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Phytochemical profiling and effect of *Andrographis echioides* (L.) Nees leaf extract on glucose uptake by 3T3-L1 cell lines – An *in vitro* study

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The human population worldwide appears to be in the midst of an epidemic of diabetes, a metabolic disorder characterised by problems in carbohydrate metabolism. The existing synthetic drugs have several limitations such as, diabetic retinopathy, nephropathy and neuropathy. Thus, in spite of remarkable progress in the treatment of diabetes by oral hypoglycemic agents, search for newer drugs continues. Hence, in the present study, *Andrographis echioides* (L.) Nees was examined for its *in vitro* antidiabetic efficacy by amylase inhibition and glucose uptake by 3T3 cell lines assays. In addition, the bioactive components were analysed by GC-MS technique. The data obtained suggests that methanol extract of *A. echioides* was effective in enhancing glucose uptake by the 3T3 cell lines *in vitro*. The GC-MS profile revealed the presence of 5-ethyl-2-imino thiazolidin-4-one recorded at 15.95min. Literature survey has proved that thiazolidin-4-one derivatives have important biological activities and thus, the antidiabetic potential of *A. echioides* can be related to its presence. Further mechanistic studies could be taken up to prove the effect *in vivo*.

Keywords: Amylase inhibition, *Andrographis echioides* (L.) Nees, Glucose uptake, GC-MS, 3T3 cell line.

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Introduction

Andrographis echioides (L.) Nees (Family Acanthaceae) is a herb commonly known as false water willow and is found throughout India and Sri Lanka. The plant is reported to be used in the treatment of fever and snake bite (external application of leaf-paste) and also has antioxidant potential[1,2]. It has also been studied for its use in goitre, liver diseases, fertility problems, Malaria, and bacterial and fungal diseases[3,4]. In spite of its varying medicinal properties, the plant still remains unexplored for various potential applications like anticancer, antidiabetic, antiviral and anti-hyperlipidemic activities.

Diabetes is a chronic disease, which occurs when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin it produces. This leads to an increased concentration of glucose in the blood (hyperglycemia). Type 1 diabetes (previously known as insulin-dependent or childhood-onset diabetes) is characterized by lack of insulin production. Type 2 diabetes (formerly called non-insulin dependent or adult-onset diabetes) is caused by the body's ineffective use of insulin. Gestational diabetes is hyperglycaemia that is first recognized during pregnancy. In 2014, the global prevalence of diabetes as estimated by the World Health Organization, to be 9 % among adults aged above 18 years. There are basically 5 classes of oral antidiabetic drugs available for the treatment of type 2 diabetes, whereas insulin acts as a sole agent for type 1 diabetes. The major problem accompanying these indeed excellent antidiabetic agents is their side effects. Most common side effects observed are hypoglycemia and hypersensitivity (Sulfonylureas)[5], Gastrointestinal symptoms (Biguanides and alpha glucosidase inhibitors)[6], weight gain and oedema (Thiazolidinediones)[7]. Even insulin therapy does not restore a permanent normalised pattern of glucose homeostasis and carries an increased risk of atherogenesis and hypoglycemia[8]. Medicinal plants have the advantage of having no or only a few side effects and are being used in traditional systems of medicine for several decades in many countries of the world[9]. Thus, the present study is the first attempt to investigate the possible antidiabetic property of *A. echioides*.

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Materials and Methods

Collection and extraction of plant material

The fresh plants of *A. echioides* were collected from Thandarai forest, Chengalpet**,** Tamil Nadu, India and taxonomically identified by Prof P Jayaraman, Plant Anatomy Research Centre (PARC), Chennai, Tamil Nadu, India and a voucher specimen (PARC/2015/3179) was deposited there. The leaves were cleaned, washed with tap water, rinsed with distilled water, and shade dried for 15 days. The shade dried leaves were coarsely powdered and subjected to soxhlet extraction using methanol for 3 days. The methanol extract was further fractionated with hexane to separate the low polar compounds. The methanol and hexane fractions were concentrated and stored for further use.

Phytochemical profile

The phytochemical profile of *A. echioides* was studied both qualitatively and quantitatively following the standard techniques prescribed by Harborne[10]. In addition, the bioactive components were analysed by GC-MS (Thermo Scientific TSQ QUANTUM XLS) using TG-5MS column (30 m X 0.25 mm X 0.25 µm). The injector and interface temperatures were maintained at 280 °C, flow rate 1 mL/min, split ratio 10, and injection volume 1 µL. The mass spectral scan range was set to 40–600 m/z and compared to the standard in NIST-011 library.

