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Article

Antioxidant, Antidiabetic and Lipid Profiling of *Spermadicyton Suaveolens* in Streptozotocin (STZ) Induced Diabetic Rats

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Diabetes is a life-threatening disease, and currently available synthetic medicines for treating diabetes are associated with various side effects. Therefore, there is an unmet need to develop herbal remedies against diabetes as an alternative to synthetic medicines. Although local healers use the roots of *Spermadicyton suaveolens* (SS) to manage diabetes, there is negligible research to validate its antidiabetic properties. The present investigation aims to the assess the antioxidant, antidiabetic, and antihyperlipidemic potential of the ethanolic extract of *S. Suaveolen's* roots (EESS) on streptozotocin (STZ) induced diabetic rats. The extract was screened for *in vitro* antioxidant and antidiabetic activity. The *in vivo* antidiabetic potential of EESS (at 200 and 400 mg/kg) was studied on STZ-induced diabetic rats for 20 days. The EESS displayed significant ($p<0.05$) antidiabetic and antioxidant properties. The administration of 200 mg/kg and 400 mg/kg EESS in STZ-induced diabetic rats significantly reduced hyperglycemia, and restored antioxidant enzymes and lipid profile–a high density lipoprotein (HDL) increased by the administration of a single dose of streptozotocin. Thus, EESS could be a promising herbal medicine in the treatment of diabetes and hyperlipidemia.

Keywords: *Spermadicyton suaveolens* extract. Antioxidant. Antidiabetic. Hyperlipidemic. Histopathology.

LIST OF ABBREVIATIONS

SS–*Spermadicyton Suaveolens*
STZ–Streptozotocin
EESS–Ethanolic extract of *Spermadicyton Suaveolens*
CESS–Chloroform extract of *Spermadicyton Suaveolens*
PESS–Petroleum extract of *Spermadicyton Suaveolens*
AESS–Aqueous extract of *Spermadicyton Suaveolens*
NO–Nitric oxide
SOD–Superoxide radical scavenging
HDL–High-density lipoprotein
CAM–Complementary and alternative medicine
NBT–Nitro blue tetrazolium
DMSO–Dimethyl sulfoxide
P-NPG–Para-nitrophenyl–α–D-glucopyranoside
Na_2CO_3–Sodium carbonate
OGTT–Oral glucose tolerance test
MDA–Malondialdehyde
GSH–Glutathione
CAT–Catalase
HDL–High-density lipoprotein
LDL–Low-density lipoprotein
VLDL–Very low-density lipoprotein

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INTRODUCTION

Management and treatment of chronic illnesses are one of the most significant difficulties faced by healthcare societies. Despite significant advances in the sector, clinicians have not been able to eradicate severe chronic illnesses such as diabetes mellitus (Asif *et al.,* 2019). Diabetes is the third-most reason for mortality. As per a 2021 report, approximately 537 million adults are living with diabetes. This number is projected to grow to 643 million by 2030 and 783 million by 2045. Globally, more than 90% of people have type 2 diabetes (Cho *et al.,* 2018; IDF Atlas 2021).

Recognising this severity, investigators worldwide emphasise the need to discover complementary and alternative medicine for diabetes. Over 80 percent of the population in the economically growing and emerging nations use herbal remedies to treat their diseases. Natural antidiabetic products are a game-changer in the treatment of diabetes due to the higher costs and adverse effects associated with allopathic therapy. (Birdee, Yeh, 2018; Falguni *et al.,* 2017; Uddin *et al.,* 2018)

Diabetes mellitus is a fuel-metabolism disorder caused by increased blood glucose due to starving insulin secretion or inability to meet the demand of target tissues. Hyperglycemia is the first clinical sign, followed by metabolic interruption of biomolecules. Prolonged hyperglycemia causes chronic microvascular and macrovascular complications, such as neuropathy, nephropathy, retinopathy, and arteriosclerosis (Satyanarayana, Chakrapani, 2012; Prabhakar, 2016).

Oxidation is the fuel for the development of diabetes by lipid autoxidation, DNA, and protein damage. The development of molecular oxygen is an integral part of routine work, and incomplete reduction is responsible for the generation of oxygen free radicals. Antioxidants neutralise the free radicals and reactive oxygen species. The imbalance between generation and neutralisation leads to oxidative stress which increases risk of complications like cardiovascular diseases, diabetes, and neurogenerative diseases, etc. (Gudise, Chowdhury, Manjappa 2019; Kunwar, Priyadarsini, 2011).

Streptozotocin is a diabetes inducer. Its administration causes a burst of free radicals due to metabolism, leading to a cytotoxic action on pancreatic β-cells. This phenomenon brings on hyperglycemia in rodents, just like diabetic people. The oxidative stress disrupts endoplasmic reticulum function leading to cell necrosis. Hyperglycemia engenders variance in the metabolism of proteins and lipids (Prabhakar 2016; Kunwar, Priyadarsini, 2011; Sajid *et al.,* 2020).

The *Spermadictyon suaveolens* (SS) commonly called as *Forest Champa, Van-Champa*, *Gidesa, Jitsaya,* etc. has diversified therapeutic applications. It is found in Maharashtra, the Himalayan region, Kashmir, and the Northern Areas of Pakistan. Local healers in Maharashtra use the herb's stem and root for various ailments like bone and muscle wounds, herpes, diabetes, etc. Ayurvedic practitioners or *vaidyas* use this plant's stem powder to treat viral ailments like herpes and diabetes (Musmade, Rakshe, Mokat, 2016).

The methanolic, chloroform, and petroleum ether extract of bark and leaves exhibit potent antioxidant and antimicrobial action. The roots were evaluated for their wound healing properties in Wistar rats (Ajaib, Khalid, Hanif, 2016). The stem and leaf contain phytoconstituents including Azulene, Tetratetracontane, n-hexadecanoic acid, Ergost–5-en–3–ol,22,23-dimethyl-,acetate,(3β), Phenol,2-methoxy-4-(1-propenyl)-,(E)(9), etc., and they have reported antioxidant, anti-inflammatory, antibacterial, and antiulcerogenic activity (Kulkarni, Sathe, 2013). The flower and leaf contain 3,7,11,15–tetramethyl–2–hexadecen–1–ol, Phytol, 3,4–dianhydro–2–deoxy–. beta–d–lyxo–hexo–pyranose, 3,7,11,15–tetramethyl–2–hexadecen–1–ol, etc., and has shown analgesic, antimicrobial, anticancer, anti-diuretic, anti-inflammatory, and antipyretic properties. (Papitha, Ravi, Selvaraj, 2017).

