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IN-VITRO ANTIDIABETIC ACTIVITY OF 2-(3,4-DIHYDROXYPHENYL)-3,5,7-TRIHYDROXY-4H-CHROMEN-4-ONE ISOLATED FROM THE METHANOLIC EXTRACT OF *ANDROGRAPHIS ECHIOIDES* LEAVES

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Keywords:

Andrographis echioides, Leaves, 2-(3, 4-dihydroxyphenyl)-3, 5, 7-trihydroxy-4H-chromen-4-one, Alpha-amylase inhibitory activity, Alpha-glucosidase inhibitory activity

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ABSTRACT: In the present study the 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one isolated from the methanolic extract of leaves of *Andrographis echioides* was studied for alpha-amylase and alpha-glucosidase inhibition using an *in-vitro* model. The isolation was done using column chromatography using gradient elution with different mobile phase. Structural elucidation was carried out on the basis of spectral analysis. The study revealed that the 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one exhibited significant α-amylase and α-glucosidase inhibitory activities with an IC_{50} value of 36.2 ± 0.42 and 41.4 ± 0.34% respectively and well compared with standard acarbose drug. The infra-red spectrum specific absorption bands for 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one *viz.* 3401.71 (O-H stretching vibration of phenol), 1666.95 (C=O Aryl ketonic stretch), 1612.42 (C-C Aromatic ring stretch), 1519.96 (C=O aromatic stretch), 1463.09 (C=C aromatic stretch), 1380.89 (O-H bending of phenols), 1318.98 (C-H bond in Aromatic hydrocarbon), 1246.04 (C-O stretch of Aryl ether), 1213.26 (C-O stretch of phenol), 1169.06 (C-CO-C stretch and bending in ketone), 932.62, 807.89, 702.92, 638.65 (C-H bending of aromatic hydrocarbons). Structural elucidation of 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one was done by spectrum analysis such as ^{13}C and ^{1}H depth nuclear magnetic resources. Mass spectra of 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one showed a parent molecular ion [M+] peak at m/z 303 which corresponds to molecular formulae $C_{15}H_{10}O_7$. Therefore, it is suggested that 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one is a potential source for natural antidiabetic compounds and could have potential use in the management of diabetes mellitus.

INTRODUCTION: Diabetes mellitus is a chronic metabolic disorder that affects the metabolism of carbohydrates, fat, and protein. It is characterized by hyperglycemia, in which blood sugar levels are elevated either because the pancreas does not produce enough insulin or cells of the body do not respond properly to the insulin produced [1].

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Type 1 diabetes results from the inadequate synthesis of insulin by β-cells of the pancreas, while type II diabetes is characterized primarily by insulin resistance (a condition in which peripheral cells do not respond normally to insulin) or β-cell dysfunction [2].

The treatments for diabetes is a reduction of the demand for insulin, stimulation of insulin secretion, enhance the mode of action of insulin at the target tissues and inhibition of degradations of oligo- and disaccharides. The enzymes alpha-glucosidase are responsible for the breakdown of oligo- and disaccharides to monosaccharides. The inhibitory action of these enzymes leads to a decrease in blood glucose level [3].

Inhibitors of α-amylase and α-glucosidase delay the breaking down of carbohydrates in the small intestine and diminish the postprandial blood glucose excursion [4]. Recently, herbal medicines are getting more importance in the treatment of diabetes as they are free from side effects and less expensive when compared to synthetic hypoglycemic agents [5, 6].

Andrographis echioides belongs to *Acanthaceae* family, used for various medicinal purposes in South Asia particularly India and China. Based on the literature, this plant possesses pharmacological properties to include antimicrobial activity, anti-inflammatory, diuretic, anthelmintic, analgesic, antipyretic, hepato-protective activities, and antioxidant effect. It contains plenty of phytochemical constituents such as flavonoids, flavones, steroids, tannins, carbohydrate, glycosides and alkaloids [7, 8]. The leaf juice of *Andrographis echioides* is used to cure fevers. Genus of *Andrographis* family plants is used to cure various diseases like goiter, liver diseases, fertility problems, bacterial, malarial and fungal disorders [9]. *Andrographis echioides* boiled with coconut oil is used to decrease the falling and graying of hair [10].

