Extraction of Gingerol from Ginger Rhizome Using Co-Solvent Method and Its Antimicrobial Activity against Biofilm Forming Pathogens

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Abstract: Medicinal plants include a various types of plants used in herbalism and some of these plants have a medicinal activities .These medicinal plants consider a rich resources of ingredients, which can be used in drug development and synthesis. Moreover, some plants consider as important sources of nutrition and as result of that, these plants recommended for their therapeutic values.The *Zingeber officinale* or ginger, used as traditional medicine over 2500 years. It has been found to possess a number of pharmachological properties including antimicrobial. The following study was conducted to investigate the antibacterial properties of ginger on foodborne pathogens, which are *S.aureus, B.cereus, E.coli* and *S.typhimurium*. In the present study, the bioactive compounds from *Zingiber officinale* and *Curcuma armada* was extracted the Total phenol and Anti-inflammatory activities were studied. The application of ginger as coating agent on raw chicken on sample and the Shelf Life analysis was calculated and found to be good preservative agent.

Keywords: *Zingeber officinale,* foodborne pathogens, preservative agent

1. INTRODUCTION

Medicinal plants include a various types of plants used in herbalism and some of these plants have a medicinal activities .These medicinal plants consider a rich resources of ingredients which can be used in drug development and synthesis. Moreover some plants consider as important sources of nutrition and as result of that these plants recommended for their therapeutic values .

There are mainly two types of ginger – *Zingiber officinale* (Ginger) and *Curcuma amada* (Mango ginger)

***Zingiber officinale*:**

Zingiber officinale belonging to family a Zingiberaceae used around the world as Spice in culinary , beverages and Indian herbal medicinal practices to treat wide range of disorders . *Zingiber officinale* commonly called Ginger and it is also has various names such as Adrak in Hindi and Urdu, Sunthi in Marathi and many more.The medicinal part of ginger is rhizomes which are used in traditional medicine for treatment of wide range of ailments which have been treated with Ginger include colds , arthritis ,nausea ,hypertension, migraines and many more. *Zingiber officinale* is now widely grown as a commercial crop in south and southeast Asia , Tropical Africa , Latin America. Ginger has been used as a spice and medicine in India and China since ancient times. Ginger is a well known Tropical herbs whose root is used in both Traditional Chinese Medicine and Western Herbal Medicine . It has a long history of medicinal used dating back 2500 years in China and India.

Nutritional composition of fresh ginger contains 80.9% moisture, 2.3% protein, 0.9% fat, 1.2% minerals , 2.4% fibre and 12.3% carbohydrates .The minerals present in ginger are iron, calcium and phosphorous, it also contains vitamins such as thiamine, riboflavin, niacin and vitamin C. Ginger rhizome contains 2 subclasses of constituents. The essential oil, which give the aroma, Gingerol, which considered as main pungent principle. The volatile oil and gingrol and other pungent principles not only give ginger its pungent aroma, but are the most medically powerful because they inhibit prostaglandin and leukotriene formation, which are products that influence blood flow and inflammation.

The gingerols were identified as the most abundant bioactive compound in ginger with various pharmacological effects including antioxidant, analgesic , anti-inflammatory and antipyeric properties .Ginger contains upto 3% of an essential oil that causes the fragnance of the spice. Although ginger essential oils is yellow, the intensity of colour , aroma and

taste varies according to the originated place of cultivation. The pungent taste of ginger is due to non-volatile phenylpropanoid derived compounds Gingerols and Shogaols.The Shogaols are formed from gingerols when ginger is dried or cooked. Zingerone also produced from gingerols during this process.

Zingiber officinale compounds has potential multifunctional bioactivities such as antimicrobial, anti-inflammatory, antioxidant, Total phenolic and many more. *Zingiber officinale* has strong antibacterial activity it showed remarkable antibacterial activity against *Bacillus subtilis, Staphylococcus aureus, Salmonella typhimurium* and to some extent antifungal properties. It can generally inhibit the growth of pathogen that harm human life. Inflammatory plays important role in the body. The anti- inflammatory effects of ginger essential oil were evaluated using Protein denaturation method and Trypsine method. The rhizome are used for the treatment of inflammatory conditions as a household remedy on empirical basis. It was there for decided to screen their extract for anti-inflammatory activity. *Escherichia coli* shows inhibitory action against *Aspergillus niger , Sacharomyces cervisiae* and many more. Free radical as we know it is one factor could promoted many diseases and antioxidant is an important bioactivity due to which it improve our health. Antioxidant activity can be analysed using many methods i.e, DPPH Follin-Ciocalteau , Total phenolic Assay and many more. DPPH is a stable radical and it is commonly used for investigation of antioxidant property. By use of DPPH as an antioxidant assay, the reduction form of DPPH radical become DPPH-H under electron transformation mechanism can be monitored. Antioxidant can protect the human body from free radicals ROS effects and retard the progress of many chronic diseases as *Zingiber officinale* havinh high antioxidant property.A biofilm may be described as a microbial-derived sessile community characteried by cells that adhere to an interface.Presence of pathogenic bacteria in biofilm matrix increases their virulence and resistance to antibiotics. Adhesion of bacteria and biofilm formation is due to many factors such as hydrogen bonding, electrostatic interactions and many more. The essential oil of ginger have been used for preventing biofilm formation by *Pseudomonas aeruginosa , Klebsiella pneumonia.*

Essential oil are used as a medicine with indicators against several problems such as for swelling , sores, loss of appetite , stomach ache, diarrhoea, tooth ache , asthmatic respiratory disorders and in pharmacological potential of ginger extract including preventing blood clots , reducing cholesterol , and triglyceride levels and increasing high density lipoprotein. It also

possessing anti-inflammatory activity.. Food-borne diseases are considered an extremely important health concern risk to the food industry and consumers, mainly due to food contamination caused by two pathogens *Escherichia coli* and *Staphylococcus aureus.* The essential oil has tested for food preservation in chicken by coating the gingerol extract on surface of chicken.