As far as we know, this is the first work to provide insight into the antioxidant and hypoglycemic effects of EESS. The current investigation was based upon the evaluation of *in vitro* and *in vivo* hypoglycemic action on STZ-NA induced rats. Besides, serum investigation of biomarkers along with *in vivo* antioxidant behavior was evaluated. Thus, we have investigated the efficiency of crude extract in diabetes-related complications *in vivo*.

MATERIAL AND METHODS

Plant Material

The root of SS was collected in the month of November from the hilly vicinity of Panhala Fort in Kolhapur district, Maharashtra. The plant was identified and authenticated by taxonomist Dr. Kavale from the Department of Botany, Shivraj College, Gadhinglaj, Maharashtra. A voucher specimen was deposited (SCG/BOT/HERB/07-2019) in Shivrai College. After collecting the roots, they were chopped into small pieces and dried before being used as a raw material for the treatment. The dried roots were pulverized on a Rising Automatic DP Pulverizer and later powedered. The powder was then surpassed through a 40# numbered sieve and stored in an airtight container.

Soxhlet extraction

The solvents employed for the extraction process were selected based on their polarity. The four different extracts of SS–the aqueous extract of SS (AESS), ethanol extract of SS (EESS), chloroform extract of SS (CESS), and petroleum ether extract of SS (PESS)–were prepared using four solvents with different polarity. Thus, the solvents' systematic extraction of plant material had increasing polarity. Successive extraction was done using 50 g powdered root material via the soxhlet apparatus (Figure 1). The PESS using petroleum ether (250 mL, 45ºC 15 cycles) was obtained, and after successive extraction, CESS using chloroform (250 mL, 45ºC 15 cycles) and EESS employing ethanol (250 mL, 60ºC 15-17 cycles) were obtained. After completing the extracting and filtering, the concentration of the residue weighing 50 ml in the water bath. The extract was then transferred to a previously weighed evaporating dish. The total weight of the evaporating dish containing the extract was measured and placed in the water bath for evaporation until it became viscous. The difference in weight was calculated every 10 minutes until a constant weight was obtained.

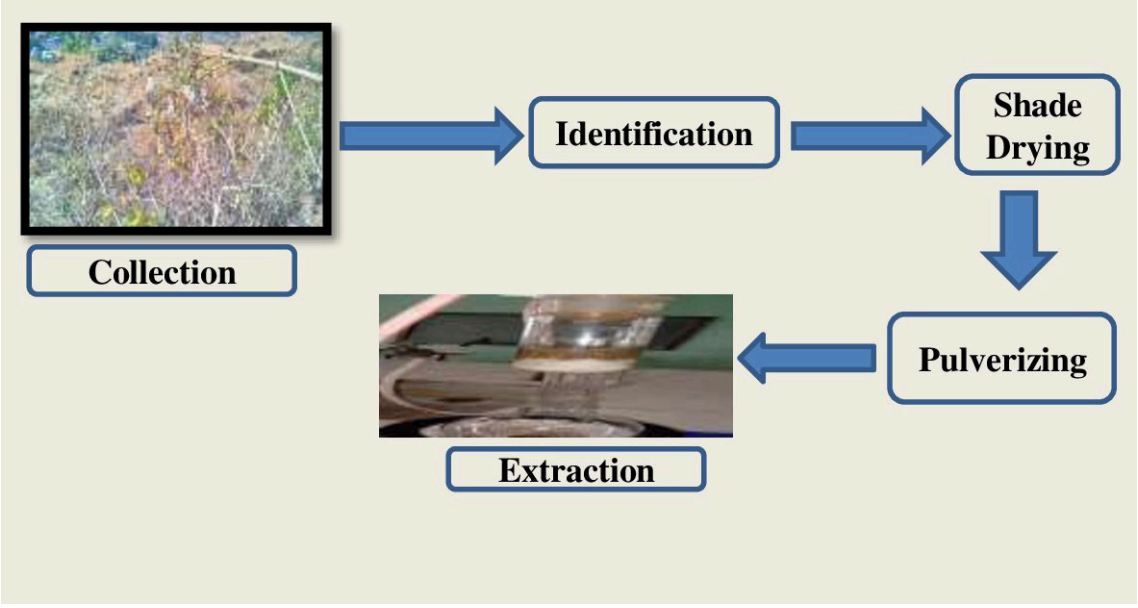

FIGURE 1 - Schematic representation of Soxhlet extraction.

In-vitro antioxidant

Nitric oxide scavenging assay

The Griess Illosvory reaction was utilised to assess free radical scavenging. The Griess Illosvory reagent commercially contains naphthyl ethylene diamine dihydrochloride (0.1% w/v), 1 mL of 10 mm sodium nitroprusside in 0.5 mL phosphate buffer saline (pH 7.4) was mixed with 1 ml each of extract of different concentrations (200, 400, 600, 800, 1000 µg/mL). The mixture was incubated at 25ºC for 150 min. After incubation, the reaction mixture was mixed with 1.0 ml of pre-prepared Griess reagent (1.0 mL sulfanilic acid reagent 0.33% in 20% glacial acetic acid at room temperature for 5 min with 1 mL of naphthyl ethylenediamine dichloride 0.1% w/v). The mixture was then incubated at room temperature for 30 minutes, and the nitrite concentration was estimated at 546 nm using nitrite solutions as the control. Buffer solution served as blank, whereas the reference solution was ascorbic acid (Patel *et al.*, 2010). The decreasing absorbance indicates a high nitric oxide scavenging activity. The percentage inhibition was determined using the equation,

$$\% \text{ Inhibition} = \frac{\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Test}}}{\text{Absorbance}_{\text{Control}}} \times 100$$

Superoxide radical scavenging assay (SOD)

The dioxide ion engulfed propensity of the extract was calculated using the alkaline DMSO process, which was slightly modified from Elizabeth and Rao's (1990) method. The decreased nitro blue tetrazolium (NBT) was evaluated by this method. Both extract (30 µL) and standard were dissolved in DMSO, 100 µl of alkaline DMSO, and 10 µl of NBT were added to fulfil the volume 140 µL. Finally, the reading was taken at 560 nm by a microplate reader. Ascorbic acid served as a positive control (Harput *et al.*, 2011).

