

STUDY OF PRELIMINARY PHYTOCHEMICAL ANTI-DIABETIC PROPERTY OF CLEOME GYNANDRA

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Article Received on
04 August 2017,

Revised on 25 August 2017,
Accepted on 16 Sept. 2017

DOI: 10.20959/wjpr201712-9549

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ABSTRACT

In present times, growing and increasing interest in herbal medicines, consequently herbal medicines have established greater attention as an alternative to clinical therapy leading to increasing demand. The exclusive use of herbal drugs, prepared and dispensed by unscientifically trained herbalists, for treatment of diseases is very common in some rural communities. *Cleome gynandra*. Linn of Cleomaceae (Capparaceae) family is an annual herb, popular in the Ayurveda, Siddha systems of medicine. *Cleome gynandra* is an abundantly available species and grows as a weed in common barren land and in crop fields throughout India. The aim of this study is to

determine the phytochemical constituents of Ethanol extracts of *Cleome gynandra* (EECG) and aqueous extract of *Cleome gynandra* (AECG), Phytochemical screening of whole plant crude extracts of *Cleome gynandra* in solvents like ethanol, aqueous extract. The quantitative studies revealed that whole plant of *Cleome gynandra* possessed alkaloids, carbohydrates, glycosides, saponins, phytosterols, proteins, flavonoids and lignin. The *in vitro* α -amylase and α -glucosidase inhibitory studies established EECG and AECG had inhibitory activity of intestinal digestive enzyme. The α -amylase inhibitor may be valued as novel therapeutic dietetic agents. Hence EECG was potent inhibition of α -amylase and α -glucosidase intestinal digestive enzyme; it may be the beneficial effect of the type 2 diabetes. The present study concludes that the leaves of *Cleome gynandra* can be utilized as an alternative source of useful drugs. This study justifies the use of *Cleome gynandra* in the treatment of ailments like diabetic mellitus.

KEYWORDS: EECG (Ethanol extracts of *Cleome gynandra*), Aqueous Extract of *Cleome Gyandra* (AECG), α -amylase.

INTRODUCTION

Cleome gynandra is used as a medicinal plant and can be found in all over world. It grows as a weed in paddy fields and also in road sides and in open grass lands. In India it is never cultivated but grows rainy season everywhere. Different species of cleome can be found in all states of India.

Cleome gynandra belongs to Cleomaceae family. Oral administration of a decoction or an infusion of the boiled leaves or the leaf-juice has been recorded to facilitate child birth, to relieve stomach pain, beneficial in constipation, thread-worm infection, conjunctivitis, oral ailments, and convulsions and in certain bilious disorders.^[1,2] Extracts of the leaves and certain isolated flavonoids have been reported to possess antibacterial, antifungal, anti-neoplastic and anti-arthritic properties and improved the levels of endogenous antioxidants and also modulated glucose metabolizing enzyme activity.^[3]

The plant possess the activities like anti-inflammatory,^[4] free radical scavenging,^[5] anticancer,^[6] and immune modulatory effects.^[7] Preliminary phytochemical screening of the powdered leaf revealed the following compounds Carotenoids, Cardiac glycosides, Cyanogenic Glycosides, Flavonoids, Saponins, Triterpenes, sugars, Tannins.^[10]

The isolation of oleic acid, linolic acid, palmitic acid, stearic acid, arachidic acid and a phytosterol from seeds oil of *Cleome pentaphylla* Linn The root consists of two glycoflavonones as naringenin-4-galactoside-1 and dihydrokaemferol-4f-galactoside-2.^[11]

In some part of leaves and roots are used by some tribal and traditional healers as an antidiabetic drug. Although the hypoglycaemic properties are not yet studied or proved, *Cleome gynandra* is believed to have the efficacy of lowering blood sugar. The possibility of cleome gynandra to use in Diabetes may be reasoned for its anti-oxidant properties, Immunomodulatory properties and due to its nutritive value. The glucose oxidation enhancing bioactivity, due to the active phytochemicals are likely to be phenolic in nature also available in *Cleome gynandra* in plenty.^[8]

One such ethno botanically important plant, *Cleome gynandra* (Cleomaceae), a plant drug of traditional systems of medicine in India^[9] i.e., Ayurveda and siddha is used for the treatment

of diabetes mellitus. In the present study made an effort to pharmacologically evaluate the plant for its anti-diabetic property.

