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PHARMACOGNOSY OF SOUTH INDIAN DATEPALM – *PHOENIX PUSILLA*

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ABSTRACT

The present investigation was carried out to evaluate the physicochemical parameters of raw sample and phytochemical analysis of fruit and seed methanolic extract of *Phoenix pusilla.* The presence of primary and secondary metabolites was also quantified. The raw powder of fruit contains high moisture when compared to seed powder. The presence of crude protein, lipid and fibre is appreciable with both the samples. The fruit and seed powder showed high extractive value in alcohol showing their high solubility in alcohol. The methanolic extract of fruit showed high content of carbohydrate, pigment and protein whereas the lipid content is high in seed extract.

KEYWORDS: Pharmacognosy, *Phoenix pusilla*, Phytochemicals, Phenolics, Flavanoids

INTRODUCTION

Medicinal plants have been an integral part of life in various regional communities for food and drugs. India has more than 3,000 years of medicinal heritage based on medicinal plants. Medicinal plants are largely used by all divisions of the population either directly as folk medications or indirectly in the preparation of recent pharmaceuticals. Fossil records date human use of plants as medicines at least to the Middle paleolithic age some 60,000 years ago.[1] From that point the development of traditional medical systems incorporating plants as

a means of therapy can be traced back only as far as recorded documents of their likeness. However, the value of these systems is much more than a significant anthropologic or archeologic fact. Medicinal plants continue to provide valuable therapeutic agents, both in modern and in traditional medicine.[2] Traditional medicines are gaining importance and are now being studied to find the scientific basis of their therapeutic actions. Palms "the princess of the plant Kingdom", represents one of the most important plant family with respect to human use. Coconut and palm kernel oils were recognized as health oils in Ayurvedic medicine almost 4000 years ago.[3,4] Genus phoenix is one of the most widely cultivated groups of palms around the world, different parts of this genus are widely used in traditional medicine for the treatment of various disorders which include memory disturbances, fever, inflammation, paralysis, loss of consciousness, nervous disorders.[5] The fruits of some species are used as a detersive and astringent in intestinal troubles, to relieve fever, cystisis, gonorrhea, edema, liver and abdominal troubles and to counteract alcohol intoxication.[6]

Phoenix pusilla Gaertn., (Family: Arecaceae) a multipurpose palm species closely related to the date palm, is commonly known as the small date palm in India, as it only grows to 100 cm tall.[7] 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. Just like the true date palm, Phoenix dactylifera it is dioecious, producing male and female flowers on separate trees. It grows wild in dry areas in India at low elevations. Its flowering season starts in November and runs through January. Clusters of edible orange-red fruits turn into black drupes in the months of July and August.[8] The pulp of the fruit is fleshy, sweet and mealy. The tender part of the palm is often eaten by the poorer people as a meal called kanji. The leaflets are woven into mats and the split petioles into baskets. Brooms were also made out of the leaves of this palm. Its fruit is used in herbal medicines, as it is sweet, sour, cooling and laxative, cardiotonic, aphrodisiac, carminative and roboranto. The fruit is also used for hyperdipsia, burning sensation, fevers, consumption, cardiac debility, seminal weakness, gasteropathy and general debility. Hence, to the possible extent sincere efforts have been made to collect relevant literature of the study. After thorough reviewing of all possible sources, it was observed that very few studies have been conducted earlier on certain dimensions of the present study.

MATERIALS AND METHODS

PREPARATION OF THE SAMPLE

The fruit pulp and seed of *Phoenix pusilla* were separated after drying the whole fruit. They were soaked in methanol separately [25g in 100ml] and left undisturbed for 24hrs. Then the solution was filtered and to the filtrate methanol was added repeatedly till 72hrs for every 24 Hrs. The filtered solution were pooled and dried for the evaporation of the solvent. Then they were dissolved in methanol at concentration of 1mg/ml and used for further analysis.