In-vitro antidiabetic activity

α- glucosidase activity

The α-glucosidase inhibitory activity of SS extracts was analysed using a standard method with little modifications. Briefly, the concoction of SS extracts (50 µL), glutathione (50 µL), 10 µL α-glucosidase in phosphate buffer was mixed in a 96-well plate and incubated for 15 minutes at 37°C. After incubation, 20 µL P-NPG (5 mM) was added and then incubated for 15 min at 37°C. The addition of 50 µL Na2CO3 (0.1 M) terminated the reaction. The blank was prepared using a similar process but without adding enzyme (α - glucosidase) solution. Without a test sample, each procedure was done thrice to act as a control. The sample and blank absorbances were read at 400 nm. The p-nitrophenol produced from p-NPG determined the α-glucosidase activity. Acarbose was positive control (Telagari, Hullatti, 2015).

Animals

Male Wistar rats weighing between 190- 220 gm were chosen for the experiment. They were placed in rat cages made of stainless steel. Except where fasting was needed, food and water were given ad libitum. The rats were kept at a constant temperature of 20-25^{0}C and were subjected to a regular 12-hour light and 12-hour dark periods. They were given a two-week acclimatisation period before the experiment.

Acute toxicity study

The lethal dose of plant extract was investigated using OECD guidelines (test 423: Acute oral toxicity-acute toxic class method, 2002). (OECD Library, 2002). Before the trial, the animals were split into four classes (n=6) and were kept on fasting overnight. The EESS was administered orally, starting from 5, 50, and 300 to increasing to 2000 mg/kg body weight. At 30 minutes and 2, 4, 8, and 24 hours after the dosage, all groups were closely monitored for the occurrence of any clinical or toxicological effects and there after every 24 hours for 14 days. The animals were monitored for

14 days for the long-term possible lethal outcome. The body weights of the animals were measured on days 1, 7, and 14.

Oral glucose tolerance test (OGTT)

Healthy rats were chosen for the OGTT. The rats were feed in an interval of 12 hours and were divided into 4 groups.

The OGTT was done after starving normal rats for 2 hours. Distilled water, EESS extract (200 mg/kg and 400mg/kg), and glibenclamide (2 mg/kg) were administered to all the four groups of rats. Glucose (2 g/kg) was fed 30 minutes after the pretreatment using distilled water, EESS, and glibenclamide. To measure the blood sugar level, blood was taken from the tail vein at 0 minutes (as a baseline before the glucose solution loading), and then at 30, 60, and 120 minutes after glucose loading, to evaluate the outcome of the extract on glucose level. The serum sugar was estimated by blood glucose test strips and glucometer (Changsha sinocare Inc; China).

Induction of Diabetes (Streptozotocin-Nicotinamide induction)

For the induction T2DM, firstly intraperitoneal (i.p.) injection of nicotinamide (NA); 120 mg/kg in saline was administered in animals. After completion of 15 min of nicotinamide administration, freshly prepared streptozotocin in 0.1M citrate buffer (pH 4.5), was administered in single dose 60 mg/kg to the animals (Furman *et al*., 2021) To avoid death from STZ-NA induced hypoglycemia, all rats were given a 10% glucose solution for 12 hr. After three days rats with fasting plasma glucose > 170 mg/dL were diabetic and were integrated in this study. The STZ-NA-induced diabetic rats were split into different subgroups, each with six animals. (Furman *et al*., 2021).

Effect of long-term treatment with EESS on glycemic control

The animals were grouped into 5 groups and 6 rats were placed in each group.

Group- I *Normal control*: Animals receiving normal diet
Group-II Positive control (*Diabetic control)*: Diabetic animals receiving normal diet and free from any treatment
Group-III *Standard (glibenclamide) treated*: Diabetic animals treated with glibenclamide at a dose of 5 mg/kg body weight
Group-IV Test (*EESS treated at a dose of 200 mg)*: Diabetic animals treated with ethanolic extract of *SS* at a dose of 200 mg/kg body weight
Group-V Test *(EESS treated at a dose of 400 mg):* Diabetic animals treated with ethanolic extract of *SS* at a dose of 400 mg/kg body weight

Both standard and test samples were given to the animals administered to the rats by gastric incubation using oral gavage. Briefly, glibenclamide was administered to the animals of group III at a dose of 5 mg/kg. Similarly, EESS was administered to the animals of group IV and V at dose of 200 and 400 mg/kg respectively. The blood samples were taken from tail vein before the experiment and on the 1st, 10th and 20th day of the treatment and fasting blood glucose level was estimated. Also, body weights of animals were recorded.

Biochemical parameters measurement

I. Estimation of triglycerides (TG)

The estimation was performed using a GPO-TOPS AGAPPE kit. Briefly, the enzyme reagent (1 mL) was added to 10 µL of the sample. The mixture was then shaken well and incubated at 37°C for 10 min. Finally, the optical density (OD) was recorded at 546 nm using a spectrophotometer.

II. Estimation of total cholesterol (TC) and HDL cholesterol (HDLC)

The TC and HDLC estimation were performed as per the previously reported protocol using a CHOD-PAP (Cholesterol oxidase phenol4-aminoantipyrine) method kit. After a brief gap, 1 mL of the enzyme reagent was added to 10 µL of EESS. The mixture was shaken well and incubated at 47°C for 5 minutes and optical

density (OD) reading was recorded at 505 nm using a spectrophotometer. (Palaniappan *et al.,* 2020).

III. Estimation of VLDL and LDL cholesterol (Friedewald et al.,1972)

The level of VLDL and LDL cholesterol were calculated based on the parameters calculated in the two procedures using a Friedewald's formula.

Tissue markers of oxidative stress

Preparation of Tissue Homogenate

The liver tissue collected from the animals were homogenated using a phosphate buffer (200 mM; pH 6.6). This obtained liver tissue homogenate was centrifuged and used for analysis.

