followed by 1 ml of 1% thiobarbaturic acid to the tubes. Tubes were heated in a water bath maintained at 75ºC for 30 min and cooled. Absorbance was measured at 534 nm and the PLE and PLH extracts was assessed for hydroxyl radical scavenging activity [23].

2.4.5.8 Extent of inhibition of *in vitro* lipid peroxidation

Aliquots containing different concentrations (20-100 µg) of PLE and PLH were taken in different tubes. To 50 µl of 5% goat liver homogenate, extracts were added. Added 50 µl of ferrous sulphate to induce oxidation and the final volume was made up to 500 µl with cold TBS (10 mM Tris, 0.15 M sodium chloride pH 7.4). Control was prepared for each sample, containing respective extract (150 µl), and liver homogenate (50 µl) and made up to a final volume of 500 µl with cold TBS. A blank was set containing no plant extract, no liver homogenate but only ferrous sulphate and TBS. The final volume was made up to 500 µl with distilled water. A medium corresponding to 100% oxidation was prepared by adding all constituents except the plant extracts and volume was made up to 500 µl. The experimental medium corresponding to auto oxidation contained only liver homogenate and TBS made up to final volume. All tubes were incubated at 37ºC for 1 hour. After that 500 µl of 70% alcohol was added to stop the reaction. One ml of 1% TBA was added to all the tubes, followed by boiling in a hot water bath for 20 min. After cooling the tubes were centrifuged. To the clear supernatants collected *in toto* added 500 µl of acetone. Thio barbituric acid reactive substance (TBARS) was measured at 535 nm and the inhibition of *in vitro* lipid peroxidation was calculated [24].

3. RESULTS AND DISCUSSION

Pancreatic and intestinal glucosidases are the key enzymes of dietary carbohydrate digestion and inhibitors of these enzymes may be effective in retarding glucose absorption [25]. This is because only monosaccharides are readily taken up from the intestine and all other carbohydrates have to be broken-down enzymatically before they can be absorbed [26].

The aqueous extract of *Polyalthia longifolia* bark exhibited very good inhibition of the enzymes α- amylase and α- glucosidase with an inhibition of 96.4% and 94.2% at a volume of 200 µl. The inhibition was found to increase upon increasing the volume and it reached saturation after 200 µl. α-amylase and α-glucosidase inhibition of *Polyalthia longifolia* bark aqueous extracts and standard acarbose is shown in Fig.1a, Fig.1b, Fig.2a and Fig 2b.

