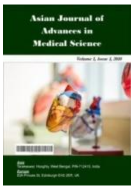
Asian Journal of Advances in Medical Science

2(1): 110-117, 2020



COMPARATIVE STUDY OF ANTIMICROBIAL ACTIVITY ON FRESH AND DRIED *Zingiber officinale* Rosc

G. GAYATHRI[1*], S. GOMATHI[1], V. AMBIKAPATHY[1] AND A. PANNEERSELVAM[1]

[1]Department of Botany and Microbiology, A.V.V.M. Sri Pushpam College (Autonomous) Poondi, Thanjavur, Tamilnadu, India.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Received: 20 October 2020
Accepted: 24 December 2020
Published: 11 January 2021

Original Research Article

ABSTRACT

The present study to investigate the antimicrobial activity, from rhizome fresh and dried *Zingiber officinale* Rosc. In the present study to observe the antibacterial activity using the microorganisms such as *E. coli*, *Staphylococcus aureus*, *K. pneumoniae* and *Pseudomonas aeroginosa* were studied by using disc diffusion method. The maximum zone of inhibition were observed in *K. pneumoniae* (25 mm), followed by *Staphylococcus aureus* (24 mm), *Pseudomonas aeroginosa* and *E. coli* each showed 22 mm. The antifungal activity carried out by using the microorganisms *Aspergillus flavus*, *A. terreus*, *Penicillum* sp and *Fusarium* sp were studied by using agar well diffusion method. The maximum zone of inhibition were observed at the concentration of 100 µg of fresh sample against *Fusarium* sp (14 mm) followed by *A. flavus* (12 mm), *A. terreus* (10 mm) and *Penicillum* sp (10 mm).

Keywords: *Zingiber officinale* Rosc; rhizome fresh and dried; antibacterial; antifungal activity.

1. INTRODUCTION

Herbs and plants have been in use as a source of therapeutic compounds in traditional medicinal system since ancient time. Medicinal plants play an important role in traditional heath care systems as well as in international herbals and pharmaceutical markets. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body [1].

Medicinal plants are generally known as Chemical Goldmines‖ as they contain natural chemicals, which are acceptable to human and animal systems. All these chemicals cannot be synthesized in laboratories. Many secondary metabolites of plant are commercially important and find use in a number of pharmaceutical compounds [2]. Human beings have been dependent on plants for their health care needs since the beginning of civilization. of the 2,50000 higher plant species on earth, more than 80,000 are medicinal in Nature. Ginger scientifically known as *Zingiber officinale* Roscoe, belonging to family Zingiberaceae is one of the most important plant with several medicinal, nutritional and ethanomedical values therefore, used extensively worldwide as a spice, flavoring agent and herbal remedy. Traditionally, *Z. officinale* is used in Ayurveda, Siddha, Chinese, Arabian, Africans, Caribbean and many other medicinal systems to cure a variety of diseases viz, nausea, vomiting, asthma, cough, palpitation, inflammation, dyspepsia, loss of appetite,

**Corresponding author: Email: gayuganesan07@gmail.com;*

constipation, indigestion and pain Species from Zingiberaceae family have been widely used as spices. Ginger (*Zingiber officinale* Rosc) is used in traditional oriental medicine for common cold, digestive disorders, and rheumatism [3].

Traditionally, *Z. Officinale* is used in Chinese, Arabian, Africans, India and many other traditional systems to cure a variety of diseases viz., nausea, vomiting, asthma, palpitation, inflammation, dyspepsia, loss of appetite, constipation, digestion and pain. In last few decades, *Z. Officinale* is extensively studied for its medicinal properties by advanced scientific techniques and a variety of several compounds has been isolated from the different parts of the plants and analysed pharmacologically. The plant is reported for antimicrobial activity anticancerigenous, antioxidative, antidiabetic activity, hepatoprotective activity, and anti-inflammatory activity and immunomodulatory activities. The purpose of this study is to evaluate the phytochemical characterization of *Zingiber officinale* with hexane extracts with ethyl acetate, methanol, and ethanol. On the other hand, evaluate antimicrobial activities of its essential oil [3]. The increased usage of antibiotics has induced microorganisms to acquire resistance factors which have become a burning predicament. As a result there is an urgent need to find the alternative of chemotherapeutic drugs in diseases treatment particularly those of plant origin which are easily available and have considerably less side effects. The antimicrobial activity of spices is due to certain phytochemicals or essential oils present in ginger [4].

