Int. J. Pharm. Investigation, 2021;11(2):154-157 Original Article

Assessment of Antioxidant and Pancreatic Lipase Activity of *Smilax zeylanica* Roots: An *in vitro* Analysis

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ABSTRACT

Objectives: It is well established fact that the phytochemicals with phenolic and flavonoids origin have antioxidant property. Thus in this study it was aimed to determine the *in vitro* antioxidant potential of various extracts of *Smilax zeylanica* L. stems. **Methods:** The Photochemical screening was done following the standard procedures. The Antioxidant activity was tested using several *in vitro* assays, viz., 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. The Total phenol and flavonoid contents were determined by colorimetric method. Further porcine lipase activity was determined. **Results:** The phytochemical screening revealed the presence of phenols and flavonoids the extracts. The Methanolic extract showed significant antioxidant potential than other extracts in all assays. The IC_{50} value of cyclo hexane extract , ethyl acetate extract and methanol extract of *Smilax zeylanica* was 29.14 ± 0.39 µ g/mL, 78.41 ±5 .53 µg /mL and 120.30 ±3 .32 µg /mL respectively in DPPH assay. Porcine pancreatic lipase inhibition assay Ishowed dose-dependent effect. The % Inhibition of cyclohexane extract (SZH) was 37.52% 54.44% and 77.07% at the highest dose 200 µg/ml. The highest inhibition was obtained in the methanolic extract at 200 µg/ml conc which was close to the standard orlistat i.e. 82.39 at 200 µg/ml. **Conclusion:** The methanolic extracts showed potential pancreatic lipase inhibition.

Key words: Antioxidant activity, DPPH, *Smilax zeylanica*, Pancreatic lipase, Phytochemical Screening, Alkaloids.

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DOI: 10.5530/ijpi.2021.2.28

INTRODUCTION

Oxidation is basic to many living creatures for the creation of energy to fuel natural cycles. Nonetheless, the uncontrolled creations of oxygen-inferred free extremists harm the cells and their capacities yet can likewise cause chain responses. Subsequently, the chain responses encourage the manufacture of new free revolutionaries. Practically all living beings are very much secured against free extremists by chemicals, for example, superoxide dismutase and catalase or dietary cancer prevention agent mixes, for example, ascorbic corrosive and tocopherols.[1] At the point when the components of cell reinforcement assurance become uneven, weakening of physiological capacities may bring about degenerative or obsessive cycles, for example, maturing, malignancy, coronary heart infections, and rheumatoid joint inflammation.[2] As of late, therapeutic plants have gotten a lot of consideration as wellsprings of naturally dynamic substances including cancer prevention agents, antimutagens and anticarcinogens.[3] It is vital to make reference to that a huge number of normal cancer prevention agents have been disengaged from various types of plant materials, for example, oilseeds, organic products, leaves, and roots just as from various vegetables, oat yields, flavours and spices.[4] Notwithstanding, logical data on cancer prevention agent properties of different plants having rare use in culinary and medication is still scant. In this way, the evaluation of such properties remains an intriguing and valuable errand, especially for finding new wellsprings of regular cell reinforcements, useful nourishments and nutraceuticals.[5] *Smilax zeylanica* L. (Family: Smilacaceae), privately known as 'Kumarilata' in Bangladesh, is an equipped or unarmed woody climber and broadly dispersed in South Asian areas, China and a few sections in Africa. Leaves, roots and rhizomes of *S. zeylanica* are generally utilized in the administration of a few infirmities. In the folkloric arrangement of medication, this plant is utilized in the treatment of venereal sicknesses, skin problems, injuries, swellings, sore and furthermore applied for stiffness and agony in lower limits.[6] Additionally, it is utilized in impotency and general shortcoming. The phytoconstituents revealed in the leaves and underlying foundations of *S. zeylanica* are dioscin (spirostanol triglycoside) and steroidal saponin glycosides, for example, diosgenin, smilagenin and sarsapogenin.[7,8] The roots and rhizomes of *S. zeylanica* have been accounted for to display antiepileptic and cancer prevention agent action, while the leaves show antidiabetic, anthelmintic and cell reinforcement potential.[6,9-12] Murali *et al.*[13] have distributed a paper about the methanolic concentrate of roots and rhizomes of *S. zeylanica* L. for hepatoprotective impact. In a short correspondence, Bari *et al.*[14] have written about the chloroform and methanolic concentrate of the leaf, stem and foundation of *S. zeylanica* L. for pesticidal action on Cryptolestes pusillus.[14] Nonetheless, as of recently no logical examination on the stems of this plant has been accounted for. Taking this in view and as a component of our continuous examination on restorative plants.[15,16] our investigation was meant to assess the cell reinforcement and cytotoxic capability of the methanolic concentrates and oil ether concentrates of *S. zeylanica* stems. Different *in vitro* measure frameworks were utilized. For cancer prevention agent potential, DPPH (1,1-diphenyl-2-picrylhydrazyl) free revolutionary searching measure, nitric oxide rummaging test, hydrogen peroxide searching test, cupric-diminishing cell reinforcement limit, ferric-decreasing cell reinforcement power, all-out cancer prevention agent limit and assurance of all-out phenol and flavonoid content were utilized. For cytotoxic potential, saline solution

