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Cytotoxicity, antioxidant and antimicrobial assay of *Elaeocarpus ganitrus* (Rudraksha) seeds

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Abstract
Objective: Pharmacological study of Rudraksha seed extract in Cytotoxicity, Antioxidant and Antimicrobial assay.
Method: Cytotoxicity of the Rudraksha seed extract on HCT116 cell line established by MTT Assay. The total antioxidant activity test was performed with help of the phosphomolybdenum method. The antimicrobial activity was calculated by following the zone inhibition technique.
Result: No significant Cytotoxicity was found in the extract up to 100µg/ml. The total antioxidant activity in 1 mg (1000 µg) extract is equivalent to 59.28 µg Ascorbic acid. The antimicrobial assay proves that Rudraksha seed extract has noteworthy antibacterial action against *Staphylococcus Aureus* bacteria.
Conclusion: These scientific researchers agree on the traditional use of Rudraksha seed for medicinal use.

Keywords: *Elaeocarpus ganitrus*, rudraksha, cytotoxicity, antioxidant, antimicrobial assay

Introduction

Rudraksha seeds are usually obtained from a variety of plant species of Elaeocarpus which are species like *Elaeocarpus ganitrus, Elaeocarpus sphaericus, Elaeocarpus reticulatus*, etc [1]. Wearing Rudraksha seeds cure illnesses like stress, anxiety, insomnia, nervousness, lack of concentration, depression heart diseases like hypertension, rheumatism, sterility, immune modulator property. The plant usually originates up to 2000 meters above sea level. The plants belong to the genus Elaeocarpus possesses above 360 known species worldwide. Cytotoxicity is one important scientific technique used in indicators for biological assessment *in vitro* study. Pharmacologically chemicals like drugs and pesticides have dissimilar cytotoxicity mechanism that shows the demolition of cell membranes, avoidance of protein separation, irreversible binding to body receptors, etc [2]. A wide range of cytotoxicity assays techniques are currently used in the subject of toxicology and pharmacology For evaluation of the cell death caused by this damage, a Scientist needs an inexpensive, dependable, and reproducible short-term cytotoxicity and cell viability evaluation technique. Cytotoxicity assays were amongst the primary *in vitro* bioassay techniques used to predict the toxicity of substances to various tissues [3]. *In vitro* cytotoxicity testing provides a vital way for the safety evaluation and screening, and also for arrangement compounds determination [4]. The selection of a particular cytotoxicity assay technology may be inclined by precise research objective calculation. Four main categories of assays are used to ensure the retort of cultured cells after handling with potential toxicants. Antioxidant activity studies in a conventionally used medicinal plant variety is a technique of systematic justification of the medicinal characteristics use by native Peoples. Antioxidants play a vital part in our lives today. Antioxidants assist our body to neutralize or destroy "Reactive Oxygen Species" (ROS) or free radicals previous to their damage to our cells [3]. Antioxidant assay of the plant mostly due to its lively phytoconstituents Posses in a variety of medicinal Plants [5]. Antimicrobial assay testing is used for drug discovery, research expansion, antibiotic testing epidemiology, and prediction of a useful result [6]. The Zone of inhibition is a spherical area in the region of the spot of the antibiotic somewhere the microorganism does not grow. The zone of inhibition technique shows the vulnerability of the microorganism towards the antibiotic. A Zone of Inhibition Test is a qualitative technique used clinically to calculate antibiotic resistance and scientifically to test the capability of solids and textiles to inhibit microbial expansion. It is a rapid method to evaluate the antimicrobial action of a substance or solution relative to a target microorganism. Zone of Inhibition test now a day's being use throughout manufacturing to assess the inhibitory character next to an assortment of dissimilar microorganisms [7]. These tests perform best if the antimicrobial is capable to percolate out of the material. It is a rapid investigation to monitor an antimicrobial substance for inhibitory action against a panel of dissimilar microorganisms In this method Materials tested characteristically consist of leachable antimicrobial which has been entrenched in plastics, solids, surfaces, liquid, etc. also in an antibiotic solution [8]. The Zone of Inhibition test is a general and basic microbiological analysis normally used right through the medical mechanism and pharmaceutical industry.

