



Studies on anti-hyperglycemic effect of *Euphorbia antiquorum* L. root in diabetic rats

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ABSTRACT

Background/Aim: To determine the anti-hyperglycemic effect of *Euphorbia antiquorum* L. root. **Materials and Methods:** The study evaluates the anti-hyperglycemic effect of *E. antiquorum* root in streptozotocin-nicotinamide-induced Type 2 diabetes mellitus and fructose-induced insulin resistance models. Alcohol and aqueous extracts of *E. antiquorum* root were administered at doses 200 and 400 mg/kg p.o. Serum levels of glucose, total cholesterol, triglycerides, glycosylated hemoglobin (GHb), and hepatic levels of malondialdehyde, glutathione, and glycogen were estimated. **Results:** Treatment with the alcohol and aqueous extracts of *E. antiquorum* roots resulted in significant ($P < 0.001$) lowering of serum blood glucose and GHb levels in both the models. Flavonoids, phenolic compounds, and glycosides were detected in the preliminary phytochemical screening. **Conclusion:** Root of *E. antiquorum* showed promising anti-hyperglycemic effect which may be due to the presence of important phytochemicals.

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INTRODUCTION

Diabetes mellitus is a group of heterogeneous disorders associated with hyperglycemia and glucose intolerance due to insulin deficiency, impaired effectiveness of insulin action, or both. Herbal based anti-diabetic drugs are being developed which could replace some of the currently used oral hypoglycemic drugs to ensure better therapeutic outcome and acceptability [1].

Snuhee is an important drug in Ayurveda, for which *Euphorbia neriifolia* L., is the accepted botanical source and *Euphorbia antiquorum* Linn. (Euphorbiaceae) is used as substitute [2]. The leaf, stem, latex, and root of *Snuhee* are used in Ayurveda for the treatment of abdominal disorders, diabetes, edema, psychosis, leprosy, coryza, anemia, and rheumatoid arthritis [3-5]. *E. antiquorum* is used as a sex stimulant [6], laxative [7], and anti-fertility agent [8]; in rheumatism, toothache and nerve diseases [9]; in the treatment of inflammation, swellings on

breast, and as a purgative [10]; for earache, dropsy, syphilis, and leprosy [11]. The plant is also used in veterinary practice [12,13]. In the Siddha system of medicine, *E. antiquorum* is known as *Sathura kalli* and is used in the treatment of skin diseases, urticaria, kapham, abdominal disorders, constipation, leucorrhea, and leprosy [3].

The phytoconstituents isolated from *E. antiquorum* are 3-O-angeloylgenol [14]; Eupha 7, 9 (11) 24-trien-3 β -ol ("antiquol C") and certain triterpenes from the latex [15]; terpenoids - friedelane-3 β , 30-dioldiacetate, 30-acetoxyfriedelane-3 β -ol, and 3 β -acetoxy friedelane-30-ol from the stem [16]; ingenane type of diterpene esters were isolated from 5 *Euphorbia* species [17]; a diterpene antiquorin along with friedelane-3 β -ol and taraxerol was also isolated from *E. antiquorum* [18].

The stem of *E. antiquorum* has been subjected to extensive pharmacological evaluations including

anti-hyperglycemic [19]; anti-inflammatory and anti-arthritic [20]; antibacterial [21]; antitussive [22]; antibacterial and antifungal [23]; hepatoprotective and antioxidant [24] activities. Anti-hyperglycemic and aldose reductase inhibition activity studies have been reported on some isolated terpenoids [25]. In the present study, evaluation of anti-diabetic property of *E. antiquorum* root has been undertaken since no such studies are reported.

MATERIALS AND METHODS

Plant Material

Roots of *E. antiquorum* were collected from the forest surroundings of Tirunelveli, Tamil Nadu, India, during March 2011. The plant material was identified and authenticated by Dr. S. N. Yoganasimhan, Plant Taxonomist, following various floras [26,27]. Voucher herbarium specimen (Sri Lalitha 045) along with a sample of the drug tested has been deposited at the herbarium and crude drug museum of Faculty of Pharmacy, M. S. Ramaiah University of Applied Sciences, Bengaluru, Karnataka, India.