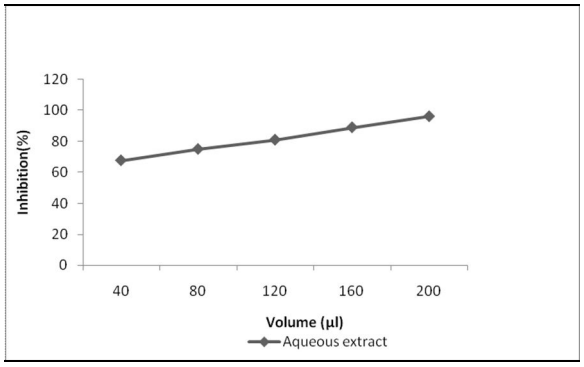

Fig 1a: Alpha amylase inhibition by *Polyalthia longifolia* bark extract

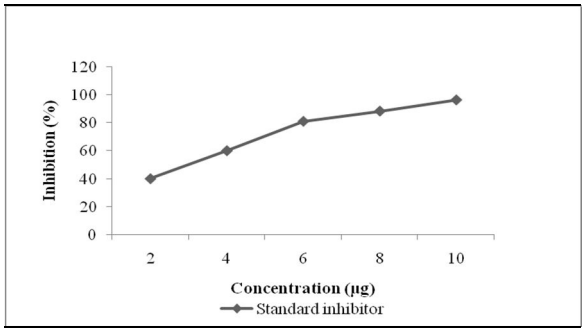

Fig 1b: Alpha glucosidase inhibition by *Polyalthia longifolia* bark extract

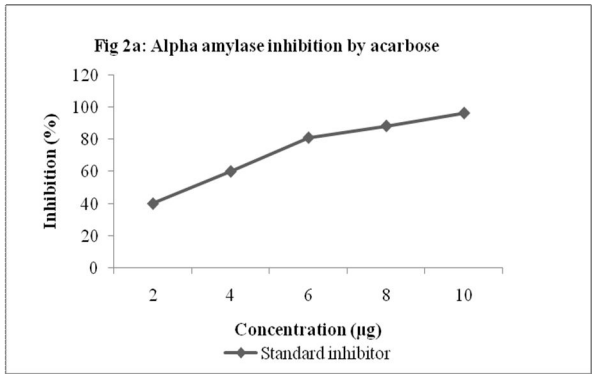

Fig 2a: Alpha amylase inhibition by acarbose

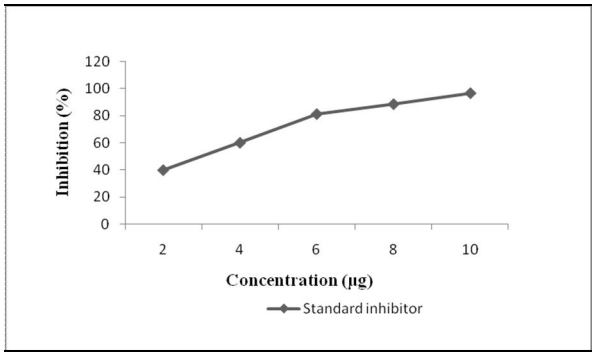

Fig 2b: Alpha glucosidase inhibition by acarbose

Free radicals are chemical entities that can exist separately with one or more unpaired electrons. The generation of free radicals can bring about thousands of reactions and thus cause extensive tissue damage. Lipids, proteins and DNA are all susceptible to attack by free radicals [27]. Antioxidants may offer resistance against oxidative stress by scavenging the free radicals or protecting the antioxidant defense mechanisms. Antioxidant effectiveness is measured by monitoring the inhibition of oxidation of a suitable substrate. Various methods are available for determining free radical scavenging effects.

The bark of *P.longifolia* extracted in various solvents *viz* aqueous, ethanol and hexane was tested for its antioxidant potential using FRAP assay. Free radical scavenging activities of *P.longifolia* bark was tested using various models *viz* DPPH, ABTS, NO, OH, SO and inhibition of *in vitro* lipid peroxidation.

The transformation of Fe^{3+} into Fe^{2+} in the presence of various fractions was measured to determine the reducing power ability. The reducing ability of a compound generally depends on the presence of reductones, which exert the antioxidant activity by breaking the free radical chain by donating a hydrogen atom [28]. The antioxidant potential of the bark of *P.longifolia* is expressed as µg equivalence of ascorbic acid. 1g of *P.longifolia* extract was found to be equivalent to 870 µg of ascorbic acid in terms of antioxidant activity (Table 1). The antioxidant principles present in *P.longifolia* caused the reduction of Fe^{3+} / ferricyanide complex to the ferrous form, and thus proved the reducing power ability.

The 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical is widely used as a model system to investigate the free radical scavenging activities of several plant extracts [29]. DPPH is a stable nitrogen-centred free radical the colour of which changes from violet to yellow upon reduction by either the process of hydrogen- or electron-donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers [30]. The reduction in the number of DPPH molecules can be calculated with the number of available hydroxyl groups. The scavenging activity of PLE and PLH extract were found to increase in a concentration dependent manner (Fig.3). PLE extract exhibited the scavenging activity of 93.4% at a concentration of 160 µg/ml. All the extracts attained saturation in the scavenging activity with further increase in the concentration. However PLH extract exhibited a maximum of 23.0 % scavenging of DPPH radical at a concentration of 120 µg/ml. PLE extract was found to be very good scavenger of the stable DPPH free radical in comparison with standard ascorbic acid. The

DPPH and ABTS free radical scavenging activity by ascorbic acid is shown in Figures 4a and 4b.

