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Original Article

Phytochemical Analysis of *Cleome viscosa* Active Polyphenolic Compounds Possessing Antidiabetic Activity

Suresh Yarrappagaari[1], Lavanya Thopireddy[2], Srinivasulu Cheemanapalli[3], Venkata Rami Reddy Narala[4], Kakarla Chandra Mohan[2], Rajeswara Reddy Saddala[1,*]

Suresh Yarrappagaari[1], Lavanya Thopireddy[2], Srinivasulu Cheemanapalli[3], Venkata Rami Reddy Narala[4], Kakarla Chandra Mohan[2], Rajeswara Reddy Saddala[1,*]

[1]Division of Ethnopharmacology, Department of Biotechnology, School of Herbal Studies and Naturo Sciences, Dravidian University, Kuppam, Andhra Pradesh, INDIA.
[2]Department of Zoology, Kasireddy Venkatareddy Government College for Women (A), Kurnool, Andhra Pradesh, INDIA.
[3]Regional Ayurveda Research Institute, Itanagar, Arumachal Pradesh, INDIA.
[4]Department of Animal Sciences (Zoology), Yogi Vemana University, Kadapa, Andhra Pradesh, INDIA.

Correspondence

Dr. Saddala Rajeswara Reddy

Assistant Professor, Division of Ethnopharmacology, Department of Biotechnology, School of Herbal Studies and Naturo Sciences, Dravidian University, Kuppam-517426, Andhra Pradesh, INDIA.
Email id: drsrr2017@gmail.com
ORCID iD: 0000-0002-2928-1446

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ABSTRACT

Background: The counteractive action of diabetes by plant polyphenols along with their utilization in the treatment of diabetic difficulties is of expanding significance from the epidemiological information with *in vitro* and *in vivo* examinations. **Objectives:** We assessed the antihyperglycemic impacts of methanolic (MeCV), aqueous (AqCV), ethyl acetate (EaCV) and n-hexane (NhCV) extracts of *Cleome viscosa* whole plant. **Materials and Methods:** Qualitative phytochemical analysis and antidiabetic activities by glucose diffusion, inhibitory movement of starch processing enzymes i.e. α-glucosidase and α-amylase, glucose uptake by yeast cell method and also the non-enzymatic action (glycosylation of Hb). The identification of polyphenols in bioactive extracts was categorized by using HPLC/UV. **Results:** To evaluate the comparative power of these extracts, the concentrations required for 50% and 100% inhibition of enzyme movement were resolved. Greatest enzyme inhibition was utilized to evaluate an inhibitor's relative viability. Results demonstrated that MeCV strongly diffused by glucose and inhibited both α-glucosidase and α-amylase enzyme movement and great impact on the glucose uptake by yeast cells and non-enzymatic glycosylation of Hb with equivalent intensity respectively than acarbose. While NhCV was showed less impact on glucose diffusion, α-glucosidase, α-amylase, glucose uptake by yeast cells and glycosylation of Hb. Gallic acid (2.45%), protocatechuic acid hexoside (0.83%), chlorogenic acid (0.77%), catechin (0.53%), quercetin (0.80%), p-hydroxybenzoic acid (0.78%), and p-coumaric (0.07%) were the main compounds of the MeCV. **Conclusion:** Based on our results we concluded that the MeCV firmly impacts on enzymatic and non-enzymatic activities due to rich content of polyphenols present in MeCV.
Key words: Enzyme inhibition, α-Glucosidase, α-Amylase, *Cleome viscosa*, HPLC/UV.

INTRODUCTION

Glycemic management is an effective, long-term treatment for people with non-insulin-dependent diabetes mellitus (NIDDM) or type-2 diabetes, diminishing the danger of both cardiovascular and neurological entanglements in the advancement of the disease.[1,2] Glucosidase inhibitors are regularly recommended by diabetics to decrease occurring or made later than a meal higher blood glucose levels instigated by the processing of polysaccharides in the little intestine.[3] These inhibitors are intended to target α-amylase and α-glucosidase, two individuals from exo-acting glycoside hydrolase chemicals (glucosidases) that originate in the intestinal territory that is basic for the processing of starches. The general impact of inhibition is to lessen the progression of glucose from complex dietary sugars into the circulatory system, decreasing the postprandial impact of starch utilization on blood glucose levels.[4] Be that as it may, the main glucosidase inhibitors are acarbose and miglitol drugs are regularly answered to create looseness of the bowels and other intestinal unsettling influences, with comparing intestinal torment and flatulence.[5] Randomized restricted preliminaries with glucosidase inhibitors report these gastrointestinal reactions as the most widely recognized purpose behind defiance and premature issue for departure.[6]

The utilization of plants or plant-based enhancements might be a progressively adequate wellspring of glycemic control (glucosidase inhibitors) because of minimal effort and relative security, including a low occurrence of gastrointestinal side-effects.[7] Polyphenolic portions from plants have been appeared to restrain α-amylase and α-glucosidase action and take into consideration more strongly control of blood glucose for example diffusion effect on glucose, haemoglobin glycosylation in addition to glucose take-up capacity.[8,9] In recent times, much consideration has been centred on the strong antidiabetic properties of polyphenolics and the

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other hand their belongings in the avoidance of different complications related to diabetes. The counteractive action of diabetes by plant polyphenols along with their utilization in the treatment of diabetic difficulties is of expanding significance from the epidemiological information with *in vitro* and *in vivo* examinations.[10]

Cleomaceae have been accounted for to be the biggest group of plants with a significant number of its individuals appearing to have pharmacological exercises which contained significant phytochemicals including polyphenolics, saponins and terpenoids.[11,12] *Cleome viscosa* L. (Wild mustard/dog mustard), family (Cleomaceae) is a clammy herb that originates as a typical weed in fields of India, South Africa, Pakistan, China, Ceylon, and all through the tropical areas of the world yearly. *C. viscosa* has a place with this family and has been recently portrayed to be rich in polyphenolics.[13-15] This plant containing rich sources of important phytoconstituents was showed antimicrobial, hepatoprotective, antiemetic, analgesic, antidiarrhoeal, antitumor and psychopharmacological activities.[16] In this examination, *in vitro* antidiabetic properties of four various solvent extracts, were explored and polyphenols were distinguished and portrayed by utilizing HPLC investigation from the whole plant of *C. viscosa*.