Antimicrobials are substances with the capacity to selectively inhibit or kill microorganisms [5]. Unfortunately, humans to develop the bacterial multi-resistance to antibiotic treatments that were originally effective for the treatment of infection caused by that microorganism. Misuse of antibiotics has resulted in the emergence of resistance against them, which is another problem affecting public health [6,7]. Bacteria have a remarkable ability to adapt to adverse environmental conditions [8] that lead to the emergence of resistant bacteria, which is recognized as a major problem in the treatment of microbial infections in hospitals and in the community [9]. Resistance to antibiotics is time-consuming generating a problem of global public health. Many studies show that pathogenic bacteria are increasing and becoming multi-resistant. Therefore, the search for new preventive measures to slow down this process is necessary to overcome this public health problem [10]. An alternative to antibiotics commonly used in medicine may be natural products of plant origin widely distributed in nature [11]. In the Mediterranean region, *Rosmarinus officinalis* and *Salvia sclarea* (Lamiaceae), *Zingiber officinale* (zingiberaceae), *Melaleuca alternifolia* and *Syzygium aromaticum* (Myrtaceae) and *Cymbopogon winterianus* (Poaceae) have been studied extensively for their anti-inflammatory, anticancer, anticholinesterase and radical scavenging activities [12,13,14,15].

Fresh as well as dried forms of ginger have been used both in medicine and in culinary for flavor and pungency. India is the largest producer (380.0 thousand tonnes) and consumer of ginger and contributing 35% of the world production. Antioxidants from natural resources are associated with health benefits against heart diseases, malaria, neuro-degenerative diseases, AIDS, cancer and longevity. Solvent extraction is the commonly used method for the extraction of bioactive components from plant sources. The selection of solvent system for the extraction will depend on the purpose of extraction, nature of the compounds, safety concerns and soon. The antioxidant-enriched fraction from ginger and to evaluate antioxidant potential. Ginger extract was prepared from dried ginger and antioxidants were enriched using solvent partition. Extracts and fractions were evaluated for their antioxidant potential in different *in vitro* model systems.

Systematic position	
Division	Monocotyledons
Order	Zingiberales
Family	Zingiberaceae
Genus	*Zingiber*
Species	*officinale* Roscoe.

2. METRIALS AND METHODS

2.1 Collection of Sample

The fresh plant materials rhizome of *Zingiber officinale* Rosc. were collected from Thanjavur District, Tamil Nadu.

2.2 Preparation of Sample

The collected ginger samples were air dried. After air dried the sample was ground in grinding machine made for the laboratory. Exposure direct sunlight was avoided to prevent the loss of active components. These powdered materials were used for further analysis.

2.3 Determination of Antimicrobial Activity (Perez et al., 1990)

2.3.1. Test microorganisms

The following bacterial and fungal strains were used for the screening of antimicrobial activity. All the microbial strains of human pathogens used were procured from IMTECH, Chandigarh and procured microbes are the Gram – negative bacteria, viz. *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeroginosa* and the Gram – positive bacteria, *Bacillus cerres* and *Staphylococcus aureus*, and fungi viz., *Aspergillus flavus*, *A. niger*, *A. terreus*, *Fusarium* sp, and *Pencillium* sp were selected for this study.

