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Research Paper

Antibacterial activities of wild rhizomatous plants - *Zingiber officinalis, Zingiber zerumbet* (Zingiberaceae) and synergistic effects of both collected from southern Western Ghats, India

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Abstract

Rhizome extracts of *Zingiber officinalis* and *Zingiber zerumbet* (Zingiberaceae) from southern Western Ghats of Tamil Nadu, India were investigated for their antibacterial activity by agar well diffusion method against bacterial human pathogens such as *Escherichia coli, β -haemo streptococci, Klebsiella penumoniae, Staphylococcus aureus, Salmonella typhi, Bacillus subtilis and Pseudomonas aeruginosa*. The study revealed that all the pathogens were inhibited by various extracts (Methanol, Ethanol, n-Butanol and Acetone) of *Zingiber* spp. sometimes far better than the control antibiotic Amoxicillin. These results clearly elucidates that *Zingiber officinalis* and *Zingiber zerumbet* collected from wild have higher antibacterial activity than that of standard antibiotic.

Keywords: Antibacterial activity, *Zingiber officinalis, Zingiber zerumbet*, Western Ghats, India

Introduction

People have used plants since the beginning of civilization. Plants provide people with food, medicines, as well as materials for construction and the manufacture of crafts and tools and many other products like fuel, paints and poisons[1]. Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. Natural products of plants have been considered as the active ingredients of most of the modern medicines. The potential of higher plants as source for new drugs is still largely unexplored. Among the estimated 250,000 – 500,000 plant species, only a small percentage has been investigated phytochemically and the fraction submitted to biological or pharmacological screening is even smaller[2-3].

Zingiber officinale Rosc. belongs to the family Zingiberaceae. It is an herbaceous perennial and erect herb with horizontal jointed tuberous rhizomes. The stems are leafy, erect and about 15-150 cm long. The leaves are lanceolate and glabrous beneath flowers are bisexual and are borne solitary in heads. Each flower has three yellowish-orange petals with an additional purplish, liplike structure. The fruit is a capsule. The seeds are many, endospermic and arillate. The fresh or dried rhizomes, which are greyish-brown with a wrinkled surface and pale yellow inside, are grown commercially. The exact country of origin is uncertain. But it was thought to be originally native of tropical south East Asia before it spread to Africa. It is now grown abundantly in North Nigeria. The rhizomes of ginger are

used as spice in food and beverages and in traditional medicine as carminative, antipyrexia and treatment of waist pain rheumatism and bronchitis[4].

Zingiber zerumbet (L.) Rosc. is also belongs to the family Zingiberaceae. It is known by various names, for example, “Bon adha” (Bangladesh), “Ghatian” and “Yaiimu” (India), “Lempoyang” (Malaysia and Indonesia), “Awapuhi” (Hawaii), “Zurunbah” (Arab), “Hong qiu jiang” (China), and “Haeo dam” or “Hiao dam” (Northern Thailand)[5-8]. It is a wild ginger, widely cultivated plant in village gardens throughout the tropics for its medicinal properties[9-10]. Rhizomes are yellowish inside, tuberous. Leaves are sessile or shortly petiolate, ligule entire leaf blade lanceolate or oblonglanceolate. Inflorescences arise from rhizomes, conical or ovoid oblong. Its rhizomes are used in the traditional medicine as a cure for swelling, loss of appetite, lumbago, diabetes, inflammation, chest pain, rheumatic pains, bronchitis, dyspepsia and sore throat[5-7,11]. The juice of the boiled rhizomes has also been used in indigenous medicine for worm infestation in children[12-13]. From the pharmacological point of view, *Z. zerumbet* has been reported to inhibit colon and lung carcinogenesis in mice[14] and CXCL12-induced invasion of breast and pancreatic tumor cells[15], apoptosis in liver cancer[16], suppress phorbol ester-induced expression of multiple scavenger receptor genes in THP-1 human monocytic cells and Epstein-Barr Virus activation[17-18]. The volatile oils of the rhizomes have been shown to contain zerumbone, humulene and camprene. Bioactive compound(s) isolated and identified from various types of extracts of *Z. zerumbet*, only zerumbone has been studied extensively. Zerumbone has been demonstrated to possess in vivo antinociceptive[19] and anti-inflammatory[11,20-21] activities. While in the in vitro studies, zerumbone has been reported to exhibit antiproliferative[19] and antiplatelet aggregation[22] activities.

