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Characterization and in Vitro Antioxidant, Anti-Cancer, and Anti-Neurogenerative Properties of Rudraksha Alkaloids Rich Extract

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ABSTRACT:

The present study investigated the antioxidant, anticancer, and anti-neurodegenerative potential of Rudraksha alkaloid rich extract. The antioxidant activity of RARE was measured by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and 2, 2'-azino-bis3-ethylbenzthiazoline-6-sulfonic acid (ABTS) free radical scavenging activities while anti-cancer and, anti-neurodegenerative properties was evaluated through MTT assay, and *acetylcholinesterase* enzyme inhibition activities respectively. The study outcomes demonstrated a comparable dose-dependent response of RARE with that of reference standards for the tested biomedical properties. The IC_{50} values for antioxidant, anti-cancer, and anti-neurodegenerative activities were obtained. Further, *in vivo* safety and efficacy assessments are warranted to confirm their *in vitro* biomedical applications.

Keywords: Alkaloid, Rudraksha bead; Antioxidant; and Anti-neurodegenerative

1. Introduction

In traditional Indian culture, Rudraksha beads hold great holistic significance, offering both spiritual and therapeutic advantages (1, 2, 3, 4, and 5). *Elaeocarpus ganitrus*, as it is scientifically named, is a member of the genus *Elaeocarpus* and family *Elaeocarpaceae*. There are over 360 species of *Elaeocarpus* known to exist in the world, with 25 of them species occurring in India (6, 7, and 8). Rudraksha beads are traditionally worn for their health advantages, as well as taken orally in the form of powder, extract, or aqueous leachate. Oral consumption of Rudraksha formulations is credited to the existence of a spectrum of phytoconstituents, whilst the physical wearing of Rudraksha rosaries offers health benefits because of its inherent electromagnetic property (9, 10, and 11). Research has shown many pharmacological properties of formulations containing rudraksha, such as anti-microbial, anti-hypertensive, anti-diabetic, analgesic, anxiolytic, cardioprotective, and antioxidant properties (12, 13) One of our previous studies reported the quantitative outcomes of phytochemicals including flavonoids, tannins, phenolics, anthocyanin, ascorbic acid, saponins, alkaloids, and terpenoids (10). Among several phytoconstituents, alkaloids' biological activities and therapeutic applications have prompted a great deal of study. Alkaloids fall into various classes. Their biosynthetic precursor and heterocyclic ring system serve as the basis for this classification. These comprise the following: imidazoles, quinolizidines, indoles, piperidines, pyrrolidines, tropanes, isoquinoline purines, and pyrrolizidines. In case of Rudraksha, it is reported to contain the indolizidine type of alkaloids with characteristic five-membered ring joined with a six-membered ring sharing a nitrogen atom. A recent study reported diverse alkaloids like Elaeokanine C, (+)-elaeocarpine, Elaeocarpenine, Isoelaeocarpine, Grandisine types A-G Isoelaeocarpiline, Elaeocarpidine, Isoelaeocarpicine, and Habbemine A and B in the Rudraksha beads (*Elaeocarpus sphaericus Schum*) collected from Java, Indonesia (14). The present study aims to characterize the Rudraksha Alkaloids Rich Extract (RARE) and evaluate its *in vitro* antioxidant, anti-cancer, and anti-neurodegenerative potential. The characterization of RARE is done by LC-MS and functional group analysis is done using FT-IR. The antioxidant activity of RARE is evaluated by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging, ferric reducing antioxidant power (FRAP), and xanthine oxidase inhibition assays, while anti-cancer and anti-neurodegenerative properties are assessed through MTT assay and acetylcholinesterase enzyme inhibition activity respectively.

2. Material and Methods

Materials

Rudraksha beads were collected from the repository of Kunwar Shekhar Vijendra Ayurvedic Medical College and Research Center, Shobhit University, Gangoh. 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were procured from Sigma Aldrich Pvt. Ltd. The human lung cancer (A549, CCL-185), human colorectal cancer (HCT-116, CCL-247), human breast cancer (MCF-7, HTB-22), human prostate cancer (PC3, CRL-1435) cell lines were procured from American Type Culture Collection (ATCC), USA. F12K, McCoy's 5A, EMEM, DMEM, Penicillin/streptomycin and Fetal Bovine serum (FBS) were procured from Gibco. Cisplatin was procured from a pharmacy. Chemicals like ferric chloride hexahydrate ($FeCl_3.6H_2O$), xanthine, xanthine oxidase, 2, 4, 6-tri (2-pyridyl)-s-triazine (TPTZ), ferrous sulphate heptahydrate ($FeSO_4.7H_2O$) were purchased from Himedia.

Preparation of Rudraksha Alkaloids Rich Extract (RARE)

The beads were adequately washed with sterile water and crushed into a coarse powder using regular mixer grinder and defatted with n-hexane. The marc was soaked overnight in 70%

ethanol at room temperature. The ethanolic extract was filtered and evaporated under reduced pressure yielding a dark reddish-brown viscous residue which was dissolved in 5% HCl, filtered, and partitioned using dichloromethane. The aqueous layer was adjusted to pH 9 using NH_4OH and then fractionated with dichloromethane. The dichloromethane layer was washed with water and evaporated under reduced pressure at 40°C, yielding a reddish-brown alkaloid rich powder which was stored at room temperature in dark glass bottle.