shrimp lethality bioassay and MTT cell suitability test were utilized to comprehend the capability of this plant for use in phytomedicine. In spite of the fact that the cell reinforcement movement of roots, rhizomes and leaves from this plant has been accounted for in past investigations, this is the main report on the stem's cancer prevention agent action and cytotoxic potential.

METHODS

Collection of plant material

The plant *Smilax zeylanica* was collected in June from Yamuna biodiversity park, New Delhi. The formal authentication and identification was done in department of botany Ch. Charan Singh University, Meerut. The roots were allowed to air dry, away from sunlight. The dried material was grounded coarsely to a powder and transferred to labelled brown bottles until required.

Extraction procedure

The fresh and dried roots of *Smilax zeylanica* were crushed by using the grinder. The dried powdered drug passed through a 20 mesh sieve to remove excessive of mucilaginous hair. The dried, powdered plant material (500g) was extracted with cyclohexane, ethyl actate and methanol respectively for 72 hr with intermittent stirring. The collected mass was subjected to drying to evaporate the excess of solvent in a rotary evaporator. The percentage yield all the extracts were calculated using the mathematical formula [Weight of the concentrated extract obtained/ Weight of the powdered plant drug taken initially ×100].

Phytochemical screening

Preliminary Phytochemical qualitative analysis of different extracts of *Smilax zeylanica* indicated the presence of alkaloids, Saponins, flavanoids, tannins, phenol compounds in the extract Total phenolic content and total flavonoid content of plant extract was determined by the method described by elsewhere.[17]

DPPH free radical assay

The radical scavenging activity was done by already predetermined methods vis, DPPH radical scavenging assay. The results were expressed as % radical scavenging activity.100µl of various concentrations (50-1000 µg/ml) of different extracts and 100µl solution of DPPH (100 mM in methanol) was incubated at 37°C for 30 min and change in absorbance of reaction mixture was read at 517nm. An equal concentration of methanol and DPPH serves as control. The experiment was performed in triplicate and percentage radical scavenging activity was calculated by formula given below:[18]

$$\% \text{ of inhibition} = \frac{(\text{A of control} - \text{A of test}) \times 100}{\text{A of control}}$$

Preparation of Extract for *in vitro* Assay

The tested extracts were initially dissolved in DMSO to give five different stock solutions with a concentration range of 0.625-10.0 mg/mL (0.625, 1.25, 2.5, 5.0 and 10mg/mL). Subsequently, 20 µL aliquot of each stock solution was used in the reaction mixture to give a final concentration range of 12.5- 200 µg/mL (12.5, 25, 50, 100 and 200 µg/mL). Similarly orlistat was prepared as standard.

Porcine pancreatic lipase inhibition assay

Porcine pancreatic lipase, p-NPB, morpholine propane sulphonic acid, were purchased from Sigma Aldrich. Porcine pancreatic lipase (PPL) inhibitory assay was performed with minor modification. The enzyme solutions was prepared immediately before use, by suspending crude porcine pancreatic lipase powder type II (Sigma, EC 3.1.1.3) in Tris-HCl buffer (50 mMTris, 150 mMNaCl, 1 mM EDTA, 10 mM MOPS, pH 7.6) to give a concentration of 5 mg/mL (200 units/mL). The solution was then centrifuged at 1,500 rpm for 10 min and the clear supernatant was recovered. The plant extracts were then pre incubated with 200 µL of PPL solution for 5 min at 37°C, before the addition of 5 µL PNPB substrate solution (10 mM in acetonitrile). The total reaction volume was made to 1 mL using the Tris-HCl buffer before measuring the absorbance at 410 nm against blank using denatured enzyme. The denatured enzyme was prepared by boiling the enzyme solution for 5 min. Orlistat was used as a reference drug. The extract was dissolved in DMSO at a final concentration not exceeding 1% (v/v) which will not affect enzyme activity.

