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LEMON GRASS- A HERBAL THERAPEUTIC AGENT

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ABSTRACT

The aim of the present work is to evaluate the anti-tumour potential of Lemon grass through preliminary cytotoxic analysis. Cytotoxic potential of Lemon grass fresh leaves extract was evaluated on chicken liver cell line through cell viability assay. Antioxidant study was also carried out to proof it as a safe source of herbal drug. TLC fingerprinting data reveals that the positive results of Lemon grass for cytotoxic and antioxidant potential may be due to presence of separated phytochemicals.

KEY WORDS: Lemon grass, cytotoxicity, antioxidant, TLC.

INTRODUCTION

Cancer is one of the most dreaded diseases of the 20th century and spreading further with continuance and increasing incidence in 21st century. Thus, advances in the clinical research for anticancer drugs have been increased over the years (Merina *et al*., 2012). According to World Health Organization, 80% of the people depend on medicinal herbs as primary healthcare system since they are commonly available, safe and comparatively economical. Some herbs protect the body from cancer by enhancing detoxification functions of the body. Compounds derived from medicinal plants are known to inhibit growth of cancer by modulating the activity of specific hormones and enzymes, by reducing the toxic side effects of chemotherapy and radiotherapy. Scientists all over the world are concentrating on the herbal medicines to boost immune cells of the body against cancer and designing herbal formulations to attack the cancerous cells without harming normal cells of the body (Sakarkar and Deshmukh, 2011).

Lemon grass *Cymbopogon citratus* is an aromatic perennial tall grass with rhizomes and densely tufted fibrous root. It has short underground stems with ringed segments, coarse, green slightly leathery leaves in dense clusters (Carlin, *et al*., 1986). The plant is a native herb from India and is cultivated in other tropical and subtropical countries. (Figueirinha *et al* 2008). It is used in different parts of the world in the treatment of digestive disorders, nervous disorders, fevers, menstrual disorder, rheumatism and other joint pains .The infusion or decoction of aerial parts of Lemon Grass has wide spread used in folk medicine. The major phytoconstituents of the plant are essential oils, flavonoids and phenolic compounds. The plant also contains alkaloids, saponins, tannins, anthraquinones and steroids (Nambiar and Matela, 2012). In the present cytotoxic and antioxidant potential of Lemon grass was studied.

MATERIAL AND METHODS

Preparation of plant extract

Plant material was collected from the wild area of Karjat region, Maharashtra. Methanol extract of (30 g) of fresh leaves of Lemon grass was prepared using cold extraction method by continuous shaking in orbital shaker at 100 rpm (25°C). After 24 hours, the extract was filtered and residue of the sample was re-extracted by the same procedure using methanol as the solvent for the period of one week (Umachigi *et al*., 2007). After one week, the collected filtrates were concentrated by evaporating the methanol at 50°C. Dried residue of the sample was re-dissolved in methanol and used for the further analysis.

Cytotoxic study

Freshly harvested liver from chicken was used for preparation of liver cell lines. For this the liver was initially cleaned with sterile saline and the four lobes were separated using sterile scissors aseptically. One of the lobe was transferred to Petri dish containing sterile Hanks Balanced Salt Solution (HBSS) and washed properly with the salt solution. This was chopped into small pieces and again washed with HBSS. The slices were then treated with 0.25% trypsin-EDTA solution and shaken to loosen the cells. The cell suspension was then filtered through cheese cloth to remove debris. The suspension was centrifuged at 5000 rpm for 3 min. The supernatant was discarded and the cells were dispensed in growth medium [DMEM enriched with 10% FBS, Penicillin (100 units/ml), Streptomycin (100 µg/ml) and amphotericin B (2.5 µg/ml)]. The cells were dispensed into T-25 cm^2 tissue culture flasks and incubated at 37°C. The cells were then trypsinised and used for viability assay (Senan *et al*., 2013). Viability is a measure of the metabolic state of a cell population, which is indicative of

the potential of the cells for growth. The prepared liver cell line (2000 cells/tube) were treated with different concentrations of methanol extract of lemon grass (2, 4, 6, 8 and 10 mg/ml) dissolved in DMSO and incubated at 37°C for 24 hrs. Each treatment was carried out in triplicate. DMSO was used as solvent blank. The cells were then trypsinised using 100 µl of 0.25% Trypsin EDTA solution for 1 minute and neutralized using 100 µl of FBS. The viability was determined by Trypan blue dye exclusion method (Talwar, 1994). The percentage of cells that were not stained with Trypan blue (viable cells) is the measure of the viability. The percentage viability of cells over the control was calculated using the formula, Percentage Viability = (Number of viable cells in drug treated /Mean number of viable cells in control) X 100 Percent Cytotoxicity = 100- Percent Viability

Antioxidant study

Total antioxidant activity

Total antioxidant activity of methanol extract of Lemon grass leaves was determined according to the method of Prieto *et al*. An aliquot of leaf extract of 1.0 ml (1.0 mg/ml) was combined with 1.0 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated in a boiling water bath at 95 °C for 90 min. Then, the samples were cooled to room temperature and the absorbance was measured at 695 nm against blank prepared in the same conditions by replacing sample with 1.0 ml of solvent. All the analyses were performed in triplicate and the results were averaged. Antioxidant capacity was expressed as Ascorbic acid equivalents (mmol/mg) (Prieto *et al*., 1999).

