

Chapter 2

Phytochemical analysis, toxicity to *Artemia salina* and Thin Layer Chromatography of leaves of *Chrozophora rottleri* (Geiseler) Spreng. (Euphorbiaceae)

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Abstract: The increasing prevalence of cancer and the limitations of current treatments highlight the need to explore alternative therapeutic strategies, including the screening of medicinal plants for anticancer properties. *Chrozophora rottleri*, a plant species of the Euphorbiaceae family, has been traditionally used to treat various ailments and exhibits a rich diversity of bioactive compounds. This study focuses on the phytochemical analysis, cytotoxicity evaluation using *Artemia salina*, and Thin Layer Chromatography (TLC) of *C. rottleri* leaves, aiming to identify potential anticancer leads and contribute to the development of novel cancer therapies.

Keywords: Bioactive compounds, cancer, chromatography, herbs

Introduction

Cancer is a leading cause of death worldwide, and the development of effective treatments remains a significant challenge (Baskar et al., 2012; Nagai and Kim, 2017). Despite advances in modern medicine, many cancer therapies are

associated with severe side effects, and the search for safer, more targeted treatments continues (Schilsky et al., 2010; Kroschinsky et al., 2017; Anand et al., 2024). Plants have been a rich source of bioactive compounds with potential therapeutic benefits (Pradhan et al., 2025), and screening plant extracts for anticancer properties has become an important area of research (Khan et al., 2019; Devi et al., 2024). Plants have been used for centuries in traditional medicine (Chandrashekar et al., 2025), and many modern cancer treatments are derived from plant-based compounds (Shrihastini et al., 2021). The diversity of plant species and the complexity of their phytochemical profiles (Murmu et al., 2016) offer a vast array of potential leads for cancer therapy.



Figure 1: Habitat of *Chrozophora rotlieri* near Mahanadi River areas

Chrozophora rotlieri (Figure 1), a less known plant species of the Euphorbiaceae family (Prema et al., 2023). It has ethnomedicinal uses (Prema et al., 2023) and

in Odisha state, India, leaves paste is used to cure skin infections. As per its ethnomedicinal uses and less unexplored, an attempt has been made to carry out qualitative phytochemical analysis, cytotoxicity, and TLC studies of the leaf of *C. rottlerin*.



Figure 2: Leaves of *C. rottlerin* & collection for experimental works

Methodology

The leaves of *C. rottleri* were collected from nearby Mahanadi areas of Cuttack district, Odisha, India (Figure 2-3). The plant species was identified by the authors. The Maceration extraction method was adopted using three solvents (n-hexane, ethanol and aqueous) for phytochemical analysis (Figure 3-4). Detection of nine secondary metabolites was conducted using standard methods (Devi et al., 2023; Jena et al., 2024; Marndi et al., 2024). A herbarium

specimen is deposited to the Herbarium unit, Ambika Prasad Research Foundation, Odisha (Figure 5). Details of phytochemical analysis is given below.



Figure 3: Dried leaves powder of *C. rottleri*

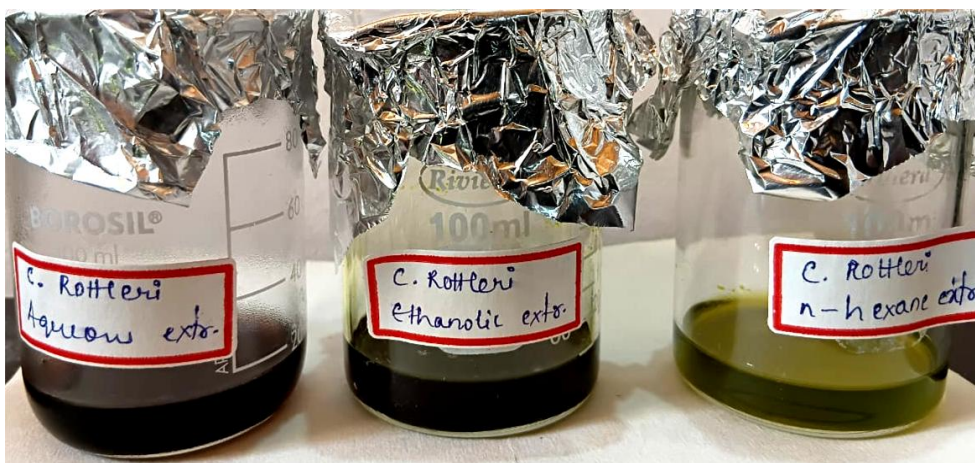


Figure 4: Filtrate extracts of *C. rottleri* leaves for experimental analysis

Test for tannin: 1 ml of leaves extract was taken in a test tube, and 3-5 drops of 10% lead acetate solution was added to it. The gelatinous precipitate formation confirmed the presence of tannin.

