Research Article

Evaluation of Hemolytic Activity, ATPase Inhibitory Activity and Antitumor Activity of TLC Extract of Lemon Grass (Cymbopogon Citratus)

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ABSTRACT
Oil of lemon grass (Cymbopogon citratus) was extracted and one of its component obtained by TLC were tested for hemolytic activity, ATPase inhibitory activity and Cell viability assessment by MTT assay using MCF-7 cell lines. The results showed that at concentrations of less than 0.08 µg/mL the TLC extract showed no hemolysis. ATPase activity inhibition assay indicated that at concentrations above 0.125ug/mL there was no significant change in inhibitory activity of the ATPase and MTT assay using MCF-7 cell lines showed that there was no cytotoxic effect at concentrations below 0.2ug/mL.

Keywords: antimicrobial, hemolytic, ATPase inhibition, MTT

INTRODUCTION
The practice of traditional medicine using medicinal plants is as old as the origin of man1. Substances found in medicinal plants are known as the active principles. These compounds have been extracted and used in different forms such as infusions, syrups, decoctions, infused oils, essential oils and creams2. Plant-derived natural products such as flavonoids, terpenes, anthraquinones, saponins, tannins, steroids, lactones and volatile oils received considerable attention in recent years due to their diverse pharmacological properties, including cytotoxic and chemo-preventive effects3. Active compounds of plants are used in folk, traditional and alternative medicine to treat diseases like cancer, cardiovascular, Alzheimer's, Parkinson's disease4-7 and have antioxidant; anti-inflammatory, anti-diarrheal, antimicrobial, Anti-parasitic, Antiviral activities, etc.8. Since most plants have medicinal properties, it is of utmost importance that their efficacy and toxicity risks are evaluated1.

Cymbopogon species (Lemon grass) is one such plant and is used as an addition to tea, and in preparations such as kadha, which is a traditional herbal 'soup' used against coughs, colds, etc. This is called Ushir in Shanskrit and Nepali and Khaskhas in Hindi. It has medicinal properties and is used extensively in Ayurvedic medicine. It is supposed to help with relieving cough and nasal congestion.14

The compounds identified in lemon grass oil are mainly terpenes, alcohols, ketones, aldehydes and esters which may be responsible for various beneficial bioactivities observed. Among them the presence of citral α, citral β, nerol, geraniol, citronellal, geranyl acetate and myrcene is commonly reported for Cymbopogon species. The study on the isolation of the pure components and the pharmacological properties will help scientific community to screen the components responsible for different bioactivities observed with the lemon grass extract.

The present study was carried out to evaluate the hemolytic activity, ATPase inhibitory activity, and Cell viability assessment by MTT assay of the TLC extracts of lemon grass oil.

MATERIALS AND METHODS
Extraction of lemongrass oil
Oil of lemon grass was extracted as per the procedure described by Masamba et al.15 The concentrated extract was obtained by simple distillation using diethyl ether. The concentrated extract was then subjected to preparative TLC to isolate the individual components as described by Nigam et al.16 using Benzene-methanol (10:1) mixture as a solvent. The spots were observed using vanillin sulfuric acid method. And the Rf values were calculated.

The component corresponding to an RF value of 0.8 which was indicative of geranial16 was used to test its hemolytic, ATPase inhibitory and anticancer activity.

Hemolysis assay
Many plants contain chemical substances that might have a hemolytic or anti-hemolytic effect on human erythrocytes. Several reports indicate that the membranes of human erythrocytes from blood types have varying stability as determined from the mean corpuscular fragility17. Plant extracts can positively affect the red cell membrane18 and many plants have serious adverse

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effects, which include induction of hemolytic anemia. Therefore, many of the commonly used plants need to be evaluated for their potential hemolytic activity.

The Hemolysis assay was done as described by Henkelman S. et al. 5 mL of blood was collected from healthy volunteers in the tubes containing 5.4 mg of EDTA to prevent coagulation and centrifuged at 1000 rpm for 10 min at 4°C. Plasma was removed carefully and the white buffy layer was completely removed by aspiration with a pipette with utmost care. The erythrocytes were then washed for additional three times with 1X PBS, pH 7.4 for 5 min. Washed erythrocytes were stored at 4°C and used within 6 h for the hemolysis assay. 50 uL of 10 dilution (100 uL Erythrocytes suspension: 900 uL 1XPBS) of erythrocytes suspension was mixed with 100 uL of test samples (TLC isolated component from lemon grass extract with concentrations ranging from 0.01µg/mL to 1µg/mL), 100 uL of 1XPBS

