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## EVALUATION THE ANTIDIABETIC, ANTIOXIDANT AND ANTIFUNGAL PROPERTIES OF A POLYHERBAL FORMULATION (*Cissus quadrangularis* & *Cynado dactylon*) IN VITRO STUDY

MARIMUTHU M, SIVA SANKARI T, Dr.KANNIKAPARAMESWARI N

Department of Biochemistry, Dr. N.G.P Arts and Science College, Coimbatore, Tamil Nadu, India.

### ABSTRACT:

The medicinal plants play an important role in the management of diseases. In India, almost 45,000 plant species are growing naturally and being cultivated. Dependence on plants as the source of medicine is prevalent in developing countries where traditional medicine plays a major role in health care. The rural population of a country is more disposed to traditional treatments because of their easy availability and lower cost. Herbal therapy, although still an unwritten science, is well established in some cultures and traditions and has become a way of life for almost 80% of the people in rural areas, especially those in Asia According to the present study, we conclude that plant medicines (*Cissus quadrangularis* and *Cynadon dactylon*) contain more than 15 chemical compounds and have a chance of showing better inhibitory activity when compared to the individual plants. Natural medicines have fewer or no side effects when compared to synthetic inhibitors. After comparing the results of this investigation with the synthetic inhibitor  $\alpha$ -amylase (acarbose) and the  $\alpha$ -glucosidase (ascorbic acid standard), we may infer that *Cissus quadrangularis* and *Cynadon dactylon* exhibit superior antidiabetic, antioxidant, and antifungal action.

**Key words:** Herbal medicine, *Cissus quadrangularis*, *Cynadondactylon*, Antidiabetic, Antioxidant, Antifungal activity

### INTRODUCTION

Plants have been used as medicines throughout history. Indeed, studies of wild animals show that they also instinctively eat certain plants to treat themselves for certain illness. In Asia, the practice of herbal medicine is extremely well established and document. As a result, most of the medicine plants that have international recognition come from this region, particularly from China and India. In Europe and North America, the use of herbal medicine in increasing fast, especially for correcting imbalances caused by modern diets and

lifestyles. Herbs and plants make up a large portion of the ingredient used by people throughout the ages to help combat disease and illness. Many of these medicinal plants are still used today all around the world. The preventive curative and corrective approaches of the health is the basic strength of the Indian system of medicine (ISM), Which are mostly plant based and comprise over 8000 medicinal and aromatic plant species. In India about 1.5 million practioners of ISM have found 25000 effective plantbased formulation. An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfer electrons from a substance to an oxidizing agent to produce free radicals. In turn, these radicals can start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions. *Cynodon dactylon* (L.) Pers. is a perennial grass belonging to family Poaceae that has a variety of medicinal properties. It is native to north and east Africa, Asia and Australia and southern Europe. It is cultivated throughout the tropics and subtropics. In Ayurveda *Cynodon dactylon* shows many pharmacological activities like antidiabetic, antioxidant, antidiarrheal, hepatoprotective, antiulcer, immunomodulator, CNS depressant, antimicrobial.

## MATERIALS AND METHODS

### COLLECTION OF PLANT MATERIALS

The plants of *Cissus quadrangularis* L. -VITACEAE and *Cynodon dactylon* (L.) Pers. - POACEAE were collected during January 2019 from the local area. The plants were identified by Dr. C.Murugan, Scientist 'E' & Head of Office, Botanical Survey of India, southern Regional Centre, Coimbatore. BSI/SRC/5/23/2019/Tech/3305 and BSI/SRC/5/23/2019/Tech/3306.

### SAMPLE PREPARATION

*Cissus quadrangularis* and *Cynodon dactylon* plants are thoroughly washed to remove the impurities, and they were shade dried at room temperature for 5-10 days. The dried plants were ground to a powder with the help of an electric blender, and the powder was stored in airtight container.

### EXTRACTION PREPARATION

*Cissus quadrangularis* and *cynodon dactylon* was washed with distilled water, air dried and made to fine powder using a sterile electrical blender. The fine powder was stored in amber bottles {airtight} at room temperature. 5gms of the *cissus quadrangularis* powder and 5gms of *cynodon dactylon* powder was taken in 100 ml of the solvents mentioned below.

