



# Characterisation, Antioxidant, Antibacterial and Anticancer activity of Essential oil of *Cymbopogon citratus* and its food preservative effect on Anchovy fish (*Stolephorus indicus*)

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**Abstract:** In the present study, the attention was on employing the plant essential oil extracts, to be efficiently used as antioxidant and antibacterial agents with an ultimate objective of developing replacements for the synthetic chemical preservatives in foods. For this study, the essential oil was extracted from *Cymbopogon citratus* by hydrodistillation method using ethyl acetate. It has been reported that phenolics and flavonoids are important indicators of antioxidant capacity which plays a vital role in improving food quality and preservation. *C.citratus* oil have been proved to have antibacterial activity against the tested food spoilage pathogens and antioxidant activity, which is generally attributed to high phenolic and flavonoid compounds owned by this oil.

The FT-IR spectrum of lemongrass essential oil showed the presence of functional groups like alkanes, amines, aldehydes, esters and lactones, phenols, anhydride, alcohols, imine / oxime, nitro compounds and organic halogen compounds which prove to exhibit medicinal properties. The study was also extended to find the anticancer ability of *C.citratus* oil against breast cancer MCF 7 cell line. The preservation study was carried out on easily perishable food like Indian anchovy fish (*Stolephorus indicus*) with a focus for maintenance of quality and prolong shelf life. The results indicate that *C.citratus* oil treated fish sample stored on ice remained fresh for 7 days and hence could be used as natural preservative thus replace or partly replace the synthetic food preservatives.

**Index Terms:** Antioxidant, Anticancer, Antibacterial activity, Natural preservatives, Indian anchovy.

## INTRODUCTION

*Cymbopogon citratus* also known as lemon grass, a native herb from India. It is an aromatic, perennial grass grown in many temperate areas. The leaves of *C.citratus* have been used in traditional medicine and are usually found in herbal supplements and teas. Laboratory studies have shown cytoprotective, antioxidant, antifungal and anti-inflammatory properties (Figueirinha, 2010) while the essential oil is used as carminative, depressant, analgesic, antipyretic, antibacterial, and antifungal agent. Essential oil of *C.citratus* have a wide range of medicinal and therapeutic properties. They have been shown to promote healthy sleep, relieve headaches, and alleviate pain. Studies have shown that their physicochemical properties enable them to have great and diverse biological activities. The use of plant essential oils, as alternative antimicrobial and pharmaceutical agents has attracted considerable interest recently. They have been reported to inhibit several plant and human pathogens as well as their usefulness in food and pharmaceutical industries. (Elshafie & Camele, 2017).

The use of plant essential oils as food preservative agents is of concern because of several reported side effects of synthetic oils and chemicals used in artificial preservatives. Seafood's like fish are extremely perishable because of its chemical composition. For storage and to extend shelf life, chemical substances are widely used which has been linked to potential health hazards. In this regard, natural preservatives with excellent antioxidant and antimicrobial properties have been extensively searched and implemented as safe alternatives, in order to prevent or slow down the deterioration of food.

In this study, essential oil of *C.citratus* have been employed in seafood to maintain the quality, as well as to extend the shelf-life. For this, we used Indian anchovy (*Stolephorus indicus*) fish, also known as Hardenberg's anchovy, is a species of oceanodromous fish in the family Engraulidae. Fresh and dried anchovies are a popular part of the cuisine in Kerala and other south Indian states, where they are referred to as netholi or chooda (nethili in Tamil Nadu) and provide a cheap source of protein in diet (Pharmaceutical Journal, 2018).

The essential oil was extracted from *C. citratus* by hydrodistillation method. It is the traditional and conventional method for extracting oil. Ethyl acetate is used as a solvent as it is environmentally safe and acceptable for food applications. Studies have shown that the oil

extraction using ethyl acetate possess high polyphenol content and antioxidant activity (Hacke *et al.*, 2018). The contents of the phenolic compounds and flavonoids were considerably higher in ethyl acetate fraction. This may be due to the higher solubility of such compounds in ethyl acetate.

## II. MATERIALS AND METHODS:

### II.A. Plant collection

Fresh leaves of *Cymbopogon citratus* were collected from nearby region in Annur, Coimbatore 641653 (11.2362° N, 77.1051° E) on 2/1/2020 between 10.00 AM to 11:15 AM. The leaves were washed in water to remove dust and shade dried for about 2-3 days.

