J. Biodivers. Conservation 9(3): 17-27 ISSN: 2457-0761 (online)
2025 http://www.aprfjournal.in

Research Article

Analysis of bioactive compounds, cytotoxic potential, and TLC profile of fruits of *Chloroxylon swietenia* DC. (Rutaceae)

Banshidhar Behera[1], Gajender Singh[2], Saraswati Majhi[3], Satish Sampatrao Tambe[4], Subhalakshmi Rout[5] and Shalini Mudalkar[6*]

[1]Post Graduate Department of Dravyaguna, Ayurvedic and Unani Tibbia College and Hospital, Karolbagh, New Delhi, India

[2]Department of Pharmacognosy, PK University, Shivpuri, Madhya Pradesh, India

[3]P.G. Department of Botany, Shailabala Women's Autonomous College, Cuttack, affiliated to Rama Devi Women's University, Bhubaneswar, Odisha, India

[4]Department of Botany and Research Centre, MGV's Loknete Vyankatrao Hiray Arts, Science and Commerce College, Panchavati, Nashik, Maharashtra, India

[5]Biodiversity and Conservation Lab., Ambika Prasad Research Foundation, Odisha, India

[6]Department of Tree Breeding and Improvement, Forest College and Research Institute, Mulugu, Siddipet, Hyderabad, Telangana, India

*Email-id: shalini.mudalkar@gmail.com; ORCID: https://orcid.org/0000-0003-0595-6485

DOI: https://doi.org/10.5281/zenodo.16916614

Article Details: Received: 2025-06-16 | Accepted: 2025-07-18 | Available online: 2025-08-13

Abstract: *Chloroxylon swietenia* DC. (Rutaceae) is a plant species known for its medicinal properties, with its fruits being used in traditional medicine for various ailments. This study aimed to investigate the presence of metabolically active compounds, cytotoxic activity, and TLC profile of the fruits of *C. swietenia*. Phytochemical analysis revealed the presence of various bioactive compounds, including tannins, saponins, reducing sugars, and alkaloids. The cytotoxic potential of the fruit extract was evaluated using the Brine shrimp assay, which showed significant cytotoxic activity. TLC profiling was used to identify and characterise the bioactive compounds present in the fruit extract. The results of this study provide scientific evidence for the traditional use of *C. swietenia* fruits and highlight their potential as a source of bioactive compounds with cytotoxic properties. Further research is warranted to isolate and characterise the bioactive compounds responsible for the observed cytotoxic activity.

Keywords: Brine shrimp, *Chloroxylon swietenia*, phytochemical, traditional, toxicity

Introduction

Plants have been a rich source of bioactive compounds with medicinal properties, and their use in traditional medicine has been well-documented across various cultures (Nasim et al., 2022). *Chloroxylon swietenia* DC. (Rutaceae), a plant species native to the Indian subcontinent has been traditionally used for its medicinal properties (Naveen et al., 2014). The fruits of *C. swietenia* have been reported to possess various biological activities, including anti-inflammatory, antioxidant, and antimicrobial properties (Kumar et al., 2006). However, the bioactive compounds responsible for these activities and their potential cytotoxic effects have not been extensively studied (Yuan et al., 2022). The identification and characterisation of bioactive compounds from natural sources are crucial for the development of new therapeutic agents (Sasidharan et al., 2010). Phytochemicals, such as tannins, saponins, reducing sugars, and alkaloids, are known to exhibit a range of biological activities, including antioxidant, anti-inflammatory, and cytotoxic effects (Zhang et al., 2015). The cytotoxic potential of these compounds is of particular interest, as they may have applications in the treatment of cancer and other diseases (Chunarkar-Patil et al., 2024). Thin-Layer Chromatography (TLC) is a widely used technique for the separation and identification of phytochemicals in plant extracts (Kowalska and Sajewicz, 2022). TLC profiling can provide valuable information on the presence and distribution of bioactive compounds in plant extracts, allowing for the identification of potential lead compounds for further study (Zahiruddin et al., 2021). In the present study, the bioactive compounds, cytotoxic potential, and TLC profile of the fruits of *C. swietenia* were investigated. By analysing the bioactive compounds and cytotoxic potential of *C. swietenia* fruits, this study aims to provide insights into their potential therapeutic applications and contribute to the understanding of the medicinal properties of this plant species. The findings of this study may also have implications for the development of new therapeutic agents and the conservation of *C. swietenia*, highlighting the importance of further research into the properties and potential uses of this plant species.

