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Elaeocarpus ganitrus: Competence of Antioxidant Assets by In-Vitro Evaluation Study



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ABSTRACT

In list, Ethnomedicine Elaeocarpus ganitrus (Family: Elaeocarpaceae) have highly ornate stony endocarp known as bead or nut and regularly termed known as Rudraksha in India. It's having huge pharmacology activities be like analgesic, anti-inflammatory, CNS activities, behavioral actions, sedative, tranquillizing, hypnosis antiasthmatic, potentiation, antidepressant, hydrocholeretic, antidiabetic, stress, anxiety, depression, palpitation, nerve pain, epilepsy, migraine, lack of concentration, asthma, hypertension, arthritis and liver diseases. It's also having one of the rare elementary antioxidant properties. Oxidation is a chemical reaction that transfers electrons from one molecule to an oxidizing agent. Oxidation reactions are known to produce free radicals. Oxidative stress plays a key role in causing various human diseases. Antioxidant defenses induction or endogenous ROS/RNS levels reduction is a rapid and clear oxidative stress indicator. In this research work identification of bark Phytochemical Elaeocarpus ganitrus, screening Petroleum, Phytochemical screening of Chloroform, Phytochemical screening of Methanol is performed. The Elaeocarpus ganitrus bark methanolic extract showed significant antioxidant activity of standard ascorbic acid. And in the overall extract, methanolic extract was exhibited effective antioxidant activities.

INTRODUCTION

Table No. 1: Elaboration of *Elaeocarpus ganitrus*

Elaeocarpus ganitrus	
	The flowers of Rudraksha are white with fringed petals and they
	come into view in April-May. The fruits of Rudraksha come in
	June and ripen by August-October. Rudraksha is bulbous in
	shape with a fat outside. Rudraksha beads are enclosed by an
	outer shell of blue color on fully ripening. Hence Rudraksha
Morphology	beads are also called blueberry beads. The tree starts giving fruit
	after 7 years and fruits for a long time after the 7-year period. A
	single Rudraksha tree bears beads in all different faces or
	mukhis at the same time, the higher mukhis or faces are very
	rare. The most common Rudraksha bead is the five faceted or
	panchmukhi.
	Chattu sampangi, Rudraksha, Bhutnasan, Rudraki, Rudraksha
	Utrasum Bead Tree, Wooden Begger bead, Rudraksha,
Synonym	Rudrakya, Rudrakshi mara, Rudraksh, Rudraksha, Rudraksam,
Synonym	Rudraksha — — — — — — — — — — — — — — — — — — —
	Rudrai, Ludrok, Udrok, Sivaksha, Sarwaksh, Paawan,
	Nilkanthaksha, Haraksha, Sivpriye.
	Elaeocarpus sphaericus commonly known as a rudraksha is a
	large evergreen broad-leaved tree found in tropical and
	subtropical areas at an altitude ranging from seacoast to 2,000
	meters above sea level. The Elaeocarpus consists of about 12
	genera and 350 species of trees and shrubs and is distributed in
Geographical Source	the tropical and subtropical regions but mainly it has about 36
Geographical Source	sister species including rudraksha. The Rudraksha tree inhabits
	areas starting from Manila, Philippines, and passing through
	Myanmar to entire North-East India, Bangladesh, Nepal, and
	Bhutan. However, in the present era, the Rudraksha tree is
	localized only in Eastern Nepal due to suitable climatic
	conditions. Around 70% of the Rudraksha trees are found in

