

# Alpha amylase and Alpha glucosidase inhibitory effects of aqueous stem extract of *Salacia oblonga* and its GC-MS analysis

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Our present investigation deals with the phytochemical screening, estimation of total flavonoids, terpenoids and tannin contents to evaluate the anti-diabetic activities of *Salacia oblonga* stem followed by GC-MS analysis. It explores the natural compounds and the potential  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory actions of stem extracts. The aqueous stem extract was selected from other extracts (ethanol, acetone, petroleum ether and chloroform) for the *in vitro* study of anti-diabetic activity by alpha amylase and alpha glucosidase inhibitory assays. The stem extract was also analyzed by gas chromatography mass spectrometry to identify the natural chemical components. Phytochemical analysis of aqueous stem extract showed major classes of secondary metabolites such as phenols, flavonoids, alkaloids, terpenoids, tannins, saponins. The total flavonoid, terpenoid, and tannin contents were quantified as 19.82±0.06 mg QE/g, 96.2±0.20 mg/g and 11.25±0.03 mg TAE/g respectively. The percentage inhibition of assays showed maximum inhibitory effects (59.46±0.04% and 68.51±0.01%) at a concentration of 100 mg/mL. The IC<sub>50</sub> values of stem extract was found to be 73.56 mg/mL and 80.90 mg/mL for alpha amylase and alpha glucosidase inhibition. Fifteen chemical constituents were found by GC-MS analysis. This study suggest the aqueous stem extract of *Salacia oblonga* might be considered as potential source of bio active constituents with excellent antidiabetic activity.

**Keywords:** *Salacia oblonga*. Antidiabetic.  $\alpha$ -Amylase.  $\alpha$ -Glucosidase. Terpenoids.

## INTRODUCTION

Floral species were got an important place in the pharmaceuticals, because of their therapeutic efficacy. This is due to the availability of potentially active natural chemical constituents. Natural products are secondary metabolites which are produced by plants through metabolic pathway. These phytochemical compounds have the capacity to cure fever, inflammation, cancer, cardiac disease, neurological disease, obesity, asthma, ulcer, urinary infections and viral disease (Sharma, Sharma, 2014). Diabetes mellitus (DM) is one of the non-communicable life threatening diseases. These are non-infectious and non-transmissible. This is largely due to physical inactivity, unhealthy diets, obesity, raised blood cholesterol and glucose (Singh, Kumar, 2015). DM is a chronic disease, characterized

by metabolic disorder associated with high blood sugar (Pallavi *et al.*, 2015). It occurs due to the pancreas does not produce enough insulin or when the body cannot use the insulin effectively (Arumugam, Manjula, Paari, 2013). It can be classified into four types (Type 1 diabetes mellitus, Type 2 diabetes mellitus, Gestational diabetes mellitus and Non-classical causes of DM) on the basis of the pathogenic process which leads to hyperglycemia (Powers, 2011). Type 1 diabetes mellitus is known as insulin dependent DM. It is a catabolic disorder caused by an auto immune reaction, resulting in selective beta cells destruction which produces severe insulin deficiency due to the inability of beta cells to respond any insulinogenic stimuli. Nowadays children or young adults are suffered very much. Type 2 diabetes mellitus is previously known as non-insulin dependent DM, a heterogeneous group of disorders characterized by insulin resistance, where the cells in the body do not respond to insulin (Mamun-or-Rashid *et al.*, 2014). Gestational diabetes occurs during pregnancy period. This is due to the resistance of insulin by placenta and placental

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hormones. Non classical causes of DM can be either genetic or acquired. DM is one of the main killers within the next 25 years. The number of people from worldwide multiplies with diabetes. Thus it is a global concern until the successive treatment is discovered. Nowadays many synthetic medicines are discovered, but they can produce many side effects (Ranjith, Rajasree, Sankar, 2012) Hence it is required for the search of herbal treatment with low cost and no side effects. Natural herbal medicines were investigated to control the glucose production or absorption.