Curcuma amada

Curcuma amada is a unique spice having morphological resemblance with ginger (*zingiber officinale*) but imparts a raw mango (*mangifera indica)*flavour. Curcuma amada is well known as mango ginger. It is a perennital rhizomatous aromatic herb which belongs to the genus Curcuma and family Zingiberaceae. The genus named Curcuma was coined by Linnaeus in 1753 in his species Plantarum. The word probably derives from the Arabic word Kurkum which means yellow colour.

This family is composed of 70-80 species which is found from India to Thailand, Indochina, Malaysia, Indonesia and Northern Australia.Curcuma amada is found in the world parts of West Bengal and is cultivated in Gujarat, Uttarpradhesh,Kerala, Karnataka, Tamilnadu and North Eastern States.Mango ginger contains small amount of fat contents and gives less caloric contents. It is well known for source starch, carbohydrate and certain mineral contents and also contains significant amount of ash, fibre, glycerides and more. Mango ginger has a good aromatic smell due to the presence of essential oil contents and the plant has mango flavouring as the rhizome of mango ginger have the smell of raw mango.Mango flavour is mainly attributed to presence of car-3-ene(3-carene) and cis- ocimene. Among the 68 volatile aroma components present in the essential oli of Mango ginger rhizome.

C. amada has long history of traditional use in folk medicine. In diverse ethnic groups and as an ingredient in culinary preparations in Indian subcontinent. Mango ginger is widely used in food flavour teas and in special dishes as salads. Mango ginger extracted can be potentially used as natural food preservatives to control the growth of food borne pathogens. Curcuma is used medicinally as a coolant, aromatic and astringent and used to promote digestion A rhizome paste has traditionally been used for healing wounds, cuts and itching. The external use of the rhizome paste for sprains and skin diseases is also an old practice. The rhizome has carminative properties as well as being useful as a stomachic. It effectively treats skin allergies, stomach problems and high blood cholestrol.

Curcuma amada has many potential metabolic activity such as Antimicrobial, anti-inflammatory, antioxidant , total phenolic content and many more. It shows good result in these properties as like *Zingiber officinale* and it is good preservative in food industry.

2. AIM AND OBJECTIVE

In the present study the Gingerol was extracted from two Ginger and its application were studied.

- ✓ To study the bioactive property of *Zingiber officinale* and *Curcuma amada*.
- ✓ To find out the compounds using – UV-visible spectrophotometer and Thin Layer Chromatography studies.

- ✓ To evaluate the antioxidant and Anti-inflammatory activity.
- ✓ To check the antimicrobial and Biofilm activity against pathogens.
- ✓ To find out the application of coating agent on chicken and studied the Shelf life analysis

3. METHODOLOGY

3.1 Collection of *Zingiber officinale* and *Curcuma amada*

Healthy fresh rhizomes of *Z.officinale* and mango ginger were purchased from the local market .The material were washed with tap water and they were peeled, and cut into small pieces and it was grinded using masher bowl.

3.2 ISOLATION OF GINGEROLS FROM *Z.officinale* AND *C.amada*

3.2.1 Preparation of Ethanol Extract

10 g of crushed sample of *Z.officinale* and 10g mango ginger each was taken and placed in respective conical flask and 50ml of 95% Ethanol was added to the each conical flask and put on a rotatory shaker at ---- rpm for 24 h. The crude extract were obtained by filtration through muslin cloth into a sterilized conical flask of 100ml and stored. The stored crude was centrifuged at 7000rpm for 10 minutes and pellet was discarded and supernatant was collected and stored in room temperature.

3.2.2 Preparation of Methanol Extract

10 g of crushed sample of *Z.officinale* and 10g mango ginger each was taken and placed in respective conical flask and 50ml of Methanol was added to the each conical flask and put on a rotatory shaker for 24 h. The crude extract were obtained by filtration through muslin cloth into a sterilized conical flask of 100ml and stored. The stored crude was centrifuged at 7000rpm for 10 minutes and pellet was discarded and supernatant was collected and stored in room temperature.

3.3 UV –VIS SPECTROPHOTOMETRIC ANALYSIS

The methanol and ethanol extract of both *Z.officinale* and Mango ginger was examined under UV Visible spectral analysis. The extracts were scanned in the wavelength ranging from 200-800 nm using UV-Visible spectrophotometer and the characteristic peaks were detected. Methanol and Ethanol was used as a blank for respective extract.

3.4 THIN LAYER CHROMATOGRAPHY

Thin Layer Chromatography method was used for the identification of compound. The filtered ethanol and methanol extract of *Z.officinale* and mango Ginger was used for this study. The chromatographic sheet was set with 5cm width and 8cm length and spotted the sample 1cm above from the bottom using capillary tubes (about 50 µl). Sample placed and

this was run in solvent system of methanol, acetic acid, water, chloroform (2:1:2:1). After 1cm from the top of the plate this was taken out from the solvent and dried to visualise the compound, the same method was fused for the TLC study. Rf value was calculated after visualisation of the compound as spot in plate. Rf value calculation formula is ;

$$\text{Rf value} = \frac{\text{Distance moved by solute}}{\text{Distance moved by solvent}}$$