Aim of the study was to investigate the *Cleome gynandra* plant for its anti-diabetic property to investigate the hypoglycemic effect of *Cleome gynandra* ethanol, aqueous whole plant extract. To Determination the phytochemical analysis of whole plant extract of *Cleome gynandra*.

MATERIALS AND METHODS

The plan of the work for the study of *Cleome gynandra*.Linn Was carried out as follows

1. Identification, collection and drying of *Cleome gynandra*

Fresh aerial plant parts (shoots, leaves, flowers and fresh pods) believed to have antidiabetic activity were collected from cultivated lands around from the town Komarapalayam, Namakkal (Dt),Tamilnadu, India. The plant material was identified and authenticated by Dr. M. Palanisamy, Scientist-D, Botanical survey of India, Plant anatomy research center, Coimbatore, Tamilnadu. The collected and identified plant materials were brought to the laboratory, washed with fresh distilled water, cut into small pieces, and air-dried under shade at room temperature. The dried plant materials were crushed into small pieces and blended using a portable electric blender to reduce them into powder form and weight of the powder taken.

1.1. Preliminary Phytochemical Studies

Extraction

Extraction was done with ethanol& aqueous extraction.

Soxhlet extraction

A predetermined amount (30gm) of the dried plant powder was put in a Soxhlet apparatus to which enough aqueous was added to submerge the powder and continuously extract it, until the extracting solvent became clear in the thimble indicating that the extraction of the phytochemicals soluble in the solvent was aqueous complete. The volume was noted; the aqueous extract was dried in an oven at a temperature of 60 °C centigrade, and the weight of the dry extract taken.

A predetermined amount (30gm) of the dried plant powder was put in a Soxhlet apparatus to which enough ethanol, (70 % v/v) was added to submerge the powder and continuously

extract it, until the extracting solvent became clear in the thimble indicating that the extraction of the phytochemicals soluble in the solvent was ethanol complete. The volume was noted; the ethanol extract was dried in an oven at a temperature of 60 °C centigrade, and the weight of the dry extract taken.

1.2. Qualitative phytochemical analysis

Test to find out the following phytochemical

Alkaloids, Saponins, Glycosides, Carbohydrates, Tannins, Flavonoids, Steroids, Phenolic compounds, Proteins and amino acids, Fixed oils and fatty acids, Gums and mucilage.

1.3. Pharmacological studies

Anti diabetic activity

- The study of *in vitro* antidiabetic activity has shown that ethanol and aqueous extracts
- The results were expressed in terms of IC₅₀, which is the concentration of the tested fraction to inhibit respective enzyme by 50 percentages.
- The Ethanol extract of *Cleome gynandra* (EECG) and aqueous extract of *Cleome gynandra* (AECG), elicited a dose dependent inhibition of α -amylase enzyme activity. The α -amylase inhibitory effect of the EECG& AECG was studied at concentrations 10 –100 μ g/ml.

1.4. Drugs, chemicals and instruments: UV-Visible Spectroscopy, Shimadzu 1800 UV-Vis spectrophotometer, Shimadzu. Japan.

1.5. Chemicals and Drugs used: Ethanol (Changshu yangyuan. Fine Chem, Ltd., India), Dimethyl sulfoxide,(LOBA Fine Chem, Ltd., India), Dinitro salicylic acid(Research Lab, Fine Chem, Ltd., India.) α -amylase, α - glucosidase, Research Lab,Mumbai, India. (Research Lab, Mumbai, India), Maltose (LOBA Fine Chem, Ltd., India), Potato starch, Acarbose (Glucobay 25, Bayer, india).

1.6. Plant Materials

The whole of *Cleome gynandra* L was collected from the town Komarapalayam, Namakkal (DT), Tamilnadu, India. The plant material was identified and authenticated by Dr. M. Palanisamy, Scientist-D, Botanical survey of India, Plant anatomy research center, Coimbatore, Tamilnadu.

2.0. Pharmacognostical Evaluation; Physicochemical Parameters

A. Ash Values

Ash values are practically much useful in ascertaining the purity and quality of plant derived crude drugs, particularly in the powdered form.

