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PHYTOCHEMICAL EVALUATION AND IN-VITRO ANTIDIABETIC ACTIVITY OF ETHANOLIC EXTRACT OF *GLOCHIDION ZEYLANICUM (GAERTN) A.JUSS.*

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Abstract : The current study was investigated the in vitro anti-diabetic activity of leaves of *Glochidion zeylanicum (Gaertn) A.Juss.* Medicinal plants have been proposed as rich yet unexploited potential sources for anti-diabetic drugs, even though used since ancient times for the treatment of diabetes mellitus. *Glochidion zeylanicum (Gaertn) A.Juss.* is a herbal medicinal plant belonging to Family Phyllanthaceae and mentioned in Ayurveda, Siddha, and Chinese medicinal system for treatment of various disorders. The literature survey confirms that the anti-diabetic activity of *Glochidion zeylanicum (Gaertn) A.Juss.* has not been scientifically investigated. Hence, the present study is under taken for the in vitro anti-diabetic activity of the whole plant of *Glochidion zyelanicum (Gaertn) A.Juss*. to evaluate its traditionally claimed anti-diabetic activity. The whole plant of *Glochidion zeylanicum (Gaertn) A.Juss.* which belongs to family Phyallanthaceae have been investigated in a systemic way covering extraction, qualitative phytochemical analysis, invitro anti-diabetic activity. The powdered material (120 gm) was subjected to solvent extraction in Soxhlet apparatus with ethanol as solvent. The colour of ethanolic extract was green and its yield is 8gm. The ethanolic extract of *Glochidion zeylanicum (Gaertn) A.Juss.* was subjected for the preliminary phytochemical analysis and found for the presence of flavonoids, steroids, alkaloids, terpenoids, glycoside, tannins and saponin. The anti-diabetic activity of ethanolic extract of the plant was done α-amylase inhibitory methods. From the results it was observed that the ethanolic extract of *Glochidion Zeylanicum (Gaertn) A.Juss.* was showed inhibition of alpha amylase can lead to reduction in post prandial hyperglycemia in diabetic conditions.

Keywords: Glochidion Zeylanicum (Gaertn) A.Juss., Phytochemical Evaluation, Anti-diabetic activity.

INTRODUCTION

Medicinal plants continue to be an important therapeutic aid for alleviating ailments of human kind. Over the last 2500 years, there have been very strong traditional systems of medicine such as Chinese, Ayurvedic, and the Unani, born and practiced, more in the eastern continent. These traditions are still flourishing, since; approximately 80% of the people in the developing countries rely on these systems of medicine for their primary health care needs.[1]

In terms of morbidity and death, diabetes mellitus is thought to be the most common endocrine metabolic illness, affecting 25% of the global population.[2] This is a combination of heterogeneous disorders commonly presenting with episodes of hypeglycemia and glucose in tolerance, as a lack of insulin, defective insulin action, or both.[3] Many different therapeutic approaches are available for treating diabetes and one of the treatment includes retarding absorption of glucose by inhibiting the carbohydrate hydrolysing enzymes like amylase and glucosidases.[4,5,6] This Anti-diabetic drug shows the action by decreasing the effects of a protein or enzyme (by the inhibition of this protein or enzyme) on the pancreas at the level of release of glucagon (diminishes its release). For patients with glycosylated hemoglobin (HbA1c) greater than 9% or fasting plasma glucose (FPG) greater than 11.1 mmol/L, insulin should be chosen to reduce blood glucose levels quickly and relieve the effect of hyperglycemia on apoptosis, dedifferentiation and trans differentiation of islet beta cells, and recovery islet cell function.[7]

It is the first-line medication for the treatment of type 2 diabetes. Metformin is the compound belongs to the class of organic compounds known as biguanides. Biguanides are the organic compounds containing two N-linked guanidine. It is a biguanide antihyperglycemic agent used for treating noninsulin dependent diabetes mellitus (NIDDM). It improves glycemic control by decreasing hepatic glucose production, decreasing glucose absorption and increasing insulin mediated glucose uptake. Metformin is the only oral antihyperglycemic agent that is not associated with weight gain.[8]

