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SYNERGISTIC ACTION OF *ZINGIBER OFFICINALE* EXTRACT WITH ANTIBIOTICS

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Abstract
The emergence of resistant strains of bacteria has seriously limited our ability to treat bacterial illness, and new antibiotics are desperately needed. In this study, the plant extracts were prepared by using diff. solvents (Acetone, EtOH and MeOH). Antimicrobial activity of plant extracts, Antibiotics (Amoxicillin, Ceftazidime, Ciprofloxacin, Amphotericin-B and Fluconazole) and combinations has been done by Agar well Diffusion method. The results of this study showed that ethanol and methanol extract show higher activity against selected pathogenic species. HPTLC has been also performed to check if there any new compound formed or not by combination of extract and antibiotics. The findings of this study suggest that combining plant extract with antibiotics may be effective in battling newly developed drug-resistant microbes.

Keywords: *Zingiber officinale*, Extracts, HPTLC, Antibiotics, Antimicrobial.

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, 2022

Introduction:

Plants make and utilize secondary metabolites, which are antimicrobial compounds, as a kind of defense. There are several applications for these secondary metabolites in both medicine and food (Tiwari and Rana, 2015). Scientists have paid a lot of attention to plant antimicrobial compounds as components in human medications (De Zoysa *et al.*, 2019; Maharjan *et al.*, 2019); of particular interest is the prevention of spoilage and the pathogenic microorganisms that cause food borne illnesses and intoxications (Sofia *et al.*, 2007; Thong son *et al.*, 2005). Due to the present level of antimicrobial resistance found in the food chain, which poses a serious risk to public health, this is especially crucial (Bennani *et al.*, 2020; Oniciuc *et al.*, 2019). Herbs and spices have historically been utilized in various cultures to treat a variety of illnesses, including as antibacterial agents (Dog, 2006; Leja and Czaczyk, 2016). Due to their extensive history of use in food preparation, herbs and spices are typically recognised as safe for humans when compared to conventional antibiotics. It has been claimed that a number of herbs and spices are powerful antimicrobials. Studies have demonstrated, for instance, that grape seed, pomegranate peel, oregano, clove, and cinnamon stick extracts all exhibited antibacterial action against foodborne pathogens, with Gram-positive bacteria being more sensitive than Gram-negative bacteria. According to report of (Shan *et al.*, 2007, 2009), *S. aureus* was the most susceptible, whilst *E. coli* was the most hardy. The phenolic content of the extracts was closely correlated with their antibacterial activity. The Zingiberaceae family includes ginger (*Zingiber officinale*). It is extensively cultivated as a spice and medicinal plant in the tropics of Asia, Africa, America, and Australia. Research on the variety of ginger have revealed that the main bioactive chemicals are greatly influenced by environmental conditions and that grown ginger displays differences in rhizomes and vegetative character (Kizhakkayil and Sasikumar, 2011).

The antibacterial properties of ginger and garlic have been the subject of numerous investigations (Akintobi *et al.*, 2013; Indu *et al.*, 2006; Khashan, 2014; Mohammed *et al.*, 2019). The ginger rhizome exhibits a broad spectrum of antibacterial action against a variety of Gram negative and Gram positive microorganisms. Yet, contradicting information regarding the antibacterial potency of ginger against bacteria has been found in several sources (Abdalla and Abdallah, 2018). Ginger is also said to have antibacterial and antiviral properties (Gebreyohannes and Gebreyohannes, 2013). The most potent antibacterial compound found in ginger rhizome oils is zingiberene (El-Barotyet *al.*, 2010); It has been shown that the extraction solvent, technique, and concentration of bioactive components each have an impact on the antibacterial properties of ginger and garlic (Hasan*et al.*, 2012; Bakht *et al.*, 2011). According to reports, Chinese garlic showed excellent promise as a culinary ingredient because of its high allicin level (Sommano *et al.*, 2016). The effectiveness of plant products is also confirmed to be significantly influenced by the extraction process, antimicrobial assay settings, genetic variations, bacterial strains, and the sources of the product. Moreover, geographical variances, environmental conditions, and physiological factors have an impact on plants' production of bioactive phytochemical substances (Abdalla and Abdallah, 2018).