Plants play vital role in the medicinal field, because of its healing capacity. Phytochemical screening is the major tool to bring about the wonderful potential of plants. Plant metabolites such as alkaloids, terpenoids, phenols, flavonoids, tannin, saponin were responsible for potential activities (Nitty, 2016). Flavonoids might be responsible for multi potential activities. The seasonal change of flavonoids may alter the anti-diabetic activity of herbal plants. Nowadays so many potentially active anti-diabetic herbal plants were identified by researchers (Stalin, Vivekanandan, Bhavya, 2013; Arumugam, Gunasekaran, Perumal, 2014). But, the growing population creates demand for herbal medicines. This is due to cheap and has no side effects. The present study was carried out to investigate the anti-diabetic herbal activity of aqueous stem extract of *S. oblonga* and the identification of natural chemical constituents which were responsible for the bioactivity. Gas Chromatography Mass Spectroscopy is most commonly used method for the identification of new phytochemical compounds and for the quantification purpose. In this method the availability of unknown organic compounds can be found by matching the spectra with reference spectra.

*Salacia oblonga* (family: Clasteraceae) is a traditional herbal plant widely available in Srilanka, tropical regions of Africa and southern regions of India. It is a woody climber, commonly known as ekanayaka, saptrangi and ponkoranti. A wide variety of species of *Salacia* grow in India (Ramamoorthy *et al.*, 2010). It is rich in antioxidants, secondary metabolites like alkaloids, terpenoids, flavonoids, steroids, tannins, saponins and phenolic compounds. It is reported that the root part is mainly used for the treatment madhumeha, the ancient name of diabetes. It is also used for curing rheumatism, skin disease, gonorrhoea and fever. The nutrient content present in the *S. oblonga* can be used as a liver tonic. It has very good antimicrobial and anti-inflammatory activities (Ismail *et al.*, 1997). The root extract was used as a source for the treatment of diabetes (Sujata, Mamta, Rachana, 2013). The natural chemical constituents of *Salacia* were used to prevent sugars in food from being

absorbed by the body. It inhibits the breakdown of glucose and also prevents the deposition of extra fat in our body. Our present study reveals the qualitative and quantitative measurement of potential natural constituents and quantification flavonoids, terpenoids and tannins to investigate the efficacy of *in vitro* anti-diabetic activity of the aqueous stem extract of *S. oblonga*.

## MATERIAL AND METHODS

### Chemicals

Ethanol, acetone, ethanol, methanol, petroleum ether, chloroform, aluminium chloride, dinitrosalicylic acid, sodium potassium tartrate,  $\alpha$ -amylase, dimethyl sulfoxide, *p*-nitrophenyl- $\alpha$ -glucopyranoside,  $\alpha$ -glucosidase, and other chemicals used for the analysis were received from HIMEDIA laboratory, Mumbai, India.

### Collection and identification of plant material

The healthy plants of *S. oblonga* were collected from different regions of Western Ghats (India). The collected plants were authenticated by Dr. Vijaya Kumar, Associate professor, Department of Botany, S.T. Hindu College, Nagercoil, 629002. The stem was washed thoroughly by tap water to remove all impurities. Then it was cut and dried under shadow for four weeks. The dried stems were powdered by ball mills and maintained at Sathyabama University, Chennai-600 119, Tamil Nadu, India.

### Preparation of plant extract

Extraction of the plant samples was done according to the method (Boulekbache-Makhlouf, Slimani, Madani, 2012). About 1 g of dried fine powder (humidity 6.6%) of *S. oblonga* stem samples were extracted with 15 mL acetone, ethanol (75%), chloroform, petroleum ether and water for 1 min using an Ultra Turrax mixer (T10 basic, 13,000 rpm) and soaked overnight at room temperature. The sample was then filtered through Whatman No.1 filter paper in a Buchner funnel. The filtered solution was evaporated under vacuum in a rotaevaporator at 40 °C to a constant weight and then dissolved in respective solvents. The concentrated extracts were stored in airtight container in refrigerator below 10 °C.

### Phytochemical screening of *S. oblonga*

Screening of secondary metabolites of stem extracts of *S. oblonga* were determined by the standard method

(Priya, Chellaram, 2014). Phytochemical screening was carried out on the stem extracts using different solvents (aqueous, ethanol, acetone, petroleum ether and chloroform) to identify the major natural chemical groups such as tannins, saponins, flavonoids, phenols, alkaloids, terpenoids, glycosides, cardiac glycosides, coumarins and steroids. General reactions revealed the presence or absence of these compounds in the tested stem extracts.

## Qualitative analysis of phytochemicals

### Test for flavonoids

About 0.5 mL of aqueous stem extract of *S.oblonga* was shaken with pet ether to remove the fatty materials. The defatted residue was dissolved in 20 mL of 80% ethanol and filtered. Then 3 mL of the filtrate was mixed with 4 mL of 1% KOH. A dark yellow colour was observed, which indicates the presence of flavonoids.