### II.B. Oil extraction

25gm of dried leaves were subjected to hydrodistillation. Weighed leaves were dissolved in distilled water and heated on a water bath for 3 hours at 90°C. After heating it is filtered and the filtrate is treated with 30ml of ethyl acetate and is placed on the orbital shaking incubator for 48 hours. As a result the essential oil float on the top of the flask along with the ethyl acetate. The distillate that is, the oil was collected using micropipette and transferred into a centrifuge tube and used for analysis. The volume of essential oil obtained was 25ml. This method was found to be the most suitable for the extraction of oil, since it allows obtaining the oil without altering the quality.

## II.C. CHARACTERISATION STUDIES

### i) UV studies

The UV-VIS analysis was performed for identification of phytoconstituents present in the sample. The extracted oil of *Cymbopogon citratus* was characterised using UV-VIS spectrophotometer (ELICO SL159 Microprocessor UV-VIS spectrophotometer) in the range of 200nm to 700nm and the peaks were observed. For this purpose, 5 mL of oil was kept in the UV-VIS cuvette.

### ii) Fourier Transform Infrared Spectroscopy

Fourier transform infrared spectroscopy spectrum of *C. citratus* oil extract was obtained using SHIMADZASU FTIR System. FTIR used for chemical identification as each molecule and chemical structure creates a unique spectra. The functional groups present in the extracts were determined by comparing the vibration frequencies in wave numbers of the sample spectrograph obtained from an FT-IR spectrophotometer with those of an IR correlation chart. The measurement was carried out in the range of 500–4000  $\text{cm}^{-1}$  at a resolution of 4  $\text{cm}^{-1}$ .

## II.D. INVITRO ANTI-OXIDANT ACTIVITY:

### i) DPPH Assay

In vitro DPPH radical scavenging assay was measured by the spectrophotometric method according to (Dhanasekaran *et al.*, 2011). *C. citratus* oil of different concentrations (50 $\mu\text{l}$ , 100 $\mu\text{l}$  and 250 $\mu\text{l}$ ) were taken in test tubes and 0.1ml of 0.1M DPPH solution was added and mixed well. After 5 minutes of incubation 0.4ml of 50mM TrisHCl was added and make up to 2ml with distilled water and incubated in dark room for 30 minutes. Reading was taken at 517 nm using spectrophotometer. Ascorbic acid was used as a standard and the percentage inhibition was calculated using the following formula.

$$\text{Percentage inhibition} = (\text{Abs Control} - \text{Abs Sample}) / \text{Abs Control} \times 100$$

### ii) Total Flavonoid content

Total flavonoid content was quantified as described by (Sarabjot Kaur *et al.*, 2014). 0.1ml of 10% aluminium chloride was added to 1ml of sample. To this mixture, added 0.1ml of 1M Potassium sodium tartrate and 2.8ml distilled water. Mixed well and incubated at room temperature for 30 minutes. After incubation, the absorbance of reaction mixture was measured at 415nm. Quercetin was used as a standard to calculate the amount of flavonoids present in the sample using the following formula.

$$\text{Total Flavonoid Content (TFC)} = C \times V/M$$

C = concentration determined from standard curve (mg/ml), V = volume used during the assay (mL), and M = mass of the extract.

### iii) Total phenol content

Total phenolics assay was quantified according to (Monisha *et al.*, 2017). 1ml of the sample was mixed with 0.2ml of Folin-Ciocalteu phenol reagent. After 5minutes, 1ml of 20% sodium carbonate was added to the mixture and incubate at 45°C for 45 minutes. After incubation, the absorbance of reaction mixture was measured at 765nm using spectrophotometer. Gallic acid was used as a standard and the amount of phenol present in the sample was calculated using the following formula.

$$\text{Total Phenol Content (TPC)} = C \times V/M$$

## II.E. INVITRO ANTI-BACTERIAL ACTIVITY:

### Agar well diffusion method

The agar well diffusion method was used for the antibacterial assay. This was performed according to (Jesteena Johney *et al.*, 2017). Petri plates were prepared by pouring 20 ml of Muller Hinton Agar medium and allowed to solidify. After solidification, 70 $\mu\text{l}$  of bacterial culture *Escherichia coli*, *Bacillus cereus*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were poured onto the medium

and swabbed uniformly. Ampicillin was used as a positive control and methanol was taken as negative control. Wells were loaded with 50µl and 100µl of *C. citratus* oil and incubated at 37°C for 24 hours. After incubation zone of inhibition was measured.

