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Preliminary pharmacognostic, phytochemical and DPPH radical scavenging activity of *Elaeocarpus ganitrus* seed (Rudraksha)

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Abstract
Elaeocarpus ganitrus plant is a traditional medicinal plant that has a long-established history of healing diseases in medicinal science since ancient times. Wearing Rudraksha seeds cures sicknesses such as stress, sleeplessness, nervousness, lack of concentration, depression, heart diseases such as hypertension, rheumatism and sterility, etc. In the present research, we have conducted preliminary pharmacognostic, phytochemical studies and Di-phenyl picryl hydroxyl radical scavenging assay (DPPH) of Rudraksha seed. Rudraksha seeds are used for phytochemical screening. The existence of secondary metabolites like alkaloids, terpenes and flavonoids is then determined from seed extracts and, also so, antioxidants' ability to reduce the DPPH radical is measured in this test. Screenings of phytochemicals in various extracts indicate the presence of various constituents like alkaloids, tannins, flavonoids, steroids, glycosides, saponins, phytosterols, etc. The antioxidant activities in extracts of the seeds of *Elaeocarpus ganitrus* show hydro-alcohol and ascorbic acid had IC50 values of 59 ± 0.56 and 50 ±1.58 g / ml.

Keywords: Rudraksha, *Elaeocarpus ganitrus*, DPPH, phytochemical screening

Introduction
Preliminary phytochemical screening is a significant method in detecting the bioactive compounds found in medicinal plants, which can show the way to drug discovery and development. Rudraksha seeds, also called Rudraksh, are the dried seeds of a tree that only grows in a few places [1]. Rudraksha (*Elaeocarpus ganitrus*) is a very tall plant that can reach heights of 45 to 200 feet. Depending on the geography and the conditions, this tree can reach a height of 15 to 30 meters. Rudraksha seeds are hard and woody on the outside, with a light chocolate color in the berry's pulp [2]. These seeds are dirty white, yellowish, reddish-brown and brownish-black, among the many colors available. Each Rudraksha seed has a different number of vertical lines flowing down from the outside to form the 'Mukhs' or face of the seed. The Rudraksha seeds are given their names based on these faces, or 'mukhs.'. Plant extracts are commonly tested for antioxidant activities using the DPPH free radical scavenging technique. In the DPPH assay, adding the extract to a violet-colored DPPH solution causes it to be reduced to a yellow-colored product by diphenylpicryl hydrazine [3, 4]. Antioxidant assay of the plant is mostly conducted due to its lively phytoconstituents present in a variety of medicinal plants. Antioxidant activity studies in a conventionally used medicinal plant variety are a technique of systematic justification of the medicinal characteristics used by native people. Antioxidants assist our body to neutralize or destroy “Reactive Oxygen Species” (ROS) or free radicals previous to their damage to our cells [5].

Fig 1: *Elaeocarpus ganitrus* plant [6]