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, 2022

Fig. 1 *Zingiber officinale*

From ancient times, *Zingiber officinale* (ginger) has been used in traditional medicines to cure a variety of ailments, such as cardiovascular disease, cough, vomiting, nausea, the common cold, and inflammation. Together with the aforementioned benefits, ginger also has zingiberene, gingerols, shogaols, and paradols, which together contribute to *Zingiber officinale*'s anti-oxidant characteristics. The biomolecules in the ginger extract cap and decrease zinc ions to form the nanoparticles (Mishra *et al.*, 2012; Terry *et al.*, 2011; Zhang *et al.*, 2013; Barui *et al.*, 2018; Rajeshkumar and Sandhiya, 2020). In spite of contemporary medicine's breakthroughs, germs remain to be one of the biggest threats to people's health. Microbially produced antibiotics have completely transformed antibacterial therapy since Fleming's 1929 discovery of penicillin. In fact, penicillin replaced other antibiotics as the primary treatment for infectious infections. Furthermore, that finding sparked a new line of research into the development of antibacterial drugs from bacteria and fungi, which has given medicine access to a large number of novel, highly potent antibiotic compounds. The widespread use of penicillin, however, by the 1940s led to the rise of new microbe strains that could destroy the medication and counteract its effects. As per (Abraham and Chain, 1940; Rammelkamp and Maxon, 1942), In a similar manner, germs have become resistant to numerous other widely used antibiotics. (Davies and Davies, 2010)The World Health Organization (WHO) views this new trend as maybe the most serious problem facing medical research. That is alarming. (Gill *et al.*, 2006) Drug consumption affects the balance of microbial populations in the gut and may cause a variety of negative effects while still treating particular conditions. By completely resisting the medication, certain bacteria may survive longer than susceptible populations. Non susceptibility to at least one agent in more than two of the recognised categories for antimicrobials is known as multidrug resistance (MDR). (Magiorakos *et al.*, 2012) Pathogens that are known to be extensively drug-resistant (XDR) are only vulnerable to two or fewer antibiotic classes, and as a result, pose a serious threat to human health. There has been a comparable decline in antimicrobial discovery along with the rise in bacterial resistance to antibiotics. Because to this, researchers are now focusing on alternative treatments such as bacteriophage therapy, conventional plant-based medications, and combinational therapies. In doing so, we draw attention to the utilization of plant natural products and plant extracts as having special promise for quickly generating novel, efficient treatment modalities available to battle bacteria resistant to current antibiotic regimens, particularly when combined in synergistic ways.

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, 2022

Materials & Method:-

Collection of Plant Material

Fresh ginger rhizomes were purchased from different area of Northern Gujarat. The respective samples were sorted to remove bad quality, soaked in tap water, washed and rinsed under running water. They were cut, dried, powdered, stored in sterile condition and used for further studies.

Extraction

With the use of a magnetic stirrer and 150 ml of a suitable solvents (methanol, ethanol, and acetone), 40 grams of finely ground plant material were extracted for 6, 12, and 24 hours at room temperature. Extracts were dried after centrifuged.

Time	Methanol	Ethanol	Acetone
6 hr	1.024 gm	0.978gm	0.801gm
12 hr	1.332 gm	1.104gm	0.996gm
24 hr	1.673 gm	1.399gm	1.206gm

Preparation of combination:-

The combination was prepared by combining Antibiotics with diff. extracts.
Antibiotic and plant extract were kept at a 1:1 ratio.

Antimicrobial Activity

Agar well diffusion method suggested by (Arodiya *et al.*, 2021) used to evaluate the antimicrobial activity of *Zingiber officinale* extracts, Antibiotics, and their Combinations. Agar media was prepared by using Muller Hinton Agar. The agar plate surface was inoculated by spreading the selected microbial (*Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Aspergillus niger* and *Candida albicans*) over the entire agar surface. Then, wells, a diameter of 8 mm were punched with a sterile cork borer and 50 µL of the tested solution (Z.O. diff. solvent extracts, antibiotics and combinations) at desired concentrations introduced into the well. Then, agar plates were incubated at 37 °C for 24 hrs. The ZOI was used to express the antimicrobial activity in mm.

HPTLC

Prewashing of HPTLC plates

HPTLC plates (10 cm×10 cm) were washed with methanol and activated at 120 °C for 15 min using a TLC plate heater III.

Preparation of standards

Standard solutions of *Zingiber officinale* methanol extract, antibiotic and their combinations were prepared with methanol at a concentration of 0.1 mg/mL. A mixture of the standards was also prepared by combining equal volumes of each standard solution.

