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INVITRO- ANTIDIABETIC, ACTIVITY OF ETHANOL EXTRACT OF *GLOCHIDION ELLIPTICUM*

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ABSTRACT

The study was aimed at evaluating Antidiabetic activity were evaluated by In vitro amylase inhibitory activity and Glucose uptake in Yeast cells. It was proposed that inhibition of α-amylase and glucose uptake by yeast cell delay the degradation of carbohydrate, which would in turn cause a decrease in the absorption of glucose, as a result the reduction of post pranial blood glucose level elevation. In the present study the ethanol extract inhibit the enzymes namely α-amylase than the aqueous extract of Glochidion ellipticum effectively.

KEYWORDS: *Glochidion ellipticum*, Antidiabetic activity.

INTRODUCTION

In spite of the overwhelming influences and our dependence on modern medicine and tremendous advances in synthetic drugs, a large segment of the world population still likes drugs from plants. In many of the developing countries the use of plant drugs is increasing because modern life saving drugs are beyond the reach of three quarters of the third world's population although many such countries spend 40-50% of their total wealth on drugs and health care. As a part of the strategy to reduce the financial burden on developing countries, it is obvious that an increased use of plant drugs will be followed in the future. Majority of crude herbs come from wild sources and it is collected to assess quality parameters by which presence of various phytochemicals can be confirmed. Standardization of natural products is complex task due to their heterogenous composition in form of whole plant. authentication, pharmacognostic evaluation, phytochemical analysis are few basic protocols for standardization of herbals.[1,2]

Since the dawn of the human civilization, the importance of medicinal plants in the treatment of variety of human ailments has been immense. Herbal medicines have recently attracted much attention as alternative medicines useful for treating or preventing life style related disorders and relatively very little knowledge is available about their mode of action. There has been a growing interest in the analysisof plant products which has stimulated intense research on their potential health benefits. Plants are the essential and integral part in Complementary and Alternative medicine and due to this they develop the ability for the formation of secondary metabolites. Plants are the best source of active secondary metabolites which are beneficial to mankind in treating many diseases (Sandhya. S, 2011). Genus Glochidion have been used for a varied of biological activities in traditional medicine and also have been using by many ethnic groups. It is a vast genus in which many plants explored chemically, but most of the species in this genus were not standardized pharmacognostically.[2,-5]

Detailed literature review states that the plant has broad spectrum of the activities which were claimed traditionally and some are proven scientifically. Most of species in this genus were explored on the basis of the chemical constituent but not on pharmacognostical and pharmacological basis.

Medicinal plants are rich in Secondary metabolites, less in quantity with more value compounds and are potential sources of drugs and essential oils. Many of these compounds are having tremendous values in treatment of various ailments. Traditional herbal practices are now-a-days becoming familiar due to the Natural drugs having no side effects when compared to that of chemical drugs.

Natural products from medicinal plants are known to be chemically balanced, effective and least injurious with none or much reduced side effects as compared to synthetic medicines. Natural products, which come out from medicinal plants are also important for pharmaceutical research and for drug development as a sources of therapeutic agents. India is perhaps the largest producer of medicinal herbs and rightly called the botanical garden of the world, which are used for thousands of years in the indigenous system of medicine like Ayurveda, Siddha and Unani. Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being. Their role is twofold in the development of new drugs either for

development of a medicine or used for the treatment of diseases. Since ancient times many plants have been utilized by man for extracting and utilizing secondary metabolite products.

The Euphorbiaceae are mostly monoecious herbs, shrubs, and trees, sometimes succulent and cactus-like, comprising one of the largest families of plants with about 300 genera and 7,500 species that are further characterized by the frequent occurrence of milky sap. The leaves are mostly alternate but may be opposite or whorled and they are simple, or compound, or sometimes highly reduced. Stipules are generally present but may be reduced to hairs, glands or spines. The flowers are unisexual and usually actinomorphic. They may be highly reduced by suppression of parts, in the extreme form consisting of a naked stamen as a male flower and a naked pistil as a female flower. A specialized type of miniature inflorescence called a cyathium occurs in about 1,500 species comprising the genera Euphorbia and Chamaesyce. The cyathium consists of a single naked pistillate flower surrounded by cymes of naked staminate flowers, each consisting of a single stamen. These flowers are all enclosed in a cup-like involucre that typically is provided with peripheral nectaries and petaloid appendages such that the whole aggregation closely resembles a single flower. In other members of the family the flowers and inflorescences are more ordinary in appearance, with male and female flowers typically bearing a 5-merous calyx and corolla of distinct segments, although the corolla is sometimes absent. In these forms the androecium most commonly consists of 5, 10 or sometimes numerous distinct or monadelphous stamens. The gynoecium of female flowers consists of a single compound pistil of typically 3 carpels, an equal number of styles or primary style branches, and a superior ovary with typically 3 locules, each bearing 1 or 2 collateral, axile-apical pendulous ovules. The fruit is usually a capsular schizocarp.[6-7]

