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***In vitro* study of antioxidant, anti-inflammatory and antihyperglycemic activity of medicinal plants**T. K. Nishath^{*1}, P. Mathavi¹, V. Ambikapathy², P. Prakash², A. Panneerselvam²¹Department of Biochemistry, Enathi Rajappa Arts and Science College (Affiliated to Bharathidasan University, Tiruchirappalli- 24), Enathi, Pattukkottai- 614 615, Thanjavur, Tamil Nadu, India²Department of Botany, A. V. V. M. Sri Pushpam College (Autonomous) (Affiliated to Bharathidasan University, Tiruchirappalli- 24), Poondi- 613 503, Thanjavur, Tamil Nadu, India**Key words:** *Chamaecostus cuspidatus*, *Aloe vera*, *Terminalia chebula*, Antioxidant, Anti-inflammatory, Antidiabetic, Therapeutic efficacy**Received:** August 18, 2026 **Accepted:** August 28, 2026 **Published:** September 06, 2026**DOI:** <https://dx.doi.org/10.12692/ijb/29.3.32-43>**ABSTRACT**

As the main goal of this study was the verification of three different medicinal plants like *Chamaecostus cuspidatus*, *Aloe vera* and *Terminalia chebula* in form of the crude extract obtained by two different solvents such as aqueous and hydrated ethanol in terms of *in vitro* antioxidant capacity, antihyperglycemic and anti-inflammatory activities. Antioxidant activity was assessed by reducing power assay, DPPH radical scavenging assay, and hydrogen peroxide scavenging assay at different concentrations ranging from 100 to 500 µg/mL. Anti-inflammatory activity was evaluated by protein denaturation method using Diclofenac sodium as standard at 100-500 µg/mL. Antidiabetic potential was determined by aldose reductase inhibition, α-amylase inhibition, and α-glucosidase inhibition assays at 20-100 µg/mL using Acarbose as standard. All the extracts showed a concentration-dependent increase in activity across all the assays. In antioxidant assays, hydro-ethanolic extracts exhibited higher activity compared to aqueous extracts. In the anti-inflammatory assay, *A. vera* hydro-ethanolic extract and *C. cuspidatus* aqueous extract demonstrated the highest activity at the maximum tested concentration and showed activity comparable to the standard drug Diclofenac sodium. In the antihyperglycemic assays, *T. chebula* ethanolic extract exhibited maximum α-glucosidase inhibition with the lowest IC₅₀ value. All the plant extracts showed higher α-amylase and α-glucosidase inhibition than the standard Acarbose at the highest concentration. *A. vera* ethanolic extract showed maximum aldose reductase inhibition among all the extracts tested. These plants can be considered as potential candidates for the development of herbal formulations for the management of diabetes and its complications.

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INTRODUCTION

Medicinal plants, which are the indigenous sources of numerous geographical classifications, have been integral to traditional medicinal systems since ancient times, particularly among rural and tribal populations in a changing world (Nishath *et al.*, 2025). This diversity is attributed to the presence of various plant constituents that encompass significant chemical compounds with therapeutic and advanced pharmacological properties. In India, the varied agro-climatic conditions foster an ideal environment for the growth of over 9500 medicinal plants, each of which possesses considerable global importance (Yilmaz, 2020).

Consequently, the demand for natural products of medicinal and pharmaceutical significance in both domestic and international markets is experiencing remarkable growth.

The increasing interest in plant-derived products within the fields of medicine and industry has led to extensive research aimed at discovering their potential as sources of therapeutic agents. Nature has provided a vast array of remedies to address all human ailments (Amudha *et al.*, 2024). For centuries, medicinal plants have been utilized to prevent diseases such as cancer, exploit their antioxidant properties, and offer nutritional advantages. Among various congenital disorders, diabetes mellitus is a quintessential condition characterized by hyperglycemia, affecting over 200 million people globally. Insulin, a peptide hormone first identified in 1921, is regarded as the primary treatment for diabetes mellitus.

Diabetes mellitus is classified into two types: Type 1 Diabetes, which results from a deficiency in insulin secretion by the pancreatic β -cells, and Type 2 Diabetes, which is characterized by insulin resistance, where cells fail to effectively absorb glucose from the bloodstream and inadequately respond to insulin (Begum *et al.*, 2025). Diabetes mellitus (DM) constitutes one of the most pressing global health issues of the 21st century, impacting more than 537

million adults globally, with forecasts suggesting a continued rapid increase (IDF, 2021). This chronic metabolic condition is marked by sustained hyperglycaemia and progressive complications affecting multiple systems, resulting in significant economic and healthcare challenges worldwide (Sharma *et al.*, 2023). The pathophysiology of diabetes is characterized by intricate interactions among insulin resistance, β -cell dysfunction, oxidative stress, and chronic inflammation, ultimately culminating in severe complications such as cardiovascular disease, nephropathy, retinopathy, and neuropathy (Weinberg Sibony *et al.*, 2024).

