

Summary

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There is a continuing search for anticancer drugs from medicinal plants. Nanotechnology holds a promise to offer many new drug delivery particles and devices. There are many routes of synthesis of nanoparticles such as physical, chemical and biological methods. The green route for synthesis of nanoparticles (biological method) using plant extracts offers advantages such as less toxicity, eco-friendly and safety which can be applied for drug delivery. Thus the objective of the research work was to quantitate the phytochemicals, study the volatile profile, antioxidant, antibacterial, phytotoxic, cytotoxic and genotoxic activities of *Mallotus tetracoccus* and *Tabebuia rosea* and then to phytosynthesize nanoparticles from plant extracts and compare the anticancer activity of the silver nanoparticles prepared from the plants as well as crude plant extracts.

The extract recovery yields for *M. tetracoccus* and *T. rosea* were found to be higher in ethanol among other extracts. The organoleptic characteristics were studied according to Indian Pharmacopeia which indicated that *M. tetracoccus* had higher amount of ash values than *T. rosea*. Qualitative phytochemical analysis of *M. tetracoccus* revealed the presence of sugars, tannins, alkaloids, flavonoids, steroids, terpenoids and phenolic acids. Qualitative phytochemical analysis of *T. rosea* displayed the presence of tannins, alkaloids, flavonoids, saponins, steroids and phenolics. Quantitative analysis revealed that the *M. tetracoccus* fractions possessed higher amount of total phenolics (TPC) and total flavonoids than *T. rosea*. Carotenoids were found to be higher in *T. rosea* fractions than in *M. tetracoccus*. Among the solvents used, aqueous ethanol and acetone proved to be efficient solvents for extracting the phenolics and flavonoids contents from the plant extracts of *M. tetracoccus* and *T. rosea*.

UV-Vis spectral analysis revealed the presence of phenols and phenolic acids in *M. tetracoccus* fractions and flavonoids and carotenoids in *T. rosea* fractions. The volatile profile of *M. tetracoccus* reported the presence of compounds such as fatty acid esters (54.91 %), sugars (2.61 %), alcohols (13.49 %), terpenoids (3.88 %), alkanes (3.03 %) and alkenes (13.31 %). The volatile profile of *T. rosea* clearly

demonstrated the presence of compounds such as quinoids (7 %), benzenoids (8 %), flavonoids (6 %), phenols (19 %), carboxylic acids and aldehydes (21 %), terpenoids (8 %) and sugars (13 %).

The radical scavenging activities of *M. tetraococcus* of aqueous ethanol fraction exhibited IC₅₀ value similar to standard ascorbic acid. The reducing power and metal chelating activities of all fractions increased with increase in concentrations. The total antioxidant activities of the extracts were similar to standard ascorbic acid. Lipid peroxidation studies by FTC and TBA methods revealed that there was high percentage inhibition by *M. tetraococcus* fractions than by *T. rosea* fractions.

Correlation studies clearly explained that there was high level of correlation between TFC and total antioxidant activity in *T. rosea* fractions. There was high correlation in *M. tetraococcus* fractions between TPC and reducing activity. The high reducing power and lipid peroxidation inhibition activity of *M. tetraococcus* fractions can be due to the presence of TFC, which is evidenced by UV-Vis spectral studies and GC-MS studied.

Among all the fractions, *M. tetraococcus* and *T. rosea* ethanolic extracts had highest activity on *Pseudomonas aeruginosa* and *Bacillus subtilis* at a dose dependent manner. Lower activities were observed for *Staphylococcus aureus* by all fractions. The fractions were most effective against gram negative microbes than gram positive microbes which may be attributed due to the action of different bioactive compounds on microbial proteins or enzymes in their cell walls.

The cytotoxicity of various fractions of *M. tetraococcus* and *T. rosea* studied by using brine shrimp larvae revealed that highest cytotoxic activities were observed in ethanolic fraction (LD₅₀ < 10 µg/mL) followed by ethyl acetate fractions (LD₅₀ < 20 µg/mL). All other fractions exhibited LD₅₀ values lesser than 50 µg/mL, which proves the pharmacological potential of these two plants. Thus two plant fractions possessed significant cytotoxic activity.