PLANT PROFILE:

Fig 1. Glochidiaon zeylanicum A. Juss

Glochidion zeylanicum A. Juss:

The genus Glochidion commonly called as cheese trees or button wood trees consisting of 300 species.[9] Several triterpenoids, triterpenoid glycosides and alkaloids are known to be constituents of the plants belonging to the genus Glochidion.[10] Biological investigations of Phyllanthus species revealed that many members of the genus possess anti-tumor promoting ability, antiviral activity against hepatitis B virus, anti-angiogenic, lipid lowering activity, antidiabetic, antiherpetic activity, anti-HIV, antiplasmodial and other activities.[11] However, no biological studies of G. zeylanicum A. Juss have been found in literature to date. Hence, in the present study, we carried out in-vitro antidiabetic activity of solvent extract of G. zeylanicum A. Juss.

Synonym: Phyllanthus zeylanicus (Gaertn.) Mull.Arg., nom. illeg.

Family: Phyllanthaceae

Vernacular Names: Neeru kukke (Kannada); Kumbal (Tamil); Itepulla, Pageri, Itepulla (Telugu); kalchia, berlu (Oria); Neevetti, Pannimutti (Malayalam).

Others common names: Ghoda/ Askand/ Ashwagandha
Hang Kong Abacus Plant
Kokamani moram- Tamil

Habitat: Evergreen and semi-Evergreen forests, also in the plains

Uses: System medicines used in Folk medicine, Siddha

METHODS AND MATERIALS:

The plant materials of Glochidion Zeylanicum (Gaertn) A.Juss. were freshly collected from Sahyadri forest, The plants materials were then identified and Authentified by Department of Botany, Yashwantrao Chavan College of Science, Karad. A herbarium was prepared and deposited in the Dept. of botany, for further reference. The plant was identified as Gochidion Zeylanicum (Gaertn) A.Juss. and was certified under Voucher No: SBF 002.

EXPERIMENTAL METHOD:

Preparation of plant Extraction:

Glochidion Zeylanicum (Gaertn) A.Juss. leaves were collected washed and shallow dry. *Glochidion Zeylanicum (Gaertn) A.Juss.* leaves were powdered using a grinder and powder of 0.40 micron was collected using specific sieves. 120 gm of powder was packed in the thimble of muslin cloth in the extractor. Mouth of the extractor was fitted to bulb type condenser and neck was packed with sealing wax. Heating was continued with continuous flow of water through the condenser. For all the extractions temperatures was kept nearer to the boiling range of the respective solvent. Extraction cycle was observed continuously for Eighteen (14) cycles till completion of extraction. Solvent was recovered by distillation and extract heating mental. Finally dried extract were weighted and preserved in the air tight containers. Percent extract were calculated. Soxhlet apparatus was used for continuous extraction of the powdered crude drug. The material was packed in the apparatus and allowed to get extracted with hot solvent that continuously percolates from top to bottom. Condensed fresh solvent percolates every time through the powder and is the major advantages with these techniques.

Fig 2: Soxhlet Extraction of *Glochidion Zeylanicum*

1.1. Preliminary phytochemical screening:

The crude solvent extracts were screened for detection of phytochemicals viz.,alkaloids, flavonoids, terpenoids, steroids, glycosides, tannins and saponins by standard phytochemical analysis. The screening test 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 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.

III. 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.

IV. 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% Fecl3 solution and dilute HCL were added to the extract. 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.

PHYSICAL EVALUATION
- ***Determination of Ash values***

a) Determination of total ash value
Procedure:
2 gm of powdered drug was placed into the tared silica crucible. Then crucible placed in furnace and heated until vapours almost cease to be evolved, lower the dish and heated more strongly until all the carbon is burnt off. Then dish had placed in desiccators for cooling. Then calculated total ash value.