2.3.2 Agar well – diffusion method

Agar well – diffusion method was followed to determined the antimicrobial activity. Nutrient agar (NA) and Potato Dextrose Agar (PDA) plates were swabbed (sterile cotton swabs) with 24 hours culture and 48 hours old – broth culture of respective bacteria and fungi. Agar wells (5 mm diameter) were made in each of these plates using sterile cork borer. About 100µl of different solvent leaf extracts were added using sterilized dropping pipettes into the wells and plates were left for 1 hour to allow a period of pre – incubation diffusion in order to minimize the effects of variation in time between the applications of different solutions the plates were incubated in an upright position at 37°C ± 2°C for 24 h for bacterial pathogens and 28°C ± 2°C for fungi. The organic solvents alone were acted as a negative control. Results were recorded, as the presence or absence of inhibition zone. The inhibitory zone around the well indicated absence of tested organism and it was reported as positive and absence of zone is negative. The diameters of the zones were measured using diameter measurement scale. The effect of plant extract was compared with standard antibiotics. Triplicates were maintained and the average values were recorded for antimicrobial activity.

2.3.3 Media used

Nutrient Agar (NA) and Potato Dextrose Agar (PDA) were used for testing the antibacterial and antifungal activity.

2.3.3.1 Composition of nutrient agar (g/l)

Ingredients	g / Litre
Peptone	- 5.0 g
Beef extract	- 3.0 g
Sodium chloride	- 5.0g
Agar	- 15.0 g
Distilled water	- 1000 ml
Final Ph	- 7.0 ± 0.2

2.3.3.2 Composition of potato dextrose agar (g/l)

Ingredients	g / Litre
Potato Infusion	- 200 g
Dextrose	- 20.0 g
Agar	- 20.0g
Distilled water	-1000 ml
Final Ph	- 5.5 ± 0.5

3. RESULTS

3.1 Antibacterial Activity of Dried Sample

Antibacterial activity of dried sample of *Zingiber officinale* against some bacterial species such as *E. coli, Staphylococcus aureus, Klebsiella pnemoniae* and *Pseudomonas aeroginosa* were studied by using disc diffusion method. The maximum zone of inhibition were observed in *E. coli* (17 mm) followed by *Pseudomonas aeroginosa* (16 mm) *Staphylococcus aureus* (15 mm) and *Klebsiella pnemoniae* (14 mm) at the concentration of 100µg of dried sample. Increased concentration of the sample, the zone of inhibition increased and decreased concentration, the zone of inhibition also decreased against the bacterial species (Table 1).

3.2 Antibacterial Activity of Fresh Sample

Antibacterial activity of fresh sample of *Zingiber officinale* against some bacterial species such as *E. coli*, *Staphylococcus aureus*, *K. pneumoniae* and *Pseudomonas aeroginosa* were studied by using disc diffusion method. The maximum zone of inhibition were observed in *K. pneumoniae* (25 mm), followed by *Staphylococcus aureus* (24 mm), *Pseudomonas aeroginosa* and *E. coli* each showed 22mm. Increased concentration of the sample, the zone of inhibition also increased and decreased concentration the zone of inhibition are decreased against the bacterial species. Fresh and dried sample used to test the antibacterial activity, the maximum zone of inhibition were observed in fresh sample when compared to dried sample of *Zingiber officinale* (Table 2).

3.3 Antifungal Activity of Dried Sample

Antifungal activity of dried sample of *Zingiber officinale* at the concentration of 25,50,75 and 100µg against some fungal species such as *Aspergillus flavus*, *A.terreus*, *Penicillum* sp and *Fusarium* sp were studied by using agar well diffusion method. The maximum zone of inhibition were observed at the concentration of 100 µg of dried sample against *Fusarium* sp (19 mm), followed by *A. terreus* (12 mm), *Penicillum* sp (10 mm) and

Aspergillus flavus (9 mm). The minimum zone of inhibition were observed at the concentration of 25µg of dried sample against *Fusarium* (10 mm) and N0 zone of inhibition were observed against *A. flavus*, *A.terreus* and *Penicillum* sp respectively (Table 3).

3.4 Antifungal Activity of Fresh Sample

Antifungal activity of fresh sample of *Zingiber officinale* at the concentration of 25,50,75 and 100 µg against some fungal species such as *A. flavus*, *A. terreus*, *Penicillum* sp and *Fusarium* sp were studied by using agar well diffusion method. The maximum zone of inhibition were observed at the concentration of 100 µg of fresh sample against *Fusarium* sp (14 mm) followed by *A. flavus* (12 mm), *A.terreus* (10 mm) and *Penicillum* sp (10 mm). The minimum zone of inhibition was observed at the concentration of 25 µg fresh sample against *Fusarium* (8 mm) and *A. terreus* (7 mm). No zone of inhibition was observed against *A. flavus* and *Penicillium* sp respectively (Table.4). The values were recorded for antimicrobial activity.