Materials and Methods

Plant collection and extraction

The rhizomatous plants were collected randomly from the regions where plenty of water is present. *Zingiber officinalis* and *Zingiber zerumbet* were collected for the study of antibacterial activity from southern Western Ghats. *Zingiber officinalis* and *Zingiber zerumbet* were collected from Paechiparai of Kanyakumari district of Tamil Nadu, India. The plant species were collected by hand or by using a knife. The plant species were transported to the laboratory in polythene bags. The unwanted plant materials were removed by using knife and the rhizome part is taken and washed with fresh water to remove the soil particles.

Extraction of compounds

The extraction was carried out with different solvents such as Methanol, Ethanol, n-Butyl alcohol and Acetone. Ten gram of the dried powder was soaked in 50 ml of different solvents with periodic shaking at room temperature. The extraction with different solvents were carried out individually for each sample. Each extracts were filtered through muslin cloth and collected in separate test tubes.

Test on human pathogens

The bacterial strains were identified strains, which obtained from Scudder laboratory, Nagercoil. The bacterial strains used for the study *were Escherichia coli, β-haemo streptococci, Klebsiella penumoniae, Staphylococcus aureus, Salmonella typhi, Bacillus subtilis* and *Pseudomonas aeruginosa*.

Preparation of test microorganisms

A loop full of the bacterial strains were inoculated in 20 ml of nutrient broth kept in a conical flask and incubated at 37^{o}C for 18 hours to activate the bacterial strains. This was used as inoculums for the further study.

Preparation of natural disc

Sterile discs were obtained and stored at 4^{o}C. Discs were handled using a pair of presterilized forceps. The extract was loaded on to the disc carefully using capillary tube, without spreading out. Thus, the disc completely saturated with the extract was used for testing antibacterial activity.

Culture of Test microorganisms

Solid media of nutrient agar was prepared by dissolving 2.8 gm of nutrient agar in 100 ml of distilled water. About 25ml of nutrient agar media was poured in to a petridish, allowed to solidify. The

inoculum of bacteria was transferred to petriplates containing solid nutrient agar media using a sterile swab.

Implementation of disc

Antibacterial activity was evaluated using agar disc diffusion technique. When the culture medium was solidified, dried test discs impregnated with extracts and synthetic discs were transferred on bacterial lawn under aseptic conditions using spirit flame sterilized forceps. The petridish was incubated at room temperature for 24 hours. The resulting, zones of inhibition around the disc were observed and recorded as positive and negative results. The inhibitory zone around the discs indicates absence of bacterial growth and was recorded as positive test (+), the absence of zones as negative test (-).

Results and Discussion

Extract of *Zingiber officinale* using Ethanol, Methanol, n-Butyl alcohol and Acetone showed varying activities. A perusal of Table 1 indicates the varied activities as measured by zone of inhibition with extracts made with Ethanol on selected human pathogens such as *Escherichia coli* (12 mm), *β*-haemo streptococci (10 mm), *Klebsiella pneumoniae* (10mm), *Staphylococcus aureus* (10mm), *Salmonella typhi* (10 mm), *Bacillus subtilis* (15 mm) and *Pseudomonas aeruginosa* (20 mm). Besides, the zone of inhibition measured with Methanol extract on *Escherichia coli* (20 mm), *β*-haemo streptococci (16 mm), *Klebsiella pneumoniae* (12 mm), *Staphylococcus aureus* (10 mm), *Salmonella typhi* (12 mm), *Bacillus subtilis* (10mm) and *Pseudomonas aeruginosa* (10 mm). In addition the zone of inhibition measured with n-butyl alcohol extract on *Escherichia coli* (10 mm), *β*-haemo streptococci (15 mm), *Klebsiella pneumoniae* (10 mm), *Staphylococcus aureus* (12 mm), *Salmonella typhi* (20 mm), *Bacillus subtilis* (12 mm) and *Pseudomonas aeruginosa* (12mm). As well as, the zone of inhibition measured with Acetone extract on *Escherichia coli* (17 mm), *β*-haemostreptococci (10 mm), *Klebsiella pneumoniae* (10mm) *Staphylococcus aureus* (10mm), *Salmonella typhi* (10mm), *Bacillus subtilis* (10 mm) and *Pseudomonas aeruginosa* (10 mm).

