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Antioxidant, Antidiabetic and Anti-inflammatory Potential of *Phoenix pusilla* Root- An Unexplored Folklore Plant.

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Abstract: *Medicinal importance of Phoenix pusilla is unexplored. In the present study, antioxidant potential, α-amylase and α-glucosidase inhibitory effect of ethanol root extract were evaluated. Also the anti-inflammatory potential was checked by two invitro methods. IC50 values of the extract against enzymes showed the ability of lowering glucose level (post prandial) at low concentration. The inhibitory action was high against α -amylase than towards α-glucosidase. Similarly the anti-inflammatory assays showed 88.62% and 67.51 % of inhibition. Thus, in vitro study revealed that the root extract have potential to act against free radicals and posess hypoglycemic and anti-inflammatory activity as determined by membrane stabilisation and proteinase inhibitory activity.*

Keywords: *Phoenix pusilla, free radical scavenging potential, Alpha amylase inhibitory effect, anti-inflammatory potential.*

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I. Introduction

The genus *Phoenix* has been reported to contain 13 species. Different parts of the plant *Phoenix* are used to treat various metabolic disturbances and diseases like fever, paralysis, inflammation, nervous disorders, loss of consciousness, memory disturbances, cystitis, gonorrhoea, oedema, abdominal problems and in counteracting alcohol intoxication [1]. Most of the species are used for ornamental purposes. The fruits of the plant known as dates, from approximately eighty percent of the species are edible and are often consumed as food and medicine throughout the world [2]. *Phoenix pusilla* (PP) Gaertn. (small date palm) a closely related species of date palm is found in India and Srilanka. It is a beautiful shrubby suckering palm with a very short stem enveloped in persistent leaf sheaths. A crown of about 15-17 leaves is produced every year. It was identified that this palm can withstand low (4°C) to high (48°C) temperatures. PP found to have ornamental, medicinal and soil conservation value [3]. In Sri Lanka it is commonly known as indi-gaha. It is distributed in the dry forests of Kerala, Karnataka and Eastern Ghats of Tamilnadu in India, at low elevations, ridges and hills. However, it is also found to be present inland at the margins of marshes and raised banks along borders of paddy fields, up to an altitude of 700 m. At the times of food shortage, trunk serves as the major source of edible starch [4].

Free radicals and other reactive oxygen species by-products are generated by living cells as results of physiological and biochemical processes. It can cause oxidative damage to lipids, proteins and DNA, eventually leading to many chronic diseases [5]. Polyphenols are identified as an effective antioxidant agent to fight against disease like diabetes, cancer, neurodegenerative diseases [6]. Flavonoids act as an antioxidant by inhibiting the enzymes of free radical generation and lipid oxidation. It also showed anti-inflammatory, antibacterial, anticancer, antiviral activities [7]. The other secondary metabolites like carotenoid, stilbenes and tannins also found to have antioxidant potential [8].

Diabetes mellitus is the chronic metabolic disorder found prevalent among young age people nowadays due to improper dietary life style. Salivary and pancreatic amylase breaks down the dietary large carbohydrates into absorbable smaller molecules. Similarly α-glucosidase found in the small intestine helps in the digestion of carbohydrates. Inhibitor of these enzymes delays the digestion and thereby reduces the postprandial glucose level [9]. So in this present study, the post prandial glucose jump regulating enzymes are targeted. Inflammation is a non specific immune response to injury, infection or destruction which leads to the secretion of chemokines, prostaglandins and histamines. This in turn causes the signs and symptoms of inflammation. Inflammation process is associated with increase in vascular permeability, protein denaturation and membrane alteration [10]. The mechanism of inflammation involves the release of arachidonic acid from the membrane of neutrophils and converted to prostaglandins, leukotrienes. Cyclooxygenase and lipooxygenase are the enzymes involves in the synthesis prostaglandins, leukotrienes respectively. So these enzymes can be targeted in inflammation

regulation [11]. Anti-inflammatory agents act either by inhibiting COX enzyme or protecting lysosomal membrane breakdown thereby hindering prostaglandin synthesis [12].