Fourier-Transform Infrared Spectroscopy (FT-IR)

Functional groups identification in RARE was carried out through Fourier transform infrared (FTIR) spectrophotometer (Agilent Cary 630, Perkin Elmer). Briefly, RARE was mixed with KBr in the ratio of 1:8 and converted into 13mm pellets using pelletizing die and hydraulic pressing at 3000 psi pressure. The pellets were kept into solid sample holder and the spectrum was evaluated for full wavelength range in pre-calibrated FT-IR (700-4000 cm^{-1}) at room temperature. After blank subtraction, the remaining absorption spectra was compared with the reference absorption peak in in-built library available for the active functional group.

Liquid Chromatography-Mass Spectroscopy (LC-MS/MS) Analysis

Rudraksha alkaloids rich extract was reconstituted in acetonitrile and water in the ratio of 1:1 and was filtered using polytetrafuoroethylene (PTFE) membrane filter (0.45 µm size). Five microliters (5 µL) of the filtrate were injected into the liquid chromatographical system (Agilent 6470 Triple Quadrupole LC/MS system). The sample was run in a C18 column (2.7 µm x 2.1 mm × 100 mm) at a flow rate of 0.3 mL/min. The temperature of the column was fixed at 55°C. The mobile phases used were 5mM ammonium formate in 0.1% formic acid in water (A) and 0.1% formic acid in 100% methanol (B). A ratio of 95 : 5 (A : B) was maintained for 1minute, followed by 60 : 40, 40 : 60, 5 : 95, 90 : 10, and 95 : 5 for consequent 1-10, 10-12, 13–16, 17-19, and 19-20 minutes respectively. The MS was set with a fragmentation voltage of 500 V in an automatic mode with 225°C probe temp, 12 mL/min flow rate, and 50 psi nebulizer gas. The identification of the various compounds present in the plant material was done based upon retention time against the pre-mix standard.

in Vitro Anti-Cancer Assay

The cell viability of RARE was evaluated in four cells lines viz. human prostate cancer (PC3), human non-small cell lung carcinoma (A549), human colorectal cancer (HCT116), and human breast cancer (MCF-7). PC3 and A549 (F-12K Medium (Kaighn's Modification of Ham's F-12 Medium), MCF7 (Eagle's Minimum Essential Medium (EMEM)), and HCT116 (McCoy's 5A Medium) cell lines were cultured with 10% FBS and 1% Penicillin/streptomycin. 5000 cells were seeded in 96 well culture plate overnight supplemented with 10 % FBS, 100 µg/mL penicillin/streptomycin. Next day, cells were treated with different concentrations of RARE (6.25, 12.5, 25, 50 and 100 µg/mL) for 72 h. Cisplatin at 5 µg/mL was used as positive control across the cell lines. Post 72 h treatment, 25 µL of MTT per well was added and the plate was incubated for four hours in CO_2 incubator. 100 µL of solubilisation solution was added and absorbance readout was taken at 570 nm. The cell viability for the treatment groups were calculated considering the value of control cells 100 % viability and an IC_{50} were computed.

in Vitro Antioxidant Activity

DPPH free radical scavenging capacity of RARE was assessed at different concentrations (6.25, 12.5, 25, 50, and 100 µg/mL) with gallic acid as a standard. Briefly, DPPH was dissolved in methanol (1mg/mL) and mixed with RARE or gallic acid followed by vigorous shaking and incubation in dark for 30 min. The samples were adequately diluted and absorbance was taken at 517 nm (15). Ferric reducing antioxidant power assay was carried as described elsewhere

(16). Standard curve was derived from different concentrations of ferrous sulphate heptahydrate ($FeSO_4.7H_2O$) serial dilution while ascorbic acid was used as standard. The samples and standard were read at 593 nm and the results were expressed as mM Fe (II) per gram of RARE. For xanthine oxidase inhibitory activity, different concentrations of RARE (6.25, 12.5, 25, 50, and 100 µg/mL) or standard allopurinol were prepared using 2% Tween 20. Samples or standard (0.1 ml) was mixed with 50 mM phosphate buffer solution, pH 7.5 (1.9 mL) and incubated for 15 min followed by addition of enzyme xanthine oxidase. The reaction was stopped by adding 0.5 M HCl after 15 mins and absorbance was read at 290 nm against blank. Data was represented as percentage xanthine oxidase inhibition (17).

In Vitro Anti-Neurodegenerative Activity

The *in vitro* anti-neurodegenerative potential was assessed using *acetylcholinesterase (AChE)* inhibition activity using method described by Ellman et al. (1961) (18). Donepezil was used as reference standard.

Statistical Analysis

All *in vitro* experiments were conducted in triplicate and data was represented as Mean ± Standard Error using Student's T test. The inhibition concentration (IC_{50}) that was calculated by constructing a dose-response logarithmic function curve using non-linear regression by GraphPad Prism 6.0 software.

3. Results and Discussion

The present study provided for the first time the comprehensive characterization outcomes of RARE to the best of our knowledge. The LC-MS/MS analysis showed the presence of a total of 44 components out of which 34 components (77%) were diverse group of alkaloids (Figure 1 and Table 1). These outcomes confirm the preparation of Rudraksha alkaloids rich extract. The analytical method known as liquid chromatography-mass spectrometry (LCMS) combines the mass spectrometer's detection selectivity with the physical separation powers of liquid chromatography (19). It separates the sample's components, and a mass spectrometer detects the charged ions. The outcome can be used to determine the molecular weight, structure, identification, and quantity of particular sample components. Additionally, the compounds are separated according to how they interact with the stationary phase of the particles' chemical layer and how the solvent elutes through the column (mobile phase). A broader spectrum of components, including proteins, high polarity or high molecular mass compounds, and thermolabile chemicals, can be analyzed using this approach. Apart from the alkaloids, the RARE also indicate the presence of components like benzene, nucleotide, hydrobenzoic acid, flavonoids, terpenoids, antibiotics, and quinoline.

