Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2016; 5(6): 80-84
Received: 10-09-2016
Accepted: 11-10-2016

Sujila V
Department of Pharmaceutical chemistry, Devaki Amma Memorial College of Pharmacy, Chelembra, Kerala, India.

Dr. Biju CR
Vice principal and HoD Devaki Amma Memorial College of Pharmacy. Chelembra, Kerala, India.

Dr. G Babu
Principal and HoD Devaki Amma Memorial College of Pharmacy, Chelembra Kerala, India

Correspondence
Dr. Biju CR
Vice principal and HoD Devaki Amma Memorial College of Pharmacy. Chelembra, Kerala, India.

Evaluation of antidiabetic activity of bioactive constituent of *Sauropus androgynus* in alloxan induced diabetic Rats and effect on inhibition of α-glucosidase enzyme

Sujila V, Dr. Biju CR and Dr. G Babu

Abstract
The antidiabetic effect of bioactive constituent isolated from alcoholic extract of the leaves of *S. androgynus* investigated in alloxan induced diabetic rats. The antidiabetic potential tested by docking studies using isolated compound as ligand and intestinal α-glucosidase as receptor. The isolate and the standard hypoglycaemic drug glibenclamide (10mg/kg) were dissolved in isotonic saline solution (NaCl, 0.9%w/v) and were administered orally. Diabetes was induced in by a single intraperitoneal injection of 150mg/kg alloxan monohydrate. Hypoglycaemia was confirmed by the elevated glucose levels determined at 72 hr after alloxan injection. The rats having blood glucose levels above 200mg/dL were used for the antidiabetic study. The isolate and standard drug were administered orally for 14 days. The fasting blood samples were collected by retro orbital sinus puncture under mild ketamine xylazine anaesthesia. Fasting blood glucose estimation was measured on day 0, 7 and 14 of the study using glucometer. The result reveal that the isolate at both dose levels (200 and 400mg/kg) cause a significant ($p < 0.001$) decrease in the blood glucose levels after oral administration at 7 and 14 days when compared to the diabetic control group.
The isolated compound tested for their antidiabetic potential by docking studies using intestinal α-glucosidase as receptor. Chemically the isolated compound is a bound flavonoid which binds with the α-glucosidase with docking score of -9. The drug receptor interaction studies also shows excellent hydrogen bond and hydrophobic interactions. The result indicates that the isolated compound is potent α-glucosidase inhibitor with good antidiabetic potential.

Keywords: Antidiabetic activity, *Sauropus androgynus*, Alloxan induced Diabetic Rats, α-glucosidase enzyme, Docking

1. Introduction
Diabetes means a siphon or running through, mellitus means sugar. Therefore, diabetes mellitus is a clinical state which is associated with flow of sugar. In 2014, the International Diabetes Federation estimated that diabetes resulted in 4.9 million deaths. The WHO estimated that diabetes resulted in 1.5 million deaths in 2012, making it is the 8th leading cause of death. The greatest increase in rates was expected to occur in Asia and Africa, where most people with diabetes will probably live in 2030. Chemical agents are available in the market to control and to treat diabetic patients but are unsafe. Present trend manages this deadly disorder through nutraceutical approaches as it is safe without any serious side effects (Sincy *et al*., 2016) [20].
Diabetes mellitus is a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. Diabetes cause wide spread pathological changes such as thickening of capillary basement membrane, increase in vessel wall matrix and cellular proliferation, resulting in vascular complications like lumen narrowing, early atherosclerosis, sclerosis of glomerular capillaries, retinopathy, neuropathy and peripheral vascular insufficiency.
Currently available treatment of Diabetes includes lifestyle modifications, medications and surgery. Presently, there is growing interest in herbal remedies due to the side effects associated with the oral hypoglycaemic agents for the treatment of diabetes. In the light of the literature on *S. androgynus*, we made an attempt for the first time to study the effect of bioactive constituent isolated from *S. androgynus* alcoholic leaf extract in antidiabetic activity by alloxan induced diabetes rat model. The isolated compound was tested for antidiabetic potential by docking studies using intestinal α- glucosidase enzyme as receptor.

