

IN VITRO ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF PHOENIX PUSILLA ROOT EXTRACTVIJAYA BHARATHI S^{1*}, ANURADHA V², RUBALAKSHMI G³

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ABSTRACT

Objective: The objective of this study was to analyze the bioactive compound presence, *in vitro* antioxidant and antimicrobial potential of *Phoenix pusilla* root extract.

Methods: Extracts were prepared with ethanol, acetone, and hexane by Soxhlet method. Then, the extracts were checked for phytochemicals presence or absence. *In vitro* antioxidant activity was analyzed by checking its scavenging ability and reducing property. Antibacterial was assayed by well diffusion method and antifungal activity using sabouraud dextrose broth.

Results: The results showed that the ethanolic root extract contains alkaloids, flavonoids, saponin, tannin, and triterpenoids have potent antioxidant activity and inhibitory activity against certain Gram-positive, Gram-negative, and fungi.

Conclusion: The present study revealed the significance of the plant as a drug. Further, *in vivo* study is needed to be used as curative agent for different health illness.

Keywords: *Phoenix pusilla*, Phytochemicals, Ethanol extract, Antioxidant potential.

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INTRODUCTION

Nowadays, secondary metabolites such as alkaloids, glycosides, resins, volatile oils, gums, and tannins of medicinal plants are the chemical compounds which are utilized to develop drugs [1,2]. Dietary intake of antioxidants can improve the protection against free radicals. Antioxidants are substances that can prevent the damage caused by reactive oxygen species [3]. Various studies revealed that the presence of phenolic compounds shows antioxidant activity. Phenolic compounds are found to have reducing activity, metal chelating properties, and a hydrogen donor function [4].

Phoenix pusilla (PP) is a dioecious plant, belongs to Arecaceae family found to propagate through seeds. Arecaceae, the palm family, is a monophyletic group, including 183 genera and 2364 species. At present, most palms are distributed in the tropics with a few species reaching subtropical areas. Arecaceae is composed by five strongly supported subfamilies: Arecoideae, Calamoideae, Ceroxyloideae, Coryphoideae, and Nypoideae with Calamoideae as sister group to the rest of the palms and Nypoideae as the next branch [5]. Various ethnobotanical surveys prove the existence of PP in Sri Lanka and South India [6]. Leaves, fruits, and roots of PP are found to have medicinal importance. Roots are utilized in asthma and bronchitis treatment [7]. Nucleated succession study of PP reveals that it cools the soil as well as found to decrease the radiation and so it was identified as better nurse plant to improve the biodiversity conservation [8]. In this present study, the bioactive compounds and *in vitro* antioxidant, antimicrobial ability of PP root extracts were analyzed. This is the first report on PP root. Hence, far only the unripe fruits of this plant were studied for its therapeutic value.

METHODS**Plant collection**

The roots of PP were collected in January from Veeramangudi village, Thanjavur District. The collected plant material was authenticated by

Prof. Jayaraman, PARC/2017/3396. The collected plant material was washed thoroughly and shade dried. The dried materials were then powdered and used for extraction.

Extraction

50 g of dried PP root powder was extracted with ethanol (PPE), acetone (PPA), and hexane (PPH) by Soxhlet method for 12 h. The extracts were evaporated, concentrated, and stored.

Phytochemical analysis

Extracts were analyzed for bioactive compounds by standard procedure [9].

Antioxidant assay

PPE, PPA, and PPH were assayed for antioxidant potential by *in vitro* methods. All the assays were carried out as triplicate. In all the assay methods, five different concentrations of extracts were analyzed (100, 200, 300, 400, and 500 µg/ml).

2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The DPPH free radical which is purple in color when reduced to hydrazine changes to yellow. The DPPH assay is based on both electron transfer and hydrogen atom transfer reactions [10]. DPPH (0.1 mM) in ethanol was prepared. Then, 10 µl of different extracts of different concentrations were added to 200 µl of DPPH solution, ascorbic acid was used as standard and incubated for 30 min, color intensity was read at 517 nm [11,12].

Scavenging of nitric oxide (NO) radical

At physiological pH, sodium nitroprusside spontaneously generates NO, which interacts with oxygen to produce nitrite ions, which can be estimated by the use of modified Griess-Ilosvay reaction. In the present investigation, Griess-Ilosvay reagent is modified by replacing 1-naphthylamine (5%) with naphthyl ethylene diamine dihydrochloride

(NEDD) (0.1% w/v). Nitrite ions react with Griess reagent, which forms a purple azo dye. If the test sample has scavengers the nitrite formation decreases. Nitrite reduction reflects in the reaction sample and the intensity of color was read at 562 nm.