Species	Indonesia, 25% in Nepal, and 5% in India. Considered a major stress reliever, reducing circulatory problems and of course, as the best beads, the berry (Elaeocarpus ganitrus) was first spotted in Indonesia and is now grown in Nepal and Hardware. The tree is considered a threatened plant in the northeastern region of India. Elaeocarpus aberrans, Elaeocarpus acuminatus, Elaeocarpus amoenus, Elaeocarpus angustifolius, Elaeocarpus apiculatus, Elaeocarpus blascoi, Elaeocarpus coorangooloo, Elaeocarpus coriaceus, Elaeocarpus crassus, Elaeocarpus dentatus, Elaeocarpus eumundii, Elaeocarpus ganitrus rudraksh tree, Elaeocarpus gaussenii, Elaeocarpus grandifloras, Elaeocarpus hartleyi, Elaeocarpus hedyosmus, Elaeocarpus williumsianus, Elaeocarpus variabilis, Elaeocarpus timikensis, Elaeocarpus taprobanicus, Elaeocarpus sylvestris, Elaeocarpus stipularis,
	Elaeocarpus sikkimensis, Elaeocarpus serratus, Elaeocarpus robustus, Elaeocarpus obovatus, Elaeocarpus neobritannicus, Elaeocarpus photiniaefolius, Elaeocarpus montanus, Elaeocarpus miegei, Elaeocarpus lanceifolius.
Types	One Face Rudrakasha, Two Faces Rudrakasha, Three faces Rudrakasha, Four Faces Rudrakasha, Five Faces Rudrakasha, Six Faces Rudrakasha, Seven Faces Rudrakasha, Eight Faces Rudrakasha, Nine Faces Rudrakasha, Ten Faces Rudrakasha, Eleven Faces Rudrakasha, Twelve Faces Rudrakasha, Thirteen Faces Rudrakasha, Fourteen Faces Rudrakasha, Fifteen Faces Rudrakasha, Sixteen Faces Rudrakasha, Seventeen Faces Rudrakasha, Sixteen Faces Rudrakasha, Nineteen Mukhi Rudrakasha, Twenty Mukhi Rudrakasha, Twenty One Mukhi Rudrakasha, Trijuti/Tribhagi Rudrakasha, Gauri Shankar Rudrakasha, Ganesh Rudrakasha
Chemical Constitutes	Elaeocarpidine, isoelaeocarpine, epiisoelaeocarpiline, rudrakine, flavonoids, quercetin, phytosterols, fat, alkaloids, carbohydrates,

	ethanol, protein, tannins, gallic acid, ellagic acid				
Bioavailability effects	Stomach ache, stress, skin diseases and anxiety, Hormonal inequality in the body, mental insecurity and whooping cough, Muscular dystrophies, Brain related and many other types of disease, Epilepsy, and gynecological problems, Memory lapse and body functional disorders, Mental harmonization, and loss of power, Sexual and behavioral disorders.				
Potential effect by chitosan-based aqueous extract Elaeocarpus ganitrus by producing a hypoglycemic effect normal rats. At 100 mg/kg body weight doses of given ora formed clinically significant hypoglycemia aqueous extract a 200mg/kg dose are comparable with the standard antidiabe drug glimeperide 20mg/kg dose body weight, ethanol extractiv were shown effective against anxiety at all doses, but a dose 200 mg/kg of ethanol extractive was at equality with that diazepam as clear from the statistical equivalence between results of this dose and that manifested by diazepam fru extracts of Elaeocarpus sphaericus at a dose level of 200 mg, body weight was studied in rat paw edema using differ					
Pharmacological activities	Antihypertension activity, Anti-inflammatory activity, Antimicrobial activity, Antiulcerogenic activity, Antioxidant activity, Cytotoxic activity, Antidiabetic activity, Antianxiety activity, Antidepressant activity, Antiasthmatic activity, analgesic, anti-inflammatory, CNS activities, typical behavioral actions, sedative, tranquillizing, hypnosis potentiation, antiasthmatic.				

MATERIALS AND METHODS

Collection of material

The *Elaeocarpus ganitrus* was the 'ARANYA BHAWAN CG FOREST HEAD QUATERS' Police Colony, Moudhapara, Raipur, Chhattisgarh, India.

Identification of material

Identification: The plant studies of the plant were identified by Dr. Praveen Kumar Joshi,

Head of the Department of Botany from Shri Narayan Prasad Awasthi Govt. Ayurved

College, Raipur (C.G.). Authentication Reference No. SRIP/Research-63/2018-19/157.

Material: *Elaeocarpus ganitrus* bark

Require Quantity: 150 gm

Extraction of *Elaeocarpus ganitrus*

1. Firstly, *Elaeocarpus ganitrus* bark was powdered coarsely.

2. The powdered *Elaeocarpus ganitrus* bark was weighed accurately (100g) and extracted

with petroleum ether at 50-55°C by using a hot extraction process with the plant of Soxhlet

apparatus for 12-15 Hours prior to removing fatty substances from the plant material.

