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PHYTOCHEMICAL STUDIES ON NUT OF VATAD (PRUNUS AMYGDALUS L)

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ABSTRACT

In the present era *Ayurvedic* medicines are becoming popular. To get encouraging results from a herb and the world revving over the issues of safety and efficacy, the prescribed portion of the ingredient- the quantity and quality of the same to be expected in the final product seems to gain top priority. The first step to answer this challenge seems to be the phytochemical screening where in, routine components of the herbal vegetation is traced.Present study deals analysis of active phytochemicals of nut of *Vatad (Prunus amygdalus L.)*

Keyword: *Ayurveda, Vatad ,Prunus amygdalus* L.,Phytochemistry

INTRODUCTION

'Phytochemistry' or the 'Chemistry of Natural Products' may be strategically place somewhere in between natural product organic chemistry and plant biochemistry[1-6] Phytochemistry[8-9] or Plant chemistry has developed in recent years as a distinct discipline. It is concerned with enormous variety of organic substances, their biosynthesis, turnover and metabolism, their norm distribution and their biological function. Development in the field of phytochemistry has increases the remedies for chronic diseases. This had created a new enthusiasm in researchers to develop herbal drugs[7]

Standardization starts right from the collection of raw materials to the extreme clinical application. In case of Ayurvedic medicines, the therapeutic efficacy is a total effect of its chemical constituents. So, the quality and purity refers to the total profile of the drug rather than any of its character. Therefore, a multidimensional approach is essential for standardizing an Ayurvedic drug. This multidimensional approach should cover every minute aspect of Ayurvedic drug specifically the name, botanical source, and geographical source, Organoleptic, morphological, anatomical, physical, chemical and biological.

Aims and Objectives of the Study

The Aims and Objectives of this study is analysis of active phytochemicals of nut of *Vatad (Prunus amygdalus L.)*

MATERIALS AND METHODS

1. Qualitative examination of Inorganic matters:

It involves qualitative examinations of electrolytes, which are present in ash of sample. Higher plants, for growth and reproduction require sixteen or Seventeen elements. Elements required in relatively large quantities are termed as macronutrients, where as nutrients

required in small amounts are termed as micronutrients. All such elements are used up in various metabolic processes. Variety of organic compounds synthesized by the plants incorporates these elements in their chemical constitutions. Therefore, their presence can be detected by simple chemical analysis. The test methods of main elements are as follows:

Preparation of Test Sample

Take 5 gm of powder of nut of *Vatad (Prunus amygdalus L.)*) in crucibles. The crucibles are kept in muffle furnace at 550°C for nearly 6 hours. After 6 hours, the ash is removed from the muffle furnace. Dissolve the ash in 5 ml of slightly acidic distilled water and use the solution for detecting the presence of mineral elements.

Calcium: Take 0.5 ml of test sample and 2 drops of conc.H_2SO_4. Formation of white precipitate indicates presence of Calcium.

Iron: Take 0.5 ml of test sample and 3 drops KSCN reagent. Formation of red color indicates presence of Iron.

Manganese: Take 0.5 ml of test solution and add 1 mL of 1% KOH solution then 5 drops of Benzedine reagent. Formation of blue color shows presence of Manganese.

Phosphorus: Take 0.5 ml of test solution and two drops of Ammonium Molybdate reagent. Formation of yellow color indicates presence of Phosphorus.

Potassium: Take 0.5 ml of test solution and 2 drops of 15% $HClO_4$ solution. Formation of $KClO_4$ crystals indicate presence of Potassium.

Sulphur: Take 0.5 ml of test solution and 2 drops of $BaCl_2$ Formation of white ppt. of $BaSO_4$ indicate presence of Sulphur.

Megnesium: Take 0.5 ml of test solution and a drops of of magnesium reagent. Formation of crystals indicate presence of megnesium

2. Determination of Heavy Metals:-

COBALT COMPONDS

Dissolve 20 mg of the ash of the drug in about 0.5 mL of distilled water, and acidify with a few drops of dilute hydrochloric acid. Add a few drops of dilute solution of sodium hydroxide. A blue ppt. is formed which turns pink on warming.

COPPER COMPOUDS

Dissolves 20 to 25 mg of the drug in 1 mL of distilled water and add dilute ammonia solution, drop wise until a clear blue solution is obtained. Heat to boiling and add drop wise 2% W/V alcoholic solution of α-benzoinoxime. A green ppt. is formed.

