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Research Article

Identification and isolation of gallic acid in Elaeocarpus ganitrus (Roxb.)

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ABSTRACT

Introduction: Elaeocarpus ganitrus Roxb., commonly known as Rudraksha, holds a prominent place in traditional Indian medicine due to its wide array of therapeutic properties. This study investigates the phytochemical composition of Rudraksha seeds, with a particular focus on the presence and quantification of gallic acid, a well-known antioxidant.

Methods: Hydroethanolic extracts of Rudraksha seeds were analysed for the presence of major phytochemicals using Thin Layer Chromatography (TLC). The total polyphenol content was determined using the Folin - Ciocalteu method, High-Performance Liquid Chromatography (HPLC) was employed to quantify gallic acid content in the extract. A 4year old male child presented with complaints of speech delay since age appropriate. The assessment was done using REELS, COMDEALL and ALD scorings and the case was diagnosed. Rudraksha churna along was honey was administered as an add-on to speech-language therapy.

Results: TLC confirmed the presence of flavonoids, triterpenoids, alkaloids, and saponins in the extract. The total polyphenol content was found to be significant, and HPLC analysis revealed that the extract contained 0.329% gallic acid. The case was re-assessed after 6 weeks of intervention and there was significant improvement in scores of REELS, COMDEALL and ALD.

Discussion: The presence of gallic acid and other phytochemicals supports the traditional use of Rudraksha in managing oxidative stress and related health conditions. These findings reinforce existing knowledge of Rudraksha's anti-inflammatory, antimicrobial, anti-asthmatic, and immune-enhancing properties, indicating its potential for development into plant-based therapeutic agents and health supplements.

Conclusion: This study highlights the rich natural composition of Elaeocarpus ganitrus (Rudraksha) seeds, revealing the presence of valuable compounds like gallic acid, flavonoids, triterpenoids, alkaloids, and saponins. The notable amount of gallic acid points to strong antioxidant properties, lending scientific support to Rudraksha's long-standing use in traditional medicine for managing oxidative stress and related conditions. These insights not only deepen our understanding of Rudraksha's health benefits but also open the door to its potential use in creating natural health supplements and plant-based therapeutic products.

Keywords: *HPLC, Hydroethanolic extract, Rudraksha, Spectrophotometer, TLC.*

INTRODUCTION

Elaeocarpus ganitrus, *Rudraksha*, is a member of the *Elaeocarpaceae* family and has been valued in Indian traditional medicine for centuries[1]. This species is predominantly found in India, especially in the Himalayan and Gangetic plains, as well as in countries like Nepal, Indonesia, and Java. The medicinal efficacy of *Elaeocarpus* species is largely linked to the presence of various bioactive compounds. Research has shown that extracts made using petroleum ether, ethanol, and water from *Elaeocarpus* species contain a variety of chemical constituents, including alkaloids (such as elaeocarpidine, elaeocarpine, and rudrakine), polyphenols (e.g., flavonoids, quercetin, tannins), phytosterols, fats, proteins, carbohydrates, and organic acids like gallic and ellagic acid[2]. Key alkaloidal compounds that have been isolated include isoelaeocarpine, epiisoelaeocarpiline, epielaeocarpiline, alloelaeocarpiline, and pseudo-epiiso-elaeocarpilline[3].

Rudraksha has traditionally been associated with a wide range of health benefits. It is therapeutically used in conditions such as anxiety, stress, insomnia, dermatological disorders, hysteria, hyperglycaemia, leucorrhoea, reproductive issues, asthma, high blood pressure, arthritis, rheumatism, and diseases of the cardiovascular and hepatic systems[4] Scientific literature supports numerous pharmacological effects of Rudraksha, including sedative, analgesic, anticonvulsant, anti-inflammatory, antioxidant, antipyretic, antihypertensive, antidiabetic, antimicrobial, anxiolytic, anticancer, anti-asthmatic, nephroprotective, immunostimulatory, and even electromagnetic activity[5]

The plant contains abundant bioactive compounds in different concentrations and polarity. The combination of different analytical techniques, such as High-performance liquid chromatography (HPLC) and Spectrophotometer method can be applied to detect bioactive constituents in bead extracts.

