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Antioxidant and antidiabetic properties of isolated fractions from methanolic extract derived from the whole plant of *Cleome viscosa* L.

Yarrappagaari Suresh[1], Gutha Rajasekar[1], Thopireddy Lavanya[2], Benne Lakshminarsimhulu[1], Kesireddy Sathyavelu Reddy[2] and Saddala Rajeswara Reddy[1*]

Abstract

Background: *Cleome viscosa* is considered as an important medicinal plant extensively used in India, China, Bangladesh, and a few countries in Africa. In the present study, in vitro anti-radical and antidiabetic potential of isolated fractions of methanolic extract of *C. viscosa* whole plant (MeCV) has been investigated. The identification of polyphenols and their related functional groups in the bioactive fraction was categorized by using HPLC and FT-IR.

Results: The total phenolic and flavonoid contents of F-D were higher than those of F-A, F-B, and F-C. The F-D exhibited superior antioxidant capacity when compared with the remaining three fractions. However, the F-D showed the highest glucose diffusion activity over the 30 min–27 h incubation period and also inhibited both α-glucosidase and α-amylase enzyme activity. HPLC analysis revealed the presence of the two known compounds (protocatechuic acid hexoside, rutin) and six unknown compounds in the F-D. FTIR spectrum confirmed the presence of phenol group.

Conclusion: The isolated F-D obtained from MeCV displayed superior antioxidant and antidiabetic activity which indicate the presence of polyphenols in the fraction. The data findings of the present study support the traditional uses of the whole plant of *C. viscosa* as a promising natural source of biological medicines for oxidative stress and diabetes.

Keywords: *Cleome viscosa*, Isolated fractions, Antioxidant activity, Antidiabetic activity, HPLC, Polyphenols

Background

Diabetes is rapidly spreading and resulting in the death of around 4 million by the year of 2017 in Europe, Middle East, North Africa, North America, Caribbean, South and Central America, South East Asia, and Western Pacific countries [1]. About 90% of all cases of diabetes in these countries are noninsulin-dependent diabetes mellitus (NIDDM), also known as type-2 diabetes (T2D). This could be because of lifestyle and dietary prospects, such as physical inactivity, smoking, consumption of alcohol, and lesser consumption of vegetables, fruits, and natural products [2]. NIDDM is also complicated by insulin conflict, hyperinsulinemia, impede insulin discharge, glucose uptake, and utilization [3]. Thus, so many factors make diabetes a significant medical issue which requires a search for other viable and safe enzyme inhibitors from natural medicine to treatment of diabetes.

In enterocytes of the small intestine, carbohydrate can be absorbed only as monosaccharides (glucose and fructose). Pancreatic α-amylase (E.C. 3.2.1.1) is the major enzyme in the digestive system and it catalyzes the

* Correspondence: drsrr2017@gmail.com
[1]Division of Ethnopharmacology, Department of Biotechnology, School of Herbal studies and Naturo Sciences, Dravidian University, Kuppam, Andhra Pradesh 517426, India
Full list of author information is available at the end of the article

hydrolysis of starch and various oligosaccharides into low-molecular-weight products such as glucose, maltose, and maltotriose units [4]. α-Glucosidase catalyzes the hydrolysis of starch to simple sugars like glucose for intestinal absorption which in turn leads to an increase in blood glucose levels. Thus, resulting in higher postprandial blood glucose levels [5]. This process of dietary starch digestion takes place quickly and results in a postprandial rise in blood sugar. The higher in postprandial blood glucose level is related to the α-amylase and α-glucosidase action of the small intestine [6]. Therefore, inhibition of these two enzymes could play a role in controlling diabetes by hindering the absorption of starch and expanding intestinal sugar holding time. The rate of glucose absorption declines by inhibiting α-amylase and α-glucosidase action and resulting decrease in the rate of increment of postprandial blood glucose levels [7]. Thus, the retardation of the action of α-amylase and α-glucosidase by any inhibitors may be one of the most effective approaches to control NIDDM [8]

Several antidiabetic medicines, for example acarbose, voglibose, and miglitol, may lessen the ingestion rate of glucose by hindering sugar processing, causing a decline in postprandial serum glucose [9]. However, a good number of these synthetic hypoglycemic specialists are toxic in addition to cause side effects [10]. A part of these side effects integrates the inflammation of gastrointestinal, distress of stomach, and looseness of the bowels and tooting [11, 12]. An alternative methodology like the incorporation of natural foods in the diet may show viable inhibitory action against α-glucosidase and α-amylase with lesser side effects. Various therapeutic plants have indicated some relationship to the hindrance of starch handling catalysts [13, 14].

Oxidative stress is an important issue faced by several diabetics which is caused due to overproduction of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), $O_2^{-}\bullet$ and OH• [15]. These ROS are responsible for the damage of most of the biomolecules such as lipids, proteins, and DNA and also cause the death of cell [16]. Plant components can be utilized to control the steadiness between free radicals and antioxidants and might be a lesser harmful choice when compared to synthetic antioxidants [17]. Due to several harmful side effects caused by synthetic antioxidants, the contemporary world is now looking for the potentially safe therapeutic plants in the treatment of several oxidative stress-related diseases.

Consequently, a few contemporary studies have been focused on the finding of promising novel antioxidants, fundamentally phenolics, and flavonoids from natural resources, which have similar properties and less-toxic impact having obvious biological actions. Plants have standard antioxidant properties of antioxidants, which arrange a good agreement of compounds, for example, a few classes of flavonoids (anthocyanins, flavonols, and flavones) and non-flavonoids (phenolic acids, lignins, terpenoids and stilbenes, and so on). These compounds are unlike in structure, the amount of phenolic hydroxyl groups, and their position is important to deviation in their antioxidative and biological actions [18]. Herbs and vegetables reach in antioxidant agents and extra phytochemicals are able to delay oxidative pressure and the other related issues [19]. The extraction, separation of the bioactive compound, pharmacological screening, toxicological assessment, and clinical assessments are the important steps involved in an evaluation of any biologically active compound from the plant resources. Hence, the present research work is also designed in the same manner involving sequential steps such as extraction, isolation, and separation of bioactive compound from plants extracts by widespread phytochemical screening assay, chromatographic techniques, such as column, thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), and also study of biological activities by in vitro methods.

In this present work, we have selected the traditional medicinal plant namely *Cleome viscosa*. L, which is commonly known as Asian spider flower or Yellow spider flower and locally known as "Bagra" in Hindi. It is spread all over the major parts of Asia, Africa, North America, South America, and Australia nations. In Indian Ayurvedic and Chinese medicine system, it is considered as an important traditional medicine for malaria, hypertension, rheumatic arthritis, neurasthenia, and snake bite [20]. Various researchers have documented for anti-inflammatory, hepatoprotective, and hypoglycemic activities of *C. viscosa* plant various extracts [21, 22]. Therefore, the present study is aimed to evaluate the preliminary phytochemical screening, estimation of total phenolic and flavonoid content, in vitro antioxidant activity, and in vitro antidiabetic activity of isolated pooled fractions obtained from MeCV.

Methods

Chemicals and reagents

The chemicals and standards were obtained from Himedia and Sigma–Aldrich (Bangalore, India). All other reagents, polar, and non-polar solvents were used for an analytical grade. Merck grade Aluminum TLC plate (silica gel coated with fluorescent indicator F254) size 20 × 20 cm were used for thin-layer chromatography. Column (60 cm height × 5 cm diameter) and silica gel (60–120 mesh) were used for column chromatography.

Collection of plant material and extraction

The well-grown and healthy *Cleome viscosa* whole plant material was collected, shade dried, and powdered. The

powder was used for the extraction of the bioactive compounds into methanol solvent. The dried powder material (500 g) was extracted in 5 L methanol using a Soxhlet apparatus at ambient temperature for 72 h. Later, the extract was filtrated under vacuum, concentrated in a rotary evaporator, and then lyophilized. Then, the obtained crude methanolic extract (MeCV) was used for the fractionation [23].

Preliminary phytochemical screening of MeCV

Gibbs method [24] was used to analyze phenols, steroids, alkaloids, and lignin; Peach and Tracey method was adapted for flavonoids analysis; Trease and Evans procedure [25] was used for tannins screening; Kokate method [26] was used for glycosides analysis; Rizk method [27] was adopted to saponins, quinines, and coumarins screening; and Trim-Hill reagent test for terpenoids screening of MeCV.

Isolation process

Fractionation of the MeCV by column chromatography

The 60 × 5cm column was used to elute components from MeCV based on the Khoo et al. (2015) method [28]. The column was rinsed by hexane and dried before exercise. The column was filled with $3/4^{th}$ of n-hexane and $2/3^{rd}$ of silica gel. The packing silica gel was utilized subsequently for activating at 100 °C for 1 h by using hexane. The column was run through various solvent combinations like 80%:20%; 60%:40%; 40%:60%; 20%:80% of methanol:n-hexane, methanol:ethyl acetate, and methanol:aqueous along with 100% methanol (me), 100% aqueous (aq), 100% ethyl acetate (ea), and 100% hexane (nh) combinations were also utilized, after loading with MeCV (20 g) in the activated silica gel (30 g). Further, 1 mL/min flow rate was maintained at collected fractions and every fraction was concentrated using a rotary evaporator under reduced pressure and we were calculating the percentage of yielding of fractions by using R/S × 100 (R = weight of obtained fractions residues; S = weight of test sample used) formula.

