



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 Aseibai²

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

²Department 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.

Received: April 25, 2018; Published: May 21, 2018

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], larvicidal 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], larvicidal activities against dengue and filariasis vectors [20], anti-helminthic [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, anti-diarrhoea, 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], Epedi., *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 placed 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 for the statistical analysis. Descriptive statistics (Mean \pm 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 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. 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%
<i>Zingiber officinale</i> (ginger)	<i>Bacillus subtilis</i>	9.33 ± 0.33a	7.33 ± 0.33a	0.00 ± 0.00a	0.00 ± 0.00
	<i>E. coli</i>	10.67 ± 0.33ab	8.67 ± 0.33b	7.00 ± 0.00b	0.00 ± 0.00
	<i>Staphylococcus aureus</i>	10.67 ± 0.33ab	8.67 ± 0.33b	7.33 ± 0.33b	0.00 ± 0.00
<i>Cymbopogon citratus</i> (lemon grass)	<i>Bacillus subtilis</i>	10.67 ± 0.67ab	8.67 ± 0.33b	0.00 ± 0.00a	0.00 ± 0.00
	<i>E. coli</i>	9.00 ± 0.58a	7.33 ± 0.33a	0.00 ± 0.00a	0.00 ± 0.00
	<i>Staphylococcus aureus</i>	10.00 ± 0.58ab	8.67 ± 0.33b	7.33 ± 0.33b	0.00 ± 0.00
Synergy of ginger and lemon grass	<i>Bacillus subtilis</i>	10.67 ± 0.67ab	7.33 ± 0.33a	0.00 ± 0.00a	0.00 ± 0.00
	<i>E. coli</i>	11.67 ± 0.67b	8.33 ± 0.33ab	4.67 ± 2.33b	0.00 ± 0.00
	<i>Staphylococcus aureus</i>	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, Myrcene 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 -myrcene 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.

Bibliography

- Ekpenyong CE, *et al.* "Phytochemical Constituents, Therapeutic Applications and Toxicological Profile of *Cymbopogon citratus* Stapf (DC) Leaf Extract". *Journal of Pharmacognosy and Phytochemistry* 3.1 (2014): 133-141.
- Sherwani SK, *et al.* "Evaluation of *In Vitro* anthelmintic activity of *Cymbopogon citratus* (lemon grass) extract". *International Journal of Pharmacy and Life Sciences* 4.6 (2013): 2722-2726.
- Silva NCC and Fernandes JA. "Biological properties of medicinal plants: a review of their antimicrobial activity". *The Journal of Venomous Animals and Toxins including Tropical Diseases* 16.3 (2010): 402-413.
- Kigigha LT, *et al.* "Activities of *Aframomum melegueta* Seed Against *Escherichia coli*, *S. aureus* and *Bacillus* species". *Point Journal of Botany and Microbiology Research* 1.2 (2015): 23-29.

