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COMPARATIVE EVALUATION OF ANTIBACTERIAL ACTIVITY OF *Zingiber officinale* AND *Curcuma longa*

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ABSTRACT

Humans have been using natural products for medicinal use for ages. Natural products of therapeutic importance are compounds derived from plants, animals, or any microorganism. Ginger and Turmeric are also used as most commonly used condiments and Natural drugs. These are traditional medicine, having some active ingredients used for the treatment of many diseases and killing of gram negative bacteria as well as gram positive i.e. *E. coli*. Turmeric (*Curcuma longa*) and ginger (*Zingiber officinale*) has been used in cooking, and in herbal remedies. It's possible mechanism of action was examined in terms of antioxidant availability during actual cooking conditions and in therapeutic applications using standardized extracts. The assays involve different extract of ginger and turmeric show Antibacterial activity killing of bacteria by well diffusion method. The Aim of this Comparative Evaluation of Antibacterials activity of *Zingiber officinale* and Curcumalongain their result of mortality in Enterobacteriaceae, water, acetone, alcohol, chloroform test extracts of *Zingiber officinale* and *Curcuma longa* were prepared. The amoxicillin and azithromycin antibiotic drugs are used as standard drug and performed antibacterial tests. To determine the zone of inhibition 3mm. Standardization of isolates were obtained after incubating for 48hours and colony counting *Zingiber officinale* and *Curcuma longa* has been shown on the antibacterials activity and found ginger have more antibacterial power then turmeric.

Keywords: -

Zingiber officinale , *Curcuma longa* Anti – bacterial test, *E- coli.*

INTRODUCTION

Humans cannot survive without food. Microbes can easily grow on foods and contaminated results food poisoning and wastage of foods. [1] Food poisoning means of illness resulting from ingestion of contaminated food. Food poisoning and food wastage can be inhibiting by using preservatives. [2] Preservatives are the substances, which are used to prevent food spoilage from microorganisms. [3]

Food poisoning bacteria s are ever present they cannot be complexly destroy but prevented with proper care and handling of food products. [4]. Food poisoning caused by various bacteria parasites. Like Gram negative bacteria like salmonella typhi, Escherichia coli, pseudomonas aeruginosa Gram positive bacteria like staphylococcus aureus, bacillus cereus, Fungus like aspergillus niger.[5]

E. coli bacteria is the rod-shaped gram-negative bacteria and one of the members of Enterobacteriaceae family that is survive on gastrointestinal tracts of humans and gut of warm-blooded animals. E. coli transmitted via food like raw milk, spinach, alfalfas sprouts. Drinking and swimming in unchlorinated water and direct person to person contact. Most *E. coli* is harmless and act as good bacteria for our digestive system but some strains can cause diarrhoea, food poisoning, pneumonia, cramps, vomiting, dysentery, and acute kidney failure. Their most common symptoms are abdominal cramps, nausea, constant fatigue and diarrhoea. [6]

Bacillus cereus is a gram-positive bacterium that release harmful toxins. There are two types of bacillus cereus intestinal and non-intestinal. Intestinal bacillus cereus food poisoning, diarrheal syndrome and emetic syndrome. Bacillus cereus can cause illness by fish, dairy, meat sauces, soups, stews and starchy food such as pasta, pastry, potatoes and sushi, soil, plants, dust and water. [7]

Aspergillus niger is a fungus and member of the most common species of the genus Aspergillus.it dangerous diseases called black mould on grapes, onion, and peanuts and aspergillosis, allergic aspergillus sinusitis, aspergilloma, chronic aspergillosis, invasive aspergillosis. The common symptoms of *Aspergillus niger* infections are similar to asthma like wheezing, shortness of breath, cough, coughing up blood, fever, chest pain, runny nose, headache, reduced ability to smell. [8]

The use of Chemical preservative and natural preservatives can prevent above types food born disease (food poisoning) and food spoilage. [9] Chemical preservatives are harmful for us they prevent foods from microbes but produces harmful diseases for human health even cancer. This is increases the demand of natural source of preservatives. [10]

