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Bioactivity of *Cymbopogon Citratus* aqueous extract against measles virus and some bacterial isolates

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ABSTRACT

This study was designed to evaluated the anti- cancerous, antiviral and antibacterial effects of *Cymbopogon citratus* aqueous leave extracts. Methyl thiazolyl tetrazolium (MTT) staining assay has been applied to detect the cytotoxicity and the antiviral properties against measles virus (MV) using cervical cancer cell line (HeLa). The antibacterial potency of the extract against *Staphylococcus aureus* (*staph. aureus*) and *Klebsiella pneumoniae* (*Kleb. pneumoniae*) was determined using disc diffusion method by means of agar overlay assay. The results showed that *C. Citratus* extract effectively destroyed Hela cell line after 72 h of exposure. The half maximal inhibitory concentration (IC_{50}) value for the extract was found to be 500 µg mL^{-1} which exhibited the concentration of 600 µg mL^{-1} potent antivirus activity. The extract demonstrated antibacterial potency against *Staph. aureus* with mean inhibition zone 16.4 mm which was higher than that produced by *Kleb. pneumoniae* with mean inhibition zone of 10.7 mm. *C. citratus* was a favourable candidate as a natural herb to treat cervical cancers in vitro, restricted the MV replication and has potent antimicrobial activity against gram-positive and negative bacteria isolated from the respiratory tract.

Keywords: *Cymbopogon citratus*, aqueous extract, antivirus, Measles virus, anti-bacteria.
Article type: Research Article.

INTRODUCTION

Cymbopogon citratus is a perfumed plant usually known as lemongrass, fits the Poaceae family. It grows naturally all over the world, mainly in the tropical and subtropical areas (Tatiana *et al.* 2016). *C. citratus* is usually recycled in useful food as well as in traditional and unconventional medicine in Latin America, Africa, and Asia (Viktorova *et al.* 2020). At this intervaldealings with plant-based medicine seems to be an alternative manner due to the development of antibiotic resistance and to the adverse effects allied with the use of artificial drugs (Salhi *et al.* 2019; Chambers *et al.* 2020; Bayas-Morejón *et al.* 2020; Porusia & Septiyana 2021; Assi *et al.* 2022). Phytochemical investigation of *C. citratus* presented that carbohydrates, proteins, alkaloids, flavonoids, steroids, glycosides, tannins, saponins, resins, reducing sugars, terpenoids, and oils were present at diverse concentrations, whereas acid compounds were lacking (Méabed *et al.* 2018). Measles is an extremely infectious disease caused by measles virus and despite the effective live-attenuated vaccines, MV remains a serious threat among young children. These facts improve the development of new therapeutics that could be effective and used for the management of the virus (Miller 2002). The anti-proliferative potential of *C. citratus* aqueous extracts was tested on five different cancer cells: human colon carcinoma, breast carcinoma, ovarian carcinoma, and a normal liver cell line, exhibiting a concentration-dependent tendency (Shanthala *et al.* 2018). Moreover, (Thangam *et al.* 2014) *C. citratus* polysaccharide fractions (1-4) linked β-D-xylofuranonose showed potential cytotoxic and apoptotic effects on cervical cancer and prostate cancer cells. *C. citratus* owns good antibacterial, anti-inflammatory activity, and encourage phagocytic properties for *Salmonella typhi*, though, it could be used as a substitute

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treatment for enteric fever. Syarif *et al.* (2020) and Suma & Tanija (2016) stated its ability to cure transferrable diseases associated with respiratory system. It aids in removing bacteria from the oral cavity and avoids teeth and gum diseases such as plaque, gingivitis, and periodontitis (Ambade & Deshpande 2019). This study aimed to explore the selective cytotoxicity of *C. citratus* aqueous extract against the Hela tumour cell line and their enhanced antivirus and antimicrobial properties against Measles virus (MV) and some bacterial isolates

MATERIALS AND METHODS

The study was directed at the department of biology laboratories, College of Science, Mustansiriyah University, and its protocol was accepted by the Ethical Committee of Mustansiriyah and Middle Technical Universities, Baghdad, Iraq.