MERCURY COMPOUNDS

Dissolve 20 to 25 mg of the ash of the drug in 1 mL of distilled water, and add 2 M sodium hydroxide until solution becomes strongly alkaline. A dense yellow ppt. is formed.

Dissolve 20 to 25 mg of the ash of the drug in 1 mL of distilled water, and add potassium iodide solution. A red ppt. is formed that dissolves in an excess of the reagent.

NICKEL COMPOUNDS

Dissolve 20 mg of the ash of the drug in about 0.5 mL of water, acidify with a few drops of dilute hydrochloric acid, and then add drop by drop a dilute solution of sodium hydroxide. A blue ppt. is formed which turns green on warming.

SILVER COMPONDS

Dissolve 20 to 25 mg of the drug in 2-3 mL of distilled water and add 0.2 mL of 7 M hydrochloric acid. A curdy white ppt. is formed that is soluble in 3 mL of 6 M ammonia. Add a few drops of a 10% W/V aqueous solution of Potassium iodide a yellow ppt. is developed.

ZINC COMPOUNDS

Dissolve 20 to 25 mg of the ash in 2 to 3 mL of distilled water, and add 0.2 mL of

10 M sodium hydroxide. A white ppt. is formed which dissolves in 2 mL of 10 M sodium hydroxide solution. Add about 5 mL of 2 M Ammonium chloride followed by 0.1 mL of sodium sulphide solution. A flocculent, white ppt. is produced.

3. Determination of Total Ash

Five Silica Crucibles were cleaned, dried well and then weighed to constant weight and labeling was made. Drug sample were then weighed accurately and placed in the Silica Crucibles respectively. These crucibles were placed in a muffle furnace at a temperature of 450°C ±5°C till were become totally free from Carbon. The time taken for this process was about 6 hrs. The crucibles containing the ash were allowed to be cooled in a desiccator and subsequently weighed to constant weight.

Calculation

Wt. of Empty Silica Crucible = A_1 gm

Wt. of Sample (X) = X gm

Wt. of the Crucible with Ash = A_2 gm

Percentage of Total Ash = $\frac{A_2 - A_1}{x} \times 100$

The process was repeated five times for each drug sample and the Average Total Ash value was calculated.

4. Qualitative Examination of Organic matter

Organic substances	Carbohydrates Molisch's	Starch	Tannin	Protein	Saponin	Phenol	Glycoside	Alkaloids
Test Applied	Molisch's reagent	Iodine solution	Vanillin solution	Ninhydrine solution	Shaking with water	FeCl3 solution	Killer killani test	Dragondroff's reagent

5. Determination of Extractive values:

The organic substances of the different parts of *Nut of Vatad (Prunus amygdalus L.)* show their solubility in various, solvents in different quantities. So for this purpose of determination of extractive values six main solvents were selected according to polarity.

(Hexane ,Chloroform, Ethyl acetate, Acetone, Methanol & Water)

Coarsely powdered air dried drug material is accurately weighed and taken in a glass stopper conical flask. Solvent is added to the flask and the flask is attached to a reflux condenser and boiled for 6 hrs, on water bath. After 6 hrs, the flask is allowed to cool and the content is filtered through filter paper. The filtrate is transferred to a pre-weighed flat bottomed dish and evaporated to dryness on a water bath. Then the dish is kept in oven for six hours for the contents to get dried fully. The Dish is cooled by keeping in a desiccator for 30 minutes and weighed without delay.

The residual mass remained in filter paper is dried as such and is collected fully. This mass in again put into the conical flask and added with next solvent according to polarity, and fitted with reflux condenser, and extract is prepared in the same method used above. This procedure is repeated with all the six solvents. The content of the extractable matter is calculated in mg per gm of air dried material in the following manner.

Calculations:

Weight of the drug material = Xgm

Weight of the empty petridish= W_1gm

Weight of the pertidish with dried extract = W_2gm

Percentage of extractive value = $\frac{(W_2-W_1)}{X} X100$

The procedure was carried out with same drug sample with different solvent taken in the order of polarity.

6. **THIN LAYER CHROMATOGRAPHY (T.L.C.)**

Thin layer chromatography is a technique to separate the compounds from a mixture based on adsorption principle. It has the advantage of faster runs, better separations, and the choice between different adsorbents. Different compounds in the sample mixture travel different distances according to how strongly they interact with the adsorbent. This allows the calculation of an Rf value and can be compared to standard compounds to aid in the identification of an unknown substance.

