

Phytochemical Study of Akarkara Root (*Anacyclus Pyrethrum DC*)

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Abstract

In the present era *Ayurveda* medicines are becoming popular and are being used world wide on account of natural origin and lack of side effects . Commercialization of traditional medicine has led to use of adulterants and low cost substitutes . Key objectives of phytochemical study of Ayurveda herbs are standardization and quality control , identification of bioactive compounds and detection of adulterants . The process bridges ancient traditional knowledge with modern science by ensuring quality, safety and efficacy in herbs. Present study deals analysis of active phytochemicals of Root of *Akarkara (Anacyclus pyrethrum DC)*

Keyword: Ayurveda, Akarkara , *Anacyclus pyrethrum*, phytochemistry

Introduction

‘Phytochemistry’ is the scientific study of chemicals derived from plants , known as phytochemicals . Phytochemical studies of medicinal plants identify bioactive compounds such as alkaloids, flavonoids and tannins essential for developing new drugs, ensuring safety and validating traditional medicine uses .

Standardization starts right from the collection of raw materials to the extreme clinical application. In case of Ayurveda 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, organo-leptic, 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 Root of *Akarkara (Anacyclus pyrethrum DC)*

Materials And Methods

In india as akarkara two species are mostly used *Anacyclus pyrethrum* and *spilanthes acmella*. In present study Roots of *Anacyclus pyrethrum* was used .

Phytochemical Analysis-

The root contains pellitorine(also called pyrethrine) ,anacyclin, flavonoids, phenols, tannins, Inulin, sesamin and various sugars. Pyrethrine the active and intensely pungent constituent of the root is a mixture of isobutylamides and produces the sialagogue effect. Anacyclin has no sialagogue it has insecticidal effect towards the grain insects



In plants two types of phytochemical are present inorganic and organic

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 Root of *Akarkara (Anacyclus pyrethrum DC)* 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 5ml of slightly acidic distilled water and use the solution for detecting the presence of mineral elements.

- Calcium:** Take 0.5ml of test sample and 2 drops of conc. H₂SO₄. 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 1ml 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% HClO₄ solu. Formation of KClO₄ crystals indicate presence of potassium
- Sulphur:** Take 0.5 ml of test solution and 2 drops of BaCl₂ Formation of white ppt. of BaSO₄ indicate presence of Sulphur.

2. Determination of Heavy Metals:-

COBALT COMPOUNDS

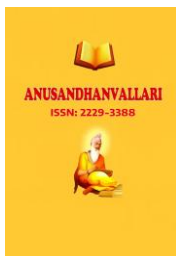
Dissolve 20 mg of the ash of the drug in about 0.5 mL of distilled water, and acidify with a few drops of dilute hydrochloric acid. Add a few drops of dilute solution of sodium hydroxide. A blue ppt is formed which turns pink on warming.

COPPER COMPOUNDS

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 COMPOUNDS

Dissolves 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 formed.

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 $450^{\circ}\text{C} \pm 5^{\circ}\text{C}$ 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

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

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

Results And Discussions

1. Qualitative Analysis of the Root of *Akarkara* (*Anacyclus pyrethrum* DC)

S. No.	Name of Minerals / Electrolyte	Results
1	Calcium	-
2	Iron	+
3	Manganese	+
4	Phosphorus	+

5	Potassium	-
6	Sulphur	+
7	Sodium	+

2. Qualitative Analysis of the Root of *Akarkara (Anacyclus pyrethrum DC)*

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 Root of *Akarkara (Anacyclus pyrethrum DC)*

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] ×100
1.	5.031 gm	32.124 gm	32.478 gm	7.03 %
2.	5.068 gm	31.962 gm	32.329 gm	7.24 %
3	5.1520 gm	37.899 gm	32.281 gm	7.41 %
4	4.993 gm	30.602 gm	30.970 gm	7.37 %
5	5.126gm	44.726 gm	45.102 gm	7.33 %

Total ash value average 7.276 %

4. Observation of Qualitative Analysis of Organic matter *Anacyclus pyrethrum* Root

Chemical constituent	Reducing sugars	Starch	Tannin	Protein	Saponin	Phenol	Glycoside	Alkaloids
Test Applied	Fehling solution	Iodine solution	Vanillin solution	Ninhydrin solution	Shaking with water	FeCl ₃ solution	FeCl ₃ +H ₂ SO ₄ solution	Dragendorff's reagent
Results	+	-	+	-	+	+	+	+

5. Determination of Extractive values:

The organic substances of the **Anacyclus pyrethrum Root** 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 and dried extract of the root powder was made separately

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

Calculations:

Weight of the drug material = Xgm

Weight of the empty petridish = W₁gm

Weight of the petridish with dried extract = W₂gm

Percentage of extractive value = $\frac{(W_2 - W_1)}{X} \times 100$

The procedure was carried out with same drug sample with different solvent taken in the order of polarity. observation of Extractive values of Root of *Akarkara (Anacyclus pyrethrum DC)*

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	93.530 gm	93.588 gm	0.116 %
2.	Dichloromethane	50gm	41.072 gm	41.287 gm	0.43 %
3.	Ethyl acetate	50gm	40.378 gm	40.630 gm	0.50 %
4.	Acetone	50gm	40.594 gm	40.870 gm	0.55 %
5	Methanol	50gm	47.276 gm	50.428 gm	06.30 %
6	Water	50gm	86.408 gm	97.051 gm	21.28 %

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 R_f value and can be compared to standard compounds to aid in the identification of an unknown substance.

