Research Article

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Green Synthesis of Silver- and Gold Nanoparticles Using *Justicia tranquebariensis* Root Extract and their Anticancer Activity Against MOLT-4 Leukemia Cells

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ABSTRACT

This research focuses on the environmental friendly synthesis of silver- and gold nanoparticles (AgNPs and AuNPs) using root extracts of *Justicia tranquebariensis* and evaluates their cytotoxic effects against the MOLT-4 leukemia cell line. FT-IR analysis revealed the presence of phenolics and flavonoids in the plant extract, which play a crucial role in the reduction and stabilization of metal nanoparticles. The morphology and elemental composition of the fabricated nanomaterials were confirmed using SEM and EDAX techniques. Cell viability assays and morphological examinations showed a concentration-dependent decrease in MOLT-4 cell survival, with the crude root extract reducing viability to 22.76% at 300 µg L^{-1}. AgNPs and AuNPs enhanced the anticancer activity by lowering viability to 20.96% (JT-Root-Ag) and 18.53% (JT-Root-Au), respectively, at just 15 µg L^{-1}. These results suggest that *J. tranquebariensis* root-mediated nanoparticles offer a promising, sustainable approach for developing effective anticancer agents.

Keywords: Green synthesis; Anti-cancer activity; Leukemia cells; *Justicia tranquebariensis* extract.

1. INTRODUCTION

Green-synthesized gold and silver nanoparticles have gained considerable attention because plant extracts serve as natural reducing and stabilizing agents (Velmurugan *et al.* 2014 and Dayana *et al.* 2024), eliminating the need for toxic chemicals (Nayak *et al.* 2015). Biogenic nanoparticles generally exhibit superior biocompatibility and enhanced functional properties compared to those produced through conventional methods. The AuNPs synthesized using plant extracts are widely explored for drug delivery, molecular imaging, biosensing and photo-thermal therapy (Goutam *et al.* 2018). Similarly, green-synthesized AgNPs are well known for their strong antimicrobial (Yousefzadi *et al.* 2014), antioxidant and catalytic activities (Mala *et al.* 2017). Phytochemicals such as polyphenols, flavonoids and alkaloids play a crucial role in controlling nanoparticle formation, morphology and stability, making plant-based synthesis a sustainable and eco-friendly strategy (Pavithra *et al.* 2017). Green synthesis using plant extracts aligns with current trends in environmentally responsible nanotechnology and expands the practical applications of metal nanoparticles in biomedical, environmental and industrial fields.

Justicia tranquebariensis plant belongs to Acanthaceae family and is commonly found in the tropical regions of India, particularly across Tamil Nadu. The plant has been used for a long time in Siddha and traditional folk medicine (Dhandapani *et al*. 2025), where its leaves and roots are employed to treat inflammation, skin disorders, fever and respiratory ailments (Sukalingam *et al.* 2018). Although it has been valued in indigenous healing practices for centuries, modern scientific studies on this species have increased only in recent years. Petrovska (2012) identified the bioactive phytochemicals in *Justicia tranquebariensis* and evaluated their preliminary pharmacological activities.

Phytochemical analyses of *Justicia tranquebariensis* have revealed that the plant contains a rich array of bioactive metabolites, including flavonoids, phenolic compounds (Jayashika, 2025), phytosterols, glycosides and several naturally occurring antioxidants that contribute to its therapeutic potential (Hari *et al.* 2022). Biological evaluations of its extracts have demonstrated notable antioxidant, antimicrobial, anti-inflammatory and cytoprotective activities, providing scientific support for its traditional medicinal applications. Additionally, recent microscopic and histochemical investigations have clarified the distribution of key metabolites across different plant tissues (Abirami and Bhavadharaniparkavi, 2024), offering valuable insights that enhance its pharmacognostic characterization and quality standardization (Krishnamoorthi and Ratha, 2015).

Previous reports on *J. tranquebariensis* indicate that comprehensive scientific validation of this

species remains limited. Further investigations are needed to identify its major bioactive constituents, elucidate their mechanisms of action, assess toxicity and safety, and evaluate potential pharmaceutical applications. Some of the earlier works focused on addressing these gaps by expanding knowledge of the plant's phytochemical makeup, investigating its biological activities and exploring its broader pharmacological potential (Krishnamoorthi, 2015).

The roots of *Justicia tranquebariensis* have long been used to treat fever, skin infections and inflammatory conditions, largely due to their rich phytochemical profile. Pharmacognostic and histochemical studies indicate that the roots contain high levels of phenolics, flavonoids, tannins and glycoside compounds known for their strong antioxidant, antimicrobial and anti-inflammatory properties (Saritha and Brindha, 2013). A recent study by Dhandapani *et al.* (2025) reported substantial accumulation of phenolic compounds and tannins in the root tissues, further supporting their therapeutic significance. Saritha and Brindha (2013) reported the ethnobotanical surveys on *Justicia tranquebariensis*. Also, the frequent use of its root-based preparations in managing common ailments is documented (Sivasamy *et al.* 2021). These findings suggest that *J. tranquebariensis* root extract is a valuable natural source of bioactive molecules that supports its long-standing traditional applications.

