The Journal of Phytopharmacology 2023; 12(5):326-333
Online at: www.phytopharmajournal.com

The Journal of Phytopharmacology
(Pharmacognosy and phytomedicine Research)

Research Article

ISSN 2320-480X
JPHYTO 2023; 12(5): 326-333
September- October
Received: 26-08-2023
Accepted: 26-10-2023
©2023, All rights reserved
doi: 10.31254/phyto.2023.12508

Shilpa KJ
Department of Applied Botany, Mangalore University, Mangalagangothri - 574199, Mangalore, Karnataka, India

Krishnakumar G
Department of Applied Botany, Mangalore University, Mangalagangothri - 574199, Mangalore, Karnataka, India

Karunakar Hegde
Department of Pharmacology, Srinivas College of Pharmacy, Valachil, 574143, Mangalore, Karnataka, India

Correspondence:
Dr. Shilpa KJ
Department of Applied Botany, Mangalore University, Mangalagangothri - 574199, Mangalore, Karnataka, India
Email: kshilpaj@gmail.com

Comparative study on the Antidiabetic activity of the bark extracts of *Syzygium caryophyllatum* (L.) Alston and *Syzygium zeylanicum* (L.) DC

Shilpa KJ, Krishnakumar G, Karunakar Hegde

ABSTRACT

The study evaluated the potential antidiabetic property of methanol extracts of S. *caryophyllatum* and *S. zeylanicum* in alloxan and streptozotocin - induced diabetic rats. Glibenclamide and extract at 5mg/kg, 200 and 400 mg/kg body weight respectively were used. Animals were divided into 7 groups of 6 each. The treatment was carried out in each group of animals for both the models for 21 days. Blood samples were withdrawn under mild anesthesia from retro - orbital of the overnight fasted animals on 1st, 7th, 14th, and 21st day. Estimation of SGPT and SGOT and the intensity of the coloured complex was performed. Body weight of animals in all the groups was recorded at 0, 7th, 15th and 21st day. There was no mortality amongst the dosed groups of animals and the extract did not show toxicity at a dose level of 2000 mg/kg. The extracts of both the species at 400 mg / kg body weight possessed a remarkable fasting blood – glucose lowering potential, significant increase in body weight and reduced levels of SGPT and SGOT in alloxan and streptozotocin induced diabetic rats. The extracts also exhibited improved glucose utilisation. The UPLC analysis of the extract revealed the presence of phenols and flavonoids with antidiabetic potential. The results support the use of *Syzygium* species in traditional system of medicine to treat diabetes mellitus.

Keywords: Albino rat, Diabetes mellitus, Streptozotocin, UPLC, LC-MS/MS.

INTRODUCTION

Diabetes mellitus, a disease widespread in the developing and developed countries is characterised by hyperglycaemia and alterations in carbohydrate, lipid and protein metabolism, associated with absolute or relative deficiencies in insulin secretion and / or insulin action, leading to polyuria, polyphagia, polydypsia, ketosis, weight loss, retinopathy and cardiovascular disorders [1,2,3]. Among the types of diabetes, gestational diabetes which occurs at the late stages of pregnancy, accounts for 5 %, type 1 diabetes represents 10 % and is characterised by lack of insulin production and type 2 diabetes which is characterized by metabolic abnormalities such as insulin resistance and decreased pancreatic β - cell function accounts for more than 85 % of all the cases [4, 5]. The disease is rapidly increasing and according to the World Health Organization, there will be 300 million diabetics by 2025 [6]. Diabetes brings complications like systemic vascular disease, cataract, kidney damage leading to renal failure and damage to peripheral nerves [7]. Diabetes is treated through insulin and other hypoglycaemic agents. But the high cost and associated side effects such as liver problems, lactic acidosis and diarrhoea has necessitated to turn on to traditional and other alternate system of treatments. Plants have always served as excellent source of drugs since these are more often less toxic and with lesser side effects in comparison to synthetic ones [8]. The active principles of a large number of antidiabetic plant drugs were isolated during 18th century and were administered in standardized dosage forms [9]. World Health Organisation supports evaluation of traditional knowledge of diabetes treatment, their efficacy, non - toxicity and reduced side effects [10]. *Syzygium* species are well known to possess antidiabetic property. Traditionally, a decoction of 60 gm of stem bark of *Syzygium caryophyllatum, Syzygium samarangens* and *Syzygium cumini* is prepared and about 120 ml is given twice a day for diabetics. The seeds of *S.cumini* are roasted in a wide – mouthed earthen pot, pounded into powder and 2.5 grams in hot water is given twice a day. Decoction from the fresh stem bark (120 g) of *Syzygium malaccense* in 120 ml water is given twice a day [3]. The traditional practitioners use *S.cumini* in treating diabetes [11]. It is listed among the most common and effective antidiabetic medicinal plants of Indian origin [12]. The methanol extract of the leaf of *S.caryophyllatum* possess antidiabetic effect on alloxan induced antidiabetic albino mice [13]. We report the antidiabetic activity and toxicity risk associated with the bark methanol extracts of *S.caryophyllatum* and *S.zeylanicum* in alloxan and streptozotocin induced diabetic rats. The efficiency of the extracts was compared to a standard hypoglycaemic drug, Glibenclamide.