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, 2022

Plate development and derivatization

The Linomat 5 semi-autosampler was used to apply the individual standard solutions and standard mixture as 2-lL bands in five tracks, 1 cm from the plate's base, with a bandwidth of 5 mm and spacing between bands of 2 mm. All tracks 1–5 on the plates received samples in the following order: antibiotic, MeOH extract, antibiotic+ MeOH extract, EtOH extract, and antibiotic+ EtOH extract. 10 mL of mobile phase were pre-saturated in HPTLC twin trough chambers (10 cm x 10 cm) for 15 minutes. Over a migration distance of 5 cm, the mobile phase was employed to resolve the adsorbed standard and standard mix after being dispersed equally across the twin trough chamber. The mobile phase was composed of ethyl acetate:methanol:acetone:toluene:ammonia (1:5:7:0.5:0.5). At the end, plates were allowed to dry and analysed.

RESULT AND DISCUSSION

Zingiber officinale extracts were compounded with API and exposed to antimicrobial property. ZOI is expressed in mm.

Table 1: Antibacterial activity of pure extract

Bacteria	**MeOH Extract**				**EtOH Extract**				**Acetone Extract**			
	Conc. in µg/ml											
	1000	500	250	125	1000	500	250	125	1000	500	250	125
S. aureus	14	13	12	10	13	13	12	10	11	10	10	09
B. subtilis	13	12	11	10	12	12	10	10	10	10	08	08
P. aeruginosa	15	14	12	11	13	12	11	09	12	11	10	10
E. coli	16	15	14	13	16	15	14	13	14	13	12	12

Table 2: Antibacterial activity of extract+Amoxicillin

Bacteria	**Amoxicillin**	**MeOH extract + Amoxicillin**				**EtOH extract + Amoxicillin**				**Acetone extract + Amoxicillin**			
	Conc. in µg/ml												
	100	1000	500	250	125	1000	500	250	125	1000	500	250	125
S. aureus	31	32	31	29	29	31	30	29	28	30	30	28	28
B. subtilis	35	34	33	33	32	33	32	31	30	32	32	31	30
P.aeruginosa	19	21	20	19	19	20	20	19	18	19	18	18	17
E. coli	20	22	21	20	19	22	21	20	20	21	20	19	18

Table 3: Antibacterial activity of extract+Ceftazidime.

Bacteria	**Ceftadizime**	**MeOH extract + Ceftazidime**				**EtOH extract + Ceftazidime**				**Acetone extract + Ceftazidime**			
	Conc. in µg/ml												
	100	1000	500	250	125	1000	500	250	125	1000	500	250	125
S. aureus	01	11	11	10	10	10	10	09	08	10	09	08	08
B. subtilis	02	10	09	09	08	10	09	08	07	09	09	08	08
P. aeruginosa	05	13	12	12	11	12	10	10	09	12	11	09	08
E. coli	15	16	16	15	15	16	15	14	13	15	14	14	12

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, 2022

Table 4 : Antibacterial activity of extract+Ciprofloxacin.

Bacteria	**Ciprofloxacin**	**MeOH extract + Ciprofloxacin**				**EtOH extract + Ciprofloxacin**				**Acetone extract + Ciprofloxacin**			
	Conc. in µg/ml												
	100	1000	500	250	125	1000	500	250	125	1000	500	250	125
S. aureus	24	23	22	21	21	22	22	21	20	22	21	21	20
B. subtilis	29	28	28	27	26	28	27	27	26	27	27	26	26
P. aeruginosa	27	27	26	26	25	27	26	25	25	26	26	25	25
E. coli	27	28	27	27	26	28	28	27	27	27	26	26	25

Table 5 : Antifungal activity of pure extract.

Fungus	**MeOH extract**				**EtOH extract**				**Acetone extract**			
	Conc. in µg/ml											
	1000	500	250	125	1000	500	250	125	1000	500	250	125
A. niger	13	12	11	10	13	12	12	11	12	12	10	10
C. albicans	11	11	10	10	11	10	10	09	10	10	09	09

Table 6: Antifungal activity of extract+Amphotericin-B.

Fungus	**Amphotericin-B**	**MeOH extract + Amphotericin-B**				**EtOH extract + Amphotericin-B**				**Acetone extract + Amphotericin-B**			
	Conc. in µg/ml												
	100	1000	500	250	125	1000	500	250	125	1000	500	250	125
A. niger	22	21	21	20	19	20	20	19	19	20	19	19	18
C. albicans	24	22	21	21	20	22	22	21	21	21	20	19	19

Table 7: Antifungal activity of extract+Fluconazole.