All assays were run at 37°C and reported results were the average of three replicates that were blank subtracted. Orlistat was used as a positive control. DMSO was used as a negative control and the activity was also examined with and without the inhibitor. Inhibition of the lipase activity was expressed as the percentage decrease in the activity when PPL was incubated with the test compounds. Lipase inhibition (%) was calculated according to the following formula:[19]

$$I\% = \left(\frac{B}{A}\right)^{*}100$$

Where *A* is the activity of the enzyme without inhibitor, *B* is the activity of the enzyme with inhibitor.

RESULTS

The percentage yield of various extracts of *Smilax zeylanica* was determined. The percentage yield for the cyclohexane extract was found to be 1.99%, ethyl acetate extract was 2.26%, Methanol extract was found to be 4.13%.

The Preliminary phytochemical screening of crude methanolic extract and petroleum ether extracts of the stems of *Smilax zeylanica* revealed the substantial presence of Phenols, flavonoids and glycoside (Table 1).

The total Phenolic content of *Smilax zeylanica* cyclohexane extract was found to be 136.55µg/mg of gallic acid, for ethyl acetate extract it was 187.11µg/mg of gallic acid and it was highest i.e., 203.11µg/gm of gallic acid for methanol extract of *Smilax zeylanica*. The total Flavonoid Content of *Smilax zeylanica* cyclohexane extract was found to be 22.06 mg/g quercetin equivalent, for ethyl acetate extract it was 27.06 mg/g quercetin equivalent and for methanol extract of *Smilax zeylanica* it was 31.9mg/g quercetin equivalent.

The extracts showed dose-dependent scavenging of DPPH radical when compared to ascorbic acid (Figure 1). The IC_{50} value of cyclohexane extract, ethyl acetate extract and methanolic extracts of *Smilax zeylanica* and petroleum ether were 35.76 µg /mL, 52.74 and 70.43 µg /ml, respectively, while the IC_{50} value for the reference ascorbic acid was 77.8 ±0 .24 µg/ml.

The extracts showed dose-dependent effect in porcine pancreatic lipase inhibition assay (Figure 2). The % Inhibition of cyclohexane extract (SZC) was 37.52% 54.44% and 77.07 at the highest dose 200 µg/ml. The highest inhibition was obtained in the methanolic extract at 200 µg/ml conc which was close to the standard orlistat i.e., 82.39 at 200 µg/ml.

DISCUSSION

In this study *Smilax zeylanica* was analysed for its potential. Primary phytochemical screening uncovered the presence of flavonoid, tannin and glycosides in both methanolic and oil ether removes. Polyphenolic mixes, similar to flavonoids, tannins and phenolic acids, ordinarily found in plants have been accounted for to have different organic impacts, including cancer prevention agent action.[20,21] Flavonoids and

Table 1: Photochemical screening of the extracts of *Smilax zeylanica*.

S.No.	Phytoconstituents present	Cyclo hexane extract of *Smilax zeylanica*	Ethyl acetate extract of *Smilax zeylanica*	Methanol extract of *Smilax zeylanica*
1.	Alkaloids	(+)	(+)	(+)
2.	Tannins	(+)	(+)	(+)
3.	Saponins	(+)	(++)	(++)
4.	Proteins	(-)	(+)	(+)
5.	Flavanoids	(+)	(++)	(++)
6.	Carbohydrates	(-)	(-)	(+)
7.	Anthraquinones	(+)	(+)	(-)
8.	Phenols	(+)	(+)	(+)
9.	Steroids	(-)	(+)	(+)

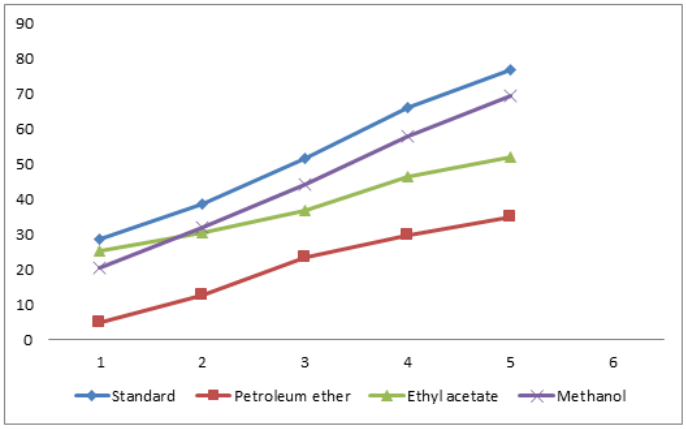

Figure 1: DPPH %age free radical- scavenging capacity for standard and extracts of *Smilax zeylanica*

