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ORIGINAL ARTICLE

A Study of *In-vitro* Hypoglycemic and Glucose Uptake Activity of Isolated Compound from Ethanolic Leaf Extract of *Amaranthus tristis* Linn.

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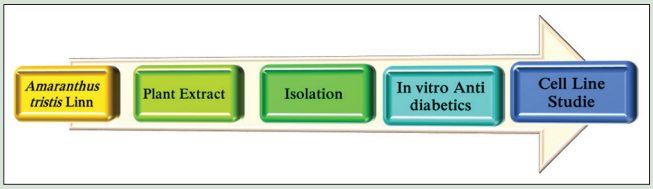
ABSTRACT

Background: Natural bioactive compounds have a long history to use effectively in the treatment of Diabetes Mellitus. *Amaranthus tristis* Linn (Amaranthaceae) have been used for the treatment of various ailments. **Objective:** The present work was undertaken to study the effect of an isolated bioactive compound from the leaf of *Amaranthus tristis* Linn on glucose uptake in cell lines like 3T3-L1 cell lines and inhibition of alpha-glucosidase and alpha-amylase enzymes. **Materials and Methods:** The Ethanolic leaf extract of *Amaranthus tristis* Linn subjected to preliminary phytochemical screening and isolated compound were tested for its cytotoxicity study by MTT assay. Isolated compound was selected further glucose uptake assay based on cytotoxicity concentration CTC_{50} value. NMR technique was used to identify the structure of the compound. **Results:** Phytochemical analysis showed the presence of alkaloids, flavonoids, glycosides. The Isolated compound showed moderate cytotoxic activity to 3T3-L1 cell line. Compounds identified as rutin In *in-vitro* glucose uptake assay of rutin showed an increase in glucose uptake. **Conclusion:** *A. tristis* Linn. showed significant antidiabetic activity in dose-dependent manner.

Key words: 3-(4,5-dimethylthiazol-2-yl) 5-diphenyltetrazolium bromide assay, 3T3-L1 cell lines, *Amaranthus tristis* Linn.

SUMMARY

- The Isolated compound of Rutin isolated from *Amaranthus tristis* Linn
- Rutin isolated from *Amaranthus tristis* Linn increses in the glucose uptake under *in-vitro* conditions
- Isolated compound of rutin significantly antidiabetic activity by using 3T3-L1 cell lines
- The evaluation of antidiabetic activity of isolated compound of Rutin in dose-dependent manner increase in percentage inhibitory activity against alpha-amylase and alpha-glucosidase.



Abbreviations Used: MTT: 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide, FBS: Fetal Bovine serum, PBS buffer: Phosphate Buffered Saline, DMEM: Modified Eagle's Medium, EDTA: Ethylenediaminetetraacetic acid, IBMX: 3-isobutyl-1-methylxanthine, TLC: Thin-layer chromatography, FTIR: Fourier-transform infrared spectroscopy, ^{13}CNMR: nuclear magnetic resonance, ^{1}H NMR: Proton nuclear magnetic resonance, GC-MS: Gas chromatography–mass spectrometry, DNSA: Dinitro salicylic acid.

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INTRODUCTION

Diabetes mellitus is a systemic endocrine disorder characterized by the decrease in both secretion of insulin and insulin action. It is commonly associated with the development of micro- and macro-vascular diseases such as neuropathy, nephropathy, and cardiovascular diseases.[1] The condition is related to reduced quality of life and also increased risk factors of heart, kidney, brain, etc., The commercial formulation used for the treatment of diabetics like sulfonylurea's, biguanides, and α-glucosidase inhibitors are more expensive and associated with various side effects, but natural herbal drugs have been found to have lesser side effects and provide long term effect for therapy in treating diabetes.[2]

Amaranthus tristis Linn. is monoecious plant and is around 100–400 cm in height. It is cultivated in India and China. The common name of this plant is known as Green Amaranth in English. This plant is becoming under an extensive variety of climatic conditions, and they can produce leafy home remedy vegetables.[3] Industrial and public use of *A. tristis* Linn. plant has been linked not only to its recognized rich nutritional value of food material but also to its potential beneficial to the therapeutic agent in diets for hyperglycemic-susceptible individuals.[4]

A. tristis Linn. is used in indigenous system of medicine since many years; In this plant material used as food supplementary in traditional area in India and the ethnobotanical report reveals the plant having more antioxidant potential in nature, so from this study to develop antidiabetics activity of selected plant.[5]

MATERIALS AND METHODS

Collection of medicinal plant

The plant of *A. tristis* Linn. collected in the place of Yercaud Hills, Tamil Nadu, India. In the long stretch of September 2017, the plant material was taxonomically recognized, affirmed, and authenticated as *A. tristis* Linn. by Dr. K. Madavacheety, Venkateswara University, Tirupati, and the voucher specimen was retained in our laboratory for future reference.