- a) Distilled water.
- b) Ethanol.
- c) Chloroform.

## PHYTOCHEMICAL SCREENING

### DETAILS OF PROCEDURE FOR QUALITATIVE ANALYSIS

#### 1. Detection of carbohydrates

Benedict's test: One mL of filtrate solution is treated with Benedict's reagent and heated gently. Reddish precipitate indicates the presence of carbohydrates.

## 2. Detection of proteins

Ninhydrin test: To the extract, 0.25% w/v ninhydrin reagent is added and boiled for few minutes. Formation of blue-violet color indicates the presence of proteins.

## 3. Detection of alkaloids

The crude extract powder is dissolved in 2 N Hydrochloric acid and filtered.

Mayer's test: Filtrate portion is treated with few drops of Mayer's reagent (potassium mercuric iodide). Formation of a yellow colored precipitate indicates the presence of alkaloids.

## 4. Detection of flavonoids

Lead acetate test: Extract sample is treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.

## 5. Detection of tannins

Gelatin test: To the extract, 1% gelatin solution containing NaCl is added. Formation of white precipitate indicates the presence of tannins.

## 6. Detection of steroids

Salkowski test: The extract sample is dissolved in chloroform and equal volume of concentrated sulphuric acid is added. Bluish red, cherry red and purple color in chloroform layer indicates the presence of sterols.

## 7. Detection of saponins

Froth test: Crude dry powder of extract is vigorously shaken with 2 mL of distilled water and is allowed to stand for 10 min. If stable froth appears, it indicates the presence of saponins.

## 8. Detection of cardiac glycoside

Keller-Kiliani's test: A portion of dry extract is treated with 1 mL of  $\text{FeCl}_3$  reagent (1 volume of 5%  $\text{FeCl}_3$  and 99 volume of glacial acetic acid). To this solution a few drops of concentrated  $\text{H}_2\text{SO}_4$  is added. The presence of greenish blue color within a few minutes indicates the presence of desoxy sugar of cardiac glycosides.

## 9. Detection of Terpenoids

Take 1ml of the filtrate and add 2ml  $\text{CHCl}_3$  and carefully add few drops of conc  $\text{H}_2\text{SO}_4$ . An interface with a reddish brown colouration is formed showing presence of terpenoids.

## 10. Detection of Anthraquinones

To 1ml of the filtrate add 10ml benzene, filter and add 5ml of 10% {v/v} ammonia to the filtrate and shake well. Development of pinkish coloured solution indicates the presence of anthraquinones.

## 11. Test for Phenols:

To 2 ml of each extract, 2 ml of 5% aqueous ferric chloride were added; formation of blue colour indicates the presence of phenols in the sample extract.

### Invitro $\alpha$ -amylase inhibitory assay

#### Reagents

1. Sodium phosphate buffer - pH 6.9
2.  $\alpha$ -amylase solution -0.5mg/mL
3. Starch solution -1%
4. Dinitrosalicylic acid (DNS) reagent

#### Method

This assay was carried out using a modified procedure of McCue and Shetty. A total of 250  $\mu$ L of extract (1.25–10mg/mL) was placed in a tube and 250  $\mu$ L of 0.02M sodium phosphate buffer (pH 6.9) containing  $\alpha$ -amylase solution (0.5mg/mL) was added. This solution was pre incubated at 25°C for 10 min, after which 250  $\mu$ L of 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9) was added at timed intervals and then further incubated at 25°C for 10min. The reaction was terminated by adding 500  $\mu$ L of dinitrosalicylic acid (DNS) reagent. The tubes were then incubated in boiling water for 5min and cooled to room temperature. The reaction mixture was diluted with 5mL distilled water and the absorbance was measured at 540 nm using spectrophotometer.