Figure 1: Hemolysis assay of the TLC extract

Figure 2: ATPas inhibition assay
was used as negative control and 100 uL of 1% SDS as positive controls. Reaction mixture was incubated at 37°C water bath for 60 min. The volume of reaction mixture was made upto 1 mL by adding 850 uL of 1XPB. Finally it was centrifuged at 300rpm for 3min and the resulting hemoglobin in supernatant was measured at 540 nm by spectrophotometer to determine the concentration of hemoglobin. The percentage hemolysis was calculated as follows:

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\% \text{ Hemolysis inhibition} = 100 - \frac{\text{Sample}}{\text{Control}} 
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ATPase inhibition assay

The ATPase activity inhibition was measured using the malachite green reagent method described by Martin G. Rowlands et al. The method uses a colorimetric assay for the measurement of inorganic phosphate forming the phosphomolybdate complex followed by its reaction with malachite green to produce the bluish green coloured product which is read at 620nm.

The TLC isolated component from lemon grass extract having concentration of 10μL/mL was diluted using 0.1% DMSO to get final concentrations ranging from 0.015μg/mL to 1μg/mL. 5μL of each dilution was added to the reaction wells. The source of ATPase used in the reaction was the 40% ammonium sulphate precipitated protein extract of S.cerevisiae. In all the reactions 10μL of 1mg/mL protein (estimated by Lowry’s method) was used. The substrate ATP, 2.5mM prepared using the assay buffer (100mM Tris - HCl containing 20mM KCl and 6mM MgCl2, pH 7.4) was used at 10μL. The reaction blank was prepared with 0.1% DMSO, protein and the substrate ATP. All reactions were carried out in duplicates in a 96 well plate and the plate was incubated at 37°C for 3hours. After 3hours of incubation reaction was stopped by adding 80μL of malachite green reagent and 34% sodium citrate.

Cell viability assessment by MTT assay

Cell viability assay with MCF-7 cells treated with the TLC isolated component from lemon grass extract was done as per the protocol described by Mosmann T. T. Trypsinized 70-80% confluent Breast cell lines MCF-7 (50,000 cells/well) were incubated for 24 hrs at 37°C, 5% CO₂ incubator. The different concentrations of TLC extract (0.01μg/mL to 1μg/mL) to be tested were taken in DMEM without FBS & were incubated for 24 hr. After incubation with different concentrations of lemongrass extract, the media was removed from the wells and fresh plain media was added. After incubation with MTT reagent, the media was removed from the wells and 100 μL of DMSO was added to rapidly solubilize the formazan. The Absorbance was measured at 590 nm. A wide range of concentrations of the TLC extracts was taken to determine the viable concentration of the extract.

RESULTS AND DISCUSSION

Hemolysis assay

The TLC isolated component from lemon grass extract with concentrations ranging from 0.01µg/mL to 1µg/mL was used to determine its hemolytic activity. It was observed that at concentrations of less than 0.08 µg/mL the TLC extract showed no toxicity below 0.08 µg/mL. Hemolysis is due to red blood cells destruction which resulted from lysis of membrane lipid bilayer. This hemolysis relates to concentration and potency of extract. The results indicate that the TLC extract shows no toxicity below 0.08 µg/mL.
**ATPase inhibition assay**

The TLC isolated component from lemon grass extract having concentration of 10µl/mL was diluted using 0.1% DMSO to get final concentrations ranging from 0.015µg/mL to 1µg/mL. The results indicate that at concentrations above 0.125ug/mL there was no significant change in inhibitory activity of the ATPase. (Fig. 2)

**Cell viability assessment by MTT assay**

Evaluation of citral’s effect on breast cancer cells was done using MTT assay. The different concentrations of TLC extract (0.01µg/mL to 1µg/mL) to be tested were taken in DMEM without FBS & were incubated for 24 hr. After incubation with MTT reagent, the media was removed from the wells and 100 µl of DMSO was added to rapidly solubilize the formazan and the absorbance was measured at 590 nm. It was found that at a concentration less than 0.2ug/mL(200ng/mL) lemon grass extract did not show cytotoxicity towards breast cancer cells. (Fig. 3).

The results obtained indicate that the TLC extract of lemon grass at concentrations in the range of 0.01-0.125ug/mL could be studied for its pharmacological potential as an anti-cancer agent.

**REFERENCES**