3. After 12-15 hours the marc obtained from the extraction process was methanol at this

time using Soxhlet apparatus for 18-2 hours or until the color of the resin faded completely.

4. The extract was concentrated up to 1/4th of its original volume; a brown semi-solid mass

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was obtained.

Organoleptic Evalution [18]

The morphology of the crude drug was determined by sensory organs i.e. consistency, size,

shape, nature, color by 1% solution of both the drugs was determined by using a digital pH

meter.

Table No. 2: Identification Procedure of Physicochemical properties

Identification Procedure of Physicochemical properties			
Properties	Procedure		
Foreign organic matter	50g of the <i>Elaeocarpus ganitrus</i> was weighted accurately. The individual sample was spread on a glass plate uniformly without overlapping. The sample was inspected with naked eyes. After complete separation, the matter was weighed and % w/w present in the sample was determined.		
LOD (Loss of drying) or Moisture content	1.5g of the powdered drug was weighed accurately into a porcelain dish. It was dried in an oven at 100°C or 105°C till a constant weight was achieved. It was cooled in a desiccator. The loss in weight was recorded as moisture.		
Total Ash Value	A thin porcelain dish was weighed and ignited. 2g of the airdried powdered crude drug was weighed into the porcelain dish. The porcelain dish with the drug was heated, till vapors almost ceased and heated more strongly until all the carbon was burnt off. It was cooled until in a desiccator. The ash obtained was weighed and the percentage yield of the total ash with reference to an air-dried sample of the crude drug was calculated.		
Acid Insoluble Ash Value	Thin porcelain dish weighed and ignited. 2g of the air-dried powdered crude drug was weighed into the porcelain dish. The porcelain dish with drug heated, till vapors almost ceased and heated more strongly until all carbon was burnt off. It was cooled in a desiccator. The ash obtained was weighed and the percentage yield of the total ash with reference to an air-dried sample of the crude drug was calculated. Using 25 ml of dilute hydrochloride acid, it was washed from the dish used for the total ash into a 100ml beaker. The resulting mixture was boiled for minutes. The boiled mixture was filtered with an "ashless" filter paper, the residue was washed twice with hot water. Crucible was ignited in the flame, cooled, and weighed. The filter paper and residue were put together in the crucible; it was		

	heated gently until vapor ceased completely and then heated
	more strongly until all carbon had been removed. It was cooled
	in desiccators. The residue was weighed and acid-insoluble ash
	of the crude drug was calculated.
	The porcelain dish was weighed and ignited. 2g of the air-dried
	powdered crude drug was weighed into the porcelain dish. The
	porcelain dish with the drug was heated, till the vapors almost
	ceased and heated more strongly until all carbon was burnt off.
	It was cooled in desiccators. The ash obtained was weighed
	and the percentage yield of the total ash with reference to an
	air-dried sample of the crude drug was calculated. Using 25 ml
	of distilled water, the ash was washed from the dish used for
Water Soluble Ash	total ash into a 100 ml beaker. The resulting mixture was
Value	
	boiled for five minutes. The boiled mixture was filtered with
	an "ashless" filter paper; the residue was washed twice with
	hot water. Crucible was ignited in the flame, cooled, and
	weighed. The resulting filtrate was taken in the crucible; it was
	heated gently until vapor ceased completely. Crucible with
	residue was cooled in desiccators.
	The residue was weighed and water-soluble ash of the crude
	drug was calculated.
	1g of the drug taken in a 25 ml stoppered cylinder. Water was
Swelling Factor	added to make the volume up to 25 ml marking. It was shaken
~ " •g = w•••	occasionally for 23 hours. It was kept for one hour. The
	volume occupied by the swollen crude was measured.
	5g of the powdered drug was weighed in a weighing bottle and
	transferred to a dried 250 ml. conical flask. A 100 ml.
	graduated flask was filled up the delivery mark with distilled
Water Soluble	water. The weighing bottle was washed; the washing was
Extractive Value	poured together with the remaining solvent into the conical
	flask. The flask was corked and set aside for 24 hours with
	frequent shaking. The resulting mixture obtained from the
	maceration solution was filtered into a 50 ml cylinder. When

sufficient filtrate had been collected, 25ml, of the filtrate was transferred to a weighed, thin porcelain dish. The filtrate was evaporated to dryness on a water bath and drying was completed in an oven at 100°C. It was cooled in a desiccator and weighed. The percentage w/w of the extractive to the airdried drug was calculated.

