

## FTIR analysis and antifungal study of *Elaeocarpus ganitrus* seeds (Rudraksha)

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### Abstract

**Objective:** Fourier-transform infrared spectroscopy (FTIR) Analysis and Antifungal Study of fungus *Candida albicans* by *Elaeocarpus ganitrus* Seeds (Rudraksha)

**Method:** FTIR analysis of Rudraksha seed Extract were analyzed with PerkinElmer spectrum version 10.03.06 and the anti-fungal activity checked by following the zone inhibition method By MHA plates.

**Result:** FTIR analysis results were recorded in the range of 4000-400 wave numbers. Significant Antifungal activity obtained in fungus *C.albicans*.

**Conclusion:** FTIR analysis results show that important bioactive compounds are present in Rudraksha seed extract. Antifungal activity study shows that Rudraksha seed can be used to stop the fungal growth of *C.albicans*.

**Keywords:** FTIR, *Elaeocarpus ganitrus*, Rudraksha, zone inhibition method, *Candida albicans*

### Introduction

#### *Elaeocarpus ganitrus* Seeds (Rudraksha)

Rudraksha seeds are recognized as Rudraksh and these are the dried seeds of a tree that only grows in a few places. Rudraksha (*Elaeocarpus ganitrus*) plant is a 50-200-foot-tall huge enormous plant [1, 2].

Depending on the area and the type of climate, this tree can reach a height of 14.60 meters to 29.20 meters. Rudraksha seeds' character is that it is irregular and woody on the exterior, with a light brownish-black color within the berry's pulp. Seeds can be filthy white, yellowish, golden-brown, or brownish-black in appearance. Each Rudraksha seed has a varying quantity of vertical lines that goes down from its outside that form 'Mukhs' or face of the seed [3]. The Rudraksha seeds are named delightful on a base of these faces or 'much. The Rudraksha bead serves as a self-protective shield, shielding the wearer from dangerous evil energies [4].

Wearing Rudraksha seeds heal sicknesses like worry, sleeplessness, nerviness, lack of focus, despair, heart problems like hypertension, rheumatism, sterility, immune modulator character, also it is observed that it has also property like asthma and anti-aging property [5, 6]. The polarity of Rudraksha beads is Electromagnetic, Paramagnetic, Diamagnetic, and Dynamic.

Since of their electromagnetism, Rudraksha also has anti-aging properties. Rudraksha Beads interrelate and integrate with the human body at a subtle level of awareness while worn. Rudraksha Beads persuade and affect positive change in the Bio Electrical System of the Human Body in a life-supporting way due to their Dielectric and Magnetic property. Positive change in our Bio-Chemical System occurs as a result of this alteration in our body's electrical composition [7, 8].



Fig 1: Rudraksha seed [9].

#### Fourier-transform infrared spectroscopy (FTIR):

FTIR (Fourier-transform infrared spectroscopy) is a method for acquiring an infrared spectrum of the absorption or emission of a solid, liquid, or gas. At the same time, an FTIR spectrometer collects high-resolution spectral data over a wide spectral range [10]. The preferred method of infrared spectroscopy is known as Fourier Transform Infra-Red (FTIR). Infrared spectroscopy involves passing IR photons through a sample. The sample absorbs some of the infrared light and passes part of it through (transmitted). The resulting spectrum depicts the sample's molecule absorption and transmission, resulting in a molecular fingerprint [11, 12]. No two unique molecule configurations produce the same infrared spectrum, just like fingerprints. As a result, infrared spectroscopy can be used for a variety of purposes. For both organic and inorganic component analysis, FTIR is widely used in a range of industries. It can tell you what chemicals are in solids, liquids, and gases. The FTIR technique is

mostly used to identify compounds that are unknown. Quantitative data, such as additives or pollutants, and kinetic data, such as infrared absorptions increasing or decreasing [13]. The study needs preparations for understanding the processes and mechanisms driving drug release are met by FTIR spectroscopy. The use of a combination of attenuated total reflectance-FTIR imaging and Nano-FTIR with chemometric to overcome the limitations of conventional infrared detection is a powerful technique. The use of FTIR in drug characterization, drug quality control, and bio-sample detection has immense promise [14].

