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SUMMARY AND CONCLUSION

Cancer is a primary public health problem that is considered as one of the leading causes of morbidity and mortality around the world. It is a multifactorial disease with growth abnormalities rising from unusual cell division caused by the combination of genetic and environmental factors. However, understanding the genetic, molecular, and cellular basis of cancer can lead to novel therapeutic targets and strategies. The conventional cancer therapies including chemotherapy, radiotherapy, surgery, immunotherapy, are associated with serious side effects and development of multi drug resistance. Furthermore, many anticancer medicines are not able to attain their target site in adequate concentrations to exhibit their pharmacological activity properly without inflicting irreversible harm to healthy tissues and cells.

Nanotechnology-based therapeutic and diagnostic techniques have shown promising strategies in the improvement of cancer treatment in recent years. It provides a range of techniques for treating cancer by overcoming biological barriers and directly delivering therapeutic chemicals. Metal NPs, particularly silver nanoparticles, have exclusive physicochemical qualities such as a high surface-to-volume ratio, broad optical properties, simplicity of manufacturing, and surface functionalization, which open up new possibilities for cancer therapies. In addition, the use of nano particulate drug carriers might improve solubility of the drug, extend half-life of the drug in the bloodstream, and reduce adverse side effects at non-target organs. Thus targeted therapies with nano sized formulations might be a valuable approach to render cytotoxicity towards the proliferating cancerous cells.

Plants are an intriguing source of new therapeutic entities, and they contribute to drugs with potential application as anti-cancer agents. Phyto-constituents are promising alternatives for producing green silver nanoparticles, which have a lot of potential in treating chronic disorders. In combination with various nanoparticles and phytochemicals, recent advancements in the fields of nanobiotechnology have proven to cure cancer with greater potency and tissue-specificity. Several plants and their parts and products have been successfully used for efficient and rapid green synthesis of AgNPs in non-hazardous

ways. The medicinal plant selected for the present study, *T. roseo-alba*, in spite of its known uses in traditional medicines, no documented evidence was found on the phytochemical characterization, anticancer effects and their possible molecular mechanisms of action. Therefore the present study aims to characterize the bioactive components and also to examine the apoptotic inducing ability and anti-proliferative effect of green synthesized silver nanoparticles using *T. roseo-alba* on lung cancer.

The study was executed in four phases. In phase I, preliminary phytochemical screening was done and bio active phyto constituents present in *T. roseo-alba* were characterized using spectral analysis. An attempt was also made to evaluate the free radical scavenging potential of different solvent extracts of *T. roseo-alba* leaves. The second phase was intended to produce the silver nanoparticles from the ethanolic extract of *T. roseo-alba* and they were characterized. In the third phase, the ability of ethanolic extract of *T. roseo-alba* and its AgNPs were assessed for their antiproliferative and apoptotic effect. In the final phase, *in silico* molecular docking analysis was done to study the interactions between the identified phytoconstituents present in *T. roseo-alba* and selected apoptotic, cancer targets.

The salient findings of the study are summarized as follows: In phase 1, the bioactive components present in *T. roseo-alba* leaves rendering the antioxidant and anti-proliferative activity were identified. Different solvent extracts of varying polarity namely chloroform, petroleum ether, ethanol and water extracts were prepared from the leaves of *T. roseo-alba* and they were screened for the presence of phytoconstituents. Among the various extracts screened, the ethanolic extract was found to be the best source of various phytochemicals (alkaloids, carbohydrates, flavonoids, phenols, tannins and terpenoids). The spectral bands observed in the UV absorption spectrum of the ethanolic extract of *T. roseo-alba* revealed the presence of multiple bioactive components. The results of FTIR spectra revealed the presence of various functional groups such as -OH and -NH which may be owing to the presence of phytoconstituents such as phenols and flavonoids. From HPTLC fingerprinting analysis using different solvent systems, the presence of the possible active constituents like Quercetin, Rutin, Nicotinic acid and Colchicine were recorded. Various major and minor peaks observed in Gas Chromatography/Mass spectroscopy (GC-MS) spectra showed the presence of active metabolites like phytosterols, fatty acids and terpenoids.