PHYSICOCHEMICAL EVALUATIONS

Proximate analysis

Proximate analysis of extract was done using the method of Association of official analytical chemist (AOAC, 2000).[9]

Moisture content

About 2 g of the extract was weighed and placed in a crucible of constant weight. This was placed in an oven at 105°C then dried; the weight was measured carefully to get a constant weight. The loss in weight indicates the moisture content.

Ash content determination

Crucible used for ash content determination was weighed and dried in a hot air oven at 110^{0}C to a constant weight. About 2 g of each extract was weighed and placed in the crucible and weight of the crucible and extract was taken. This was placed in a furnace and ignited for 3 hr at 55^{0}C till the samples have a cotton wool like texture and then it was cooled in a desiccator and weighed using balance.

Crude protein

About 1 g of the sample was weighed into the Kjeldahl flask. About 0.1 gm of Ca_2SO_4 was added into the flask with 20 ml Conc.H_2SO_4. The flask was then placed in a slanting position on Kjeldahl heating mantle in the fume cupboard. Digestion continued until there was a color change from black to bluish green which indicating that digestion has ended. It was set up against blank, the digest were removed and allowed to cool and was then diluted with water and made up to 200 ml on ice. About 50 ml of aliquot of each digest were poured into a distillation flask. About 30 ml of NaOH were carefully layered into solution in order to make it a strong alkaline and 50 ml of 0.1 N H_2SO_4 measured and kept in a beaker with 2 drops of methyl red as an indicator. The H_2SO_4 acted as a receiving flask. About 150 ml was distilled

over heat was put off to avoid drop in pressure. The distillate was titrated with 0.1M NaOH in the burette. This was done for extract and blank and % of nitrogen was calculated.

Lipid content

About 1 g of sample was weighed into a thimble of known weight. About 150 ml of petroleum ether (60-80^{0}C) was poured into 250 ml conical flask using measuring cylinder. The soxhlet extractor were the sack and its content had been introduced was fitted and solvent boiled under reflux. The extraction process lasted for 8 h. and sack with its content were removed dried in an oven for 2 h. and then weighed with a balance.

Crude fibre

This is organic residue which remains after the materials have been treated with standardized conditions with light petroleum, boiled diluted H_2SO_4 boiled diluted HCl, alcohol and ether. The crude fibre consists largely of cellulose together with little lignin and it can extrapolate as: 100 – (Moisture % + ash % + lipid % + protein %).

EXTRACTIVE VALUE

Solubility values of crude drugs are useful for their evaluation especially when the constituents of a drug cannot be readily estimated by any other means. Further, these values indicate the nature of the constituents present in a crude drug. The raw materials were dried and powdered and the powdered materials were used for analysing different parameters.

Alcohol soluble extractive (ASE)

Four gram of air dried and coarsely powdered tissue was macerated and placed in a glass stopper flask with 100 ml of 90% ethanol for 24 h. The contents were frequently shaken for the first 6 h. and allowing to stand for 18 h. in 90% ethanol. Then the contents were filtered rapidly with taking precautions against loss of ethanol. 25 ml of the filtrate was allowed to dry on a water bath using tared flat bottomed petri plate/shallow dish. The petri plate was dried at 105°C for 1h. in a hot air oven and removed and cooled in a desiccator and weighed. The process was repeated till the concordant weight was obtained and the percentage of ethanol-soluble extractive value was calculated using the following formula[10]

$$\text{\% of alcohol soluble extractive value} = \frac{B - A \times 4 \times 100}{W}$$

Where, A= empty wt. of the dish (g)

B = wt. of dish + residue (g)

W= wt. of plant material taken (g)

Water soluble extractive (WSE)

Four gram of the air dried and coarsely, powdered tissue was macerated with 100 ml of 5% chloroform water in a glass stopper conical flask for 24 h. the contents were shaken frequently during the first 6 h. Thereafter the contents were filtered rapidly by decanting the water extract. 25 ml of the filtrate was evaporated to dryness on a water bath in tarred flat bottomed petri plate/shallow dish. 2 ml of alcohol was added to the dry residue and the contents were shaken and dried again on water bath. It was then dried at 105°C for 1 h. in the hot air oven and cooled in a desiccator for 30 mins and weighed. The process was repeated till the concordant weight is obtained. The % of WSE was calculated using the formula mentioned for calculation of alcohol soluble extractive value.[10]