Traditional drug treatment against diabetes found to work by inhibiting the action of carbohydrate digestive enzyme like amylase, glucosidase or by reducing the activity of other factors that increases blood sugar level. According to Mamun-or-Rashid et al., 2014 review report, plants of 56 families were commonly used in diabetes treatment and also among the different parts utilised for treatment, fruits were found to be commonly used [13]. Antiinflammatory potential of medicinal plants is found to be due to the inhibition of 15-lipooxygenase, nitric oxide synthesis, cyclooxygenase- 1 or 2, phospholipase- A2, proinflammatory cytokines [14]. Various scientific reports reveal that the anti-inflammatory potential may be due the presence of flavonoid, alkaloids, triterpenoids, volatile oils etc [15]. Purified compounds like quercetin, allicin, ferulic acid showed excellent anti-inflammatory activity [16]. Various species of *Phoenix* are known to possess anti-microbial, anti-oxidant, antidiabetic, antitumor and hepatoprotective activities [5]. Hence in this study, the *in vitro* antioxidant activity, anti-inflammatory and antidiabetic activity of ethanolic extract of *Phoenix pusilla* root was demonstrated using various invitro methods. Standard drugs are used to compare the activity of the extract.

II. Materials And Methods

Sample Preparation

Roots of PP were collected, authenticated, dried and ethanol extract (PPE) was prepared using Soxhlet apparatus and following the method as given by Jiji *et al*, 2016 [17].

Total antioxidant activity and Free radical Scavenging Assays

The total antioxidant and radical scavenging activity of the ethanolic extract of *Phoenix pusilla* root was determined by standard protocol. *In vitro* antioxidant and radical scavenging activities such as DPPH assay, Hydrogen peroxide activity, Nitric oxide scavenging assay, Ferric reducing power assay, Deoxyribose non-site specific hydroxyl radical scavenging activity, Superoxide radical, ABTS assay and estimation of lipid peroxidation using egg yolk, β carotene linoleic acid assaywere carried out based on the protocol followed by Suganya *et al.,* 2017a, 2017b, 2017c [18,19,20].

α -Amylase inhibitory assay

200 µl of root extract and positive standard (acarbose) of concentration ranging from 250-1250µg/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 minutes. Then 200 µl of 1% starch solution was added, incubated for 3 minutes. Finally the reaction was stopped by adding 3,5-dinitrosalicylic acid (DNSA), kept in boiling water bath for 10 minutes, cooled and the absorbance was measured at 540nm [21]. Control was also treated similarly without the extract. The % α-amylase inhibition and IC50 value were calculated.

%αamylase inhibition=100×Abs100%control−AbsSampleAbs100%Control

α –Glucosidase inhibitory assay

20 µl of different concentration ranging from 250-1250µg/ml of PPE and Standard drug Acarbose were taken and same volume of 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 all the tubes and incubated at 37°C for 20minutes 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 [22].

Anti-inflammatory activity

HRBC (Human red blood cell) membrane stabilization

Blood was collected from healthy volunteers, mixed with equal volume of Alsever's solution and the HRBC suspension was made. To 1ml of suspension equal volume of extract (200- 1000 µg/ml) was added, incubated and centrifuged. Diclofenac sodium (Positive standard) and the control (without extract) was also treated similarly and simultaneously. The haemoglobin content was estimated spectrometrically at 560nm [10]. The percentage protection was calculated

(Abs of blank -Abs of extract) / Abs of control x 100.

Proteinase inhibitory activity

2ml of trypsin, 1ml of Tris HCl buffer, 1ml of different concentration of extract and standard was incubated at 37° C for 5 min. Then 1ml of casein was added (incubated for 20 minutes) and finally the 2ml perchloric acid was added as an arresting agent. The obtained cloudy suspension was centrifuged the absorbance of the supernatant was measured at 210 nm [23]. The percentage inhibition of proteinase inhibitory activity was calculated.

Percentage inhibition = (Abs control –Abs sample) X 100/ Abs control.