Methodology

Collection of samples and preparation of extracts

The fruits of *C. swietenia* were collected from Latehar Forest Division, Jharkhand, in May 2025 (Figure 1). The plant was identified by Dr. Sanjeet Kumar. The Soxhlet extraction method used three solvents (aqueous, ethanol, and n-hexane) for phytochemical analysis (Devi et al., 2023; Jena et al., 2024; Figures 2 & 3). Nine different bioactive compounds were detected using standard methods (Kumar et al., 2013).

Qualitative phytochemical analysis for secondary metabolites

Test for tannin: 2-5 drops of 0.1% lead acetate solution were added to one millilitre of the filtrate of the fruit extract. The white gelatinous precipitate provided a positive result for the presence of tannins.

Test for saponin: One millilitre of distilled water was added to one millilitre of the filtrate of the fruit extract and shaken vigorously. The persistent froth formation after shaking indicated the presence of saponin.

Test for flavonoids: Two millilitres of 2% NaOH solution and three drops of dilute HCl were added to one millilitre of the filtrate of the fruit extract. The colour of the filtrate initially turned to an intense yellow colour with NaOH solution, and later turned colourless. This colour change confirmed the presence of flavonoids.

Test for terpenoids: One millilitre of the filtrate of fruit extract was added with six drops of chloroform and placed in the water bath for a few minutes. Then six drops of concentrated H_2SO_4 were added. The reddish-brown interface confirmed the positive result for the presence of terpenoids.

Test for phenolic compounds: One millilitre of the filtrate of fruit extract was taken, and a few drops of 5% Ferric chloride solution were added. The bluish-black colour provided the positive result of the phenolic compounds.

Test for reducing sugars: One millilitre of the filtrate of fruit extract was taken, and two 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: One millilitre of the filtrate of the fruit extract was mixed with one millilitre of chloroform, and one millilitre of concentrated H_2SO_4 was added to it. The appearance of upper red and lower yellow with green fluorescence provided a positive result of the test for steroids.

Test for alkaloids: One millilitre of the filtrate of the fruit 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.

Cytotoxicity analysis

For the cytotoxicity test, the initial step was the hatching of Brine shrimp cysts (MAF Peqon Artemia). Standardisation of the optimal salinity was carried out at 2.0%, 3.5%, and 5.0% saline concentrations to determine the best salinity for proper hatching of Brine shrimp (*Artemia salina*) cysts (Devi et al., 2024). The brine shrimp cysts were then incubated in 3.5% saline water with adequate aeration, at room temperature (28-35°C), and under light for 72 hours. Proper hatching was observed within 24-72 hours, depending on the quality of cysts, aeration, and light conditions. Different concentrations (15.62, 31.25, 62.5, 125, and 250 mg/mL) of *C. swietenia* fruit extracts were prepared, and 3.5% saline water was used to achieve a total volume of 2 mL in each test tube. The crude extracts were dissolved in 1% DMSO. Ten live nauplii were selected and introduced into five test tubes containing the extracts. Positive and negative controls were prepared using 3.5% saline water and vincristine sulphate (5 mg ml^{-1}), respectively, each with a volume of 2 ml. The live larvae, being highly motile, were easily distinguished from unhatched cysts. The surviving nauplii were counted, and the percentage of mortality was calculated (Marndi et al., 2024).

TLC analysis

Using silica gel powder, TLC plates were prepared on 9 cm glass slides (SILICA GEL G, CAS 112926-00-8, Spectrochem Pvt. Ltd.). Slides were washed with a clinical laboratory detergent and dried. Clean and dry slides were wiped with ethyl acetate to remove surface adherents. Three grams of silica were taken in 20 ml of distilled water, and a slurry was prepared by constant stirring before being poured over the slides. The slides were left undisturbed for at least 24 hours to dry the silica layer. Afterwards, 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 the mobile phase (Kumar and Jena, 2014). Then, spots were visualised and the retention factor (RF) values recorded.

Results and discussion

The qualitative phytochemical analysis of *Chloroxylon swietenia* fruits reveals the presence of various bioactive compounds across different extracts. Results show that tannins, saponins, and reducing sugars are consistently present in all three extracts, viz., n-hexane, ethanol, and aqueous extracts, indicating these compounds are abundant in the fruit and may contribute to its medicinal properties (Table 1). However, alkaloids are only found in the ethanol and aqueous extracts, not in the n-hexane extract, suggesting they are more polar and soluble in polar solvents like ethanol and distilled water. The presence of tannins, saponins, and reducing sugars may influence the medicinal potential of *C. swietenia* fruits. Tannins are known for their astringent and antioxidant effects, saponins for their anti-inflammatory and antimicrobial activities, while reducing sugars could enhance the nutritional value (Fraga-Corral et al., 2021; Sharma et al., 2023; Figures 4-6). Some researchers have worked on the bioactivity of the plant, *C. swietenia.* Jayaprasad et al., (2016) have evaluated the anti-diabetic potential of *C. swietenia* bark extracts, supporting the use of plant extracts for treating diabetes traditionally. Ravishankara et al., (2021) have studied the analgesic and anti-inflammatory activities of fruit ethanol extract of *C. swietenia* in rats, which are due to the presence of the phytocompounds in the fruits.

