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Qualitative and Quantitative Analysis of Bioactive Constituents Present in Seed Extracts of *Phoenix sylvestris*

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Abstract: ***Seeds are fertilised , mature ovules- the result of sexual reproduction in plants . Seeds are of immence biological and economic importance .They contain high protein ,starch , oil reserves that help in the early stage of growth and development in plants. The present study was carried out to test the presence of various phytochemicals in the seed extract of the plant and also to estimate the total phenol and flavonoids in the ethanolic seed extract. Phytochemical analysis of the seed extract of Phoenix sylvestris revealed the presence of most of the biochemicals tested for such as carbohydrate, protein, alkaloid, Glycosides, flavonoid , phenol,steroids and saponin. The total phenolic content of the ethanolic seed extract is found to be 13.56 mg/g and the total flavonoid contents of the seed extract is found to be 18.56 mg/g. The presence of various phytochemicals in the tested plant reveals that this plant may be a good source for production of new drugs for various ailments.***

Keywords: Silver date palm , *Phoenix sylvestris* Roxb., Seeds , Phytochemical analysis , Preliminary screening

1. Introduction

Bioactive compounds in plants can be defined as secondary plant metabolites eliciting pharmacological or toxicological effects in man and animals. Secondary metabolites are produced within the plants beside the primary biosynthetic and metabolic routes for compounds associated with plant growth and development, and are regarded as products of biochemical "side tracks" in the plant cells and not needed for the daily functioning of the plant. Several of them are found to hold various types of important functions in the living plant. For example, flavonoids can protect against free radicals generated during photosynthesis. Terpenoids may attract pollinator or inhibit competing plants. Alkaloids usually ward off herbivore animals or insect attacks .So, every secondary metabolite has different function in the plants.

Phoenix sylvestris Roxb. (Silver date palm, Indian date palm etc.) belongs to the family Arecaceae. The word „*Phoenix'* in the Latin name came from Greek and means "purple", while „*sylvestris'* means "wild" .It is an unbranched, erect, tall, dioecious, evergreen tree. This genus having 17 species found in diverse habitats, swamps, deserts and mangroove sea coats (Zaid and Jimenez 2002). *P. sylvestris* is widely distributed in South Asia from Pakistan to Myanmar, across India, Nepal, Bhutan and Bangladesh (Barrow 1998, Henderson 2009). In presents-day India, it is commonly found on low ground in the Sub-Himalayan tract, along river banks on the Deccan plateau(south-central India), in forest up to elevations of 1350m in Himachal Pradesh, and especially on lower hill survives in disturbed areas, such as waste lands or seasonally inundated areas(Parmar & Kaushal 1982). Apart from its distribution in a "wild" state, *P. sylvestris* is also cultivated in parts of South Asia, mostly in its eastern and south eastern parts according to the literature: West Bengal (including Kolkata, the Coromandel coasts), Andhra Pradesh (south eastern India) and Chittagong (eastern Bangladesh) (Parmar & Kaushal 1982,Chowdhury et al.2008), in Punjab and Sindh provinces of Pakistan .

Many parts of this plant are used for their medicinal properties (Parmar & Kaushal 1982). This plant has been identify as a component of traditional medicine against various ailment. *P.sylvestris* have also been reported for its traditional medicinal use by the tribal peoples (Chowdhury et al, 2010; Gandhimathi and Sreedevi, 2012; Salvi and Katewa,2012) and also have been reported for its nutritive and protective activity. Seed extract of *P.sylvestris* shows anti-bacterial activity (Kothari ,2011).

2. Material and Method

2.1 Plant Material

Seeds of *Phoenix sylvestris* were purchased from local market of Bhopal (M.P), India in the months of September 2015. These seeds were authenticated by Dr. Zea Ul Hasan,H O D , Department of Botany, Safia College, Bhopal and preserved in the herbarium (specimen No. 357/Bot./Safia/15)of the Dept. of Botany Safia College, Bhopal (M.P.)