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Preparation of plant extract

The dried leaves were made into coarse powder with the help of dry mechanical grinder and passed through the sieve number 60. The powdered leaves extracted using soxhlation method. The powder was defatted with petroleum ether (40°C –60°C) and extracted with ethanol. Extracts subjected to rotary evaporator to remove solvents. This extract used to perform preliminary phytochemical marker analysis was done using the standard procedure.[6]

Chemicals

3-(4,5-dimethylthiazol-2-yl) 5-diphenyltetrazolium bromide (MTT) reagent and fetal bovine serum (FBS), phosphate-buffered saline buffer, Dulbecco's modified Eagle's medium (DMEM), and trypsin were collected from Fisher Scientific India; ethylenediaminetetraacetic acid, glucose, and antibiotics from E. Merck Ltd., Laboratories Ltd., Mumbai; and dimethyl sulfoxide (DMSO) and propanol from E. Merck Ltd., Mumbai, India.

Isolation of compound

The compound obtained from ethanolic extract of *A. tristis* Linn. with various fractions of chloroform: ethanol solvent system in a column chromatography with gradient elution technique. Isolated compound was confirmed phytochemical test and spectral studies.[7,8]

Cell line

3T3-L1 cell line is used for evaluating *in vitro* anti-adipogenic activity which done by National Centre for Cell Science, Pune, India.

Adipocyte differentiation in 3T3-L1 cells

3T3-L1 cells line were collected from Murine Swiss 3T3 cells of embryos cells. 3T3-L1 cells were seeded in 96-well plates (5×10^3 cells/well). The estimation of glucose take-up assay was performed by utilizing DMEM/F12 medium with 10% FBS. After 2 days, the medium is changed to division medium [DMEM/F12 + 2% FBS containing 10 µg/mL of insulin, 0.5 mM isobutylmethylxanthine (IBMX) 3-isobutyl-1-methylxanthine, and 1.0 µM dexamethasone (sigma)]. 3T3-L1 cells was treated with a blend of dexamethasone, IBMX, and insulin embrace as an adjusted combination. Cells stayed to separation medium for 4 days with media renewed each 48 h. Following 48 h, separation medium was supplanted with medium such as DMEM/F12 + 2% FBS and substance facilitates assessment of glucose uptake assay.[9]

Glucose uptake assay

The Glucose uptake assay was performed using 3T3-L1 cell line with different concentration range (10, 20, 40 and 80 µg/ml) of isolated compound of rution was added respective wells and inhubated for 5hrs with and without insulin condition, the test was performed triplicates.

The supernatant discarded and the cell lysate was used to determine the glucose content by DNSA (Dinitro salicylic acid) method. The plate was read at 570 nm.

The readings of the test samples scored by comparison with the zero control. Percent viability of the test substances is calculated in comparison with the control using MTT assay.[10]

$$\text{Percent increase} = \frac{t}{c} \times 100$$

where t – absorbance of test substance and c – absorbance of the vehicle control.

In vitro antidiabetic screening of isolated compound by alpha-amylase enzyme

The Starch solution (0.1% w/v) was prepared by adding 100mg of starch with 100ml of sodium acetate buffer (16 mM). The sodium acetate buffer was prepared by adding 36.2 g of sodium acetate and 14.8 ml of glacial acetic acid and the volume was made upto 100 ml with distilled water. The enzyme solution was prepared by 27.5 mg of alpha-amylase dissolved in 100ml of distilled water. The colorimetric reagent was prepared by adding sodium potassium tartrate with 3,5-dinitrosalicylic acid solution. Colour development was preformed adding control and isolated compoumd of rution individually with starch solution followed by enzyme solution and coloring reagent in alkaline condition at 25°C for 3 min. The formation of maltose was estimated by the reduction of 3,5-dinitrosalicylic acid convert to 3-amino-5-nitro salicylic acid. This reaction is measured at 540 nm.[11]