MATERIALS AND METHODS

Plant material collection and authentication

Healthy *Cleome viscosa* whole plant (family: Cleomaceae) material was collected during the morning session. The collected whole plant was authenticated (No. NY/531) by Prof N. Yasodamma, Department of Botany, Sri Venkateswara University, Tirupati, India.

Chemicals and reagents

Dialysis tubes (6cm x 5mM), glucose, α-amylase were purchased from Himedia (Bangalore, India). α- glucosidase and acarbose were purchased from Sigma–Aldrich (Bangalore, India). 3, 5-dinitro salicylic acid, starch, CMC (Carboxy methylcellulose), haemoglobin, gentamycin, metformin, acetonitrile, methanol and HPLC grade distilled water and analytical grade acetic acid was purchased from Merck (Bangalore, India). All other chemical reagents and buffer solutions used analytical grade.

Extracts preparation

The well-grown and healthy *Cleome viscosa* whole plant material were collected, shade dried and powdered. The powder was used for the extraction of the bioactive compounds in methanol, aqueous, ethyl acetate and n-hexane solvents. Dried powder of *C. viscosa* (500 g) was extracted in 5L of each solvent by using a soxhlet apparatus at room temperature for 72hr. Later, the extracts were filtrated under vacuum, concentrated in a rotary shaker, and then lyophilized.

Qualitative phytochemical assessment of four different extracts of *C. viscosa*

Standard procedures Gibbs method used to phenols, steroids, alkaloids and lignin estimation, Peach and Tracey method adapted for flavonoids analysis, Trease and Evans procedure used for tannins screening, Kokate method used for glycosides analysis, Rizk method were adopted to saponins, quinines and coumarins screening and Trim-Hill reagent test for terpenoids screening of four various extracts of *C. viscosa* whole plant.

In vitro antidiabetic action of four different extracts of *C. viscosa*

Assay of the glucose diffusion method

A simple model system was used to evaluate the effects of four various extracts from *C. viscosa* on the movement of glucose *in vitro*. The model was adapted from a method described by Edwards *et al*. (1987) which involved the use of a sealed dialysis tube into which 15mL of a solution of glucose and sodium chloride (0.15M) was introduced and the appearance of glucose in the external solution was measured.[16] The model used in the present experiment consisted of a dialysis tube (6cmX15mm) into which 1mL of 50g/L each fraction in 1% CMC and 1mL of 0.15M sodium chloride containing 0.22M D-glucose was added. The dialysis tube was sealed at each end placed in a 50mL centrifuge tube containing 45mL of 0.15M sodium chloride. The tubes were placed on an orbital shaker and kept at room temperature. The movement of glucose into the external solution was monitored at different time intervals (30min, 1hr, 3hr, 7hr, 24hr and 27hr).

Study of α- Glucosidase inhibition assay

1mg of the α-glucosidase enzyme (isolated from Saccharomyces cerevisiae) was suspended with 100mL neutral PBS buffer which contains the 200mg of bovine serum albumin.[17] The various concentrations (20, 40, 60, 80 and 100µg/mL) of four various extracts of *C. viscosa* was added with reaction mixture (10µL of pH 6.8 phosphate buffer; 490µL of 5mM p-NPG (p-nitrophenyl α-d glucopyranoside). The reaction mixture was incubated at 37°C for 5min then adds 250µL of α-glucosidase (0.15unit/mL) and again incubated at 37°C for 15min. Then cool the reaction and add 2mL of sodium carbonate (200mM) to stop the reaction. The activity of enzyme inhibition was measured at 405nm and acarbose was utilized as a reference compound.

Study of α-Amylase inhibitory assay

In 1% phosphate buffer and the starch solution was prepared and incubated with 500µL enzyme (α-amylase) for 10min at 37°C. 1mL of (20, 40, 60, 80 and 100µg/mL) of four various extracts of *C. viscosa* was added to the enzyme solution. 2M of NaOH is applied to stop the reaction process. 1mL of Dinitro salicylic acid is mixed and the reaction is maintained in the hot water bath for 5min. After completion of incubation, test tubes were cooled by running tap water, the final volume of test solution was makeup to 10mL using sterile distilled water and absorbance was measured at 540nm. Acarbose was used as a reference substance.[18]

Assay of Non- enzymatic glycosylation of haemoglobin (HbA1c) method

In vitro antidiabetic activity of four various extracts of *C.viscosa* was scrutinized with the HbA1c method.[19] The combination of 2 % glucose, 0.06 % haemoglobin and 0.02 % sodium azide solutions were organized in 0.01 M phosphate buffer (pH 7.4). 1mL of different concentrations (20, 40, 60, 80 and 100 µg/mL) of four various extracts of *C. viscosa* was mixed with the above combination. The reaction mixture was incubated in a dark place at room temperature for 72 hr. The levels of HbA1c were measured at 520 nm. Metformin was used as a reference drug for this assay.

Assay of glucose uptake by yeast cell method

Assurance of antidiabetic capacity was performed by four various extracts of *C. viscosa* utilizing glucose uptake by yeast cell method was adapted by Gupta *et al*.[20] Yeast was purchased from the local market, washed with double distilled water by centrifugation at 2,500rpm for 5min and the process was repeated twice. The upper layer (supernatant) was isolated and 10% (v/v) of suspension was prepared. The four various extracts of *C. viscosa* (20-100µg/mL) was taken and incubated by adding with 1mL of three different concentrations of glucose (5, 10 and 25mM) at 37°C for 10min. 100µL of yeast was added to the mixture and to begin the experiment, again kept for incubation at 37°C for 1h. Following

60 min, tubes were centrifuged at 3000rpm for 5 min and glucose was assessed in the supernatant. Metronidazole was utilized as a standard drug for this assay.