Material and Method

Collection of authenticating *Elaeocarpus ganitrus* seeds (Rudraksha) was done by purchasing online from Rudra Center (https://www.rudraksha-ratna.com/) and further confirmed its originality by *CSIR-NISCAIR's* Research & Academics *in* Science & Technology Communication *New Delhi. Authentication No.-NISCAIR/RHMD/Consult/2019/ 3436-37*. The Rudraksha seeds are coarsely pulverized in a cutter and grinding mill. For preparation Hydroalcoholic

crude extract 100gm of Rudraksha seeds powder mix with 1000ml of Solvent Mixture (80% Methanol 1% HCl in distilled water). Then it In cubates in a shaker for 24 hours. Extracts centrifuged to get clear supernatant and dried the extract in an oven at 40-60 ºC. For ether extraction, we Weighed 10gm of Plant Powder and mix with 100 ml of Solvent (Petroleum Ether). Incubated in a shaker for 24 hours. Filter the extracts with Whatman filter paper 1 and dry the extract in the oven at 40-60ºC. The extracts used for the experiment of Cytotoxicity, Antioxidant and Antimicrobial assay.

Fig 1: *Elaeocarpus ganitrus* seeds

Fig 2: *Elaeocarpus ganitrus* seeds powder

Cytotoxicity Assay

Cytotoxicity of Rudraksha seed extract on the HCT116 cell line was determined by MTT Assay [9]. The cells (10000 cells/well) were cultivated in 96 well plates for 24 h in RPMI medium supplemented with 10% FBS and 1% antibiotic solution at 37°C with 5% CO2. The next day cells were treated from 62.5-1000 µg/ml of the hydroalcoholic extract [10]. After incubation for 24 hours, MTT (a final concentration of 0.5 mg/ml) was added to cell culture and further incubated for 2 h. At the end of the experiment, culture supernatant was removed and cell layer with matrix was dissolved in 100 µl DMSO (dimethyl sulfoxide) and read in an Elisa plate reader model (BioTek Instruments Inc, Vermont, USA) at 550 nm and 660 nm light range [11].

Total Antioxidant Assay

The total antioxidant activity was determined by the phosphomolybdenum method, it is based on the reduction of MO (VI) to MO (V) by the sample and subsequence formation of a green Phosphate/ MO (V) complex at pH below 7. The absorbance is measured at 695nm using a UV/Vis spectrophotometer. The antioxidant ability was articulated as Ascorbic acid alike (AAE) by using the standard Ascorbic acid solution [12]. For this Standard solution needed Ascorbic acid (1 mg/ml),ether extract (1 mg/ml), Phosphomolybdenum Reagent (0.6M Sulfuric Acid, 28mM Sodium Phosphate, 4mM Ammonium Molybdate. 50-250µg/ml concentration of standard & extract solution was prepared and 200 µl of each sample/standard were dispensed in a test tube. To all the tubes add 1.8 ml of Phosphomolybdenum reagent was added. 200 µl of water and 1.8 ml of reagent alone served as control. All the tubes were incubated at 90°C for 90 minutes. Samples were chilled to room temperature and the absorbance was measured at 695nm using a UV/Vis spectrophotometer next to the blank solution. The antioxidant capacity was expressed as Ascorbic acid equivalent (AAE) by using the standard Ascorbic acid.