Extract of *Zingiber zerumbet* using Ethanol, Methanol, n – Butyl alcohol and acetone showed varying activities. A perusal of Table 1 indicates the varied activities as measured by zone of inhibition with extracts made with Ethanol on selected human pathogens such as *Escherichia coli* (NZI), *β*-haemostreptococci (10 mm), *Klebsiella pneumoniae* (10 mm), *Staphylococcus aureus* (10 mm), *Salmonella typhi* (10 mm), *Bacillus subtilis* (10 mm) and *Pseudomonas aeruginosa* (10mm). In addition, the zone of inhibition measured with Methanol extract on *Escherichia coli* (NZI), *β*-haemostreptococci (10 mm), *Klebsiella pneumoniae* (15 mm), *Staphylococcus aureus* (10mm), *Salmonella typhi* (10mm), *Bacillus subtilis* (10 mm) and *Pseudomonas aeruginosa* (10mm). Moreover, the zone of inhibition measured with n – butyl alcohol extract on *Escherichia coli* (10mm), *β*-haemostreptococci (15mm), *Klebsiella pneumoniae* (10 mm), *Staphylococcus aureus* (10mm), *Salmonella typhi* (10 mm), *Bacillus subtilis* (22 mm) and *Pseudomonas aeruginosa* (10 mm). The zone obtained from inhibition measured with Acetone extract *Escherichia coli* (NZI), *β*-haemostreptoocci (10 mm), *Klebsiella pneumoniae* (10 mm), Staphyloccocus aureus (20 mm), *Salmonella typhi* (NZI), *Bacillus subtilis* (15 mm) and *Pseudomonas aeruginosa* (15 mm).

Combined extracts of *Zingiber officinale* and *Zingiber zerumbet* using Ethanol, Methanol, n-Butyl alcohol and Acetone showed varying activities. A perusal of Table 1 indicates the varied activities as measured by zone of inhibition with extracts made with Ethanol on selected human pathogens such as Escherchia coli (10 mm), *β*-haemostreptococci (10 mm), *Klebsiella pneumoniae* (15 mm), *Staphylococcus aureus* (15 mm), *Salmonella typhi* (NZI), *Bacillus subtilis* (NZI) and *Pseudomonas aeruginosa* (NZI). Besides, the zone of inhibition measured with Methanol extract *Escherichia coli* (15 mm), *β*-haemostreptococci (10 mm), *Klebsiella pneumoniae* (12 mm), *Staphylococcus aureus* (NZI), *Salmonella typhi* (10 mm), *Bacillus subtilis* (NZI) and *Pseudomonas aeruginosa* (NZI). The zone obtained from inhibition measured with n-Butyl alcohol extract on *Escherichia coli* (10mm), *β*-haemostreptococci (10 mm), *Klebsiella pneumoniae* (12 mm), *Staphylococcus aureus* (NZI), *Salmonella typhi* (10 mm), *Bacillus subtilis* (NZI) and *Pseudomonas aeruginosa* (NZI). In addition, the zone of inhibition measured with Acetone extract on *Escherichia coli* (12mm), *β*-haemostreptococci (10 mm), *Klebsiella pneumoniae* (10 mm), *Staphylococcus aureus* (NZI), *Salmonella typhi* (NZI), *Bacillus subtilis* (10 mm) and *Pseudomonas aeruginosa* (NZI).

In our results, *Pseudomonas aeruginosa* alone was inhibited to Ethanolic extract of *Z. officinale* with the zone of inhibition measuring 20mm. *Bacillus subtilis* and *Escherichia coli* were susceptible to the

extract of *Z. officinale* with the zone formation above 10mm. *Escherichia coli*, *β*-haemo streptococci, *Klebsiella pneumoniae* and *Salmonella typhi* were inhibited by Methanolic extract of *Z. officinale* with the zone of inhibition measuring above 12mm. The maximum zone formation was observed against *Escherichia coli* with the zone of inhibition measuring 20mm. *β*-haemo streptococci, *Staphylococcus aureus*, *Salmonella typhi*, *Bacillus subtilis* and *Pseudomonas aeruginosa* were inhibited to n-Butyl alcohol extract of *Zingiber officinale* with the zone of inhibition measuring above 10mm. Of these, maximum zone formation was observed against *Salmonella typhi* with the zone of inhibition measuring 20mm. *Escherichia coli* alone were inhibited by Acetone extract of *Z. officinale* with the zone of inhibition measuring 17mm.