In vitro method used in antidiabetic screening of isolated compound using alpha-glucosidase enzyme

One milliliter of starch stock solution (2% w/v) was mixed with 0.2 M tris buffer solution at the pH 8.0 and different concentration range of 0.2-1.0 mg/ml was prepared from the isolated compound and heated for 5 min at 37°C. The response started by adding 1 ml enzymatic solution of alpha-glucosidase (1 U/ml) to it followed by heated for 40 min at 35°C. At that point, the response terminated by the added of 2 ml of 6N HCl. Optical density was measured at 540 nm.[12]

The concentration of the isolated compound required scavenging of half of the radicals (IC_{50}). Rate restraint (I%) was figured by I% = (Ac − As)/Ac × 100, where Ac is the optical density of the control and As is the optical density of the sample.[13]

RESULTS AND DISCUSSION

The preliminary phytochemical tests for the ethanol extract of *A. tristis* Linn. leaves revealed the presence of carbohydrates, alkaloids, flavonoids, tannins, steroidal glycosides, and phenols. Isolation was performed using column chromatography with different concentration of various fractions of solvent system used from that chloroform: ethanol (9:1) solvent system used for isolate the compound the presence of bioactive compounds was confirmed by total leukocyte count and melting point, and fraction showed positive test for flavonoids [Table 1]. The structure of isolated compound confirmed with help of spectral studies such as Fourier transform infrared spectroscopy, ^1H, ^{13}C nuclear magnetic

Table 1: ^{13}C nuclear magnetic resonance interpretation value of isolated compound from *Amaranthus tristis* Linn. with reference rutin

Isolated compound	Reference rutin	Compound-I (isolated rutin)	Reference rutin
144.72 (C2)	144.8	116.26 ($C6^{I}$)	116.36
133.31 (C3)	133.41	101.20 (C^{III})	101.32
177.35 (C4)	177.48	74.07 ($C2^{II}$)	74.18
161.20 (C5)	161.33	76.47 ($C3^{II}$)	76.57
98.65 (C6)	98.53	68.19 ($C4^{II}$)	68.33
156.56 (C7)	156.73	75.91 ($C5^{II}$)	76.01
93.55 (C8)	93.69	66.97 ($C6^{II}$)	67.09
100.70 (C9)	100.84	101.20 ($C1^{III}$)	101.0
156.40 (C10)	156.55	70.57 ($C2^{III}$)	70.52
121.56 ($C1^{I}$)	121.70	70.35 ($C3^{III}$)	70.48
116.26 ($C2^{I}$)	116.70	70.01 ($C5^{III}$)	70.67
144.72 ($C3^{I}$)	144.84	68.19 ($C6^{III}$)	68.55
148.38 ($C4^{I}$)	148.5	17.67 C-CH3	17.72