5. Kigigha LT, et al. "Phytochemical and antibacterial activities of Musanga cecropioides tissues against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus* and *Bacillus* species". *International Journal of Applied Research and Technology* 5.1 (2016): 100-107.
6. Kalunta CG. "Antimicrobial effect of different seed extracts of *Piper nigrum* against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*". *Biotechnology Research* 3.3 (2017): 71-76.
7. Kigigha LT and Kalunta CG. "Antimicrobial efficacy of leaf extracts of *Piper nigrum* against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*". *Journal of Basic Pharmacology and Toxicology* 1.2 (2017): 32-36.
8. Ranitha A. "Extraction and characterization of essential oil from ginger (*Zingiber officinale* Roscoe) and lemongrass (*Cymbopogon citratus*) by microwave-assisted hydrodistillation (MAHD)". Thesis submitted in fulfillment of the requirements for the award of degree of Bachelor of Chemical Engineering, Universiti Malaysia Pahang (2012).
9. González-Guevara JC., et al. "Physicochemical characterization of medicinal essential oil from the rhizome of *Zingiber officinale* (ginger), grown in San Carlos, Costa Rica". *Journal of Applied Pharmaceutical Sciences* 4.1 (2017): 9-18.
10. Izah SC., et al. "Advances in preservatives and condiments used in zobo (a food-drink) production". *Biotechnological Research* 2.3 (2016): 104-119.
11. Lawal TO., et al. "Antibacterial Potentials of Three Common Spices against Selected Pathogens". *Journal of Natural Sciences Research* 4.18 (2014): 78-84.
12. Gaurav K., et al. "A Review on Pharmacological and Phytochemical Properties of *Zingiber officinale* Roscoe (Zingiberaceae)". *Journal of Pharmacy Research* 4.9 (2011): 2963-2966.
13. Al-Amin ZM., et al. "Anti-diabetic and hypolipidemic properties of ginger (*Zingiber officinale*) in streptozotocin-induced diabetic rats". *British Journal of Nutrition* 96.4 (2006): 660-666.
14. Zhao X., et al. "Effects of ginger root (*Zingiber officinale*) on laying performance and antioxidant status of laying hens and on dietary oxidation stability". *Poultry Science* 90.8 (2011): 1720-1727.
15. Bhargava S., et al. "Zingiber Officinale: Chemical and phytochemical screening and evaluation of its antimicrobial activities". *Journal of Chemical and Pharmaceutical Research* 4.1 (2012): 360-364.
16. Indu S and Nirmala AM. "Comparative chemical composition and antimicrobial activity of fresh and dry ginger oil". *International Journal of Current Pharmaceutical Research* 2.4 (2010): 2-4.
17. Thomson M., et al. "The use of Ginger as a potential anti-inflammatory and antithrombotic agent". *Prostaglandins, Leukotrienes and Essential Fatty Acids* 67.6 (2012): 475-478.
18. Sylvanus U., et al. "Antimicrobial activities of volatile oil compound from the rhizome of *Zingiber officinale* using solvent method extraction". *Pharmaceutical and Biological Evaluations* 2.3 (2015): 47-51.
19. Li F., et al. "Isolation of quinine reductase (QR) inducing agents from ginger rhizome and In vitro anti-inflammatory activity". *Food Research International* 44.6 (2011): 1597-1603.
20. Rabha B., et al. "Larvicidal activity of some essential oil hydrodistillates against dengue and filariasis vectors". *E3 Journal of Medical Research* 1.1 (2012): 014-016.
21. AL-Ballawi ZFS., et al. "In Vitro Studies of Some Medicinal Plants Extracts for Antiviral Activity against Rotavirus". *IOSR Journal of Pharmacy and Biological Sciences* 12.2 (2017): 53-58.
22. Haniadka R., et al. "A review of the gastroprotective effects of ginger (*Zingiber officinale* Roscoe)". *Food and Function* 4.6 (2013): 845-855.
23. Shah G., et al. "Scientific basis for the therapeutic use of *Cymbopogon citratus*, stapf (Lemon grass)". *Journal of Advanced Pharmaceutical Technology and Research* 2.1 (2011): 3-8.
24. Bharti SK., et al. "Essential Oil of *Cymbopogon citratus* against Diabetes: Validation by in vivo Experiments and Computational Studies". *Scientific Reports* 2.3 (2013): 1-9.
25. Ewansiha JU., et al. "Antimicrobial Activity of *Cymbopogon citratus* (lemon grass) and its Phytochemical Properties". *Frontiers in Science* 2.6 (2012): 214-220.
26. Cheel J., et al. "Free radical scavengers and antioxidants from Lemongrass (*Cymbopogon citratus* (DC.) Stapf.)". *Journal of Agricultural and Food Chemistry* 53.7 (2005): 2511-2517.
27. Melo SF., et al. "Effect of the *Cymbopogon citratus*, *Maytenus ilicifolia* and *Baccharis genistelloides* extracts against the stannous chloride oxidative damage in *Escherichia coli*". *Mutation Research* 496.1-2 (2001): 33-38.
28. Onawunmia GO., et al. "Antibacterial constituents in the essential oil of *Cymbopogon citratus* (DC.) Stapf". *Journal of Ethnopharmacology* 12 (1984): 279-286.
29. Syed M., et al. "Essential oils of Graminae family having antibacterial activity Part 1. (*Cymbopogon citratus*, *C. martinii* and *C. jawarancusa* oils)". *Pakistan Journal of Scientific and Industrial Research* 33 (1990): 529-531.
30. Koh PH., et al. "Antioxidant potential of *Cymbopogon citratus* extract: alleviation of carbon tetrachloride-induced hepatic oxidative stress and toxicity". *Human and Experimental Toxicology* 31.1 (2012): 81-91.
31. Ullah N., et al. "*Cymbopogon citratus* protects against the renal injury induced by toxic doses of aminoglycosides in rabbits". *Indian Journal of Pharmaceutical Sciences*. 75 (2013): 241-246.
32. Tchoumboungang F., et al. "In vivo Anti malaria Activity of Essential Oils from *Cymbopogon Citratus* and *Ocimum Gratissimum* on Mice Injected with *Plasmodium Berghei*". *Planta Medica* 71.1 (2005): 20-23.
33. Cheesbrough M. "District Laboratory Practice in Tropical Countries". Low price Edition part 2. Cambridge press, England (2004).
34. Lino A and Deogracious O. "The invitro antibacterial activity of *Annona senegalensis*, *Sacurideae longipendiculata* and *Steganotaema araliacea*. Uganda medicinal plants". *African Journal of Health Sciences* 6.1 (2006): 31-35.
35. Doherty VF., et al. "Antimicrobial activities of *Aframomum Melegueta* (Alligator pepper)". *International Journal of Biology* 2.2 (2010): 126-131.
36. Agu GC and Thomas BT. "Antibacterial Activities of Ethanol and Aqueous Extracts of Five Nigerian Medicinal Plants on Some Wound Pathogens". *Natural Sciences* 10.2 (2012): 78-84.