Preparation of *C. citratus* crude aqueous extracts

C. citratus leaves were washed several times with distilled water for the removal of dust and unwanted particles. Leaves were air-dried for 3-4 days and then crushed with a mechanical grinder. Hot aqueous extract of dried leaves was prepared as recommended by (Al Manhel & Niamah 2015). A total of 10 grams of plant leaves powder was added to 100 mL of boiled distilled water in sterile conical flask and put on hot plate magnetic stirrer for thirty minutes, then, left at room temperature for 24 h. The solution obtained was filtered through a filter paper (twice), evaporated and the remaining materials were collected, kept in refrigerator until use. Preparation of stock solution: The aqueous extract that used in this study was done by disbanding 1 gram of plant powder in 10 mL distilled water and stored at -20°C after sterilized by Millipore filter (0.45 µm).

Cell culture and Measles virus

Cervical cancer cell line (HeLa) and live attenuated measles virus vaccine (MV) were provided by the cell Bank Unit, Experimental Therapy Department, Iraqi Centre for Cancer, and Medical Genetic Research (ICCMGR).

Cytotoxicity

Hela cell line were cultured in RPMI media containing 10% foetal calf serum in flasks (25 cm) for 24-48 h, collected using a 0.25% solution of Trypsin-EDTA, diluted with media to approximately 10^4 cells $well^{-1}$ (counted by hemocytometer) and dispensed into 96 wells plate. Incubated in 5 % CO_2 at 37 °C till confluent sheet is formed. Ten different concentrations of the aqueous extract (100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 µg mL^{-1}) were prepared by using Free serum RPMI medium, added to the plate (after discarded the medium). Three replicates for each concentration were done, and column No. 11 in the plate was kept as control (cells with no treatment). The cells were incubated at 37 °C under a humidified atmosphere containing 5% CO_2 for 72 h.

Cell viability

Cell viability was measured after 72 h by removing the media, adding 100 µL of Methyl thiazolyl tetrazolium (MTT) solution (Chiang Wand Chang 2002) to distinguish the viable cells from the dead cells, incubating for two h at 37 °C, (MTT) solution was removed and 100 µL $well^{-1}$ of Dimethyl sulphoxid (DMSO) were add, incubated at room temperature for 15 min, then the absorbency was read at 492 nm. The inhibition rate of cell growth (the percentage of cytotoxicity) was calculated (Repetto 2003).

Antiviral assay

Two concentrations of the plant extract (500 and 600 µg mL^{-1}) were selected and used in antiviral screening. The Measles virus was diluted with serum-free media at the rate of 0.1 MOI (virus titre: 4×10^7 $TCID_{50}$ mL^{-1}) and used to infect Hela cells in 96 micro-titration plate which incubated for one hour at 37 °C to allow attachment of the virus, whereas the control cell was treated with media only. Virus was removed from the wells using micropipette and plant extract with different concentration diluted with serum-free media (three replicates for each concentration) were added. Plates incubated at 37 °C, CO_2 5% for 72 h (Omilabu *et al.* 2010). The percentage inhibition was calculated from measured cell viability by using MTT assay.

Pathogenic strains

Strains of *Staph. aureus* and *Kleb. Pneumoniae* (25 each) were isolated from sputum culture and diagnosed according to Levinson (2010) in Al-yarmuk Hospital, Baghdad in December 2019.

Adjustment of bacterial inoculum

Pure culture of each isolate was prepared, single colonies were picked and inoculated in 2 mL of Brain Heart

Infusion (BHI) broth and incubated 24 h at 37 °C. The inoculum size of each isolate was adjusted to 0.5 McFarland turbidity scale under septic conditions according to CLSI (2012).