Extraction procedure (Mukharjee, 2007).
Following procedure was adopted for the preparation of methanol extracts from the shade dried and powdered herbs*:*

2.2 Defatting of Plant Material

Seeds of *Phoenix sylvestris* were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether in a soxhlet apparatus. The extraction was continued till the defatting of the material had taken place.

2.3 Extraction by hot continuous percolation process

50 gms. of *Phoenix sylvestris* dried seeds were exhaustively extracted with various solvent chloroform, ethyl acetate, and ethanol using different drug: solvent ratios using hot continuous percolation for different time. The extracts were

evaporated above their boiling points finally the percentage yields were calculated of the dried extracts

2.4 Determination of Percentage yield

Calculation of percentage yield

The percentage yield of each extract was calculated by using formula:

$$\text{Percentage yield} = \frac{\text{Weight of extract x 100}}{\text{Weight of powdered drug take}}$$

2.5 Qualitative Phytochemical Tests (Khandelwal, 2005, Kokate, 1994)

The extracts were subjected to various qualitative tests to detect the presence of plant constituents. The results have been shown in table.

2.5.1 Preparation of Test Solution

The test solution was prepared by taking 1 g of the extract in 25 ml of methanol.

A. Test for Carbohydrates

Following tests were carried out for carbohydrates.

a) **Molisch's test:** In a test tube containing extract of drug, added two drop of freshly prepared 20% alcoholic solution of α- napthol and mixed concentrated sulphuric acid along the sides of the test tube. If carbohydrate present purple color or reddish violet color produce at the junction between two liquids.
b) **Benedict's test:** In a test tube containing extract of drug add benedict"s solution, mix well, boiled the mixture vigorously for two minutes and then cooled. Formation of red precipitate due to presence of carbohydrates.
c) **Barfoed's test:** The barfoed"s solution added to 0.5 ml of solution under examination, heated to boil. Formation of red precipitate of copper oxide was indicated the presence of carbohydrates.
d) **Anthrone test:** To the two ml of anthrone test solution, add the extract of drug. A green or blue colour indicated the presence of carbohydrate.

B. Test for Alkaloids

a) **Dragendorff's Test**: Few mg of extract of the drug dissolved in 5 ml of water added 2 M hydrochloric acid until an acid reaction occurred; 1 ml of dragendorff"s reagent (potassium bismuth iodide solution) was added an orange red precipitate indicated the presence of alkaloids.
b) **Wagner's test**: Acidify the extract of drug with 1.5 % v/v of hydrochloric acid and added a few drop of Wagner"s reagent (iodine potassium iodide solution). Formations of reddish brown precipitate indicated the presence of alkaloids.
c) **Mayer's Test**: Two ml of extract solution was treated with 2 - 3 drops of Mayer"s reagent was added (potassium mercuric iodide solution) formation of dull white precipitate indicated the presence of alkaloid.
d) **Hager's Test**: Extract of the drug solution was treated with 3 ml of Hager"s reagent (saturated solution of picric acid) formation of yellow precipitate confirmed the presence of alkaloids.

C. Test for Steroids and Sterols

a) **Liebermann's Burchard reaction**: The test extract solution was dissolved in 2 ml of chloroform in a dry test tube. Now 10 drops of acetic anhydride and 2 drops of concentrated sulphuric acid were added. The solution became red, then blue and finally bluish green in color.
b) **Salkowsky test**: The extract of test solution dissolved in chloroform and equal volume of conc. sulphuric acid was added. Bluish red cherry, red and purple color was noted in chloroform layer, whereas acid assumes marked green fluorescence.

