ISSN 2395-650X

International Journal of Life Sciences Biotechnology Pharma Sciences

E-mail: editorijlbps@gmail.com editor@ijlbps.org

International Journal of Life Sciences Biotechnology Pharma Sciences

Volume7 Issue4,Dec2019

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

G.Prem Kumar

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 parametersviz. 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 :

Gas chromatography-mass spectrometry (GC-MS) has been used successfully in recent years to determine the precise chemical composition of plant extracts and biological materials.The volatile matter, long-chain branching hydrocarbons, alcohols, acids, and esters may all be reliably identified by GC-MS (1,2).Thirdly, octadecadienoic acid, hexadecanoic acid, and oleic acid were found in the greatest concentrations using GC-MS. These substances have been earlier reported as exhibiting hypoglycemic and hypolipidemic actions. (5,4)Hyperglycemia due to abnormalities in insulin production, action, or both define the metabolic syndrome known as diabetes mellitus. It is widely recognized that long-term damage, malfunction, and ultimate failure of organs, notably the eyes, kidneys, nerves, heart, and blood arteries, are connected with chronic hyperglycemia in diabetes. (6) Diabetes mellitus is a syndrome characterized by impaired insulin secretion, hyperglycemia, and an altered metabolism of lipids, carbohydrates, and proteins, as well as pancreatic cell damage and an increased risk of complications from vascular diseases; it is the result of a complex interaction between genetic predisposition and environmental factors.(7) The production of diabetes by streptozotocin (STZ) is a common experimental paradigm for investigating the effects of insulin and glucose on plasma lipids and glucose levels.It has been reported that a wide variety of herbs, spices, and other plant materials may be used to treat diabetes (9,10).The onset of diabetic cardiovascular problems is facilitated by hyperlipidemia.(11)

Department of Pharmacology, C. L. Baid Metha College of Pharmacy, Chennai, (T.N.) - India 2, Department of Pharmacology

There has been a surge in study into the possible health advantages of plant products as their analysis has gained popularity. The medicinal plants may be used as a complement to current treatments or as a starting point for the creation of novel medicines due to the presence of oral hypoglycemic and antihyperglycemic chemicals. (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 thepresence 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 wasobtained.

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'sEthical 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 overnightby 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 250mg kg_1 of ethanolic extract of GU for six weeks.

Group IV: diabetic-induced animals were fed with 500mg 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. Mathematical dissection

Graph Pad Prism was used to conduct a one-way analysis of variance (ANOVA) followed by a Dunnett's test in order to compare the results from the control group with the STZ-treated group. A significance level of p 0.05 was used.

What We Learned and WhyFlavonoids, Triterpene, steroids, phenols, and palmitic acid ester were all found to be present in the extract during preliminary phytochemical testing. GC-MS analysis of this plant yielded the following table of phytoconstituents, indicating that the ethanol extract of GU is a complex combination of numerous chemicals.Rats were divided into control and experimental groups, and their respective blood glucose levels are shown in table 1.After 28 days of diabetes induction, diabetic rats had a mean FBG level of 280.82.29 mg/dL, whereas control rats had a level of 82.832.39mg/dL. The average fasting glucose (FBG) level of the treatment group that got the gold standard medication (glibenclamide) was 1382.43mg/dL. Compared to the diabetic group, the FBG levels of the groups treated with either 250 or 500 milligrams per kilogram of body weight (mg/kg) with Grewiaumbelifera extract dropped by 222.3 and 5.06 milligrams per deciliter (mg/dL), respectively.In other words, 118.8 3.99 mg/dL. Total cholesterol, serum creatinine, and blood urea levels are also affected by Grewiaum belifera ethanolic extract, as shown in Table 1. Total cholesterol levels were 91.51.435 mg/dL, serum creatinine levels were 0.500.104 mg/dL, and blood urea levels were 291.311 mg/dL in the control groups.The mean (standard deviation) total cholesterol, serum creatinine, and blood urea levels in the diabetic control group were 172.51.532, 1.600.201, and 41.661.826, respectively. These levels dropped considerably after receiving Grewiaumbelifera extract.Current research focuses on the impact of various dosage of the extract and comparison with that of standard anti- diabetic medicine (glibenclamide) in induced diabetic state. At 500 milligrams per kilogram of body weight in mice, the extract showed considerable hypoglycemic action.