Calculation:
Weight of empty crucible = 14.35gm
Weight of drug taken = 3gm
Weight of crucible + ash = 15.30gm
Weight of ash = 0.95gm

$$\%\text{Total Ash Value} = \frac{\text{Weight of total Ash}}{\text{Weight of crude drug taken}} \times 100$$

Total ash value of sample = 100x 0.95/2 = 47.5%
The total ash value was found to be 47.5 % of drug.

b) Determination of acid soluble ash value

Procedure:

The ash obtained from above procedure and it was washed by 30ml of dil HCL in 1 beaker and boiled for 5 min. Then filtered and residue was washed with water. Then residue was taken into crucible; then heated gently until vapours cease to be evolved and then heat more strongly until all carbon has been removed. The crucible had placed in desiccators for cooling. Then calculated acid soluble ash value.

Calculation:

Weight of ash = 0.95gm

Weight of residue = 0.66gm

$$\% \text{ Acid insoluble Ash Value} = \frac{\text{Weight of acid insoluble Ash}}{\text{Weight of crude drug taken}} \times 100$$

% of acid insoluble ash value = 100x 0.66/2 = 33%

The % of acid insoluble ash was found to be 33%

c) Determination of Alcoholic soluble extensive value

Procedure:

5 gm of the powdered drug into a dry 250mL of conical flask. Filled with solvent 100mL alcohol (90%). Corked the flask and had set aside for 24 hours, shacked frequently (Maceration) filter into a 50mL cylinder. When sufficient filtrate had collected transferred 25mL of the filtrate to weighed, thin porcelain dish, as used for the ash values determination. Evaporated to dryness on a water bath and completed the drying in an oven at 100^0 c. Cooled in desiccators and had taken weight. Approximately 95% w/w of extractives with reference to the air-dried drug.

d) Determination of moisture content (loss of drying)

Procedure:

1gm of the powdered drug into flat and thin porcelain dish. Dried in the desiccators and watched. Percent loss on drying was calculated using the following formula.

$$\% \text{ Loss on drying} = \frac{\text{Loss in weight of sample}}{\text{Weight of sample}} \times 100$$

Calculation:

Weight of the empty crucible = 66.020 gm

Weight of drug taken = 1gm

Weight of crucible + drug = 67.020gm

Weight of crucible + drug after drying = 67.100gm

Total moisture content of sample = 100x 0.92/1 = 9.2%

The total moisture content was found to be 9.2% of drug.

Table 1: Macroscopic characteristics of whole plant of *Glochidion Zeylanicum (Gaertn) A.Juss.*

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

IN VITRO ANTIDIABETIC ACTIVITY: [12]

Principle:

In humans, the digestion of starch involves several stages. Initially, partial digestion by the salivary amylase results in the degradation of polymeric substrates into shorter oligomers. Later on in the gut these are further hydrolyzed by pancreatic alpha amylases into maltose, maltotriose and small malto-oligosaccharides. The digestive enzyme (alpha-amylase) is responsible for hydrolyzing dietary starch (maltose), which breaks down into glucose prior to absorption. Inhibition of alpha amylase can lead to reduction in post prandial hyperglycemia in diabetic condition. Treatment of diabetes include: improvement of the activity of insulin at the objective tissues, with the utilization of sensitizers (biguanides, thiozolidinediones); incitement of endogenous insulin discharge with the utilization of sulfonylureas (glibenclamide, glimepiride), and decrease of the interest for insulin utilizing particular enzyme inhibitors (acarbose, miglitol).

Procedure:
500 µL of the test sample of Glochidion Zeylanicum A. Juss. extract was allowed to react with 500 µL of 0.1M phosphate buffer pH 6.9 containing 0.5% α-amylase enzyme. After 10-minute incubation at 25^0C, 500 µL of 1% starch soluble, Extra pure in 0.1M phosphate buffer pH6.8 was added. Again incubated at 25^0c for 10 min. The same was performed for the controls where 500 µL of the enzyme was replaced by buffer. After incubation, 1000 µL of added to both control and test. Standard acarbose (α-amylase enzyme inhibitor) used as standard drug. They were kept in boiling water bath for 10 min and cooled. The absorbance was recorded at 540 nm using spectrophotometer and the percentage inhibition of α-amylase enzyme was calculated using the formula,

