

Preliminary phytochemical, microscopic analysis and metabolite profiling of *Phoenix pusilla* root

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ARTICLE INFO

Received on: 08/04/2019
Accepted on: 30/06/2019
Available online: 05/03/2020

Key words:

GC-MS, *Phoenix pusilla* root, phytochemical analysis, microscopic study, shelf-life.

ABSTRACT

Palms are considered as familiar strangers. Various studies reported that the genus *Phoenix* was mostly cultivated, traditionally used plants as medicine, and also as an ornamental plant. In this present study, preliminary phytochemical analysis, the study of microscopical structures and the chemical constituents of *Phoenix pusilla* root was evaluated. Transverse Section of *P. pusilla* root was studied by sectioning the two different thickness roots and also powder microscopy was carried out. Non-polar, volatile constituents of crude ethanolic extract of root were analyzed by GC-MS. Primary and secondary metabolites quantification revealed that the carbohydrate content and total phenolics concentration were higher than the other metabolites. The physicochemical analysis showed the moisture content was 3.78 ± 0.54 , the ash content value was 2.46 ± 0.95 , and the crude fiber content was higher than protein, fat content. Microscopy study showed the presence of unique tracheids. Metabolite profiling revealed the presence of 96 different constituents and more than 10 found to have biological importance. Pharmacognostic study is an important step of research which reveals about shelf-life of the drug, adulterations, etc.

INTRODUCTION

Phoenix puscilla (PP) commonly known as dwarf date palm was found to grow in lowland areas and ridges (Sankar and Shoba, 2014). All the parts of this plant found to have folklore medicinal value. Ceylon Date Palm is used in the treatment of bladder stones, piles, fevers, dysentery, rectal prolapses, pies, cough, and urinary tract infections. Porridge made from pith is used in the treatment of gonorrhoea, gleet, and fractures and is an antidote for snake bites. Seeds are used as vermifuge with cow milk. Pith is used in the treatment of gonorrhoea, gleet, and fractures, which also acts as an antidote for snake bite. Roots are antibacterial and used as a disposable toothbrush (<http://www.asia-medicinalplants.info/phoenix-pusilla-gaertn/>). Roots of PP are fibrous which anchors the palm strongly to the soil. The leaves are woven into ornamental baskets, sleeping mats, and pouches. The split petioles are used for making baskets. An edible starch is obtained from the stems in times of food shortage.

The leaves are used locally for making baskets, mats, etc. An edible starch is obtained from the trunk. It is used in times of scarcity. The leaves are woven into ornamental baskets, sleeping mats, and pouches. The split petioles are used for making baskets (Sudharsan, 2004).

Drug standardization studies have become a vital part of pharmacognostic studies because this study gives the exact information (macroscopic, microscopic, physiochemical parameters, etc.) of plants. The crude drug has to be evaluated to analyze the biochemical changes, any defect raised during storage and the adulteration. The microscopic analysis utilizes various techniques to check the presence or absence of hairs (trichomes), oil glands, canals, particular cell types, seed or pollen morphology, and vascular traces (Lachumy *et al.*, 2012). Microscopic evaluation includes qualitative and quantitative observations. Qualitative evaluation comprises transverse and longitudinal section of plant parts with or without staining. Quantitative microscopy gives information about the vein number, stomatal number, palisade ratio, etc. Standardization of drugs is an important study which reveals the adulteration and contamination in the herbal preparation (Alamgir, 2017). Importance of the microscopic study of plants

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is to identify and differentiate the plants that resemble the same (Vidya Shriram Patil *et al.*, 2016).

GC–MS is the technique used to identify the non-polar, volatile compounds (Nishaa *et al.*, 2013). GC–MS is utilized in many fields of research including food science, environmental analysis, explosives investigation, unknown sample identification, etc. (Hussain and Maqbool, 2014). Ethnobotanical survey of various places shows that the PP have habitat commonly in southern India (Eswari *et al.*, 2017; Rekha *et al.*, 2013). Xenic and metaxenic study of PP showed that the pollen of PP fertilizes date palm as like pollen of date palm and significant difference were seen in the fruits and seeds developed (Sudharsan *et al.*, 2010). Fruits and roots of *P. puscilla* were reported to have pharmacological application in traditional medicine. Hence, in this present study, microscopical and metabolite analysis is done to authenticate the structural features and to identify the active constituents present in the extract.

