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Radical scavenging activity of *Saraca indica* bark extracts and its inhibitory effect on the enzymes of carbohydrate metabolism

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ABSTRACT

Total antioxidant potential of various solvent extracts of Saraca indica bark (Water, Ethanol, Ethyl acetate, Hexane and Chloroform) were assessed by FRAP assay. Ethanol extract and hexane extract showed highest antioxidant potential when compared to other extracts. *In vitro* radical scavenging properties of hexane and ethanol extract have been investigated using various model systems viz., DPPH, ABTS, NO, SO, OH and inhibition of *in vitro* lipid peroxidation. Ethanol extract is found to possess highest DPPH, ABTS, nitricoxide, superoxide and hydroxyl radical scavenging activities and inhibition of in vitro lipid peroxidation when compared to the hexane extracts. Ethanol extracts exhibited good inhibition on the enzymes alpha-amylase and alpha-glucosidase compared to hexane extracts. Enzyme inhibition and radical scavenging was found to occur in a concentration dependent manner. This study indicates significant alpha amylase and alpha-glucosidase inhibition and antioxidant potential of *Saraca indica* bark which can be exploited for the treatment of various free radical mediated ailments like diabetes mellitus. This is the first study reporting the *in vitro* alpha-amylase, alpha-glucosidase inhibitory potential of *Saraca indica* bark. Studies are being done to isolate the potent compounds with enzyme inhibitory property, study the mechanism of inhibition and antioxidant potential.

Keywords: *Saraca indica*, alpha-amylase, alpha-glucosidase, antioxidant, chloroform, hexane.

1. INTRODUCTION

Free radicals are unstable molecules with unpaired or odd electrons. Free radicals are highly reactive and they attack molecules by capturing electrons and thus modifying chemical structure and thereby causing damage [1]. Free radicals play an important role in the pathogenesis of chronic degenerative diseases including cancer, diabetes, autoimmunity, inflammatory, cardiovascular, neurodegenerative diseases and aging [2].

Antioxidants are substances that may protect cells from the oxidative damage caused by unstable molecules known as free radicals. Antioxidants are substances which scavenge these free radicals and combat the damage. Studying the antioxidant properties of medicinal plants have become most important part of research for the choice of good candidatures for pre-clinical studies in the areas of diabetes mellitus and cancer [3]. Inhibition of carbohydrate metabolizing enzymes viz., alpha-amylase and alpha-glucosidase helps in reducing the gastrointestinal absorption of glucose thereby becoming important for the management of diabetes mellitus which is a metabolic disorder associated with derangement of carbohydrate, fat and protein metabolism. It is characterized by hyperglycemia due to the defects in insulin secretion, insulin action, or both. Alpha amylase and glucosidase inhibitors are used for developing new target drugs for the treatment of diabetes, obesity and hyperlipidaemia [4].

Drugs from plant sources are being used by about 80% of the world population. Herbal medicines have stood the test of time for their safety, efficacy, acceptability and lesser side effects. They are used since time immemorial and have long history of being used for the treatment of disorders like diabetes, particularly in developing countries in traditional and alternative system of medicine [5]. Scientific investigation of these plants is needed for proving their effect.

Saraca indica L. (Family: Leguminosae) is a medium sized evergreen tree up to 9 m in height with numerous spreading and drooping glabrous branches. It is referred commonly as Asoka. The bark of the tree is dark brown to grey or black; flowers are fragrant, numerous, and orange or red color; leaves are pinnate, 15-25 cm long having 4-6 pairs of oblong-lanceolate leaflets. The plant is popularly known as Asok or Asoka (Hindi, Oriya, Bengali, Gujrati, Assamese, Marathi and Punjabi), Ashokadamara (Kannada), Asogam (Tamil), Asokam (Malayalam), Ashokapatta (Telugu) [6]. *Saraca indica* is one of the important indigenous medicinal plants and found throughout India. Bark of the plant is bitter and traditionally used as astringent, anthelmintic, demulcent, biliousness, colic, piles, ulcers, fractures, menorrhagia, metropathy, dyspepsia, visceromegaly. Stem bark of *Saraca indica* is astringent, antileucorrhoeic, antibilious and uterine sedative; Chemical investigation found the presence of different catechols, sterols, tannins, flavonoids, glycosides, leucopelargonidin and leucocyanidin. The antidiabetic, oxytocic, anticancer, peptic ulcer, antimicrobial, antibacterial and antioxidant activities of the plant have been reported [7, 8].

