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In-Vitro Antifungal Efficacy of *Zingiber Officinale*

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ABSTRACT:

Synthetic medicines are being replaced by those derived from natural resources because of cost burden and side effects of synthetic medicines. Zingiber officinale (Ginger) has been act as home remedy against the microbial infections. Its fresh juice has radio protective action that could be helpful in avoiding gamma radiation which exert harmful effects during the period of cancer treatment. It exhibited chemo defensive and anti-neoplastic action as well as has been useful to cure the inflammation in the body. Present study investigated in vitro antifungal efficacy against Aspergillus niger, Aspergillus oryzaea, Fusarium oxysporum and Fusarium tricinctum using disc diffusion technique. Maximum inhibitory zone against Aspergillus niger produced after 96 hours i.e. 46.66 ±2.5mm and minimum after 48 hours i.e. 41.66 ± 2.5mm. Maximum inhibitory zone against Aspergillus oryzaea was produced after 96 hours i.e. 23.33±1.73 mm and minimum at 48 hours i.e. 17.66 ± 1.73 mm. Maximum inhibitory zone against Fusarium oxysporum was produced after 96 hours i.e. 24.00 ± 1.5mm and minimum at 48 hours i.e. 18.00 ±1.5mm. Maximum inhibitory zone against Fusarium tricinctum was produced after 96 hours i.e. 38.33 ± 2.00 mm and minimum at 48 hours i.e. 35.00 ± 2.00 mm. These concentrates may have been proved to be effective antimicrobials and can be exploited in controlling superfluous microbial growth.

Keywords: *Aspergillus oryzaea, Aspergillus niger, Fusarium tricinctum, Fusarium oxysporum, Zingiber officinal, Antimicrobial activity, Inhibitory activity.*

INTRODUCTION

Zingiber officinal (Ginger) is herbal plant that has been cultivated broadly throughout the world for various purposes. Rhizome or root part of ginger is medicinal properties and aids in various ailments e.g. motion

sickness, vomiting, gastrointestinal ulcers, diabetes, fever, nausea, arterial tension, dry mouth/xerostomia, rheumatoid arthritis, migraine headache, sore throat, cancer and other minor respiratory problems (Teles et al., 2019) . Ginger has been in use since ancient times due to its antiseptic properties for thousands of years in Asian cultures (Ernst and Pittler, 2000; Weil, 2005). Europe is famous for the ginger and is a daily use spice and a natural medicine (Sasidharan and Menon, 2010). Due to its (*Zingiber officinale*) medicinal properties, consumption of wild ginger rhizome to normalize menstruation and heart beat is well known among inhabitants of America (Holtmann et al., 1989).

Ali et al. (2008) reported that *Zingiber officinale* is a medicinal plant widely used for a broad range of unrelated diseases e.g. cramps, hypertension, arthritis, indigestion, constipation, sprains, sore throats, muscular aches, pains, dementia, vomiting, rheumatism, fever and other infectious diseases all over the world. Curative plants are used as pharmaceuticals, nutraceuticals, cosmetics and food supplements (Sharma et al., 2010). There are some qualities of Ginger. A few include simply available, universally suitable, relatively low-priced and well accepted by public. *Zingiber officinale* is member of the Zingiberaceae family (Sharma et al., 2010). Tuberous and non-tuberous rhizomes have sturdy aromatic and medicinal properties (Chen et al., 2008).

Zingiber officinale extract obtained are reported to repress the growth and stimulate apoptosis of variety of cancer types including colon, oral, skin, cervical, ovarian, breast, renal, prostate, pancreatic, liver, gastric and brain cancer. *Zingiber officinale* have antioxidant, anti-inflammatory, anti-mutagenic actions and biological efficacies (Srinivasan, 2014). Therefore, it is not wrong to say that it can be used to treat the bacterial infections (Tan and Vanitha, 2004). It is abundantly grown in several countries such as Indonesia, India, Japan, China, Nigeria, Australia (Queensland), Jamaica, Sierra Leone and other West Indies islands. Ginger is considered to act directly on the digestive system for controlling queasiness (Foster, 2011) and motion sickness (Holtmann et al., 1989).