S. androgynus endemic to India, many people use this plant as traditional medicine to relieve fever and treat urinary problems. The anti-inflammatory and antioxidant activity of *S. androgynus* plant was report by Ida Christi, 2014 [19]. The *S. androgynus* is a tropical shrub, used as a leafy vegetable. The people in southern regions of India believed that this plant has good potential to resist diabetes. Hence, an attempt has been made to explore the antidiabetic potential of leaves of *S. androgynus.*

Materials and methods

Plant materials and extraction

S. androgynus (Euphorbiaceae) leaves were collected. The leaves (plant material) were dried, coarsely powdered and extracted (2kg) separately with ethanol (70%) in a soxhlet extractor for 2hr. The extracts were concentrated to dryness. The nature and yields of the extract were noted and stored in a refrigerator at 4 ^{0}C for further studies.

The column chromatographic technique is widely used for separation, isolation and purification of natural compounds. The fractions were chromatographed over silica gel (100-200) mesh of column length 1m and diameter 1.5 inch. Elution was carried out with solvent and mixtures of increasing polarities. The fractions were collected and monitored on TLC (silica gel G, adsorbent with suitable solvent system) and similar fractions were pooled together based upon their TLC profile. The flavonoidal compound isolated from ethyl acetate: ethanol fraction give a single spot with R_f value and it was subjected to IR, ^{13}C NMR, ^{1}H NMR and Mass spectral analysis to confirm the structure of compound.

In-vivo studies

Experimental animals

Healthy Albino rats of wistar strain of either sex, weighing 180-220g, were purchased from the Animal house, Kerala Agriculture University College of Veterinary and Animal science, Mannuthy, Thrissur, Kerala and stock maintained in the Animal house of Devaki Amma Memorial College of Pharmacy, Chelembra, Malappuram, Kerala. The animal house was well ventilated and animals had 12±1h day and night schedule. The animals were housed in large spacious hygienic cages during the course of the experimental period and room temperature was maintained at 25±1 ^{0}C. The animals were fed with standard rat feed and water ad libitum. The experiments were conducted as per the guidelines of CPCSEA.

Alloxan induced diabetes (Shilali *et al*., 2015) [13].

Preparation of test samples

The isolated compound and the standard hypoglycaemic drug glibenclamide (10mg/kg) were dissolved in isotonic saline solution (NaCl, 0.9%w/v) and were administered orally.

Induction of diabetes in rats by Alloxan monohydrate

Diabetes was induced in overnight fasted adult Wistar albino rats weighing 180-220g by a single intraperitoneal injection of 150mg/kg alloxan monohydrate (CHEMCO) freshly dissolved in normal saline (0.9%w/v). Hyperglycemia was confirmed by the elevated glucose levels determined at 72hr after alloxan injection. The rats having blood glucose levels above 200mg/dL were used for the antidiabetic study.

After induction of diabetes the experimental animals were divided in to 5 groups, each consisting of six rats.

Group I: Normal control (saline)
Group II: Diabetic control (Alloxan, 150mg/kg, ip)
Group III: Alloxan (150mg/kg. ip) + Glibenclamide, 10mg/kg
Group IV: Alloxan (150mg/kg. ip) + Isolated compound (200mg/kg, p.o)
Group V: Alloxan (150mg/kg. ip) + Isolated compound (400mg/kg, p.o)

The isolated compound and standard drug were administered orally for 14 days. The fasting blood samples were collected by retro orbital sinus puncture under mild Ketamine xylazine anaesthesia. Fasting blood glucose estimation was measured on day 0, 7, and 14 of the study using glucometer (Mark Inc).

Statistical analysis

The results are presented as mean ± SEM with n = 6. Statistical analysis was performed using one-way ANOVA followed by Dunnett's method. The *p* value was reported to denote the degree of significance. Graph pad prism software was used for statistical analysis.

Drug-receptor interaction studies

The study was carried out with intestinal α-glucosidase as receptor and isolated compound as drug. Miglitol was selected as the standard drug for comparison of results.

Preparation of protein molecule

Crystallographic structure of intestinal α-glucosidase was obtained from PDB. The inhibitors and water molecules attached to protein were removed using Argus Lab software.

