

Research Article

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

Vinjamuri .S*, Syeda Afshan, Sowmya Shekar, V Jaya Saraswathi

Department of Biotechnology, BMSCE, Bangalore (autonomous college under VTU)

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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, alkaloids, 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, antidiarrheal, antimicrobial, Anti-parasitic, Antiviral activities, etc.⁸⁻¹³. 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 R_f values were calculated.

The component corresponding to an R_f value of 0.8 which was indicative of geraniol¹⁶ 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

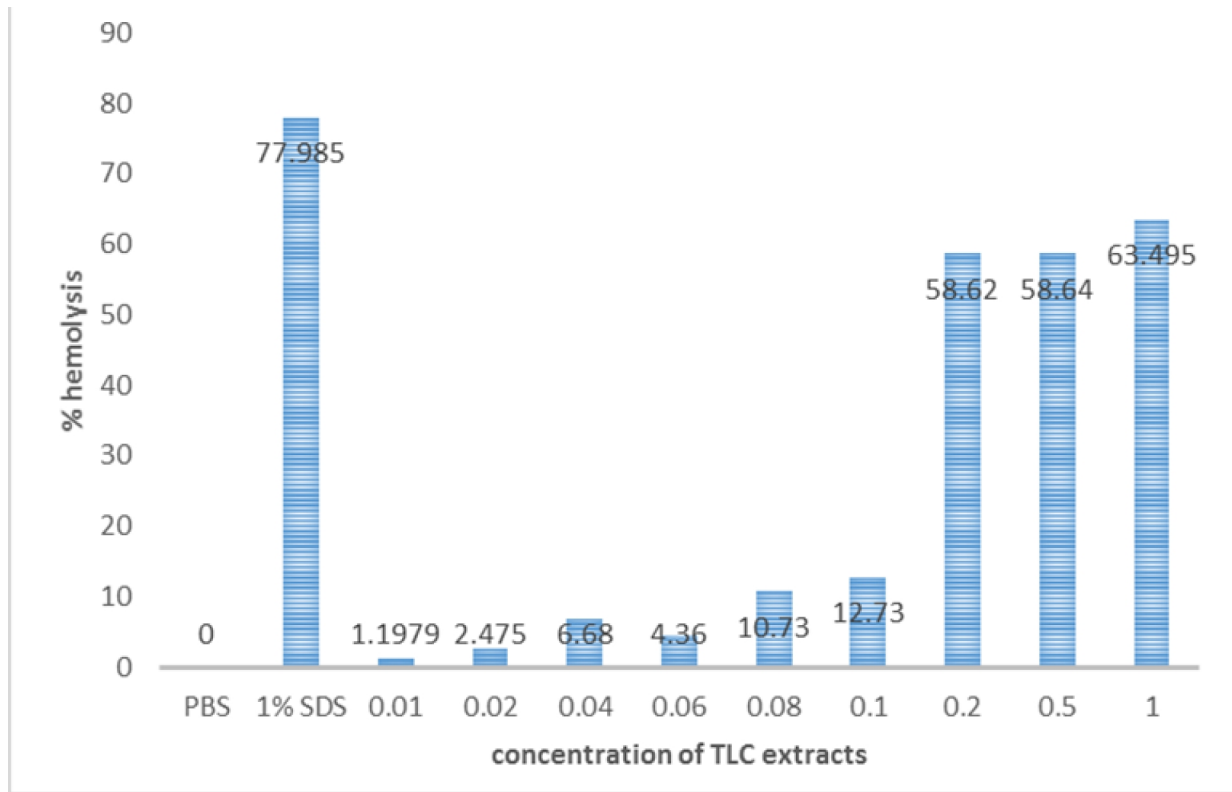


Figure 1: Hemolysis assay of the TLC extract

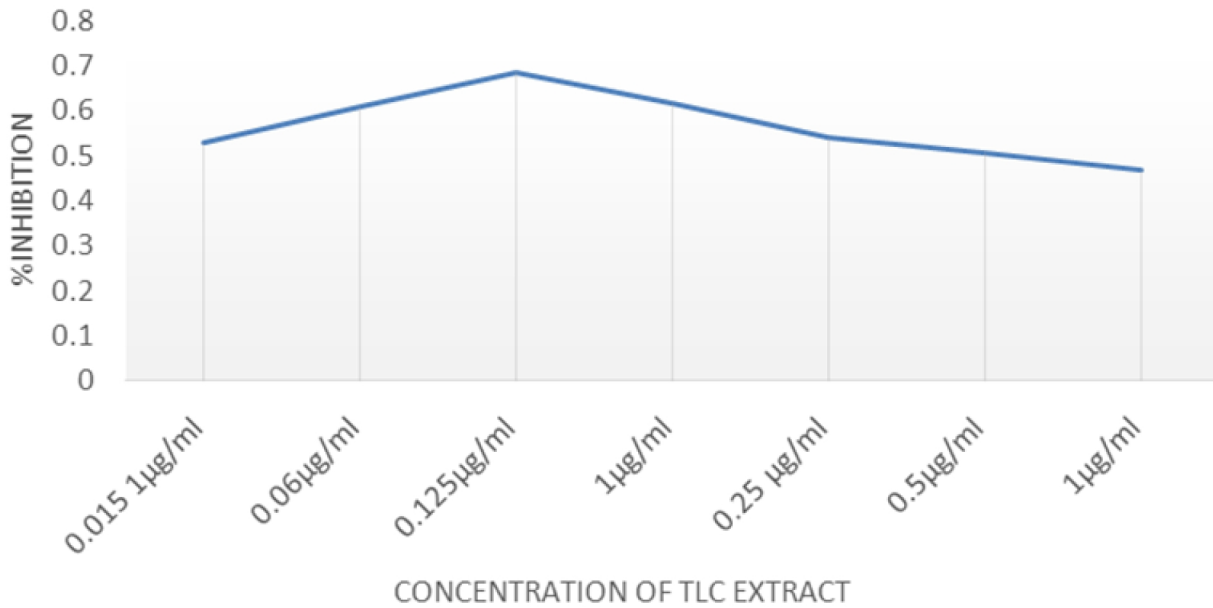


Figure 2: ATPas inhibition assay

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*¹⁹. 5mL 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

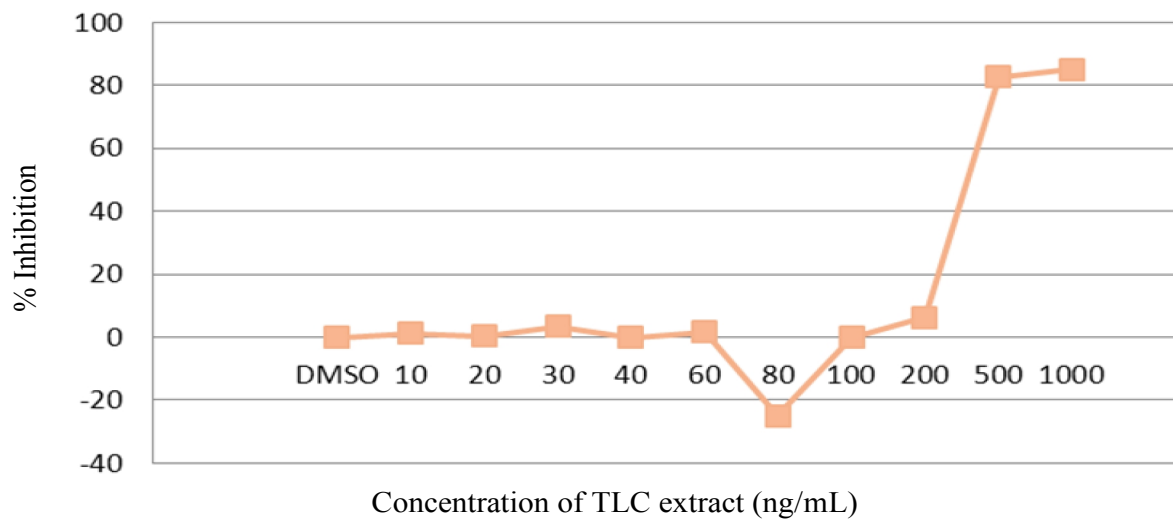


Figure 3: Cell viability assessment by MTT assay

was used as negative control and 100 μ L 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 μ L 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:

$$\% \text{ Hemolysis inhibition} = 100 - [\text{Sample} / \text{Control}] \times 100$$

ATPase inhibition assay

The ATPase activity inhibition was measured using the malachite green reagent method described by Martin G. Rowlands *et al.*²⁰. 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 10 μ L/mL was diluted using 0.1% DMSO to get final concentrations ranging from 0.015 μ g/mL to 1 μ g/mL. 5 μ L 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 10 μ L 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 10 μ L. 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 80 μ L 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.²¹. 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 (0.01 μ g/mL to 1 μ g/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 μ L 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

TLC isolated component from lemon grass extract with concentrations ranging from 0.01 μ g/mL to 1 μ g/mL was used to determine its hemolytic activity.

It was observed that at concentrations of less than 0.08 μ g/mL the TLC extract showed no hemolysis (Fig.1).

The erythrocyte model has been widely used for as it presents a direct indication of toxicity of injectable formulations as well as general indication of membrane toxicity. Another advantage of erythrocytes model is that blood is readily available and that cells are easy to isolate from the blood; moreover, its membrane has similarities with other cell membrane²³. 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 μ g/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.

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