Alcohol Soluble Extractive Value

5g of the powdered drug was weighed in a weighing bottle and transferred to a dried 250 ml. conical flask. A 100 ml graduated flask was filled up the delivery mark with methanol. The weighing bottle was washed; the washing was poured together with the remaining solvent into the conical flask. The flask was corked and set aside for 24 hours with frequent shaking. The resulting mixture obtained from the maceration solution was filtered into a 50 ml cylinder. When sufficient filtrate had been collected, 25ml, of the filtrate was transferred to a weighed, thin porcelain dish. The filtrate was evaporated to dryness on the water-bath and drying was completed in oven 100°C. It was cooled in a desiccator and weighed. The percentage w/w of the extractive to the air-dried drug was calculated.

Phytochemical screening

Preliminary phytochemical screening was carried out by using the following method;

Test for Carbohydrates:

a) Molisch's Test:

A few drops of α -naphthol solution in methanol were added to 2-3 drops of Methanolic extract. The resulting solution was shaken and concentrated H_2SO_4 was added from the sides of the test tube. A violet-colored solution or violet-colored ring was formed at the junction of two liquids.

b) Fehling's Test:

1ml of Fehling's A and 1ml of Fehling's B solution was mixed and boiled for one minute. An equal amount of the methanolic solution was added to the above solution. The resulting solution was heated in a boiling water bath for 5-10 minutes. Firstly yellow, then the brick-red precipitate was observed. The appearance of the brick red color indicates the presence of reducing sugar.

c) Benedict's test:

An equal volume of Benedict's reagent and the test solution was mixed in a test tube. The resulting mixture was heated in a water bath for 5 minutes. After 5 minutes solution was observed for the formation of the reddish-brown color precipitate, indicating the presence of the carbohydrates.

Test for Glycosides:

a) Killer Killani Test (Test for deoxysugars):

To 2ml. of Methanolic extract, a few drops of glacial acetic acid, 1drop of FeCl₃, and H₂SO₄ were added. The reddish-brown color was observed at the junction of the 2 liquid layers, and the upper layer appeared bluish-green.

b) Borntrager's Test (Test for anthraquinone glycosides):

To 3ml. of methanolic extract dilute H₂SO₄ was added. The resulting mixture was boiled and filtered then the filtrate was cooled. An equal volume of benzene or Chloroform was added and shaken well. The organic solvent was separated and ammonia was added. The ammonical layer turns pink or red in color.

Test for Tannins and Phenolic compounds:

a) FeCl₃:

To 2ml. of the methanolic extract, a few drops of FeCl₃ solution were added. The solution color was turned blue-black color or there was an appearance of blue-black color.

b) Bromine water test: To 2ml. of methanolic extract, a few drops of bromine water were added. There was discoloration of the bromine water.

- c) Lead Acetate test: To 2ml. of the methanolic extract, a few drops of Lead Acetate solution (5%) were added. The white precipitate was observed.
- **d) Acetic Acid solution test**: To 2ml. of the methanolic extract, a few drops of dilute Acetic An acid solution was added. The red color of the solution was observed.
- e) Dilute HNO₃ Test: To 2ml. of the methanolic extract, a few drops of the dilute HNO₃ solution were added. Reddish-yellow color of the resulting solution was observed.
- **f) Dilute Iodine Solution Test:** To 2ml. of the methanolic extract, a few drops of the dilute solution of Iodine (5%) were added. The red color of the resulting solution was observed.
- **g) Dilute Potassium Permanganate Solution:** To 2ml. of the methanolic extract, a few drops of dilute potassium permanganate (10%) were added. Discoloration of potassium permanganate solution was observed.
- h) NaCl Solution Test: The test solution was treated with 10% sodium chloride solution and it was observed for the cream color.