From the leaves extract of *Andrographis echioides* various chemical constituents were isolated dihydro echioidinin, skullcap avone 1 2'-methyl ether, echioidinin, echioidin, skullcap avone 1 and 2'-O-bD-glucopyranoside [11]. Some of the other chemical constituents present in the *Andrographis echioides* are more than 17 compounds such as borneol, cyclohexanol 2,4 dimethyl phenol, 3,4 altroson, deconoic acid, Squalene, vitamin E, Methoprene, 2-nonenlol Oxirane,octyl-, 2, 2-cyclopentene-1-undecanoic acid, ketone, 1,5-methylbicyclo [2.1.0] pent-5-ylmethyl and 2,5-cyclohexadiene-1,4-dione, 2, 5- dihydroxy-3-methyl -6- (1-methyl ethyl) bicycle heptan -3- one [12]. However, no single method was found in the literature to our knowledge to detect 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one in methanolic extract of leaves of *Andrographis echioides*.

Inhibition of alpha-amylase and alpha-glucosidase enzymes can be an important strategy in the management of post prandial blood glucose level in type 2 diabetes patient [13]. However, Therefore, in the present study, the antidiabetic activities of 2-(3,4-dihydroxyphenyl)-3, 5, 7-trihydroxy-4H-chromen-4-one isolated from the methanolic leaves extract of *Andrographis echioides* were evaluated employing *in-vitro* assay methods.

MATERIALS AND METHODS:

Collection of Plant Material: The leaves of *Andrographis echioides* were collected in May from the mullipatti, pudukkottai, Tamil Nadu, India. The plant was identified, and leaves of *Andrographis echioides* were authenticated and confirmed from Dr. S. John Britto, Director, Rapinat herbarium, St. Joseph College, Tiruchirapalli, and Tamil Nadu for identifying the plants. The voucher specimen number SGP001 (7.06.2017).

Chemicals and Reagents: Alpha (α)-Glucosidase, porcine pancreas alpha (α)-amylase, *p*-nitrophenyl-α-D-glucopyranose (*p*-NPG), 3,5-dinitrosalicylic acid (DNS) and acarbose were purchased from Sigma Chemical Co. (St. Louis, MO, USA).2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one(purity 99%), was purchased from Sigma-Aldrich, New Delhi. All the chemicals, including solvents such as n-hexane, ethyl acetate, chloroform, methanol, anisaldehyde sulphuric acid reagents (0.5 ml p-anisaldehyde in 50 ml glacial acetic acid and 1ml conc. sulfuric acid. Heat to 105 °C until maximum visualization of spots) were of analytical grade and were procured from E. Merck, India. All the chemicals used including the solvents were of analytical grade.

Preparation of Methanol Extracts: The leaves of *Andrographis echioides* were washed in running water, cut into small pieces and then shade dried for a week at 35-40 ºC, after which it was ground to a uniform powder of 40 mesh size. The methanol extracts were prepared by soaking 1.5 kg each of the dried powder plant materials in 1.5 L of methanol using a soxhlet extractor continuously for 10 h. The extracts were filtered through Whatman filter paper no. 42 (125 mm) to remove all unextractable matter, including cellular materials and other constitutions that are insoluble in the extraction solvent. The entire extracts were concentrated to dryness using a rotary evaporator under reduced pressure. The final dried samples were stored in labeled sterile bottles and kept at -

20ºC. The filtrate obtained was used as a sample solution for the further isolation [14].

Isolation of 2-(3, 4-dihydroxyphenyl)-3, 5, 7-trihydroxy-4H-chromen-4-one by column chromatography: The condensed methanol extract of leaves (986 g) of the sample was subjected to column chromatography over TLC grade silica gel. Elution of the column first with n-hexane, an increasing amount of ethyl acetate in n-hexane and finally with methanol yielded some fractions. The preparation of solvent systems used to obtain 2- (3, 4- dihydroxyphenyl)- 3, 5, 7- trihydroxy- 4H-chromen-4-one (283mg/786g) were ethyl acetate-methanol (70:30) from fraction 9. The compounds were detected on TLC plates by spraying with Libermann- Burchard reagent and heated at 100 °C for 10 min [15].

Purification of Isolated Compounds by HPTLC and High-Performance Liquid Chromatography:
Preparative Thin-layer chromatography (TLC): The isolated pure compound was dissolved in appropriate solvents. 5 µl of isolated compounds 2-(3, 4-dihydroxyphenyl)- 3, 5, 7-trihydroxy- 4H-chromen-4-onewere applied to silica gel plates, Merck (Germany) 20 × 20 cm, 0.25 mm in thickness. Plates were developed using the solvent system ethyl acetate-methanol (70:30 v/v) for 2-(3, 4- dihydroxyphenyl)- 3, 5, 7- trihydroxy- 4H-chromen-4-one. The separated zones were visualized with freshly prepared Libermann - Burchard reagent and heated at 100 °C for 10 min. Chromatograms were then examined under daylight within 10 min [16].