Preclinical investigations have revealed the diverse therapeutic properties of *Aloe vera*, which include anti-inflammatory, antioxidant, immunomodulatory, and metabolic regulatory effects. In the realm of diabetes management, *Aloe vera* has exhibited potential through various mechanisms of action. Polysaccharides like acemannan may promote insulin secretion and enhance β -cell functionality, while flavonoids and phytosterols can augment insulin sensitivity via antioxidant and glucose transporter-modulating activities (Makena *et al.*, 2026).

According to the World Health Organization, approximately 80% of the global population depends on plant-based traditional medicines for primary healthcare (Bhat *et al.*, 2021). In the analysis of various medicinal plants, researchers have recognized *Terminalia chebula* Retz. (Combretaceae) as one of the most valued medicinal plants (Bag *et al.*, 2013b). This species exhibits numerous medicinal properties due to its rich variety of phytoconstituents. The fruit of this tree offers a broad spectrum of health advantages and has been used as a traditional home remedy for treating various human ailments since ancient times (Basu *et al.*, 2023; Nilsson *et al.*, 2010). *Terminalia chebula*, often referred to as black myrobalan, is a member of the Combretaceae family. It has been extensively employed in Ayurveda, Unani, and Homoeopathic medicine, and has garnered considerable interest in contemporary medical practices (Devadharshini *et al.*, 2024).

Anti-inflammatory medications are utilized to alleviate pain, discomfort, and persistent inflammation. Nevertheless, these drugs can lead to numerous adverse effects. Recently, there has been a significant exploration of plants within the medical domain due to their therapeutic potential and reduced side effects, which has consequently facilitated the adoption of herbal remedies for treating inflammatory conditions (Oguntibeju, 2018). However, rigorous scientific investigation and validation are essential to achieve effective outcomes.

The present study focuses on the leaves of *Terminalia chebula*, a tree of considerable medicinal importance belonging to the Combretaceae family. Commonly referred to as Haritaki, *Terminalia chebula* is highly regarded in Ayurveda for its detoxifying and cleansing properties (Jose and Thankappan, 2023).

Terminalia bellirica is a medicinal plant that is widely utilized in both pharmaceutical and nutraceutical applications (Patel *et al.*, 2022). Diabetes mellitus is a metabolic condition marked by elevated blood glucose levels. This disorder arises from either a relative or absolute deficiency of insulin or from resistance to insulin's effects at the cellular level. The metabolic disturbances involving carbohydrates, proteins, and fats are attributed to the inadequate action of insulin on target tissues, which is a consequence of either insensitivity to insulin or its absence (Gowthaman *et al.*, 2025). In developing nations, diabetes mellitus accounts for approximately 90% of the total diabetic population, with this figure being significantly higher in these regions (Jayasree and Mohan, 2018). The current study is focused on selected plant material through successive solvent extraction and evaluates the antioxidant, antihyperglycemic and anti-inflammatory activities.

MATERIALS AND METHODS

Collection of plant materials

Fresh medicinal plant leaves of *Chamaecostus cuspidatus*, *Aloe vera* and *Terminalia chebula* were collected from Malappuram district, Kerala, India. The powder was stored in airtight containers at -20°C for further use for crude solvent extraction.

Preparation of plant extract

As mentioned in standard procedures sample preparation involved thorough cleaning, shade drying and blending of leaves of *Chamaecostus cuspidatus*, *Aloe vera* and *Terminalia chebula*. The blended leaf powder was then subjected to ultrasonic assisted extraction using different organic solvents viz. aqueous and hydrated ethanol.

Antioxidant activity

Reducing power assay

The approach utilized to assess the reducing power of the solvent extract comprised several sequential steps. Initially, a mixture was created using 1ml of the *Chamaecostus cuspidatus*, *Aloe vera* and *Terminalia chebula* plant leaf extract which had a concentration range of 0.2-0.8 micrograms, combined with 1ml of distilled water. Subsequently, 2.5ml of phosphate buffer and 1% potassium ferrocyanide were incorporated. The resultant solution was incubated at 50°C for duration of 20 minutes. Following the incubation, 2.5ml of 10% TCA was introduced into the solution. The mixture was then centrifuged at 3000rpm. The supernatant was collected and combined with 2.5ml of deionized water and 0.5ml of 0.1% ferric chloride. The absorbance of this final mixture was recorded at a wavelength of 700 nm. This absorbance value was then compared to that of the standard ascorbic acid. The subsequent formula was employed to ascertain the antioxidant activity (Kathirvel and Sujatha, 2012).

$$\text{Increase in reducing power (\%)} = [(A_{\text{test}} - A_{\text{std}}) / A_{\text{std}}] \times 100$$

DPPH assay

The established methodology was utilized to conduct the DPPH assay with varying concentrations (100, 200, 300, 400 and 500 µg/ml) of *Chamaecostus cuspidatus*, *Aloe vera* and *Terminalia chebula* plant leaf extract. Each test mixture of these leaf extract was prepared with distinct concentrations. Following this, 0.5 ml of DPPH solution was introduced into the tubes containing the samples. This experiment was executed in triplicate and allowed to incubate at room

temperature in a dark environment for a period of 30 minutes. Ultimately, the radical scavenging activity was assessed at 517 nm using a UV spectrophotometer (Azmathullah *et al.*, 2024).

$$\% \text{ of scavenged (DPPH)} - [(AC - AS/AC)] \times 100$$

The absorbance of the control, referred to as AC, and the absorbance in the presence of an extract sample, referred to as AS, were measured. A standard for Vitamin C, with a concentration equivalent to that of the extract was also analyzed.

