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## **Chapter-6**

### **Summary and Conclusion**

Cancer research is progressing toward the identification of anticancer agents from natural sources that may be employed as medicines or as supplements to existing treatment modalities. This is promoted since, as compared to standard chemotherapeutic drugs, they have less adverse effects. The fungus selected for present study is *S. stipitatum*, a very rare termite fungus that can be found only from old undisturbed termite nests. It has great significance among tribal people because of its medicinal properties. They used to preserve this fungus to treat various diseases like jaundice, cholera, stomach pain, arthritis etc.. But due to its rare occurrence and difficulty in locating them only limited studies have done in this species. The specialty of this fungus includes its exclusive habitat, total lack of spores and its inability to survive in an open environment. It becomes dead within a day when it encounters the open environment. Presently, it is included in the genus *Xylaria* but our molecular studies on fresh specimen reveals that it cannot be treated as any *Xylaria* sp. *S. stipitatum* has a unique separate species entity.

Secondary metabolite in plants and fungi are now being considered with high significance because of its medicinal properties. Preliminary phytochemical screening on *S. stipitatum* shows the presence of most number of compounds in its ethanol extract. They are alkaloids, flavonoids, phenols, saponin and aleurone grains. And this extract was chosen for the further studies. The GC-MS analysis conducted for volatile compounds and LC-MS analysis conducted for the non- volatile compounds reveals the presence of many bio- active compounds that are pharmaceutically very important.

To investigate the anticancer potential of the fungus, initially in- vitro cytotoxicity was done by trypan blue dye exclusion method in DLA and EAC cells. Percentage cytotoxicity was better in DLA cells. Then moved on to the animal

experiments. It was all conducted in Amala Cancer Research Center, Thrissur. Acute toxicity study was conducted to determine the toxic effects of the drug and the drug showed no sorts of toxicity. So then in-vivo antitumor study was conducted. Two models were chosen for the study. DLA induced solid tumor model and EAC induced ascites tumor model. In the former one, the solid tumor volume was measured and the percentage inhibition of tumor was calculated in comparison with the control untreated group. And the drug seems to be effective and shown 70.35% inhibition of tumor and standard group shown 76.74% inhibition. So, the activity of drug was almost comparable with the standard. In case of EAC induced ascites model the percentage increase in the life span was calculated. And the ethanol extract at higher dose shows 55% increase in percentage life span while the standard cyclophosphamide treated group showed only 44.4% percentage increase in life span. It reveals that the ethanol extract of *S. stipitatum* is very efficient as an anti-tumor agent.

Majority of human cancers are currently considered to be caused by environmental factors. Exogenous and endogenous sources of free radicals responsible for the genetic instability; have been correlated with augmented malignant potential. Hence antioxidants have significant importance in cancer chemoprevention. The in vivo antioxidant study was conducted to find the antioxidant potential of the extract. The assays were conducted in the blood and liver tissues of sodium fluoride stress induced mice of which some groups are pretreated with standard and ethanol extract. In the ethanol extract treated groups the antioxidant enzymes show elevated values than that of the untreated group. The study proves that the extract provides the ability to withstand stress by enhancing the level of antioxidants in order to scavenge the free radicals. The decreased level of lipid

peroxidation in treated group further proves the ability of the extract to overcome stress.

Even though inflammation is a vital defense mechanism to cope up with injuries and infection, may become severe and contribute to many chronic diseases even cancer. The in vivo anti-inflammatory activity of the extract was studied by the acute carrageenan induced inflammation and chronic formalin induced inflammation. Here the percentage inhibition of inflammation is calculated after inducing the inflammation. Two groups were pretreated with higher and lower dose of ethanol extract and another group was pretreated with standard drug diclofenac. One group used as control was left untreated. And the inflammation was measured and percentage inhibition was calculated. The extracts significantly reduced the paw edema in both models in comparison with control untreated group.

Mycosynthesis of nanoparticles are now on the interest list of researchers since it is an economical and less harmful way of nanoparticle synthesis in comparison with physical and chemical synthesis. It is widely used in pharmaceutical industry for various purposes including drug preparations are for targeted delivery of drug to specific locations. So, silver nanoparticles were synthesized using the aqueous extract of *S. stipitatum* and its characterization was done to confirm the presence of silver nanoparticles.

Using the macro fungus *S. stipitatum*, nanoparticles with sizes ranging from 12-28 nm were produced extracellularly, as verified by SEM and TEM. In UV spectra, these nanoparticles had a distinct absorption peak at 440 nm. XRD verified the crystalline structure of silver nanoparticles.

To conclude, the current study elucidates the molecular characterization of *S. stipitatum*, anticancer potential of the ethanol extract of the drug, and the ability for

synthesis of nanoparticles. The chemical screening reveals many bioactive compounds with excellent medicinal properties. So, more studies are required to disclose the entire medicinal uses to isolate the compounds and to prepare new drug formulations. Furthermore, it explains the necessity of conserving this rare fungus.