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. Recent reports suggest that one of the therapeutic approaches for decreasing post-prandial hyperglycaemia is to prevent absorption of glucose by the inhibition of carbohydrate-hydrolysing enzymes, such as alpha-glucosidase and alpha-amylase [9]. Oral hypoglycemic agents / drugs may be effective for glycemic control, but they come with their attendant side effects such as liver disorders, flatulence, abdominal pain, renal tumours, hepatic injury, acute hepatitis, abdominal fullness and diarrhea [4, 9 & 10]. Therefore, there is an increasing need for the development of a natural and safe product without side effects. Research on the properties and effect of amylase and glucosidase inhibitors from plants is gaining interest as it is one of the possible mechanisms for maintaining glucose homeostasis [11]. 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 extensively studying about the antidiabetic property using animal models. Hence the present study was considered to evaluate the extent of alpha-amylase and alpha-glucosidase inhibition and *in vitro* antioxidant property of *Saraca indica* bark for its potential use in prevention and treatment of diabetes mellitus using various *in vitro* assays and various methods for determining free radical scavenging effects by monitoring the inhibition of oxidation of a suitable substrate.

2. MATERIALS AND METHODS

2.1 Plant collection

The bark of *Saraca indica* was collected from Central Institute for Medicinal Plant Heritage (CIMH), identified and authenticated by the botanist from Botanical Survey of India, Tamil Nadu Agricultural University campus, Coimbatore, Tamil Nadu, India.

2.2 Chemicals

Curcumin and ascorbic acid were procured from High media. DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS (2,2-azinobis (3-ethyl benzothiazoline-6-sulfonic acid) were purchased from Sigma Aldrich company Ltd. 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 *Saraca indica* was shade dried, ground to a fine powder and stored at room temperature. The dried bark was crushed using a mechanical grinder into a coarse powder and stored in air tight containers. The bark powder was extracted in various solvents, *viz* aqueous, ethylacetate, ethanol, hexane and chloroform.

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 lyophilized to get the dry form of the extract (SIA) and calculated the yield percentage.

2.3.1.2. Ethyl acetate extract

One part of the powdered bark was macerated in five parts of ethyl acetate, kept in shaker for 48 hours at 40°C. Filtered and collected the solvent. The solvent was evaporated to obtain the ethanol extract of *Saraca indica* (SIEA) and calculated the yield percentage.

2.3.1.3. 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 *Saraca indica* (SIE) and calculated the yield percentage.

2.3.1.4. 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 *Saraca indica* (SIH) and calculated the yield percentage.

2.3.1.5. Chloroform extract

One part of the powdered bark was macerated in five parts of chloroform, kept in shaker for 48 hours at 40°C. Filtered and collected the solvent. The solvent was evaporated to obtain the chloroform extract of *Saraca indica* (SIC) and calculated the yield percentage.

2.4. Analytical methods

2.4.1. Total antioxidant activity

The total antioxidant activity of various extracts of *Saraca indica* viz SIA, SIEA, SIE, SIH, SIC were assayed by FRAP method (Ferric Reduction Antioxidant Power) [12]. 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 37ºC. A standard solution of ascorbic acid was tested in parallel.

2.4.2. Alpha-amylase inhibitor assay

α-amylase inhibitory properties of various solvent extracts were determined by the method of Bernfield [13] with slight modifications as described below. The working enzyme solution was prepared by dissolving 1 mg of porcine pancreatic α-amylase enzyme in 10 ml of phosphate buffer (pH 6.9). 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. 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 different concentration (25- 200 µg) of the various solvent extracts.

2.4.3 Alpha-glucosidase inhibitor assay

The α-glucosidase inhibitory activity was determined in the most potent extracts with good antioxidant potential and alpha-amylase inhibition by measuring the release of 4-nitrophenol from paranitrophenyl α-D glucopyranoside [14]. The assay mixtures for these experiments contained 0.3 ml of 10 mM paranitrophenyl 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 different concentration (25- 200 µg) of the solvent extracts.

2.4.4 Total phenol content

The total phenol content was determined with Folin-Ciocalteau reagent using pyrocatechol as the standard. To 0.1 ml of ethanolic extract of *Saraca indica* added 0.5 ml of diluted Folin Ciocalteau reagent followed by 2.0 ml of 20% Na_2CO_3 solution after 10 min. Mixed thoroughly and measured the absorbance at 660 nm after 30 min. From the standard graph calculated the amount of polyphenols and expressed as mg of phenols per g of the sample [15].

