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PHARMACOGNOSTICAL AND PHYTOCHEMICAL ANALYSIS OF *RUDRAKSHA (Elaeocarpus ganitrus* ROXB.EX G.DON*)*

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ABSTRACT

Rudraksha (Elaeocarpus ganitrus Roxb.ex G.Don*)* (syn. *Elaeocarpus sphaericus*) has been in use as a herbal preparation both as preventive and curative medicine. It bears a great religious, spiritual and materialistic significance. Traditionally, the plant is used to treat various diseases such as mental diseases, hypertension, epilepsy etc. **Aim**: To evaluate pharmacognostical and phytochemical study of *Rudraksha (Elaeocarpus ganitrus* Roxb.ex G.Don*).* **Material & Method**: Fresh fruits of *Rudraksha* were collected from its authentic source and pharmacognostical and phytochemical analysis was done by following the standard protocol. **Result**: *Rudraksha* was evaluated for different standardization parameters which showed Foreign matter (0%), moisture content (6.39%), pH value (5.1), Alcohol extractive value (10.33 %), aqueous extractive value (18.81%), Total ash (1.01%), acid insoluble ash (0.29 %), water soluble ash (0.35%). Phytochemical analysis showed presence and absence of various phytoconstituents in two different extracts i.e., Aqueous and ethanol. **Conclusion**: Each plant has countless medicinal values which can be used to treat different diseases but this can be only possible if we are aware of its pharmacognostical and phytochemical properties.

KEYWORDS: *Rudraksha*, Hypertension, Epilepsy, Pharmacognostical, Phytochemical

INTRODUCTION

Herbal drugs are being used in medicine from times immemorial and the oldest available document is the "*Oushadhi Sukta"* in *Rigveda*.

In earlier times, the drugs were identified and their properties were learnt with the help of the forest dwellers, cowherds, hunters and shepherds who may have had the opportunity of witnessing their effects.[1](*Ch. Su.*1/121)

Nowadays, Analytical study of herbal drugs are carried out for the standardization of drugs. Pharmacognostical studies aim to develop a rational relationship between the chemical moieties of naturally occurring drugs and biological and therapeutic effects they generate Chemical analysis helps in determining the chemical constituents of the drug which are responsible for the drug action. Biological assays are being used for the therapeutical evaluation of the drug, where the action of the drug is observed on a healthy animal and the results are made to pathological states.

Ayurveda is the most prominent indigenous system of medicine, with historical roots in the Indian subcontinent. It is believed to have been passed on to humans from the God themselves, which dates back to more than five thousand years. The focus of *Ayurveda* is not only to promote good health but to cure ailments also.[2]

Drug *Rudraksha (Elaeocarpus ganitrus* Roxb.ex G.Don***)*** has been used by people as a folk medicine for treating different diseases like hypertension, epilepsy etc. According to Hindu mythology, *Rudraksha* beads bear a great religious, spiritual and materialistic significance.

MATERIAL AND METHODS

Fresh fruits of *Eleaocarpus ganitrus* Roxb.ex G.Don was collected from Itharna village, District- Dehradun, State –Uttarakhand in the month of July 2020. Sample was authenticated by BSI (Botanical Survey of India, specimen no. 394), Dehradun. Pharmacognostical & Phytochemical study was performed at Uttarakhand ayurveda university, Harrawala, Uttarakhand. Phytochemical screening of Aqueous and ethanolic extracts of *Eleaocarpus ganitrus* was done to detect presence or absence of various phytoconstituents like alkaloids, carbohydrates, flavonoids, phenols, saponins etc.

Chemical reagents

10% formalin solution, Glycerine, Safranin, Dilute Ferric chloride, Methylene blue, HCl, Phloroglucinol, Iodine solution, Molisch's reagent, Benedict's reagent, Barfoed's reagent, Fehling solution, Mayer's reagent, Dragendorff's reagent, Picric Acid, Nitric Acid, Ferric Chloride, Potassium Dichromate, Ninhydrin, Chloroform, Ammonia Solution, Copper sulphate, Sodium Hydroxide, Millon's reagent, Sulphuric Acid, Lead Acetate, Acetone, Benzene, etc.