### Invitro $\alpha$ -glucosidase inhibitory assay

#### Reagents

1. p-Nitrophenyl glucopyranoside (pNPG) :Phosphate buffer (pH 6.9) -20mM
2.  $\alpha$ - glucosidase -1.0U/mL
3. Na<sub>2</sub>CO<sub>3</sub> -0.1M

#### Method

The effect of the plant extracts on  $\alpha$ -glucosidase activity was determined according to the method described by Kim et al., using  $\alpha$ -glucosidase from *Saccharomyces cerevisiae*. 100  $\mu$ L of  $\alpha$ - glucosidase (1.0U/mL) was pre incubated with 50  $\mu$ L of the different concentrations of the extracts for 10min. Then 50  $\mu$ L of 3.0mM pNPG as a substrate dissolved in 20mM phosphate buffer (pH 6.9) was then added to start the reaction. The reaction mixture was incubated at 37°C for 20min and stopped by adding 2mL of 0.1M Na<sub>2</sub>CO<sub>3</sub>. The  $\alpha$ - glucosidase activity was determined by measuring the yellow-colored paranitrophenol released from pNPG at 405 nm.

### DPPH radical scavenging assay

#### Reagents

1. DPPH solution: DPPH 2mg/mL of Ethanol.
2. Ascorbic acid-Std: 1mg/mL of Ethanol.
3. Plant extract: 3 mg/mL of DMSO.
4. Solvent: Ethanol.

## Method

The hydrogen donating ability of extracts was examined in the presence of DPPH stable radical. One millilitre of 0.3 mM DPPH ethanol solution was added to 2.5 mL of sample solution of different concentration and allowed to react at room temperature. After 30 minutes the absorbance values were measured at 517 nm. Ethanol (1.0 mL) and plant extract solution (2.5mL) was used as a blank, DPPH solution (1.0mL, 0.3 mM) and ethanol (2.5mL) served as negative control. Ascorbic acid (100µL) was used as a positive control (Subhashini et al., 2011).

## ANTIFUNGAL ACTIVITY

### Microbial cultures and growth conditions

The plant extracts were assayed for antifungal activity against the fungal strain *A. niger*, obtained from Microbial Culture Collected from department of microbiology in Dr.NGP arts and science college,coimbatore. This fungus was grown on PDA plate at 28°C and maintained with periodic sub-culturing at t 40C.

### Reagents

1. Potato Dextrose Agar (PDA) Medium (pH 6.7)
2. Potato - 250g
3. Dextrose - 15g
4. Agar - 18g
5. Distilled water - 1000ml

## Method

Every 15ml of sterile potato dextrose agar medium was poured into sterile petridishes after flaming the top of the conical flask. 100, 200, 300, 400, 500µl ethanolic extract was added respectively. The solution in each petridish was gently swirled and allowed to solidify. The extract amended medium in the petridishes were inoculated separately at the centre with each test fungus and incubated at room temperature (a sterile needle was used to inoculate fungal strains to these petridishes). The medium without extract served as control. Incubation period of 24- 48hours at 28°C was maintained for observation of antifungal activity of plant extracts. The antifungal activity was evaluated by measuring zones of inhibition of fungal growth surrounding the plant extract. The complete antifungal analysis was carried out under strict aseptic conditions.

## RESULTS AND DISCUSSION

### PRELIMINARY SCREENING OF PHYTOCHEMICAL TEST IN PLANT EXTRACTS

The phytochemical screening of the *Cissus quadrangularis* and *Cynadon dactylon* plant extracts done to identify different chemical compounds. The different tests performed with three different solvents such as aqueous, chloroform, ethanol plant extract showed the presence of different compounds and revealed positive result for the various constituents were presented in **Table 1**.

**Table 1:** Preliminary qualitative phytochemical screening in three different solvents of plant extracts of *Cissus quadrangularis* L. and *Cynadon dactylon* (L). Pers.