Percentage calculation

The percentage inhibition (I %) of α-glucosidase, α-amylase, glycosylation of Hb and estimation of glucose uptake by yeast cells was calculated by the following formula:

$$I(\%) = \frac{(\text{Control}_{Abs} - \text{Test sample}_{Abs})}{(\text{Control}_{ABs})} \times 100$$

Whereas; Abs= absorbance at the testing wavelength

Scrutiny of phenolic and flavonoid compounds by analytical HPLC/UV

Test samples preparation

25 mg of extract was dissolved in 25mL of methanol (1mg/1mL) and the test solution was filtered using syringe discs (0.45µm) before subjecting the analysis of HPLC.

Instrumentation and Chromatographic Conditions

Twofold Waters 600 E siphon furnished framework with Waters 2996 photodiode cluster indicator connected to the array processor was utilized for this investigation. Most elevated pressure of 2500 psi and least of 1500 psi pressure was kept up and the investigation of results for confirmation of standard outcomes by utilizing Empower framework programming. The chromatographic partition was achieving section was utilizing a column C_{18} segment (250 × 4.6) 5 µm, Waters) at 25°C. Test arrangement and the portable stage comprised of acetonitrile (solvent system A) and water with 0.2% formic acid (solvent system B). The stream rate was kept at 0.7 mL/min. The angle program was as per the following: A-35%/B-65% (0– 6 min), A-60%/B-40% (6– 9 min), A-80%/B-20% (9– 14 min), A-100% (14– 25 min), A-35%/B-65% (25– 30 min). The infusion volume was 20µL and peaks were observed at 280 nm. Peaks were distinguished by standard compounds.

Statistical analysis

Mean± SE considered for every experimental value (n=3); dissimilarities between control groups and tested groups were investigated by one-way ANOVA pursued by Tukey's post-hoc ($P < 0.05$) and Dunnett test ($P<0.001$) using SPSS version 16.0 (SPSS Inc. Chicago, IL, USA) were considered for significance.

RESULTS

Soxhlet extraction

As appeared in Table 1, the extraction procedures seeming a huge impact on the absolute yield of extracts from *C. viscosa*. Given the outcomes, the maximum extract yield has gotten by MeCV (18.6% w/w), followed by AqCV (18% w/w), EaCV (2% w/w) and NhCV (0.6% w/w). These outcomes are as per the normal principle that a high polarity solvent demonstrates a higher rate yield whereas low polarity solvent demonstrates a low level of yield.

Qualitative phytochemical assessment of four different extracts of *C. viscosa*

The present investigation revealed that the different MeCV, AqCV, EaCV and NhCV of the whole plant of *C. viscosa* contained flavonoids, phenols, steroids, alkaloids, lignin, tannins, glycosides, terpenoids, saponins, quinines and coumarins (Table 2). Flavonoids and phenols were identified in most of the tests in MeCV and AqCV of *C. viscosa* and the steroids, alkaloids and coumarins were found in all extracts. Lignin and tannins were available at an extremely low level in all extracts. Besides MeCV and AqCV of the whole plant of *C. viscosa* demonstrated the nearness of a rich assortment of secondary metabolites pursued by the NhCV indicated a low assortment of secondary metabolites. Contrasted with all other solvents extracts, MeCV had a higher number of secondary metabolites with a high level of precipitation.

Table 1: The percentage of yield obtained from MeCV, AqCV, EaCV and NhCV extracts through soxhlet extraction method.

Name of the extract	Extract weight	Percentage of yielding (W/W)
MeCV	93g	18.6%
AqCV	90g	18%
EaCV	10g	2%
NhCV	3g	0.6%

Table 2: Qualitative phytochemical analyses of C. viscosa whole plant four various solvent extracts.

Phytochemicals		MeCV	AqCV	EaCV	NhCV
Flavonoids	Ferric chloride test	+	+	+	_
	Shinoda's test	+	+	+	_
	Zinc-Hcl reduction test	+	+	+	_
Phenols	Lead- acetate test	+	+	_	_
	Phenols test	+	+	+	+
	Ellagic acid test	+	+	+	+
Steroids	Salkowski test	+	+	+	+
	Liebermann- burchard	+	+	+	+
Alkaloids	Mayer's test	_	_	_	_
	Wagner's test	+	+	+	+
	Dragendorff's test	+	+	_	_
Lignin	Labat test	+	_	+	_
	Lignin test	_	_	_	_
Tannins	Ferric chloride test	+	_	_	_
	Lead acetate test	+	+	_	_
Glycosides	Kellarkiliani test	_	+	_	_
	Conc.H_2SO_4 test	+	+	+	_
Terpenoids	Moisch's test	+	+	_	+
	Trim- hill reagent test	+	+	_	_
	Saponins	+	+	_	_
	Quinines	+	+	_	_
	Coumarins	+	+	+	+

+=Presence; - = Absence.

In vitro antidiabetic action of four different extracts of *C. viscosa*

Inhibition of Glucose Diffusion method

The impact of the selected plant extracts (MeCV, AqCV, EaCV and NhCV) on impeding glucose diffusion over the dialysis film is represented in Figure 1 and Figure 2. The rate of glucose diffusion/dispersion was found to increase with time from 30 min to 27 hr. Amid

the examination, the development of glucose over the dialysis layer was checked once in 30min till 27 hr. It was seen that all plant extracts exhibited significant inhibitory impacts on the development of glucose into outside arrangement crosswise over dialysis film when contrasted with control. The inhibition of glucose dispersion by MeCV extract was essentially higher (p<0.001) than the remaining three extracts. The impacts were reflected with a higher GDRI value for MeCV than AqCV, EaCV and NhCV.