METARIAL AND METHODS

Collection of samples:

Rudraksha- E. ganitrus seeds were harvested from the herbal garden at an Ayurveda teaching hospital, Mysuru, Karnataka, India.

Preparation of hydroethanolic extract preparation:

The collected samples were rinsed in water and air dried under shade conditions until all moisture content was gone. The hydroethanolic extract was prepared by adding 70 % ethanol (10 ml) and incubated for 1 week at room temperature. (Fig 1 and Fig 2)

Fig.1 Dried seeds of Rudraksha

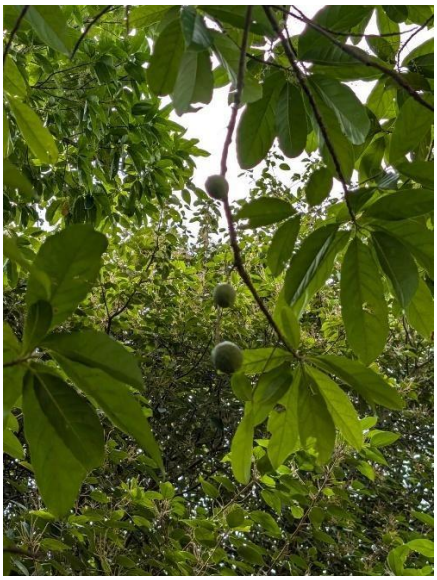


Fig.2 Rudraksha tree

1. TLC profile of *Rudraksha Churna* Methanol Extract

By TLC presence of flavonoids, triterpenoids, alkaloids and saponins are confirmed.

2. Estimation of Total Polyphenols in *Rudraksha Churna* sample extract using Folin-Ciocalteu phenol reagent by Spectrophotometer method:

Reagents:

- Purified water
- Folin-Ciocalteu phenol reagent (10% V/V)
- Sodium carbonate solution (7.5% W/V)

- Gallic acid standard stock solution (100µg/mL)

Preparation of Gallic acid standard stock solution (100µg/mL):
Weighed 10 mg of Gallic acid standard into a 100 mL volumetric flask, added 50mL of water and sonicated to dissolve, further made up the volume to 100mL with water.

Linearity Solutions:
The Linearity standard solutions in the concentration ranging from 2.5, 5.0, 10.0, 25.0, 50.0µg/mL were prepared by respective dilutions of the Gallic acid standard stock solution.

Sample Solution:
Weighed 20 mg of extracted sample into 10mL volumetric flask, dissolved with water and made up to the mark.
Procedure:

- Transferred 1.0mL of all five Gallic acid Linearity solutions (3.0) into separate 10mL volumetric flasks.
- Transferred 1.0mL of sample solution (4.0) into a 10mL volumetric flask and mark it as a sample.
- Transferred 1.0mL of purified water into a 10mL volumetric flask and mark it as a blank.
- Added 5.0mL of Folin–Ciocalteu phenol reagent (10% V/V) into each flask (5 linearity solutions, sample and blank).
- Added 4.0mL of sodium carbonate solution (7.5%) into each flask within 3-8 minutes after the addition of Folin–Ciocalteu phenol reagent.
- Allowed the solutions for about 60minutes to attain room temperature and then measured the absorbance at 765nm using UV- Visible spectrophotometer.

