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Research Article

Antibacterial and Synergistic Activities of Methanolic Leaves Extract of Lemon Grass (*Cymbopogon citratus*) and Rhizomes of Ginger (*Zingiber officinale*) against *Escherichia coli, Staphylococcus aureus* and *Bacillus subtilis*

Sylvester Chibueze Izah[1]* and Ebinyo R Aseibai[2]

[1]*Department of Biological Sciences, Faculty of Science, Niger Delta University, Wilberforce Island, Bayelsa state, Nigeria*

[2]*Department of Science Foundation, School of Foundation Studies, Bayelsa state College of Health Technology, Otuogidi, Nigeria*

***Corresponding Author:** Sylvester Chibueze Izah, Department of Biological Sciences, Faculty of Science, Niger Delta University, Wilberforce Island, Bayelsa state, Nigeria.

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Abstract

Cymbopogon citratus (lemon grass) and *Zingiber officinale* (ginger) are common food spices that are used in preparing decoctions for certain disease condition by traditional medicine practitioners. This study assessed antibacterial and synergistic activities of methanolic leaves extract of lemon grass and rhizomes of ginger. The plant materials were dried and extracted using methanol for 48 hours. Agar well diffusion method was adopted for the sensitivity testing. Results showed that zone of inhibition for *Bacillus subtilis, E. coli* and *S. aureus* was 9.33 mm, 10.67 mm and 10.67 mm, respectively for ginger, 10.67 mm, 9.00 mm and 10.00 mm, respectively for lemon grass and 10.67 mm, 11.67 mm, 11.67 mm, respectively for synergy of lemon grass and ginger. At 95% concentration of the extracts, the mean zone of inhibition showed by *Bacillus subtilis, E. coli* and *S. aureus* was 7.33 mm, 8.67 mm and 8.67 mm, respectively for ginger, 8.67 mm, 7.33 mm and 8.67 mm, respectively for lemon grass, and 7.33 mm, 8.33 mm, 8.67 mm, respectively for synergy of lemon grass and ginger. There was significant difference ($P < 0.05$) among the various isolates, and plants and its combinations at 100%, 95% and 90% extract concentrations. The findings showed that the synergy had slight superior potency against the tested isolates.

Keywords: Antimicrobial; Disease Condition; Cymbopogon citratus; Medicinal Plants; Zingiber officinale

Introduction

Herbal medicine has served as therapeutic/prophylactic products for the treatments of diseases throughout history [1]. According to Sherwani., *et al.* [2], the use of plant as medicine can be traced back to human history. Silva and Fernandes [3] also reported that plant is used to flavor and conserve food, to treat health disorders and to prevent diseases including epidemic.

The knowledge of medicinal potentials of plants is transmitted from one generation to another over the centuries [3]. Currently, phytomedicine and herbal medicine practices have been accepted globally [1]. The medicinal potentials of plant have been linked to its phytochemical composition and toxicological profile [1,4-7]. Silva and Fernandes [2] reported that bioactive compounds produced during secondary vegetal metabolism are usually responsible for the biological properties.

Several plants (herbs) used as food and or/ spices have been widely studied for their pharmacological properties. Ekpenyong., *et al.* [1] reported that research is still being carried out to elucidate the putative phytochemical and toxicological profiles of some herbs. Furthermore, the role of some medicinal plants against some certain disease and/or pest has been commercialized. Some of the common herbs that have pharmacological potentials that is used as food include *Myristica fragrans* (nutmeg), *Vernonia amygdalina* (bitter leaf), *Ocimum gratissimum* (scent leaf), *Zingiber officinale* (ginger), *Aframomum melegueta* (alligator pepper), *Cymbopogon citratus* (lemon grass), *Piper nigrum* (climbing pepper).

Zingiber officinale Roscoe (ginger) is a perennial herb that grows up to about 3 - 4 feet high [8]. Ginger is native to India [9,9], China, Java, and several African countries [8] such as Nigeria, Sierra Leone etc. Ginger is commonly used as ornamentals, spices/ flavoring agents (food), and medicine [8,10-12] for several diseases. Some medicinal properties of ginger include anti-diabetic and hypolipideamic [13], antioxidants [14], antimicrobial [15-17], anti-inflammatory [18,19], lavicidal activities against dengue and filariasis vectors [20], anti-viral infection against rotavirus infection [21]. Haniadka., *et al.* [22] reported that rhizomes of ginger in used by traditional medicine practitioners for the treatment of several ailments including arthritis, rheumatism, sprains, muscular aches, pains, sore throats, cramps, hypertension, dementia, fever, infectious diseases, catarrh, nervous diseases, gingivitis, toothache, asthma, stroke and diabetes. The authors further reported that its used for the treatment of gastric ailments (viz: constipation, dyspepsia, belching, bloating, gastritis, epigastric discomfort, gastric ulcerations, indigestion, nausea and vomiting)

Cymbopogon citratus (lemon grass) belongs to Poaceae family. Lemon grass is also native to Sri Lanka [23] and India [8,23] and its now cultivated in tropical regions of Asia, America [23], Africa. Lemon grass monocotyledonous aromatic perennial plant [2] that can grow up to 90 cm in height and 5 mm wide [1]. Studies have shown that bioactive constituents of lemon grass are responsible for its wide range of pharmacological and physiological properties [1]. Lemon grass is used as medicine, cosmetic and food probably due to the composition [1].

