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# In-vitro Evaluation of Antioxidant and Glycemic Control Properties of Selected Plant Extracts

Rajkumar Patsker[1*], Naveen Gupta[2], Vikash Gupta[3], Dharmendra Rajput[2]

[1]Research Scholar, Patel College of Pharmacy, Madhyanchal Professional University, Bhopal, MP, India.
[2]Professor, Patel College of Pharmacy, Madhyanchal Professional University, Bhopal, MP, India.
[3]Professor, Kailash Narayan Patel College of Pharmacy, Bhopal, M.P.
*Corresponding Author: Rajkumar Patsker, Email: rajkumarpatsker@gmail.com

## ABSTRACT

Recent studies indicated that 89.8 million Indians suffer from diabetes, in the year 2024, about 3.4 million people died directly because of diabetes. The number of diabetics is gradually increasing over the last few decades. Diabetes, being a degenerative disease, has become the 3rd most lethal disease, it is associated with many complications, some of which are cardiovascular disease, liver dysfunction, and diabetic nephropathy, closely linked to oxidative stress. This oxidative stress causes an imbalance between free radicals and the body's antioxidant defense, this further contributes to the development and progression of the other diabetes associated complications. This research study was aimed to evaluate invitro properties of selected plant extracts, i.e., stem bark of Ficus racemose, leaves of Tecoma stans, leaves of Bougainvillea spectabilis, and seeds of Cyamopsis tetragonoloba to quantify their antioxidant potential by DPPH, ABTS radical and hydrogen peroxide scavenging activity and glycemic control properties by inhibition of α-amylase and α-glucosidase. After detailed research study it was demonstrated that ethanolic extract of all four-plant extract showed significantly stronger antioxidant activity compared to aqueous extract. Whereas as in-vitro glycemic control study showed that both ethanolic and aqueous extracts showed a dose-dependent inhibitory effect on both α-amylase and α-glucosidase enzymes. The α- glucosidase enzyme was more potently inhibited by all four plant extracts than the α-amylase enzyme. This correlation is often attributed to a higher concentration of phenolic compounds, terpenoids and flavonoids in the more active extract, as these compounds are well-known to function as both radical scavengers and enzyme inhibitors. In conclusion, the antioxidant potential of four plants ethanolic extracts is integral to its overall anti-diabetic efficacy, acting as a prophylactic measure against ROS-mediated β-cell destruction and complication development, while the enzyme inhibition of α-amylase/glucosidase provides immediate postprandial glucose management. Thus in vitro study provided scientific evidence supporting the traditional use of these selected plant extracts.

**KEYWORDS**: Invitro, antioxidant, diabetes mellitus, α-amylase, α-glucosidase, antidiabetic

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## INTRODUCTION

According to the 11th edition of the Diabetes Atlas (2024-25), the International Diabetes Federation's most recent studies indicate that 89.8 million Indians suffer from diabetes, and this number is projected to be 156.9 million by 2050. According to official data, there are about 589 million people worldwide with diabetes, the majority of whom are from lower to middle-income countries. In the year 2024, about 3.4 million people died directly because of diabetes. The number of diabetics has been gradually increasing over the last few decades. Diabetes mellitus has been reported to be a major global health concern, with 1 trillion dollars in health expenditure, a 338% increase has been reported in the previous 17 years. Diabetes, being a degenerative disease of mankind, has become the third most lethal disease, and it is increasing rapidly in an uncontrolled manner. All three types of diabetes are increasing, of which type 2, in particular, has increased by 150%. The prevalence of diabetes is approximately 8.9% of the population, with 6.5% of adults being under 50 years old, and 15.3% of patients being prediabetics. Looking at the current uncontrolled situation, there is a global and top-priority need to stop the progression of diabetes. Diabetes Mellitus (DM) is associated with complications, such as cardiovascular disease, liver dysfunction, and diabetic nephropathy, and these are closely linked to oxidative stress. Oxidative stress causes an imbalance between free radicals and the body's antioxidant defense. This is a central mechanism that contributes to the development and progression of the above-mentioned and other diabetes associated complications [1]. Research has also shown that chronic hyperglycemia increases the production of reactive oxygen species (ROS), overwhelms the body's natural antioxidant defense systems which overwhelming the body's natural antioxidant defense systems, leading to further cellular damage as well as insulin resistance [2]. The major mechanisms by which hyperglycemia generates damaging ROS include: mitochondrial overproduction, where excess glucose flux in endothelial cells leads to the overproduction of mitochondrial superoxide. Polyol pathway flux, here increased glucose shunted through the polyol pathway, consumes NADPH, it is a critical cofactor that is needed to regenerate the potent cellular antioxidant, reduced glutathione (GSH). This basically depletes the cell's antioxidant reserves. Lastly, high glucose accelerates the non-enzymatic glycation of proteins, forming Advanced glycation end-products (AGEs). The binding of AGEs to their receptor (RAGE) activates pro-inflammatory and pro- oxidant pathways, further generating ROS [3, 4]. Thus, the vicious cycle continues.