Preparation of Extracts

Total alcohol extract was prepared by soxhlation with 95% v/v ethanol (yield 15.4% w/w). The total aqueous extract was prepared by maceration with chloroform water (0.25% v/v of chloroform in distilled water) (yield 13.8% w/w). The alcohol and aqueous extracts were suspended in 2% w/v acacia solution in distilled water for pharmacological studies.

Phytochemical Studies

The dried extracts were subjected to preliminary phytochemical screening to detect the presence of various phytochemical constituents and the extracts were further standardized by high-performance thin-layer chromatography (HPTLC) [28]. Camag HPTLC system equipped with Linomat V applicator, TLC scanner 3, Reprostar 3 with 12 bit CCD camera for photo documentation, controlled by WinCATS-4 software was used. All the solvents used were of HPLC grade obtained from Merck, India. All weighing were done on Precisa XB 12A digital balance. The extract concentration used was 5 mg/ml and pre-coated aluminum plates with silica 60 F₂₅₄ (10 cm × 10 cm) as stationary phase was used. Ethyl acetate:pyridine:water:methanol (80:20:10:5) was used as the mobile phase. Developed plates were then scanned under the wavelengths 254 nm, 366 nm, and 425 nm using deuterium, mercury and tungsten lamps, respectively and photo documented using Camag Reprostar 3.

Pharmacological Studies

Animals

Albino rats (Wistar strain) of either sex 8-12 weeks old, weighing 170-250 g were used in acute toxicity and anti-diabetic studies. The animals were maintained as per Committee for the Purpose of Control and Supervision of Experiments on Animals

guidelines and kept at 12 h dark/12 h light cycle. This study was approved by the Institutional Animal Ethics Committee of the institution (IEAC certificate no. MSRCP/M-40/2011).

Acute Toxicity

Acute toxicity studies were carried out following OECD guidelines 420 [29].

Fructose-Induced Insulin Resistance

Insulin resistance was induced in rats by chronic fructose feeding (40% fructose + 60% normal rat chow, 25 g/100 g b.w/day) for a period of 21 days. After 21 days fasting, serum glucose levels were checked and animals with moderate diabetes having serum glucose ≥ 180 mg/dl were further grouped into the positive control, standard and extract treated groups. Vehicle treated non-diabetic rats were assigned as the normal control group (Group I). Diabetic rats were divided into six groups of six animals each. Untreated diabetic rats served as the positive control group (Group II). Group III was administered standard anti-diabetic drug pioglitazone (10 mg/kg, b.w, p.o). Groups IV-VII were administered the alcohol and aqueous extracts at doses 200 and 400 mg/kg, respectively for 28 days. After 28 days animals were fasted overnight and on the 29th day, blood samples (<1 ml) were collected from the retro-orbital sinus under ether anesthesia. Serum was separated from the clotted blood by centrifugation at 12,000 rpm for 10 min and used for the estimations [30,31].

Streptozotocin (STZ)-Nicotinamide (NA) Induced Type 2 Diabetes Mellitus (NIDDM)

Diabetic mellitus (NIDDM) was induced by a single injection of freshly prepared solution of STZ (65 mg/kg b.w. intraperitoneal [i.p.]) in 0.1 mol/L cold citrate buffer (pH 4.5), 15 min after the administration of NA (230 mg/kg b.w. i.p). After 14 days, fasting serum glucose levels were checked for the development of diabetes. Animals with fasting serum glucose levels ≥ 180 mg/dl were further grouped into the positive control, standard and extract groups. Vehicle treated non-diabetic rats were assigned as the normal control group (Group I). Group II was the positive control, in which vehicle-treated diabetic rats were included. Group III was the standard group which was administered with glimepiride 0.5 mg/kg. Groups IV and V were administered the alcohol extract at dose 200 and 400 mg/kg, respectively, and Groups VI and VII were administered the aqueous extracts of *E. antiquorum* roots at doses 200 and 400 mg/kg respectively. Each group consisted of six animals. The treatment schedule was once daily for 28 days by oral administration. On the 29th day, blood (<1 ml) was withdrawn by retro-orbital puncturing under ether anesthesia. The animals were kept for overnight fasting prior to blood withdrawal [32-34].

Glucose [35], total cholesterol (TC) and triglycerides (TG) [36], glycosylated hemoglobin (GH_b) [37] were tested in serum for both models using commercial diagnostic kits.

