

HPTLC Fingerprinting & Phytochemical Analysis of *Elaeocarpus Ganitrus* Plant

Deepika Singh¹, Priyanka Dixit², and Rajiv Gupta^{3*}

¹. M.S. Pharm, Sr. Assistant Professor, and Department of Pharmaceutics, School of Pharmacy, BBD University (Formerly Babu Banarasi Das National Institute of Technology and Management), Ayodhya, Lucknow-226 028(U.P.), India.

². M. Pharm, Sr. Assistant Professor and Department of Pharmaceutical Chemistry, School of Pharmacy, BBD University (Formerly Babu Banarasi Das National Institute of Technology and Management), Ayodhya, Lucknow-226 028(U.P.), India.

^{3.*}Ph.D., Principal & Dean, and Department of Pharmacognosy, School of Pharmacy, BBD University (Formerly Babu Banarasi Das National Institute of Technology and Management), Ayodhya, Lucknow-226 028(U.P.), India.

Corresponding Author: Prof. (Dr.) Rajiv Gupta, Principal & Dean, School of Pharmacy, BBD University, Lucknow-226 028 (U.P.) INDIA.

Abstract

Elaeocarpus ganitrus commonly known as Rudraksha in India and grown in the Himalayan region belongs to the Elaeocarpaceae family. The terms Rudraksha is derived from words Rudra (रुद्र) and Aksha (अक्ष). Various diseases like, diabetes, cancer, depression, epilepsy, liver illnesses, asthma, hypertension, arthritis, palpitations, stress etc. Rudraksha also plays an important role in a wide range of pharmacological properties, including strong antibacterial, hypoglycemic, analgesic, anti-inflammatory, antioxidant and hypoglycemia properties. Phytochemical study shown that Rudraksha contain gallic acid, proteins, amino acids, carbohydrates, glycosides, saponins, phenolic compounds, phytosterols, and tannins. It was found that the plants contain gallic acid and quercetin that is antioxidants in nature and it is further used for anticancer studies.

Keywords: Cancer, *Elaeocarpus ganitrus*, HPTLC, Rudraksha.

1. Introduction

Rudraksha is known as *Elaeocarpus ganitrus* belonging to Elaeocarpaceae family. Medium-sized *Elaeocarpus ganitrus* trees are grown as ornamental trees throughout India and can be found in Nepal, Bihar, Bengal, Assam, Madhya Pradesh, and Bombay.^{[1],[2],[3]} Cancer is defined as a fatal disease because it may metastasize to various organs. It was reported that 90% of cancer related death occur due to direct or indirect effects of metastatic dissemination.^{[4],[5]} It is well known that all over the world cancer is one of the most serious health problems that affect the duration and quality of the individual's life. As the conventional therapeutic strategies fail to fulfill the major requirements for a successful cancer therapy, the use of naturally developed anticancer agents has evolved as an alternative safe, low cost, and convenient one.^[6]

Medicinal plants play a golden role not only as traditional medicine but also as trade commodities, meeting the demand of distant markets for the development of new drugs^{[7], [8]}. They have phytochemicals, responsible for various disease management and treatment. According to WHO approximately 80% of the world population relies on plant-based medicines for primary health care. Acceptance & demand for medicinal plants progressively increases in the present time because they are safe^{[9],[10]} HPTLC study of Rudraksha shown that they have gallic acid and it was reported that quercetin and both phytochemicals are antioxidant in nature^{[11],[12],[13]}. There are various health issues, like heart disease, cancer, and aging, in which antioxidants are, used to combat oxidative stress and inflammation

[14],[15],[16]. This research paper could be useful for researchers working on preparing plant-based antioxidant formulations and further study can be on anticancer activity.

2. Objectives

The objective of this research work was to study the HPTLC fingerprinting and phytochemical analysis of *Elaeocarpus ganitrus*.

3. Methods

3.1 Collection of plant parts and Authentication

Selected plant materials *Elaeocarpus ganitrus* (Elaeocarpaceae) for research work was personally collected from BBD group campus and NBRI garden, Lucknow in the month of August 2020. Selected plant material was washed and dried in cool, airy & shady place from the same batch, leaves are selected and pressed to prepare respective herbarium sheet, and Authentication was successfully carried out for selected plant part by CSIR institute NISCAIR (Raw material & Herbarium Div.) New Delhi.

3.2 Macroscopic analysis

The plant's morphological characteristics, consisting of shade, taste, form, and length, had been studied morphologically using the techniques that have been documented [17], [18].