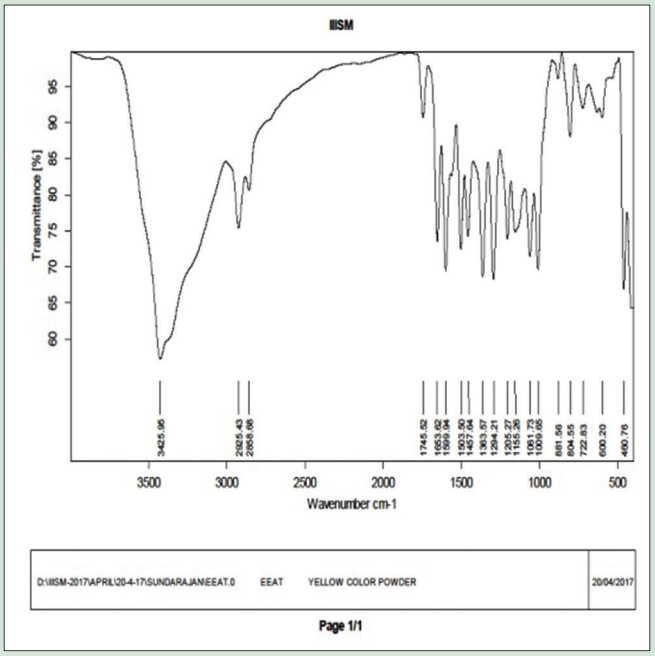

Figure 1: Infrared spectra

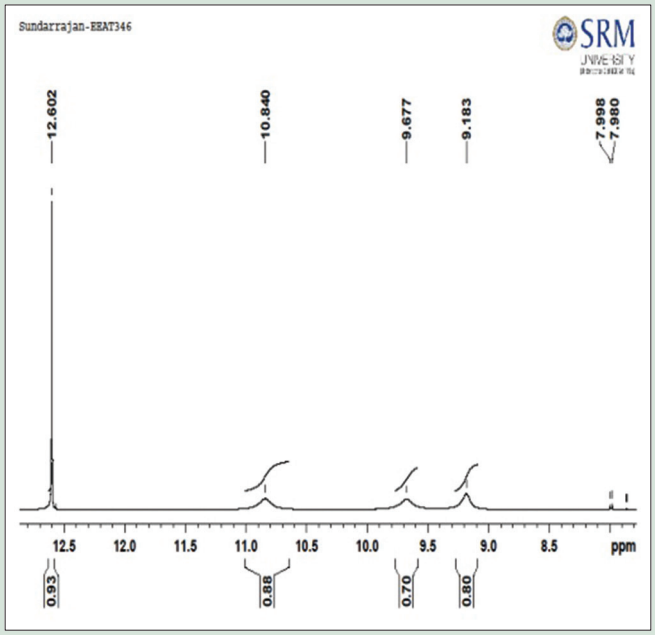

Figure 2: ^{1}H nuclear magnetic resonance spectra

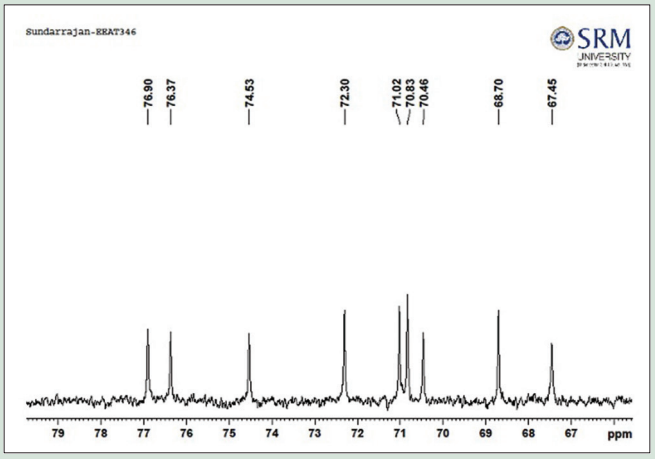

Figure 3: ^{13}C nuclear magnetic resonance of isolated compound

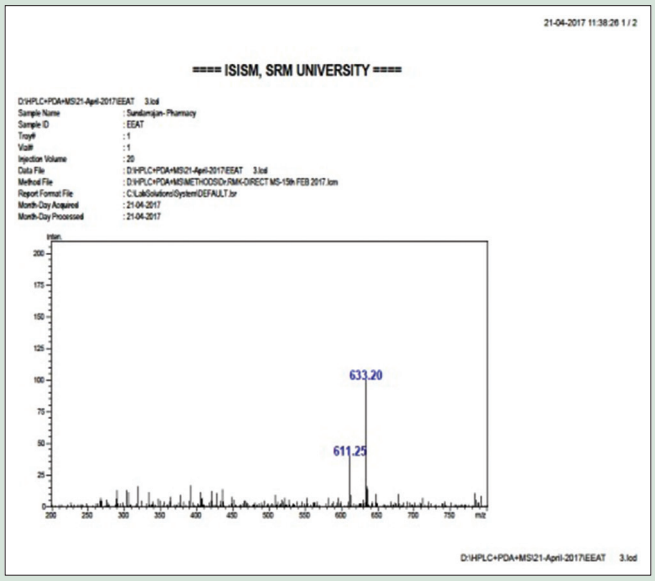

Figure 4: Gas chromatography–mass spectrometry spectra

resonance, and gas chromatography-mass spectrometry. Interpretation data reveal that positive with rutin [Figures 1-4].

Spectral characterization

Insulation resistance values

3423 OH (bonded), 2906 CH stretch, 1457(C-H Bend) 1656 (C = O), 1505 (C = C) C = C aromatic, 1360 C-O-C, 1295 C-O-C, 1200 C-O-C, 1060 C-O-C, 810 substituted aromatics other fingerprint bands characteristics to rutin are seen following 970, 880, 730, and 700.