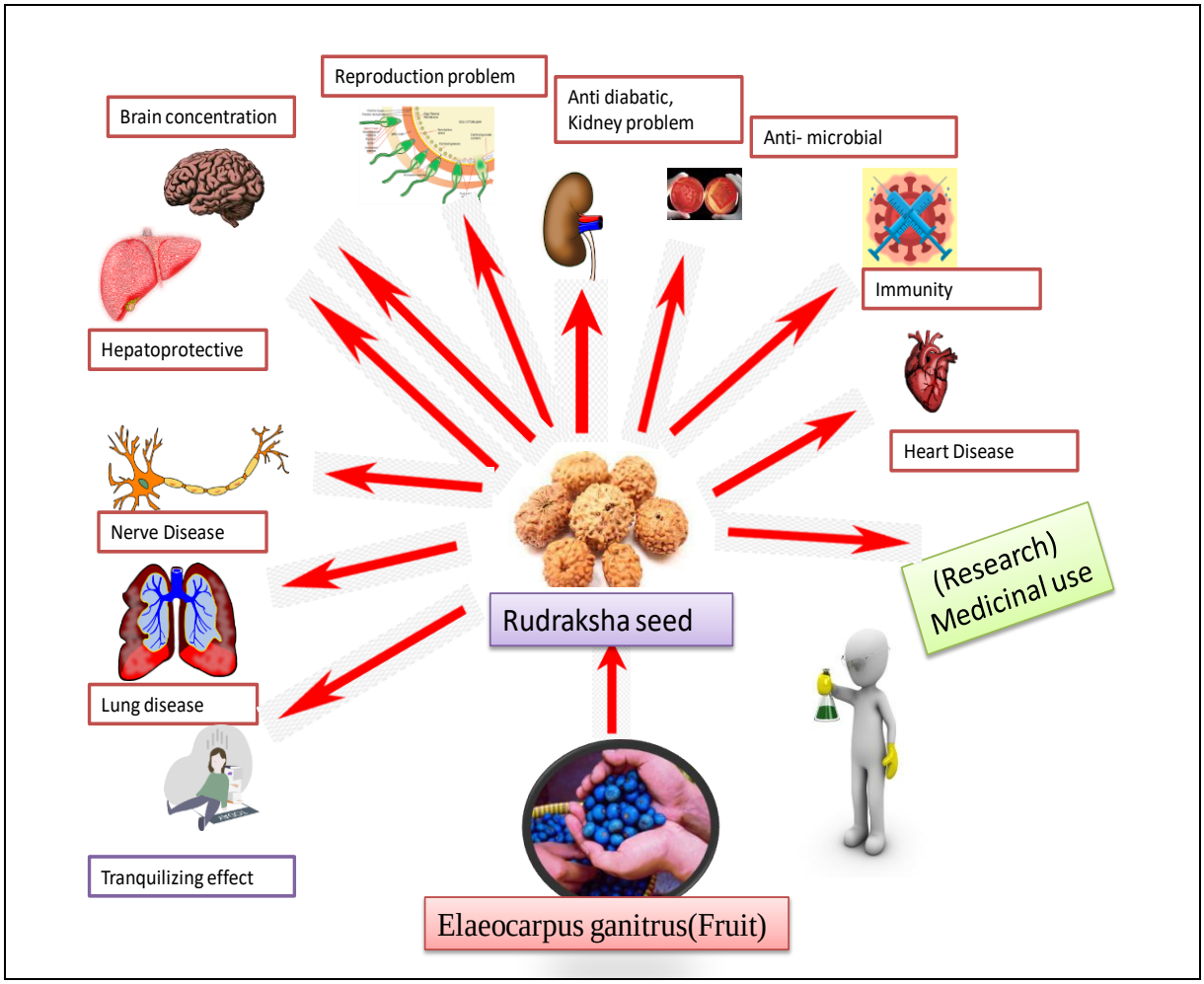

Fig 2: Rudraksha common medicinal uses

Material and Method

Collection and Authentication

The collection of authenticated *Elaeocarpus ganitrus* (Rudraksha) was done by purchasing online from the Rudra Center (https://www.rudraksha-ratna.com/). Preliminary X-ray analysis was conducted to find that Rudraksha seed internal structure has five compartments, which signifies that Rudraksha seeds obtained from *Elaeocarpus ganitrus* are five face Rudraksha seeds. We further confirmed its originality by CSIR-NISCAIR's, Research & Academics in Science& Technology, Communication New Delhi.
Authentication No: (NISCAIR/RHMD/Consult/2019/3436-37).

Extraction of *Elaeocarpus ganitrus*

After the Collection of the plant *Elaeocarpus ganitrus* seeds, it was completely air-dried and then ground into a very fine powder [7, 8]. For the microscopic study, Rudraksha fruit was taken in (Formalin-5 ml + Acetic acid- 5 ml + 70% Alcohol-90 ml). Afterward, microscopic slides were set following the standard plant anatomy protocols. The transverse cross-sections of the fruit were obtained by slicing the paraffin-embedded specimen using a Rotary Microtome [9].
The width of the sections was calculated at almost around 10–12 µm. De waxing of the thin Rudraksha sections is carried out by regular procedures. Later, the thin sections were marked with a toluidine blue indicator. Rudaraksha sections of the fruit were cleaned with 30% sodium hypochlorite solution and stained with safran in solution. Microscopic categorization of fruit done by Labored LX-200 Binocular Microscope, powder microscopy performed following standard procedure microchemical test conducted for the histological region. A microscope was used to view the slide.
A known quantity of Rudraksha seed (2000gm) was taken and extracted by using petroleum ether, Ethyl Acetate, ethanol, and distilled water successively in the Soxhlet Extractor. Finally, the solvent was evaporated by a rotary evaporator to obtain crude dried extract [10]. Crude extract material was collected and stored for further use. On a slide, a few drops of chloral hydrate solution were applied to a sample of powdered plant material, which was then covered with a glass slip and gently heated over a Bunsen burner at low temperature. Boiling at a high temperature was avoided [11].