A) Determination of malondialdehyde (MDA)

The liver tissue homogenate 0.4 mL (10%) was compounded with sodium dodecyl sulphate (1.5 mL; 8.1%). The above mixture, acetate buffer pH 3.5 (1.5 mL; 20%), and TBA solution (1.5 mL; 0.8%) were mixed and the mixture was completely vortexed. It was left to stand after cooling until organic and aqueous layers were split, 5 mL of n-butanol-pyridine was added in the process (15:1). Finally, the absorbance of organic layer was recorded at 532 nm using a UV-visible spectrophotometer (Sani, Kouhsari, Moradabadi, 2012; Merghem, Dahamna, Khennouf, 2019).

B) Determination of catalase enzyme activity

The catalase enzyme activity was determined by mixing 200 mL of diluted liver tissue homogenate with 1.0 mL of phosphate buffer, 0.4 mL of distilled water, and 0.5 mL H_2O_2. To terminate the reaction, 2 mL potassium dichromate acetic acid was added after 1 minute of incubation at 37°C. Finally, the samples were maintained in a boiling water bath for 15 minutes (Sani, Kouhsari, Moradabadi, 2012; Merghem, Dahamna, Khennouf, 2019).

C) Determination of reduced glutathione (GSH)

For this assay, 1 mL liver tissue homogenate was mixed with 3mL of DTNB solution in phosphate buffer (0.1M pH 8) and 7.5 mL 0.3 mM NAPDH (pH 7.3). The resultant solution was then diluted with 4 mL 0.1 M phosphate buffer (pH 8). Finally, the absorbance was taken at 412 nm against blank solution prepared (Sani, Kouhsari, Moradabadi, 2012; Merghem, Dahamna, Khennouf, 2019).

Histopathological analysis

The animal pancreas was removed at the end of the experiment and fixed in buffered formalin (10%) at room temperature. Then it was stained with Hematoxylin and Eosin (H and E) stains, and observed using a 40X magnifications under a microscope on albumenized glass slides (Bancroft, Gamble, 2008).

Statistical Analysis

The outcomes of samples were displayed as mean ± standard error means. The statistical analysis was performed by using GraphPad Prism software version 5 (GraphPad Software, Inc., La Jolla, CA, USA). The results were analyzed by a one-way analysis of variance. Values of $p < 0.01$ and $p < 0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION-

Extraction

The four extract of *S. Suaveolens* were prepared by hot continuous (soxhlet extraction) process. These extract were further characterized for *in vitro* and *in vivo* activities.

In vitro antioxidant

Nitric oxide scavenging assay

Nitric oxide is a highly reactive compound which in the presence of oxygen generates the stable compounds

(nitrates and nitrite). The Griess reagent was used for its measurement. The concentration of nitrous acid is decreased due to the ability of scavenging action of test compounds. The antioxidant potential of extracts is depicted in (Figure 2A). The obtained results revealed increased antioxidant potential with an augment in the concentration. The EESS showed more potency (354.69 ± 0.92 µg/mL) for elimination of free nitrite radical as compared to other. However the EESS (354.69 ± 0.92 µg/mL) scavenges less free radical than the standard (73.06 ± 0.42 µg/mL).

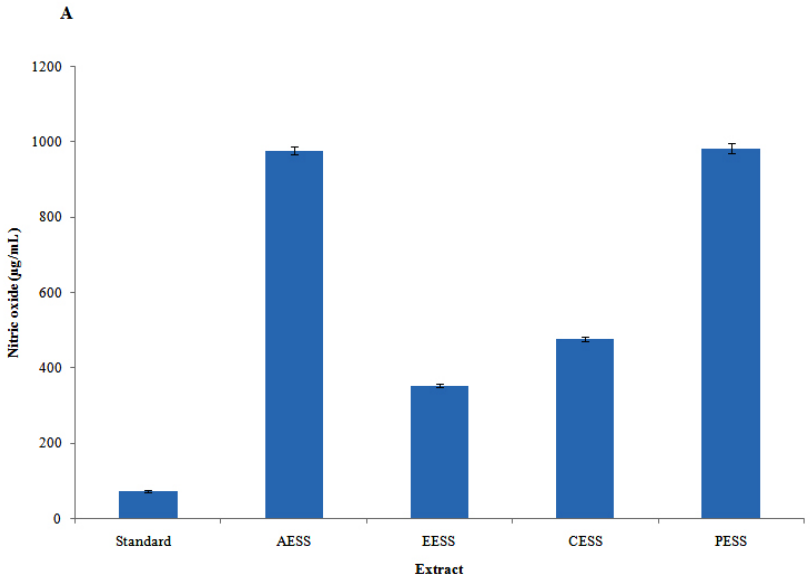

FIGURE 2A - Scavenging potential of different extracts of *Spermadicyton suaveolens* against Nitric oxide.

Nitric oxide (NO) is synthesized by vascular endothelial cells, phagocytes, and neurons which cause harmful effects from amino acid L-arginine. When radical superoxide responds, the lethality of NO is enhanced due to the formation of another reactive compound peroxynitrite anion ($ONOO^-$). Naturally sourced antioxidants showed promise in the reduction of oxidative stress and therefore can be used as an choice to the synthetic antioxidants in the treatment of diabetes (disease associated with oxidative stress) (Habu, Ibeh, 2015). In the current investigation, EESS and CESS demonstrated good nitrite free radical scavenging action in a dose dependent manner which is comparable with standard (ascorbic acid). Among these two extracts, EESS displayed potent nitrite free radical scavenging action than CESS that may be due to the presence of more polyphenolic contents extracted by ethanol from SS through the better penetration into cell membrane of plants (Boora, Chirisa, Mukanganyama, 2014).

Superoxide radical scavenging

This assay measured the dioxide scavenging potential of extracts. The nonenzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS/NADH) system produces dioxide ions, which lessen NBT to a purple formazan. The generated superoxide was reduced to nitro blue tetrazolium, which forms purple formazan at 560 nm. Among the four extracts, EESS displayed significant ($p<0.01$) superoxide radical scavenging activity (low IC_{50} value: 0.278 ± 0.028 µg/mL) when compared to AESS, CESS and PESS. However, the free radical scavenging potential of EESS was observed to be less than standard (IC_{50}: 0.092 ± 0.011) depicted in (Figure 2B).