Further, *in vitro* radical scavenging ability of various solvents extracts of *T. roseo-alba* leaves was assessed. Though all extracts were capable of scavenging all tested radicals in a dose dependent manner, they were competently scavenged to a greater extent by the ethanolic extract of *T. roseo-alba*. From the study, an effective dose dependent radical scavenging potential was evidenced in the ethanolic extract and it was comparable with the standard ascorbic acid. The antioxidant potential of the extract might be positively correlated with the presence of phenols and flavonoids. This remarkable radical scavenging potential might be the reason for amelioration of the damages caused by various reactive oxygen species.

In phase II, using ethanolic extract of *T. roseo-alba* silver nanoparticles were produced under controlled conditions. The colour change from pale yellow to dark brown, which may be due to the excitation of surface plasmon resonance, proved the formation of silver nanoparticles. It was further confirmed based on the peaks observed in UV-Vis spectra of the green synthesized silver nanoparticles, exhibiting the plasmon resonance between 300-350nm.

FTIR analysis was carried out to figure out the various functional groups present in the *T. roseo-alba* extract that are involved in the bio reduction of silver ions to AgNPs and their subsequent coating and stabilization. The various band intensities at different regions of IR spectra for the *Tabebuia roseo- alba* extract and its silver nanoparticles were investigated. The absence of several fundamental peaks in the IR spectrum of silver nanoparticles validated the involvement of the functional groups of the phytoconstituents in the plant extract in the formation and subsequent capping of silver nanoparticles.

The crystallinity and purity of green synthesised AgNPs were evaluated by powder X-ray diffraction. The peaks observed in the diffractogram are corresponding with face-centred cubic phase of metallic Ag**.** The diffraction peaks observed at the 2θ positions of 38.15^0, 44.34^0, 64.50^0 and 77.48^0 confirmed the bragg reflections corresponds to (111), (200), (220) and (311) planes and revealed the face centred cubic phase formation of the silver nanoparticles [JCPDS Card No; 03-065-2871]. The average crystallite size was calculated with respect to the major intensity peak corresponding to the (111) plane and it was estimated to be around 27 nm which falls in the acceptable feature of nanoparticles.

Further, elemental composition of the silver nanoparticles was analysed using energy-dispersion X-ray (EDX) and the result showed the presence of C, Ag, O, and S elements. In addition, the strong signal at 3 KeV corresponding to the metallic silver nanocrystals, was recorded from EDX analysis and the weight percentage of silver was recorded as 48.34. The presence of other weak signals characteristics for oxygen and carbon may be due to the surface passivation of silver nanoparticles by the plant extract.

Moreover, to investigate the size and morphology of the green synthesized AgNPs, the scanning electron microscopy and transmission electron microscopy was performed. The SEM image revealed the presence of relatively spherical shaped nanoparticles and the presence of some larger particles may be because of the accumulation of nanoparticles during sample preparation. The TEM image revealed the narrow size distribution of nanoparticles, in the range of 5-100nm.

In phase III, in order to determine the cytotoxic effect of the ethanolic extract of *T. roseo-alba* and its AgNPs, MTT assay was performed in Lung cancer A549 cell line. The cells were incubated with increasing concentrations of the samples and the viability of the cells was assessed using MTT assay. The experiment was performed using different concentrations of the ethanolic extract of *T. roseo-alba* and its AgNPs ranging from 50, 100, 150, 200, 300, 400 and 500µg. The results revealed that the ethanolic extract of *T. roseo-alba* and its AgNPs were able to induce cell death in a concentration dependent manner as evident from the decrease in the percentage viability. Though both the samples were showing promising cytotoxic activity, AgNPs of *T. roseo-alba* was found to be more potent than the ethanolic extract.