PHYTOCHEMICAL	AQUEOUS	CHLOROFORM	ETHANOL
Alkaloids	–	+	–
Flavonoids	+	–	–
Terpenoids	–	+	+
Steroids	–	+	+
Saponins	+	+	–
Tannins	+	+	+
Glycosides	–	+	+
Phenol	+	+	+
Anthrocyanin	+	–	–
Protein	+	–	+
Carbohydrate	+	–	–

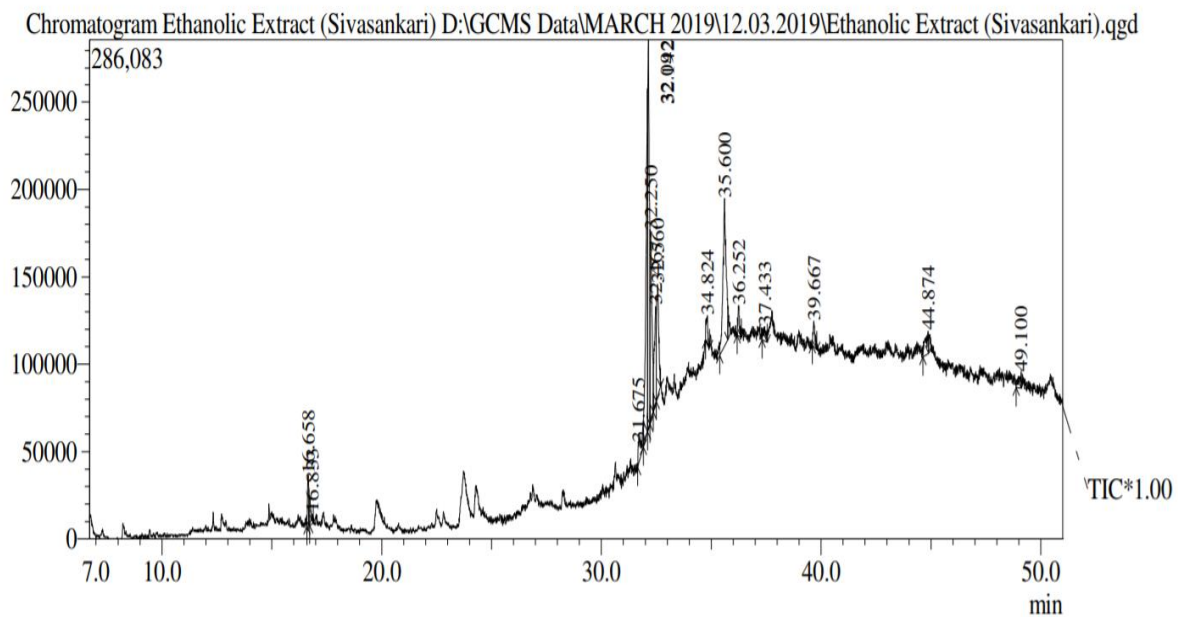
\* + Presence, - Absence

The phytochemical analysis of *Cissus quadrangularis* and *Cynadon dactylon* plants revealed the presence of alkaloids, flavanoids, terpenoids ,steroids ,saponins, tannins, glycosides, phenol, anthocyanin, protein and carbohydrate in aqueous,chloroform,ethanol extracts. These active compounds of the plant could be responsible for the different pharmacological activities.

#### QUALITATIVE ANALYSIS BY GC-MS

0.20 ml of the ethanolic extract of *cissus quadrangularis* and *cynodon dactylon* was prepared as the same as the previous plant extraction for GC-MS analysis.GC-MS analysis is carried out a shimadzu GC-MS (Model Number: QP2010S) instrument employing the following conditions: COLUMN(Rxi sil MS: 30 meter length: 0.25 µm thickness), operating in the electron impact mode at 70Ev;helium(99.999%) was used as carrier gas at a constant flow of 1ml/min.The oven temperature was programmed from 70<sup>0</sup>C with an increase to260<sup>0</sup>C /min at 10<sup>0</sup>C/min.Diluted samples of 0.20 ml were injected manually. The studies on the active principles in the *cissus quadrangularis* and *cynadon dactylon* extract by GC-MS analysis clearly showed the presence of 15 compounds. The active principles with their Retention Time (RT),Area,Area %,Height,Height 5% arev presented in the table. The GC-MS chromatogram of the peaks obtained shown in **Figure 8**.