3. High-Performance Liquid Chromatography (HPLC) analysis

The Shimadzu 2010A-HT was employed for the study, which has an integrated vacuum degasser, automatic sample manager, ultra-performance Quaternary solvent manager. A C-18 stationary phase (Shimadzu Shim packs C-18, 250×4.6mm, 5µm) was used for chromatographic separation and detection was carried by a Photodiode array detector (PDA). (Table 1)

Tabel 2. Materials for High-Performance Liquid Chromatography (HPLC) analysis

Sl. No.	Parameters	Conditions
1.	Instrument Model	Shimadzu LC-2010A-HT
2.	Column	Shim pack C-18 Column (250×4.6mm, 5µm)
3.	Mobile phase	Acetonitrile: 0.1% Tri fluoro acetic acid: (10 : 90)
4.	Run time	10.0 minutes
5.	Flow rate	1.2 mL/min
6.	Injection volume	10µL
7.	Column oven temperature	45°C
8.	Wavelength	270nm
9.	Gallic acid eluted at	4.1 min

Preparation of solutions:

a) Preparation of standard stock (100ug/mL):
Weighed 10 mg of Gallic acid standard into a 100 mL volumetric flask, added methanol and sonicated to dissolve, further made up to the volume with methanol.

b) Preparation of Linearity standard solutions:
The Linearity standard solutions in the concentration ranging from 1, 5, 10, 25, 50, 100µg/ml were prepared by respective dilutions of the standard stock of Gallic acid.

c) Preparation of sample solution:
Transferred 100mg of sample extract into a 10mL volumetric flask, added 5mL of methanol and sonicated to dissolve, further made up to the volume with methanol.

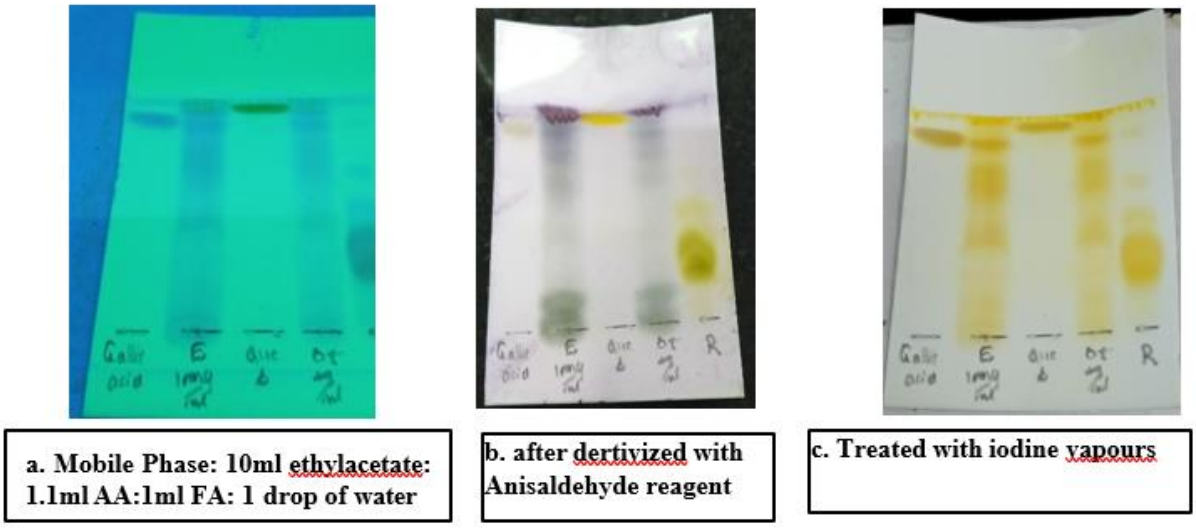
PILOT CASE STUDY:
A 4-year-old male child presented with complaints of speech delay since age appropriate. The child is able to speak 2-3 words with meaning. The child was diagnosed with Language disorder with the assessment of REELS, COMDEALL and ALD. *Rudraksha churna* was administered internally with honey after food twice daily as an add-on to speech-language therapy weekly 3 times for 6 weeks.