Zingiber officinale has great value as a trendy medicine against nausea during conceiving (Langner et al., 1998). Different types of problems e.g. upset stomach, bloating, flatulence, heartburn, dyspepsia, sickness, colic, diarrhea, gas and hungriness can be cured by *Zingiber officinale*. Ayurvedic medicinal system suggests Zingiber officinale as increasing agent for digestion of food (Ali et al., 2008). *Zingiber officinale* is considered as relief for various kinds of pains e.g. low back pain, arthritis pain, stomach, muscle soreness, menstrual pain and chest pain and as well as helpful to alleviate cough, upper respiratory tract diseases and bronchitis. It (*Zingiber officinale*) is suggested for joint problems due to its anti-inflammatory

action (Shukla and Singh, 2007). Active ingredients of ginger are helpful for laxative and antacid medication also protect from skin burn. Regulating the body circulation and lowering high blood pressure are also included in their uses. There are many applications of *Zingiber officinale* e.g, flavoring compound in foodstuffs and as fragrance in soaps as well as in cosmetics (Alam, 2013). *Zingiber officinale* contains the element caprylic acid, which has powerful antifungal activity. Gingerols present in it (*Zingiber officinale*) have analgesic, sedative, antipyretic, antibacterial and gastro intestinal tract motility effects (Azu et al., 2007). *Zingiber officinale* has strong aromatic and medicinal properties (Chen, 2008). It has been exploited for the cure of many infectious in many countries (Ali et al., 2008; Tan and Vanitha, 2004). The spicy taste of *Zingiber officinale* is due to gingerols, shagaols and zingerone (Mascolo et al., 1998) and therefore possess a broad array of pharmacological characteristics (Ficker et al., 2003; Habsah et al., 2000). Unfortunately, East Pakistan is now separated and is has a big source of this crop yield. Now in Pakistan extensive amount of *Zingiber officinale* is imported from foreign countries. So, there is necessity to develop appropriate regions in Pakistan for the cultivation of this expensive crop (Ahmad et al., 1983).

Diseases caused by infectious organisms including viruses, bacteria, protozoa, fungi and different multicellular parasites are called as infectious diseases (Zubairu and Gwa, 2020). Currently, plant infections are limiting factor in crop production. Crops infections lead to field losses and become popular as world population increases. About 50% of the deaths in equatorial countries happen due to infectious diseases (Khosravi and Behzadi, 2006).

The present study was focused on evaluation of the antifungal efficacy of fresh juice of *Zingiber officinale* against four different fungi i.e. *Aspergillus niger, Aspergillus oryzaea,* Fusarium oxysporum and Fusarium tricinctum to determine the most suitable, non-hazardous and inexpensive method for controlling fungal strains.

MATERIALS AND METHODS

Study Area

The present study was conducted in Department of Biology, Lahore Garrison University, Lahore, Pakistan and University of the Punjab, Lahore, Pakistan to study the Isolation, Identification, Antifungal susceptibility pattern of *Aspergillus niger, Aspergillus oryzaea, Fusarium oxysporum and Fusarium tricinctum.* These strains were obtained from Institute of Agricultural Sciences; University of the Punjab, Lahore. The pure cultures were sub cultured on Potato Dextrose Agar (PDA) and kept at 4°C until ready for the further analysis.

Preparation of the Zingiber officinale fresh juice

The rhizome was washed with fresh water. The outer layer of the *Zingiber officinale* was peeled through knife and the fat part of the plants was rewashed with distilled water. Ginger was cut into different sections. About 30.0gm of the ginger rhizome was used to form juice. After making juice, it is ready for its antifungal efficacy (Adeshina et al., 2012).