MATERIALS AND METHODS

All the chemicals and reagent used were of laboratory grade and were procured from manufactures ofResearch lab fine chemicals, Mumbai., Loba Chemie, Mumbai, Sigma-Aldrich, Mumbai., Hi Media Lab Mumbai, Finar reagents, Ahmadabad, Merck, Mumbai, Genuine Chem., Mumbai, Labin, Mumbai, Moly Chem, Mumbai).

Collection of plant

The plant was collected from the forest regions of Koyna dam of karad dist, satara. It was authenticated by Dr. Sanjay S. Sathe, Asso. Professor, Dept. of Botany, PDVP, mahavidyalaya Tasgaon, Dist-Sangli. A herbarium was prepared and deposited in the Dept.

of Pharmacognosy for further reference. The plant was identified as *Gochidion elipticum*. (Euphorbiaceae) and was certified under Voucher No: RCP-SNG/ ph'cog/ 2009-10/003.

EXTRACTION METHODS

- **Preparation of various extract of medicinal plants**
- **Aqueous extraction**

Aqueous extracts *Gochidion elipticum* were carried out by cold maceration. In this process, solid ingredients were subjected to cold maceration with chloroform: Water I.P (2:98) (**Indian Pharmacopoeia (I.P.); 1996**). Powder was placed in 2 liters round bottom flask for about 7 days at room temperature in a warm place. The flask was securely plugged with absorbent cotton and was shaken periodically with frequent agitation until soluble matter is dissolved. The mixture was filtered and after most of the liquid has drained, the filtrate was concentrated to residue at constant temperature bath at temperature 50^0C.

Note: Chloroform water I.P.
2.5 ml of chloroform was shaken with 900 ml of water until dissolved and diluted to 1000 ml with water.

- **Successive solvent extraction**

The dried leaves of the plant of *Gochidion elipticum* were reduced to coarse powder (40 size mesh) and around 200 gm of powder was subjected to successive hot continuous extraction (soxhlet apparatus) with petroleum ether (60-80^0C), chloroform, ethyl acetate and ethanol to about 10 cycles per batch for 1 batches. The extraction was continued until the solvent in the thimble became clear. Each time before extracting with next solvent the powdered material was dried at room temperature.

After the effective extraction, solvent was distilled off using rotary vacuum evaporator and the extracts were concentrated at low temperatures. The dried concentrated extracts were used for phytochemical investigation, isolation, pharmacological activity.[8,9]

- **Alcoholic extraction**

About 500 gms of fresh air-dried Leaves and stem bark of *Gochidion elipticum* were extracted with ethanol by using soxhlet extractor. The extract was filtered and concentrated with the help of rotary vacuum evaporator.

Antidiabetic activity

In vitro amylase inhibitory activity

The inhibitory activity of ethanol and aqueous extract against amylase activity was tested amylase (Diastase (fungal) by following method) the enzyme (0.5%) was prepared in phosphate buffer (pH 6.8). briefly, In the test tube control and standard was replaced with buffer and acarbose. The remaining test tube containing 500 µl of different concentration of extract (0-50mg/ml) and 500 µl of 0.0 M phosphate buffer (pH 6.8) containing amylase were incubated at 25OC for 10 min. after preincubation, 500 µl of 1% starch solution in 0.1 M Phosphate buffer (pH 6.8) was added to each tube and further incubated at 25OC for 10 min. the reaction was stopped by addition of 1 ml DNS (dinitro salicylic acid reagent). The test tube were placed in a boiling water bath for 10 min and cooled. The absorbance was measured at 540nm. The percentage inhibition was calculated.

II) Glucose uptake in Yeast cells

Yeast cells were prepared according to the method of Yeast cells[9] briefly, commercial baker's yeast was washed by repeated centrifugation (3,000×g; 5 min) in distilled water until the supernatant fluids were clear and a 10% (v/v) suspension was prepared in distilled water. Various concentrations of extracts (1–5 mg) were added to 1mL of glucose solution (5, 10 and 25 mM) and incubated together for 10 min at 37°C. Reaction was started by adding 100 µl of yeast suspension, vortex and further incubated at 37°C for 60 min. After 60 min, the tubes were centrifuged (2,500 × g, 5 min) and glucose was estimated in the supernatant. Metformin was taken as standard drug. The percentage increase in glucose uptake by yeast cells was calculated using previously published protocol. All the tests were performed in triplicate.[10]