Table 1. Antibacterial activity of dried sample of *Zingiber officinale* Rosc. against some bacteria

S. No	Name of the bacteria	Zone of inhibition (mm)			
		25 µg	50 µg	75 µg	100 µg
1	*Escherchia coli*	10	12	15	17
2	*Klebsiella pneumonia*	-	7	12	14
3	*Pseudomonas aeroginasa*	5	8	12	16
4	*Staphylococcus aureus*	10	12	15	17

Table 2. Antibacterial activity of fresh sample *Zingiber officinale* Rosc. against some bacteria

S. No	Name of the bacteria	Zone of inhibition (mm)			
		25 µg	50 µg	75 µg	100 µg
1	*Escherchia coli*	18	19	20	22
2	*Klebsiella pneumonia*	22	24	25	25
3	*Pseudomonas aeroginasa*	19	21	21	22
4	*Staphylococcus aureus*	20	23	23	24

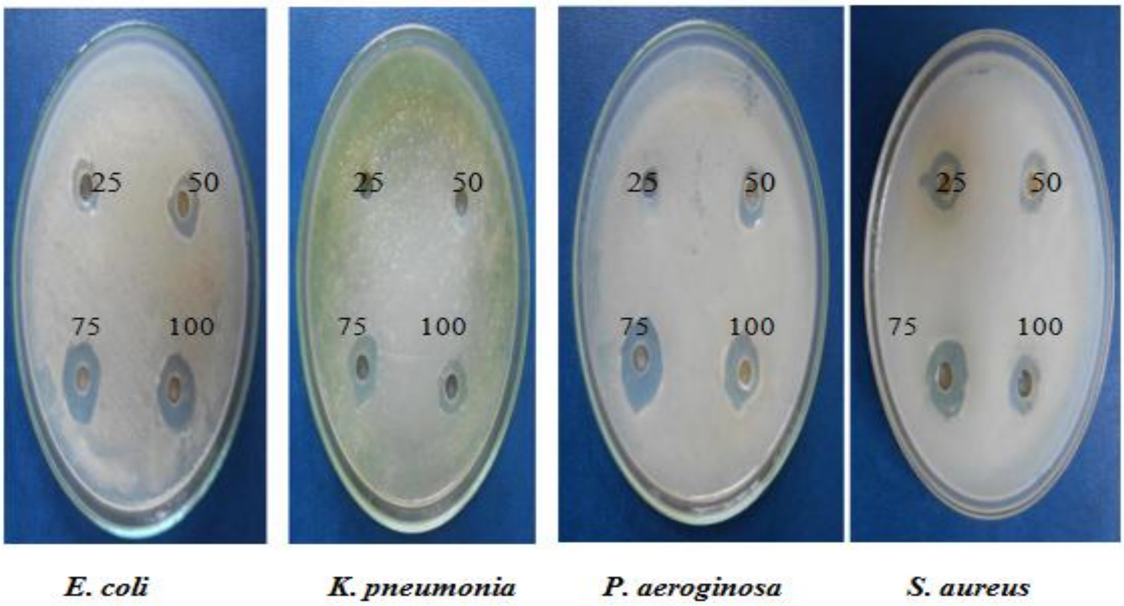


