Comparative Antimicrobial Activity of Lemon Grass (Cymbopogon citratus) and Garlic (Allium sativum) Extracts on Salmonella typhi

O. F. Olukunle¹ and O. J. Adenola¹*

¹Department of Microbiology, Federal University of Technology, Akure, Nigeria.

Authors’ contributions

This work was carried out in collaboration between both authors. Author OJA designed the study, performed the statistical analysis. Author OFO wrote the protocol. Author OJA wrote the first draft of the manuscript. Author OFO managed the analyses of the study. Author OJA managed the literature searches. Both authors read and approved the final manuscript.

ABSTRACT

In the genesis of using plants for treatment of diseases, Garlic and lemon grass were some of the earliest plants that have proven effective. In this study the antimicrobial effect of Cymbopogon citratus (lemon grass) and Allium sativum (garlic) extracts on clinical isolate of Salmonella typhi was examined. Water, ethanol and methanol were used as extraction solvent. Antimicrobial effects of conventional antibiotics was assayed using disc diffusion method while antimicrobial activity of lemon grass and garlic extracts were carried out using agar well diffusion method and the zones of inhibition was measured in millimeter (mm). Comparatively, the highest percentage yield of extract was observed in water extract of garlic (21.83%) and lemon grass (6.67%). Ciprofloxacin and Perfloxacin have the highest zones of inhibition of 19.73±0.24mm and 14.47±0.31mm respectively against the clinical isolates of Salmonella typhi. Two out of the twelve clinical isolate of Salmonella typhi used were multi drug resistant. Lemon grass extracts did not show any antimicrobial effects on the clinical isolates of Salmonella typhi while garlic extracts showed antimicrobial effect at the...
1. INTRODUCTION

Salmonella remains a primary cause of food poisoning worldwide, and massive outbreaks have been witnessed in recent years. *Salmonella* spp. are Gram negative, heterotrophic, mesophilic bacteria that present in warm-blooded animal hosts and are an important human pathogen associated with poultry and poultry products. Up to now, *Salmonella* is the most commonly identified bacterial agent causing illnesses, such as typhoid fever in humans [1]. Typhoid fever still causes substantial illness and deaths in many parts of the world, especially in developing nations [2]. According to a report published in World Health Organization bulletin in 2000, the estimated global incidence of typhoid fever was about 21.6 million and mortality rate was up to 216,000 per year. The highest incidence rate of typhoid fever (more than 100/100,000 cases per year) has been reported in south-central Asia and south-east Asia [3]. In 2008, it was reported that the incidence of typhoid fever is 451/100,000 per year in Pakistan [4]. Multi drug resistant (MDR) *Salmonella typhi* showed resistance to all three first line drugs (Ampicillin (AMP), Chloramphenicol (C), and Trimethoprim-sulfamethoxazole (SXT)) [5]. MDR *Salmonella typhi* emerged somewhere in the mid 1990s and are now reported in different regions of the world [6,7]. Under these circumstances, third generation cephalosporin namely, ceftriaxone (CRO) appears to be the most reliable choice for MDR and nalidixic acid resistant isolates of *Salmonella typhi* (NARST) [8,9]. This continuous spread of MDR pathogens and cost effectiveness of drug regimen has become a serious threat to public health and infection control practitioners. The multiple and repeated difficulties with antibiotics has prompted researchers to explore alternate agents. The plant world is an immense store of pharmacologically active chemical compounds which exist as secondary phytoconstituents. The beneficial medicinal effect of plant materials typically result from the combination of secondary products present in the plant. The medicinal action of plants are unique to particular plant species or a related group [10,11,12,13,14]. This concept is consistent with the fact that the combinations of secondary products in a particular plant are often taxonomically distinct. The secondary products can have a variety of functions in plants at the cellular level as plant growth regulators, modulators of gene expressions, in signal transduction and also have protective actions in relation to abiotic stresses [15]. So, it is likely that their ecological function may have some bearing on potential medicinal effects for humans and animals as well. The antimicrobial properties of *Cymbopogon citratus* (lemon grass) and garlic (*Allium sativum*) extract has led to the selection of these plants for these research. Lemon grass belongs to the section of *Andropogon* called *Cymbopogon* of the family Gramineae. A very large genus of the family, including about 500 described species out of which eight species occur in Iraq. Due to the production of lemon grass oil as major component, two of the species i.e. *Cymbopogon citratus* and *Cymbopogon flexuosus* are generally called Lemon grass [16]. Medicinal use of lemon grass is known to mankind since antiquity. Its oil has been used to cure various ailments like cough, cold, spitting of blood, rheumatism, lumbago, digestive problems, bladder problems, leprosy, and as mouth wash for the toothache and swollen gums. It is also been claimed to be stimulating, diuretic, antiprurigative and sudorific to reduce fever [17]. Similarly pharmacological investigation on the essential oil of *C. citratus* revealed that it has a depressant effect on the Central Nervous System [18]. It has analgesic and antipyretic properties. The extract juice from the lemon grass contains inhibitor of the promotion stage of carcinogenesis induced by cotton oil. It is an oral anti tumor drug for the cancer and in combination with cyclodextrin lengthened the survival time [19] and [20]. Gallstone dissolving preparations have been made of oil [21]. The lemon grass contains high percentage of Vitamin C, which is a characteristic of plants used as drug e.g., belladonna and jaborandi. Lemon grass oils show activity towards the phyto pathogenic fungi. Phytotherapeutic agents like garlic (*Allium sativum*) which is frequently used in alternative medicine has gained immense interest in medical literature [4,22]. Recently, garlic has been found to be an effective agent for its concentration of 800 mg/ml, water extract of garlic has the highest zone of inhibition (14.00 mm) against the tested isolates. Therefore garlic extract could be used for the formulation of drug and treatment of *Salmonella typhi* infection.