Table 1. Qualitative phytochemical analysis of *C. swietenia* fruits using different extracts

Bioactive compounds	n-Hexane	Ethanol	Aqueous
Tannin	Positive	Positive	Positive
Saponin	Positive	Positive	Positive
Flavonoids	Negative	Negative	Negative
Terpenoids	Negative	Negative	Negative
Phenolic compounds	Negative	Negative	Negative
Reducing sugars	Positive	Positive	Positive
Steroids	Negative	Negative	Negative
Alkaloids	Negative	Positive	Positive

Cytotoxicity testing of *C. swietenia* fruits using Brine shrimp nauplii reveals differing toxicity levels among extracts. The n-hexane and ethanol extracts both show 100% mortality at all tested concentrations (15.62-250 mg/ml), indicating strong cytotoxic activity comparable to the positive control, Vincristine sulphate. In contrast, the aqueous extract exhibits a concentration-dependent increase in mortality, ranging from 20% to 80% across the same concentration range (Table 2). These results suggest that the compounds responsible for cytotoxic activity are likely non-polar, given the high potency of the n-hexane and ethanol extracts. Although the aqueous extract shows some activity, it appears less potent. These findings have implications for the development of therapeutic agents from *C. swietenia* fruits, particularly those targeting cancer or other diseases involving cytotoxic activity (Figures 7-10). Some scientists have also worked on the cytotoxic potential of *C. swietenia*. For example, Senthilkumar and Venkatesalu, (2013) have studied the antifungal and cytotoxic effect of the essential oil of *C. swietenia* on Vero cells by MTT assay. Kamble et al., (2022) investigated the anti-breast cancer effect of *C. swietenia* leaves on the human breast cancer cell line after revealing the cytotoxicity of the plant extracts.

Table 2. Cytotoxicity analysis of *C. swietenia* fruits using Brine shrimp nauplii

Extracts	Concentration (in mg/ml)	Initial number of nauplii	Number of deaths of nauplii (after hours)				Death rates (%)
			1	2	3	4	
n-Hexane	15.62	10	10	10	10	10	100
	31.25	10	10	10	10	10	100
	62.5	10	10	10	10	10	100
	125	10	10	10	10	10	100
	250	10	10	10	10	10	100
	3.5% Saline	10	0	0	0	0	0
	Vincristine sulphate	10	10	10	10	10	100
Ethanol	15.62	10	10	10	10	10	100
	31.25	10	10	10	10	10	100
	62.5	10	10	10	10	10	100
	125	10	10	10	10	10	100
	250	10	10	10	10	10	100
	3.5% Saline	10	0	0	0	0	0
	Vincristine sulphate	10	10	10	10	10	100
Aqueous	15.62	10	0	0	2	2	20
	31.25	10	0	0	4	4	40
	62.5	10	0	4	4	4	40
	125	10	2	3	6	6	60

	250	10	4	4	6	8	80
	3.5% Saline	10	0	0	0	0	0
	Vincristine sulphate	10	10	10	10	10	100

The TLC analysis of the ethanolic- aqueous extract indicates no detectable spots in any of the three mobile phases used i.e., Methanol: Chloroform (1: 1), Ethyl acetate: Chloroform (1: 9), and Ethyl acetate: Water: Acetic acid (8: 1: 1). This absence suggests that the compounds either do not separate well under these chromatographic conditions or are present in quantities too low for visualisation (Table 3). TLC studies of *C. swietenia* have been carried out by some researchers, like Agrawal et al., (2017) carried out TLC analysis of *C. swietenia* leaves and found the presence of quercetin and gallic acid in different extracts. Nagaraju et al., (2018) have conducted TLC showing the presence of different chemical constituents like flavonoids, tannins, glycosides, and saponins with coloured spots.