### Test for saponins

About 0.5 mL of stem extract was dissolved in 2 mL of boiling water in a test tube, allowed to cool and shaken to mix thoroughly. Foam appeared indicating the presence of saponins.

### Test for alkaloids

About 0.5 mL of stem extract was mixed with about 8 mL of 1% HCl, warmed and filtered. Then 2 mL of filtrate was treated separately with Mayer's reagent. Turbidity was observed to indicate the presence of alkaloids.

### Test for tannins

About 0.5 mL of extract was boiled with 20 mL of distilled water in a test tube and then filtered. 0.1% FeCl<sub>3</sub> was added to the filtrate. Appearance of brownish green coloration showed the presence of tannins.

### Test for coumarins

About 0.5 mL of aqueous stem extract was taken in a small test tube and covered with filter paper moistened with 1 N NaOH. The test tube was placed for few minutes in boiling water. Then the filter paper was removed and examined in UV light for yellow fluorescence to indicate the presence of coumarins.

### Test for anthocyanin and betacyanin

To 2 mL of the stem extract, 1 mL of 2N sodium hydroxide was added and heated for 5 min at 100 °C. Formation of yellow colour indicates the presence of betacyanin.

### Test for glycosides

About 2 mL of stem extract was mixed with 3mL of chloroform and 10% ammonium solution was added. Formation of pink colour was not identified, which indicates the absence of glycosides.

### Test for cardiac glycosides

To 0.5 mL of the stem extract, 2 mL of glacial acetic acid and few drops of 5% ferric chloride were added. This under layered with 1 mL of concentrated sulphuric acid. Formation of brown ring at interface indicates the presence of cardiac glycosides.

### Test for terpenoids

To 0.5 mL of the stem extract, 2 mL of chloroform was added and concentrated Sulphuric acid was added carefully. Formation of red brown colour at the interface indicates the presence of terpenoids.

### Test for phenols

To 1 mL of the stem extract, 2 mL of distilled water followed by few drops of 10% ferric chloride was added. Formation of blue colour indicates the presence of phenols.

### Test for quinones

To 1 mL of the stem extract, 1 mL of concentrated sulphuric acid was added. Formation of red colour indicates the presence of quinones.

### Test for steroids

To 0.5 mL of the stem extract, 2 mL of chloroform and 1 mL of sulphuric acid were added. Formation of reddish brown ring at interface indicates the presence of steroids.

## Estimation of total flavonoid contents

Total flavonoid contents present in the aqueous stem extract was determined by the aluminum chloride colorimetric method (Mervat *et al.*, 2009). About 0.5 mL of stem extract of *S. oblonga* at a concentration of 1 mg/ mL was taken and the volume made up to 3 mL with methanol. Then 0.1 mL 10% AlCl<sub>3</sub>, 0.1 mL of potassium acetate and 2.8 mL distilled water were added sequentially. The test solution was vigorously shaken. The solution was incubated for 30 minutes. Absorbance was recorded by using UV-Visible spectrophotometer UV-2450 (Shimadzu) at 415 nm. A standard calibration plot was generated using known concentrations of quercetin. The concentrations of flavonoid in the test samples were calculated from the calibration plot and expressed as mg QE/g of sample.

### Estimation of total tannin contents

Tannin content present in the aqueous stem extract of *S. oblonga* was carried out by the standard method (Babu, Samundeeswari, Chitti Babu, 2015). One mL of aqueous stem extract was mixed with 0.5 mL of folin-ciocalteau's reagent, followed by the addition of (20%) sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) solution (1 mL) and distilled water (8 mL). The reaction mixture was allowed to stand for 30 min at room temperature. The supernatant was obtained by centrifugation using 6,000 rpm and absorbance was recorded at 725 nm using UV-Visible Spectrophotometer. Different concentrations of standard tannic acid were prepared and the absorbance of various tannic acid concentrations was plotted for a standard graph. The tannin content was expressed as mg TAE/g of the sample.