Evaluation of glucose uptake by 3T3 L1 cell lines

Chemicals and reagents

Dulbecco's Modified Eagle's Medium (DMEM) and Foetal Calf Serum (FCS) for cell culture were purchased from GIBCOBRL, USA and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) kit was from Promega, USA. Chemicals and solvents were procured from Merck, India.

Measurement of glucose uptake using 6-NBDG

The 3T3 L1 preadipocytes were grown on DMEM medium supplemented with 2 mM glutamine, antibiotics (penicillin 120 U/mL, streptomycin 75 µg/mL, gentamycin 160 µg/mL, amphotericin B 3 µg/mL) and 10 % FCS at 37 °C in a humidified incubator with 5 % CO_2. The grown cells were differentiated to adipocytes after attaining confluency. Glucose uptake experiments were performed as described earlier with slight modifications[11]. Briefly, 3T3-L1 adipocytes were serum-starved in DMEM for 16 h, washed with PBS (pH 7.4) and subsequently incubated for 30 min in the following media: DMEM alone, DMEM containing either 100 nM insulin or plant extract (varying concentration) or Rosiglitazone. Cells were then washed with PBS containing 0.3 mM phloretin and thereafter lysed in 1 mL of 1N NaOH for scintillation counting. The lysates were quantitated using scintillation counter and the results expressed as % glucose uptake. The uptake effects were compared with the positive control rosiglitazone (50 µM).

$$\% \text{ Glucose uptake} = (\text{OD control} - \text{OD test}) / \text{OD control} \times 100$$

Where OD control is the absorbance of control without extract, insulin or Rosiglitazone, OD test is absorbance of sample treated with plant extract, insulin or Rosiglitazone.

Statistical Analysis

Data are presented as mean±SE. Statistical significance of treatments was determined using the paired t test (*indicates $P < 0.05$; **indicates $P < 0.01$).

Results and Discussion

Phytochemical profile

The qualitative phytochemical profile of methanol extract of *A. echioides* revealed the presence of alkaloids, phenols, flavonoids, tannins, and terpenoids (Table 1). The GC-MS profile depicted in Fig. 1 and Table 2 shows presence of 13 significant peaks at retention times, 15.95, 16.23, 16.35, 16.46, 16.53, 16.85, 16.62, 19.72, 25.93, 26.30, 26.39, 26.46, and 26.53, respectively. The compound of interest is 5-ethyl-2-imino Thiazolidin-4-one (Fig. 2) with the molecular formula $C_5H_8N_2OS$ (CAS#1762692) recorded at 15.95 min. Literature survey revealed that thiazolidin-4-one derivatives have important biological activities such as antidiabetic[12], anti-inflammatory, anti-tuberculosis, anticancer, antitumor, anti-HIV, antioxidant, antiviral, anticonvulsant, antihistaminic activity[13]. Other obtained compounds did not have any

Table 1—Qualitative phytochemical profile of methanol extract of *A. echioides* leaves

S. No.	Compound	Result
1	Alkaloids	+
2	Phenols	+
3	Flavonoids	+
4	Tannins	+
5	Glycosides	-
6	Saponins	-
7	Reducing Sugar	-
8	Proteins	-
9	Terpenoids	+