Statistical analysis

All the experiments were carried out as triplicates, the data were analysed and expressed as mean ±SEM. P significance value less than 0.05 was considered significant [24].

III. Results And Discussion

Various parts of the plant have been described by various ancient Ayurvadacharyas for the treatment of diseases. In Kerala (India), the fruits of *Pheonix pusilla* are used as Parushaka. Charaka demonstrated and prescribed the use of fruits alone or a decoction of the leaves, roots and bark to treat fever, cough, diseases of the spleen, alcoholism and rheumatism. The fruit is small, fleshy and sweet with flavour like chestnuts that possess laxative, cardiotoinc, aphrodisiac, carminative, purgative and roborant properties. The fruit is also used for hyperdipsia, burning sensation, fever, consumption (a wasting disease especially pulmonary tuberculosis), cardiac debility, seminal weakness, gasteropathy and general debility. Sushruta prescribed fruits to have cooling and appetizing effects and be used as astringent for treating haemoptysis. In folk-medicine the root-bark is used internally and externally in rheumatism. A refreshing summer drink, well known as Sharbat-e-phaalsaa (Unani squash) is used as a cardiac tonic and appetizer. Leaves were found to be effective against pustular eruptions [25, 26].

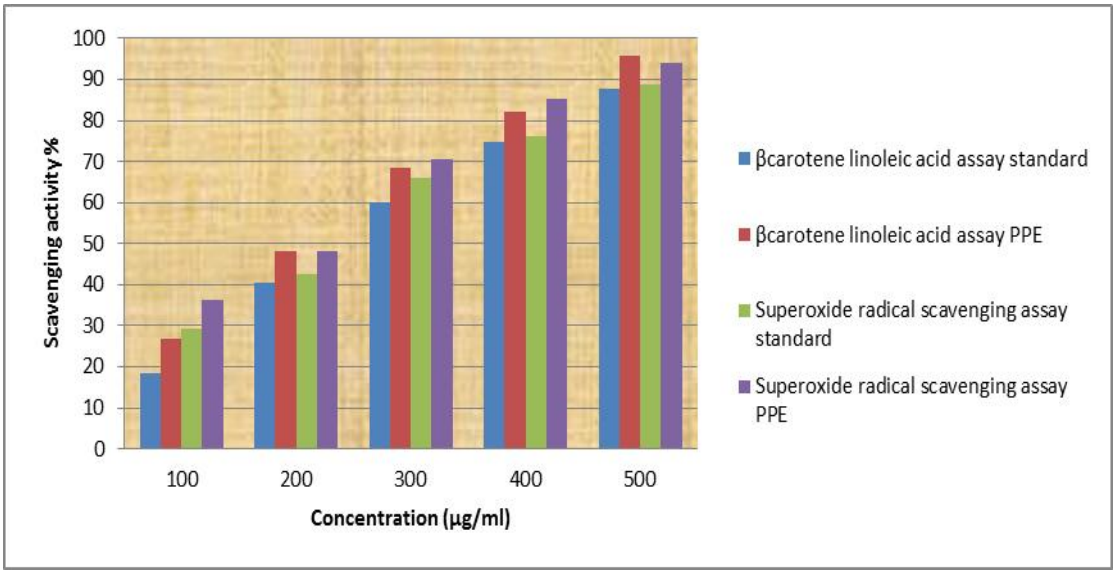

Figure 1: β carotene and SO Radical Scavenging Activity of *Phoenix pusilla* root Extract

