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Phytoconstituents evaluation by GC-MS and therapeutic efficacy of *Grewiaum bellifera* on *streptozotocin (STZ)*-induced diabetic rats

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Abstract

Grewiaum bellifera is an Indian traditional medicinal plant of the *Tiliaceae* family. The aerial plant part extract is much more useful in treatment like spleen damage, liver complications and cardio disorders. The rats were treated orally with the extract of *Grewiaumbellifera* at 250mg and 500 mg / kg body wt.for 28days. Biochemical parameters viz. fasting blood glucose, blood urea, serum creatinine and total cholesterol were analyzed. Phytocontituents like *Triterpene, Asarone, Diterpene, linoleic acid ester, Flavonoid compound, Steroid compound* were present in GC-MS analysis. It was also observed that fasting blood glucose showed a significant decrease at a dose of 500mg/kg body wt. (From 280.8 ± 2.29 to 118.8 ± 3.99mg/dl) when compared with that of standard drug glibenclamide. The results shows in this study clearly indicates that the extract possess anti-hyperglycemic activity and may be promising for the development of phytomedicine for diabetes mellitus.

Key-Words: *Grewiaum bellifera,* Diabetes, Therapeutic uses

Introduction

In recent years, gas chromatography–mass spectrography (GC–MS) has been applied unambiguously to identify the structures of different phytoconstituents in plant extracts and biological samples with great success.(1,2) GC–MS is a reliable technique to identify the constituents of volatile matter, long-chain branched hydrocarbons, alcohols, acids, and esters .(3) Generally, the highest concentration of octadecadienoic acid, hexadecanoic acid, and oleic acid was detected with GC–MS. These compounds have been already reported as exerting hypoglycemic and hypolipidemic effects. (5,4)

Diabetes mellitus is a group of metabolic alterations characterized by hyperglycemia resulting from defects in insulin secretion, action, or both. It is well-established that chronic hyperglycemia in diabetes is associated with long-term damage, dysfunction, and eventually the failure of organs, especially the eyes, kidneys, nerves, heart, and blood vessels. (6)

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Diabetes mellitus is a syndrome resulting from a variable interaction and environmental factors and is characterized by depleted insulin secretion, hyperglycemia, and altered metabolism of lipids, carbohydrates, and proteins, in addition to damaged cells of the pancreas and increased risk of complications of vascular diseases.(7) Streptozotocin (STZ) induction of diabetes is an experimental model widely used to study glycemic and lipedemic changes in plasma.(8)

A multitude of herbs, spices, and other plant materials have been described for the treatment of diabetes globally (9,10).Hyperlipidemia contributes to the development of cardiovascular complications related to diabetes.(11)

There has been a growing interest in the analysis of plant products which has stimulated intense research on their potential health benefits. The medicinal plants provide a useful source of oral hypoglycemic and antihyperglycemic compounds for the development of new pharmaceuticals as well as a dietary supplement to existing therapies. (12)

A number of herbs are traditionally used in different countries during drug or toxin induced in hepatic, renal and cardiac disorders (13). *Grewiaumbelliferea* (Tiliaceae) (GU) is herbaceous medicinal plant that has been distributed in Kanniyakumari district, Tamilnadu, India (14).

Extensive phytochemical investigations shows that the presence of many chemical constituents including palmitic and linoleic acid such as n-Hexadecanoic acid, 9,12-Octadecatrienoic acid (Z,Z,Z) -, and oleic acid, which are considered significant for Hypocholesterolemic property (15) (16) (17). It is used as CNS depressant (18), hypotension and antidiuretic agent (19).

This investigation was undertaken to study the phytoconstituents by GC–MS and the antihyperglycemic and antihyperlipidemic activities of ethanol extract of *Grewia umbellifera* in STZ-induced diabetic rats.

Material and Methods

Plant material

Grewiaum belifera's aerial part plant collected and authenticated by Dr.V.Chelladurai (Research Officer) Botany (C.C.R.A.S) Government of India. Voucher specimen (SIVET C-453/2012-2013) has been retained in the Dept of Biochemistry, S.I.V.E.T College of Arts & Science, Chennai. Materials were cleaned with water and dried in the shade until a constant weight was obtained.

Animals

Studies were carried out using Wistar albino male rats (150–200 g), maintained at animal house SBST VIT, Vellore, Tamilnadu, India. The animals were housed in polyacrylic cages (38 cm_23 cm_10 cm) and maintained under standard laboratory conditions (temperature 25_20_C) with dark/light cycle (12/12 h). The animals were fed with standard pellet diet and fresh water ad *liibitum*. All the animals were acclimatized to lab conditions for a week before commencement of the experiment. All the procedures described were reviewed and approved by the Animal's Ethical Committee.