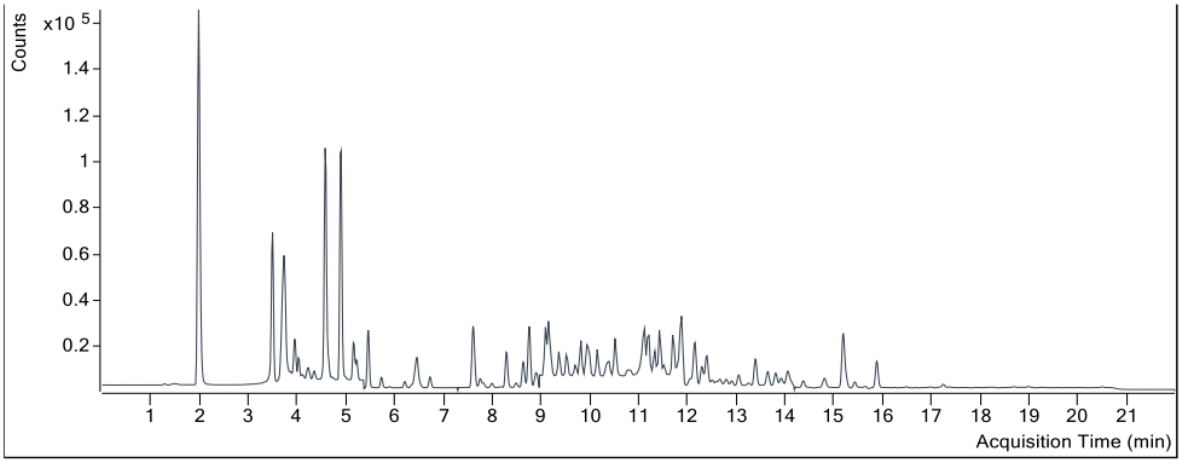

Figure 1. LC-MC analysis of RARE

FTIR

The FTIR spectra of RARE with typical functional groups are shown in Figure 2 and Table 2. Results indicate sharp peaks in between 3005-3094 cm^{-1} of C-H stretching or alkene compound; strong and broad O-H stretching of carboxylic acid usually cantered on 3000 cm^{-1} and strong and broad N-H Stretch band of amine salt in between 2800-3000 cm^{-1} ; medium peaks in between 2600-2800 cm^{-1} representing C-H stretching or aldehyde; strong peak at 2926.2991 cm^{-1} representing C-H (str.) or alkane; weak peak at 2150 cm^{-1} indicating C=C=O stretching or ketene; peak at 2255.9806 cm^{-1} indicating CΞN stretching or nitrile ; peak at 2067.9572 cm^{-1} indicating C=C=N stretching or ketenimine; medium peak in between 1900-2000 cm^{-1} indicates C=C=C stretching or allene. Further the peak at 1186.2347 cm^{-1} represent C-O stretching and ester; strong peaks at 1118.2390 cm^{-1} and 1091.7711 cm^{-1} indicating C-O stretching and secondary alcohol; strong, broad peak at 1049.7162 cm^{-1} and 1036.0117 cm^{-1} indicating CO-O-CO stretching; anhydride. Medium peak at 853.5980 cm^{-1} shows C=C bending; trisubstituted; strong peak at 723.1412 indicates C=C bending disubstituted (cis); alkene that in line with the previously reported studies for pure isolated alkaloid (20). Alkaloids extracted from different plants have major functional groups O-H, N-H, C=O and C-H stretching groups of these compounds and confirming the presence of alkaloids in these plants (20).

Peak in between 1600- 1720 cm^{-1} indicates C=O bond which further indicating the presence of Noscapine alkaloid; peaks in between 600-1550 cm^{-1} and strong peak at 1281 cm^{-1} and 1243 cm^{-1} representing C-H (out of plane bend.); C-N stretching and C-O stretching or alkyl aryl ether which combinedly indicates the presence of murrayanine alkaloids (21). Peaks in between 600-1550 cm^{-1} and strong peak at 1281 cm^{-1} and 1243 cm^{-1} representing C-H (out of plane bend.); peak in between 1600- 1720 cm^{-1} indicates C=O bond; strong peak at 1444 cm^{-1} C=N; OCH3; sharp peaks in between 3005-3094 cm^{-1} indicate the C-H stretching or alkene compound combined resembling the harmaline and harmine. Presence of the N-H Stretch band or amine in between 2800-3000 cm^{-1}; C-H (out of plane bend.) in between 600-1550 cm^{-1}, C=O bond in between 1600- 1720 cm^{-1}, C=C alkene at 723 cm^{-1} and 853 cm^{-1}; C-N at 1281 cm^{-1}; CO-O-CO stretching; anhydride at 1049 and 1036 indicating piperidine alkaloid (22).