Preparation of ligand

The ligand compounds were drawn using ACD/Chemsketch as MDL MOL file and imported to Argus Lab after adding hydrogen bonds.

Docking

The docking of ligand and target were done in Argus Lab software. The docking score and best pose of isolated compound with targets were noted. The hydrogen bond interaction and hydrophobic interaction noted by Molegro molecular viewer.

Results

The leaves (plant material) were dried, coarsely powdered and extracted (2kg) separately with ethanol (70%) in a soxhlet extractor for 2hr. The extracts were concentrated to dryness and subjected to phytochemical identification tests to confirm the various phytoconstituents.

Table 1: Preliminary phytochemical studies on *Sauropus androgynous*

Sl.No.	Phytoconstituents	Hydro alcohol extract	Isolated compound
1.	Carbohydrate	P	P
2.	Amino acids & proteins	P	A
3.	Oils & fat	P	A
4.	Flavonoids	P	P
5.	Tannins	P	A
6.	Saponins	P	A

P: Present, A: Absent

The structural elucidation of isolated compound carried out by IR, ^{13}C NMR, ^{1}H NMR and Mass spectral analysis. The structure of isolated compound found to be,

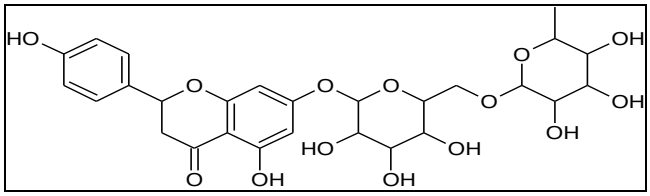


Fig 1: Flavanone-7-O-glycoside with Aglycone moiety: Naringenin, Glycone moiety: Rutinose, Molecular weight: 580.54 g mol^{-} and Molecular formula: C_{27} H32O14

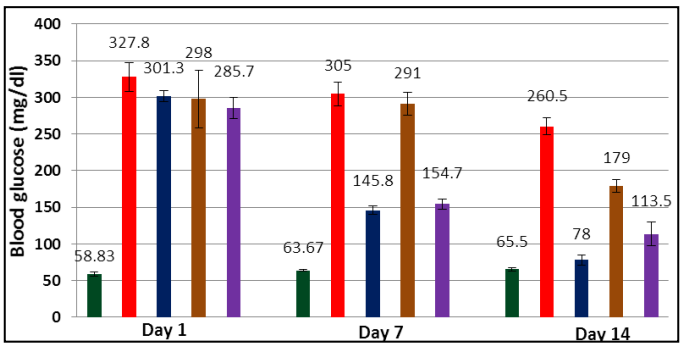
Effect of isolated compound on blood glucose level

The results of the blood glucose level are given in Table. 2 and Figure. 2. The data reveals that the isolated compound at both the dose levels (200 and 400mg/kg) show a significant ($p < 0.001$) decrease in blood glucose level after oral administration at 7 and 14 days when compared to the diabetic control group.

Table 2: Glucose levels of animals during Antidiabetic study

Sl. no	Groups	Glucose level (mg/dl)		
		1st day	7th day	14thday
1	Normal	58.83 ± 2.372	63.67±1.282	65.5 ±2.217
2	Alloxan induced	327.8 ± 19.29 ***	305 ± 16.24 ***	260.5±11.81 ***
3	Alloxan induced+ Glibenclamide treated	302.3 ± 7.688 ns	145.8 ± 5.73 ***	78 ± 6.787 ***
4	Alloxan induced+ 200 mg/kg isolate treated	298 ± 16.07 ns	291±15.7 ns	179 ± 8.91 ***
5	Alloxan induced+ 400 mg /kg isolate treated	2 85 ± 14.15 ns	154.7 ± 6.922 ***	113 ± 16.5***

Values are expressed as mean±SEM (n=6). Values are significantly different from group 2, alloxan induced; ns: non significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (One way ANOVA followed by Dunnett's test.