High-Performance Liquid Chromatography (HPLC): The analytical HPLC system (Shimadzu) was equipped with a diode array detector, a 20µl loop, 200 × 4.6 mm C18 column, methanol (HPLC grade, 0.2mm filtered) used as a mobile phase. The isolated 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H- chromen- 4- one compound were separated using a mobile phase of methanol: water (75:25 v/v) at a flow rate of 1.0 ml/min, column temperature 30 °C. Injection volume was 40 µl and detection was carried out at 346 nm [17].

Structural Elucidation Study of Isolated Compound: Different spectroscopic methods including UV, FT-IR, ^{1}H- NMR, ^{13}C-NMR and GC-MS were used to elucidate the structure of isolated compounds. The UV-visible spectrum of the isolated compounds in methanol was recorded using a Shimadzu 160A UV–visible spectro-photometer. The Fourier Transform Infrared (FT-IR) spectra were recorded with a nominal resolution of 4 cm^{-1} and a wave number range from400 to 4000 cm^{-1} using the KBr pellet technique. 1H and 13CNMR spectra were acquired on Bruker WP 200 SY and AM200 SY instruments (1H, 200.13 MHz; 13C, 50.32 MHz) using TMS as internal standard and $CDCL_3$ as a solvent. GC-MS analysis of the extract was performed using a Perkin-Elmer GC Clarus 500 system and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with an Elite-I, fused silica capillary column (30 mm × 0.25 mm 1D × 1 µ Mdf, composed of 100% Dimethyl polysiloxane) [15-17].

In-vitro* Antidiabetic Activity of 2-(3,4-dihydroxyphenyl)-3, 5, 7-trihydroxy-4H-chromen-4-one from Leaves of *Andrographis echioides:
Alpha-Amylase Inhibitory Assay: This assay was carried out using a modified procedure of McCue and Shetty, 2004 [18]. A total of 250 µL of isolated compound 2- (3,4-dihydroxyphenyl)- 3, 5, 7-trihydroxy-4H-chromen-4-one (20-100 µg/ml) was placed in a tube and 250 µL of 0.02M sodium phosphate buffer (pH 6.9) containing α-amylase solution (0.5 mg/mL) was added. This solution was preincubated at 25 ºC for 10 min, after which 250 µL of 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9) was added at timed intervals and then further incubated at for 25 ºC for 10 min. The reaction was terminated by adding 500 µL of dinitro-salicylic acid (DNS) reagent. The tubes were then incubated in boiling water for 5 min and cooled to room temperature. The reaction mixture was diluted with 5 mL distilled water, and the absorbance was measured at 540 nm using a spectrophotometer. A control was prepared using the same procedure replacing the extract with distilled water.

The α-amylase inhibitory activity was calculated as percentage inhibition:

% Inhibition = [(Abs control – Abs 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H chromen-4- one) / Abs control] × 100

Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC_{50}) were determined graphically.

Alpha-Glucosidase Inhibitory Assay: The effect of the isolated compound 2-(3,4-dihydroxyphenyl)-3, 5, 7- trihydroxy- 4H- chromen- 4- one on α-glucosidase activity was determined according to the method described by Kim *et al*., 2005 [19] using α-glucosidase from *Saccharomyces cerevisiae*. The substrate solution p-nitro phenyl glucopyranoside (p-NPG) was prepared in 20 mM phosphate buffer, and pH 6.9. 100 µL of α- glucosidase (1.0 U/mL) was preincubated with 50 µL of the different concentrations (20-100 µg/ml) of the isolated compound 2- (3, 4- dihydroxyphenyl)- 3, 5, 7- trihydroxy-4H-chromen-4-one for 10 min. Then 50 µL of 3.0 mM (p-NPG) as a substrate dissolved in 20mM phosphate buffer (pH 6.9) was then added to start the reaction.

The reaction mixture was incubated at 37 ºC for 20 min and stopped by adding 2 mL of 0.1M Na_2CO_3. The α-glucosidase activity was determined by measuring the yellow-colored para-nitrophenol released from p-NPG at 405 nm. The results were expressed as a percentage of the blank control.

The α-glucosidase inhibitory activity was calculated as percentage inhibition:

% Inhibition = [(Abs control – Abs 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one) / Abs control] × 100

Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC_{50}) were determined graphically.

Statistical Analysis: All assays were conducted in triplicate. Statistical analyses were performed with SPSS 16.0 for an analysis of variance (ANOVA) followed by Duncan's test. Differences at $P<0.05$ were considered to be significant.

RESULTS AND DISCUSSION:

Structural Elucidation of Isolated Compounds: 2- (3, 4-dihydroxyphenyl)- 3, 5, 7-trihydroxy- 4H-chromen-4-one is yellow compound with melting point 314-315 °C, MW: 302.238 g/mol which correspond to the molecular formulae $C_{15}H_{10}O_7$. The UV λ_{max} value of compound 2-(3, 4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one was 250 and 374 nm **Fig. 1**.