### Zone inhibition method for microorganism

Zone of Inhibition Test, commonly known as a Kirby-Bauer Test, is a qualitative method for measuring antibiotic resistance in humans and for testing the ability of solids and fabrics to prevent microbial growth in the industry [15]. The Zone of Inhibition is a circular area surrounding the antibiotic's active site where microorganism colonies do not grow. The zone of inhibition can be used to determine a microbe's sensitivity to an antibiotic. Image processing can be used to automate the process of measuring the diameter of this Zone of Inhibition [16]. Approximately one million cells from a single strain are disseminated onto an agar plate using a sterile brush, which is subsequently incubated in the presence of the antimicrobial substance. If the bacterial or fungal strain is responsive to the antimicrobial treatment, a zone of inhibition emerges on the agar plate. If the antimicrobial agent is resistant, there will be no zone. Antimicrobial susceptibility testing can be utilized in drug development, epidemiology, and therapeutic outcome prediction. The Zone of Inhibition test is currently widely used in industry to determine the inhibitory nature of a variety of microorganisms. The antibiotic must be able to seep out of the material for this test to be effective [17].

### Material and Method

Collection of authenticating *Elaeocarpus ganitrus* (Rudraksha) was done by purchasing online from Rudra Center (<https://www.rudraksha-ratna.com/>) and 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. The Rudraksha seeds are coarsely pulverized in a cutter and grinding mill. *Elaeocarpus ganitrus* Seeds were used to make the alcoholic extract. 500g Rudraksha seed powder extracted with 10 liters of methanol at reflux temperature for 15 hours and cool to room temperature, filtered concentrated under vacuum. This Extract is used for experimental purposes in this research. The extract was analyzed with PerkinElmer spectrum version 10.03.06 and results were recorded in the range from 4000-400 wave number. For the Anti-fungal- Zone Inhibition test of *Candida albicans*, we used MHA Plates, Fungal cultures ( *C. Albicans*,), Sterile discs (6 mm), Amphotericin B discs (positive control), Solvent (vehicle control), the Concentration range of samples – (0 to 500µg/ml). The anti-fungal activity was checked by following the zone inhibition method. The MHA plates were spread inoculated with 100 µl of log cultures of fungal culture (adjusted to 0.5 McFarland Unit) and fungus followed by placing the discs containing 20µl of different dilutions concentration (0 to 500 µg/ml). Each disc was loaded with 10µl of solvent (DMSO) so that extract can diffuse to medium. One disc was loaded with solvent alone which served as vehicle control and an Amphotericin B disc (5 mg/ml) was taken as the positive control. The plates of *C.albicans* were incubated at 37°C for 24 hours, and clear zones created around the discs were measured and recorded.



