

COMPARATIVE STUDIES ON QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF ERANDA MOOLA (RICINUS COMMUNIS LINN.) IN DIFFERENT SEASON

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ABSTRACT

Ayurveda has several unique to good maintain health and cure the ailment condition like herbs preparations. Plants have served human being as a natural source for managements and therapies from earliest times, amongst them medicinal herbs have gain consideration because of its beneficial of health comprehensive use and less side effect. All medicinal plants from the most important natural resources base of the Indian. *Dravya Guna sangraha* or gathering of drug has been given prominence in the study of *dravya Guna shastra*. The *Dravya* should be collected according to Ayurvedic method. In the current years plant research has improved throughout the world and a massive amount of

evidence have been collected to show immense potential of curative plants used in various traditional system, thus in the current exploration the phytochemical analysis of *Ricinus communis* Linn. Was accepted out as these plants have been evidenced to be one of the important medicine for treatment of *Vrsya* and *Vatahara* properties. Acharya have been given suggestion of season, during which they should be gathering and specified parts to be selected for medicinal purposes will contain more micro and macro or chemical active principles. According Ayurveda and advance science the plant quantitative, qualitative and phytochemical value are affected by the *rutu*, rainy season, hit, cold, altitude, technique of cultivation, duration of day light, collection of wild area, effect of lunar cycle and variation of soil conditions. HPTLC, qualitative and quantitative analysis etc. were done as per pharmacopoeial standards and the results were documented.

INTRODUCTION

Eranda (*Ricinus communis* Linn.), of Euphorbiaceae family is a very common herbs said in ayurvedic classics from Vedic period Eranda roots mostly used in Vrshya and Vatahara ailment. *Kala* (season) are the affected all universe and decide potency and growth of the plants. Plant development and potency of plants alterations as per season (*Kala*). The collection of plant *moola* (root) in different season. According Charaka collection of plant *moola* in *Grishma*,^[1] and Raj *Nighantu* collection of plant *moola* in *Shishira ritu*.^[2]

The plant Eranda *moola* qualitative and quantitative phytochemical variation depends upon season. The human and plant both are affected by season.^[3] Present time we have to recommend this overlooked and untouched part of Ayurveda. The ecological factor like earth, temperature, rainfall, time and other factor are also effect on drug. So here an attempt is been made to find out authenticity behind the effect of diverse region on quality and action of drug which are collected from different regions and different type of soil.

Vedic and Ayurveda announces each and every materials in this world, if used for health and cure illnesses and thus has medicinal qualities.^[4] the standardization of Ayurvedic medicaments is “of broad and current interest”. Presently it has become mandatory to give due to deliberation to all the dynamics which affect the potency of the medicinal plant consideration. Calibration of drug of plant origin is need of the hour in order to approve its effective therapeutic worth and stand out in crowded global market. Calibration of this embraces their authentication climatic zones, collection season and such others. Among these site of collection of the useful part of the plant plays imperative role to assure the superiority of drug.

MATERIAL AND METHOD

Drug *Ricinus communis* Linn. Was collected from two different season for qualitative and quantitative phytochemical examination.

Collection of root (*Ricinus communis* Linn.) raw materials

A) Sample no. 1

- *Grishma* (Summer) *ritu* ---- *Sadharana desa*
- Place ---Uttar Pradesh
- The Soil is fertile alluvial soils (Sandy to clayey loam)

- The temperature start rising from May and will be on peak in June and July around 45⁰C and average temperature will be around 33.88⁰C.

B) Sample no. 2

- *Shishira* (Late winter)--- *Sadharana desa*
- Place – Uttar Pradesh
- The Soil is fertile alluvial soils (Sandy to clayey loam)
- The temperature in February to march average 19.44⁰C

Sampling of the drug

The botanically identified and authentication sample of *Eranda* roots (*Ricinus communis* Linn.), (Compared with BARO 123450010899, 10902) from Department OF Botany FACULTY OF SCIENCE, THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA Vadodara Gujrat.

Analytical study (Qualitative and quantitative), (HPTLC) study was carried out in VASU research center (A division of Vasu healthcare Pvt. Ltd) A2/624-625/2 GIDC, MAKARPURA, VADODARA-10 GUJARAT.