Inhibition of α- glucosidase assay

In the present investigation, the various extracts of *C. viscosa* plant had the option to exhibit inhibitory movement against α-glucosidase when contrasted with standard acarbose drug. In this test; the MeCV displayed the most elevated inhibitory movement of 59.87% at 100µg/mL concentration when contrasted with the remaining three extracts. The NhCV had a low inhibitory action of 37.29% observed at 100µg/mL concentration when contrasted with the remaining extracts. The other extracts had demonstrated a moderate level of α-glucosidase inhibition movement at a similar concentration (Figure 3). The IC_{50} for acarbose, MeCV, AqCV, EaCV and NhCV were 21.70±0.67µg/mL, 63.13±0.97µg/mL, 68.51±1.25µg/mL, 119.81±1.64µg/mL and 125.08±1.54µg/mL, respectively (Table 3).

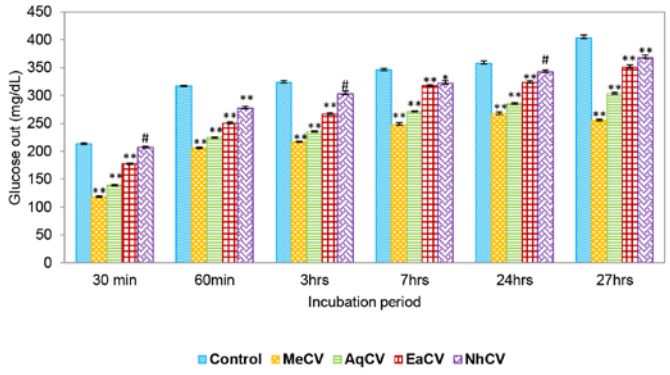

Figure 1: Effect of four various extracts (50g/L) of *C. viscosa* on the movement of glucose out of the dialysis tube over the incubation of 27 hr. Qualities in the Figure spoke to as mean ± SE for triplicates, implying in the segments contrast altogether contrasted with control test (without test sample) at *P<0.001 (Performed One Way ANOVA pursued by Dunnett test). #-indicates there is no significance when compared to control.

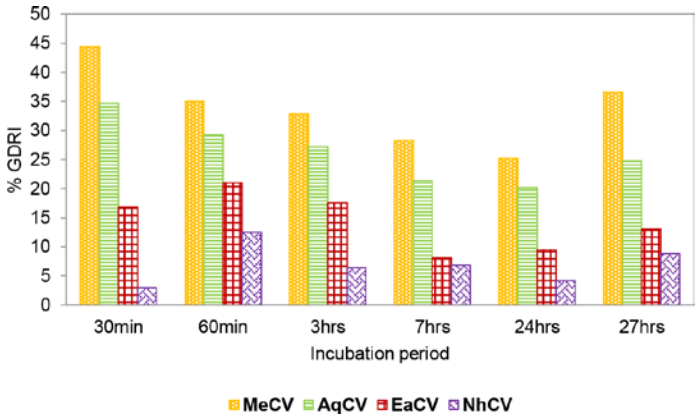

Figure 2: Effect of various extracts obtained from *C. viscosa* on the percentage of GDRI.

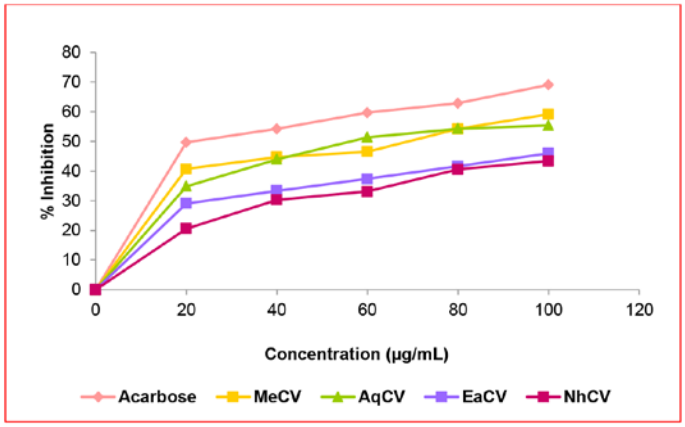

Figure 3: α-glucosidase inhibition activity of four various extracts of *C. viscosa* whole plant. Values were Mean±SE (n=3).

Table 3: *In vitro* antidiabetic activities of four different extracts obtained from *C. viscosa* whole plant.

Name of the assay	Test samples name	IC_{50} (µg/mL)
α-glucosidase inhibition	Acarbose	21.70±0.67[a]
	MeCV	63.13±0.97[b]
	AqCV	68.51±1.25[b]
	EaCV	119.81±1.64[c]
	NhCV	125.08±1.54[c]
α-amylase inhibition	Acarbose	21.43±0.32[a]
	MeCV	37.90±0.64[b]
	AqCV	45.67±0.64[c]
	EaCV	70.69±1[d]
	NhCV	74.54±1.38[d]
HbA1c assay	Metformin	11.78±0.62[a]
	MeCV	31.44±0.77[b]
	AqCV	40.42±1.30[c]
	EaCV	72.05±2.07[d]
	NhCV	78.88±1.33[e]
Glucose uptake by yeast cell (25mM)	Metronidazole	57.55±0.76[a]
	MeCV	59.83±1.18[a]
	AqCV	62.12±0.84[a]
	EaCV	68.95±1.40[b]
	NhCV	72.09±1.23[b]
Glucose uptake by yeast cell (25mM)	Metronidazole	52.02±1.07[a]
	MeCV	51.39±0.87[a]
	AqCV	55.43±1.11[a]
	EaCV	64.49±1.64[b]
	NhCV	70.53±1.99[b]
Glucose uptake by yeast cell (25mM)	Metronidazole	57.74±1.64[a]
	MeCV	69.10±0.79[b]
	AqCV	69.62±1.05[b]
	EaCV	76.48±1.21[c]
	NhCV	78.56±1.51[c]

Qualities in the table are spoken to as mean ± SE for triplicates, Means ±SE not sharing a typical superscript in the line contrast fundamentally at p<0.05 (Performed One Way ANOVA pursued by Tukey's post hoc).