Ginger has been used medicinally since ancient China and India. It is also used as a culinary spice and herbal remedy. Sanskrit literature, the Chinese pharmacopoeias, and the Ayurvedic Sesruta teachings all mention the usage of ginger. [11–16] Other names for it include black ginger, zingiberis rhizoma, and ginger root. Austria, China, Egypt, India, Great Britain, Japan, Switzerland, and the Netherlands are among the nations whose official pharmacopoeias include ginger. [16-21]

Turmeric a native of south-east asia, is used as a food additive (spice), preservative and colouring agent in Asian countries including China, Bangladesh, Burma, Nigeria, Australia, West indies, Peru, Jamaica and some other Caribbean and Latin American Countries. [23-24] Turmeric has also been used for centuries in ayuevedic medicine, which integrates the medicinal properties of herbs with food the extraordinary herbs have found into the spotlight in the west and rest of globe, because of its wide range of medicinal benefits. uses of turmeric dates back nearly 4000 years to the vedic culture in India it is extensively used ayurveda and siddha medicine as home remedy for various diseases. turmeric derived from rhizomes *Curcuma longa* (family *zingiberaceae*) is a perennial plant having short stem with large oblong leaves, and bears ovate, pyriform or oblong rhizomes, which are often branched and brownish- yellow in colour. [25-27]

This study purposed to determine the bioactivity of ginger and turmeric in an effort to establish its use in the medicinal and scientific study and which one is showed more antibacterial activity with scientific study.

MATERIALS AND METHODS

Chemicals

For this study, Nutrient agar, Chloroform and Acetone (CDH Fine Chemical, India), Ethanol (Changshu Hongsheng Fine Chemical, China) of AR grade were used. The water used for the extraction was double

distilled prepared in the laboratory while for antimicrobial study, the sterile water, prepared in the laboratory, was used.

Plant Materials

Zinziber officinalis rhizomes were collected from local market Durg Chhattisgarh region (21.19664˚ N, 81.21988˚ E). Further, the collected plant material was washed thoroughly with water to remove any surface dust particles and undesired materials followed by drying in shade and grinding to fine powder through 60 mm sieve.

Macroscopic Evaluation

For the macroscopic evaluation of leaves, firstly the collected rhizomes were clean with the water and surface was dried gently by using tissue paper. The colour, odour, taste, size, shape and texture were examined.

Physical Evaluation

Determination of ash values

The accurately weighed powdered drug was incinerated using incinerator with increasing temperature up to 650 °C until the carbon free ash obtained as 'total ash'. The resulted total ash was further used for the determination of 'water soluble' and 'acid insoluble' ash using dilute hydrochloric acid. The process was repeated thrice and average value is calculated.

Determination of extractive values

For the determination of the extractive values, accurately weighed 5 g of drug powder was macerated with 100 ml alcohol (90%) for 24 hours with occasional shaking for first 6 hours, evaporated, dried at 105 °C and percentage w/w of alcohol soluble extractive value was calculated. For the determination of water-soluble extractive value, instead of alcohol, chloroform water was used as a solvent in the above procedure. Besides that, the extractive values obtained through soxhelation were also determined.

Determination of foaming index

After confirmation of presence of saponin glycosides by the preliminary phytochemical screening given subsequently, the foaming index was determined. The test for foaming index was performed as per the standard procedure using decoction of plant material. We used the parameter as, by measuring the height of each test tube (number of test tube used were 10), if the height of foam in every test tube is less than 1cm then foaming index become less than 100. If it's more than 1 cm in every test tube then it will be over 1000 and if the height of the foam in any one test tube become 1 cm then following formulae was used for the determination of foaming index.

$$\textbf{Foaming index} = \frac{\textbf{1000}}{\textbf{a}}$$

a- Volume of plant material's decoction (ml) in the test tube showing 1 cm height

Determination of loss on drying (LOD)

For the determination of LOD, the gravimetric method was used. In this method, the drying of accurately weighed powder drug was carried out at 105 °C using hot air oven until the constant weight obtained and the percentage of volatile substance along with moisture was determined.