The ash content of crude drug is generally taken to be the remaining residue after the incineration of powdered material. Ash standards are recognized for a number of official drugs. The standards are usually getting a higher limit on the total ash or acid insoluble ash permitted.

The residue of total ash is the remaining after incineration. The acid insoluble ash is the division of the total ash, which are insoluble in diluted hydrochloric acid.

The residue or ash yielded by organic chemical compounds as a rule, the amount of inorganic matters is a measure of presence as an impurity. In general, the inorganic matters are present in relatively trace amounts. The purification process, which is difficult to remove such impurities and practically acceptable if only trace amount, is present. The determination of the quality and purity of ash values are helpful in the crude drugs in powder form.

The detailed procedures given in Indian pharmacopoeia were used to determine the crude drug's ash values such as total ash and acid insoluble ash.

2.1. Determination of Total Ash Value

Accurately weighed about 3 gm of dried powdered whole plant parts of the *cleome gynandra* was taken in a silica crucible and incinerated in a muffle furnace at a temperature not exceeding 450°C until free from carbon, cooled and weighed, the charred mass exhaust with hot water, residue was collected on ash less filter paper, and incinerate the residue and filter paper until the white ash or nearly so. Calculate the total ash percentage with reference to the air dried drug.

2.2. Determination of Water Soluble Ash Value

The total ash was obtained and boiled with 25 ml of water for 5 minutes. Then the insoluble matter was collected on an ash less filter paper, washed with hot water for 15 min at a temperature not exceeding 450°C. The insoluble matter was subtracted from the weight of total ash. The variation in weight represents the water soluble ash. The water soluble ash percentage was calculated with reference to the air dried drug.

2.3. Determination of Acid Insoluble Ash Value

The obtained ash directed under total ash was boiled with 25 ml of 2M HCL for 5 minutes. To collect insoluble matters on ash less filter paper and washed with hot water and weighed, then acid insoluble ash percentage was calculated with reference to the air dried drug.

2.4. Determination of Extractive Values

Extractive values of coarse powdered crude drugs are useful for their evaluation, the extraction of the drug with an exacting solvent yield a solution containing different phytoconstituents. The interested phytoconstituents of composition in that depends upon the drug nature and solvent used. Further, this value indicates the present crude drug in nature constituents.

2.5. Determination of Alcohol Soluble Extractive Value

Weigh accurately 5 gm of air-dried coarse powder of whole plant parts of *cleome gynandra* was soaked with 100 ml of ethanol (90%) in a closed flask for 24 hours, frequently agitated during the first 6 hours and stand to allow for 18 hrs. Then it was filtered immediately against the loss of the solvent. The 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105°C and weighed. The percentage of ethanol soluble extractive value was calculated with reference to the air-dried drug.

2.6. Determination of Water Soluble Extractive Value

Weigh accurately 5 gm of coarsely powdered whole plant parts of the *cleome gynandra* and macerate with 100 ml of water in closed flak for 24 hours, shaking frequently during the first 6 hours and allow to stand for 18 hours. Thereafter, it was filtered rapidly taking precautions against loss of the solvent. Then filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105°C and weighed. The water soluble extractive percentage was calculated with reference to air dried drug.

2.7. Loss on Drying

The percentage loss on weight while drying determines the amount of volatile matter of any type (including water) that can be driven off under the condition specified (Hot air oven or Desiccators). If the sample is large sized crystal form, then reduce the particle size by quick crushing into a powder.

Procedure

The powdered drug of 1.5 gm. was weighed accurately in a porcelain dish, which was formerly dried at 105°C in hot air oven to constant and then weighed. From the weight difference, then calculate the percentage loss on drying with reference to the air dried substance.

Extraction(A) Crude Extraction

The powdered plant materials were initially subjected to Soxhlet extraction. Separately powdered 30 grams of dried powdered whole plant parts of *cleome gynandra* was extracted with ethanol in a Soxhlet extractor for 36 hours. Then dried powder (30 gm.) was extracted and concentrated to yield a reddish brown solid. All the extracts were concentrated to dryness by evaporation under controlled temperature (40-50°C). All the extracts were stored in a refrigerator at 4°C until further use.