Following blood withdrawal, the animals were sacrificed by an excess of anesthesia and liver was isolated. The liver was washed and used for preparation of homogenates - 10% w/v liver homogenate in 0.15 M potassium chloride buffer, used for the estimation of malondialdehyde (MDA) [38]; 10% w/v liver homogenate in 0.25% w/v sucrose in phosphate buffer (pH 7.4), used for the estimation of glutathione (GSH) [39]; 1% w/v liver homogenate in 5% trichloroacetic acid, used for the estimation of the liver glycogen [40].

Statistical Analysis

The data were expressed as mean \pm SEM and tested with one-way analysis of variance followed by Tukey Kramer multiple comparison test.

RESULTS

Phytochemical Analysis

Preliminary phytochemical analysis revealed the presence of carbohydrates and glycosides; phenolic compounds and tannins; flavonoids.

HPTLC Studies

The alcohol extract at 254 nm revealed 6 phytoconstituents with no characteristic fluorescence [Figure 1]. At 366 nm, 3 phytoconstituents were revealed of which, one spot having R_f 0.59 exhibited blue fluorescence and another with R_f 0.66 exhibited light blue fluorescence. At 425 nm, 1 phytoconstituent having R_f 0.92 was revealed.

The aqueous extract revealed 18 phytoconstituents at 254 nm with no characteristic fluorescence [Figure 2]. At 366 nm, the aqueous extract revealed 10 spots and those with R_f values 0.64 and 0.75 were prominent. Spot with R_f 0.64 exhibited light blue fluorescence, whereas the one with R_f 0.75 exhibited dark blue fluorescence. The alcohol extract revealed 3 phytoconstituents with R_f values 0.03, 0.34, and 0.92 at 425 nm.

Acute Toxicity

Both the alcohol and aqueous extracts were found to be safe up to 2000 mg/kg.

Fructose-Induced Insulin Resistance

Administration of fructose for 21 days caused the development of hyperglycemia (≥ 180 mg/dl) in all the animals. The treatment with extracts of *E. antiquorum* roots 200 and 400 mg/kg resulted in significant ($P < 0.001$) lowering of serum blood glucose levels compared to the untreated diabetic control animals.

Serum of the diabetic control animals showed significantly ($P < 0.001$) increase in the TC levels. Serum TG levels were also high in the untreated diabetic animals. Treatment with extracts significantly reduced the elevated lipid levels.

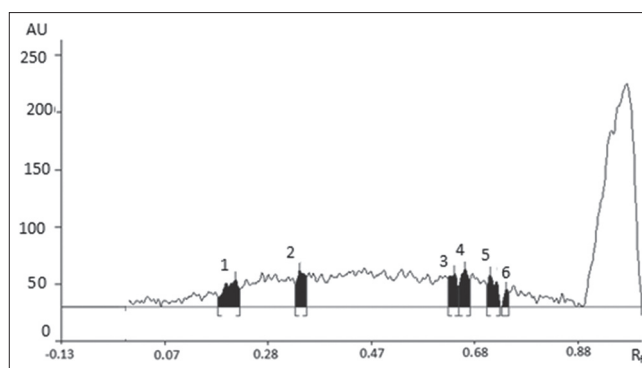


Figure 1: High-performance thin-layer chromatography fingerprint of alcohol extract of the root of *Euphorbia antiquorum* at 254 nm

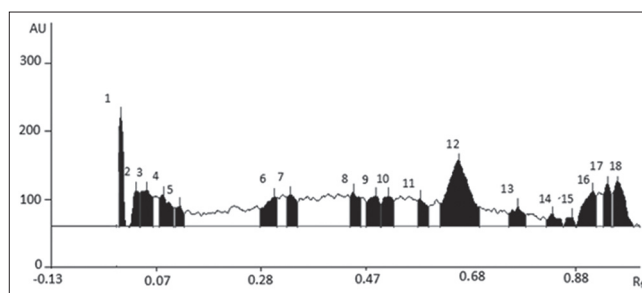


Figure 2: High-performance thin-layer chromatography fingerprint of aqueous extract of the root of *Euphorbia antiquorum* at 254 nm

Significant ($P < 0.001$) reduction in TC levels was observed in the groups treated with 400 mg/kg dose of both extracts. However, TG levels were significantly lowered with the dose of 200 mg/kg as well. GH_b levels were significantly ($P < 0.001$) lowered in the all test drug-treated groups when compared to control [Table 1].