Ferric reducing antioxidant power

The reducing power of methanol extract of Lemon grass leaves and Ascorbic acid was determined according to the method of Oyaizu (1986). 2.0, 4.0, 6.0 and 8.0 mg/ml of the extracts and 0.2, 0.4, 0.6, and 0.8 mg/ml of standard (ascorbic acid) was mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1%). The mixture was incubated at 50°C for 20 minutes. 2.5 ml of trichloroacetic acid (10%) was added to the 2.5 ml of the reaction mixture, which was then centrifuged at 3000 g for 10 minutes. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%) and the absorbance was measured at 700 nm against blank prepared in the same conditions by replacing sample with 1.0 ml of solvent. All the analyses

were performed in triplicate and the results were averaged. Increased absorbance of the reaction mixture indicated increasing reducing power.

TLC fingerprinting

Methanol extract of Lemon grass leaves was analysed by TLC for the presence of different components. Samples were prepared according to the method of Wagner and Bladt (1996). Silica gel G_{60} F_{254} TLC precoated plate (Merck) was used as adsorbent. The plate was developed using Toluene: ethylacetate (9:1) as mobile phase. The number of bands present in the samples was detected by spraying the plate with Anisaldehyde sulphuric acid reagent.

RESULT AND DISCUSSION

Cytotoxic study

Figure 1, showed the cytotoxic effects of methanol extract of Lemon grass leaves on liver cells and the results were found to be significant. Cell viability was found to decrease as the concentration of extract increases and cytotoxic effect was found to increase. At 10 mg/ml of extract concentration the effect was found to be better. Cytotoxic effect of plant extract determines its anti-tumour effect Cytotoxicity testing is important for the sole purpose of determining the potential toxicity of the compounds being studied. Cytotoxic agents unselectively kill and damage cancerous cells by interfering with either, the cellular process or mechanical process. Cytotoxicity studies with normal culture systems (tissue culture) of local plant extracts or folk medicinal plant extracts has not been studied extensively and this is vital for the safety evaluation or any herbal preparations. Thus the significant results for cytotoxic effect of Lemon grass, can reveals its further use as a safe herbal anti-cancer agent (Hanisa *et al*., 2014).

Antioxidant Study

Total antioxidant activity

Total antioxidant activity is a quantitative assay, since the antioxidant activity is expressed as the number of equivalents of Ascorbic acid. The assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of a green phosphate/Mo (V) complex at acidic pH with the maximal absorption at 695nm (Prieto *et al*., 1999). The linear equation of ascorbic acid for total antioxidant activity was found to be y=2.676x with r^2= 0.9979 (Fig. 2). The antioxidant activity of leaf extract was found to be 0.13±0.02 mM Ascorbic acid/mg extract of lemon grass leaves.

Ferric reducing antioxidant power

The reducing ability of a compound generally depends on the presence of reductants which have been exhibited antioxidative potential by breaking the free radical chain, donating a hydrogen atom (Saha *et al*., 2008). The presence of reductants (i.e. antioxidants) in plant extract causes the reduction of the Fe3+/ferricyanide complex to the ferrous form. Therefore, the Fe2+ can be monitored by measuring the formation of Perl's Prussian blue colour at 700 nm. Increase in absorbance at 700 nm reflects an increase in reductive ability (Duh *et al*., 1999). As the concentration of leaf extract of Lemon grass and Ascorbic acid increases the ferric reducing antioxidant power increases (Fig. 3). The reducing power of extract of was very potent and it increases as the quantity of sample increases.

TLC Fingerprinting

In TLC finger printing seven bands were separated in the methanol extract of Lemon grass leaves with the Rf values of 0.08, 0.21, 0.38, 0.48, 0.58, 0.71 and 0.84 (Figure 4). TLC fingerprinting helps in the separation of phytoconstituents present in the extract. Positive results for cytotoxicity and antioxidant activities may be due to the presence of these phytoconstituents.