In the IR spectrum of isolated compound **Fig. 2** a very broad peak at 3401.71 (O-H stretching vibration of phenol), 1666.95 (C=O Aryl ketonic stretch), 1612.42 (C---C Aromatic ring stretch), 1519.96 (C=O aromatic stretch), 1463.09 (C=C aromatic stretch), 1380.89 (O-H bending of phenols), 1318.98 (C-H bond in Aromatic hydrocarbon), 1246.04 (C-O stretch of Aryl ether), 1213.26 (C-O stretch of phenol), 1169.06 (C-CO-C stretch and bending in ketone), 932.62, 807.89, 702.92, 638.65 (C-H bending of aromatic hydrocarbons).

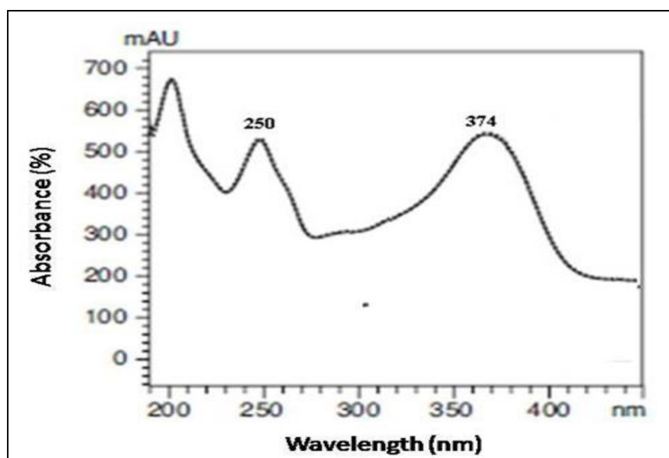


FIG. 1: UV SPECTRA OF THE ISOLATED COMPOUND

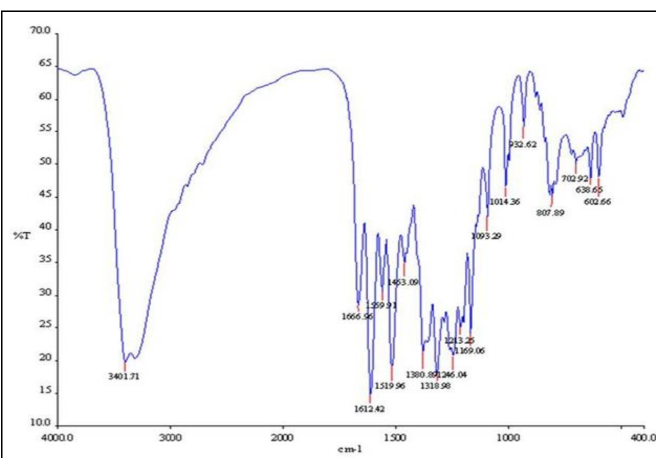


FIG. 2: IR SPECTRA OF THE ISOLATED COMPOUND

In the proton ^1H NMR spectra of 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one **Fig. 3** showed 9.61 (1H, s, OH-3), 12.50 (1H, s, OH-5), 6.19 (1H, d, J = 2.0 Hz, H-6), 10.79 (1H, s, OH-7), 6.40 (1H, d, J = 2.0 Hz, H-8), 7.67 (1H, d, J = 2.0 Hz, H-2'), 9.32 (1H, s, OH-3'), 9.39 (1H, s, OH-4'), 6.87 (1H, d, J = 8.5 Hz, H-5'), 7.53 (1H, dd, J=2.0, 8.0 Hz, H-6').

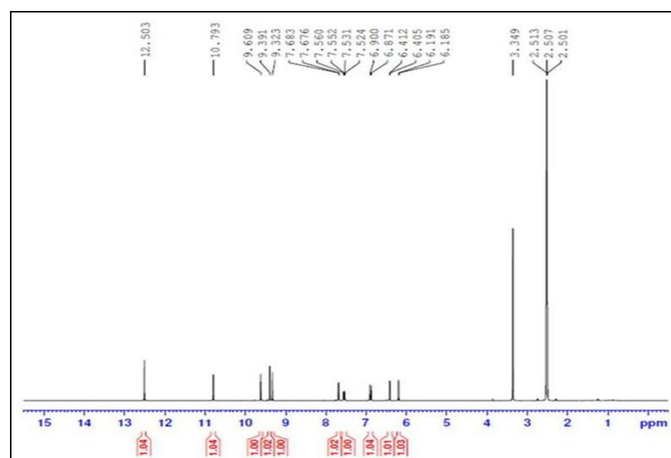


FIG. 3: ^{1}H NMR SPECTRA OF THE ISOLATED COMPOUND

In the ^{13}C NMR spectra of 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one **Fig. 4** showed 147.2 (C-2), 136.1 (C-3), 176.3 (C-4), 161.1 (C-5), 98.6 (C-6), 164.3 (C-7), 93.8 (C-8), 156.5 (C-9), 103.4 (C-10), 122.4 (C-1'), 115.5 (C-2'), 145.25 (C-3'), 147.2 (C-4'), 115.5 (C-5'), 120.4 (C-6'). The structure was confirmed by comparison with literature data.