3.5 SHELF LIFE ANALYSIS (Turbidity assay)

Shelf life was estimated in order to check how long the product will remain in presence of the bacteria. 10 ml of LB Broth was prepared and to 2 ml of LB broth , 10 µl of *Escherichia coli* was inoculated. To that 100µl of sample was added and incubated at 37ºC in specific time interval. Then OD was measured at each time interval at 600 nm using colorimeter

3.6 ANTIOXIDANT ACTIVITY

3.6.1 DPPH Assay

Free radical scavenging activity of the extracts was determined by DPPH assay. DPPH solution (0.004%, w/v) was prepared in methanol. Stock solution (1 mg/ml) standard ascorbic acid (0.05 g/ml) were prepared using methanol. 0.5ml of the sample solution and 1ml of DPPH solution was added along with 0.4 ml of 50mM tris Hcl buffer, tube was incubated in dark for 30 minutes and the reading was measured at 517nm using spectrophotometer (LT 291 labtronics microprocessor). Methanol was used as a blank, and the mg/g of the DPPH was calculated by using the ascorbic acid as a standard.

3.7 TOTAL PHENOL CONTENT

Total phenol content was measured by Folin-Coicalteu assay method. The 1ml of the extract was mixed with 0.5ml of 10% folin-ciocalteu reagent and 2ml of the 20% Na_2CO_3 solution, the mixture was allowed to mix and incubated in shaking incubator at 45^0C for 15minutes. After incubation the OD value was measured at 765nm under spectrophotometer. Distilled Water was used as a standard to calculate the mg/g of the phenol content.

3.8 ANTI-INFLAMMATORY ACTIVITY (Patel and Desai, 2016)

3.8.1 TRYPSIN METHOD

The reaction mixture (2 ml) contain 0.06 mg of trypsin, 1 ml 20 mM Tris HCl buffer (pH 7.4) and 1 ml test sample/ standard drug, Diclofenac sodium, of different concentration 100- 600 g/ml. The mixture is incubated at 37°C for 5 min and then 1 ml of 0.8% (w/v) casein was added. The mixture was incubated for an additional 20 min. 2 ml of 70% perchloric acid was added to terminate the reaction. Cloudy suspension was centrifuged at 3000 rpm for 5 minutes , and the absorbance of the supernatant was read at 210 nm against buffer as blank. The percentage inhibition of proteinase inhibitory activity was calculated using the following equation.

Percentage inhibition = (Abs control –Abs sample) X100/ Abs control.

3.8.2 PROTEIN DENATURATION ASSAY

Albumin denaturation assay wascarried out by using the protocol of Sakat et al., 2010. 5 concentrations of the sample from 25, 50, 75, 100, 125mg was dissolved in 1% Bovine serum albumin fraction and incubated at 37oC for 20 minutes followed by incubated at 51oC for 20 minutes in water bath. The tubes were allowed to cool in room temperature and the measurement was taken spectrophotometrically in the nm of 660 using microprocessor spectrophotometer LT 291. The experiment was performed in triplicate and percentage of inhibition of protein denaturation was calculated.

$$\%\ of\ inhibition = \frac{Absorbance\ of\ control\ OD - Absorbance\ of\ sample\ OD}{Absorbance\ of\ control\ OD}\ X100$$

3.9 ANTIBACTERIAL ACTIVITY

Antibacterial activity of the sample was identified by using well diffusion method against the bacteria. Mueller hinton agar (39gm in 1000ml) was prepared and swabbed 70µl of the bacterial culture (*Bacillus subtilis*, *Staphylococcus aureus* ,*Salmonella typhi*) using cotton swab and well were made with corck borer followed by the sample was added (50µl). Antibiotic disc (Amoxycillin / Sulbactan 30/1530mcg) was placed as a positive control, the plate was incubated 37^0C for 24 hrs. After incubation anti bacterial activity of the sample was measured based on the zone of inhibition in mm.

3.10 BIOFILM ASSAY

The effect of extracts on biofilm was evaluated in 96-well polystyrene flat bottom plates. Briefly, 300µl of inoculated fresh trypticase soya yeast broth (TSY)(final concentration 10^6 CFU/mL) was aliquoted into each well of microplate and cultured in presesnce of sublethal concentrations (75,50, and 25% of MBC) previously determined . Wells containing medium and those without extracts and only with methanol were used as controls. Plates were incubated at 37ºC for 48h. After incubation, supernatant was removed and each well was washed thoroughly witth sterile distilled water to remove free-floating cells: thereafter plated were air-dried for 30min and the biofilm formed was stained during 15 min at room temperature with 0.1% aqueous solution of crystal violet. Following incubation, the excess of stain was removed washing the plate three times with sterile distilled water. Finally , the dye bound to the cells was solubilized by adding 250µl of 95% ethanol to each well and after 15min of incubation , absorbance was measured using microplate reader at a wavelength of 570 nm. Biofilm determination was made using the formula SBF= (AB – CW)/G , where SBF is the specific biofilm formation, AB is the OD570 n of the attached and stained bacteria, CW is the OD570 nm of the stained control wells containing only bacteria-free medium, and G is the OD630 nm of cell growth in broth.

3.11 GINGEROL AS BIOPRESERVATIVE IN CHICKEN

The chicken used in the experiment was purchased from the market. Three pieces of chicken was taken and placed in sterile petri plates. 40 µl of sample was added to the surface of chicken and incubated for 24 hours at room temperature. After 24 hours , surface of the chicken was swabbed using sterile cotton swab and it was inoculated into 10 ml nutrient broth and incubated for 24hours at 37ºC. on the next day OD vales are measured using colorimeter. This procedure was repeated for 4 days.

