EXPLORING THE HYPOGLYCEMIC POTENTIAL OF DATE SEED FLOUR (PHOENIX DACTYLIFERA L.) THROUGH IN VITRO METHODS AND PRODUCT INNOVATION

Project Reference No.: *47S_MSC_0173*

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Keywords:

Date seed flour, hypoglycemia, product innovation, anti-diabetic

Introduction:

Treatment options for diabetes mellitus include oral hypoglycemic medications and insulin. These medications temporarily restore normal glucose balance and hence must be taken at frequent intervals. Common adverse effects include hypoglycemia, renal disorders, gastrointestinal issues, hepatotoxicity, cardiovascular risks, and insulinoma. These medications must be taken indefinitely. Plant-based medications, which are eco-friendly, bio-friendly, cost-effective, and reasonably safe, have transitioned from the periphery to the mainstream due to enhanced research in traditional medicine over the last several decades.

Tribal groups in India continue to use their traditional knowledge for treating various illnesses and disorders due to its safety, efficacy, and cost-effectiveness. Traditional treatments are becoming more popular among people in urban and rural regions, particularly in India and China (Katewa et al., 2004). In addition to being used for sustenance and healing purposes, plants and their many components have importance in tribal ceremonies and play a significant role in their societal and spiritual practices. Traditional plant knowledge is the foundation of ethnobotanical study in India (Jagtap et al., 2006).

Phoenix dactylifera L. is an ancient, cultivated fruit crop in Africa, Arabian Peninsula, Middle East and the Indian subcontinent. It is an exceptional source of nutrition, containing dietary fibres, carbohydrates, proteins, lipids, vitamins, minerals and bioactive compounds. The main purpose of the study will be to evaluate the antioxidant and antidiabetic activities of the date seed flour prepared from fresh and roasted date seeds.

Objectives:

- Standardization of the method for preparation of date see flour.
- To determine the antioxidant property in date seed flour by malondialdehyde (MDA) test, DPPH radical scavenging activity, hydrogen peroxide scavenging activity and total phenol test.
- Evaluation of hypoglycaemic activity in date seeds using various in vitro methods:
    1. α-amylase inhibitory test
    2. Pancreatic lipase (PPL) inhibition assay
    3. *In vitro* glucose diffusion inhibitory assay
- To determine the total nutrient content of sun dried dates seeds.
- Development of a product using date seed powder helpful for diabetic patients.
- Sensory evaluation of the product on the nine-point hedonic scale.

Methodology:

The preparation of seed flour was first standardized. For this, seeds were separated, washed, boiled (80 – 90 ^{o}C), sun dried, and roasted (6 h at 100 ^{o}C in a hot air oven). Pulverized seeds were finely ground flour using a Cutting Mill. Phytochemical screening performed to determine the best solvent for the extraction of the bioactives (Harborne et al., 1999; Khandelwal, 2009).

Total soluble sugar, total reducing sugar, total protein and total phenolic content was determined. The antioxidant activity was evaluated using malondialdehyde, DPPH radical (Braca et al., 2002), and hydrogen peroxide scavenging (Ruch et al., 1989) assays. The anti-diabetic activity was determined using *in vitro* glucose diffusion inhibitory assay (Edwards et al., 1987), where the drug metformin was used as standard. α-amylase and α-glucosidase inhibitory effect (Zhang et al., 2014) was assayed using acarbose as control. Pancreatic lipase inhibition assay (Bustanji et al., 2019) was used to evaluate the anti-diabetic activity with the drug orlistat as standard. Nutritional evaluation was performed by estimating the amount of amount of gluten was determined.

Conclusion:

Primary and secondary metabolism in plants produces phytochemicals, which fundamentally have therapeutic properties. The date seed powder was extracted by utilizing methanol. The qualitative investigation of the phytochemicals uncovered the presence of alkaloids, terpenoids, phenols, flavonoids and saponins (Table 1). Anthraquinone glycosides were not present in the seed powder. Based on the colour intensity, the extract had maximum concentration of alkaloids; followed by saponins, tannins, terpenoids. Flavonoids were present in low amounts (Table 1).

Table 1: Qualitative phytochemical composition of *Phoenix dactylifera* seed powder

Sl.no	Phytochemical analysed	Test performed	Result
1.	Alkaloids	Mayer's test	+++
		Wagner's test	+++
		Dragendroff's test	+++
2.	Glycosides	Keller-Killiani test	-
3.	Saponins	Foam test	-
4.	Terpenoids	Salkowski's test	+++
5.	Phenols	Ferric chloride test	+++
6.	Flavonoids	Lead acetate test	+
7.	Proteins and amino acids	Xanthoproteic test	++
		Ninhydrin test	++
8.	Carbohydrate	Molisch test	+++
		Benedict's test	+++
		Fehling test	+++
9.	Phytosterols	Salkowski's test	+++

PARAMETERS	DATE SEED EXTRACT
Total soluble sugar (µg/ml)	1.77±0.5
Total reducing sugar (µg/ml)	3.17±0.01
Total protein content (µg/ml)	3.64±0.02

The hydromethanolic extract of P. dactylifera seed flour and metformin were subjected to this assay to determine glucose diffusion and GDRI% across the dialysis membrane. The rate of glucose diffusion across the membrane in the presence of metformin increased between 30 – 90 min and then gradually declined, while the extract demonstrated a much slower but persistent increase during the period of observation (Table 2). GDRI, a means to predict the effect of a fiber on delaying glucose absorption in the gastrointestinal tract declined steadily with increase in time. High value of GDRI corresponds to higher retardation index of glucose by the sample. Presence of fiber in the sample is implicated in inhibition in movement by encapsulating starch and enzyme.