Extraction and phytochemical screening

The powdered plant material was extracted with solvents- chloroform, acetone, ethanol and water using maceration methods. The liquid extracts thus obtained were filtered through Whatman filter paper no. 1. The extracts were further concentrated and dried using water bath and subjected to phytochemical screening for the presence of carbohydrates, proteins, alkaloids, saponins, anthraquinone glycosides, steroids, flavonoids, terpenoids, tannins and amino acids along with antimicrobial study. For the phytochemical screening, various standards procedures were followed as given below [17-19].

Tests for carbohydrates

Molisch's test (General test) To 2-3 ml of aqueous extract, add few drops of alpha-napthol solution in alcohol, shake and add conc. H_2SO_4 from sides of the test tube. Violet ring is formed at the junction of two liquids.

Fehling's test (For reducing sugars) Mix 1 ml of Fehling's A and 1 ml of Fehling's B solution, boil for one minute. Add equal volume of test solution. Heat in boiling water bath for 5-10 minutes. First yellow, then brick red precipitate is observed.

Benedicts test Mix 2ml test solution with 5 ml benedict solution shakes it well. Heat it for 3 mintes in water bath. Red or yellow ppt observed.

Iodine test Mix 3 ml test solution. Add few drops of iodine solution. Purple of dark colour observed.

Tests for alkaloids

For the alkaloidal test, 2 ml of dilute HCl added to 1 g of dry extracts, shaken well, filtered and use for following tests.

Mayer's Test To 3 ml of the filtrates add 1 ml of Mayer's reagent (Potassium mercuric iodide). The creamy precipitate indicates the presence of alkaloids.

Wagner's Test To 3 ml of the filtrates add 1 ml of Wagner's reagent (Iodine in potassium iodide). The reddish-brown precipitate indicates the presence of alkaloids.

Dragendroff's Test To 3 ml of the filtrates add 1 ml of Dragendroff's reagent (Potassium bismuth iodide). The appearance of orange brown precipitate indicates the presence of alkaloids.

Tests for saponins

Foam test Shake little quantity of extract with water. The persistent foam for 10 minutes confirms the presence of saponins.

Tests for steroids

Salkowski's test To the 2 ml test solution, add 2 ml chloroform and 2 ml conc. H_2SO_4. Shake well. Chloroform layer appears red and acid layer shows greenish yellow fluorescence.

Legal's test (For cardenolides) To the 1 ml of test solution, add 1 ml pyridine and 1 ml sodium nitroprusside solution. Pink to red colour appears.

Tests for flavonoids

Shinoda tests Dissolve extract in 5 ml of 95% v/v ethanol and add few drops of conc. HCl and 0.5 g of magnesium turnings. The pink, crimson or magenta colour represents flavonoids.

Tests for tannins

Ferric chloride test with the 5% ferric chloride solution extract gives dark green or deep blue colour.

Lead acetate test Add 10% w/v solution of basic lead acetate in distilled water to extract. Precipitate is obtained.

Potassium dichromate test with the extract, potassium dichromate solution produce dark precipitate.

Microbial strains

The microbial strains, to evaluate the antimicrobial activity of leaves extracts of *Zingiber officinale* food borne pathogenic and spoilage microorganisms, were obtained from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India. This study was conducted using *Escherichia coli* (MTCC 42/Gram positive bacteria).

Antimicrobial assay and minimum inhibitory concentration (MIC)

The antimicrobial assay was carried out using agar well diffusion method, in brief; the sterilized nutrient agar medium plates were spread with bacterial suspension (10^6 CFU/ml), prepared in sterile water, using sterile glass spreader. Further, the holes were punched using sterile cork borer and volume of 50 µl extract prepared in sterile water was filled in each bore. Here, sterile water was used as control. The plates were incubated at 35-37 °C for 24 hours (for *Escherichia coli*).