4. RESULT AND DISCUSSION:

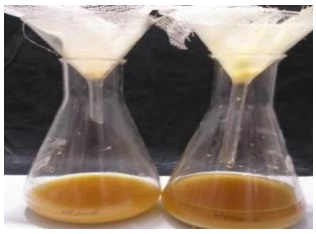
4.1. PEPARATION OF SAMPLE:

4.1.1 *Zzigiber officinale*

1)Fresh ginger purchased from market & washed

2)Skin was peeled off and crushed

3) Preparation of ethanol methanol extract



4)Filteration and Centrifugation

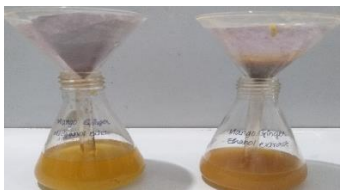
5) Extracted Gingerol

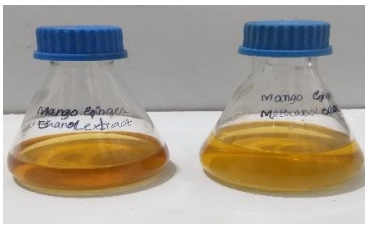
Fig 1: Preparation of sample (*Zigiber officinale)*

4.1.2 *Curcuma amada*

1)Fresh mango ginger purchased

2)Crushed mango ginger





3)Filteration and centrifugation

4)Extracted gingerol

Fig 2: Preparation of sample (*Curcuma amada)*

4.2 UV VIS SPECTROPHOTOMETER ANALYSIS :

4.2.1 Zingiber officinale – Methanol extract

Table 1: UV spectrophotometer readings (*Z.officinale)*

SL NO.	COMPOUND	nm

I	Compound 1	205.0 nm
II	Compound 2	215.0 nm
III	Compound 3	225.0 nm
IV	Compound 4	235. 0 nm
V	Compound 5	250.0 nm
VI	Compound 6	275.0 nm
VII	Compound 7	325.0 nm
VIII	Compound 8	360.0 nm

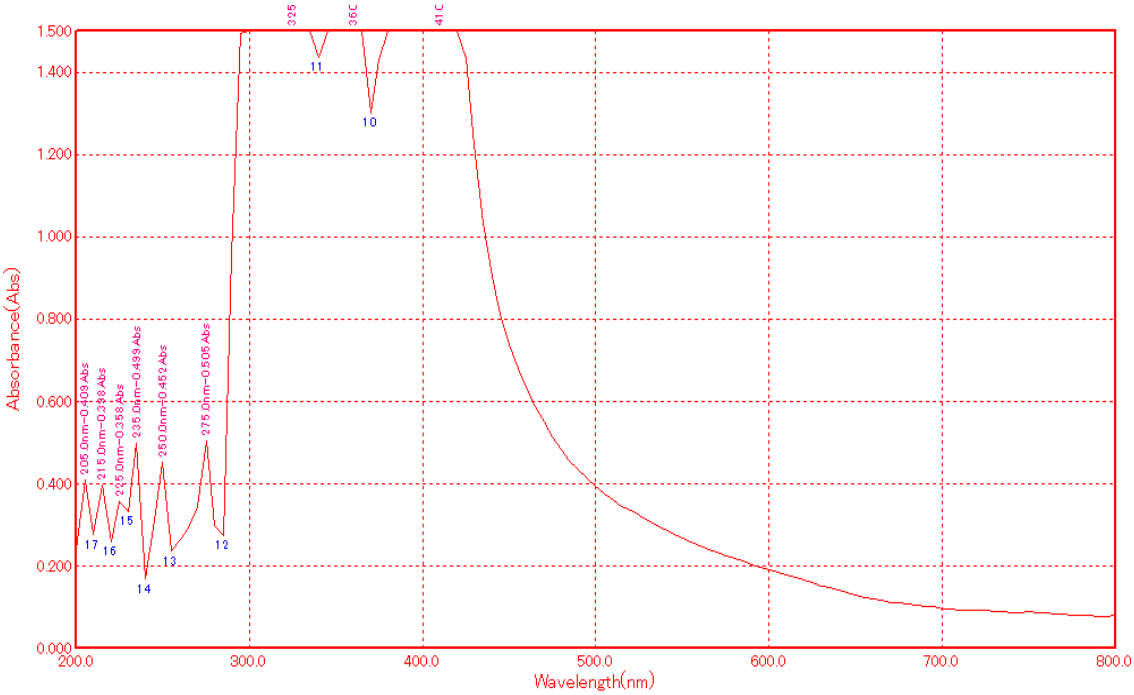


Fig 3: Graph showing *Z.officinale* Peaks

4.2.2 Curcuma amada

Table 2: Showing UV Spectrophotometer reading for *Curcuma amada*

SL NO.	COMPOUND	nm

I	Compound 1	205.0 nm
II	Compound 2	215.0 nm
III	Compound 3	230.0 nm
IV	Compound 4	250. 0 nm
V	Compound 5	270.0 nm
VI	Compound 6	355.0 nm
VII	Compound 7	385.0 nm
VIII	Compound 8	715.0 nm