MATERIALS AND METHODS

Animals

Healthy Wistar albino rats (150 – 200 g) of either sex were used for the experiments. They were maintained under standard conditions (temperature 22 ± 2° C, relative humidity 60 ± 5 % and 12 h light/dark cycle). The animals were housed in sanitized polypropylene cages containing paddy husk as bedding. They had free access to standard pellet diet and water *ad libitum*. Experiments were conducted between 10:00 to15:00 h. All experimental protocols were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) prior to the initiation of the experiment and the care of the laboratory animals were taken as per the CPCSEA regulations (Ref No: CSCP/CPCSEA/P13/F212/2013 dated: 5/12/13)

Preparation of extracts

The bark of *S. caryophyllatum* was collected from around the Mangalore University campus. The bark of *S. zeylanicum* was collected from the river sides of Sullia Taluk. The samples were shade dried and Soxhlet extracted with methanol at 30° C for 5 hours. A small amount of sodium sulphate was added to remove the moisture content and the supernatant was filtered in a three – layered muslin cloth. The clear supernatant was evaporated to dryness. The extract obtained was stored at 4° C until use.

Determination of acute toxicity

Acute toxicity study was performed as per OECD - 425 guidelines [14]. Wistar albino rats of either sex weighing 150 - 200 g were selected by random sampling technique for the study. The animals were fasted overnight providing only water and extracts were administered orally at a dose level of 2000 mg/kg body weight. Animals were under close observation for the first 24 h and monitored for 14 days for mortality, changes in general behaviour, signs of discomfort and other physical activities.

Assessment of anti - diabetic activity in alloxan - induced diabetic rats

Hyperglycaemia was induced by a single i.p. injection of 100 mg/kg of body weight of freshly prepared Alloxan monohydrate in normal saline. After 3 days, the hyperglycaemic rats (glucose level > 200 mg/dl) were divided into seven groups with six rats in each group for the anti - diabetic study. The treatment (p.o.) was started on the same day except for diabetic control. The rats had free access to feed and water *ad libitum*. Fasting blood glucose level was determined after depriving food for 16 h with free access to drinking water.

Group 1: Vehicle control (normal saline)

Group 2: Diabetic control (Alloxan100 mg/kg, i.p.)

Group 3: Diabetic + Glibenclamide (5 mg/kg, p.o.)

Group 4: Diabetic + *S.caryophyllatum* extract (200 mg/kg, p.o.)

Group 5: Diabetic + *S.caryophyllatum* extract (400 mg/kg, p.o.)

Group 6: Diabetic + *S.zeylanicum* extract (200 mg/kg, p.o.)

Group 7: Diabetic + *S.zeylanicum* extract (400 mg/kg, p.o.)

Assessment of anti-diabetic activity in Streptozotocin (STZ) induced diabetic rats:

Hyperglycaemia was induced by a single i.p. injection of 50 mg/kg of STZ in citrate buffer, freshly prepared and injected within 5 minutes of preparation to prevent degradation. After administration of streptozotocin, the animals had free access to feed and water *ad libitum*. The development of hyperglycaemia in rats was confirmed by fasting blood glucose estimation 72 h post streptozotocin injection. The rats with fasting blood glucose level of above 200 mg/dl at 72 h after STZ injection were considered diabetic and included in the study. Fasting blood glucose was determined after depriving food for 16 h with free access to drinking water.

Experimental design:

Animals were randomly divided into 7 groups of 6 each and assigned as below.

Group 1: Vehicle control (Citrate buffer)

Group 2: Diabetic control (Streptozotocin 50 mg/kg, i.p.)

Group 3: Diabetic + Glibenclamide (5 mg/kg, p.o.)

Group 4: Diabetic + *S.caryophyllatum* extract (200 mg/kg, p.o.)

Group 5: Diabetic + *S.caryophyllatum* extract (400 mg/kg, p.o.)

Group 6: Diabetic + *S.zeylanicum* extract (200 mg/kg, p.o.)

Group 7: Diabetic + *S.zeylanicum* extract (400 mg/kg, p.o.)

Collection of blood and serum samples:

The above treatment was carried out in each group of animals for both the models for 21 days. Fasting blood glucose level was measured using single touch glucometer. Blood samples were withdrawn under mild anesthesia from retro - orbital of the overnight fasted animals on 1st, 7th, 14th, and 21st day. On 21st day the blood was collected for biochemical estimations by retro orbital puncture. The serum was obtained by centrifugation at 3000 rpm for 10 min and they were used for estimation of SGPT (Serum Glutamic Pyruvic Transaminase), SGOT (Serum Glutamic Oxaloacetic Transaminase) by using a corresponding kit from Agappe Diagnostics Pvt. Ltd and the intensity of the coloured complex formed after treating with these reagents were estimated in semi - auto analyzer (Chem – 400).

Oral glucose tolerance test:

The oral glucose tolerance test was performed in overnight fasted normal rats. Rats were divided into six groups (each group containing six animals). After 30 minutes of extract / standard drug administration, glucose at a concentration of 2g / kg body weight was fed to all the groups. Blood was withdrawn from the retro - orbital sinus just prior to the glucose administration and at 30, 60, 120, 180 and 240 minutes after which glucose loading and glucose levels were measured.

Group 1: Vehicle control (Citrate buffer)

Group 2: Diabetic + Glibenclamide (5 mg/kg, p.o.)

Group 3: Diabetic + *S.caryophyllatum* extract (200 mg/kg, p.o.)

