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PHYTOCHEMICAL AND *IN-VITRO* ANTIDIABETIC SCREENING OF *SPERMADICYTON SUAVEOLENS*

Pratik Maske [1, * 2], Popat Kumbhar [2], John Disouza [2], Maya Sharma [1, 4] and Ashok Wali [5]

Pacific Academy of Higher Education and Research [1], Udaipur - 313001, Rajasthan, India.
Department of Pharmaceutical Chemistry [2], Genesis Institute of Pharmacy, Sonyachi Shiroli, Kolhapur - 416212, Maharashtra, India.
Tatyasaheb Kore College of Pharmacy [3], Warananagar, Kolhapur - 416113, Maharashtra, India.
Pacific College of Pharmacy [4], Pacific University, Udaipur - 313003, Rajasthan, India.
Hon. Shri. Annasaheb Dange Ayurved Medical College [5], Walawa, Sangli - 416301, Maharashtra, India.

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Correspondence to Author:
Mr. Pratik P. Maske

Research Scholar,
Pacific Academy of Higher Education and Research, Udaipur - 313001, Rajasthan, India.

E-mail: ppmaske.genesis@gmail.com

ABSTRACT: The discovery of alternative medications in diabetes management is of huge importance to achieve better safety and efficacy. The natural antioxidant and carbohydrate hydrolyzing enzyme inhibitors are gaining too much importance in the management of diabetes. Lack of scientific data is available on antioxidant and antidiabetic activities on root extract of *Spermadicyton suaveolens*. Therefore, we aimed to investigate the *in-vitro* antioxidant and α- amylase potential of *Spermadicyton suaveolens.* The roots were extracted by the successive Soxhlet extraction process. The extracts were evaluated for the presence of chemical constituents. Besides, the extracts were assessed for their antioxidant potential using a DPPH assay and α- amylase assay by the DNSA method. Phytochemical analysis acknowledged the presence of secondary metabolites alkaloid, phenol, saponins, steroids respectively. All the extracts showed moderate to high antioxidant and enzyme inhibition potential. Amongst all extracts, ethanolic extract showed the highest free radical scavenging potential (IC_{50}= 324 mg/mL). Moreover, the ethanolic extract also showed better α- amylase enzyme inhibition action (IC_{50}= 602 mg/mL). The finding from the study confirmed the traditional application of *spermadicyton suaveolens* as an antidiabetic agent, which is obvious through inhibition of enzyme action involved in carbohydrate metabolism and triggering antioxidant mechanism.

INTRODUCTION: Diabetes mellitus (DM) is one of the chief metabolic disorders that have severely impacted human health and wellness. The prevalence of diabetes in India in 2019 was 10.4%, an expected rise of 11.2% in 2030.

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India is the hub for diabetes after china, which was found 73 million diabetes carriers and it is projected that by 2045 the number is barely twice that of 134 million [1].

One of the basic menaces to human health is Type 2 diabetes, due to increased prevalence, chronic conditions, and debilitating consequences. Therefore, there is an unmet need to find out the effective treatment of this deadly and curable illness [2]. Inhibition of enzymes is a key therapeutic for hyperglycemia. Two types of enzymes are associated with the digestion of carbohydrates

namely α- amylase & α-glucosidase. The former is secreted by the pancreas and salivary gland while the latter one is secreted in the small intestine. α-amylase is a digestive enzyme and initiates the process of carbohydrate digestion [3]. It acts on carbohydrates such as starch and cleaves the α -1,4-glycosidic bond leading to the formation of oligosaccharide maltotriose and maltose. These oligosaccharides on further degradation forms glucose which enter systemic circulation lead to increased blood glucose level. This leads to increased postprandial hyperglycemia. Hence, the α- amylase plays a vital role in delaying carbohydrate digestion leading to the decreased glucose level in serum which decreases glucose serum level [4].

Furthermore, numerous studies linked diabetes mellitus and oxidative stress to each other. The overproduction of reactive oxygen species (ROS) like superoxide radical, hydroxyl radical, hydrogen peroxide, etc. was the cause of oxidative stress. Oxidative stress was negative regulatory effects on insulin resistance and secretion, due to an imbalance between formations and neutralizing of ROS is disturb. The biomolecules such as proteins, lipids, DNA, etc. are greatly affected due to ROS which causes permanent disturbances of cell function leads to cell death. The production and neutralization of ROS are well maintained by antioxidants. The externally supplied synthetic antioxidants are more harmful than plants derived products [5, 6, 7].