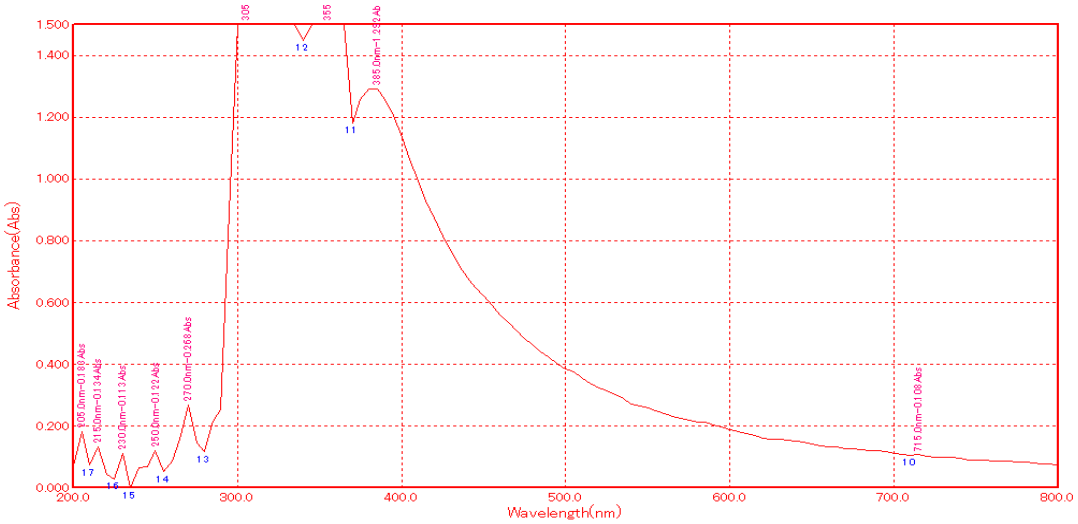


Fig 4: Graph showing *C.amada* Peaks

4.3 THIN LAYER CHROMATOGRAPHY:

Gingerol is analysed for retention factor. Gingerol is separated using Thin Layer chromatography. The components can be separately visible by exposure to iodine vapours to the TLC plate. The iodine vapours will change the colour to yellow where the components are present.

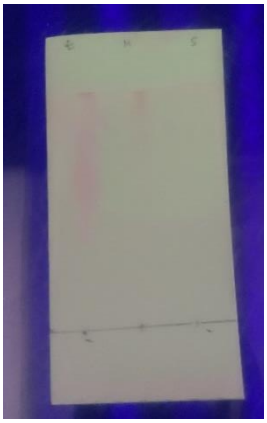


Fig 5: Standard Gingerol in TLC Plate

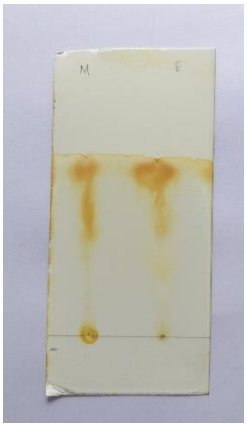


Fig6: TLC plate of *Zingiber officinale*

Fig 7 : TLC plate of *Curcuma amada*

Table 3: Rf value for Ethanol and Methanol extracts of *Zingiber officinale*

Solution	Solvent Front Height(cm)	No. of spots	Spot height(cm)	Rf value
Ethanol Extract	6.7	1	4.6	0.65
Methanol Extract	6.7	1	4.4	0.68

Table 4: Rf value for Ethanol and Methanol extracts of *Curcuma amada*

Solution	Solvent Front Height(cm)	No. of Spots	Spot Height(cm)	Rf value
Ethanol extract	7.5	1	5.5	0.73
Methanol Extract	7.5	1	5.4	0.72

According to the Rf value obtained it was found that Ethanol extract (Rf = 0.68 , 0.73) of both the ginger shows best result . Comparing *Z.offinale* and *C.amada* it shows that *C.amada* is the best.

4.4 SHELF LIFE ANALYSIS

Table 5: Shelf life analysis of the sample

SL NO.	TIME INTERVALS	OD VALUES	
		Z.officinale	*C.amada*
1	1 hour	0.72	0.68
2	2 hour	0.74	0.78
3	3 hour	0.74	0.78
4	24 hour	0.63	0.72
5	Control	1.63	2.41

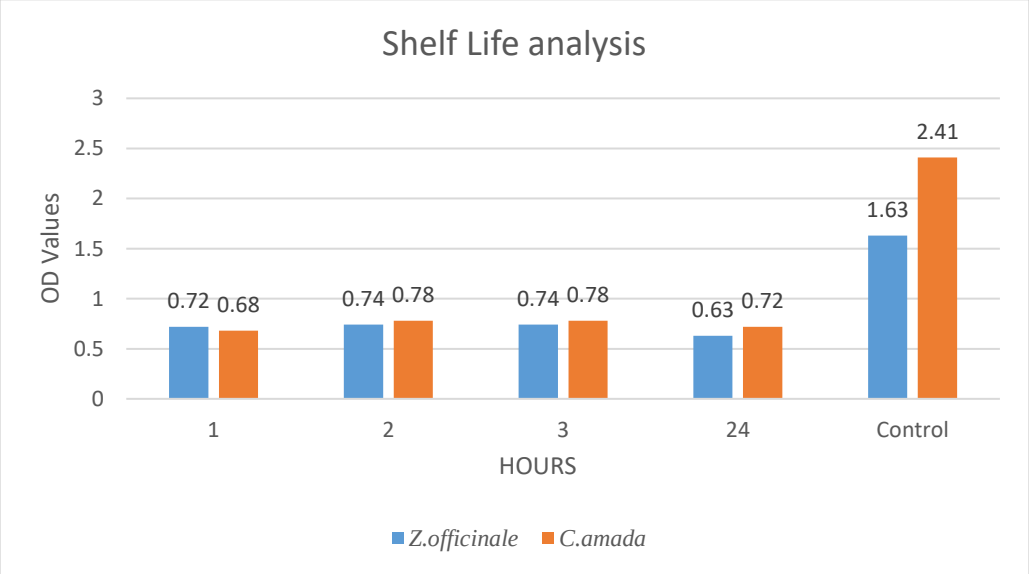


Fig 8: Shelf life analysis of the sample in the presence of bacteria

The shelf life analysis shows how long the product will remain in the presence of bacteria. The result shows that *Z.officinale* and *C.amada* remains stable for 2nd hr and 3rd hr but after 24 hours the shelf life of both the sample get declined.