Antimicrobial activity assay (Zone Inhibition Test) [13]

This test was conducted on MHA (Mueller-Hilton Agar) plates/ PDA Plates. For Bacterial cultures (*Staphylococcus aureus (gram +ve)*, *Pseudomonas aeruginosa (gram -ve)*), Fungal Culture (*Aspergillus spp.*) chosen. Ciprofloxacin discs (positive control) for Bacterial Sample chosen for the test. Solvent (vehicle control) Protein Sample (Different dilution of peptides (currently provided sample), 50-1000 µg/ml) [14]. The antimicrobial activity is checked by following the zone inhibition method. The MHA/PDA plates were spread inoculated with 100 µl of log cultures of all the bacteria and fungus followed by placing the discs containing 50µl of different dilutions concentration (50 - 1000 µg/ml). The antimicrobial activity was checked by following the zone inhibition method. The MHA/PDA plates were spread inoculated with 100 µl of log cultures of all the bacteria and fungus followed by placing the discs containing 50µl of different dilutions concentration (50 - 1000 µg/ml). Each disc was loaded with 50 µl of solvent so that the extract can diffuse to medium. One disc was loaded with solvent alone which served as vehicle control and a ciprofloxacin disc (30 mcg) was taken as a positive control. The plates were incubated at 32°C for 24 h and halo zones shaped in the region of the discs were calculated and recorded.

Results

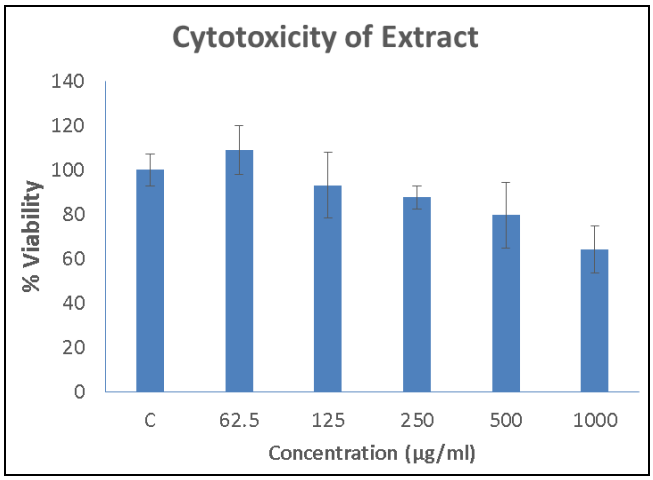

Fig 3: Cytotoxicity study

No significant Cytotoxicity was found in the extract up to 100µg/ml. The total antioxidant activity in 1 mg (1000 µg) extract is equivalent to 59.28 µg Ascorbic acid.

Table 1: Antimicrobial assay

				Size of Zone		
S. NO	**Solution Conc**	**Stock Conc**	***Staphylococcus Aureus***		***Pseudomonas***	***Trichoderma***
	(µg/ml)	**(mg/ml)**				
Extract 1	50	1	12	12	12	0
Extract 2	100	2	11.5	11.5	0	0
Extract 3	250	5	12	12	12	0
Extract 4	500	10	12	11	12	0
Ciprofloxacin		2	28	34	25	0