The powdered plant materials were initially subjected to Soxhlet extraction. Separately powdered 30 grams of dried powdered whole plant parts of *cleome gynandra* was extracted with aqueous in a Soxhlet extractor for 36 hours. Then dried powder (30 gm.) was extracted and concentrated to yield a reddish brown solid. All the extracts were concentrated to dryness by evaporation under controlled temperature (40-50°C). All the extracts were stored in a refrigerator at 4°C until further use.

3.0. Qualitative Phytochemical Analysis

A complete and systematic study of crude drugs should include a complete investigation of both primary and secondary metabolites of plant metabolism. The different qualitative chemical tests using various chemical reagents are to be performed for establishing chemical aspect of extracts for their nature of chemical composition.

3.1. A) Test for carbohydrates

1. Molisch Test: Small portion of extracts treated with α -naphthol and concentrated sulphuric acid along the sides of the test tube. Reddish violet or purple colour was produced at the junction between two liquids.

2. Fehling's Test: To the extracts equal quantity of Fehling's solution A and B are added. While heating gently; the brick red precipitate is obtained.

3. Benedict's test: To the 5 ml of Benedict's reagent, add 8 drops of solution under examination. Mix well, boiling the mixture vigorously for 2 minutes and then cool. Red precipitate was obtained.

4. Barfoed's test: Add 0.5 ml of solution under examination to the 5 ml of the Barfoed's solution, red precipitate is formed while heat to boiling.

3.2. B) Test for Alkaloids

1. Dragendroff's Test: To the extract, add 1 ml of Dragendroff's reagent orange red precipitate is produced.

2. Wagner's test: To the extract add Wagner's reagent. The reddish brown precipitate is produced.

3. Mayer's Test: To the extract add 1 ml or 2 ml of Mayer's reagent. The dull white precipitate is produced.

4. Hager's Test: To the extract add 3 ml of Hager's reagent yellow Precipitate is produced.

3.3. C) Test for Steroids and Sterols

1. Liebermann Burchard test: Dissolve the test sample in 2 ml of chloroform in a dry test tube then add 10 drops of acetic anhydride and 2 drops of concentrated sulphuric acid. The solution becomes red, then blue and finally bluish green in colour.

2. Salkowski test: Dissolve the test solution in chloroform and add equal volume of conc. sulphuric acid. Bluish cherry red and purple color is noted in chloroform layer, whereas acid assumes marked green fluorescence.

3.4. D) Test for Glycosides

1. Legal's test

Sample is dissolved in pyridine; sodium nitroprusside solution is added to it and made alkaline. Pink red colour is produced.

2. Baljet test

To the drug sample, sodium picrate solution is added. Yellow to orange colour is produced.

3. Borntrager test: Add a few ml of dilute sulphuric acid to the test solution. Boil, filter and extract the filtrate with ether or chloroform. Then organic layer is separated to which ammonia is added, pink, red or violet colour is produced in organic layer.

4. Killer Killani test: Sample is dissolved in acetic acid containing traces of ferric chloride and transferred to the surface of concentrated sulphuric acid. At the junction of liquid reddish brown color is produced which gradually becomes blue.

3.5. E) Test for Saponins

Foam test: About 1 ml of alcoholic sample is taken in a graduated cylinder and it is diluted to 20 ml with distilled water and shaken for 15 min. The formation of 1 cm layer of foam indicates the presence of saponins.

3.6. F) Test for Flavonoids

Shinoda test: To the sample, magnesium turnings and then concentrated hydrochloric acid is added. Red colour is produced.

3.7. G) Tests for Tannins and Phenolic Compounds

The Phenol content in the raw material was estimated by using following reagents.

To 2-3 ml of extract, add few drops of following reagents

a). **5% FeCl₃ solution:** Deep blue-black color.

b). **Lead acetate solution:** White precipitate.

c). **Gelatin solution:** White precipitate.

d). **Acetic acid solution:** Red color solution.

e). **Dilute iodine solution:** Transient red color.

f). **Dilute HNO₃:** Reddish to yellow color.

3.8. H) Test for Fixed Oils and Fatty acids

1. Spot test: A small quantity of the extract is placed between two filter papers. Oil stain produced with any extract shows the presence of fixed oils and fats in the extracts.