Disc Diffusion assay

This method for antimicrobial susceptibility test was done to ensure the antifungal action of the plant extracts. Fungal cultures were placed on potato dextrose agar plates uniformly using a cotton swab. The disc diffusion assay was performed according to the method of (Tepe et al., 2005). The discs were dipped into different plant extracts and then placed on the dried potato dextrose agar plates for the susceptibility test. Each plate containing one disc incubated at 30°C and then checked the zone of inhibition after different time intervals.

Experimental design

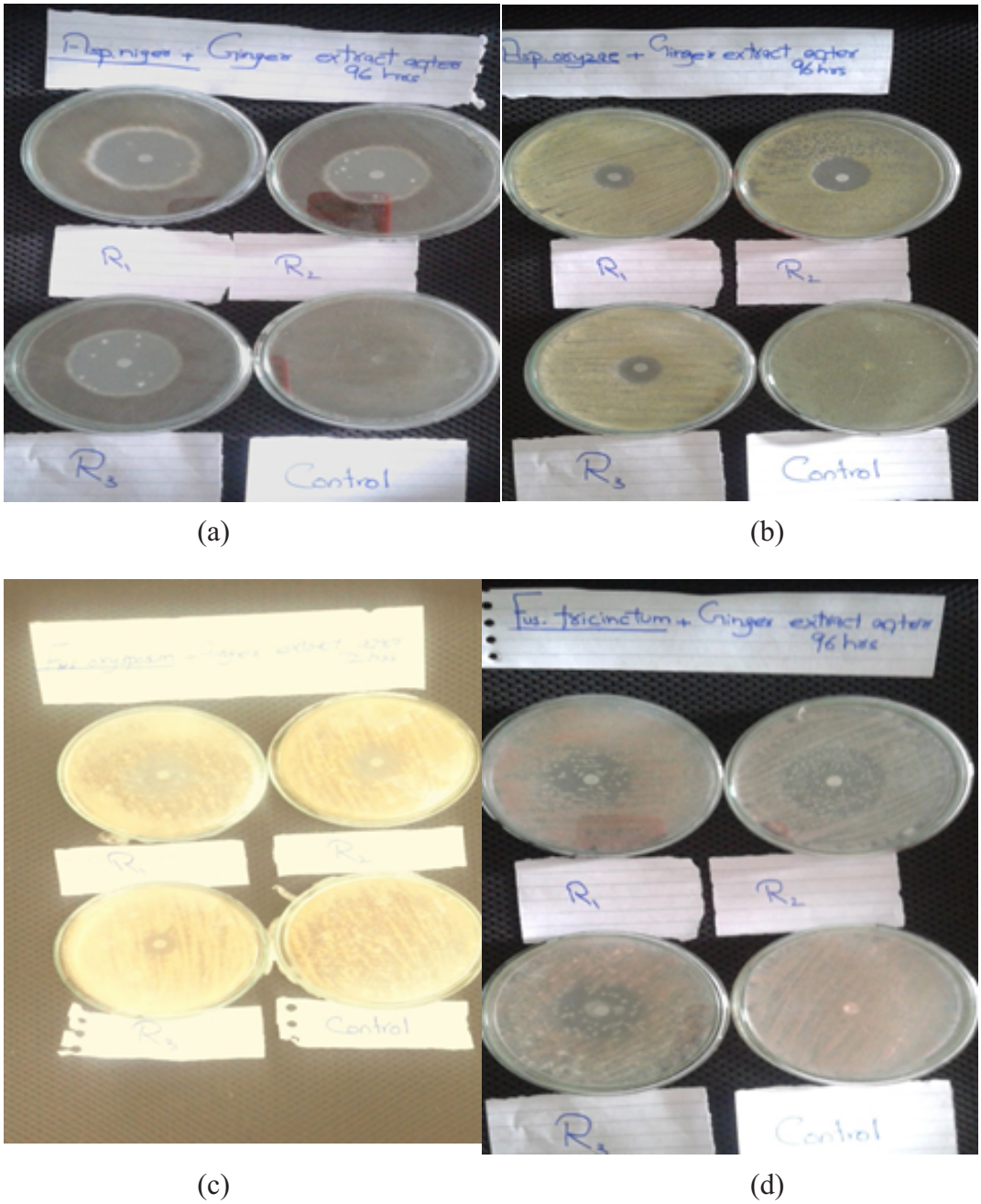
Antifungal assay of prepared petri plates having discs of fresh juice (*Zingiber officinale*) was done to observe the results. Petri plates were labeled properly as R1, R2, R3 with fourth test tube being the control. Zone of inhibition was observed after 48 hours. Plates showing zone of inhibition were captured and measured in millimeters.