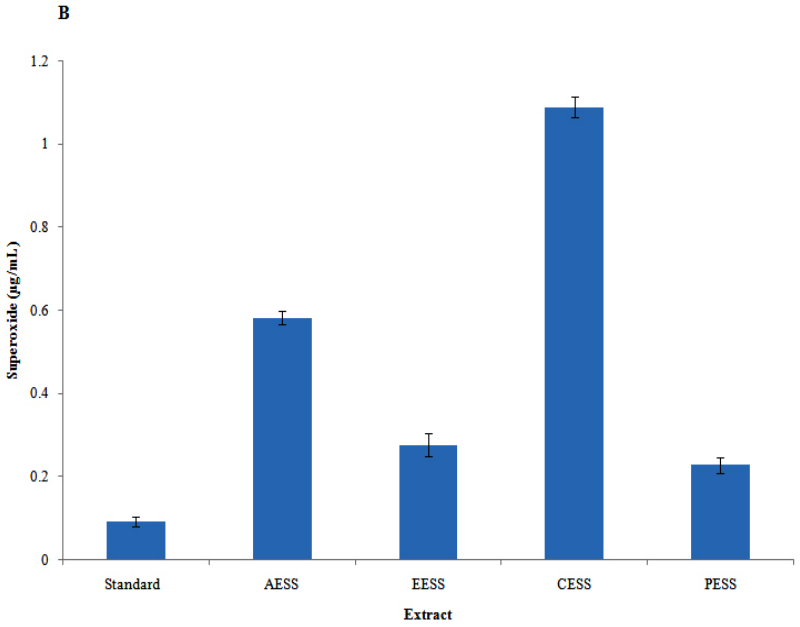

FIGURE 2B - Scavenging potential of different extracts of *Spermadicyton suaveolens* against Superoxide.

The extracts were tested for the NBT assay to determine its ability to remove superoxide anions. Superoxide anion is the prevalent free radical in living system and since it is starting material for other reactive oxygen species such as hydroxyl radical, hydrogen peroxide etc. causes tissue damage with potential reaction with biomolecules. The concentration of superoxide anion is the prime for oxidative stress (Hazra, Biswas, Mandal, 2008). In addition, at the cellular level, superoxide offers much more toxic effects. The extracts and standard was demonstrated increase in superoxide scavenging action with increase in concentration. The superoxide scavenging action of standard (ascorbic acid) was found to be higher than that of EESS and PESS.

In vitro antidiabetic activity

Diabetes mellitus is associated with malfunctioning of the metabolic system which becomes chronic that leads to several complications. Due to the constraints of publicly known pharmacological mediators for the management of diabetes, there is an unmet need to discover novel antidiabetic medicines with assorted modes of action (Adedayo *et al.,* 2014).

Several novel bioactive phyto-constituents (secondary metabolites) derived from plants with hypoglycemic and anti-hyperglycemic activities showed remarkable anti-diabetic efficacy that is comparable to, and often even more effective than currently available marketed oral hypoglycemic medications (Rai *et al.,* 2013; Watal *et al.,* 2014).

α-glucosidase activity

The half-maximal inhibitory activity of SS extracts on α-glucosidase was represented (Figure 3). There was a significant difference ($P < 0.05$) in the inhibitory potential of the standard (acarbose) and all extracts tested on α-glucosidase activities. The EESS displayed good inhibitory potential against α-glucosidase (IC_{50} 727 ± 0.94 µg/mL) than other extracts but not promising while compared to standard acarbose (IC_{50} 94.18 ± 0.692 µg/mL).

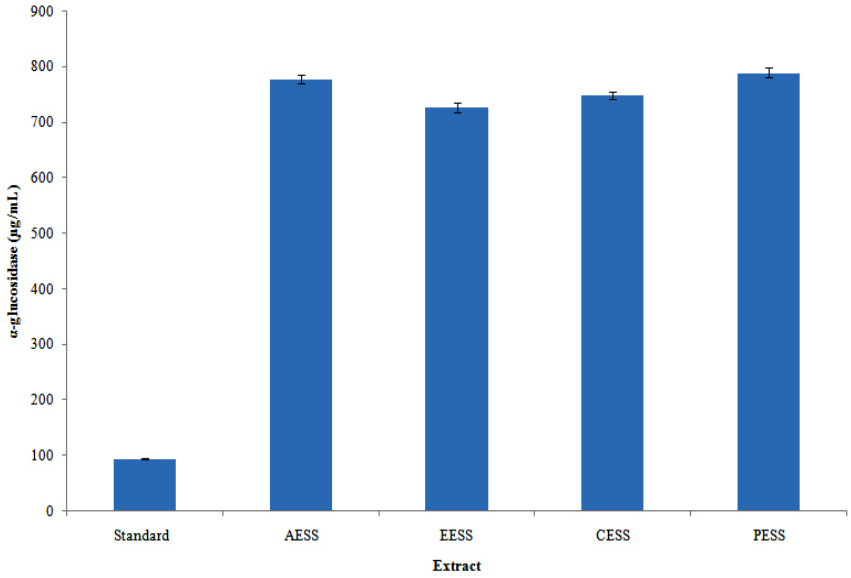

FIGURE 3 - Inhibitory effects of different extracts of *Spermadicyton suaveolens* on the α-glucosidase.

Carbohydrates are long chain compound that undergoes breakdown into the monosaccharide like glucose in the digestive tract through enzyme namely α-glucosidase. Moreover, this enzyme is responsible for the reabsorption of glucose in the intestine. Therefore, this enzyme can serve as a chief target in the treatment of diabetes. Thus, enzyme suppression is one of the effective approaches used to reduce postprandial hyperglycemia in diabetics by slugging the absorption of intestinal glucose. So, the enzyme inhibitors are vital in management and treatment of diabetes (Khan *et al.,* 2016). In this study, EESS showed strongest α-glucosidase inhibitory action when compared to other extracts however; it was less than standard (Alimi, Ashafa, 2017). Thus, better antidiabetic activity of the EESS extract against T2DM could be attributed to the delaying of carbohydrate digestion due to the significant inhibition of α-glucosidase activity.

Acute toxicity study

Oral administration of the crude EESS was found to be safe at a dose of 2 g/kg body weight, which showed no any toxicity. Thus, after 14 days, no animal mortality was seen with EESS. The lethal dose of EESS was considered to be more than 2 g/kg. The 200 mg/kg and 400 mg/kg doses were considered for study.