2. Saponification test

Few drops of 0.5N alcoholic potassium hydroxide are added to the extract with a few drops of phenolphthalein solution. Later the mixture is heated on a water bath for 1-2 hour soap formation indicates the presence of fixed oils and fats in the extracts.

3.9. I) Test for Proteins and Amino acids

1. Biuret test: Add 1 ml of 40% sodium hydroxide and 2 drops of 1% copper sulphate to the extract, a violet colour indicates the presence of proteins.

2. Ninhydrin test: Add 2 drops of freshly prepared 0.2% Ninhydrin reagent to the extract and heat. A blue colour develops indicating the presence of proteins, peptides or amino acids.

3. Xanthoproteic test: To the extract, add 20% of sodium hydroxide and conc. HNO₃. Yellow or yellowish orange colour indicates presence of aromatic amino acid while gently heating.

4.0. *In vitro* Anti diabetic activity

Diabetes is a disease, which still eludes effective and satisfactory cure. It may be possible that the immensely rich plant resources of our country can definitely provide effective hypoglycemic agents and it has been estimated that more than 3000 species of plants have been used throughout the world to treat diabetes. A novel antidiabetic drug should possess hypoglycemic at low concentration against β cells and should be safe against higher concentrations.

The study of *in vitro* antidiabetic activity has shown that ethanol and aqueous extracts is potent. Hence, ethanol extract is selected and fractioned with different solvents; the ethyl acetate and n-butanol and the fractions were tested for *in vitro* antidiabetic activity; inhibition of α -amylase. The results were expressed in terms of IC₅₀, which is the concentration of the tested fraction to inhibit respective enzyme by 50 percentages.

5.0. Statistical analysis

All the values were expressed as mean \pm Standard Error of Mean (SEM) and were analyzed for significance by ANOVA and groups were compared with Dunnett test, using In Stat v.2.02 software (GraphPad Software Inc.). Differences between groups (p Value) were considered significant at P<0.05 level.

RESULTS AND DISCUSSION

Cleome gynandra belonging to family Cleomaceae was selected for our project, on the basis of ethanobotanical information which reveals its uses against one of the most hazardous disease. We felt worthwhile to validate scientifically, the folk claim for its therapeutic activity. We have also taken its detailed Pharmacognostical and preliminary phytochemical

investigations to prove its appropriate identification and rationalize its use as drug of therapeutic importance.

6.0. Pharmacognostical studies

Analytical parameters

The analytical parameters were investigated and reported as, total ash value (10.4%w/w), water soluble ash value (2.5 %w/w), acid insoluble ash value (1.7%w/w), sulphated ash value (3.7 %w/w), water soluble extractive value (3.6 %w/w), alcohol soluble extractive value (5.4 %w/w), loss on drying (12.6 %w/w). The above studies were enabled to identify the plant material for future investigation and form an important aspect of drug studies. The results were given in table 3 and 4.

6.1. Ash values

Table. 1: Data for ash values for powdered whole plant of *Cleome gynandra*.

S. No	Parameter	%w/w
	Ash values	
1.	Total Ash	10.4
2.	Water Soluble Ash	2.5
3.	Acid Insoluble Ash	1.7
4.	Sulphated Ash	3.7

Table. 2: Extractive values and loss on drying.

Analytical parameter	Percentage (w/w)
Water soluble extractive	3.6 %
Alcohol soluble extractive	5.4 %
Loss on drying	12.6 %

6.2. Preliminary phytochemical studies

The whole plant of *Cleome gynandra* was subjected for hot continuous extraction using ethanol as solvent. The yield was found to be 5.27% w/w. the extracts obtained were subjected to various phytochemical tests, to identify the active constituents, which showed the presence of phytosterols, carbohydrate, fatty acids and proteins, flavonoids and phenolic compounds. The results were given in table 3.

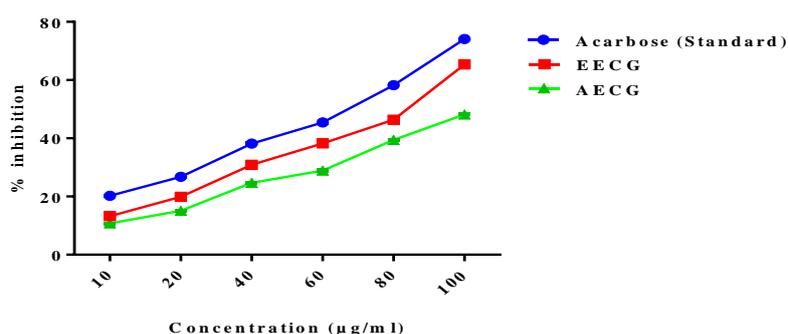
Table. 3: Presence of phytoconstituents in whole plant of cleome gynandra.