Hepatic GSH levels decreased significantly ($P < 0.001$) in the positive control rats. This was significantly ($P < 0.05$ and $P < 0.001$) increased in animals treated with the higher dose of alcohol and aqueous extracts respectively. Liver glycogen levels decreased significantly ($P < 0.001$) in the positive control group. In groups treated with the alcohol extract, liver glycogen levels increased significantly ($P < 0.01$, $P < 0.001$ for 200 and 400 mg/kg, respectively). The aqueous extract at 400 mg/kg dose also showed significantly ($P < 0.001$) increase in hepatic glycogen levels. Hepatic MDA levels were significantly ($P < 0.001$) high in the diabetic control rats, indicating lipid peroxidation. However, treatment with extracts significantly ($P < 0.001$) reduced the extent of lipid peroxidation [Table 2].

STZ-NIDDM

Fasting serum glucose levels of positive control rats were significantly ($P < 0.001$) higher than the normal rats. The alcohol and aqueous extracts of *E. antiquorum* roots exhibited significant anti-hyperglycemic effects. There was a significant ($P < 0.001$) decrease in serum glucose levels with 200 and 400 mg/kg of alcohol and aqueous extracts. GH_b levels were significantly increased in the diabetic control animals and were

significantly ($P < 0.001$) lowered in the extract treated groups [Table 3].

Serum TC levels were significantly ($P < 0.001$) increased in the positive control group. There was a significant ($P < 0.001$) decrease in the cholesterol levels in animals treated with the higher dose of alcohol and aqueous extracts. The serum TG levels were also significantly ($P < 0.001$) high in the diabetic control group, and this was significantly ($P < 0.001$) controlled in the extract treated groups.

Hepatic GSH levels were significantly reduced ($P < 0.001$) in the positive control animals and significant ($P < 0.001$) increase was observed in both the extract treated groups. Administration of STZ and NA caused extensive lipid peroxidation which was evidenced by the significant ($P < 0.001$) increase in hepatic MDA levels in the diabetic control animals. Lipid peroxidation was also significantly ($P < 0.001$) lowered in the groups treated with the alcohol and aqueous extracts of *E. antiquorum* roots. A significant increase in liver glycogen levels was observed in the

alcohol ($P < 0.01$, $P < 0.001$) and aqueous ($P < 0.001$) extract treated groups [Table 4].

DISCUSSION

Administration of both STZ and NA by i.p. injection induces experimental diabetes in rats. STZ (2-deoxy-2-([methyl (nitroso) amino] carbonyl) amino)- β -D-glucopyranose) is a naturally occurring compound, produced by *Streptomyces achromogenes*, and it causes pancreatic β -cell damage. NA is administered partially to protect the insulin-secreting cells against STZ [41].

The anti-hyperglycemic activity of *E. antiquorum* extracts was compared with glimepiride, the second generation anti-hyperglycemic drug. Oral administration of *E. antiquorum* extracts and glimepiride to STZ-NA-induced diabetic rats decreased the serum glucose levels.

Increase TC and TG levels were observed in the untreated diabetic control rats. In diabetic rats treated with *E. antiquorum*

Table 1: Effect of *E. antiquorum* root extracts on serum parameters in fructose-induced insulin resistance

Treatment/groups	Glucose	TC	TG	GH _b
Normal control	101.14 \pm 2.54	41.95 \pm 1.69	70.69 \pm 1.67	12.40 \pm 0.27
Positive control	249.82 \pm 7.42 ^a	77.26 \pm 8.01 ^a	80.90 \pm 4.38 ^{ns}	17.86 \pm 0.80 ^{ns}
Standard (pioglitazone)	141.17 \pm 9.09***	32.05 \pm 2.16***	36.27 \pm 2.71***	4.54 \pm 0.37***
Alcohol extract 200 mg/kg	181.29 \pm 3.87***	69.74 \pm 2.90 ^{ns}	75.22 \pm 2.36***	9.29 \pm 2.35***
Alcohol extract 400 mg/kg	154.68 \pm 6.46***	71.23 \pm 6.51***	67.79 \pm 3.92***	9.08 \pm 1.35***
Aqueous extract 200 mg/kg	190.54 \pm 4.56***	67.19 \pm 5.20 ^{ns}	81.40 \pm 2.66***	8.34 \pm 0.77***
Aqueous extract 400 mg/kg	136.56 \pm 12.62***	41.62 \pm 4.56***	58.84 \pm 1.93***	7.45 \pm 1.35***