MATERIALS AND METHODS

Sample preparation

Roots of PP were collected, authenticated, dried, and ethanol extract (PPE) was prepared using Soxhlet apparatus (Jiji *et al.*, 2016). Briefly, 50 g of dried *Phoenix puscilla* (PP) root powder was extracted with ethanol (PPE) by Soxhlet method for 12 hours. The extracts were evaporated, concentrated, and stored.

Organoleptic evaluation

Sensory screenable parameters like color, odor, taste, size, and shape of the PP root sample were evaluated (Mondal *et al.*, 2013).

Physicochemical evaluations

Proximate analysis (Moisture content, ash content, crude protein fiber, and fat content) and extractive value were done using the method of Association of official analytical chemist (AOAC Guidelines, 2016). For the determination of extractive value, 1 g of the root powder is soaked in 100 ml of each solvent, namely, Alcohol, water, and ether and left for 24 hours with frequent shaking. Then, the suspension is filtered and the filtrate is evaporated, dried, and weighed.

Determination of physicochemical characteristics

Physical characteristics of the test sample were determined which includes bulk density, tapped density, compressibility index, pH, and Hausner ratio that indicate the flow properties of the drug (Bahuguna *et al.*, 2014). The presence of bitterness value, swelling index, and foaming index was done. The pharmacological activity of the drug has been applied to evaluate and standardize the drug (WHO Guidelines; Tarunkumar, 2013).

Heavy metal analysis

Samples were tested for the presence of heavy metals like arsenic, cadmium, chromium, lead, and mercury (Banares, 2015; Gilreath and Esmarch, 1954).

Fluorescence analysis

The root dry powder was treated with various solvents (Table 6) and was then observed under UV/visible chamber (Kumar *et al.*, 2013).

Quantitative determination of primary metabolites and secondary metabolites

The quantitative determination of primary metabolites, such as carbohydrates, protein, total chlorophyll content, and lipid content was evaluated by various methods (Deshmukh *et al.*, 2018; Laveena *et al.*, 2017) and secondary metabolites such as total phenolics, tannins, and total flavonoid were also determined (Shibu and Dhanam, 2015).

Transverse section of roots

Roots of two different thicknesses were studied. Roots were collected, dried, and fixed in FAA (Formalin-5 ml + acetic acid-5 ml+ 70% ethanol). After 24 hours of fixing, the specimens were dehydrated with graded series of butanol and filtered by adding paraffin wax. Then, the specimen was sectioned with a microtome to the thickness of 10–12 μ m. The sections were stained with toluidine blue, Safranin, and fast green (Azwanida, 2015).

Powdered microscopy

Shade-dried roots were finely powdered, a little amount was placed on a slide, and three drops of chloral hydrate were added, then covered with a cover slip and observed under a microscope (Akbar *et al.*, 2014).

GC–MS analysis

One milliliter of ethanolic extract of the sample was subjected to GC–MS to analyze the chemical composition. GC–MS was carried out using normal phase C18 silica column by maintaining the oven temperature at 60°, injection temperature at 250°, and the pressure maintained at 56.7 kPa. Mass spectrum was interpreted using National Institute Standard and Technology library (Patel *et al.*, 2017).

RESULTS AND DISCUSSION

Therapeutic efficacy of medicinal plants depends upon the quality and quantity of chemical constituents. Pharmacognostic studies ensure plant identity, lays down standardization parameters which will help and prevent adulterations (Chanda, 2014). Among the primary metabolites, carbohydrates were high than the remaining and the lipid concentration was found to be 0.43 ± 0.08 mg/g dw which was very low than the other primary phytoconstituents (Table 1).

Table 1. Quantification of primary metabolites.

S. No	Primary metabolites	Sample weight (mg/g dw)
1	Carbohydrates	14.08 \pm 0.21
2	Chlorophyll	3.85 \pm 0.11
3	Proteins	2.03 \pm 0.13
4	Lipids	0.43 \pm 0.08

The values are mean of Triplicate.