Keywords: *Salmonella typhi*; *Cymbopogon citrates*; *Allium sativum*; antimicrobial effects.
2.1 Source and Identification of Test Microorganism

Stock cultures of Salmonella typhi used in this study were collected from Don Bosco Hospital, Akure, Ondo State, Nigeria. Each isolate was sub-cultured on salmonella shigella agar (SSA) and identified based on their colonial morphology and specific biochemical reactions.

The suspected colonies of Salmonella typhi that developed on Salmonella Shigella Agar were sub-cultured by streaking on a freshly prepared nutrient agar plates until pure colonies were obtained according to the conventional procedure as highlighted by [27]. This was followed by characterization of the isolates using [28]. Salmonella typhi colonies that identified were cultured on double strength nutrient agar slant and incubated at 37°C for 24 hours, growth was observed and the slants were stored in the refrigerator to preserve the bacterial isolate. The isolate on slants were sub-cultured on freshly prepared double strength nutrient agar slant subsequently.

2.2 Source of Plants Used in this Study

Fresh garlic (A. sativum) was purchased from Oba’s market, Akure, Ondo State, Nigeria while Lemon grass was collected within FUTA South Gate area. The lemon grass was separated from stems, washed in clean water and dried at room temperature. The dried grasses were milled to a fine powder, and stored in airtight container at room temperature until when required. The cloves of garlic were separated and peeled to obtain the edible portion. Four hundred and thirty five grams of the edible portion of garlic was chopped using pestle and mortar and was divided into three equal weights.

2.3 Preparation of Water, Ethanol and Methanol Extracts of Lemon Grass and Garlic

Dried lemon grasses were extracted by weighing samples of 264 g of finely grounded dried lemon grass into three equal sizes. Each portion was homogenized in 900 ml of sterile distilled water, ethanol and methanol. The homogenate were kept in a covered sterile container for three days. The garlic extracts were prepared according to the methods of [22], four hundred and thirty-five grams of the edible portion of garlic was chopped using pestle and mortar and was divided into three equal weights. Each of the three weighed chopped garlic was homogenized in 700 mls of sterile distilled water, ethanol and methanol respectively in a blender. Sterile muslin cloth was used to remove the large particles from the homogenate and then filtered using Whatman No. 1 filter paper. Extracts obtained were then concentrated in vacuum using rotary evaporator to remove the solvents [29]. The extraction efficiency was quantified by determining and comparing the weight of each of the extracts yield.

2.4 Standardization of Inoculum Using McFarland Turbidity Standard

McFarland turbidity standard which was used to measure the density of bacterial cells was prepared according to the method of [30]. The
standardized inoculum was used for antibacterial assay.

