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 break down 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, Embilica officinalis, Terminalia chebula, Terminalia bellerica, Piper nigrum, Piper longum, Raphanus sativus, Terminalia chebula etc.*

NMR Spectroscopy

Spectra were obtained using 700 MHz NMR (Varian) spectrometer. One dimensional study using ^{1}H & ^{13}C nuclei and 2D (COSY, TOCSY and HMBC) were carried out. The following were the acquisition parameters: Proton spectra were 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 at relaxation delay 2s, data point 1024 and no. of scan 8. Formulations with higher cytotoxicity were selected for NMR study. Five gram of KG formulation was extracted in 10 ml of distilled water and 5 ml of the GTK formulation was diluted to make concentration of 10 mg/ml. The extracts were then centrifuged at 10,000 rpm for 10 minutes at 20ºC to settle the non-polar, water insoluble compounds followed by filtered through Whatman's paper no. 1. 560 µl of sample was taken in 5mm NMR tube with 1mM TSP in 30 µl D_2O solution in coaxial NMR insert. NMRshiftDB and SBDS (Integrated Spectral Data Base System for Organic Compounds, Japan) data library were used for assignments of the peaks [3].

LIB Spectroscopy

The 4-channel spectrometer was used (Ocean optics LIBS 2000+). The wavelength range was studied from 200-1100 nm. The laser energy employed was 175-mJ and repetitive rate was 2 Hz use to acquire the spectra (Figure 3). Intensity ratio was calculated with respect to C and O.

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 (IC_{50}) 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).

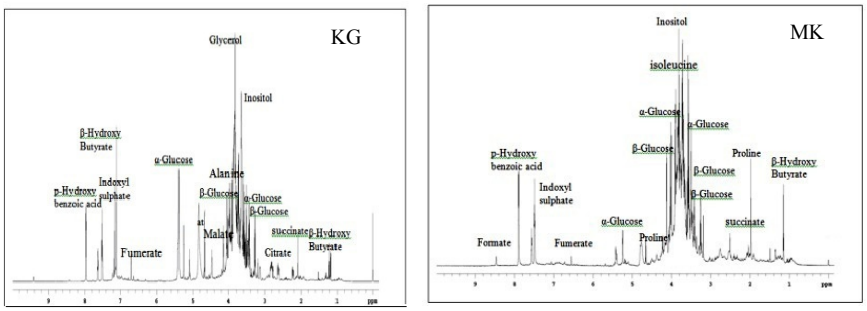

Figure 1: Proton NMR spectra of KG and MK formulations

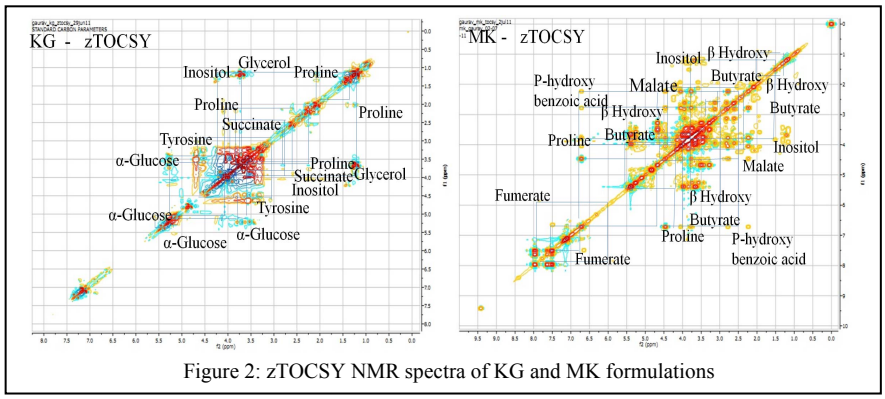

Figure 2: zTOCSY NMR spectra of KG and MK formulations

Results

The maximum cytotxicity 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. [5]. The LIBS spectra shows the such as Mg, Ca, H, O, C and N in both formulations.

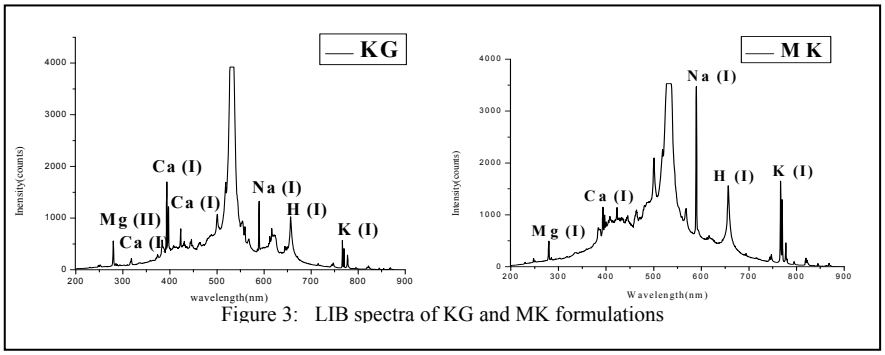

Figure 3: LIB spectra of KG and MK formulations

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 metabonomic 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

[1] Sandhya T et al. *Cancer Lett* 2006; 18,238:304-13.

[2] Lee KW et al. *Nat Rev Cancer* 2011; 11:211-8.

[3] Kim, HK et al. *Nat Protoc* 2011; 29:267-75.

[4] Kim, HJ et al. *J Ethnopharm* 2011; 14,136:197-203.

[5] Chatterjee S et al. *Phytochem* 2010; 71:1085-94.