Conventionally various approaches have been available for treatment associated with noninsulin-dependent diabetes mellitus, stimulation of insulin secretion, and triggering insulin action on target tissues through sulfonylurea, biguanide, *etc*, delaying the absorption of glucose through enzyme inhibition respectively [8, 9]. The pharmacological mediators like acarbose inhibit the action of α-amylase, however; their applications are limited due to severe gastrointestinal adverse effects of bloating, abdominal discomfort, diarrhea, and flatulence respectively. Therefore, herbal remedies are gaining more attention in the treatment of diabetes due to the lack of side effects and cost-effectiveness as compared to synthetic drugs [10, 11]. In the current investigation, we have selected *Spermadictyon suaveolens* commonly called *Forest champ.* It is distributed occasionally in Maharashtra, the Himalayas region Kashmir region, and the Northern Areas of Pakistan. Traditionally it was widely used in various ailments bone, wound healing, herpes, diabetes, *et* [12]. The plant has been reported for its antimicrobial, antioxidant, wound healing properties. The root of this plant was investigated for its wound healing action on Wister rats [13]. The stem and leaf exhibited antioxidant, anti-inflammatory, antibacterial, antiulcerogenic activity [14]. Herein, without an acknowledged clinical review on its *in-vitro* antioxidant and anti-diabetic potential, the current study was designed to evaluate antioxidant and anti-diabetic activities of *S. suaveolens* root extracts through *in-vitro* models.

MATERIAL & METHODS:

Collection and Authentication of Plant Materials: The plant samples were submitted for botanical verification by a recognized taxonomist. The *Spermadicyton suaveolons* roots were chopped into small pieces and dried before being used as raw material. To acquire powder, the roots were pulverized on a Rising Automatic DP Pulverizer. The ensuing powder became surpassed through a 40 # sieve and stored in an airtight container

Preparation of Plant Extract by Soxhlet Extraction: Successive extraction was done using 50 g of powdered material of root rind in the soxhlet apparatus. The solvent petroleum ether (250 mL, 45 °C 15 cycles) was used for extraction, and after successive extraction with petroleum ether, chloroform (250 mL, 45 °C 15 cycles) and ethanol (250 mL, 60 °C 15-7 cycles). The aqueous extract was prepared by the maceration process. After completion of the extraction process, the extract was filtered and the filtrate was concentrated to about 50 mL on a water bath. The extract was transferred in a previously weighed evaporating dish. The total weight of evaporating dish containing extract was measured and the extract was further placed on a water bath for evaporation till it becomes viscous. The difference in weight was calculated every 10 minutes until a constant weight was obtained. All the four extract AESS (Aqueous extract of *Spermadicyton suaveolens*), EESS (Ethanolic extract of *Spermadicyton suaveolens*), CESS (Chloroform extract of *Spermadicyton suaveolens*) and PESS

(Petroleum ether extract of *Spermadicyton suaveolens*) were screened for *in-vitro* potential

Phytochemical Analysis: The preliminary investigation of secondary metabolite in the ethanolic root extract was confirmed by performing qualitative analysis as per standard protocol. Simply by the visual aids like a colour change or frothing is a signal for the presence or absence of secondary metabolites [15, 16, 17].

Tests for Anthraquinone: To 3 mL extract, 5 mL dil. H_2SO_4 was added. The mixture was boiled in a water bath, cooled and filtered. The cold filtrate was extracted with benzene or chloroform and the organic solvent was separated. Then, an equal volume of ammonia was added to the above extract. The ammonical layer was turned pink to red colour due to the presence of anthraquinone moiety.

Test for Saponins: 2 g of extract was mixed with 10 mL of distilled water and transferred to the graduated cylinder, agitated vigorously. The setup was left aside 15 min for persistent frothing.

Test for Alkaloid: 2 g of extract was mixed with 10 mL of 1% hydrochloric acid warmed on the water bath for 5 min the mixture was filtered through Whatman filter paper. Then 3 drops of Wagner's reagent were added to the 3 mL filtrate. A reddish-brown coloured precipitate is a confirmation of the presence of an alkaloid.

Test for Cardiac Glycoside: 0.3 g of extract was dissolved in 3mL glacial acetic acid containing 5% ferric chloride solution. Then 2 mL concentrated sulphuric acid was added slowly from the sides of the test tube. Development of reddish-brown layer, changes to bluish green on the standing indication of the presence of cardenolides.

Test for Flavonoids: An equimolar volume of extract and 2M hydrochloric acid was heated on a water bath for 30 min, filtered through Whatman filter paper.

The filtrate was then extracted with ethyl acetate, concentrated to dryness to which 5 mL of concentrated hydrochloric acid was added. The formation of red colour followed by purple is an indication of the presence of flavonoids.

