“Isolation, Separation, Identification and Characterization of Active Ingredients from Anticancer Medicinal Plants”

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ABSTRACT

The present investigation on “Isolation, Separation, Identification and Characterization of Active Ingredients from Anticancer Medicinal Plants” was carried out at FTL and Department of Biotechnology, Junagadh Agricultural University, Junagadh. The four medicinal plants i.e., Euphorbia tirucalli- stem (ET), Cadaba fruticosa-Leaf (CF), panicum maximus-Roots (PA) and Annona muricata- fruit (AMF) and Leaf (AML) were collected from different regions of Gujarat. The plants were authenticated by Dr. S. G. Nagar, Assistant professor, Department of Botany, M.S University, Baroda.

This study was performed to investigate the antimicrobial, antioxidant as well as anticancer properties from selected medicinal plants on four cancer cell lines (HeLa 229, KB 3-1, ACHN and A549 carcinoma cell lines) and Isolation, identification and characterization of various bioactive compounds responsible for its biological activity was done. This investigation was achieved by extraction of medicinal plants in different solvents (Hexane, Chloroform, Methanol, water and 80% Methanol), preliminary phytochemical and physicochemical screening, antimicrobial activity, antioxidant activity, anticancer activity, separation and identification of phytochemicals, proteomics analysis of cancer cell line treated with bioactive extracts.

Results of physicochemical analyses of all four medicinal plants with one plant having two distinct economic parts revealed from the present study can be used as a
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diagnostic tool for the standardization and identification of plant. Elemental analysis would be beneficial for determining effectiveness of all medicinal plants in treating various diseases which occur due to mineral deficiency. In all medicinal plants, highest total phenol content was found in *Euphorbia tirucalli*- stem (49.55 mg/g) and *Annona muricata*- fruit (58.94 mg/g), while total alkaloid was higher in *Cadaba fruticosa*-Leaf (54.46 mg/g), *panicum antidotale*-Roots (42.62 mg/g) and *Annona muricata*-Leaf (35.79 mg/g). In four medicinal plants with one plant having two distinct economic parts plants, % extraction yield was found higher in 80% methanol. Phytochemical screening revealed that most of the secondary metabolites (i.e. alkaloids, phenol, flavonoids, saponins, steroids, terpenoids etc.) were harvested in polar solvents like methanol, water and 80% methanol.

Antimicrobial activity was performed by agar well diffusion against five bacteria and five fungi. Out of 25 extracts 18 extracts inhibited bacterial growth of *Bacillus subtilis*, in which the highest inhibition was recorded by 80% Methanol extract of ET plant. *Pseudomonas aeruginosa* was inhibited by Hexane, chloroform, Methanol, water extract of ET, CF and PA. AML water extract showed the maximum antifungal activity against *Macrophomina Phaseolina*. Growth of *Sclerotium Rolfsii* was highly inhibited by water extract of PA. *In vitro* antioxidant activity of the extracts was carried out by DPPH free radical scavenging activity. Lowest IC$_{50}$ was observed in PA 80% MeOH extract (11.07µg/ml) followed by PA MeOH extract (16.04µg/ml), AMF 80% MeOH extract (17.48µg/ml).

All twenty five plant extracts were investigated for their anticancer activities by trypan blue cell exclusion assay, MTT reducing cytotoxic assay and Fluorescent staining by AO/PI method. The results exhibited dose- and time-dependent killing capabilities in various human cancer cell lines. The killing activity was specific toward cancer cells, as it changed cell line to cell line. Cell death caused by the plant extracts is via apoptosis. 80% MeOH extract of PA showed particularly strong anticancer capabilities. ET Hexane and chloroform, CF water extract, AML water extract and AMF MeOH extract were scrutinized as a bioactive extracts out of 25 extracts. The results suggested that those selected bioactive extracts of plants showed promising anticancer activity.

All selected bioactive extracts were separated by Sepbox 2D-2000 except ET hexane and Chloroform extracts, because non polar extracts could not separate in sepbox. So both the ET extracts were having good anticancer activity directly identified in GC-MS. After separation through Sepbox, high intensity fractions were selected from sepbox chromatogram and again tested by MTT reduction cytotoxic assay. The fractions with the
greatest MTT activity were identified and confirmed by LC-Q-TOF-MS. In ET hexane extract 10 and in chloroform extract 12 anticancer compounds were identified. In CF water extract 22 anticancer compounds were identified from bioactive fractions. From bioactive fractions, 23 anticancer compounds were identified in PA 80% MeOH extract. In AML water extract 13 anticancer compounds were identified from bioactive fractions. In bioactive fractions, 23 anticancer compounds were identified in PA 80% MeOH extract. In AML water extract 13 anticancer compounds were identified from bioactive fractions. In AMF MeOH extract 27 anticancer compounds were identified from bioactive fractions. In proteomics study, KB 3-1 cell line was treated with four bioactive extracts and compared with protein spots with Control. This study was assessed for the correlation between anticancer activity and change in abundance of proteins between control and bioactive extracts treated cell lines. Change in protein expression revealed the effect of bioactive extracts. Protein expression were up and down regulated, while some of them totally suppressed or some were newly expressed compared to control. The results provide reasonable evidence that the selected bioactive extracts contains compounds with cytotoxic activity or anti cell proliferative activity.

This study provides some evidence that selected bioactive extracts have great potent anticancer properties. Those bioactive extracts inhibit four different carcinoma cell lines. Inhibition could be achieved by deregulation of metabolic processes or act as cytotoxic compound which induce apoptosis and finally result in cell death.