3.3 Microscopic studies

The leaf is cut into loose-hand transverse slices. This slicing technique helps in obtaining thin sections of the leaf that can be easily mounted for microscopy. The transverse leaf slices are treated with a saturated solution of chloral hydrate. Chloral hydrate is a chemical that acts as a clearing agent in microscopy [19].

3.4 Powder microscopy

Powdered crude drugs include cell fragments within the form of identifiable tissues, and the dried powder mass after passing via sieve #40 became used for powder microscopical studies. Surface constants such as fibers, lignified vessels, epidermal cells, crystals of calcium oxalate, starch grains, etc. are studied. The drug, which is powdered, is placed on a slide and stained under a microscope with the use of unique reagents along with safranin, iodine solution, phloroglucinol, etc [20].

3.5 Preparation of plant extract

Following an intensive cleaning and fast drainage of the smooth, ingesting water used to clean the freshly gathered *Elaeocarpus ganitrus* the leaves were shaded and dried to allow any residual moisture to evaporate. After being dried for seven to fourteen days, the leaves were milled right into a fine powder.

Then the powder was used for the preparation of the extract. The powdered material of shortlisted plant was extracted with the aid of the warm method, the Soxhlet extraction method, and the usage of hydroalcoholic solvent (3:7 ratio) [21].

3.6 Preliminary phytochemical screening

After that, more processing was carried out on the phyto extract to determine and filter its bioactive chemical composition, which included proteins, amino acids, carbohydrates, glycosides, saponins, phenolic compounds, phytosterols, and tannins. Extracts of the investigated medicinal herbs had been screened using a standard method [22]. All of the plant leaf materials were extracted using a hydroalcoholic solvent using the Soxhlet extraction method after being color-dried, coarsely floor, and sieved via sieve #40 [23].

3.7 HPTLC Study

A study using HPTLC was conducted on a specific medicinal plant extract.

On TLC glass plates measuring 20 cm, HPTLC was used.

MERCK employed a 200 μ m thick layer of silica gel 60F254 as a stationary phase for precoating. Phase of mobility: Formic acid: Ethyl acetate: Toluene. (5: 5:1.v/v/v.) Developing distance: 70 mm from the plate's bottom edge [23],[24],[25],[26].

4. Results

4.1 Morphological assessment

Morphological assessment was conducted. Fig.1 indicates about the different parts of leaf of *Elaeocarpus ganitrus*.



Fig.1 Leaf of *Elaeocarpus ganitrus* showing different parts and dorsal & ventral view of leaf.

4.2 Powder Microscopy

Powder of *Elaeocarpus ganitrus* was evaluated for various parts and fig. 2 indicates the epidermal cells and stone cells of *Elaeocarpus ganitrus* respectively.

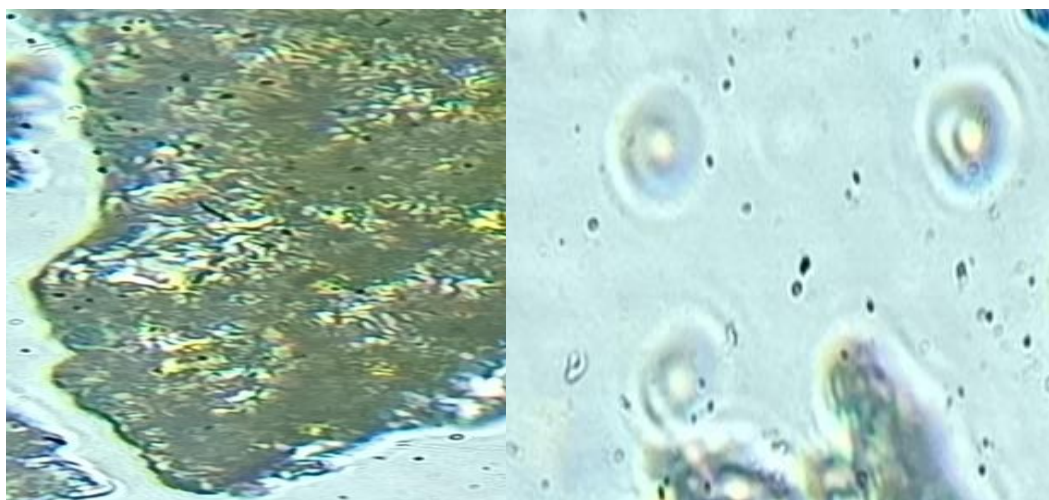


Fig.2 Powder microscopy of *Elaeocarpus ganitrus* showing epidermal cell and stone cells respectively.