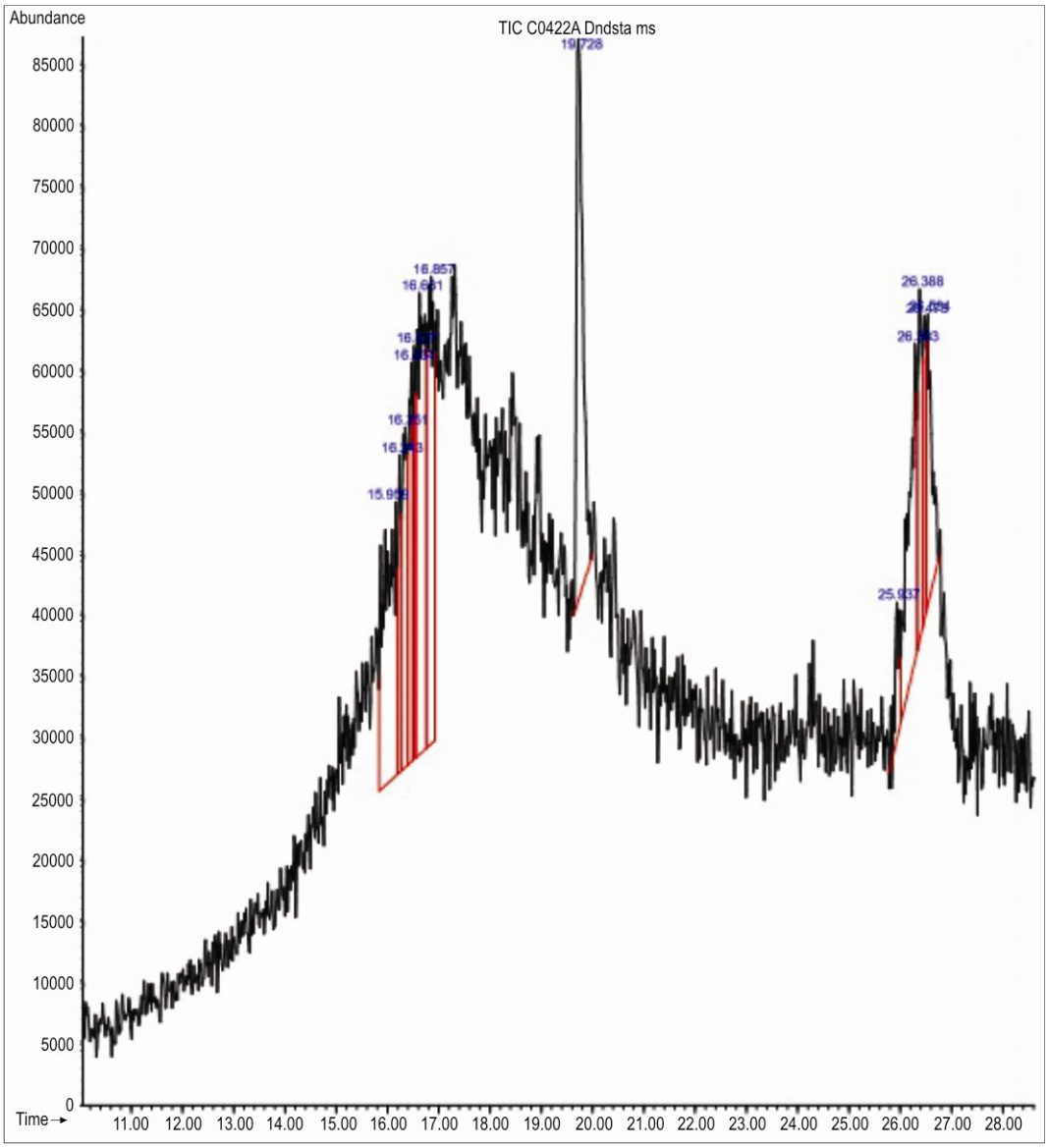

Fig. 1—GC-MS profile of methanol extract of *Andrographis echioides* leaves

scientifically validated antidiabetic potential. Thus, the presence of thiazolidinone in the studied extract could presumably attribute to the glucose uptake. However, the specific compound responsible for the antidiabetic potential of *A. echioides* can only be drawn after further detailed mechanistic studies.

A recent study was conducted by Bhat *et al.* on a series of novel Protein tyrosine phosphatase 1B (PTP1B) inhibitors that contain a thiazolidinone-substituted biphenyl scaffold[14]. PTP1B is an enzyme that dephosphorylates insulin receptor and leads to down-regulation of insulin signalling. These PTP1B inhibitors are significant therapeutic agents for the treatment of diabetes which normalizes increased plasma glucose level by enhancing the insulin sensitivity. Bhat also reported that introduction of the 4-oxothiazolidine-2-thione moiety increased the inhibitory activity against PTP1B[15]. Thiazolidine-2, 4-diones derivatives with carboxylic ester moieties at N-3 and benzyl and heteroaryl substituents at C-5 have also been screened for their anti-hyperglycemic activity[14].