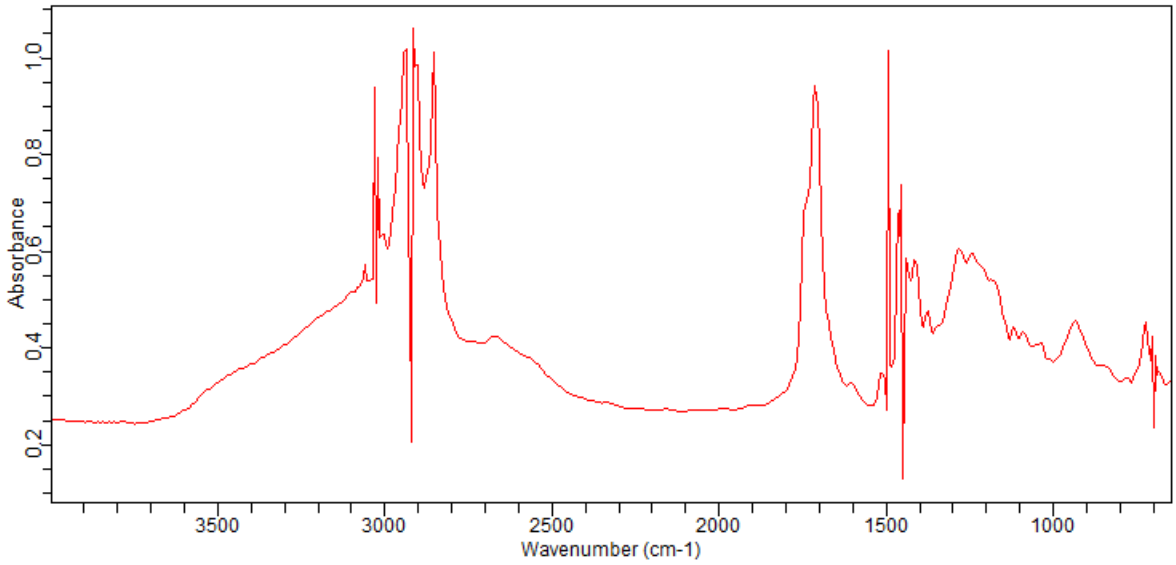

Figure 2. FT-IR analysis of RARE

Peak At	Peak Height	Functional Group and compound Name
3094.6317	0.7330	sharp peaks in between 3005-3094 indicating the C-H stretching – alkene compound
3078.7434	0.7638	sharp peaks in between 3005-3094 indicating the C-H stretching – alkene compound
3060.3326	0.8850	sharp peaks in between 3005-3094 indicating the C-H stretching – alkene compound
3030.5139	2.0032	sharp peaks in between 3005-3094 indicating the C-H stretching – alkene compound
3022.6827	2.0049	sharp peaks in between 3005-3094 indicating the C-H stretching – alkene compound
3005.7779	1.0528	O-H stretching; carboxylic acid; usually centered on 3000 cm-1 Strong and Broad
2926.2991	0.7453	Strong Peak at 2926.2991; C-H (str.),; alkane
2736.0542	0.4676	Medium, C-H stretching, aldehyde, Doublet
2673.3672	0.4958	Medium, C-H stretching, aldehyde, Doublet
2343.6296	0.1336	Peaks in between 2330-2350 O=C=O stretching; carbon dioxide
2330.3015	0.1331	O=C=O stretching; carbon dioxide
2255.9806	0.1040	Weak, CΞN stretching; nitrile
2155.4178	0.0992	C=C=O stretching; 2150, ketene; Weak, CΞN stretching; nitrile 2255.9806
2067.9572	0.0975	C=C=N stretching; ketenimine
1986.1063	0.1010	Medium; C=C=C stretching; allene
1901.0552	0.1184	Medium; C=C=C stretching; allene
1550.6855	0.1243	C-H group
1514.8428	0.2924	C-H group
1494.0977	2.0429	C-H group
1464.3543	1.1657	C-H (in plane bend.)
1454.9795	1.3304	C-H group

1444.6634	1.0087	C-H (in plane bend.)
1412.8493	0.9140	C-H (in plane bend.)
1377.9479	0.6372	C-H (in plane bend.)
1281.8654	0.9709	Strong, C-N stretching;
1243.9143	0.9422	Strong, C-O stretching, alkyl aryl ether
1186.2347	0.7994	Strong; C-O stretching; ester
1118.2390	0.5458	Strong; C-O stretching; secondary alcohol
1091.7711	0.5180	C-H (out of plane bend.)
1049.7162	0.4468	strong, broad; CO-O-CO stretching; anhydride
1036.0117	0.4597	C-H (out of plane bend.)
935.1476	0.5768	C-H (out of plane bend.)
853.5980	0.3392	C-H (out of plane bend.); medium; C=C bending; trisubstituted
782.2515	0.2705	C-H (out of plane bend.)
755.5577	0.2865	C-H (out of plane bend.)
723.1412	0.5746	C-H (out of plane bend.); strong; C=C bending; alkene; disubstituted (cis)
704.7304	0.4913	C-H (out of plane bend.)
692.2683	0.4162	C-H (out of plane bend.);

Table 1: Peak analysis for FTIR analysis of Alkaloid extract

Antioxidant

Alkaloid extract was used for anti-oxidant activity by using DPPH, FRAP and Xanthine oxidase assay at different concentration. The result demonstrated in figure 3 – 5 represent the outcome of DPPH, FRAP and Xanthine oxidase assay. In DPPH assay, alkaloid extract showed excellent response and was statistically significant compared to Gallic acid as standard. In FRAP activity and Xanthine assay, alkaloid extract did not show the activity.