Thin layer chromatographic study

As per the usual basic climbing manner, we were using 60F254 (Merck), 7 × 6 cm pre-coated TLC plates. We cut TLC plate on 1 cm between two tracks by ordinary household scissors based on the plate markings. Capillary tubes were used to spot the sample (1 μL). Hexane: ethyl acetate:acetic acid (2:7:1 ratio) solvent system was utilized [29]. Spot the fractions on TLC plate and developed in TLC chamber. After developing the plates to allow the air dry in addition to spray the iodine vapors to detect the bands. After its retention, factor (R_f) values were calculated as follows:

$$R_f = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

Determination of phytochemical content in the isolated pooled fractions of MeCV

Total content of phenols

Total phenolic content (TPC) was calculated according to the standard Singleton and Rossi (1965) procedure [30]. Then, 100 mg/mL of isolated pooled fractions of MeCV was mixed with Folin–Ciocalteu's reagent (0.5 mL) and 20% of sodium carbonate (2.5 mL) in addition to 6.0 mL of distilled water. The resulting solution was mixed well and incubated at 40 °C for 30 min by using water bath shaker. The reaction mixture absorbance was measured at 760 nm. A calibration curve was prepared by using gallic acid standard.

Total content of flavonoids

For the determination of total flavonoid content (TFC), aluminum chloride colorimetric method was employed by using Chang et al. (2002) [31]. The 50–100 mg/mL concentration of quercetin standard was used for the construction of the calibration curve. In brief, 100 mg of isolated pooled fractions of MeCV was dissolved in 1 mL of distilled water individually. Further, 0.5 mL of this solution was mixed with 95% ethanol (1.5 mL), 10% aluminum chloride (0.1 mL), and 1 M potassium acetate (0.1 mL) in addition to distilled water (2.8 mL). After incubation at room temperature (26 ± 2 °C) for 30 min, the reaction mixture absorbance was measured at 415 nm.

In vitro antioxidant activity of isolated pooled fractions of MeCV

DPPH radical scavenging assay

The antioxidant activity of the isolated pooled fractions of MeCV was measured based on the DPPH free radical scavenging activity according to Brand-Williams et al.'s (1995) method with slight modifications [32]. Three milliliters of 60 μM DPPH in methanol was mixed with 1 mL of isolated pooled fractions from MeCV of various concentrations (20, 40, 60, 80, and 100 μg/mL). Consequently, 1 mL of methanol and 1 mL of DPPH was used as control and gallic acid was used as a reference standard. The reaction was conceded in three times and the absorbance was measured at 517 nm after incubation of 30 min in dark.

$ABTS^{\bullet+}$ radical scavenging assay

The assay of ABTS radical scavenging activity was adopted from Re et al. (1999) [33]. The stock solution included 7 mM of ABTS and 2.4 mM of potassium persulfate. One milliliter of varying concentrations (20, 40, 60, 80, and 100 μg/mL) of isolated pooled fractions of

MeCV and standard ascorbic acid was permitted to react with 1 mL of ABTS stock solution and the absorbance was measured at 734 nm after the incubation for 7 min.

Hydrogen peroxide scavenging assay

The ability of the isolated pooled fractions of MeCV to scavenge hydrogen peroxide (H_2O_2) was determined according to the Ruch et al.'s (1989) method [34]. Then, 2 mmol/l of H_2O_2 solution was prepared in neutral phosphate buffer. Isolated pooled fractions (20, 40, 60, 80, and 100 μg/mL) were added to 0.6 mL of H_2O_2 solution. The absorbance of H_2O_2 was measured at 230 nm after incubation for 10 min against blank and the radical scavenging activity was compared with ascorbic acid.

Superoxide radical (O_2^-) scavenging assay

Superoxide radicals are generated in phenazine methosulfate (PMS), nicotinamide adenine dinucleotide (NAD) systems by oxidation of nicotinamide adenine dinucleotide and assayed by the reduction of nitroblue tetrazolium (NBT) [35]. In this experiment, the superoxide radicals were generated in 3 mL of 16 mM Tris HCl buffer (pH 8.0) containing 1 mL of 50 μM NBT, 1 mL of 7 8μM NAD, and isolated pooled fractions (20–100 μg/mL). The reaction was started by adding 1 mL of 10 μM PMS solution to the mixture. The reaction mixture was incubated at 25 °C for 5 min; absorbance was measured at 560 nm using a spectrophotometer against the blank.

Ferric reducing antioxidant power assay

The ferric reducing antioxidant power (FRAP) assay was conducted according to the method reported by Benzie and Strain [36]. FRAP reagent was prepared freshly by mixing three solutions a, b, and c, 300 mM sodium acetate buffer, pH = 3.6 (solution a), 10 mM TPTZ solution in 40 mM Hcl (solution b), and 20 mM ferric chloride ($FeCl_3$) (solution c) in the proportions of 10:1:1 (v/v/v). The reagent was kept in darkness for 30 min to complete the reaction. For the assay, 0.1 mL of isolated pooled fractions from MeCV and ascorbic acid (20–100 μg/mL) was mixed with 2.9 mL of FRAP reagent separately. All the samples were prepared in triplicate and vortexed for 1 min and incubated in dark for 30 min at 37 °C. The increase in absorbance of the reaction mixture was measured for each sample on UV–Visible spectrophotometer at 593 nm. FRAP activity was calculated as μM ascorbic acid equivalents (AAE) in a gram of sample dry weight.

In vitro antidiabetic activity of isolated pooled fractions of MeCV

Inhibition of glucose diffusion method

A simple model system was used to evaluate the effects of isolated pooled fractions from MeCV on the movement of glucose in vitro. The model was adapted from a method described by Edwards et al. (1987) [37] which involved the use of a sealed dialysis tube into which 15 mL of a solution of glucose and sodium chloride (0.15 M) was introduced and the content of glucose in the external solution was measured. The model used in the present experiment consisted of a dialysis tube (6 cm × 15 mm) into which 1 mL of 50 g/L each fraction in 1% CMC and 1 mL of 0.15 M sodium chloride containing 0.22 M D-glucose was added. The dialysis tube was sealed at each end placed in a 50 mL centrifuge tube containing 45 mL of 0.15 M 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 (30 min, 1 h, 3 h, 7 h, 24 h, and 27 h).

Study of α- Glucosidase inhibition assay

One milligram of the α-glucosidase enzyme (isolated from Saccharomyces cerevisiae) was suspended with 100 mL neutral PBS buffer which contains the 200 mg of bovine serum albumin [38]. The various concentrations (20, 40, 60, 80, and 100 μg/mL) of isolated pooled fractions from MeCV were added with reaction mixture (10 μL of pH 6.8 phosphate buffer; 490 μL of 5 mM p-NPG (p-nitro phenyl α-d glucopyranoside)). The reaction mixture was incubated at 37 °C for 5 min then added 250 μL of α-glucosidase (0.15 unit/mL) and again incubated at 37 °C for 15 min. Then cool the reaction mixture and add 2 mL of sodium carbonate (200 mM) to stop the reaction. The activity of enzyme inhibition was measured at 405 nm 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 500 μL enzyme (α-amylase) for 10 min at 37 °C. Then, 1 mL of (20, 40, 60, 80, and 100 μg/mL) isolated pooled fractions from MeCV was added to the enzyme solution. Further, 2 M of NaOH is applied to stop the reaction process. One milliliter of Dinitro salicylic acid is mixed and the reaction is maintained in the hot water bath for 5 min. After completion of incubation, test tubes were cooled by running tap water, and the final volume of test solution was to make up to 10 mL using sterile distilled water and absorbance was measured at 540 nm. Acarbose was used as a reference substance [39].

Percentage calculation

Percentage of free radical scavenging and α-amylase and α-glucosidase inhibition was measured by using the following formula: % Inhibition/ scavenging activity = $[(A_{control} - A_{sample})/ (A_{control})] \times 100$

where A = absorbance

Characterization of active F-D fraction

Identification of phenolic and flavonoids by high-performance liquid chromatography

Instrumentation Twofold Waters 600 E pump equipped the system with Waters-2996 photodiode array detector linked to arrangement processor was used for the analysis. A maximum force of 2500 psi and minimum of 1500 psi was retained. C18 column Symmetry (250 × 4.6) was used in chromatographic separation at 25 °C.

Chromatographic conditions In this analysis, two types of solvents were prepared, i.e., solvent-A (mobile phase: acetonitrile) and solvent-B (water with 0.2% formic acid). The stream velocity was held in reserve at 0.7 mL/min. The ascent series was as pursued: 35% A/65% B at 0–6 min, 60% A/40% B at 6–9 min, 80% A/20% B at 9–14 min, 100% A at 14–25 min, and 35% A/65% B at 25–30 min. The 20 μL was considered as injection volume and peaks were scrutinized at 280 nm. Peaks were identified by congruent retention times (RT) compared with previously reported standards.

Identification of functional groups by Fourier transform infrared The Fourier transform infrared (FT-IR) spectra of purified isolated fractions powder were recorded on a Thermo Nicolet 330 FTIR spectroscopy (Madison, WI, USA). To find good indicators to resonance percentage, isolated fractions were taken in the range 400–4000 cm^{-1} and the resolution was kept as 4.0 cm^{-1}.

Statistical analysis All investigation values were represented mean ± SE and mean ± SD. The data was investigated by one-way ANOVA followed by Tukey's post hoc and Dunnett's test (SPSS version 16.0, SPSS Inc. Chicago, IL, USA). The dose-response curves were plotted between the percentages of scavenging activity versus concentrations.

Results

Preliminary phytochemical screening of MeCV

In the view of extraction outcome, the yield of MeCV was 18.6% w/w with the weight of 93 g. The results obtained from the preliminary phytochemical tests are represented in Table 1. The study showed the presence of flavonoids, phenols, steroids, alkaloids, lignin, tannins, glycosides, terpenoids, saponins, quinines, and coumarins in the MeCV.