### Estimation of total terpenoids

Total terpenoid content in the aqueous stem extracts were measured by the method (Indumathi *et al.*, 2014). About 1 g of *S. oblonga* stem powder was taken separately and soaked in 15 mL of alcohol for 24 hours. Then it was filtered and the filtrate was extracted with petroleum ether, the ether extract was treated as total terpenoid. Total terpenoids were measured by the following formula,

$$\frac{(\text{Final weight of the sample} - \text{Initial weight of the sample})}{\text{Weight of the sample}} \times 100$$

### Alpha-amylase inhibitory assay

The alpha-amylase inhibitory activities of the stem extract of *S. oblonga* were carried out according to the standard method (Nickavar, Yousefian, 2009). The starch solution (0.5% w/v) used as the substrate was prepared by boiling potato starch in distilled water for 15 min. The enzyme solution was prepared by dissolving 1mg of porcine pancreatic alpha amylase in 20 mM phosphate buffer (100 mL, pH 6.9). The sample solutions were prepared in DMSO (dimethyl sulfoxide) in different concentrations (10 to 100 mg/mL). The DNS solution (20 ml 96 mM 3,5-dinitrosalicylic acid, 12 g sodium potassium tartrate in 8 ml of 2 M NaOH and 12 mL deionized water) was used as the colouring reagent of reaction. Three sets of experiments were conducted for test, blank and control. A mixture of 1 mL of each of the test and enzyme solutions, in a test tube was incubated at 25 °C for 30 min. Then, after taking out 1 mL from this mixture, 1 mL of the above-mentioned starch solution was added and the mixture was incubated at 25 °C for 3 min. Finally, 1 mL of the DNS

solution was added. The tube was then covered and heated in water bath at 85 °C for 15 min. After cooling the tube, the reaction mixture was diluted with distilled water (9 mL). It was mixed well and the absorbance was recorded at 540 nm. In case of blank, the DNS solution was added prior to the addition of the starch solution, while rest of the method was same as for the test. For control, all procedure was again the same except that plant extract was replaced by 1 mL of DMSO. Acarbose, a well-known anti-diabetic medicine, was used as a positive control. The percentage inhibition was calculated by the formula:

$$\% \text{ Inhibition} = [(A_c - A_s) / A_c] \times 100$$

$A_c$ -absorbance for control;  $A_s$ -absorbance for standard

### Alpha-glucosidase inhibitory assay

The  $\alpha$ -glucosidase inhibition was determined by the following modified methods (Matsui *et al.*, 1996; Bräunlich *et al.*, 2013). The  $\alpha$ -glucosidase reaction mixture contained 2.9 mM p-nitrophenyl- $\alpha$ -glucopyranoside (pNPG), varying concentrations (10 mg/mL to 100 mg/mL) of *S. oblonga* stem extract and 1.0 U/mL  $\alpha$ -glucosidase in sodium phosphate buffer, pH 6.9. Control tubes contained only DMSO, enzyme and substrate, while in positive controls acarbose replaced the sample extract. Mixtures without enzyme, sample extract and acarbose served as blanks. The reaction mixtures were incubated at 25 °C for 5 min, after which the reaction was stopped by boiling for 2 min. Absorbance of the resulting p-nitro phenol (pNP) was determined at 405 nm using spectrophotometer and was considered directly proportional to the activity of the enzyme. The  $\text{IC}_{50}$  values were determined from plots of percentage inhibition *versus* log inhibitor concentration and were calculated by non-linear regression analysis from the mean inhibitory values. Acarbose was used as the reference drug for alpha glucosidase inhibition assay. All the tests were performed by triplicates.

### Gas chromatography - mass spectrometry analysis

The stem extract of *S. oblonga* were subjected to GC-MS analysis. This method is used for the identification of biologically active natural chemical constituents. It was carried out by using GC-MS-5975C Agilent system composed of an auto sampler and a gas chromatograph interfaced with a mass spectrometer (GC-MS) instrument. It was worked with the following

conditions: column Elite-1 fused silica capillary column (300.25 mm ID×1EM df, composed of 100% dimethyl poly siloxane), operating in electron impact mode at 70 eV. A constant flow of 1.51 mL/min and an injection volume 1 µL of helium (99.999%) gas was used as carrier gas (split ratio 10:1). The temperature of injector and ion source were maintained at 240 °C and 200 °C respectively. The oven temperature was programmed from 70 °C (isothermal for 2min) with an increase of 10 °C per minute to 300 °C per minute, ending with a 9 minute isothermal at 300 °C. Mass spectra were taken at 70 eV, with a scan range 40-1000 m/z. Solvent cut time was 5 min; MS start time being 5 min; MS end time being 35min; Ion source temperature set to 200 °C and interface temperature being 240 °C.

### Identification of chemical constituents

The chemical compounds were identified by comparing the spectral data obtained on the Gas Chromatograph Mass Spectroscopy with the data base of National Institute Standard and Technology (NIST) having more than 62000 patterns. The name of the chemical component, molecular weight and the chemical structure of the stem extract of *S.oblonga* were identified.