as compared to the diabetes control group for a period of 28 days (Table 1). Certain flavonoids [15] and phytol [16] are known to have hypoglycemic effect and are credited with the regeneration of pancreatic beta cells [17]. Moreover, sterols have been demonstrated to lower blood sugar in animal models of the disease [18]. Therefore, the inclusion of flavonoids, Triterpene, steroids, phenols, and palmitic acid ester, or the synergistic effects of these compounds, may account for the extract's potent anti-diabetic action. According to Table 1, there was a significant elevation in blood urea. compared to control rats, diabetic rats have higher levels of blood creatinine and other indications of renal impairment [19]. Blood urea and serum creatinine levels were considerably reduced in diabetic rats given the extract.

This provides more evidence for the extract's efficacy in treating renal issues related to diabetes and lends credence to the traditional use of this plant to treat kidney illness among indigenous peoples. It has been shown that hyperglycemia states are accompanied by hyperlipidemia [20], which is a major coronary risk factor for cardiovascular illnesses [21]. Linoleic acid ester [22] in the extract of Grewia umbelifera may be responsible for the dramatic reduction in total cholesterol seen in the treatment group.

Preliminary research indicates that Grewia umbelifera extract lowers blood glucose levels in diabetic rats.

Further investigations need to be carried out to explore the anti-diabetic principle found in this extract, isolate the same and describe the component, so that it may be employed as a phytomedicine for anti-diabetic therapy in future.

References

Prasain, J.K., C.-C. Wang, and S. Barnes. 2004. Mass spectroscopic methods for the determination of flavonoids in biological samples. Free Radical Biology & Medicine 37: 1324–50.

De Rijke, E., P. Out, W.M.A. Neissen, F. Ariese, C. Gooijer, and U.A. Brinkman. 2006. The analytical separation and detection methods for flavonoids. Journal of Chromatography A 1112: 31–63.

Anjali, R., T. Rasika, T. Amruta, P. Vedavati, and D. Nirmala. 2009. GC–MS study of a steam volatile matter from Mimusops elengi. International Journal of ChemTech Research, 1: 158–61.

Dadu Khan Burdi, M. Qasim Samejo, M. Iqbal Bhanger, and Khalid M. Khan. 2007. Fatty acid composition of Abies Pindrow (West Himalayan fir). Pakistan Journal of Pharmaceutical Science20: 9–15.

Hussain, Z., A. Waheed, R.A. Qureshi, D.K. Burdi, J.V. Eugen, N. Khan, and H. Masooda. 2004. The effect of medicinal plant of Islambad and Muree rejoin of Pakistan on insulin secretion from INS-1cells. Phytotherapy Research 18: 73–7.

American Diabetes Association (ADA). 2005. Diagnosis and classification of diabetes mellitus. Diabetes Care 28: 37–42.

Davis, S.N., and D.K. Granner. 1996. Insulin, oral hypoglycemic agents, and the pharmacology of the endocrine pancreas. In The pharmacological basis of therapeutics, 9th ed., J.G. Hardman, L.E. Limbird, P.B. Molinoff, R.W. Ruddon and A.G. Gilman's, Chap. 60, 1487–518. New York: McGraw-Hill.

Kurup, S., and R. Bhonde. 2000. Combined effect of nicotinamide and streptozotocin on diabetic status in partially pancreatictomized adult BALB/C mice. Hormone and Metabolic Research. 32: 330–4.

Marles, R.J., and N.R. Fransworth. 1995. Antidiabetic plants and their active constituents. Phytomedicine 2: 137–89.

Kesari, A.N., R.K. Gupta, and G. Watal. 2005. Hypoglycemic effects of Murraya koenigii on normal and alloxon diabetic rabbits. Journal of Ethanopharmacology 11: 223–31.

Nabel, E.G. 2003. Cardiovascular disease. The New England Journal of Medicine 349: 60–72.

Ohno, T., F. Horio, S. Tanaka, M. Terada, T. Namikawa, and J. Kitoh. 2000. Fatty liver and hyperlipidemia in IDDM of streptozotocin treated shrews. Life Sciences 66: 125–31.

