Abstract

The green part of the universe is the backbone for the existence of all life. Utilization of plants for survival is practiced since ages. Evidences of use of plants for therapeutic purposes and well-being of mankind are there in ancient history and literature. India, being a country with mega diversity, has rich indigenous knowledge of natural world and their uses. The present study aims to explore these age-old knowledge and natural resources to fight against diseases like leishmaniasis and related challenges of fungal infections of modern era.

At the onset of this study, a survey was conducted among the rural and tribal communities belonging to Jalpaiguri district and parts of Alipurduar district of West Bengal. The plants used by them for therapeutic purposes against leishmaniasis (Kala-azar) and fungal infections were enlisted, from which four plants, *Rauvolfia serpentina*, *Moringa oleifera*, *Nyctanthes arbor-tristis* and *Clausena excavata* were chosen for further study. A thorough literature survey was conducted on the shortlisted plants to know their reported bioactive compounds.

Phytochemical analysis of leaf extracts of plants showed presence of secondary metabolites like alkaloid, flavonoid, terpenoid, tannin and phenolics. Quantitative estimation of phenolic compounds showed that all the four tested plants contained good amount of phenolics with *N. arbor-tristis* and *C. excavata* recording the highest and lowest content respectively. Flavonoid content was found to be highest in *M. oleifera* extract. Plant leaf extracts were further studied for antioxidant activity by DPPH free radical scavenging assay. While all the tested plants showed positive results, *C. excavata* extract was found to be most potential antioxidant.

Antifungal activities of the crude leaf extracts were tested by agar cup assay against *Candida albicans*. In addition, the antifungal compound excavarin-A was also tested by similar method. This compound was purified from the dichloromethane extract of *C. excavata* leaves by bioassay guided fractionation following silica gel column chromatography. The identity of the compound was confirmed through UV–VIS, IR and NMR (1H- and 13C-) spectroscopic analysis. All the plants showed positive results and the MIC value was lowest for excavarin-A (0.078mg/ml) followed by *R. serpentina* (0.156mg/ml).

Anti-leishmanial activity of the selected botanicals was studied against the protozoa *Leishmania donovani*. For *in vitro* screening, the pathogen, cultured in 96-well plate, were treated with graded doses of test substances (crude leaf extracts of *R. serpentina, C. excavate* and the molecule excavarin-A). The percentage of growth inhibition of parasitic promastigotes were calculated to estimate anti-leishmanial activity. Results showed that all the test substances had antileishmanial activities *in vitro*. The IC_{50} values for excavarin-A and the crude leaf extracts of *R. serpentina* and *C. excavata* was recorded as 1.24 mg/ml, 4.04 mg/ml and 32.1 mg/ml respectively. The purified compound was found to be much more effective than the crude extracts. The selected botanicals were also tested on amastigotes cultured in hamster macrophage in laboratory condition. Excavarin-A was found to be most efficacious followed by crude extracts of *R. serpentina* and *C. excavata.* However, both *C. excavata* leaf extract and excavarin-A purified from its leaves showed cytotoxicity in test for viability of hamster macrophages in the range of effective therapeutic doses. On the other hand, *R. serpentina* leaf extract was not found cytotoxic in its effective range of concentrations.

Botanicals showing good antipathogenic potentiality *in vitro* were also tested *in vivo,* excluding those that were found to be cytotoxic

in the therapeutic range of doses. In a preliminary *in vivo* experiment, infections were created on the skin of experimental animals (male albino rats) using cell suspension of *C. albicans.* The experimental candidiasis models were treated with crude extracts of *R. serpentina* and *M. oleifera.* Both the tested leaf extracts significantly decreased and cured the infection upon superficial application. *R. serpentina* surfaced as a potentially better antifungal agent. The elevation in total count of WBC which was found by haematological analysis of blood samples of treated animals, suggested that the leaf extracts of both the plants have protective roles in improving host defence to counter fungal attack.

Crude leaf extract of *R. serpentina* was also tested for its antileishmanial activity *in vivo*. The test animals, Syrian golden hamsters, were first infected with *L. donovani* parasite, and after development of infection, the graded doses of leaf extract were administered intramuscularly. Parasitic burden of spleen and liver of infected hamsters were reduced after treatment in a dose-dependent manner. *R. serpentina* extract was also found to be hepatoprotective as evident from the plasma levels of SGOT and SGPT enzymes.

Studies on mechanisms involved in antileishmanial activity showed that the action of the *R. serpentina* extract is mediated through inhibition of leishmanial superoxide dismutase (SOD) evident by measuring the inhibition of pyrogallol autoxidation rate. Further an enhanced release of toxic superoxide radical which was measured spectrophotometrically through the formation of blue formazan was also observed. Polyacrylamide gel electrophoresis of *Leishmania* promastigote lysate confirmed these results. On gel activity staining through non-denaturing PAGE as well as SDS-PAGE showed degeneration of SOD bands with increasing concentration of *R. serpentina* extract. The results obtained from these experiments suggest that inhibition of SOD and simultaneous release of

superoxide radicals impose toxic effects to destroy intracellular parasites during experimental visceral leishmaniasis. The findings of this study may be significant in the field of development of new therapeutic agents from natural resources against fungal infections and leishmaniasis.