Test for Steroids: Mixed 3 mL extract with 3 mL acetic anhydride warmed on a water bath and cooled the resulting solution. Then few drops of concentrated sulphuric acid were added to the above mixture. Blue coloration is a positive indication of the presence of steroids.

Test for Tannins: 10% sodium chloride containing 1% gelatin solution was added to a 2 mL extract. A white precipitate was formed, revealing the presence of tannins in the extract.

Test for Phenol: 1 g of extract was mixed with 10 mL of ethanol, boiled in a water bath for 10 min. and then filtered through Whatman filter paper. 3 mL 5% ferric chloride solution was added into the above-cooled solution. The formation of a green precipitate indicates the presence of phenol.

Test for Triterpenes: 1 g of extract was mixed with 10 mL chloroform and then filtered through Whatman filter paper. Few drops of concentrated sulphuric acid were added to the filtrate, stirred, and allowed to stand for 5 min. The development of golden yellow colour has confirmed the triterpenes.

***In-vitro* Antioxidant:**

DPPH Radical Scavenging Assay: The free radical scavenging activity of AESS, EESS, CESS and PESS extracts was estimated *in-vitro* by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay as per Brand-Williams method with slight modifications. Briefly, the methanolic DPPH solution of 0.5 mM was prepared. The extract was dissolved in DMSO solution. 2 mL of 0.5 mM methanolic DPPH solution was mixed with 2 mL extracts of various concentrations (100, 200, 400, 600, 800 and 1000 µg/mL). The resultant mixtures were mixed and kept aside at room temperature in a dark for 60 min. The ascorbic acid (AA) was served as a positive control. The DPPH was reduced due to the donation of hydrogen by the antioxidant compound. The absorbance was measured at 517 nm and the reaction was conceding three times [18].

α- amylase Activity: *In-vitro* amylase inhibition was studied by the method of Bernfeld. The α-amylase inhibition assay was carried out using the 3, 5-dinitrosalicylic acid (DNSA) method. Various concentrations of 10 to1000 µg/mL were prepared by dissolving root extract in 10% DMSO solvent.

The mixture of 400 µL of α-amylase and extract was prepared by dissolving in 10% DMSO solution equally. The resultant solution was incubated at 30 °C for 10 min.

Then 200 µL of 1% starch solution was added after 20 min of incubation followed by the addition of 200 µL of DNSA reagent to complete the reaction.

The mixture was then boiled at 85-90 °C on a water bath for 10 min, cooled, and diluted with 5 mL distilled water. Finally, the absorbance was measured at 540 nm using a UV-Visible spectrophotometer. Acarbose solution is considered a positive control. The α-amylase inhibitory activity was expressed as percent inhibition [19].

Statistical Analysis: The outcomes of samples were displayed as mean ± standard error means of triplet analysis. Statistical analysis was performed in a one-way analysis of variance. Values of $p < 0.01$ and $p < 0.05$ were considered to be statistically significant.

RESULT:

Preparation and Phytochemical Analysis of the Extract: The four extracts were prepared by successive Soxhlet extraction. All the extracts were evaluated for the presence of secondary metabolites. The extracts showed the presence of various secondary metabolites such as alkaloid, phenol, saponins, steroids, *etc*. respectively. The outcome of the screening was shown in **Table 1.**

TABLE 1: PHYTOCHEMICAL SCREENING OF EXTRACT OF *SPERMADICYTON SUAVEOLENS*

Photochemical	Ethanolic Extract (EESS)	Chloroform Extract (CESS)	Petroleum ether Extract (PESS)
Anthraquinone	--	--	--
Saponins	++	++	++
Alkaloid	++	--	++
Cardiac Glycoside	++	++	--
Flavonoids	--	--	--
Steroids	++	++	++
Tannins	--	--	--
Phenol	++	++	++
Triterpenes	--	--	--

++: Detected, --: Not detected.

DPPH Radical Scavenging Assay: The free radical scavenging action of four solvent extracts was evaluated by DPPH assay and was expressed in % inhibition and compared with standard antioxidant ascorbic acid.

The free radical scavenging action of all the extracts **Table 2** indicated the % inhibition of DPPH assorted with polarities of solvent.

TABLE 2: FREE RADICAL SCAVENGING POTENTIAL OF *SPERMADICYTON SUAVEOLENS*

Extract	DPPH (mg/mL)
AESS	455.84 ± 0.97
EESS	324 ± 0.32
CESS	500 ± 1.02
PESS	522.72 ± 0.87
Std.	269.6 ± 0.25

Values are expressed as mean ± SD (n=3).