The root extract of *Justicia tranquebariensis* has recently gained recognition for its effectiveness as a natural reducing and stabilizing agent in the green synthesis of AgNPs and AuNPs. The presence of phenolics, flavonoids and tannins allow for the effective and rapid reduction of metal ions such as Ag^{+} and Au^{3+}, leading to the formation of stable nanoparticles accompanied by distinct colour changes and characteristic surface plasmon resonance peaks (Moulton *et al.* 2010). Dayana *et al* (2024) reported that AgNPs synthesized using this root extract exhibited strong antibacterial, antioxidant, antifungal and anticancer activities, demonstrating the functional potential of its bioactive constituents. Although direct studies on the synthesis of AuNPs using *J. tranquebariensis* root extract remain limited, research on related Justicia species shows efficient AuNP formation through similar phytochemical-driven mechanisms, suggesting comparable potential for this plant. The authors found that the root extract of *J. tranquebariensis* offers a sustainable and biologically active approach for producing silver and AuNPs with promising therapeutic applications.

This study presents a novel method that employs the root extract of *Justicia tranquebariensis*, a medicinal plant rarely investigated, for the green synthesis of AgNPs and AuNPs. It further highlights the role of plant-derived bioactive compounds in reducing and stabilizing metal ions, thereby eliminating the need for toxic chemicals commonly used in conventional nanomaterial fabrication. Comprehensive characterization using FT-IR, scanning electron microscopy (SEM) and EDAX techniques offers detailed insights into the nanostructure and chemical features of the nanomaterial fabrication. Additionally, the investigation evaluates the anticancer potential of these nanomaterial fabrications against the MOLT-4 leukemia cell line, providing new perspectives on their therapeutic applications. This integrated approach combining green synthesis with biological evaluation represents a significant contribution to sustainable nanomedicine research involving *J. tranquebariensis*.

2. MATERIALS AND METHODS

2.1 Synthesis of Silver and Gold Nanoparticles from *J. tranquebariensis* Root Extract

Silver nitrate ($AgNO_3$), chloroauric acid ($HAuCl_4$), sodium borohydride ($NaBH_4$), and *p*-nitrophenol were purchased from Sigma-Aldrich, India. All solutions were prepared using high-purity double-distilled or Millipore water. Prior to use, all glassware and reaction vessels were cleaned with freshly prepared aqua regia (HCl: HNO_3 = 3:1) and thoroughly rinsed with distilled water to eliminate any residual contaminants. All chemicals, reagents and solvents used in this study were of guaranteed reagent grade to ensure experimental accuracy.

The roots of *Justicia tranquebariensis* were collected from the Seelapadi village, Dindigul, and Tamilnadu, India and thoroughly washed under running tap water to remove surface impurities. The cleaned roots were then gently crushed and transferred to a beaker, followed by the addition of distilled water. The mixture was heated for 30 minutes to facilitate the release of water-soluble phytoconstituents. After extraction, the mixture was filtered through Whatman filter paper to obtain a clear root extract, which was subsequently used for the synthesis of AgNPs and AuNPs, designated as JT-Root-Ag and JT-Root-Au, respectively. The details of the anticancer activity procedure are provided in the supplementary information.

3. RESULTS AND DISCUSSION

3.1 FT-IR Results of JT-Root Extract

The FT-IR spectra of JT-Root, JT-Root-Ag and JT-Root-Au extracts are shown in Fig. 1 and the values are tabulated in Table 1. The peaks observed at 3491, 3477 and 3565 cm^{-1} represent the –OH vibration in JT-Root extract, JT-Root-Ag and JT-Root-Au, respectively.

The aliphatic C-H stretching frequencies are observed at 2922, 2923 and 2923 cm^{-1} in JT-Root extract, JT-Root-Ag and JT-Root-Au, respectively. In a literature work, the peaks at 2853, 2852 and 2852 cm^{-1} were ascribed to C-H stretching vibration of JT-Leaf extract, JT- Leaf-Ag and JT-Leaf-Au, respectively.

The characteristic peaks of C=O stretching vibration are positioned at 1637, 1638 and 1641 cm^{-1}, respectively. All other peaks can be similarly corroborated to relevant vibrations as seen in Table 1.

3.2 Morphology Studies of JT-Root-Ag NPs and JT-Root-Au NPs

The SEM studies of the JT-Root-Ag sample, illustrated in Fig. 2, provides a clear view of the nanostructures at magnifications of 200, 20, 10 and 2 µm. At 200 µm, the particles display irregular, branched patterns resembling tree-like formations, which may result from clusters formed during the synthesis process.

When observed at higher magnifications, the particles become more distinct, revealing predominantly spherical shapes. This transition in appearance suggests that the phytochemical constituents of the *Justicia tranquebariensis* root extract play a crucial role in directing particle formation and preventing excessive aggregation, thereby contributing to the development of relatively uniform spherical nanoparticles.