Sample were labeled as follow

Table no. 1

Sample collected in <i>Grishma rtu</i> (summer)	Sample No 1
Sample collected in <i>Shishira rtu</i> (late winter)	Sample No 2

Time of collection

Root of *Ricinus communis* Linn. Were collected in the month of

Sample 1—10 June 2018

Sample 2--- 12 February 2018

Method of preparation of sample

- The fresh sample of *Ricinus communis* root were taken from two different season and were dried in shade for 3 weeks.
- After that dried samples (roots two different season) breakup the small particle by the help Mortar and pestle (iron *khalva*) after were ground to fine powder distinctly with the help of electric grinder.

- The samples were carefully stored in airtight polythene bags and labeled as sample 1, sample 2 with mentioned the date of collection and season respectively.

OBJECTIVE

Phytochemical analysis of different seasonal samples of *Ricinus communis* Linn. Roots.

Phytochemical analysis^[5]

The Phyto-chemical study was carried out for the assessment of raw drug help in the identification of a drug as well as chemical constituents, qualitative and quantitative of the drug. Phytochemical study is done by partition of various content with help of chemical and physical apparatus. The physical values help in the assessment of raw drugs. The physical values of the drug can be measured by evaluate of loss on drying, Ash value, acid insoluble ash, water soluble ash etc. the extractive value in various solvent and ash value are significant in identification and standardization of single drugs.

A) Determination of moisture content (Loss of drying)^[5]

Method of determination of loss of drying

- Weight about 1.5 gm of the *Eranda* root powdered drug in to weighted flat and thin porcelain dish.
- Dry in oven at 100⁰C or 105⁰
- Cool in a desiccator and watch.
- The loss of weight is usually recorded as moisture.
- Weight of the Unfilled petri dish = W 1 gm
- Weight of the *Erand* root sample = A gm
- Weight of the Petri dish with *Eranda* root before drying (W3)= (W1+A)
- Weight Petri dish after drying =W2 gm
- Loss on drying in % =(W3-W2/A)*100

Ash value

Determination of total ash^[5]

- Two crucibles were cleaned, dried well and then weight to constant weight and labeling was made Sample 1(*grishma rtu*), Sample and Sample 2 (*Shishira rtu*).
- 5 gm (*Eranda* root) of the powdered drug sample were then weighted and placed in the silica Crucibles respectively.

- Then are kept in Muffle furnace at 450°C approximately. These are heated on burner using a flame about 2 cm. high and auxiliary the crucible about 7 cm. above the flame.
- The crucible containing the Ash were allowed to be cooled in a desiccators and weighted the samples Ash and calculated the percentage of total Ash with reference to the air dried samples of raw drug.

Calculation

Wt. of unfilled Silica Crucible = A

Wt. of sample (A) = B

Wt. of the Crucible + Ash (after complete incineration) = C

'E' g of the raw drug gives (C-A) g of the Ash

of the raw drug gives $100/E \times (C-A)$ g of the Ash

Percentage of total Ash of the samples = $100(C-A)/E\%$

Extractive values

Determination of alcohol-soluble extractive^[5]

- About 5 gm of *Eranda* root powdered drug weighted in a weighing bottle and shifted in to a dry 250 ml. tapering flask. (This process repeated in three chance because three samples are available).
- 100 ml graduated flask is filled to delivery mark with the solvent (90% alcohol). The weighing bottle is washed and poured the washings, jointly with the remainder of the solvent into the tapering flask.
- Cork the flask and set aside for 24 hours, trembling frequently.
- Filtered in to a 50 ml cylinder. After enough filtrate is collected, 25 ml of the filtrate is shifted to a weighted, thin ceramic dish.
- Evaporated to aridity on a water- bath and thorough the drying in an oven at 100°C and then chilled in a desiccators and weighted.
- The percentage of w/w of extractive with reference to the air dried drug is calculated.

Calculation

25 ml. of alcoholic extract gives = X g of residue

100 ml. of alcoholic extract gives = 4X g of residue

5 g of air dried drug (different seasonal samples) gives-4X g of alcohol (90%) soluble residue.