37. Epidi JO., *et al.* "Antibacterial and synergistic potency of tissues of *Vitex grandifolia*". *Biotechnology Research* 2.2 (2016): 69-76.
38. Epidi JO., *et al.* "Antibacterial and Synergistic Efficacy of Extracts of *Alstonia boonei* Tissues". *British Journal of Applied Research* 1.1 (2016): 0021-0026.
39. Izah SC., *et al.* "Antibacterial Efficacy of Aqueous Extract of *Myristica fragrans* (Common Nutmeg)". *EC Pharmacology and Toxicology* 6.4 (2018): 291-295.
40. Ogodo AC., *et al.* "Activity of Leave and Stem Bark Cuttings of *Ocimum gratissimum* Extracts on Foodborne Pathogens". *AASCIT Journal Bioscience* 3.2 (2017): 5-11.
41. Okigbo RN., *et al.* "Antimicrobial potentials of (UDA) *Xylopi aethopica* and *Ocimum gratissimum* L. on some pathogens of man". *International Journal of Molecular Medicine and Advance Sciences* 1.4 (2005): 392-397.
42. Shirin APR and Jamuna P. "Chemical composition and antioxidant properties of ginger root (*Zingiber officinale*)". *Journal of Medicinal Plants Research* 4.24 (2010): 2674-2679.
43. Bello OO and Adeleke O. "Antimicrobial Effects of Spices on Spoilage Organisms of Moin-Moin". *Advances in Bioresearch* 3.2 (2012): 60-65.
44. Braide W., *et al.* "Perspectives in the hurdle techniques in the preservation of a non alcoholic beverage, Zobo". *African Journal of Food Science and Technology* 3.2 (2012): 46-52.

Volume 1 Issue 6 June 2018

© All rights are reserved by Sylvester Chibueze Izah and Ebinyo R Aseibai.