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RESEARCH ARTICLE

In Vitro Anti-oxidant and Antidiabetic Activity of the Leaves of *Stereospermum sauveolens* (Roxb.) DC

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Abstract

Background: Diabetes mellitus is a metabolic condition characterised by a persistent rise in blood glucose levels. Diabetes is just one of the many disorders that can be treated using natural remedies made from plants. *Stereospermum suaveolens* (Roxb.) DC, belongs to the family Bignoniaceae, commonly known as Padri. It is employed as a folk remedy in southern India to cure diabetes. **Aim:** The aim of this study is to assess the potential anti-diabetic and antioxidant properties of the hydroalcoholic extract of *S. suaveolens* (Roxb.) DC leaves through phytochemical screening and *in vitro* assays. **Methods:** Leaves of *S. suaveolens* (Roxb.) DC were collected and processed to obtain a hydroalcoholic extract. The extract was subjected to qualitative analysis to identify the presence of various phytochemical constituents. The free radical scavenging activity of the extract was evaluated using Nitric oxide radical scavenging assay and DPPH racial scavenging assay. *In vitro* anti-diabetic studies were performed through α-amylase inhibition assay and glucose uptake assay. **Results:** The hydroalcoholic extract of *S. suaveolens* (Roxb.) DC leaves exhibited the presence of bioactive compounds. The extract demonstrated dose-dependent free radical scavenging activity in the *in vitro* antioxidant assays. The extract showed remarkable inhibitory activity against α-amylase, suggesting its potential in controlling carbohydrate digestion. Also the extract significantly enhanced glucose uptake by yeast cells, indicating its potential to regulate blood glucose levels. **Conclusion:** The findings of this study indicate that the hydroalcoholic extract of *S. suaveolens* (Roxb.) DC leaves possesses both antioxidant and anti-diabetic properties. These results suggest its potential as a natural medicinal agent for the management and treatment of type 2 diabetes mellitus.

Keywords: Antidiabetic Activity, Antioxidant Activity, *Stereospermum suaveolens*

1. Introduction

A metabolic condition called Diabetes mellitus is characterized by a persistent rise in blood glucose levels. Nearly 300 million individuals worldwide have diabetes, which is caused by the body's inability to produce enough insulin to meet its own needs, either due to impaired insulin production, impaired insulin action, or both[1]. Type 1 diabetes mellitus, type 2 diabetes mellitus, and gestational diabetes mellitus are the three categories under which diabetes mellitus is categorised. Anti-glutamic acid decarboxylase antibodies, which cause local anti-inflammatory action in and around islets and result in the loss of the beta cells, are the hallmarks of type 1 diabetes, also known as juvenile diabetes. Up to 80 – 90 % of all instances of Diabetes mellitus are type 2 diabetes. Type 2 diabetes is distinguished by decreased insulin production or the hormone's peripheral resistance effect. Obesity, advanced age, family history of diabetes, and inactivity are the main risk factors for type 2 diabetes. Medication, exercise, and dietary modifications can all be used to treat type 2 diabetes. Instead of being a pathophysiologic disorder, gestational diabetes mellitus is an operational

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classification. Women who have type 2 diabetes mellitus are diagnosed when they are pregnant[2].

The elevation in blood glucose level can cause blurred vision, very high level of blood sugar can cause coma and even death[3]. About 75% of deaths in diabetes are due to coronary artery disease the 1 complication of diabetes can cause damage to eyes, kidneys and nerves. There is no satisfactory treatment for diabetes in modern medicine. However, there are many synthetic drugs for the treatment of diabetes, but continuous use of these drugs can cause many side effects and are highly expensive[4]. Sulfonylureas and non-sulfonylureas like alpha glycosides inhibitors, meglitinide analogues, thiazolidinediones and biguanides are the list of drugs which currently available as oral anti-diabetic drugs. In recent years the use of natural products obtained from plants gained importance worldwide due to their low side effects and low cost[5]. Medicinal plants play an important role in the development and effective treatment of modern herbal medicine in the treatment of many diseases such as cancer, liver disease, arthritis and diabetes. Many plants are being used as folk medicine for the treatment of diabetes.