In the present study, the lung cancer cell lines A549 was treated with the ethanolic extract of *T. roseo-alba* and its AgNPs and analysed for their apoptosis inducing ability using (Annexin V/FITC staining) flow cytometer. The results obviously indicated the presence of increased early and late apoptotic cell population upon treatment and thus proving the ability of ethanolic extract and AgNps to induce apoptotic cell death.

Mitochondrial membrane depolarization is considered to be an early event in apoptosis. Thus, the level of mitochondrial membrane potential was measured in A549 cells using a flow cytometer after the treatment with ethanolic extract of *T. roseo-alba* and its AgNPs. The results revealed the ability of ethanolic extract and its AgNPs to activate the intrinsic apoptotic signaling pathway validating their cytotoxic behaviour.

Further, apoptosis was confirmed by DNA fragmentation analysis which showed dose dependent increase in the DNA fragmentation levels in A549 cells upon treatment with the ethanolic extract of *T. roseo-alba* and its AgNPs.

Since Caspase 3 plays a vital role in the execution of programmed cell death, the expression of caspase 3 was examined through western blotting. From the present study, an up-regulation on the active forms of caspases 3 was observed upon treatment with ethanolic extract of *T. roseo-alba* and its silver nanoparticles and thus evidencing the Bax/Bcl pathway mediated apoptosis in Lung cancer A549 cell line.

Deregulation of the cell cycle components has been proven to persuade apoptosis and thus cell cycle checkpoints link cell cycle to Apoptosis. Hence the effect of ethanolic extract of *T. roseo-alba* and its silver nanoparticles on the cell cycle distribution of A549 cells were studied using a flow cytometer. The cells treated with ethanolic extract at their IC_{50} concentration were arrested at G2/M transition phase and an increase in the Sub G1 population was found. Further treatment with silver nanoparticles arrested the cell cycle in G0-G1 check point and the cells were quiescent.

These findings shed light on the anticancer ability of the ethanolic extract of *T. roseo-alba* and its AgNPs to be used as the potential source for the development of cancer therapeutic drugs with a targeted efficiency.

In phase IV, *in silico* molecular analysis of four different compounds, identified from *T. roseo-alba,* namely Gallic acid, Nicotinic acid, Colchicine and Quercetin have been taken for computing against apoptotic and lung cancer targets. Colchicine was predicted to be the most potent inhibitor amongst all the known selected natural compounds against Bcl 2 family members with the highest binding energy values and quercetin was effective in docking against the lung cancer targets, mutant *K-ras* and EGFR, when compared to other compounds. The results of the present study showed that the phytoconstituents of *T. roseo-alba* were able to interact with members of Bcl2 and lung cancer targets which suggested these compounds induced apoptosis via intrinsic apoptotic pathway and also these might be able to influence the cell signaling pathway associated with proliferation of cell by targeting *K-ras* and EGFR.

To conclude, the results of the present study clearly establishes the strong evidence for the antioxidative, apoptotic efficacy and anti-proliferative effect of *T. roseo-alba*. Our results also revealed that AgNPs of *T. roseo-alba* are more effective than

ethanolic crude extract. So, leaf extract mediated silver nanoparticles synthesised from *T. roseo-alba* could be considered as the promising anticancer agent and it might provide a lead for the designing and the development of novel drugs for lung cancer treatment.

Suggestions for the further research

The outcome of the present study has opened a number of avenues for future research. Some of them, which can be pursued for future active research, are given below.

- Future studies are required to be carried out to assess and ascertain the efficient anticancer properties in other lung cancer cell lines and cell strains.
- *In vivo* studies can be carried out to validate the anti-cancer efficiency of the drug using animal models.
- Clinical trials can be carried out using human volunteers for their pharmacological validation.
- Effect of the drug can be studied at molecular level to establish its impact on apoptotic and oncogenic targets of other types of cancer including liver, kidney, breast and ovary.