Table 1: Growth Inhibition of Human pathogenic Bacteria by Rhizome of extracts of *Zingiber officinale* and *Zingiber zerumbet* (diameter of zone in mm)

Human Pathogens	Solvents	*Zingiber officinale*	*Zingiber zerumbet*	*Zingiber officinale with Zingiber zerumbet*	Amoxicillin
Escherchia coli	Mt	20	-	15	19
	Et	12	-	10	
	n-But	10	10	10	
	Ac	17	-	12	
β-haemostreptococci	Mt	16	10	10	-
	Et	10	10	10	
	n-But	15	15	10	
	Ac	10	10	10	
Klebsiella pneumoniae	Mt	12	15	12	12
	Et	10	10	15	
	n-But	10	10	10	
	Ac	10	10	10	
Staphylococcus aureus	Mt	10	10	-	-
	Et	10	10	10	
	n-But	12	10	-	
	Ac	10	20		
Salmonella typhi	Mt	12	10	10	-
	Et	10	10	-	
	n-But	20	10	-	
	Ac	10	-	-	
Bacillus subtilis	Mt	10	10	-	10
	Et	15	10	-	
	n-But	12	22	15	
	Ac	10	15	10	
Pseudomonas aeruginosa	Mt	10	10	-	16
	Et	20	10	-	
	n-But	12	10	10	
	Ac	10	15	-	

(-) = NZI – No Zone of Inhibition/Absence of susceptibility, Mt – Methanol, Et – Ethanol, n-But - n-Butanol, Ac - Acetone

The methanolic extract of *Zingiber* spp. showed better inhibition against *β*-haemo streptococci, *S. aureus* and *S. typhi* than the standard antibiotic. Of course, standard antibiotic has no effect on these bacteria. Similarly, *Z. officinale* showed better inhibition of growth of *E. coli* than the standard antibiotic amoxicillin. However, the methanolic extract of *Z. zerumbet* showed better inhibition of growth of *K. pneumoniae* than the standard antibiotic amoxicillin. *B. subtilis* inhibited equally by all the three as well standard antibiotic. *P. aeruginosa* was inhibited by standard antibiotic whereas the botanicals' performance is far poor than the standard (Figure – 1). The ethanolic extract of *Zingiber* spp. showed better inhibition against *β*-haemo streptococci, *S. aureus* and *S. typhi* than the standard antibiotic amoxicillin which has no effect on these bacteria. *Bacillus subtilis* and *P. aeruginosa* were inhibited by *Z. officinale* whereas *K. pneumoniae* was inhibited by both *Z. officinale* and *Z. zerumbet* combined. *E. coli* was inhibited by standard antibiotic whereas the botanicals' performance is far poor than the standard (Figure 2).

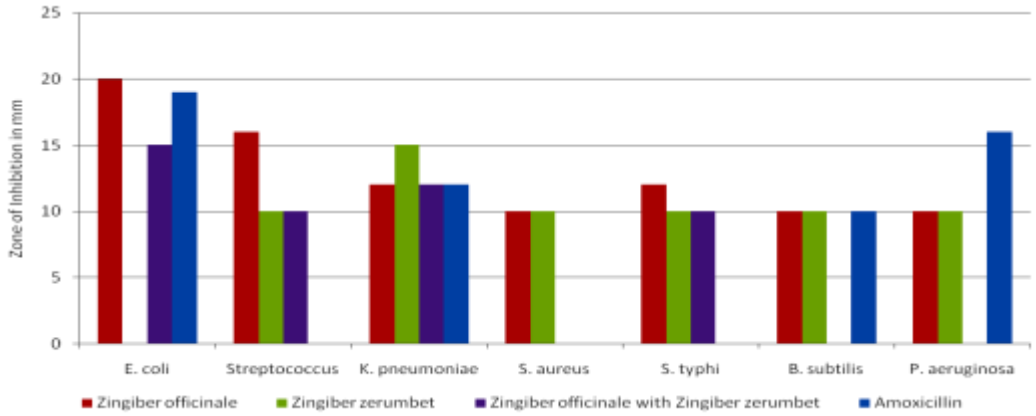

Figure 1: Sensitivity pattern of pathogenic bacteria with methanolic extract of *Zingiber* spp.

