Research Article

Phytochemical Evaluation and *In vitro* Antidiabetic Activity of Ethanolic Extract of *Viscum articulatum*

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ABSTRACT

Natural products are the major mine for discovering promising lead candidates, which play an important role in future drug development programs. Ease of availability, least side effects and low cost make the herbal preparations are the main key player of all available therapies, especially in rural areas. Since centuries, many plants are considered a fundamental source of potent anti-diabetic drugs. *Viscum articulatum* is a herbal medicinal plant belonging to Family *Viscaceae*, and mentioned in Ayurveda, Siddha, and Chinese medicinal system for treatment of various disorders. The literature survey confirms that the anti-diabetic activity of *Viscum articulatum* has not been scientifically investigated. Hence, the present study is under taken for the in vitro anti-diabetic activity of the whole palnt of *Viscum articulatum* to evaluate its traditionally claimed anti-diabetic activity. The whole plant of *Viscum articulatum* which belongs to family viscaceae have been investigated in a systemic way covering extraction, qualitative phytochemical analysis, invitro anti-diabetic activity. The powdered material (100 gm) was subjected to solvent extraction in soxhlet apparatus with ethanol as solvent. The colour of ethanolic extract was green and its yield is 7. 1gm. The ethanolic extract of *Viscum articulatum* was subjected for the preliminary phytochemical analysis and found for the presence of flavonoids, steroids, alkaloids, terpenoids. The anti-diabetic activity of ethanolic extract of the plant was done by alpha amylase inhibitory method and IC_{50} value of extract was found to be 53.79. From the results it was observed that the ethanolic extract of *Viscum articulatum* is moderately suitable for the control diabetic conditions.

Keywords: *Viscum articulatum*, anti-diabetic activity, IC50, alpha amylase inhibitory method, Herbal products.

INTRODUCTION

A medicinal plant is any plant in which one or more of its organs contains substances that can be used for therapeutic purpose or which are precursors for the synthesis of useful drugs. Medicinal plants have always been considered a healthy source of life for all people. Therapeutically properties of medical plants are very useful in healing various diseases and the advantage of these medicinal plants is being 100% natural [1]. Herbal products are often perceived as safe because they are "natural". In India, in recent years, there is increased research on traditional ayurvedic herbal medicines on the basis of their known effectiveness in the treatment of ailments for which they have been traditionally applied. Considerable efforts have been directed towards the discovery and development of natural products from various plant and animal sources which have antiplatelet, anticoagulant, antithrombotic, and thrombolytic activities etc.

The term diabetes is the shortened version of the full name diabetes mellitus. Diabetes mellitus is derived from the Greek word diabetes meaning siphon- to pass through and the Latin word mellitus meaning honeyed or sweet. This is because in diabetes excess sugar is found in blood as well as the urine. It was known in the 17th century as the "pissing evil". The term diabetes was probably coined by Apollonius of Memphis around 250 B.C [2-3]. In 1910, Sir Edward Albert Sharpey-Schafer suggested that people with diabetes were deficient in a single chemical that was normally produced by pancreas – he proposed called this substance 'insulin', from the Latin Insula, meaning Island, in reference to the insulin- producing islets of Langerhans in the pancreas [4-6].

Natural products are the major mine for discovering promising lead candidates, which play an important role in future drug development programs. Ease of availability, least side effects and low cost make the herbal preparations are the main key player of all available therapies, especially in rural areas [7-9]. Since centuries, many plants are considered a fundamental source of potent anti-diabetic drugs. Although, synthetic oral hypoglycemic together with insulin are the main route for controlling diabetes. However, they exhibited prominent side effects and failed to reverse the course of its complications. This constitutes the major force for finding alternatives, mainly from plant kingdom that are of less severe or even no side effects [11,12].

Viscum articulatum is an herbal medicinal plant belonging to Family *Viscaceae*, and mentioned in Ayurveda, Siddha, and Chinese medicinal system for treatment of various disorders. It has many medicinal values and used traditionally and ethnobotanical for the treatment of rheumatism arthritis, bone fracture, cuts, wound healing, fever, fatigue, liver diseases, epilepsy, blood diseases, etc. The literature survey confirms that the anti-diabetic activity of *Viscum articulatum* has not been scientifically

investigated. Hence, the present study is under taken for the in vitro anti diabetic activity of the whole palnt of *Viscum articulatum* to evaluate its traditionally claimed anti diabetic activity [13-16].