Antibacterial efficacy

The disc diffusion method was prepared using agar overlay assay (Emmanuel *et al.* 2016). In sterile tubes, each containing 4 mL of sterile melted agar (0.75% agar) pre-warmed to 50 °C for 10 min then cooled to 45 °C. Then 0.1 mL of each isolate was inoculated, mixed the melted agar well, and poured out immediately over the pre-warmed plate (at 37 C for 2 hours) containing a layer of blank Muller Hinton Agar (MHA), allowed the top agar to solidify then placed 6 mm sterile filter paper discs on the surface of the top agar. One disc was socked with 50 µL of the plant extract, a second disc soaked with 50 µL sterile distilled water (solvent) as a negative control and a disc of gentamicin 10 µg $disc^{-1}$ as a positive control. The plates were incubated at 37°C for 24 h. The test was done in triplicate for each isolate.

RESULTS

The cytotoxicity of *C. citratus* aqueous extract was assessed on cervical cancer cell lines (HeLa) which treated with ten different concentrations of the extract and after 72 hours of incubation at 37 °C. The results revealed that *C. citratus* leave extract had the highest inhibition rate (68.7%) in the concentration of 1000 µg mL^{-1} and decreased to 20.7% in 100 µg mL^{-1}. The inhibition was directly proportional to the concentration of the extract and the half maximal inhibitory concentration 50 (IC_{50}) was found to be 500 µg mL^{-1} (Table 1; Fig. 1). The results showed that using 500 and 600 µg mL^{-1} of extract as antivirus lowered the percentage inhibition of the cell growth to 61.2 and 58.1 respectively, while the virus positive control inhibited the cells viability to 68.4 (Table 2). The cytopathic effects of MV characterized by partial loss of the monolayer, rounding and shrinkage of the cells with granular appearance in the cytoplasm of Hela cells (Fig. 2).

Table 1. Growth inhibition (%) of *C. citratus* extract on Hela cell line after 72 h.

Conc. (µg mL^{-1})	Inhibition (%)
100	20.7
200	27.5
300	35.6
400	42.5
500	46.7
600	54.2
700	57.2
800	59.6
900	61.7
1000	68.7