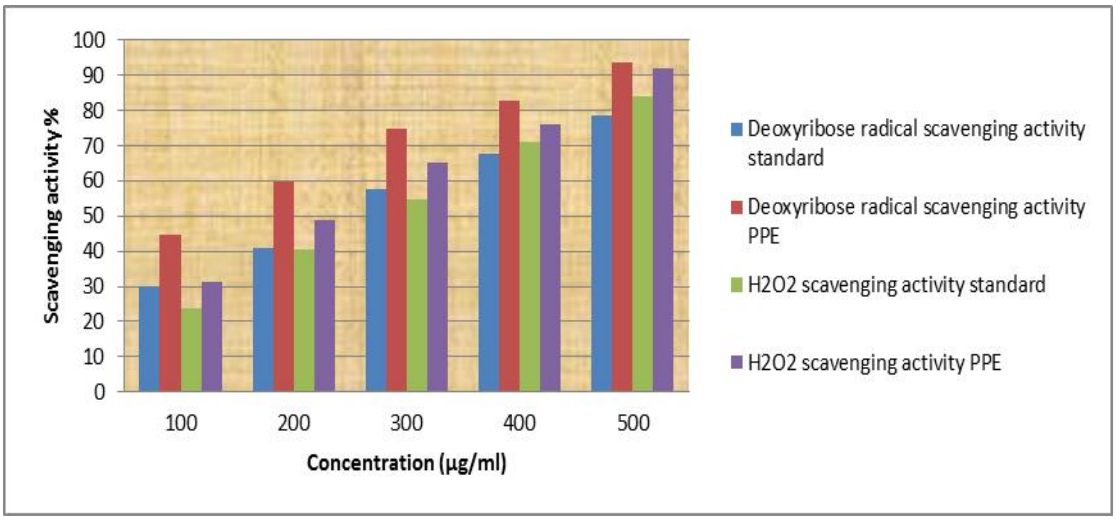

Figure 2: Deoxy Ribose and H_2O_2 Radical Scavenging Activity of *Phoenix pusilla* root Extract

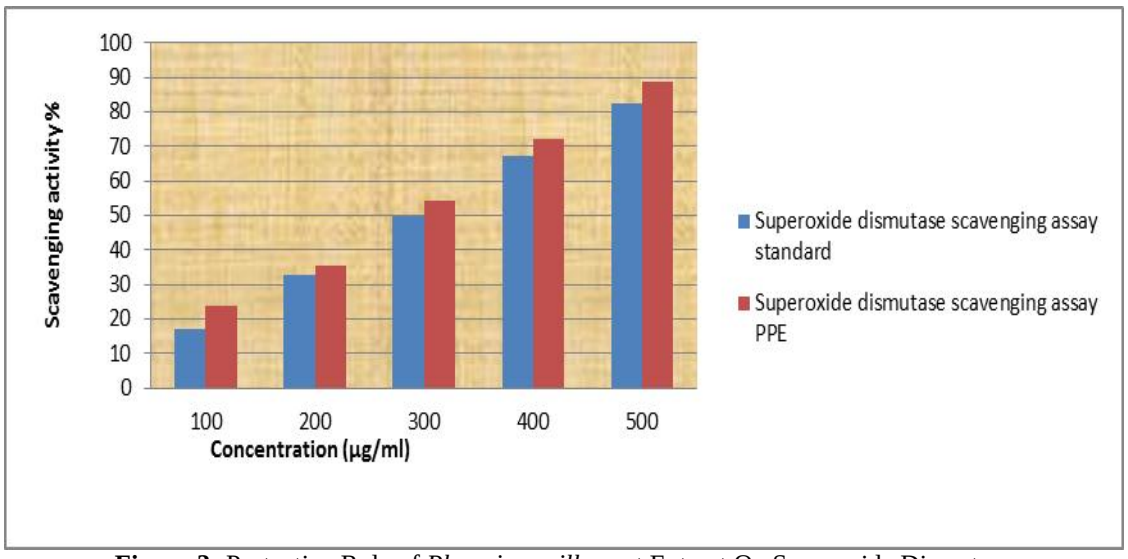

Figure 3: Protective Role of *Phoenix pusilla* root Extract On Superoxide Dismutase