Further, the elemental confirmation is obtained from the EDAX spectrum and mapping analysis shown in Fig. 3 and 4. The EDAX results identify carbon (1.70%), nitrogen (5.53%) and oxygen (12.14%), originating from plant-derived compounds that adhere to the nanoparticle surface and serve as natural stabilizers. Silver constitutes the majority of the elemental composition (80.63%), indicating successful reduction of silver ions by the bioactive components of the extract. The elemental distribution maps further verify that silver is evenly dispersed throughout the sample.

The SEM images of the JT-Root-Au NPs (Fig. 5) show the surface morphology of synthesized particles at various magnifications, including 10, 5, 2, 1, 500 and 200 nm. Across all magnification levels, the particles exhibit an irregular, flaky structure, indicating a heterogeneous and non-uniform morphology. This texture likely results from the influence of bioactive compounds in the *Justicia tranquebariensis* root extract, which modulate the formation and aggregation of AuNPs. The SEM analysis highlights the extract's role in controlling nanoparticle shape and assembly, leading to predominantly non-spherical particle formations.

Table 1. FT-IR spectral values of JT-Root, JT-Root-Ag and JT-Root-Au extracts

S. No.	Band Position (cm^{-1})			Assignment
	JT-Root	JT-Root-Ag	JT-Root-Au	
1	3491	3477	3565	–OH or NH_2 vibration
2	2922	2923	2923	Aliphatic C-H stretching
3	2853	2852	2852	C-H stretching
4	1637	1638	1641	C=O stretching vibration
5	1464	1468	1458	C-O-H function group
6	1413	1459	1437	Bending vibration of C-O
7	1382	1384	1384	C=C stretching vibration
8	1333	1337	1337	Vibration of alcohol C-O moiety
9	1269	1271	1268	C-N stretching of amine
10	1113	1114	1120	C-O bending vibration
11	1060	1052	1081	C-H stretching of aromatic ring
12	1058	-	1040	C-N stretching of amine
13	680	671	672	Wagging vibration of C-O-H groups

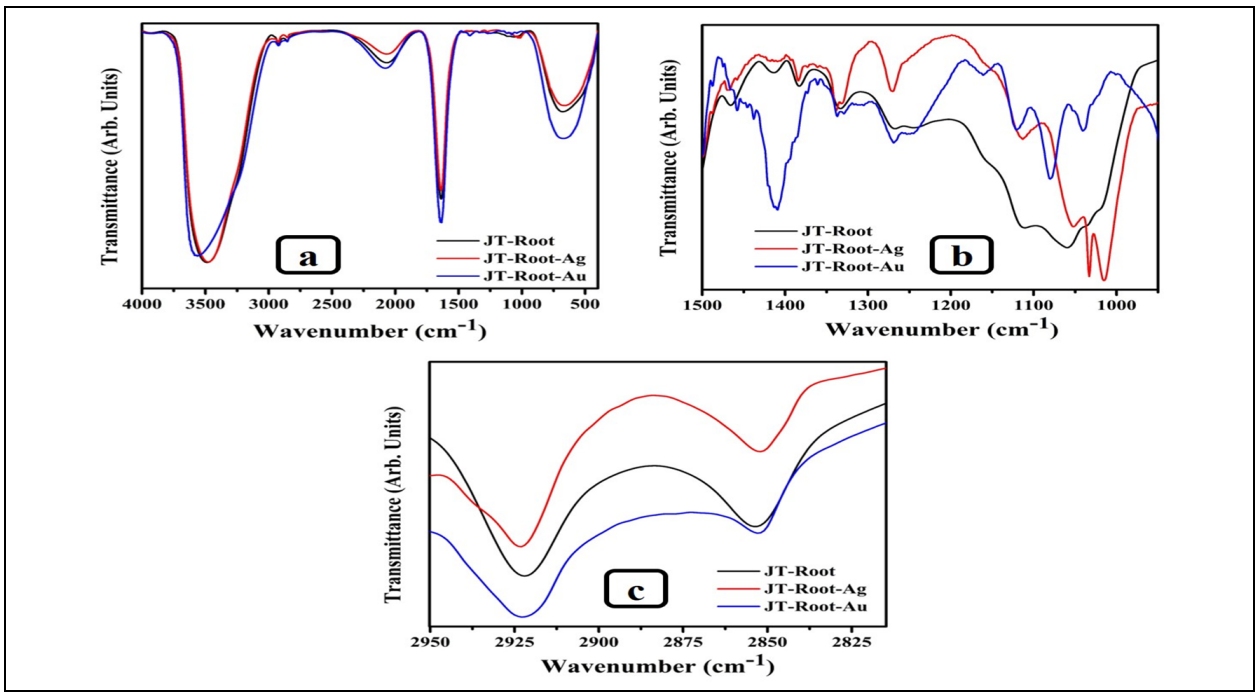


Fig. 1: FT-IR spectra of JT-Root extract, JT-Root-Ag NPs and JT-Root-Au NPs (a) 4000 – 400 cm^{-1}, (b) 1500 – 950 cm^{-1} and (c) 2980 – 2815 cm^{-1}