MATERIALS AND METHODS

Materials

The plant materials of *Viscum articulatum* were freshly collected from Talakona forest and nearby villages of Andhra Pradesh state. The plants materials were then identified and Authentified by Dr.Madharachetty department of botany, Venkateswara University, Tirupati, Andhra Pradesh. A voucher specimen of *Viscum articulatum* has been deposited in the Herbarium of the department of botany for the further reference. All the solvents and reagents used in the present work are of analytical grade. Required chemicals were procured from Qualigeris chemicals pvt. Ltd, Mumbai.

Experimental methods

Preparation of plant extraction

The freshly collected plant materials are washed, and then dried in hot air oven at a temperature not more than 50° C. The dried materials were coarsely powdered and the powder (100 g) was packed in soxhlet apparatus and successively extracted with ethanol. Finally extracts was concentrated in rotary evaporator at a temperature not more than 50^0C and then, dried under vacuum dessicators. Thus, obtained extract was used for further experiments. In the current research we have used the ethanolic extract of *Viscum articulatum.* The plant material was air-dried and reduced to coarse powder the powdered material 100g was subjected to solvent extraction in soxhlet apparatus with ethanol the extraction procedure was continued until the colorless solution was obtained and the solution was concentrated with rotary evaporator under reduced pressure and the dried extract was weighed the percentage yield were calculated. The collected extracts were stored and then used for further analysis [17].

Figure 1: Soxhlet extraction of *Viscum articulatum*

Preliminary phytochemical screening[18]

Preliminary phytochemical screening was done using the specified protocols for the qualitative analysis of Alkaloids, carbohydrates, fixed oils, flavonoids, glycosides, phytosterol/terpenes, saponins, and tannins/phenols. The screening tests as follows,

I. Test for Alkaloids: About 50 mg of solvent-free extract was stirred with little quantity of dilute hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloid reagents as follows.

a) Dragendorff's Test: To few ml of the extract, 1 or 2 ml of Dragendorff's reagent (potassium bismuth iodide solution) was added and shaken well. A prominent reddish brown precipitate indicates the positive test.

b) Mayer's Test: To few ml of the extract, two drops of Mayer's reagent (potassium mercuric iodide solution) was added along the sides of the test tube. Formation of white or creamy precipitate confirms the test as positive.

c) Wagner's Test: To few ml of the extract, few ml of wagner's reagent (Iodine- potassium iodide) was added along the sides of the test tube. Formation of reddish brown precipitate indicates the positive test.

d) Hager's Test: To few ml of the extract, few drops of Hager's reagent. (Saturated aqueous solution of picric acid) was added. Formation of Yellow precipitate indicates the positive test.

II. Test for Carbohydrates:

a) Molisch's Test: To 3ml of the extract, few drops of freshly prepared α-napthol solution in alcohol was added and shaken well. Then 1 ml of Conc.H_2SO_4 was added along the sides of the test tube under the tap water for cooling. A violet ring at the junction indicates the presence of carbohydrates.

b) Benedict's Test: To few ml of the extract, few drops of Benedict's reagent was added. The mixture was heated on a water bath for 2 minutes. Development of red precipitate indicates the presence of reducing sugars.

c) Fehling's Test: To 1ml of the extract equal quantity of Fehling's solution A and B were added and heated on water bath for 5-10 min. Formation of Brick red precipitate indicates the presence of sugars.

d) Barfoed'sTest: To 1ml of test solution (extract), 1ml of Barfoed's reagent was added and heated on water bath for 2 minutes. Formation of red precipitate indicates the presence of sugars.

III. Test for Glycosides:

1. Anthraquinone glycosides:

a) Borntrager's Test: A few ml of dilute sulphuric acid was added to the extract solution, boiled, filtered and treated the filtrate with chloroform and shaken well. Then separated the chloroform layer, and tested with a few ml of ammonium solution. The pink or red colored ammonical layer formation represents the glycosides.

b) Modified Borntrager's Test: A few ml of 5% $Fecl_3$ solution and dilute Hcl were added to the extract. Then

boiled for 5min, cooled and shaken well with organic solvents like benzene, ether or chloroform. The organic layer so formed is separated with pipette. Then added equal quantity of dilute ammonia. Formation of pinkish red colour ammonical layer represents the glycosides.