Extraction

The whole plant was dried under shade and then powdered with a mechanical grinder to obtain a coarse powder. Equal quantity of powder was passed through 40 mesh sieve and extracted with ethanol (95% v/v) in Soxhlet's apparatus at 60_C.The solvent was completely removed and obtained dried crude extract which was used for investigation. (20)

GC–MS analysis of ethanol extract of GU for the identification of chemical composition

The identification of chemical composition of ethanol extract of GU was performed using a GC–MS spectrograph (Agilent 6890/Hewlett–Packard 5975) fitted with electron impact (EI) mode. The ethanol extract (2.0 mL) of was GU injected with a Hamilton syringe to the GC–MS manually for total ion chromatographic analysis in split mode. In quantitative analysis, selected ion monitoring (SIM) mode was employed during the GC–MS analysis. SIM plot of the ion current resulting from very small mass range with only compounds of the selected mass were detected and plotted.

Experimental induction of diabetes

Diabetes was induced in the animals fasted overnight by a single intraperitoneal (ip) injection of freshly prepared solution of STZ (Sigma, USA) 35 mg kg^{-1} body weight in 0.1M cold citrate buffer pH4.5 (21,22,23) The animals were allowed to drink 5% glucose solution to overcome the drug-induced hyperglycemia. (24) Control rats were injected with citrate buffer (0.1M) alone as a placebo. Animals were considered diabetic if the blood glucose values were >250 mg dL^{-1} on the third day after STZ injection. After a fortnight, rats with moderate diabetes having glycosuria (indicated by Benedict's test for urine) and hyperglycemia with blood glucose range of 200 – 300 mg dL^{-1} were used for the experiment. Blood was collected from the eyes (venous pool) by sinoocular puncture.

Experimental design

Rats were divided into five groups as follows after the induction of STZ-induced diabetes. Diabetes was induced in rats two weeks before starting the treatment.

Group I: animals were considered as control rats.

Group II: animals were treated as diabetic STZ-induced rats.

Group III: diabetic-induced animals were fed with 250 mg kg_1 of ethanolic extract of GU for six weeks.

Group IV: diabetic-induced animals were fed with 500 mg kg_1 of ethanolic extract of GU for six weeks.

Group V: diabetic rats were given glibenclamide orally (0.6 mg kg_1) in distilled water daily for six weeks.

Treatment with the plant extract was started from the 5th day after the STZ injection for 28 days till the end of the study. After 28 days of treatment fasting blood sample was collected from retro- orbital puncture technique under light ether anesthesia and used for biochemical analysis using standard enzymatic methods in an auto analyzer.

Statistical analysis

Results were analyzed for statistical significance using one way ANOVA followed by Dunnett's test using the graph pad statistical software for comparison with control group and STZ-treated group. A $p < 0.05$ was considered as significant.

Results and Discussion

Preliminary phytochemical screening of the extract gave positive tests for flavonoids, Triterpene , steroids, phenols and palmitic acid ester. The ethanol extract of GU was a complex mixture of many constituents and 16 compounds were identified in this plant by GC–MS. Phytoconstituents as follows in the table.

The blood glucose level in control and all the experimental groups of rats were analyzed and are shown in table 1.

The mean FBG level in control rats was 82.83±2.39mg/ dL, in diabetic rats it was 280.8±2.29 mg/dL after 28 days of induction of diabetes. The group that received the standard drug (glibenclamide) showed 138±2.43mg/dL as mean FBG level. When compared with the diabetic group, the groups which were treated with the extract of *Grewiaumbelifera's* 250mg/kg b. wt. and 500mg/kg. b. wt. showed a significant decrease in FBG levels 222.3±5.06 mg/dL& 118.8 ±3.99 mg/dL respectively. Table 1 also shows the effect of ethanolic extract of *Grewiaum belifera's* on total cholesterol, serum creatinine and blood urea. In control groups the values were 91.5±1.435 mg/dL (total cholesterol), 0.50±0.104 mg/dL (serum creatinine) and 29±1.311 mg/dL (blood urea).

These values were significantly elevated in the diabetic control group 172.5±1.532 mg/dL in total cholesterol, 1.60±0.201 mg/dL in serum creatinine and 41.66±1.826 mg/dL in blood urea. Administration of *Grewiaumbelifera's* extract significantly lowered these values.

Current study focused on the effect of different dose of the extract and comparison with that of standard anti-diabetic drug (glibenclamide) in induced diabetic condition. The extract exhibited a significant hypoglycemic activity at a dose of 500 mg/kg b. wt. in 28 days, when compared with the diabetic control group (Table 1). It is well known that certain flavonoids [15] and phytol [16] exhibit hypoglycemic activity and are also known for their ability of beta-cell regeneration of pancreas [17]. Sterols have also shown to decrease blood sugar in experimental animal models [18]. Thus the significant anti-diabetic effect of the extract may be due to the presence of flavonoids, Triterpene, steroids, phenols and palmitic acid ester.or their synergistic properties. The results in Table 1 showed significant increase in the level of blood urea and serum creatinine which are markers of renal dysfunction [19] in the diabetic rats when compared to control rats. The diabetic rats treated with the extract the level of blood urea and serum creatinine were significantly decreased.