4 ml of sodium nitroprusside (10 mM), 1 ml of phosphate buffer saline (pH 7.4), and 1 ml of extract of different concentration or standard solution (rutin) were taken and incubated at 25°C for 150 min. After incubation, 0.5 ml of the reaction mixture containing nitrite ion was removed, 1 ml of sulphanic acid reagent was added, mixed well, and allowed to stand for 5 min for completion of diazotization. Then, 1 ml of NEDD was added, mixed, and allowed to stand for 30 min in diffused light. Then, the color intensity was read spectrometrically [13].

Scavenging of 2, 2'-azino-bis (3-ethylbezothiazoline-6-sulfonic acid)diammonium salt (ABTS) radical cation assay

The ABTS generates free radical when it is oxidized with persulfate (blue/green color) which will be reduced by antioxidants in the sample that leads to decolorization [9]. 2 mM ABTS was prepared with 17 mM potassium persulfate and kept in dark before use. The reaction mixture was prepared by pipetting 0.2 ml of various concentrations of the extracts and standard (rutin), 1.0 ml of distilled DMSO, and 0.16 ml of ABTS solution, final volume was made to 1.36 ml. Absorbance was measured spectrophotometrically, after 20 min at 734 nm [13].

Lipid peroxidation inhibition activity

Reactive oxygen species may cause lipid peroxidation which leads to the oxidation of biomolecules and results in cellular damage. In this method, 1 ml of egg lecithin, 0.02 ml of ascorbic acid, 0.02 ml of ferric chloride, and 0.1 ml of different concentration of extract, standard were taken and incubated at 37°C (1 h). Then, 2 ml of 15% trichloroacetic acid and 2 ml of 0.37% thiobarbituric acid were added and boiled for 15 min, cooled, centrifuged, and absorbance of the supernatant was measured at 532 nm [14].

Antibacterial activity

Antibacterial activity was determined using agar well diffusion method. The *in vitro* antibacterial activity of the ethanolic root extract was compared with the standard antibiotic levofloxacin (10 mg/ml). The bacterial culture (Gram-positive, Gram-negative) was swabbed on the Mueller-Hinton Agar. Using well cutter, five wells (for four different concentration of test sample and positive control) were made in those plates at require distance. 50 µl of test sample (25, 50, 75, and 100 mg/ml) were added into the test well, levofloxacin disc was added to the center well. Then, the plates were kept in the incubator

and incubated at 37°C for overnight. After overnight incubation, the plates were checked for inhibition zones and its diameter (mm) was measured [15].

Antifungal screening

In this antifungal screening, the strains used were grown in the sabouraud dextrose broth and the OD was adjusted (600 nm) by dilution for proper growth. Then, the sabouraud dextrose agar plates were prepared by pouring 20 ml of the same. In the plate wells were made, and 50 µl of the different concentration plant extract (ethanol extract), fluconazole (positive control) were added to the respective wells. The plates were then incubated at 27°C for 48 h [16].

RESULTS AND DISCUSSION

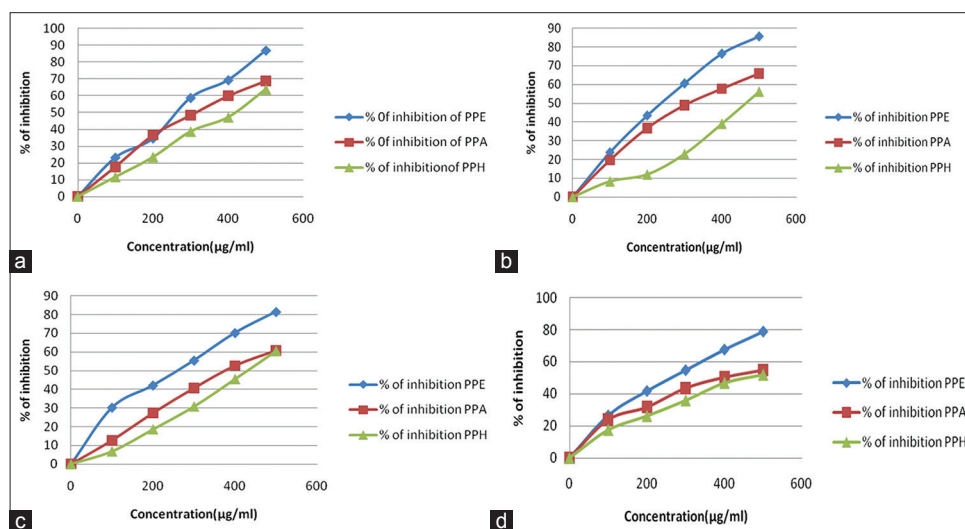
Various root extracts of PP, phytochemical analysis revealed that the ethanolic extract showed the maximum secondary metabolites presence when compared with acetone and hexane extracts. PPE showed the presence of alkaloids, flavonoids, triterpenoids, saponin, tannin, phenolic compounds, glycosides, and primary metabolite like carbohydrates (Table 1).