2.5 Antimicrobial Susceptibility Test for Salmonella typhi

Antibiotic susceptibility patterns of Salmonella typhi was determined by disc diffusion method with the use of Mueller-Hinton agar, according to the Bauer-Kirby method [31]. The following clinical antibiotics, with their concentrations given in parentheses were used as recommended by [32]; Tarivid Ofloxacin (30 μg), Gentamicin (20 μg), Chloramphenicol (30 μg), Augmentin (30 μg), Ciprofloxacin (10 μg), Amoxacillin (30 μg), Streptomycin (10 μg), Sparfloxacin (10 μg), Septrin (30 μg), and Pefloxacin (10 μg). The zone of inhibition was noted and recorded in millimeter.

2.6 Antibacterial Activity of Plant Extracts

Each of the plant extracts was screened for antimicrobial activity by performing agar well diffusion assay following the method of [33]. The recovered plant extracts were being reconstituted using 30% v/v Dimethyl sulfoxide and sterilized (by filtration) using autoclavable sterile injection filters of 0.22 μm pore size. The various plant extracts to be screened were reconstituted to concentration of 800 mg/ml and about 0.5 ml each were introduced to the agar wells in each of the seeded agar. The negative control for the experiment was 30% aqueous DMSO while Ciprofloxacin (50 mg/ml) was used as the positive control. All the plates were incubated at 37°C for 24 hours after which the zone of inhibition was measured.

2.7 Data Analysis

Data were statistically analyzed using SPSS (Statistical Package for Social Science) version 20, mean zones of inhibition were separated using new Duncan’s Multiple Range Test and significant differences were value at p≤ 0.05.

3. RESULTS

3.1 Morphological, Biochemical Characterization and Identification of the Clinical Isolates

All bacterial isolates showed different biochemical reactions and were characterized and identified. Thirty eight [34] isolates were identified as Salmonella species in which twelve [12] were Salmonella typhi and twenty six [26] were identified as other Salmonella species.

Table 1. Effects of extraction solvent on percentage yield of plant extract

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Garlic (%)</th>
<th>Lemon grass (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>16.48±0.01a</td>
<td>4.15±0.08b</td>
</tr>
<tr>
<td>Ethanol</td>
<td>16.31±0.57a</td>
<td>3.69±0.12a</td>
</tr>
<tr>
<td>Water</td>
<td>21.83±0.58b</td>
<td>6.67±0.11c</td>
</tr>
</tbody>
</table>

Values are presented as % mean ± SE, values in the same column with same superscript are not significantly different according to Duncan’s multiple range test at p≤0.05; Key: SE- Standard Error, %: Percentage

Fig. 1. Antibiotics susceptibility test of clinical isolates Salmonella typhi (isolates A-F)
Table 2. Antimicrobial susceptibility test of the plants extracts

<table>
<thead>
<tr>
<th>Salmonella isolates</th>
<th>Ciprofloxacin (50 mg/ml)</th>
<th>GM (800 mg/ml)</th>
<th>GE (800 mg/ml)</th>
<th>GW (800 mg/ml)</th>
<th>LM (800 mg/ml)</th>
<th>LE (800 mg/ml)</th>
<th>LW (800 mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>7.00±0.17&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.50±0.18&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.50±0.00&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.00±0.63&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.00±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>K</td>
<td>7.00±0.57&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.00±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00±0.58&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.00±0.05&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.00±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SE; values in the same row with same superscript are not significantly different according to Duncan’s multiple range test at p≤0.05


Fig. 2. Antibiotics susceptibility test of clinical isolates Salmonella typhi (isolates G-L)
Table 3. Minimum inhibitory concentration (MIC) of plants extracts on clinical isolate of Salmonella typhi

<table>
<thead>
<tr>
<th>Salmonella isolates</th>
<th>Ciprofloxacin</th>
<th>GM</th>
<th>GE</th>
<th>GW</th>
<th>LM</th>
<th>LE</th>
<th>LW</th>
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<tr>
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<td>K</td>
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<td>600.00</td>
<td>500.00</td>
<td>150.00</td>
<td>1300</td>
<td>1350</td>
<td>1450</td>
</tr>
</tbody>
</table>


Table 4. Minimum bactericidal concentration (MBC) of plants extracts on clinical isolate of Salmonella typhi