## II.F. INVITRO ANTI-CANCER ACTIVITY:

### Preparation of cell lines media

DMEM media (19.75 g of DMEM, 3.7g of sodium carbonate, 4.5g of glucose) was prepared in T- flask and the addition of MCF 7 cell lines to the flask and was incubated in CO<sub>2</sub> incubator with 5% CO<sub>2</sub>, 37°C temperature, at 70-80% of humidity for 24-72hrs.

### MTT Assay

The anticancer activity of oil sample on MCF 7 breast cancer cells was determined by the MTT (3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide) assay was used to assess the cytotoxicity (Horiuchi et al., 1988). *C. citratus* oil of different concentration (10µl, 20µl, 30µl, 40µl) were taken and 100µl of cell lines were added. DMSO was taken as blank and cell lines were used as a control. The plate was incubated for 24 hours in CO<sub>2</sub> incubator. DMSO and trypsin was used to lyse the cells and also for the washing. After washing, 20µl MTT dye was added to the wells and incubated for 24 hours. OD values were measured using ELISA reader (Robonic read well touch ELISA plate reader). Percentage of cell death was calculated using the following formula;

Percentage of cell death =  $\frac{\text{Abs of Control} - \text{Abs of Sample}}{\text{Abs of Control}} \times 100$

## II.G. SHELF-LIFE EXTENSION STUDIES

For this research, Anchovy fish (*Stolephorus indicus*) purchased near Ukkadam Lake, Coimbatore was used for this study. Four anchovies of same size were collected. Control samples were wrapped by aluminium foil. Fish sample was stored at room temperature and on ice. They were sprayed with 5ml and 10ml concentration of *C. citratus* oil extract in two separate aluminium foils. Allowed them to dry for few minutes. After drying, wrapped the remaining fishes (tests) and stored on ice. The wrapped fishes were left undisturbed for the shelf life study.

### i) Physical changes during storage

Parameters like eyes, gills, skin, odour, muscle texture and weight were observed with naked eye between fresh and rotten fish during 3<sup>rd</sup> and 7<sup>th</sup> days of storage.

### ii) Microbial evaluation

The samples were swabbed using a sterile cotton swab on 3<sup>rd</sup> and 7<sup>th</sup> day of storage and were inoculated into nutrient broth. After incubation of 24 hours, the absorbance was read in spectrophotometer.

## III. RESULTS DISCUSSION:

### III.A. CHARACTERISATION STUDIES:

#### i) UV studies

The identification and characterisation of the bioactive compounds was analysed by using UV Visible study for *C. citratus* essential oil. The Plasmon peak was observed in the range of 200 nm to 700 nm for the sample. Graph was shown below in Figure 1. It is easy to identify the presence of compounds based upon the peak, showing different peak in each nanometre. The oil extract showing peak at 205nm, 220nm, 230nm, 240nm, 260nm, 275nm, 320nm, 335nm, 360nm, 535nm.