**Figure 8:** GC-MS chromatogram of ethanolic extract of *cissus quadrangularis* and *cynadon dactylon***Table 2:** GC-MS chromatogram of ethanolic extract of *cissus quadrangularis* and *cynadon dactylon*

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	16.658	95929	1.86	28948	3.37	E3-TETRADECENYLACETATE	109.15
2	16.833	69343	1.35	5710	0.66	BICYCLO[3.1.0]HEXAN-3-ONE, 2,2-DIMETHYL-	67.10
3	31.675	154999	3.01	12739	1.48	OCTADECANOIC ACID, ETHYL ESTER	88.05
4	32.092	1066000	20.69	196384	22.86	.alpha.-Farnesene	124.10
5	32.142	1029510	19.98	223259	25.99	.gamma.-Sitosterol	55.05
6	32.250	657970	12.77	108357	12.61	.beta.-Sitosterol	231.05
7	32.467	335973	6.52	55883	6.51	STIGMASTANE-3,6-DIONE, (5.ALPHA.)-	53.05
8	32.560	421440	8.18	64304	7.49	Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate	331.20
9	34.824	79308	1.54	16350	1.90	ISOCHIAPIN B %2<	57.10
10	35.600	817007	15.86	84515	9.84	Cycloartenol acetate	69.10
11	36.252	83189	1.61	17583	2.05	2-methyltetracosane	57.05
12	37.433	63864	1.24	8229	0.96	Phenyl-1-thio-.alpha.-d-glucufuranoside	127.10
13	39.667	85287	1.66	14850	1.73	Sulfurous acid, 2-propyl tetradecyl ester	57.05
14	44.874	128609	2.50	13000	1.51	4-METHYL-N-(5-NITRO-1,3-THIAZOL-2-YL)-1,3-OXAZOLE-5-CARBOXAMIDE	110.15
15	49.100	63417	1.23	8856	1.03	LINOLEIC ACID TMS II	117.00
		5151845	100.00	858967	100.00		

Interpretation on mass spectrum of GC-MS was done using the database of National Institute Stanard and Technology (NIST 11) and WILEY 8 having more than 62,000 patterns. The mass spectrum of the unknown compound was compared with the spectrum of the known components stored in the NIST 11 and WILEY 8 library (Admas et al., 2001).

GC-MS analysis of *cissus quadrangularis* and *cynadon dactylon* of ethanolic extract revealed the existence of the compounds such as

- E3-TETRADECENYLACETATE
- BICYCLO[3.1.0]HEXAN-3-ONE, 2,2-DIMETHYL-
- OCTADECANOIC ACID, ETHYL ESTER

- .alpha.-Farnesene
- .gamma.-Sitosterol
- .beta.-Sitosterol
- STIGMASTANE-3, 6-DIONE, (5.ALPHA.)-
- Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate
- ISOCHIAPIN B %2<
- Cycloartenol acetate
- 2-methyltetracosane
- Phenyl-1-thio-.alpha.-d-glucofuranoside
- Sulfurous acid, 2-propyl tetradecyl ester
- 4-METHYL-N-(5-NITRO-1,3-THIAZOL-2-YL)-1,3-OXAZOLE-5-CARBOXAMIDE
- LINOLEIC ACID TMS II