Equipment, Glassware and Consumables

Distilled Water, Ethanol, Autoclave, Scale, Cotton Swab, separating funnel, Measuring cylinder, Metal holder, Spray bottle, Petri dish, Hotplate, Water bath, Beaker, Funnel, Test tube, Conical flask, Ultra-Sonicator, Volumetric flask, Crucible, Round bottom flask, T.L.C plate, T.L.C chamber, Reagent bottle etc.

❖ PHARMACOGNOSTICAL STUDY[3]

In the 19th century, the term 'Materia Medica' was used for the subject now known as "Pharmacognosy". While studying *Sarsaprilla*, it was Seydler, a German scientist, who coined the term "Pharmacognosy' in 1815. Pharmacognosy is derived from two Greek words viz. *Pharmakon* (a drug) and *Gignosco* (to acquire knowledge). It is the science having to do with the recognition of the nature and value of drugs, and more especially of drugs of plant origin. The subject matter includes the plant source, the gross and microscopic appearance of the medicinal portion of the plant and the chemical nature of its active principles.

- **Macroscopic study**

The collected sample of *Eleaocarpus ganitrus* Roxb.ex G.Don was studied organoleptically with naked eye & magnifying lens, with the help of Pharmacognostical procedure and Shape, Size, Surface, Taste, Odour and Colour findings were recorded.

- **Microscopic study**

Powder Microscopy

Procedure

✓ Sufficient amount of powder of *Eleaocarpus ganitrus* Roxb.ex G.Don and different chemical reagents on a slide was taken for examining the characters of powder. Slide was warmed over a low flame for short time. A drop of glycerine was put on the slide and covered with the cover slip. Then slide was observed under the microscope.

✓ Chemical reagents used for staining of the powder samples were as follows.

Safranin, Methylene blue, HCl Phloroglucinol.

❖ PHYSICOCHEMICAL ANALYSIS[4]

- **Determination of Foreign Matter**

The sample must be free from visible signs of mold growth, sliminess, stones, rodent excreta, insects or any other noxious foreign matter when examined as given below. A representative portion from a large container was taken and was spread in a thin layer in a suitable dish or tray. Sample was examined in day light with unaided eye. Suspected particles, if any, were transferred to a petri dish and examined with 10x lens in day light**.**

- **Determination of pH value**

5 gm of powdered drug was taken in a beaker with 50 ml fresh distilled water. It was stirred well with the help of a glass rod and allowed to stand for 2 hours. It was then filtered and pH was determined using pH meter at 25 degrees Celsius.

- **Determination of Moisture Content**

Moisture content is a water holding capacity of sample, higher moisture content in sample show that it may decrease stability.

Moisture content was determined by placing weighed *Eleaocarpus ganitrus* Roxb.ex G.Don of 5gm of drug in oven at 105ºC for 5 hours, and weight of sample was calculated for every 30 minute, until the weight of the samples came out to be constant, no variation of weight was recorded. This sample was allowed to cool at room temperature in a desiccator for 1 hour before weighing.

Calculation

Weight of the empty petridish = W_1gm

Weight of the drug sample = X gm

Weight of the petridish with drug before drying (W_3) = (W_1 + X)

Weight of petridish after drying = W_2gm

Loss on drying in % = W_3-W_2x100/X

- **Determination of Extractive values**

It is a gravimetric analysis (Maceration Process), the extraction of any crude drug with a particular solvent yields a solution containing different phytoconstituents. The composition of these phytoconstituents in that particular solvent depends upon the nature of the drug and solvent used.