The allelopathic potential of plant extracts studied using radish seed phytotoxicity assay revealed that the highest root growth inhibitions were exhibited by chloroform and ethyl acetate fractions of *M. tetraococcus* and *T. rosea* respectively. All the extracts exhibited toxicity in radish seed bioassay at 10,000 and 1000 ppm. The relative germination rates were found to be higher in petroleum ether and ethanol fractions of *M. tetraococcus* and *T. rosea* respectively. Thus the germination index of radish seeds confirmed higher activity for petroleum ether and ethanol fractions of *T. rosea* extracts. Very low germination indices were observed for *M. tetraococcus* fractions which proved the phytotoxic potential of *M. tetraococcus* to inhibit radish seeds. In genotoxicity studied by cytokinesis block micronucleus assay on peripheral blood lymphocytes and MOLT-4 cells, absence of genotoxicity was evidenced among *M. tetraococcus* and *T. rosea* extracts. Thus the extracts were found to have a protective effect on human cells *in vitro*.

To the need to develop environment friendly and economically feasible nanoparticles, green synthesis gives a helping hand for researchers. Many biomolecules in plants such as proteins/enzymes, amino acids, polysaccharides, alkaloids, alcoholic compounds and vitamins could be involved in bioreduction, formation and stabilization of metal nanoparticles have been proved in this study. Reduction potential of ions and reducing capacity of plants which depend on the presence of polyphenols, enzymes and other chelating agents present in plants have critical effects on the amounts of nanoparticle production.

Silver NPs were prepared biologically using *M. tetraococcus* and *T. rosea* extracts. The synthesized NPs were standardised using different pH and temperature. Further NPs were characterized using UV-Vis spectral studies, AFM, FTIR, SEM and EDX analysis. The formation of brown color solution after addition of silver nitrate solution to plant extract was analysed by measuring the absorption spectrum of AgNPs at 420 nm using UV-Vis spectrophotometer, which confirmed the formation of silver nanoparticles. The formation of AgNPs were standardised at pH 7 and temperature of 60 °C for both samples. The FTIR analysis of NMT and NTR samples revealed that the hydroxyl functional groups of phenols and carboxyl functional groups could be involved in the reduction of silver nitrate to silver metal,

resulting in the formation of silver nanoparticles. The particles sizes analysed using AFM of NMT and NTR were found to be in the range of 49-98 nm and 46-100 nm, with an average size of 70 and 73 nm respectively. The EDX analysis for the presence of inorganic substances in nanosamples proved pure strong silver signals at 3 KeV in NMT and NTR.

The cytotoxicity of AgNPs synthesized using plant extracts NMT, NTR and crude plant extracts were studied against human fibroblast cell line (L929) and human ductal breast carcinoma cell lines (T47D) using MTT assay. The results clearly demonstrated that the NMT had higher level of cytotoxicity (77 to 85 %) on cancer cells and very less cytotoxicity on normal cells (5-10 %) at the concentrations studied. The ethanolic plant extracts of *M. tetraococcus* and *T. rosea* displayed less cytotoxicity (21 to 63 %) when compared to nanosamples on tumor cells. Though NTR was found to have higher anticancer activity than NMT, it failed to exhibit less activity on normal cells. Thus NMT may be suggested for further anticancer studies and also in drug delivery. Thus the nanoparticles are capped and stabilized by the active phytochemicals present in the plant extracts.

Thus the ethanolic fractions (polar fractions) have highest bioactivity acting as a more potent extract when compared with the other fractions (non-polar). It could be concluded that *M. tetraococcus* and *T. rosea* plants are of phytopharmaceutical importance possessing significant antioxidant, radical scavenging activity, antibacterial, cytotoxicity and phytotoxic activities. Thus the presence of active phytochemical compounds in the extracts of *M. tetraococcus* and *T. rosea* can have beneficial use in pharmaceutical industries. The silver nanoparticles along with plant extracts offer an excellent drug delivery system for cancer therapy.