Ether- soluble extractive (ESE)

1 g of air dried coarsely powdered was macerated with 100 ml of ether in a closed flask for 24 hr with frequent shaking. It was filtered rapidly with taking precautions against loss of ether. 25 ml of filtrate was then evaporated in a tarred flat bottom shallow dish, dried at 100^0C and weighed. The percentage of ether soluble extractive was calculated using the formula mentioned for calculation of alcohol soluble extractive value.[10]

Organoleptic evaluation

Organoleptic features of the plant were evaluated by observing colour, odour, taste, size, shape of morphology.

Fluorescence analysis

The *Phoenix puscilla* (Fruit pulp and seed) powder was treated with various acidic and basic solvents like methanol, ammonia, potassium hydroxide, picric acid, distilled water, petroleum ether, 50% sulphuric acid, 50% nitric acid and 50% hydrochloric acid for fluorescence analysis and were then observed under UV/ visible chamber simultaneously and then observed under visible light and ultra violet light.

PHYTOCHEMICAL SCREENING OF EXTRACTS

Methanol extract of *Phoenix puscilla* (Fruit pulp and seed) were used for preliminary phytochemical analyses using standard procedures.[11]

QUANTITATIVE DETERMINATION OF PRIMARY METABOLITES IN METHANOLIC EXTRACT

Determination of Carbohydrate

100 mg of sample was hydrolysed in a boiling tube with 5 ml of 2.5 N HCl in a boiling water bath for a period of 3 hours. It was cooled to room temperature and solid sodium carbonate was added until effervescence ceases. The contents were centrifuged and the supernatant was made to 100 ml using distilled water. From this 0.2 ml of sample was pipetted out and made up the volume to 1 ml with distilled water. Then 1.0 ml of phenol reagent was added followed by 5.0 ml of sulphuric acid. The tubes were kept at 25-30°C for 20 min. The absorbance was read at 490 nm.[11]

Estimation of Total Chlorophyll content

100 mg leaf tissues were soaked in 10 ml of DMSO: acetone mixture (1:1) for overnight incubation (in the dark) and absorbance read at 663 and 645 nm and total chlorophyll content was calculated using the following equations.

Chlorophyll a (Ca) = $(12.25 \times \text{OD at } 663) - (2.79 \times \text{OD at } 645) \times 10/(1000 \times \text{wt})$

Chlorophyll b (Cb) = $(21.50 \times \text{OD at } 645) - (5.10 \times \text{OD at } 663) \times 10/(1000 \times \text{wt})$

Total Chlorophyll (C) = $(7.15 \times \text{OD at } 663) + (18.71 \times \text{OD at } 645) \times 10/(1000 \times \text{wt})$

Determination of Protein

The dried and powdered samples was extracted by stirring with 50 ml of 50% methanol (1:5 w/v) at 25 °C for 24 h and centrifuged at 7,000 rpm for 10 min .0.2 ml of extract was pipette out and the volume was made to 1.0 ml with distilled water. 5.0 ml of alkaline copper reagent was added to all the tubes and allowed it to stand for 10 min. Then 0.5 ml of Folin's Ciocalteau reagent was added and incubated in dark for 30 min. The intensity of the colour developed was read at 660 nm.[11]

Estimation of Total Lipid content

10 gm sample was used to extract lipids with 150 ml of petroleum ether for 16 hr, at a solvent condensation rate of 2–3 drops/sec according to AOAC approved method with minor modifications of sample size and extraction time. The obtained extract was concentrated and evaporated at room temperature to dryness. The weight of extract gives the total lipid content which was expressed as mg/g dry matter.[11]