Table 1 Preliminary phytochemical screening of MeCV

Phytochemicals		MeCV
Flavonoids	Ferric chloride test	+
	Shinoda's test	+
	Zinc-Hcl reduction test	+
	Lead-acetate test	+
Phenols	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	+
	Moisch's test	+
Terpenoids	Trim- hill reagent test	+
Saponins		+
Quinines		+
Coumarins		+

+ = presence; − = absence

Analysis of column chromatography

We have collected 16 purified fractions from the isolation and purification process of MeCV by using column chromatography. The collected fractions were all concentrated by using rotary evaporation (100 rpm) at 40 °C (H-Biomedical-EV311 plus-v). After completion of this process, we collected semi-solid and solid material and describe the color and yield (in g) of fractions. Percentage of yielding values of the isolated fractions was represented in Table 2.

Thin layer chromatographic analysis

Table 3 describes the retention factor (R_f) values of all 16 fractions. The similar fractions were pooled into four main categories (F-A, F-B, F-C, and F-D) based on their R_f values. The pooled F-A, F-B, F-C, and F-D fractions were tested for total phenolic, total flavonoid content, in vitro antioxidant, and in vitro antidiabetic activity.

Determination of phytochemicals content in isolated pooled fractions of MeCV

In our current results, F-D demonstrates the high content of TCP (104.89 ± 4.52 mg GAE/g) and TCF (97.35 ± 2.81 mg QRE/g) when compared to the rest of the fractions. At that same time, F-A demonstrates the low content of TCP (33.77 ± 0.69 mg GAE/g) and TCF (38.28 ± 1.36 mg QRE/g). TCP content has

Table 2 MeCV isolated fractions extractive values

Number of fraction collected	Solvents used	Color appearance	Yielding (in g)	Yielding Percentage (%w/w)
1-Fraction	Me : Nh (80:20)	Light green	2.244	11.22
2-Fraction	Me : Nh (60:40)	Light green	1.852	9.26
3-Fraction	Me : Nh (40:60)	Light green	1.097	5.48
4-Fraction	Me : Nh (20:80)	Light green	1.784	8.92
5-Fraction	Me :Ea (80:20)	Light brown	1.730	8.65
6-Fraction	Me :Ea (60:40)	Light brown	2.033	10.16
7-Fraction	Me :Ea (40:60)	Light brown	2.725	13.62
8-Fraction	Me :Ea (20:80)	Light brown	2.423	12.11
9-Fraction	Me :Aq (80:20)	Dark brown	3.594	17.97
10-Fraction	Me :Aq (60:40)	Dark brown	3.157	15.78
1-1Fraction	Me :Aq (40:60)	Dark brown	3.468	17.34
12-Fraction	Me :Aq (20:80)	Dark brown	3.541	17.70
13-Fraction	Me-100%	Brown	3.615	18.07
14-Fraction	Aq-100%	Dark brown	3.600	18.00
15-Fraction	Ea-100%	Light brown	3.568	17.84
16-Fraction	Nh-100 %	Light green	3.347	16.73

Me methanol, *Nh* n-hexane, *Ea* ethyl acetate, *Aq* aqueous

rich source than TCF in all fractions (Fig. 1). TCP was calculated by standard gallic acid equation curve, i.e., $Y = 3.536x + 0.496$; $R^2 = 0.997$and TCF was calculated by standard quercetin equation curve, i.e., $Y = 2.950x + 0.997$; $R^2 = 0.983$.

Table 3 R_f values and number of spots detected of MeCV isolated fractions

Name of fraction	No. of spots detected	R_f values
1-Fraction	02	0.96, 0.20
2-Fraction	04	0.90, 0.86, 0.34, 0.32
3-Fraction	04	0.90, 0.86, 0.34, 0.32
4-Fraction	04	0.90, 0.86, 0.34, 0.32
5-Fraction	02	0.88, 0.18
6-Fraction	04	0.88, 0.86, 0.20, 0.18
7-Fraction	04	0.88, 0.86, 0.20, 0.18
8-Fraction	03	0.88, 0.20, 0.16
9-Fraction	03	0.80, 0.17, 0.15
10-Fraction	04	0.80, 0.77, 0.17, 0.15
11-Fraction	04	0.80, 0.77, 0.17, 0.15
12-Fraction	03	0.77, 0.17, 0.15
13-Fraction	04	0.83, 0.27, 0.26, 0.22
14-Fraction	05	0.85, 0.83, 0.27, 0.26, 0.22
15-Fraction	05	0.85, 0.83, 0.27, 0.26, 0.22
16-Fraction	05	0.85, 0.83, 0.27, 0.26, 0.22

In vitro antioxidant activity of isolated pooled fractions of MeCV

DPPH radical scavenging activity

In the current study, MeCV isolated pooled fractions (F-A, F-B, F-C, and F-D) showed considerable movement in the scavenging of DPPH, reaching up to 85%, 85.95%, 82.02%, and 82.5% at 100 μg/mL concentration. Figure 2a demonstrates the dose-response curve of DPPH scavenging activity of four fractions when compared with gallic acid. The IC_{50} of F-D was 38.54 ± 0.36 μg/mL while the IC_{50} values of gallic acid standard, F-A, F-B, and F-C were 34.47 ± 0.81 μg/mL, 47.16 ± 1.02 μg/mL, 44.35 ± 0.47 μg/mL, and 40.36 ± 0.96 μg/mL (Fig. 3). The DPPH scavenging assay is one of the regularly used important methods to scrutinize the presence of antioxidant compounds in plant and plant-based ingredients. The results revealed that the F-D showed potential DPPH scavenging activity when compared to the rest of the fractions.

ABTS radical scavenging activity

The MeCV isolated F-A, F-B, F-C, and F-D fractions were rapid and efficient scavengers of ABTS (Fig. 2b) and this was validated by the ascorbic acid standard. It exhibited potent scavenging effect against those of ABTS radicals with an IC_{50} values of F-A (39.73 ± 0.70 μg/mL), F-B (35.69 ± 0.25 μg/mL), F-C (32.18 ± 0.10 μg/mL), and F-D (30.31 ± 0.54 μg/mL) (Fig. 3). F-D was exhibited high potent scavenging effect significantly

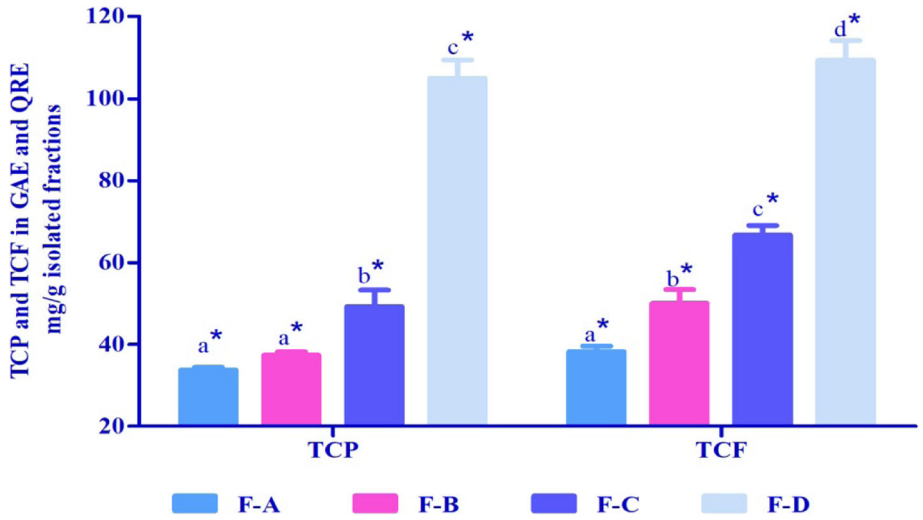

Fig. 1 TCP and TCF in the isolated pooled fractions of MeCV. Each vertical bar represents Mean ± SE for $n = 3$. The vertical bars having the same alphabet do not differ significantly, whereas the bars having the different alphabet differ significantly at $P < 0.05$(one-way ANOVA followed by Tukey's post hoc test)