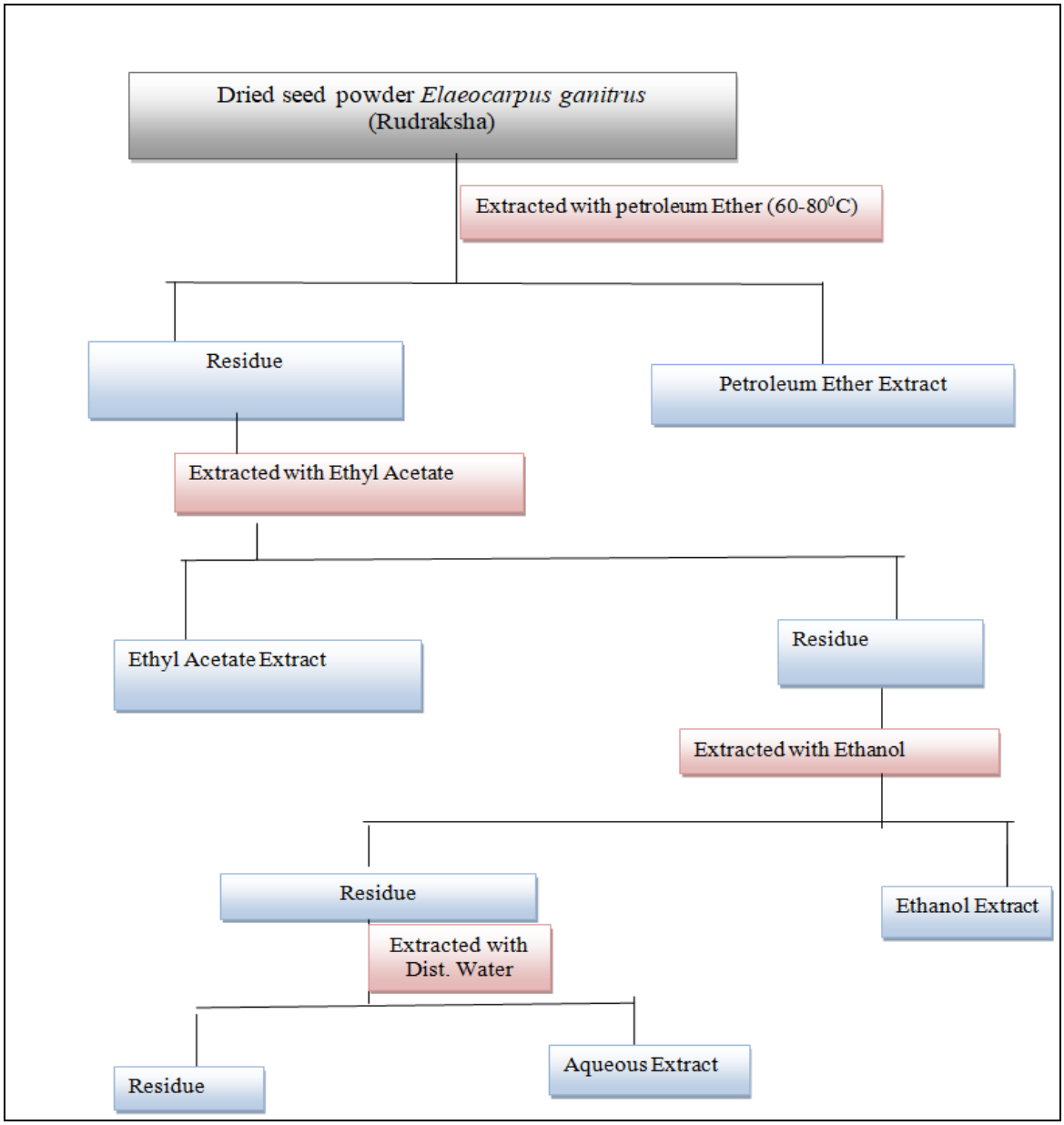

Fig 3: Successive extraction of dried seeds powders of *Elaeocarpus ganitrus* (Rudraksha)

Preliminary Phytochemical tests

All the extracts of *Elaeocarpus ganitrus* (Rudraksha) were subjected to qualitative tests for the identification of various active Phytoconstituents [12]. The powder content was treated with a variety of chemical reagents to detect phytoconstituents by observing color changes in natural light, as well as the color and consistency of extracts using the standard procedure [13]

Test of Alkaloids [14]

The solvent-free extract was stimulated with a few ml of dilute hydrochloric acid (HCl) and then it was clean. The filtrate was tested attentively with a range of alkaloid testing reagents.

Mayer's Test: To the few ml of filtrate, one to two drops of Mayer's reagent were added by the side of the test tube. A white or creamy precipitate indicates the presence of alkaloids.

Wagner's test: Couples of Wagner's reagent were introduced by the side of the test tube to a few ml of filtrate. The positive result was verified by a brick-red precipitate.

Hager's test: 1 or 2 ml of Hager's reagent (saturated aqueous solution of picric acid) was added to a few ml of filtrate. The presence of a bright yellow precipitate indicated that the test was positive.

Dragendorff's test: 1 to 2 ml of Dragendorff's reagent was added to a few ml of filtrate. The presence of a large amount of brown precipitate indicated that the test was positive.

Test of Carbohydrates [15]
The extract was filtered after being dissolved in 5 ml of water. The filtrate was put through a series of tests.

Molash's test: a few drops of alcoholic naphthol solution were added to 2 ml of filtrate, the mixture was agitated well, and 1 ml of concentrated sulphuric acid was slowly added along the walls of the test tube and allowed to stand. The presence of carbohydrates was indicated by a violet ring [16].

Fehling's test: 1 ml filtrate was boiled with 1 ml Fehling solutions A and B on a water bath. The presence of sugar was indicated by a crimson precipitate.