2. Test for Cardiac glycosides

c) Legal Test: About 50 mg of extract was dissolved in pyridine, sodium nitro- prusside solution was added and made alkaline with 10% sodium hydroxide solution. Presence of glycosides is indicated by the development of pink colour.

3. Test for Saponin glycosides:

d) Foam formation Test: To the small quantity of extract 20ml of distilled water was added and shaken in graduated cylinder for 5min. Formation of 2cm foam layer represents the saponin glycosides.

IV. Test for Proteins

a) Ninhydrin Test: To 3ml of the extract 3 drops of freshly prepared 0.2% ninhydrin reagent (0.1% solution in n-butanol) was added and heated on water bath for few minutes. Development of purple or blue colour indicates the presence of proteins.

b) Biuret Test: To 3ml of the extract, few drops of 40% NaoH and 2 drops of 1% copper sulphate solution were added. Violet or pink colour indicates the presence of proteins.

c) Millon's Test: To 3ml of the extract, few ml of millon's reagent was added. Formation of white precipitate indicates the presence of proteins.

V. Test for Fats and Fixed oils

a) To the 5 drops extract, 10ml of $CuSO_4$ solution and 10% NaOH were added. Development of a clear blue solution shows the presence of glycerine in the sample.

b) To the 5 drops of extract, a pinch of sodium hydrogen sulphate was added. Pungent odour emanates indicates the presence of glycerine.

VI. Test for Steroids

a) Libermann-Burchard's Test: The extract was dissolved in few ml of acetic anhydride, heated to boiling, and then 1ml Conc. Sulphuric acid was added along the sides of the test tube. Development of red, pink or violet at the junction of the liquids indicates the presence of steroids.

b) Salkwoski test: The extract was dissolved in few ml of chloroform, and then equal volume of sulphuric acid was added. Development of blue or red colour represents the steroids.

VII. Test for Flavonoids

a) Shinoda Test: To the ethanolic extract, magnesium turnings were added followed by the addition of Conc. Hydrochloric acid. Presence of flavonoids produces the crimson red (cherry-red) or pink colour.

b) Alkane test: To the ethanolic extract 10% NaOH solution or ammonium hydroxide was added. Development of dark yellow coloration (fluorescence) represents the flavonoids Which decolorizes after addition of acid.

Determination of Total Ash Values

Weigh accurately 2gm of feed sample prepared in a tarred crucible. Char at low temp. first and then incinerate the material in a muffle furnace for 4 hrs or more until free from all carbonaceous materials and ash is white or grayish white. Cool the crucible and ash partly on asbestos sheet and then in a desiccators and weight. Repeat the process of ignition in the muffle furnace cooling and weighing at half an hour interval until the different between two successive weighing is less than 1mg.Note the lowest weight [19-21].

Total ash % by weight = 100x (W2-W)/W1

Where, W2= weight in gram of the crucible with ash

W1=weight in gram of the sample taken

W=weight in gram of the empty crucible.

Determination of acid- insoluble ash

To the ash obtained in the total ash determination, add 2mL of HCl and heat on asbestos sheet for 10min. Allow to cool and filter the contents of the crucible through Whatman no. 42 filter paper or its equivalent. Wash the residue and the crucible with water until the washings are free from acid. Return the residue to the crucible and collected the filtrate in a volumetric flask for mineral estimation. Keep in an electric air-oven maintained at 135 ±2°C for about 3hrs. Ignite in a muffle furnace at 500±20 for 3hrs and cool the dish in desiccators and weigh. Return the process of ignition in the muffle furnace, cooling and weighing at half an hour interval until the different between two successive weighing is less than 1mg. Note the lowest weight.

Acid insoluble ash, % by weight= 100x (W2-W)/W1

Where, W2= weight in gram of the crucible and acid insoluble ash

W1=weight in gram of the sample taken for analysis

W=weight in gram of the empty crucible

Determination of water- soluble ash[22]

Boil the total ash for 5 minutes with 25 ml of water; collect the insoluble matter in a Gooch crucible or on an ash less filter paper, wash with hot water, and ignite to constant weight at a low temperature. Subtract the weight of insoluble matter from the weight of the ash; the difference in weight represents the water- soluble ash. Calculate the percentage of water- soluble ash with reference to the air dried drug.

Extractive values[23, 24]

Extractive values of crude drugs are useful for their evaluation, especially when the constituents of a drug

cannot be readily estimated by any other means. Further, these values indicate the nature of the constituents present in a crude drug.