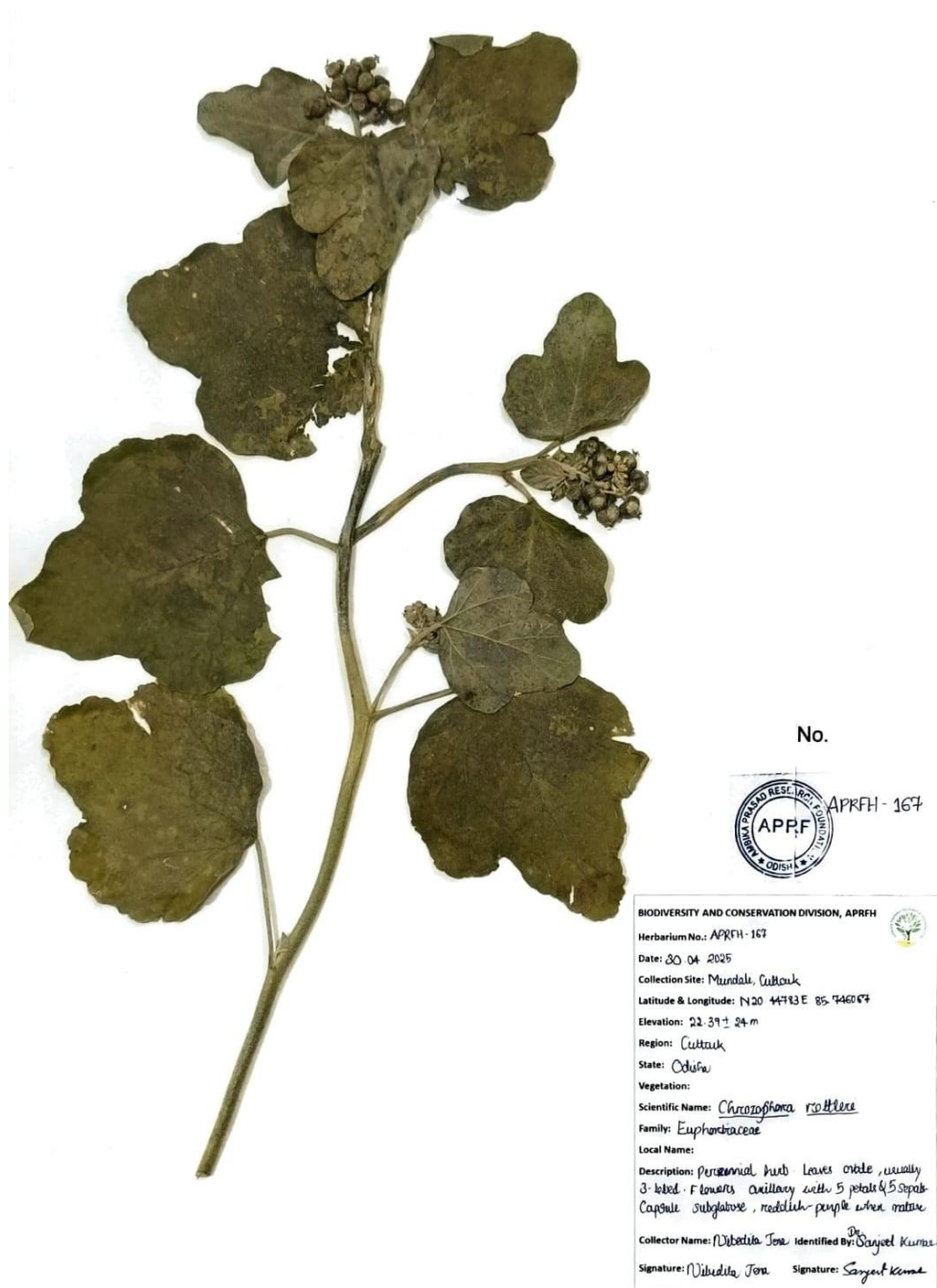


Figure 5: Herbarium specimen of *C. rotthlei*

Test for saponin: About 1 ml of distilled water was added to 1 ml of the leaves extract and shaken well. The formation of persistent froth was observed, confirming the presence of saponin.

