NMR Metabolomic and LIBS elemental profiling of anticancer herbal formulation
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Introduction
Profiling of metabolites is a rapidly expanding area of research for resolving metabolic pathways. Metabolic fingerprinting in medicinally important plant based formulations is critical to establishing the quality of herbal medicines. Metabolic and elemental profiling of selected herbal formulations in Indian system of medicine was carried out using NMR spectroscopy and laser induced breakdown spectroscopy (LIBS) techniques. The anticancer activity and antioxidant potential of formulations were evaluated on Hep G2 cancer cell line of four herbal formulations using MTT assay and FRAP assay respectively [1,2].

Materials and Methods
Four polyherbal formulations KG, VK, GTK and MK were selected for the anticancer and antioxidant study. While the first was in tablet form and other three were aqueous solution. Each formulation was made up of several ingredients in a definite composition. These ingredients were medicinal plants such as Comiphora mukul, Emblica officinalis, Terminalia chebula, Terminalia bellirica, Piper nigrum, Piper longum, Raphanus sativus, Terminalia chebula etc.

NMR Spectroscopy
Spectra were obtained using 700 MHz NMR (Varian) spectrometer. One dimensional study using 1H & 13C nuclei and 2D (COSY, TOCSY and HMBC) was acquired with no. of scans 64, data point 32768k and relaxation delay 2s. Carbon spectra were acquired with relaxation delay 2s at 5000 scans. zTOCSY and HMBC was acquired with no. of scans 64, data point 32768k and relaxation delay 2s.

Cytotoxic studies
These studies were carried out using Hep G2 (liver) cancer cell line using MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay. The inhibitory concentration (IC50) values were calculated taking the viability of untreated cells (which was 100%) as reference. Anticancer potential of these formulations were being assessed by flow cytometry [4].

Antioxidant studies
The antioxidant studies were carried out using FRAP (Ferric Reducing Antioxidant Power) assay. The ferric reducing antioxidant capacity was maximum for VK (17.0 µM Fe++ g-1) followed by KG (13.99 µM Fe++ g-1) and minimum for MK (3.17 µM Fe++ g-1).

Results
The maximum cytotoxicity was observed for KG (22.48 µg/ml) followed by MK (27.58 mg/ml). So these two formulations were selected for characterization through NMR spectroscopy. Figures 1 shows the 1D spectrum of KG and MK. The peaks were assigned using the 2D spectra (figures 2). It can be seen that these formulations contain aromatic compounds, sugars, amino acids and some other metabolites such as choline, glycerol, inositol, β Hydroxy Butyrate, Indoxl Sulphate and p-Hydroxy Benzoic acid. The LIBS spectra shows the such as Mg, Ca, H, O, C and N in both formulations. These results obtained with LIBS show that formulations which have higher concentration of Mg and Ca also have more antioxidant properties.

Discussion
The current study supports that the KG and MK formulations inhibit the cell proliferation significantly and have good antioxidant activity among all formulation. NMR metabolomic study revealed chemical identification of metabolites to explore their pharmacological action. The results obtained with LIBS showed that formulations which have higher concentration of Mg and Ca also have more antioxidant properties.

References