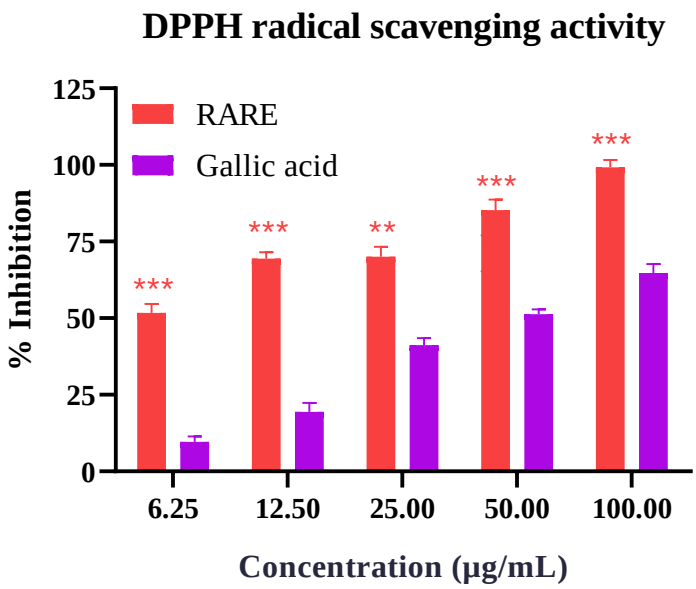

Figure 3: DPPH radical scavenging activity for RARE

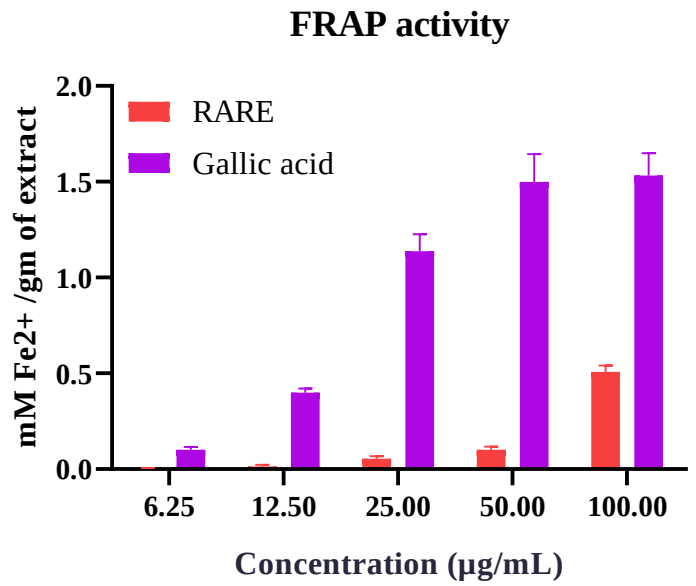

Figure 4: FRAP activity for RARE

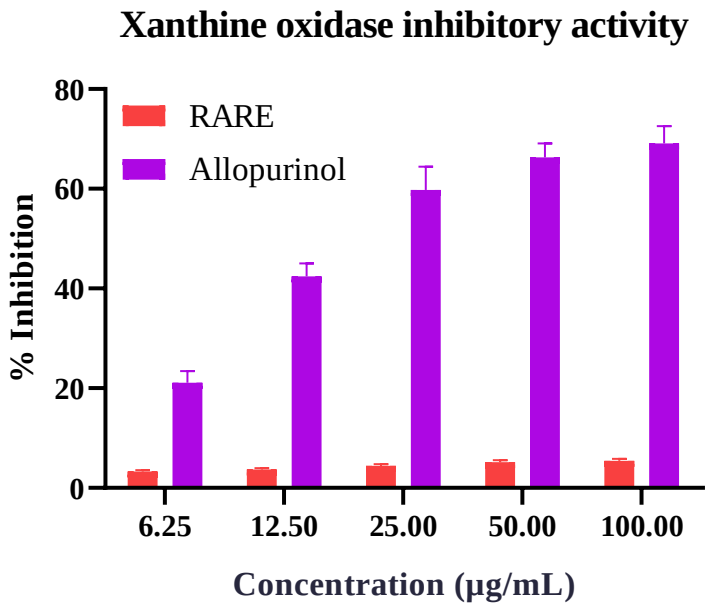

Figure 3: Xanthine oxidase inhibitory activity for RARE

Anti-Cancer Activity

To understand the anti-cancer activity of alkaloid extract of Rudraksha beads, cell viability assay was performed in four cancer cell lines related to human prostate cancer, human non-small cell lung carcinoma, human colorectal cancer, and human breast cancer. The results demonstrated in Figure 6 to 9 represent the outcomes of the MTT cell viability assay for varied concentrations of alkaloid extract and cisplatin as standard reference. In all the tested seven cell lines, the percent cell viability was dose dependent and decreased with an increased concentration of alkaloid extract. The IC50 values are 8.65 (A549), 18.26 (HCT-116), 18.39 (PC-3) and 25.98 (MCF-7)

The results from the *in vitro* cell viability study provided strong rationale to assess an *in vivo* anti-cancer efficacy of a selected dose of these extracts of Rudraksha bead in mouse tumor model (23, 24).