Test for Terpenoids and Steroids:

a) Salkowski: To 2ml. of the methanolic extract, 2ml. of chloroform was added, mixed well, and then 2ml. of concentrated H₂SO₄ was added. It was shaken vigorously, the chloroform layer showed red color, and the acid layer was shown greenish-yellow fluorescence.

Test for Alkaloids:

- a) Mayer's Reagent Test: To 2ml. of the methanolic extract, a few drops of Mayer's reagent were added; addition of Mayer's reagent was given precipitate.
- **b) Dragendorff's Test**: To 2ml. of the methanolic extract, a few drops of the Dragendorff's reagent were added. There was a formation of an orange-brown precipitate.
- c) Hager's Test: To 2ml. of the methanolic extract, a few drops of the Hager's reagent were added. It gave yellow precipitate.

Test for Saponins:

a) Foam Test: 2ml. methanolic extract of the crude drug was shaken vigorously with water, persistence foam was formed.

Test for Volatile oil:

a) Filter Paper Test: Volatile oils have a characteristic odor. When the dried extract was pressed between two filter papers, there was a spot of oil observed which disappeared after some time.

Test for Proteins:

- **a) Precipitation Test:** 2ml. methanolic extract of the crude drug was given white colloidal precipitate with the following reagents:
- I. 5% Lead Acetate Solution
- II. 5% Ammonium Sulphate Solution
- III. 5% CuSO₄ Solution

Test for Flavonoids:

- a) Shinoda Test: To 2ml. of the methanolic extract, 5ml. of 95% ethanol, few drops of HCl and 0.5g of magnesium was added, pink color was observed.
- b) Dilute Ammonia Solution test: Methanolic extract of the crude drug and 5ml. of the dilute ammonia solution were mixed well. Then concentrated H₂SO₄ was added, resulting in a yellow-colored solution, on standing there was the disappearance of the yellow color.
- c) NaOH and HCl Test: Small amount of the extract was treated with aqueous NaOH and HCl. Then it was observed for the formation of yellow-orange color.

Chromatographic Analysis

Chromatography: Chromatography is a method of separation of a mixture into individual components using a stationary phase and mobile phase.

RESULTS AND DISCUSSION:

The organoleptic evaluation of crude drugs has been done to determine the consistency, color, odor, taste, and physical nature of the crude drug.

Table No. 3: Organoleptic or morphological evaluation of *Elaeocarpus ganitus*:

	Crude drug	Plant part	Observed parameters
1.	Elaeocarpus ganitrus	Bark	Consistency –rounded with a rough surface Nature – Rough Wood Colour – Greenish Brown Taste - Pungent Odour – Odourless

The physicochemical properties like the presence of foreign matters, loss on drying, total ash value, extractive values have been determined.

Table No. 4: Physicochemical screening of Elaeocarpus ganitrus

S. No.	Test.	Elaeocarpus ganitrus
1.	Foreign matter	13.2%
2.	LOD	22% w/w
3.	Total Ash Value	12.9g % w/w
4.	Acid Insoluble Ash Value	5.25% w/w
5.	Water Insoluble Ash Value	8.67% w/w
6.	Selling Factor	0.2ml
7.	Water Soluble Extractive Value	35% w/w
8.	Alcohol Soluble Extractive Value	19.23% w/w

Phytochemical screening has been performed to determine the type and presence of chemical constituents in its composition.

Table No. 5: Phytochemical screening of Petroleum of *Elaeocarpus ganitrus*

S. No.	Test for	Test name	Observation	Inference
1.	Carbohydrates	Molisch Test	No violet ring formed at the junction of two liquids	Positive Test
2.	Glycosides	Killer Killani Test	The reddish-brown color at the junction of two layers	Negative Test
3.	Tannins	FeCl ₃ Test	The appearance of blue-black color	Positive Test
4.	Alkaloids	Mayer's reagent Test	Cream precipitate	Presence of alkaloids
5.	Flavonoids	Shinoda Test	The pinkish-red color was observed	Positive Test