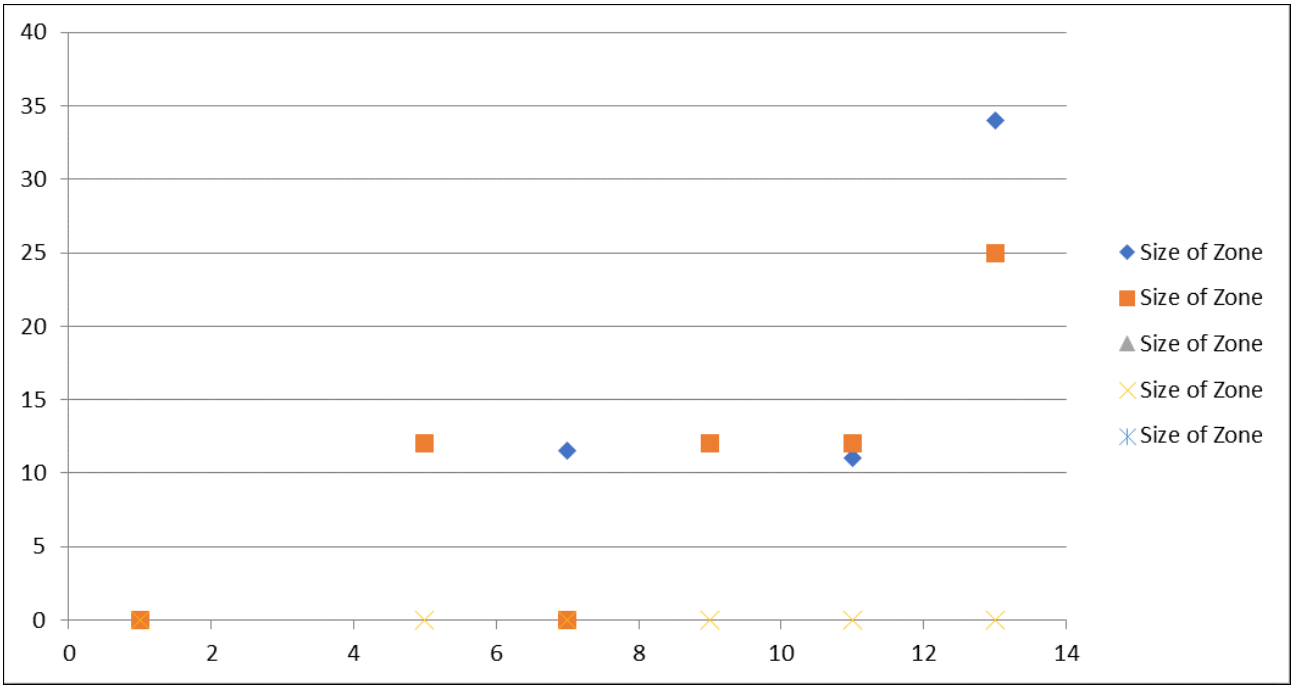

Fig 4: Graphical presentation of Antimicrobial assay of Rudraksha

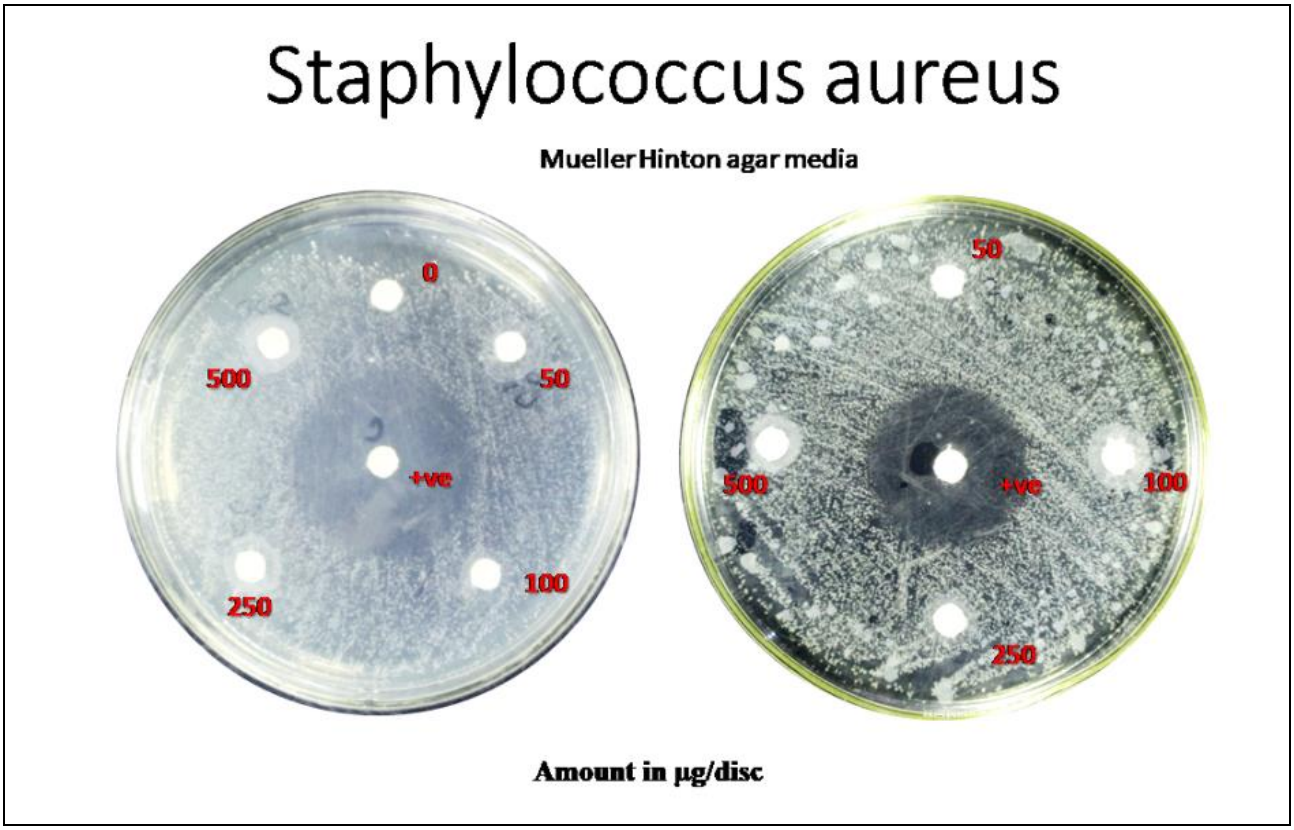

Fig 5: Antimicrobial assay of *Staphylococcus aureus*

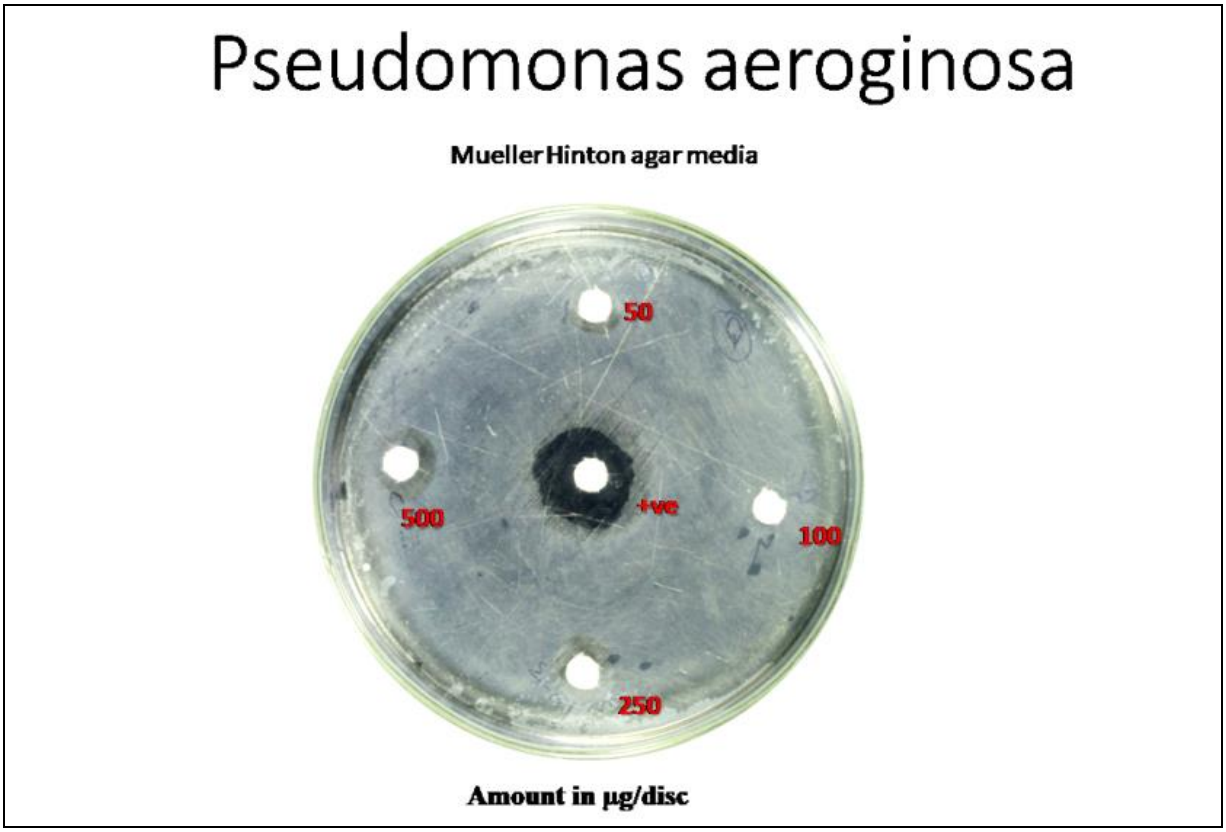

Fig 6: Antimicrobial assay of *Pseudomonas aeruginosa*

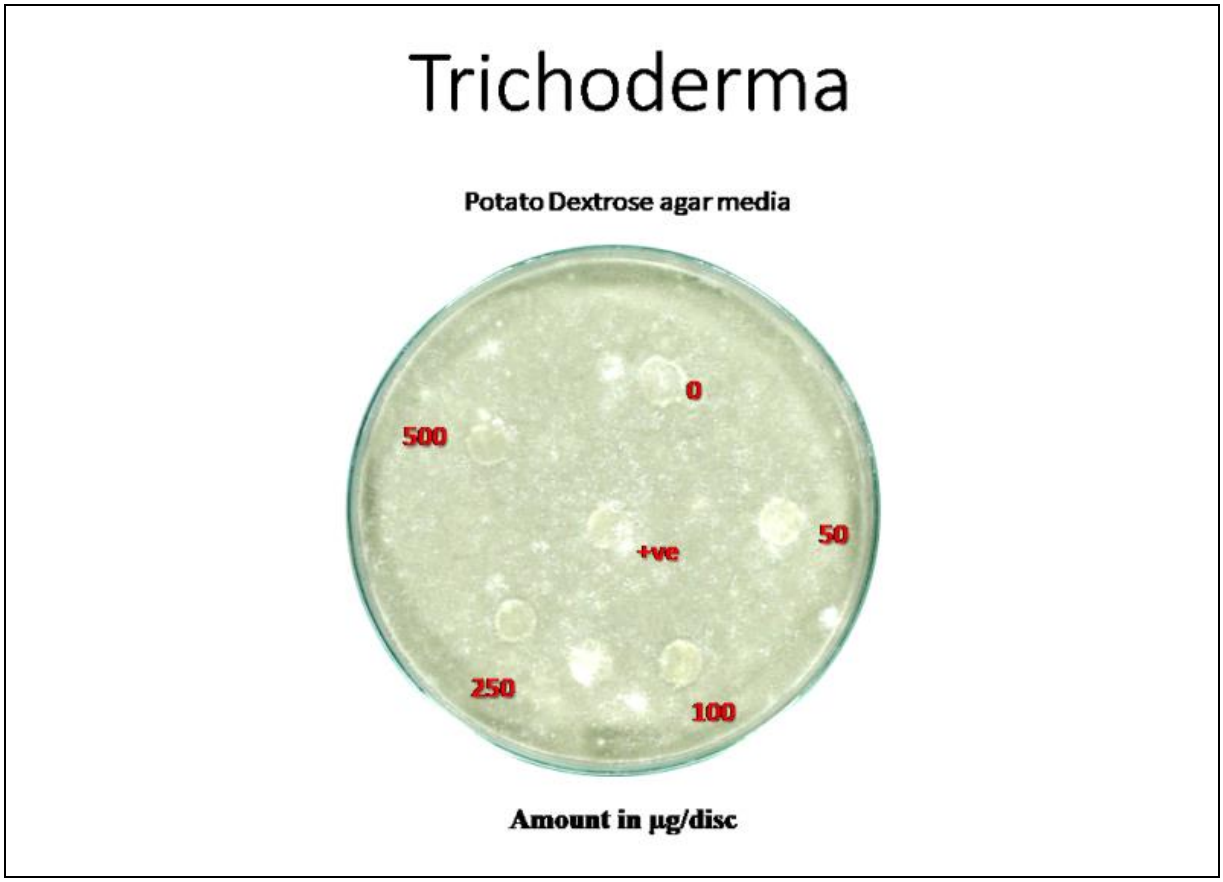

Fig 7: Antimicrobial assay of *Trichoderma*

Discussion

Cytotoxicity assays really on various cell functions. There is a dissimilar division for these assays which are dye exclusion assays, fluorometric assays, colorimetric assays, luminometric assays, etc. Selecting the suitable method among these assays is significant for getting accurate and dependable results [15]. Statistical data of *Elaeocarpus ganitrous* seed extract show no significant toxicity and the