All the three extracts showed the presence of flavonoids and tannin, whereas the steroids existence was identified only in the hexane extract. Like ethanol extract, PPA showed phenolic compound and saponin presence. Flavonoids are found to have antioxidant, antibacterial,

Table 1: Phytochemical analysis of *Phoenix pusilla* root extracts

S. No.	Compounds	Ethanolic extract	Acetone extract	Hexane extract
1	Alkaloid	+	-	-
2	Flavonoid	+++	+	+
3	Triterpenoid	+	-	-
4	Phenolic compound	++	+	-
5	Protein	-	-	-
6	Carbohydrates	+++	-	-
7	Saponin	+++	++	-
8	Steroids	-	-	+++
9	Glycosides	+	-	-
10	Amino acid	-	-	-
11	Tannin	+	+	+
12	Oil	-	-	-
13	Gums and mucilage	-	-	-
14	Chlorogenic compound	-	-	-

+: Less, ++: Moderate, +++: High, -: Negative



Graph 1: (a-d) Antioxidant potential of different extracts of *Phoenix pusilla* root (a. DPPH method, b. ABTS method, c. Nitric oxide method, d. Lipid peroxidation inhibition method)

antiviral, anticancer, and anti-inflammatory activity [17,18]. Apart from having activities similar to flavonoids, tannin also utilized for immediate relief of sore throats, diarrhea, dysentery, hemorrhaging, fatigue, and skin ulcers [19]. Tannin is used as an ingredient in most of the Ayurveda, Siddha, and Unani formulations such as Churna, Rasa, Bhasma, and Khurs [20]. Saponin has commercial application in food, cosmetics, agriculture, and pharmaceutical industries [21]. *In vitro* antioxidant assay showed that the ethanol extract possesses potential scavenging property than the other two extracts (Graph 1). In DPPH assay, PPE showed 86.9 ± 6.8% which is comparatively higher than PPA and PPH. Similarly, the reducing power of iron and scavenging activity of NO, ABTS, lipid peroxide was high in PPE. IC50 calculation represents that the concentration of extract needed for 50% inhibition was less for ABTS (260 µg/ml), NO scavenging assay (272 µg/ml), followed by DPPH (277.56 µg/ml), and LPI (284 µg/ml) assay. Ascorbic acid was used as a standard in DPPH and LPI method, whereas rutin as standard in ABTS and NO scavenging method. Non-enzymatic antioxidants such as flavonoids, phenolics, tannins, and carotenoids presence may be the reason for antioxidant activity [22,23]. Flavonoids and tannin presence in all the three extracts might be the ground for the activity against the free radicals. The existing scientific proof reveals that the tannins can be

used as a food additive [24]. Hence, the future study has to focus on isolating the tannin, analyzing its free radical scavenging potential and can be used as food additive.

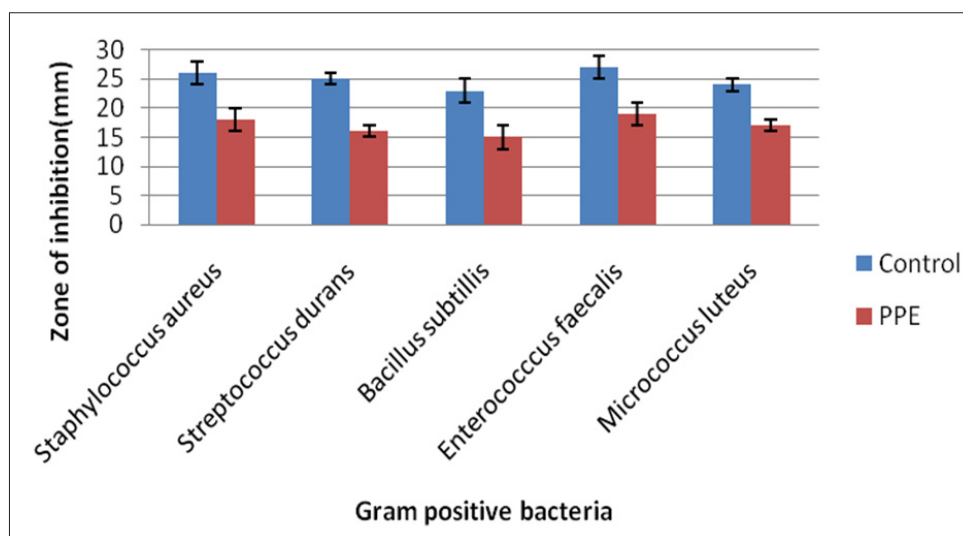
Among the 5 Gram-positive bacteria assayed, *Enterococcus faecalis* showed maximum zone of inhibition with 19 mm (Graph 2), whereas Gram-negative bacteria such as *Escherichia coli*, *Serratia marcescens* showed the same value of inhibition at the concentration of 100 mg/ml (Graph 3). Antifungal assay showed that the *Candida albicans* (16 mm) was more susceptible to ethanol extract of PP (Graph 4).