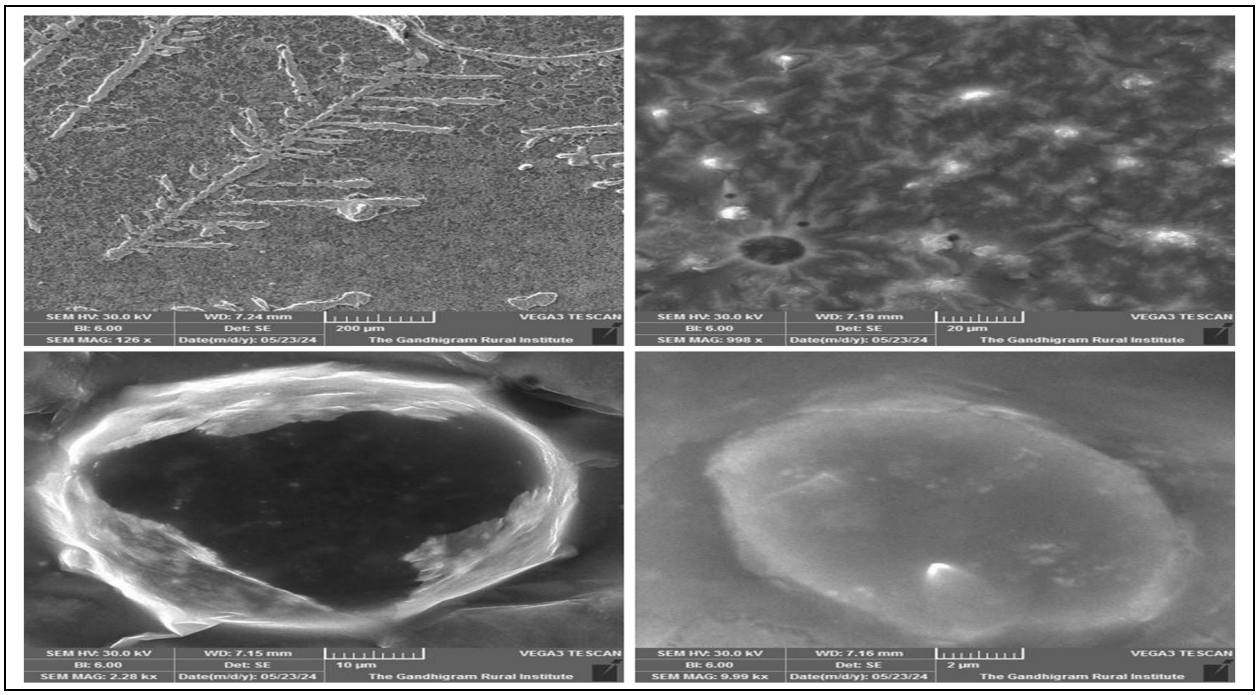


Fig. 2: Images of JT-Root-Ag NPs at various magnifications (200, 20, 10 and 2 µm)