Test of Glycosides [17]
The extract was hydrolyzed for 2 hours on the water in strong hydrochloric acid, filtered, and the hydrolysis was subjected to the following tests.

Barfoed's test: Combine 1 ml of Barfoed's reagent with 1 ml of filtrate in a boiling water bath for 2 minutes. The development of crimson precipitate revealed the presence of sugar.

Test of Saponins by Foam Test
The filtrate was marked up to 20 ml by diluting it with distilled water. For 15 minutes, the suspension was traumatized in a graded cylinder. Saponins were detected in a few centimeters of foam.

Test of Proteins and Amino Acids
The filtrate was diluted in 10 ml distilled water and filtered through Whatman No.1 filter paper, being subjected to protein and amino acid analysis.

Million's test: a few drops of Million's reagent were applied to 2 ml of filtrate. The presence of proteins was shown by a white precipitate [18].

Biuret test: A drop of 2 percent copper sulfate solution was added to an aliquot of 2 ml filtrate. 1 ml ethanol (95%) was added to this, followed by an excess of potassium hydroxide pellets; the pink color in the Ethanolic layer indicates the presence of proteins.

Test of Phytosterols
Salkowski's reagent Test: Salkowski's reagent was used to treat the extract. The presence of phytosterols is indicated by the yellowish tint with a green fluorescence look.

Test of Fixed Oils and Fats [18]
Spot test: Filtrate was placed between two filter papers in a modest amount. The presence of solidified oil was indicated by an oil stain on the paper.

Saponification test: A tiny amount of extract was mixed with a few drops of 0.5 N alcoholic potassium hydroxide solutions and a drop of phenolphthalein. The mixture was heated in a water bath for almost two hours. The presence of fixed oils and fats can be seen in the formation of soap or partial alkali neutralization.

Test of phenolic compounds and Tannins [19]
Ferric chloride Test: In 5 ml of distilled water, the extract was dissolved. A few drops of neutral ferric chloride solution (5% ferric chloride) were added to this. The presence of phenolic chemicals was indicated by a dark green apple color.

Lead acetates test: After dissolving the extract in distilled water, 3 ml of a 10% lead acetate solution was added. The presence of phenol compounds was indicated by a bulky white precipitate.

Test of Gum and Mucilage
The extract was dissolved in 10 ml of distilled water and to this; 25 ml of absolute alcohol was added with constant stirring. White or cloudy precipitate indicated the presence of gums and mucilages.

Fluorescence analysis of powder and Rudraksha extracts
The fruit extracts were examined and analyzed for fluorescence using standard methods in the daytime, short, and long UV light.

Estimation of inorganic constituents
1 g of dried Rudraksha seed powder was digested with concentrated nitric acid and perchloric acid (3:1) until a clear solution was obtained to estimate the content of the inorganic element. The solution was then rendered up

to a given volume with de-mineralized water and analyzed in a Perkin Elmer atomic absorption spectrophotometer after cooling.

Physicochemical parameters

Loss on drying was calculated at 25°C, pH of 1% aqueous soluble portion of the fruit was calculated, and ash and extractive values were calculated using the standard protocol. Fruit extracts were analyzed, using Thin Layer Chromatography (TLC) according to normal procedures.

Estimation of Heavy Metals in Rudraksha

This test was conducted by Nitric-Hydrochloric Acid (1:3) Digestion technique. To the 10 mg sample, 10 ml of a freshly prepared acid mixture of 1:3 mixtures of 65 % HNO_3 and 37 % HCl was added (2.5 ml nitric acid and 7.5 ml hydrochloric acid). The mixture was boiled gently over a water bath (95 °C) for 4–5 h (or until the sample had completely dissolved) (Srivastava *et al*., 2006). Sample Acquisition in ICP-OES method Volume of the sample was made up to 14 ml before acquisition in PerkinElmer® Optima™ 8000 ICP-OES. Results were reported as µg/gm [20].

Thin-layer Chromatographic Studies (TLC) of *Elaeocarpus ganitrus* seeds

Thin-layer chromatography was carried out on all fractions using TLC pre-coated plates by using an ascending chromatography technique. The seed extracts of each solvent were subjected to TLC. All spots are colorless in daylight, but they are colored under UV light. TLC chromatography is used for preliminary phytochemical screening [21]. TLC is a solid-liquid type chromatographic technique in which the solid stationary phase and the liquid mobile phase are used for chromatographic separation. Different solvent extracts of *Elaeocarpus ganitrus* were used for TLC. For the stationary phase, slurry was prepared using silica gel [22, 23]. A thin layer of silica gel slurry was applied to the TLC plate. The TLC plate was activated at 1100C in the oven for one hour. Different solvent extracts of Rudraksha seed powder applied on TLC plate and Rf Factor calculated by displacement of solute front to the displacement of solvent front.

$$\mathrm{Rf} = \frac{\text{Distance from baseline travel by solute}}{\text{Distance from baseline travel by solvent}}$$

2.5. DPPH radical scavenging activity

The antioxidant activity of hydroalcoholic extracts of *Elaeocarpus ganitrus* seeds was tested using the DPPH free radical scavenging activity technique. With a few modifications, this antioxidant assay was put into practice. The antioxidants' ability to reduce the DPPH radical is measured in this test [24]. The reaction was monitored every 15 minutes for up to 90 minutes, with the absorption measured using a UV spectrometer. The absorbance of the mixture was estimated at 517 nm utilizing a UV-Vis spectrometer. The absorbance of the mixture was estimated at 517 nm using UV VIS spectrometry [25].