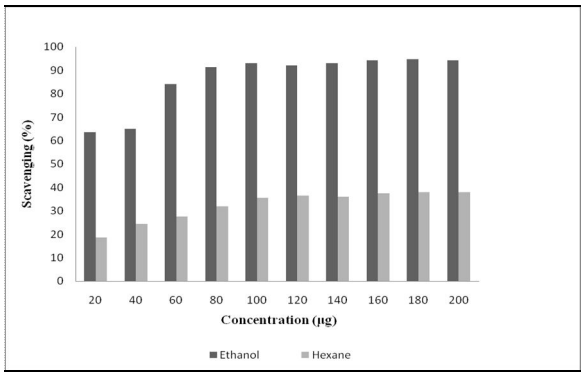

Fig 3: DPPH free radical scavenging activity of *Polyalthia longifolia* bark extracts

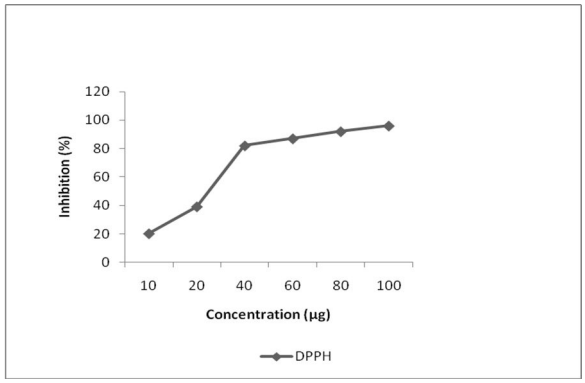

Fig 4a: DPPH scavenging activity of ascorbic acid

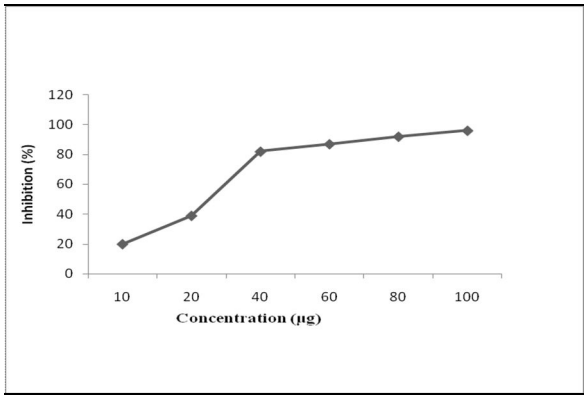

Fig 4b: ABTS scavenging activity of ascorbic acid

The ABTS assay is based on the inhibition of the absorbance of the radical cation $ABTS^{+}$, which has a characteristic long wavelength absorption spectrum. The ABTS radical scavenging activities of *P.longifolia* extract is depicted in Fig.5. The scavenging activity of PLE and PLH extract were found to increase in a concentration dependent manner. PLE showed a maximum scavenging of 95.0 % at a concentration of 160 µg/ml. PLH showed a maximum of 38% scavenging activity at a concentration of 180 µg/ml. The scavenging activity attained saturation with further increase in the concentration of extracts. The results imply that PLE was found to be potent inhibitor and scavenger of the ABTS radical compared to the PLH extract.

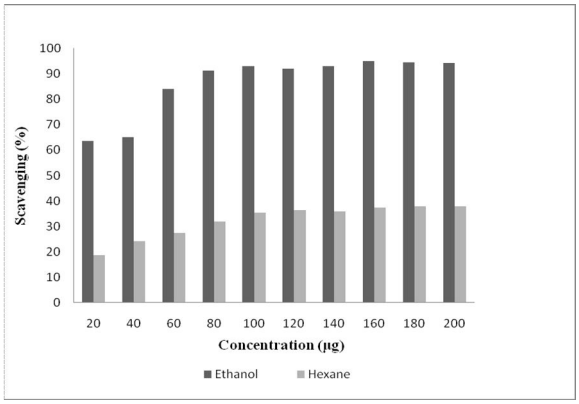

Fig 5: ABTS free radical scavenging activity of *Polyalthia longifolia* bark extracts