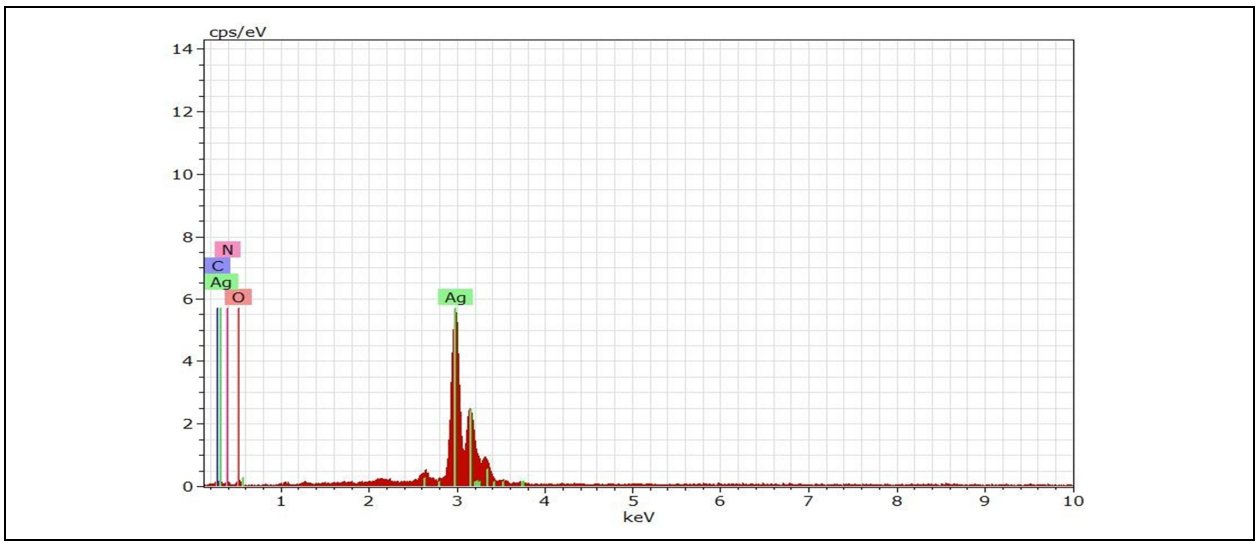


Fig. 3: EDAX analysis of JT-Root-Ag NPs

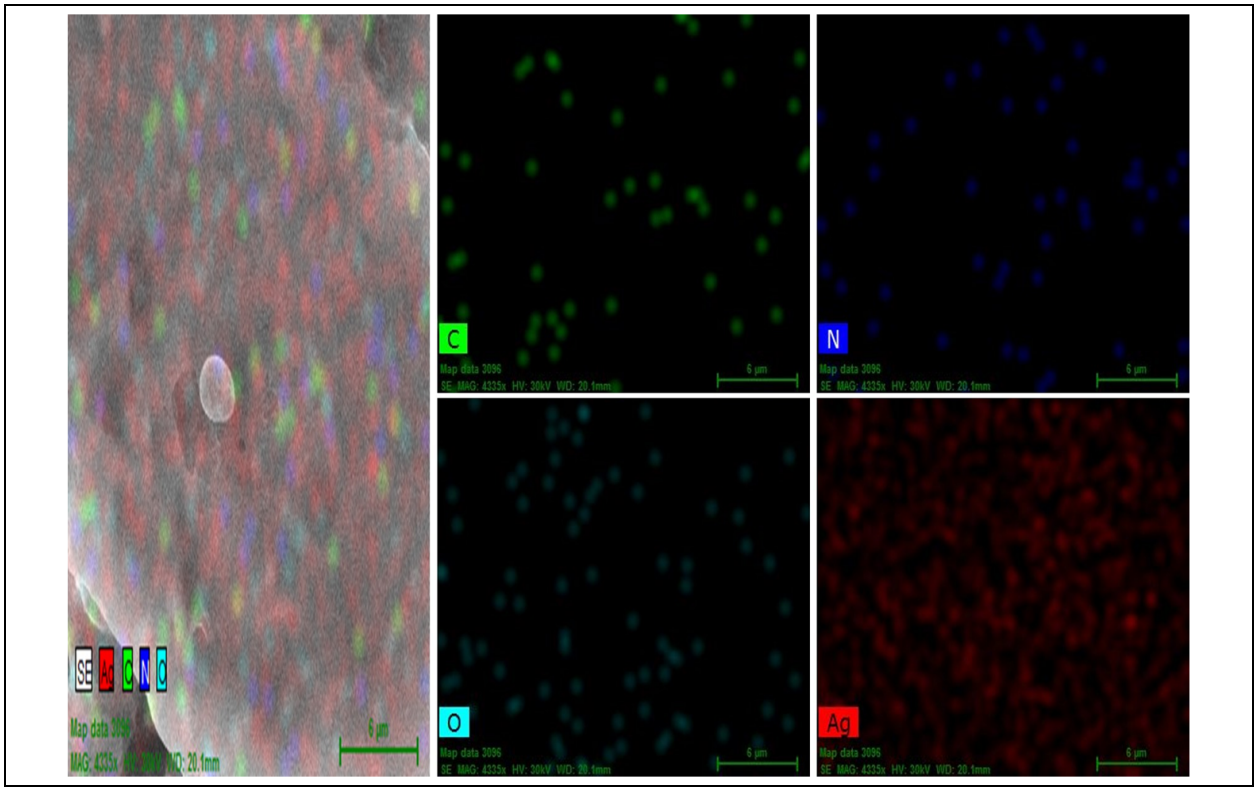


Fig. 4: EDAX mapping analysis of JT-Root-Ag NPs

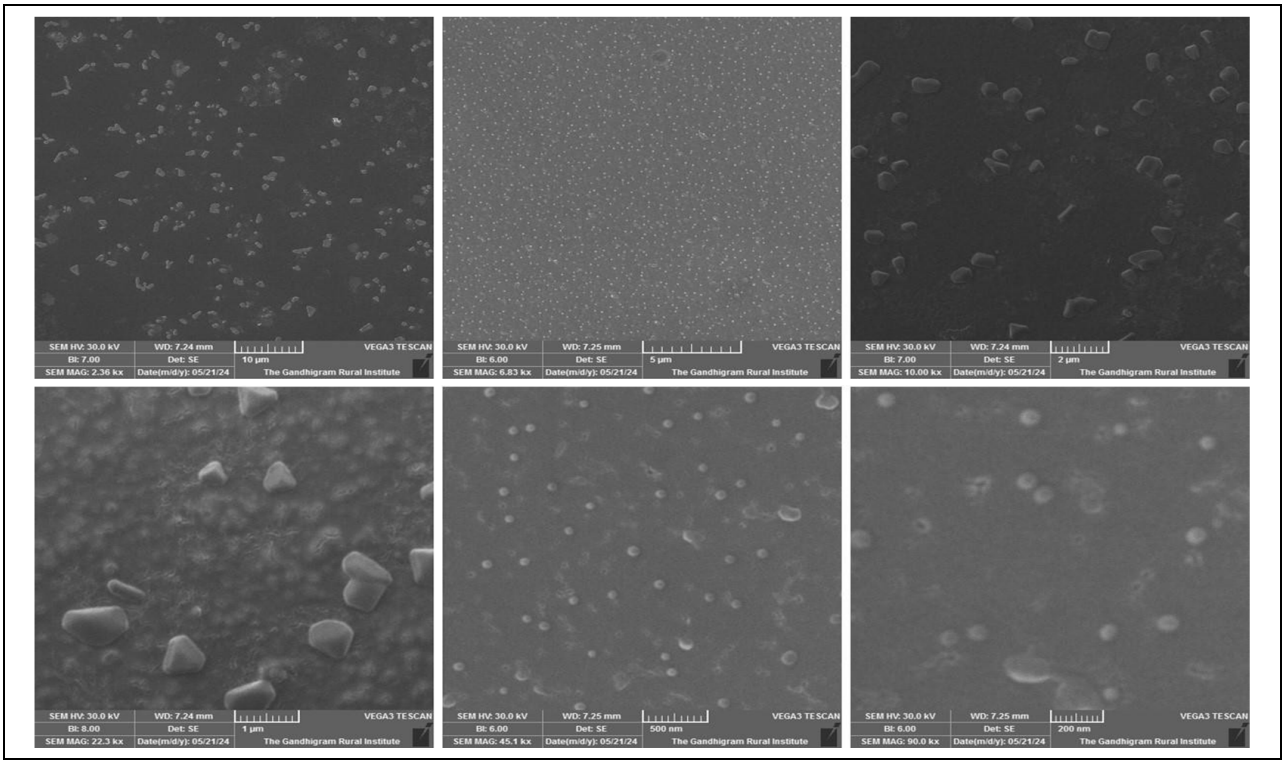


Fig. 5: Images of JT-Root-Au NPs at various magnification (10, 5, 2, 1 µm and 500, 200 nm)

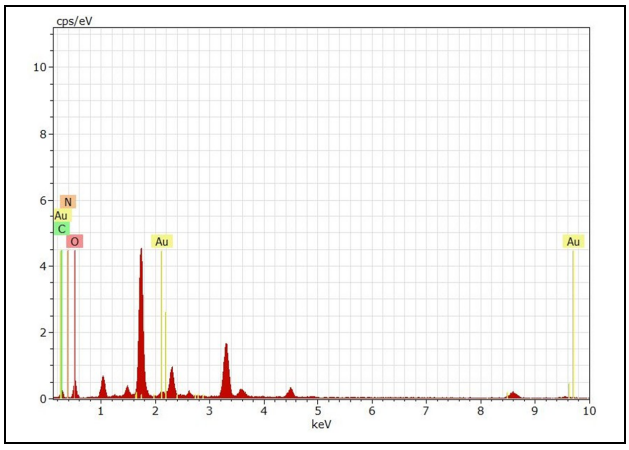


Fig. 6: EDAX analysis of JT-Root-Au NPs

The energy-dispersive X-ray spectroscopy (EDAX) analysis, presented in Fig. 6 and 7, confirms the elemental composition of the JT-root-Au NPs. The data reveal significant amounts of carbon (24.55%), nitrogen (10.39%) and oxygen (63.20%), which are attributed to organic molecules such as proteins, phenolics and flavonoids present in the extract. These biomolecules act as natural stabilizing agents by binding to the nanoparticle surfaces and preventing excessive agglomeration. Gold content was detected at 1.87%, confirming the successful incorporation of metallic gold into the nanoparticles. Elemental mapping further demonstrates a relatively uniform spatial distribution of these elements, emphasizing the effective synthesis and stabilization of AuNPs using the root extract of *J. tranquebariensis*.

3.3 Anti-cancer Activity of JT-Root, JT-Root-Ag and JT-Root-Au Samples

Table 2 provides the cell viability data obtained from the anticancer evaluation of the JT-Root, JT-Root-Ag and JT-Root-Au samples. The JT-Root extract (Fig. 8) exhibits a distinct concentration dependent reduction in cell viability across the tested range of 50 – 300 µg L^{-1}.

At the lower end of the concentration scale (50 µg L^{-1}), the extract maintains a high viability of 90.08%, indicating that it is relatively non-toxic and exerts minimal harmful effects on the cells at this level. When the concentration increases to 100 µg L^{-1}, viability decreases to 75.28%, marking the initial onset of cellular stress or early metabolic disturbance. A more noticeable decline is observed at 150 µg L^{-1}, where viability falls to 56.91%, suggesting that the bioactive constituents of the extract begin to exert stronger inhibitory effects as their intracellular accumulation increases. At higher concentrations, the cytotoxic impact becomes more pronounced. Viability drops to 40.97% at 200 µg L^{-1} and further decreases to 32.35% at 250 µg L^{-1}, indicating substantial impairment of cellular processes such as membrane integrity and metabolic activity function. The lowest viability value, 22.76% at 300 µg L^{-1}, demonstrates that the extract exhibits marked

cytotoxicity at elevated doses. This confirms that the JT-Root extract shows mild to moderate cytotoxicity at lower concentrations and strong inhibitory effects at higher levels, reflecting typical dose-dependent biological behavior commonly associated with phytochemical rich plant extracts.