The body weights of animals of the control and EESS treated groups were increased progressively throughout the study period (Table I). Moreover, behavioral observations of the all animals treated at different doses of EESS were found to be normal as control group animal behaviour (Table II). Thus, these obtained results revealed safety and tolerability (absence of toxicity) in the animals treated with various doses of EESS.

TABLE I - Effect of extract on body weight of rats in Acute toxicity study

Group	Body Weight (gm)		
	1st Day	7th Day	14th Day
Control	180.33 ±0.39	187.5 ±0.49	200.16 ±0.69
EESS 300 mg/kg	184.66 ±0.42	187.5 ±0.37	198.5 ±0.45

TABLE I - Effect of extract on body weight of rats in Acute toxicity study

Group	Body Weight (gm)		
	1st Day	7th Day	14th Day
EESS 1000 mg/kg	181.33 ±0.33	186.33 ±0.44	200.33 ±0.38
EESS 2000 mg/kg	184.16 ±0.41	185.66 ±0.57	195.66 ±0.47

TABLE II - Behavioral observations of rats on EESS administration

Parameters	Dose	Behavioural observations						
		30 min	2 H	4 H	8 H	24 H	7th Day	14th Day
Salivation	Control	Normal	Normal	Normal	Normal	Normal	Normal	Normal
	EESS 300 mg/kg	Normal	Normal	Normal	Normal	Normal	Normal	Normal
	EESS 1000 mg/kg	Normal	Normal	Normal	Normal	Normal	Normal	Normal
	EESS 2000 mg/kg	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Sleep	Control	Normal	Normal	Normal	Normal	Normal	Normal	Normal
	EESS 300 mg/kg	Normal	Normal	Normal	Normal	Normal	Normal	Normal
	EESS 1000 mg/kg	Normal	Normal	Normal	Normal	Normal	Normal	Normal
	EESS 2000 mg/kg	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Mortality	Control	Not found	Not found	Not found	Not found	Not found	Not found	Not found
	EESS 300 mg/kg	Not found	Not found	Not found	Not found	Not found	Not found	Not found
	EESS 1000 mg/kg	Not found	Not found	Not found	Not found	Not found	Not found	Not found
	EESS 2000 mg/kg	Not found	Not found	Not found	Not found	Not found	Not found	Not found

Hypoglycemic action of SS extract on Oral Glucose loaded rats

The effect of EESS on glucose tolerance in rats is depicted in **(**Table III**).** Considerable change in blood glucose level was observed after administration of glucose orally. The EESS at concentration of 200 mg/kg and 400 mg/kg evoked significant ($p<0.01$) hypoglycemic effects after 120 min as compared to control.

TABLE III - Hypoglycemic action of *S. Suaveolens* on Oral Glucose loaded rats

Group No.	Treatment	Blood glucose level (mg/dL)			
		0 min	30 min	60 min	120 min
	Control	72.83± 1.47	133.16± 1.16	127.33± 1.21	111.33± 1.21
	Std (Glibenkamide 2mg/kg)	82.16± 2.13*	121.16± 1.16*	97.33± 1.36**	89.16± 1.47**

TABLE III - Hypoglycemic action of *S. Suaveolens* on Oral Glucose loaded rats

Group No.	Treatment	Blood glucose level (mg/dL)			
		0 min	30 min	60 min	120 min
	EESS 200 mg/kg	88.5± 2.07*	122.16 ± 1.60*	92.33 ± 1.75*	89.66± 1.21**
	EESS 400mg/kg	90.83± 1.16*	136.66± 1.63*	95.83 ± 1.72*	93.5 ± 1.37*

An oral glucose tolerance test identifies how your body handles glucose before and after the meal. OGTT is considered a '24 carat gold sensitive' test for screening and diagnosis of glucose utilization those other tests miss. In the current OGTT study, the EESS shut down the blood glucose from 60 min as compared to control. This confirms the appropriate utilization of glucose by rats. The capability of EESS to reduce postprandial glucose may be attributed to a decrease in glycogen split up and synthesis of glucose, which shoot up the glucose absorption. The obtained results clearly revealed the capability of the extract to minimize hyperglycemia-related problems in diabetes (Kifle, Yesuf, Atnafie, 2020).

Anti-diabetic action

Change in body weight

The body weights of all groups of rats treated with different formulations was measured. Diabetic control rats (group II) gained significant ($p<0.05$) lower weight than normal control rats (group I). Both groups (IV and V) treated with EESS at (200 mg/kg and 400 mg/kg) showed elevated body weight as compared to diabetic control however, it is less when compared to standard (Table IV). Thus, the diabetic rat displayed frequently decrease in the body weight.

Glucose is the body's carbohydrate currency. In diabetes mellitus, body cells fail to exploit this currency for the generation of energy instead of nutrient material used as an energy source. Ultimately reduction in protein storage leads to a reduction in body weight. Throughout the experimental process, STZ-induced diabetic rats showed a consistent drop in body weight. On the other hand, the oral administration of the ethanolic extract to diabetic rats marked upgrading in body weight as compared to others. Thus, obtained results proved the utilization of glucose for the generation of energy instead of protein (Pedro *et al.,* 2004).

TABLE IV - Effect of *S. suaveolens* extract on body weight

Group No.	Administration/ Treatment	Body weight (g)		
		1st	10th	20th
1	Normal	200.16 ± 15.62	187.5 ± 11.48	180.83 ± 11.83
2	Diabetic control	198.5 ± 14.78	187.5 ± 12.51	174.66 ± 12.69
3	Standard	198.33 ± 15.81*	196.83 ± 17.42**	197.66 ± 11.84**
4	EESS 200 mg/kg	200.33 ±12.42*	181.33 ± 11.03	186.33 ± 10.57*
5	EESS 400mg/kg	195.16 ± 2.60*	184.16 ± 3.93*	185.66 ± 8.18**

Change in blood glucose level after ethanolic extract treatment.

The blood sugar levels were analysed on the first day of the study. The STZ-induced rats' group (II, III, IV and V) showed momentous blood sugar concentration when compared to the normal control group (I) (Table V). On the 10th day after induction, the diabetic control group exhibited a marked augment in blood glucose level. In contrast, the group treated with standard showed a considerably lower blood sugar concentration than EESS. Furthermore, groups IV and V treated with EESS displayed remarkably reduced blood glucose levels compared to diabetic control. After 20 days of study, STZ-induced diabetic control group showed significantly high blood glucose level (224.5±5.68 mg/dL) compared to normal control rats (84.16± 2.31 mg/dL).