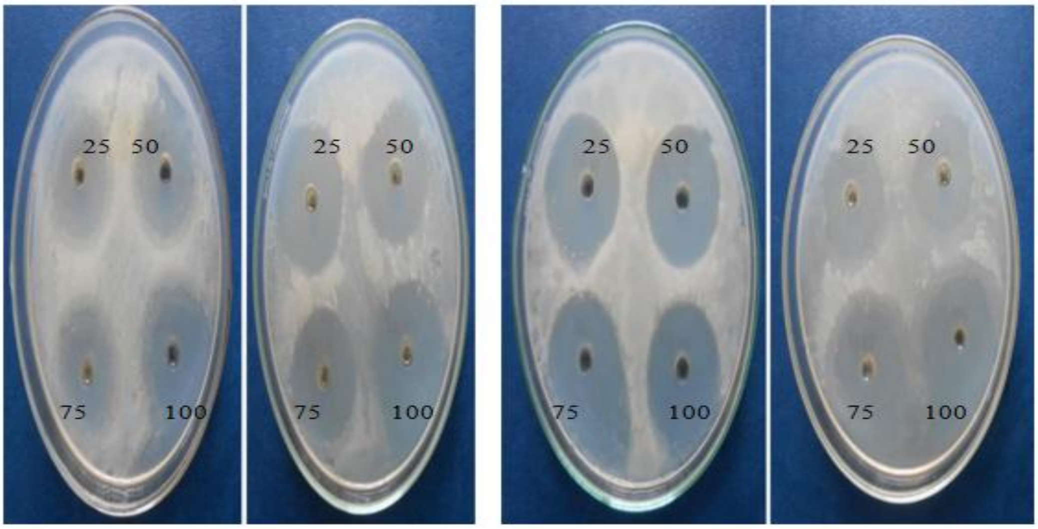
Plate 1. Antibacterial activity of dried sample in *Zingiber officinale* Rosc.

Table 3. Antifungal activity of dried sample of *Zingiber officinale* Rosc. against some fungi

S. No	Name of the fungi	Zone of inhibition (mm)			
		25 µg	50 µg	75 µg	100 µg
1	*Aspergillus flavus*	-	-	8	9
2	*A. terreus*	-	9	11	12
3	*Penicillium* sp.	-	-	9	10
4	*Fusarium* sp.	10	14	15	19

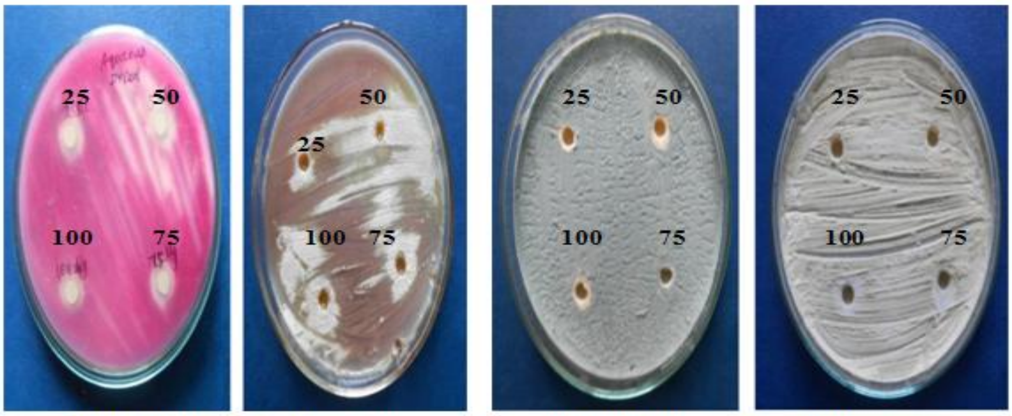
Table 4. Antifungal activity of fresh sample *Zingiber officinale* Rosc. against some fungi

S. No	Name of the fungi	Zone of inhibition (mm)			
		25 µg	50 µg	75 µg	100 µg
1	*Aspergillus flavus*	-	-	11	12
2	*A. terreus*	7	8	8	10
3	*Penicillium* sp	-	-	9	10
4	*Fusarium* sp	8	9	10	14