Table 3**.** TLC analysis of *C. swietenia* fruits in different mobile phases

	Mobile phases	Rf value of spots
Ethanolic-aqueous extract	Methanol: Chloroform (1:1)	-
	Ethyl acetate: Chloroform (1:9)	-
	Ethyl acetate: Water: Acetic acid (8:1:1)	-

Figure 1: Fruit sample of *C. swietenia*

Figure 2: Coarse powder of *C. swietenia* fruits for experimental analysis

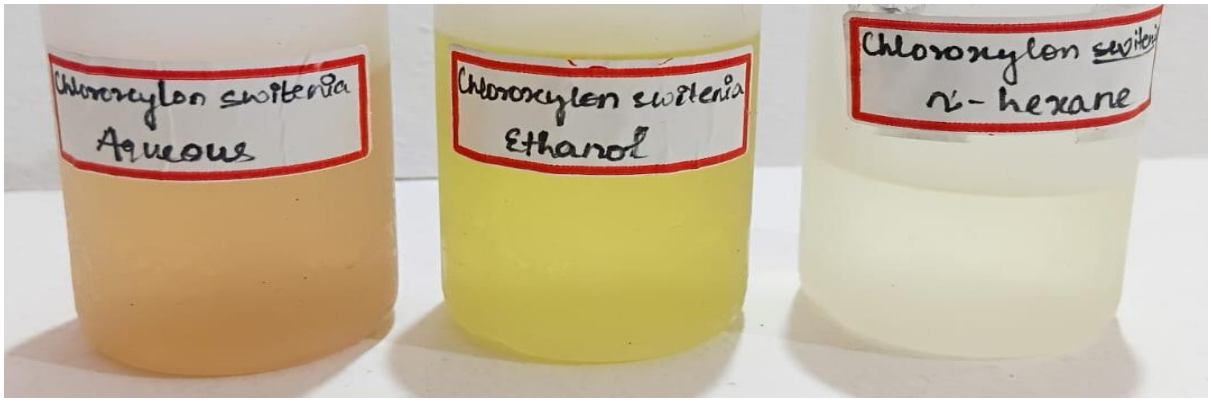

Figure 3: Extracts of *C. swietenia* fruits in different solvents for experimental analysis

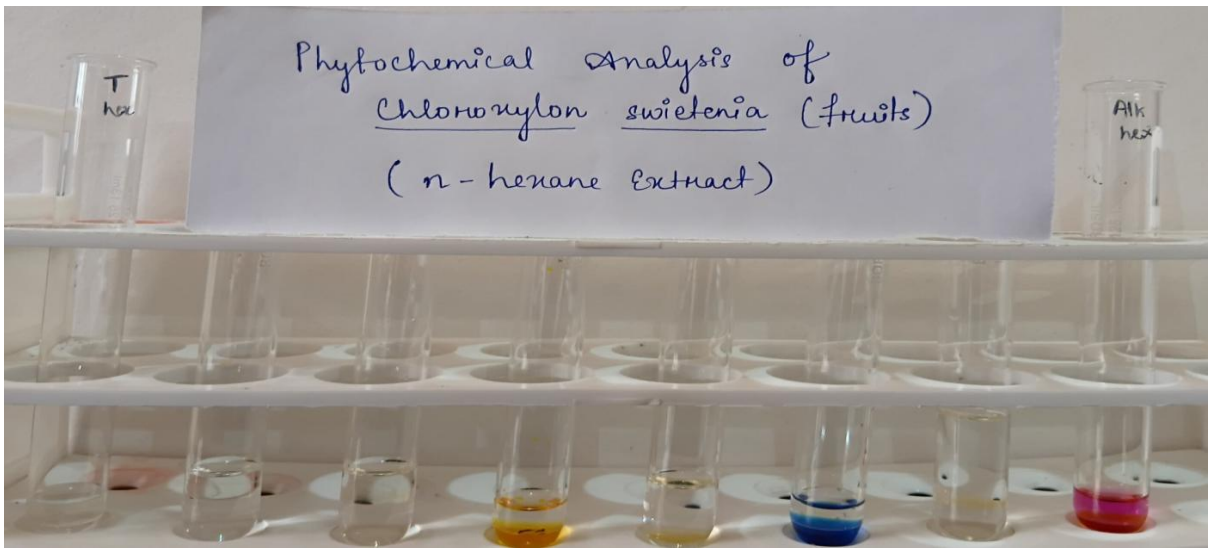

Figure 4: Detection of secondary metabolites of *C. swietenia* fruits using n-hexane extract