➢ **Determination of Alcohol Soluble Extractive:**

5 gm coarsely powdered air-dried drug was macerated with 100 ml of Alcohol of the specified strength in a closed flask for twenty-four hours. It was then continuously shaken for six hours using rotary shaker and allowed to stand for eighteen hours. The content was filtered using filter paper. The filtrate was transferred to a pre-weighed flat-bottomed dish and evaporated to dryness on a water bath. Then the dish was kept in oven at 105ºC, to constant weight and weigh. The percentage of alcohol-soluble extractive was calculated with reference to the air-dried drug.

Calculations

Weight of the drug material = X gm

Weight of the empty petridish = W_1gm

Weight of the petridish with dried extract = W_2gm

Percentage of extractive value =W_2-W_1x100/X

The procedure was repeated three times and the mean value was calculated.

➢ **Determination of Water-Soluble Extractive**

Procedure was same as that of alcohol soluble extractive value and it was proceeded using distilled water instead of alcohol.

I. Determination of Total Ash

Ash is a quantity analysis technique for determining siliceous material and inorganic substance in sample. Acid Insoluble Ash shows siliceous material and heavy metals. Water Soluble Ash shows quantity of water inorganic Substance.

The total ash method is designed to measure the total amount of material remaining after ignition. This includes both physiological ash which is derived from the plant tissue itself and non-physiological ash which is the residue of the extraneous matter (e.g., sand and soil) adhering to plant surface.

Silica Crucible was cleaned, dried well, labelled with glass pencils and then weighed to constant weight. 3 gm of powdered drug sample (*Elaeocarpus ganitrus* Roxb.ex G.Don) was put in the Silica crucible. The drug was spread evenly in to a thin layer. This crucible was placed in a muffle furnace and ignited at a temperature of 450°C for about 6 hrs or more until the ash was totally free from Carbon. The crucible containing the ash was allowed to be cooled in desiccators and subsequently weighed to constant weight. The percentage of ash with reference to the air-dried drug was calculated.

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 = [A_2 - A_1 /X] x 100

II. Determination of Water-soluble Ash

Water – soluble ash value determined as per Pharmacopoeia of India 1996. Boiled the total ash for 5 minutes with 25 ml of water; collected the insoluble matter in a Gooch's Crucible or on an ash less filter paper, Washed with hot water and ignite for 15 minutes at a temperature not exceeding 450^0C. Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight represented the water – soluble ash. Calculate the percentage of water – soluble ash with reference to the air - dried drug.

Calculation

Wt. of drug sample = X gm

Wt. of total ash = A gm

Wt. of Crucible = G1 gm

Wt. of Crucible with insoluble Ash = G2 gm

Wt. of insoluble ash (G3) = G2-G1

Water soluble ash (G4) = Wt. of Total Ash A gm- Wt. of insoluble Ash (G3)

Percentage of water-soluble Ash = A – [(G3)/X] x 100

I. Determination of Acid Insoluble Ash

Acid insoluble Ash value determined as per Pharmacopoeia of India, 1996. Boiled the total ash with 25 ml of 2M hydrochloric acid for 5 minutes, collected the insoluble matter in a Gooch crucible or on an ashless filter paper, washed with hot water, ignite, cool in a

desiccator and weighed. Calculate the percentage of acid - insoluble ash with reference to the air - dried drug.

Calculation

Wt. of drug sample - X gm

Wt. of Crucible = G1 gm

Wt. of Crucible with insoluble Ash = G2 gm

Wt. of insoluble ash (G3) = G2-G1

Percentage of acid insoluble ash = G3/X×100

❖ PHYTOCHEMICAL ANALYSIS[5]

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. A plant cell produces two types of metabolites: primary metabolites involved directly in growth and metabolism (carbohydrates, lipids and proteins), and secondary metabolites not involved in metabolic activity (alkaloids, phenolics, sterols etc) but act as defence chemicals.

Drug samples were tested for the presence of various active phytocompounds like phenols, tannins, flavonoids, proteins, reducing sugar, carbohydrates, lipids, saponins, alkaloids.