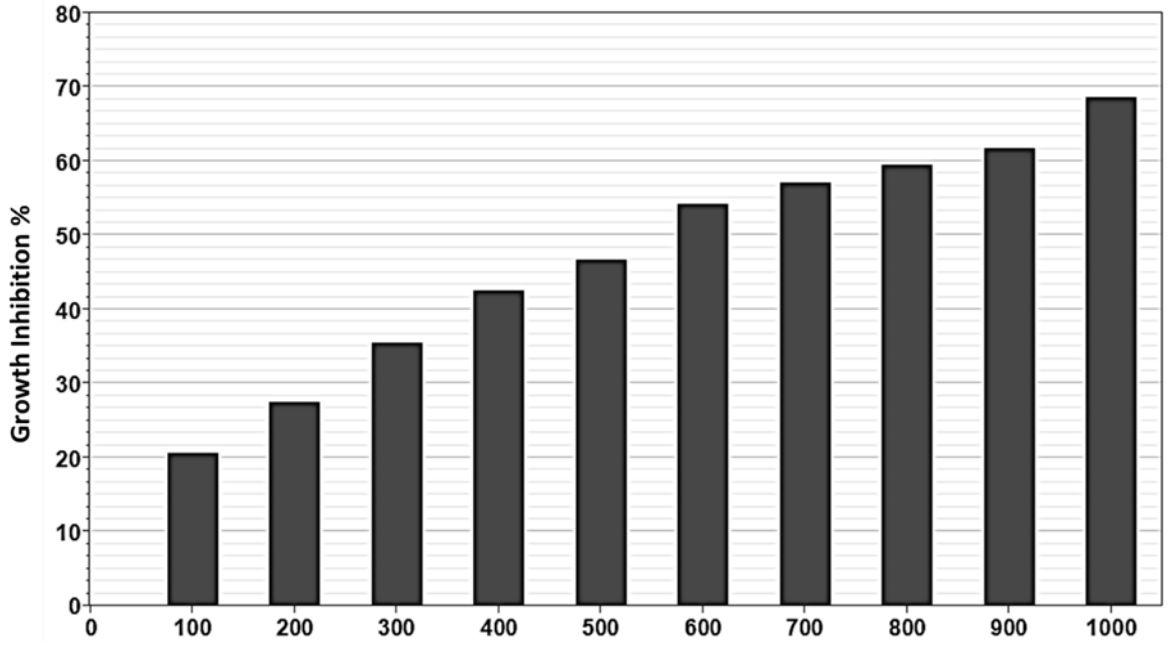

Fig. 1. Growth inhibition percent of *C. citratus* extract on Hela cell line after 72 h post exposure.

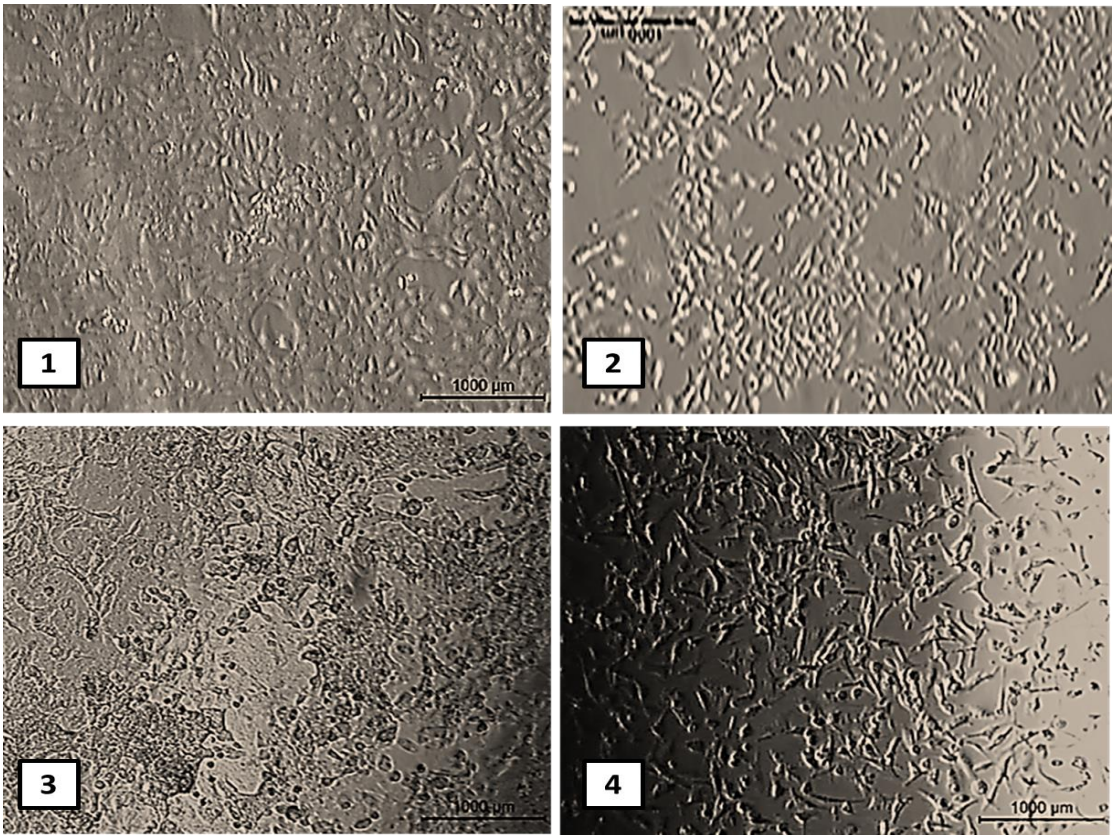

Fig. 2. Cytopathic effects in Hela cell line after 72 h; 1: cell control; 2: *C. citratus* extract **(**600 µg mL^{-1}); 3: virus control; 4: MV + *C. citratus* extract **(**600 µg mL^{-1}).