1	Pre-assessment	REEL: RLA: 4.6-5 yrs ELA:12-18 mts COMDEALL: GM:42-48 mts FM: 42-48 mts ADL: 42-48 mts RL: 42-48 mts EL: 42-48 mts CS: 42-48 mts ES: 42-48 mts SS: 42-48 mts ALD: RLA: 4.6-5 yrs ELA: 12-18 mts
2	Post-assessment	REEL: RLA:4.6-5 yrs ELA: 4.6-5 yrs COMDEALL: GM:54-60 mts FM: 54-60 mts ADL: 54-60 mts RL: 54-60 mts EL: 54-60 mts CS: 54-60 mts ES: 54-60 mts SS: 54-60 mts **ALD:** RLA: 4.6-5 yrs ELA: 4.6-5 yrs

RESULTS

The aqueous methanol extraction, the hydroethanolic extract of *Elaeocarpus ganitrus* seeds yielded 10 g of semisolid material, corresponding to 120% w/w. Preliminary phytochemical screening using Thin Layer Chromatography (TLC) confirmed the presence of flavonoids, triterpenoids, alkaloids, and saponins. Each class of compound was identified using specific mobile phases and detection reagents. Flavonoids were visualized using ethyl acetate, acetic acid. formic acid, water (10:1.1:1:0.1) and anisaldehyde reagent under iodine vapours. Triterpenoids were detected under UV light (366 nm) using hexane, ethyl acetate (7:3) and anisaldehyde reagent. Alkaloids were identified using toluene, ethyl acetate (7:3) and iodine vapours, while saponins were detected using chloroform, methanol, water, acetic acid (6:1.2:0.05:2) and anisaldehyde reagent. (Fig 3 to Fig 6)

Fig.3 TLC profile of Flavonoids

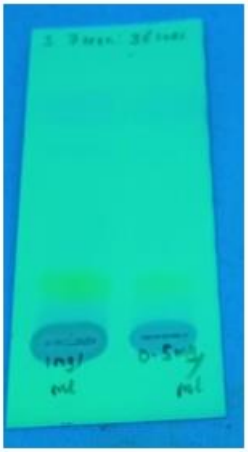


a. Mobile Phase: 10ml ethylacetate: 1.1ml AA:1ml FA: 1 drop of water

b. after dertivized with Anisaldehyde reagent

c. Treated with iodine vapours

Fig.4 TLC profile of Triterpenoids



TLC Mobile Phase: 7 hexane:3 Ethylacetate

Under UV 366nm

Treatment with Anisaldehyde reagent

Fig.5 TLC Profile of Alkaloids

7 Toluene :3 Ethyl acetate

Treatment with Iodine vapours

Fig.6 TLC Profile of Saponins

6ml Chcl3:1.2ml Methanol:1 drop water: 2ml Acetic acid

Detection: Plate treated with Anisaldehyde reagent.

Quantitative estimation of total polyphenols was performed using the Folin–Ciocalteu method. A calibration curve was generated using gallic acid standards ranging from 2.5 to 50 µg/mL, with corresponding absorbance values from 0.056 to 0.843. The sample extract showed an absorbance of 0.419, indicating substantial polyphenol content.

CALCULATION

The concentration of polyphenols in the sample was determined from the calibration curve using the regression equation:

y = 0.016x + 0.005

Where y= Absorbance of sample,

Where x= unknown concentration of polyphenols in sample.