Vehicle control, Alloxan induced diabetic rats, Alloxan induced + Glibenclamide treated rats, Alloxan induced + Isolate 200 mg/kg treated rats, Alloxan induced + Isolate 400 mg/kg treated rats

Fig 2: Graphical representation of Antidiabetic study

Drug-receptor interaction studies

The docking studies carried out using Intestinal α-glucosidase with PDB Id: 3l4w as receptor and isolated compound as drug.

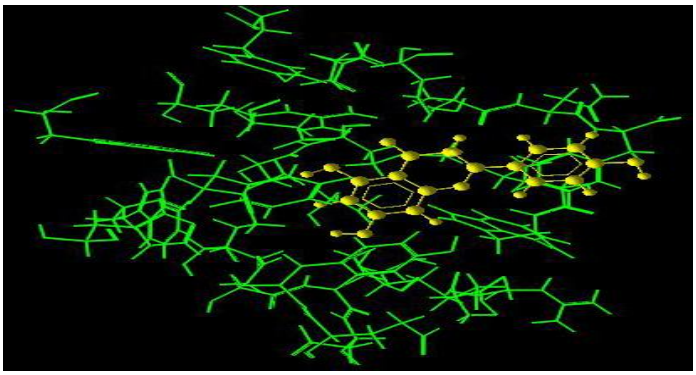


Fig 3: Docking image of isolated compound and Intestinal α-glucosidase receptor with Docking score = -9

The docking score revealed that isolated compound has better α-glucosidase inhibitory activity than Miglitol (docking score: -7.35)

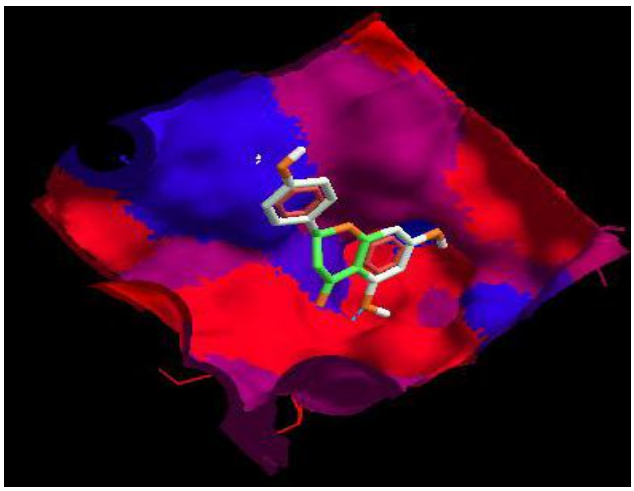


Fig 4: Hydrophobic interactions of Isolated compound and Intestinal α-glucosidase receptor

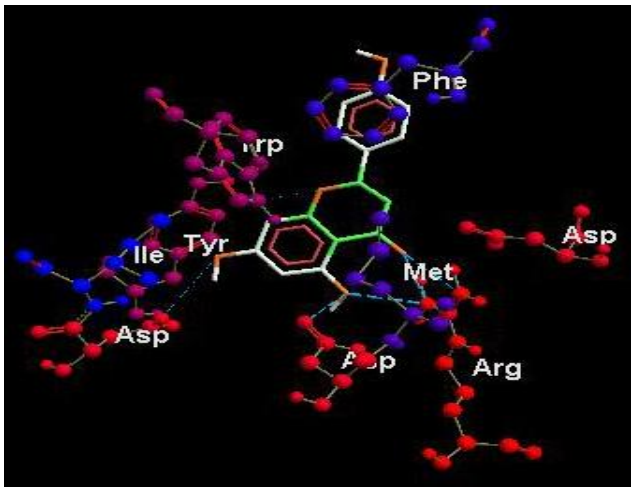


Fig 5: Hydrogen bond interactions of isolated compound and Intestinal α-glucosidase receptor

The isolated compound shows better hydrophobic and hydrogen bond interaction than Miglitol. The drug-receptor interaction study confirms that the isolated compound is a potent α-glucosidase inhibitor with higher antidiabetic potential than Miglitol.

Discussion

The increasing worldwide incidence of diabetes mellitus in adults constitutes a global public health burden. It is predicted that by 2030, India, China and the United states will have the largest number of people with diabetes (Wild *et al*., 2004) [16].