On the other hand, the group treated with standard and EESS extract at both concentrations demonstrated a noteworthy reduction in the blood glucose level compared diabetic control group. Besides, the antidiabetic activity of EESS at 400mg/kg dose was equivalent to the standard treatment. Thus, the obtained results revealed the antidiabetic potential of SS.

TABLE V - Hypoglycemic activity of *S. Suaveolens* in diabetic animals

Group No.	Administration/ Treatment	Blood glucose (mg/dL)		
		1st	10th	20th
1	Normal	81.83 ± 3.12	83.66 ± 3.26	84.16 ± 2.31
2	Diabetic control	209.83 ± 7.49	216.83 ± 7.54	224.5 ± 5.68
3	Standard	159.33 ± 25.37*	143.16 ± 18.36*	128 ± 9.95*
4	EESS 200 mg/kg	182.66 ± 3.98*	167.33 ± 3.20*	148.66 ± 3.01**
5	EESS 400mg/kg	179.66 ± 5.60*	153.5 ± 4.67*	137.66 ± 3.14*

Change in the serum lipid levels

The HDL, LDL, VLDL, triglycerides, and total cholesterol levels in the serum of different groups on the last day of the experiment were measured and noted in Table VI. The diabetic control group exhibited elevated total cholesterol, triglycerides, HDL-cholesterol, VLDL-cholesterol, and LDL-cholesterol levels than the regular group. The groups treated with standard EESS at both concentrations demonstrated a remarkable reduction in total cholesterol, triglycerides, HDL-cholesterol, VLDL-cholesterol, and LDL-cholesterol. EESS showed antihyperlipidemic activity in a dose-dependent manner. The antihyperlipidemic activity of EESS at 400 mg/kg dose was at par with standard treatment.

TABLE VI - Effects of *S. Suaveolens* extract on lipid profile parameters

Group No.	Administration/ Treatment	Concentration in mg/dL				
		Total Cholesterol	Triglycerides	LDL -cholesterol	VLDL-cholesterol	HDL -cholesterol
1	Normal	78.33 ± 1.44	105.86 ± 1.06	41.35 ± 1.42	21.72 ± 0.21	12.78 ± 1.00
2	Diabetic control	162.56 ± 1.53	177.44 ± 1.13	87.38 ± 1.68	35.48 ± 0.22	87.56 ± 2.66

TABLE VI - Effects of *S. Suaveolens* extract on lipid profile parameters

Group No.	Administration/ Treatment	Concentration in mg/dL				
		Total Cholesterol	Triglycerides	LDL -cholesterol	VLDL- cholesterol	HDL -cholesterol
3	Standard	92.62 ± 0.76**	85.63 ± 1.27*	51.33 ± 1.72*	17.12 ± 0.25**	24.15 ± 2.09*
4	Ethanol extract 200 mg/kg	121.97 ± 1.00*	140.33 ± 1.24*	64.98 ± 2.37*	28.06 ± 0.24 **	28.92 ± 2.08*
5	Ethanol extract 400mg/kg	97.79 ± 1.17*	121.08 ± 0.96**	51.53 ± 1.95*	24.21 ± 0.19**	22.03 ± 2.86*

Diabetes mellitus is characterised by metabolic manifestation; among these lipid metabolism affects the most. Dyslipidemia is a condition of elevated triglycerides and cholesterol levels, a warning sign for clinical manifestation of a coronary heart disease (Elberry *et al.,* 2015). Triglycerides serves as a fuel reserve of the body and are stored in the adipose tissue. Under normal physiological conditions, triglycerides hydrolyse to free fatty acids through lipase. However, in diabetes, triglyceride level is higher than normal due to the inactivation of lipase triggered by insulin. The serum TC, TG and LDL concentrations rose significantly, and HDL levels decreased. In this study, lipid biomarkers of diabetic rats showed a higher level of TC, TG and LDL, and a reduced HDL. Administration of EESS led to a decreased level of TC, TG, and LDL, and an increased level of HDL. The effect of EESS proved effective in lowering lipid biomarkers by improving their metabolism rate close to the standard metabolism rate. This finding suggests that the extract is capable of reducing dyslipidemia condition. (Seedevi *et al.,* 2020).

Defensive In-vivo antioxidant action

In STZ-induced diabetic rats the level of SOD, CAT, and GSH are decreased (32.92 ± 2.45, 4.32 ± 0.08 and 7.74 ± 0.34), while the level of MDA (1.43 ± 0.04) increased in liver tissue, when compared to normal rats (14.17 ± 3.41, 3.92 ± 0.05, 5.78 ± 0.24 and 2.91 ± 0.11). Treatment of diabetes using EESS with a loading dose 200 mg and 400 mg/kg body weight revitalised the levels of all these biochemicals (SOD, CAT and GSH) to that of normal. The treatment using EESS with a 400 mg/kg dosage was found to be niftier to revitalise the levels of biochemicals, than the 200 mg/kg dosage. (Table VII).

TABLE VII - Protective outcomes of EESS on antioxidant enzymes

Group No.	Administration/ Treatment	MDA (moles MDA/mg proteins/mL)	SOD (unit/ mg proteins)	Catalase µmol/ mg pr.min	GSH (nmol per minute per milligram)
1	Normal	1.43 ± 0.04	32.92 ± 2.45	4.32 ± 0.08	7.74 ± 0.34
2	Diabetic control	2.91 ± 0.11	14.17 ± 3.41	3.92 ± 0.05	5.78 ± 0.24
3	Standard	1.54 ± 0.04**	32.92 ± 2.92*	4.29 ± 0.06**	7.12 ± 0.49*
4	Ethanol extract 200 mg/kg	1.70 ± 0.07**	32.50 ± 4.18*	3.74 ± 0.06**	6.78 ± 0.18*
5	Ethanol extract 400mg/kg	1.57 ± 0.04**	32.92 ± 1.88*	3.89 ± 0.06**	7.29 ± 0.20*