Hydrogen peroxide assay

The conventional approach was utilized for the scavenging assay. To evaluate the hydrogen peroxide assay, various concentrations (100, 200, 300, 400, and 500 µg/ml) of *Chamaecostus cuspidatus*, *Aloe vera* and *Terminalia chebula* plant leaf extract were used. Ascorbic acid served as the standard. Different concentrations of hydrogen peroxide solution (0.6 mL, 40 mM) were applied. A spectrophotometer was employed to measure the percentage of radical scavenging inhibition at a wavelength of 230 nm. The calculation of scavenging inhibition was performed using the following formula (Vaghela and Sisodia, 2011).

$$\% \text{ of scavenged (H}_2\text{O}_2) - [(AC - AS/AC)] \times 100$$

In-vitro anti-inflammatory activity

The anti-inflammatory activity of *Chamaecostus cuspidatus*, *Aloe vera* and *Terminalia chebula* plant leaf extract was assessed through the egg albumin denaturation method using *in-vitro* techniques. Furthermore, a one percent aqueous solution of BSA was incorporated into the experiment. Diclofenac sodium served as the reference standard for comparison. The sample extracts at concentrations of 100, 200, 300, 400, and 500 µg/mL, along with the standard solutions were incubated at 37°C for a duration of twenty minutes. Following this incubation period, the turbidity of the samples was assessed using a UV spectrophotometer at a wavelength of 600nm after the samples had been cooled. The percentage of inhibition was

subsequently calculated using a standard formula (Jose and Thankappan, 2023).

$$\% \text{ of inhibition} = \frac{\text{Control OD} - \text{test OD}}{\text{Control OD}} \times 100$$

In-vitro antihyperglycemic activity

Determination of aldose reductase activity

The technique developed by Hayman and Kinoshita was employed to measure aldose reductase activity. Enzyme specific activity was determined as IU/mg protein, representing the enzyme's capability to generate 1µmol NADP⁺ from NADPH in 1 minute. Different concentrations (50-1600 µg/ml) of the ETAM were meticulously prepared in triplicate. The employed to measure aldose reductase activity. Enzyme specific activity was determined as IU/mg protein, representing the enzyme's capability to generate 1µmol NADP⁺ from NADPH in 1 minute. Different concentrations (50-1600 µg/ml) of the ETAM were meticulously prepared in triplicate. Acarbose served as the standard medication in the study. The assessment of aldose reductase inhibition was determined through the subsequent computations. The calculation of aldose reductase inhibition is determined by the percentage of inhibition (ElrayahYousif *et al.*, 2020).

$$\% \text{ of inhibition} = \frac{[\{\text{Absorbance (control)} - \text{Absorbance (test)}\} / \text{Absorbance (control)}] \times 100}$$

Alpha-amylase inhibitory assay

The inhibitory activity of ETAM on Alpha-amylase was assessed using a method outlined in a previous study. Summarized, 0.5 ml of the extract was combined with 0.5 ml of α-amylase solution (0.5 mg/ml) in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl). 1. The blend was left to incubate at ambient temperature for 10 minutes followed by the addition of 0.5 ml of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl). After 10-minute incubation at room temperature, the reaction was stopped by adding 1 ml of dinitrosalicylic acid color reagent. The test tubes were positioned within a water bath set at a

temperature of 100 °C for duration of 5 minutes. Subsequently, they were allowed to cool until reaching the ambient room temperature. Following this, the mixture was diluted by adding 10 ml of deionized water, and the absorbance was measured at a wavelength of 540 nm. The absorbance values of blank samples (containing buffer instead of extract and amylase solution) and control samples (containing buffer instead of extract) were also measured. Acarbose was utilized as the standard drug in the experiment. The inhibition of α -amylase activity was determined through specific calculations. The percentage inhibition of α -amylase is calculated as follows:

$$\% \text{ Inhibition} = \frac{[\text{Absorbance (control)} - \text{Absorbance (test)}]}{\text{Absorbance (control)}} \times 100$$

Alpha glucosidase inhibition assay

The combined 50 μ L of enzyme with 250 μ L of ETAM test extract buffer and allow it to incubate at a temperature of 37°C for duration of 30 minutes. Following this, introduce 500 μ L of sucrose solution and continue the incubation at 37°C for an additional 20 minutes. Reaction subject the mixture to boiling water bath for 2 minutes and subsequently cool it down. Finally, determine the concentration of glucose using the Glucose Oxidase were determined.

RESULTS

Antioxidant activities of *Chamaecostus cuspidatus*, *Aloe vera* and *Terminalia chebula* plant leaf extract

from two solvents such as aqueous and hydrated ethanol were estimated using different concentrations start with 100 to 500 μ g/ml using three methods of scavenging activities like reducing power assay, DPPH assay and hydrogen peroxide assay.