4.5 DETERMINTION OF DPPH RADICAL SCAVENGING ACTIVITY:

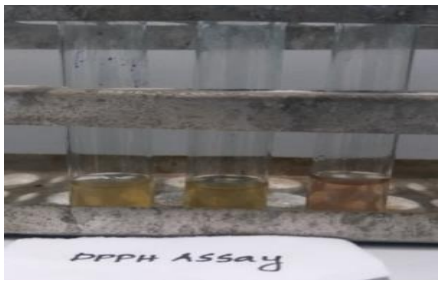


Fig 9: DPPH ASSAY

The antioxidant activity of *Z.officinale* ethanol extract was better than *C.amada* extract for all equivalent solvent. The antioxidant activity was highest in Ethanol followed by methanol.For, *Z.officinale* ethanol extract showed the highest antioxidant activity with 1.036 followed by methanol 0.710. For *C.amada* Methanol extract showed the highest antioxidant activity with 0.130 followed by ethanol 0.124.

Table 6: DPPH Assay values:

SL NO.	SAMPLE	METHANOL EXTRACT	ETHANOL EXTRACT	CONTROL
1	*Z.officinale*	0.710	1.036	0.501
2	*C.amada*	0.130	0.124	0.501

4.6 DETERMINATION OF TOTAL PHENOLIC CONTENT(TPC)

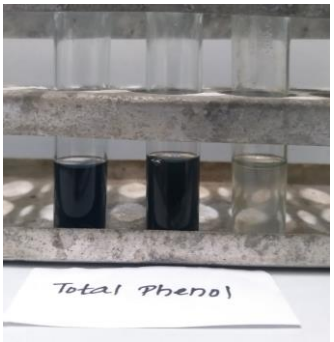


Fig 10. Total Phenolic Assay

Table 7: Total phenolic content readings

SL NO.	SAMPLE	METHANOL EXTRACT	ETHANOL EXTRACT	CONTROL
1	*Z.oficinale*	1.5	1.5	0.214
2	*C.amada*	0.614	1.234	0.214

For ethanol extracts values for *Z.officinale* and *C.amada* were 1.5 and 1.234 respectively. For methanol extract values for *Z.officinale* and *C.amada* were 1.5 and 0.614 respectively. All *Z.officinale* were significantly better result when compared to the *C.amada* . Ethanol Extract of *Zingiber officinale* gave the highest TPC reading.

4.7 ANTI INFLAMMATORY:

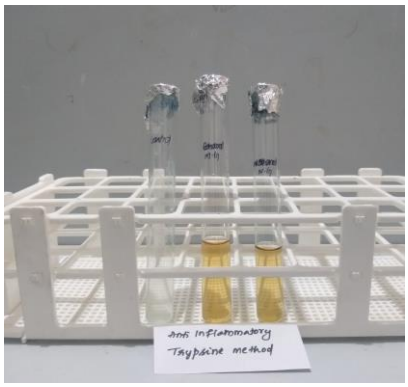


Fig 11 : Trypsin method

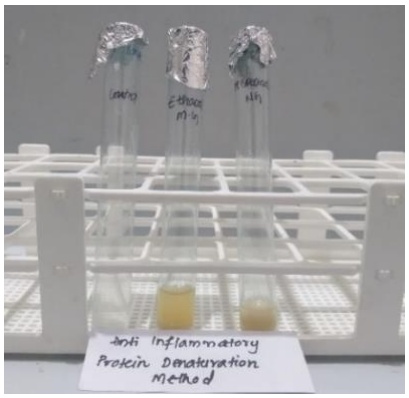


Fig 12: Potein denaturation method

I.TRYPSINE METHOD:

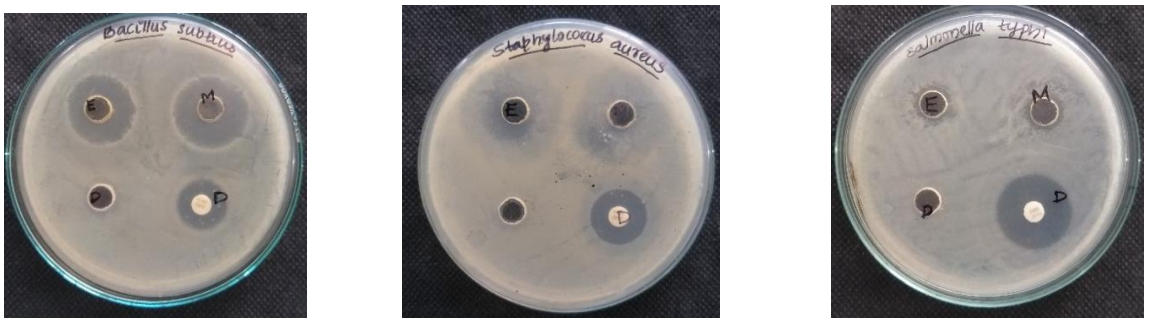
- *Z.officinale* of Methanol extract - 0.137
- *C.Amada* of Ethanol extract - 0.135
- Control - 0.314

II.PROTEIN DENATURE METHOD:

- *Z.officinale* of Methanol extract - 0.137
- *C.amada* of Ethanol extract - 0.135
- Control - 2.16

In trypsin method and protein denaturation method *Z.officinale* of methanol extract shows the best result compared with *C.amada*.. *Z.officinale* of 0.137 for both the method.