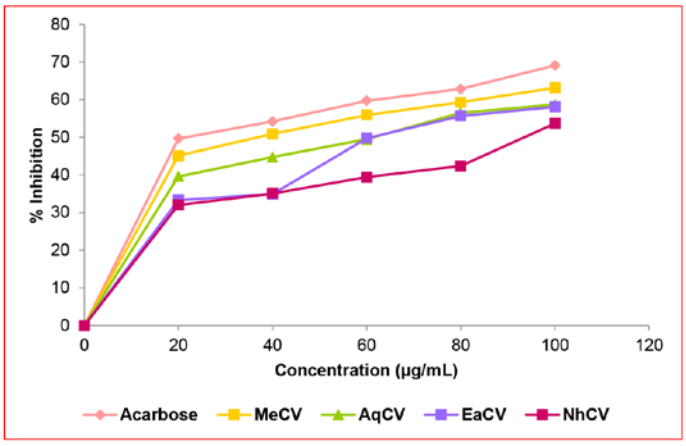

Figure 4: Inhibition of the α-amylase activity of four various extracts of *C. viscosa* whole plant. Values were Mean±SE (*n*=3).

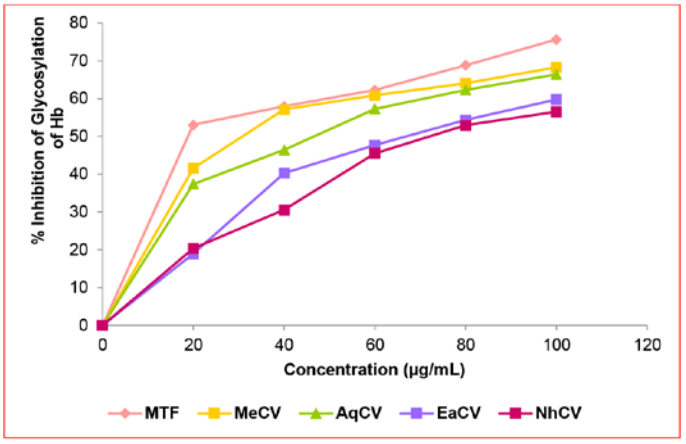

Figure 5: HbA1c inhibition activity of four various extracts of *C. viscosa* whole plant. Values were Mean±SE (*n*=3). MTF- Metformin.

Inhibition of α-amylase assay

The *in vitro* inhibitory exercises of α-amylase of the four various solvent extracts of *C. viscosa* were measured. The outcomes demonstrated that at the dose-dependent inhibition action was monitored (Figure 4). The MeCV make use of highest inhibitory action of α-amylase of 63.23% at 100µg/mL concentration when contrasted by the remaining three extracts. The concentrations at 20-100µg/mL differed significantly (p<0.05) for all extracts. A lower inhibition rate of 58.94% was observed for α-amylase enzyme inhibition to NhCV when compared with remaining extracts. The IC_{50} values of acarbose, MeCV, AqCV, EaCV and NhCV extracts were 21.43±0.32µg/mL, 37.90±0.64µg/mL, 45.67±0.64µg/mL, 70.69±1µg/mL and 74.54±1.38µg/mL respectively (Table 3).

Given the above outcomes, four different extracts of *C. viscosa* plant demonstrated a weaker α-glucosidase inhibition contrasted with the outcomes revealed for inhibition of α-amylase enzyme action (Figure 3, Figure 4 and Table 3).

Assay of HbA1c method

The IC_{50} values of HbA1c activity of four dissimilar extracts of *C. viscosa* whole plant and diabetic reference drug metformin were represented in Table 3. Metformin, MeCV, AqCV, EaCV and NhCV showed HbA1c inhibition activity with IC_{50} values 11.78±0.62µg/mL, 31.44±0.77µg/mL, 40.42±1.30µg/mL, 72.05±2.07µg/mL and 78.88±1.33µg/mL respectively. Among these four extracts, MeCV was significantly (p<0.05) showed most potent HbA1c inhibition activity. While NhCV has shown poorer HbA1c inhibition activity. The percentage inhibition of HbA1c is dependent on determining dose (20-100 µg/mL) when the dose of the tested extract was increased at the same time formation of glucose-haemoglobin binding decreased and action inhibition was increased (Figure 5).

Glucose uptake by yeast cells method

The rate of glucose uptake by cell membrane in yeast cell system at three glucose concentrations (25mM, 10mM and 5mM) were showed in Table 3. The MeCV exhibited significantly higher (p<0.05) activity than AqCV, EaCV and NhCV in all concentrations. The 10mM glucose concentration showed better inhibitory activity in four different extracts of *C. viscosa* whole plant and standard metronidazole. However, the higher percent increase and IC_{50} of glucose uptake by yeast cells were observed at the concentration of 10mM, it was found to be dose-dependent of the plant extracts (Figure 6, 7 and Figure 8).

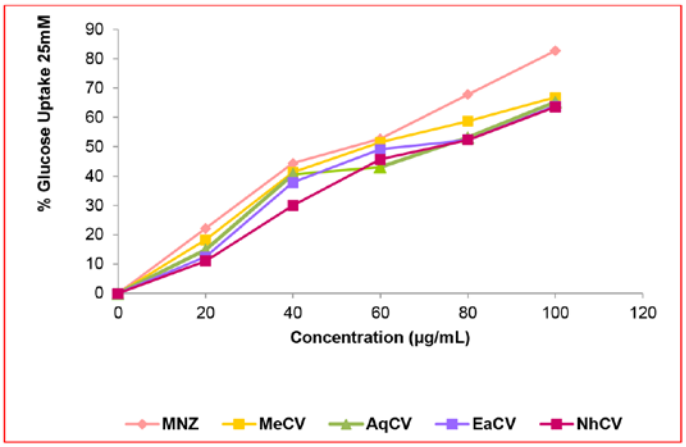

Figure 6: 25mM of glucose uptake by yeast cells activity of *C. viscosa* whole plant four various extracts. Values were Mean±SE (*n*=3). MNZ- Metronidazole.