These 15 chemical compounds responsible for the therapeutical activity

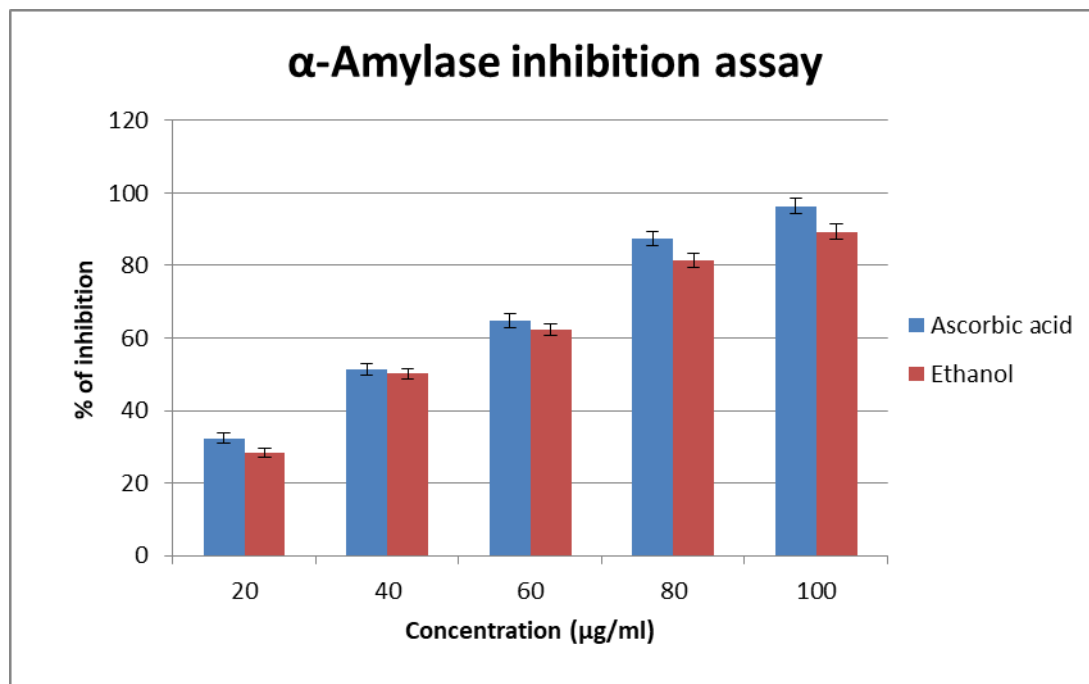
### IN VITRO $\alpha$ -AMYLASE INHIBITORY ASSAY

$\alpha$ -amylase catalyzes the hydrolysis of  $\alpha$ -1, 4-glucosidic linkages of starch, glycogen, and various oligosaccharides and simplifies the availability of sugars for the intestinal absorption. Inhibition of this enzyme activity in the digestive tract of humans is considered to be effective to control diabetes by diminishing the absorption of glucose decomposed from starch by this enzyme. There was a dose-dependent increase in percentage inhibitory activity against alpha- amylase enzyme. At a concentration of 20( $\mu\text{g}/\text{ml}$ ) of plant extract showed a percentage inhibition  $28.5 \pm 1.21\%$  and for 100 ( $\mu\text{g}/\text{ml}$ ) plant extract showed inhibition of  $96.1 \pm 2.01\%$  (**Table 1**)

**Table 3:** In vitro antidiabetic activity of alpha-amylase method

S.NO	Concentration of sample( $\mu\text{g}/\text{ml}$ )	Ascorbic acid	% of inhibition
1	20	$32.4 \pm 1.34$	$28.5 \pm 1.21$
2	40	$51.34 \pm 1.53$	$50.2 \pm 1.45$
3	60	$64.82 \pm 1.92$	$62.3 \pm 1.67$
4	80	$87.49 \pm 2.03$	$81.4 \pm 1.94$
5	100	$96.41 \pm 2.13$	$89.32 \pm 2.01$