Group 4: Diabetic + *S.caryophyllatum* extract (400 mg/kg, p.o.)

Group 5: Diabetic + *S.zeylanicum* extract (200 mg/kg, p.o.)

Group 6: Diabetic + *S.zeylanicum* extract (400 mg/kg, p.o.)

Body Weight

Body weight of animals in all the groups was recorded at 0, 7th, 15th and 21st day.

UPLC analysis of Phenols and Flavonoids

Chemicals and Reagents

Phenolic acid standards namely ferulic acid, 2,4 dihydroxy benzoic acid, caffeic acid, gallic acid, gentisic acid, o - coumaric acid, p - coumaric acid, p - hydroxy benzoic acid, protocatechuic acid, salycylic acid, syringic acid, t - cinnamic acid, vanillic acid, chlorogenic acid and flavanoid standards namely catechin, hesperitin, apigenin, neringenin, myrcetin, rutin, luteoline, quercetin, umbelliferone were acquired from Sigma Chemical Co., USA. The standard solutions were prepared in 80 % ethanol. The organic solvents used as the mobile phases for liquid chromatography were of chromatographic / MS grade. All mobile phases were filtered through membrane filters with a pore size of 0.45 µm. The standard curve for individual Phenolic acids and flavonoids were made by using the known concentrations of individual compounds which were identified and quantified by their molecular weight (parent mass m/z) and most abundant fragmented daughters

Equipment

An Acquity UPLC - H class coupled with TQD - MS/MS from M/S Waters, USA with ESI source was used in the Phenolic acids and flavonoids determinations, equipped with a degasser, quaternary pump, automatic injection system (0 – 10 µL), with a diode array detector and a temperature control compartment for the analytical column. The detection system allowed for the simultaneous detection at various wavelengths and MRM for individual masses. The overall system was controlled by the Mass lynx software, which also administered the data collection and treatment system.

LC and MS - MS conditions

The mobile phase consisted of an aqueous phase of 0.1 % formic acid in water (A) and organic phase of 0.2 % formic acid in methanol (B). The total run was for 15 min with gradient function. Initial gradient was composed of 90 % A and 10 % B for 2.5 min, 70 % A, 30 % B at 4 min, and 60 % A 40 % B at 5 min and 80 % A 20 % B at 10 min. At 12 min the gradient was changed to 90 % A and 10 % B and maintained up to 15 min. The flow rate was 0.3 mL/min. The analytical column was 2.1 X 50 mm UPLC BEH - C18 column (Waters) with 1.7 µm particle size protected by a Vanguard BEH C - 18 with 1.7 µm particle size (Waters) and the column temperature was maintained at 25°C. The sample injection volume was 2 µl each time for both phenolic acids and flavonoids. The metabolites eluted were monitored using the UPLC column. Effluent was pumped directly without any split into the TQD - MS/MS (Waters, USA) system, optimized for the Phenolic acids and flavonoids analysis.

Statistical analysis

Results of biochemical estimation were reported as mean ± SEM. The total variation present in the data was analyzed by one way analysis of variance (ANOVA) followed by Dunnett's 't' test. P value lesser than 0.05 was considered statistically significant.

RESULTS

Determination of acute toxicity

There was no mortality or change in general appearance and signs of discomfort amongst the dosed groups of animals and the extract did not show toxicity at a dose level of 2000 mg/kg.

Assessment of anti - diabetic activity in alloxan and streptozotocin - induced diabetic rats

The fasting blood glucose level in normal, glibenclamide and extract treated rats are summarized in Table 1 and Table 2. Fasting blood glucose (FBG) level was within the range of 80 - 95 mg/dl in all the groups prior to diabetic induction.Treatment with Alloxan (100 mg/kg, i.p) had increased the FBG level above 200 mg/dl after 72 h. Treatment with Glibenclamide and extracts significantly normalized the elevated blood glucose level. Fasting blood glucose (FBG) level was within the range of 80 - 90 mg/dl in all the groups prior to STZ administration. Treatment with STZ (50 mg/kg, i.p) had increased the FBG level above 300 mg/dl after 72 hrs. Treatment with extracts significantly normalized the elevated blood glucose level.

Oral glucose tolerance test (OGTT) in Wistar rats:

The result of the OGTT in Wistar rats in diabetic, control and normal rats treated with extracts and glibenclamide after oral administration of glucose for 30, 60, 120 and 240 minutes is shown in Table 3. The glucose level in the control rats increased to the peak at 30 minutes after glucose load and decreased to near normal levels at 240 minutes. The extract and glibenclamide treated diabetic rats showed an increase at 30 minute and then a significant reduction ($P < 0.05$ & $P < 0.01$) at 60, 120 and 240 min.

Serum biomarkers:

After 21days of experiment, serum biomarkers such as SGPT and SGOT level were significantly elevated in diabetic control group. In animals treated with extracts SGPT and SGOT levels were decreased significantly ($P < 0.001$, $P < 0.01$ respectively) (Table 4).

Body Weight

The body weight in the normal rats ranged between 178 – 188 grams and which increased to 189 – 198 grams on 21st day. On the other hand, the diabetic rats showed a significant decrease in body weight which weighed 155 grams on 21st day of the experiment. Treatment with extracts showed a significant increase in body weight as compared to diabetic control group in both the models (Table 5 and Table 6). Similar results were observed among the rats treated with the extracts in both alloxan induced and streptozotocin induced models.