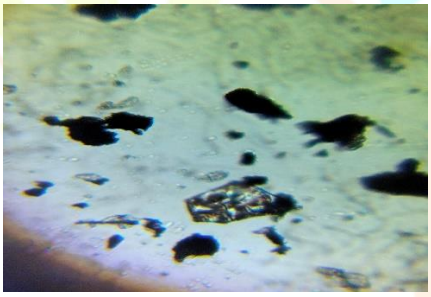
$$\text{Inhibition (\%)} = \frac{\text{Abs 540 (control)} - \text{Abs 540 (extract)}}{\text{Abs 540(control)}} \times 100$$

RESULTS:
Present extractive of different extract of Glochidioan zeylanicum A. Juss is depicted (Table…). In Glochidioan zeylanicum A. Juss leaves, maximum percent extractive value was observed for ethanol extractive (25.0%).

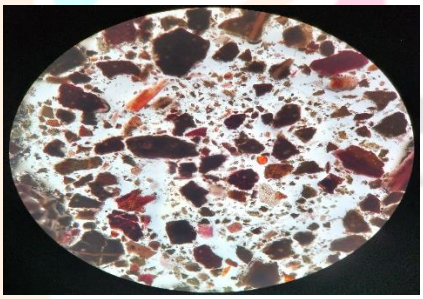
Macroscopic Charecteristics:
The detail macroscopic characters of fresh plant of Glochidioan zeylanicum A. Juss was moted including special features such as Colour, Size, and Shape etc., IT is a slender, perennial herb, growing up to 1 m in height often dichotomously branched. Odour is Aromatic, pungent and Taste is bitter.

Microscopic Charecteristics:
The preliminary phytochemical screening of ethanol extract of Glochidioan zeylanicum A. Juss whole plant revealed the presence of all secondary metabolites. While the ethanolic extract exhibited the presence of alkaloid, flavonoid, saponin, phenol, tannin, glycoside and terpenoid.

Powder Charecteristics:



[A]



[B]

Results of physicochemical paramers and its evaluation:

Table 2: Physicochemical parameters of whole plant of *Glochidion Zeylanicum (Gaertn) A.Juss.*

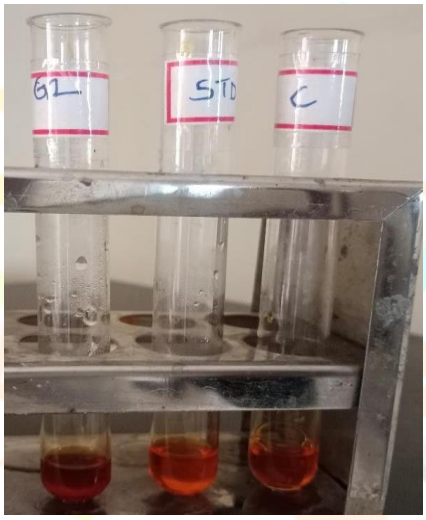
Sr. No.	Name Of Parameters	%w/w
1.	Ash values Total ash Acid in soluble ash	 47.5% 33%
2.	Extractive values Alcohol soluble	 95%
3.	Loss on drying	9.2%

Results for Qualitative Chemical Investigation of alcoholic extract of Glochidioan zeylanicum A. Juss.:

Table 3: Qualitative phytochemical analysis of ethanolic extract of *Glochidion Zeylanicum (Gaertn) A.Juss.*

Sr. No.	Chemical Constituents	Ethanolic extracts
1.	Alkaloids	Presents
2.	Steroids	Absents
3.	Flavonoids	Presents
4.	Glycosides	Absents
5.	Tannins	Presents
6.	Saponins	Presents

IN VITRO ANTIDIABETIC ACTIVITY:



These observations confirm that Ethanolic of the Glochidion zeylanicum A. Juss leaves have anti diabetic activity. The digestive enzyme (alpha-amylase) is responsible for hydrolyzing dietary starch (maltose), which breaks down into glucose prior to absorption. Inhibition of alpha amylase can lead to reduction in post prandial hyperglycemia in diabetic condition. The Glochidion zeylanicum A. Juss extract was showed poor inhibition of amylase enzyme at the concentration of 1mg/ml when compared to acarbose standard.