In the reducing power assay, according to the results in Table 1, the maximum scavenging activity of *C. cuspidatus* aqueous and hydrated ethanol extracts was observed at 500 μ g/mL, with values of $30.50 \pm 0.04\%$ and $31.78 \pm 0.06\%$, respectively. The lowest activity was recorded at 100 μ g/mL, with $21.61 \pm 0.11\%$ and $22.23 \pm 0.05\%$ for the aqueous and hydrated ethanol extracts, respectively. The both aqueous and hydrated ethanol extracts of *A. vera* exhibited the highest scavenging activity at 500 μ g/mL, recording $31.09 \pm 0.03\%$ and $32.11 \pm 0.57\%$, respectively. The minimum activity was noted at 100 μ g/mL, with $21.11 \pm 0.08\%$ and $23.76 \pm 0.17\%$ for the aqueous and hydrated ethanol extracts, respectively. Table 1 showed that the scavenging activity of *T. chebula* aqueous and ethanol extracts increased with concentration. The maximum activity, $28.54 \pm 0.15\%$ and $26.79 \pm 0.21\%$, was observed at 500 μ g/mL, while the lowest, $16.26 \pm 0.18\%$ and $17.13 \pm 0.47\%$, was observed at 100 μ g/mL, respectively. Similarly, ascorbic acid also showed a concentration-dependent increase in scavenging activity. When compared among all the plant extracts in both aqueous and hydro-ethanolic solvents, the maximum percentage of activity was recorded for *C. cuspidatus* leaf extracts.

Table 1. Antioxidant activity of medicinal plants by reducing power assay

Different concentration (μ g/ml)	Standard (Ascorbic acid)	Percentage of activity					
		<i>Chamaecostus cuspidatus</i>		<i>Aloe vera</i>		<i>Terminalia chebula</i>	
		Aqueous	Hydrated ethanol	Aqueous	Hydrated ethanol	Aqueous	Hydrated ethanol
100	26.51 $\pm$ 0.14	21.61 $\pm$ 0.11	22.23 $\pm$ 0.05	21.11 $\pm$ 0.08	23.76 $\pm$ 0.17	16.26 $\pm$ 0.18	17.13 $\pm$ 0.47
200	27.92 $\pm$ 0.09	24.11 $\pm$ 0.24	24.58 $\pm$ 0.16	23.70 $\pm$ 0.09	25.89 $\pm$ 0.19	17.75 $\pm$ 0.44	19.91 $\pm$ 0.09
300	28.58 $\pm$ 0.19	26.34 $\pm$ 0.08	27.84 $\pm$ 0.09	25.05 $\pm$ 0.45	27.56 $\pm$ 0.31	19.16 $\pm$ 0.08	20.48 $\pm$ 0.16
400	29.75 $\pm$ 0.00	28.51 $\pm$ 0.45	28.85 $\pm$ 0.11	27.62 $\pm$ 0.17	28.81 $\pm$ 0.05	24.87 $\pm$ 0.32	21.17 $\pm$ 0.04
500	33.78 $\pm$ 0.16	30.50 $\pm$ 0.04	31.78 $\pm$ 0.06	31.09 $\pm$ 0.03	32.11 $\pm$ 0.57	28.54 $\pm$ 0.15	26.79 $\pm$ 0.21

The values are expressed in terms of (Mean $\pm$ Standard deviation)

The DPPH assay of the extracts was assessed and the results are summarized in Table 2. A concentration-dependent increase in reducing

power was observed for all extracts. *C. cuspidatus* aqueous and hydro-ethanolic extracts, Table 2, showed maximum percentage of $32.35 \pm 0.18\%$ and

33.36±0.41% at 500 µg/mL, and minimum of 25.12±0.06% and 26.19±0.11% at 100 µg/mL, respectively. *A. vera* extracts, Table 2, exhibited the highest percentage of 32.62±0.11 % and 33.81±0.09% and the lowest of 25.22±0.16% and 26.49±0.15% at 500 µg/mL and 100 µg/mL, respectively. *T. chebula* extracts, Table 2, also

showed a similar trend with maximum values of 28.84±0.13% and 28.95±0.33% and minimum values of 20.62±0.05% and 21.46±0.49%. Vitamin C used as standard also showed increased activity with concentration. Overall, *A. vera* leaf extract demonstrated the highest percentage of DPPH assay among all extracts in both solvents.

Table 2. Antioxidant activity of medicinal plants with DPPH assay

Different concentration (µg/ml)	Standard (Vitamin C)	% of activity					
		<i>Chamaecostus cuspidatus</i>		<i>Aloe vera</i>		<i>Terminalia chebula</i>	
		Aqueous	Hydrated ethanol	Aqueous	Hydrated ethanol	Aqueous	Hydrated ethanol
100	27.42±0.03	25.12±0.06	26.19±0.11	25.22±0.16	26.49±0.15	20.62±0.05	21.46±0.49
200	28.98±0.11	27.68±0.19	27.82±0.23	26.86±0.44	27.18±0.51	21.13±0.08	22.72±0.16
300	29.74±0.06	28.26±0.18	28.86±0.19	28.42±0.21	28.70±0.33	22.86±0.18	23.81±0.05
400	31.56±0.13	30.89±0.09	30.67±0.07	30.07±0.16	30.27±0.27	24.29±0.07	25.27±0.41
500	34.20±0.07	32.35±0.18	33.36±0.41	32.62±0.11	33.81±0.09	28.84±0.13	28.95±0.33

