

products. Thus, the functionalization of plumbagin with an appropriate nanomaterial is a promising strategy to overcome the problems associated with plumbagin when used alone, and to enhance the therapeutic potential of plumbagin in its use for therapeutic applications. Silver nanoparticles (AgNPs) have garnered significant interest of the scientific community for synthesis of “hybrid drug molecules” as they act as good nanocarriers of the drugs leading to targeted drug delivery. Furthermore, the unique physical, chemical, optical and biological properties of AgNPs have made them excellent candidates in biomedical applications as antimicrobial and anticancer agents.

Here, we report a preliminary study conducted to synthesize plumbagin functionalized AgNPs to be used in antimicrobial applications. In this study plumbagin was extracted from the roots of *Plumbago indica* and crude plumbagin was purified by recrystallization.¹ The identity and purity of plumbagin were confirmed by GC/MS, FT-IR and UV-Vis spectra. AgNPs were synthesized by reduction of silver nitrate with hydroxylamine hydrochloride following the Leopold-Lendl method.² Synthesized AgNPs gave rise to the characteristic surface

plasmon resonance (SPR) absorption peak at 410 nm in the UV-Vis spectrum. Vortex mixing of AgNPs with plumbagin in 1: 2×10⁵ molar ratio at 1600 rpm for 2 hours at room temperature led to the successful functionalization of AgNPs with plumbagin, resulting a significant difference in the UV-Vis and FT-IR spectra. Since both materials possess notable antimicrobial properties, an enhanced antimicrobial potential is expected from plumbagin functionalized AgNPs due to the synergistic effect. Investigation of the antimicrobial potential of plumbagin functionalized AgNPs is currently in progress.

Keywords

Plumbagin, Silver nanoparticles, Functionalization, Antimicrobial

References

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Cytotoxic potential and apoptotic effect of *Barringtonia asiatica* seed kernel against HepG2 cell line

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Liver cancer causes significant morbidity and mortality among humans worldwide due to lack of effective therapeutic strategies for control and treatment. According to World Health Organization, 7.5 % of male deaths in Sri Lanka are due to liver cancer and mortality rate due to liver cancer is increasing each year¹. The need for pharmacological agents has necessitated the search for newer therapies from natural products. *Barringtonia asiatica* is a species of *Barringtonia* native to mangrove habitats along tropical coasts and islands of the Indian Ocean and is grown as an ornamental plant in Sri Lanka. Crude methanolic extract (CME ;15 g powder / 40 mL MeOH; 24 hrs; dried at 45oC)) and an isolated fraction (MPLCBA-3 fraction) of CME of *B. asiatica*, using medium pressure liquid chromatography (MPLC) have showed cytotoxicity against HepG2 cell line². The present study investigated the mechanisms of cytotoxicity of CME and MPLCBA-3 fractions from

Barringtonia asiatica seed kernel against HepG2 cell line. Cytotoxicity was assayed with LDH assay with CME (10, 25, 50, 75, 100, 125 ppm) and MPLCBA-3 fraction (2.5, 5, 10, 15, 20, 25 ppm). Mechanisms of action were determined using DNA fragmentation analysis with CME (25, 50 and 100 ppm) and MPLCBA-3 fraction (5, 10 and 20 ppm) and fluorescence microscopic analysis with CME (10, 20, 40 and 80ppm) and MPLCBA-3 (5, 10, 20, 40 ppm] against the liver cancer cell line using standard procedures^{3,4,5}. The total LDH activities in the medium when HepG2 cells were treated with CME (10-125 ppm) and MPLCBA-3 fraction (2.5-25 ppm), increased from 53.1%-87.3% [34.2%] and 53.9– 66.4% [12.5%], respectively against the controls. LDH activity increased with increasing concentration of the cytotoxic fraction confirming cell membrane damage by CME and MPLC fractions. However, MPLCBA-3 fraction caused more damage compared to CME. AO/EB staining