**Pathological Consequences of Oxidative Stress**

Chronic oxidative stress (OS) is reported to be a crucial factor in the development of both β-cell dysfunction and insulin resistance, which further leads to the progression and complications of diabetes. The insulin-producing β-cells are highly susceptible to oxidative stress due to their low intrinsic antioxidant defense system. ROS induce cellular damage (apoptosis), reducing the total mass of functional β-cells and thus impairing insulin secretion. ROS interfere with the insulin signaling pathway in peripheral tissues, present in like muscle and fat cells, impairing the translocation of GLUT4 transporters to the cell surface, thus reducing glucose uptake. OS is reported to be the underlying link for microvascular complications like diabetic retinopathy, nephropathy, and neuropathy, and macrovascular diseases, atherosclerosis, and cardiomyopathy by causing endothelial dysfunction and inflammation in blood vessels [1, 2, 5].

**Rationale and Aim**

Modern available treatments for type 2 diabetes mellitus (T2DM) often involve synthetic drugs, which may have significant side effects [6]. Whereas, treatment with traditional natural plant- based compounds, such as polyphenols and flavonoids, is known for their potent antioxidant and anti-diabetic activities, offering a safer therapeutic alternative [7]. The primary mechanism by which postprandial (after-meal) hyperglycemia is managed includes inhibition of carbohydrate- hydrolyzing enzymes, specifically α-amylase and α-glucosidase [8]. Thus, this research study aimed to perform an in vitro evaluation of selected plant extracts (e.g., stem bark of Ficus racemose, leaves of Tecoma stans, leaves of Bougainvillea spectabilis, and seeds of Cyamopsis tetragonoloba) to quantify their antioxidant potential (radical scavenging ability) and glycemic control properties (inhibition of α-amylase and α-glucosidase).

## MATERIALS AND METHODS

**Plant Material and Extraction**

After a thorough literature review stem bark of Ficus racemose, leaves of Tecoma stans, leaves of Bougainvillea spectabilis, and seeds of Cyamopsis tetragonoloba were identified and authenticated by a botanist of Vindhya Herbal Testing and Research Laboratory, Van Parisar, Barkheda Pathani, Bhopal, M.P., India. After cleaning, the plant materials were dried and ground into a powder, maintaining proper particle size. Powdered plant parts were defatted using petroleum ether. After drying, 80% ethanol was used as a solvent, followed by distilled water for successive extraction using a Soxhlet apparatus. After complete extraction, the extracts were collected and concentrated under reduced pressure using a vacuum to yield the crude extract, which was stored at 4 °C until used.

*Ficus racemosa* Linn.

*Cyamopsis tetragonoloba* (L.) Taub.

*Tecoma stans*

*Bougainvillea spectabilis*

**Invitro Antioxidant Assays**

**DPPH Radical Scavenging Assay**

The antioxidant activity of the ethanolic and aqueous extracts of all four plants was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay method. Different concentrations of the plant extracts were mixed with a DPPH methanolic solution and incubated in the dark. The characteristic colour of DPPH is purple, gets fades when they are neutralized

by an antioxidant compound. Using a spectrophotometer, absorbance was measured and noted at 517 nm against a blank. Ascorbic acid was used as the positive control. The results were expressed as the IC50 value, i.e., concentration required to scavenge a percentage (%) of the DPPH radicals [9].