Table no. 4 Effect of compounds by using alpha amylase inhibition assay

		α-amylase enzyme inhibition assay		
Sample code	**Concentration**	**Absorbance at 540nm**	**Mean**	**% inhibition**
Control	**1mg/ml**	**0.73** **0.67** **0.70**	**0.70**	**12**
Standard Acarbose	**1mg/ml**	**0.20** **0.21** **0.23**	**0.21**	**70**
Sample – GZ extract	**1mg/ml**	**0.63** **0.60** **0.65**	**0.62**	**11.42**

CONCLUSION:

At present research an attempt has been made to find out the therapeutic activity like anti-diabetic for the Glochidioan zeylanicum A. Juss plant. From the literature review the whole plant of Glochidioan zeylanicum A. Jusss (Phyallantaceae) was selected for the study and the following parameters were studied.

- Selection, identification and collection,
- Extraction, preliminary phytochemical analysis and GC-MS
- In vitro antidiabetic activity

REFERENCES:

1. Tsay HS, Agrawal DC. Tissue Culture Technology of Chinese Medicinal Plant Resources in Taiwan and their Sustainable Utilization. Int J App Sci Eng 2005; 3:215-223.
2. Anju Singh, Rakhi Mishra, Avishikta Ray, Surabhi Tripathy, Satkar Prasad, Reenu Yadav. (2023). Development And Evaluation Of Anti Diabetic Activity Of Phytosomes For Better Therapeutic Effect Of Extract. *Journal of Pharmaceutical Negative Results*, 1330–1343.
3. World Health Organisation. Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications, Report of a WHO Consultation Part 1. Geneva: Diagnosis and Classification of Diabetes Mellitus; 1999:2.
4. Afifi AF, Kamel EM, Khalil AA, et al. Purification and characterization of a-amylase from Penicillium olsonii under the effect of some antioxidant vitamins. Glob J Biotech Biochem. 2008;3:14e21.
5. De Melo EB, Da Silveira Gomes A, Carvalho I. a and b-Glucosidase inhibitors: chemical structure and biological activity. Tetrahedron. 2006;62:10277e10302.
6. Raptis SA, Dimitriadis GD. Oral hypoglycemic agents: insulin secretagogues, a-glucosidase inhibitors and insulin sensitizers. Exp Clin Endocrinol Diabetes. 2001;109(2):S265eS287.
7. Shi C, Zhang R, Bai R, Liu D, Wang Y, Zhang X, Hao Wang, Jianling Du; "Efficacy and safety of sitagliptin added to metformin and insulin compared with voglibose in patients with newly diagnosed type 2 diabetes." *CLINICS* 2019;74;736.
8. P. Chengalva, A. Parameswari S, Aruna G. "Development And Validation Of Rp-Hplc Method For Metformin Hydrochloride And Nateglinide In Bulk And Combined Dosage Form"*Int.J.PharmSci*,2016;8(4);267-271.
9. Sandhya S, Chaintanya RSNAKK, Vinod KR Rao KNV, Banji1 D, Sudhakar K and Swetha R (2010) An updated review on the Genus Glochidion Plant. Archives of Applied Science Research 2 (2):309-322.
10. Jawarkar SV and Kane SR. Phytochemical and Anthelmintic Investigation of Leaves of Glochidion ellipticum Linn. European Journal of Experimental Biology. 7 (2:9).2017, 1-3.
11. A.T.M. Zafrul Azam, Abdullah Al Hasan, Md. Gias Uddin, Mohammad Mehedi Masud and Choudhury Mahmood Hasan, "Antimicrobial, Antioxidant and Cytotoxic Activities of Glochidion multiloculare (Roxb. ex Willd.) Müll. Arg. (Euphorbiaceae)" Dhaka Univ. J. Pharm. Sci. 11(2): 117-120, 2012.
12. A. A. Muchandi, A. S. Jadhav, S. B. Patil, S. A. Patil, and N. B. Jadhav, "Antioxidant and *In Vitro* Antidiabetic Activities of Methanol Extract of *Piper cubeba* L.," *International Research Journal of Pharmacy and Medical Sciences (IRJPMS)*, Volume 1, Issue 3, pp. 1-4, 2018.