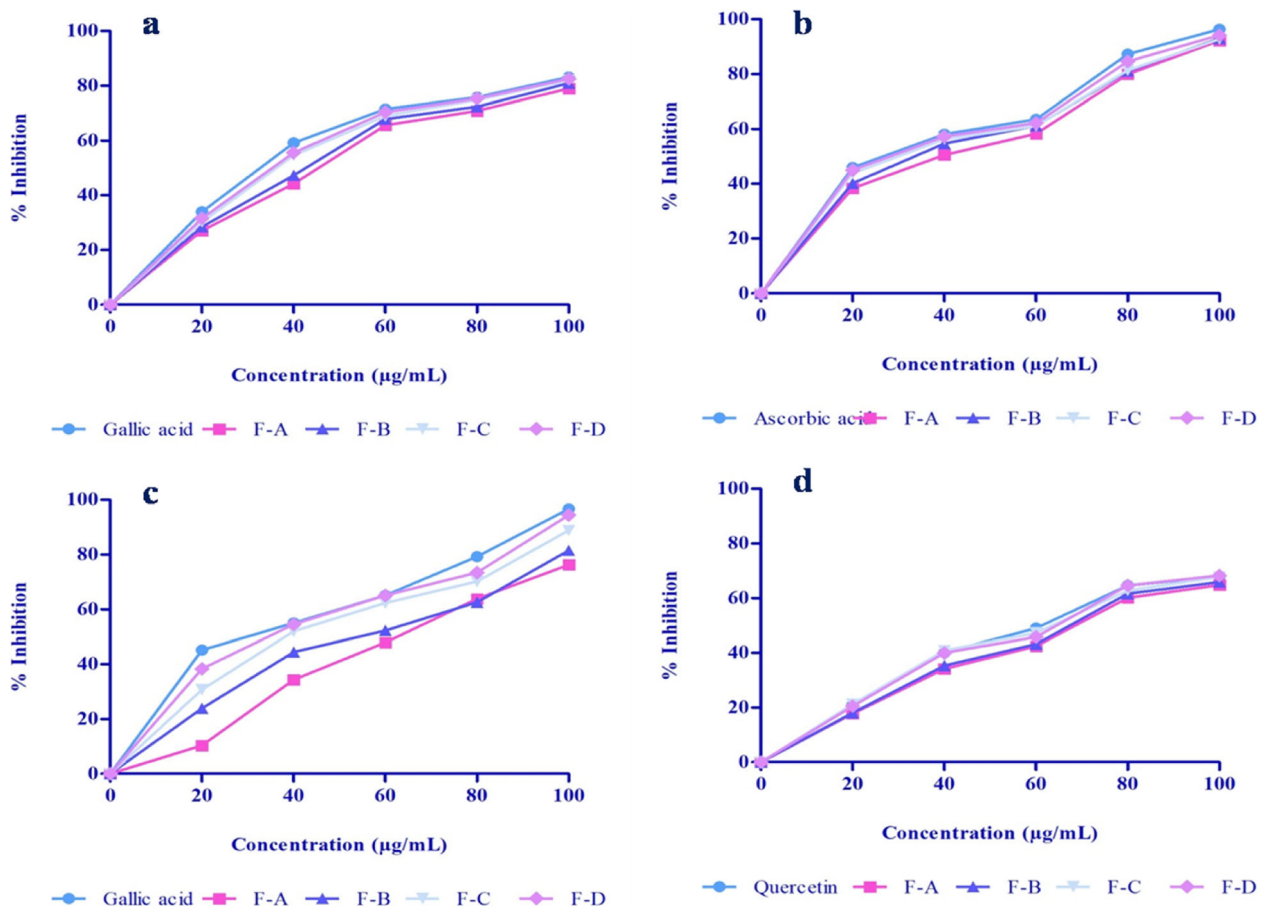

Fig. 2 **a** DPPH, **b** ABTS, **c** H_2O_2, and **d** superoxide radical scavenging activities of four pooled fractions and standards. The data demonstrates the mean of triplicates of five doses used in the graph

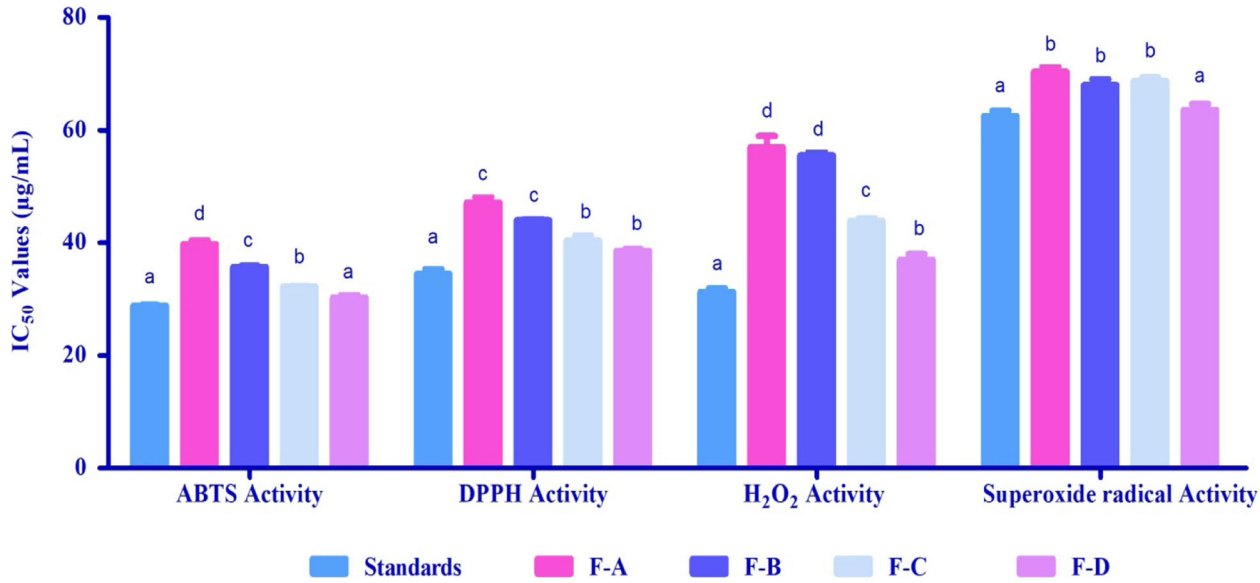

Fig. 3 IC_{50} values of free radical scavenging activities of four pooled fractions of MeCV and standards. Each vertical bar represents mean ± SE for $n = 3$. The vertical bars having the same alphabet do not differ significantly whereas the bars having the different alphabet differ significantly at $P < 0.05$ (one-way ANOVA followed by Tukey's post hoc test)

equivalent to that of ascorbic acid (28.87 ± 0.15 μg/mL). The ABTS scavenging activity percentage was 92.24% (F-A), 93.02% (F-B), 92.82% (F-C), and 94.18% (F-D) at 100 μg/mL concentration, while ascorbic acid at the similar dose exhibited 96.41% scavenging.

H_2O_2 scavenging activity

As shown in Figs. 2c and 3, MeCV isolated F-A, F-B, F-C, and F-D also verified the activity of corrosion of H_2O_2 in concentration-dependent manner with 76.26%, 81.56%, 88.88%, and 94.44% respectively at 100 μg/mL concentration, while gallic acid was 96.71% at the same concentration. The IC_{50} values of corrosion of H_2O_2 activity of F-A, F-B, F-C, and F-D were 57.59 ± 2.91 μg/mL, 55.52 ± 0.86 μg/mL, 43.84 ± 0.48 μg/mL, and 37.60 ± 1.36 μg/mL while the standard gallic acid was 31.27 ± 0.65 μg/mL. The H_2O_2 scavenging activity by the MeCV isolated pooled fractions may be due to their polyphenols, which could be due to the electron transfer to H_2O_2 resulting in the formation of H_2O.

Superoxide radical scavenging activity

In the current assay, the MeCV isolated pooled F-A, F-B, F-C, and F-D was studied for its inhibitory effects on the formation of superoxide radicals. Figure 2d exemplifies a significant decrease in the formation of superoxide radicals due to the scavenging knack of pooled fractions and quercetin standard. The F-A, F-B, F-C, and F-D showed the maximum inhibitory capacity of 64.75%, 65.85%, 67.68%, and 68.28% respectively at 100 μg/mL, while quercetin standard at the same dose demonstrates 67.94%. This assay revealed that the isolated fractions have a significant potent (in particularly F-D) scavenging property of superoxide radicals. In contrast, IC_{50} values of F-A, F-B, F-C, F-D, and quercetin were 70.35 ± 0.95 μg/mL, 68.53 ± 0.68 μg/mL, 68.65 ± 0.71 μg/mL, 63.61 ± 1.04 μg/mL, and 62.54 ± 0.92 μg/mL (Fig. 3). The results indicated that the F-D was significantly as potent as quercetin standard.

Ferric reducing antioxidant power

The ferric reducing antioxidant ability of the isolated pooled fractions indicated good ferric reducing antioxidant power (FRAP) activity. Among the pooled fractions, the highest FRAP activity was noted for F-D (584.58 ± 9.15 μmol AAE/g DW) followed by F-A (229.21 ± 2.77 μmol AAE/g DW), F-B (346.11 ± 4.91 μmol AAE/g DW), and F-C (495.11 ± 6.10 μmol AAE/g DW) (Fig. 4). According to these results, F-A had significantly ($P < 0.05$) lower FRAP activity. The activity of FRAP was calculated by standard ascorbic acid equation curve, i.e., $Y = 0.005x + 0.282$; $R^2 = 0.975$.

In vitro antidiabetic activity of isolated pooled fractions of MeCV

Inhibition of glucose diffusion

The inhibition rate of glucose diffusion was established to increase from 30 min to 27 h. After 27 h, the control group showed 403.33 ± 5.20 mg/dL in the external glucose solution while F-A, F-B, F-C, and F-D showed 273.33 ± 2.60 mg/dL, 259 ± 3.21 mg/dL, 229.67 ± 3.48 mg/dL, and 219.33 ± 4.33 mg/dL respectively (Fig. 5). At the all-time intervals, i.e., 30 min, 60 min, 3 h, 7 h, 24 h,

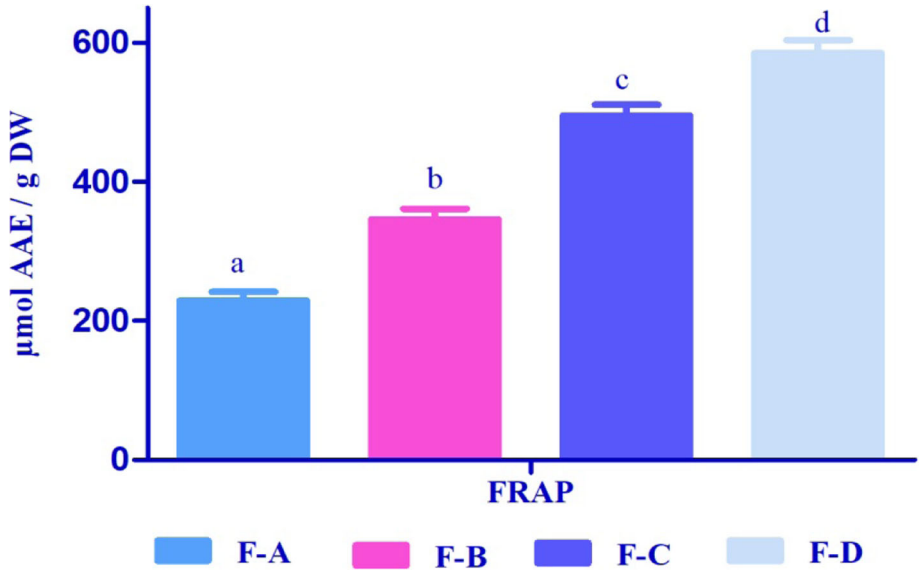

Fig. 4 FRAP activity in the isolated pooled fractions of MeCV. Each vertical bar represents mean ± SE for $n = 3$. The vertical bars having the same alphabet do not differ significantly, whereas the bars having the different alphabet differ significantly at $P < 0.05$ (one-way ANOVA followed by Tukey's post hoc test)

and 27 h, F-D was showed 127.77%, 189.04%, 140.69%, 119.03%, 83.56%, and 83.89% of maximum GDRI while F-A was showed 73.43%, 84.66%, 77.65%, 56.25%, 45.06%, and 47.56% of less GDRI and remaining fractions demonstrated moderate GDRI percentage at the same time intervals (Fig. 6).

