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ANTI-DIABETIC ACTIVITY OF AQUEOUS AND METHANOLIC LEAF EXTRACTS OF *SCHLEICHERA OLEOSA* LOUR.

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ABSTRACT

Compounds obtained from plants may be good substitutes of the conventional drugs for the effective treatment of diabetes. *Schleichera* is genus of plant that belongs to the family Sapindaceae. The present study was undertaken to investigate the antidiabetic details of the methanolic and aqueous leaf extract of *Schleichera oleosa.* Freshly prepared extracts of the powdered leaves were subjected to phytochemical analyses. The amylase inhibition assay was performed using the chromogenic (Dinitro salicylic acid) DNSA method. The methanolic leaf extract of *Schleichera oleosa* showed 26.191±0.001 percentage of amylase inhibition at 10 µg concentration, 50.131±0.002 percentage of amylase inhibition at 50 µg of concentration and 68.888±0.002 percentage amylase inhibition against 100 µg concentration and aqueous leaf extract of *Schleichera oleosa* showed 24.123±0.040%, 33.112±.004 % and 55.471±002 % amylase inhibition against 10 µg, 50 µg and 100 µg extract concentration respectively while Acarbose showed 99.833±0.288, 100.133±0.230, and 100.233±0.404 percentage of amylase inhibition against 10 µg, 50 µg and 100 µg the concentrations respectively. Thus we can assume that *Schleichera oleosa* is a good anti-diabetic agent and can be used in the treatment of diabetic mellitus.

KEYWORDS: *Schleichera oleosa*, amylase inhibition, antidiabetic activity, Acarbose.

INTRODUCTION

From the beginning of human history, plants are used as exemplary sources of drugs for treatment of various ailments .[1]due to their biodiversity and most certainly the abundance of phytochemicals and secondary metabolites.[2] In remote areas of our world especially of India where modern health facilities and access to established treatments are limited, crude products of plants/herbs are used extensively for treatment and are undeniably the mainstay of the health care system.[3] Apart from that, current research in medicinal plants lend adequate acceptance to their effectiveness and potency.[4] In most cases products of medicinal plants are preferred over and above the existing conventional and chemotherapeutic options predominantly because it cures degenerative diseases including diabetes mellitus without adverse side effects.[5] The Indian literature has the mention the utilization of plants in treatment of all kinds of human ailments.[6] India has about 45 000 plant species and among them, several thousands are claimed to possess medicinal properties.[7]

Diabetes causes severe long term health complications connected to it and is therefore a serious problem of the present modern society. Especially, type 2 diabetes mellitus (T2DM) is the most common form of diabetes, which is accountable for above 80% of the total cases of diabetes.[8,9] Disturbances in the metabolism of Glucose are the main factors that are responsible for diabetes. It is a well known fact that the insulin released by the pancreatic β-cells is the hormone responsible for glucose balance in the blood.[10,11] Insulin stimulate hepatocytes, myocytes, and adipocytes to uptake glucose from the circulatory system. Depending on need, glucose can either be used as an energetic source by glycolysis, or alternatively, stored as glycogen inside muscle or liver cells.[12] When the insulin is used in an inapt method, it leads to insulin resistance, in which cells become incapable of responding to normal levels of insulin circulating in the blood which ultimately leads to the occurrence of the disease.[13] Present antidiabetic treatment is commonly based on conventional synthetic drugs that most often have side effects. Thus, there is an incessant need to develop alternative and better pharmaceuticals for the improved management and treatment of the diabetes.

Compounds obtained from plants may be good substitutes of the conventional drugs for the effective treatment of diabetes, because they may have the quality to even reduce the risk of the diseases and a large amount of the product can be consumed everyday without being aware of the risks as these natural products will have no side effects. The number of plants and natural bio products having antidiabetic quality are many and are discussed elaborately in the literature. In the ancient

history there are various records of plants being used for the treatment of diseases with similar symptoms as that of diabetes.[14] Their massive advantage is that they can be consumed in everyday diet. In recent times, more attention is given to the study of natural products.[15]

Schleichera is a genus of plant that belongs to the family Sapindaceae. *S. oleosa* is a common tree found in the forests and country side in Indian subcontinent and Southeast Asia and they are commonly known as Kusum in 'Hindi' and are used for culture of lac insect. The villagers of India make use of this tree in numerous ways from time immemorial. Its leaves, twigs and seed-cake are used as fodder to feed cattle. The wood is very good as firewood and forms excellent charcoal. 'kusum oil' extracted from its seed is used for cooking and illumination purpose in villages where electrification has not yet reached and other modern facilities are still a far distant dream. They are used by the village healers to cure of itching, acne, burns, other skin troubles, rheumatism (external massage), hair dressing and for promoting hair growth.[16]