Fig 2: *Elaeocarpus ganitrus* seeds powder

### Results

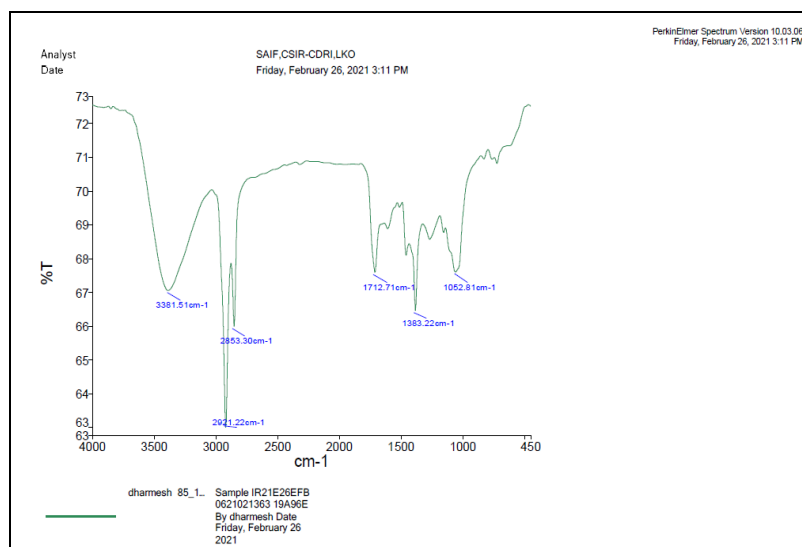


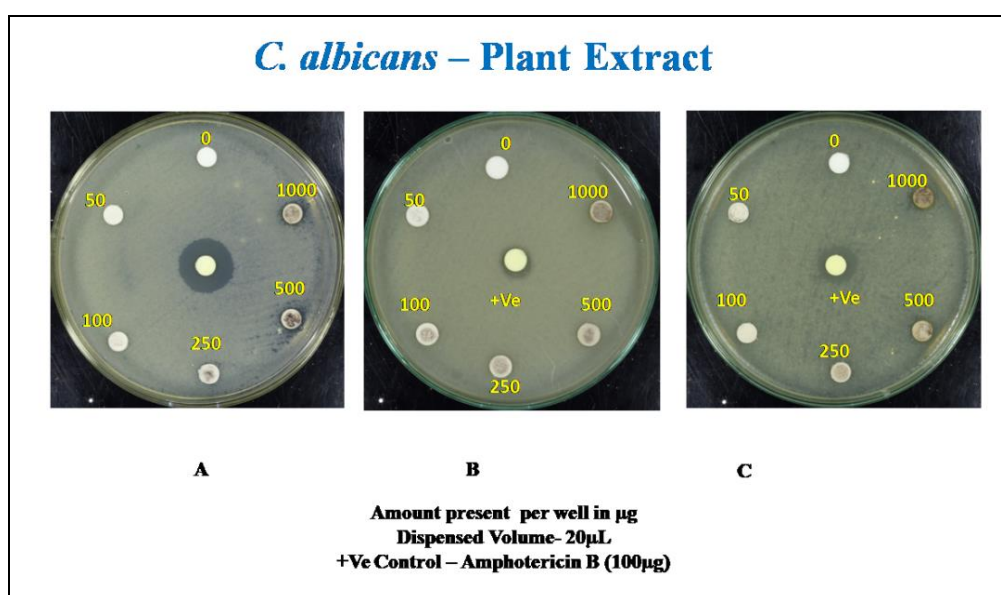
Fig 3: FTIR graph spectrum

**Table 1:** FTIR pear Area/Height data table of sample

List of peak Area/ Height		
Peak Name	X( $\text{cm}^{-1}$ )	Y(%T)
1	3381.51	67.07
2	2921.22	63.01
3	2853.3	66
4	1712.71	67.61
5	1383.22	66.47
6	1052.81	67.63

**Table 2:** FTIR graph interpretation of compound

Sr. No.	Wave Number		Assignment	Possible Compound Type
	Exp.	Literature		
1.	3381.51 $\text{cm}^{-1}$	3400–3200	Normal ‘‘polymeric’’ OH stretch	Alcohol and hydroxy compound
		3400–3380	Aliphatic primary amine, NH stretch	Ether and oxy compound
2.	2929.22 $\text{cm}^{-1}$	2935–2915	Methylene C-H asym./sym. Stretch	Saturated Aliphatic (alkene/alkyl)
3.	2853.30 $\text{cm}^{-1}$	2935–2915/2865–2845	Methylene C-H asym./sym. Stretch	Saturated Aliphatic (alkene/alkyl)
4.	1712.71 $\text{cm}^{-1}$	1725–1705	Ketone	Carbonyl compound
		1725–1700	Carboxylic acid	Carbonyl compound
5.	1383.22 $\text{cm}^{-1}$	1420–1370	Organic sulfates	Simple hetero-oxy compounds
6.	1052.81 $\text{cm}^{-1}$	1055–1020	Organic siloxane or silicone (Si-O-Si)	Simple hetero-oxy compounds
		~1050	Primary alcohol, C-O stretch	Alcohol and hydroxy compound
		1150–1000	Aliphatic fluoro compounds, C-F stretch	Aliphatic organohalogen compound
		1100–1000	Phosphate ion	Common inorganic ions
		1100–900	Silicate ion	Common inorganic ions

**Fig 4:** Anti-fungal- Zone inhibition test (*C. albicans* – Plant Extract)**Table 3:** Test organism *C. albicans* (Zone Inhibition in mm.)