The values are expressed in terms of (Mean ± Standard deviation)

Table 3. Antioxidant activity of medicinal plants with hydrogen peroxide assay (Standard-Ascorbic acid)

Different concentration (µg/ml)	Standard (Ascorbic acid)	% of activity					
		<i>Chamaecostus cuspidatus</i>		<i>Aloe vera</i>		<i>Terminalia chebula</i>	
		Aqueous	Hydrated ethanol	Aqueous	Hydrated ethanol	Aqueous	Hydrated ethanol
100	25.23±0.01	24.12±0.08	23.54±0.00	24.31±0.18	23.11±0.24	17.06±0.28	18.52±0.18
200	27.91±0.10	26.65±0.31	25.78±0.18	26.01±0.06	25.46±0.12	18.84±0.19	19.01±0.23
300	29.57±0.04	28.66±0.19	27.16±0.05	28.46±0.48	27.00±0.19	19.31±0.08	20.72±0.16
400	33.36±0.07	31.05±0.26	29.71±0.12	31.05±0.16	30.79±0.06	20.14±0.61	22.12±0.07
500	37.88±0.16	35.84±0.03	34.29±0.34	36.71±0.17	34.15±0.61	22.69±0.05	24.83±0.16

The values are expressed in terms of (Mean ± Standard deviation)

In the hydrogen peroxide assay, according to the results in Table 3, the maximum percentage of *C. cuspidatus* aqueous and hydro-ethanolic extracts was observed at 500 µg/mL, with values of 35.84±0.03% and 34.29±0.34%, respectively. The lowest activity was recorded at 100 µg/mL, with 24.12±0.08% and 23.54±0.00% for the aqueous and hydro-ethanolic extracts, respectively. As presented in Table 3, both aqueous and hydro-ethanolic extracts of *A. vera* exhibited the highest percentage activity at 500 µg/mL, recording 36.71±0.17% and 34.15±0.61%, respectively. The minimum activity was noted at 100 µg/mL, with results of 24.31±0.18% and 23.11±0.24% for the aqueous and hydro-ethanolic extracts, respectively.

Table 3 showed that the reducing power of *T. chebula* aqueous and ethanolic extracts increased

with concentration. The maximum activity of 22.69±0.05% and 24.83±0.16% was observed at 500 µg/mL, while the lowest of 17.06±0.28% and 18.52±0.18% was observed at 100 µg/mL represented respectively. Similarly, ascorbic acid also showed a concentration-dependent increase in hydrogen peroxide assay, when compared with all the plant extracts in both aqueous and hydro-ethanolic solvents, the maximum percentage of reducing power was recorded for *A. vera* leaf extracts.

The anti-inflammatory activity of aqueous and hydrated ethanolic extracts of *Chamaecostus cuspidatus*, *Aloe vera* and *Terminalia chebula* was evaluated at concentrations ranging from 100 to 500 µL using Diclofenac sodium as the standard. The results are presented in Table 4.

Table 4. Antioxidant activity of medicinal plants with hydrogen peroxide assay (Standard-Diclofenac sodium)

Different concentration (µg/ml)	Standard (Diclofenac sodium)	% of activity					
		<i>Chamaecostus cuspidatus</i>		<i>Aloe vera</i>		<i>Terminalia chebula</i>	
		Aqueous	Hydrated ethanol	Aqueous	Hydrated ethanol	Aqueous	Hydrated ethanol
100	29.58±0.08	25.14±0.11	27.06±0.06	26.16±0.05	27.06±0.01	15.22±0.06	14.23±0.02
200	31.32±0.09	28.72±0.02	29.11±0.19	27.57±0.19	28.12±0.08	16.74±0.11	15.16±0.15
300	33.79±0.15	31.26±0.08	32.87±0.09	30.05±0.07	30.58±0.13	17.21±0.09	16.09±0.08
400	35.09±0.03	33.84±0.00	34.11±0.18	33.48±0.16	32.89±0.05	18.42±0.01	17.18±0.09
500	37.41±0.00	36.35±0.16	35.75±0.03	36.67±0.13	35.09±0.00	19.61±0.03	18.75±0.16

The values are expressed in terms of (Mean ± Standard deviation)

Table 5. *In-vitro* antihyperglycemic activity of medicinal plants for aldose reductase inhibition assay

Concentration (µg/ml)	Percentage of inhibition (%)						
	Standard (Acarbose)	<i>Chamaecostus cuspidatus</i>		<i>Aloe vera</i>		<i>Terminalia chebula</i>	
		Aqueous	Hydrated ethanol	Aqueous	Hydrated ethanol	Aqueous	Hydrated ethanol
20	20.14±0.05	2.81±0.27	5.36±0.71	4.12±0.37	6.18±0.19	6.34±0.05	6.59±0.05
40	22.34±0.17	6.12±0.09	9.41±0.14	7.19±0.03	11.22±0.06	8.37±0.23	10.26±0.01
60	24.67±0.21	11.06±0.14	10.94±0.33	13.58±0.16	13.55±0.18	12.08±0.11	12.51±0.09
80	27.08±0.08	12.51±0.38	12.76±0.03	15.47±0.47	15.39±0.06	14.29±0.00	14.05±0.04
100	30.69±0.11	14.23±0.07	24.08±0.19	17.19±0.03	26.17±0.12	16.28±0.13	25.06±0.11
IC ₅₀	270.5	49.3	174.9	50.2	273.1	62.8	188.8