In this study, *S. suaveolens* (Roxb.) DC plant was selected for the anti-diabetic study. *S. suaveolens*, belongs to the family Bignoniaceae, commonly known as Padri. It is a tree that is deciduous and is likely found throughout India. Asthma, discomfort, and inflammation are all treated with a decoction of the root. Hiccups can be controlled by administering flowers and honey orally. It is employed as a folk remedy in southern India to curediabetes[6-8]. The bark of *S. suaveolens* (Roxb.) DC has an anti-diabetic activity in STZ-induced diabetic rats[9]. Dinatin-7-glucuronide and 6-*o*-*β*-D-Glucosylscutellarein were isolated from the leaves of the plant[10-15]. However, there is no scientific report regarding the antidiabetic ability of the plant leaves based on the literature review. Hence, an attempt is made to evaluate the anti- diabetic potential of the leaves of the plant *S. suaveolens* by *in vitro* assays.

2. Materials and Methods

2.1 Plant Material

The leaves of the plant *S. suaveolens* was collected from Tripathi, Andhra Pradesh. The leaves were identified and authenticated by Professor P.Jayaraman, Plant Anatomy Research, Tambaram, Chennai, by referring to the voucher specimen number [PARC/2012/1080] and deposited at the Department of Pharmacognosy, Sri Ramachandra Faculty of Pharmacy, Sri Ramachandra Institute of Higher Education and Research, Chennai.

2.2 Preparation of Hydro-alcoholic Extract

Stereospermum suaveolens (Roxb.) DC leaves were collected, cut into small pieces, dried in the shade, and ground into a coarse powder. By cold macerating the powdered material for 72 hours, 48 hours, and 24 hours, 50% ethanol was used to extract it in an aspirator container. After decantation and filtration, distillation utilising a rotary vacuum evaporator was used to extract about 80% of the solvent. The extract was also dried in vacuum desiccators.

2.3 Phytochemical Analysis

Preliminary phytochemical analysis of the powdered leaves and hydroalcoholic extract of *S. suaveolens* (HASS) were performed for the identification of constituents like terpenoids, flavonoids, steroids, anthraquinone, glycosides, carbohydrates, quinones, saponins, protein and amino acids using standard methods[12-19].

2.4 Fluorescence Analysis

The fluorescence character of the powdered leaves and HASS were studied in both the daylight and UV light.

2.5 Antioxidant Determination[20-23]

2.5.1 Nitric Oxide Radical Scavenging Assay

Different concentrations of the extract (10, 50, 100, 200, 400, 800, 1000 µg/ml) were added to a reaction mixture of 3 ml containing sodium nitroprusside and 10 mM in phosphate-buffered saline. The reaction mixture was then incubated at 37°C for 4 hours. Each extract concentration was made in a duplicate. The control sample was maintained in the same way as the test sample. The aforementioned solution was mixed with 0.5 ml of Griess reagent, and the absorbance was calculated at 546 nm. By excluding the extract from the reaction mixture, ascorbic acid was produced in triplicate and utilised as the standard.

2.5.2 DPPH Radical Scavenging Assay

About 100 ml of ethanol was used to make a stock solution containing 25 mg of DPPH (200 M), and 0.05 ml of extract was combined with various ethanol concentrations of 10, 50, 100, 200, 400, 800, and 1000 µg/ml. The standard was ascorbic acid, which was made in triplicate and used in place of the extract. Spectrophotometric analysis was used to measure the DPPH scavenging activity at 517 nm.