The wood of the tree is pinkish-brown which is heartwood and is very hard, strong and outstanding to make utensils, axles, tool handles, plows, and rollers of sugar mills and oil presses and other furniture. In India, it is used in lac culture industry as host for the lac insect [Laccifer lacca (Karr)].[17] The product of lac culture is called Kusum lac and is a good quality product for making various biogredable materials. In parts of southern India, it is a prominent bee plant for nectar.[18] It also has many medicinal uses and is used in conventional medicine for several indications. The seeds are powdered and are applied to wounds and ulcers of cattle to remove maggots and flies. The bark is used against various infections, skin inflammations, ulcers, acne itching, and other skin infections.[17] It is generally used as a pain killer, antibiotic and against diarrhea and dysentery.[19] In recent times, it was reported that the bark along with water is used to treat menorrhea.[20] It is widely distributed throughout India.[19]

The present study was undertaken to investigate the antidiabetic details of the methanolic and aqueous leaf extract of *Schleichera oleosa.*

MATERIALS AND METHODS

Collection of plant material

The fresh mature leaves of the plant *Schleichera oleosa* were collected, washed and disinfected with HgCl2 and washed again. The leaves were dried in shade under room temperature for six to seven days and then crushed into coarse powder using electric grinder. The powder was sieved to get fine powder using fine plastic sieve which was stored in air tight bottle in the laboratory until required.[21]

Extract preparation

50g of the powder was subjected to extraction by soxhlet using methanol and distilled water separately. The extracts obtained were filtered, concentrated after dryness in rotary flash evaporator maintained at 45ºC.percentage yield of each extract was calculated and the dried extracts were stored in airtight containers at room temperature for further studies.[22,23]

Phytochemical analyses

Freshly prepared extracts of the powdered leaves were subjected to phytochemical analyses to find the presence of the following phyto-constituents such as flavanoids, alkaloids, carbohydrates, glycosides, polysaccharides, tannins, saponins, steroids, proteins, lipids, oils by standard methods.[24] The details have been described elsewhere by Jose Sophy *et al.*[25]

Amylase inhibition assay

One gm of 3,5-Dinitrosalicylic acid (DNS) dissolved in 2N NaOH, 30 gm of potassium sodium tartarate was added to the solution and whole quantity was made up to 100 ml. The inhibition assay was performed using the chromogenic DNSA method [338.] The total assay mixture composed of 1400 µl of 0.05 M sodium phosphate buffer (pH 6.9), 50 µl of amylase (Diastase procured from HiMedia, Mumbai, Cat No. RM 638) and samples at concentration 100, 250 and 500 µg were incubated at 37°C for 10 min. After pre-incubation, 500 µl of 1% (w/v) starch solution in the above buffer was added to each tube and incubated at 37°C for 15 min. The reaction was terminated with 1.0 ml DNSA reagent, placed in boiling water bath for 5 min, cooled to room temperature and the absorbance measured at 540 nm. The control amylase represented 100% enzyme activity and did not contain any sample of analysis. To eliminate the absorbance produced by sample, appropriate extract controls with the extract in the reaction mixture in which the enzyme was added after adding DNS. The maltose liberated was determined by the help of standard maltose curve and activities were calculated according to the following formula.

$$\text{Activity} = \frac{\text{Conc. of Maltose liberated X ml of enzyme used}}{\text{Mol. wt of maltose X incubation time (min)}} \text{ X dilution factor}$$

One unit of enzyme activity is defined as the amount of enzyme required to release one micromole of maltose from starch per min under the assay conditions. The inhibitory induction property shown by the sample was compared with that of control and expressed as percent induction/inhibition. This was calculated according to the following formula.

$$\% \text{ inhibition/induction} = \frac{\text{Activity in presence of compound}}{\text{Control Activity}} \times 100$$

Analysis of Acarbose as standard inhibitor: Acarbose was used as a standard inhibitor and it was assayed at above mentioned leaf extract concentrations. The assay method was similar to the above mentioned procedure, instead of test samples, acarbose was added. The results were compared to that of test sample.

