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**Phytoconstituents evaluation by GC-MS and anti-hyperglycemic activity of *Cynodon dactylon* on streptozotocin induced diabetes in rats**

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**ABSTRACT**

Many herbs are known to be hypoglycemic, *Cynodon dactylon* is an indigenous medicinal plant used in Indian herbal medicine for the treatment of diabetes. The aim of this study was to identify the phytoconstituents of *Cynodon dactylon* extract by Gas Chromatography–Mass Spectrography (GC-MS) and to evaluate its *in vivo* hypoglycemic effect in streptozotocin induced diabetic rats. The rats were treated orally with the extract of *C.dactylon* at 200mg and 400 mg / kg bwt. for 28 days. Biochemical parameters viz. fasting blood glucose (FBG), blood urea, serum creatinine and total cholesterol were analyzed. Phytoconstituents like phytol, Octadecanoic acid, squalene, thymol, n-Hexadecanoic acid were present in GC-MS analysis. It was also observed that FBG showed a significant decrease at a dose of 400mg/kg b.wt. (From  $283\pm 2.49$  to  $117.8\pm 4.69$ mg/dL) when compared with that of standard drug glibenclamide. The results obtained 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:** hypoglycemic effect, GC-MS, streptozotocin, *Cynodon dactylon*, squalene, phytol.

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**INTRODUCTION**

Diabetes mellitus is an endocrine disorder that is characterized by hyperglycemia. It is the most important non - infective epidemic to hit the globe in the present millennium. By the year 2025, India shall have the maximum number of diabetes in the world making it, the “Diabetes capital of the world” [1]. Clinically, the onset and rate of progression of diabetic complications, including cataract, corneal epitheliopathy, microangiopathy, nephropathy, neuropathy, and retinopathy, appear to be dependent upon both the duration and the severity of the diabetes [2].

Since ancient times natural products of plant origin and numerous dietary constituents have been known. About 800 plant species have been reported to possess anti-diabetic properties. Several plant species have been used for prevention or management of diabetes by the Native Americans, Chinese, South Americans and Asian Indians [3]. A number of investigations have been conducted on oral anti hyperglycemic agents from plants used in traditional medicine, and many of the plants were found with good activity [4]. The WHO has also recommended the evaluation of the plant's effectiveness in condition where we lack safe modern drugs [5]. The list of drugs available for management of diabetes is short and more drugs are still needed. Therefore, the discovery of more drugs which may have new modes of action is very pertinent. Traditional plant remedies have always provided sources of useful hypoglycemic agents and therefore, should continue to be investigated for possible drug alternatives. This has led to an increasing demand of research on anti-diabetic natural products which produces minimal or no side effects [6].

*Cynodon dactylon* (L.) Pers. (family –Poaceae), which is commonly known as Bermuda grass or Durva in Hindi is traditionally used for diabetes [7], kidney problems, urinary disease, gastrointestinal disorder constipation and abdominal pain [8]. Whole plant is used for-diuretic, dropsy, syphilis, wound infection, piles [9]. The juice of the plant is astringent and is applied externally to fresh cuts and wounds. It is used in the treatment of catarrhal ophthalmia, hysteria, epilepsy, insanity, chronic diarrhea and dysentery. The plant is folk remedy for calculus, carbuncles, cough, hypertension, snake bites and gout [10]. The ethanolic extract of aerial parts of *C. dactylon* showed marked protection against convulsions induced by chemo convulsive agents in mice [11].

In the light of the above information the present investigation was undertaken to evaluate the phytoconstituents by GC-MS and to assess the anti-diabetic effect of *C.dactylon* using Wistar rats as animal models.

## EXPERIMENTAL SECTION

### Plant material and preparation of extract

*C.dactylon* was collected locally in the district of Chennai, Tamil Nadu, India. The taxonomical identification and authentication was done by Dr.Sasikala Ethirajulu, Assistant Director Pharmacognosy, Siddha Central Research Institute (SCRI), Chennai, Tamil Nadu. The fresh green plants were first washed, air dried and made into a coarse powder and then extracted with 80% ethanol, the resulting dark greenish brown extract was filtered and concentrated in rotavapour under reduced pressure. The concentrated extract was lyophilized to get a powder and this was used for the studies.