Lemon grass have been widely reported to have anti-diabetic [24], anti-microbial [25-29], anti-oxidants [26,30], renal healing [31], anti-malaria [32], lavicidal activities against dengue and filariasis vectors [20], anti-helmintic [2], anti-viral infection against rotavirus infection [21]. In a review study, Shah., *et al.* [23] reported that lemon grass is potent against anti-amoebic, anti-microbial, antidiarrhoea, antifilarial, hypotensive, anticonvulsant, analgesic, antiemetic, antitussive, antirheumatic, antiseptic, anti-inflammatory antimalarial, antimutagenicity, antimycobacterial, antioxidants, hypoglycemic and neurobehavioral potentials. The authors have further reported that decoctions made with lemon grass are used in several countries to treat different type of ailments. Sherwani., *et al.* [2] have reported that lemon grass is used for the treatment of gastrointestinal disturbances, nervous, hypertension, fever, elephantiasis, coughs, flu, headache, gingivitis, malaria, leprosy, ophthalmia, vascular disorders and pneumonia [2].

The antimicrobial potentials of lemon grass and ginger have been widely reported in literature using different solvent for extraction. But information about the synergistic efficacy of both lemon grass and ginger is scanty in literature. Hence, this study aimed at assessing the synergistic efficacy of methanolic extracts of lemon grass and rhizome of ginger.

Materials and Methods

Samples procurement, preparations and extraction

Samples of ginger rhizomes (Figure 1a) were purchased from Swali market in Yenagoa metropolis, Bayelsa state, Nigeria. While the leaves of lemon grass (Figure 1b) was obtained from Ndemili in Ndokwa west local government Area of Delta state, Nigeria. The lemon grass leaf was shade dried, and the ginger rhizomes was cut into pieces. Then after, the samples were separately macerated using pestle and mortar. 40g of the powered samples were soaked in 100 ml of methanol for 48 hours. Furthermore, 20g of each of the powered lemon and ginger mixed together making the mixture 40g, and it was soaked in methanol for 48 hours as well. After the soaking period, it was filtered using muslin cloth, and the filtrate was re-filtered using Whatman filter paper. The solvent was allowed to evaporate in a water bath.

[a] Ginger rhizome

[b] Lemon grass

Figure 1: Rhizome of ginger and Lemon grass.

Dilution of the extracts

Extract dilution previously described by Kigigha., *et al.* [4] was adopted for this study. The extract was considered as 100% concentration and then further diluted into 95%, 90% and 85% using sterile water.

Source of microbes

The microbial isolates (*Staphylococcus aureus, E. coli*, and *Bacillus subtilis*) used in this study were obtained from Medical Microbiology Department, Federal Medical Centre, Yenagoa, Bayelsa state. The purity and characteristics of the bacteria isolates was determined following the method provided by Cheesbrough [33]. The isolates were inoculated into prepared sterile peptone water and incubated for 24 hours prior to use.

Antimicrobial screening of the extract

Agar well diffusion method previously described by Lino and Deogracious [34] cited in Doherty., *et al*. [35] with slight modification by Agu and Thomas [36], Kigigha., *et al*. [4,5], Epidi., *et al.* [37,38] was used for this study. Approximately 20ml of prepared nutrient agar was poured onto sterile Petri dish, and it allowed to solidify. Then after, 0.3ml of the organisms in peptone water was place in the solidified agar plates and spread over the surface of the agar. Wells of 6mm were made in agar plate using cork borer. 3 ml of the extracts were dispensed into the agar wells and it was incubated for 24 hours. The resultant zones of inhibition were measured using metre rule.

Statistical analysis

SPSS software version 20 was used to for the statistical analysis. Descriptive statistics (Mean ± standard error) was carried out. One-way analysis of variance was carried out at P = 0.05. Waller-Duncan multiple range test statistics was used for mean separations.

Results and Discussion

The zone of inhibition of methanolic leaves extract of *Cymbopogon citratus* and rhizomes of *Zingiber officinale* at different concentrations is presented in table 1. The zone of inhibition decline as dilution with distilled water increases. At 100% concentration, the mean zone of inhibition showed by *Bacillus subtilis, E. coli* and *S. aureus* was 9.33mm, 10.67 mm and 10.67 mm, respectively for ginger, 10.67 mm, 9.00 mm and 10.00 mm, respectively for lemon grass and 10.67 mm, 11.67 mm, 11.67 mm, respectively for synergy of lemon grass and ginger. At 95% concentration of the extracts, the mean zone of inhibition showed by *Bacillus subtilis, E. coli* and *S. aureus* was7.33 mm, 8.67 mm and 8.67 mm, respectively for ginger, 8.67 mm, 7.33 mm and 8.67 mm, respectively for lemon grass, and 7.33 mm, 8.33 mm, 8.67 mm, respectively for synergy of lemon grass and ginger. At 90% concentration of the extracts, the mean zone of inhibition showed by *Bacillus subtilis, E. coli* and *S. aureus* was 0.00 mm, 7.00 mm and 7.33 mm, respectively for ginger, 0.00 mm, 0.00 mm and 7.33 mm, respectively for lemon grass, and 0.00 mm, 4.67 mm, 5.00 mm, respectively for synergy of lemon grass and ginger. There was significant difference ($P < 0.05$) among the various isolates, and plant and its combinations at 100%, 95% and 90% concentration.