Similarly, secondary metabolites quantification showed that the total flavonoids were 8.21 ± 0.22 mg/g dw, tannins concentration was 6.74 ± 0.24 mg/g dw, and total phenolics amount was 28.54 ± 0.16 mg/g dw (Table 2). Various studies reported that the phenol compounds have potent antifungal, antiviral, antibacterial activity, anti-aging, skin renewal property, etc. (Dzialo *et al.*, 2016). For isolation of pure compounds, secondary metabolites quantification is important (Geetha and Geetha, 2014).

Organoleptic evaluation revealed that the root was light brown in color, odorless but has a bitter taste (Table 3). The bitter taste may be due to the presence of tannins. Tannins are polyphenols found to have astringent, bitterness property. Tannins are also used in tanning, dyeing, photography, and in refining beer, wine (Ashok, 2012). It was also found that the root was about 2.4–5.3 cm long and it was rod-shaped. Proximate analysis showed that the moisture content was low, which indicated the long shelf-life nature of the drug. Stability of the drugs depends on the moisture content (Chandel, 2011).

Fiber content was high than the protein and the fat content (Table 4). Total ash content implies that the inorganic content was less. Usually, the total ash content determines the foreign substance and the other adulterations (Ibrahim *et al.*,

2012). Extractive values indicated that the more soluble contents were in ethanol with $15.26 \pm 0.12\%$, which was followed by ether (4.85 ± 0.19), and water (3.15 ± 0.03) stands last (Table 5). Higher alcohol soluble index shows that the alcohol would be the better extractive solvent (Ajazuddin and Saraf, 2010).

Bulk density of the test sample was 0.013 g/ml, whereas tapped density was found to be 0.783 g/ml. The compressibility index, Hausner ratio, and pH obtained were 18.033%, 2.803, and 6, respectively (Table 6). Foaming index was not found and bitterness value was 0.135 (Table 6). The heavy metals were not present in the test sample which indicates that the sample was in pure form which can be used for further analysis (Table 7).

Fluorescent analysis showed varied colors, such as light brown, dark brown, yellow, pink, violet, etc., on treating with different solvents. Some substances fluoresce in normal light and others in UV light (Table 8). There are other substances which can be converted to fluoresce (Gayathri and Kiruba, 2015). Authentication of plant materials includes macroscopic, microscopic, chemical analysis, and DNA fingerprinting. Due to recent advancements in technology, the microscopical analysis has become a cheap, easy, and convenient method (Lachumy *et al.*, 2012). Thin root has a thick stellar cylinder surrounded by a wide cortical cylinder. Cortical part is ridged and furrowed deeply to varying degrees. The cortical cylinder includes an outer thick continuous layer of compact small thick-walled cells. Thinner cortex has wide, radially elongated air chambers divided by thin seriate partition filaments. A compact prominent fiber bundle was seen in the partition filaments. Central stellar cylinder was 15 mm in diameter. It consists of an endodermal layer of small spindle-shaped cells with the inner tangential walls and radial walls. Stellar cylinder

Table 2. Quantification of secondary metabolites.

S. No	Secondary metabolites	Sample weight (mg/g dw)
1	Total phenolics	28.54 ± 0.16
2	Tannins	6.74 ± 0.24
3	Total flavonoids	8.21 ± 0.22

The values are mean of Triplicate.

Table 3. Organoleptic features.

S. No	Organoleptic features	Sample
1	Color	Light brown
2	Odor	Odorless
3	Taste	Bitter
4	Size	2.4-5.3 cm long
5	Shape	Rod

Table 4. Proximate composition.

S. No	Composition	Sample % dry weight
1	Moisture content	3.78 ± 0.54
2	Ash content	2.46 ± 0.95
3	Crude protein	1.34 ± 0.42
4	Crude fiber	4.08 ± 0.72
5	Fat content	1.11 ± 0.30

The values are mean of Triplicate.

Table 5. Extractive values.

S. No	Parameters	Sample
1	Alcohol soluble extractive %	15.26 ± 0.12
2	Water soluble extractive %	3.15 ± 0.03
3	Ether soluble extractive %	4.85 ± 0.19

The values are mean of Triplicate.