100 g of air dried drug (different seasonal samples) gives- 80X of alcohol (90%) soluble residue.

Alcohol (90%) soluble extractive value of the sample =80x%

Determination of water soluble extractive^[5]

- About 5 gm of powdered drug (Different seasonal samples) weighted in a weighing bottled and shifted in to a dry 250 ml. tapering flask.
- 100 ml graduated flask is complete to delivery mark with the solvent The weighing bottle is washed and poured the washings, jointly With the residue of the solvent into the tapering flask.
- Cork the flask and set aside for 24 hours, trembling frequently.
- Filtered in to a 50 ml cylinder. After enough filtrate is collected, 25 ml of the filtrate is shifted to a weighted, thin ceramic dish.
- Evaporated to dryness on a water- bath and thorough the drying in an oven at 100⁰C and then chilled in a desiccators and weighted.
- The percentage of w/w of extractive with reference to the air dried drug is calculated.

Phytochemical analysis^[5]

Alkaloids

With dragendroff's reagent

The *Eranda* root is treated with few drops of dilute 2N HCL and 0.5ml Dragendroff's reagent. Brown precipitate is attained in all two samples 1(*Grishma*), 2 (*Shishira*).

Flavonoids

If the *Eranda* root powder mix with neutral lead acetate gives yellow, orange, red or brick color precipitation. But all three different samples of *Eranda* roots are absent in this character.

Triterpenoids

Salkowski test

A red purple color appears when a chloroform solution of sterol (*Eranda* root sample) is treated with an equal volume of conc. H₂SO₄.

Present triterpenoids in all two different samples of *Eranda* roots.

Tannins

Take aqueous extract of *Eranda* root sample. Add very dilute solution of the ferric chloride, blue color changes to olive-green.

If the *Eranda* root mix with 5% lead acetate solution tannins give precipitate which turns red on addition of KOH solution on excess addition precipitate is dissolved.

Present tannin in all two different samples of *Eranda* roots.

Saponins

To an aqueous of *Eranda* root sample MG add solution of lead acetate, formation of white precipitate indicates the presence of saponins. But all two different samples of *Eranda* roots absent in this characters.

Carbohydrate**Fehling test**

To 5gm *Eranda* roots sample add equal volume of Fehling A and Fehling B mixture. Place in boiling water bath for 5-6 minutes a red precipitate formed. Present Carbohydrates in all two different samples of *Eranda* roots.

Iodine test

Acidify the *Eranda* roots sample with HCL and add 1 drop of the mixture to a solution of Iodine in KI. The formation of blue color indicates the presence of starch; a red color indicates the presence of glycogen.

Steroids

Salkowski's test: to 2 ml of chloroform extract of the drug, 1ml of concentrated H_2SO_4 was added with *Eranda* roots sample through side of the test tube.

Absent steroids in all two different samples of *Eranda* roots.

Liebermann- Burchad's test: To 1ml of petroleum ether extract of the drug in chloroform 2ml acetic anhydride solution was added with *Eranda* roots sample followed by 1ml of concentrated Sulphuric acid solution.

Absent steroids in all two different samples of *Eranda* roots.

Chromatographic techniques

Chromatography is a method in which a chemical combination carried by a liquid or gas is divided into components as result of differential spreading of the solutes as they run around or over a stationary liquid or dense phase.

High performance thin layer chromatography (HPTLC)

High- performance thin layer chromatography (HPTLC) is an enhanced.

Form of thin-layer chromatography (TLC). A number of enhancements can be made to the basic process of thin-layer chromatography to automate the various steps, to rise the resolution achieved and to allow extra perfect quantitative measurements.

Mechanization is useful to overcome the doubtful in droplet size and position when the sampled is applied to the TLC plate by hand. One recent approach to mechanization has been the use of piezoelectric devices and inkjet printers for applying the sample.