2.6 Evaluation of Anti-Diabetic Activity Using *In Vitro* Assay

2.6.1 α – Amylase Inhibition Assay[20-24]

The alpha-amylase inhibitory assay was carried out using the chromogenic DNSA method. 500µl of enzyme solution was mixed with 1 ml of different concentrations of the extract and incubated at 37°C for 10 min. Thereafter 500µl of starch solution was added to each test tube and incubated at 37°C for 10mins. The reaction was terminated by the addition of 1ml of the DNSA reagent and incubated in boiling water for 5 minutes. It was then cooled, diluted with 10ml of water and measured at 540 nm. Acarbose was used as the standard. The % inhibition was calculated using the formula:

$$\%\,alpha\,amylase\,inhibition = \frac{EC-(ET-TC)}{EC}\times 100$$

Where, EC- Enzyme activity of control, ET- Enzyme activity of test, TC- Test control.

2.6.2 Glucose Uptake Capacity of Yeast Cells[25]

Yeast suspension was made by repeatedly centrifuging 25mM yeast at 3000 rpm in distilled water until the supernatant liquid was crystal clear. Various extract concentrations (250, 500, 750, and 1000 µg) were combined with about 1 ml of a glucose solution (5, 10, and 25 mM), which was then incubated for 10 minutes at 37°C. The reaction was initiated by adding 100 l of the yeast suspension, vortexed, and continued for an additional hour at 37°C. The reaction mixture was centrifuged for five minutes at 2500 rpm after an hour, and the amount of glucose inthe supernatant was calculated. Metformin was consumed as usual. The following formula was used to determine the per cent increase in yeast cells' absorption of glucose:

$$\%\,increase\,in\,glucose\,upcake = \frac{Absorbance(sample)-Absorbance(control)}{Absorbance(sample)}*100$$

3. Results

3.1 Phytochemical Analysis

Table 1 shows the results of a preliminary phytochemical examination of the powdered leaf material and HASS, which revealed the presence of terpenes, flavonoids, steroids, tannins, carbohydrates, quinones, phenols, saponins, and proteins.

3.2 Fluorescence Analysis

The fluorescence properties of the powdered plant material and HASS revealed the existence of fluorescence ingredients like flavonoids and helped in the identification of the plant. Tables 2 and 3 display the outcome.

3.3 *In Vitro* Antioxidant Activity

The antioxidant potential of the HASS was studied by using an in vitro assay method. In the nitric oxide radical scavenging assay, the hydroalcoholic extract showed 8.52 – 61.24 %. in DPPH radical scavenging assay the hydroalcoholic assay showed a maximum of 84.46% of antioxidant activity for a concentration of 1000µg/ml. The result is shown in Table 4 and Figures 1 and 2.

3.4 *In Vitro* Anti-diabetic Activity

Anti-diabetic activity of the leaves was screened by in vitro method and the alpha-amylase inhibition and glucose uptake by yeast cell assay method were presented in Table 5 and Figures 3 and 4.

4. Discussion

Numerous plants are regarded as important sources of strong anti-diabetic medications. Although a number of oral hypoglycemics are used in conjunction with insulin to treat diabetes. However, they exhibit several adverse effects and were unable to address the root of the difficulties[5]. This study examined the leaves of *S. suaveolens* for their antioxidant and anti-diabetic properties. The antioxidant activity was screened through nitric oxide scavenging activity [61.24%] and

Table 1. Preliminary phytochemical analysis of powdered leaves and HASS

S. No.	Test	Reagent	Result	
			Leaf powder	HASS
1	Terpenoids	Tin metal + Thionyl chloride	+	-
2	Steroids	Sulphuric acid	+	-
3	Flavanoids	Megnesium turnings + Conc. Hydochloric acid	+	+
4	Alkaloids	Wagner's Reagent	-	-
5	Quinones	Sodium hydroxide	+	+
6	Carbohydrates	Fehling's A and B	+	+
7	Glycosides	Anthrone +Sulphuric acid	+	+
8	Tannins	Lead acetate	+	+
9	Anthraquinones	Strong Ammonia	-	-
10	Phenols	Ferric chloride	+	+
11	Saponins	Water	+	+
12	Protiens	Copper sulphate + Sodium hydroxide	+	+