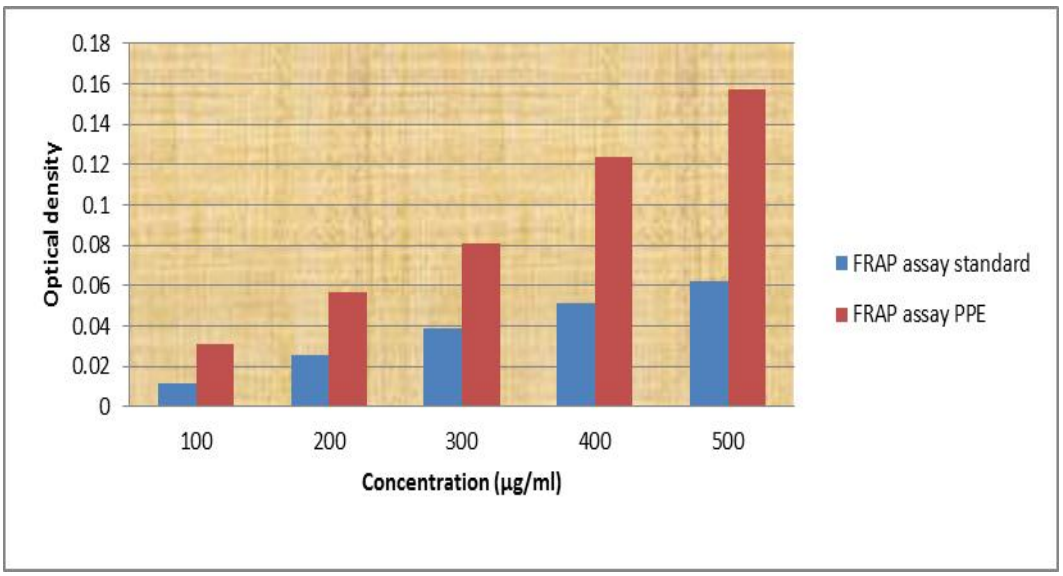

Figure 4: FRAP Radical Scavenging Activity of *Phoenix pusilla* root Extract

Among the antioxidant assays analysed it was identified that β- carotene linoleic acid assay showed highest percentage of antioxidant activity (95.85%) followed superoxide radical scavenging (94.15%), deoxyribose radical scavenging (93.57%), H2O2 scavenging assay (91.82%) and SOD (88.93%) (Figure: 1 to Figure 3). Extract of PP also showed effective antioxidant property by reducing ferric ions (Figure: 4) . In almost all the assay method, the PPE showed effective antioxidant and radical scavenging activity when compared with the standards used. Saha *et al*, 2017 reported that among the methanol, ethanol and acetone extracts of *Phoenix sylvestris*, the acetone extract showed strong inhibitory effect towards oxidants [27]. In Vivo study of ethanol unripen *Phoenix pusilla* fruit extract also showed effective antioxidant [28]. Similarly the study with fruit extract of *Phoenix dactylifera* also revealed the effective free radical scavenging activity and also the effective antioxidant activity was due to the presence of flavanoids [29].