The minimum inhibitory concentration (MIC) represents the lowest concentration of substance, as an antimicrobial, that can inhibit the visible growth of microorganism after overnight incubation [20, 21]. In order to determine the MIC's of extracts, the initial extracts concentration of 50 mg/ml were evaluated for growth inhibition. Further, those extracts showed inhibition zone were checked for lower concentrations while the concentrations were increased for not showing visible inhibition zone. Overall, for the MIC's of extracts, the concentrations used were in the range of 10-100 mg/ml. The experiment was repeated thrice and diameter of inhibition zone, in mm, by respective extracts was noted down as mean ± standard deviation.

RESULTS AND DISCUSSIONS

The plant materials of *Zingiber officinale* and *Curcuma longa* is belonging to family Zingiberace used in the current study, to determine its effectiveness as antimicrobial agent against food borne pathogenic and spoilage microorganisms.

Table no. 1 : Physical evaluation of ginger and turmeric

S. N.	PARAMETERS	%COMPOSITIONS	
1.	Extractive Value – Ethanol Water Acetone Chloroform	Ginger 0.1, 0. 18 0.1 0.05	Turmeric 0.7 0.13 0.6 0.3
3.	Foaming index	166.66 mm	500mm
4.	Swelling Index	3cm	5cm
5.	loss on drying for given crude drug sample.	13.75	4.14
6.	Total ash value	60.66%	0.19%
7.	Acid Insoluble Ash Value	1.35	4.20
8.	Water soluble Ash Value:	1.67	1.00

Ethanol

Acetone

Water **Chloroform**

Figure no. : 1 Extractive value ginger and turmeric with ethanol, turmeric water and chloroform solvents

Figure no. : 2 Foaming index of ginger and turmeric

Figure no. : 3 Swelling index of ginger and turmeric

Figure no. : 4 loss on drying of ginger and turmeric

Figure no.: 5 Ash value of ginger and turmeric

The extractive values of ethanol, water, acetone, chloroform of ginger was found to be 0.1, 0. 18, 0.1, 0.05 and turmeric 0.7, 0.13, 0.6, 0.3 respectively. These extractive values clearly indicated the presence of more polar constituents then non polar in the rhizomes. The foaming index of ginger and turmeric was found to be 166.66mm, 500mm respectively which gives idea about the presence of higher percentage saponins presents in turmeric, besides preliminary phytochemical screening. The swelling index of ginger and turmeric was found to be 3cm and 5 cm respectively that indicates higher swelling power in turmeric. The loss on drying of powdered drug was 13.75% and 4.20 respectively, as the volatile components reported in the rhizomes. The ash value of ginger and turmeric was found to be 60.66%, 0.19% respectively that indicates presence of inorganic materials in it. The acid soluble ash value were found to be 1.35, 3.20 and water soluble ash were found to be 1.67, 1.00 respectively (Table no.1).

Table no. 2 : Phytochemical investigation of ginger and turmeric

S.NO.	Category	Test Name	Procedure	Ginger	Turmeric
1.	Carbohydrates	Molish test	2-3 ml sample + few drops of molish regent shake it + conc. H2SO4 from sides of tube violet ring	present	present
		Fehling test	1ml sample + equal amount of fehling's solution A + fehling solution B + heat , Brick red ppt	absent	present
		Iodine test	3ml sample + few drops of iodine solution Blue color (Disappear on boiling)	present	absent
		Benedict test	1ml sample + 5ml benedict solution shake + heat it for 3 mint (in water bath) Red or Yellow color	absent	present
2.	Alkaloids	Dragandraff's test	2-3 ml sample + few drops of dragandraff's reagent (potassium bismuth iodide) Orange Brown ppt	present	present
		Mayer's test	3ml of sample + few drops of mayer's reagent Cream colour ppt	present	present
		Wagner's test	3ml sample + few drops of wagner's reagent Reddish brown colour ppt	Present	Present
3.	Tannins	Ferric chloride test	1ml sample + ferric chloride solution dark blue\ green\ black colour	Present	Present
		Potassium permanganate test(KMNO4)	1ml sample + KMNO4 colourless	Present	Present

Figure no. 6 : Phytochemical investigation of ginger and turmeric

On phytochemical investigation of ginger and turmeric of various extracts, carbohydrates, and alkaloids and tannins were present in ginger and turmeric extract. This phytochemical investigation indicates alkaloids may be responsible for antimicrobial activity of ginger and turmeric.