**Figure 9:** In vitro antidiabetic activity of alpha-amylase method

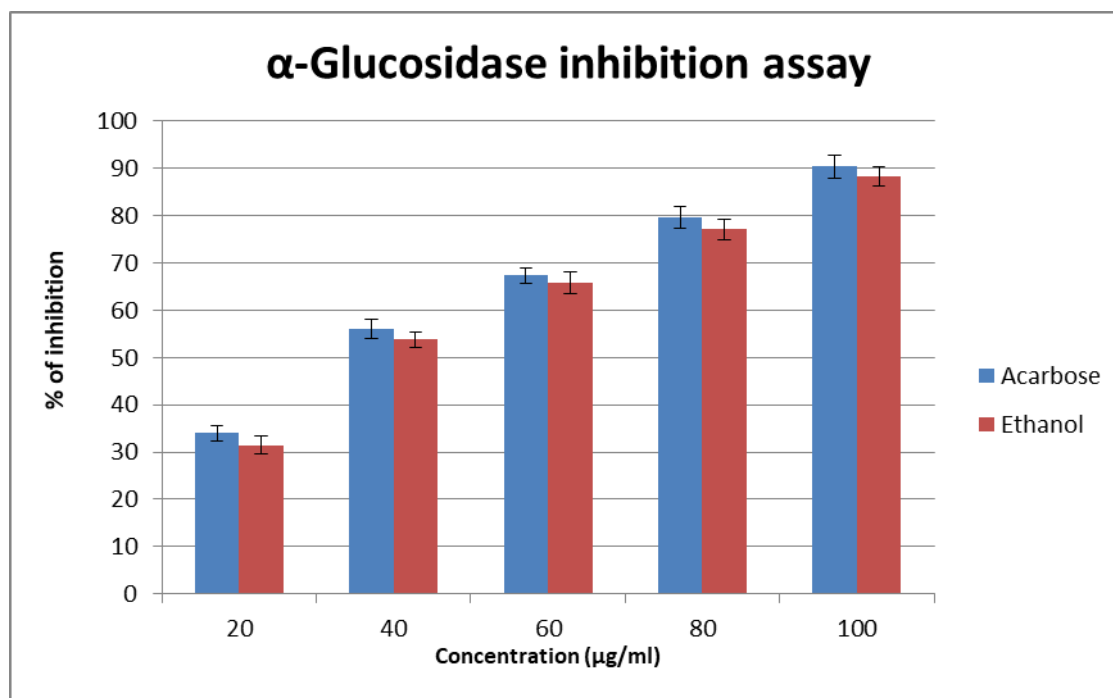
### IN VITRO $\alpha$ -GLUCOSIDASE INHIBITORY ASSAY

The *Cissus quadrangularis* and *Cynadon dactylon* ethanol extract revealed a significant inhibitory action of alpha-glucosidase enzyme. The percentage inhibition at 20-100 ( $\mu\text{g/ml}$ ) concentrations of *Cissus quadrangularis* and *Cynadon dactylon* extract showed a dose dependent increase in percentage inhibition. The percentage inhibition varied from  $31.7 \pm 1.94\%$ - $89.4 \pm 2.01\%$  for lowest highest concentration to the highest concentration (**Table 4**).

**Table 4:** In vitro antidiabetic activity of alpha glucosidase method

S. No	Concentration of sample ( $\mu\text{g/ml}$ )	acarbose	% of inhibition
1	20	$34.03 \pm 1.58$	$31.5 \pm 1.94$
2	40	$56.08 \pm 1.93$	$53.8 \pm 1.64$
3	60	$67.32 \pm 1.63$	$65.9 \pm 2.28$
4	80	$79.57 \pm 2.31$	$77.2 \pm 2.16$
5	100	$90.41 \pm 2.51$	$88.3 \pm 2.01$