This further shows the ability of the extract in treating diabetes associated renal complications and also supports the usage of this plant by tribal's for kidney diseases. Hyperlipidemia has been reported to accompany hyperglycemia states [20] and it is important coronary risk factors for heart diseases [21]. The level of total cholesterol in *Grewia umbelifera* treated group was also decreased significantly this may be due to the presence of Linoleic acid ester [22] in the extract. It can therefore be concluded that the preliminary study shows that the extract of *Grewia umbelifera* decreases the blood glucose level in diabetic animals.

Further studies are to be carried out to investigate the anti-diabetic principle present in this extract, isolate the same and characterize the compound, so that it may be used as a phytomedicine for anti-diabetic treatment in future.

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Table 1: Chemical composition of ethanolic GU aerial part extract by GCMS Activity of Phyto Components identified in the Plant extract [GC MS study]

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %	Compound Nature	**Activity
1.	2.32	Glycerin	$C_3H_8O_3$	92	0.57	Alcoholic compound	Antimicrobial Preservative
2.	3.79	Carbamic acid, hydroxyl-, ethyl ester	$C_3H_7NO_3$	105	0.64	Ester compound	No activity reported
3.	3.91	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144	0.49	Flavonoid fraction	Antimicrobial Antiinflammatory
4.	8.00	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-	$C_4H_9NO_5$	151	8.24	Nitrogen compound	Antimicrobial
5.	9.52	Asarone	$C_{12}H_{16}O_3$	208	2.95	Asarone	Anticonvulsant; Antipyretic Antispasmodic CNS-Depressant Cardio depressant Emetic Fungicide Mutagenic Myorelaxant Pesticide Psychoactive Sedative Tranquilizer
6.	9.77	Megastigmatrienone	$C_{13}H_{18}O$	190	1.13	Ketone compound	No activity reported
7.	9.85	Alpha-l-rhamnopyranose	$C_6H_{12}O_5$	164	1.41	Sugar moiety	Preservative
8.	11.00	Phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy- [Synonyms: Coniferol]	$C_{10}H_{12}O_3$	180	1.69	Phenolic compound	Antimicrobial Antiinflammatory Antioxidant
9.	12.00	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	2.19	Terpene alcohol	Antimicrobial Antiinflammatory
10.	13.90	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	1.21	Palmitic acid ester	Antioxidant, Pesticide, Hypocholesterolemic, Nematicide, Lubricant, Antiandrogenic, Flavor, Hemolytic
11.	13.95	Benzenepropanoic acid, 2,5-dimethoxy-	$C_{11}H_{14}O_4$	210	0.56	Aromatic acid	Antimicrobial
12.	15.45	Phytol	$C_20H_{40}O$	296	6.92	Diterpene	Antimicrobial Antiinflammatory Anticancer Diuretic
13.	16.14	11,14-Eicosadienoic acid, methyl ester	$C_{21}H_{38}O_2$	322	0.55	Unsaturated fatty acid ester	Anticholesterol Cardio protective
14.	16.23	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- [Synonyms: Linolenic acid, methyl ester]	$C_{19}H_{32}O_2$	292	0.97	Linoleic acid ester	HypocholesterolemicNematicideAntiarthriticHepatoprotective Anti androgenic Hypocholesterolemic 5-Alpha reductase

							inhibitor Antihistaminic AnticoronaryInsectifuge AntieczemicAntiacne
15.	21.17	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330	0.53	Ester compound	No activity reported
16.	21.45	Pentadecanal-	$C_{15}H_{30}O$	226	0.51	Aldehyde	Antimicrobial
17.	25.35	Squalene	$C_{30}H_{50}$	410	2.18	Triterpene	Antibacterial, Antioxidant, Antitumor, Cancer preventive, Immunostimulant, Chemo preventive, Lipoxygenase-inhibitor Pesticide
18.	29.90	Vitamin E	$C_{29}H_{50}O_2$	430	5.96	Vitamin E	Antiageing, Analgesic, AntidiabaticAntiinflammatory, Antioxidant, Antidermatitic, Antileukemic, Antitumor, Anticancer, Hepatoprotective, HypocholesterolemicAntiulcerogenic, Vasodilator, Antispasmodic, Antibronchitic, Anticoronary
19.	33.16	Hexadecanoic acid, 2,3-bis(acetyloxy)propyl ester	$C_{23}H_{42}O_6$	414	53.91	Fatty acid ester	Antioxidant, Pesticide, Hypocholesterolemic, Nematicide, Lubricant, Antiandrogenic, Flavor, Hemolytic.
20.	33.67	á-Sitosterol	$C_{29}H_{50}O$	414	4.17	Steroid	Antimicrobial Anticancer Antiinflammatory Antiasthma Diuretic
21.	35.02	á-D-Mannofuranoside, farnesyl-	$C_{21}H_{36}O_6$	384	1.73	Sugar compound	Preservative
22.	35.9	Lupeol	$C_{30}H_{50}O$	426	1.47	Triterpene compound	Antimicrobial Antiinflammatory Anticancer Antiviral Cytotoxic Pesticide Antimalarial