**ABTS radical scavenging activity**

Different concentration of test extracts was mixed with ABTS working standard, and the free radical scavenging activity was calculated. After mixing the solution, exactly after one minute and then up to 6 minutes, the characteristic colour of ABTS, which is green gets fades when they are neutralized by an antioxidant compound. Thus, a decrease in the absorbance at 745 nm was observed and calculated. The results were expressed as the IC50 value [9].

**Hydrogen peroxide scavenging activity**

Hydrogen peroxide itself is not a free radical, but it is a major, stable ROS intermediate that is produced in abundance in hyperglycemic conditions. The abilities of the different tests, extracts, and standards to directly neutralize or reduce H2O2 (known concentration) were measured. The amount of residual hydrogen peroxide is then measured using a spectrophotometer at 230 nm. A decrease in the detected H2O2 indicated the test sample's scavenging activity [10].

**Invitro Glycemic Control Assays**

**α-Amylase Inhibition Assay**

The inhibitory effect on porcine pancreatic α-amylase was evaluated. Here, the plant extract was preincubated with the enzyme before the addition of starch solution (substrate). The reaction was terminated, and the reducing sugar, i.e., maltose, released was calculated using the DNSA (dinitrosalicylic acid) method. Acarbose was used as the positive control or standard. Absorbance was measured at 540 nm [11, 12].

**α-Glucosidase Inhibition Assay**

The inhibition of yeast α-glucosidase was measured using p-nitrophenyl-α-D-glucopyranoside (NPG) as the substrate. The release of p-nitrophenol due to enzymatic activity was measured spectrophotometrically at 405nm. Plant extracts were tested at various concentrations, and the results were reported as IC50 values, with Acarbose serving as the positive control [11, 12].

**IC50 value**

IC50 was calculated by plotting the percentage of inhibition against concentration. Here, each experiment was repeated three times at each concentration to get the mean. The antioxidant capacity of test samples was expressed as IC50. It is the concentration necessary for a 50% reduction of DPPH, hydrogen peroxide, and ABTS radical. It is assessed by plotting an x-y graph and fitting the data with a straight line (linear regression). The IC50 value was then estimated by using the fitted line, i.e, $Y = a * X + b$, $IC50 = (50 - b)/a$.

**Statistical Analysis**

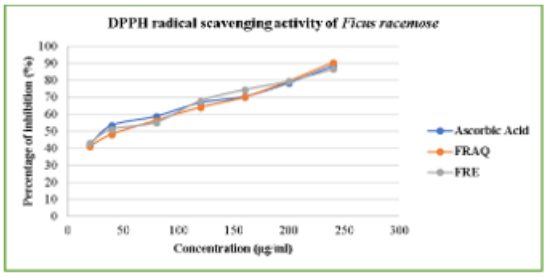
Data were analyzed using one-way ANOVA, and results were presented as Mean ± Standard Error of the Mean (SEM). IC50 values were calculated using a linear regression analysis.