Nuclear magnetic resonance values

Chemical shifts of rutin in dimethyl sulfoxide-D6 with respect to tetramethylsilane

δ ppm Assignment 1.12 d, 3H, CH_3 3.38-3.56 m, 8H, rutinoside protons and CH_2O 3.62 dd, 1H, CH-CH_3 rhamnose 3.82 d, 1H, CH-CH_2O glucose 4.51 d, 1H, OCHO rhamnose 5.10 d, 1H, OCHO glucose 6.20 d, 1H, Ar-H 6.39 d, 1H, Ar-H 6.89 d, 1H, Ar-H 7.61-7.67 m, 2H, Ar-H.

Mass spectrum

The mass spectrum of rutin obtained by desorption chemical ionization using ammonia as a reactant gas show a molecular ion M at m/e 633 amu.

3T3-L1 cell line

Antidiabetic activity of the given compound showed remarkable glucose uptake when insulin was accompanied. Without insulin conditions, the activity was threefold less glucose uptake is shown in Table 2. It was evident that the high concentration (80 μg/ml) showed less glucose uptake when compared with (20 μg/ml) low concentration.

The little cytotoxicity found when drug concentration increased due to this phenomenon the less glucose uptake observed at higher concentration.

Table 2: Antidiabetic potential of isolated compound from *Amaranthus tristis* Linn. on 3T3-L1 cells by glucose uptake assay

Concentration (µg/ml)	Glucose uptake percentage	
	Insulin absent	Insulin present
10	85.71429±1.24	138.2022±1.24
20	91.83673±1.23	158.427±0.34
40	77.55102±1.24	137.6404±1.32
80	61.22449±0.34	116.2921±1.34

Values are represented as mean±SEM of percentage of inhibition (n=5).
SEM: Standard error of mean

Table 3: *In vitro* antidiabetic activity of isolated compound from *Amaranthus tristis* Linn. by alpha-amylase method

Concentration of sample (mg/ml)	Percentage inhibition of rutin
0.2	21±0.05
0.4	59±0.07
0.6	65±0.03
0.8	81±0.06
1.0	98±0.04
IC_{50}	0.49

Values are represented as mean±SEM of percentage of inhibition (n=5).
SEM: Standard error of mean

Table 4: *In vitro* antidiabetic activity of isolated compound from *Amaranthus tristis* Linn. alpha-glucosidase method

Concentration of sample (mg/ml)	Percentage inhibition of rutin
0.2	33.6±0.14
0.4	55.1±0.17
0.6	70.1±0.12
0.8	82.5±0.09
1.0	91.2±0.11
IC_{50}	0.49

Values are represented as mean±SEM of percentage of inhibition (n=5).
SEM: Standard error of mean

In vitro antidiabetic activity

The antidiabetic activity was evaluated in the isolated compound of rutin using alpha-amylase method the percentage inhibitory activity was measured. The concentration of 0.2 mg/ml of showed the percentage inhibition of 21% and the 1.0 mg/ml concentration of isolated rutin showed the alpha-amylase enzyme inhibition of 98%. Tables 3 and 4 show the study in which antidiabetic activity was evaluated *in vitro* alpha-amylase and alpha-glucosidase activity of isolated compound of rutin. The rutin showed significant inhibition activity. The potential of isolated compound of rutin obtained from *A. tristis* Linn. was studied by inhibiting alpha-glucosidase and alpha-amylase.

CONCLUSION

The isolated compound of rutin from *A. tristis* Linn. is protected and furthermore utilized as a part of glucose take-up mechanism. The significant glucose transporter component present in fat tissue and skeletal muscle is GLUT-4 is translocated from an intracellular layer stockpiling site to the plasma membrane.

The results showed that the isolated compound of rutin from *A. tristis* Linn. increases in the glucose uptake under *in vitro* conditions. It may occur due to the presence isolated compound of rutin from *A. tristis* Linn. or due to its effect on the receptors present in the cell membrane.

In this study of evaluation of *in vitro* alpha-amylase and alpha-glucosidase activity of the isolated compound of rutin from *A. tristis* Linn., it showed significant antidiabetic activity in dose-dependent manner. It may be used as novel antidiabetic target new drug design.

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Conflicts of interest

There are no conflicts of interest.

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