UPLC (Ultra High-Performance Liquid Chromatography) analysis of phenols and flavonoids

The methanol extract of the bark of *S.caryophyllatum* and *S. zeylanicum* extracts were analysed for the presence of following phenolic acids: Syringic acid, Ferulic acid, Caffeic acid, Gallic acid, Vanillic acid, p – coumaric acid, p – hydroxyl – benzoic acid, Salicylic acid, Gentisic acid, Protocatechuic acid, O – Coumaric acid, 2,4 – Dihydroxy benzoic acid and Chlorogenic acid. All the phenolic acids analysed were detected in *S.caryophyllatum* except Chlorogenic acid. The major phenolics were Vanillic acid (170.83 µg/mg), Syringic acid (166.57 µg/mg) and Ferulic acid (74.17 µg/mg).The *S.zeylanicum* extract showed the presence of Caffeic acid, Gallic acid, Vanillic acid, p – hydroxyl benzoic acid, Salicylic acid, O – Coumaric acid, and 2, 4 – Dihydroxy benzoic acid and Gallic acid constituted 508.89 µg / mg (Table 2.8). The following were the Flavonoids analysed: Rutin, Myricetin, Catechin, Kempherol, Naringenin, Quercetin, Hesperedin, Apigenin, Umbelliferon and Luteolin. Among the Flavonoids analysed Rutin, Myricetin, Catechin and Umbelliferon were detected in *S. caryophyllatum* extract and Myricetin, Catechin, Naringenin, Apigenin, Umbelliferon and Luteolin in extract of *S.zeylanicum*. Catechin was present in higher concentration in both the species i.e. 84.63 µg/mg and 91.14 µg/mg in *S.caryophyllatum* and *S.zeylanicum* respectively (Table 7 and Table 8).

DISCUSSION

Although diabetes is considered to be a non - curable disease, it is yet a controllable one. Various studies have been conducted and are still being carried out *in vivo* using experimental rats against diabetes. Plants are providing us opportunities to identify the potential antidiabetic drugs to not only lower the fasting blood glucose level but also in improving the diabetic complications [15]. Hence the present study aimed at identifying the antidiabetic properties of *S.caryophyllatum* and *S.zeylanicum* in alloxan and streptozotocin – induced diabetic rats.

The findings on acute toxicity suggested that the extracts were safe and hence 1/10th and 1/5th of LD 50 cut off value of the extracts (200 and 400 mg/kg body weight) were selected as screening dose. Similar results were observed in a study carried out in *Syzygium cumini* seeds where mortality was not recorded at a dose level of 2,000 mg / kg body weight (Kumar et al., 2008 16). The *Colocasia esculenta* leaf extract [17] and *Pandanus fascicularis* [18] root extracts were safe at a dose level of 5000 mg / kg body weight.

Alloxan brings about hyperglycaemia by causing a massive reduction in insulin release by destroying the β – cells of the islets of langerhans, resulting in the decreased utilization of glucose [19,20]. It is also known to generate free radicals of oxygen resulting in extensive DNA damage [21], hence treatment with alloxan (100 mg/kg, i.p) increased the FBG level above 200 mg / dl after 72 h and reached up to 264.09 mg / dl on 21st day in diabetic control group. Glibenclamide was used as a standard drug as it is known to stimulate insulin secretion from pancreatic β – cells, hence treatment with Glibenclamide brought about a significant reduction in the blood glucose level which dropped to 103.65 mg / dl ($P < 0.001$). Streptozotocin – induced diabetes mellitus in rats is one among the widely used animal model and is known to cause selective pancreatic islet beta cell cytotoxicity [22]. It is taken into the pancreatic β – cells by glucose transporter 2 [23]. Hence treatment with STZ (50 mg/kg, i.p) had increased the FBG level above 300 mg / dl after 72 hrs which reached up to 351.02 mg / dl on 21st day in diabetic control. Even the extracts were effective and significant in normalizing the blood glucose level. The *S. zeylanicum* extract was comparatively more effective than *S.caryophyllatum* in normalising the blood glucose level.

From the Oral Glucose Tolerance Test, it has been observed that the extracts exhibited improved glucose utilisation. The extracts were capable of lowering the glucose near to normal. Among all the extracts *S.caryophyllatum* at a concentration of 200 mg / kg body weight effectively prevented the glucose rise in diabetic rats without causing hypoglycaemic state. This significant improvement in utilisation of glucose could be due to the insulin mimetic activity of the plant extracts by restoring the delayed insulin response [24].

The normal rats showed an increase in body weight from day 0 to day 21. Over the same period of time, the diabetic rats showed a decrease in body weight. In the diabetic rats, decrease in the rate of protein synthesis in the liver and muscle resulted in weight loss [25]. Weight loss, one among the major complications in type I diabetes arises due to the impairment in insulin action in converting glucose to glycogen, fat catabolism and inhibition of lipid breakdown [26]. The administration of extracts and glibenclamide resulted in the increase in body weight and is attributed to the reversal of gluconeogenesis and glycogenolysis [27]. Dexamethasone induced diabetic rats which underwent weight reduction was prevented by the aqueous extract of *Erythrina indica* [28]. Savitha and Padmavathy, (2013) [13] have reported a significant gain in body weight by *S.caryophyllatum* leaf extract treated diabetic rats.