Table 6. Physical characteristics.

Physical characteristics	Sample
Bulk density (g/ml)	0.013 ± 0.061
Tapped density (g/ml)	0.783 ± 0.132
Compressibility index (%)	18.033 ± 0.156
Hausner ratio	2.803 ± 0.001
pH range	6.0

The values are mean of Triplicate except pH.

Table 7. Pharmacological evaluation.

Parameters	Sample
Bitterness value/g	0.135 ± 0.008
Foaming index	Not found

The values are mean of Triplicate.

Table 8. Heavy metal determination.

Heavy metals	Test sample
Arsenic	Negative
Cadmium	Negative
Chromium	Negative
Lead	Negative
Mercury	Negative

consists of densely distributed wide circular, thin-walled vessels, and the ground tissue with lignified cells. The metaxylem and protoxylem vessels are located along the periphery of the vascular cylinder (Fig. 1). The xylem was within the protoxylem. Phloem was seen in between the metaxylem elements.

Thick root was up to 2.5 mm in diameter. It has a thick continuous cylinder of the sclerenchymatous cortex. The cortical cylinder is followed by the inner cortex. Major part of the cortex was sclerenchymatous with numerous polygonal air-chambers formed by one or two layers of partition filaments. Dark stained fiber bundles were randomly seen in the partition filament. Fiber bundles consist of compact aggregation of angular thick walled lignified fibers (Fig. 2). The central stele has a typical monocot root structure. On the outer zone of the stele, numerous solitary vessels were seen which were wide with the inner region and narrow towards the endodermal cylinder. The vessels were either circular or ovate in outline and measuring about 15–20mm in diameter. Towards the outer zone, smaller metaxylem with narrow protoxylem elements was seen. Phloem elements were wide, compact, and thin walled.

Very long uniformly narrow unique types of tracheids were occasionally seen. Tracheids were 10 μ m thick. Unique

types of tracheid bundles were found to be long, blunt at the ends, and unique in thickness (Fig. 3). Tracheids have dense multiseriate and elliptic horizontal bordered pits (Fig. 4). Tracheids were 500 μ m long and 60 μ m wide. Also found abundant libriform fibers which were either narrow or wide. Narrow fibers were thick walled, 550 μ m long, and 10 μ m wide. Wide fibers were prominent, wide lumened, and thin walled. Wide fiber was 1.1 μ m long and 20 μ m wide. Vessels were either short or barrel-shaped or long and cylindrical. Vessels have very dense multiseriate elliptical opposite and lateral wall bordered pits (Fig. 5). Vessel elements have circular, wide horizontal end wall perforations. Parenchymal cells seen were rectangular or cylindrical in shape.

GC–MS analysis of ethanolic roots (PP) extract results revealed the presence of 96 compounds (Table 9 and Fig. 6). The major constituents belong to fatty acids, sugar, sugar alcohol, phenolics, and organic acids. The compound with high molecular weight was SILIKONFETT SE30; similarly, the compound which showed high retention time (30.775) was dioctyl phthalate. Among the 96 compounds, Xanthosine showed the highest peak area (15.79%). Xanthosine was found to have wound healing, antibacterial property, and also have application

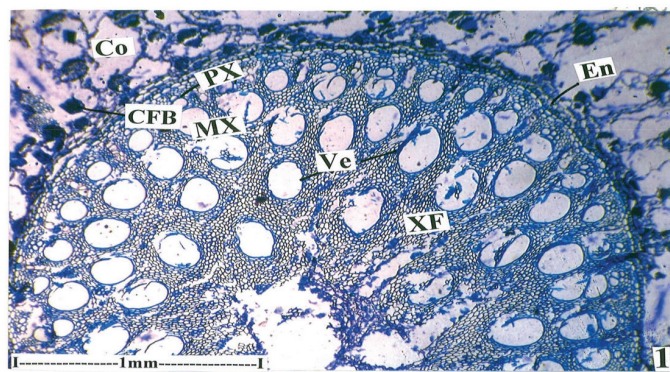


Figure 1. T.S of thin root showing stellar vascular elements. Co = Cortex, CFB = Corticle fibre bundle, PX = Protoxylem, MX = Metaxylem, Ve = Vessels, En = Endodermis, XF = Xylem fiber.