### Materials & Chemicals

Streptozotocin and glibenclamide was obtained from Sigma-Aldrich, USA. All the chemicals and solvents used were of analytical grade.

### Preliminary phytochemical screening

The presence of preliminary phytochemicals in the extract was estimated using colour reactions method [12].

**Gas Chromatography- Mass Spectrum Analysis (GC-MS)**

GC-MS technique was carried out at Indian Institute of Crop Processing Technology (IICPT) Thanjavur GC-MS technique was used in this study to identify the phytochemicals present in the extract., Tamil Nadu. The powdered leaf material (20g) was soaked in 50ml of 80% alcohol for 12 hours and then filtered through Whatmann filter paper No.41 along with 2g sodium sulphate to remove the sediments and traces of water in the filtrate. Before filtering the filter paper along with sodium sulphate was wetted with absolute alcohol. The filtrate was then concentrated by bubbling nitrogen gas into the solution and concentrated to 1 ml. Then the plant extract (2 $\mu$ L) was injected in the Gas Chromatography- Mass Spectrometer.

Gas chromatography technique was carried out in GC Clarus 500 Perkin Elmer with column Elite -1 (100% Dimethyl poly siloxan). Mass detector used was gold-Perkin Elmer and nitrogen gas was used as a carrier gas. The temperature of the column was maintained at 200-280°C. Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technique (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The Name, Molecular weight, Structure of the component of the test material was ascertained.

**Experimental animals- Hypoglycemic activity**

Male Wistar rats (180-220 g body weight) were selected for the experiment. The study was approved and conducted in the Department of Pharmacology, Siddha Central Research Institute, Chennai, with due permission from Institutional Animal Ethics Committee (registration number 73/PHARMA/CRIS/2009). The animals were fed on a pelleted diet and water. The animals were maintained in their respective groups. All the studies were conducted in accordance with the National Institute of Health's guide for the care of Laboratory Animals [13].

**Experimental induction of diabetes**

Diabetes was induced by a single intraperitoneal injection of freshly prepared STZ (60 mg/kg b. wt.) in 0.1M citrate buffer (pH 4.5) to overnight fasted rats. FBG level was estimated at the time of induction and after 5 days. Animals having marked hyperglycemia (>250 mg/dL), were selected for the study.

**Experimental design**

Animals were classified in to 5 groups, of six animals in each group. Food and water were provided *ad libitum* to the animals. The groups are as follows:

*Group I - control rats were treated with vehicle alone [(Tween 80) 0.2 ml /100 g b. wt.]*

*Group II - Diabetic control.*

*Group III - Diabetic rats + glibenclamide (10 mg/kg b. wt. per day).*

*Group IV - Diabetic rats were treated with C. dactylon suspension (200 mg/kg b wt.)*

*Group V - Diabetic rats were treated with C. dactylon suspension (400 mg/kg b wt.)*

Treatment with the plant extract was started from the 5<sup>th</sup> 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

One- way Analysis of Variance (ANOVA) with Dunnett post test was used for statistical analysis of collected data. Differences were considered significant at  $P < 0.05$ . Values are expressed as Mean  $\pm$  SEM for 6 different preparations.

## RESULTS

Preliminary phytochemical screening of the extract gave positive tests for flavonoids, saponins, tannins, steroids, phenols, coumarins and glycosides. GC-MS study of *C. dactylon* leaves has shown the presence of many phytochemicals which contribute to the medicinal activity of the plant (Figure 1). This analysis revealed that *C. dactylon* leaves contain Glycerin (38.49%), 9, 12-Octadecadienoyl chloride, (Z, Z)-(15.61%), Hexadecanoic acid, ethyl ester (9.50%), Ethyl  $\alpha$ -D-glucopyranoside (8.42%), Linoleic acid, ethyl ester (5.32%), Squalene (1.94%) and Phytol (4.89%) Figure 2 and 3 shows the mass spectrum and the structure of phytol and squalene respectively.