Table no. 3 : in vitro Antibacterial study of ginger and turmeric extracts

Drug Name	Concentration of drug	Counted growth of Bacteria
Amoxicillin	10%	3
Azithromycin	10%	4
Water Turmeric	10%	8
Water Ginger	10%	3
Acetone Ginger	10%	1
Acetone Turmeric	10%	16
Alcohol Ginger	10%	5
Alcohol Turmeric	10%	8
Chloroform Ginger	10%	2
Chloroform Turmeric	10%	3

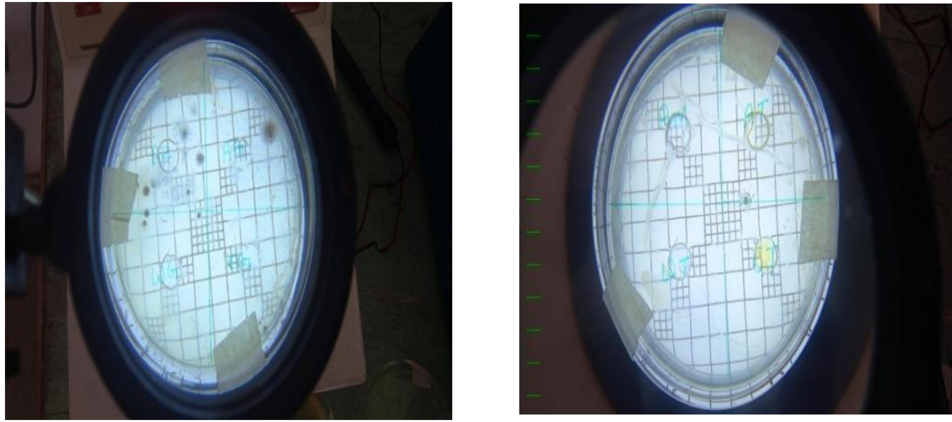

Figure no. 7 : Antimicrobial assay of ginger and turmeric

The antimicrobial assay revealed the significance of some extracts against the tested food borne pathogenic and spoilage bacteria. The antimicrobial assay clearly indicated that, the chloroform extract of both ginger and turmeric extracts and water and alcohol ginger extracts have lowest growth inhibition of microbial strains. of *E. coli* of and respectively , while water turmeric and acetone turmeric were found to show no particular inhibition to tested microorganisms up to the 10 % concentration of (Fig. 7 and Table 3)

Overall, the present study revealed that, chloroform extract of ginger and turmeric both are effective towards inhibition of food borne pathogenic and spoilage microorganisms and aqueous and ethanolic extracts of ginger also showed lowest growth of *E coli* bacteria so according to antibacterial study ginger extracts showed more effective antibacterial activity then turmeric extracts.

CONCLUSION

Food poisoning and spoilage are common problems nowadays that can threaten the health of humans. Modernization and scientific approach helped us to control such situation by the use of artificial preservatives. But due to increased capacity of microorganisms to resist such preservatives and toxicity chances, we need some better option that can be efficiently effective against them. In search of better options, researchers are continuously studying the use of plant extracts with antimicrobial action as preservatives. In continuation of such explorations, in this study, the antimicrobial activity of *Zingiber officinalis* and *Curcuma longa* extracts were evaluated against *Escherichia coli* responsible for most cases of food poisoning and spoilage. *E. coli* this

study concluded that, the *Zingiber officinalis* and *Curcuma longa* extracts can be used as preservatives to control the food poisoning and spoilage.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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