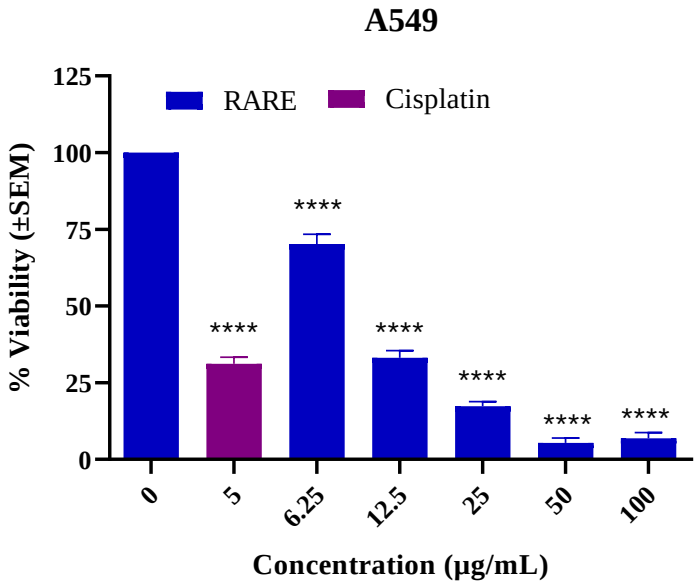

Figure 4: Cell viability of A549 cells upon RARE treatment

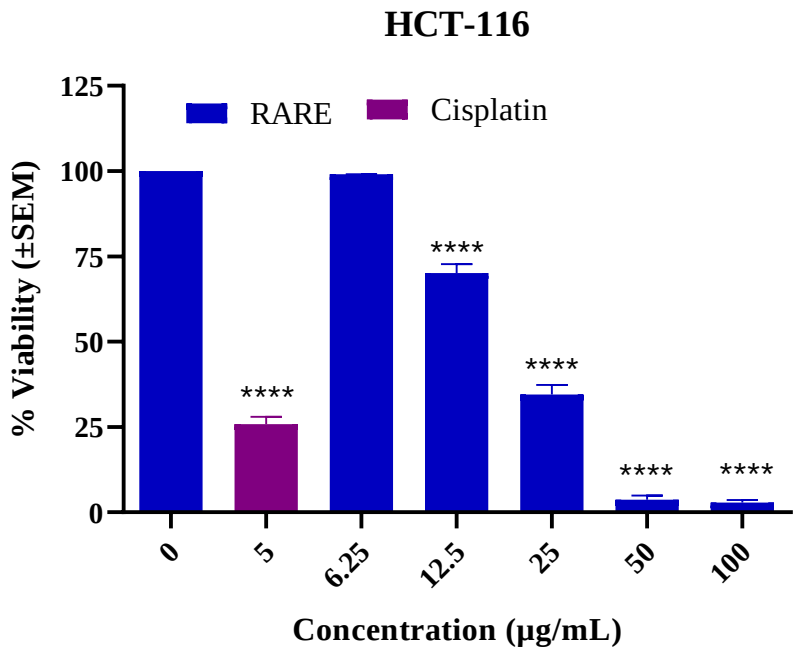

Figure 5: Cell viability of HCT-116 cells upon RARE treatment

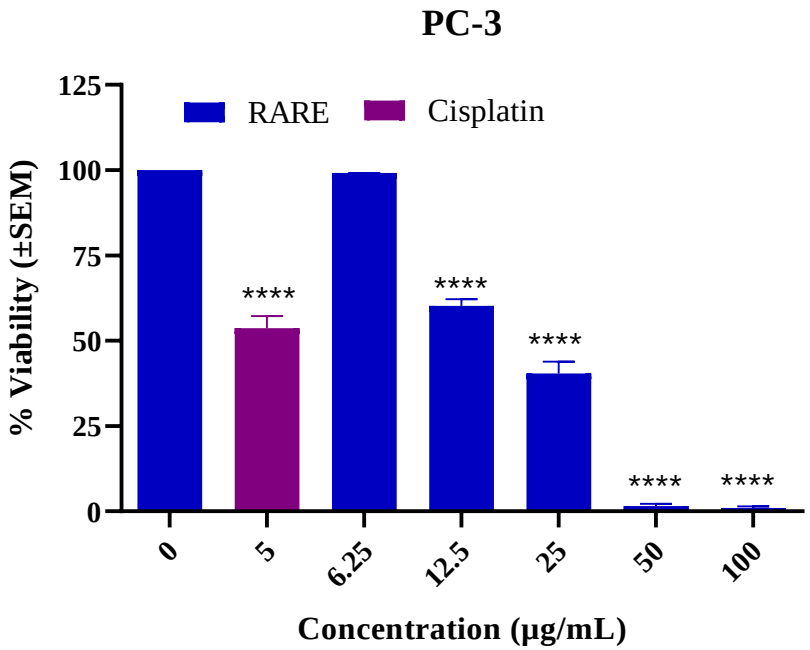

Figure 6: Cell viability of PC-3 cells upon RARE treatment

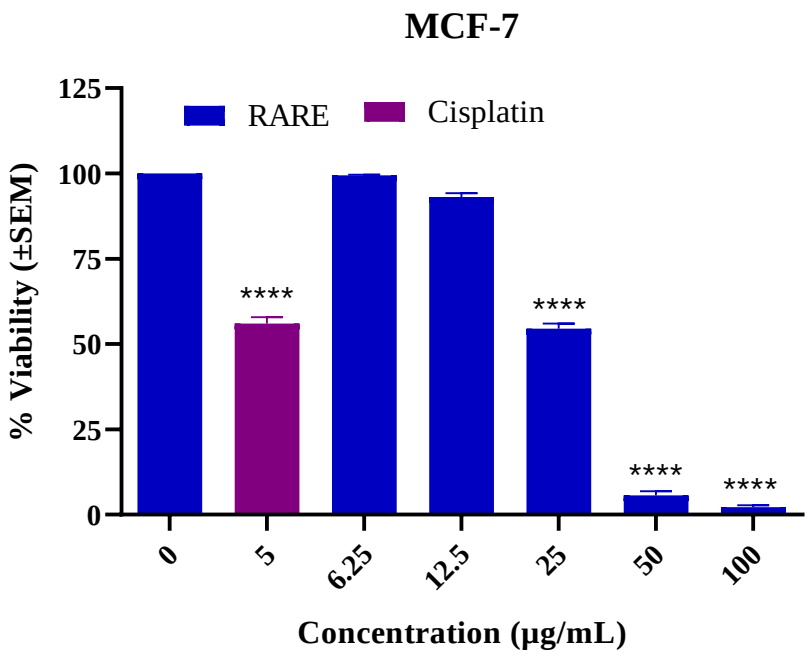

Figure 4: Cell viability of MCF-7 cells upon RARE treatment

Anti-Neurodegenerative

The cholinergic neurotransmission process relies on the acetylcholinesterase enzyme, as do several non-cholinergic processes, in which the enzyme hydrolyzes the neurotransmitter acetylcholine into choline and acetate (25, 26). The degeneration of specific acetylcholine neurotransmitter synapses in the cerebral cortex has been documented in the neurodegenerative condition known as Alzheimer's disease, attributable to insufficient acetylcholine neurotransmission. Consequently, the inhibition of the acetylcholinesterase enzyme has been established as the primary treatment approach for Alzheimer's disease. In Figure 10, the inhibitory effect of RARE on acetylcholinesterase is illustrated (IC50= 16.71), showing a level of inhibition comparable to the standard drug donepezil (IC50= 43.14).

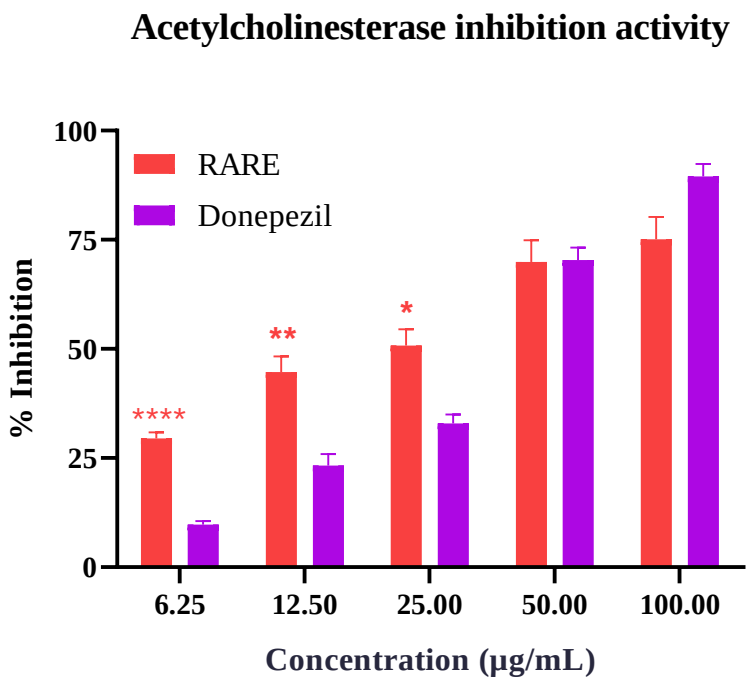

Figure 4: Acetylcholinesterase inhibition activity for RARE

4. Conclusion