Table 2: Glucose dialysis retardation index (GDRI) in glucose diffusion

	Glucose content in dialysate (mM)					
	30 min	60 min	90 min	120 min	150 min	180 min
Control (Metformin)	58.8 ± 2.9	80.6 ± 5.1	65.8 ± 7.2	68.57 ± 6.9	58.2 ± 7.3	48.18 ± 5.2
Date seed extract	14.7 ± 1.6	19.3 ± 4.4	21.9 ± 6.3	31.4 ± 5.4	40.9 ± 6.2	60.0 ± 4.8

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 hydrolysed by pancreatic amylases into maltose, maltotriose and small malto-oligosaccharides. The digestive alpha amylase is responsible for hydrolysing dietary starch, which breaks down into glucose prior to absorption. Inhibition of alpha amylase can lead to reduction in post prandial hyperglycemia in diabetic condition (Roux et al., 2001., Lonkisch et al., 1998). The extract of date seed possesses anti-diabetic activity comparable with Acarbose standard (Figure 3).

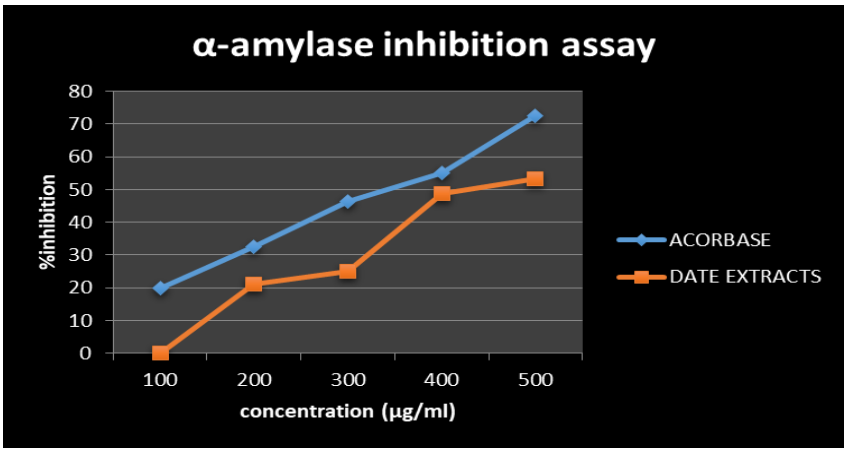

Figure 3: Inhibition of α-amylase by date seed extract

The inhibitory effect date seed extracts against pancreatic lipase were compared to that of orlistat, a lipase inhibitor used as positive control (Figure 4). The inhibitive capacities of the seed extract against the lipase activity might be perfectly coincident with their total phenolics compounds. In fact, various natural products containing polyphenols have been reported to have anti-obesity and anti-diabetic effects by inhibiting lipase activity and α-glucosidase activity.

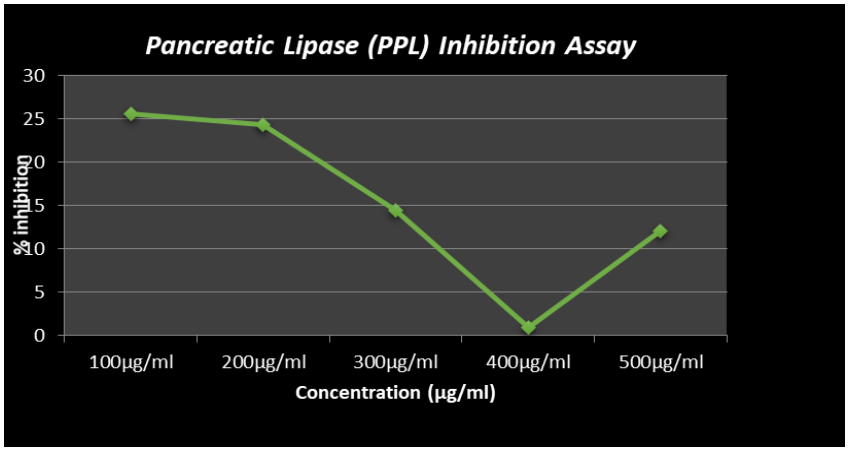

Fig 4: Inhibition of pancreatic lipase by date seed extract

The free radical scavenging activity of Phoenix dactylifera is shown in Figure 5. The antioxidant activity of date seed extract was compared with Ascorbic acid where the percentage of antioxidant property of Ascorbic acid was found to be 80% and the percentage of antioxidant property of date seed extract was found to be 13.75%. This indicates that date seed has significant antioxidant effect when compared with ascorbic acid. Anthocyanins and other flavonoids, phenolic acids and vitamins like

ascorbic acid and vitamin E contribute to the protective effect against oxidative damage to the cell. Since antioxidant activity of individual dietary compounds cannot be evaluated, the determination of total antioxidant activity allows a more realistic evaluation of the potential protective effect of a food.