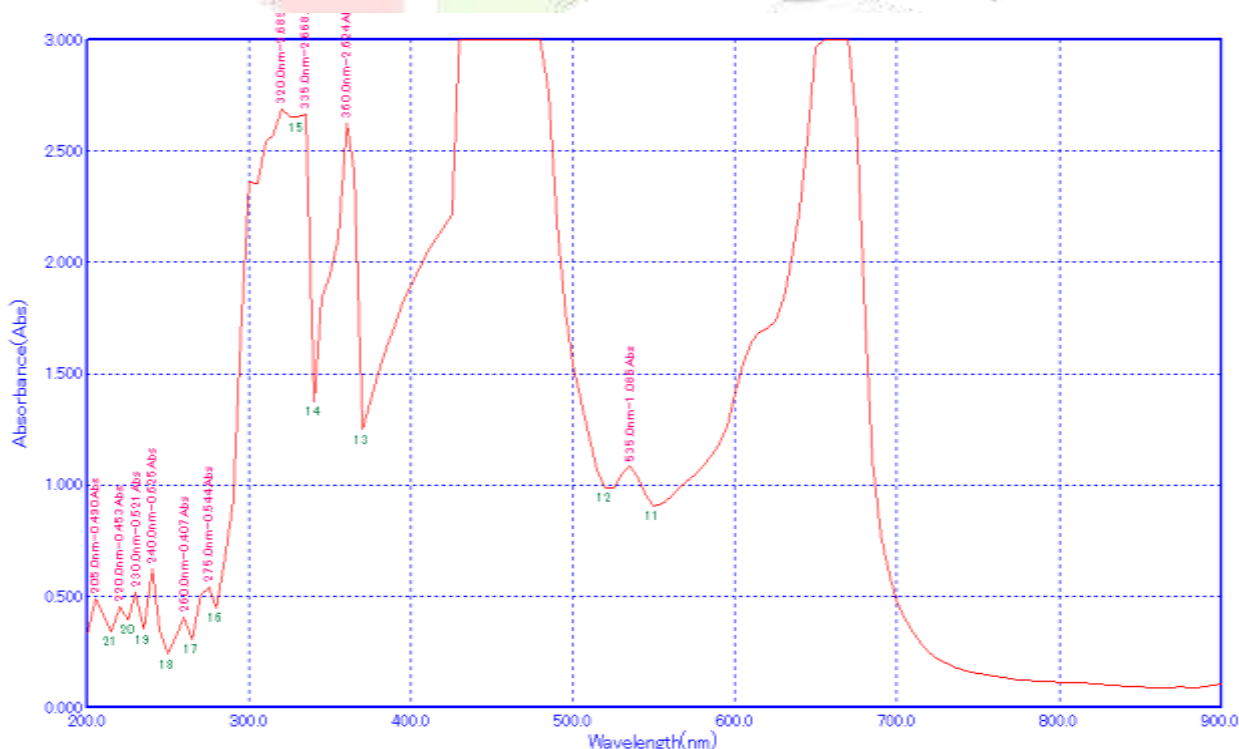


Figure 1: UV studies on *C. citratus* essential oil

### Fourier Transform Infrared Spectroscopy

In the FT-IR spectrum of lemongrass essential oil, the absorption band or frequency from 1735.93, 1373.32, 1234.44, 1041.56, 2985.81, 848.68, 1512.19, 786.96 are C=O stretching, O-H bending, C-N stretching, CO-O-CO stretching, C-H stretching, C-Cl stretching, N-O stretching and C-H bending showed the presence of the functional groups viz. alkanes, amines, aldehydes, esters and lactones, phenols, anhydride, alcohols, imine / oxime, nitro compounds and organic halogen compounds. All these compounds belong to secondary plant metabolites as per researcher explanations (Skoog, 2007). The presence of these characteristic functional groups could be responsible for the various medicinal properties of *C. citratus* oil. The graph was depicted in Figure. 2