The spot capacity (analogous to peak capacity in HPTLC) can be increased by developing the plate with two dissimilar solvents, using 2 –dimensional Chromatography. The technique begins with development of sample- loaded plate with first solvent. The TLC plate which was ready for the thin layer chromatography were placed inside the HPTLC visualizing chamber under UV rays for detected of spot. Then it is shifted to the HPTLC scanner for scanning the TLC plate and auto report generation under 254, 366 and 540 nanometer.

Chromatography condition

The following chromatographic condition was established by trial and mistake and was kept constant all over the experimentation.

Qualitative analysis Observation and Result

Phytochemical constituent	Sample 1(Grishma)	Sample 2 (Shishira)
Alkaloids	++	++
Flavonoids	-	-
Steroids	-	-
Triterpenoids	+	+
Tannins	+	+++
Saponins	-	-
Carbohydrates	+	+

Quantitative analysis observation and result

S.N	Physico-chemical parameter	Sample 1	Sample 2	API
1	Loss on Drying	5.78	4.99	Not available
2	Total Ash	6.99	4.99	Not more than 8%
3	Acid insoluble Ash	0.95	1.45	Not more than 1%
4	Water soluble extractive	8.36	9.57	Not less than 9%
5	Alcohol soluble	4.37	4.68	Not less than 3%
6	Assay of Alkaloid	0.34	0.50	Alkaloid Ricinine

Hptlc (High performance thin layer chromatography) 3d overlay chromatogram @ 254nm

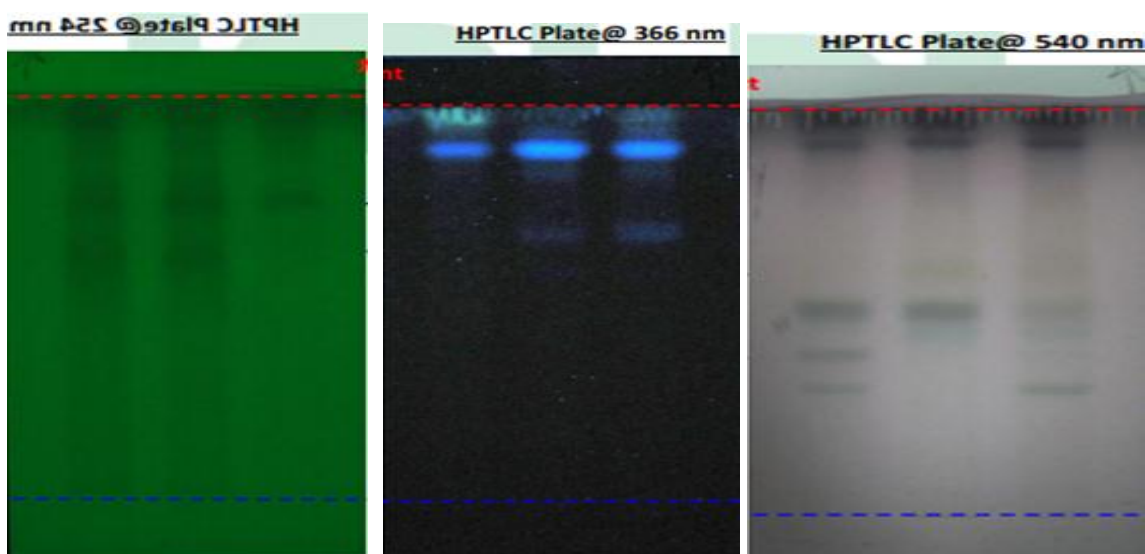
Spot no	Sample 1 (Grishma)	Sample 2 (Shishira)
1	--	0.60
2	0.74	0.74

3D overlay chromatogram@366nm

Spot no	Sample 1 (Grishma)	Sample 2 (Shishira)
1	---	0.68
2	---	0.83
3	0.90	0.90

3D Overlay chromatogram@540nm

Spot no	Sample 1 (Grishma)	Sample 2 (Shishira)
1	0.32	0.32
2	0.40	0.41
3	-	0.45
4	-	0.61
5	0.92	0.92



DISCUSSION

Phyto-chemical and physico-chemical study

The Phyto-chemical study of the drug such as loss on drying, Ash value, alcohol soluble extract, water soluble extract, high performance thin layer chromatography done as per the method mentioned in API. (Ayurvedic pharmacopeia of India) The different contents were detected with dissimilar value in all the two samples.