Test for flavonoids: About 1 ml of the leaves extract was taken, and 2 ml of NaOH solution and 3-5 drops of dilute HCl were added to it. The color initially turned to an intense yellow color with NaOH solution and later became colorless, which confirmed the presence of flavonoids.

Test for terpenoids: To 1 ml of the leaves extract, 5-6 drops of chloroform were added. Then, 4-6 drops of concentrated sulphuric acid were added to it. The reddish-brown interface confirmed the presence of terpenoids.

Test for phenols: About 1 ml of the leaves extract was taken. A few drops of 5% ferric chloride solution were added. The dark bluish-black appearance confirmed the presence of phenolic compounds.

Test for reducing sugars: About 1 ml of the leaves extract was taken. 2 drops of Fehling's solution A followed by Fehling's solution B were added and kept in the water bath for a few minutes. The presence of red-orange precipitate confirmed the presence of reducing sugars.

Test for steroids: To 1 ml of the leaves extract, 1 ml of chloroform followed by 1 ml of concentrated sulphuric acid were added. The appearance of upper red and lower yellow with green fluorescence indicated the presence of steroids.

Test for alkaloids: About 1 ml of the leaves extract was taken, and 3-4 drops of Dragendroff's reagent were added to it. The formation of a reddish-brown precipitate confirmed the presence of alkaloids.

Test for carbonyl compounds: About 1 ml of leaves extract was taken, and 3-4 drops of 2,4-Dinitrophenylhydrazine (DNPH) reagent were added. The formation of yellow crystals confirmed the presence of carbonyl compounds.

Cytotoxicity analysis: For cytotoxicity test, hatching of Brine shrimp cysts was the initial process. Standardization of the optimum salinity was done at 2.0, 3.5 and 5.0 % saline concentration to check the optimum salinity for proper hatching of Brine shrimp (*A. salina*) cysts (Kumar et al., 2012). The brine shrimp cysts are then incubated in 3.5 % saline water with proper aeration, room temperature or between (28-35°C) and light for 72 hours. Proper hatching could be observed within 24-72 hours depending upon the quality of cysts, proper aeration and light provided. Different concentrations (15.62mg ml⁻¹, 31.25 mg ml⁻¹, 62.5mg ml⁻¹, 125 mg ml⁻¹ and 250 mg ml⁻¹) of the *C. rottleri* leaves extracts were taken and 3.5% saline water were used to make the volume 2 ml in each test tube. 1 % DMSO was used to dissolve the crude extracts. Ten nauplii were selected and introduced to five test tubes of the extracts. Positive and negative controls were prepared using 3.5% saline water and vincristine sulphate (5 mg ml⁻¹) respectively with a volume of 2 ml. The live larvae are highly mobile and thus differentiates from the unhatched cysts. The survivor nauplii were counted and analyzed the death % (Nayak et al., 2021).

Chromatographic analysis: TLC plates were prepared on 9 cm glass slides using silica gel powder. Slides were washed with clinical laboratory detergent and dried. Clean and dried slides were wiped with ethyl acetate for removing surface adherents. 3 g of silica was taken in 20 ml of distilled water and slurry was prepared by constantly stirring and finally poured over the slides. The slides were left undisturbed till the drying of silica layer. Then the slides were activated at 50 °C for 20 minutes before running the TLC (Mishra and Bhatnagar, 2024).

Methanol: chloroform (1:1), ethyl acetate: chloroform (1:9) and ethyl acetate: water: acetic acid (8:1:1) were used as mobile phases. Then spots were visualized under UV chamber and recorded the RF (Retention Factor) values (Kumar and Jena, 2014).

Results and discussion

The phytochemical analysis of *C. rottleri* leaves revealed the presence of various bioactive compounds, including tannins, saponins, reducing sugars, and alkaloids in different extracts (Table 1; Figures 6-8). The aqueous extract showed the presence of phenolic compounds and terpenoids, which are known for their medicinal properties. The presence of these compounds suggests that *C. rottleri* leaves may possess potential therapeutic benefits.