In the light of the literature on *S. androgynus*, we made an attempt for the first time to study the effect of bound flavonoid compound isolated from ethanolic extract of *S. androgynus* in antidiabetic activity by Alloxan induced diabetes rat model and the isolated compound tested for antidiabetic potential by docking studies.

Alloxan induces diabetes by damaging the insulin secreting pancreatic β cells, resulting in a decrease in endogenous insulin release. Numerous studies demonstrated that a variety of plant extracts effectively lowered the glucose level in alloxan induced diabetic animals (Ji Su Kim *et al*., 2006) [11].

The present study revealed that the isolated compound at both the dose levels (200mg/kg and 400mg/kg) showed a significant ($p < 0.001$) decrease in blood glucose level after oral administration at 7 and 14 days when compared to the diabetic control group.

The docking of α-glucosidase enzyme and isolated compound showed docking score of -9 and good hydrophobic interaction as well as hydrogen bond interaction. The enzyme α-glucosidase is the final enzymes for the digestion of carbohydrates in the brush border of small intestine mucosa. It slows down and decreases digestion and absorption of polysaccharides and sucrose. The drug-receptor interaction studies confirmed that the isolated compound is a potent α-glucosidase inhibitor.

Conclusion

The present work revealed that the bound flavonoidal compound isolated from *S. androgynus* has good antidiabetic activity. The antidiabetic effect comparable to that of Glibenclamide in *in vivo* studies and Miglitol in drug-receptor interaction studies. I hope this study will help to develop flavonoid or flavonoid based semi synthetic compound as antidiabetic agent in future.

Reference

1. Farnsworth NR, Soejarto DD. Global importance of medicinal plants. Cambridge University Press, Cambridge, UK. 1991; 25-51
2. Senthamarai Selvi V, Govindaraja G, Anusha Basker. Antifungal activity and Phytochemical Analysis of *Cympogon citratus*, *Sauropus androgynus* and *Spilanthus acmella* Plants. World Journal of Fungal and Plant Biology. 2011; 2(1):6-10.
3. Catherine N, Jasper K, Michael W. Flavanoid content in ethanolic extracts of selected raw and traditionally processed indigenous foods consumed by vulnerable groups of Kenya: antioxidant and type II diabetes- related functional properties. International Journal of Food Sciences and Nutrition. 2011; 62(5):465-473.
4. Harborne JB. Phytochemical Methods. Chapman and Hall, London. 1983; 3:1-39.
5. Santoso U, Fenita Y. The effect of *Sauropus androgynus* Extract and Lemuru Oil on Fat deposition and Fatty acid composition of Meat in Broiler chickens. Journal of Indonesian Trop. Anim. Agric. 2010; 35(1):48-54.
6. Shubha, Prathibha KY, Manjula AC. Environment Friendly Strategy to Increase Antioxidant content and produce *Sauropus androgynus*. Journal of Life Sciences. 2011; 6(4):567-569.