I. Tests for Carbohydrates

- Molisch's test
- Benedict's
- Fehling solution test
- Iodine test

Molisch's test

2 ml of test Solution was taken in a test tube and 2 ml of the Molisch's reagent was added and shaken carefully and then about 1ml. of conc. H_2SO_4 is poured from side of the test tube and allowed to stand for one 1 minute. A Purple colour ring at the junction of the two layers if formed indicated the presence of Carbohydrate.

Benedict's test

It is used for reducing sugars and composed of mainly Copper sulphate and sodium hydroxide. To the 4 ml of aqueous solution of drug, 1 ml of Benedict's solution was added and heated almost to boiling. Formation of green, yellow, orange, red or brown colour in

order of increasing concentrations of simple sugar in the test solution, due to formation of cuprous oxide.

Fehling solution test

It is generally used for reducing sugars and composed of two solutions, which are mixed in situ. Fehling solution A composed of 0.5% of copper sulphate whereas Fehling solution B composed of Sodium Potassium tartrate.

Equal volumes of Fehling A and Fehling B solutions were mixed (1 ml each) and 2 ml of aqueous solution of drug was added followed by boiling for 5-10 minutes on water bath.

Iodine test

1 drop of Iodine solution was added in 2 ml of test sample. Presence of blue colour shows Polysaccharide.

II. Tests for Alkaloids

- Mayer's reagent test
- Dragendorff's test

Mayer's reagent test

2 ml of test Solution was taken in a test tube to which and 2 ml of the Mayer's reagent (Potassium Mercury Iodide solution) was added. A White or Pale Yellow precipitate if formed indicated presence of Alkaloids except with Alkaloids of the Purine groups and few others.

Dragendorff's reagent test

2 ml of test Solution was taken in a test tube in which 2 ml of the Dragendorff's reagent (Mixture of Potassium Iodide and Bismuth sub nitrate solution) was added. An orange precipitate if formed indicated presence of Alkaloids.

III. Test for Amino acid

Ninhydrin test

The Ninhydrin test is used to detect the presence of alpha-amino acids and proteins containing free amino groups. Protein solution when heated with ninhydrin molecules, it gives characteristic deep blue or pale yellow colour due to the formation of complex between two ninhydrin molecule and nitrogen of free amino acid.

IV. Tests for Proteins

- Biuret test
- Millon's test
- Xanthoproteic test

Biuret test

A few mg of the residue was taken in water and 1 ml of 4% sodium hydroxide solution was added to it, followed by a drop of 1% solution of copper sulphate. Development of violet or pink colour indicates the presence of proteins.

Millon's test

A small quantity of test sample was taken and 2 to 3 ml of millon's reagent was added. The white precipitate slowly turning to pink, indicate the presence of proteins.

Xanthoproteic test

A small quantity of test sample was taken with 2 ml of water and 0.5 ml of Conc. Nitric acid was added to it. Appearance of Yellow colour indicates the presence of protein.

V. Test for saponin

Foam test

A small quantity of the test sample was taken in a test tube and shaken vigorously with a small amount of sodium bicarbonate and water. A stable, characteristic honeycomb like froth indicates the presence of saponins.

VI. Test for glycosides

- Borntrager's Test
- Modified Borntrager's Test
- Killer-killani Test
- With dilute HNO_3

Borntrager's Test

1 ml of Benzene and 0.5 ml of dilute ammonia solution was added to the ethanolic extract and was observed for the formation of reddish pink colour.

Modified Borntrager's Test

2-3 drop of 5% $Fecl_3$ & 5ml of dilute HCL was added in 3ml of test sample. Heat for 5 mint & shake then add dilute ammonia. Ammoniacal layer shows pinkish red colour.

Killer-kilani Test

Add 0.4 ml of glacial acetic acid and a few drops of 5% ferric chloride solution to a little of drug extract. Further add 0.5 ml of Conc. $H2SO_4$. The formation of blue colour in acetic acid layer confirmed the test.

With dilute HNO_3

2-3 drop of test sample was added in 1ml dilute HNO_3. Yellow precipitate shows presence of Glycoside.