Table 2. Growth inhibition (%) of the combination (extract + MV) in Hela cell line after 72 h post infection.

Extract conc. (µg mL^{-1})	Virus control	Combination (virus + extract)
500	68.4	61.2
600	68.4	58.1

Antibacterial efficiency

Twenty-four isolates only were sensitive to the extract used as presented in Table 3. The reduction in bacterial growth around each disc was used to determine the inhibition zone in mm. The mean diameter of inhibition zone for sensitive bacterial isolates was presented in Table 4 and Fig. 3.

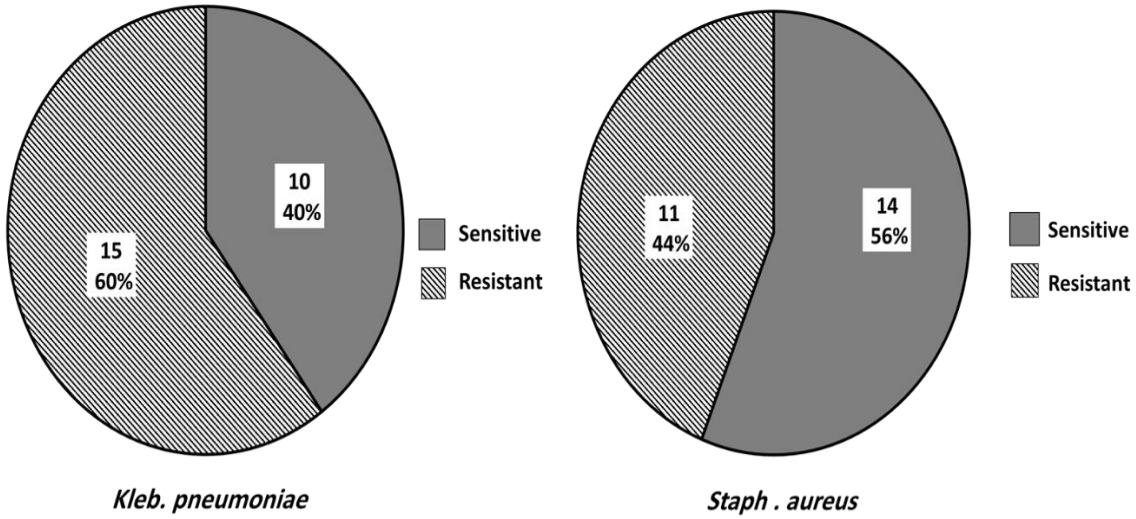

Fig. 3. Antimicrobial outcome of the extract on examined pathogenic bacteria.

Table 3. Antimicrobial outcome of the extract on tested pathogenic bacteria.

Bacterial species	Total no. of isolates	Sensitive	%	Resist	%
Staph. aureus	25	14	56	11	44
Kleb. Pneumonia	25	10	40	15	60

Table 4. Mean zone of inhibition for the sensitive isolates.

Bacterial species	50 µL Extract	Gentamicin (10 µg disc^{-1})	Distilled water (50 µL)
Staph. aureus	16.4	22.0	-
Kleb .pneumoniae	10.7	15.2	-

Note: (-) Means no zone of inhibition produced.