QUANTITATIVE DETERMINATION OF SECONDARY METABOLITES

Determination of Total Phenolics and Tannins

Ten microliter aliquots of the extracts (2 mg/2 ml) was taken in test tubes and made up to the volume of 1 ml with distilled water. Then 0.5 ml of Folin-Ciocalteu phenol reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) were added sequentially in each tube. Soon after vortexing the reaction mixture, the test tubes were placed in dark for 40 min and the absorbance was recorded at 725 nm against the reagent blank. The analysis was performed in triplicate and the results were expressed as tannic acid equivalents. Using the same extracts, the tannins was estimated after treatment with polyvinyl polypyrrolidone (PVPP). One hundred milligrams of PVPP was placed in a test tube and to this 1 ml distilled water and then 1 ml of the sample extracts were added. The contents were vortexed and kept in the test tube at 4°C for 4h. Then it was centrifuged (3000 rpm for 10 min at room temperature) and the supernatant was collected. This supernatant has only simple phenolics other than tannins (the tannins would have been precipitated along with the PVPP). The phenolic content of the supernatant expressed as the content of non-tannin phenolics (tannic acid equivalents) on a dry matter basis.[12] From the above results, the tannin content of the sample was calculated as follows: Tannin (%) = Total phenolics (%) – Non-tannin phenolics (%).

Determination of Total Flavonoid content

The flavonoid content was determined by the use of a slightly modified colorimetry method. A 0.5 ml aliquot of appropriately (2 mg/2 ml) diluted sample solution was mixed with 2 ml of distilled water and subsequently with 0.15 ml of 5 % $NaNO_2$ solution. After 6 min, 0.15 ml of 10% $AlCl_3$ solution was added and allowed to stand for 6 min, and then 2 ml of 4% NaOH solution was added to the mixture. Immediately, water was added to bring the final volume to 5 ml, and then the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was determined at 510 nm versus water blank. The analysis was performed in triplicate and the results were expressed as rutin equivalent.

RESULTS AND DISCUSSION

Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of such economic materials as tannins, oils, gums, precursors for the synthesis of

complex chemical substances. In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies.[13]

Physicochemical evaluation

Physicochemical evaluation includes tests for moisture content, ash content, crude fibre, lipid content, crude fibre and solubility value of both the fruit pulp and seed sample. In the present investigation, proximate composition of both the fruit pulp and seed sample were analyzed and shown in Table 1. The moisture content of pulp was 24.91 ± 0.32% dry weight. The ash content was 0.81 ± 0.17 % dry weight. The crude protein and fat content were 2.52 ± 0.61 and 0.31 ± 0.47% dry weight, respectively and the crude fibre was 0.51 ± 0.13 % dry weight. The low moisture content in the seeds indicated that they have capacity to prevent microbial attack and allows for high storage capacity. Though the seed has low moisture content, it is found to contain an equal appreciable amount of all the other proximate parameters.

Table 1: Proximate Analysis of Methanolic Extract of Fruit Pulp and Seed.

S.NO	Composition	Fruit pulp % Dry weight	Seed % Dry weight
1	Moisture content	24.91 ± 0.32	8.71 ± 0.19
2	Ash content	0.81 ± 0.17	1.03 ± 0.05
3	Crude protein	2.52 ± 0.61	2.19 ± 0.53
4	Crude fiber	0.51 ± 0.13	0.68 ± 0.39
5	Fat content	0.31 ± 0.47	0.43 ± 0.71

The ash content is generally recognized as a measure of quality for the assessment of the functional properties of foods. Ash in food contributes the residue remaining after all the moisture has been removed as well as the organic material (fat, protein, carbohydrates, vitamins, organic acid, *etc*). Ash content is generally taken to be a measure of the mineral content of the original food. The moderate amount of protein present in the fruit indicates that the plant can form a part of human diet.

Extractive values

Different plant species would obviously have different chemical profile. Chemical present in the plant material could be dissolved in different solvent for the purpose of further analysis. Therefore, three solvents - water, alcohol and ether were selected to determine the soluble substance. Water-soluble extractive value plays an important role in evaluation of crude drugs. Less extractive value indicates addition of exhausted material, adulteration or incorrect processing during drying or storage or formulating. The water-soluble extractive values of

fruit pulp is 4.07 ± 0.23%, alcohol soluble extractive values is 8.79 ± 0.03% and ether soluble extractive values is 1.93 ± 0.14%. [Table 2].