4.8 ANTIBACTERIAL ACTIVITY:



1)*Bacillus subtilis* **3)*Staphylococcus aureus*** **3) *Salmonella typhi***

Fig 13: Antibacterial Activity

Table 8: Antibacterial activity measurement

SL NO.	ORGANISM	ETHANOL EXTRACT	METHANOL EXTRACT	DISC	DISTILLED WATER
1	*Bacillus subtilis*	0.8mm	0.9mm	0.5mm	nil
2	*Staphylococcus aureus*	10mm	11mm	0.5mm	nil
3	*Salmonella typhi*	0.2mm	0.2mm	0.8mm	nil

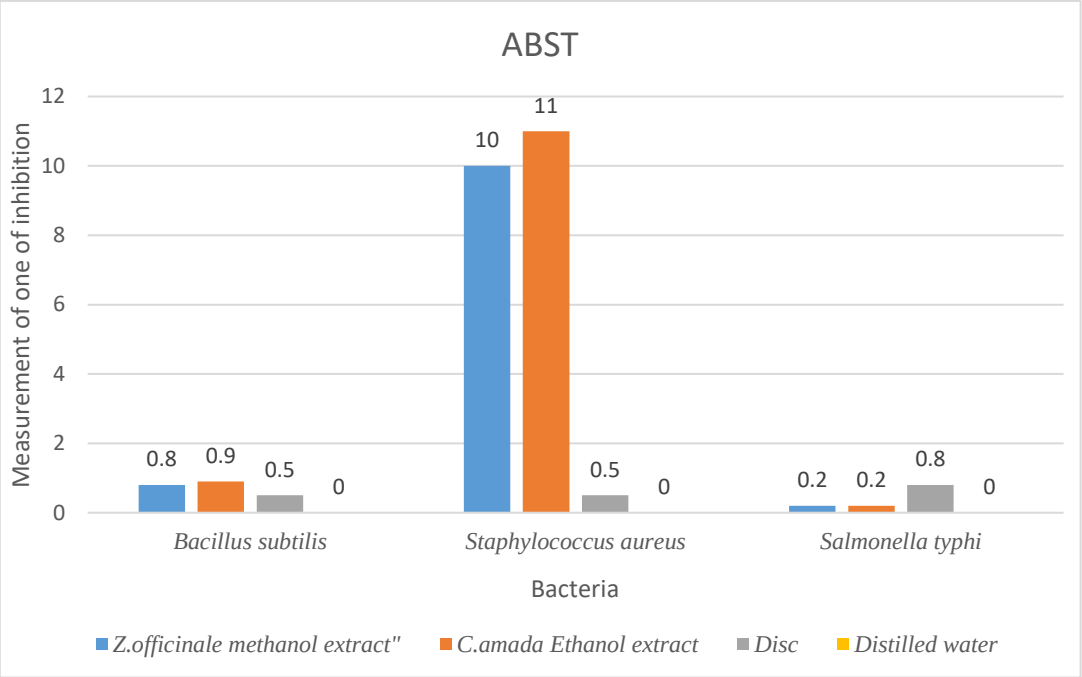


Fig 14:ABST Graph

Antimicrobial sensitivity Test is used to determine the efficacy of potential antimicrobials from *Z.officinale* extracts against *Bacillus subtilis, Staphylococcus aureus* and *Salmonella typhi.* Methanol extract showed higher result compared to ethanol extract. Antimicrobial activity showed more active against *Staphylococcus aureus* .

4.9 BIOFILM ASSAY:

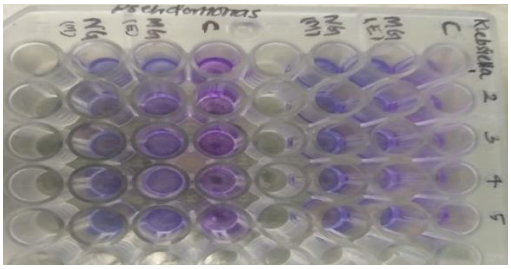


Table 9:showing biofilm for *Klebsilla pnuemoniae*

SL NO.	SAMPLE	DILUTIONS				
		50	100	150	200	250
1	*C.amada*	0.603	0.524	0.394	0.246	0.191
2	*Z.officinale*	0.520	0.513	0.305	0.202	0.192
3	Control	0.817	0.847	0.887	0.887	0.857

Table 10: showing biofilm for *Pseudomonas aeruginosa*

SL NO.	SAMPLE	DILUTIONS				
		50	100	150	200	250
1	*C.amada*	0.644	0.629	0.524	0.311	0.280
2	*Z.officinale*	0.681	0.529	0.420	0.311	0.218
3	Control	0.796	0.789	0.765	0.762	0.739