Phytoconstituents	Ethanol extract	Aqueous extract
Alkaloids	-	-
Saponins	-	-
Glycosides	-	-
Carbohydrates	+	+
Tannins	-	-
Flavonoids	+	+
Steroids	+	+
Phenolic compounds	+	+
Proteins and amino acids	+	+
Fixed oils and fatty acids	+	+
Gums and mucilage	+	+

6.3. Pharmacological studies: *In vitro* antidiabetic activity.**Table. 4: *In vitro* α -amylase inhibitory activity of *Cleome gynandra* extracts.**

Test substance	Concentration ($\mu\text{g/ml}$)% inhibition						IC ₅₀ ($\mu\text{g/ml}$)
	10	20	40	60	80	100	
Acarbose (Standard)	20.25 \pm 0.02	26.75 \pm 0.05	38.17 \pm 0.05	45.42 \pm 0.03	58.24 \pm 0.02	74.12 \pm 0.05	55.00\pm0.25
EECG	13.21 \pm 0.04	19.90 \pm 0.05	30.091 \pm 0.02	38.21 \pm 0.02	46.38 \pm 0.05	65.34 \pm 0.03	77.53\pm0.15
AECG	10.76 \pm 0.03	15.13 \pm 0.04	24.71 \pm 0.04	28.90 \pm 0.05	39.42 \pm 0.05	48.59 \pm 0.02	123.5\pm0.40

The Ethanol extract of *Cleome gynandra* (EECG) and aqueous extract of *Cleome gynandra* (AECG), elicited a dose dependent inhibition of α -amylase enzyme activity. The α -amylase inhibitory effect of the EECG was found to be ranging from 13.21 % to 65.34% when studied at concentrations 10 –100 $\mu\text{g/ml}$. At the same concentration range the inhibitory effect of AECG was found to be ranging from 10.76% to 48.59%, whereas the effect of standard drug acarbose ranged from 20.25% to 74.12%. The IC₅₀ of EECG was found to be 77.53 \pm 0.15 $\mu\text{g/ml}$, whereas AECG showed at 123.5 \pm 0.40 $\mu\text{g/ml}$. The IC₅₀ of acarbose was found to be 55.00 \pm 0.25 $\mu\text{g/ml}$ (Table-4 and Figure-1).

**Figure. 1: *In vitro* α -amylase inhibitory activity of *Cleome gynandra* extracts.**

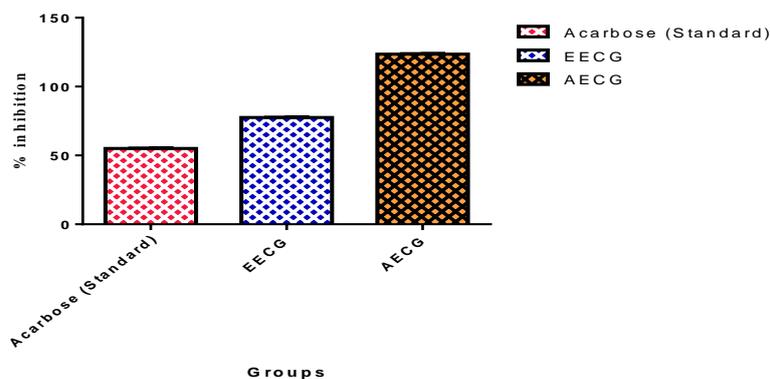


Figure. 2: IC₅₀ value of *in vitro* α -amylase inhibitory activity of *Cleome gynandra*.

Extracts

In vitro inhibition of α -glycosidas: The extract of EEGG and AECG, elicited a dose dependent inhibition of α -glycosidase enzyme activity.

Table. 5: *In vitro* α - glycosidase inhibitory activity of *Cleome gynandra* extracts.