VII. Test for Lipid & Fat

Greasy spot test

Take 1 drop of test sample in filter paper for different solution. Oily spot indicates presence of Lipid & Fat.

VIII. Test for Flavonoids

Shinoda's test

A small quantity of test sample was dissolved in 5 ml ethanol (95%v/v) and reacted with few drops of concentrated hydrochloric acid and 0.5 gm of magnesium metal. Appearance of pink, crimson or magenta colour within a minute or two indicates the presence of flavonoids.

IX. Test for Steroids

Salkowski's reaction

Few mg of extract was taken in 2 ml of chloroform and 2 ml of concentrated sulphuric acid was added from the side of test tube. The test tube was shaken for few minutes. The development of red colour indicates the presence of steroids.

X. Test for Tannins

- Ferric chloride solution
- Lead acetate
- Dilute HNO_3 test

Ferric chloride solution

A 5 percent solution of ferric chloride in 90 % alcohol was prepared. Few drops of this solution were added to a little of the above filtrate. Appearance of dark green or deep blue colour indicates the presence of tannins.

Lead acetate

A 10 percent w/v solution of basic lead acetate in distilled water was added to the test filtrate. Reddish brown bulky precipitate indicates of the presence of tannins.

Dilute HNO_3 test

2-3 drop of dilute HNO_3 was added in 2 ml of test sample. Presence of reddish to yellow colour indicate tannin.

XI. Test for Phenolic Compound

The extract was taken in water and warmed; to this 2 ml of ferric chloride solution was added and observed for the formation of green and blue colour.

❖ CHROMATOGRAPHIC STUDY

Thin layer Chromatography

Thin layer Chromatography is a tool for separation and identification of chemical constituent. Thin-layer chromatography is a technique in which a solute undergoes distribution between two phases, a stationary phase acting through adsorption and a mobile phase in the form of a liquid. The adsorbent is a relatively thin, uniform layer of dry finely powdered material applied to a glass, plastic or metal sheet or plate. Glass plates are most commonly used. Separation may also be achieved on the basis of partition or a combination of partition and adsorption, depending on the particular type of support, its preparation and its use with different solvent.

Identification can be affected by observation of spots of identical Rf value and about equal magnitude obtained, respectively, with an unknown and a reference sample chromatographed on the same plate. A visual comparison of the size and intensity of the spots usually serves for semi-quantitative estimation.

➢ Chromatography plates

T.L.C. plate coated with 0.25 mm layer of silica gel 60 F_{254} with fluorescent indicator was used. (Each plate dimension is 10 cm long and 2 cm width)

➢ **Activation of pre-coated Silica gel 60 F_{254}**

Plates were dried in hot oven at 105^0 C for one and half hour.

➢ **Preparation of mobile solution**

Toluene: Ethyl Acetate: Formic acid (6:3.5:0.5)

➢ **Preparation of test solution**

4 gm powdered drugs were extracted with 100 ml of ethanol (90 percent) in a Soxhlet apparatus consecutively three times. Extract was filtered and concentrated to 10 ml.

➢ **Sample application**

Samples were applied with the help of capillary 1(one) cm above the base of T.L.C. plate. Then it was dipped in mobile solution. T.L.C. plate was removed from the mobile solution immediately after the spot reached the 1(one) cm below the top of the T.L.C. plate.

➢ **Visualization**

Iodine Vapour

➢ **R_f Value**

Measured and recorded the distance of each spot from the point of its application and calculated R_f value by dividing the distance travelled by the spots by the distance travelled by the front of the mobile phase.

➢ **Calculation of R_f Value**

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

OBSERVATIONS AND RESULT

❖ **Pharmacognostical study**

1. Organoleptic study

Character	**Seed of *Elaeocarpus ganitrus* Roxb.ex G.Don**
Shape	Spherical, stony, obovoid or oval
Size	1 or 2 cm.
Surface	Longitudinally grooved, tubercled
Colour	Brown
Texture	Hard
Odour	Aromatic
Taste	Sour

2. **Powder Microscopic study:** Powder microscopy showed the presence calcium oxalate crystals, fibres, cork cells, sclereids, starch, vessels.