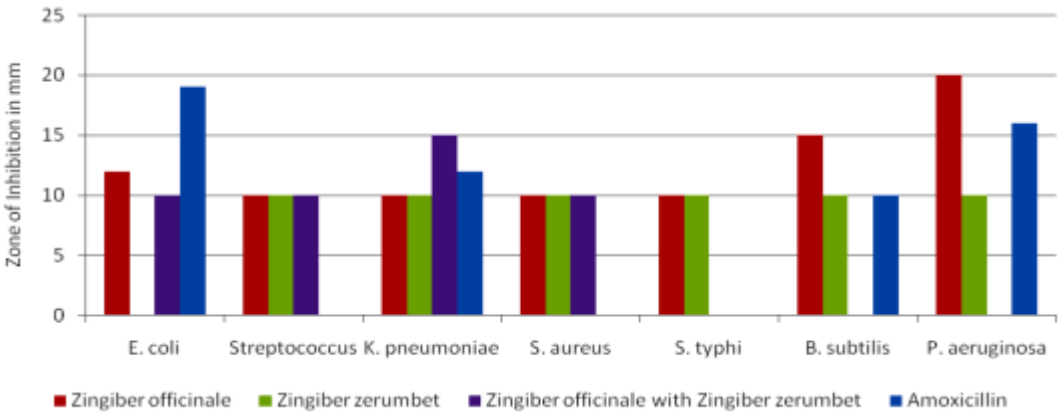

Figure 2: Sensitivity pattern of pathogenic bacteria with ethanolic extract of *Zingiber* spp.

The n-butanol extract of *Zingiber* spp. showed better inhibition against *β*-haemo streptococci, *S. aureus* and *S. typhi* than the standard antibiotic amoxicillin which has no effect on these bacteria. *E. coli, K. pneumoniae* and *P. aeruginosa* were inhibited by both *Z. officinale* and *Z. zerumbet* than the standard antibiotic. *B. subtilis* was inhibited by standard antibiotic whereas the botanicals' performance is far poor than the standard (Figure 3). The acetone extract of *Zingiber* spp. showed better inhibition against *β*-haemo streptococci, *S. aureus* and *S. typhi* than the standard antibiotic amoxicillin which has no effect on these bacteria. Especially *S. aureus* and *B. subtilis* were inhibited by *Z. zerumbet* and *S. typhi* was inhibited by *Z. officinale* whereas *β*-haemo streptococci was inhibited by all the three acetone extracts. Acetone extract has poor effect on *E. coli, K. pneumoniae* and *P. aeruginosa* than the standard antibiotic amoxicillin (Figure 4). The present study was in correlation with the results of previous study which found out that Ginger extract was effective against all of the microorganisms tested. Diameter of the inhibition zones were ranged in between 13.0-19.0mm[23].

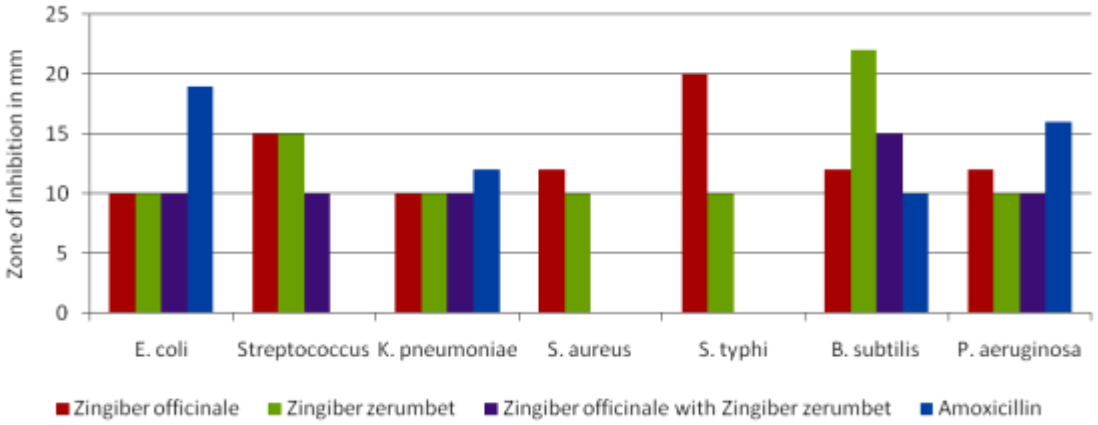

Figure 3: Sensitivity pattern of pathogenic bacteria with n-butanol extract of *Zingiber* spp.