Sample code	Amount loaded ( $\mu\text{g}$ )	Plate A	Plate B	Plate C	Average	SD	SEM
Plant Extract	Control	16	11	13	13.33333	2.516611	1.452966
	0	0	0	0	0	0	0
	50	0	0	0	0	0	0
	100	0	0	0	0	0	0
	250	7	0	6	4.333333	3.785939	2.185813
	500	10	7	10	9	1.732051	1
	1000	11	8	11	10	1.732051	1

Amount present per well in  $\mu\text{g}$  Dispensed Volume- 20 $\mu\text{L}$ +Ve Control – Amphotericin B (100 $\mu\text{g}$ )

## Discussion

Our study is nevertheless a valuable contribution to the field of herbal plant science for structural elucidation of the functional group present in *Elaeocarpus ganitrus* seed (Rudraksha) and it suggests that Rudraksha seed extract can be used for an organic fungicide of *C. albicans* at higher

doses. FTIR analysis of a methanolic extract of Rudraksha seed extract by PerkinElmer spectrum version 10.03.06 gives the spectra presented in Figure.3 of this manuscript. Six major functional group present at peak obtained in the spectrum which are 3381.51 $\text{cm}^{-1}$ , 2929.22  $\text{cm}^{-1}$ , 2853.30  $\text{cm}^{-1}$ , 1712.71  $\text{cm}^{-1}$ , 1383.22  $\text{cm}^{-1}$ , 1052.81  $\text{cm}^{-1}$  which are

present in Table.2. The graph interpretation of FTIR was conducted in Table.2 and interpreted that the six major peak areas contain Alcohol and hydroxy compound, Ether and oxy compound, Saturated Aliphatic (alkene/alkyl) compound, Carbonyl compound, Simple hetero-oxy compounds. Alcohol and hydroxy compound, Aliphatic organohalogen compound, and common inorganic compound. The current study represents one of the largest studies to date on the microbiological assay of the herbal extract by the Zone of Inhibition test. *Elaeocarpus ganitrus* seed extract used for Antifungal Study using Amphotericin as standard. Amount present per well in µg Dispensed Volume- 20µL and Zone Inhibition in mm expressed in the Figure.4. In the experiment, we found that Rudraksha plant extracts on effective in higher doses above 100 µg and the experimental result of antifungal action is expressed in Table.3 of this manuscript. Some of the broad conclusions drawn from the results that Rudraksha seed extract can be used as a fungicide agent for fungal species like *C. albicans* at higher doses.

### Conclusion

Rudraksha seeds have been used traditionally for medicinal purposes like Antiageing, stress, anxiety, insomnia, and mental concentration. This research constitutes a contribution towards the solution of finding the key functional group of Rudraksha seed by FTIR analysis. We find that major functional groups are Alcohol and hydroxy compound, Ether and oxy compound, Saturated Aliphatic (alkene/alkyl) compound, Carbonyl compound, Simple hetero-oxy compounds. Alcohol and hydroxy compound, aliphatic organohalogen compound, and common inorganic compound. The main conclusion that can be drawn from this work that the above functional group compounds may be responsible for Rudraksha's therapeutic property. Antifungal Study of Rudraksha seeds against fungal species *Candida albicans* shows that Rudraksha seed is only effective for fungicidal action at higher doses. This highlights the potential usefulness of Rudraksha fungicide action at present time.

### 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 Prepared the article under the supervision of Dr. Arun Kumar Mishra and Dr. Amit Mishra.

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