One-way analysis of variance. The values are expressed as mean \pm SEM; $n=6$ animals in each group. Tukey-Kramer multiple comparison test *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ versus positive control; ^a $P < 0.001$ versus normal control group. SEM: Standard error of mean, TC: Total cholesterol, TG: Triglycerides, GH_b: Glycosylated hemoglobin, *E. antiquorum*: *Euphorbia antiquorum*

Table 2: Effect of *E. antiquorum* root extracts on hepatic parameters in fructose-induced insulin resistance

Treatment/groups	Liver weight	MDA	GSH	Glycogen
Normal control	4.03 \pm 0.45	1.09 \pm 0.10	61 \pm 1.53	1079.32 \pm 73.26
Positive control	3.35 \pm 0.22 ^b	2.15 \pm 0.12 ^a	32.10 \pm 1.57 ^a	606.46 \pm 19.97 ^a
Standard (pioglitazone)	3.94 \pm 0.09 ^{ns}	0.18 \pm 0.01***	69.75 \pm 1.95***	2062.55 \pm 59.62***
Alcohol extract 200 mg/kg	3.69 \pm 0.18*	1.19 \pm 0.17***	42.42 \pm 1.75 ^{ns}	942.51 \pm 104.73**
Alcohol extract 400 mg/kg	3.78 \pm 0.12 ^{ns}	0.45 \pm 0.03***	53.67 \pm 10.57*	1237.98 \pm 13.26***
Aqueous extract 200 mg/kg	3.35 \pm 0.14***	0.98 \pm 0.07***	46.25 \pm 0.97 ^{ns}	836.17 \pm 36.79 ^{ns}
Aqueous extract 400 mg/kg	3.54 \pm 0.09***	0.58 \pm 0.02***	65.22 \pm 1.20***	1072.92 \pm 16.16***

One-way analysis of variance. The values are expressed as mean \pm SEM; $n=6$ animals in each group. Tukey-Kramer multiple comparison test *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ versus positive control; ^a $P < 0.001$, ^b $P < 0.01$ versus normal control group. SEM: Standard error of mean, MDA: Malondialdehyde, *E. antiquorum*: *Euphorbia antiquorum*, GSH: Glutathione

Table 3: Effect of *E. antiquorum* root extracts on serum parameters in STZ-NIDDM

Treatment/groups	Glucose	TC	TG	GH _b
Normal control	101.14 \pm 2.54	41.95 \pm 1.69	70.69 \pm 1.67	12.40 \pm 0.27
Positive control	221.5 \pm 20.89 ^a	83.31 \pm 2.49 ^a	101.45 \pm 3.35 ^a	18.90 \pm 0.58 ^a
Standard (glimepiride)	86.98 \pm 1.05***	40.94 \pm 2.28***	41.14 \pm 1.70***	4.94 \pm 0.42***
Alcohol extract 200 mg/kg	142.16 \pm 2.60***	78.15 \pm 2.0 ^{ns}	65.11 \pm 2.84***	9.59 \pm 0.67***
Alcohol extract 400 mg/kg	107.70 \pm 2.16***	52.72 \pm 3.8***	68.69 \pm 2.70***	9.59 \pm 0.67***
Aqueous extract 200 mg/kg	160.36 \pm 3.82***	77.49 \pm 2.4 ^{ns}	74.10 \pm 2.30***	11.95 \pm 0.72***
Aqueous extract 400 mg/kg	151.51 \pm 3.56***	33.00 \pm 3.07***	83.75 \pm 2.30***	5.69 \pm 0.46***

One-way analysis of variance. The values are expressed as mean \pm SEM; $n=6$ animals in each group. Tukey-Kramer multiple comparison test *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ versus positive control; ^a $P < 0.001$ versus normal control group. SEM: Standard error of mean, TC: Total cholesterol, TG: Triglycerides, GH_b: Glycosylated hemoglobin, *E. antiquorum*: *Euphorbia antiquorum*

Table 4: Effect of *E. antiquorum* root extracts on hepatic parameters in STZ-NIDDM