Inhibitory effects of carbohydrate-metabolizing enzymes

The carbohydrate-metabolizing enzymes, i.e., α-glucosidase and α-amylase, inhibitory strength of MeCV isolated F-A, F-B, F-C, and F-D fractions are described in the Fig. 7a, b. The F-D exhibited significantly higher α-glucosidase and α-amylase inhibition with IC_{50} 48.76 ± 0.96 µg/

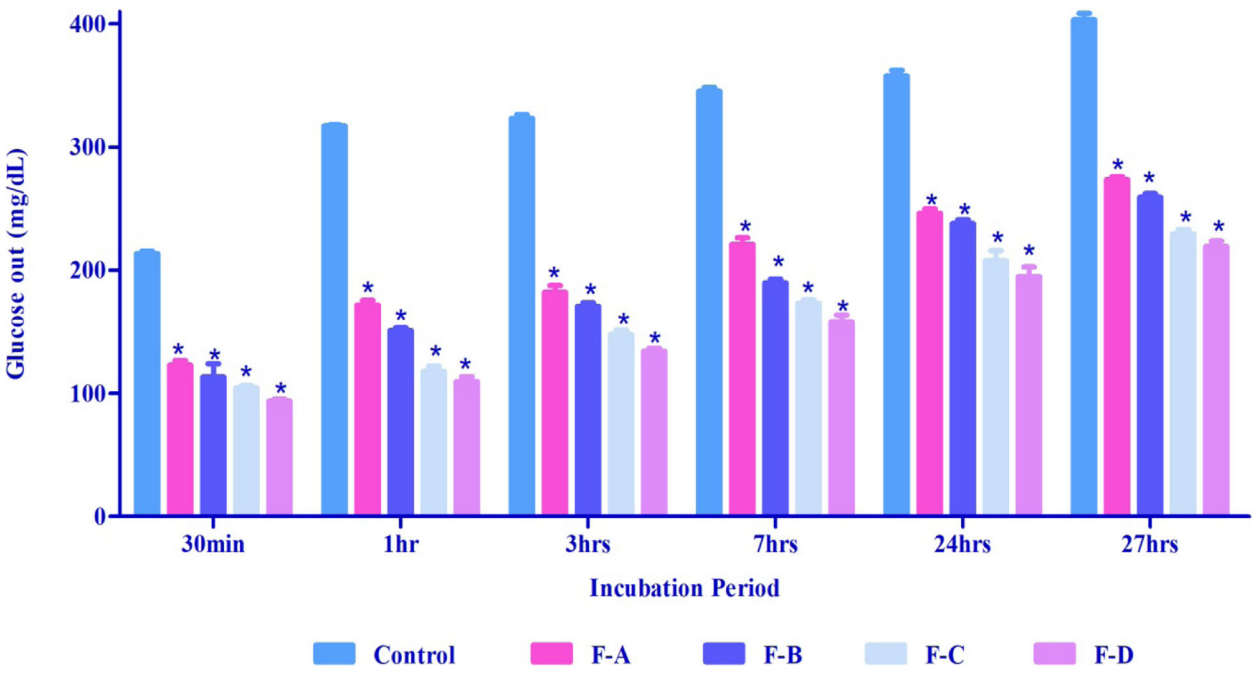

Fig. 5 Effect of isolated pooled fractions (50 g/L) obtained from MeCV on the movement of glucose out of dialysis tube over 27-h incubation. Each vertical bar represents mean ± SE ($n = 6$) the vertical bars differ significantly compared to the control group at $^*P < 0.001$ (one-way ANOVA by Dunnett's test)

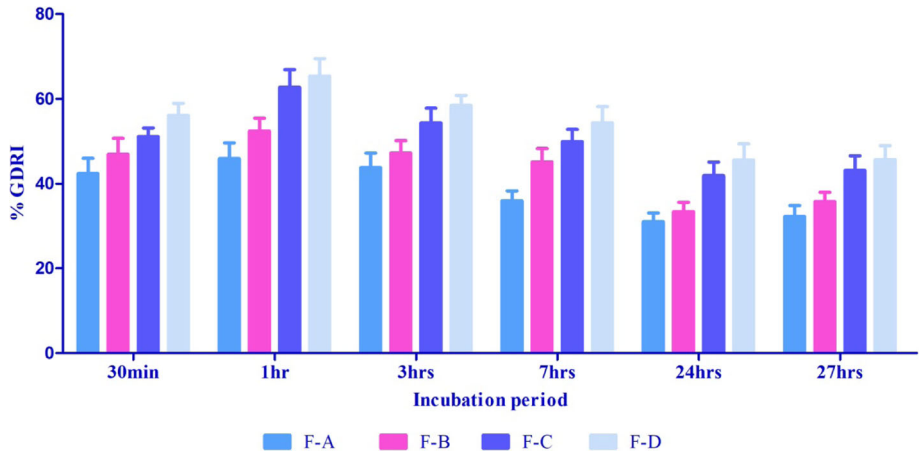

Fig. 6 Effect of isolated pooled fractions from MeCV on the percentage of GDRI. The vertical bars represented as mean ± SD for $n = 6$

mL and 25.93 ± 0.83 μg/mL followed by F-A (IC_{50}; 105.29 ± 0.96 μg/mL and 85.56 ± 0.32 μg/mL), F-B (IC_{50}; 104.67 ± 1.82 μg/mL and 64.54 ± 0.99 μg/mL), and F-C (IC_{50}; 62.57 ± 0.58 μg/mL and 38.70 ± 0.32 μg/mL) were showed lesser and moderate α-glucosidase and α-amylase inhibition while acarbose IC_{50} was 21.70 ± 0.67 μg/mL and 21.43 ± 0.32 μg/mL (Fig. 8). These results suggest that the F-D has strong α-amylase inhibition than α-glucosidase enzyme inhibition while F-A has lesser inhibition in both enzymes.

The data demonstrates the mean of triplicates of five doses shown in the graph.

Correlation study of TCP, TCF, in vitro antioxidant activity and in vitro antidiabetic activity

Plant secondary metabolites, in particularly polyphenols, could transform an assortment of enzymes and resistant cells inside the human organization, apart from their potentiality of antioxidants [40]. The Pearson correlation analysis was functionalized to TCP, TCF, DPPH scavenging activity, ABTS radical scavenging activity, H_2O_2 scavenging activity, superoxide radical scavenging activity, FRAP, α-glucosidase, and α-amylase enzyme inhibition activity (Table 4). In F-A, TCP exhibited a nonlinear correlation with TCF, DPPH, ABTS, H_2O_2, FRAP, and α-glucosidase and a linear correlation with O_2^- scavenging activity and α-amylase enzyme inhibition activity. TCF exhibited a linear correlation in DPPH, ABTS, H_2O_2, O_2^-, FRAP, and α-glucosidase and nonlinear correlation in α-amylase enzyme inhibition activity. α-Amylase exhibited nonlinear correlation in all activities; at the same moment, H_2O_2 exhibited a nonlinear correlation with O_2^-. A linear correlation was observed in DPPH with ABTS, H_2O_2, O_2^-, FRAP, and α-glucosidase. In linear correlation of ABTS with H_2O_2, O_2^-, FRAP, α-glucosidase. A linear correlation of H_2O_2 with O_2^-, FRAP, α-glucosidase, in O_2^-, with FRAP and α-glucosidase along with FRAP was exhibited linear correlation in α-glucosidase.

TCP exhibited a non-linear correlation with TCF and FRAP while remaining activities were linearly correlated in F-B. TCF exhibited a linear correlation with O_2^- and α-glucosidase while the rest of the activities exhibited

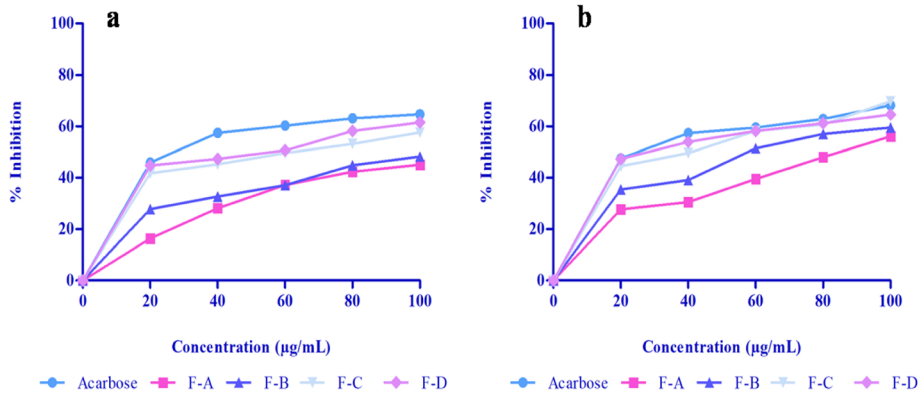

Fig. 7 **a** α-Glucosidase and **b** α-amylase inhibition activity of MeCV isolated pooled fractions and acarbose standard