The antioxidant activity was anticipated as follow

$$\text{Inhibition}(\%) = \frac{\mathrm{Ac} - \mathrm{A1}}{A1}$$

Ac = is the absorbance of the control
A1= Absorbance of the extractives/standard.
The percent of inhibition was then plotted against the concentration, and the graph was created.
IC50 values were used to calculate the antioxidant activity of the sample (the inhibition concentration of the sample reduce 50 percent the absorbance of DPPH). The standard was ascorbic acid, which was utilized at the same concentration as the sample.

Results

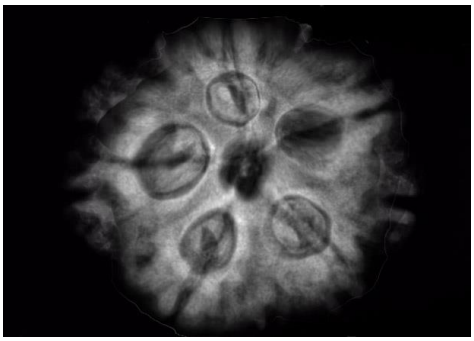

Fig 4: X-Ray analysis of Rudraksha seed

Table 1: Physicochemical values of the seed of *Elaeocarpus ganitrus*

Parameters	Results
1. Organoleptic Property.	
Appearance	Coarse powder
Colour	brownish-white
Odor	Characteristic
2. Loss of drying	12.5%w/v
3. pH values	
a pH of 1% aqueous solution	5.7
4. Ash values (%)	
Total ash	2.92%
Acid insoluble ash	0.74%
Sulphated ash	3.012%
Water-soluble matter (%)	34.026%
Alcohol soluble matter (%)	31.48%
5. Successive solvent extractives (%)	
Petroleum ether extract	0.68%
Ethyl acetate extract	0.48%
Ethanol extract	16.23%
Aqueous extract	10.67%
6. Foreign organic matter	0.6% w/w
7. Estimation of Heavy Metals(µg/gm Extract)	
Zn	5.6
Fe	45.5
Cu	36.4
Mn	15.4
Cr	11.2
Mg	1279.6
As	40.6
Ni	Not detected
Cd	Not detected
Pb	Not detected

Table 2: TLC fingerprinting of different extracts of seeds of *Elaeocarpus ganitrus*

Different solvent Extract	Detection	Spots	R_f Value
Petroleum ether extract	UV lamp	4	0.37,0.45,0.54,0.62
Ethyl acetate extract	UV lamp	2	0.64,0.75
Ethanol extract	UV lamp	3	0.38,0.45,0.58
Aqueous extract	UV lamp	2	0.81,0.85

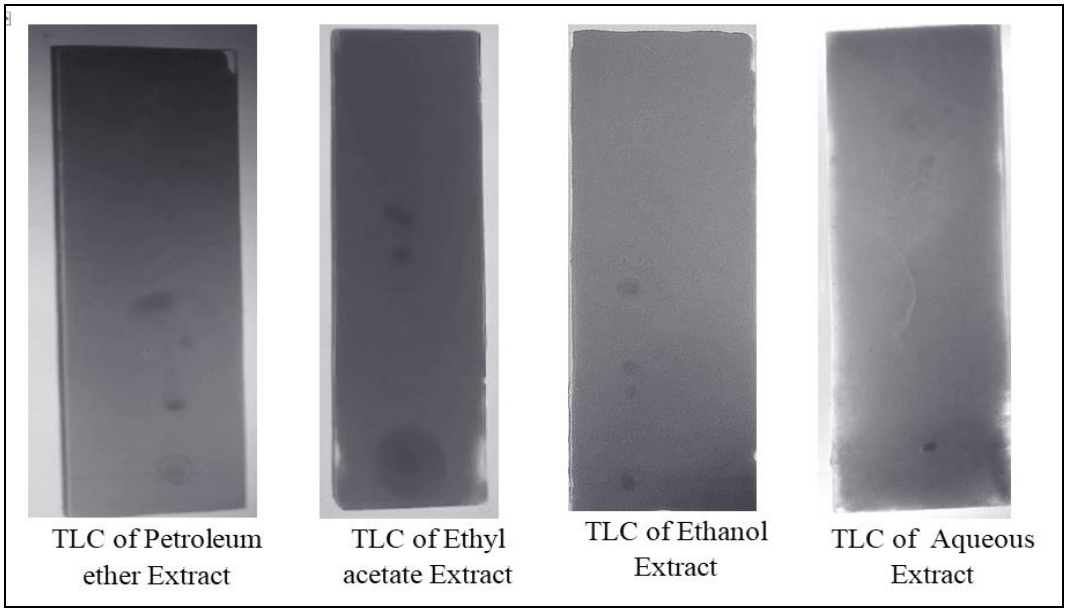

Fig 5: TLC fingerprinting image of different extracts of seed of *Elaeocarpus ganitrus*