E. coli *K. pneumonia* *P. aeroginosa* *S. aureus*

Plate 2. Antibacterial activity of fresh sample in *Zingiber officinale* Rosc



Fsarium sp *A. terreus* *Penicillum* sp *A. flavus*

Plate 3. Antifungal activity of dried sample in *Zingiber officinale* Rosc

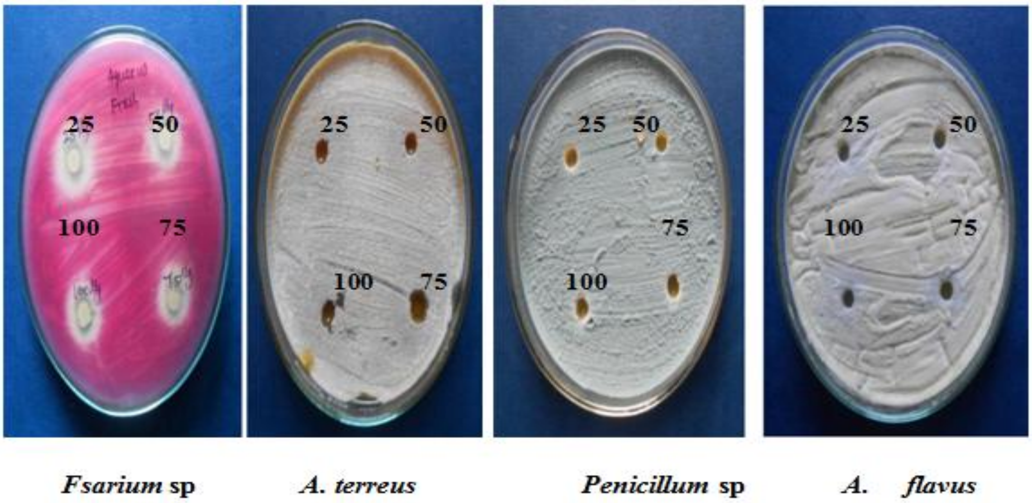


Plate 4. Antifungal activity of fresh sample in *Zingiber officinale* Rosc

4. DISCUSSION

The activity studies show that fresh ginger oil (FG) was on par with standard antibiotic against Aspergi*llus niger, candida* and *Pseudomonas aeruginosa*, weaker towards *saccharomyces cerevisiae* and inactive against *Bacillus subtilis*, *Pencillium* sp. and *Trichoderma* sp. Dry ginger oil (DG) was more active towards *Pseudomonas aeruginosa* on par with standard towards *Candida*, weaker than standard against *Bacillus subtilis, Aspergillus niger, Pencillium spp, Saccharomyces cereviseae*. The composition of fresh ginger oil shows that it contains more of oxygenated compounds (29%) compared to dry ginger oil (14%).The higher content of geranial and other oxygenated compounds makes fresh ginger oil more potent than dry ginger oil. The content of hydrocarbon compounds are more in dry ginger oil compared to fresh ginger oil. Earlier studies 1-2 have reported that monoterpene compounds are more active than sesquiterpene compounds. Dry ginger oil had higher content of sesquiterpene hydrocarbons. Hydrocarbon compounds are reported to have less activity compared to oxygenated compounds 1-2 [16].

The effect of antibacterial activity of *Zingiber officinale* evaluated against bacterial strains in aqueous extract by using agar well diffusion methods were followed with Muller Hindon agar plants were prepared only by organic solvents extraction. Here that 25 µg to 100 µg was analysed. The maximum antibacterial activity were observed in 100 µg/ml when compared with lower concentration of extracts. The *Zingiber officinale* fresh sample was extraordinary performance when compared with dried sample with higher conc. of excellent properties against clinical bacteria. So, it has been conformed the activity of phytochemicals and the importance of such reaction of *Zingiber officinale*. Four bacteria such as *E.coli*, *Staphylococcus aureus*, *Klepsiella pneumonia* and *Psuedomonas aeroginosa* were tested. The maximum zone of inhibition at *Klepsiella pneumonia* from 25 µg to 100 µg concentration of *Zingiber officinale* fresh materials than the dried materials.