Source: Dr.Duke's Phytochemical and Ethnobotanical Databases

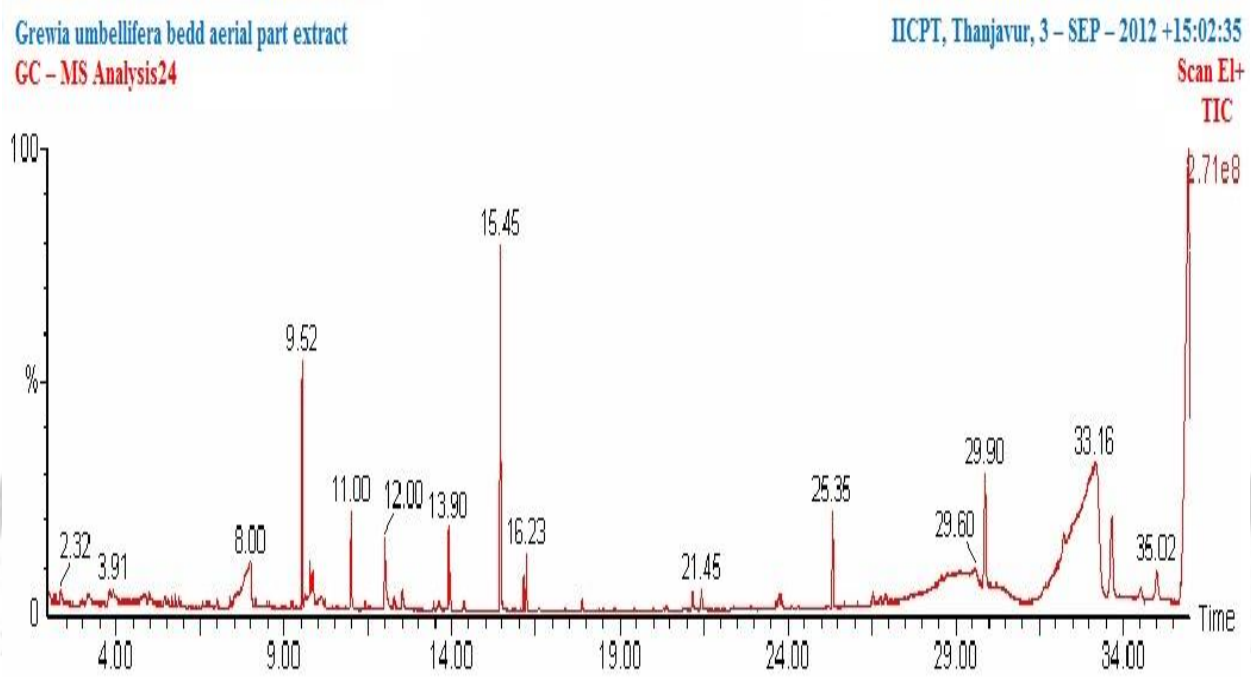

Fig. 1: GC-MS analysis of ethanolic GU aerial plant extract, The chromatogram showing n- Hexadecanoic Acid (53.91), 1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-(8.24),Phytol (6.92) and ç-Sitosterol (4.17) peaks detected by GC-MS

Table 2: Effect of administration of ethanol extract of *Grewia umbellifera* on biochemical constituents in normal, diabetic and treated rats

Experimental groups	FBG 28thday after induction(mg/dL)	Total cholesterol(mg/dL)	Serum Creatinine(mg/dL)	Blood Urea(mg/dL)
Group I	82.83 ± 2.39**	91.5±1.435**	0.50 ± 0.104**	29 ± 1.311**
Group II	280.8 ± 2.29	172.5±1.532	1.60 ± 0.201	41.66 ± 1.826
Group III	222.3 ± 5.06**	129.7±1.793**	1.40 ± 0.127^{ns}	36.33 ± 1.701*
Group IV	118.8 ± 3.99**	105 ±1.2**	0.68 ± 0.037**	31.61 ± 1.101**
Group V	138 ± 2.43**	111.3±2.331**	0.9 ± 0.06**	35.63 ± 287**