Nitric oxide is a free radical involved in the regulation of various physiological processes [31]. Excess concentration of NO is associated with several diseases. *In vitro* inhibition of nitric oxide radical is a measure of antioxidant activity of plant drugs. Scavenging of nitric oxide radical is based on the generation of nitric oxide from sodium nitroprusside in buffered saline, which reacts with oxygen to produce nitrite ions that can be measured by using Griess reagent [32]. The absorbance of the chromophore is measured at 546 nm in the presence of the extracts. The nitric oxide scavenging activities of the extracts of *P.longifolia* are presented in Fig.6. The scavenging activity of PLE and PLH were found to increase in a concentration dependent manner. PLE showed a scavenging activity of 85% at 140 µg/ml PLH recorded scavenging activity of 26.0% at 180 µg/ml concentration attaining saturation with further increase in the concentration of extracts. Ethanolic extract of *P.longifolia* decreased the amount of nitrite generated from the decomposition of sodium nitroprusside *in vitro*. This may be due to the antioxidant principles in the extracts which compete with oxygen to react with NO, thereby inhibiting the generation of nitrite ions. Curcumin was used as a reference compound. The IC_{50} values of *P.longifolia* extract PLE was found to be 21 µg respectively, whereas the IC_{50} values of curcumin was 20.4.

The hydroxyl radical scavenging activity is measured as the percentage of inhibition of hydroxyl radicals generated in the Fenton's reaction mixture by studying the competition between deoxyribose and the extract for hydrogen radicals generated from Fe^{3+}/ EDTA/ H_2O_2 systems. The hydroxyl radicals attack deoxy

ribose which eventually results in TBARS formation. The NO, SO, OH radical scavenging activity of standard curcumin is presented in Fig 7a. The hydroxyl radical scavenging activity of PLE and PLH of *P.longifolia* was found to increase in a concentration dependent manner (Fig.7b). PLE recorded the highest scavenging activity of 88.0 % at 50 µg/ml while PLH recorded the scavenging activity of 42% at 100 µg/ml. The extracts attained saturation in the scavenging activity with further increase in concentration. PLE was found to be very good scavenger of hydroxyl radical in comparison with standard curcumin [33].

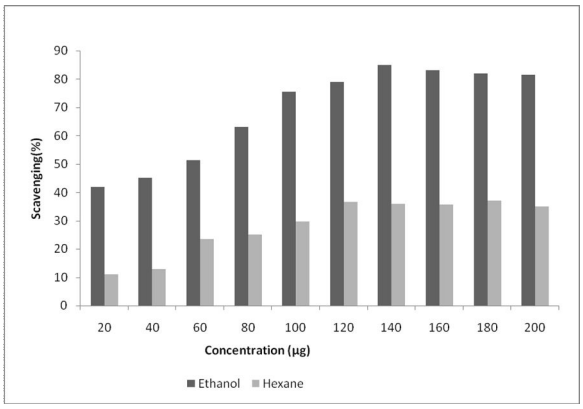

Fig 6: Nitric oxide scavenging activity of *Polyalthia longifolia* bark extracts

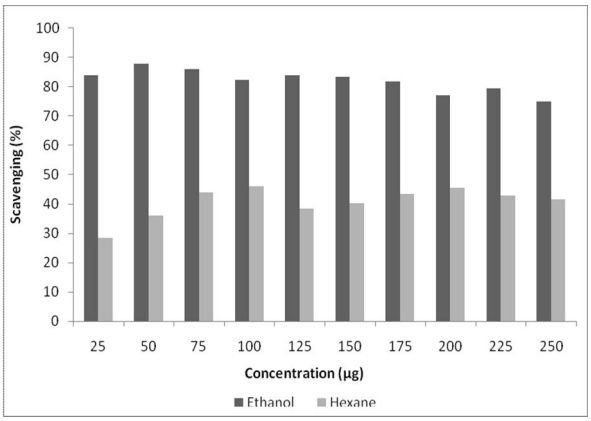

Fig 7a: Hydroxyl radical scavenging activity of *Polyalthia longifolia* bark extracts