### Statistical analysis

Each result was repeated three times and represented by Mean ± Standard Deviation. The statistical significance was evaluated by the analysis of variance (ANOVA)

followed by Duncan Multiple Range Test (DMRT) using SPSS ver.18.0. P value less than 0.01 is statistically significant.

### RESULTS

The preliminary phytochemical screening of stem extracts of *S.oblonga* revealed the presence of potentially bio active secondary metabolites. Among the different solvent extractions aqueous stem extract showed strong positive for the natural constituents such as phenols, flavonoids, tannins, saponins, alkaloids, terpenoids, steroids, cardiac glycosides and coumarins. The results were tabulated in Table I. Thus the aqueous stem extract was used for the quantification of phytochemicals. Flavonoids, terpenoids and tannins were responsible for the anti-diabetic activity. Total flavonoids, tannins and terpenoids were estimated as 19.82±0.06 mg QE/g, 96.2±0.20 mg/g, 11.25±0.03 mg TAE/g respectively (Table II).

*In vitro* anti-diabetic activity was carried out in the aqueous stem extract of *S. oblonga* by using alpha amylase and alpha glucosidase inhibitory assays. *S. oblonga* showed potential inhibitory effects on these enzymes. Acarbose, a synthetic drug has the ability to inhibit alpha amylase and alpha glucosidase enzymes. Thus acarbose act as a standard for both assays.

Different concentrations (10, 20, 40, 60, 80, 100 mg/mL) of aqueous stem extracts were subjected to alpha amylase inhibitory assay. The maximum percentage of

**TABLE I** - Qualitative phytochemical analysis of stem extracts of *S. oblonga*

Phytochemicals Tested	Solvent extractions of <i>Salacia oblonga</i> stem				
	Aqueous	Ethanol	chloroform	Petroleum ether	Acetone
Tannins	+	-	-	-	-
Saponins	++	++	-	+	+
Quinones	++	++	-	-	-
Terpenoids	+++	+	-	-	-
Steroids	+	+	-	+	-
Flavonoids	+++	+	+	+	-
Phenol	++	+	+	+	+
Alkaloids	+	+	-	-	-
Glycosides	-	-	-	-	-
Cardiac glycosides	+	-	-	+	-
Coumarins	++	+	-	-	-
Antho cyanin	-	-	-	-	-
Beta cyanin	+	+	-	-	-

+positive; ++strong positive; -negative

**TABLE II** - Quantification of Flavonoids, Tannins and Terpenoids

S. No	Phytochemicals	Quantity
1	Flavonoids (mg QE/g)	19.82±0.06
2	Tannins (mg TAE/g)	11.25±0.03
3	Terpenoids (mg/g)	96.2±0.20

mg QE/g- milli gram quercetin equivalent per gram; mg TAE/g- milligram tannic acid equivalent per gram; mg/g- milli gram per gram.

inhibition (59.46±0.04%) was obtained at a concentration of 100 mg/mL, where as the standard drug shown 74.03±0.03% at a concentration of 100 µg/mL. The IC<sub>50</sub> values were calculated for acarbose and stem extract as 64.95 µg/mL and 80.90 mg/mL respectively. The effective inhibition of alpha glucosidase, tested for the standard and aqueous stem extract of *S.oblonga*. Different concentrations (10, 20, 40, 60, 80, 100 mg/mL) of aqueous stem extracts were subjected to alpha glucosidase inhibitory assay. The maximum percentage of inhibition (68.51±0.01%) was obtained at a concentration of 100mg/mL of stem extract. The standard drug acarbose was shown maximum percentage of inhibition (84.15±0.03%) at a concentration of 100 µg/mL. The IC<sub>50</sub> values were calculated for acarbose (58.21 µg/mL) and stem extract of *S.oblonga* (73.56 mg/mL).