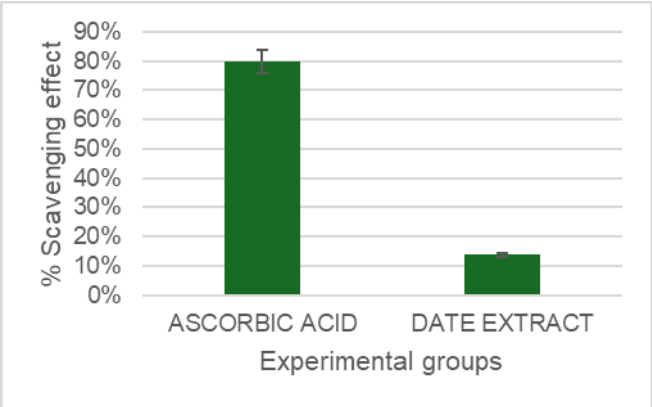

Figure 5: Graphical representation of Free radical scavenging activity of date seed extract

The hydrogen peroxide scavenging activity of date seeds extract was determined and compared with the standard Ascorbic acid by calculating the percentage inhibition (Figure 6). The percentage inhibition of date seed extract was found to be 98.22% at 500µg/ml of the concentration.

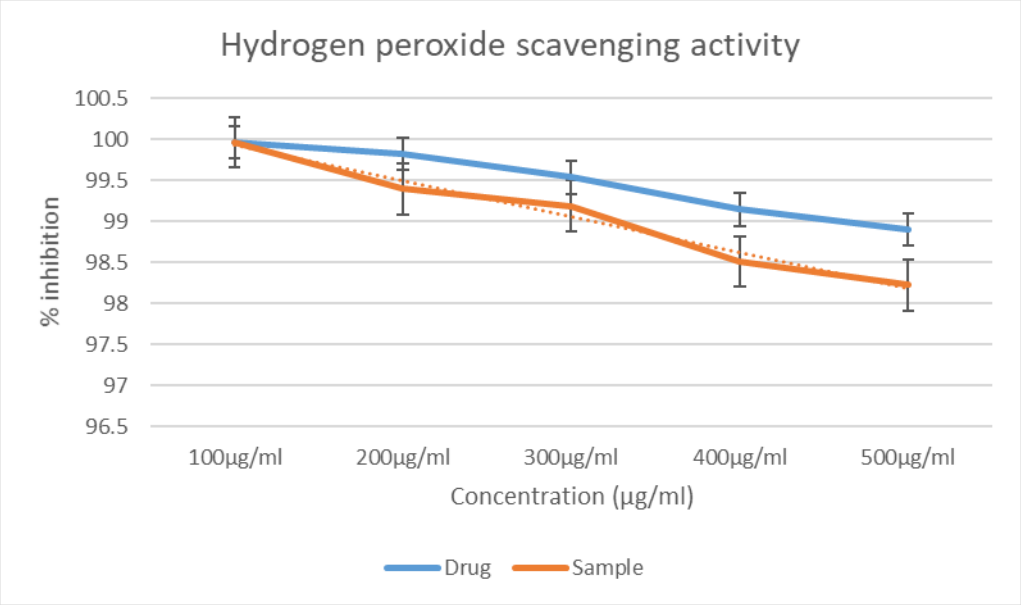

Figure 6: H2O2 scavenging activity of date seed extract and standard Ascorbic acid

The percentage of gluten found in date seed flour was 0.646%. The amount of seed powder taken for the analysis was 5g. The weight of the dough formed was 6.72g and after drying, the dry weight was 3.49g.

Scope for future work:

The expected outcome of incorporating date seed flour into hypoglycemic products involves several potential benefits. Firstly, due to its rich content of bioactive compounds such as phenolics and fibers, date seed flour may contribute to improved

glycemic control by slowing down the absorption of glucose from the digestive system, thus helping to regulate blood sugar levels. Secondly, the presence of antioxidants in date seed flour could offer protective effects against oxidative stress, a common complication associated with diabetes. Additionally, the inclusion of date seed flour may enhance the nutritional profile of the products by adding dietary fiber, vitamins, and minerals, which can support overall health and well-being, especially for individuals managing diabetes. Lastly, sensory evaluations are expected to indicate acceptable taste and texture profiles, ensuring consumer satisfaction and adherence to dietary recommendations. Overall, the study aims to provide scientific substantiation for the utilization of date palm seed flour as a functional food ingredient targeting metabolic syndrome and diabetes management, alongside consumer-friendly product development.