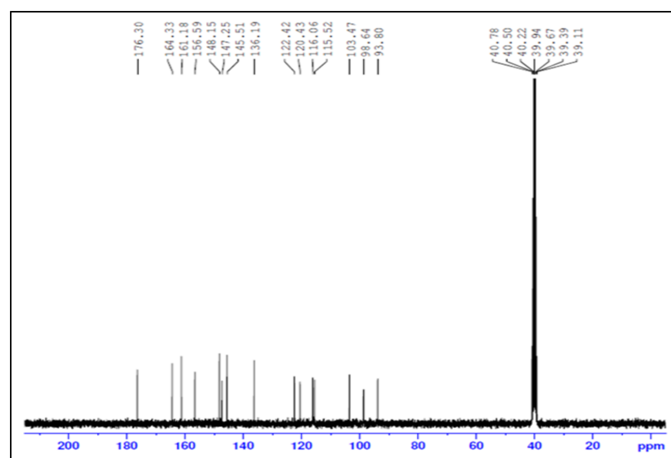


FIG. 4: ^{13} C NMR SPECTRA OF THE ISOLATED COMPOUND

Mass spectrum of isolated compound 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one showed parent molecular ion [M^+] peak at mlz 303 which corresponds to the molecular formula $C_{15}H_{10}O_7$. The GC-MS spectra of these isolated compounds revealed the characteristic fragments m/z with % abundance 285.2 (69), 257.3 (100), 229.4 (42), 271.3 (14), 247.1 (30), 161.2 (35.5), 135.2 (11), 153.1 (7), 147.1 (5.5). The molecular weight and fragmentation pattern indicate that the compounds presenting 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one respectively.

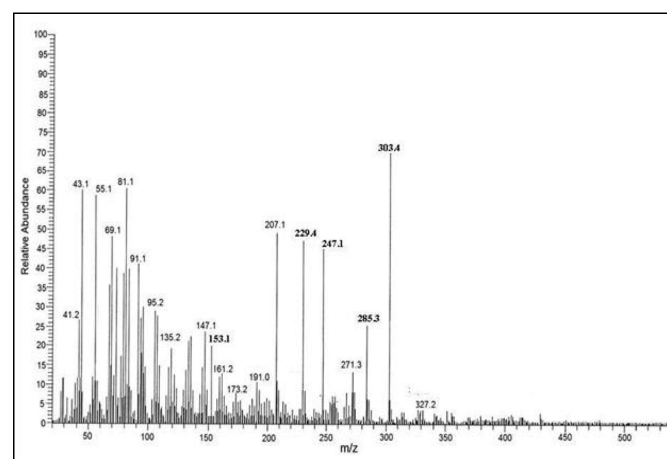
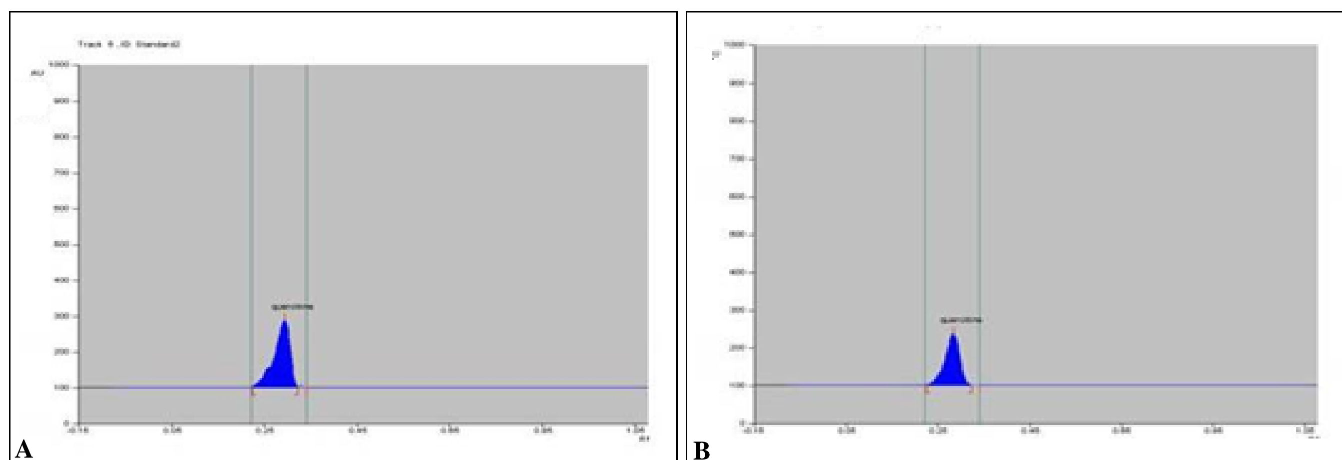