2.4.5 Free radical scavenging activity

A stock solution of 1 mg / ml of *Saraca indica* ethanol extract (SIE), hexane extract (SIH) was prepared. This was diluted to get various concentrations (20-100 µg/ml) in the final volume of reaction mixture. SIE was dissolved in ethanol, while SIH 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 linolenic acid micelles 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 SIE and SIH on DPPH scavenging activity was estimated as per the following method [16]. Aliquots containing different concentrations (20-100 µg) of ethanol extract (SIE); hexane extract (SIH) 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 37ºC 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 88 µl 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 (SIE), hexane extract (SIH) 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 SIE and SIH on ABTS scavenging activity was determined [17].

2.4.5.3. Nitric oxide scavenging activity

Aliquots containing different concentrations (20-100 µg) of SIE and SIH were taken in different tubes. One ml of sodium nitro prusside (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 SIE and SIH extracts was assayed [18].

2.4.5.4. Super oxide anion scavenging activity

Aliquots containing different concentrations (20-100 µg) of SIE and SIH 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 100 µl 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 SIE and SIH extracts was calculated [19].

2.4.5.5. Hydroxyl radical scavenging activity

Aliquots containing different concentrations (20-100 µg) of SIE and SIH 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 37º C 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 75º C for 30 min and cooled. Absorbance was measured at 534 nm and SIE and SIH extracts were assessed for hydroxyl radical scavenging activity [20].

2.4.5.6. Extent of inhibition of *in vitro* lipid peroxidation

Aliquots containing different concentrations (20-100 µg) of SIE and SIH were taken in different tubes. To 50 µl of 5% 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 [21].

3. RESULTS AND DISCUSSION

The yield percentage of the various solvent extracts of *Saraca indica* is expressed in Table 1. The yield percentage was highest for *Saraca indica* ethanolic extract.

Table – 1: Percentage yield of *Saraca indica* extracts.

Extract	Yield %
SIA	4.0
SIE	8.84
SIEA	1.8
SIC	0.96
SIH	0.38

Values are mean of triplicates

A comparison of alpha-amylase and alpha-glucosidase inhibition of *Saraca indica* extracts is shown in Table 2.

Table – 2: Alpha-amylase inhibition and alpha-glucosidase inhibition of *Saraca indica* bark extracts.

Extract	Alpha-amylase inhibition (%)	Alpha-glucosidase inhibition (%)
SIA	82	80
SIE	95	92
SIEA	85	83
SIC	53	51
SIH	61	62

Values are mean of triplicates

Saraca indica ethanol extracts followed by aqueous and ethyl acetate extracts showed very good inhibition of the enzymes alpha-amylase and alpha-glucosidase. SIE exhibited very good inhibition of the enzymes α- amylase and α-glucosidase with an inhibition of 95.0% and 92.0% at a concentration of 120 µg. The inhibition was found to increase upon increasing the concentration and it reached saturation after 200 µg. α-amylase and α-glucosidase inhibition by standard acarbose is shown in Fig 1.

Alpha-amylase and α-glucosidase inhibitory activity of some of the traditional plant parts used for the treatment of diabetes like *Syzigium cumini* seed extracts and *Psidium guajava* leaves [22], crude ethanol extract of *Cissus arnottianai* [23], *Polyalthia longifolia* bark [24] have been reported. This might be one of the possible reasons for their hypoglycemic activity. Search for low molecular weight α-glucosidase inhibitors in medicinal plants has become important for inhibiting absorption of intestinal glucose at the brush border.

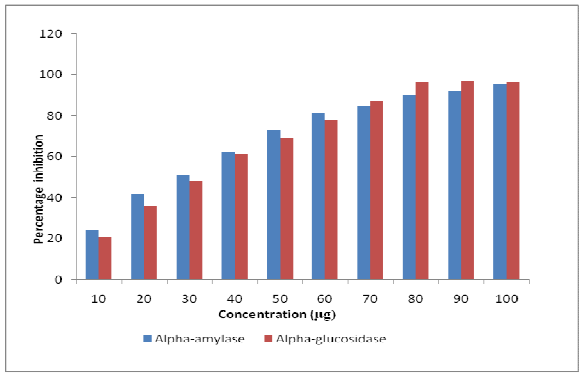

Figure - 1: α-amylase and α-glucosidase inhibition by acarbose.