Test substance	Concentration ($\mu\text{g/ml}$)% inhibition						IC ₅₀ ($\mu\text{g/ml}$)
	10	20	40	60	80	100	
Acarbose (Standard)	17.65 \pm 0.09	26.99 \pm 0.08	35.18 \pm 0.05	42.84 \pm 0.06	53.73 \pm 0.06	64.99 \pm 0.09	66.64\pm0.25
EECG	10.14 \pm 0.03	19.48 \pm 0.03	32.24 \pm 0.05	36.11 \pm 0.04	48.62 \pm 0.07	58.48 \pm 0.05	83.21\pm0.15
AECG	9.23 \pm 0.05	17.10 \pm 0.07	26.21 \pm 0.07	35.84 \pm 0.06	44.32 \pm 0.05	53.67 \pm 0.09	95.88\pm0.40

The α -glycosidase inhibitory effect of the EEGG was found to be ranging from 10.14 % to 58.48% when studied at concentrations 10–100 $\mu\text{g/ml}$. At the same concentration range the inhibitory effect of AECG was found to be ranging from 9.23% to 53.67%, whereas the effect of standard drug acarbose ranged from 17.65% to 64.99%. The IC₅₀ of EEGG was found to be 83.21 \pm 0.28 $\mu\text{g/ml}$, whereas AECG showed at 95.88 \pm 0.17 $\mu\text{g/ml}$. The IC₅₀ of acarbose was found to be 66.64 \pm 0.51 $\mu\text{g/ml}$ (Table-5 and Figure-3).

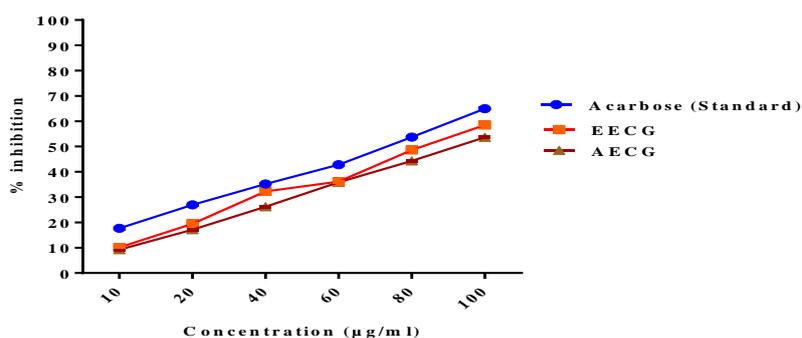


Figure. 3: *In vitro* α -glucosidase inhibitory activity of *Cleome gynandra* extracts.

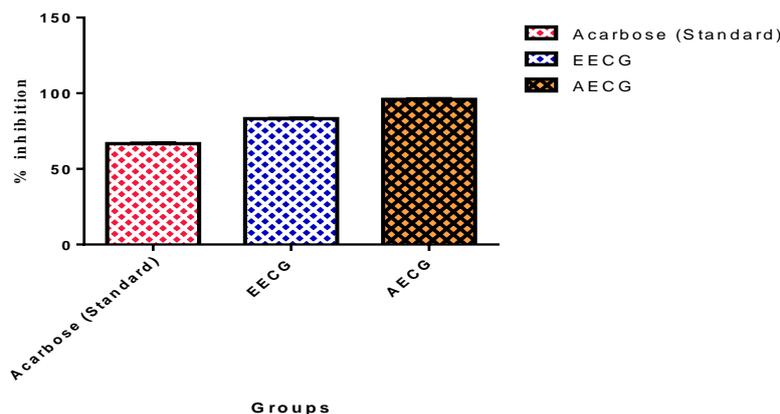


Figure. 4: IC₅₀ value of *in vitro* α -glucosidase inhibitory activity of *Cleome gynandra*.

Extracts

A. *In vitro* Inhibition of α -amylase

Preparation of test and standard solutions: The Ethanol extract of *cleome gynandra* (EECD) and aqueous extract of *cleome gynandra* (AECD) and the standard Acarbose (20 mg each) were separately dissolved in 20 ml of freshly prepared 5% distilled DMSO. These solutions were serially diluted with freshly prepared distilled DMSO to obtain the lower dilutions.