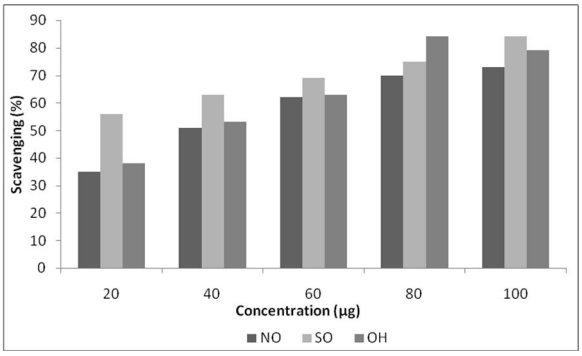

Fig 7b: NO, SO and OH radical scavenging activity of curcumin

Superoxides are produced from molecular oxygen due to oxidative enzymes [34] of body as well as via non-enzymatic reaction such as antioxidation by catecholamines [35]. The decrease in absorbance at 560 nm with antioxidants thus indicates the consumption of superoxide anion in the reaction mixture. The superoxide scavenging activity of PLE and PLH was found to increase in a concentration dependent manner (Fig.8). PLE showed maximum scavenging of 82.0% at a concentration of 140 µg/ml and PLH reported 28.3% scavenging activity at a concentration of 160 µg/ml.

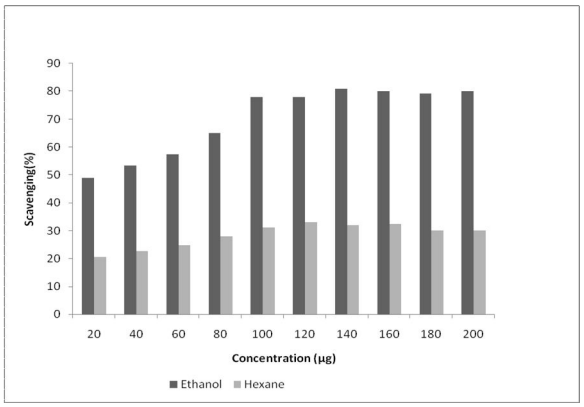

Fig 8: Super oxide scavenging activity of *Polyalthia longifolia* bark extracts

PLE was found to be very good scavenger of superoxide radical in comparison with standard curcumin. The IC_{50} values of *P.longifolia* extract PLE on superoxide radical scavenging activity was found to be 15 µg respectively, and that of curcumin was 6.0. The results indicate that the extracts of *P.longifolia* have a potent scavenging activity with increasing percentage inhibition. The probable mechanism of scavenging the superoxide anions may be due to the inhibitory effect of the extract towards generation of superoxides in the *in vitro* reaction mixture.

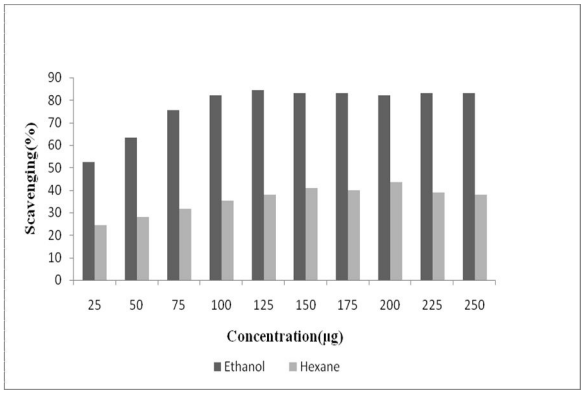

Fig 9: Inhibition of *in vitro* lipid peroxidation by *Polyalthia longifolia* bark extracts