Figure 10: Alpha amylase inhibition assay



### DPPH RADICAL SCAVENGING ACTIVITY

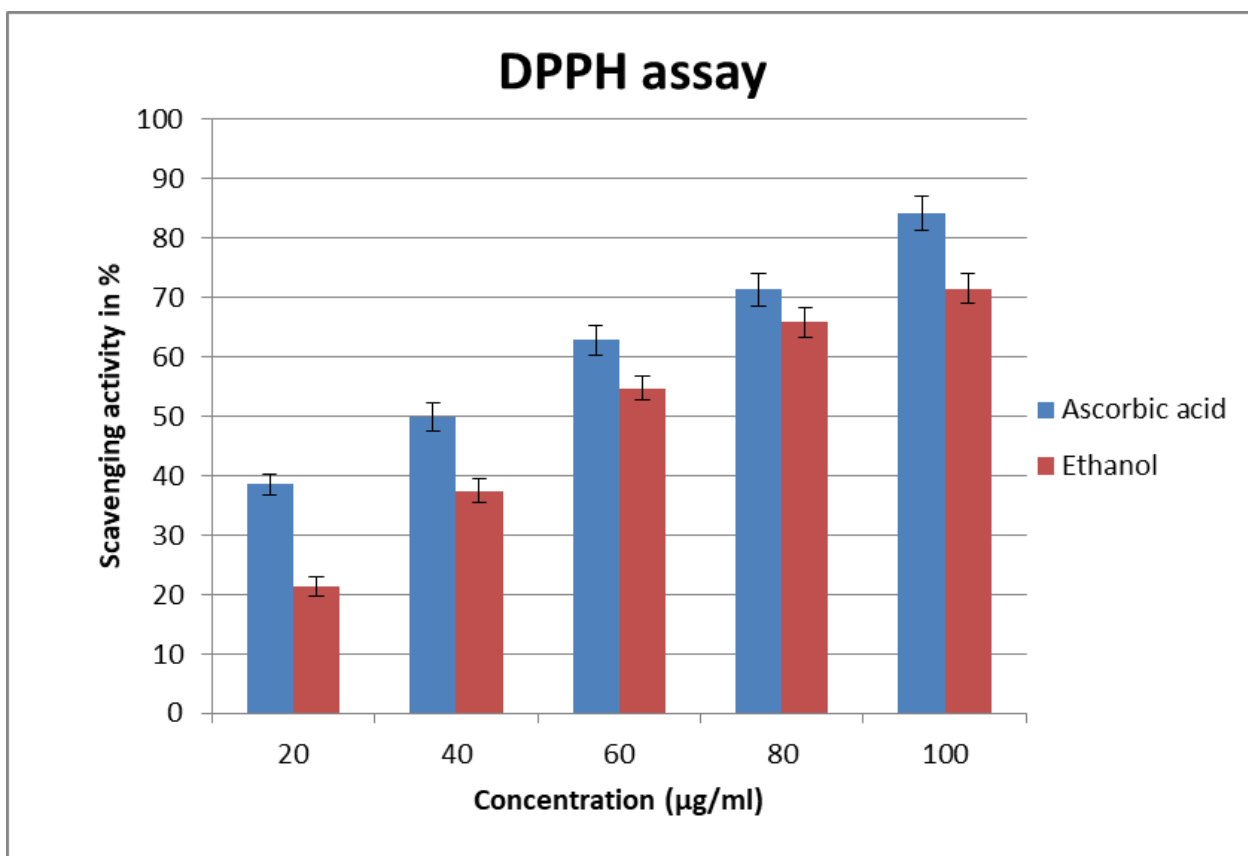
Antioxidants are compounds that protect cells against damaging effects of reactive oxygen species (ROS) which can neutralize free radicals before they can do harm and induce some damage to the cells.

The present study the plants extracts were tested in different concentrations against DPPH to find the radical scavenging activity. The antioxidant activity was found to be maximum for the 100(µg/ml) of extract that was used in the DPPH assay. DPPH radical scavenging activity shown in **Table 5** and graphical representation is shown in **Figure 11**.

**Table 5: DPPH radical scavenging activity**

S. No	Concentration of sample(ml)	Ascarobic acid	% of inhibition
1	20	38.5±1.85%	31.5±1.71%
2	40	49.92±2.32%	53.8±1.93%
3	60	62.83±2.51%	65.9±2.03%
4	80	71.32±2.81%	77.2±2.41%
5	100	84.13±2.92%	90.3±2.51%

**Figure 11: DPPH radical scavenging activity**



## ANTIFUNGAL ACTIVITY

The *Cissus quadrangularis* and *Cynodon dactylon* ethanol extract revealed a significant inhibitory action of fungal strain (*Aspergillus Niger*). The zone of inhibition is maximum at 500µl of plant extract is shown **Table 6**.

**Table 6:** Evaluate the antifungal activity

S.NO	ORGANISM	100µl	200µl	300µl	400µl	500µl
1	Control(Without plant extract)	0.5	0.5	0.5	0.5	0.5
2	<i>Aspergillus Niger</i>	1.2	1.5	1.8	1.9	2.3

## CONCLUSION

The present study we conclude that the combination of *Cissus quadrangularis* and *Cynodon dactylon* medicines contain more than 15 chemical compounds, which may contribute to their better inhibitory activity compared to the individual plants. Additionally, natural medicines like these are suggested to have lesser or no side effects compared to synthetic inhibitors.

Specifically, the study suggests that the combination of *Cissus quadrangularis* and *Cynodon dactylon* shows better antidiabetic, antioxidant, and antifungal activity compared to acarbose (a synthetic inhibitor) for  $\alpha$ -glucosidase and ascorbic acid (a standard) for  $\alpha$ -amylase. This indicates the potential of these plant medicines as effective alternatives for managing diabetes and related conditions.

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