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  $84.83 \pm 2.89$  mg/dL, in diabetic rats it was  $283.8 \pm 2.49$  mg/dL after 28 days of induction of diabetes. The group that received the standard drug (glibenclamide) showed  $136 \pm 3.43$  mg/dL as mean FBG level. When compared with the diabetic group, the groups which were treated with the extract of *C. dactylon* 200mg/kg b. wt. and 400mg/kg. b. wt. showed a significant decrease in FBG levels  $224.3 \pm 6.06$  mg/dL &  $117.8 \pm 4.69$  mg/dL respectively. Table 1 also shows the effect of hydroalcoholic extract of *C. dactylon* on total cholesterol, serum creatinine and blood urea. In control groups the values were  $93.5 \pm 1.335$  mg/dL (total cholesterol),  $0.53 \pm 0.094$  mg/dL (serum creatinine) and  $30 \pm 1.211$  mg/dL (blood urea). These values were significantly elevated in the diabetic control group  $178.5 \pm 1.232$  mg/dL in total cholesterol,  $1.60 \pm 0.198$  mg/dL in serum creatinine and  $43.66 \pm 1.626$  mg/dL in blood urea. Administration of *C. dactylon* extract significantly lowered these values.

## DISCUSSION

Streptozotocin (STZ) selectively destroys the insulin secreting beta-cells of the pancreas [14]. 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 400 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 phenolics, flavonoids and phytol 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 *C.dactylon* treated group was also decreased significantly this may be due to the presence of squalene [22] in the extract.

It can therefore be concluded that the preliminary study shows that the extract of *C.dactylon* 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 phyto-medicine for anti-diabetic treatment in future.

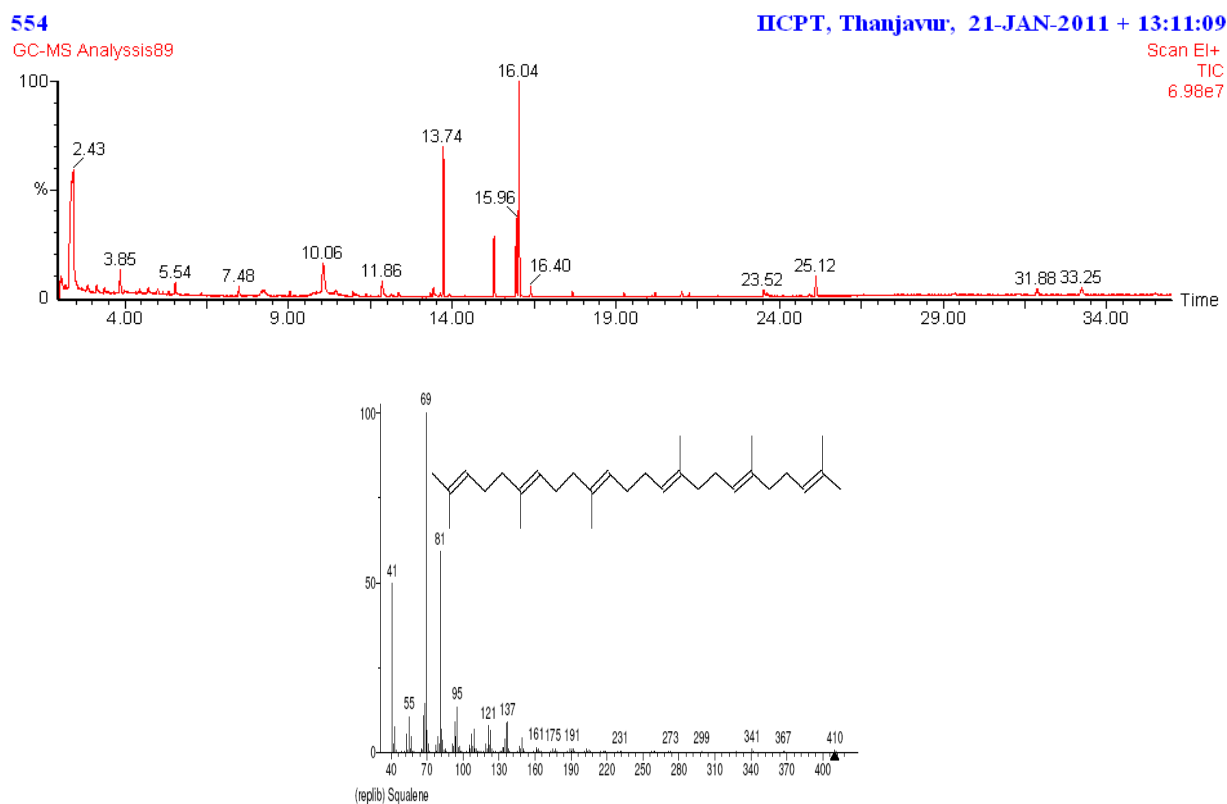
**Table: 1 Effect of administration of hydro alcoholic extract of *Cynodon dactylon* on biochemical constituents in normal, diabetic and treated rats**