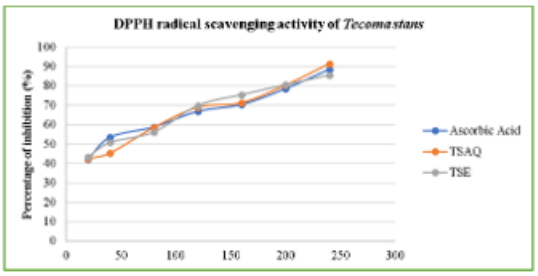
## RESULTS

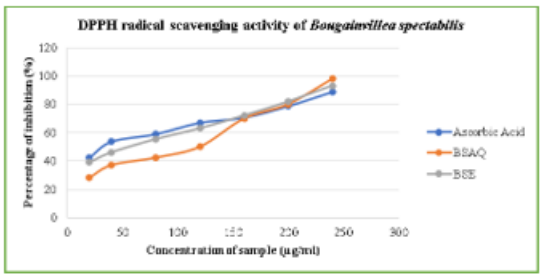
**Antioxidant Activity**

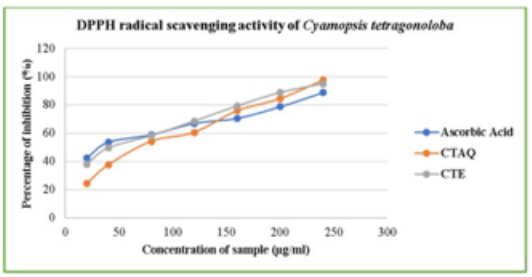
The ethanolic extract of Ficus racemose (FR), Tecoma stans (TS), Bougainvillea spectabilis (BS), Cyamopsis tetragonoloba (CT), demonstrated significantly stronger DPPH scavenging activity, i.e., IC50 was noted to be 43.21, 39.79, 61.23, and 50.83 compared to the aqueous extract, which was comparatively higher. The ethanolic extract of FR, TS, BS, and CT demonstrated significantly stronger hydrogen peroxide scavenging activity, i.e., IC50 was noted to be 38.87, 49.85, 65.89, and 45.54 compared to the aqueous extract, which was comparatively higher. The ethanolic extract of FR, TS, BS, and CT demonstrated significantly stronger ABTS scavenging activity, i.e., IC50 was noted to be 23.68, 56.86, 25.80, and 23.94 compared to the aqueous extract, which was comparatively higher.

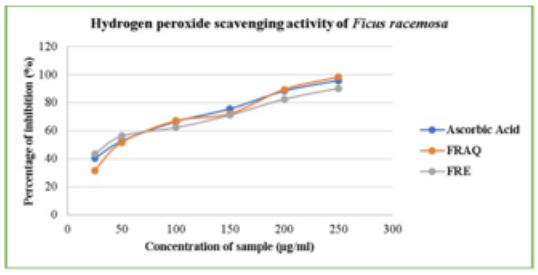
| Plants | Extract | DPPH IC50 (µg/mL) | H2O2 IC50 (µg/mL) | ABTS IC50 (µg/mL) |
|---|---|---|---|---|
| *Ficus racemose* | Ethanol | 43.21±1.54 | 38.87±1.68 | 23.68±1.65 |
| | Aqueous | 55.09±1.38 | 62.24±1.39 | 54.69±1.62 |
| *Tecoma stans* | Ethanol | 39.79±1.48 | 49.85±1.11 | 56.86±1.23 |
| | Aqueous | 51.34±1.25 | 58.01±1.65 | 87.58±1.68 |
| *Bougainvillea spectabilis* | Ethanol | 61.23±1.50 | 65.89±1.61 | 25.80±1.63 |
| | Aqueous | 96.37±1.46 | 83.08±1.07 | 40.95±1.30 |
| *Cyamopsis tetragonoloba* | Ethanol | 50.83±1.49 | 45.54±1.48 | 23.94±1.44 |
| | Aqueous | 84.48±1.35 | 73.03±1.19 | 54.23±1.59 |
| Ascorbic Acid | | 39.83±1.16 | 46.67±1.34 | 43.29±1.65 |

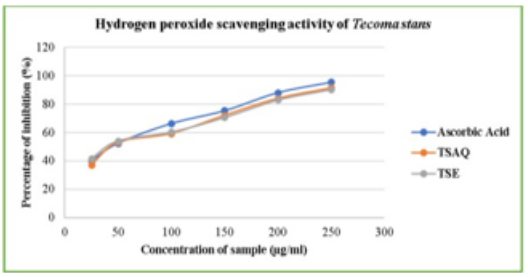

DPPH radical scavenging activity of Ficus racemose
Percentage of Inhibition (%)
Concentration (µg/ml)
Ascorbic Acid
FRAQ
FRE

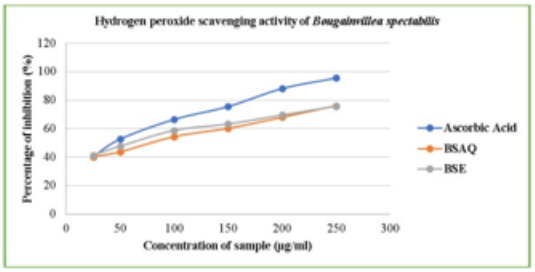

DPPH radical scavenging activity of Tecoma stans
Percentage of Inhibition (%)
Ascorbic Acid
TSAQ
TSE