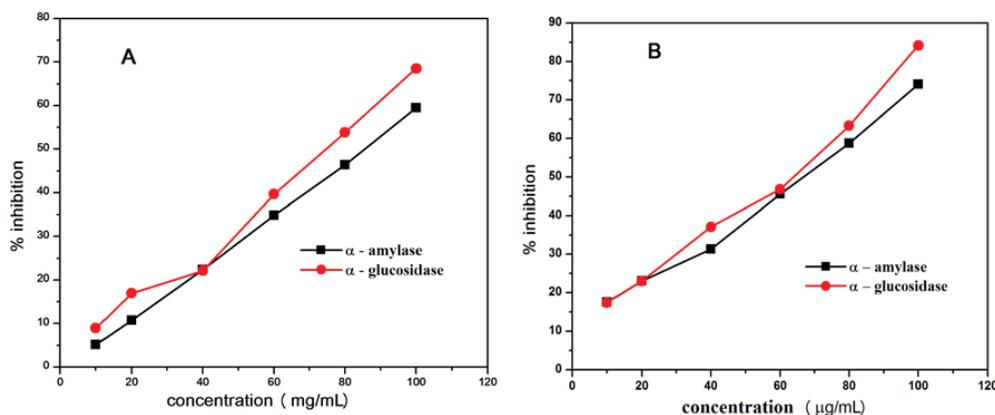
From the results it was shown that the aqueous stem extract of *S. oblonga* effectively inhibits the action of both alpha amylase and alpha glucosidase enzyme by a dose dependent manner. The percentage inhibition of alpha amylase enzyme varied from 5.14±0.04% to 59.46±0.04% at a concentration ranges from 10 to 100 mg/mL of stem extract. Like that, the percentage inhibition of alpha glucosidase enzyme varied from 8.92±0.02% to 68.51±0.01% at a concentration of 10 to 100 mg/mL

of stem extract of *S. oblonga*. The percentage inhibition of alpha amylase and alpha glucosidase against various concentrations of acarbose and *S. oblonga* were compared and graphically represented in Figure 1. It indicates that the stem extract has greater tendency for the inhibition of alpha-glucosidase than alpha-amylase.

Qualitative analysis of chemical compounds might be required for the identification of potential chemical constituents. Gas chromatogram combined with mass spectroscopy is an important tool for such studies. It provides the qualitative information about the chemical constituents and was characterised by mass spectrum. Fifteen therapeutic important compounds were identified from the stem extract of *S. oblonga* through GC-MS analysis (Figure 2). The name of chemical components, molecular weight, molecular formula and their potential activities were shown in Table III.

## DISCUSSION

Diabetes mellitus is a metabolic disorder, a life threatening disease which is increasing day by day. Insulin is key player to regulate carbohydrate, fat and protein metabolism. Insulin deficiency may affect the above important metabolisms. The enzyme alpha amylase and alpha glucosidase may be responsible for the breakdown of carbohydrates into glucose. Alpha amylase is responsible for hydrolysing the starch, which breaks down into glucose before absorption (Abhijit *et al.*, 2014). Inhibition of alpha amylase can lead reduction in post prandial hyperglycemia (Raman *et al.*, 2012). Alpha glucosidase is an enzyme present in the small intestine, used for the cleavage of disaccharides in to glucose. There are many natural constituents available in plants with alpha glucosidase inhibition activity. *S. oblonga* is considerable example having



**FIGURE 1** – Comparison of α-amylase and α-glucosidase inhibition. A: % inhibition of α-amylase and α-glucosidase against various concentrations of *S.oblonga*. B: % inhibition of α-amylase and α-glucosidase against various concentrations of acarbose