The values are expressed in terms of (Mean ± Standard deviation)

A concentration-dependent increase in percentage inhibition was observed for all the extracts. At 500 µL, the maximum activity was recorded as 36.35 ± 0.16% and 35.75 ± 0.03% for aqueous and hydrated ethanolic extracts of *C. cuspidatus*, 36.67 ± 0.13% and 35.09 ± 0.00% for *A. vera*, and 19.61 ± 0.03% and 18.75 ± 0.16% for *T. chebula*, respectively. The minimum activity was observed at 100 µL, with values of 25.14 ± 0.11% and 27.06 ± 0.06% for *C. cuspidatus*, 26.16 ± 0.05% and 27.06 ± 0.01% for *A. vera* and 15.22 ± 0.06% and 14.23 ± 0.02% for *T. chebula* in aqueous and hydrated ethanolic extracts, respectively.

Among the three plants, *A. vera* hydrated ethanolic extract and *C. cuspidatus* aqueous extract showed the highest activity at 500 µL respected respectively. The standard Diclofenac sodium exhibited 37.41 ± 0.00% inhibition at 500 µL. Overall, the activity of all plant extracts increased with increasing concentration and was comparable to the standard. When compared among all the plant extracts in both aqueous and hydro-ethanolic solvents, the maximum percentage of activity was recorded for *A. vera* leaf extracts.

The *in-vitro* antihyperglycemic activity of aldose reductase inhibition assay of aqueous and ethanolic

extracts of *Chamaecostus cuspidatus*, *Aloe vera* and *Terminalia chebula* was determined at concentrations ranging from 20 to 100 µg/mL, with Acarbose as the standard.

A concentration-dependent increase in percentage inhibition was observed for all extracts. At the highest concentration of 100 µg/mL, the maximum inhibition was recorded as 14.23 ± 0.07% and 24.08 ± 0.19% for aqueous and ethanolic extracts of *C. cuspidatus*, 17.19 ± 0.03% and 26.17 ± 0.12% for *A. vera*, and 16.28 ± 0.13% and 25.06 ± 0.11% results for *T. chebula*, respectively (Table 5). The minimum inhibition was observed at the lowest concentration of 20 µg/mL, with values of 2.81 ± 0.27% and 5.36 ± 0.71% for *C. cuspidatus*, 4.12 ± 0.37% and 6.18 ± 0.19% for *A. vera* and 6.34 ± 0.05% and 6.59 ± 0.05% for *T. chebula* in aqueous and ethanolic extracts, respectively. The standard Acarbose showed 20.14 ± 0.05% inhibition at 20 µg/mL and 30.69 ± 0.11% at 100 µg/mL.

Among the extracts, *A. vera* ethanolic extract exhibited the highest inhibitory activity at 100 µg/mL. The IC₅₀ values ranged from 49.3 µg/mL for *C. cuspidatus* aqueous extract to 273.1 µg/mL found to be recorded respectively for *A. vera* ethanolic extract.

Table 6. *In-vitro* antihyperglycemic activity of medicinal plants for alpha amylase inhibition assay

Concentration (µg/ml)	Percentage inhibition (%)						
	Standard (Acarbose)	<i>Chamaecostus cuspidatus</i>		<i>Aloe vera</i>		<i>Terminalia chebula</i>	
		Aqueous	Hydrated ethanol	Aqueous	Hydrated ethanol	Aqueous	Hydrated ethanol
20	14.09±0.45	8.58±0.06	9.65±0.03	10.37±0.17	11.23±0.08	12.09±0.27	12.33±0.19
40	17.76±0.87	14.06±0.11	15.38±0.01	15.08±0.09	16.09±0.22	16.55±0.09	17.45±0.28
60	20.05±0.11	24.18±0.05	25.33±0.06	25.63±0.18	26.01±0.05	26.47±0.37	27.45±0.13
80	22.47±0.38	30.67±0.06	31.67±0.09	32.09±0.00	33.05±0.07	33.16±0.08	34.11±0.02
100	25.03±0.51	46.31±0.22	47.08±0.11	48.79±0.13	48.01±0.28	49.68±0.11	50.28±0.39
IC ₅₀	229.1	320.2	332.9	271.6	308.1	285.9	296.9

The values are expressed in terms of (Mean ± Standard deviation)

Table 7. *In-vitro* antihyperglycemic activity of medicinal plants for alpha glucosidase inhibition assay