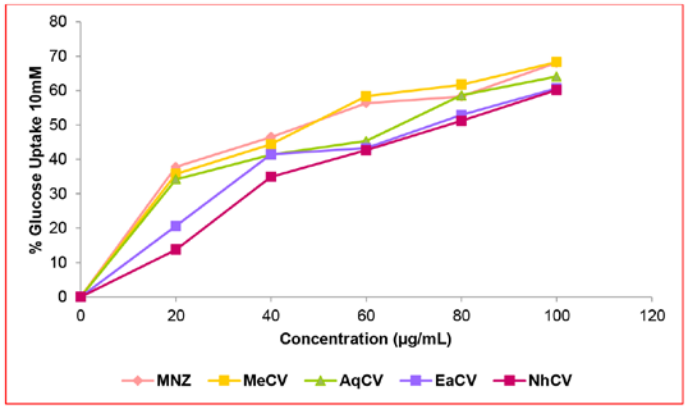

Figure 7: 10mM of glucose uptake by yeast cells activity of *C. viscosa* whole plant four various extracts. Values were Mean±SE (*n*=3). MNZ- Metronidazole.

Scrutiny of phenolic and flavonoid compounds by analytical HPLC/UV

The above-obtained results indicate that the *C. viscosa* whole plant of MeCV was more potent than the remaining extracts, based on the results

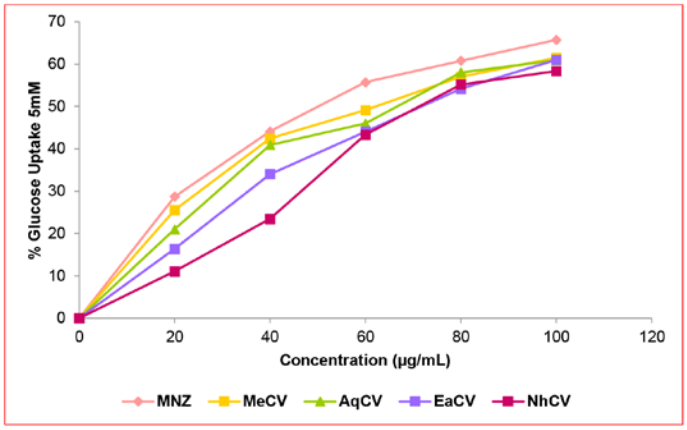

Figure 8: 5mM of glucose uptake by yeast cells activity of *C. viscosa* whole plant four various extracts. Values were Mean±SE (*n*=3). MNZ- Metronidazole.

Table 4: MeCV extract HPLC Validation data for phenols and flavonoid standards.

Peak No	R_t (min)	Compound name	% compounds
1	0.31	Unknown	0.54
2	0.58	Unknown	1.99
3	0.85	Unknown	7.38
4	2.07	Unknown	38.80
5	2.77	Unknown	25.88
6	3.50	Unknown	11.80
7	4.12	Unknown	3.18
8	4.32	Unknown	2.77
9	4.75	Unknown	1.36
10	6.03	Gallic acid	2.45
11	7.10	Protocatechuic acid hexoside	0.83
12	8.19	Chlorogenic acid	0.77
13	8.79	Catechin	0.53
14	12.16	Quercetin	0.80
15	13.54	p-Hydroxybenzoic acid	0.78
16	25.21	p-Coumaric	0.07

we decided to scrutinize the major bioactive compounds (phenolic and flavonoid compounds) responsible for the antidiabetic activity of MeCV. The major peaks identified by comparison with authentic standards in previous literature.[21-25]

Table 4 demonstrated a nearness of 16 compounds numbered by their retention time (RT). Actually, the portrayal of this MeCV extract qualified seven compounds (Figure 9), in particular, gallic acid (2.45%), protocatechuic acid hexoside (0.83%), chlorogenic acid (0.77%), catechin (0.53%), quercetin (0.80%), p-hydroxybenzoic acid (0.78%), and p-coumaric (0.07%). However, alternate compounds were unknown. The unknown compounds characterized by Peak 4 and 5 (Figure 9) were available in the most noteworthy sum in the extract with the level of 38.80% and 25.88%. The greater part of known compounds referenced in Table 4 has been found by a few researchers as great antioxidant, antimicrobial, α-glucosidase inhibitor, anti-hemolytic, antidiabetic and anticancer chemicals.[21,22,24]

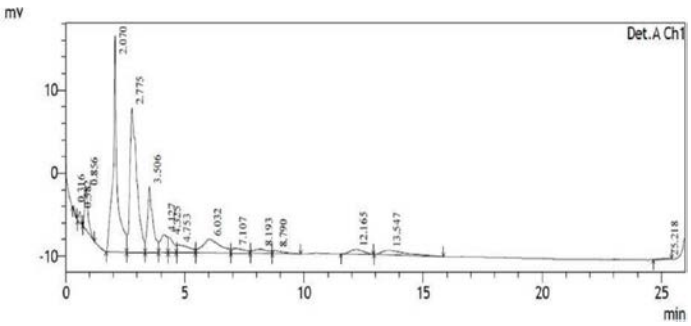

Figure 9: HPLC chromatogram of MeCV of *C. viscosa* whole plant.