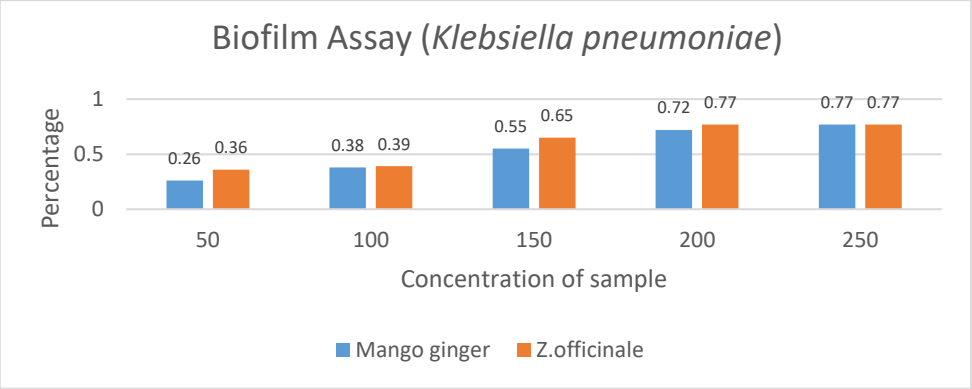


Fig 15: Biofilm Assay (*Klebsiella Pnuemoniae)*

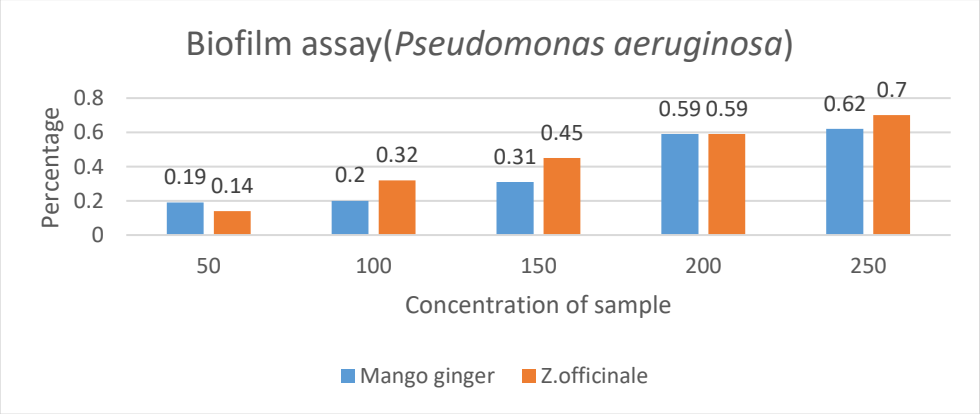


Fig 16: Biofilm Assay (*Pseudomonas aeruginosa)*

4.10 GINGEROL AS BIOPRESERVATIVE IN CHICKEN:

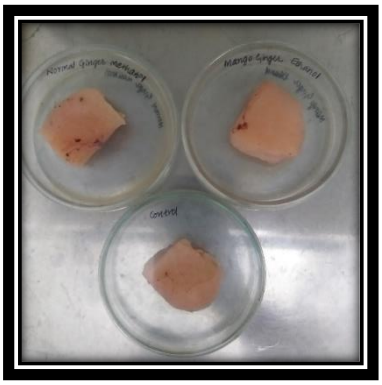


Fig 17:Sample Coated chicken

Fig 18: Shelf Life Analysis (Swab method)

Table 11: Readings of Shelf life analysis

SL NO.	DAYS	OD VALUES		
		CONTROL	*Z.officinale*	*C.amada*
1	I	1.22	1.05	0.74
2	II	0.76	0.66	0.59
3	III	0.91	0.69	0.57
4	IV	1.25	0.77	0.68

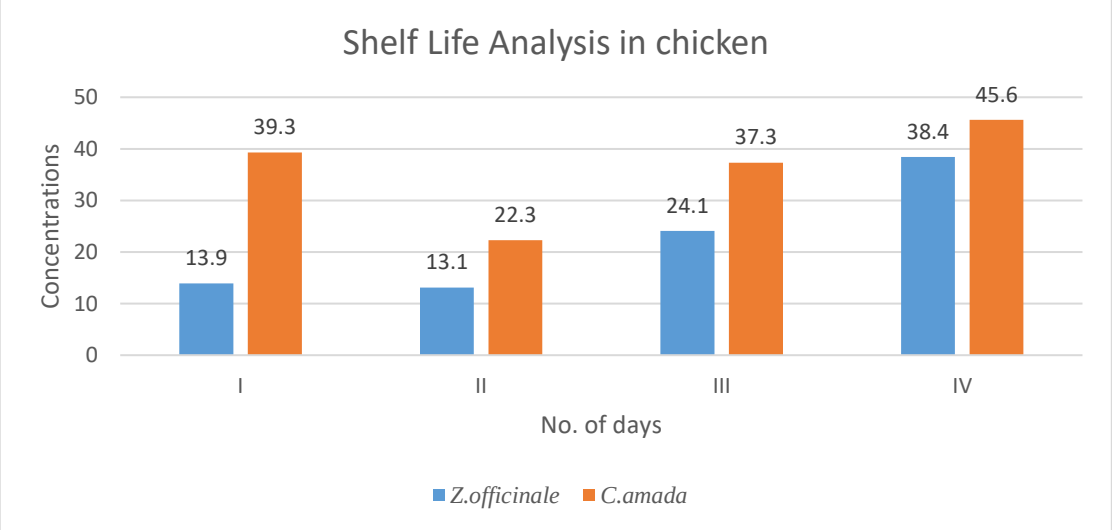


Fig 19: Graph showing shelf life analysis

According to the graph, *Z.officinale* shows high shelf life on 4th day while *C.amada* shows high shelf life on 1st and 4th day. This indicate that the *C.amada* shows best result in cimoare with *Z.officinale.*

SUMMARY AND CONCLUSION

In the present study the bioactive compounds from *Zingiber officinale* and *Curcuma amada* was extracted the Total phenol and Anti-inflammatory activities were studied It shows best results for Total Phenol and Anti-Inflammatory. The UV-VIS , study confirmed the presence of various compounds and the peak at 200-800 nm confirmed the analysis also confirmed the compound (Rf value std 0.72; sample 0.72 & 0.68).The antibacterial study against bacteria and found more active against *S.aureus*. The application of ginger as coating agent on raw chicken on sample and the Shelf Life analysis was calculated and found to be good preservative agent.

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