HASS – Hydro alcoholic extract, (+) Present (-) Absent

Table 2. Fluorescence analysis of powdered leaves of *S. suaveolens* (Roxb.) DC

S. No.	Reagent Treatment	Daylight	UV Light	
			Short (254 nm)	Long (365 nm)
1	Powder alone	Pale brown	Dark green	Dark brown
2	Water	Yellow	Green	Dark brown
3	1N Hydrochloric acid	Pale yellow	Green	Dark brown
4	1N Nitric acid	Yellow	Green	Brown
5	1N Sulphuric acid	Pale yellow	Green	Brown
6	1N Sodium hydroxide	Reddish brown	Green	Dark green
7	Alcoholic Sodium hydroxide	Colourless	Green	Brown
8	1N Potassium hydroxide	Brown	Pale green	Dark green
9	Alcoholic Potassium hydroxide	Pale yellow	Pale green	Dark green
10	Strong Ammonia	Brown	Pale green	Dark green

Table 3. Fluorescence analysis of HASS

Extract	Daylight	UV light	
		Short (254 nm)	Long (365 nm)
Hydro alcohol	Dark brown	Green	Brown

DPPH scavenging activity [74.57%] *in vitro*. It was discovered that DPPH radical scavenging activity was the most effective. When compared to ascorbic acid, the hydro alcoholic extract of *S. suaveolens* leaves (HASS) demonstrated dose-dependent free radical scavenging activity. Between the two methods, the DPPH radical scavenging assay had the highest % inhibition. Antioxidants, along with Reactive Oxygen Species (ROS), are crucial in the development of many diseases and disorders. Following that, plants are the primary source of antioxidant molecules that have the power to either eliminate or neutralise ROS. The presence of phytoconstituents including phenols, tannins, and flavonoids can demonstrate the leaves' strong antioxidant qualities.

Based on the various parts employed and their mode of action, some medicinal plants have anti-diabetic potential[8]. One of the mechanisms by which the plant exhibits anti-diabetic action is enzyme

Table 4. *In vitro* antioxidant activity of HASS

Conc. In µg/ml	Percent Inhibition			
	Nitric oxide radical scavenging method		DPPH radical scavenging method	
	Hydro alcoholic extract (HASS)	Ascorbic acid	Hydro alcoholic extract (HASS)	Ascorbic acid
50	8.5248	34.5173	7.5624	69.0348
100	12.6084	47.5698	16.5426	70.1416
200	16.6047	52.1987	23.0678	72.1171
400	29.2750	57.2149	44.2345	75.0803
800	41.1400	65.4375	59.5748	78.3767
1000	61.2453	78.2415	74.5716	84.4698

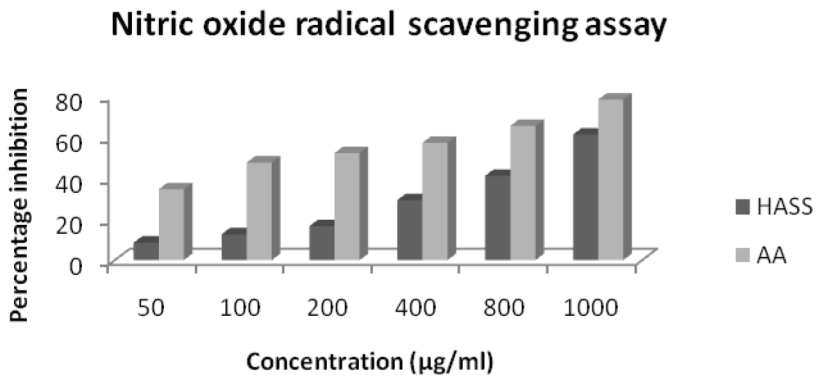

Figure 1. Nitric oxide radical scavenging activity of HASS.