Calculation of Rf Value

$$Rf = \frac{\text{Distance traveled bysolute from origin line}}{\text{Distance traveled bysolvent from origin line}}$$

7. High pressure liquid chromatography (HPLC)

HPLC is a form of column chromatography used frequently in biochemistry and analytical chemistry to separate, identify, and quantify compounds. HPLC utilizes a column that holds chromatographic packing material (stationary phase), a pump that moves the mobile phase(s) through the column, and a detector that shows the retention times of the molecules. Retention time varies depending on the interactions between the stationary phase, the molecules being analyzed, and the solvent(s) used.

Sample Preparation Of *Vatad* (*Prunus amygdalus L.*)

2 gm of *Vatad (Prunus amygdalus L.)* was powdered & made a diluted suspension in 50 mM phosphate buffer pH 7.2 then Protein concentration was measured by spectrophotometer at 280 nM then Diluted sample was filtered with 0.2 micron filter to avoid any suspended particle then20 µl (50µl) of *Vatad (Prunus amygdalus L.)*sample was injected into the Vydac C-18 column

8. **U.V.Sphectrophotometric**

In physics, spectrophotometry is the quantifiable study of electromagnetic spectra. It is more specific than the general term electromagnetic spectroscopy in that spectrophotometry deals with visible light, near-ultraviolet, and near-infrared. Also, the term does not cover time-resolved spectroscopic techniques.

Spectrophotometry involves the use of a spectrophotometer. A spectrophotometer is a photometer (a device for measuring light intensity) that can measure intensity as a function of the color, or more specifically, the wavelength of light. There are many kinds of spectrophotometers. Among the most important distinctions used to classify them are the wavelengths they work with the measurement techniques they use, how they acquire a spectrum, and the sources of intensity variation they are designed to measure. Other important features of spectrophotometers include the spectral bandwidth and linear range.

RESULTS AND DISCUSSIONS

1. Qualitative Analysis of the Nut of *Vatad (Prunus amygdalus L.)*

S.No.	Name of Minerals / Electrolyte	Results
1	Calcium	+
2	Iron	+
3	Meganese	+
4	Phosphorus	+
5	Potassium	+
6	Sulphur	+
7	Sodium	+
8	Magnesium	+

2. Qualitative Analysis of the Nut of *Vatad (Prunus amygdalus L.)*

S.No.	Name of the Heavy Metal	Results
1	Cobalt	-
2	Copper	+
3	Mercury	-
4	Nickel	-
5	Zinc	+
6	Silver	-

3. Observations for determination of total ash of Nut of *Vatad (Prunus amygdalus L.)*

S. No	Weight of the sample (X)	Weight of empty crucible (A_1)	Weight of Crucible with ash (A_2)	Percentage of total ash $[(A_2-A_1)/X] \times 100$
1.	5.00 gm	1.6997gm	45.1034gm	8.04%
2.	5.00 gm	31.1323gm	31.6124gm	9.60%
3	5.00gm	32.1949gm	32.6509gm	9.12%
4	5.00 gm	30.9173gm	31.3712gm	9.08%
5	5.00gm	46.7311gm	47.1284gm	7.95%

4. Observation of Qualitative analysis of Organic matter in *Nut of Vatad (Prunus amygdalus L.)*

S. No.	Chemical constituent	Test Applied	Result
1.	Carbohydrates	Molisch's reagent	+
2.	Reducing sugar	Fehling solution	+
3.	Starch	Iodine solution	+
4.	Tannin	Vanillin solution	+

5.	Protein	Ninhydrin solution	+
6.	Saponin	Shaking with water	-
7.	Phenol	$FeCl_3$ solution	+
8.	Glycoside	$FeCl_3$ + H2so4 solution	-
9.	Alkaloid	Dragondroff's reagent	-

5. Determination of Extractive values of *Nut* of *Vatad (Prunus amygdalus L.)*

S. No	Solvent	Weight of the drug (X)	Weight of Empty Petri dish (W_1)	Weight of Petridish+Dried extract(W_2)	Percentage of Extract $(W_2- W_1)/X$ □100
1.	Hexane	50gm	92.521gm	104.179 gm	23.32%
2.	Chloroform	50gm	20.491gm	24.235 gm	7.89%
3.	Ethyl acetate	50gm	40.571gm	42.244 gm	3.35%
4.	Acetone	50gm	40.640gm	420.816 gm	0.352%
5	Methanol	50gm	33.580gm	46.369gm	25.58%
6	Water	50gm	76.100gm	80.054gm	27.91%