In this antimicrobial assay, the zone of inhibition was found to dose-dependent. The compounds responsible for antimicrobial activity have to be isolated which may reduce the doses of extract used as antimicrobial agent [16].

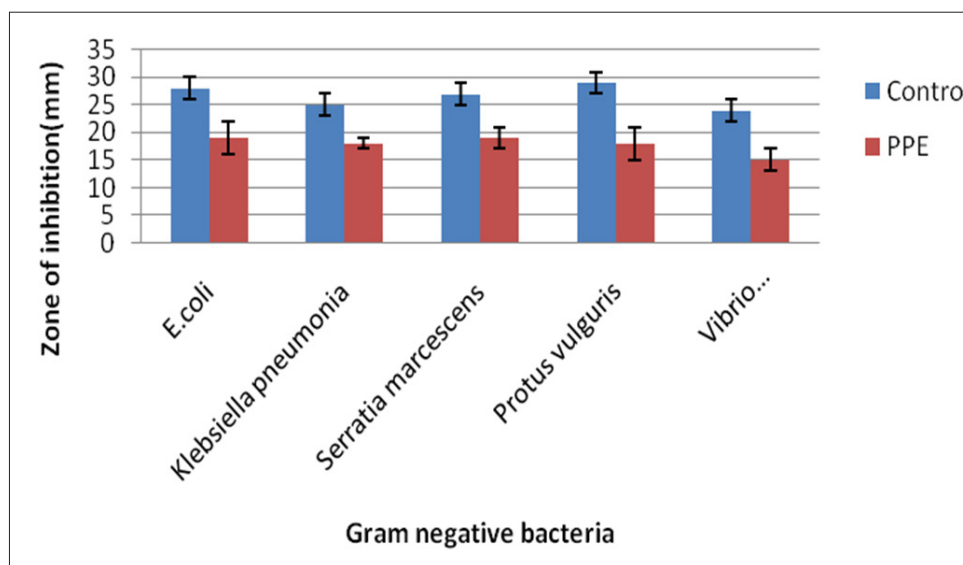
Statistical analysis by t test (n=3) using mean±SEM revealed that the p < 0.05, so the obtained result was considered as statistically significant.

CONCLUSION

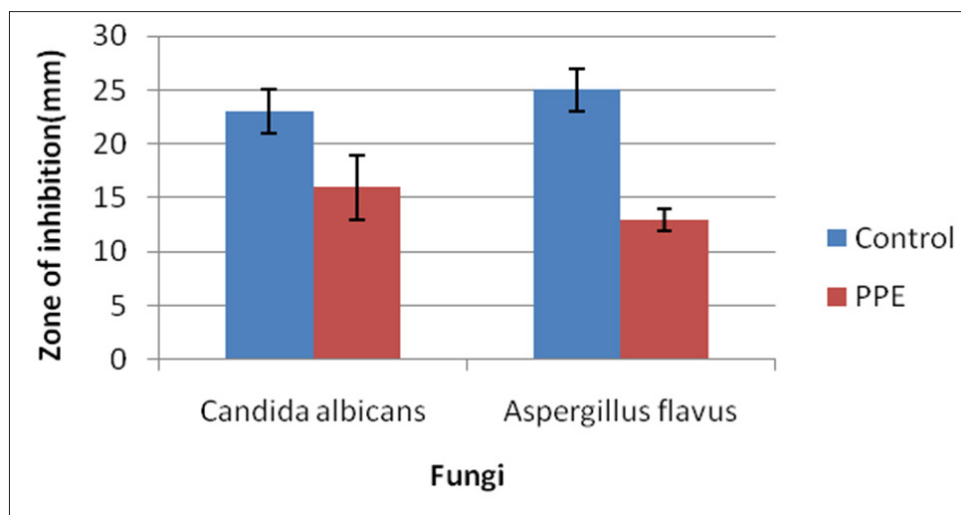
Present finding reveals that this plant is enriched with therapeutic value. Since PP root extracts exhibited better antioxidant, antimicrobial



Graph 2: Zone of inhibition of *Phoenix pusilla* ethanol root extract against Gram-positive bacteria



Graph 3: Zone of inhibition of *Phoenix pusilla* ethanol root extract against Gram-negative bacteria



Graph 4: Zone of inhibition of *Phoenix pusilla* ethanol root extract against fungi

activity further study is needed to explore its importance in various diseases treatment and raise the medicinal importance of this plant to next level.

AUTHORS CONTRIBUTION

All the authors contributed equally.

CONFLICT OF INTEREST

There is no conflict of interest.

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