result shows in figure3. Figure. 3 graph plotted for cytotoxicity study taking a concentration of extract in µg/ml at X-axis and percentage of viability of cell in Y-Axis. In this science experiment, we found that Rudraksha powder does not possess cytotoxicity activity. No significant Cytotoxicity was found in the extract up to 100µg/ml. The total antioxidant activity in 1 mg (1000 µg) extract is equivalent to 59.28 µg Ascorbic acid. Antimicrobial assay of (*Staphylococcus aureus (gram +ve)*, *Pseudomonas aeruginosa (gram -ve)*), Fungal Culture (*Aspergillus spp*.) result expressed in table1. The graphical presentation of data of the table.1 expressed in Fig.2. The antimicrobial assay by zone inhibition method shows that Rudraksha powders have some anti microbiological action. Different solution concentration shows that Rudraksha powders have potential anti microbiological action against *Staphylococcus Aureus* and *Pseudomonas* but against fungal specimen, *Trichoderma* Rudraksha seed extract did not show any anti-microbiological property. Fig.5, fig.6, and fif.7 expressed the Antimicrobial assay zone inhibition of *Staphylococcus aureus*, *Pseudomonas aeroginosa*, and *Trichoderma* respectively.

Conclusion

This scientific experiment exposed that *Elaeocarpus ganitrus* seed (Rudraksha) has no noteworthy Cytotoxicity in the extract up to 100µg/ml. The total antioxidant activity in 1 mg (1000 µg) of the extract is equivalent to 59.28 µg Ascorbic acid. *Elaeocarpus ganitrus* seeds (Rudraksha) hydroalcoholic extract have antimicrobial property against *Staphylococcus Aureus* and *Pseudomonas* but against fungal specimen *Trichoderma.* Rudraksha seed extract fails to show any anti-microbiological characteristics. Rudraksha seeds have been used traditionally for stress, anxiety, anti-microbiological, anti-aging, and immunomodulatory with the antihypertensive property. This experiment agrees on the seeds amazing medicinal property and it requires further scientific experiments to further explore their medicinal property.

Consent for Publication

Not applicable.

Conflict of Interest

The authors affirm no conflict of interest, financial or otherwise

Author Contribution

The main author is Mr. Subhashish Tripathy. This research paper was prepared by Mr. Subhashish Tripathy under the supervision of Dr. Arun Kumar Mishra and Dr. Amit Mishra.

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