ABSTRACT

Medicinal plants are the most important source for the pharmaceutical industries. Thus the discovery of new drugs from the plants sources is being researched till date. Based on this, the study has been concentrated on four plants for their pharmacological activity. Among the four plants leaves tested for the phytochemical constituents namely *A. alnifolia, B. maderaspatensis, I. staphylina* and *D. volubulis, B. maderaspatensis* showed the maximum amount of phytochemicals. Five different solvent fractions namely chloroform, petroleum ether, acetone, methanol and ethyl acetate were tested for total phytochemical content using spectrophotometry method. Based on the polarity of the solvent checked, Methanol fraction showed the maximum amount of phytochemical constituents in the *Blepharis maderaspatensis*.

In methanol fraction of leaf of *B. maderaspetensis*, higher content of flavanoid were observed followed by *A. alnifolia, D. volubulis and I. staphylina* leaf extracts. Among the medicinal plants tested, *B. maderaspatensis* showed the maximum flavanoid content of 30.9mg/g of dry weight of leaf powder and total phenol content of 186.4mg/g of dry weight of leaf powder in methanol fraction. The total saponin content was 21.3mg/g of dry weight in methanol fraction. The total carotenoid content was 22.6mg/g of dry weight of leaf powder. The total tannin content was 30.2mg/g in methanol fraction. The thin layer chromatography fingerprint showed distinct bands for all the tested medicinal plants. Bioautography of experimental plants were conducted and among the four experimental plants, *B. maderaspetensis* showed good antioxidant content than the other plant leaf extracts. Based on these data, methanolic leaf extract of *B. maderaspetensis* selected for
further studies. The anti-inflammatory activities both in vitro and in vivo models were studied and reported on many medicinal plant in India as well as worldwide.

An extensive variety of In vitro methods are available for proving antioxidant activity and among thus ABTS, DPPH, Nitric oxide free radicals are useful to ascertain antioxidant ability of certain substances from natural and synthetic sources. Free radicals of ABTS, DPPH nitric oxide are highly stable until exposed in air or any oxidative substances. These free radicals are green and purple colored and substances activity is determined based on its ability to decolorize at its own wave length to light yellow to forming a stable molecule.

In the antioxidant activity, B. maderaspatensis showed the maximum ABTS scavenging activity in methanol of concentration of 750μg/ml. In DPPH scavenging activity, it showed a maximum of 91.75% inhibition with methanol and in NO scavenging activity, B. maderaspatensis showed a maximum of 87.45% inhibition with methanol. In vitro cytotoxicity assay on Vero cell line, 50μg/ml showed the least growth. The concentrated fractions were taken for the bioassay with in vitro cytotoxicity on normal Vero cell line using six different concentrations at 12, 24, 48 and 72hrs incubation. Till 48hrs, 12.5, 6.2, 3.1 and 1.5 μg of isolated compound does not show any cytotoxicity activity. The results clearly indicated absence of toxicity between 25 to 45 μg/ml of protocatachuic acid isolated from B. medaraspantensis. In ethyl acetate: methanol combination was used as a mobile phase to separate the compounds on TLC. The separated TLC was used for bioautography study, the better antioxidant activity was observed. Further, these compounds were concentrated under reduced pressure.

Hence, the methanolic fraction of B. madaraspetensis was taken for purification of active principle. In silica gel column chromatography, the solvents
chloroform, petroleum ether, acetone, ethyl acetate and methanol were used for fractionation using various proportions in a step wise manner. Totally 170 fractions were collected and pooled based on the band on TLC with respective solvent as mobile phase. The fractions 130 - 148 showed similar spot on TLC of bioautography under UV light. These fractions were tested for antioxidant activity. The isolated antioxidant compound showed two bands (270nm, 300nm) in UV-Vis spectrum. The purity of the fraction was analyzed by HPLC and it showed a single peak at the retention time of 29.3min. The structural elucidation of purified compound was analyzed by UV-VIS spectrophotometry ($\lambda$270, 300 nm), FTIR, LCMS, $^1$HNMR and $^{13}$CNMR and GC-MS. With the spectral and mass value of the compound was identified and as protocatechuic acid. The isolated compound from *B. madaraspetensis* was identified as protocatechuic acid by comparing their physical and spectral data with those reported in the previous literature while by comparing with authentic sample. Phytochemicals are reported to control various points in inflammation. Over the past few decades many modern therapeutic drugs were developed or obtained from natural products.

Many medicinal plants were explored for its phytochemical contents and as well as its anti-inflammatory activity using different *in vitro* and *in vivo* model and discussed by many reviewers. Some of the phytochemicals against herbivorous insects also end up being harmful to humans nucleic acid, carbohydrate and lipid metabolism, neurochemicals, neuropeptides, hormones and neurotransmitters; agonistic activity on neurotransmitter systems, cholinesterase. In this study, cytotoxicity was determined using various concentrations at different time interval. The purified fraction was further analyzed for cytokines expression in three different concentrations 10, 20 and 30$\mu$g/ml. In the cytotoxicity assay, 20$\mu$g of PCA was found to show the cytokinin expression level of LPS-induced iNOS, COX2, TNF-$\alpha$,
IL1 and IL-6 in the cell lines. The increasing concentrations of purified fractions treated cells were used for RNA isolation and cDNA construction for RT-PCR analysis, which gave better reduction of cytokines expression while compared with LPS induced cytokines expression. The western blot study also supported well the RT-PCR results and the protein levels of cytokines are reduced in treated cells. In this technique, 20\mu g of protocatachuic acid isolated from *B. maderaspatensis* treated showed effective inhibition.

Further, the protocatachuic acid was analyzed for anticancer activity using cancer cell lines **MCF-7 (Human breast adenocarcinoma cells)**, **HCT-116 (Human colon cancer cells)** and **SNU-182 (Hepatocellular carcinoma cells)**. The increasing concentrations of protocatachuic acid and increasing incubation period shows good inhibitory activity using MTT assay. Among cancer cell line, SNU-182 shows susceptible than the other two cell line. The IC\textsubscript{50} concentrations were also calculated in this study. The 72hrs treated cells were stained with AOED for observing apoptosis activity and all the cancer cell line shows better apoptotic cells under microscope. DNA fragmentation study showed the SNU-182 cell line form the ladder in gel electrophoresis than the other two cancer cells; In Flow cytometry, higher apoptotic cells was observed in SNU-182 and also reduced expression of MMP 2&9 in SDS-PAGE - zymogram.

From the above presented results, *B. maderaspetensis* leaf has potential activity of anti-inflammatory and anticancer activity.