Table 1. Qualitative phytochemical analysis of *Chrozophora rottleri* leaves using different extracts

Bioactive compounds	n-hexane	Ethanol	Aqueous
Tannin	Present	Present	Present
Saponin	Present	Present	Present
Flavonoids	Absent	Absent	Absent
Terpenoids	Absent	Absent	Present
Phenolic compounds	Absent	Absent	Present
Reducing sugars	Present	Present	Present
Steroids	Absent	Absent	Absent
Alkaloids	Present	Present	Absent
Carbonyl compounds	Absent	Absent	Absent

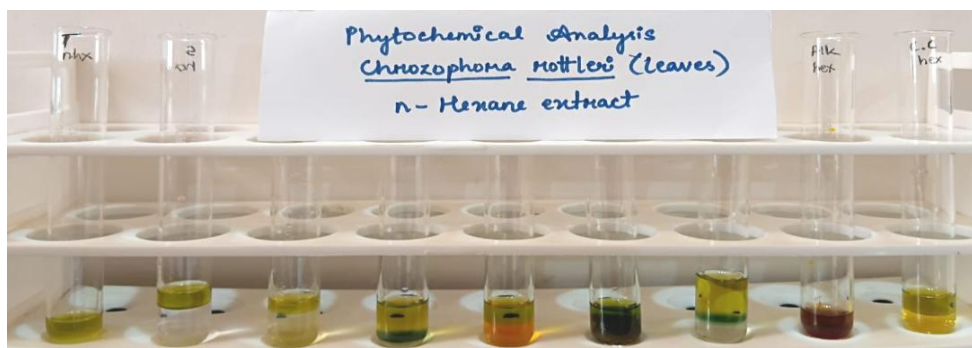


Figure 6: Detection of secondary metabolites of *C. rotterii* leaves using n-hexane extract

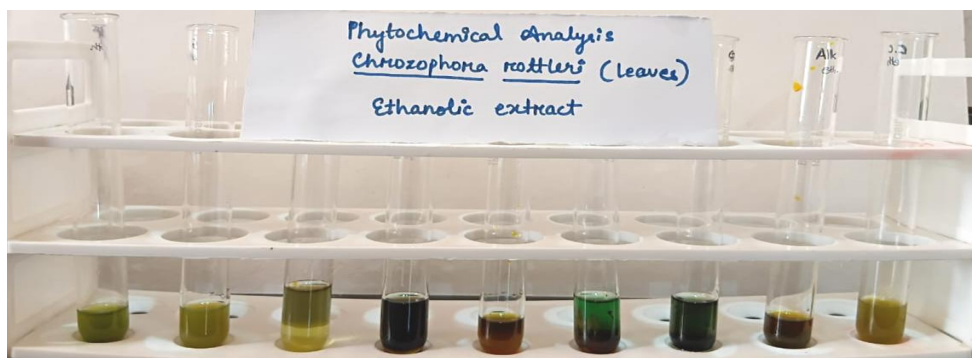


Figure 7: Detection of secondary metabolites of *C. rotterii* leaves using ethanolic extract



Figure 8: Detection of secondary metabolites of *C. rotterii* leaves using aqueous extract

The cytotoxicity evaluation using *Artemia salina* showed significant mortality rates for n-hexane and ethanol extracts, indicating potential anticancer properties (Table 2; Figures 9-11). In contrast, the aqueous extract exhibited minimal cytotoxicity, suggesting that the bioactive compounds present in this extract may have a more targeted or gentle effect (Figure 12). These findings highlight the importance of further investigating the potential therapeutic applications of *C. rottleri*.