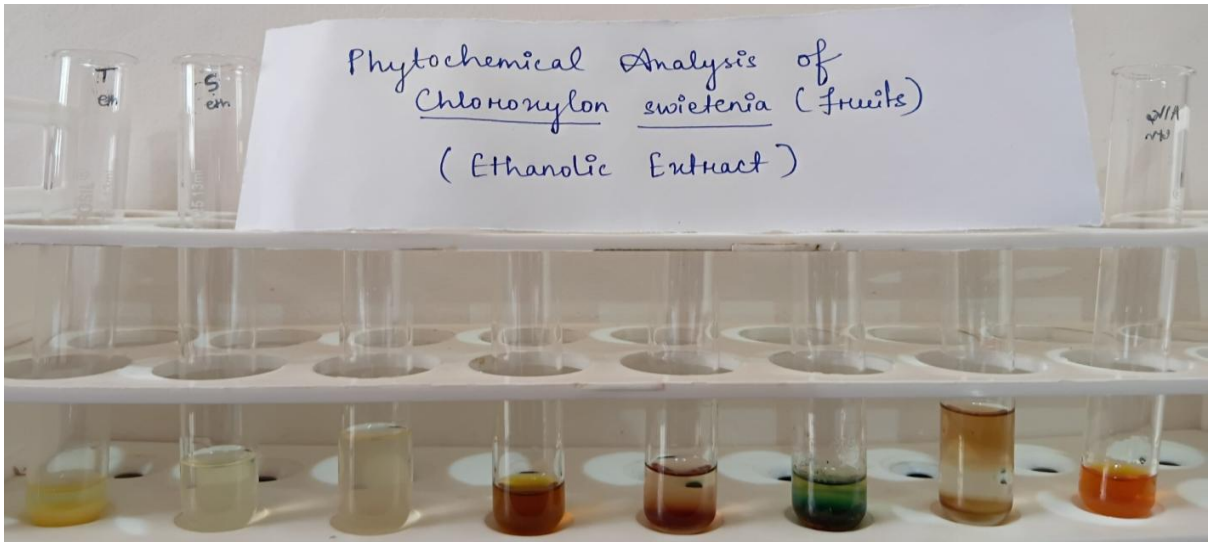

Figure 5: Detection of secondary metabolites of *C. swietenia* fruits using ethanolic extract

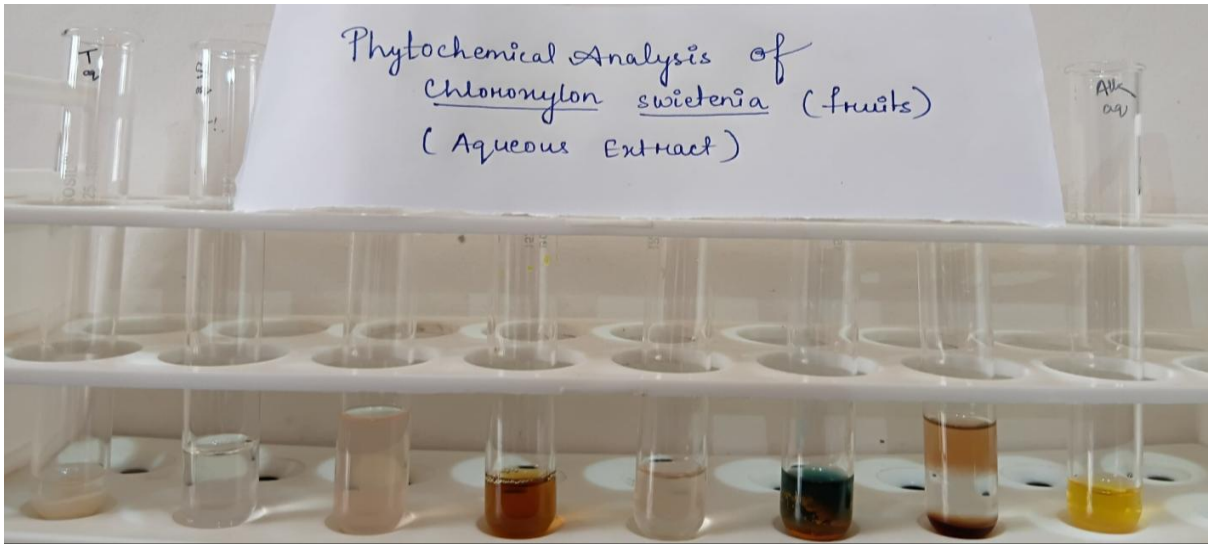

Figure 6: Detection of secondary metabolites of *C. swietenia* fruits using aqueous extract

Figure 7: Toxicity analysis of n-hexane extract of *C. swietenia* fruits using Brine shrimp assay