DISCUSSION

Dried *C. citratus* leaves are widely used as a lemon flavour ingredient in herbal teas, prepared either by decoction or infusion (Tilaye *et al.* 2018). We measured the optical density (OD) after treatment of Hela cell line with different concentrations of the extract. The 500 µg mL^{-1} is the IC_{50}. *C. citratus* extract exhibited the IC_{50} at 500 µg mL^{-1} due to an alteration in mitochondrial membrane potential and could greatly inhibit the growth in HeLa cell line consequently increased cytotoxicity observed (Nakamura *et al.* 2003). (Van *et al.* 2016) observed that *C. citratus* essential oil displayed potent activity against the human lung carcinoma (A549) cell line and moderate effects on the HeLa cells. However, it was inactive against human hepatocellular carcinoma (Hep3B) cell line. (Rabbani *et al.* 2006) stated that an increase in the reactive oxygen species (ROS) production in *C. citratus*-treated cancer cells causing cell death equated to control cells. ROS production is a purpose shared by all non-surgical therapeutic systems for cancers, including radiotherapy, photodynamic, and chemotherapy usage. Moreover, (Wang *et al.* 1875) reviewed that *C. citratus* emulsion recruits the cancer cell damage through declined cell proliferation, amplified intracellular ROS, transformed mitochondrial membrane and prompted apoptosis in HeLa cell line and highly-invasive squamous (ME-180) cell. Hela cells demonstrated mean inhibition of approximately 61.2 and 58.1% at 500 and 600 µg mL^{-1}, respectively. The inhibition (%) was recede when used the combination of virus and extract than the virus alone which mean that the extract affected the proliferation of the virus in the cells. the majority of *C. citratus* extract fraction had moderate antiviral activity (++) against (MV). (Chiamenti *et al.* 2019; Chambers *et al.* 2020; Viktorova *et al.* 2020) pointed out that the lemongrass essential oil has hopeful activity against antibiotic-resistant bacteria and chemotherapeutic-resistant tumours by inhibiting the overexpression of the transmembrane efflux pump which carry the drug outside the cells and therefore decrease its intracellular concentration and make them sensitive. The difference in susceptibility of both tested bacteria to the extract may be related to the difference in genetic composition, physiology, and metabolism of each isolate (Izah & Aseibai 2018) or may be related to the solubility of leaves contents, extract concentration, type of medium used and the diffusion rate in the medium (Vyshali *et al.* 2016). The mean zone of inhibition produced by *Staph. aureus* was larger than that produced by *Kleb. pnenmoniae* and this may be due to the differences in the chemical composition of the cell membrane. In Gram-negative (*Kleb. pnenmoniae*) the outer membrane acts as an impediment to the small molecules due to its high lipid content (Lambert 2002). Our results were concordant with several researchers who adduce the antibacterial performance of *C. citratus* leaves water extract against numerous Gram- positive and negative bacteria (Basera & Lavania 2019). Different marbles were published about the active component present in *C. citratus* leaves water extract which had antibacterial efficacy**,** (Komiya *et al.* 2006) said that tannins contents had a modulatory action on bacterial antigenic receptors while (Adukwu *et al.* 2012) related that to citral content and the to the alkaloids, tannins and flavonoids content as stated by (Gopinath *et al.* 2013), or to essential oils acids, alkaloids, tannins, and steroids as announced by (Faraja *et al.* 2018). The phenolic compounds including flavonoids contain polar hydroxyl groups that were responsible for antioxidants, free radical scavenger, anti-bacterial and anti-inflammatory actions (Oladeji *et al.* 2019). Also (Ashraf *et al.* 2016) believed that the inhibitory impact of the bioactive components (terpenoids and phenols) is due to the interaction and disruption of enzymes and proteins useful for microbial metabolism. *C. citratus* and polyphenol-rich fractions inhibit Lipopolysaccharide (LPS) and induced activation of the nuclear factor (NF)-kB pathway, consequently repressed the cytokine production on human macrophages. Chlorogenic acid was the main phenolic acid of the *C. citratus* infusion, and at least in part, liable for that effect. Moreover, it was shown that *C. citratus* and polyphenol-rich fractions inhibited the proteasome activity, a complex that controls NF-kB activation, having CGA a strong influence (Francisco *et al.* 2013). This reveals that *C. citratus* infusion composites are capable to reduce

inflammation and peripheral pain in vivo (Garcia *et al.* 2015). However, the inhibition is still high which is may be due to cytotoxicity of the extract (Micol *et al.* 2005).

CONCLUSION

The results directed that the *C. citratus* aqueous extract could be a hopeful candidate for the natural basis of anticancer and antiviral. It possesses good antibacterial activity thus it could be used as an additional therapy to cure diseases correlated to the respiratory system.

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