D. Test for Glycosides

a) **Legal's test**: Extract solution dissolved in pyridine then sodium nitroprusside solution was added to it and made alkaline. Pink red colour indicated the presence of glycosides.
b) **Baljet's test**: To the drug extract, sodium picrate solution was added, yellow to orange colour was indicated the presence of glycosides.
c) **Borntrager's test**: Few ml of dilute sulphuric acid solution, the test solution of extract was added. It was filtered and the filtrate was boiled with ether or chloroform. Then organic layer was separated to which ammonia was added, pink, red or violet colour was produced in orange layer confirmed the presence of glycosides.
d) **Keller Kiliani test**: Methanolic extract was dissolved in glacial acetic acid containing trace of ferric chloride one ml concentrated sulphuric acid was added carefully by the side of the test tube. A blue colour in the acetic acid layer and red colour at the junction of the two liquid indicated the presence of glycosides.

E. Test of Saponins

a) 1 ml of alcoholic extract was diluted with 20 ml distilled water and shaken in graduated cylinder for 15 minutes. One cm layer of foam indicated the presence of saponins.

F. Test for Flavanoids

a) **Shinoda test**: In the test tube containing alcoholic extract of the drug added 5 - 10 drops of dil. hydrochloric acid followed by the small piece of magnesium. In presence of flavonoids a pink, reddish pink or brown color was produced.

G. Test for Tannins

a) To the sample of the extract, ferric chloride solution was added appearance of dark blue or greenish black colour indicated the presence of tannins.
b) To the sample of extract, potassium cyanide was added, deep red colour was confirmed the presence of tannins.
c) To the sample of extract, potassium dichromate solution was added, yellow precipitate was produced.

H. Test for Triterpenoids

a) In the test tube, 2 or 3 granules of tin was added, and dissolved in 2 ml of thionyl chloride solution and test solution was added. Pink colour was produced which indicates the presence of triterpenoids.
b) Two ml of acetic anhydride solution was added to 1 ml of extract of drug in chloroform followed by one ml of conc.

sulphuric acid, a violet colored ring was formed indicating presence of triterpinoid.

I. Test for Protein and Amino acid

a) Biuret‟s test: To 2 - 3 ml of the extract of drug added in 1 ml of 40 % sodium hydroxide solutions and 2 drops of 1 % copper sulphate solution mix thoroughly, a purplish - violet or pinkish - violet colour produced that indicates the presence of proteins.
b) Ninhydrin‟s test: Two drops of freshly prepared 0.2 % ninhydrin reagent was added to the extract and heated to boiling for 1 - 2 min. and allow cooling. A blue colour developed that indicating the presence of proteins, peptides or amino acids.
c) Xanthoprotein test: To the extract in a test tube, add conc. nitric acid. A white precipitate was obtained and upon heating turns to yellow and cool the solution carefully. Added 20 % of sodium hydroxide solution in excess orange colour indicated presence of aromatic amino acid.
d) Millon‟s test: The small quantity of extract of the drug dissolved in distilled water added 5 - 6 drop of millon‟s reagent. A white precipitate was formed which turned red on heating, indicated the presence of proteins.
e) Lead Acetate test: The extract was taken and two ml of 40 % sodium hydroxide solution was added and boiled, glacial acetic acid was added and cooled than added 1 ml of lead acetate solution, gray black precipitate was formed which indicated presence of sulphur containing amino acid.

J. Test of Resins

Dissolved the extract in the acetone and pore the solution in the distilled water. Turbidity indicated the presence of resin.

K. Test of Fats or Fixed oils

a) Using sodium hydroxide: The extract was mixed in one ml 1 % of copper sulphate solution then added 10 % sodium hydroxide solution a clear blue solution was obtain which showed glycerin present in sample.
b) Using sodium hydrogen sulphate: The extract was taken in test tube added a pinch of sodium hydrogen sulphate pungent odour was formed which showed glycerin present in sample.
c) Saponification: Four ml of 2 % sodium carbonate solution was taken and the extract was added. Shaked vigorously and boiled. A clean soapy solution was formed cooled and added few drops of conc. HCl and observed that fatty separate out and float up.

Quantitative analysis of bioactive compound content in all methanolic extracts

Total phenolic content estimation (Olufunmiso *et al.*, 2011).