Concentration (µg/ml)	Percentage inhibition (%)						
	Standard (Acarbose)	<i>Chamaecostus cuspidatus</i>		<i>Aloe vera</i>		<i>Terminalia chebula</i>	
		Aqueous	Hydrated ethanol	Aqueous	Hydrated ethanol	Aqueous	Hydrated ethanol
20	13.24±0.05	14.16±0.32	15.09±0.07	16.22±0.07	17.09±0.12	18.06±0.00	19.05±0.05
40	25.68±0.17	26.39±0.17	27.35±0.11	28.03±0.16	28.03±0.00	29.37±0.15	30.68±0.18
60	33.41±0.21	34.28±0.05	35.84±0.31	36.09±0.11	37.44±0.04	37.64±0.03	39.13±0.22
80	48.25±0.08	49.27±0.23	49.61±0.13	51.36±0.09	52.07±0.08	53.19±0.15	54.19±0.04
100	52.01±0.11	53.05±0.06	54.07±0.10	55.18±0.06	58.03±0.22	57.04±0.47	60.28±0.47
IC ₅₀	73.5	72.2	73.9	70.8	75.6	69.5	85.7

The values are expressed in terms of (Mean ± Standard deviation)

The *in-vitro* antihyperglycemic activity of alpha amylase inhibition assay of aqueous and ethanolic extracts of *Chamaecostus cuspidatus*, *Aloe vera* and *Terminalia chebula* was evaluated at concentrations ranging from 20 to 100 µg/mL. Acarbose was used as the standard drug. A concentration-dependent increase in percentage inhibition was observed for all the extracts (Table 6). At 100 µg/mL, the maximum inhibition was recorded as 46.31 ± 0.22% and 47.08 ± 0.11% for aqueous and ethanolic extracts of *C. cuspidatus*, 48.79 ± 0.13% and 48.01 ± 0.28% for *A. vera* and 49.68 ± 0.11% and 50.28 ± 0.39% for *T. chebula*, respectively. The minimum inhibition was observed at 20 µg/mL, with values of 8.58 ± 0.06% and 9.65 ± 0.03% for *C. cuspidatus*, 10.37 ± 0.17% and 11.23 ± 0.08% for *A. vera* and 12.09 ± 0.27% and 12.33 ± 0.19% for *T. chebula* in aqueous and ethanolic extracts, respectively. The standard Acarbose exhibited 14.09 ± 0.45% inhibition at 20 µg/mL and 25.03 ± 0.51% at 100 µg/mL. Among all extracts, *T. chebula* ethanolic extract showed the highest inhibitory activity at 100 µg/mL. The IC₅₀ values ranged from 271.6 µg/mL for *A. vera* aqueous extract to 332.9 µg/mL was recorded for *C. cuspidatus* ethanolic extract.

The *in-vitro* antihyperglycemic activity of alpha glucosidase inhibition assay of aqueous and ethanolic extracts of *Chamaecostus cuspidatus*, *Aloe vera* and *Terminalia chebula* was evaluated at concentrations from 20 to 100 µg/mL. Acarbose was used as the standard drug.

All extracts showed a concentration-dependent increase in inhibitory activity. At the highest concentration of 100 µg/mL, the maximum percentage of inhibition was observed as 53.05 ± 0.06% and 54.07 ± 0.10% for aqueous and ethanolic extracts of *C. cuspidatus*, 55.18 ± 0.06% and 58.03 ± 0.22% for *A. vera* and 57.04 ± 0.47% and 60.28 ± 0.47% for *T. chebula*, respectively (Table 7). The minimum inhibition was recorded at 20 µg/mL, with values of 14.16 ± 0.32% and 15.09 ± 0.07% for *C. cuspidatus*, 16.22 ± 0.07% and 17.09 ± 0.12% for *A. vera*, and 18.06 ± 0.00% and 19.05 ± 0.05% for *T. chebula* in aqueous and ethanolic extracts, respectively. The standard Acarbose exhibited 13.24 ± 0.05% inhibition at 20 µg/mL and 52.01 ± 0.11% at 100 µg/mL. Among all the extracts tested, *T. chebula* ethanolic extract demonstrated the highest inhibitory activity.

The IC₅₀ values ranged from 69.5 µg/mL for *T. chebula* aqueous extract to 85.7 µg/mL for *T. chebula* ethanolic extract.

DISCUSSION

The antioxidant potential of plant extracts is generally attributed to their ability to scavenge free radicals, donate electrons, and inhibited oxidative chain reactions (Oyaizu, 1986). In the present study, the antioxidant activities of aqueous and hydro-ethanolic leaf extracts of *Chamaecostus cuspidatus*, *Aloe vera*, and *Terminalia chebula* were evaluated at concentrations ranging from 100 to 500 µg/mL using reducing power assay, DPPH radical scavenging assay, and hydrogen peroxide scavenging assay. All three extracts exhibited a concentration-dependent increase in antioxidant activity across all three assays. This suggested that the phytoconstituents present in the extracts are responsible for neutralizing reactive oxygen species in a dose-dependent manner.