The elevation of serum biomarker enzymes such as SGPT and SGOT was observed in diabetic rats. The serum biomarkers are the enzymes which indicate the liver toxicity and their elevated levels confirm the damage caused to the liver cells. The elevated serum level reduced significantly in extract treated diabetic rats in comparison to the diabetic control.

UPLC analysis of Phenols and Flavonoids

Among all the phenolic acids detected Vanillic acid (170.83 µg /mg) and Syringic acid (116.57 µg /mg) were the major ones in *S.caryophyllatum* and in *S.zeylanicum,* gallic acid (508.89 µg /mg) was the major phenolic acid present. Studies on hepatoprotective effect of Syringic acid and Vanillic acid on Concanavalin – induced liver injury in mice have shown that both the acids were capable of suppressing Con A - induced liver inflammation and damage in mice [29]. Gallic acid is reported to induce apoptosis in pre - adipocyte cells via a Fas - and mitochondrial mediated pathway [30]. It is also known to inhibit the saturation of odd – chain polyunsaturated fatty acid [31]. Catechin was one of the major polyphenols detected in both the species. Gallic acid and Caffeic acid were the phenols identified in the methanol extract of *S. polyanthum* leaves [32]. Gallic acid, Chlorogenic acid, Caffeic acid, Rutin, Quercetin and Kaempferol were detected in aqueous extract of *S. jambos* [33]. Six flavonoid compounds viz. 4 - hydroxybenzaldehyde, myricetin – 3 – O - rhamnoside, europetin - 3 – O - rhamnoside, phloretin, myrigalone - G and myrigalone - B were isolated from the ethanolic leaf extracts of *S. aqueum* [34]. Anthocyanins and flavonoid rich extracts are known to lessen the hyperglycaemic symptoms [35]. Kamalakkannan and Prince (2006) [36] have reported rutin and quercetin to have antidiabetic property against type II diabetes.

Table 1: Serum glucose level in Alloxan induced diabetic rats

	Blood glucose level (mg/ dl)				
Groups	Before diabetic induction	Day 0	Day 7	Day 14	Day 21
Normal Control	85.97 ± 2.81	86.8 ± 1.0	87.52 ± 1.39	87.81 ± 1.61	89.05 ± 1.87
Diabetic Control	86.69 ± 4.8	210.60 ± 4.62	229.81 ± 3.23	251.19 ± 2.94	264.09 ± 3.39
Glibenclamide (5 mg/kg	85.13 ± 1.53	209.32 ±3.14	165.02 ± 4.10 ***	135.27 ± 3.93***	103.65 ± 3.04***
SCME (200 mg/kg)	87.91 ± 4.307	215.83 ± 5.65	195.021 ± 5.23**	172.71 ± 6.28**	145.81 ± 5.19**
SCME (400 mg/kg)	89.78 ± 2.82	217.21 ± 4.22	184.425 ± 6.12***	153.23 ± 4.92***	130.14 ± 3.25**
SZME (200 mg/ kg)	88.02 ± 4.307	213.63 ± 5.59	185.29 ± 6.33**	162.73 ± 5.23**	133.31 ± 5.39**
SZME (400 mg/kg)	88.78 ± 1.72	216.34 ± 6.23	179.47 ± 5.02***	149.25 ± 5.02***	123.19 ± 2.95***

Note: SCME denotes *Syzygium caryophyllatum* methanol extract; SZME denotes *Syzygium zeylanicum* methanol extract. Values are expressed as mean ± SEM; n = 6. One way ANOVA followed by Dunnett's't' test. **P < 0.01 and ***P < 0.001 compared with Diabetic control

Table 2: Serum glucose level in STZ induced diabetic rats

	Blood glucose level (mg/ dl)				
Groups	Before diabetic induction	Day 0	Day 7	Day 14	Day 21
Normal Control	86.52 ± 2.36	86.20 ± 1.66	87.14±1.99	87.81 ± 1.61	89.05 ± 1.87
Diabetic Control	87.28 ± 3.58	315.66 ± 12. 46	327.36±10.62	251.19± 2.94	351.02 ± 8.22
Glibenclamide (5 mg/kg	88.33 ± 1.62	319.66±11.25	241.20 ± 8.54 ***	135.27 ± 3.93***	122.09 ± 4.25***
SCME (200 mg/kg)	86.46 ± 3.57	215.83 ± 5.65	290.53 ± 7.32**	172.71 ± 6.28**	152.26 ± 5.67**
SCME (400 mg/kg)	86.25 ± 2.92	324.02±10.46	258.40 ± 7.07**	190.04 ± 5.21***	138.36 ± 2.244**
SZME (200 mg/ kg)	87.06± 2.52	319.18 ± 8.06	282.51 ± 6.02**	209.15 ± 6.35**	140.26 ± 6.15**
SZME (400 mg/kg)	87.95 ± 3.01	323.22 ± 9.86	249.10 ± 7.70***	181.27 ± 4.23***	130.16 ± 3.244***

Note: SCME denotes *Syzygium caryophyllatum* methanol extract; SZME denotes *Syzygium zeylanicum* methanol extract. Values are expressed as mean ± SEM; n = 6. One way ANOVA followed by Dunnett's't' test. **P < 0.01 and ***P < 0.001 compared with Diabetic control