STATISTICAL ANALYSIS

The results were mentioned as Mean ± Standard Deviation (S.D). Data were assessed through analysis of variance (IBM SPSS version 21).

RESULTS AND DISCUSSION

Zingiber officinale has an antifungal activity against all fungus. Fig. 1 shows zone of inhibition against such fungi with three replicates and control, after 96 hours. Fig. 2 shows the graphical representation of antifungal activity after different time intervals and it indicates that *Aspergillus niger* had maximum growth while *Aspergillus oryzaea* had minimum growth after 96 hours. Fig. 1 represents that mean inhibition zone (mm) of *Zingiber officinale* against *Aspergillus niger* is greater than other fungus. Fig. 2 represents that inhibition zone increases with the passage of time i.e. 48, 72 and 96 hours. Table 1 describes that maximum inhibitory zone against *Aspergillus niger* produced after 96 hours i.e. 46.66 ±2.5mm and minimum after 48 hours i.e. 41.66±2.5mm. Maximum inhibitory zone against *Aspergillus oryzaea* was produced after 96 hours i.e. 23.33 ± 1.73 mm and minimum at 48 hours i.e. 17.66 ± 1.73 mm. Maximum inhibitory zone against *Fusarium oxysporum* was produced after 96 hours i.e. 24.00 ± 1.5mm and minimum at 48 hours i.e. 18.00 ±1.5mm. Maximum inhibitory zone against *Fusarium tricinctum* was produced after 96 hours i.e. 38.33 ± 2.00 mm and minimum at 48 hours i.e. 35.00 ± 2.00 mm.



(a) (b)

(c) (d)

Fig. 1: Zone of inhibition produced against (a) Aspergillus niger (b) *Aspergillus oryzaea (c) Fusarium oxysporum (d) Fusarium tricinctum* with four replicates after 96 hour.

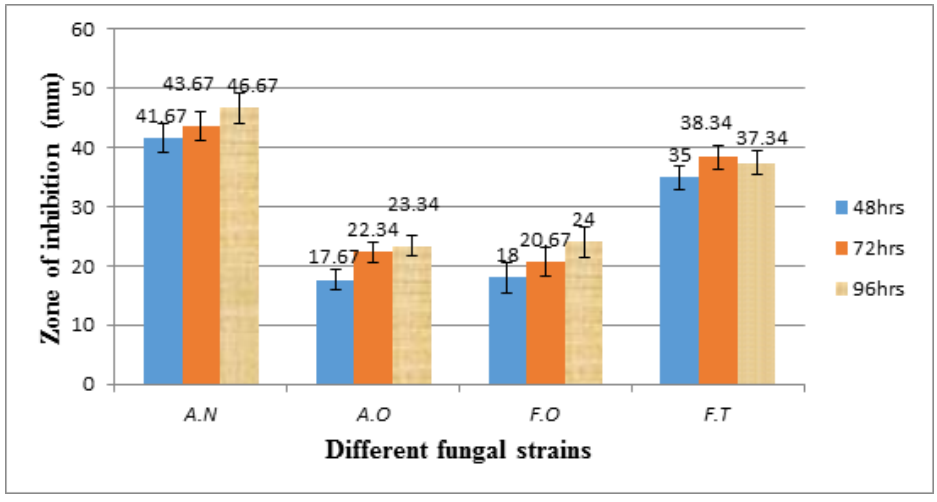


Fig. 2: Activity of Ginger (*Zingiber officinale*) against various fungi i.e. A.N (*Aspergillus niger*), A.O (*Aspergillus oryzaea*), F.O (Fusarium oxysporum) and F.T (*Fusarium tricinctum*) after different time intervals.