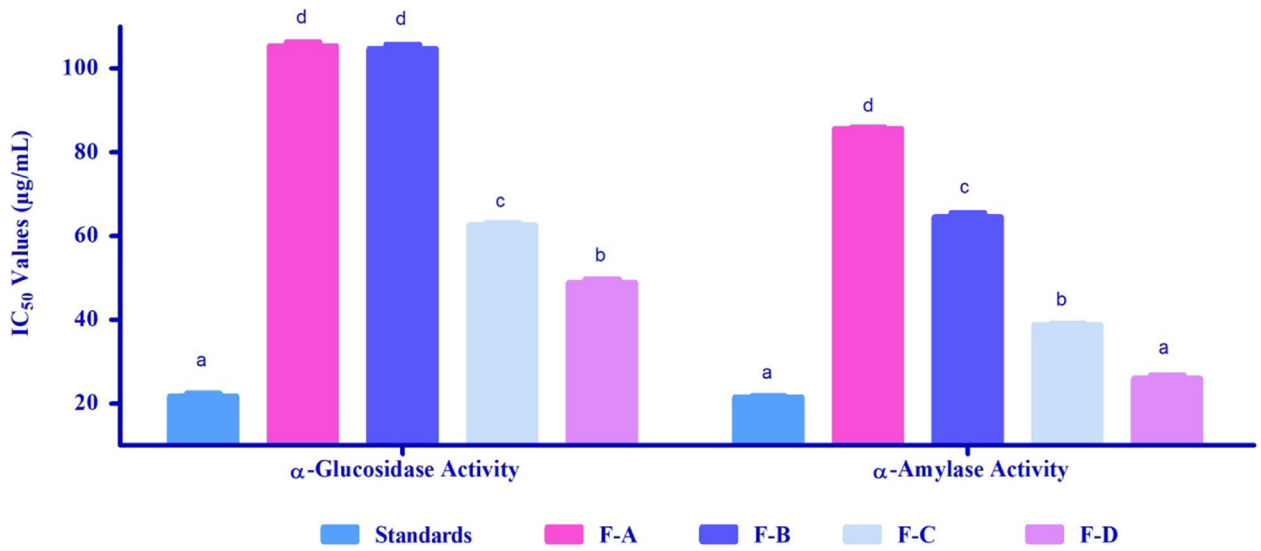

Fig. 8 IC_{50} values of α-glucosidase and α-amylase inhibition activities of four pooled fractions of MeCV and acarbose standard. Each vertical bar represents mean ± SE for $n = 3$. The vertical bars having the same alphabet do not differ significantly, whereas the bars having the different alphabet differ significantly at $P < 0.05$ (one-way ANOVA followed by Tukey's post hoc test)

nonlinear correlation. At the same time, O_2^- exhibited a nonlinear correlation with DPPH, ABTS, and H_2O_2 along with this the α-glucosidase was also a nonlinear correlation with ABTS, H_2O_2, O_2^-, FRAP, and linear correlation with α-amylase.

In F-C and FRAP exhibited nonlinear correlation in TCP, TCF, DPPH, ABTS, H_2O_2, O_2^-, FRAP, and α-Glucosidase while FRAP exhibited significant ($P < 0.05$) linear correlation with α-amylase. DPPH exhibited nonlinear correlation in H_2O_2. In this fraction, α-glucosidase and α-amylase exhibited linear correlation in all in vitro activities (except FRAP) while a linear correlation occurred in between the TCP, TCF, DPPH, ABTS, H_2O_2, and O_2^-.

F-D demonstrates that the TCP was a nonlinear correlation with DPPH and ABTS while TCF exhibited a nonlinear correlation with ABTS. DPPH exhibited a nonlinear correlation with FRAP and linear correlation exhibited with α-glucosidase, α-amylase, and the rest of the in-vitro antioxidant activities. ABTS demonstrated a strong nonlinear correlation with H_2O_2, O_2^-, FRAP, and α-amylase while H_2O_2 exhibited a linear correlation with O_2^-, FRAP, α-glucosidase, and α-amylase.

On the whole, TCP and TCF demonstrated a strong linear correlation with the majority of antioxidant and antidiabetic activities in F-D while F-C, F-B, and F-A demonstrated the moderate and lesser linear correlation and strong nonlinear correlation. This strength may be attributed to the polyphenolic compounds under revealing in high-performance liquid chromatography (HPLC) analysis. The scavenging ability of free radical is a significant relation to avoid the oxidative stress interconnected in the direction of hyperglycemia [41]. The carbohydrate-metabolizing enzymes inhibitory activities were linearly correlated to TCP and TCF of F-D; though, it was also related to polyphenolic compounds [42]. The current correlation investigation specified that the α-glucosidase and α-amylase inhibition capability of the deliberated F-D might be accredited to their DPPH, ABTS, H_2O_2, O_2^- scavenging, and FRAP assets.

Characterization of bioactive F-D fraction

Detection of polyphenols by HPLC analysis

In this study, the MeCV was purified by silica gel column chromatography. The obtained fractions were analyzed by TLC and bands were recognized. The bands among similar R_f was pooled and concentrated. Then among the four pooled fractions, F-D showed potent in vitro antioxidant and in vitro antidiabetic activity. Based on these outcomes, the bioactive F-D was subjected to scrutinize its purity by HPLC. Table 5 and Fig. 9 confirmed eight compounds by their retention time (RT). The unknown compounds characterized by peak 1-5 and 8 (Table 5) were available in the most noteworthy in the fraction. Major peaks were observed at 3.001 min (36.22%) and 4.523 min (39.05%) along with known compounds were also identified, i.e., protocatechuic acid hexoside (10.33%) and rutin (4.08%). This analysis could be qualified to the supportive liveliness between the various compounds [43, 44]. The eluted compounds were further conforming to partial structural elucidation through FTIR study.

Table 4 Correlation coefficients of Pearson analysis to exhibit a linear correlation between phytochemical content, in vitro antioxidant and in vitro antidiabetic assays in MeCV isolated four pooled fractions

Test samples	TCP	TCF	DPPH	ABTS	H_2O_2	O_2^-	FRAP	α-glucosidase	α-amylase
F-A									
TCP		− 0.90	− 0.80	− 0.16	− 0.99*	0.33	− 0.16	− 0.02	0.90
TCF			0.98	0.57	0.92	0.10	0.58	0.45	− 1.00**
DPPH				0.71	0.84	0.28	0.71	0.60	− 0.98
ABTS					0.22	0.87	1.00**	0.99	− 0.57
H_2O_2						− 0.27	0.22	0.08	− 0.92
O_2^-							0.87	0.93	− 0.10
FRAP								0.98	− 0.58
α-glucosidase									− 0.45
F-B									
TCP		− 0.46	0.94	0.63	0.24	0.01	− 0.33	0.73	0.85
TCF			− 0.73	− 0.98	− 0.97	0.87	− 0.67	0.25	− 0.85
DPPH				0.85	0.56	− 0.32	0.004	0.46	0.98
ABTS					0.90	− 0.76	0.51	− 0.05	0.94
H_2O_2						− 0.96	0.82	− 0.47	0.71
O_2^-							− 0.94	0.68	− 0.50
FRAP								− 0.88	0.20
α-glucosidase									0.28
F-C									
TCP		0.84	0.56	0.89	0.35	0.99	− 0.93	0.97	0.92
TCF			0.04	0.99	0.79	0.79	− 0.98	0.94	0.98
DPPH				0.14	− 0.57	0.64	− 0.23	0.35	0.21
ABTS					0.72	0.85	− 0.99	0.97	0.99*
H_2O_2						0.26	− 0.66	0.56	0.68
O_2^-							− 0.89	0.94	0.88
FRAP								− 0.99	1.00*
α-glucosidase									0.98
F-D									
TCP		0.41	− 0.05	− 0.98	0.97	0.77	0.98	0.06	0.21
TCF			0.88	− 0.25	0.60	0.89	0.27	0.93	0.97
DPPH				0.27	0.16	0.58	− 0.20	0.99	0.96
ABTS					− 0.92	− 0.65	− 1.00*	0.10	− 0.04
H_2O_2						0.89	0.93	0.28	0.42
O_2^-							0.67	0.68	0.78
FRAP								− 0.08	0.06
α-glucosidase									0.98

Correlations significance considered at *$P < 0.05$ and **$P < 0.01$. *TCP* total content of phenols, *TCF* total content of flavonoids, *DPPH* DPPH radical scavenging activity, *ABTS* ABTS radical scavenging activity, H_2O_2 H_2O_2 scavenging activity, O_2^- superoxide radical scavenging activity, *FRAP* ferric reducing antioxidant power, α-*glucosidase* α-glucosidase inhibition activity, α-*amylase* α-amylase inhibition activity

Functional groups identification by FT-IR analysis

The IR-spectrum of bioactive F-D was recorded by Thermo Nicolet-330 FTIR spectroscopy and results obtain the range under 400–4000 cm^{-1} IR region. From the absorption spectrum, the vibration assignments, wavenumber (cm^{-1}), along with intensities of main peaks were documented. The main peaks (see Fig. 10) of bioactive F-D signify the occurrence of various functional groups of compounds such as phenols, alcohols, alkanes and alkyls, amides, aliphatic amines, alkyl halides, and alkanes. The more strong bands occurring at 3298.28 cm^{-1}, 2924.09 cm^{-1}, 2854.65 cm^{-1}, 1595.13 cm^{-1},

Table 5 HPLC validation data of bioactive F-D for polyphenol standards

Peak No	RT (min)	Compound name	% Compound
1	3.001	Unknown	36.22
2	4.523	Unknown	39.05
3	5.214	Unknown	5.92
4	5.982	Unknown	5.32
5	7.090	Unknown	5.24
6	7.434	Protocatechuic acid hexoside	10.33
7	10.283	Rutin	4.08
8	11.465	Unknown	3.16

RT Retention time

1357.89 cm^{-1}, 1195.87 cm^{-1}, 1080.14 cm^{-1}, and 1043.49 cm^{-1} related to the stretch of O–H, H-, C–H, N-H, C–H wag (–CH2X), and C-N respectively (Table 6).