Essential oil of *Z. officinale* inhibited the growth of 15 bacterial strains: six Gram-positive and nine Gram-negative strains. The Gram-positive were the most susceptible when presenting the highest inhibition halos (*S. aureus* cc: 32.66 ± 2.01 mm, *S. aureus* FES-C: 30.0 ± 3.48 mm, *S. aureus* FES-I: 24.0 ± 0.00 mm, *S. epidemidis* ATCC 12228: 21.00 ± 1.41 mm and E. faecalis ATCC 14506: 22.00 ± 2.16 mm) and lower MIC values, including the multiresistant *S. aureus* 23 MR species. CMI values for gram positive strains were found between 0.25 and 0.5 mg/mL, except for *E. faecalis* ATCC 14506 for which a MIC value of 1.0 mg/mL was obtained. Antibacterial effect of essential oil showed significant differences in the inhibition of Gram-positive and Gram-negative bacteria ($p < 0.0001$), being most susceptible Gram positive strains, suggesting that one of microbial targets of oil is wall Cell, since Gram positive bacteria have a cell wall composed of a thick layer of peptidoglycan surrounding the cytoplasm membrane Burt, S. [17] However, it may have other microbial targets, such as plasma membrane, which explains the inhibitory effect of oil on Gram negative bacteria, as the constituents of essential oils have been reported to have lipophilic properties, which interact with the membranes by altering their fluidity and permeability Berger, [18] In other studies it has been reported that essential oil of *Z. officinale* is more active on Gram positive bacteria, including aureus [19,20,21]. However, there are reports of outstanding susceptibility in Gram negative strains, mainly in *P. aeruginosa*, *E. coli*, *Enterobacter* sp., *K. pneumoniae* and *Proteus vulgaris* [22, 23,24,25] Sasidharan et al. 2010,. Antibacterial effect of essential oil on Gram positive bacteria is of great relevance, because these strains are of medical importance. The genus *Staphylococcus* has been considered one of major responsible for infectious diseases in humans such as endocarditic, food poisoning, skin infections, among others [26,27].

In the recent investigation suggests that the effect of antifungal activity of *Zingiber officinalis* of dried samples at *Fusarium* sp than the *Aspergillus flavus*, *A. terreus* and *Penicillum* sp from the higher concentration with aqueous extract, whereas fresh sample of *Zingiber officinalis* also the same trend of *Fusarium* sp was maximum zone of inhibition when compared with other fungi. In evaluation of antifungal activity, all three strains of *Candida* were sensitive to essential oil, as were the four strains of filamentous fungi *C. tropicalis* was the most susceptible of yeast strain with the highest inhibition halos (30 ± 0.00 mm) and lowest MIC (0.125 mg/mL). *T. mentagrophytes* was the most susceptible strain of filamentous fungi (CF50 = 0.08 mg/mL) Antifungal activity of *Z. officinale* essential oil is well documented, mainly in filamentous fungi such as *Penicillium* spp., *Rhizopus* sp., *A. flavus*, *A. solani*, *A. oryzae*, *A. niger*, *F. moniliforme*, *F. verticillioides* Yamamoto-Ribeiro *et al.*, 2013 and in yeast fungi such as *Saccharomyces cerevisiae* and *C. albicans*. The novel antifungal activity results in oil being the first report of activity on *C. tropicalis* and *T. mentagrophytes*, given the medical significance represented by these strains. *C. tropicalis* is responsible for 3 to 66 percent of gynecological infections in tropical countries (Chai et al., 2010). It is commonly associated with the development of systemic fungal infections and presents a considerable biological potential as an opportunistic agent in patients with cancer, leukemia and neutropenia. Meanwhile, *T. metagrophytes* can cause inflammatory skin diseases, affecting the epidermis and skin appendages [28].

5. SUMMARY AND CONCLUSION

The effect of biological activity of *Zingiber officinale* maximum responsible against some clinical isolates of bacteria and fungi were determined and the zone of inhibition also higher was depending upon the extract concentration was observed.

According to antimicrobial activity of *Klebsiella pneumoniae* and fungi *Fusarium* sp. was maximum zone of inhibition when compared other microorganisms. One of the beautiful results also determined that the low concentration (20 µg/ml) against clinical microorganisms. Another important information on fresh samples was extra ordinary properties when compared with dried samples of *Zingiber officinale*.

In the current study concluded that the *Zingiber officinale* are known beneficial therapeutic effects in traditional practice from the observation.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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