The above results showed that *Zingiber officinale* fresh juice had the best antifungal activity against the test organisms such as Aspergillus spp. and Fusarium spp. The fresh juice of *Zingiber officinale* produced the maximum value of zone of inhibition i.e. 46.66±1.5mm against *Aspergillus niger* and showed minimum value i.e. 17.66±1.73mm against *Aspergillus oryzaea* among all the fungal strains. This very high antifungal value is due to the compounds having strong antifungal potential present in the extract. This is in conformity to work by Atai et al. (2009), who observed similar activity of ethanol extract of *Zingiber officinale* (Ginger) on Candidia with relation to that of conventional antibiotics. Several modern studies prove that *Zingiber officinale* has antimicrobial properties against many bacteria, fungi and viruses. Investigators have compared its efficiency with viable recommendation antifungal disc and results show that *Zingiber officinale* is more powerful than antibiotics (Saad et al., 2014).

The susceptibility of ginger extract also confirms observation by Sasidharan and Menon, (2010), who performed an antimicrobial action of the oils (*Zingiber officinale*) against, Pseudomonas aeruginosa, *Aspergillus niger, Bacillus subtilis, Candida albicans, Trichoderma spp, Pencillium spp. and Saccharomyces cerevisia* by disc diffusion method .They analyzed a broad appliance of ginger oil in the healing of many bacterial and fungal infections. The results of Pratibha and Rajendra, (2016) also supports present work who investigated the antimicrobial efficacy of *Zingiber officinale* (Ginger) and concluded that chloroform extract of ginger exhibited highest zone of inhibition as 25.75 mm against tested microbial strain. Our results also similar to work of Supreetha et al. (2011) who evaluated

the inhibitory effect of *Zingiber officinale* extract on candida albicans under controlled conditions and found ethanol extract of *Zingiber officinale* had inhibitory effect against that fungus. The search work of Saada et al. (2015) also confirms present study but they worked on bacteria using fresh and powdered ginger extract by performing same antimicrobial test. They concluded that in comparison to tested antibiotics, fresh and powdered ginger extracts showed a higher antibacterial effect against *Streptococcus mutans* ginger oil in the healing of many bacterial and fungal infections. The results of Pratibha and Rajendra, (2016) also supports present work who investigated the antimicrobial efficacy of *Zingiber officinale* (Ginger) and concluded that chloroform extract of ginger exhibited highest zone of inhibition as 25.75 mm against tested microbial strain. Our results also similar to work of Supreetha et al. (2011) who evaluated the inhibitory effect of *Zingiber officinale* extract on Candida albicans under controlled conditions and found ethanol extract of *Zingiber officinale* had inhibitory effect against that fungus. The search work of Saada et al. (2015) also confirms present study but they worked on bacteria using fresh and powdered ginger extract by performing same antimicrobial test. They concluded that in comparison to tested antibiotics, fresh and powdered ginger extracts showed a higher antibacterial effect against *Streptococcus mutans.*

CONCLUSION

Our results suggest that *Zingiber officinale* can serve as strong antifungal agent and its consumption could be useful in the prevention of diseases. Therefore, adding ginger in preserved foodstuff can eventually increase the useful life and also keep the value of preserved food. Further research is needed towards phyto-chemical analysis of compounds present in the extracts of ginger and sugarcane which could possibly be subjugated for pharmaceutical use. Because of active phytochemicals present in *Zingiber officinale,* this plant can be helpful in designing antifungal drugs.

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