4.3 Transverse Microscopy of Plants

Microscopy in the form of transverse section of fresh sample and powder sample was investigated (following W.H.O. guidelines) to determine characteristic features which may be helpful for future researchers & also as a benchmark for checking adulteration and substitution.

The transverse- section of the leaf was divided into three regions: the epidermis, mesophyll, and vascular bundle. The epidermis was found on both the upper and lower surfaces. It was found to be single-layered, compactly arranged parenchyma cells covered externally with a cuticle.

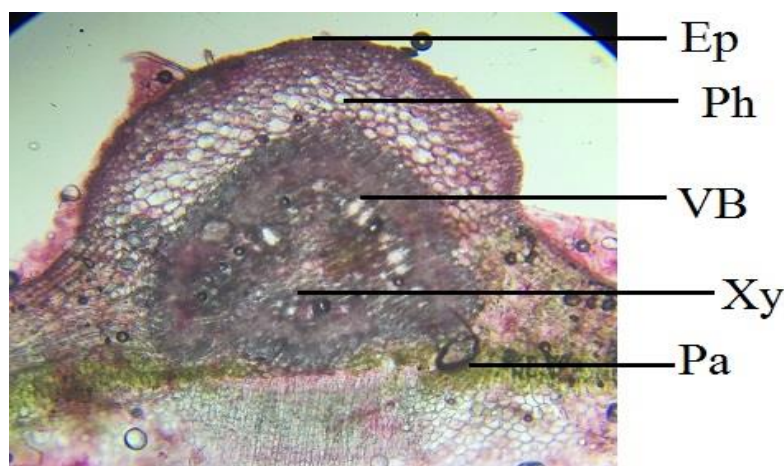


Fig.3 T. S.of Elaeocarpus ganitrus leaf showing different regions like, epidermis, vascular bundle, Phloem, Xylem, Parenchyma etc.

Pa- Parenchyma

VB- Vascular Bundle

Ph-Phloem Xy- Xylem

EP- Epidermis

4.4 Preparation of plant extract

The powdered material of shortlisted plants was extracted by hot process soxhlet extraction method using hydroalcoholic solvent. The hot process Soxhlet extraction method using a hydroalcoholic solvent has proven effective in extracting bioactive compounds from Rudraksha. This research supports its potential use in various medicinal and therapeutic applications, highlighting its role as a valuable natural resource in healthcare and pharmaceutical industries.

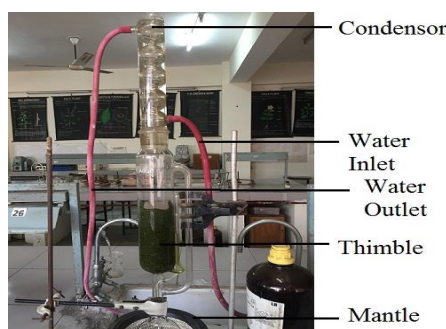


Fig. 4 Soxhlet Apparatus work on solvent reflux and siphon principle to continuously extract the solid matter by pure solvent

4.5 Phytochemical Screening

Phytochemical screening involves testing plant extracts for the presence of various chemical compounds, which can provide insights into their potential medicinal properties. Rudraksha (*Elaeocarpus ganitrus*) plants help in traditional medicine systems, and studying their phytochemical composition can shed light on their therapeutic potential.

Conducting a comprehensive phytochemical screening can provide valuable information for further research into the medicinal properties of Rudraksha. The inference drawn from phytochemical screening was as follows, major phytochemical group present-Alkaloids, Tannins, Phenolic compound, Steroids, Reducing sugar, Proteins, Flavanoids, Saponin, Amino acids etc.

4.6 HPTLC Results

The HPTLC on extract of *Elaeocarpus ganitrus* was used for an investigation, and the results showed that the plants contained the antioxidants gallic acid and quercetin. For HPTLC investigations, the following mobile phase is utilized: toluene, ethyl acetate, and formic acid (5:5:1. v/v/v).

HPTLC fingerprinting of Rudraksha extract demonstrates its potential as a source of bioactive compounds, particularly quercetin and gallic acid, with significant pharmacological activities.