Glucose uptake by 3T3-L1 cell lines

The data obtained suggests that methanol extract of *A. echioides* was effective in enhancing glucose uptake by the 3T3 cell lines *in vitro*. At 24 h, the cells treated with methanol extract of *A. echioides* exhibited 3-fold increase in glucose uptake (at the

Table 2—GC-MS profile of methanol extract of *A. echioides* leaves

S. No.	Retention time	Compound name	CAS#
1	15.953	Thiazolidin-4-one, 5-ethyl-2-imino	001762-69-2
2	16.235	5-Hexen-2-ol, 5-methyl-	050551-88-7
3	16.358	Acetoacetic acid, 1-thio-, s-allyl ester	015780-65-1
4	16.464	5-Hexen-2-ol, 5-methyl-	050551-88-7
5	16.534	5-Hexen-2-ol, 5-methyl-	050551-88-7
6	16.622	Acetic acid, (2-propenylthio)-	020600-63-9
7	16.851	2-Cyclopentene-1-undecanoic acid,(+)-	000459-67-6
8	19.721	2-(2-Nitrovinyl) furan	000699-18-3
9	25.936	3-Hydroxy-7, 8-dihydro-beta.-ionol	172705-13-4
10	26.306	Di-n-octyl phthalate	000117-84-0
12	26.394	1, 2-Benzenedicarboxylic acid, diiso octyl ester	027554-26-3
12	26.465	Phthalic acid, heptyl undecyl ester	1000308-94-0
13	26.535	1, 2-Benzenedicarboxylic acid, diiso octyl ester	027554-26-3

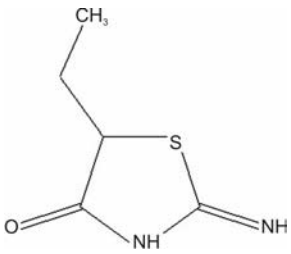

Fig. 2—Chemical structure of 5-ethyl, 2-imino thiazolidin-4-one

minimum concentration tested) compared with untreated cells. The plant extract proved to mimic up to 86 % of Rosiglitazone activity and 12 % greater than that of Insulin. Furthermore, the increase in glucose uptake was dose-dependent upto a concentration of 1 µg beyond which the glucose uptake rate showed to decrease with increasing concentration of plant extract (Fig. 3).

Several literatures have demonstrated that the treatment with thiazolidinediones (TZDs) increase the adipose tissue glucose transport activity and GLUT4 expression[16-23]. Also, 3T3-L1 and 3T3-F442A cell line studies have reported TZD-induced increase in glucose transport, although effects on glucose transporters appear to be dependent on the differentiation state[24]. It has been reported that TZD acts as agonists of nuclear receptor peroxisome

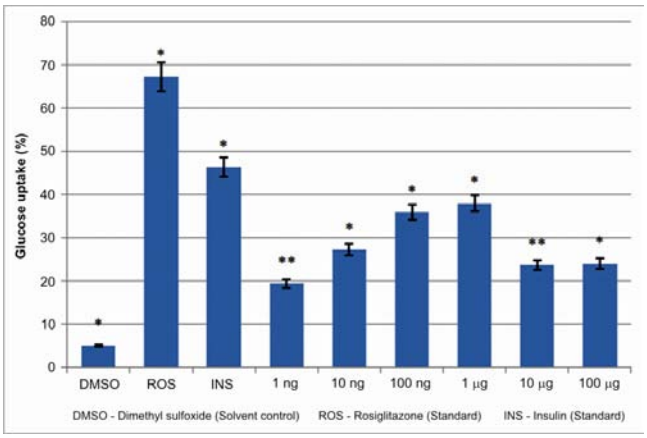

Fig. 3—Effect of methanol extract of *Andrographis echioides* leaves on glucose uptake in 3T3L1 cell lines

proliferator-activated receptor gamma (PPAR-γ) by reducing insulin resistance in the liver and peripheral tissues; increasing the intake of insulin-dependent glucose and decreasing withdrawal of glucose from the liver[25].

Conclusion

In India, diabetes is now a potential epidemic with increasing number of individuals being diagnosed with the disease every year. Therefore, it is crucial to search for safe and effective options for its treatment. Results of the present study suggest that the tested extract of *A. echioides* is effective in enhancing glucose uptake. Moreover, the glucose uptake activity could be increased by isolation and purification of single bioactive compounds from the leaves. However, further mechanistic studies are required to prove the effect *in vivo.*

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