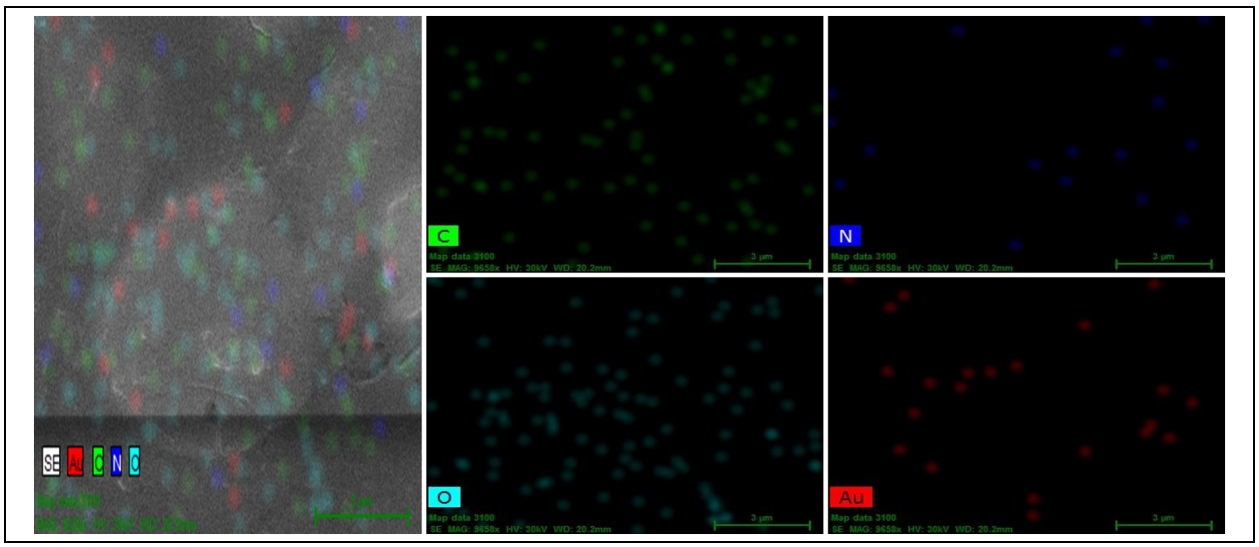


Fig. 7. EDAX mapping analysis of JT-Root-Au NPs

Table 2. Anti-cancer activity values of JT-Root, JT-Root-Ag and JT-Root-Au samples

S. No.	Concentration ($\mu g\ L^{-1}$)	Cell Viability (%)	Concentration ($\mu g\ L^{-1}$)	Cell Viability (%)	
		JT-Root		JT-Root-Ag	JT-Root-Au
1	50	90.08	2.5	83.16	86.90
2	100	75.28	5.0	71.99	69.55
3	150	56.91	7.5	65.29	50.68
4	200	40.97	10.0	47.76	43.53
5	250	32.35	12.5	36.76	35.03
6	300	22.76	15.0	20.96	18.53

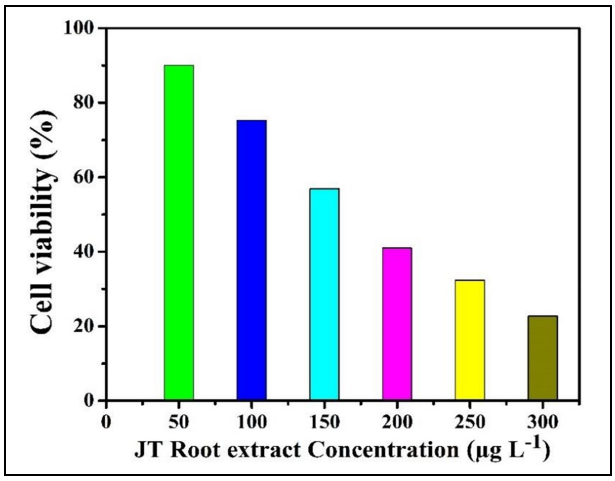


Fig. 8: Anticancer activity of JT-Root extract at various concentrations against blood cancer MOLT-4 cells for 24 hrs

The JT-Root-Ag sample (Fig. 9), which incorporates silver nanoparticles derived from the JT-Root extract, shows a substantially stronger cytotoxic response than the crude extract, even though it is tested at much lower concentrations (2.5 – 15 $\mu g\ L^{-1}$). At 2.5 $\mu g\ L^{-1}$, the cell viability is already reduced to 83.16%, indicating a more pronounced inhibitory effect compared to JT-Root at significantly higher doses.