Table 3: Blood glucose level on OGTT in Wistar rats

	Blood glucose level (mg/ dl)				
Groups	0 min	30 min	60 min	120 min	240 min
Normal	86.62 ± 1.30	137.22± 1.26	121.09±3.82	111.27±2.36	84.44 ±3.12
Glibenclamide (5 mg/kg	83.61 ± 2.58	96.30±2.36**	84.29 ±3.67 **	78.16 ± 2.46**	68.44± 1.32**
SCME (200 mg/kg)	84.39 ± 2.12	127.30±2.61*	114.26 ± 5.41**	98.05 ± 4.35*	81.60 ± 3.05*
SCME (400 mg/kg)	84.69 ± 2.09	121.61±2.31**	92.06 ± 5.40**	83.99 ± 3.65**	75.62 ± 2.05**
SZME (200 mg/ kg)	84.92± 3.02	126.81 ± 3.01*	112.36 ± 3.19**	92.35 ± 3.54*	78.20 ± 2.95**
SZME (400 mg/kg)	84.02± 3.27	123.18 ± 2.91**	90.63 ± 3.99***	82.21 ± 3.60**	73.22± 1.95**

Note: SCME denotes *Syzygium caryophyllatum* methanol extract; SZME denotes *Syzygium zeylanicum* methanol extract. Values are expressed as mean ± SEM; n = 6. One way ANOVA followed by Dunnett's't' test. *P < 0.05 and **P < 0.01 compared with normal control

Table 4: SGPT and SGOT levels in diabetic rats

Groups	Alloxan		STZ	
	SGPT	SGOT	SGPT	SGOT
Normal	58.45±1.21	67.59±1.40	57.41±2.81	64.53±3.05
Diabetic	140.51±1.95	149.38±1.60	136.40±2.38	151.22±1.11

Glibenclamide (5 mg/kg)	88.38±1.55***	85.41±2.05**	80.51±3.14***	75.51±1.84***
SCME (200 mg/kg)	110.07±2.90**	109.33±1.60**	96.14±3.05**	93.04±1.20**
SCME (400 mg/kg)	104.80±1.95***	97.01±2.05***	92.07±2.09***	81.69±1.09***
SZME (200 mg/ kg)	108.71±2.10**	103.13±1.94**	93.24±2.95**	90.41±1.92**
SZME (400 mg/kg)	100.32±2.54***	93.19±2.82***	90.78±2.91***	79.96±1.92***

SCME denotes *Syzygium caryophyllatum* methanol extract., SZME denotes *Syzygium zeylanicum* methanol extract. Values are expressed as mean ± SEM; n = 6. One way ANOVA followed by Dunnett's't' test. **P < 0.01 and ***P < 0.001 compare with Diabetic control.

Table 5: Body weight in alloxan induced diabetic rats

Groups	Body weight (Grams)			
	Day 0	Day 7	Day 14	Day 21
Normal	178.41±5.30	182.30±4.61	185.51±4.07	189.42±5.61
Diabetic	179.95±5.24	173.52±3.79	165.84±6.05	155.15±5.09
Glibenclamide (5 mg/kg)	180.95±3.51	182.30±2.26**	184.32±2.67**	187.59±3.44**
SCME (200 mg/kg)	180.31±3.11	175.51±3.63*	178.56±4.48**	182.05±5.65**
SCME (400 mg/kg)	179.19± 4.10	177.31±3.22**	180.31±4.92***	183.81±4.09***
SZME (200 mg/ kg)	178.21±4.12	174.23±4.37**	178.09±3.05**	183.01±4.08**
SZME (400 mg/kg)	180.41± 3.49	178.53±3.73**	181.76±4.08***	185.79±3.48***

Note: SCME denotes *Syzygium caryophyllatum* methanol extract; SZME denotes *Syzygium zeylanicum* methanol extract. Values are expressed as mean ± SEM; n=6. One way ANOVA followed by Dunnett's't' test. *P<0.05, **P<0.01 and ***P<0.001 compare with Diabetic control.

Table 6: Body weight in STZ induced diabetic rats

Groups	Body weight (Grams)			
	Day 0	Day 7	Day 14	Day 21
Normal	188.15±5.08	190.25±5.12	195.12±5.69	198.65±4.98
Diabetic	185.29±4.37	174.61±5.05	162.45±6.07	155.82±3.24
Glibenclamide (5 mg/kg)	187.36±4.23	181.91±4.14**	183.27±4.41***	186.22±4.63***
SCME (200 mg/kg)	186.63±3.32	175.54±3.23*	178.81±3.82**	182.91±4.36**
SCME (400 mg/kg)	187.29±4.37	177.46±3.61**	180.56±2.48***	184.45±4.15***
SZME (200 mg/ kg)	185.86±3.62	176.54±4.27*	180.42±3.56**	182.46±3.74***
SZME (400 mg/kg)	188.04±5.01	179.27±3.87**	182.42±3.83***	185.05±4.37***

Note: SCME denotes *Syzygium caryophyllatum* methanol extract; SZME denotes *Syzygium zeylanicum* methanol extract. Values are expressed as mean ± SEM; n = 6. One way ANOVA followed by Dunnett's't' test. *P < 0.05, **P<0.01 and ***P < 0.001 compare with Diabetic control.