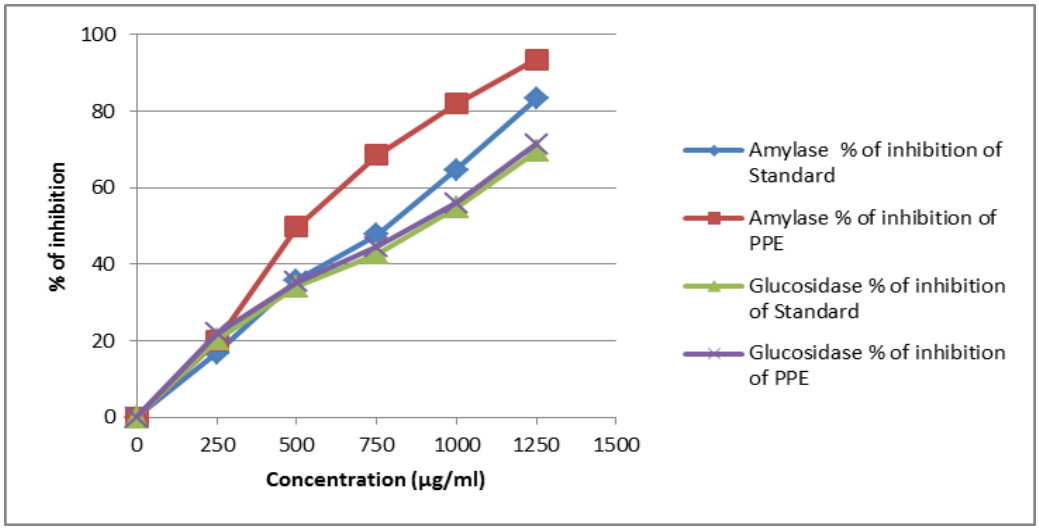

Figure 5: Antidiabetic activity of *Phoenix pusilla* root Extract

Table 1: IC50 values For Inhibition of α Amylase and α Glucosidase

Enzyme	IC 50 Value	
	Standard Acarbose	PPE
αAmylase	755.221	572.720
αGlucosidase	869.459	836.330

Alpha-amylase inhibitory activity showed that the % of enzyme inhibition (Figure 5) was concentration dependent and it was high (93.54%) at the tested higher concentration of 1250 µg/ml. The inhibition by standard drug for the same concentration revealed that PP root ethanol extract have effective inhibitory activity. IC50 value of extract was 572.720 µg/ml (Table: 1). In alpha-glucosidase inhibitory activity, the inhibitory values of the extract (71.34%) was very close upon comparison with the positive standard (69.54%) but slightly stronger than the standard. On comparing both the enzymes inhibitory activity, the PPE showed effective inhibition than the positive standard. Inhibitory property may be due to the presence of saponins, tannins, flavanoids and other phytochemicals reported in the ethanol extract [30]. Unripen fruits of *Phoenix pusilla* showed effective glucosidase inhibition than amylase inhibition which was seen reverse in roots [31]. *Phoenix sylvestris* fruits extact showed IC50 value of 5.0 µg/ml (α-amylase) and 9.0 µg/ml (α-glucosidase) which was very high inhibitory activity than the values obtained for PPE root and unripe fruit of *Phoenix pusilla* [32].

Table 2: Antiinflammatory Activity of *Phoenix pusilla* Ehanolic Extract By Proteinase Inhibitory Method

Concentration µg/ml	% of inhibition of Standard	% of inhibition of PPE
200	16.94	29.73
400	25.99	48.80
600	48.48	61.41
800	63.67	77.63
1000	73.37	88.62
IC 50 Value	657.26	446.695

Table 3: Antiinflammatory Activity of *Phoenix pusilla* Ehanolic Extract By HRBC membrane stabilization Method

Concentration µg/ml	% of inhibition of Standard	% of inhibition of PPE
200	13.69	16.23
400	24.02	25.86
600	30.16	33.19
800	43.14	50.16
1000	61.16	67.51
IC 50 Value	872.94	779.883

Ethanol extract of PP was found to have high proteinase inhibitory activity (88.62%) than the membrane stabilization action (67.51%). But when compared with the diclofenac sodium, the extract showed effective anti-inflammatory action in both the ways (Table 2 and 3). The erythrocyte membrane stabilization is

studied, as it is same as lysosomal membrane. Stabilisation of this membrane thus limits the release of the proteases and the other substances which inturn causes further inflammation. Lysosomal released enzymes are associated with acute or chronic inflammation [33]. Previously, invitro antioxidant and antimicrobial activity of different solvent extracts of *P.Pusilla* root were reported [34]. The other pharmacological studies and characterization of bioactive components are yet to discover regarding this test sample. Till date, the available anti-inflammatory drugs either steroidal or non-steroidal found to cause side effects. Hence, such plant based drugs will be an alternative to overcome the side effects.

IV. Conclusion

The results of this present study showed the effective antioxidant, hypoglycemic and anti-inflammatory activity of the crude PP ethanol extract. So further study has to be carried out to isolate the specific secondary metabolite responsible for the pharmacological activity, assessed properly for the activity and can be proposed as drug for therapeutic use.

Conflict of interest

There is no conflict of interest.

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