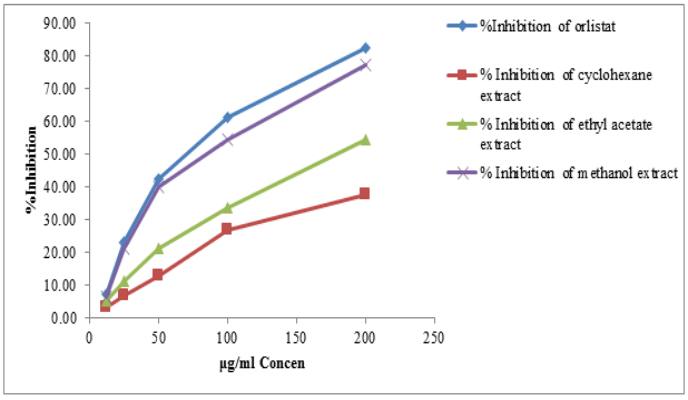

Figure 2: Porcine pancreatic lipase inhibition of *Smilax zeylanica* extracts.

tannins present in the plant extricates, as apparent from phytochemical screening, might be principally answerable for the antioxidant activity. Free extremists significantly affect the oxidation of unsaturated lipids. The DPPH revolutionary was utilized as a steady free extremist to decide pancreatic lipase activity.[22] The DPPH cell reinforcement measure depends on the capacity of DPPH, a steady free extremist, to decolorize within the sight. The odd electron in the DPPH revolutionary is liable for the absorbance at 517 nm and furthermore for the noticeable profound purple tone.[23] Along these lines, one of the potential components of methanolic remove's solid antiradical action may be described to the presence of a decent measure of phenolic and flavonoidal substance. Nitric oxide or responsive nitrogen species, shaped during their response with oxygen or superoxide's are receptive.[24] Hydrogen peroxide itself isn't responsive, yet it can in some cases be harmful on the grounds that it might offer ascent to hydroxyl extremists in cells.[25] Flavonoids have been appeared to shield mammalian and bacterial cells from cytotoxicity by hydrogen peroxide restraint, particular extracts with the o-dihydroxy phenolic structure.[26] Pancreatic lipase is a vital compound for lipid ingestion by hydrolysis of all out dietary fats. Consequently, the examination of new specialist for pancreatic lipase inhibitor is as yet required. Our finding is the first run showed solid enemy of lipase action. This recommends that these spices appear to be promising as the inhibitor of pancreatic lipase. Our outcomes indicating a critical positive relationship between are phenolic, flavonoid, alkaloid substance and restraint action, which offer solid help that these phytochemical are key for pancreatic lipase activity. The study performed showed that the plant has shown the capacity of pancreatic lipase movement. Distributed exploration additionally detailed that flavonoids and alkaloid have the potential for pancreatic lipase activity. We inferred that *Smilax zeylanica* acted as key specialists for pancreatic lipase hindrance *in vitro*.

CONCLUSION

The methanolic extracts could be considered as potential sources of natural antioxidant. It also showed potential pancreatic lipase inhibition could be explored for antilipidemic potential.

ACKNOWLEDGEMENT

The author acknowledges the I.T.S College of Pharmacy, Ghaziabad.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

DPPH: 2,2-diphenyl-1-picrylhydrazyl; **Porcine assay:** Porcine pancreatic lipase inhibition assay; **SZC:** Cyclohexane extract; **SZE:** Ethyl acetate extract; **SZM:** Methanol extract.

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Article History: Submission Date : 22-02-2021; Revised Date : 14-03-2021; Acceptance Date : 02-04-2021.
Cite this article: Mishra R, Juyal D, Kumar SS. Assessment of Antioxidant and Pancreatic Lipase Activity of *Smilax zeylanica* Roots: An *in vitro* Analysis. Int. J. Pharm. Investigation, 2021;11(2):154-7.