Table 2: Extractive values of Methanolic Extract of Fruit Pulp and Seed.

S.NO	Parameters	Fruit pulp (Values)	Seed (Values)
1	Alcohol soluble extractive %	8.79 ± 0.03	9.58 ± 0.01
2	Water soluble extractive %	4.07 ± 0.23	5.83 ± 0.13
3	Ether soluble extractive %	1.93 ± 0.14	0.74 ± 0.14

Similarly, the water-soluble extractive values of fruit seed is 5.83 ± 0.13%, alcohol soluble extractive values is 9.58 ± 0.01% and ether soluble extractive values is 0.74 ± 0.14% [Table 2]. It was observed that alcohol soluble extractives value is higher than water soluble and ether soluble extractive value. Our results were in agreement with the results of Oloyede.[14] and Nambiar and Hema Matela.[15]

Organoleptic features

Organoleptic authentication means the study of herbal medicines using various organs of senses which includes the analysis of color, odour, taste, shape, size, texture, weight, structure, *etc*. Obviously the initial visibility, odour, color, taste, sight and smell of the plant or plant extract are specific to identify itself. Organoleptic evaluation is simplest analysis but most common practice among the practitioners, herbalists, locals and herb sellers. The various characteristics were given in Table 3.

Table 3: Organoleptic Evaluation of Methanolic Extract of Fruit Pulp and Seed.

S.NO	Organoleptic features	Fruit pulp	Seed
1	**Color**	Orange-red	Dark brown
2	**Odor**	Dates flavor	Coffee flavor
3	**Taste**	Sweet	Sour and bitter
4	**Size**	1-1.5 cm long, 0.1-0.2 cm thick	0.8 cm long, 0.12-0.15 cm thick
5	**Shape**	Oval	Oblong-ventrally grooved

Fluorescence analysis

The fluorescence analysis represented the behavioural changes of powdered fruit and seed sample with different chemicals reagents under day light and UV[16,17,18] and the results are presented in Table 4.

Table 4: Fluorescence Analysis of Methanolic Extract of Fruit Pulp and Seed.

Reagents	Fruit pulp Visible	Seed Visible	Fruit pulp UV	Seed UV
Powder	Dark brown	Light brown	Dark brown	Light brown
Powder + pet ether	Black	Red	Brown	Pink
Powder + ethyl acetate	Dark brown	Chocolate brown	Light red	Pink
Powder + ethyl acetate: HCl (1:1)	Dark brown	Chocolate brown	Light red	Pink
Powder + methanol	Reddish orange	Orange	Red	Pink
Powder + chloroform	Black	Dark brown	Brown	Light red
Powder + acetone	Light green	Red	Violet	Pink
Powder + 50 % H_2SO_4	Black	Red	Black	Pink
Powder + 50 % HNO_3	Red	orange	Yellowish red	Red
Powder + 50 % HCl	Black	Black	Grape color	Grape color
Powder + 10 % NaOH	Black	Brown	Pink	Violet

Phytochemical screening of *Phoenix puscilla* (Fruit pulp and seed)

After the preparation of methanolic extract of fruit pulp and seed, the systematic analysis for phytoconstituents revealed the presence of Flavanoids, Phenolics and tannin and Carbohydrates predominantly [Table 5]. The phytochemical constituent of a plant will often determine the physiological action on the human body.[19] The most important of these bioactive constituents of plants are alkaloids, flavonoids, tannins, phenolic compounds etc.[20] Our results are comparable with those of Shankar and Shoba.[21]

Table 5: Phytochemical Analysis of Methanolic Extract of Fruit Pulp and Seed.