Determination of alcohol soluble extract[25]

5 gm. of the air-dried coarse powder of *Viscum articulatum* was macerated with 100 ml of 90% ethanol in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing standing for 18hours. Thereafter, it was filtered rapidly taking precautions against the loss of the solvent. Out of that filtrate, 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried and weighed. The percentage of ethanol soluble extractive value was calculated with reference to the air-dried drug. The results are recorded in the table.

Determination of water - soluble extract

Weigh accurately 5 gm. of coarsely powdered drug and macerate it with 100 ml of chloroform water in a closed flask for 24 hours, shaking frequently during the first 6 hours and allow to standing for 18 hours. Thereafter, it was filtered rapidly taking precautions against loss of the solvent. Then 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried and weighed [26]. The percentage of water soluble extractive was calculated with reference to the air dried drug. The results are given in the table.

***In vitro* α-Amylase inhibition assay**

α-amylase enzyme activity can be measured In vitro by hydrolysis of starch in the presence of α-amylase enzyme. This process was quantified by using iodine, in which blue color develops with starch [27]. In other words, α-amylase activity is directly proportional to the intensity of blue color in test sample.

DNS (3,5Dinitrosalicylic acid) method was performed to determine the α-amylase inhibitory activity, by quantifying the reducing sugar (glucose equivalent) liberated under the assay condition. The enzyme inhibitory activity was expressed as decrease in units of glucose liberated. Plant extract concentrations ranging from 0.2-1mg/ml was incubated with 1ml of 1 unit PPA (Porcine pancreatic alpha-Amylase) enzyme for 30 minutes at 37°C. After incubation 1ml of 1% buffered starch was added and the mixture was further incubated for 10 minutes at room temperature [28, 29]. The reaction was stopped by adding 1ml DNS reagent and the contents were heated in boiling water bath for 5minutes[30]. Blank was prepared without plant extract and enzyme which was replaced with equal quantity of 0.1M phosphate buffer [31,32].

Control representing 100% enzyme activity without plant extract was also included. The absorbance was read at 540nm using UV spectrophotometer. Standard antidiabetic drug Acarbose was used as positive control[33-35]. The antidiabetic property was determined through inhibition of α-amylase which was expressed as percentage of inhibition.

The percentage inhibition of α-amylase was calculated using the formula:

$$\text{Percentage inhibition} = \frac{Absorbance\ of\ control - Absorbance\ of\ test \times 100}{Absorbance\ of\ control}$$

Statistical analysis

In vitro data were expressed as mean percentage inhibition ±SD (N=3). IC_{50} value of percentage inhibition of enzymes was determined using nonlinear regression graph (log 10 concentration versus percentage enzyme inhibition)[36]. All analysis and IC_{50} value determination were carried out in graph pad Prism (Version 5.0) software.

RESULTS

Table 1: Macroscopic characteristics of whole plant of *viscum articulatum*

S.No.	Parameters (Physical Tests)	Observation of plant
1	Texture	Coarse powder
2	Colour	Greenish- brown
3	Odour	Aromatic
4	Taste	Astringent

Table 2: Physicochemical parameters of whole plant of *viscum articulatum*.

S.no	Name of parameter	%w/w
1	**Ash values**	
	Total ash	10.96%
	Acid in soluble ash	0.5%
	Water soluble ash	2.9%
2	**Extractive values**	
	Water soluble	28.72%
	Alcohol soluble	13.76%
3	**Loss on drying**	6.4%

Table 3: Qualitative phytochemical analysis of ethanolic extract of *viscum articulatum*

S.no	Chemical constituents	Ethanolic extract
1	Alkaloids	Present
2	Carbohydrates	Present
3	Anthraquinone glycosides	Present
4	Cardiac glycosides	Absent
5	Saponins	Present
6	Flavonoids	Present
7	Steroids and tri terpinoids	Present
8	Tannins	Present
9	Proteins	Present
10	Gums and mucilage	Absent

Table 4: Effect of ethanolic extract of *viscum articulatum* on anti-diabetic activity by alpha amylase method

Drug Conc. µg/ml	Percentage inhibition of alpha-amylase activity of test drug	Percentage inhibition of alpha-amylase activity of standard drug
20	25.51±0.33	42.45±0.26
40	45.86±0.12	60.74±0.21
60	52.27±0.30	62.19±0.54
80	63.67±0.17	75.28±0.66
100	83.42±0.22	84.17±0.59