7. Krishna kumar KR, Jayaprakash AP, Srinivasan KK. Extraction, Isolation, and Identification of Phytoconstituents present in the *Hemionitis arifolia* leaves. International Journal of Pharmacy and Pharmaceutical Sciences. 2015; 7(2):477-484.
8. OECD Guidance Document on Acute Oral Toxicity. Environmental Health and Safety Monograph Series on Testing and Assessment 2001, 24.
9. https://en.wikipedia.org/
10. Robert M Silverstein, Francis X Webster. Spectrometric identification of organic compounds. 2009; 16:200-215.
11. Ji Su Kim, Jung Bong Ju, Chang Won Chai, Sei Chang Kim. Hypoglycaemic and antihyperlipidemic effect of four Korean medicinal plants in alloxan induced diabetic rats. American Journal of Biotechnology and Biochemistry. 2006; 2:154-160.
12. Shweta yadav, Padma kumar. Production, Isolation and Identification of Flavonoids from Aerial parts of Hiptage benghalensis. International Journal of Life science and Pharma research. 2012; L:1-5.
13. Shilali K, Ramachandra YL. Antidiabetic effect of Bark Extract of Phyllanthus acidus. International Journal of Preclinical and Pharmaceutical Research. 2015; 6(1):35-38
14. Sharma BK. Spectroscopy. Goel publishing house. 2011; 21:619-693
15. Ariharan VN, Meena devi VN, Nagendra Prasad P. Antibacterial activity of *Sauropus* and *Rogynous* leaf extracts against some pathogenic bacteria. Chemical, Environmental and Pharmaceutical Research. 2013, 134-137.
16. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for 2000 and projections for 2030, Diabetes care. 2004; 27:1047-1053.
17. Trease GE, Evans WC. Pharmacognosy. Bailliere tinda, London. 1989; 13:770-771.
18. Fried B, Sherma J. Thin Layer Chromatography: Techniques and Applications. Chromatographic science series 66, Marcel Dekker, New York. 1994; 3:40-51.
19. Ida Christi VE. Study of Pharmacognostical, Anti-inflammatory and Antioxidant activity of *Sauropus androgynus* plant. International Journal of Advances in Pharmaceutical Research 2014; 5(3):198-207.
20. Sincy Joseph, Lekha Kumar, Narmatha Bai V. Evaluation of antidiabetic activity of *Strobilanthes cuspidata* in alloxan induced diabetic rats and the effect of bioactive compounds on inhibition of α-amylase enzyme. Journal of Pharmacognosy and Phytochemistry. 2016; 5(3):169-175.
21. Veerabahu, Ramasamy, Mohan. Arockia Jenecius Alphonse. *In vitro* Antioxidant activity of *Sauropus bacciformis Blume* (Euphorbiaceae). International Research Journal of Pharmacy. 2012; 3(9):256-259.
22. Silja VP, Varma KS, Mohanan KV. Ethnomedicinal plant knowledge of the Mullukurma tribe of Wayanad district, Kerala. Indian Journal of Traditional Knowledge. 2008; 7:604-612.
23. Lee Seong Wei, Wendy Wee, Julius Yong Fu Siong. Characterisation of antimicrobial, antioxidant, anticancer properties and chemical composition of *Sauropus androgynus* stem extract. ACTA MEDICA LITUANICA. 2011; 18:12-16.
24. Morgan C. Medicine of the Gods: Basic Principles of ayurvedic Medicine. Mandrake of Oxford. Oxford: U.K.