Table 2: Cytotoxicity analysis of *Chrozophora rottleri* leaves using Brine shrimp nauplii

Extracts	Concentration (in mg/ml)	Initial number of Nauplii	Number of Deaths of Nauplii (after hours)			Death rate (%)
			1	2	3	
n-hexane	15.62	10	10	10	10	100
	31.25	10	10	10	10	100
	62.5	10	10	10	10	100
	125	10	10	10	10	100
	250	10	10	10	10	100
	3.5% Saline	10	0	0	0	0
	Vincristine sulphate	10	10	10	10	100
Ethanol	15.62	10	10	10	10	100
	31.25	10	10	10	10	100
	62.5	10	10	10	10	100
	125	10	10	10	10	100
	250	10	10	10	10	100
	3.5% Saline	10	0	0	0	0
	Vincristine sulphate	10	10	10	10	100
Aqueous	15.62	10	0	0	0	0
	31.25	10	0	0	0	0
	62.5	10	0	0	0	0
	125	10	0	0	1	10
	250	10	0	2	2	20

	3.5% Saline	10	0	0	0	0
	Vincristine sulphate	10	10	10	10	100

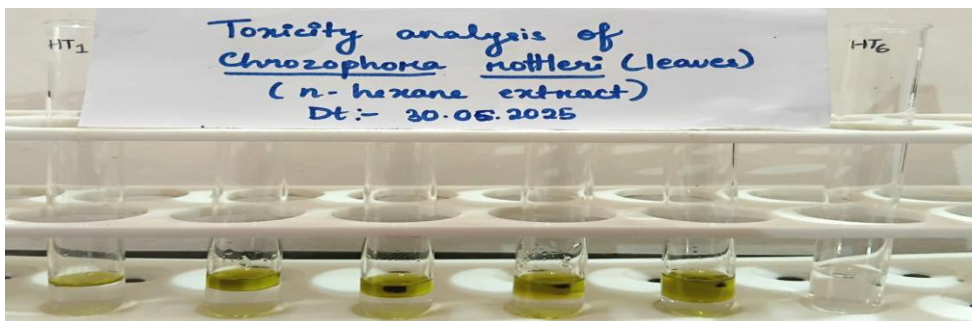


Figure 9: Toxicity analysis using Brine shrimp nauplii of n-hexane extract of *C. rottleri* leaves

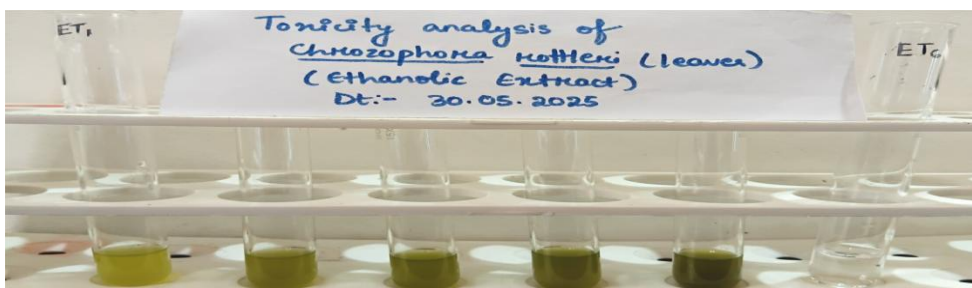


Figure 10: Toxicity analysis using Brine shrimp nauplii of ethanolic extract of *C. rottleri* leaves

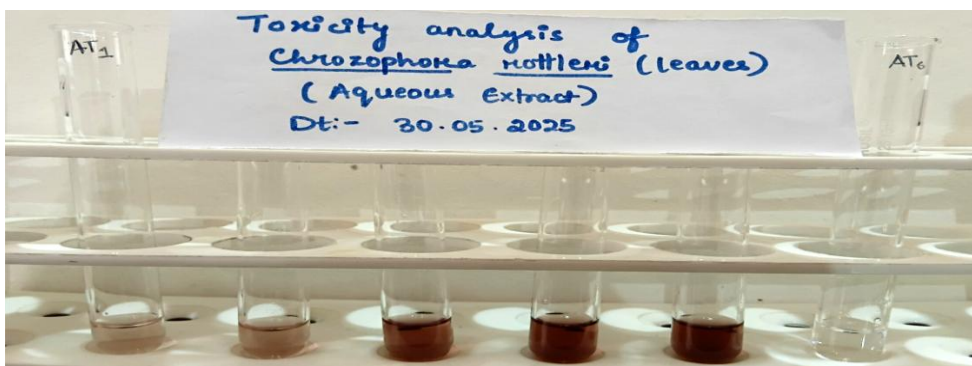


Figure 11: Toxicity analysis using Brine shrimp nauplii of aqueous extract of *C. rottleri* leaves