RESULT

Tests for phytochemicals in the methanolic and aqueous leaf extracts of *Schleichera oleosa* revealed the presence of the following phyto-constituents such as flavanoids, alkaloids, carbohydrates, glycosides, polysaccharides, tannins, saponins, steroids, proteins, lipids, oils. The methanolic leaf extract of *Schleichera oleosa* showed 26.191±0.001 percentage of amylase inhibition at 10 µg concentration, 50.131±0.002 percentage of amylase inhibition at 50 µg of concentration and 68.888±0.002 percentage amylase inhibition against 100 µg concentration and aqueous leaf extract of *Schleichera oleosa* showed 24.123±0.040%, 33.112±.004 % and 55.471±002 % amylase inhibition against 10 µg, 50 µg and 100 µg extract concentration respectively while Acarbose showed 99.833±0.288, 100.133±0.230, and 100.233±0.404 percentage of amylase inhibition against 10 µg, 50 µg and 100 µg the concentrations respectively.

Table 1. Amylase inhibition activity of methanolic and aqueous leaf extracts of *Schleichera oleosa* (mean ±SD)
(↑Significantly higher;↓ Significantly lower;# no significant difference; mean ±SD, n=3)

Concentration (µg)	*S.oleosa*Met	*S.oleosa*H2O	*Acarbose*
10	26.191±0.001	24.123±0.040	99.833±0.288
50	50.131±0.002↑	33.112±.004	100.133±0.230
100	68.888±0.002↑	55.471±002↑	100.233±0.404

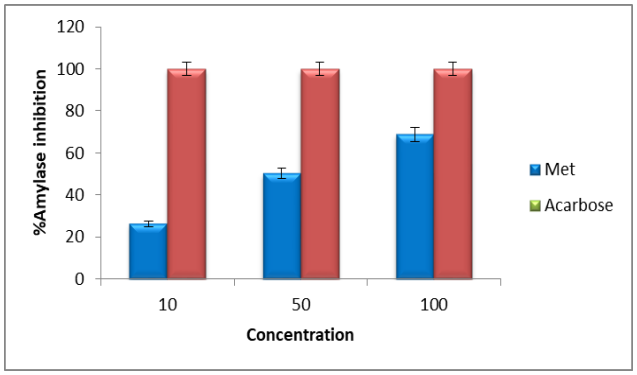

Figure: 1. Amylase inhibition activity of methanolic leaf extracts of *Schleichera oleosa* (mean ±SD).

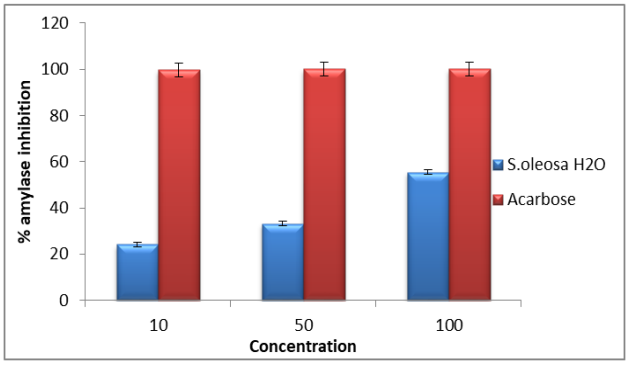

Figure: 2. Amylase inhibition activity of methanolic leaf extracts of *Schleichera oleosa* (mean ±SD).

DISCUSSION

Earlier studies have shown that various plants show antidiabetic activity such as the extracts of ginger and garlic on the isolated per fused pancreas of streptozotocin induced diabetic rats,[26] the insulinotropic character of the dietary red chilli (*Capsicum frutescens* L.),[27] insulin secretary actions of extracts of *Asparagus racemosus* root in perfused pancreas, isolated islets and clonal pancreatic beta-cells[28] and Enhancement of insulin release from the beta-cell line INS-1 by an ethanolic extract of *Bauhinia variegata* and its major constituent roseoside.[29]

Anti-diabetic efficacy of *Schleichera oleosa* studied showed 26.191±0.001of inhibition of amylase for 10 mg of methanolic leaf extract, 50.131±0.002 % for 50 mg and 68.888±0.002% for 100 mg while the aqueous extract of *S. oleosa* showed 24.123±0.040% for 10 mg, 33.112±.004% for 50 mg and 55.471±002↑% for 100 mg of the extract. The Acarbose showed 100% of inhibition of amylase for all three concentrations of the leaf extracts. From the above data it is clear that the methanolic extract has better anti-diabetic property owing to its better extracting capacity.

Hence both the extracts can be used as the anti-diabetic agent. Thus we can assume that *Schleichera oleosa* is a good anti-diabetic agent and can be used in the treatment of diabetic mellitus.

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