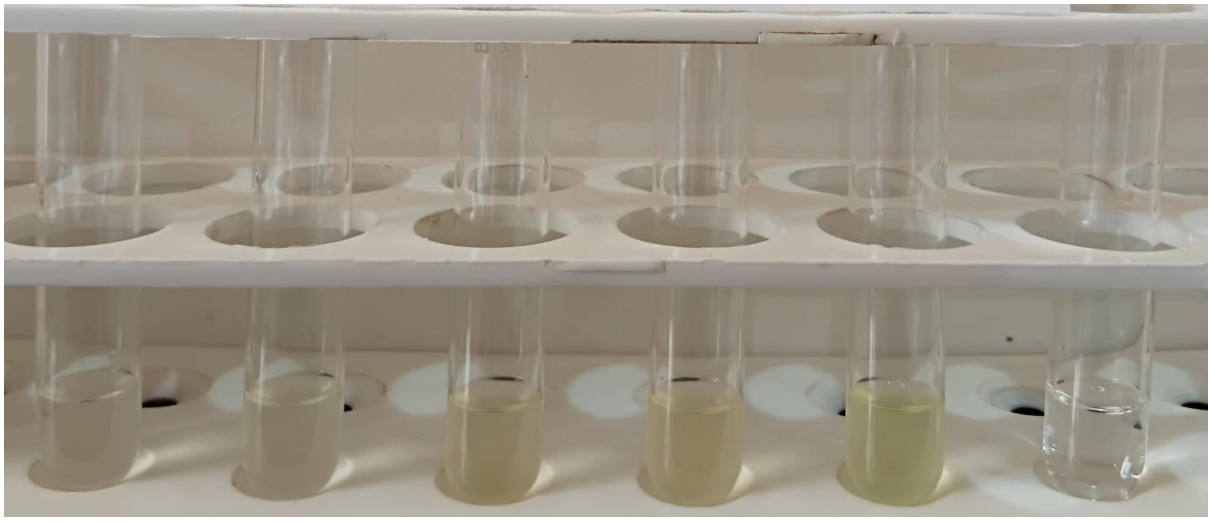

Figure 8: Toxicity analysis of ethanolic extract of *C. swietenia* fruits using Brine shrimp assay

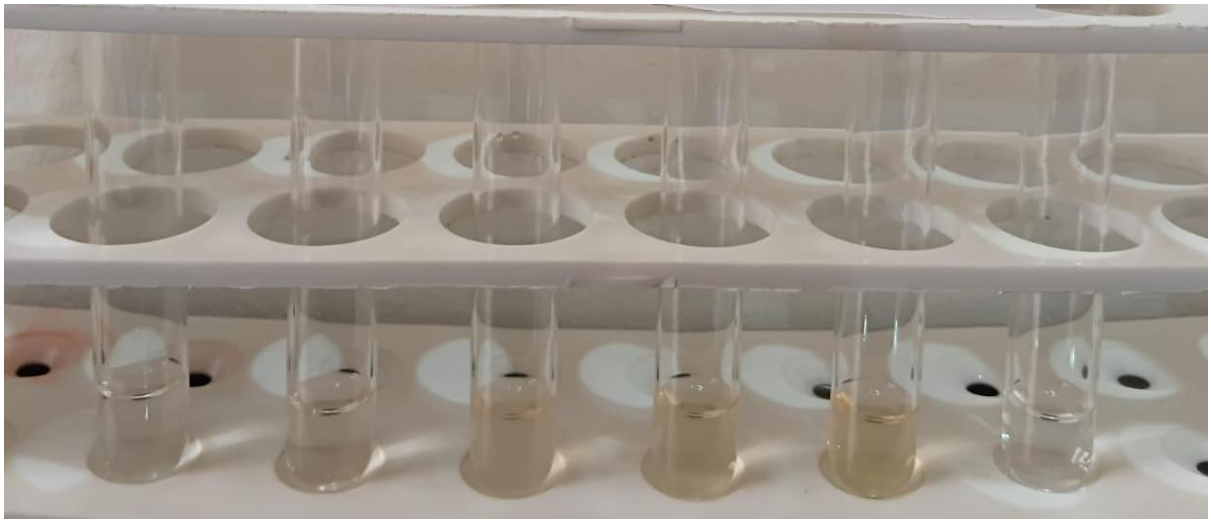

Figure 9: Toxicity analysis of aqueous extract of *C. swietenia* fruits using Brine shrimp assay