Principle: The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method.

Preparation of Standard: 50 mg Gallic acid was dissolved in 50 ml methanol, various aliquots of 25- 150 µg/ml was prepared in methanol.

Preparation of Extract:

1gm of dried powder of drug was extracted with 100 ml methanol separately for all extract in 100 ml volumetric flask, filter, and make up the volume up to 100 ml. One ml (1mg/ml) of this extract was for the estimation of flavonoids.

Procedure: 1 ml of extract or standard was mixed with 5 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 4 ml (75g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 30min at 40°C for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Total flavonoids content estimation:

Principle:

Determination of total flavonoids content was based on aluminium chloride method.

Preparation of standard: 50 mg quercetin was dissolved in 50 ml methanol, and various aliquots of 25- 150 µg/ml were prepared in methanol.

Preparation of extracts

1gm of dried powder of the drug was extracted with 100 ml methanol separately for all extract in 100 ml volumetric flask, filter, and make up the volume up to 100 ml. One ml (1mg/ml) of this extract was for the estimation of flavonoids.

Procedure: 1 ml of 2% $AlCl_3$ Methanolic solution was added to 1 ml of extract or standard and allowed to stand for 60 min at room temperature; absorbance was measured at 420 nm.

3. Results and Discussion

Phytochemical Extraction and Preliminary Screening

The different solvent extracts of the *Phoenix sylvestris* Roxb. seeds were subjected to preliminary qualitative assessment for the presence of alkaloids, glycosides, tannins, saponins, flavonoids, steroids and others separately according to Kokate‟s methods (1994). The results of phytochemical analysis are discussed in the (table 1). From the results, it is clear that the ethanolic seeds extract of *Phoenix sylvestris* shows the presence of alkaloids, phenols, flavonoids, amino acid and terpenoids, when extracted with different solvents using soxhlet extraction procedure (Table1).

Total phenolic (TPC) and flavonoids (TFC) content estimation

The content of total phenolic compounds (TPC) and to total tannin content was expressed as mg/gm of gallic acid equivalent of dry ethanolic extract sample using the equation obtained from the calibration curve: $Y = 0.004X + 0.009$, $R^2 = 0.999$, where × is the absorbance and y is the tannic acid equivalent (GAE). Total flavonoids content was calculated as quercetin equivalent (mg/g) using the equation based on the calibration curve: $Y=0.004\ X+0.001$, $R^2=0.996$, where X is the absorbance and Y is the quercetin equivalent (QE).

Quantitative determination of total phenols was done on the basis of a standard curve of gallic acid and linearity of the calibration curve was achieved between 25 to 150 µg/ml concentration for gallic acid ($r^2 = 0.996$). Quantitative determination of total flavonoids was done on the basis of a standard curve of Quercetin and linearity of the calibration curve was achieved between 25 to 150 mg/ml concentration

for quercetin (r^2 = 0.996). Ethanolic extarct of *Phoenix sylvestris* was found to contain more flavonoids content as compare to phenolic content. (table - 2).

4. Conclusion

The study proves that the ethanolic seed extract of *Phoenix sylvestris* were found rich source of Phenols and Flavonoids. The quantification of these plant extract proves high medicinal value and can be used in polyherbal Preperations.

Table 1: Results of Phytochemical Screening

S.No.	Constituents	Pet. Ether	Chloroform	Ethyl acetate	Ethanol
1.	Alkaloids	-	-	+	+
2.	Glycosides	-	-	-	-
3.	Flavonoids	-	-	+	+
4.	Phenols	-	-	-	+
5.	Carbohydrate	-	-	-	-
6.	Proteins	-	-	-	+
7.	Saponins	-	-	+	+
8.	Diterpenes	+	-	+	+

Table 2: Result of Phenolic and flavonoids content

S. No	Ethanolic Extracts	Total phenolic content (mg/gm)	Total flavonoids content (mg/gm)
1.	*Phoenix sylvestris*	13.56	18.56

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