The present research findings concluded the promising *in vitro* biomedical applications of REMAG including antioxidant, anti-inflammatory, anti-diabetic, and anti-neurodegenerative activities. Further, *in vivo* safety and efficacy assessments are warranted to confirm their *in vitro* biomedical applications.

5. References

1. Rai, D. V., Jawla, S., & Bajpai, S. (2016). The Study of Rudraksha Bead showing a Symbol of OM. Dev Sanskriti Interdisciplinary International Journal, 8, 08-13.
2. Joshi, S. C., & Jain, P. K. (2014). A review on ethnomedicinal and traditional uses of Elaeocarpus ganitrus roxb. (rudraksha). Int J Pharm Bio Sci, 5(1), 495-511.
3. Sharma, S., Rai, D.V., Rastogi, M. (2022). Physical, Physicochemical, and Structural Characteristics of Three, Four, and Five Mukhi Rudraksha. In: Mallick, P.K., Bhoi, A.K., González-Briones, A., Pattnaik, P.K. (eds) Electronic Systems and Intelligent Computing. Lecture Notes in Electrical Engineering, vol 860.
4. Singh, A. (2018). Recent trends in import and export of Rudraksha beads in India. doi: 10.54207/BSMPS2000-2018-8RT41Y
5. Balbir, Singh, Mohan, Paul, S., Ishar, Anupam, Sharma, Rohit, Arora., Saroj, Arora. (2015). Phytochemical and biological aspects of Rudraksha, the stony endocarp of Elaeocarpus ganitrus (Roxb.): a review. Israel Journal of Plant Sciences
6. Tilak, A., Gangwar, S. S., Thakur, R. N., & Sharma, R. (2017). Elaeocarpus Ganitrus (Rudraksha) Medicinal Use in Modern Time. Imperial Journal of Interdisciplinary Research (IJIR), 3(1), 1531-1538.