Table 3: Phytochemical constitute of the seed extract of *Elaeocarpus ganitrus*

Sl. No	Phytoconstituents	Tests	Hexane	Chloroform	Ethyl acetate	Ethanol
1	Alkaloids	Mayer's test	+	+	+	++
2	Glycosides	*Borntrager's test*	-	+	-	-
3	saponins	Froth forming test	-	-	-	-
4	Phenolic compound	*Lead acetate test*	-	+	+	-
5	Tannins	*$FeCl_3$ Test*	+	+	+	++
6	Phytosterols	*Libermann Buchard Test*	-	-	+	+
7	Carbohydrates	*Feelings test*	+	-	+	+
8	Proteins	Biuret test	-	-	-	-
9	Amino acid	*Ninhydrin test*	-	-	-	-
10	Flavonoids	Alkaline test	+	+	+	+
11	Quinones	Quinone test	+	+	+	+
12	Terpenoids	Salkowski test	+	+	+	+

(- Means absent) (+ Means present) (++ Means strongly present)

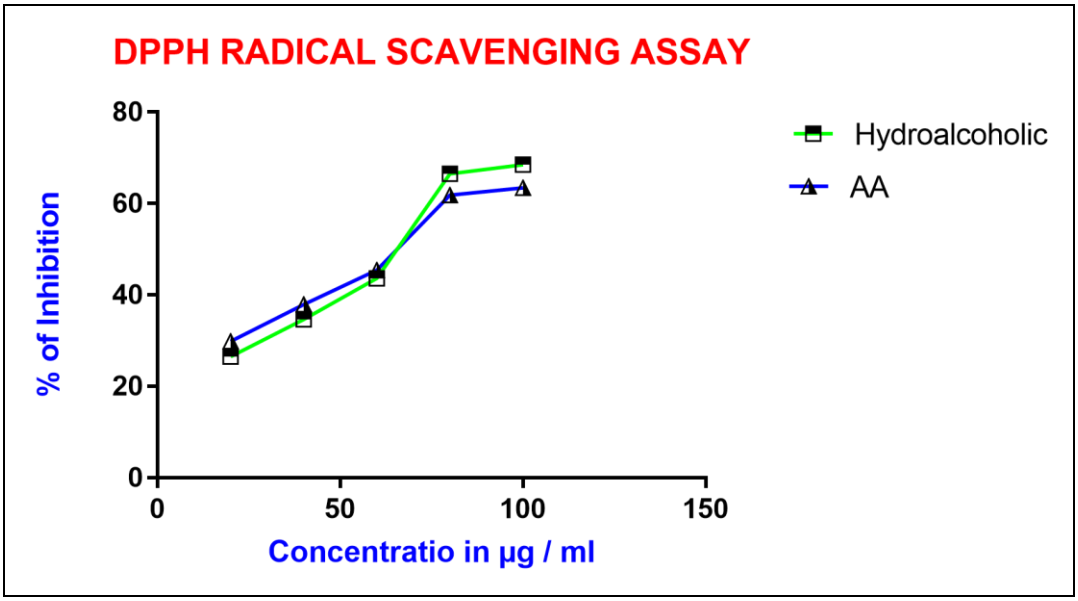

Fig 6: DPPH radical scavenging activity of *Elaeocarpus ganitrus*

Discussion

Preliminary X-ray analysis in Figure.4 shows that the Rudraksha seed internal structure has five compartments which signify that Rudraksha seeds obtained from *Elaeocarpus ganitrus* are five face Rudraksha seeds. *Elaeocarpus ganitrus* seed microscopy shows epidermis, parenchymatous cells, vascular bundle, lateral vein, unicellular Trichomes, calcium oxalate crystals, xylem and phloem that are prominent in Rudraksha microscopy. A prominent funicle can often be found at one of the hard seed's ends. A thick cellular endosperm covers the Rudraksha seed. Endosperm cells are arranged in parallel, solid rows. From the margin to the middle, rows are extended. Endosperm cells near the seed's edge are less prominent, and they gradually grow larger as the seed progresses. Seed endosperm cells contain large calcium oxalate crystals or sphaerocrystals. The vascular system and tanniniferous cells are noticeable under powder microscopy. The mesocarp cells are parenchymatous. Their shape is elongated, cylindrical, or spherical. The cellulose walls of the mesocarp are thin. The cells are tightly packed and stained darkly. The cylindrical cells are about 152 meters long and 32 meters wide. The square cells have a diameter of 70 meters. The stone cells make up the bulk of the endocarp. Endocarp walls are thick, lignified, and smooth. The lumen of the cell is larger. When examined under a microscope, the thick mass of cells in the powder exposed the presence of calcium oxalate crystals in the cells. The microscopy of Rudraksha seeds shows that these are genuine Rudraksha. Table.1shows Physicochemical values of the seed of *Elaeocarpus ganitrus*. *Elaeocarpus ganitrus* seed powder appearance was a coarse powder with a brownish-grey color having characteristic odor. Loss of drying was 12.5%w/v. The pH of the 1% aqueous solution was 5.7, which signifies that it's acidic, The total ash value was found to be 2.92 %. Successive solvent extractives with four types of solvents with different polarities, Petroleum ether, Ethyl acetate and Ethanol and Dist water were found to be 0.68%., 0.48%, 16.23% and 10.67% respectively. Foreign organic matter was found to be 0.6% w/w. Testing the estimation of heavy metal, we found various percentages of Zn, Fe, Cu, Mn, Cr and Mg while Ni, Cd and Pb were absent. Fruit pulp powder was treated with a variety of alkali and acids before being subjected to short and long UV examinations for fluorescence investigation. Under short and long UV, 1Normal HCl produced light yellow and brownish yellow, 1Normal NaOH produced yellow, brown and yellowish-grey, 50 % HNO3