DISCUSSION

Many active compounds can be found in plants, fruits and vegetables including phenolics, anthocyanins, carotenoids and tocopherols.[26] More or less 20% of known plants have been used in pharmaceutical studies, impacting the healthcare system in constructive ways such as treating cancer, diabetes and harmful diseases.[27] Plants can produce a large number of diverse bioactive compounds. Scientists have studied and analyzed the impact of different types of solvents, such as methanol, hexane, and ethyl alcohol, for biologically active extraction from various plants parts, such as fruits, flowers, leaves and seeds. To extract different phenolic compounds from plants with a high degree of accuracy, various solvents of different polarities must be used.[28] Therefore we are decided to select four solvents (the basis of decreasing polarities) for the extraction of *C. viscosa* whole plant by using a soxhlet apparatus method. This work concentrated on the discourse of the impact of four different extracts of *C. viscosa* to inhibit key carbohydrate hydrolyzing enzymes (α-glucosidase and α-amylase). Besides, the capacity of the extracts on glucose diffusion, non-enzymatic glycosylation of Hb and glucose uptake by yeast cell method was additionally assessed. The presence of phytoconstituents in the *C. viscosa* was described by using HPLC examination.

Table 1 demonstrates the yield rate of *C. viscosa* four different extracts and they were acquired utilizing Soxhlet extraction. The outcomes demonstrate that the yield rate of *C. viscosa* extracts varied when various solvents were utilized. MeCV created the most noteworthy yield of extract (18.6%), pursued by AqCV, EaCV and NhCV. The most reduced yield of extract was appeared by the NhCV (2%) because the solvent extractions are affected by the extraction ability. The extraction ability relies upon the solvents substance structure and its extremity, and these elements impact the extraction yield.[29] An investigation by Abu *et al.*[30] demonstrated that *Dendrobium sabin* flower extract utilizing the vacuum evaporation technique with aqueous as the solvent gave the most elevated yield of extract (45.8%) contrasted with methanol (18.7%). For the Soxhlet extraction technique, the temperature utilized varied for the various solvents because Soxhlet extraction was needed for the evaporated and reduced solvent to be at the solvent flouting point.

In the present study, the qualitative phytochemical screening proves that a mixture of phytoconstituents is present in four various extracts of *C. viscosa* whole plant. Particularly the MeCV and AqCV extract of *C. viscosa* whole plant which contains a massive number of components such as phenols, flavonoids, alkaloids, saponins, tannins, terpenoids etc. Numerous of these compounds contain maybe shown to fabricate potent antihyperglycemic action.

Hyperglycemic is a chronic sickness accrued by high blood sugar levels and instability in the carbohydrates, fat and proteins metabolism.[31] The insulin moved glucose uptake in adipose and skeletal muscle

tissues are serious for falling postprandial blood glucose absorption. Dysregulation of this progression is one of the significant factors, which can be measured as the main reason for high economic loss which can, in turn, hold back the growth of nations.[32] Earlier than in the presence were drugs from drug companies, natural treatments were used and they can still be used today. Medicinal plants recommend an affluent supply of potentially constructive antidiabetic drugs.[33,34] *C.viscosa* whole plant juice is recommended by Menghani *et al*.[35] for the treatment of diabetes. This plant has been recently reported as a potential anti-diabetic herb.[36]

We investigated the impact of chosen *C. viscosa* plant four various extracts on glucose entanglement *in vitro*. The impediment in glucose dissemination *in vivo* may be ascribed to the physical difficulty, insoluble fiber particles, which entrap glucose atoms inside the fiber, organize anticipating postprandial glucose rise.[37] GDRI, a helpful *in vitro* indicator to anticipate the impact of phytochemicals present in the extracts on the postponement in glucose assimilation, was determined in this examination.[38] MeCV was found to have the most elevated GDRI esteem when contrasted with AqCV, EaCV and NhCV. Also, Wood *et al.*[39] detailed that plants appearing somewhere in the range of 6 and 48% inhibitory activity on glucose dissemination over a semi-permeable layer had moderate inhibitory movement. Besides, broadly examined resources of solvent extracts having phytochemicals, for example, phenols, flavonoids, alkaloids, coumarins and some other group of compounds were found to repress somewhere in the range of glucose dissemination after 4hrs *in vitro*.[40]

We have likewise researched the effect of chosen plant extracts on starch hydrolyzing essential chemicals (α-amylase and α-glucosidase) *in vitro*. α-amylase and α-glucosidase are key starch hydrolyzing enzymes in charge of the breaking of α, 1-4 bonds in polysaccharides, improving glucose.[41] The flow of glucose observed a couple of minutes after ingestion affixed to hyperglycemia, the common for diabetes. A few logical investigations have revealed insight into the restraint of these key glycoside hydrolysis to inhibit starch processing, lessening glucose assimilation rate, and subsequently avoiding postprandial glucose flow.[42] The capacity of plant extracts to balance glucose discharge from starch and its assimilation has been demonstrated to be an appealing remedial methodology in the administration of diabetes. The mixes of phenols and flavonoids found in extracts have likewise been accounted for to communicate with proteins and hence inhibit the enzymatic movement.[43]

Results from this examination will in broad demonstrate that extracts of *C.viscosa* plant indicated a variable inhibitory impact on α-glucosidase and α-amylase *in vitro*. It was seen that MeCV, AqCV, EaCV and NhCV had share potent α-glucosidase and α-amylase inhibitory movement. From information gathered, methanol solvent extract conveyed a higher concentration of inhibitory phytochemicals as recently stated.[44] Moreover, a few scientific reports attributed the inhibitory activity of plant phytochemicals to α-amylase.[45] This will in common recommend an uncompetitive method of inhibition. Uncompetitive inhibitors attach to compound substrate complex influential an enzyme substrate-inhibitor complex.[45] This complex lessens for the catalyst active site for the substrate diminishing the predilection and postponing the rate of response.[46] It was likewise noticed that active extract uncompetitively inhibited the α-glucosidase enzyme. Moreover, α-glucosidase inhibitory test will in widespread showed that the extract of *C. viscosa* plant has intense inhibitors of α-glucosidase when contrasted with standard acarbose. In analysis, MeCV was found to pursue a combined type of inhibitor. Combined inhibitor attaches to free and to substrate-bound chemical and interferes with official and catalysis of the substrate,[47] expanding liking and diminishing the rate of response. Inhibition of glucose generation, as well as assimilation, may be significant systems in the administration of diabetes.