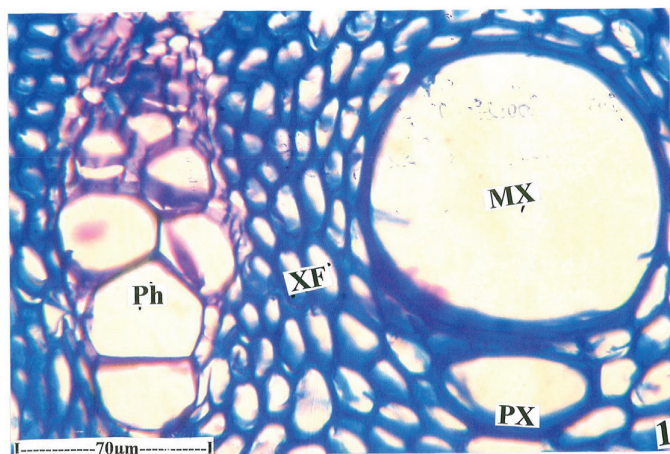


Figure 2. Thick root showing metaxylem and primary xylem in radial arrangement. Ph = Phloem, XF = Xylem fiber, MX = Metaxylem, PX = Protoxylem.

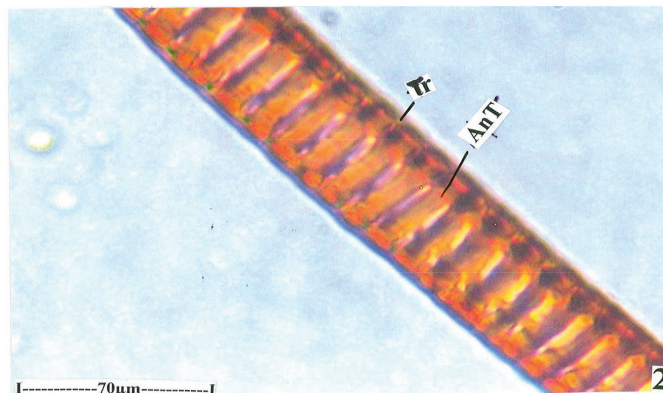


Figure 3. Tracheids showing close annular lateral wall thickening. Tr = Tracheids, AnT = Annular Tracheid.

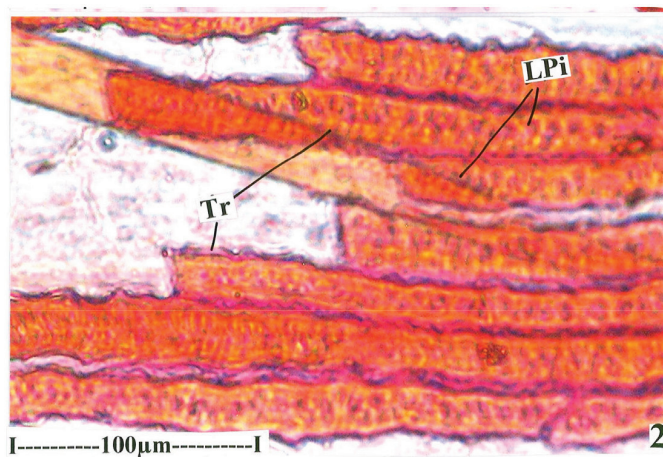


Figure 4. Tracheids showing lateral wall pits. LPi = Lateral wall pits, Tr = Tracheids.