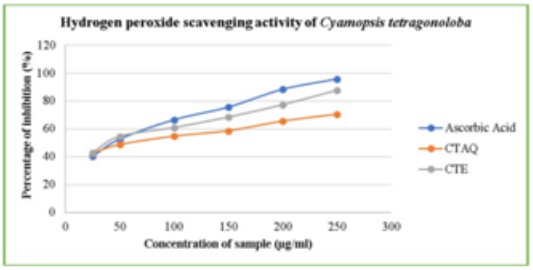

DPPH radical scavenging activity of Bougainvillea spectabilis
Percentage of inhibition (%)
Concentration of sample (µg/ml)
Ascorbic Acid
BSAQ
BSE

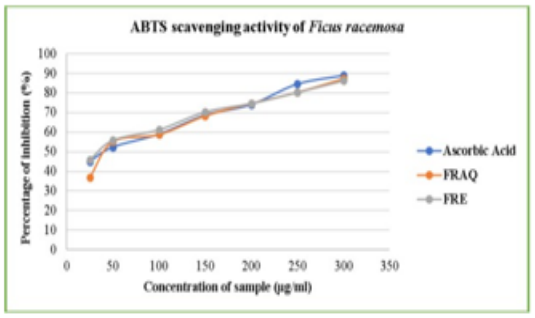

DPPH radical scavenging activity of Cyamopsis tetragonoloba
Percentage of Inhibition (%)
Concentration of sample (µg/ml)
Ascorbic Acid
CTAQ
CTE

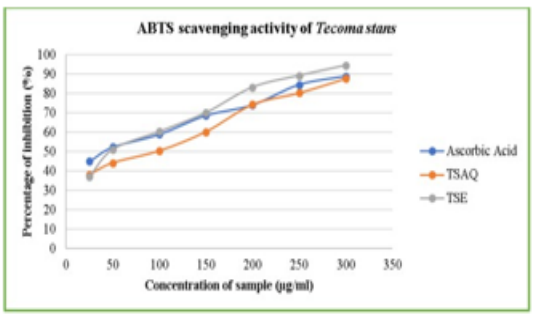

Hydrogen peroxide scavenging activity of Ficus racemosa
Percentage of Inhibition (%)
Concentration of sample (µg/ml)
Ascorbic Acid
FRAQ
FRE

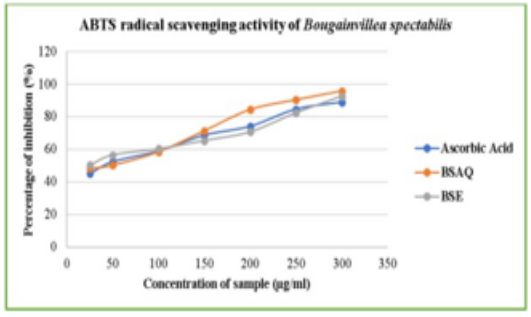

Hydrogen peroxide scavenging activity of Tecoma stans
Percentage of Inhibition (%)
Concentration of sample (µg/ml)
Ascorbic Acid
TSAQ
TSE

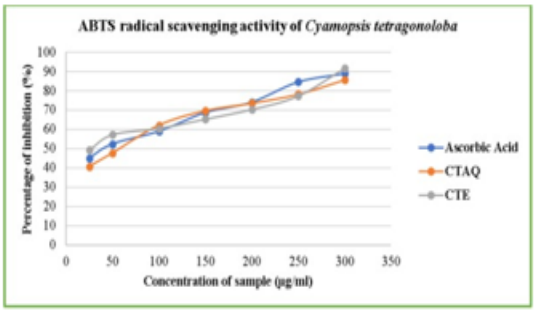

Hydrogen peroxide scavenging activity of Bougainvillea spectabilis
Percentage of Inhibition (%)
Concentration of sample (µg/ml)
Ascorbic Acid
BSAQ
BSE


