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SPECTRAL ANALYSIS AND HYPOGLYCEMIC EFFECT OF CRUDE SAPONIN EXTRACTED FROM *PHOENIX PUSILLA* ROOT

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ABSTRACT: In this present study, the objective was to extract crude saponins from the root of *Phoenix pusilla* (PP) and to check its inhibitory potential of α- amylase and α- glucosidase enzyme. Saponins were isolated by partitioning between butanol and water. Further, the presence of saponin was confirmed by the foam test. Then the crude saponins were analyzed by UV- spectroscopy in the range of 200-400 nm and Fourier transform infrared spectroscopy (FTIR). FTIR results revealed the presence of – OH, -C-H, C=O functional groups. *In-vitro* antidiabetic activity assay of isolated crude saponin with acarbose as positive standard showed efficient hypoglycemic ability. Saponins have application in the food industry, cosmetics preparation and also exhibit various pharmaceutical activities. So plant-based saponin isolation in the pure form will be a boon to human utilization. This was the first report of in vitro antidiabetic potential of crude saponins of PP root.

INTRODUCTION: Phoenix pusilla (PP) belongs to Arecaceae family native to Srilanka and India. It grows upto 3 feet, and it is commonly called as dwarf date palm [1]. Fruits of PP are used to treat stomach disease, which affects mucosal lining, fever, seminal weakness [2], pith is used by poor people for making kanji and is used to treat gleet, gonorrhea. Roots are used to treat toothache [3]. Phoenix consists of 14 species, of which 7 species are seen in India and 2 species are native to India [4]. Saponins are the phytochemicals found to be distributed in different concentrations in various parts of monocotyledon and dicotyledon plants.

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They are water-soluble, have a bitter taste and surfactant properties. Saponin has aglycone (terpenoid, C-30, steroid, C-27), glycone part and so it has amphiphatic nature [5]. Saponins showed biological activity like haemolytic action (in fish), anti-inflammatory, anti-tumor, anti-insect, *etc.* Saponin has application in soap manufacture, shampoo, cosmetics and fire extinguisher [6]. Saponins have the ability to regulate nutrient uptake by the intestine, reduce protein digestion, serum cholesterol [7].

In 1500 BC, Egyptians first recognized diabetes. In the same period, Indians identified and named it as 'madhumeha', which means honey in the urine [8]. Defect in insulin secretion or defect in insulin action, or both is the major cause of diabetes. The major impact of the defect is a rise in blood glucose levels [9, 10]. Diabetes was identified as the 8th leading cause for death in the world. Insulin is the peptide hormone secreted by β- cells of the

pancreas [11]. The role of insulin in the target cells (Adipose tissue, skeletal muscle, liver) is to promote energy storage. It affects carbohydrate, protein, and lipid metabolism. Insulin stimulates glucose uptake, promotes glycolysis and glycogenesis, and protein synthesis [12].

Saponins present in the plant have a protective effect on pancreatic islet cells and promotes insulin secretion from the remaining pancreatic β-cells. The ability of saponin to reduce elevated plasma blood glucose makes saponin an excellent candidate in the treatment of diabetes mellitus [13].

Saponins have been known to possess the anti-diabetic property and are promising compounds with the potential to be developed into new drugs for anti-diabetes. Saponins isolated from various plants have been reported against diabetes. Hence, in this present study, crude saponins of PP were isolated, characterized and *in-vitro* enzyme inhibition against alpha-amylase and Alpha-glucosidase was determined.

MATERIALS AND METHODS:

Sample Collection: Roots of PP were collected from Viramangudi village, Thanjavur district, authenticated, dried, and stored.

Crude Saponin Isolation (PPCS): 100 g of dry powder of PP was deffated with 500 ml of petroleum ether in soxhlet apparatus, the marc re-extracted with methanol for 24 h, the methanol was evaporated, concentrated and the yield was noted (5 g). The methanolic extract was then partitioned between butanol and water (1:1), left at room temperature without disturbing the mixture for 1 day. Then the organic layer was collected, the solvent was evaporated, the residue was dissolved in methanol. Finally, diethyl ether was added to precipitate the saponin [14].

Foam Test: Saponin presence was confirmed by adding a small amount of precipitate and water in a test tube. The tube was shaken vigorously [15].

FTIR: Translucent sample disc was prepared by mixing crude saponin with KBr pellet and loaded into FTIR spectroscope. The spectra were recorded in the scan range of 500 cm^{-1}-3500 cm^{-1} [16]. Chemical bonds can be identified from the IR spectrum.