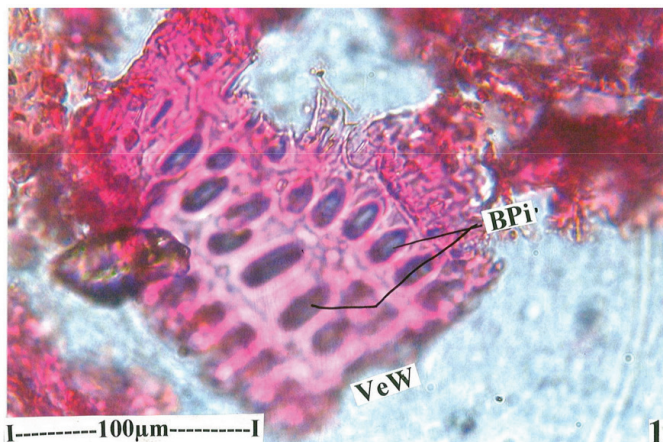


Figure 5. Powder microscopy showing a broken lateral wall of a vessel element. Bpi = Bordered pits, VeW = Vessel wall.

Table 9. Fluorescence analysis.

Reagents	Sample Visible	Sample UV
Powder	Light brown	Dark brown
Powder + pet ether	Pink	Black
Powder + ethyl acetate	Pink	Dark brown
Powder + ethyl acetate: HCl (1:1)	Brown	Dark brown
Powder + methanol	Pink	Reddish orange
Powder + chloroform	Light red	Black
Powder + acetone	Pink	Light green
Powder + 50 % H ₂ SO ₄	Brown	Black
Powder + 50 % HNO ₃	Red	Red
Powder + 50 % HCl	Yellow	Black
Powder + 10 % NaOH	Violet	Black

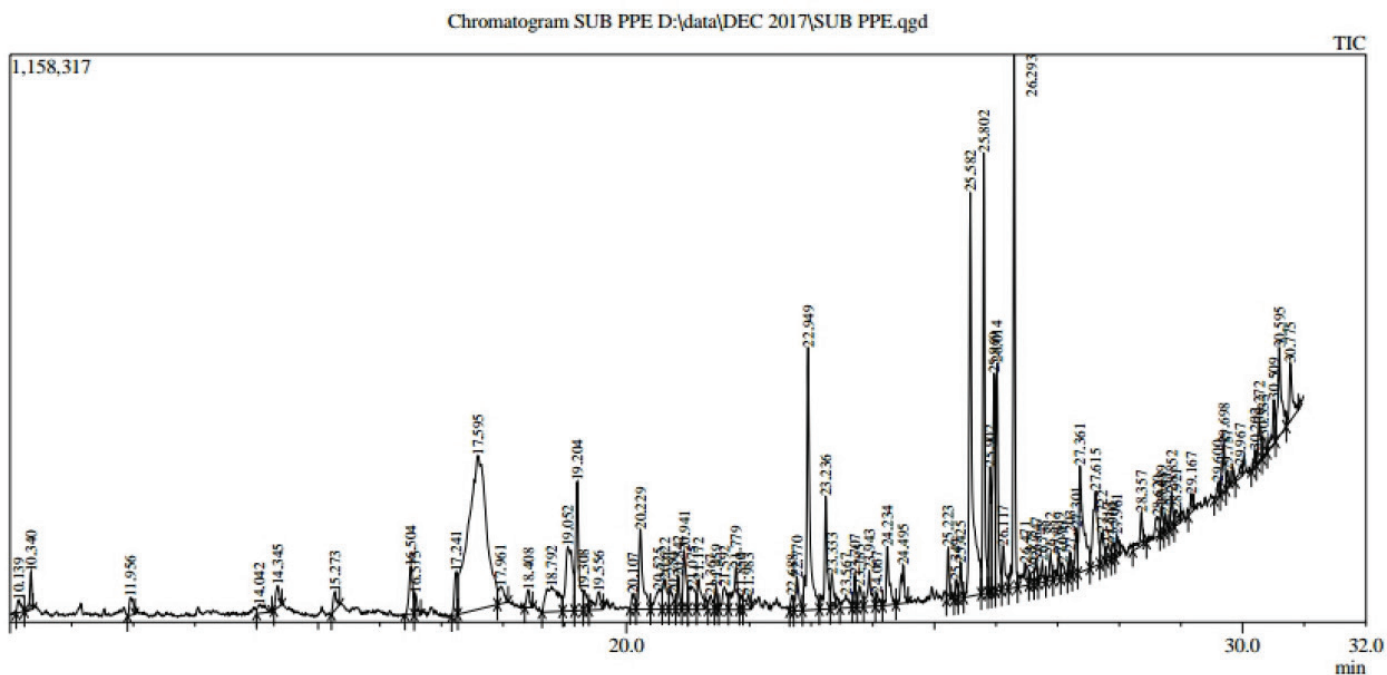


Figure 6. GC-MS compounds identification of *P. pusilla*.