Hydrogen peroxide scavenging activity of Cyamopsis tetragonoloba
Percentage of Inhibition (%)
Concentration of sample (µg/ml)
Ascorbic Acid
CTAQ
CTE


ABTS scavenging activity of Ficus racemosa
Percentage of Inhibition (%)
Concentration of sample (µg/ml)
Ascorbic Acid
FRAQ
FRE


ABTS scavenging activity of Tecoma stans
Percentage of Inhibition (%)
Concentration of sample (µg/ml)
Ascorbic Acid
TSAQ
TSE

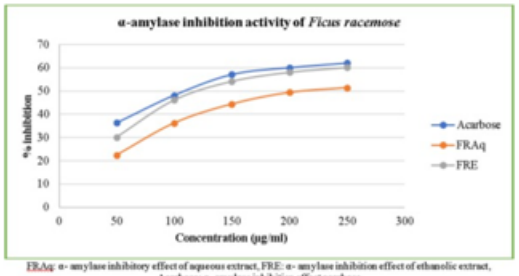

ABTS radical scavenging activity of Bougainvillea spectabilis
Percentage of Inhibition (%)
Concentration of sample (µg/ml)
Ascorbic Acid
BSAQ
BSE


ABTS radical scavenging activity of Cyamopsis tetragonoloba
Percentage of Inhibition (%)
Concentration of sample (µg/ml)
Ascorbic Acid
CTAQ
CTE

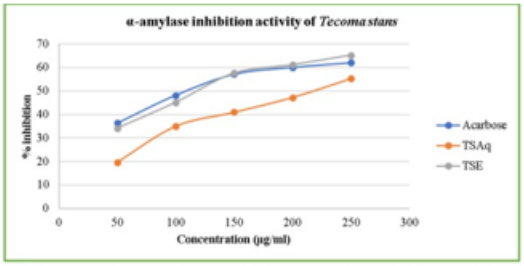
**Glycemic Control Properties**

| Plants | Extract | α-amylase inhibition activity IC50 (µg/mL) | α-glucosidase inhibition activity IC50 (µg/mL) |
|---|---|---|---|
| *Ficus racemose* | Ethanol | 151.62±1.84 | 63.27±1.62 |
| | Aqueous | 214.83±1.47 | 90.77±1.78 |
| *Tecoma stans* | Ethanol | 138.21±1.81 | 65.72±1.16 |
| | Aqueous | 212.15±1.69 | 83.93±1.82 |
| *Bougainvillea spectabilis* | Ethanol | 143.78±1.71 | 49.14±1.79 |
| | Aqueous | 188.96±1.73 | 82.41±1.74 |
| *Cyamopsis tetragonoloba* | Ethanol | 140.05±1.91 | 66.05±1.73 |
| | Aqueous | 152.78±1.61 | 81.76±1.73 |
| Acarbose | | 128.34±1.62 | 60.09±1.27 |

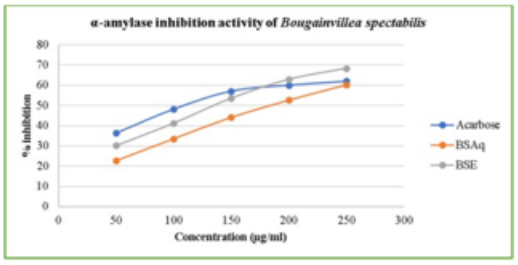
Both ethanolic and aqueous extracts showed a dose-dependent inhibitory effect on both α-amylase and α-glucosidase enzymes. The α-glucosidase enzyme was more potently inhibited by all four plant extracts than the α-amylase enzyme. The ethanol extract of Tecoma stans exhibited the highest overall anti-diabetic activity in α-amylase inhibition activity. Although its IC50 values were higher than the standard drug Acarbose. Whereas Bougainvillea spectabilis exhibited the overall lower anti-diabetic activity by α-glucosidase inhibition activity. Although its IC50 values were higher than the standard drug Acarbose. Ethanolic extract of all four plants exhibited comparatively better α-amylase inhibition activity and α-glucosidase inhibition activity than aqueous extracts.