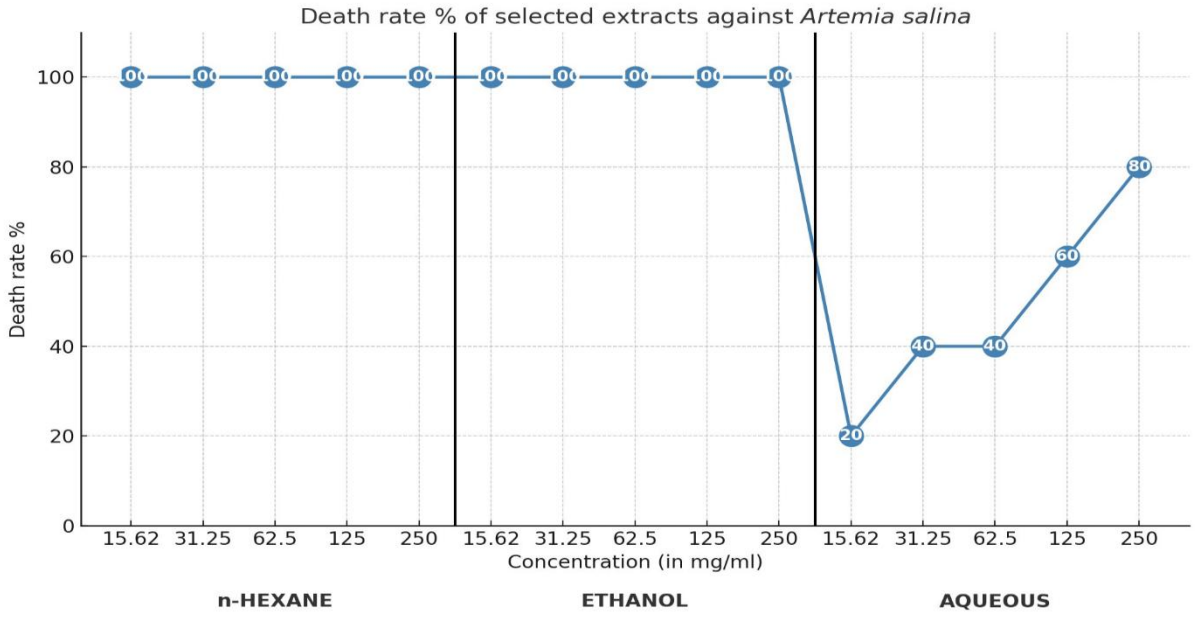

Figure 10: Death % of Brine Shrimp nauplii in different extracts

Conclusion

The present study provides valuable insights into the bioactive compounds, cytotoxic potential, and TLC profile of the fruits of *Chloroxylon swietenia*. The presence of various phytochemicals, including tannins, saponins, reducing sugars, and alkaloids, was detected in the fruit extract, which may contribute to its medicinal properties. The cytotoxic potential of the fruit extract was also demonstrated, suggesting its potential application in the treatment of certain diseases. The TLC profile of the fruit extract revealed the presence of several bioactive compounds, which can be further isolated and characterised for their therapeutic potential. The findings of this study provide scientific evidence for the traditional use of *C. swietenia* fruits and highlight their potential as a source of bioactive compounds with medicinal applications. The study contributes to the understanding of the medicinal properties of *C. swietenia* fruits and provides a foundation for further research into their potential therapeutic applications. Further studies are warranted to isolate and characterise the bioactive compounds responsible for the observed cytotoxic activity and to explore their potential use in the development of new therapeutic agents.

References

Agrawal J, Upadhayay R, Sanghi S and Soni P. (2017). Investigation of phyto-constituents & TLC of *Chloroxylon swietenia* DC. leaves. Asian Journal of Pharmaceutical Education and Research. 6(3): 70-76.

Chunarkar-Patil P, Kaleem M, Mishra R, Ray S, Ahmad A, Verma D, Bhayye S, Dubey R, Singh HN and Kumar S. (2024). Anticancer drug discovery based on natural products: from computational approaches to clinical studies. Biomedicines. 12(1): 201. DOI: 10.3390/biomedicines12010201

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: 691-697.

Devi RS, Satapathy KB, Manjula BL, Sharma BP and Kumar S. (2024). Evaluation of ethnopharmacological potential of *Oenanthe javanica* DC. Annals of Biology. 40: 135-139.

Fraga-Corral M, Otero P, Cassani L, Echave J, Garcia-Oliveira P, Carpena M, Chamorro F, Lourenço-Lopes C, Prieto MA and Simal-Gandara J. (2021). Traditional applications of tannin rich extracts supported by scientific data: chemical composition, bioavailability and bioaccessibility. Foods (Basel, Switzerland). 10(2): 251. DOI: 10.3390/foods10020251

Jayaprasad B, Sharavanan PS and Sivaraj R. (2016). Antidiabetic effect of *Chloroxylon swietenia* bark extracts on streptozotocin induced diabetic rats. Beni-Suef University Journal of Basic and Applied Sciences. 5(1): 61-69.

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: 62-70.