in stem cell research (Choudhary, 2014). The other compounds having comparatively high peak area were naphthalene (5.39%), 9,9-Perhydrobiphenanthryl (4.75%), 4-((1e)-3-hydroxy-1-propenyl)-2-methoxyphenol (4.54%), 3,6,9-triethyl-3,6,9-trimethyl tetracyclo[6.1.0.0~2,4~.0~5,7~]nonane (3.12%), and the remaining showed less than 3% of peak area. It was identified that the crude extract of PP showed the presence of biologically important compounds like hexanoic acid, Valproic acid, Nipeocotic A, Diclofenac sodium, IsochiapinB, hexadeconic acid, and few other compounds. The leaf and fruit pulp of *Phoenix sylvestris* and *P.pusilla* were explored through *invitro* studies for various pharmacological properties. Fatty acid and esters of fatty acids were reported in ethanolic extract of *P. pusilla* fruit

(Antony Thangadurai and Velavan, 2018). Antinociceptive and neuropharmacological activities of methanolic extract of *P. sylvestris* fruit was reported (Shahib *et al.*, 2015). Similarly, antioxidant and antibacterial activities were also reported (Sharma *et al.*, 2015). Antioxidant and glycosidase inhibitory potential of mesocarp of *P. sylvestris* fruit was also reported (Susmita Das *et al.*, 2016).

Metabolite profiling by GC-MS will be helpful in planning further analysis through *invitro* and *invivo* studies. Thus, the characterization of bioactive compounds by spectral analysis will pave the way to determine the probable role of this unexplored plant parts against several human diseases.

Table 10. Metabolite profile of PP root crude extract.

S. No	RT Time	Compound
1	3.131	4-methylpentanoic acid
2	3.178	3-hydroxybenzylhydrazine
3	3.250	2-methylpyrazine
4	3.417	3-(2-methoxy-ethoxymethoxy)-2-methyl-pentan-1-ol
5	4.083	1-butanol, 2-amino-3-methyl-,
6	4.275	formic acid, pentyl ester
7	4.667	6-oxabicyclo[3.1.0]hexan-3-one
8	5.542	hexanoic acid
9	5.725	aniline-1-(13)c
10	5.792	2,4-dihydroxy-2,5-dimethyl-3(2h)-furan-3-one
11	7.042	Benzenemethanol
12	7.950	3,5-dideutero pyridine-4-carboxylic acid-d1
13	8.050	Valproic acid
14	9.567	4-hydroxycyclohexyl acetate
15	10.142	pyrido[1,2-a]azepine-6,7,8,9-tetracarboxylic acid, 10-(benzoyloxy)-6,7-dihydro-, tetramethyl ester
16	10.342	acetic acid, phenylmethyl ester
17	11.958	5-hydroxymethylfurfural
18	14.042	2,4-dimethylhexan-3-ol
19	14.342	2-methoxy-4-vinylphenol
20	15.275	2,6-dimethoxyphenol
21	16.500	pyrimido[1,2-a]azepine, 2,3,4,6,7,8,9,10-octahydro

CONCLUSION

Pharmacognostic evaluation is important in determining the quality and purity of the drugs. Quantitative parameters of *P. pusilla* root will be helpful in focusing the target metabolite for further medicinal application. The determination of physicochemical parameters revealed the non-toxic nature of the test sample. GC-MS study of *P. pusilla* root showed the existence of pharmacologically active compounds. Among the active compounds, further study on Saponin has to be focused for purification, characterization, and explored for its pharmacological activity through *in-vivo* studies.

CONFLICT OF INTEREST

The authors declare that they have no conflict of Interest

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How to cite this article:

Pradeep A, Bharathi SV, Ali MS. Preliminary phytochemical, microscopic analysis and metabolite profiling of *Phoenix pusilla* root. *J Appl Pharm Sci*, 2020; 10(03):128–134.