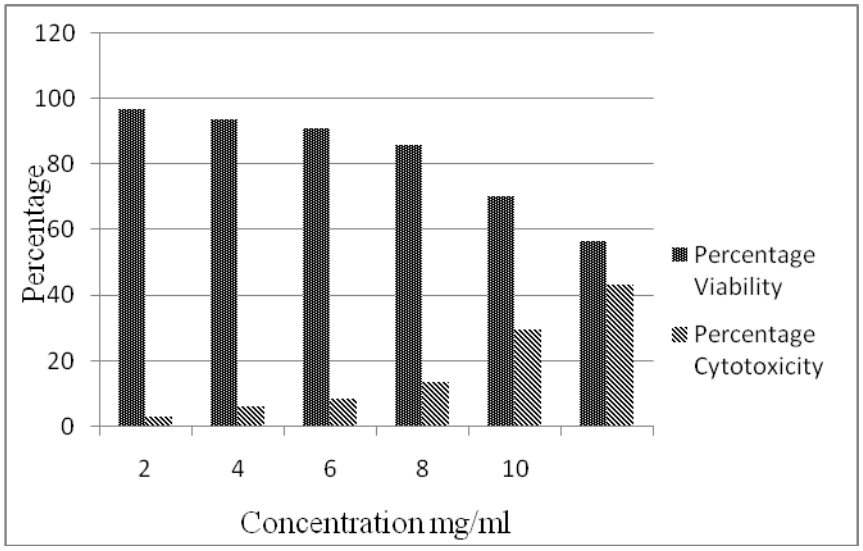

Figure 1: Cytotoxic potential of methanol extract of Lemon grass leaves

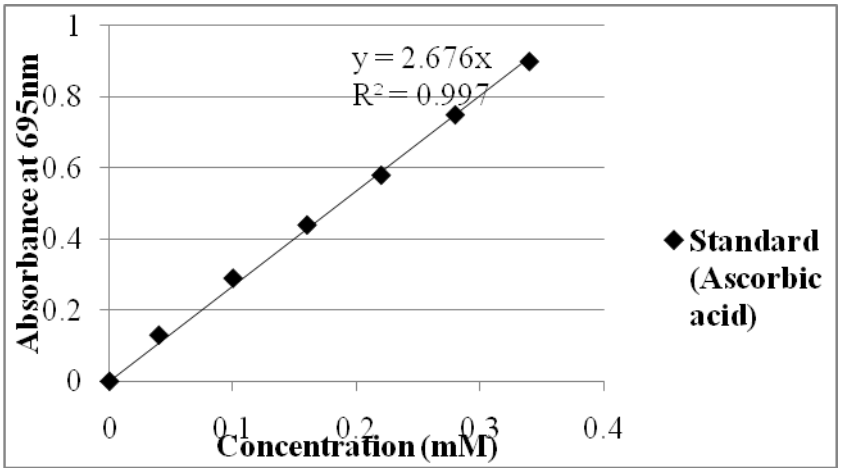

Figure 2: Standard calibration curve of Ascorbic acid for total antioxidant activity analysis of Lemon grass leaves extract

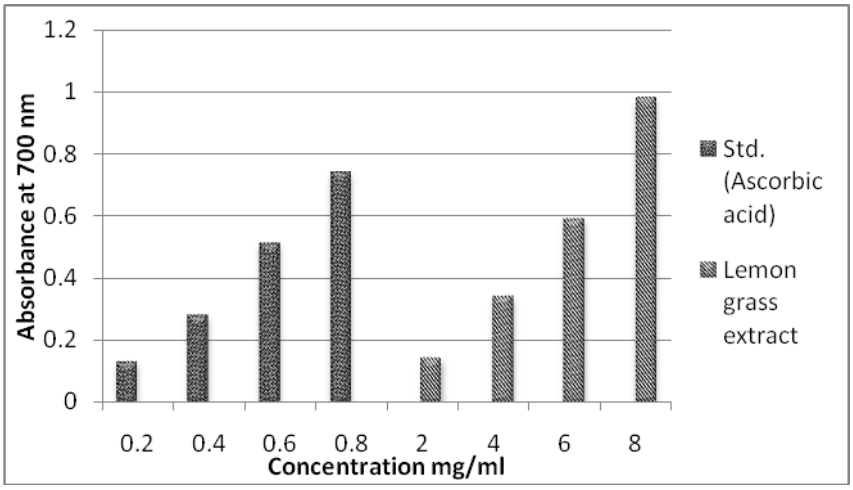

Figure 3: Ferric reducing antioxidant power of methanol extract of Lemon grass leaves and Ascorbic acid

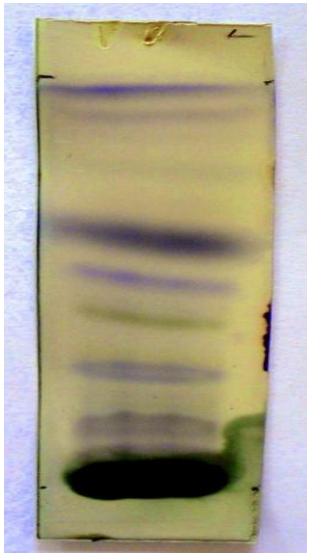

Figure 4: TLC fingerprinting of methanol extract of Lemon grass leaves

CONCLUSION

The methanol extract of Lemon grass leaves showed potent cytotoxic effect on liver cells, along with antioxidant activity, may be due to the presence of different phytoconstituents. Thus, the lemon grass can be introduced as safe source of herbal anti-tumour agent for cancer therapy. Further studies can be done on its cytotoxic effect on cancerous line along isolation of bioactive compound.

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