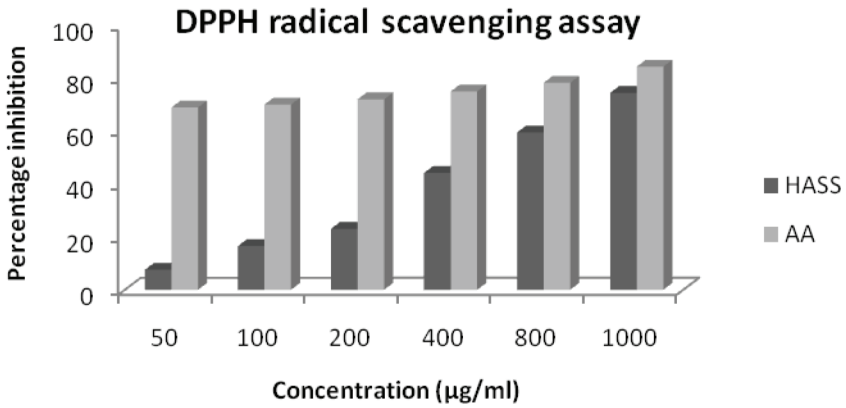

Figure 2. DPPH radical scavenging activity HASS.

inhibition. Alpha-amylase is an enzyme that breaks down polysaccharide -bonds to produce large amounts of glucose and maltose. Saliva and pancreatic juice both include the enzyme alpha-amylase. Alpha-amylase inhibitors have been shown to reduce postprandial hyperglycemia levels effectively. Several medicinal plants also contain alpha-amylase and glucosidase inhibitors, making them potent alternatives to synthetic medications[4].

According to the findings, *S. suaveolens* hydroalcoholic extract can suppress alpha- amylase. A rise in extract concentration indicates an increase in the rate of glucose absorption. Concentration gradient[21] is involved in glucose transportation. Facilitated diffusion is involved in the transport of glucose through the yeast membrane. Effectively binding to glucose, the extract is transferred across the cell membrane for further metabolism[26]. According to the research, the hydroalcoholic extract can

Table 5. *In vitro* antidiabetic activity of HASS

Conc. in µg/ml	*In vitro* antidiabetic activity			
	Alpha amylase inhibition assay (Percentage inhibition)		Glucose uptake by yeast cells method (Percentage increase in uptake)	
	Hydro alcoholic extract (HASS)	Acarbose	Hydro alcoholic extract (HASS)	Metformin
50	8.1267	52.9756	4.1578	9.6724
100	17.4528	58.9756	12.2348	14.2678
200	23.7458	62.7589	18.1679	19.4857
400	35.1768	69.1586	22.6978	25.4872
800	43.5125	75.5623	29.2978	32.1879
1000	57.2357	81.0245	44.4278	49.8304

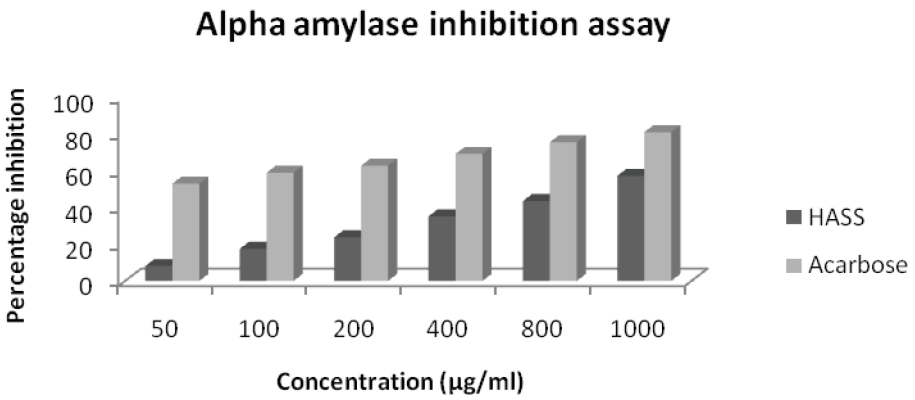

Figure 3. Alpha amylase inhibition activity of HASS.