FIG. 5: MASS SPECTRA FOR THE ISOLATED COMPOUND2-(3, 4-DIHYDROXYPHENYL)-3, 5, 7-TRIHYDROXY-4H-CHROMEN-4-ONE

Purification of Isolated Compound by HPTLC and HPLC: HPTLC fingerprint patterns have been therefore evolved to check the purity of isolated compound from the methanolic extract of sample. The R_f value of standard 2-(3,4-dihydroxyphenyl)-3, 5, 7-trihydroxy-4H-chromen-4-one 0.26 was matched with the R_f value of isolated compound was about 0.26 was shown in peak **Fig. 6**.



'FIG. 6: HPTLC CHROMATOGRAM OF PURITY OF THE ISOLATED COMPOUND (A) STANDARD2-(3,4-DIHYDROXYPHENYL)-3,5,7-TRIHYDROXY-4H-CHROMEN-4-ONE (B) ISOLATED2-(3,4-DIHYDROXYPHENYL)-3,5,7-TRIHYDROXY-4H-CHROMEN-4-ONE IN METHANOLIC EXTRACTS OF LEAVES OF *ANDROGRAPHIS ECHIOIDES*

The Retention time of 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one isolated from the methanolic extract of isolated compound 2-(3, 4- dihydroxyphenyl)- 3, 5, 7- trihydroxy- 4H-chromen-4-one was about 8.42 was shown by HPLC peak **Fig. 7**.

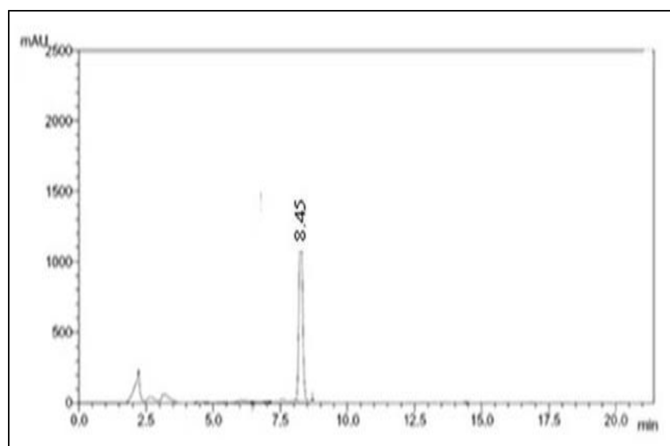


FIG. 7: HPLC SPECTRA OF PURITY OF THE ISOLATED COMPOUND

Previous studies suggested that the UV spectra of the compound, CiA in different reagent showed the presence of 5, 7, 3'4' tetrahydroxy flavonol aglycones. IR spectra reveal the presence of hydroxyl, carbonyl, aromatic and ether group. The ^{1}H NMR shows the presence of two meta coupled aromatic protons at H-6, H-8, position confirms the 5, 7- di-substituted ring A. The ^{13}C-NMR spectrum indicates the presence of carbon atoms. All the spectral data of compound CiA were found to be that of 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one [20]. 2- (3, 4- dihydroxyphenyl)- 3, 5, 7- trihydroxy- 4H- chromen- 4- one is an important constituent of *Tridax procumbens* which has number of uses such as anticancer, antidiabetic, hepatoprotective, antioxidant, *etc*. was isolated from flowers of *Tridax procumbens* using simple, rapid and convenient isolation procedure. The yield was found to be 0.072% [21]. The flavonoid 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one was isolated effectively from the leaves of *Trigonella foenum-graecum* and their antioxidant activity was studied [22]. The peaks present in the NMR spectrum showed resemblance with the pure 2- (3, 4- dihydroxyphenyl)- 3, 5, 7- trihydroxy- 4H-chromen-4-one which was also confirmed by previous literature [23, 24]. Thus, it can be confirmed that the isolated compound is found to be 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one.