Bailey, L.J., and C. Day. 1989. Traditional plant medicine as treatment for diabetes. Diabetes. Care 12: 553–64.

Vijay.T**,** M.S. Dhana Rajan, K.Sarumathy, A. Sudha .*In vitro* cytotoxicity of Grewia umbellifera**.** International Journal of Pharmacy and Life Sciences 2(12); 2011: 1293-1298.

Chelladurai, V 1972. Glossoryof Indian medicinal plants with active principles, CSIR, New Delhi, part I , pp 340-341.

Kurian GA, Philp S, Varghese T(2005). Effect of aqueous extract of Desmodiumgangeticum DC root in the severity of myocardial infarction. J Ethanopharmacol; 97:4557-61.

Hyo Ku Lee., Yang Mun Choi., Dong OukNoh andHyungJooSuh. 2005. Antioxidant Effect of Korean Traditional Lotus Liquor (*Yunyupju*)*Inter.J Food Sci& Tech.*40 (7):709 - 715.

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	$C_{20}H_{40}O$	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 AnticoronaryInsectifugeAntieczemicAntiacne
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

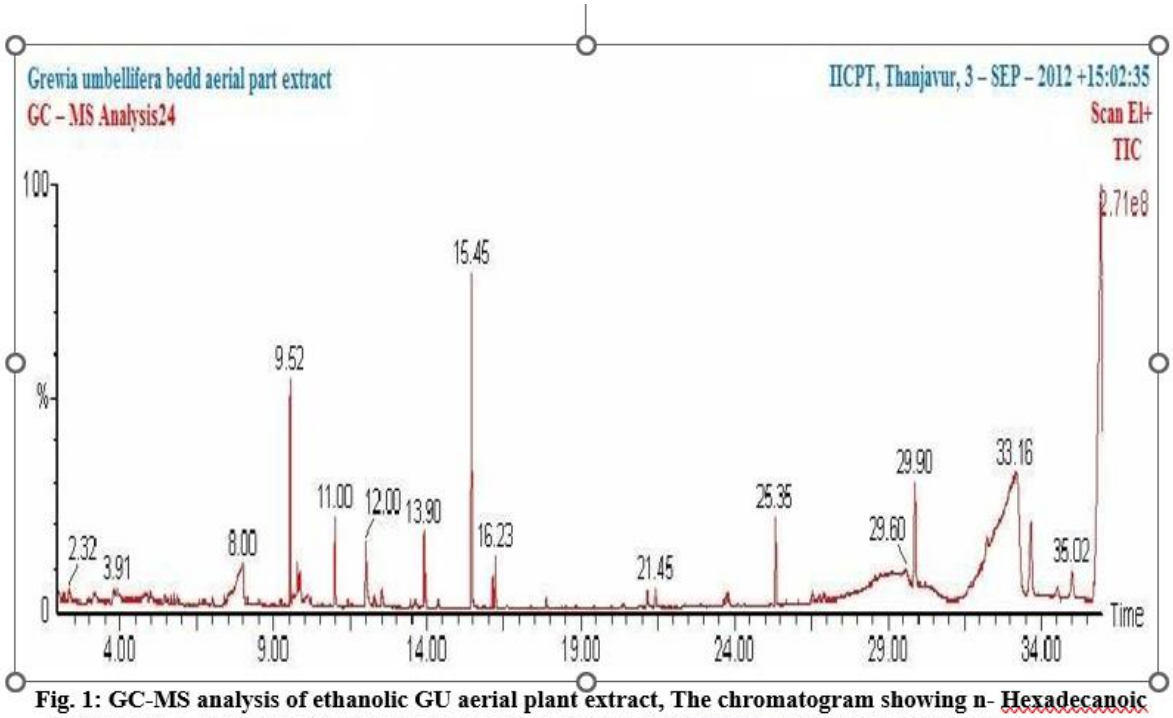

Fig. 1: GC-MS analysis of ethanolic GU aerial plant extract, The chromatogram showing n- Hexadecanoic Acid (53.91), 1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-(8.24),Phytol (6.92) and ç-Sitosterol (4.17) peaks detected by GC-MS

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

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**