Persistent hyperglycemia results in the generation of free radicals that cause a rise in oxidative stress leading damage to biomolecules, cells, and tissues, due to decreased antioxidant shielding system (Khaled *et al.,* 2011; Dahech *et al.,* 2011). Oxidation of lipids causes augments in the production of peroxides and free radicals due to a weakening defensive mechanism. In diabetes auto-oxidation and non-enzymatic glycation, resulting in an elevated level of oxygen free radicals, cripple SOD. Also, high glucose levels capable of inactivating glutathione peroxidase (GPx) due to glycation and severe oxidative stress are manifestations of GSH. Hydrogen peroxide ($H_2O_{2)}$ generated by the action of SOD on superoxide is metabolized by CAT. MDA is an unstable byproduct of lipid peroxidation. Oxidative stress is responsible for elevated MDA levels, which is an indicator for the measurement of oxidative stress. The current statistics show that STZ administration caused a significant increase in oxidative stress, as seen by a reduction in antioxidant enzyme activity. In this study, EESS displayed a marked increase in MDA and a decrease in SOD, CAT, and GSH in STZ-induced diabetic rats. The antioxidant repairing mechanism is attributed to the free radical scavenging action of EESS (Sajid *et al.,* 2020; Dworzański *et al.,* 2020)**.**

Defensive effect on the pancreas

Histo-architecture examination of the pancreas is depicted (Figure 4). The architecture of normal pancreas was well organised, while the diabetic pancreatic tissue shows degeneration in the islet due to necrosis. Also, reduction and devastation of β-cells was observed in the diabetic pancreatic cells. The standard drug glibenclamide when administered in diabetic rats repaired the normal structure of the islet. Furthermore, the administration of EESS at a dose of 200 mg/kg and 400 mg/kg to diabetic rats showed backtracked pathological changes to normal and degeneration of some islet cells.

Furthermore, the histological observations of the pancreatic tissue conclude that it is protected by the SS root extract. Recovery of the Langerhans' islet count to normal in diabetic rats with EESS may reveal the regeneration potential of the plant extract. There were findings of insulinogenic effects from different extracts of medicinal plants leading to the modulation of β-cells. An enhanced secretion of insulin from the β-cells or by rejuvenation of β-cells could be the possible cause for an antihyperglycemic effect (Prasad, Prabhu, 2012; Xue *et al.,* 2009).

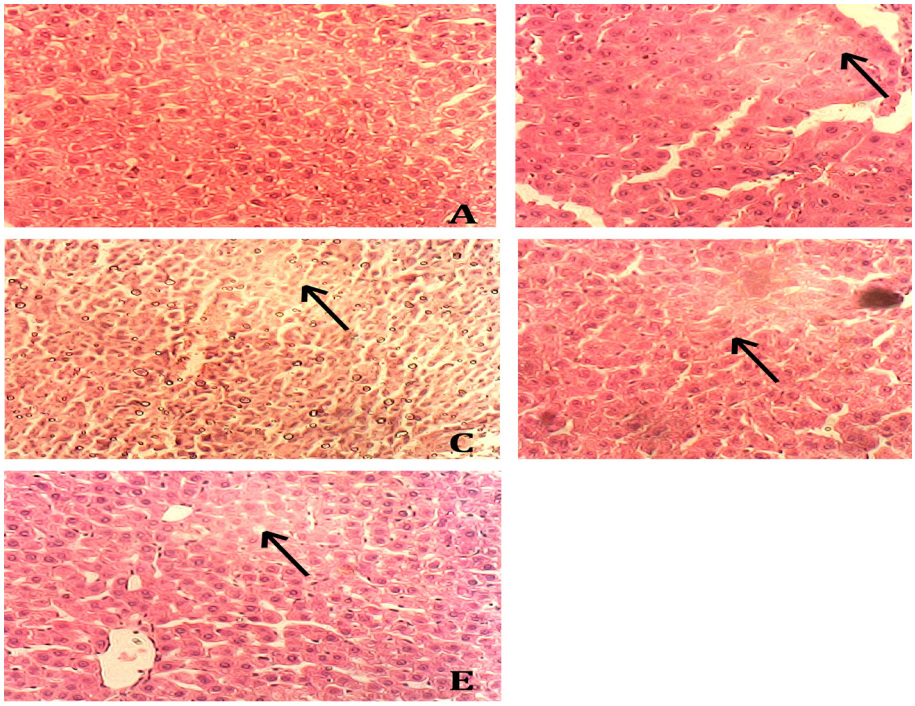

FIGURE 4 - Histo- architecture of Pancreas.

Pancreas histopathology

(A) Normal control rat pancreas showing normal islets of Langerhans with pale rounded and ovoid β-cells in the center (arrow), embedded in exocrine portion of pancreas. **(B)** Diabetic control rat pancreas showing shrinkage of islets of Langerhans with degeneration and necrosis of components cells where its nucleus appeared densely basophilic and karyolysis is evident (arrow) **(C)** Standard treated rat pancreas showing normal islets of Langerhans with pale rounded and ovoid β-cells in the center (arrow) **(D)** Pancreas of diabetic rat treated Ethanolic 200 mg/kg extract showing normal islets of Langerhans with its normal pale large round to ovoid shaped containing cells (arrow) that embedded in exocrine portion of pancreas. **(E)** Pancreas of diabetic rat treated with Ethanolic 400 mg/kg extract showing normal sized islets of Langerhans but some degeneration of the β cell in the center were noticed (arrow).

In conclusion, in the present study we evaluated the *in vitro* and *in vivo* antioxidant and antidiabetic potential of the ethanolic extract of *Spermadicyton suaveolens* using biochemical parameters. The *in vitro* antioxidants and antidiabetic activities were investigated for all extracts, among them ethanolic extract showed more dominancy over other. The EESS exerts anti-hyperglycemic potential in STZ-induced diabetic rats, with considerable effect compared to standard glibenclamide. And the serum investigation proved the anti-hyperlipidemic potential of EESS by restoring all the parameters to normal. However, future work is needed for isolation and identification of phytoconstituents using various spectral techniques. Moreover, systemic work would be needed for exploring the molecular mechanism responsible for antidiabetic action.

Ethics approval and consent to participate

The study was permitted by the Institution Animal Ethical Committee and Rajarambapu College of Pharmacy, Kasegaon (1290/PO/Re/S/09/CPCSEA, Protocol No. RCP/P-18/18-19 Dated: 16/03/2019).

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