Lipid peroxidation is the oxidative degeneration of polyunsaturated fatty acids and

involves formation of lipid radicals leading to membrane damage. Free radicals induce lipid peroxidation in poly unsaturated lipid rich areas like brain and liver [36]. Initiation of lipid peroxidation by ferrous sulphate takes place through hydroxyl radical by Fenton's reaction. The inhibition could be caused by absence of ferryl-perferryl complex or by scavenging the hydroxyl radical on the superoxide radicals or by changing the Fe^{3+}/ Fe^{2+} or by reducing the rate of conversion of ferrous to ferric or by chelating the iron itself. The Inhibition of *in vitro* lipid peroxidation of PLE and PLH was found to increase in a concentration dependent manner (Fig.9). *In vitro* lipid peroxidation was inhibited at a level of 83.7% at 175 µg/ml by PLE and 43% at 200 µg/ml by PLH. PLE was found to be good inhibitor of *in vitro* lipid peroxidation. The IC_{50} values for DPPH, ABTS, NO, SO, OH radical scavenging and inhibition of *in vitro* lipid peroxidation are presented in Table 1.

Table-1: IC_{50} values (µg/ml) for antioxidant activity of *Polyalthia longifolia* bark extract, ascorbic acid and curcumin

Extract/Standard	DPPH	ABTS	NO	SO	OH	LPO
PLE	18.5	15.7	21	17	23	74
Ascorbic acid	22	21	-	-	-	-
Curcumin	-	-	20.4	16	36	-

Values are mean of triplicates

Phenolics are diverse secondary metabolites possessing ideal structural chemistry for free radical scavenging activity, and they have been shown to be more effective antioxidants *in vitro* than tocopherols and ascorbate. Plant polyphenols are known to have multifunctional properties such as ability to act as reducing agents, and singlet oxygen quenchers, in addition to their hydrogen donating properties [37]. The total phenol content of bark of *P.longifolia* was estimated as 13.5 mg/g (Table 2). Natural phenolics exert their beneficial health effects mainly through their antioxidant activity by decreasing oxygen concentration, intercepting singlet oxygen, preventing first-chain initiation by scavenging initial radicals such as hydroxyl radicals, binding metal ion catalysts, decomposing primary products of oxidation to non-radical species, and breaking chains to prevent continued hydrogen abstraction from substances. Another mechanism underlying the antioxidative properties of phenolics is the ability of flavanoids to alter peroxidation kinetics by modification of the lipid packing order and to decrease fluidity of membranes. These changes could sterically hinder diffusion of free radicals and restrict peroxidative reactions. Moreover, it has been shown recently that phenolic compounds can be involved in the hydrogen peroxide scavenging cascade in plant cells [38-39].

Table-2: Total antioxidant potential and total phenol content of *Polyalthia longifolia* bark

Total antioxidant potential[a] Ascorbic acid equivalents	Phenol content[b](mg/g)
870±4.5	13.5±0.5

Values are mean ± SD of triplicates
Sample: a - Aqueous extract (5g / 25 ml distilled water),
b - Ethanol extract
*Table shows conversion of 1g of sample extract

Medicinal plants are known to contain a variety of antioxidants. Numerous substances have been suggested to appear as antioxidants. It has been revealed that various phenolic antioxidants such as flavanoids, tannins, coumarins, xanthones and more recently procyanidins scavenge radicals dose dependently, thus they are viewed as promising therapeutic drugs for free radical pathologies [7].

The results of the present study demonstrates the α-amylase and α- glucosidase inhibitory proerty and antioxidant potential of *P.longifolia* bark which can be exploited for the treatment of free radical mediated ailments like diabetes mellitus.

4. CONCLUSION

In summary, the bark of *P.longifolia* may be considered as good source of α-amylase and α-glucosidase inhibitors and natural antioxidants for diabetes mellitus and other free radical mediated ailments. Further investigation on the isolation, purification and identification of antioxidative and antidiabetic principles, testing the *in vivo* antidiabetic property by pre-clinical studies is being performed in our laboratory. This will certainly help to identify the possible mechanism of action and the potency of the active compounds.

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