Plant constituent	Extracts		
	Name of the test	**Sample 1**	**Sample 2**
Alkaloids	Wagner's test	-	-
Flavonoids	Shimoda, Lead acetate test	++	+
Phenolics & Tannins	Lead actetate test,	++	+
	Ferric chloride test	+	+
Steroids & Sterols	Salkowski test	+	+
Carbohydrates	Fehlings test,	++	+
	Benedicts test	++	+
Saponin	Honey comb test,	-	-
	Foam test	-	-
Glycosides	Glycosides test	+	-
Protein & amino acid	Biuret test,	++	++
	Ninhydrin test	+	+
Anthraquinone	Borntragers test	-	-

Quantification of primary metabolites

The extracts were analysed for the quantitative determination of primary metabolites. The pulp was found to contain high amount of carbohydrate, Chlorophyll, protein and Lipid when

compared to the seed [Table 6]. Quantitative analysis of primary metabolites shows that, Carbohydrate content was found to high (50.36 ± 2.53mg/g) followed by pigment (7.89 ± 0.73mg/g) and then protein (3.18 ± 0.01mg/g). Carbohydrates are one such group of carbon compounds, which are essential to life. Almost all organisms use carbohydrates as building blocks of cells and as a matter of fact, exploit their rich supply of potential energy to maintain life. Proteins are essential to maintaining the structure and function of all life and vital for growth and development. Chlorophyll is the most indispensable class of primary compounds as they are the only substances that capture sunlight and make it available to plant system for its cultivation on photosynthesis.

Table 6: Quatification of Primary Metabolites of Methanolic Extract of Fruit Pulp and Seed.

S.NO	Primary metabolites	Fruit pulp Weight (mg/g dw)	Seed Weight (mg/g dw)
1	Carbohydrates	50.36 ± 2.53	14.38 ± 0.71
2	Chlorophyll	7.89 ± 0.73	4.24 ± 0.39
3	Protein	3.18 ± 0.01	2.21 ± 0.32
4	Lipids	0.38 ± 0.16	0.84 ± 0.45

Quantification of secondary metabolites

Secondary metabolite analysis is necessary for extraction, purification, separation, crystallization, identification of various phytocompounds.

Table 7: Quatification of Secondary Metabolites of Methanolic Extract of Fruit Pulp and Seed.

S.NO	Secondary metabolites	Fruit pulp Weight (mg/g dw)	Seed Weight (mg/g dw)
1	Total phenolic	3.15 ± 0.28	1.28 ± 0.41
2	Tannin	1.23 ± 0.13	0.75 ± 0.68
3	Total flavonoids	2.38 ± 0.05	0.91 ± 0.03

The methanolic extract of fruit showed higher level of phenolics (3.15 ± 0.28 mg/g dw) than the other secondary metabolites when compared with seed extract [Table 7]. The higher amount of phenol is important in regulation of plant growth, development and disease resistance. The level of flavonoid content was 2.38 ± 0.05mg/g dw extract. Earlier reports revealed that plant phenolic compounds including flavonoids are potent antioxidants with reported antimutagenic and anticarcinogenic effects.[22] Consequently, *P.pusilla* by its richness in different secondary metabolites may have several medical importances such as

anti-tumor especially the ethanol extract due to the presence of flavonoids[23] and antioxidant due to its richness in phenolic compounds.[24]

CONCLUSION

The preliminary pharmacological evalustion of this south indian date palm, *Phoenix pusilla* show that they can be potential sources of nutraceuticals and bioactive compounds. Based on the results represented here, it can be concluded that both the samples i.e. fruit and seed have appeared as rich sources of both primary and secondary phytoconstituents and offer immense opportunities for the development of drugs targeted for various pharmaceutical purpose. Various species of *Phoenix* also have very good antioxidant properties and good amount of phytochemicals. The obtained compounds are known to possess potent antimicrobial, antitumour, hepatoprotective properties and good therapeutic potential which may help in drug development. Furthermore, the high correlation observed between the various assays employed and phenolics content indicates that these phenolics are among the predominant sources of antioxidant activity among the species of *Phoenix*. It can therefore be concluded that apart from these preliminary studies, characterization of bioactive compounds and exploring their bioactive potential is the need of this hour.

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