Table 7: UPLC quantification of phenolic acids in *S. caryophyllatum* and *S. zeylanicum* bark

Phenolic acids	Parent and Daughter ions	*S.caryophyllatum* (µg/mg)	*S.zeylanicum* (µg/mg)
Syringic acid	196.97 >153.11	116.57	ND
Ferulic acid	192.9 > 149.07	74.17	ND
Caffeic acid	178.9 >135.05	12.50	12.45
Gallic acid	168.9 >125.03	7.77	508.89
Vanillic acid	166.97 >123.07	170.83	3.41
P – Coumaric acid	162.9 > 119.05	3.32	ND
P– Hydroxy benzoic acid	136.9 > 93.01	12.99	7.41
Salicylic acid	136.9 > 93.1	2.42	0.93
Gentisic acid	152.9 > 108.98	9.25	ND
Protocatechuic acid	152.9 > 109.05	16.68	ND

O - Coumaric acid	162.9 >119.05	3.05	2.55
2,4-Dihydroxy benzoic acid	152.9 >109.0	3.98	3.57
Chlorogenic acid	352.97 >191.1	ND	ND
Total		433.53	539.21

Note: ND denotes Not Detected

Table 8: UPLC quantification of flavonoids in *S.caryophyllatum* and *S.zeylanicum* bark

Flavonoids	Parent and Daughter ions	*S.caryophyllatum* (µg/mg)	*S.zeylanicum* (µg/mg)
Rutin	609.1>300.2	18.77	ND
Myricetin	317.03>151.06	32.16	5.77
Catechin	289.03>245.15	84.63	91.14
Kempherol	284.97>145.5	ND	ND
Naringenin	271.03>151.0	ND	1.81
Quercetin	301.03>151.12	ND	ND
Hesperedin	300.97>164.05	ND	ND
Apigenin	268.97>107.04	ND	18.63
Umbeliferon	161.04>133.07	24.91	2.52
Luteolin	284.97>132.95	ND	4.96
Total		160.48	124.83

Note: ND denotes Not Detected

CONCLUSION

The extracts of *Syzygium caryophyllatum* and *Syzygium zeylanicum* at 400 mg / kg body weight possessed a remarkable fasting blood – glucose lowering potential, significant increase in body weight and reduced levels of SGPT and SGOT in alloxan and Streptozotocin induced diabetic rats. The extracts also exhibited improved glucose utilisation. The results confirm the use of *Syzygium* species in traditional system of medicine to treat diabetes mellitus.

Conflict of Interest

None declared.

Financial Support

None declared.

REFERENCES

1. Kumar P, Clark M. Diabetes mellitus and other disorders of metabolism. Clinical medicine. 2002;2:1069-71.
2. Valiathan MS. Healing plants. Curr Sc. 1998;75:1122-1127.
3. ERHSS Ediriweera, WD Ratnasooriya. A review on herbs used in treatment of Diabetes Mellitus by Sri Lankan ayurvedic and traditional physicians. Ayu. 2009;30(4):373-391.
4. WHO/IDF. Report of consultation: Definition, diagnosis and classification of diabetes mellitus and its complications, Geneva, World Health Organization, 1999.
5. Jadhav R, Puchchakayala G. Hypoglycemic and antidiabetic activity of flavonoids: boswellic acid, Ellagic acid, quercetin, rutin on Streptozotocin – nicotinamide induced type 2 diabetic rats. Int J Pharm Pharm Sc. 2012; 42(2):51-256.
6. Sy GY, Cisse A, Nongonierma RB, Sarr M, Mbodj NA, Faye B. Hypoglycaemic and antidiabetic activity of acetonic extract of *Vernonia colorata* leaves in normoglycaemic and alloxan – induced diabetic rats. J Ethnopharmacol. 2005; 98(1-2):171-175.
7. Hunt JV. Ascorbic acid and diabetes mellitus. Subcellular Biochemistry: Ascorbic Acid: Biochemistry and Biomedical Cell Biology. 1996 Jan 1:369-405.
8. Pari L, Umamaheswari J. Antihyperglycaemic activity of *Musa sapientum* flowers: effect on lipid peroxidation in alloxan diabetic rats. Phytother Res. 2000;14:1-3.
9. Wu Z, Luo JZ, Luo L. American ginseng modulates pancreatic beta cell activities. Chin Med, 2007;2:11.
10. Shokeen P, Anand P, Murali YK, Tandon V. Antidiabetic activity of 50 % ethanolic extract of *Ricinus communis* and its purified fractions. Food Chem Toxicol. 2008; 46(11):3458 - 3466.
11. 11Sagrawat HAS, Mann Kharya MD. Pharmacological potential of *Eugenia jambolana*. A review. Pharmacogn Mag. 2006;2:96 -105.
12. 12 Rizvi S I, Mishra N. Traditional Indian Medicines Used for the Management of Diabetes Mellitus J Diabetes Res. 2013;712092
13. Rabeque CS, Padmavathy S. Hypoglycaemic effect of *Syzygium caryophyllatum* (L.) Alston on alloxan induced diabetic albino mice. Asian J Pharm Clin Res. 2013;6(4):202 -205.
14. OECD. Guideline on Acute Oral Toxicity (AOT) Environmental Health and Safety Monograph Series on Testing and Adjustment, 2021, No. 425.
15. Tian YA, Johnson G, Ashcroft SJ. Sulfonylureas enhance exocytosis from pancreatic b - cells by a mechanism that does not involve direct activation of protein kinase C. Diabetes. 1998;47:1722-1726.
16. Kumawat NS, Chaudhari SP, Wani NS, Deshmukh TA, Patil, VR. Antidiabetic activity of ethanol extract of *Colocasia esculenta* leaves in alloxan induced diabetic rats. Int J Pharm Tech Res. 2010;2(2):1246 -1249.