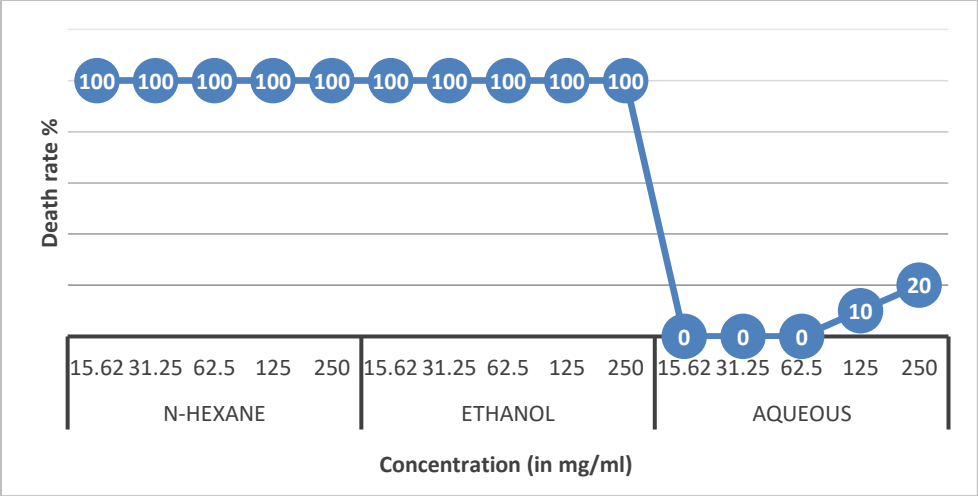


Figure 12: Death rate % of selected extracts against *Artemia salina*

The TLC analysis of the hydroethanolic extract using methanol: chloroform (1:1) as the mobile phase revealed distinct spots with different R_f values, indicating the presence of specific bioactive compounds (Table 3). The absence of spots with other mobile phases suggests that the compounds present in the extract may have specific polarities or properties.

Table 3: TLC analysis of *Chrozophora rotleri* leaves using different mobile phases

	Mobile Phases	Rf value of Spots				
		S1	S2	S3	S4	S5
Hydroethanolic	Methanol: Chloroform (1:1)	0.5	0.52	0.57	0.61	0.68
	Ethyl acetate: Chloroform (1:9)	Nil	Nil	Nil	Nil	Nil
	Ethyl acetate: Water: Acetic acid (8:1:1)	Nil	Nil	Nil	Nil	Nil

Conclusion

The phytochemical analysis and cytotoxicity evaluation of *Chrozophora rottileri* leaves suggest that this plant species possesses potential therapeutic benefits, particularly in the context of cancer treatment. The presence of bioactive compounds and significant cytotoxicity exhibited by certain extracts highlight the importance of further research to isolate and characterize the specific compounds responsible for these effects. These findings provide a foundation for future studies to explore the potential applications of *C. rottileri* in the development of novel cancer therapies.

References

- Anand U, Dey A, Chandel AKS, Sanyal R, Mishra A, Pandey DK, De Falco V, Upadhyay A, Kandimalla R, Chaudhary A, Dhanjal JK, Dewanjee S, Vallamkondu J and Pérez de la Lastra JM. (2024). Cancer chemotherapy and beyond: Current status, drug candidates, associated risks and progress in targeted therapeutics. *Genes Dis.* 10(4):1367-1401. doi: 10.1016/j.gendis.2022.02.007. Erratum in: *Genes Diseases.* 11(4):101211. doi: 10.1016/j.gendis.2024.101211
- Baskar R, Lee KA, Yeo R and Yeoh KW. (2012). Cancer and radiation therapy: current advances and future directions. *International Journal of Medical Sciences.* 9(3):193-199.
- Chandrashekar HP, Das SR, Jaiswal A, Basole SG, Bhat SS, Jena N and Kumar S. (2025). Food, Ethnomedicinal and Pharmacological Evaluation of *Streblus asper* Lour. (Fruits): A Minor Nutraceutical of India. *Annals of Agri-Bio Research.* 30(1):98-102.
- Devi RS, Manjula BL, Mukherjee S, Watakhare DN, Satapathy KB and Kumar S. (2024). Cytotoxicity Analysis of Selected Plants Using *Artemia salina*. *Annals of Agri-Bio Research.* 29(1): 45-49.
- Devi RS, Satapathy KB and Kumar S. (2023). Validation of tribal claims for formulation of future drugs through evaluation of ethno-pharmacological values of *Ludwigia adscendens*. *Medicinal Plants.* 15(4): 691-697.
- Jena N, Rout S, Devi RS and Kumar S. (2024). Phytochemical and cytotoxicity analysis of fruits of *Zanthoxylum asiaticum* (L.) Appelhans, Groppo & J. Wen: a minor fruit plant of Odisha, India. *e-planet.* 22(1): 62-70.