Discussion

The present study has been initiated to study *C. viscosa* plant as a potential antioxidant and antidiabetic agent. During this process, four isolated pooled fractions of MeCv were obtained to evaluate preliminary phytochemical screening, total phenolic and flavonoid content, and in-vitro antioxidant and antidiabetic property. The presence of phenolic compounds and their related functional groups were determined by HPLC and FTIR analysis.

The preliminary phytochemical screening was used to identify the secondary metabolites in the crude extracts of plants. In the present study, the preliminary phytochemical analysis revealed the presence of flavonoids, phenols, steroids, alkaloids, lignin, tannins, glycosides, terpenoids, saponins, quinines, and coumarins in the MeCV. Medicinal and therapeutic properties of herbs are associated with their secondary metabolites. The effectiveness of the herbal remedies depends upon the solubility of these phytocompounds in various solvents. The results of preliminary phytochemical screening showed that the methanolic extract of *C. viscosa* (MeCV) whole plant has majority of phytochemicals. This may be due to the organic nature of methanol and its higher capacity to dissolve organic and active bioactive compounds [45]. The results of the present study confirm the previous studies, which have reported that methanol is a better solvent for more consistent extraction of compounds from medicinal plants compared to other solvents [46, 47].

Plants contain numerous phytochemical constituents, mainly polyphenols which are known to be biologically more active compounds and are responsible for exhibiting various pharmacological activities. The intervene plant polyphenols are significant wellspring of

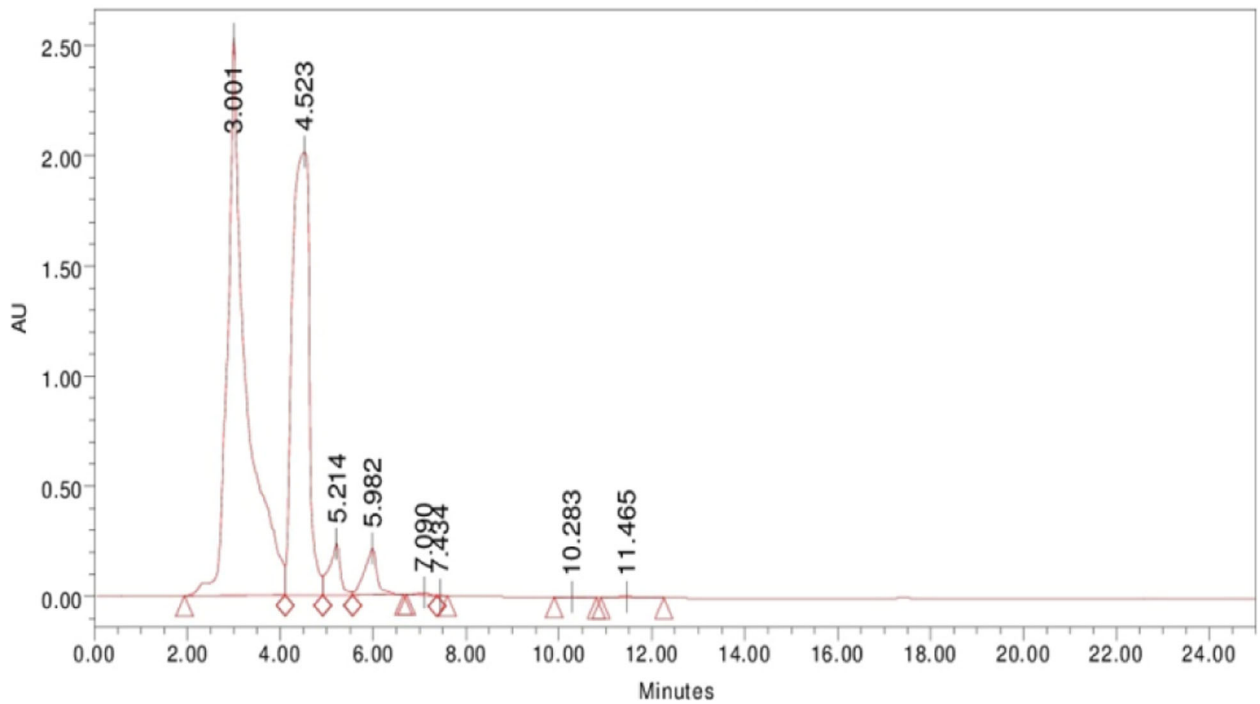

Fig. 9 Elution of polyphenols profile in bioactive F-D in HPLC. Major peaks were detected at 3.001 min and 4.523 min the elution was permitted up to 30 min

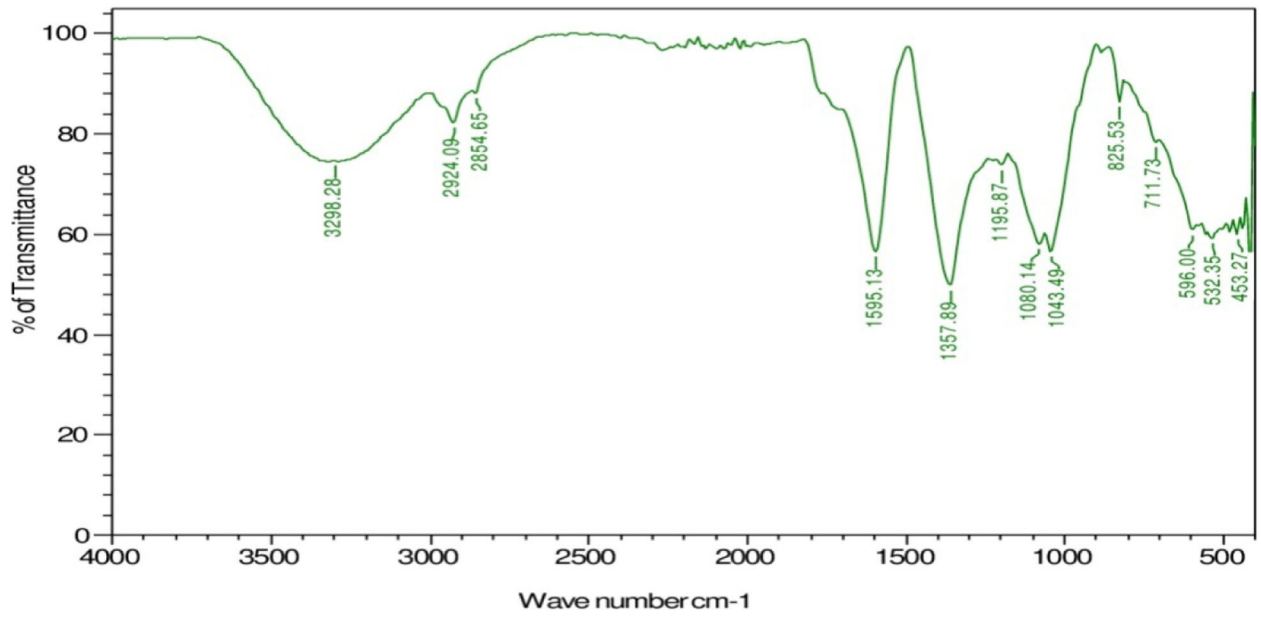

Fig. 10 FT-IR Spectrum scrutiny of bioactive F-D

natural antioxidants that are advanced larger than synthetic ones on account of safety concerns. The bioactive metabolites have been appeared to diminish the hazards and group of diseases, for example, cancer, cardiovascular, hepatotoxicity, neurodegenerative ailments, and so on by free radicals scavenging through different biological systems [48]. As indicated by Ananth et al. (2013), the free radical scavenging agents' action of polyphenols is to a great extent because of their redox properties which make them go about as lessening operators, donors of hydrogen, quenchers of singlet oxygen in addition to potential metal chelators [49]. In this examination, an impressive abnormal state of polyphenols (TCP and TCF) was seen in the F-D of the MeCV. This might clarify the broad traditional utilization of the plant.

In the current investigation, the increasing action of DPPH radical scavenging activity with increasing doses of the MeCV isolated fractions demonstrated an expanded capacity to give hydrogen particles bringing about a lighter arrangement which is corresponding to the number of electrons picked up [50]. Along these

Table 6 FT-IR peak values interpretation of bioactive F-D as follows

Peak value (cm^{-1})	Assignment of functional group	The intensity of functional group	Identified groups
3298.28	O–H stretch, H–bonded	Strong, broad	Alcohols, phenols
2924.09	C-H stretch	Strong	Alkanes and alkyls
2854.65	C-H stretch	Strong	Alkanes and alkyls
1595.13	N-H bend	Medium-strong	Amides
1357.89	C–H rock	Medium	Alkanes
1195.87	C–H wag (–CH2X)	Medium	Alkyl halides
1080.14	C–N stretch	Medium	Aliphatic amines
1043.49	C–N stretch	Medium	Aliphatic amines
825.53	C-Cl stretch	Strong	Alkyl halides
711.73	C–H rock	Medium	Alkanes
596.00	C–Cl stretch	Medium	Alkyl halides
532.35	C–Br stretch	Medium	Alkyl halides
453.27	C-I stretch	Strong	Alkyl halides

lines, it might be assumed that the MeCV isolated fractions have DPPH radicals scavenging activity, by lessening the radical to relating hydrazine because of its capacity of donating hydrogen particle. However, DPPH radical scavenging activity is lower than that of ABTS radical scavenging activity of isolated pooled fractions from MeCV. The chemical reaction of the radicals and the dissolvability of isolated fractions in various solvent systems are a few factors that have been accounted for to influence the limit of fractions to respond and quenching the various radicals [51]. Youn et al. (2019) found that the leaf extracts of *Dendropanax morbifera* have high ABTS scavenging activity than that of DPPH scavenging action [52].