6. T.L.C. of Nut of Vatad (Prunus amygdalus L.)

Hexane Extract

(Mobile phase-Solvent Chloroform:Etyleacetate::8.5:1.5,Visualization::Short Wave (254 nm) UV)

Spot No.	Distance traveled by solvent	Distance traveled by solute	RF Value
1.	7.5	1.5	0.20
2	7.5	2.5	0.33
3	7.5	5.0	0.67
4	7.5	6.5	0.87

Chloroform Extract

(Mobile phase-Solvent DCM :Tolueen::9:1,Visualization::Short Wave (254 nm) UV)

Spot No.	Distance traveled by solvent	Distance traveled by solute	Rf Value
1.	7.3	0.6	0.08
2.	7.3	1.2	0.16
3	7.3	5.1	0.69

CONCLUSION

In the present era *Ayurvedic* medicines are becoming popular. To get encouraging results from a herb and the world revving over the issues of safety and efficacy, the prescribed portion of the ingredient- the quantity and quality of the same to be expected in the final product seems to gain top priority. The first step to answer this challenge seems to be the phytochemical screening where in, routine components of the herbal vegetation is traced. The selected drug *Vatad* (*Prunus amygdalus L.*) is subjected to the phytochemical analysis, with the available facilities, the qualitative and quantitative

analysis have been done and result have been drawn.

Ash value will be great significance, because it determines the quantity of the inorganic material present in the drug and higher value is suggestive of thermo-non labile/heat stable or inorganic constituents. The average percentage of total Ash in Nut of *Vatad* (*Prunus amygdalus L.*) is 8.76 %

Extractive value is suggestive of the quantity of the constituents that can be extracted in liquid media. Hence it will be a valuable tool for the assessment of the standard drug. The substandard drugs containing a lesser amount of constituents can easily be identified. In this regard this will be a very useful and economical technique. The solubility depends on the behavior of the constituents in different media. Higher extractive value denotes more the principles. In presented work, six solvents have been used to determine the extractive values. Extractive values of the Nut of *Vatad* (*Prunus amygdalus L.*) is maximum in water and minimum in ethyl acetate.

TLC provides drug finger print. It is therefore suitable for monitoring the identity and purity of drugs and for the detection of adulteration and substitution. The TLC of hexane extract and chloroform extract was carried out by useing different conditions. The number of spots obtained in different conditions was noted and their R_f values were calculated. The Chromatography of hexane and chloroform samples under short wave radiation revealed 4 and 3 spot respectively. In *H.P.L.C study* find the six major peaks with retention time. In *UV spectrophotometer* study maximum wavelength (l_{max}) in hexane, chloroform, ethyacetate, acetone and methanol are 234.0 nm, 236 nm, 207 nm, 219.5 nm and 212.5 nm respectively.

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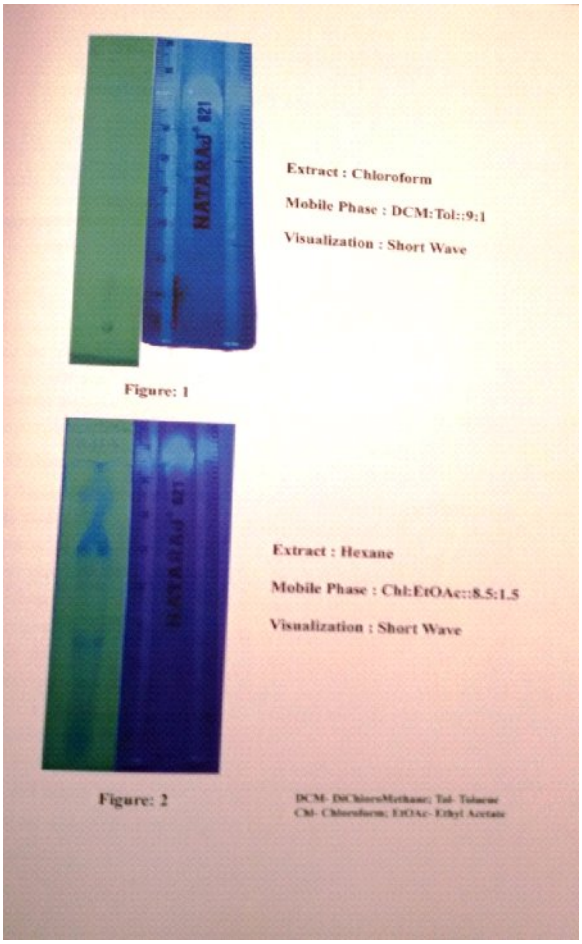
Figure1 TLC of VATAD



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