The positive control AA showed a dose-based scavenging activity of DPPH radical, at a concentration of 1000 mg/mL it scavenges 88.75% free DPPH radical. The half-maximal inhibitory concentration of AA was found to be 269.6 mg/mL. All extracts exhibited medium to excellent free radical scavenging action. The EESS showed significant ($p<0.01$) scavenging activity when compared to AESS, CESS and PESS.

α- amylase Activity: To evaluate the antidiabetic potential of the *S. Suaveleons* an *in-vitro* α-amylase activity was performed. The pharmacological mediator acarbose is used as a standard to compare the inhibitory action. The maximum inhibitory concentration (IC_{50}) values for AESS, EESS, CESS and PESS are presented in **Table 3.**

TABLE 3: A- AMYLASE INHIBITION POTENTIAL OF *SPERMADICYTON SUAVEOLENS*

Extract	α- amylase (mg/mL)
AESS	805.32 ± 3.71
EESS	602.19 ± 1.42
CESS	753.68 ± 2.23
PESS	735.35 ± 3.03
Std.	436.04 ± 9.25

Values are expressed as mean ± SD (n=3)

The EESS displayed significant ($p<0.01$) activity (IC_{50} of 602 ±1.42 mg/mL) when compared to AESS, CESS and PESS. Acarbose exhibited potent α-amylase inhibitory action with a

maximum inhibitory concentration (IC_{50}) 421.13 ± 1.75 mg/mL.

DISCUSSION: Medicines have a significant benefit for humanity as various findings demonstrated that plant extracts include not just minerals and primary metabolites, but additionally an enormous variety of secondary metabolites with tremendous healing efficiencies. The phyto-chemical studies showed the presence of various secondary metabolites. The presence of alkaloids, phenol, saponins steroids may be contributed for antioxidant and antidiabetic potential [20, 21].

Oxidative stress is one of the major causes of pathogenesis and amelioration of diabetes. Herbal antioxidants are recognized to have a significant role in the prevention of most age-related illnesses and improvement of overall health [22]. The antioxidant potential of extracts was estimated by the DPPH method *in-vitro.*

In this assay, the reduction of purple-coloured DPPH to yellow hydrazine is due to the presence of antioxidant action of the extracts. The standard compound exhibited maximum antioxidant action. On the other hand, the EESS, AESS, CESS and PESS extracts exhibited less antioxidant activity when compared with standard.

Among all the extracts, EESS displayed remarkable free radical scavenging action. These extracts showed antioxidant activity by eliminating free radicals, which can be produced under hyperglycemic conditions [21]. Furthermore, the antioxidant activity of these extracts may be attributed to the presence of diverse metabolites in *S. suaveolens* extracts. Postprandial hyperglycemia is mainly responsible for two carbohydrate enzymes (α-amylase and α-glucosidase).

The α-amylase initiates the process of digestion of carbohydrates by hydrolysis, which catalyzes disaccharides to monosaccharides through 1, 4 glycosidic linkage of disaccharides (starch, glycogen) and which leads to postprandial high blood glucose. In the management of hyperglycemia, α-amylase inhibitors are beneficial to prolong the digestion of carbohydrates and therefore lower postprandial glucose levels [23, 24]. The inhibition of enzymes is the crucial approach capable of successful therapies to major public health problems worldwide, including diabetes mellitus. Based on our results, all the extracts showed medium to elevated α-amylase inhibition potential. All three extracts exhibited dose-dependent suppression of pancreatic amylase. These α-amylase inhibition potentials of plant extracts could be due to the presence of secondary metabolites such as indole alkaloids, anthraquinones, terpenoids (diterpenes and triterpenes), flavonoids, and other phenolic derivatives. Moreover, these metabolites have the potential to delay starch digestion that can be responsible for the antidiabetic activity of plant extracts [25].

CONCLUSION: The chemical screening of extract showed the presence of a mixture of secondary metabolites such as cardiac glycoside, steroids, alkaloids, and saponins. The *S. suaveolens* extracts were screened for *in-vitro* antidiabetic and antioxidant properties. Among the four extracts prepared, the ethanolic extract of *S. suaveolens* exhibited significant *in-vitro* antidiabetic and antioxidant properties. In contrast, the antioxidant activity has been strongly correlated with the inhibitory potential α-amylase in various samples. Thus, *S. suaveolens* extracts could be promising in the effective treatment of diabetes. However, further experimental work is needed for exploring *in-vivo* antidiabetic as well as antioxidant potential.

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