showed uniform green cells in the control HepG2 cells whereas apoptotic cells in the early stage were marked by yellow-green (10 ppm of CME, 5, 10 ppm of MPLCBA-3) and apoptotic cells in the late stage were marked with concentrated and asymmetrically yellow-orange nuclei (20, 40, 80 ppm of CME, 20, 40 ppm of MPLCBA-3, 10 ppm of thymoquinone [control]) under fluorescence microscope. Thus, HepG2 cells after treatment with CME and MPLCBA-3 fractions indicated cell apoptosis. Control had intact nuclei with uniformly dispersed chromatin in HepG2 cell lines. DNA fragmentation is a feature of apoptosis. HepG2 cells treated with CME and MPLCBA-3 fraction of *B. asiatica* and thymoquinone indicated DNA fragmentation, confirmed due to smearing observed in the gel electrophoresis when compared to control. When comparing CME and MPLCBA-3 fraction higher fragmentation was observed with the MPLCBA-3 fraction. The cell membrane damage as indicated by high LDH activity also correlates to the observation made under fluorescence microscopic pictures. When considering fluorescence microscopic pictures stained with Hoechst stain, at higher concentrations of

MPLCBA-3 fraction (20 ppm), condensed nuclei were observed in the HepG2 cells. These correlated with gel picture of DNA fragmentation of MPLCBA-3 fraction in HepG2 cell lines where smearing indicated that DNA were fragmented. The morphological characteristics indicated that these extracts cause apoptosis while biochemical changes linked with apoptosis included leaching of LDH indicating membrane damage and DNA fragmentation. Thus, both crude methanolic extract and MPLCBA-3 fraction have shown high cytotoxic potential due to membrane damage and DNA fragmentation causing apoptosis of HepG2 cells.

Keywords

Barringtonia asiatica, DNA fragmentation, LDH assay, Fluorescence microscopic analysis

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Durian and rambutan peels as potential sources of antioxidants

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Rambutan (*Nephelium lappaceum* L.) and Durian (*Durian zibethinus* Murr.) are popular seasonal fruits grown in tropical countries, enriched in varieties of phytochemicals.^{1,2} However, peels of these fruits cause unpleasant odors and serious environmental problems. As the exploration of bioactive compounds may lead to novel environmentally friendly drug discovery, the main objective of this study was to investigate the use of waste materials, durian and rambutan peels, as potential sources of antioxidants. Therefore, this work focused on determination of antioxidant activities of methanol extract of durian and rambutan peels using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay and determination of its total polyphenolic contents (TPC) and total flavonoid contents (TFC).

The chemical constituents of durian and rambutan peels were first extracted using methanol. Then the methanol extract was sequentially extracted with hexane, chloroform and methanol. The antioxidant activity of extracts of durian and rambutan peels and its fractions

were investigated using DPPH radical scavenging assay.³ TPC of methanol extracts of durian and rambutan peels were determined using Folin-Ciocalteu method⁴ and TFC of the extracts were analyzed using aluminium chloride method.⁴ According to the results, all the extracts and its fractions showed antioxidant activity. The IC₅₀ values of methanol extract of rambutan peels (7.86±0.22 µg/mL), its hexane (13.49±0.52 µg/mL), chloroform (26.99±0.20 µg/mL) and methanol (30.85±0.97 µg/mL) fractions were lower than that of the control, butylated hydroxytoluene (BHT) (43.70±0.89 µg/mL). IC₅₀ values of methanol extract of durian peels (100.48±4.16 µg/mL), its hexane (>1000 µg/mL), chloroform (161.99±6.23 µg/mL) and methanol (>1000 µg/mL) fractions were higher than that of the control, butylated hydroxytoluene (BHT) (43.70±0.89 µg/mL). TPC of methanol extracts of durian and rambutan peels were found to be 2.98±0.03 and 14.80±0.21 mg GAE/g dry weight respectively. Higher TFC was observed in methanol extract of durian peels (30.87 mg Catechin /g dry weight) than in methanol