Calculation of R_f Value

$$R_f = \frac{\text{Distance traveled by solute from origin line}}{\text{Distance traveled by solvent from origin line}}$$

TLC plate coated with 0.25 mm layer of silica gel GF 254 with fluorescent indicator (Mercks) were used. Each plate having dimension 10 cm long and 2 cm width



Thin layer chromatography of Root of *Akarkara* (*Anacyclus pyrethrum* DC)

Hexane Extract

(Mobile phase-Solvent Hexene: benzene::2:8, Visualization::Short Wave (254 nm) UV)

Spot No.	Distance traveled by solvent	Distance traveled by solute	Rf Value
1.	5.6	1.8	0.32
2	5.6	2.1	0.37
3	5.6	2.4	0.42
4	5.6	2.8	0.50
5	5.6	4.5	0.80

Visualization – Long wave (366 nm) UV

Spot No.	Distance traveled by solvent	Distance traveled by solute	Rf Value
1.	5.6	0.4	0.07
2.	5.6	0.7	0.12

Dichloromethane Extract

(Mobile phase-Solvent DCM 100 %, Visualization::Short Wave (254 nm) UV)

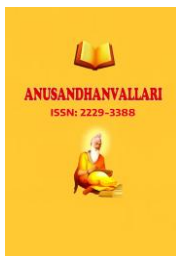
Spot No.	Distance traveled by solvent	Distance traveled by solute	Rf Value
1.	5.3	1.5	0.28
2.	5.3	2.5	0.47
3	5.3	3.4	0.64
4	5.3	4.3	0.81
5	5.3	5.0	0.94

Visualization – Long wave (366 nm) UV

Spot No.	Distance traveled by solvent	Distance traveled by solute	Rf Value
1.	5.3	0.5	0.09
2.	5.3	0.9	0.16
3	5.3	1.2	0.22

7. Spectrophotometry

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.



The photometer delivers a voltage signal to a display changes as the amount of light absorbed by the liquid changes. If development of color is linked to the concentration of a substance, in solution than that concentration can be measured by determining the extent of absorption of light at the appropriate wave length

All six successive extracts of *Anacyclus pyrethrum* root were used in concn. Of 0.5 mg/ml for spectrophotometry. The result obtained are as follows.

Spectrophotometry results showed λ max in hexane extract 236.0 nm, DCM extract 282.0 nm, Ethyl acetate 275.0 nm, acetone 335.0 nm, Methanol extract 225.0 nm and in water extract 254.5 nm.

Conclusion

With the advancement of scientific knowledge chemical study has started occupying a major role in the study of medicinal plants. It is crucial for safety, efficacy, drug discovery and validating traditional medicine.

The selected drug Root of *Akarkara* (*Anacyclus pyrethrum* DC) was taken for 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 Root of *Akarkara* (*Anacyclus pyrethrum* DC) is 7.276% (Note more than 10% API)

Extractive value is suggestive of the quantity of the constituents that can be extracted in liquid media. Extractive values of the Root of *Akarkara* (*Anacyclus pyrethrum* DC) is maximum in water and minimum in hexane.

Analysis of drug showed presence of iron, manganese, phosphorus, sulphur and sodium minerals and Heavy metals Zinc and copper.

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 Dichloromethane extract was carried out. Rf values are given in phytochemical analysis.

References

1. Ashutosh Kar -Pharmacognosy and Pharmacobiotechnology, p.19-20.
2. Nadkarni, A.K.; Indian Materia Medica, Popular Prakashan Pvt Ltd, Bombay, 3, 2312. (1998).
3. The Wealth of India Raw material. 5,101 (2001).
4. Indian materia medica (K M Nadkarni)
5. The Ayurvedic pharmacopoeia of india (Ministry of health and family welfare Govt of india)
6. Harbourn, J.B., "Phytochemical Methods- a Guide to Modern Techniques of plant analysis". Reprint 1976, Harsted press, New York, p.4-6.
7. Cooper and Gunn, In; Tutorial Pharmacy, JB Publishers, New Delhi, p.259.
8. Rawlins, E.A., Bentley's Text Book of Pharmaceutics, 8th editions, ELBS publications, New Delhi, p.180.
9. Basset, J.; Denny, J.; Jeffery, J. H.; and Mendham, J., "Vogel's Text Book of Quantitative Inorganic Analysis", 4th edition, ELBS – Longaman, Essex, UK, p.196. (1985).
10. Hebert, E., brain and Ellery, W, Kenneth, "Text Book of practical Pharmacognosy", baillore, London, p.363 (1984).



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11. Harbourne, J.B, “Phytochemical methods- A Guide to Modern Techniques of plant analysis”,2 d Edition, Ghapman and hall, London. Pp. 4-120(1984).
 12. Review of indian medicinal plants vol 2 (indian council of medical research new delhi)
 13. Indian medicinal plants (kritika and basu)
 14. Backett. A.H.; Stelaka, J.B., Practical pharmaceutical chemistry, 3rd edition, 2 CBS publishers, New Delhi; pp. 75-81