Under 366 nm

Under White Light

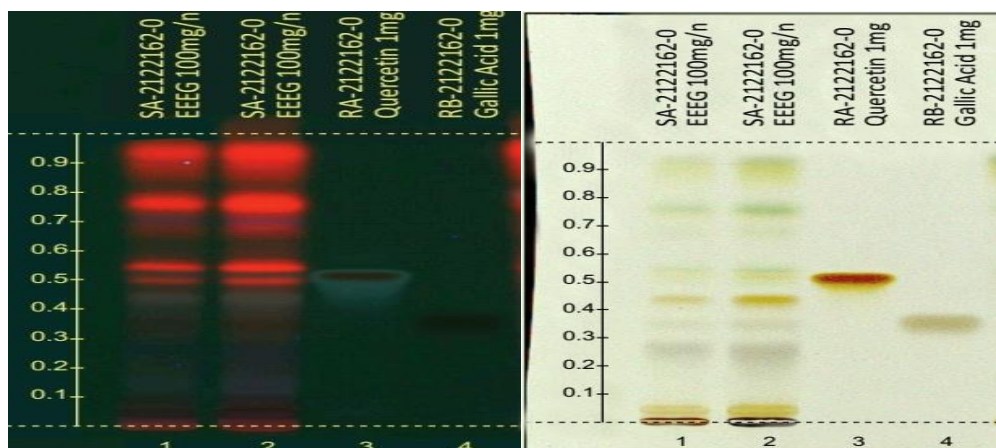


Fig.5Major bands include those corresponding to quercetin and gallic acid, confirmed by comparison with standard compounds and UV spectra.

EEEG (*Elaeocarpus ganitrus*)

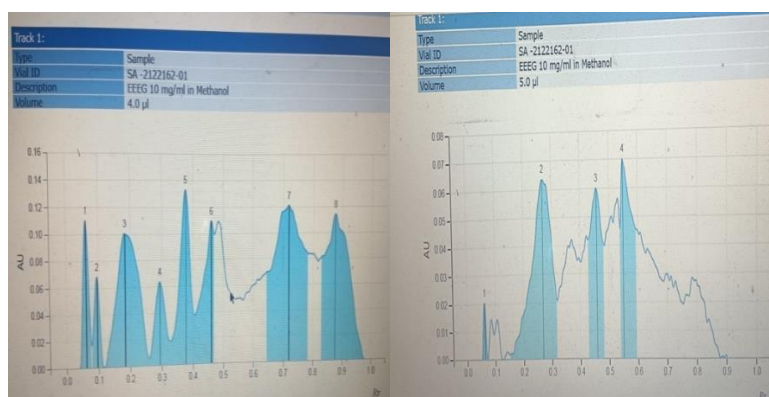


Fig. 6 Peaks were obtained for plants showing sharp peak for gallic acid

5. Discussion

Phytochemical study of rudraksh extract shows that it contains various phytochemicals that is responsible for pharmacological action. Rudraksh plants leaves contain phytochemicals such as quercetin and gallic acid, which have been identified for their potential in treating various diseases, including cancer because they having antioxidant properties. By performing HPTLC fingerprinting of leaves extract of Rudraksha it was confirm that it contain gallic acid and quercetin and it is used in therapeutic applications, and further scientific exploration. The basis for additional research and validation of the antioxidant characteristics of these plants' leaves is provided by this study.

Acknowledgement:

The authors would like to thank The Librarian, Central Drugs Research Institute - C.S.I.R.(C.D.R.I.-C.S.I.R), Lucknow, for all assistance provided during the literature search and Prof. Sri Sivakumar and Madhu Verma Ph. D Research Scholar from Department of Chemical Engineering, IIT, Kanpur for their assistance during cell line studies. The corresponding author is also thankful to AICTE-MODROBS project grant (F. No. 8024/RID/BOR/MOD-458/2009-10).