Fungus	**Fluconazole**	**MeOH extract + Fluconazole**				**EtOH extract + Fluconazole**				**Acetone extract + Fluconazole**			
	Conc. in µg/ml												
	100	1000	500	250	125	1000	500	250	125	1000	500	250	125
A. niger	02	14	13	12	11	14	12	12	11	13	12	11	11
C. albicans	00	08	08	07	07	08	07	07	06	07	07	06	06

The *Zingiber officinale* was used to make extracts that were tested against two gram-positive (*Staphylococcus aureus, Bacillus subtilis*) and two gram-negative (*Pseudomonas aeruginosa, Escherichia coli*) bacteria as well as two fungi (*Aspergillus niger and Candida albicans*) to determine their antibacterial and antifungal properties.

The extracts were prepared using the solvents viz. methanol, ethanol, and acetone and were exposed to bacterial and fungal strains for their antimicrobial potential, It was noted that the MeOH extracts exhibited the highest values of a zone of inhibition in comparison to all other extracts. The ZOI values for the plant extracts related to the theory regarding the solubility of plant products to be more in polar solvents in comparison to the non-polar solvents which can be correlated from the

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 UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, 2022

antimicrobial evaluation. The Three drugs Amoxicillin, Ceftazidime, Ciprofloxacin, and Two drug Amphotericin-B and Fluconazole were exposed to bacterial and fungal strains for their antimicrobial potential respectively. Ceftazidime did not show any positive results against *B. subtilis*in antibacterial and Fluconazole also didn't show any effect on *Candida albicans* in antifungal activity.

After examining the individual antibacterial effects of plant extract and API, their combined antimicrobial effects in a 1:1 ratio were also investigated. The results shown in the above table clearly demonstrated that utilizing a half volume of API with extract yields results that are almost identical to those obtained using a pure API sample.

Among all the combination, the combination of MeOH extract with all the drugs show highest ZOI. Combination with Amoxicillin show 34 mm ZOI against *B. subtilis* which is the highest and 21 mm ZOI against *P. Aeruginosa* which is the lowest result found. the combination of all three extracts with Ceftazidime show 16 mm ZOI which is highest in *E. coli* and 10 mm is lowest in *B. subtilis*in antibacterial for 1000 µg/ml**.** when extracts combine with Ciprofloxacin we got 28 mm ZOI same against both *B. subtilis* and *E. coli* which is highest and 23 mm ZOI against *S. aureus* which is lowest.

In antifungal activity, the combination of EtOH and MeOH extract with Amphotericin show 22 mm ZOI against *Candida albicans* which is the highest ZOI, and the combination of Acetone and ethanol extract with Amphotericin show 20 mm ZOI against *Aspergillus niger* which is lowest ZOI. in combination with Fluconazole EtOH and MeOH Extract Show 14 mm ZOI against *Aspergillus niger* which is highest and Acetone Extract Show 07 mm ZOI mm against *Candida albicans* which is lowest.

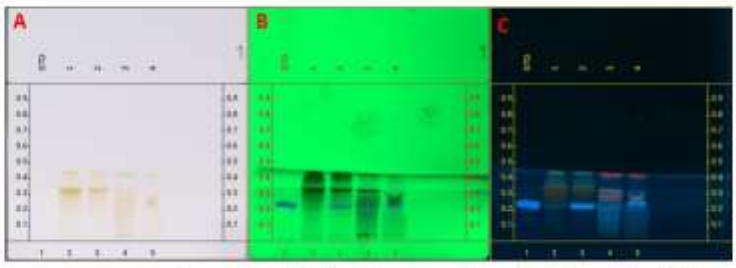

Fig. 2 HPTLC profile of Amoxicilline, Extracts and combinations at A) visible, B) UV 254nm, C) UV 366nm

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, 2022

In Fig.2 (A, B, C) represent the same HPTLC experiments result in diff. lights. The first band of Amoxicillin also present at the same Rf value in the third and fifth band which is the combination of Amoxicillin and MeOH extract & Amoxicillin and EtOH extract respectively. No any new band is visible and no old band has Disappeared which confirms no any new compound formed by combining antibiotics and extract and so why no further study require for toxicity.

CONCLUSIONS

In addition to reducing side effects by lowering antibiotic concentrations, plant extracts can be utilized to increase the antibacterial activity of antibiotics. Following adequate toxicological investigations, combinatorial chemistry can replace the usage of conventional drugs.

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