produced greenish-brown color florescence. 1Normal NaOH in Methanol formed darkish brown and brownish olive green colors. 50 percent H2SO4 sulphuric acid produced a pale reddish and reddish-brown color. Methanol in nitrocellulose formed yellow and deep green colors. TLC fingerprinting of different solvent extract images of the seed of *Elaeocarpus ganitrus* Extract expressed in Table 3. TLC fingerprinting images of different extracts are shown in Figure. 5. Petroleum ether extract shows 4 spots under UV lamps having R_f value of 0.37,0.45,0.54,0.62 Similarly, Ethyl acetate extract shows 2 spots under UV lamps having Rf value of 0.64,0.75. Ethanol extract shows 3 spots under UV lamps having R_f value of 0.38, 0.45, 0.58, and aqueous extract shows 2 spots under UV lamps having R_f Value 0.81, 0.85. The fresh fruit of Rudraksha had a sour and slightly sweaty Taste. The existence of Glucose and Fructose were the sources of carbohydrates present in the fruit, according to the qualitative chemical test study. The secondary metabolites found in Rudraksha were Alkaloids, tannin, phenol compounds, and some flavonoids and sterols are the secondary derived metabolites in the fruit. Table.3 depicts the action of powder with various chemical reagents. DPPH radical scavenging activity of Rudraksha is shown in figure.6. DPPH radical scavenging % for the hydro-alcohol extract. By quenching the DPPH radical, the extract demonstrated concentration-dependent anti-radical action. The extract was shown to have a DPPH scavenging activity of 32.95 ± 0.10 percent (20 g / ml) and 72.56 ± 0.06 percent (100 g / ml). In the concentration range of 80 g/ml, the reference standard ascorbic acid showed considerable radical scavenging action. Hydro- alcohol and ascorbic acid had IC50 values of 59± 0.56 and 50± 1.58 g / ml, respectively. The *Elaeocarpus ganitrus* had a significant scavenging power that was comparable to that of conventional ascorbic acid, according to the analytical data.

Conclusion

The main conclusion that can be drawn from this Research work is that seed purchased from online source are genuine five faced *Elaeocarpus ganitrus* (Rudraksha) seed. Microscopy of Rudraksha seed shows epidermis, parenchymatous cells, vascular bundle, lateral vein, unicellular Trichomes, calcium oxalate crystals, xylem and phloem that are prominent in this pharmacognostic study. The secondary metabolites found in Rudraksha were alkaloids, tannin, phenol compounds and some flavonoids and sterols are the secondary derived metabolites in the fruit. The Hydro-alcoholic portions have a DPPH scavenging activity of 32.95 ± 0.10 percent (20 g / ml) and 72.56 ± 0.06 percent (100 g / ml). In the concentration range of 80 g/ml, the reference standard ascorbic acid showed considerable radical scavenging action. Hydro- alcohol and ascorbic acid had IC50 values of 59± 0.56 and 50± 1.58 g / ml, respectively which shows Rudraksha significance free radical scavenging activity.