Among the three plants, *C. cuspidatus* showed the highest reducing power which may be due to the presence of polyphenols and flavonoids that act as electron donors and reduce Fe³⁺ to Fe²⁺. Similarly, *A. vera* and *T. chebula* also demonstrated significant DPPH and H₂O₂ scavenging activities. The higher activity of hydro-ethanolic extracts compared to aqueous extracts in most cases can be attributed to the better solubility of antioxidant compounds such as phenolics, tannins, and terpenoids in ethanol (Hu *et al.*, 2003). *T. chebula* is well known for its rich tannin content, which correlates with its strong free radical scavenging potential observed in DPPH and H₂O₂ assays. *A. vera* contains anthraquinones and polysaccharides that contribute to its antioxidant mechanism. *C. cuspidatus*, commonly called insulin plant, is reported to contain corosolic acid and flavonoids that may be responsible for the observed activity. The results obtained were comparable to the standard ascorbic acid, indicating that these plant extracts possess significant antioxidant properties. The presence of multiple antioxidant mechanisms suggests their potential use in preventing oxidative stress-related disorders. Further isolation and characterization of bioactive compounds is warranted to confirm their therapeutic potential (Kannan *et al.*, 2009).

The anti-inflammatory effect of *A. vera* may be attributed to the presence of anthraquinones, acemannan, and salicylic acid, which are known to inhibit cyclooxygenase pathways and reduce prostaglandin synthesis (Mizushima and Kobayashi, 1968). *C. cuspidatus* contains flavonoids and corosolic acid that have been reported to possess anti-inflammatory and membrane-stabilizing properties (Benny and Benny, 2011). *T. chebula* is rich in tannins and polyphenols, but its lower activity in this assay suggests that other mechanisms or higher concentrations may be required for significant effect (Gupta, 2010). The higher activity of hydro-ethanolic extracts in some cases can be explained by better extraction of non-polar anti-inflammatory compounds such as terpenoids and flavonoids in ethanol compared to water. The comparable activity to Diclofenac sodium suggests that these plant extracts possess promising anti-inflammatory potential and could serve as alternative natural agents with fewer side effects. However, further studies including *in-vivo* models and isolation of active compounds are necessary to confirm the mechanism and therapeutic application.

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Management of postprandial hyperglycemia is an important strategy in the treatment of type 2 diabetes (Bischoff, 1994).

Inhibition of carbohydrate-hydrolyzing enzymes such as α-amylase and α-glucosidase, and the polyol pathway enzyme aldose reductase, are key approaches to control blood glucose levels and prevent diabetic complications. In the present study, the *in-vitro* antidiabetic potential of aqueous and ethanolic leaf extracts of *Chamaecostus cuspidatus*, *Aloe vera* and *Terminalia chebula* was evaluated at 20-100 µg/mL using Acarbose as standard. All three extracts exhibited a concentration-dependent increase in inhibitory activity across all assays. The superior activity of ethanolic extracts over aqueous extracts may be due to better extraction of bioactive phytoconstituents such as flavonoids, phenols,

tannins, and terpenoids in ethanol. *T. chebula* is rich in hydrolysable tannins and chebulinic acid which are known α -glucosidase inhibitors (Van Heyningen, 1959). *A. vera* contains anthraquinones and polysaccharides reported to have hypoglycemic effects. *C. cuspidatus*, commonly called insulin plant, contains corosolic acid which has been shown to enhance glucose uptake. The comparable and in some cases higher inhibition than Acarbose suggests that these plant extracts possess significant antidiabetic potential through multiple mechanisms: delaying carbohydrate digestion and preventing accumulation of sorbitol (Shetty, 2010). Further *in-vivo* studies and isolation of active compounds are warranted to validate their therapeutic use in diabetes management (Bag *et al.*, 2013a).

CONCLUSION

The present study was conducted to evaluate the *in-vitro* antioxidant, anti-inflammatory, and antihyperglycemic potential of aqueous and hydro-ethanolic leaf extracts of *Chamaecostus cuspidatus*, *Aloe vera* and *Terminalia chebula*. All the extracts exhibited a concentration-dependent increase in activity in all the assays performed. In antioxidant assays, the hydro-ethanolic extracts showed comparatively higher activity than aqueous extracts, which may be due to the presence of more phenols, flavonoids and tannins extracted in ethanol. In the anti-inflammatory activity, *Aloe vera* hydro-ethanolic extract and *C. cuspidatus* aqueous extract demonstrated maximum inhibition at 500 μ g/mL, which was comparable to the standard drug Diclofenac sodium. This suggested their potential to inhibit protein denaturation and inflammatory mediators. In the antihyperglycemic assays, all three plants showed significant inhibition of aldose reductase, α -amylase, and α -glucosidase enzymes. Notably, *T. chebula* ethanolic extract exhibited the highest α -glucosidase inhibition with the lowest IC₅₀ value, and all extracts showed greater α -amylase and α -glucosidase inhibition than Acarbose at 100 μ g/mL. *A. vera* ethanolic extract showed the highest aldose reductase inhibition. The pharmacological activities can be attributed to the presence of bioactive phytoconstituents such as flavonoids, tannins,

corosolic acid and anthraquinones in these plants. From the results, it can be concluded that *C. cuspidatus*, *A. vera* and *T. chebula* possess significant *in-vitro* antioxidant, anti-inflammatory, and antihyperglycemic activities. These findings validate their traditional use and suggested that they can be potential candidates for the development of herbal formulations of management of diabetes mellitus and its associated complications.

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