Free radicals are chemical entities which exist separately with one or more unpaired electrons and are unstable. The generation of free radicals can result in chain of reactions and thus cause damage to lipids, proteins and DNA resulting in extensive tissue damage. Antioxidants prevent oxidative stress by scavenging the free radicals or protecting the antioxidant defense mechanisms [25]. The bark of *S.indica* extracted in various solvents *viz* aqueous, ethylacetate, ethanol, hexane and chloroform was tested for its antioxidant potential using FRAP assay. 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. The antioxidant potential of the bark of *S.indica* is expressed as mg equivalents of ascorbic acid. The results of FRAP assay revealed that the total antioxidant potential of ethanolic extract was found to be highest among all the extracts tested. Ethanolic extract from 5 g of *S.indica* bark was found to be equivalent to 120 mg of ascorbic acid in terms of antioxidant activity (Table 3). The antioxidant principles present in *S.indica* caused the reduction of Fe^{3+} / ferricyanide complex to the ferrous form, and thus proved the reducing power ability.

Free radical scavenging activities of *S.indica* ethanol and hexane bark extracts was tested using various models *viz* DPPH, ABTS, NO, OH, SO and inhibition of *in vitro* lipid peroxidation. 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. DPPH is stable, nitrogen-centered free radical which produces violet colour in ethanol solution. It was reduced to a yellow coloured product, diphenyl picryl hydrazine, with

the addition of the extracts. The reduction in the number of DPPH molecules can be calculated with the number of available hydroxyl groups. The scavenging activity of SIE and SIH extract were found to increase in a concentration dependent manner (Fig.2). SIE extract exhibited the scavenging activity of 93 % at a concentration of 40 µg/ml. All the extracts attained saturation in the scavenging activity with further increase in the concentration. However SIH extract exhibited a maximum of 31.0 % scavenging of DPPH radical at a concentration of 200 µg/ml. SIE extract was found to be very good scavenger of the stable DPPH free radical in comparison with standard ascorbic acid as reported earlier [26]. The DPPH and ABTS free radical scavenging activity by ascorbic acid is shown in Figure 3.

Table - 3: Total antioxidant potential of *Saraca indica* bark extracts.

Extract	Total antioxidant potential * (mg ascorbic acid equivalents)
SIA	85.5 ± 2.4
SIEA	15.0 ± 1.3
SIE	120.0 ± 4.8
SIC	2.43 ± 0.32
SIH	3.10 ± 0.24

Values are mean ± SD of triplicates

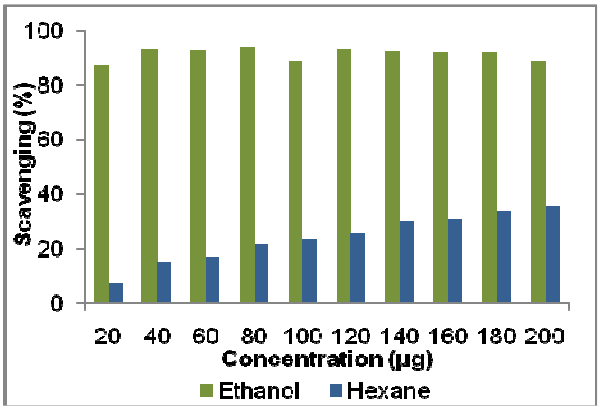

Figure - 2: DPPH radical scavenging activity of SIE and SIH.

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 *S.indica* extract is depicted in Fig.4. The scavenging activity of SIE and SIH extract were found to increase in a concentration dependent manner. SIE showed a maximum scavenging of 92.0 % at a concentration of 40 µg/ml. SIH showed a maximum of 44 % scavenging activity at a concentration of 120 µg/ml. The scavenging activity attained saturation with further increase in the concentration of extracts. The results imply that SIE was found to be potent inhibitor and scavenger of the ABTS radical compared to the SIH extract.

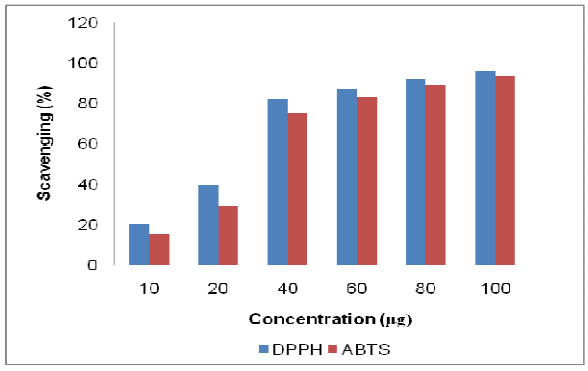

Figure - 3: DPPH and ABTS radical scavenging activity of ascorbic acid.