Conflict of Interest

The authors affirm no conflict of interest, financial or otherwise

References

1. Hardainiyan S, Nandy BC, Kumar K. Elaeocarpus ganitrus (Rudraksha): A reservoir plant with their pharmacological effects. Int J Pharm Sci Rev Res,2015:34:55-64.
2. Lal PMTA: Elaeocarpus sphaericus: A tree with curative powers: an overview. Research Journal of Medicinal Plant,2013:7(1):23-31.
3. Sharma OP, Bhat TK: DPPH antioxidant assay revisited. Food chemistry,2009:113(4):1202-1205.
4. Szabo M, Idiţoiu C, Chambre D, Lupea A: Improved DPPH determination for antioxidant activity spectrophotometric assay. Chemical Papers,2007:61(3):214-216.
5. Prihantini AI, Tachibana S: Antioxidant compounds produced by Pseudocercospora sp. ESL 02, an endophytic fungus isolated from Elaeocarpus sylvestris. Asian Pacific journal of tropical biomedicine,2017:7(2):110-115.
6. Ritu Singh MS, Divya, Upadhayay: Rudraksh, Geographical Distribution, Reason For Decline & Measures To Enhance Propagation. In. Edited by Heritage INTFAaC. New Delhi: INTACH, 2016, 1-148.
7. Das P, Kar P, Hasnu S, Nath S, Tanti B. Phytochemical screening and antioxidant activity of Elaeocarpus serratus L. of Assam. Journal of Pharmacognosy and Phytochemistry,2017:6(4):866-869.
8. Tshilanda DD, Inkoto CL, Mpongu K, Mata S, Mutwale PK, Tshibangu DS-T, Bongo GN, Ngbolua K, Mpiana PT: Microscopic studies, phytochemical and biological screenings of Ocimum canum. International Journal of Pharmacy and Chemistry,2019:5(5):61-67.
9. Dring D: Chapter III Techniques for Microscopic Preparation. In: Methods in microbiology. Volume 4, edn: Elsevier; 1971, 95-111.
10. Mayank K, Manjul Pratap S. Pharmacognostical Standardization and Pharmacological Potential of Elaeocarpus ganitrus Leaves as an Antiulcer Agent. International Journal of Pharmaceutical Sciences and Nanotechnology,2018, 11(4).
11. Vuong QV, Ngoc Thuy Pham H, Vu HT, Dang TT, Van Ngo T, Chalmers AC. Fruit characteristics, phytochemical and antioxidant properties of blueberry ash (Elaeocarpus reticulatus). Heliyon,2018:4(10):e00834.
12. Zhao J, Pawar RS, Ali Z, Khan IA. Phytochemical investigation of Turnera diffusa. Journal of natural products,2007:70(2):289-292.
13. Mojab F, Kamalinejad M, Ghaderi N, Vahidipour HR. Phytochemical screening of some species of Iranian plants. Iranian Journal of Pharmaceutical Research,2010:(2):77-82.

14. Farnsworth NR: Biological and phytochemical screening of plants. Journal of pharmaceutical sciences,1966:55(3):225-276.
15. Yadav M, Chatterji S, Gupta SK, Watal G. Preliminary phytochemical screening of six medicinal plants used in traditional medicine. Int J Pharm Pharm Sci,2014:6(5):539-542.
16. Rajendra C, Magadum GS, Nadaf MA, Yashoda S, Manjula M. Phytochemical screening of the rhizome of Kaempferia galanga. International Journal of Pharmacognosy and Phytochemical Research,2011:3(3):61-63.
17. Bhandary SK, Bhat VS, Sharmila K, Bekal MP. Preliminary phytochemical screening of various extracts of Punica granatum peel, whole fruit and seeds. Journal of Health and Allied Sciences NU,2012:2(04):34-38.
18. Jyothiprabha V, Venkatachalam P: Preliminary phytochemical screening of different solvent extracts of selected lndian spices. Int J Curr Microbiol App Sci,2016:5(2):116-122.
19. Adesuyi A, Awosanya O, Adaramola F, Omeonu A: Nutritional and phytochemical screening of Aloe barbadensis. Current Research of Journal Biological Science,2012:4(1):4-9.
20. Rai DV, Sharma S, Rastogi M: Scientific Research on Elaeocarpus ganitrus (Rudraksha) For Its Medicinal Importance. Durg V Rai, Shiva Sharma and Manisha Rastogi,2019:69:1.
21. Chakraborthy G: Phytochemical screening of Calendula officinalis Linn leaf extract by TLC. International Journal of Research in Ayurveda and Pharmacy (IJRAP),2010:1(1):131-134.
22. Kumar M, Mondal P, Borah S, Mahato K: Physico-chemical evaluation, preliminary phytochemical investigation, fluorescence and TLC analysis of leaves of the plant Lasia spinosa (Lour) Thwaites. Int J Pharm Pharm Sci,2013:5(2):306-310.
23. Kumar M, Mondal P, Borah S, Mahato K. Physico-chemical evaluation, preliminary phytochemical investigation, fluorescence and TLC analysis of leaves of the plant Lasia spinosa (Lour) Thwaites. International Journal of Pharmacy and Pharmaceutical Sciences,2013:5:306-310.
24. Chen Z, Bertin R, Froldi G: EC50 estimation of antioxidant activity in DPPH assay using several statistical programs. Food chemistry,2013:138(1):414-420.
25. Deng J, Cheng W, Yang G: A novel antioxidant activity index (AAU) for natural products using the DPPH assay. Food Chemistry,2011:125(4):1430-1435.