***In-vitro* Alpha Amylase Inhibitory Assay:** In this study the *in-vitro* alpha amylase inhibitory activities of the 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one isolated from methanolic extract of *Andrographis echioides* leaves was investigated. The result of experiment showed that, there was a dose-dependent increase in percentage inhibitory activity against alpha-amylase enzyme. The 2-(3,4-dihydroxyphenyl)-3, 5, 7- trihydroxy-4H-chromen-4-one (20-100 µg/ml) of the various concentrations exhibited potent α-amylase inhibitory activity in a dose-dependent manner. The 2- (3, 4- dihydroxyphenyl)- 3, 5, 7-trihydroxy-4H-chromen-4-one showed inhibitory activity from 23.65 ± 0.25 to 61.67 ± 0.37% with an IC_{50} value of 37.16 ± 0.35 µg/ml **Table 1**. Acarbose is a standard drug for the α-amylase inhibitor.

Acarbose at a concentration of (20-100 μg/ml) showed α-amylase inhibitory activity from 37.85 ± 0.24 to 75.97 ± 0.37% with an IC_{50} value 41.4 ± 0.34 μg/ml. A comparison of α–amylase inhibitory activity between the standard drug has been depicted in **Fig. 8**. Our results are in accordance with the previous study wherein, there is a positive relationship between the total polyphenol and flavonoid content and the ability to inhibit intestinal α-glucosidase and pancreatic α-amylase [25, 26]. The isolated compounds were tested for their antidiabetic potential *in-vitro* by inhibition of the α-amylase enzyme. Whereas for plant *Andrographis paniculata*, flavonoid extracts of the leaf have shown the tremendous alpha-amylase inhibitory effect of 62.22% at 1.5 mg/ml concentration, with a very low IC_{50} value of 0.004 mg/ml (P≤0.05) was reported [27].

TABLE 1: *IN-VITRO* ANTIDIABETIC ACTIVITY OF THE 2-(3,4-DIHYDROXYPHENYL)-3,5,7-TRIHYDROXY-4H-CHROMEN-4-ONE USING ALPHA AMYLASE METHOD AND COMPARISON WITH STANDARD DRUG ACARBOSE

S. no.	Concentrations (μg/ml)	Alpha amylase (%)	
		2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one	Acarbose
1	20	23.65 ± 0.25	37.85 ± 0.24
2	40	34.79 ± 0.14	50.21 ± 1.37
3	60	39.54 ± 0.25	61.20 ± 1.42
4	80	56.32 ± 0.36	69.25 ± 1.47
5	100	61.67 ± 0.37	75.97 ± 0.37
	IC_{50}	37.16 ± 0.35	41.4 ± 0.34

Note: Each value was obtained by calculating the average of three experiments and data are presented as mean ± SEM

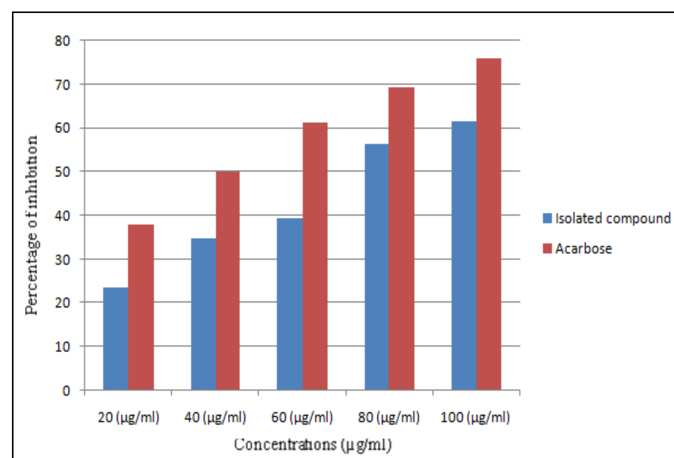


FIG. 8: α-AMYLASE INHIBITORY ACTIVITY OF ACARBOSE *vs*. 2-(3, 4-DIHYDROXYPHENYL)-3, 5, 7-TRIHYDROXY- 4H- CHROMEN- 4- ONE ISOLATED FROM *ANDROGRAPHIS ECHIOIDES* LEAVES

***In-vitro* α-glucosidase Inhibitory Assay:** The results of antidiabetic activity using α- glucosidase inhibitory assay of the 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one isolated from a methanolic extract of *Andrographis echioides* leaves are shown in **Table 2**. The 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one revealed a significant inhibitory action of the α-glucosidase enzyme. The percentage inhibition at 20-100 μg/ml concentrations of 2-(3,4-dihydroxy-phenyl)- 3, 5, 7- trihydroxy- 4H- chromen- 4- one showed a dose-dependent increase in percentage inhibition.