Concentration of total polyphenols =

x = (y – 0.005) / 0.016

x = (0.419 – 0.005) / 0.016

x = 25.875µg/mL

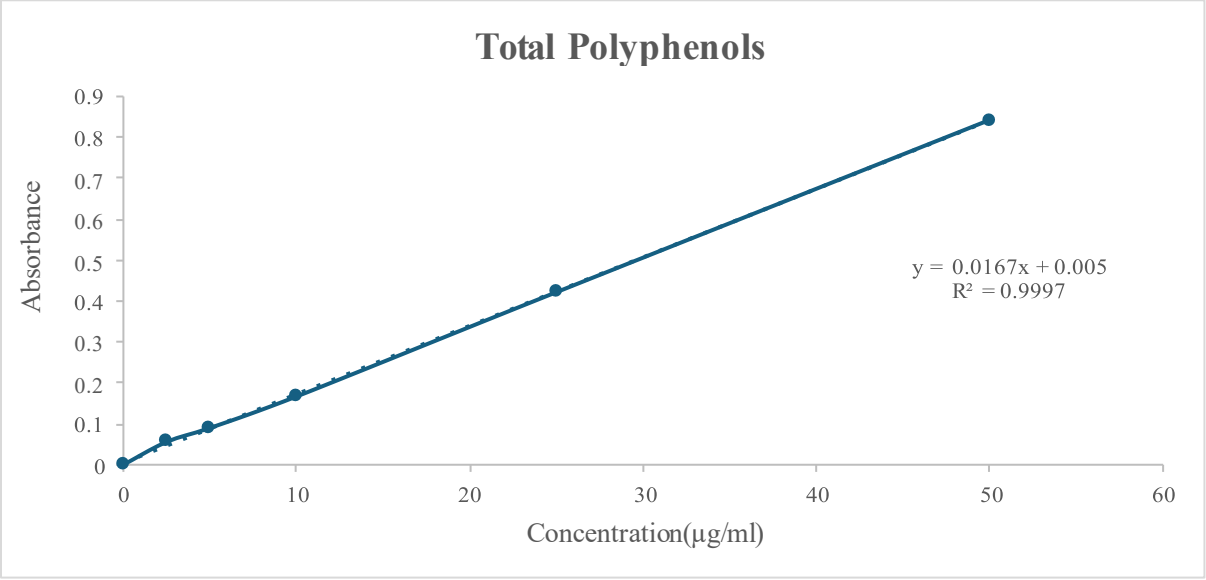
x = 258.75µg OR 0.25875mg of polyphenols per 20mg of sample x = 1.294mg of polyphenols per 100mg of sample

= 1.294 / 100 * 100= 1.294%

Report: 1.294% of polyphenols was present in the given sample extract.

Tabel 1. Results of estimation of Total polyphenols in Rudraksha *Churna* sample.

Concentration (µg/ml)	Absorbance
Blank	0.000
2.5	0.056
5.0	0.090
10.0	0.167
25.0	0.422
50.0	0.843
Sample	0.419



Graph 1. Results of estimation of Total Polyphenols in Rudraksha

High-Performance Liquid Chromatography (HPLC) analysis was conducted using a Shimadzu LC- 2010A-HT system equipped with a Shim-pack C18 column (250×4.6 mm, 5 µm). The mobile phase consisted of acetonitrile and 0.1% trifluoroacetic acid (10:90), with a flow rate of 1.2 mL/min and detection at 270 nm. Gallic acid eluted at 4.1 minutes. The sample extract produced a peak area of 944007, corresponding to a gallic acid concentration of 32.899 µg/ml. This equates to 0.32899 mg per 100 mg of extract, or **0.329% w/w** gallic acid.

Calculation:

The concentration of Gallic acid in the sample was determined from the calibration curve using the regression

equation:

y = 28119x + 18898
Where y= Peak area response of sample

Where x= unknown concentration of Gallic acid in sample

Concentration of Gallic acid = x = (y – 18898) / 28119

= (944007 – 18898) / 28119

= 32.899µg/mL

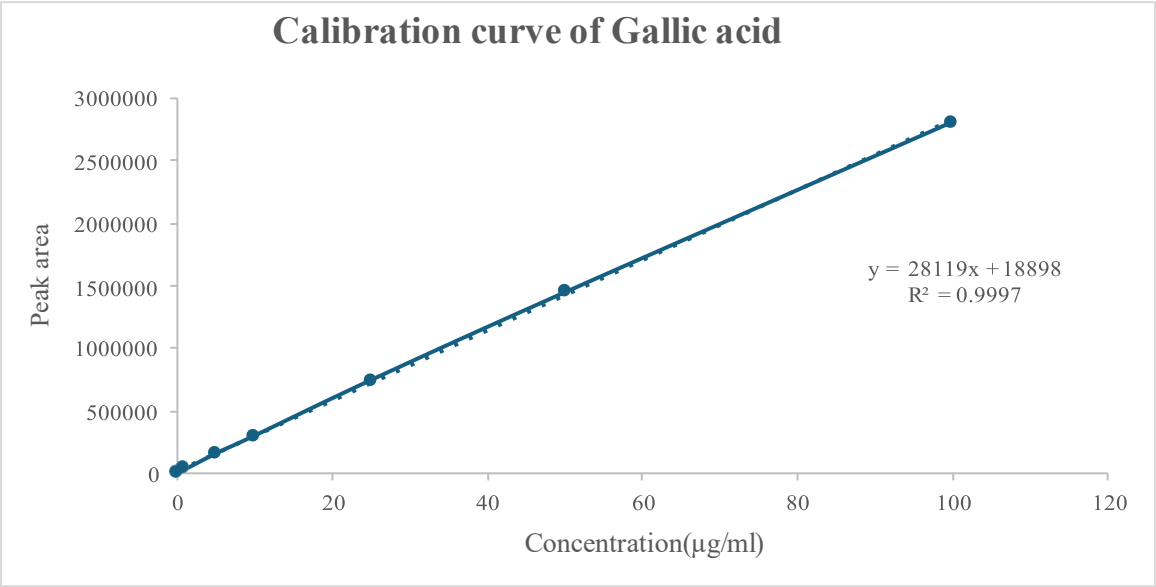
= 328.99µg OR 0.32899mg of Gallic acid per 100mg of sample

= 0.32899 / 100 * 100 = 0.32899%

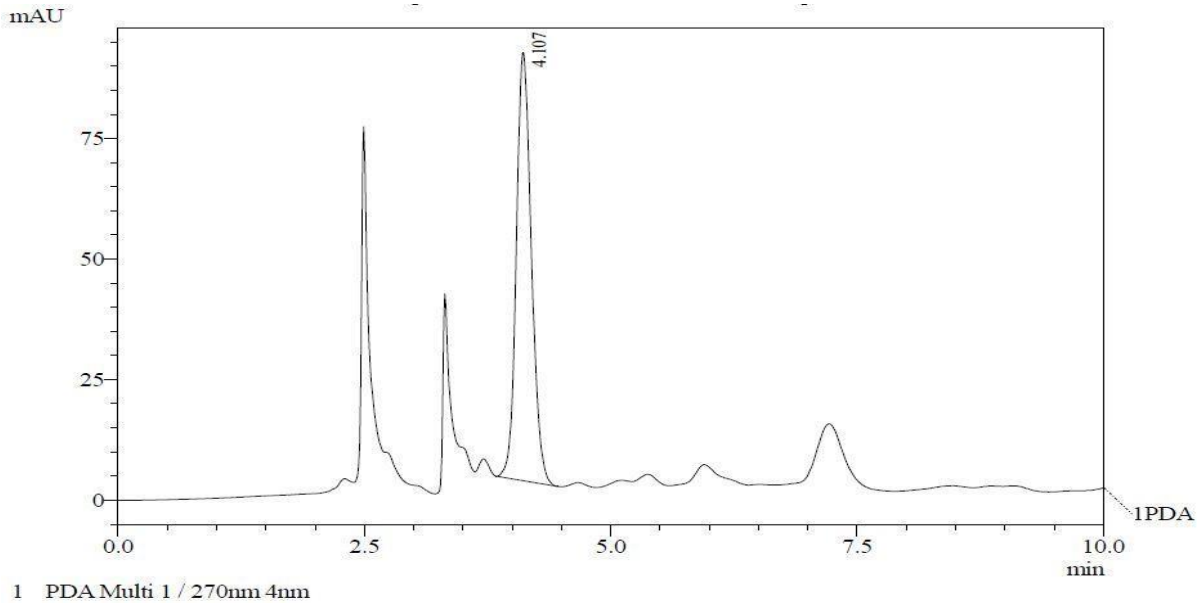
Report: The given sample *Rudraksha Churna* contains **0.329%** of Gallic acid.