As per Wu et al. (2006), the test of ABTS radical scavenging activity has been utilized as an index that revealed the antioxidant actions of test samples [53]. The ABTS radical scavenging activity of the MeCV isolated fractions in this study was observed to be as 92%; same as the standard ascorbic acid at the 100 μg/mL of maximum dose. Subramanian and Ramani (2020) also found similar observations in the ABTS radical scavenging activity of different solvent extracts of *Capparis brevispina* DC leaves [54].

Hydrogen peroxide (H_2O_2) occurs normally at low fixation levels noticeable all around, water, plants, human body, food, and microorganisms [55]. H_2O_2 is quickly decayed into O_2 and H_2O and this may create •OH (hydroxyl radicals) that can start lipid peroxidation (LPO) and cause DNA damage [56]. In the current examination, MeCV isolated fractions productively scavenged H_2O_2 which might be ascribed to the nearness of polyphenols that could give electrons to H_2O_2, resulting in subsequent deactivation of H_2O_2 to H_2O. Adebiyi et al. (2017) also reported similar outcomes in the H_2O_2 scavenging activity of different solvent extracts of *Grewia carpinifolia* [57].

Superoxide radical (O_2^-) is estimated as an important natural resource of reactive oxygen species (ROS) [58]. However, the O_2^- is a delicate oxidant; it suggests mount to potent and chain-breaking hydroxyl radicals (•OH) and singlet oxygen (O2) [59]. The aftereffects of our investigation revealed that F-D has a viable limit of scavenging capacity for O_2 and connected with total phenolic and flavonoid content along these lines recommending its antioxidant potential. Ketha et al. (2020) found the comparative impacts against superoxide radical scavenging action of methanolic solvent extracts of *Cardiospermum canescens* synthetic compounds which are in charge of their activity [60].

FRAP test was especially used to assess the measure of cell reinforcement or reductants nearness in a test sample that reacts with colorless Fe3+-TPTZ (ferric tripyridyltriazine) complex and structures a dark blue color Fe2+-TPTZ (ferrous tripyridyltriazine) complex [36]. In our present investigation, F-D demonstrated the most elevated and diminishing power followed by the F-A, F-B, and F-C. The compounds synthesized from *Anacardium occidentale* leaf extracts and *Clinacanthus nutans* whole plant have similar results [61, 62]. In this measure, redox reaction occurs in decreases Fe3+ to Fe2+ which contributes an electron in the nearness of antioxidants in the test sample.

Based on the results of above free radical scavenging activities, it is confirmed that the isolated pooled fractions obtained from MeCV acts as potential antioxidant agents. Generally, antioxidants are substances organized to scavenging or controlling the oxidation of the particles. Previously, the therapeutic field focused on antioxidant therapy in the management of various diseases, particularly diabetes. Before conducting the exploratory studies, the clinical trials have proved the capability of antioxidants in avoiding diabetic complications. In most of the cases, therapeutic plants with antioxidant action are considered for the treatment of diabetes mellitus [63].

The main objective of the treatment of diabetic patients during the period of treatment is to maintain the normal range of glucose in fasting and postprandial condition. The present study revealed the capacity of isolated pooled fraction obtained from MeCV was controlled glucose diffusion using in vitro method. In particularly, F-D demonstrates the potential inhibitory action of glucose diffusion increment that may be helpful for permit flexibility in meal plan in NIDDM. Our results are similar to Basha and Kumari (2012); the four various solvent extracts of *Psidium guajava* has a significant effect against the inhibitory impact on glucose diffusion [64].

Garg and Baliga (2002) also proved that controlling postprandial hyperglycaemia is a viable method for the management of diabetes mellitus particularly in NIDDM [65]. This can be proficient by inhibiting primary carbohydrate hydrolyzing enzymes, i.e., α-glucosidase and α-amylase (Fig. 7a and b) in the gastrointestinal tract. The capacity of the F-D to inhibit α-glucosidase and α-amylase in a dose-dependent manner might be credited to polyphenols in the fraction, which correspond with the report of Garg and Baliga (2002) [65]. The inhibitory properties of α-glucosidase and α-amylase by the F-D might be valuable in controlling the symptoms, i.e., flatulence, abdominal distention, and diarrhea. In this investigation, it very well may be reasoned that F-D restrained the movement of α-glucosidase and α-amylase (Fig. 8) in an equal manner of standard acarbose respectively. This might be credited to the rich substance of polyphenols in the part.

Taking everything into account, among the four isolated pooled fractions, F-D has a strong antioxidant property for treating diabetes and their related complications. Therefore, we have decided to choose F-D fraction for possible bioactive polyphenolic compounds detected by using HPLC analysis. The identified polyphenolic compounds were summarized in Table 5. The peaks of these compounds were recorded at 280 nm and were identified by comparison with standard compounds of polyphenols. However, the antidiabetic activity of the MeCV isolated F-D may be attributed to the presence of these compounds.

FTIR identifies and elucidates the chemical constituents which provide accurate measurements of the whole range of biological samples [66]. The F-D of MeCV exhibited phenols, alcohols, alkanes and alkyls, amides, aliphatic amines, alkyl halides, and alkanes. Identification and elucidation of polyphenols in the F-D was confirmed due to strong O–H and H–bonded stretching which is representing the phenols and alcohol functional groups. Similar results are reported by Choudhary and Mishra [67]. The peak at 2924.09 cm^{-1} and 2854.65 cm^{-1} is a strong C-H bonding stretch due to ether linkage and it indicates the adsorbed on the surface of F-D fraction. The presence of alkanes, alkyls, amides, aliphatic amines, alkyl halides, and alkanes in F-D may represent the other group of phytochemicals, which are present in the crude MeCV by preliminary phytochemical screening. These outcomes are similar with Maobe and Nyarango (2013) who stated that the functional groups of carboxylic acids, anhydrides, alcohols, phenols, amines, amides, esters, organic halogens, and carbohydrate could be responsible for the various medicinal properties of *Grewia tilifolia* [68].

However, the result we got in the present study is similar to several other documentation on the antioxidant therapy in the management of diabetes [67]. Recently, Gudise et al. (2019) also demonstrated that *Argyreia pierreana* and *Matelea denticulata* leaf extracts possess high antioxidant and antidiabetic potential which contains a high content of polyphenols [69]. The inhibitory effect on key enzymes is relevant to acute type-2 diabetes effect of ethanolic extract of *Artocarpus heterophyllus* antioxidant effects [70].

Conclusion

Findings from this investigation revealed that the MeCv isolated FD fraction showed higher glucose dispersion, α-glucosidase and α-amylase inhibitory potential, and antioxidant property. This superior activity of FD fraction might be due to their high phenolic and flavonoid content than the remaining three fractions. These results recommended that the plant of *C. viscosa* can be taken as a good natural source of remedial medicine for diabetes. However, further studies are needed to evaluate the accurate hypoglycemic and antioxidant effect of FD pooled fraction in order to utilize *C. viscosa* as a natural antioxidant and an antidiabetic agent.

Abbreviations

AAE: Ascorbic acid equivalents; ABTS: 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); ANOVA: Analysis of variance; CMC: Carboxymethyl cellulose; DNA: Deoxy ribonuclease; DPPH: 2,2-Diphenyl-1-picrylhydrazyl; DW: Dry weight; FRAP: Ferric reducing antioxidant power; FT-IR: Fourier transform infrared; GAE: Gallic acid equivalents; GDRI: Glucose dialysis retardation index; H_2O: Water; H_2O_2: Hydrogen peroxide; HPLC: High-performance liquid chromatography; IC_{50}: Inhibitory concentration at 50%; LPO: Lipid peroxidation; MeCV: Methanolic extract from traditional *Cleome viscosa*; NAD: Nicotinamide adenine dinucleotide; NBT: Nitro blue tetrazolium chloride; NIDDM: Noninsulin-dependent diabetes mellitus; O_2^{-}•: Superoxide radicals; OH•: Hydroxyl radical; PBS: Phosphate buffer saline; PMS: Phenazine methosulfate; QRE: Quercetin equivalents; R_f: Retention factor; ROS: Receptive oxygen species; RT: Retention times; SE: Standard error; SPSS: Statistical Package for the Social Sciences; TLC: Thin-layer chromatography; TPTZ: 2,4,6-Tris(2-pyridyl)-s-triazine

Acknowledgement

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Plant authentication

The *Cleome viscosa* (Family: Cleomaceae) whole plant material was identified and validating (No. NY/531) by Prof. N. Yasodamma, Taxonomist, Department of Botany, S.V. University, Tirupati, India.

Authors' contributions

YS participated in conceptualizing the study and designed the study, conducted the research, optimized methods, and participated in conceptualizing the study, drafting, and revising the manuscript. GR helped in conducting research methods, entering data and revising the manuscript. TL participated in conceptualizing the study, drafting and revising the manuscript. BLN participated in entering data and revising the manuscript. KSR participated in conceptualizing and supervising the study, revising the manuscript, and review. SRR conceptualized and designed the study, drafted and revised the manuscript, analyzed data and supervised the research work, review and editing. All authors had perused and approve the final edition of manuscript and consent to be responsible for all parts of the work in guaranteeing that questions related to the work are properly examined and resolved.

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Availability of data and materials

Data and material are available upon request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

There is no competing of interests.

Author details

[1]Division of Ethnopharmacology, Department of Biotechnology, School of Herbal studies and Naturo Sciences, Dravidian University, Kuppam, Andhra Pradesh 517426, India. [2]Department of Zoology, Sri Venkateswara University, Tirupati, Andhra Pradesh 517502, India.

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