2002; 128.
25. Anusha Bhasker, Senthamarai Selvi V. Anti-inflammatory and analgesic activities of *Sauropus androgynus (L.) Merr* (Euphorbiaceae) Plant in experimental animal models. Scholars Research Library. 2012; 4(3):782-785.
26. Ocvirk S, Kistler M, Khan S, Talukder HS, Hauner H. Traditional medicinal plants used for the treatment of diabetes in rural and urban areas of Dhaka, Bangladesh – an ethnobotanical survey. Journal of Ethnobiology and Ethnomedicine. 2013; 9:1-43.
27. Tripathi BK, Srivastava AK. Diabetes mellitus: Complications and therapeutics. Medical Science Monitor. 2006; 12(7):130-147.
28. Savithriamma N, Linga Rao M, Suhrulatha D. Screening of medicinal plants for secondary metabolites. Middle-East Journal of Scientific Research. 2011; 8:579-584.
29. Belle EA, Charlwood BV. Secondary plant products. Encyclopaedia of plant physiology. Springer Verlag Publication. 1980; 8:674-676.
30. Andrew JK, Clifford JB. Antidiabetic Agents Current Role in Type 2 Diabetes Mellitus. Drugs. 2005; 65(3):385-411.
31. Rao MU, Sreenivasulu M, Chengaiah B, Reddy KJ, Chetty CM. Herbal medicines for Diabetes Mellitus; A Review. International Journal of Pharmaceutical Technology and Research. 2010; 2(3):1884-1892.
32. Gerich J. Glucose counters regulation and its impact on diabetes mellitus. Diabetes. 1988; 37:1608-1617.
33. Eshaifol AO, Antony K, Ali A, Kong ML, Vallentina RN. Herbal Medicines and Nutraceuticals for Diabetic Vascular Complications: Mechanism of Action and Bioactive Phytochemicals. Current Pharmaceutical Design. 2010; 16:3776-3807.
34. Mishra SB, Rao CHV, Ojha SK, Vijayakumar M, Varma A. An analytical review of plants for anti diabetic activity with their phytoconstituent and mechanism of action. International Journal of Pharmaceutical Sciences and Research. 2010; 1(1):29-46.
35. Arshad Mohamed, Ashoka Shinoy, Moses Samuel Rajan AR, Shabaraya. Evaluation of the antidiabetic activity of glibenclamide in combination with ginger in streptozotocin induced diabetes. International Journal of Pharmaceutical and Chemical Sciences. 2013; 2(3):1339-1344.
36. Raju S, Hemamalini K. *In vivo* animal model screening of anti-diabetic activity. Asian journal of pharmaceutical and clinical research. 2012; 5(4):118-124.
37. Lenzen S. The mechanisms of alloxan and streptozotocin induced diabetes. Diabetologia. 2008; 51:216-226.
38. Kanchanapoom T, Chaumsri P, Kasai R, Otsucka H, Yamasaki K. Lignan and megastigmane glycosides from *Sauropus androgynus*. Phytochemistry 2003; 63:985-988.
39. Pickup JC, Williams G. Textbook of diabetes, 3rd ed., Blackwell science Ltd, USA, 2003, 103-114.
40. Laar FA, Lucassen PLBJ, Akkermans RP, Lisdonk EH, Rutten GEHM, Weel C. Alpha-glucosidase inhibitors for type 2 diabetes mellitus. Cochrane Review, The Cochrane Library, 2008; 5:105-111.
41. Cheng AYY, Fantus IG. Oral antihyperglycemic therapy for type 2 diabetes. Mellitus Can Med Assoc J, 2005; 17:213-226.
42. Etuk EU. Animals models for studying diabetes mellitus. Agric Biol J N Am, 2010; 1:130-134.
43. Grover JK, Yadav S, Vats V. Medicinal Plants of India with antidiabetic potential, Journal of Ethanopharmacology, 2002, 81-100.
44. Kirtikar KP, Basu BD. Indian medicinal plants, 2nd ed., International book distributers, India, 1987; 3:65-69.
45. Garg M, Dhar VJ, Kalia AN. Antidiabetic potential of *Phyllanthus urinaria* in albino rats. Int J Green Pharm. 2006; 1:168-170.
46. Aruna RV, Ramesh B, Kartha VN. Effects of beta carotene on protein glycosylation in alloxan induced diabetic rats. Indian J Exp Biol, 1999; 32:319-401.
47. Okoli CO, Ibiam AF, Ezike AC, Akah PA, Okoye TC. Evaluation of antidiabetic potentials of *Phyllanthus niruri* in alloxan diabetic rats. African Journal of Biotechnology, 2010; 9(2):248-259.
48. Pawlowska AM, Oleszek W, Braca A. Quali-Quantitative analysis of flavonoids of *Morus nigra L.* and *Morus Alba* (Moraceae) fruits. Food Chem, 2008; 56:3377-3380.
49. Kapoor, Sing A, Kapoor SL, Shrivastava SN. Survey of Indian medicinal plants for saponins, alkaloids and flavonoids Lioydia, 1969; 31:297-302.
50. Ghosh, Suryawanshi S. Effect of *Vinca rosea* extracts in treatment of alloxan diabetes in male albino rats. Indian Journal of Experimental Biology. 39:748-759.
51. Benjapak N, Swatsitang P, Tanpanich S. Determination of antioxidant capacity and nutritive values of *Sauropus androgynus* (L.). KKU Science Journal. 2008; 36(4):279-289.
52. Sawahata M, Ogura T, Tagawa A, Takahashi H, Yazawa T, Matsuura M *et al*. *Sauropus androgynus*- associated bronchiolitis obliterans of mother and daughter-autopsy report. Respiratory Medicine CME. 2010; 3(4):214-217.
53. Bender AE, Ismail KS. Nutritive value and toxicity of Malaysian food, *Sauropus albicans.* Plant Foods Man. 1975; (1):139-143.
54. Padmavathi P, Prabhakara Rao M. Nutritive value of *Sauropus androgynus* leaves. Plant Foods and Human Nutrition, 1990; 40:107-113.