High-Performance Liquid Chromatography (HPLC): Analytical HPLC was carried out by injecting 20 µl of crude saponin into C18 column (4.6 × 250 mm) with acetonitrile: water as mobile phase in the ratio of 25:75 at the flow rate of 0.8 ml /min [17].

Liquid Chromatography-Mass Spectroscopy (LC-MS): LC-MS is a hyphenated technique where HPLC is coupled with MS. The principle of this method is separation occurs in the liquid column, and then the separated molecule is ionized. Then the ionized molecule based on their charge/ mass ratio was detected. The main application of LC-MS is the compound can be analyzed qualitatively and quantitatively [18].

***In-vitro* Antidiabetic Activity:**

α -Amylase Inhibitory Assay: 200 µl of root crude saponin and positive standard (acarbose) of concentration range 250-1250 µg/ml were taken. To each concentration, 200 µl of α-amylase solution dissolved in phosphate buffer was added and incubated at 30 °C for 10 min. Then 200 µl of 1% starch solution was added, incubated for 3 min. Finally, the reaction was stopped by adding 3, 5-dinitrosalicylic acid, kept in boiling water bath for 10 min, cooled, and the absorbance was measured at 540 nm. Control was also treated similarly without the extract [19].

α –Glucosidase Inhibitory Assay: 20 µl of five different concentrations of PPE, acarbose were taken, and the same volume of the enzyme was added. The reaction mixture was then incubated at 37 °C for 15 min. 5 mM P-NPG (para nitrophenyl glucose, 20 µl) was added to the extract, as well as to the standard, incubated at 37 °C for 20 min and the reaction was stopped by adding 50 µl Na_2 CO_3 (0.1 M). The absorbance of the released p-nitrophenol was measured at 405 nm. Control was set up without the extract [20].

Results and Discussion: Saponin was isolated by partitioning between butanol and water in the separating funnel. After 24 h, two separation layers were seen with the lower aqueous layer and upper organic layer. Butanol upper layer (brown colour) contains the saponins, which were collected separately **Fig. 1**. The presence of saponin was confirmed by foam test and the foam persisted for 3

min **Fig. 2**. FTIR analysis showed the C=O, C-H, OH stretch presence at the frequency of 1645.47, 2873.23, 2938.91, 3364.01 cm^{-1} **Fig. 3**, **Table 1**.