Values are expressed as mean ±SD; n=3

IC_{50} = 29.45 (Acarbose)

IC_{50} = 53.79 (*Viscum Articulatum* extract)

Alpha amylase activity:

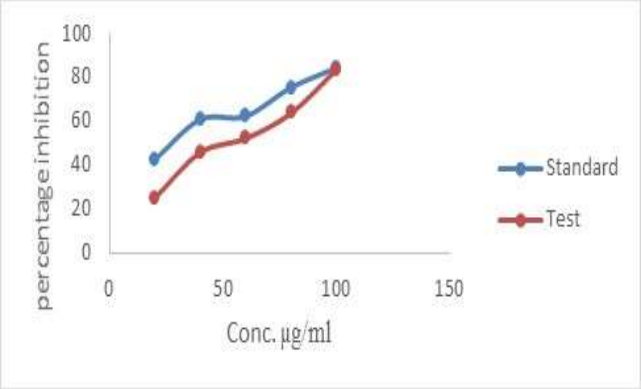

Figure 2: Effect of ethnolic extract of *Viscum articulatum* on alpha amylase inhibition activity

DISCUSSION

The whole plant of *Viscum articulatum* which belongs to family viscaceae have been investigated in a systemic way covering extraction, qualitative phytochemical analysis, invitro anti-diabetic activity.

The powdered material (100gm) was subjected to solvent extraction in soxhlet apparatus with ethanol as solvent. The colour of ethanolic extract was green and its yield is 7.1gm.

The ethanolic extract of *Viscum articulatum* was subjected for the preliminary phytochemical analysis and found for the presence of flavonoids, steroids, alkaloids, terpenoids. Alpha amylase is one of the main enzyme in human body that is responsible for breakdown of starch to more simple sugars, alpha amylase hydrolyze complex polysaccharides to produce oligosaccharides and disaccharides which are then hydrolyzed by alpha glucosidase to monosaccharides which are absorbed through the small intestine into the hepatic portal vein and increase prostaglandial glucose level. Amylase inhibitors are also known as starch blockers because they prevent dietary starch from being absorbed by the body and there by lower prostaglandial glucose levels. Slowing the digestion and breakdown of starch may have beneficial effects on Insulin resistance and glycemic index control in people with diabetes.

The anti-diabetic activity of ethanolic extract of the plant was done by alpha amylase inhibitory method. The results were presented in the table no.14 the activity was done at various concentrations of the extract and standard acarbose is used. The optical density was absorbed at 540nm. A minor dose dependent alpha amylase inhibition was observed with the extract and the percentage inhibition at 40µg/ml, 60 µg/ml and IC_{50} value of extract is 53.79 µg/ml. for the standard at 20 µg/ml, 40 µg/ml and IC_{50} value of standard is 29.45µg/ml. From the results it was observed that the ethanolic extract of *Viscum articulatum* is moderately suitable for the control diabetic conditions.

The flavonoids containing compounds like naringenin present in plant *Viscum articulatum* may account for the observed anti-diabetic effects of the extracts. Various natural compounds have been used in the treatment of diabetes to control blood sugar as the synthetic anti-diabetic drugs have adverse effects in humans.

CONCLUSION

At present research an attempt has been made to find out the therapeutic activity like anti-diabetic for the *Viscum articulatum* plant. From the literature review the whole plant of *Viscum articulatum* (Viscaceae) was selected for the study and the following parameters were studied.

- Selection, identification and collection,
- Extraction and preliminary phytochemical analysis and
- *In vitro* antidiabetic activity.

The ethanolic extract of *Viscum articulatum* was identified for the presence of flavonoids, steroids, triterpenoids, alkaloids and carbohydrates. The ethanolic extract of *Viscum articulatum* showed anti-diabetic activity by alpha amylase inhibition method and maximum percentage of inhibition was found at 100 µg/ml (83.42%). Diabetes mellitus is an alarming metabolic disorder leading to hyperglycemic which later develops to micro and macro vascular complications and becomes a major cause of death. Alpha amylase is a digestive enzyme attached to the membrane of brush border of the small intestine. Through different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes, there is an increase demand by patients to use natural products with anti-diabetic activity. The presence of above mentioned phytoconstituents may be responsible for the anti-diabetic activity.

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