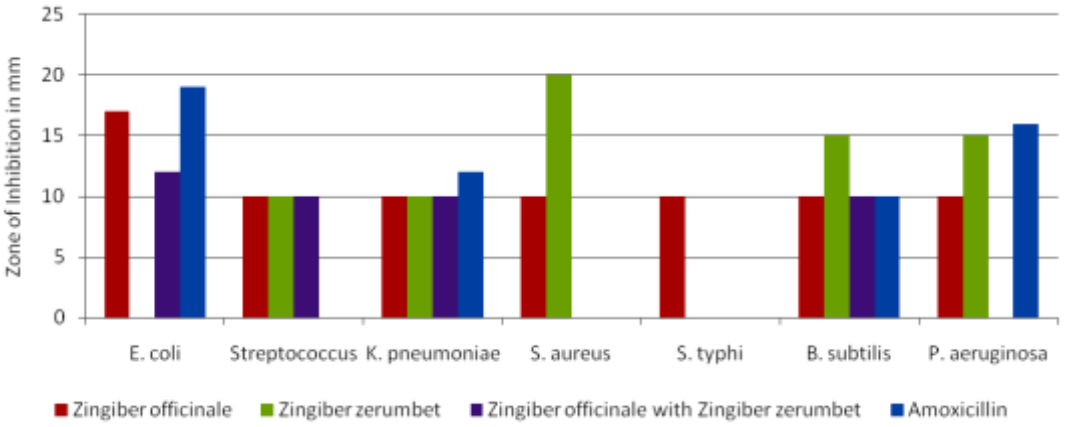

Figure 4: Sensitivity pattern of pathogenic bacteria with acetone extract of *Zingiber* spp.

E. coli was not susceptible to Ethanolic extract of *Z. zerumbet*, maximum zone of inhibition was observed against *β*-haemo streptococci, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Salmonella typhi*, *Bacillus subtilis* and *Pseudomonas aeruginosa* measuring 10mm. Methanolic extract of *Z. zerumbet* inhibited the growth of *Klebsiella pneumoniae* alone with the zone formation measuring 15mm. n-Butyl alcohol extract of *Z. zerumbet* inhibited the growth of *Bacillus subtilis* alone with the zone formation measuring 22mm. *Salmonella typhi* and *Escherichia coli* were not susceptible to Acetone extract of Zingiber zerumbet, maximum zone of inhibition was observed against *Staphylococcus aureus* measuring 20mm. Antibacterial activity of *Z. zerumbet* compound namely zederone was determined against a number of multi-drug resistant and methicillin-resistant *Staphylococcus aureus* strains and minimum inhibitory concentration (MIC) values were found to be in the range of 64-128 Mg/ml[24-25].

Salmonella typhi, *Bacillus subtilis* and *Pseudomonas aeruginosa* were susceptible to Ethanol extract of *Z. officinale* with *Z. zerumbet*, maximum inhibition zone were observed against *Klebsiella pneumoniae* and *Staphylococcus aureus* measuring 15mm. Maximum inhibition zone was observed against *Escherichia coli* measuring 15mm. *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa* were not susceptible to n-Butyl alcohol extract of *Z. officinale* with *Z. zerumbet*, maximum inhibition zone measuring 12mm diameter was observed against *Klebsiella pneumoniae*. Whereas *Salmonella typhi* and *Pseudomonas aeruginosa* were resistant to Acetone extract of *Z. officinale* with *Z. zerumbet*, maximum inhibition zone observed against *Escherichia coli* measuring 12mm. Similar work reported shows that most Zingiberaceae plant extracts exhibited antimicrobial activity against all tested food microorganisms. This study was a positive demonstration of the utility of the screening Taiwan's Endemic Zingiberaceous plants for their food and medicinal uses[26].

It was reported that the organic solvent extract of ginger rhizomes has also been shown to cause significant inhibition of skin tumour. On the basis of these common uses of this plant in traditional folk medicine and its reported activities in the literature, the anti-inflammatory and antimicrobial properties of the rhizome extract of *Zingiber officinale* in rats and mice respectively[27]. Samy (2005) used methanolic extracts of ginger which did not present antimicrobial effect against *Staphylococcus aureus* and *Escherichia coli*[28]. However, Indu *et al.* (2006) used a different method of ginger extract preparation, verified an inhibitory action against *Escherichia coli* as well as high antimicrobial activity of garlic extracts against *Escherichia coli* and *Salmonella*[29]. It was found out that the high concentration of zerumbone and α- caryophyllene in leaf and rhizome oil makes it respectively potentially useful in the medicines because they exhibit antimicrobial properties. It is worth noting that the oil of *Zingiber zerumbet* have been reported to be used in folk medicine in the treatment of inflammation, diarrhea and rheumatic pain[30].

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