Kamble SS, Choudhari J, Nimma R, Kumar TVS, Patil KK, Hese SV, Dawane BS and Gacche RN. (2022). *Chloroxylon swietenia* (Roxb.) DC induces cell death and apoptosis by down-regulating the NF-κB pathway in MCF-7 breast cancer cells: *in vitro* and *in vivo* investigations. Cancer reports (Hoboken, N.J.). 5(10): e1600. DOI: 10.1002/cnr2.1600

Kowalska T and Sajewicz M. (2022). Thin-Layer Chromatography (TLC) in the screening of botanicals-its versatile potential and selected applications. Molecules (Basel, Switzerland). 27(19): 6607. DOI: 10.3390/molecules27196607

Kumar K, Ganesh M, Baskar S, Srinivasan K, Kanagasabai R, Sambathkumar R, Kumar SS and Sivakumar T. (2006). Evaluation of anti-inflammatory activity and toxicity studies of *Chloroxylon sweitenia* in rats. Ancient Science of Life. 25(3&4): 33-43.

Kumar S and Jena PK. (2014). Chromatographic, antibacterial, and FT-IR analysis of *Dioscorea pentaphylla* L. tuber extracts. Plant Science Research. 36: 83-90.

Kumar S, Jyotirmayee K and Sarangi M. (2013). Thin layer chromatography: a tool of biotechnology for isolation of bioactive compounds from medicinal plants. International Journal of Pharmaceutical Sciences Review and Research. 18(1): 126-132.

Marndi S, Barik M and Kumar S. (2024). Phytochemical and cytotoxicity analysis of *Eulophia explanata* Lindl. (Tuber extracts). African Journal of Biological Sciences. 6: 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.

Nagaraju B, Ramana H, Shiva Krishna N, Bhaskar M and Venkateshwarao P. (2018). *In-vitro* studies on anti-diabetic and wound healing activity of *Chloroxylon swietenia* Linn (Rutaceae) leaf extract. World Journal of Pharmaceutical Research. 7(3): 578-589.

Nasim N, Sandeep IS and Mohanty S. (2022). Plant-derived natural products for drug discovery: current approaches and prospects. The Nucleus: an international journal of cytology and allied topics. 65(3): 399-411. DOI: 10.1007/s13237-022-00405-3

Naveen YP, Divya Rupini G, Ahmed F and Urooj A. (2014). Pharmacological effects and active phytoconstituents of *Swietenia mahagoni*: a review. Journal of Integrative Medicine. 12(2): 86-93. DOI: 10.1016/S2095-4964(14)60018-2

Ravishankara B, Mahmood R, Krishna V, Kumar VNM and Ajith S. (2021). Analgesic and Anti-inflammatory activity of fruit extract phytocompounds of *Chloroxylon swietenia* DC. International Journal of Botany Studies. 6(2): 152-157.

Sasidharan S, Chen Y, Saravanan D, Sundram KM and Yoga Latha L. (2011). Extraction, isolation and characterization of bioactive compounds from plants' extracts. African Journal of Traditional, Complementary, and Alternative Medicines: AJTCAM. 8(1): 1-10.

Senthilkumar A and Venkatesalu V. (2013). *In vitro* fungitoxic and cytotoxic efficacy of *Chloroxylon swietenia* DC. leaf essential oil. Journal of Essential Oil Research. 25(4): 348-353. DOI: 10.1080/10412905.2013.782472

Sharma K, Kaur R, Kumar S, Saini RK, Sharma S, Pawde SV and Kumar V. (2023). Saponins: a concise review on food related aspects, applications and health implications. Food Chemistry Advances. 2: 100191. DOI: 10.1016/j.focha.2023.100191

Yuan M, Zhang G, Bai W, Han X, Li C and Bian S. (2022). The role of bioactive compounds in natural products extracted from plants in cancer treatment and their mechanisms related to anticancer effects. Oxidative Medicine and Cellular Longevity. 2022: 1429869. DOI: 10.1155/2022/1429869

Zahiruddin S, Parveen A, Khan W, Parveen R and Ahmad S. (2021). TLC-based metabolite profiling and bioactivity-based scientific validation for use of water extracts in AYUSH formulations. Evidence-Based Complementary and Alternative Medicine: eCAM. 2021: 2847440. DOI: 10.1155/2021/2847440

Zhang YJ, Gan RY, Li S, Zhou Y, Li AN, Xu DP and Li HB. (2015). Antioxidant phytochemicals for the prevention and treatment of chronic diseases. Molecules (Basel, Switzerland). 20(12): 21138-21156. DOI: 10.3390/molecules201219753