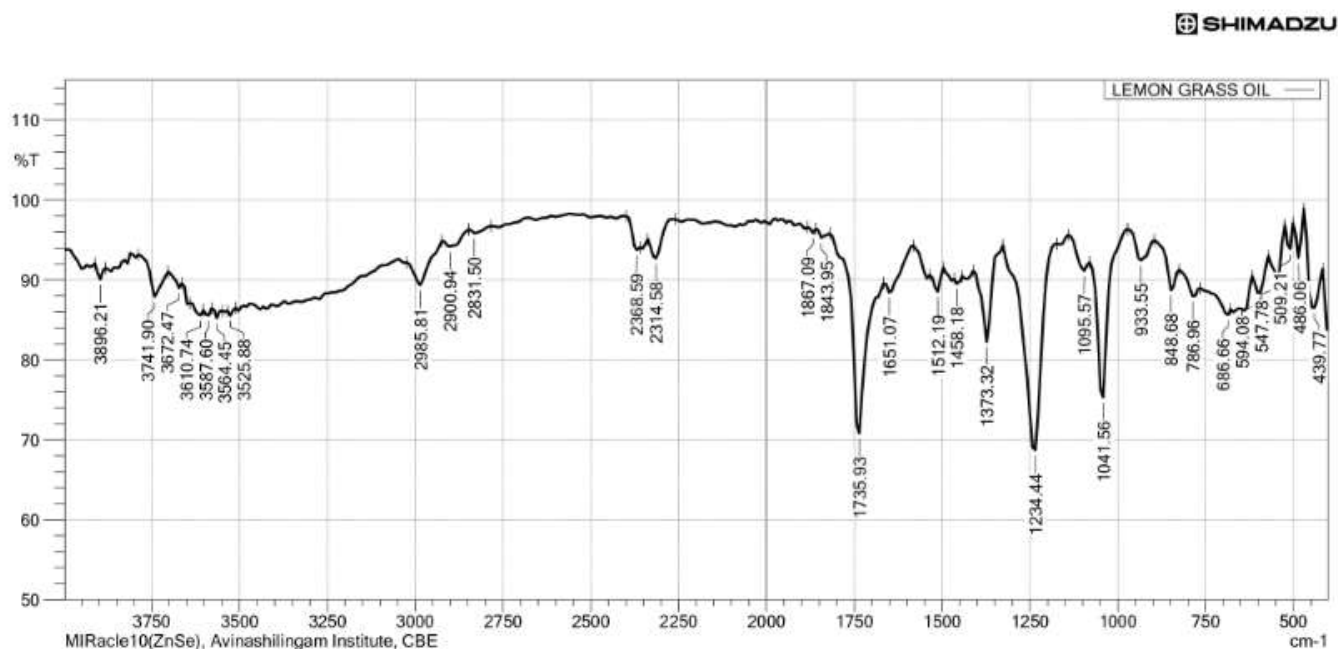


Figure 2: FTIR studies of *C. citratus* essential oil

### III.B. INVITRO ANTI-OXIDANT ACTIVITY:

#### i) DPPH assay

In this study, the antioxidant concentration from plant essential oil of *C. citratus* was determined from ascorbic acid standard curve and percentage inhibition was found. The percentage inhibition increases with increase in the concentration of plant oil. The antioxidant activity using 1,1 - diphenyl -2picryl-hydrazil (DPPH) method of *C. citratus* essential oil showed high amount of activity. Lipid oxidation is a major cause of quality deterioration in many types of natural and processed foods. One of the most effective means of retarding lipid oxidation in foods is to incorporate antioxidants as preservatives (Anbudhasan, 2014).

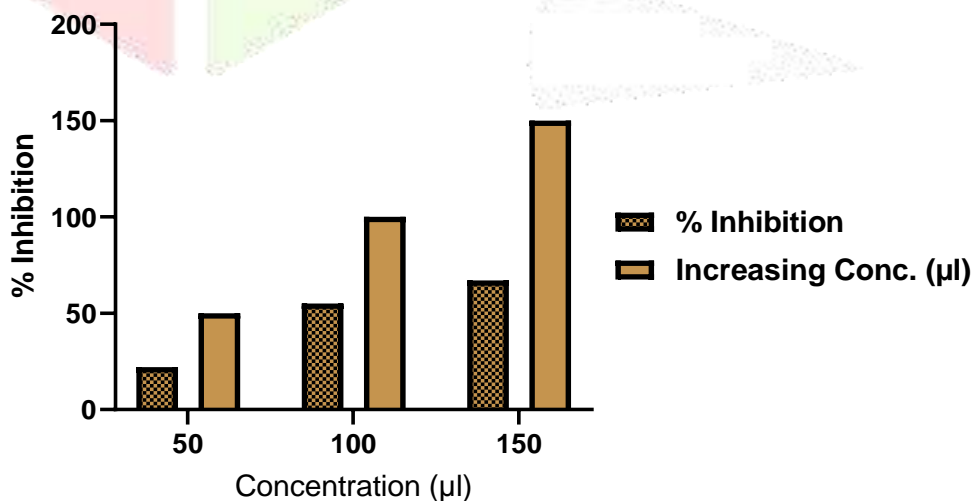


Figure 3. Comparison of percentage inhibition of *C. citratus* oil at different concentrations.

#### ii) Total Flavonoid content

Flavonoids are famous for their antioxidant properties and it is a universal plant pigment. Flavonoid content was calculated by using quercetin as standard. The total flavonoid content of *C. citratus* essential oil extract was found to be **26mg/ml**. Flavonoid is a polyphenolic compound that is ubiquitous in nature, comprising a number of hydroxyl groups attached to aromatic ring structures that determine its antioxidant properties (Lalas et al., 2002).