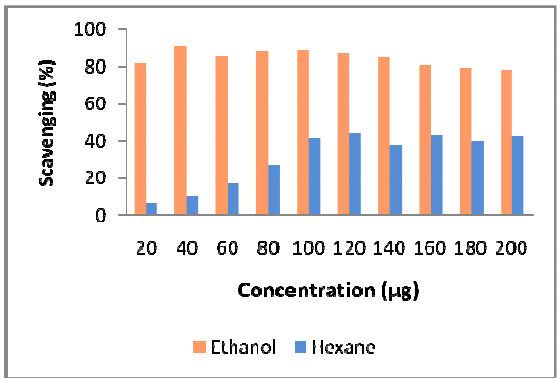

Figure - 4: ABTS free radical scavenging activity of SIE and SIH.

Nitric oxide is a free radical generated by endothelial cells, macrophages, neurons etc and involved in the regulation of various physiological processes [27]. 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 [28]. 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 *S.indica* are presented in Fig.5. The scavenging activity of SIE and SIH were found to increase in a concentration dependent manner. SIE showed a scavenging activity of 84% at 80 µg/ml while SIH recorded scavenging activity of 46.0% at 160 µg/ml concentration attaining saturation with further increase in the concentration of extracts. Ethanolic extract of *S.indica* 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.

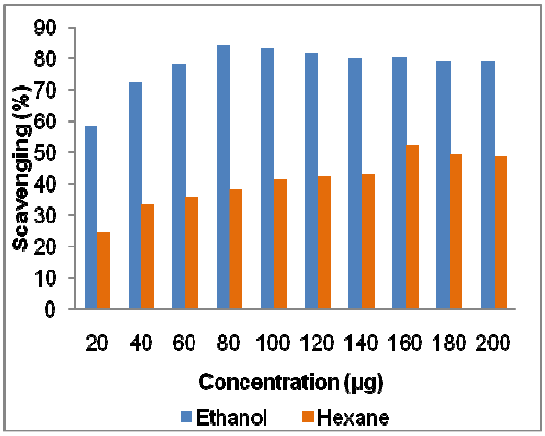

Figure - 5: NO free radical scavenging activity of SIE and SIH.

Superoxides are produced from molecular oxygen due to oxidative enzymes [29] of body as well as via non-enzymatic reaction such as antioxidation by catecholamines [30]. 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 SIE and SIH was found to increase in a concentration dependent manner (Fig.6). SIE showed maximum scavenging of 82.0% at a concentration of 140 µg/ml and SIH reported 31.0 % scavenging activity at a concentration of 60 µg/ml.

SIE was found to be very good scavenger of superoxide radical in comparison with standard curcumin. The results indicate that the extracts of *S.indica* 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.

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 [31]. The NO, SO, OH radical scavenging activity of standard curcumin is presented in Fig 7a. The hydroxyl radical scavenging activity of SIE and SIH of *S.indica* was found to increase in a concentration dependent manner (Fig.7b). SIE recorded the highest scavenging in activity of 91.0 % at 75 µg/ml while SIH recorded the scavenging activity of 43% at 250 µg/ml. The extracts attained saturation in the scavenging activity with further increase in concentration. SIE was found to be very good scavenger of hydroxyl radical in comparison with standard curcumin.

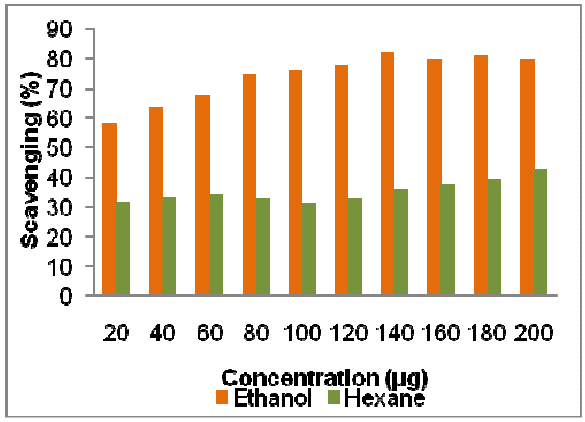

Figure - 6: Super oxide scavenging activity of SIE and SIH.