Treatment/groups	Liver weight	MDA	GSH	Glycogen
Normal control	4.03±0.45	1.09±0.10	61±1.53	1079.32±73.26
Positive control	3.35±0.22 ^b	2.16±0.09 ^a	26.88±1.62 ^a	517.96±94.03 ^a
Standard (glimepiride)	4.11±0.06 ^{**}	0.36±0.02 ^{***}	73.39±0.40 ^{***}	1550.82±26.88 ^{***}
Alcohol extract 200 mg/kg	3.61±0.06 ^{ns}	1.25±0.10 ^{***}	42.86±0.30 ^{***}	872.10±38.30 ^{**}
Alcohol extract 400 mg/kg	3.85±0.03 ^{ns}	0.71±0.02 ^{***}	49.50±2.88 ^{***}	985±56.21 ^{***}
Aqueous extract 200 mg/kg	3.74±0.06 ^{ns}	1.56±0.04 ^{***}	44.74±0.43 ^{***}	961.36±19.98 ^{***}
Aqueous extract 400 mg/kg	3.85±0.06 ^{ns}	0.77±0.04 ^{***}	62.17±1.01 ^{***}	1207.36±49.14 ^{***}

One-way analysis of variance. The values are expressed as mean±SEM; n=6 animals in each group. Tukey-Kramer multiple comparison test

***P<0.001, **P<0.01, *P<0.05 versus positive control; ^aP<0.001, ^bP<0.01 versus normal control group. NIDDM: Nicotinamide-induced Type 2 diabetes mellitus, MDA: Malondialdehyde, *E. antiquorum*: *Euphorbia antiquorum*, GSH: Glutathione

extracts and glimepiride, the levels of TC and TG were significantly lowered as compared to the diabetic control. GH_b levels increase over long periods of time in diabetes. In the diabetic condition, an excess of glucose present in the blood reacts with hemoglobin to form GH_b. The rate of glycation or glycosylation is proportional to the concentration of glucose in the blood. In the current study, the untreated diabetic rats indicated the higher levels of GH_b in blood compared to the normal rats. Serum of animals treated with the *E. antiquorum* extracts and glimepiride showed a significant decrease in GH_b levels.

The liver plays an important role in buffering the post-prandial hyperglycemia and is involved in the synthesis of glycogen. Diabetes mellitus impairs the normal ability of the liver to synthesize glycogen. Glycogen depletion causes the mobilization of fat to meet the body's metabolic demands [42]. Hepatic glycogen levels were significantly ($P < 0.001$) lowered in the untreated diabetic control group and this abnormality was brought back to near normal levels in the extract treated groups.

The untreated diabetic animals in the present study registered low levels of GSH and high levels of MDA, suggesting its increased utilization to overcome the oxidative stress, while the significant elevation of GSH levels in the treated animals coincided with a significant decline in lipid peroxidation.

Fructose is an important dietary source of carbohydrates and is a simple sugar present in fruits and honey. Fructose induces insulin resistance by obesity-associated mechanisms. Hepatic triglyceride accumulation may result in protein kinase C activation and insulin resistance due to increased uptake of free fatty acids. The high-fructose diet was found to increase the serum levels of glucose, TG and TC, a phenomenon commonly associated with diabetes mellitus [43]. These are known to be high-risk factors in the development of cardiovascular disorders including hypertension. Results of this study showed that *E. antiquorum* root extracts possess lipid-lowering effects in fructose-induced insulin resistance.

Administration of alcohol and aqueous extracts reduced the MDA level in fructose-fed rats to levels similar to those of normal rats. This finding suggests that chronic oral treatment with higher doses of alcohol and aqueous root extracts of *E. antiquorum* prevent lipid peroxidation in the fructose-induced diabetic rats. The reduction in plasma MDA levels in

normal rats treated with the extract provides further evidence that the extract possess anti-diabetic activity.

Flavonoids, phenolic compounds and glycosides were detected in preliminary phytochemical screening of the root extracts of *E. antiquorum*. Earlier evidence reveal the anti-diabetic potential of these phytoconstituents and the presence of these phytoconstituents in the extracts of *E. antiquorum* root could be responsible for their anti-diabetic activity [42,44].

The results of this study confirmed the anti-diabetic potential of *E. antiquorum* root and helps in substantiating the use of *E. antiquorum* as a potential drug in the treatment of diabetes. The study also substantiates the use of *E. antiquorum* as a substitute for *E. neriifolia* which is the accepted botanical source of the Ayurveda drug *Snuhee*.

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