This enhanced activity is likely due to the combined influence of plant derived compounds and AgNPs, which are known to generate oxidative stress and interfere with vital cellular mechanisms. As the concentration increases, the cytotoxic impact becomes more evident. Cell viability decreases to 71.99% at 5 $\mu g\ L^{-1}$ and further to 65.29% at 7.5 $\mu g\ L^{-1}$, suggesting that the AgNPs facilitate efficient cellular interaction and uptake. A major shift in activity is observed at 10 $\mu g\ L^{-1}$, where viability drops sharply to 47.76%, implying that JT-Root-Ag disrupts essential metabolic or structural

functions more effectively than the crude extract. At 15 µg L^{-1}, viability falls to 20.96%, a reduction similar to that caused by the highest JT-Root dose but achieved at only a small fraction of the concentration. These findings indicate that the integration of AgNPs significantly boosts the cytotoxic potential of the JT-Root extract by enhancing surface reactivity, improving cell penetration and intensifying metabolic stress within the cells.

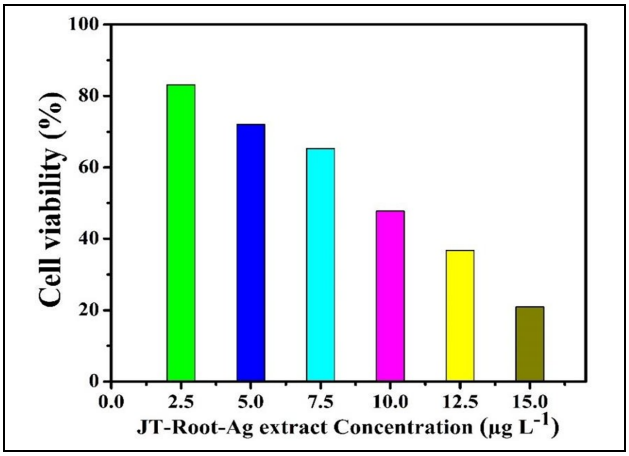


Fig. 9: Anticancer activity of JT-Root-Ag sample against blood cancer MOLT-4 cells for 24 hrs

JT-Root-Au prepared by integrating JT-Root phytochemicals with AuNPs shows a strong concentration dependent decrease in cell viability (Fig. 10). In the case of JT-Root-Au sample at the initial concentration of 2.5 µg L^{-1}, the viability remains at 86.90%, which is marginally higher than that of the silver-based formulation (JT-Root-Ag) yet still lower than the crude extract. This indicates that AuNPs contribute to enhanced cellular interaction even at very low levels. As the concentration increases, the cytotoxic response becomes more pronounced. Cell viability decreases to 69.55% at 5 µg L^{-1} and further declines to 50.68% at 7.5 µg L^{-1}, showing that JT-Root-Au begins to exert stronger inhibitory effects with increasing nanoparticle content. A substantial drop is observed at 10 µg L^{-1}, where viability reaches 43.53%, suggesting marked interference with essential cellular functions. The minimum viability value of 18.53% at 15 µg L^{-1} represents the greatest reduction among all the tested samples, indicating that JT-Root-Au is the most potent formulation at higher concentrations. This enhanced cytotoxicity may be linked to the distinctive surface properties and biocompatibility of gold nanoparticles, which can promote efficient cellular uptake, intensify metabolic disruption and increase the interaction of phytochemicals with cellular components. Overall, JT-Root-Au demonstrates the strongest cytotoxic performance compared with JT-Root and JT-Root-Ag, particularly at elevated concentrations. Among all extract exhibits the cytotoxic effect was clearly observed at higher concentration where a significant reduction in cell viability was recorded compared to lower concentrations.

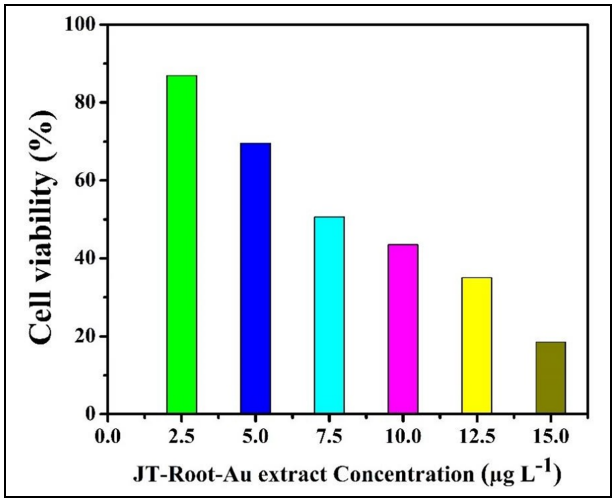


Fig. 10: Anticancer activity of JT-Root-Au sample against blood cancer MOLT-4 cells for 24 hrs

4. CONCLUSION

The *Justicia tranquebariensis* root extract and its silver (JT-Root-Ag) and gold (JT-Root-Au) nanoparticles exhibited the presence of multiple functional groups, such as phenolic compounds and flavonoids as confirmed by FT-IR analysis. These phytochemicals are believed to play a key role in the reduction of metal ions and in stabilizing the nanomaterial fabrication, thereby contributing to their biological activity. Anticancer studies showed that JT-Root-Ag and JT-Root-Au significantly decreased the viability of MOLT-4 leukemia cells in a concentration dependent manner. The viability of MOLT-4 cells decreased to 18.53% upon exposure to JT-Root-Au, indicating strong cytotoxic activity.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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