Plants	Isolates	100%	95%	90%	85%
Zingiber officinale (ginger)	*Bacillus subtilis*	9.33 ± 0.33a	7.33 ± 0.33a	0.00 ± 0.00a	0.00 ± 0.00
	E. coli	10.67 ± 0.33ab	8.67 ± 0.33b	7.00 ± 0.00b	0.00 ± 0.00
	Staphylococcus aureus	10.67 ± 0.33ab	8.67 ± 0.33b	7.33 ± 0.33b	0.00 ± 0.00
Cymbopogon citratus (lemon grass)	*Bacillus subtilis*	10.67 ± 0.67ab	8.67 ± 0.33b	0.00 ± 0.00a	0.00 ± 0.00
	E. coli	9.00 ± 0.58a	7.33 ± 0.33a	0.00 ± 0.00a	0.00 ± 0.00
	Staphylococcus aureus	10.00 ± 0.58ab	8.67 ± 0.33b	7.33 ± 0.33b	0.00 ± 0.00
Synergy of ginger and lemon grass	*Bacillus subtilis*	10.67 ± 0.67ab	7.33 ± 0.33a	0.00 ± 0.00a	0.00 ± 0.00
	E. coli	11.67 ± 0.67b	8.33 ± 0.33ab	4.67 ± 2.33b	0.00 ± 0.00
	Staphylococcus aureus	11.67 ± 0.33b	8.67 ± 0.33b	5.00 ± 2.52b	0.00 ± 0.00

Table 1: Zone of inhibition (mm) of methanolic leaves extract of Cymbopogon citratus and rhizomes of Zingiber officinale.

Different letters along the column indicate significant variation ($P < 0.05$) according to Waller-Duncan statistics.

The significant difference among the zone of inhibition may be associated to variation physiology, metabolism, nutrition, genetic composition and biochemistry of the isolates under study [5-7,37-39]. Furthermore, age of the plants, type of solvents, extract protocol, environmental condition of the area the plant was cultivated, time of harvesting may affect the sensitivity of the plants against some microbial isolates [39-41]. The synergy showed apparently increase in sensitivity level compared to the independent extracts of ginger and lemon grass.

The antimicrobial potentials of both plants could be due to the presence of phytochemical and bioactive ingredients [4-7,37-39]. Ekpenyong., *et al*. [1] reported that lemon grass contains phytoconstituents including tannins, saponins, flavonoids, phenols, anthraquinones, alkaloids, deoxysugars, and various essential oil. Shah., *et al.* [23] also reported the presence of flavonoids, phenolic compounds, terpenes, alcohols, ketones, aldehyde and esters, Citral α, Citral β, Nerol Geraniol, Citronellal, Terpinolene, Geranyl acetate, Myrecene and Terpinol Methylheptenone in lemon grass. Ranitha [8] reported essential oil of ginger contain Borneol, β-Bisabolene, Cineole, α-Cedrene, α-Curcumene, β-Farnesene (E), β-Sesquihelladiene, β-Thujene and Zingiberene, and lemon grass contain Citral, Geranic Acid, Geranyl Acetate, Linalool, Neric acid, (Z) Citral, β-mycrene and β-Thujene. Furthermore, flavonoids, tannins [9,15,42], alkaloids, saponins, and triterpenes [9,15], total polyphenol [42], phlobotannins and glycosides [15] are present in ginger. The presence of flavonoids and phenolic compounds may be responsible for the antibacterial potential of both plants.

The findings of this study are in consonance with previously word that suggested that ginger and lemon grass have anti-bacteria activities. Sylvanus., *et al.* [17] reported that hexane extract lemon grass is potent against Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi and Staphylococcus aureus. Several authors have reported anti-bacteria properties of ginger to both gram positive and gram-negative bacteria [15,43,44]. Furthermore, lemon grass also has antibacterial activity against both gram positive and negative organisms [25,27-29].

Conclusion

The field of herbal medicine and phytomedicine is gain attention probably due to search of new drugs including antimicrobial. Lemon and ginger are food common flavoring/spicing plants that are also used in preparing decoctions against some disease conditions. This study evaluated the antibacterial and synergistic efficacy of leaves extract of lemon grass and rhizomes of ginger. The results validate the existing information that both plants have broad spectrum antibacterial potentials. In addition, synergetic effects of both plants have slight apparent effects compared to separate plant extracts.

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