17. Rajeswari J, Kesavan K, Jayakar B. Antidiabetic activity and chemical characterization of aqueous/ethanol prop roots extracts of *Pandanus fascicularis* Lam in Streptozotocin - induced diabetic rats. Asian Pac J Trop Biomed. 2012;S170-S174.
18. Omamoto H, Ucgigata Y, Hiroskitckan. STZ and alloxan induces DNA strand breaks and poly (ADP ribose) synthatase in pancreatic islet. Nature. 1981; 294:284-286.
19. Fisher LJ. Drugs and chemicals that produce diabetes. Trends Pharmacol Sci. 1985; 6:72-75.
20. Murthy BK, Nammi S, Kota MK, Krishna Rao RV, Rao NK, Annapurna A. Evaluation of hypoglycemic and antihyyperglycemic effect of *Datura metel* (Linn.) seeds in normal and alloxan – induced diabetic rat. J Ethnopharmacol. 2004; 91(1):95-98.
21. Junod A, Lambert AE, Stauffacher W, Renold AE. Diabetogenic action of streptozotocin: relationship of dose to metabolic response. J Clin Investig. 1969; 48:2129-39.
22. Asada T, Ogawa T, Iwai M, Kobayashi, M. Effect of recombinant human insulin – like growth factor - I on expression of glucose transporters, GLUT 2 and GLUT 4, in Streptozotocin - diabetic rat. Jpn J Pharmacol. 1998; 78(1):63-67.
23. Teotia S, Singh. M. Hypoglycemic effect of *Prunus amygdalu* seeds in albino rabbits. Indian J Exp Biol. 1997; 35:295-6.
24. Mc Nurlan MA, Garlick. PJ. Rates of protein synthesis in rat liver and small intestine in protein deprivation and diabetes. Proc Nutr Soc. 1979; 38(3):133
25. Gillespie KM. Type 1 diabetes: pathogenesis and prevention. Can Med Assoc J. 2006;175(2):165-170.
26. Saravanan G, Pari L. Hypoglycaemic and Antihyperglycaemic effect of *Syzygium cumini* Bark in Streptozotocin - Induced Diabetic Rats. J Pharmacol Toxicol. 2008;3:1-10.
27. Kumar AY, Nandakumar K, Handral M, Talwar S, Dhayabaran D. Hypoglycaemic and anti-diabetic activity of stem bark extracts *Erythrina indica* in normal and alloxan – induced diabetic rats. Saudi Pharm J. 2011; 19(1):35-42.
28. Itoh A, Isoda K, Kondoh M, Kawase M, Kobayashi M, Tamesada M, Yagi K. Hepatoprotective Effect of Syringic Acid and Vanillic Acid on Concanavalin A - Induced Liver Injury. Biol Pharm Bull. 2009; 32(7):1215-1219.
29. Hsu CL, Lo WH, Yen GC. Gallic acid induces apoptosisin 3T3 - L1 pre - adipocytes via a fas - and mitochondrial mediated pathway. J Agric Food Chem. 2007; 55(18):7359-736.
30. Nakano N, Shirasaka N, Koyama H, Hino M, Murakami T, Shimizu S et al., C19 odd - chain polyunsaturated fatty acids (PUFAs) are metabolized to C21- PUFAs in a rat liver cell line, and curcumin, gallic acid, and their related compounds inhibit their desaturation. Biosci Biotechnol and Biochem. 2000;64(8):1641-1650.
31. Har LW, Ismail IS. Antioxidant activity, total phenolics and total Flavonoids of *Syzygium polyanthum* (Wight) Walp leaves. Int J Med Aromat Plants. 2012;2(2):219-228.
32. Bonfanti G, Bitencourt, PR, Karine S B, Priscila SS, Jantsch LB, Pigatto. et al. *Syzygium jambos* and *Solanum guaraniticum* show Similar Antioxidant Properties but Induce Different Enzymatic Activities in the Brain of Rats. Molecules. 2013;18:9179-9194.
33. Manaharan T, Appleton D, Cheng HM, Palanisamy UD. Flavonoids isolated from *Syzygium aqueum* leaf extract as potential antihyperglycaemic agents. Food Chem. 2012;132:1802-1807.
34. Sharma B, Balomajumder C, Roy P. Hypoglycemic and hypo - lipidemic effects of flavonoid rich extract from *Eugenia jambolana* seeds on Streptozotocin induced diabetic rats. Food Chem Toxicol. 2008;46:2376-2383.
35. Kamalakkannan N, Prince PSM. The influence of rutin on the extracellular matrix in Streptozotocin - induced diabetic rat kidney. J Pharm Pharmacol. 2006;58:1091-1098

HOW TO CITE THIS ARTICLE

Shilpa KJ, Krishnakumar G, Hegde K. Comparative study on the Antidiabetic activity of the bark extracts of *Syzygium caryophyllatum* (L.) Alston and *Syzygium zeylanicum* (L.) DC. J Phytopharmacol 2023; 12(5):326-333. doi: 10.31254/phyto.2023.12508

Creative Commons (CC) License-

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY 4.0) license. This license permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited. (http://creativecommons.org/licenses/by/4.0/).