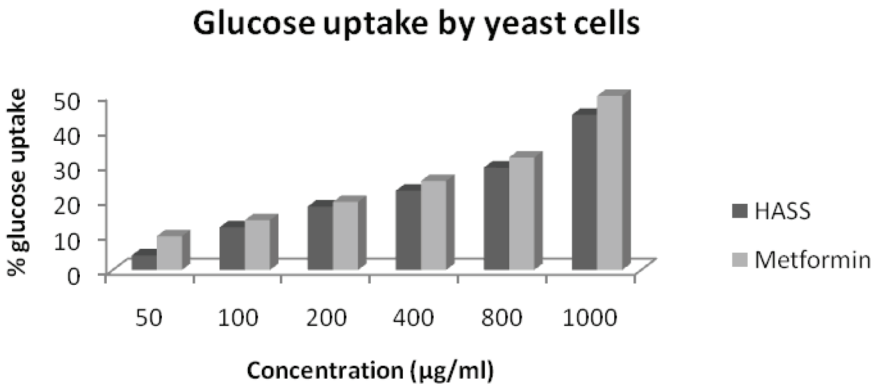

Figure 4. Glucose uptake activity of HASS.

increase glucose absorption while lowering blood glucose levels. The hydroalcoholic extract has noteworthy efficacy, inhibiting alpha-amylase by 57.23% and significantly increasing glucose absorption by 44.42%. Thus, research demonstrates that using *S. suaveolens* leaf extract can help to decrease glucose absorption. A therapeutic strategy for the control of diabetes is provided by plant-based alpha inhibition and glucose absorption.

5. Conclusion

Based on the result obtained from this study shows that the leaves of the plant *S. suaveolens* exhibit a remarkable anti-diabetic and anti-oxidant activity in the Hydro alcoholic extract of the leaves. The leaves of the plant *S. suaveolens* has the potential for the control and treatment of type 2 diabetes. Furthermore, research has

to be carried out to discovera novel effective drug for diabetes which has both anti-diabetic and antioxidant properties.