The percentage inhibition varied from 30.58 ± 0.48% to 82.71 ± 0.91% for the highest concentration to the lowest concentration. Thus the inhibition of the activity of α-glucosidase by 2-(3, 4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one would delay the degradation of carbohydrate, which would, in turn, cause a decrease in the absorption of glucose, as a result, the reduction of postprandial blood glucose level elevation. A comparison of α–glucosidase inhibitory activity between the standard drug has been depicted in **Fig. 9**.

In this study, acarbose was also used as a standard drug for the α-glucosidase inhibitor. Acarbose at a concentration of (20-100 μg/ml) showed α-glucosidase inhibitory activity from 42.70 ± 1.40 to 91.68 ± 1.38% with an IC_{50} value 45.03 ± 1.03 μg/ml. This indicates that the 2-(3,4-dihydroxy-phenyl)- 3, 5, 7- trihydroxy-4H-chromen-4-one is very potent α-amylase and α-glucosidase inhibitor in comparison with acarbose [28]. The hypoglycemic activity of crude extracts and isolated compounds (2- (3, 4-dihydroxyphenyl)-3, 5, 7-trihydroxy-4H-chromen-4-one acetate, cis-p-coumaric acid, 2-(3, 4- dihydroxyphenyl)- 3, 5, 7- trihydroxy- 4H-chromen-4-one, β-sitosterol, trans-p-coumaric acid, linoleic acid, (+)-catechin, afzelin and quercitrin) was assessed by the ability to inhibit α-amylase and α-glucosidase enzymes [29].

TABLE 2: *IN-VITRO* ANTIDIABETIC ACTIVITY OF THE 2-(3,4-DIHYDROXYPHENYL)-3,5,7-TRIHYDROXY-4H-CHROMEN-4-ONE USING α-GLUCOSIDASE METHOD AND COMPARISON WITH STANDARD DRUG ACARBOSE

S. no.	Concentrations (µg/ml)	Alpha α-glucosidase (%) 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one	Acarbose
1	20	30.58 ± 0.48	42.70 ± 1.40
2	40	38.76 ± 0.91	52.34 ± 1.37
3	60	46.28 ± 0.51	65.48 ± 1.42
4	80	64.4 ± 0.73	74.54 ± 1.47
5	100	82.71 ± 0.91	91.68 ± 0.38
	IC_{50}	42.52 ± 0.82	45.03 ± 1.03

Note: Each value was obtained by calculating the average of three experiments and data are presented as mean ± SEM

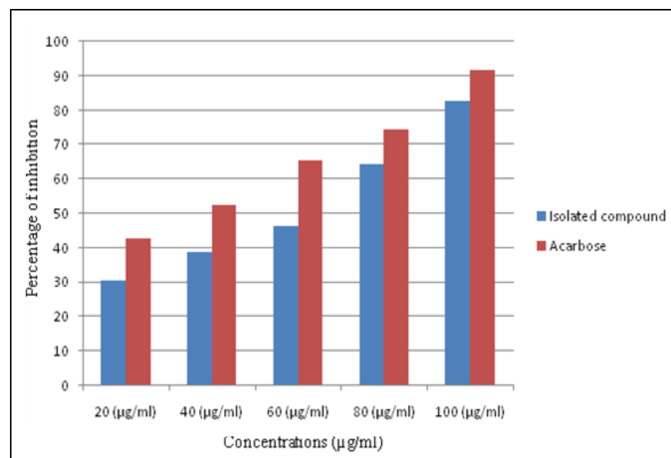


FIG. 9: α-GLUCOSIDASE INHIBITORY ACTIVITY OF ACARBOSE *vs* 2-(3, 4-DIHYDROXYPHENYL)-3, 5, 7-TRIHYDROXY- 4H- CHROMEN- 4- ONE(QUERCETIN) ISOLATED FROM *ANDROGRAPHIS ECHIOIDES* LEAVES

CONCLUSION: The plant *Andrographis echioides* showed significant enzyme inhibitory activity, so the compound 2-(3,4-dihydroxy-phenyl)- 3, 5, 7- trihydroxy- 4H- chromen- 4- one isolated and characterized which are responsible for inhibiting activity, has to be done for the usage of antidiabetic agent. To investigate the biological activities of 2- (3, 4-dihydroxyphenyl)- 3, 5, 7-trihydroxy-4H-chromen- 4- one, the antidiabetic activities of the 2-(3,4-dihydroxyphenyl)-3, 5, 7-trihydroxy-4H-chromen-4-one isolated from the methanolic extract of *Andrographis echioides* leaves has been analyzed. As a result, we found that the 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one have inhibitory activity against α-amylase and α-glucosidase and this therapeutic potentiality could be exploited in the management of post prandial hyperglycemia in the treatment of type 2 diabetes mellitus.

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