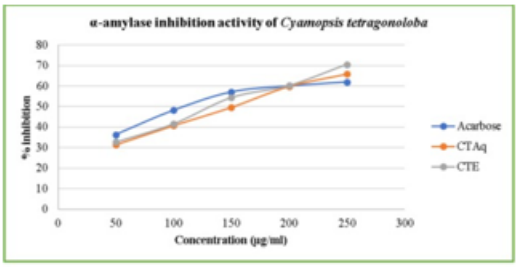

FRAq: α- amylase inhibitory effect of aqueous extract, FRE: α- amylase inhibition effect of ethanolic extract, Acarbose: α- amylase inhibition effect acarbose




TSAq: α- amylase inhibitory effect of aqueous extract, TSE: α- amylase inhibition effect of ethanolic extract, Acarbose: α- amylase inhibition effect acarbose




BSAq: α- amylase inhibitory effect of aqueous extract, BSE: α- amylase inhibition effect of ethanolic extract, Acarbose: α- amylase inhibition effect acarbose




CTAq: α- amylase inhibitory effect of aqueous extract, CTE: α- amylase inhibition effect of ethanolic extract, Acarbose: α- amylase inhibition effect acarbose

## DISCUSSION

**Correlation of Antioxidant and Anti-Diabetic Activity**

The results indicate that the ethanol extract of FR, TS, BS, and CT possesses superior antioxidant and anti-diabetic activities compared to the aqueous extract. This correlation is often attributed to a higher concentration of phenolic compounds, terpinoids and flavonoids in the more active extract, as these compounds are well-known to function as both radical scavengers and enzyme inhibitors [13, 14].

**Mechanism for glycemic control**

The observed inhibitory effect of FR, TS, BS, and CT on α-amylase and α-glucosidase suggests a potential therapeutic benefit for managing postprandial glucose levels [15, 16]. By slowing the breakdown of starches and disaccharides, the extracts could effectively blunt the rapid spike in blood glucose following a meal. The finding that α-glucosidase inhibition was stronger than α- amylase inhibition is therapeutically desirable, as partial inhibition of α-amylase is preferred to avoid side effects like gastrointestinal discomfort [17].

**The dual-action hypothesis:**
Antioxidant support for anti-diabetic activity: The high antioxidant activity measured in FR, TS, BS, and CT ethanolic extracts is not just an accompanying characteristic but a fundamental mechanism that supports and enhances their anti-diabetic effect. This is the dual-action hypothesis, direct glycemic control: phytochemicals like polyphenols act as enzyme inhibitors (e.g., against α- amylase and α-glucosidase) to slow glucose absorption [18].

Mitigation of damage (antioxidant support): Concurrently, the antioxidant activity of compounds presents in the ethanolic extract of all four plants directly addresses the root cause of diabetic progression. By scavenging free radicals and reducing hydrogen peroxide (produced in abundance during hyperglycemic conditions), FR, TS, BS, CT, and ethanolic extracts perform three critical protective functions in vivo: protect pancreatic β-Cells from ROS-induced apoptosis, helping to preserve the body's endogenous insulin production capacity. Restore insulin signaling, by neutralizing ROS the plant extracts help reduce the OS-induced damage to the insulin signaling cascade in muscle and adipose tissue, potentially reversing or mitigating insulin resistance. Prevents associated complications: The protective action on vascular endothelial cells helps maintain nitric oxide (NO) bioavailability (which is inactivated by ROS), thereby preventing the progression of micro- and macro-vascular damage [19, 20].

## CONCLUSION

In conclusion, the antioxidant potential of an FR, TS, BS, CT, ethanolic extracts is integral to its overall anti-diabetic efficacy, acting as a prophylactic measure against ROS-mediated β-cell destruction and complication development, while the enzyme inhibition of α-amylase/glucosidase provides immediate postprandial glucose management. Thus in vitro study provides scientific evidence supporting the traditional use of these selected plant extracts. The results highlight the potential of these extracts for development into novel therapeutic agents against diabetes.

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