FIG. 1: SAPONIN PARTITIONED BETWEEN WATER AND BUTANOL

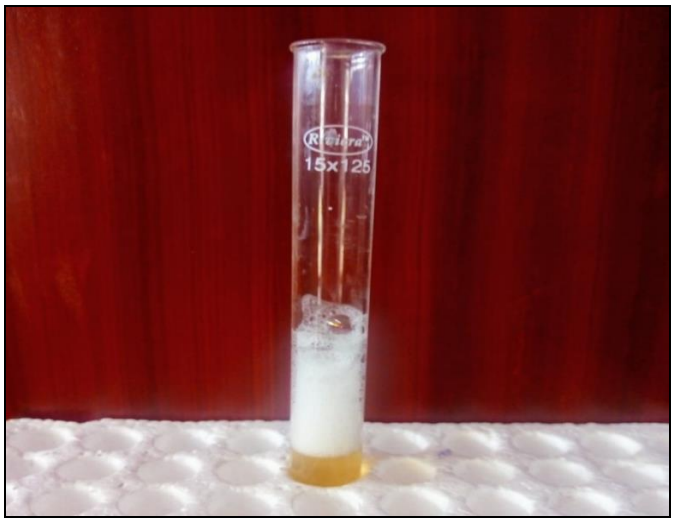

FIG. 2: FOAM TEST

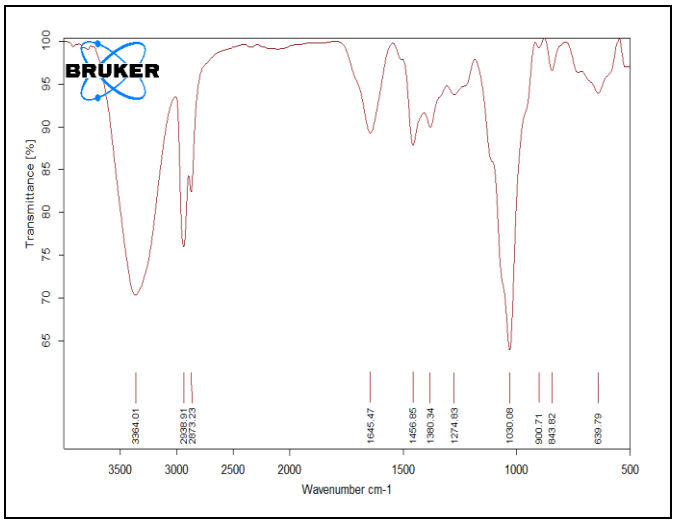

FIG. 3: FTIR SPECTRUM OF PPE CRUDE SAPONINS

TABLE 1: STRETCH AND BENDS OF CRUDE SAPONINS

S. no.	Wave number cm^{-1}	Functional group
1	3364.01	OH stretch
2	2938.91	C-H stretch
3	2873.23	C-H stretch
4	1645.47	C=O stretch

Saponins are used to treat and cure diseases like bronchitis, eczema, varicose ulcer, *etc.* [21] Crude saponins of *Terminalia arjuna* and *Prosopis spicigera* showed immunopathological activity [22]. Asha *et al.*, isolated saponin from *Rosamarinus officinalis* and showed the same type of functional group existence [23]. Saponin isolated from brittle stars showed a similar peak, which was obtained with PP crude saponin. Brittle star saponin was also found to show similarity with the standard *Quillaja saponin* [24]. IR spectra of *Sapindus emarginatus* showed the existence of a similar type of functional group [25]. HPLC chromatogram showed 19 peaks, the peak with retention time 3.381 showed maximum height, and this peak molecular weight was checked with LC-MS **Fig. 4**.

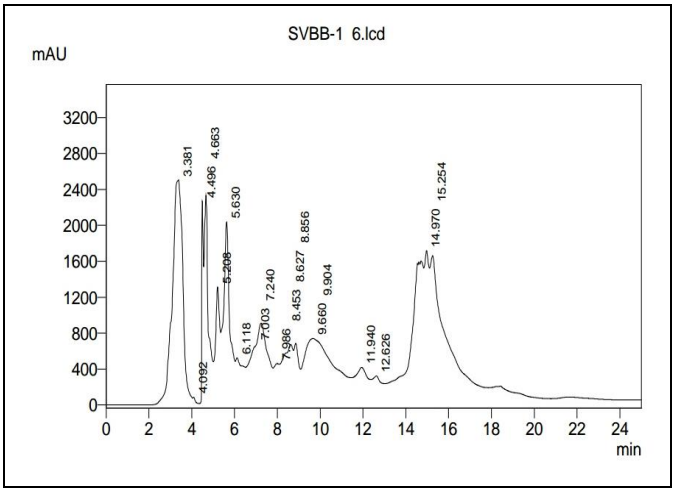

FIG. 4: HPLC CHROMATOGRAM

A normal or reverse phase column can be used for identification but for the ultimate separation reverse-phase column. The most commonly followed protocol for triterpenoid saponin separation is the utilization of C18 column and acetonitrile: water as mobile phase. Selection of stationary and mobile phase for the saponin separation is important as it plays a crucial role in separation [26]. The highest peak observed with HPLC analysis was targeted. Fraction of the highest peak was subjected for LC-MS analysis. LC-MS of fraction 1 (3.381 retention time) showed a single peak with a molecular weight 344.35.

Asha *et. al.*, isolated saponin from *Rosamarinus officinalis* and showed the same type of functional group existence as found with PPCS [23]. So it may be the same type of saponin identified in *Rosamarinus officinalis.*