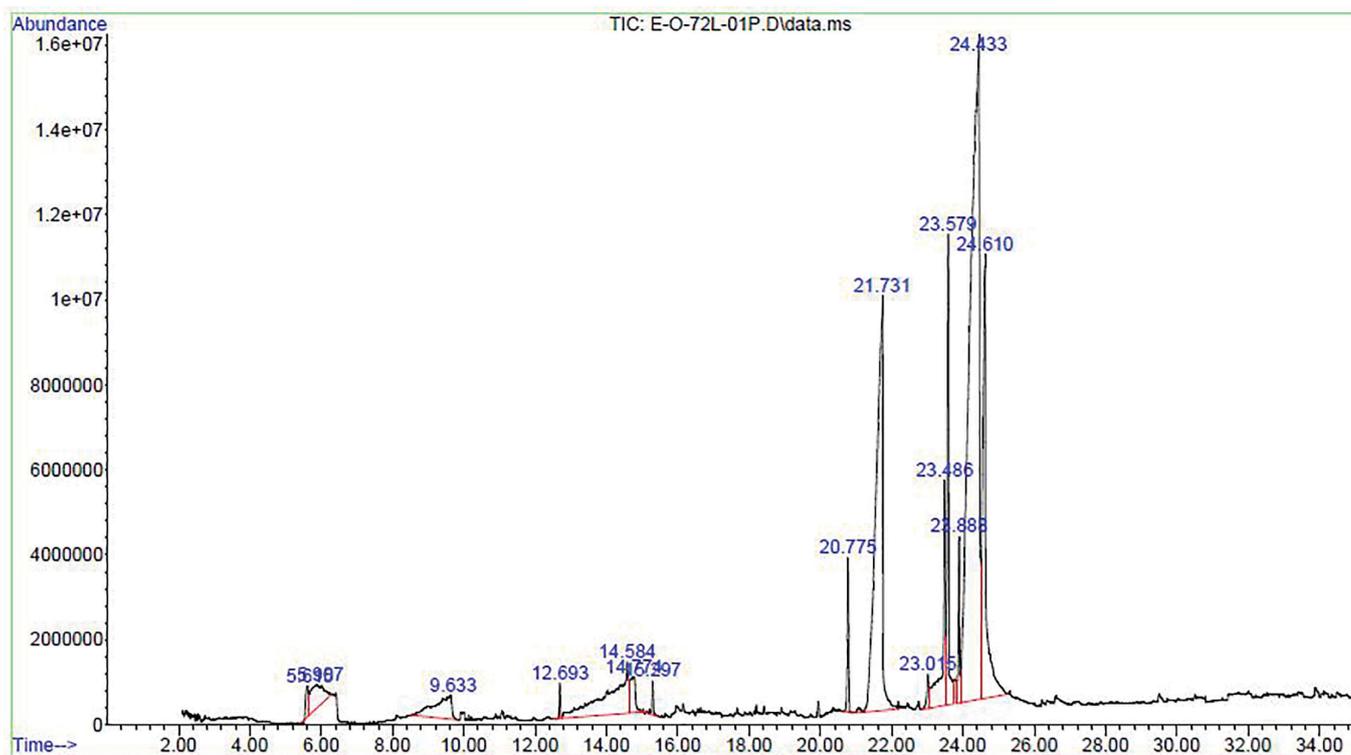


FIGURE 2 - Gas Chromatography Mass Spectrum of stem extract of *S.oblonga*.

TABLE III - Phytochemicals identified by GC-MS analysis

S. No	RT	Peak area %	Name of the chemical component	Molecular formula	Molecular weight
1	5.618	0.54	1-silacylo-2,4-hexadiene	C <sub>5</sub> H <sub>6</sub> Si	94
2	5.910	2.07	sulfuric acid, dimethyl ester	C <sub>2</sub> H <sub>6</sub> O <sub>4</sub> S	126
3	9.633	2.75	glycerin	C <sub>3</sub> H <sub>8</sub> O <sub>3</sub>	92
4	12.693	0.25	3H-pyrazol-3-one, 1,2-dihydro-1,2,5-trimethyl-	C <sub>6</sub> H <sub>10</sub> N <sub>2</sub> O	126
5	14.584	6.52	1,2,3,4-butanetetrol, [S-(R*,R*)]-	C <sub>4</sub> H <sub>10</sub> O <sub>4</sub>	122
6	14.776	1.29	1,2-ethanediol, 1-(2-phenyl-1,3,2-dioxaborolan-4-yl)-, [S-(R*,R*)]-	C <sub>10</sub> H <sub>13</sub> BO <sub>4</sub>	208
7	15.297	0.35	benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	C <sub>15</sub> H <sub>22</sub>	202
8	20.775	1.55	hexadecanoic acid, methyl ester	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270
9	21.731	19.65	<i>n</i> -hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256
10	23.015	0.49	heptadecanoic acid	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270
11	23.486	4.33	10,13-octadecadienoic acid, methyl ester	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294
12	23.579	5.47	9-octadecenoic acid (Z)-, methyl ester,	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296
		5.47	9,12-octadecadienoic acid(Z,Z)-, methyl ester	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296
13	23.888	1.64	methyl stearate	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	298
14	24.433	42.09	6-octadecenoic acid	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282
15	24.610	11.01	octadecanoic acid	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	284