6. References

1. Baynes HW. Classification, pathophysiology, diagnosis and management of diabetes mellitus. J Diabetes Metab. 2015; 6(5):1-9.
2. Wherrett D, Huot C, Mitchell B, Pacaud D. Type 1 diabetes in children and adolescents. Can J Diabetes. 2013; 37:S153-62. https://doi.org/10.1016/j.jcjd.2013.01.042 PMid:24070940
3. Gupta PD, De A. Diabetes Mellitus and its herbal treatment. Int J Res Pharmaceut Biomed Sci. 2012; 3:706-721.
4. Kazeem MI, Adamson JO, Ogunwande IA. Modes of inhibition of α-amylase and α-glucosidase by aqueous extract of *Morinda lucida* Benth leaf. BioMed Res Int. 2013; 2013. https://doi.org/10.1155/2013/527570 PMid:24455701 PMCid:PMC3884628
5. Rao MU, Sreenivasulu M, Chengaiah B, Reddy KJ, Chetty CM. Herbal medicines for diabetes mellitus: A review. Int J Pharm Tech Res. 2010; 2(3):1883-92.
6. Joshi SK, Sharma BD, Bhatia CR, Singh RV, Thakur RS. The wealth of India raw materials. Council of Scientific and Industrial Research Publication, New Delhi. 1992; 3:270-1.
7. Kirtikar KR, Basu BD. Indian medicinal plants. 2nd ed. Dehradun (India): International Book Distributors; 1988. p. 1848-49.
8. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian medicinal plants. 5th ed. New Delhi (India): National Institute of Science Communication, 1999. p. 234.
9. Balasubramanian T, Lal MS, Sarkar M, Chatterjee TK. Antihyperglycemic and antioxidant activities of medicinal plant *Stereospermum suaveolens* in streptozotocin-induced diabetic rats. J Diet Suppl. 2009; 6(3):227-51. https://doi.org/10.1080/19390210903070780 PMid:22435475
10. Anonymous Wealth of India, Council of Scientific and Industrial Research, Vol-VII, p. 51.
11. Kritikar KR, Basu. Indian Medicinal Plants, Popular Prakashan. 1999; 2:1848.
12. Rao KV, McBride TJ, Oleson JJ. Recognition and evaluation of lapachol as an Antitumor agent. Cancer Res. 1968; 28(10):1952-4.
13. Subramanian SS, Nagarajan S, Sulochana N. Flavonoids of eight bignoniaceous plants. Phytochem. 1972.
14. Joshi KC. Chemical examination of the roots of *Stereospermum suaveolens* DC. J Indian Chem Soc. 1977; 54(6):648-9.
15. Haque M, Rahman K, Begum B, Hasan C, Rashid M. Secondary metabolites from *Sterespermum chelonoides*. Dhaka University J Pharm Sci. 2005; 4(1):12-6. https://doi.org/10.3329/dujps.v4i1.201
16. Nair AG, Mohandoss S, Voirin B, Bayet C, favrebonvin j. 6-o-beta-d-glucosylscutellarein-a rare flavone glycoside from *Stereospermum suaveolens*. J of the Indian Chem Soc. 1988; 65(2):150-1.
17. Dhar ML, Dhar MM, Dhawan BN, Mehrotra BN, Ray C. Screening of Indian plants for biological activity: Part I.
18. Balasubramanian. T, Tapan Kumar Chatterjee, Mahananda Sarkar, Sundar Lal Meena. Anti-inflammatory effect of *Stereospermum suaveolens* ethanol extract in rats. Pharm Boitechnol. 2010; 48(3):318-23. https://doi.org/10.3109/13880200903127383 PMid:20645819
19. Thirumalaisamy B, Prabhakaran SG, Marimuthu K, Chatterjee TK. Antihyperlipidemic activity of the Ethyl-acetate fraction of *Stereospermum suaveolens* in Streptozotocin-induced diabetic rats. J Pharmacopunct. 2013; 16(3):23. https://doi.org/10.3831/KPI.2013.16.020 PMid:25780672 PMCid:PMC4331969
20. Latha S, Grace XF, Shanthi S, Chamundeeswari D, Seethalakshmi S, Reddy CU. *In vitro* antioxidant and anti-inflammatory activity of methanol extract of *Stereospermum colais* (Buch.-ham. ex. dillw). Sri Ramachandra J Med. 2011; 4(1):11-4.
21. Shabna RM. Ram NR. Sushmitha KP. Suchithra N. Shruti S. Sugumar M. *In vitro* biological evaluation of *Acanthophora spicifera*. Res J Pharm Technol. 2020; 13(10):4777-83. https://doi.org/10.5958/0974-360X.2020.00840.9
22. Shanthi S, Ramya maddali, Fatima Grace X, Pandi Tejaswi Alekya, Latha S, Chamundeeswari D. Pharmacognostical and preliminary phytochemical studies on stem bark of *Eugenia jambolana*. Int J Pharm Sci Rev Res. 2014; 29(1):74-6.
23. Prakash G, Yoganandam, Gopal V, Thanka J. Promising *in vitro* anti-diabetic and anti- oxidant activity of *Aavarai kudineer* - A potent polyherbal Siddha formulation. Int J Phytopharmacol. 2015; 6(4):2290-34.
24. Vijayalakshmi K, Selvaraj IC, Sindhu S, Arumugam P. *In vitro* investigation of antidiabetic potential of selected traditional medicinal plants. Int J Pharmacogn Phytochem Res. 2015; 6(4):856-61.
25. Abirami N, Natarajan B, Sagadevan E. Phytochemical investigation and *in vitro* evaluation of hypoglycemic potential of *Grewia hirsuta*. Int J Pharm Bio Sci. 2014; 5(1):76-83.
26. Rehman G, Hamayun M, Iqbal A, Ul Islam S, Arshad S, Zaman K, Ahmad A, Shehzad A, Hussain A, Lee I. *In vitro* antidiabetic effects and antioxidant potential of *Cassia nemophila* pods. BioMed Res Int. 2018; 2018. https://doi.org/10.1155/2018/1824790 PMid:29607313 PMCid:PMC5827890