7. Jyotsana, Singh, Kushwaha, Alpana, S., Joshi. (2023). in silico characterization and phylogenetic analysis of Elaeocarpus ganitrus based on ITS2 barcode sequence. bioRxiv, doi: 10.1101/2023.07.15.549003
8. Bharati, Sarjerao, Borase, S.S., Surana. (2023). A Comprehensive review on Elaeocarpus floribundus Blume. Current traditional medicine, doi: 10.2174/2215083809666230228112737
9. Sharma, S., Rai, D. V., & Rastogi, M. (2019). Effect of Four Mukhi Rudraksha on Cognitive Dysfunction in Stressed Working Females. International Journal on Emerging Technologies, 10(4), 289-295.
10. Sharma, S., Rai, D. V., & Rastogi, M. (2021). Compositional Characteristics of Rudraksha (Eleaocarpus Ganitrus Roxb.). Plant Archives, 21 (1), 627-634.
11. Anand.S. (2023). Pharmaceutical and Analytical Evaluation on Gandhaka Maritha Loha Bhasma. International Journal of Ayurveda and Pharma Research, doi: 10.47070/ijapr.v11i3.2737
12. Sudradjat, S. E, Timotius, K.H. (2022), Pharmacological properties and phytochemical components of Elaeocarpus: A comparative study, Phytomedicine Plus, 2 (4)
13. Vinod, Kumawat., B., P., Yadav., Ashwathy, Vijayan., Avinash, Jain., Jeuti, Rani, Das., Bhagwan, Sahai, Sharma., Shruti, Khanduri., Rakesh, Rana., Richa, Singhal., T, Maheshwar., N, Srikanth., K, S, Dhiman. (2022). Safety and efficacy of Rudraksha Churna in the treatment of essential hypertensiona single-arm multicentre trial. doi: 10.4103/jras.jras_50_21
14. N. Primiani, C., Pujiati, P. A., Setiawan, M. (2021), Phytochemical Constituents and Antimicrobial Activity of Elaeocarpus Sphaericus Schum Seed Extract, Tropical Journal of Natural Product Research 5 (10), 1775–1781.
15. Sousa, A., Ferreira, I.C.F.R., Barros, L., Bento, A., Pereira, J. A., Effect of solvent and extraction temperatures on the antioxidant potential of traditional stoned table olives "alcaparra", LWT - Food Science and Technology 41 (4), 739-745
16. Utarı, A.U., A., N.D., & Astuti, E. (2023). Antioxidant Activity Test of Caulerpa racemosa Extract from Takalar District Using the FRAP (Ferric Reducing Antioxidant Power) Method. Nusantara Scientific Medical Research Journal 02 (01), 45-54
17. Hudaib MM, Tawaha KA, Mohammad MK, Assaf AM, Issa AY, Alali FQ, Aburjai TA, Bustanji YK. (2011) Xanthine oxidase inhibitory activity of the methanolic extracts of selected Jordanian medicinal plants. Pharmacognosy Magazine, 7(28), 320-4.
18. Ellman GL, Courtney KD, Andres JV, Featherstone RM (1961) A new and rapid colorimetric determination of acetyl cholinesterase activity. Biochem Pharmacol 7(2):88–95
19. Baran, A., & Keskin, C. (2023). Determination of Constituents of Extract of Celtis tournefortii Lam. by LC-MS/MS, Investigation of Enzyme Inhibition, Antimicrobial and Anticancer Effects. International Journal of Pure and Applied Sciences 9(1), 56-65
20. Sanjit Karki, Kebindra Shrestha, Rajendra Gautam and Ram Narayan Jha. (2020) phytochemical screening, FT-IR and GC-MS analysis of Euphorbia hirta. Journal of Pharmacognosy and Phytochemistry 9(1), 1883-1889.
21. Torosyan, G., Keller, S., Scheuer, L., Beigang, R., and Papaioannou, E.T. (2018). Optimized spintronic terahertz emitters based on epitaxial grown Fe/Pt layer structures. Sci. Rep. 8, 1311.
22. Fachriyah, E., Ghifari, M. A., & Anam, K. (2018). Isolation, Identification, and Xanthine oxidase inhibition activity of alkaloid compound from Peperomia pellucida. IOP Conference Series: Materials Science and Engineering 349 (1), 12-17.

23. Turner, A., Bond, D.R., Vuong, Q.V., Chalmers, A., Beckett E.L., Weidenhofer, J., Scarlett C, J., (2020). Elaeocarpus reticulatus fruit extracts reduce viability and induce apoptosis in pancreatic cancer cells in vitro. Molecular Biology Reports 47, 2073–2084
24. Sagar M, Pandey P., K., Sharma S, Rastogi M., (2024). Evaluation of Anti-cancer Efficacy of Eleaocarpus Ganitrus (Rudraksha) Bead Mediated Silver Nanoparticles in Mouse Lewis lung carcinoma tumour model. African Journal of Biological Sciences. 6 (9), 4841-4852
25. Soreq H, Seidman S. (2001) Acetylcholinesterase--new roles for an old actor. Nature Reviews Neuroscience 2(4), 294-302.
26. Silman I, Sussman JL. (2005) Acetylcholinesterase: 'classical' and 'non-classical' functions and pharmacology. Current Opinion in Pharmacology. 5(3):293-302.