❖ **Physicochemical Analysis**

Test	Result	API
Foreign matter	0%	Not mentioned
Moisture Content	6.39%	Not Mentioned
pH Value	5.1	Not Mentioned
Alcohol Extractive Value	10.33%	NLT 2%
Aqueous Extractive Value	18.81%	NLT 1%
Total Ash	1.01%	NMT 1.2%
Acid Insoluble Ash	0.29 %	NMT 0.4%
Water soluble Ash	0.35%	Not Mentioned

❖ **Phytochemical Analysis**

Phytoconstituents	Aqueous extract	Ethanol extract
Carbohydrate	+ve	-ve
Alkaloids	+ve	+ve
Amino acids	-ve	+ve
Protein	+ve	-ve
Saponin	+ve	-ve
Glycosides	-ve	+ve
Tannins and Phenolic compound	+ve	+ve
Steroids	+ve	-ve
Lipid and Fat	+ve	+ve
Flavonoids	+ve	+ve

❖ **Chromatographic Study**

Mobile solution:

Toluene: Ethyl Acetate: Formic acid (6:3.5:0.5)

Sample: Ethanol Extract

Visualization: Iodine Vapour

Sample	Visualization	No. of spots	R_f Values
Elaeocarpus ganitrus Roxb.ex G.Don	Iodine Vapour	07	0.41, 0.45, 0.52, 0.57, 0.64, 0.71, 0.84

DISCUSSION

Pharmacognostical study of *Rudraksha* was done through standard protocol as mentioned in API. *Elaeocarpus ganitrus* studied organoleptically in which different features like shape, size, surface, colour, odour, taste were studied. Powder microscopy showed Calcium oxalate crystals, Fibres, cork cells, Sclerides, Starch and Vessels. In Physicochemical analysis,

Moisture content of *Elaeocarpus ganitrus* Roxb.ex G.Don was 6.39%. Moisture is one of the major factors responsible for the deterioration of drugs. Extractive value helps to indicate the nature of chemical constituents present in the drug and also helps in the identification of adulterants. Water soluble extractive value is applied for the drugs which contain water soluble constituents such as tannins, sugars, mucilage. Alcohol soluble extractive value is applied for the drugs which contain alcohol soluble constituents such as tannins, resins, alkaloids and glycosides. Water soluble extractive value of *Elaeocarpus ganitrus* Roxb.ex G.Don was 18.81%. This means it is more effective in polar vehicle than non-polar one. Alcohol extractive value of *Elaeocarpus ganitrus* Roxb.ex G.Don was 10.33%. Total ash value of *Elaeocarpus ganitrus* Roxb.ex G.Don was 1.01%. Ash is an inorganic residue that indicates total amount of minerals present. Thus, ash value is a valid parameter to assess the degree of purity of a given drug. Acid insoluble ash value of *Elaeocarpus ganitrus* Roxb.ex G.Don was 0.29%. Acid insoluble ash value indicates silica contamination. Water soluble ash value of *Elaeocarpus ganitrus* Roxb.ex G.Don was 0.35%. In phytochemical study, Aqueous extract of *Elaeocarpus ganitrus* Roxb.ex G.Don showed presence of carbohydrates, alkaloids, protein, saponin, tannins and phenolic compound, steroids, flavonoids and lipid and fat. Ethanol extract of *Elaeocarpus ganitrus* Roxb.ex G.Don showed the presence of alkaloids, amino acids, glycosides, tannins and phenolic compounds, flavonoids, lipid and fat.

CONCLUSION

Pharmacognosy of medicinal plants is very important to confirm their identity, quality, purity, potency, safety and efficacy. The present study gives imaginative information related to pharmacognostical and phytochemical study of *Rudraksha* which will be helpful in its standardization.

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