5. References

- [1] Rauniar, G.P., & Sharma, M.(2012). Evaluation of Anxiolytic Effect of *Elaeocarpus Ganitrus* in Mice. *Original article*, 10(2), 108-112.
- [2] Chaturvedi, B.K.(2004). Shiv Purana, Vidyeshwar Samhita, Chapter 25, Diamond Books (P) Ltd., New Delhi.
- [3] Garg, K., Goswami, K., &Khurana, G. (2013).A Pharmacognostical Review on *Elaeocarpus Sphaericus*.*International Journal of Pharmacy and Pharmaceutical Sciences*, 5(1), 3-8.
- [4] Park, T. J., Curran, T. (2014). Essential roles of Crk and CrkL in fibroblast structure and motility. *Oncogene*, 33 (43) 5121-5132.
- [5] Alabi, M.A.,Adebawo, O.O.,Daini , O.A.,Somari , S.B.,&Somari , R.I.(2016). HSPD1, HSPB1 and VDAC1 are over-expressed in invasive ductal carcinoma of the breast. *Int. J. Cancer Res.*, 12 (2), 82–91.
- [6] Robert, J., Michael, H., Axel, H., Jost, L., Gunter, M., Wilhelm, N., Tanja, P., & Hans J. (2017). Herbal medicinal products: Evidence and tradition from a historical perspective.*Journal of Ethnopharmacology*, 207(5), 220-225.
- [7] Tilburt, J.C.,& Kaptchuk, T.J.(2008). Herbal medicine research and global health: an ethical analysis. *Bull World Health Organ*, 86(8):594-599.
- [8] Zeng H.(2009). Selenium is an essential micronutrient: Roles in Cell Cycle and Apoptosis. *Molecules*, 14(3), 1263-1278.
- [9] Cuong, D.M., Arasu, M.V., Jeon, J., Park, Y.J., Kwon, S.-J., Al-Dhabi, N.A.,& Park, S.U. (2017). Medically important carotenoids from *Momordica charantia* and their gene expressions in different organs. *Saudi J. Biol. Sci.*,24(2), 1913–1919.
- [10]Maqsoodlou, V.,Assadpour, E., Mohebodini, H., Jafari, S.M.(2020).Improving the efficiency of natural antioxidant compounds via different nanocarriers, *Adv. Colloid Interface Sci.*, 278, 102-122.
- [11]Aghapour, F., Moghadamnia, A.A., Nicolini, A., Kani, S.N.M., Barari, L., Morakabati, P., Rezazadeh, L., &Kazemi, S.(2018). Quercetin conjugated with silica nanoparticles inhibits tumor growth in MCF-7 breast cancer cell lines. *Biochem.Biophys. Res. Commun.* 500,860–865.
- [12]Han Q.S., Wang X.H., Cai S.F., Liu X.L., Zhang Y.F., Yang L., Wang C., &Yang R.(2018). Quercetin nanoparticles with enhanced bioavailability as multifunctional agents toward amyloid induced neurotoxicity. *J. Mater. Chem. B*. 6, 1387–1393
- [13]Baite, T.N., Mandal, B.,&Purkait, M.K. (2022). Antioxidant-incorporated poly(vinyl alcohol) coating: preparation, characterization, and influence on ripening of green bananas.*ACS Omega*, 7.42320–42330.
- [14]Emmanuel, M. (2021). Chapter 31- Medicinal plants, antioxidant potential, and cancer. *Oxidative Stress and Dietary Antioxidants*. 349-357.
- [15]Garg, K., Goswami, K., &Khurana, G. (2013).A Pharmacognostical Review on *Elaeocarpus Sphaericus*.*International Journal of Pharmacy and Pharmaceutical Sciences*, 5(1), 3-8.

-
- [16] Kumar, N., Dubey, M., & Agarwal, V. (2013). Rudraksha: A Review on Mythological, Spritual And Medicinal Importance. *Global J Res. Med. Plants & Indigen Med.*, 2, 65–72.
- [17] S., & Balbir, S. (2012). Microscopy of Rudraksha Sacred Seeds-The Traditional Panacea. *International Journal of Natural Product Science*, 1, 132-138.
- [18] Donald, J. A. (2003). Burger's Medicinal Chemistry and Drug Discovery.
- [19] Mukherjee, P.K. (2002). Quality control of Herbal drugs- An approach to evaluation of botanicals 1st edn, New Delhi: Business Horizon.
- [20] Wallis T.E.(1997).Textbook of Pharmacognosy. 5th ed, New Delhi: CBS Publishers and Distributors.
- [21] Ganesh, K., Massague, J.(2021). Targeting metastatic cancer. *Nature medicine*, 27(1), 34-44.
- [22] Wagner, H.(1996). Plant Drug Analysis: A Thin Layer Chromatography Atlas 2nd ed. Springer.
- [23] Renger, B.(1993).Quantitative planar chromatography as a tool in pharmaceutical analysis. *J. AOAC Int.*, 76, 7- 13.
- [24] Renger, B. (1998). Contemporary thin layer chromatography in pharmaceutical quality control. *J. AOAC Int.*, 81, 333-339.
- [25] Andola, H.C., Purohit, V.K. (2010). High Performance Thin Layer Chromatography (HPTLC): A Modern Analytical tool for Biological Analysis. *Nat. Sci.*, 8(10), 58-61.