- Jena N, Vimala, Singh B, Patra A, Sharma BP, Hossain E and Kumar S. (2025). Methods for ethnobotanical data collection, phytochemistry, antioxidant, anthelmintic, and antimicrobial activities for pharmacological evaluation of medicinal plants. *Journal of Biodiversity and Conservation*. 9(2): 87-107.
- Khan T, Ali M, Khan A, Nisar P, Jan SA, Afridi S and Shinwari ZK. (2019). Anticancer Plants: A Review of the Active Phytochemicals, Applications in Animal Models, and Regulatory Aspects. *Biomolecules*. 10(1):47. doi: 10.3390/biom10010047
- Kroschinsky F, Stölzel F, von Bonin S, Beutel G, Kochanek M, Kiehl M and Schellongowski P. (2017). Intensive Care in Hematological and Oncological Patients (iCHOP) Collaborative Group. New drugs, new toxicities: severe side effects of modern targeted and immunotherapy of cancer and their management. *Critical Care*. 21(1):89. doi: 10.1186/s13054-017-1678-1
- Kumar S and Jena PK. (2014). Chromatographic, antibacterial, and FT-IR analysis of *Dioscorea pentaphylla* L. tuber extracts. *Plant Science and Research*. 36 (1&2): 83-90.
- Kumar S, Tripathy, PK and Jena PK. (2012). Ethnobotany and bioactive compounds in leaf of *Bixa orellana* L. and its toxicity to *A. salina* L. *Plant Science and Research*. 34 (1&2): 93-96.
- Marndi S, Barik M and Kumar S. (2024). Phytochemical and cytotoxicity analysis of *Eulophia explanata* Lindl. (Tuber extracts). *African Journal of Biological Sciences*. 6(4): 2751-2756.
- Mishra S and Bhatnagar S. (2024). Comparative analysis of methanol extract (leaf) of three species of *Sansevieria* using chemical and biological parameters. *World Journal of Pharmaceutical and Life Sciences*. 10(2): 116-118.
- Murmu P, Kumar S, Patra JK, Singh NR and Rath SK. (2016). Ethnobotanical, Nutritional, Phytochemical and Antimicrobial studies of *Garcinia xanthochymus* fruit extracts. *British Biotechnology Journal*. 13(2): 1-11.
- Nagai H and Kim YH. (2017). Cancer prevention from the perspective of global cancer burden patterns. *Journal of Thoracic Disease*. 9(3):448-451.
- Nayak SR, Choudhury R, Kumar S, Solanki HA and Biswal SK. (2021). Analysis of pharmacological values of *Vanda tessellata* and its toxicity to *A. salina* L. *International Journal of Green and Herbal Chemistry*. 10(3): 238-248.
- Pradhan S, Mohanty JN and Kumar S. (2025). Ethnomedicinal Plants Used by Santhal Community of India. *Annals of Agri-Bio Research*. 30(1): 86-97.

- Prema R, Ari HK, Logesh TG, Kumar SE, Soundariya C, Nandhita V and Kumar CS. (2023). An overview general aspect of *Chrozophora rottleri* – Suryavarti. International Journal of Pharmacology and Clinical Research.7(3): 187-194.
- Schilsky RL, Allen J, Benner J, Sigal E and McClellan M. (2010). Commentary: tackling the challenges of developing targeted therapies for cancer. *Oncologist*. 15(5):484-487.
- Shrihastini V, Muthuramalingam P, Adarshan S, Sujitha M, Chen JT, Shin H and Ramesh M. (2021). Plant Derived Bioactive Compounds, Their Anti-Cancer Effects and *In Silico* Approaches as an Alternative Target Treatment Strategy for Breast Cancer: An Updated Overview. *Cancers*. 13(24):6222. doi: 10.3390/cancers13246222