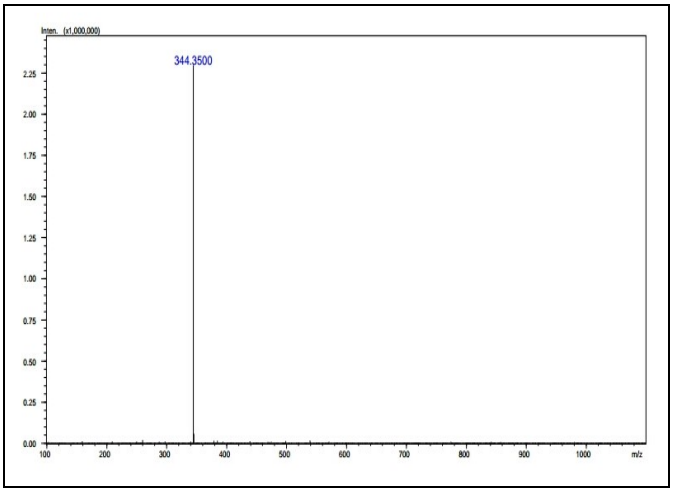

FIG. 5: LC-MS

α -amylase and α-glucosidase are key enzymes involve in carbohydrates breakdown and intestinal absorption, respectively. Inhibition of these enzymes regulates the blood glucose level increase after carbohydrates diet and can be an important strategy in the management of non-insulin-dependent diabetes mellitus (NIDDM) [27]. *The in-vitro* anti-diabetic activity revealed that the crude saponins were effective against alpha-amylase as well as glucosidase enzyme, but comparatively, α-amylase inhibition potential was high (**Fig 6**, **7**). IC_{50} value of α- amylase inhibition was 587.12 µg/ml, and α- glucosidase inhibition was 835.72 µg/ml.

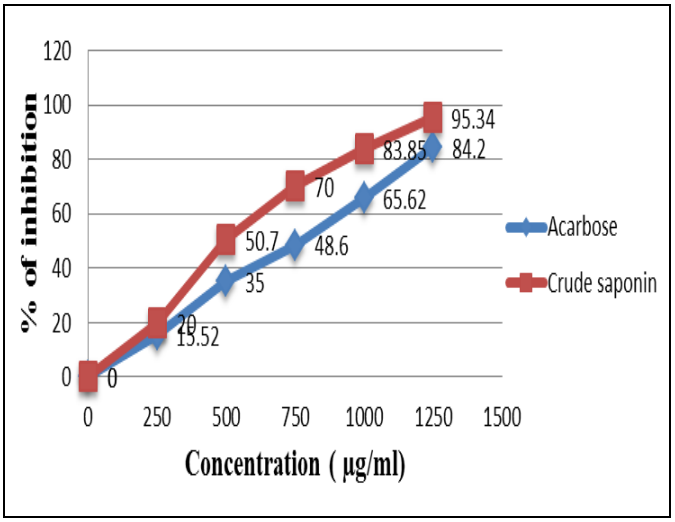

FIG. 6: AMYLASE INHIBITION ACTIVITY

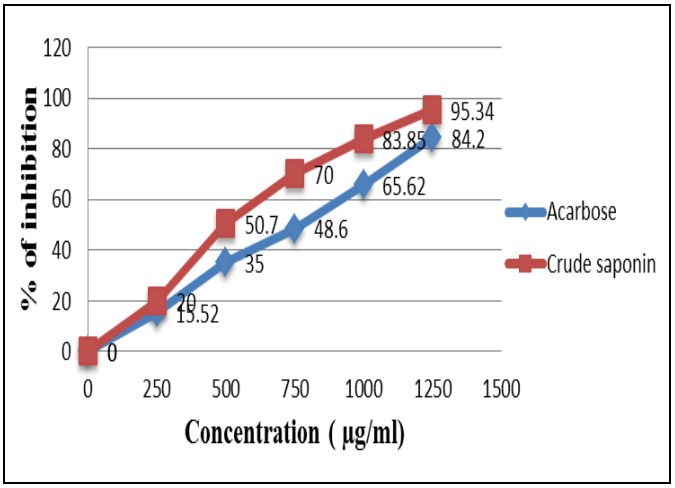

FIG. 7: GLUCOSIDASE INHIBITION ACTIVITY

Saponin was extracted from *Cochlospermum planchonii* root by liquid-liquid partition method using butanol. Inhibition efficiency against alpha-amylase and alpha-glucosidase for the extracted saponin was evaluated. It was found that the root saponin showed effective inhibition against both the enzymes which was taken for the assay. Furthermore, it was identified that the root saponin inhibits the enzyme by uncompetitive inhibition [28]. Steroidal saponin was isolated from bitter tea vernonia leaf and its inhibition potential against α-amylase and α-glucosidase was checked. It was observed that the saponin was effective against both the enzyme inhibition [29]. So it was evident that saponins possess antidiabetic property by inhibiting the enzymes associated with postprandial blood glucose regulation. In the above-mentioned findings, butanol saponin fraction or saponin isolated from butanol fraction of different parts of the plant were reported for amylase. Glucosidase inhibition, its mode of inhibition, was identified, and moreover, in the present study, butanol-based saponin isolation was carried out. In contrast, in future the exact mode of partially purified saponin of *Phoenix pusilla* root against the amylase and glucosidase inhibition has to be studied.

CONCLUSION: Saponins are a high molecular weight polar compound proved to have various biological activities. PP plant parts have folklore medicinal value, but it is exploited for another purpose. A further *in-vivo* study is needed to properly utilize crude saponins of PP root against diabetes and associated complications.

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