### iii) Total phenol content

This study revealed the presence of phenolic constituents in *C. citratus* essential oil. Polyphenols in plant extracts react with specific redox reagents (Folin's-Ciocalteu reagent) to form a blue complex that can be quantified by visible-light spectrophotometry [Schofield P et al., 2001]. Phenolic constituents are important in plants because of scavenging activity due to the hydroxyl group. Total phenol concentrations were determined from standard curve using Gallic acid as standard. *C. citratus* essential oil has higher phenol content of **25mg/ml** of oil. Different phenols have various therapeutic and protective effects which contribute to the plant quality and nutritional value. The phenolics and flavonoids are important indicators of antioxidant capacity and plays a vital role towards preventing diseases and sustaining a state of wellbeing (Mohammed and Fazilah., 2015).

### III.C. INVITRO ANTI-BACTERIAL ACTIVITY:

#### Agar well diffusion method

The zone of inhibition of bacterial organisms was shown in table 1. *C. citratus* oil in two concentrations (50µl and 100µl) has shown significant inhibition effect on growth of bacteria. *Bacillus cereus* and *Staphylococcus aureus* were inhibited more effectively by the plant oil extract than *Escherichia coli* and *Pseudomonas aeruginosa*.

Table 1: zone of inhibition of extracts against different bacteria

Microorganism used	50µl (mm)	100µl (mm)	Ampicillin disc (mm)	C
<i>S aureus</i>	5	5	5	NIL
<i>P aeruginosa</i>	2	4	4	NIL
<i>E coli</i>	3	4	4	NIL
<i>B.cereus</i>	4	8	5	NIL

### III.D. INVITRO ANTI-CANCER ACTIVITY:

The percentage of cell death or inhibition of extract in different concentrations were depicted in figure 4. *C. citratus* oil have significant anticancer activity with increasing concentrations and IC50 value was found to be 82.3µl. Thus *C. citratus* essential oil has the potential to be a powerful anticancer agent against breast cancer cell lines and hence the oil could be used for cancer prevention and treatment.

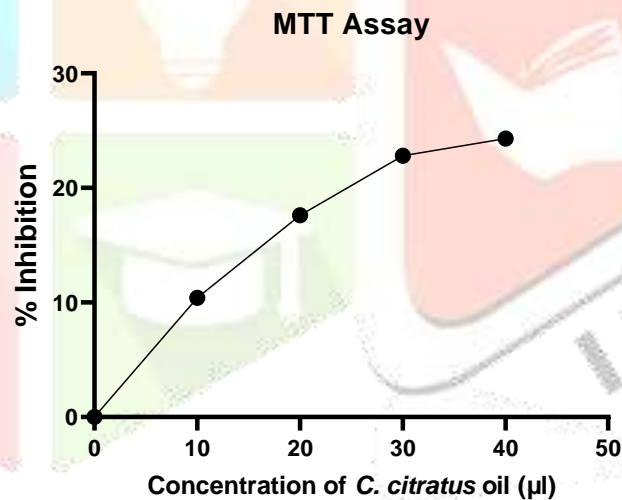
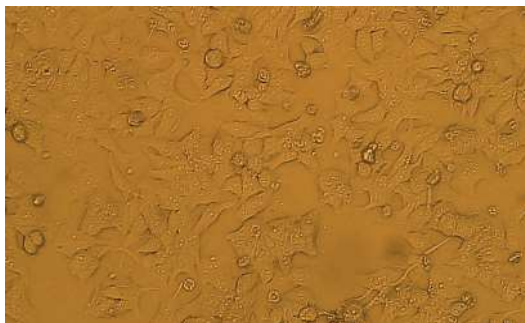
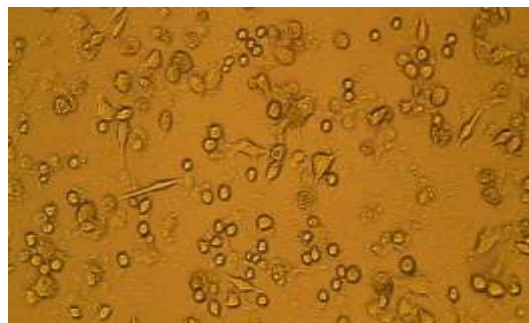


Figure 4. Percentage inhibition of sample at different concentration



Control MCF 7 cell line



Treated MCF 7 cell line

Figure 5. Control and treated cell line

**III.E. SHELF-LIFE EXTENSION STUDIES:**

The rotten smell (ammonia like smell) was observed in fish stored at room temperature (control) after 10 hours. It was unwrapped and swabbed with sterile cotton swab for microbial evaluation. The fish stored on ice (control) was rotten after 2 days. The foil was opened and swabbed. The samples without oil extract served as control. The fishes coated with 5ml and 10ml oil extracts remained fresh for 6 days. All the four samples were swabbed on 3<sup>rd</sup> and 7<sup>th</sup> day of storage. From Table 2. It was observed that the fish stored at room temperature stayed fresh for only 10 hours. It is because that both enzymatic and microbiological activity are greatly influenced by temperature. The fish stored on ice remained fresh for 2 days whereas those treated with oil extracts and ice were fresh for 7 days.