Experimental groups	FBG 28 <sup>th</sup> day after induction(mg/dL)	Total cholesterol(mg/dL)	Serum Creatinine(mg/dL)	Blood Urea(mg/dL)
Group I	84.83 ± 2.89**	93.5±1.335**	0.53 ± 0.094**	30 ± 1.211**
Group II	283.8 ± 2.49	178.5±1.232	1.60 ± 0.198	43.66 ± 1.626
Group III	136 ± 3.43**	113.3±2.231**	0.88 ± 0.07**	34.83 ± 1.887**
Group IV	224.3 ± 6.06**	130.5±1.893**	1.38 ± 0.137 <sup>ns</sup>	37.33 ± 1.801*
Group V	117.8 ± 4.69**	104 ± 1.983**	0.71 ± 0.047**	32.66 ± 1.054**

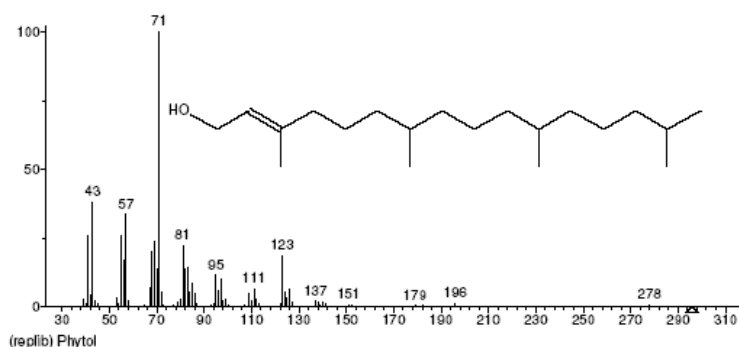
Data are expressed as Mean ± SEM, n=6, using one- way ANOVA with Dunnett post test.

\*\* P<0.01, \* P<0.05 vs. Group II (Diabetic Controls) ns- not significant P>0.05

**Figure 1: Chromatogram obtained from the GC-MS with the extract of *Cynodon dactylon***



Name: Squalene  
 Formula: C<sub>30</sub>H<sub>50</sub>  
 MW: 410 CAS#: 7683-64-9 NIST#: 256484 ID#: 7712 DB: replib  
 Other DBs: EPA, IRDB  
 Contributor: LAC, NIDDK, NIH, Bethesda, MD 20892  
 10 largest peaks:  
 69 999 | 81 590 | 41 496 | 68 143 | 95 132 | 67 108 | 55 103 | 93 88 | 137 87 | 136 84 |  
 Synonyms:  
 1.2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-  
 2.Skvalen  
 3.Spinacene  
 4.Supraene  
 5.(6E,10E,14E,18E)-2,6,10,15,19,23-Hexamethyl-2,6,10,14,18,22-tetracosahexaene #  
 6.Sqalene



Name: Phytol  
 Formula: C<sub>20</sub>H<sub>40</sub>O  
 MW: 296 CAS#: 150-86-7 NIST#: 108727 ID#: 8057 DB: replib  
 Other DBs: Fine, TSCA, RTECS, HODOC, EINECS  
 Contributor: Philip Morris R&D  
 10 largest peaks:  
 71 999 | 43 381 | 57 334 | 41 260 | 55 259 | 69 239 | 81 223 | 68 199 | 123 184 | 56 169 |

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