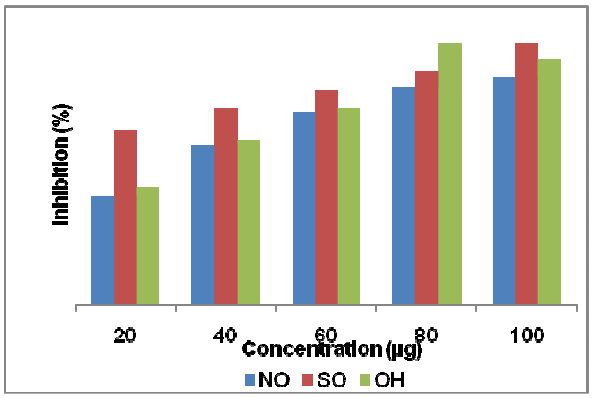

Figure - 7a: NO, SO and OH radical scavenging effect of curcumin.

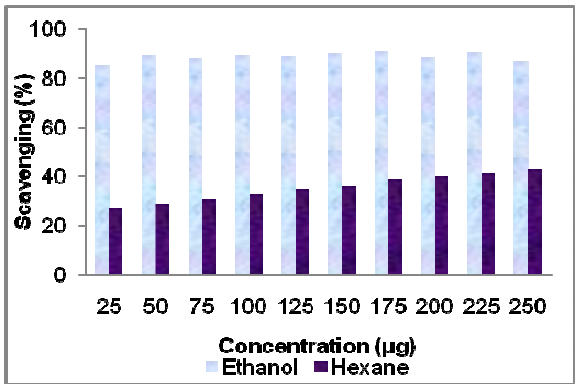

Figure - 7b: Hydroxyl radical scavenging activity of SIE and SIH.

Lipid peroxidation is the oxidative degeneration of polyunsaturated fatty acids and

Table - 4: The IC_{50} values of SIE, ascorbic acid and curcumin for DPPH, ABTS, NO, SO, OH radical scavenging and inhibition of *in vitro* lipid peroxidation

Extract /Standard	DPPH	ABTS	NO	SO	OH	*In vitro* lipid peroxidation
SIE	13.6	12.5	17	14.3	18.6	17.6
Ascorbic acid	22.0	21.0	-	-	-	-
Curcumin	-	-	20.4	6.5	36.0	-

involves formation of lipid radicals leading to membrane damage. Free radicals induce lipid peroxidation in polyunsaturated lipid rich areas like brain and liver [32]. 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 SIE and SIH was found to increase in a concentration dependent manner (Fig.8). *In vitro* lipid peroxidation was inhibited at a level of 85.0% at100 µg/ml by SIE and 43% at 175 µg/ml by SIH. SIE was found to be good inhibitor of *in vitro* lipid peroxidation.

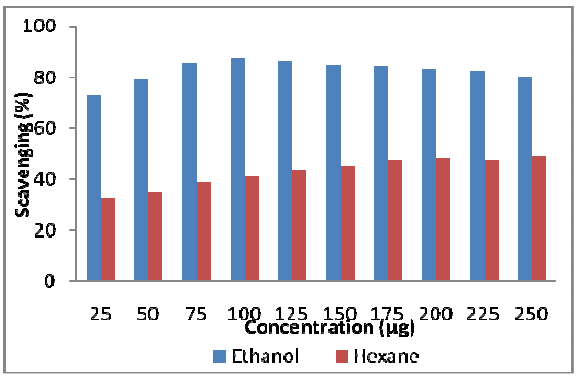

Figure - 8: Inhibition of *in vitro* lipid peroxidation by SIE and SIH.

The IC_{50} values for DPPH, ABTS, NO, SO, OH radical scavenging activity and inhibition of *in vitro* lipid peroxidation by SIE and standard ascorbic acid, curcumin are presented in Table 4.

Phenolics are diverse secondary metabolites abundant in plant tissues. Polyphenols possess ideal structural chemistry for free radical scavenging activity, and they have been shown to be more effective antioxidants *in vitro* than tocopherols and ascorbate. The total phenol content of bark of *S.indica* was estimated as 17.4 mg/g. Antioxidative properties of polyphenols arise from their high reactivity as hydrogen or electron donors, from the ability of the polyphenol derived radical to stabilize and delocalize the unpaired electron (chain breaking function) and from their ability to chelate transition metal ions (termination of the Fenton reaction) [33]. These changes could sterically hinder diffusion of free radicals and restrict peroxidative reactions.

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 [34].

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

4. CONCLUSION

In summary, the bark of *S.indica* 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 constituents, testing the *in vitro* and *in vivo* antidiabetic property using cell-lines and 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 compound.

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