Table 2. Shelf life of treated and untreated fish samples.

Samples	Shelf life
1) Fish in Room temperature	10 hours
2) Fish + Ice	2 days
3) Fish + Ice + 5ml extract	6 days
4) Fish + Ice + 10ml extract	7 days

**i) Physical changes during storage**

Colour, odour, texture and weight were compared with the fresh and rotten fish. During last day of storage, the colour of the fish become discoloured from natural colour and the texture becomes soft and less firm. Due to longer chilled storage the eyes structure become concave and cloudy and loss of weight was observed which may be due to leaching of amino acids. The muscle tissue of fishes undergoes spoilage faster than mammalian muscles. Immediately after death, several biochemical and enzymatic changes are activated in seafood muscles. Those changes along with enzymatic and microbial induced activities are involved in the degradation of muscle tissues (Pereira de Abreu *et al.*, 2010). The observed physical changes during storage was given in table 3.

Table 3. Physical changes during storage.

Parameter	Fresh	Rotten
<b>Eyes</b>	Clear, bulging/ Protruding 	Sunken, cloudy 
<b>Skin and Gills</b>	Pink to red, Shiny, Mucus absent 	Discoloured, Excessive mucus 
<b>Odour</b>	Natural, fresh	Fishy, sour, ammonia like smell
<b>Muscle texture</b>	Firm, elastic	Soft and less firm
<b>Weight</b>	20.7 g (approx.)	Weight loss – may be due to leaching of amino acids

**ii) Microbial evaluation**

Generally a nutrient broth when freshly prepared is transparent. When inoculated with bacteria, they divide and grow and hence the broth becomes turbid. The change in turbidity was measured in spectrophotometer. From Table 4. It was clear that the control samples have more microbial growth when compared to the samples (test) with extract. This could be due to the influence of extract with the fish tissues. Thus, the *C.citratus* oil has slowed or prevented the microbial growth during storage.

Table 4. Absorbance of treated and untreated samples

Samples	3 <sup>rd</sup> day (nm)	7 <sup>th</sup> day (nm)
1. Fish in room temp (control)	0.702	-
2. Fish + Ice (control)	0.349	0.738
3. Fish + Ice + 5ml extract	0.151	0.201
4. Fish + Ice + 10ml extract	0.141	0.169

Application of plant oil extract as preservative had shown to extend the shelf life of anchovy fish. Studies indicated that the fish stored on ice along with the extract had a significant increase in shelf life by 7 days. Thus, the oil of *C.citratus* could perform better in extending the shelf life of fish and hence serve as safer natural preservative.

#### IV. CONCLUSION:

Researchers have identified that *C.citratus* oil possess antidepressant, antioxidant, antiseptic, sedative, nervine, bactericidal, and fungicidal properties (Gardner, 2013). The oil has a promising anticancer activity and causes loss in tumor cell viability by activating the apoptotic process as identified by electron microscopy (Parduman Sharma. 2008).

In the present study, the *C.citratus* essential oil extracts were used to find its effectiveness in sea food preservation. The compound characterisation studies showed the presence of various bioactive compounds and its use in food preservation and therapeutics. The antioxidant activities was carried out and the Total phenol and Flavonoid content were estimated. The antibacterial activities were also studied against *E.coli*, *B.cereus*, *S.aureus*, *P.aeruginosa* bacteria. The anticancer activity was studied against MCF 7 cells and IC50 value was found. The shelf life extension study was carried out with Indian anchovies and the physical and microbial attributes were evaluated.

Our study revealed that *C.citratus* essential oil could be used as natural preservative thus replacing the synthetic chemical additives. Though much more works are still required in order to understand better about the exact mechanism of action of *C.citratus* oil and their main components, the effective dose, and the best combination strategy.

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