Total ash value

The Ash value is the occurrence of the inorganic matter in the drug on heating at exact temperature. The Ash value of two different seasonal samples 1 & 2 is found 6.99%, and 6.74% respectively. The Ash value of sample 1 (*Grishma rtu*) is highest than other samples.

Moiture content (Loss on drying)

The Moisture content signifies the presence of the water molecule in a specific drug. Here the moisture content of Sample 1 (Grishma) and Sample 2 (Shishira) is found 5.78% and 4.99% respectively. The moisture content in the sample 2, which was collected in Shishira is the peak.

Water soluble extract

The extractive worth of a drug are useful for assessment of a raw drug and gives knowledge about the nature of the chemical constituents present in a raw drug. The water- soluble extractives of the drug Sample 1 (*grishma*), sample 2 (*Shishira*) is (1) 8.36% and (2) 9.57% respectively. The sample 2 (*Shishira*) is having highest water-soluble extractive values because it is having extra quantity of chemical and active principles than the sample 1 collected in *Grishma rtu*.

Alcohol soluble extract

The study of the drug sample 1 (*Grishma*), and sample 2 (Shishira) for alcohol soluble extract shows 4.37% and 4.68% respectively. The worth of sample no 2 is greater than other samples.

The sample 2 shows higher alcohol extractive values because it is having extra quantity of chemical and active principles than sample no 1 collected in *Grishma rtu*.

Chromatographic analysis

Ricine (Alkaloid) is the active principle exist in the Root of *Ricinus communis* Linn. Which is very effective in Amavat, jwara, katishula, udara roga, act as anti-inflammatory and aphrodisiac.

The sample no 2 (*Shishira*) shows highest values of alkaloids because it is having maximum quantity of chemical and active principle than sample no 1 is collected in (*Grishma*).

Physico-chemical analysis

The preliminary phyto- chemical analysis shows the occurrence of Alkaloid, triterpenoids, carbohydrates, and tannin; The Tannin values of samples is found 1(*Grishma*) (+) 2 (*Shishira*) (+++) respectively. The tannin value of sample 2(*Shishira rutu*). Shows peak values of alkaloids because it is having extra quantity of chemical and active principle than sample no 1 is collected in (*Grishma*). Flavonoids, Steroids and Saponins are absent in all two samples

Hptlc – High performance thin layer chromatography

The TLC plate which was prepared for the thin layer chromatography were placed inside the HPTLC visualizing chamber under UV rays for observation of spot. Then it is shifted to the HPTLC scanner for scanning the TLC plate and auto report generation under 254nm, 366nm, and 544nm. The auto generation of report shows following observation in all the two samples. *Ricinoleic* acid is the active principle of the *Ricinus communis* Linn. roots the sample 2(*Shishira rtu*) shows the maximum spot present in TLC plate in different R_f values (254nm, 366nm and 540nm), hence the study show higher concentration of *Ricinoleic* acid in sample no 2 (*Shishira rtu*) than the sample no 1 (*Grishma*). Hence the study is suggested that sample no 2 is very good sample.

CONCLUSION

The sample no 2 collected in *Shishira rtu* is found better because of the extra extractive values (HPTLC) and low moisture content, high water soluble extractive values, maximum alkaloid and extra Tannin are also found in sample 2 (*Shishira*) shows extra percentage because of the occurrence of more active principles and chemical constituent. *Shishira rtu* is more powerful and found more better and superior due to occurrence of extra active principles and chemical constituent based on the Pharamacognostic and Phyto-chemical study. Hence the study suggest *Ricinus communis* Linn. Root should be collected in *Shishira*

rtu for the therapeutic and curative use. This study also represents the concept of *Shishira rtu* that it also influences the strength of the drug.

This study has proved that the *Eranda* root (*Ricinus communis* Linn.) was effected by season. Higher in *Shishira* then *varsha* while the minimum quantity was obtained in *Grishma rutu*. The study also supports the ancient method of collection of the roots as mentioned by *Raja nighantu* in *Shishira rutu*.

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