Tabel 2. Results of estimation of Gallic acid in *Rudraksha Churna* sample.

Concentration (µg/mL)	Peak area
0	0
1	31625
5	160205
10	302922
25	747136
50	1448787
100	2812368
Sample	944007



Graph 2. Calibration curve of Gallic acid



Graph 3. Chromatogram which shows Gallic acid peak at 4.1min

DISCUSSION

Plants are recognized for producing a wide array of bioactive compounds, including alkaloids, glycosides, flavonoids, phenols, terpenoids, steroids, tannins, phytosterols, quinones, and various other derivatives. These naturally occurring substances are widely utilized across industries such as pharmaceuticals, cosmetics, medicine, biochemistry, chemicals, and pesticides. The therapeutic effects observed in different plants are primarily attributed to the unique combination of these phytochemicals. In the case of Rudraksha (*Elaeocarpus ganitrus*), one of its primary active constituents is gallic acid. This compound is known for its strong antioxidant activity, playing a crucial role in scavenging free radicals and safeguarding cells against oxidative stress. The presence of gallic acid significantly contributes to the medicinal and antioxidant potential of Rudraksha.

Anti-Asthmatic Properties:

Studies in animal models have shown that fruit extracts of *Elaeocarpus granitus* possess anti-asthmatic effects. Extracts derived using petroleum ether, chloroform, acetone, and ethanol have demonstrated the ability to stabilize mast cells, indicating potential benefits for managing bronchial asthma.[6]

Antimicrobial Activity:

Leaf extracts of *Elaeocarpus ganitrus* have demonstrated broad-spectrum antimicrobial properties. These include inhibitory effects against a variety of bacterial and fungal pathogens such as *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Penicillium* species, *Aspergillus flavus*, *Candida albicans*, and *Candida tropicalis*.[7]

Immunomodulatory Potential:

Methanolic extracts of *Elaeocarpus ganitrus* seeds have been examined for their impact on the immune system. Both in vitro and in vivo assessments showed that the extract significantly influenced immune function. It enhanced the production of nitric oxide, superoxide, and lysosomal enzymes in isolated murine macrophages. These effects were dose-dependent and included both innate (phagocytic) and adaptive (cell-mediated and humoral) immune responses.[8,9.10]

Neurophysiological and Cardiovascular Benefits:

Elaeocarpus ganitrus (Rudraksha) has been traditionally recognized for its calming and regulatory effects on the nervous and cardiovascular systems. Literature reports its anxiolytic, sedative, and hypotensive properties, which align with its therapeutic use in conditions such as hypertension, insomnia, and anxiety. Studies suggest the efficacy of Rudraksha in managing neurological disorders—including stress, sleeplessness, and depression—primarily attributed to its ability to balance Vata dosha and stabilize neurochemical activity. Additionally, Rudraksha has shown promise in regulating blood pressure and improving cardiac function, offering a safer alternative to conventional antihypertensive drugs that often carry adverse effects.[11]

CONCLUSION

This study attempted to explore the presence of important bioactive compounds like flavonoids, triterpenoids,

alkaloids, and saponins through TLC, while the spectrophotometric and HPLC analysis revealed the gallic acid content as 0.329% in *Elaeocarpus ganitrus* Roxb. (Rudraksha), particularly through the analysis of its hydroethanolic seed extract. The test findings with continued research, including clinical studies and advanced compound isolation, will be essential to fully harness and standardize the medicinal benefits of E. ganitrus for broader clinical use.

Case study highlights the therapeutic impact of integrating Ayurvedic intervention —specifically *Rudraksha churna*—as an adjunct to conventional speech-language therapy. The observed improvements suggest a potential synergistic effect of *Rudraksha churna* when used alongside structured speech- language therapy. The normalization of expressive language age and enhancement in motor, cognitive, and social domains support its role in promoting neurodevelopmental integration.

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