Table No. 6: Phytochemical screening of Chloroform of *Elaeocarpus ganitrus*

S. No.	Test for	Test name	Observation	Inference
1.	Carbohydrates	Molish Test	No violet ring formed at the junction of two liquids	Negative Test
2.	Glycosides	Killer killani Test	The reddish-brown color at the junction of two layers	Positive Test
3.	Tannins	FeCl ₃ Test	The appearance of blue-black color	Negative Test
4.	Alkaloids	Mayer's reagent Test	Cream precipitate	Positive Test
5.	Flavonoids	Shinoda Test	The pinkish-red color was Observed	Positive Test

Table No. 7: Phytochemical screening of Methanol of *Elaeocarpus ganitrus*

S. No.	Test for	Test name	Observation	Inference
1.	Carbohydrates	Molish Test	No violet ring formed at the junction of two liquids	Positive Test
2.	Glycosides	Killer killani Test	The reddish-brown color at the junction of two layers	Negative Test
3.	Tannins	FeCl ₃ Test	The appearance of blue-black color	Positive Test
4.	Alkaloids	Mayer's reagent Test	Cream precipitate	Positive Test
5.	Flavonoids	Shinoda Test	The pinkish-red color was Observed	Negative Test

TABLE No. 8: R_f value of Thin layer chromatography

SOLVENT SYSTEM	EXTRACT	TRAVEL SOLUTE	TRAVEL SOLVENT	R _f VALUE
	Methanol	6.6	7.2	0.91
Hexane:Ethyl Acetate:Acetic acid	Petroleum Ether	6.4	7.3	0.87
(5:4:1)	Chloroform	6.1	6.8	0.90
	Methanol	5.6	6.2	0.90
Hexane:Toluene:Ethyl Acetate (0.8:6:3.2)	Petroleum Ether	6.2	7.4	0.83
	Chloroform	6.5	7.0	0.92
	Methanol	5.8	7	0.82
Chloroform:Methanol (8:2)	Petroleum Ether	6.5	8.4	0.82
	Chloroform	6.4	7	0.91

Table No. 9: DPPH radical scavenging activity of *Eleocarpus Ganitrus* Bark and standard antioxidant ascorbic acid

S. NO.	Eleocarpus Ganitrus Extract	Concentration (µg/ml)	Absorbance at 517nm	% Radical scavenging activity	IC50(μg)
		5	0.2086	6.81	
		10	0.1932	13.71	
STD.	Ascorbic acid	15	0.1744	22.10	
		20	0.1678	25.05	3846.15
		25	0.1439	5.73	
		5	0.2024	9.60%	
		10	0.1784	20.32%	
1.	Methanol	15	0.1633	27.06%	
		20	0.1558	30.41%	4992.10
		25	0.1553	30.63%	
		5	0.1892	15.49%	
		10	0.1816	18.89%	
2.	Petroleum ether	15	0.1768	21.03%	
		20	0.1708	23.71%	12464.75
		25	0.1704	23.89%	
		5	0.1896	15.31%	
		10	0.1889	15.63%	
3.	Chloroform	15	0.1854	17.19%	
		20	0.1746	22.01%	6239.75
		25	0.1508	32.64%	

CONCLUSION:

Elaeocarpus ganitrus bark crude drug traditionally used as antiageing, insomnia, immunomodulatory, antihypertensive, antidiabetic, anticonvulsant, sedative, antioxidant activity. Physicochemical screening result following Foreign matter 13.2%, LOD 22% w/w, Total Ash Value 12.9g % w/w, Acid Insoluble Ash Value 5.25% w/w, Water Insoluble Ash Value 8.67% w/w, Selling Factor 0.2ml, Water

Soluble Extractive Value 35% w/w, Alcohol Soluble Extractive Value 19.23% w/w. The phytochemical screening of the extract with the following solution Petroleum, Chloroform, Methanol obtained after extraction process has been done which shows the presence of carbohydrates, glycosides, tannins, alkaloids, and flavonoids. According to the result obtained it can be concluded that the various pharmacological activities reported may be due to the presence of these chemical constituents as their major active constituents. The *Elaeocarpus ganitrus* bark methanolic extract showed significant antioxidant activity of standard ascorbic acid. And overall extract, methanolic extract was showed the best antioxidant activity.

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