Procedure: The different concentrations (5–1000 μ g/ml) of EECD and AECD, standard Acarbose were prepared in 5% DMSO. 500 μ l of test/standard was added to 500 μ l of α -amylase (0.5 mg/ml) and was incubated for 10 min at room temperature. Then added 500 μ l of 1% starch solution and incubated for another 10 min. After that 1 ml of the 3, 5-dinitrosalicylic acid as a coloring reagent was added to the reaction mixture and heated in a boiling water bath for 5 min. After cooling, it was diluted with 10 ml of distilled water.

The absorbance was then measured at 540 nm against the reagent blank. The α -amylase inhibition was expressed as percentage of inhibition and the IC₅₀ values determined by linear regression plots with varying concentration of fraction against percentage inhibition. The percentage inhibition was calculated using the formula given below.

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

DISCUSSION

In present times, growing and increasing interest in herbal medicines, consequently herbal medicines have established greater attention as an alternative to clinical therapy leading to

increasing demand. The exclusive use of herbal drugs, prepared and dispensed by unscientifically trained herbalists, for treatment of diseases is very common in some rural communities. The experimental screening method is important in order to ascertain the safety and efficacy of herbal products as well as to establish the active component of these herbal remedies. The use of herbal medicines is gradually more popular now a days, the majority of herbal products is marketed without reliable scientific evidence and mandatory safety and evaluation of toxicity in most countries due to the evidence regarding side effects of herbal medicine. FDA has published about guidance documents controlling the herbal products. There is a rising demand of toxicological evaluation of herbal medicines.

The plant phytochemical studies could be provide an answer to the society seeking for better therapeutic medicine from natural sources which is supposed to be more efficient with less or no side effects.

The results obtained from the present phytochemical analysis of the whole plant of *Cleome gynandra* showed the presence of alkaloids, tannins, flavonoids, cardiac glycosides, steroids, saponins, phenols, flavonoids, phytosterols, lignins, and macronutrient analysis revealed the presence of proteins, carbohydrates, reducing sugar, fats and oil. Alkaloids and flavonoids have been used as antiviral, antibacterial, antiamebic and anticancer agents. Phenols and flavonoids are the groups of secondary metabolites are of great importance as cellular support material because they form the integral part of cell wall structure by polymeric phenolics and they can protect the human body from the oxidative stress which may cause many disease, including cancer, cardiovascular problems and ageing.

The *in vitro* α -amylase and α -glucosidase inhibitory studies established EECG and AECG had inhibitory activity of intestinal digestive enzyme. The percentage inhibition showed a concentration dependent reduction. These enzymes are accountable in hydrolyzing dietary starch into maltose and then split down into glucose prior to absorption. Since α -amylases plays important role in the breakdown of starch in human beings and animals, the presence of such inhibitors in food material may be responsible for impaired starch digestion. The α -amylase inhibitor may be valued as novel therapeutic diabetic agents.

Acarbose like drugs, that inhibit α -glucosidase present in the small intestine epithelium, have been verified to decrease postprandial hyperglycemia and improve damaged glucose metabolism without promoting insulin secretion in type 2 diabetic patients. These

medications are useful for people who have blood glucose levels are above the level considered serious for diabetes and also useful for people taking sulfonylurea medication. Therefore, the breaking and delay of carbohydrate absorption with a natural based α -glucosidase inhibitor offers a prospective therapeutic approach for the management of type 2 diabetes mellitus. Hence EECG was potent inhibition of α -amylase and α -glucosidase intestinal digestive enzyme; it may be the beneficial effect of the type 2 diabetes.

CONCLUSION

The present study concludes that the whole plant of *Cleome gynandra* can be utilized as an alternative source of useful drugs. This study justifies the use of *Cleome gynandra* in the treatment of ailments like diabetic mellitus, Non-nutrient, phytochemical content which act as antioxidant against dangerous free radicals in the body system.

In this study suggest the identified phytochemical compounds may be the bioactive constituents which are medicinally valuable. Based on the obtained results and observations, we can infer that the plant of *Cleome gynandra* L could be used for the supportive treatment of diabetes mellitus.

ACKNOWLEDGEMENT

The authors are thankful to Principal and Research Department of chemistry, SSM College of Pharmacy, Bhavani, Erode district, Tamilnadu for constant encouragement and providing necessary facilities.

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