Glycosylated haemoglobin (HbA1c) has accomplished huge noticeable quality in the present trend of medicinal science because of its utilization as a degree of long-term diabetic control. As indicated by our outcomes, extracts of *C. viscosa* plant (MeCV, AqCV, EaCV and NhCV) inhibited the HbA1c. In our examination, expansion of MeCV containing the majority of the phytochemicals may impressively inhibit the HbA1c. This is additionally one purpose behind the MeCV spoke to higher inhibition action when contrasted from remaining AqCV, EaCV and NhCV.

The scheme of glucose transport over the yeast cell layer has been accepted consideration as *in vitro* screening strategy for the hypoglycemic impact of different medicinal plants and their determined mixes.[48] The aftereffects of the rate of glucose transport crosswise over the cell layer in the yeast cells construction are spoken to in Table 3 and Figure 3 for the *C. viscosa* plant extracts. The measure of glucose that stays in the medium after a particular time temporary stop in as an indication of the glucose take-up by yeast cell layer. The examinations on the medium of nonmetabolizable sugars and certain metabolizable glycosides have recommended that sugar transport over the yeast cell layer is intervened by stereospecific film possessor. It has likewise been accounted for that in yeast cells glucose transport is incredible and it commonly concurs that glucose is transported in yeast cells by an encouraging dispersion process. The rate of glucose take-up into the yeast cells was straight in all three glucose concentrations (25mM, 10mM and 5mM) utilized in the examination. The MeCV showed significantly higher ($p<0.05$) action than the AqCV, EaCV and NhCV at all three concentrations utilized in the investigation. The percent expansion in the glucose take-up by the yeast cell was observed to be defiantly corresponding to the concentration of glucose and diminished with an expansion in the concentration of molar in the glucose arrangement.

CONCLUSION

Based on the outcomes of the whole study, MeCV exhibited higher inhibitory action on key carbohydrate hydrolyzing compounds (*α*-glucosidase and *α*-amylase), dispersion of glucose, HbA1c and glucose take-up by cell layer, which may be due to its high concentrations of phenolic and flavonoids. Phenolic mixes are naturally phenomenon mixes. The greater part of these mixes have rich sources of scavenging of free radical and cell reinforcement properties as well as therapeutic properties and have been utilized as antidiabetic medications. Flavonoids are characteristic phenolic mixes with a few natural activities. These phytochemicals with solid cell reinforcement properties have been accounted for to be great inhibitors of starch hydrolyzing proteins, and controllers of hyperglycemia and other complication of diabetes mellitus. Future work is essential to decide whether precise phytochemicals are in charge of these inhibitory impacts of MeCV on antidiabetic movement. Our team is planning the *in vivo* study to see the capability of MeCV isolated active fractions in STZ-prompted hyperglycemia in Wistar strain male rats.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

Abs: absorbance; **ANOVA:** Analysis of variance**; AqCV:** Aqueous extract of *Cleome viscosa*; **CMC:** Carboxymethyl cellulose; **EaCV:** Ethyl acetate

extract of *Cleome viscosa*; **GDRI:** Glucose dialysis retardation index; **HbA1c:** Non- enzymatic glycosylation of haemoglobin; HPLC**:** High-performance liquid chromatography; **IC_{50}:** Inhibitory concentration at 50%; **MeCV:** Methanolic extract of *Cleome viscosa*; **MNZ:** Metronidazole; **MTF:** Metformin; **NhCV:** n-Hexane extract of *Cleome viscosa;* **NIDDM:** Non-insulin dependent diabetes mellitus; **RT:** Retention times; **SE:** Standard Error; **SPSS:** Statistical Package for the Social Sciences.

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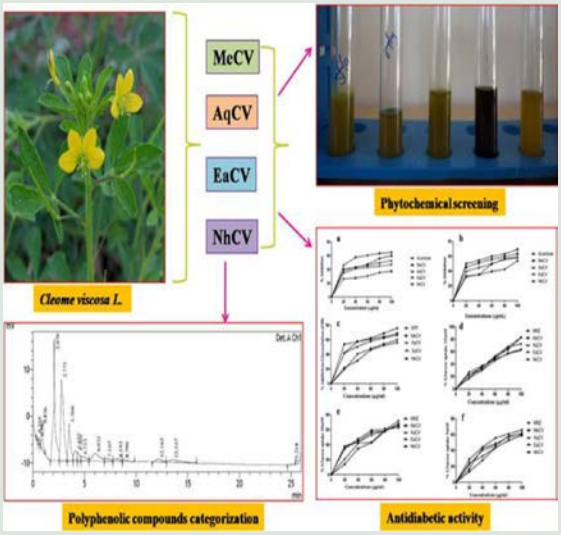
GRAPHICAL ABSTRACT



SUMMARY

- This research work assessed the antihyperglycemic impacts of methanolic (MeCV), aqueous (AqCV), ethyl acetate (EaCV) and n-hexane (NhCV) extracts of Cleome viscosa whole plant.
- Based on the results of this study, it can be concluded that the methanolic extract of Cleome viscosa (MeCV) firmly impacts on enzymatic (α-glucosidase, α- amylase inhibitory activity) and non-enzymatic activities (HbA1c and glucose uptake by yeast cell assays) due to the high presence of polyphenols in MeCV.
- Gallic acid (2.45%), protocatechuic acid hexoside (0.83%), chlorogenic acid (0.77%), catechin (0.53%), quercetin (0.80%), p-hydroxybenzoic acid (0.78%), and p-coumaric (0.07%) were the main compounds seen in the MeCV.

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