RESULT AND DISCUSSION

In vitro amylase inhibition by extract of leaves Glochidion ellipticum

Sr. no	Conc. (µg/ml)	% inhibition	
		Standard (Metformin)	Ethanol extract of leaves
1	10	60.26 ± 1.10	59.57 ± 2.13
2	20	74.58 ± 2.11	62.26 ± 2.16
3	30	77.59 ± 3.12	72.24 ± 2.14
4	40	80.24 ± 2.15	74.15 ± 2.12
5	50	82.32 ± 3.11	81.26 ± 2.11

From this Table no.91, it is indicate that In vitro amylase inhibition activity ethanol extract of Glochidion ellipticum leaves was shown good significant inhibition as compared to standard

drug metformin. The extract concentration 50 µg/ml showed 82.32% inhibition than standard 81.26%.

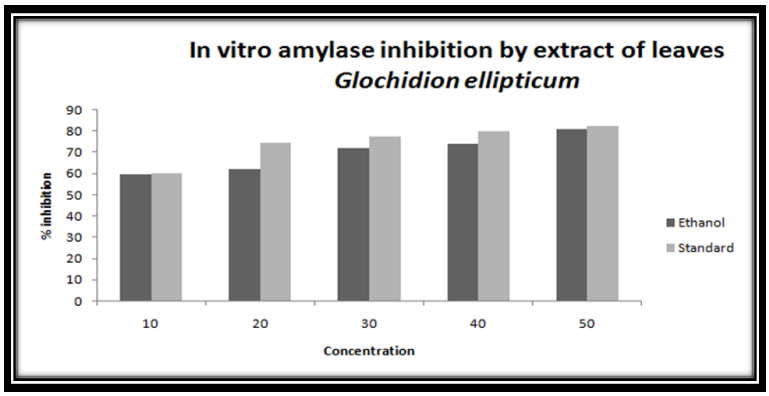

Fig. no.1 In vitro amylase inhibition by extract of leaves Glochidion ellipticum

Effect of ethanol extract of leaves Glochidion ellipticum in glucose uptake inhibition by yeast cell.

Sr. no	Conc.(µg/ml)	% inhibition	
		Standard (Metformin)	Ethanol extract of leaves
1	10	28.26 ± 2.13	37.25 ± 2.35
2	20	59.54 ± 1.40	62.25 ± 2.45
3	30	84.26 ± 3.36	83.24 ± 2.25
4	40	89.51 ± 2.25	85.54 ± 2.15
5	50	91.27 ± 3.15	89.24 ± 2.13

Values were expressed as mean ± SD From this Table no.97, it is indicate that In vitro amylase inhibition activity ethanol extract of Glochidion ellipticum leaves was shown good significant inhibition as compared to standard drug metformin. The extract concentration 50 µg/ml showed 89.24% inhibition than standard 91.27%.

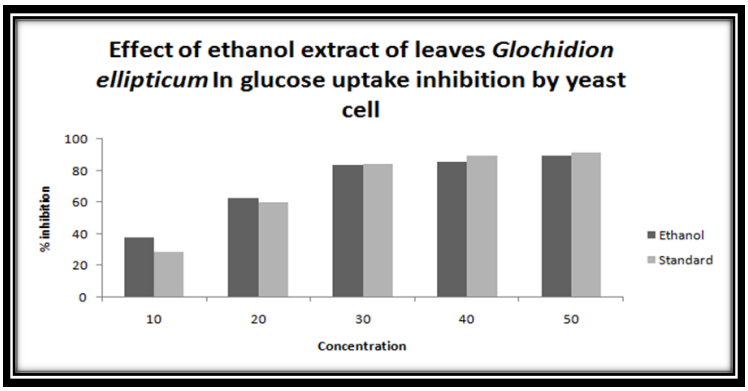

Fig. no. 2 Effect of ethanol extract of leaves Glochidion ellipticum in glucose uptake inhibition by yeast cell

CONCLUSION

In the present study, shows Antidiabetic activity were evaluated by In vitro amylase inhibitory activity and Glucose uptake in Yeast cells. Diabetes mellitus is a metabolic disorder with increasing incidence throughout the world. Insulin is a key player in control of glucose haematosis. Lack of insulin affects metabolism of carbohydrates, fats and proteins. It was proposed that inhibition of α-amylase and glucose uptake by yeast cell delay the degradation of carbohydrate, which would in turn cause a decrease in the absorption of glucose, as a result the reduction of post pranial blood glucose level elevation. In the present study the ethanol extract inhibit the enzymes namely α-amylase than the aqueous extract of Glochidion ellipticum effectively.

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