RT: Retention Time

alpha glucosidase inhibitory action. It leads to minimum absorption of mono saccharides. Thus inhibition of alpha glucosidase may be the challenging one to control diabetes (Anuradha Devi, Mallikarjuna, 2016). The methanolic and aqueous extract of *Taraxacum officinale* showed potential percentage of alpha amylase and alpha glucosidase inhibition (Amin, Sawhney, Jassal, 2015). In our findings, antidiabetic activity of aqueous stem extract of *S.oblonga* was effective against alpha amylase and alpha glucosidase enzyme. Inhibitory action of stem extracts increases with increased concentration. Similar dose dependent increasing results were obtained on the plants like *Syzygium cumini*, *Psidium guajava* (Karthic *et al.*, 2008; Manikandan, Vijaya, Durai, 2013). Most of the herbal plants and its parts have the tendency to reduce the blood glucose level. This is due to the availability of tannins, terpenoids and flavonoids (Osadebe *et al.*, 2010). Tannins and flavonoids have potential inhibitory effects on alpha amylase and alpha glucosidase (Poongunran *et al.*, 2015). The alpha amylase inhibitory action of *Tragia involucrate* was due to the availability of terpenoids (Vinodhini *et al.*, 2015). In *Melia azedarach*, the antidiabetic potential was mainly due to the presence of tannins and terpenoids (Khan *et al.*, 2014). Alpha amylase inhibitory effects of tannins were due to its ability to bind carbohydrates and proteins. The above studies proved that, the secondary metabolites like flavonoids, tannins and terpenoids were effectively inhibit alpha amylase and alpha glucosidase. In the present study, the aqueous extract of *S. oblonga* effectively inhibits alpha amylase and alpha glucosidase enzymes with IC<sub>50</sub> values 73.56 mg/mL and 80.90 mg/mL respectively. The natural chemical constituents may attribute this activity. In the aqueous stem extract potential amount of flavonoids, tannins and terpenoids were found.

Natural chemical constituents reported by GC-MS analysis were shown potential glucose reduction capacity. Various therapeutic compounds were identified through GC-MS by many researchers (Sahaya Sathish, Janakiraman, Johnson, 2012; Gopinath *et al.*, 2013). Such compounds possess antioxidant, antibacterial, antifungal, anti-inflammatory, anti-diabetic and anticancer activities. From the previous studies, it was confirmed that *n*-hexadecanoic acid is an important chemical constituent with antioxidant, hypocholesterolemic, nematocidal, pesticide, lubricant, haemolytic 5-alpha reductase inhibitor, anti-inflammatory, antibacterial and antiandrogenic characteristics (Purushoth *et al.*, 2013). 9-octadecenoic acid (Z)-, methyl ester was reported as predominant compound in *Gymnema sylvestre* (Subashini *et al.*, 2015). GC-MS analysis of methanolic extract

of *Cassia italica* shown the presence of hexadecanoic acid, methyl ester, *n*-hexadecanoic acid, octadecanoic acid and other constituents with antioxidant, flavour, hypocholesterolemic, pesticide and 5-alpha reductase inhibitor action (Sermakkani, Thangapandian, 2012). 9,12-octadecadienoic acid (Z,Z)-, methyl ester has the property of anti-inflammatory anti-inflammatory, antiarthritic, hepatoprotective, hypocholesterolemic 5-alpha reductase inhibitor, insectifuge and antiarthritic activity (Sudha, Chidambarampillai, Mohan, 2013; Omoregie, Macdonald, Ovuakporie, 2015). Among the fifteen compounds, *n*-hexadecanoic acid, 9,12-octadecadienoic acid(Z,Z)-, methyl ester and octadecanoic acid had potential glucose reduction capacity. This property insists the herbal usage of *S. oblonga* stem for pharmaceutical purpose. The above studies confirm the anti-diabetic and other bioactive nature of chemical compounds obtained in the aqueous stem extract of *S. oblonga*. The root part of *S. oblonga* also reported for potential antidiabetic activity (Williams *et al.*, 2007). But there is no report on the aqueous stem extract. Thus our study may be helpful for the new drug discovery using the stem part of *S. oblonga*.

From our findings it was concluded that the presence of natural chemical constituents in the aqueous stem extract of *S. oblonga* may be responsible for the effective inhibitory action of alpha amylase and alpha glucosidase enzymes. The inhibitory effect is more towards alpha glucosidase than alpha amylase. This activity supports the herbal usage of aqueous stem extract of *S. oblonga*. Biodiversity plays vital role for the protection of living beings from variety of diseases. Among the plant diversity *S. oblonga* stem extract shown potentially active anti-diabetic property. Traditionally the root part of *S. oblonga* was used for the pharmaceutical purpose, which may destroy the total plant and bring the herb towards an endangered position. Our present study confirmed the alpha-amylase and alpha-glucosidase inhibitory effects of aqueous stem extract of *S. oblonga* and the GC-MS analysis revealed the presence of active components. Further studies are required for the isolation of anti-diabetic compounds.

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## CONFLICT OF INTEREST

Authors declare no conflict of interest.

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