<table>
<thead>
<tr>
<th>Salmonella isolates</th>
<th>Ciprofloxacin</th>
<th>GM</th>
<th>GE</th>
<th>GW</th>
<th>LM</th>
<th>LE</th>
<th>LW</th>
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<tbody>
<tr>
<td>I</td>
<td>100.00</td>
<td>500.00</td>
<td>600.00</td>
<td>200.00</td>
<td>1350</td>
<td>1400</td>
<td>1500</td>
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<td>550.00</td>
<td>200.00</td>
<td>1400</td>
<td>1400</td>
<td>1500</td>
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</table>


3.2 Percentage Yield of the Extracts for Each Solvent

Mean percentage yield of the extracts are shown in Table 1. The result revealed that there was significant difference (p≤0.05) in the percentage yield of the extracts for the solvents ranging from 16.31% to 21.83% (Garlic) and 3.69% to 6.67% (Lemon grass). Percentage yield of water extract was significantly (p≤0.05) higher than the yield from methanol and ethanol respectively in both plants.

3.3 Antibiotics Susceptibility Test of Clinical Isolates of Salmonella typhi

The antibiotics susceptibility test of clinical isolates of Salmonella typhi were carried, the zones of inhibition of commercially available antibiotics were measured in millimeter (Figs. 1 and 2). Ciprofloxacin has highest mean zone of inhibition ranging from 19.33±0.33 to 20.00±1.00mm followed by Pefloxacin having mean zone of inhibition ranging from 13.00±0.58 to 15.00±1.7mm while Septrin, Chloramphenicol and Amoxacilin has no zone of inhibition against all the tested isolates. The zones of inhibition of all antibiotics used were significantly (p<0.05) low against isolates I and K.

3.4 Antimicrobial activity of plant extracts on Salmonella typhi

The result revealed that there was significant difference (p≤0.05) in the inhibition of growth of Salmonella typhi (Table 2). Significant inhibition was observed in garlic extracts at concentration of 800 mg/ml on Salmonella typhi . Garlic extracts inhibited the growth of isolates, 4.50, 2.50 and 7.00 mm for methanol, ethanol and water extracts respectively. There was no significant inhibition observed in lemon grass extracts at same concentration.

3.5 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Plants Extracts on Salmonella typhi

The result revealed that there were significant differences (p≤0.05) in the MIC and MBC of the plants extracts from various solvents against Salmonella typhi (Tables 3 and 4). The MIC of the methanol, ethanol and water extracts of garlic for Salmonella typhi isolate I and K were (450.00 mg/ml, 550.00 mg/ml and 150.00 mg/ml) and (600.00 mg/ml, 500.00 mg/ml and 150.00 mg/ml) respectively while the MBC were (500.00 mg/ml, 600 mg/ml and 200 mg/ml) and (650.00 mg/ml, 550 mg/ml and 200.00 mg/ml) respectively. The MIC of the Methanol, ethanol and water extracts of lemon grass for Salmonella typhi isolate I and K were higher than what was observed in garlic extracts.

4. DISCUSSION

The results obtained in this study validate that the disease caused by antibiotic resistant Salmonella typhi can be treated with natural products such as garlic. Garlic and lemon grass were extracted with methanol, ethanol and water. Percentage yield from water was significantly (p≤0.05) higher than the yield from methanol and ethanol, which it is in accordance with the findings of [35]. In this study the zone of inhibition
of commercially available antibiotics to clinical isolates of *Salmonella typhi* were measured in millimeter. Methanol, ethanol and water extract of garlic shows antimicrobial activity against the isolate of *Salmonella typhi*. Aqueous extract of garlic has the maximum inhibition against the isolates of *Salmonella typhi* followed by methanol and ethanol extracts. This is in agreement with the findings of [36] who reported that crude extract of garlic clove has maximum inhibition against *Salmonella typhi* followed by methanolic extract of garlic clove. This is also similar to the findings of [37] in the *in vitro* use of garlic, as antibacterial agent against *Salmonella typhi*. It showed that garlic has antibacterial activities against *Salmonella typhi*. There was no significant inhibition for lemon grass extracts at concentration of 800 mg/ml. Methanol, ethanol and water extracts of lemon grass shows no antimicrobial activity. This is in line with the study carried out by [38], who also reported that ethanol extract of lemon grass had no antimicrobial activity on the test organisms. However, the findings of [34], showed that chloroform extract of lemon grass demonstrated antimicrobial activity against isolate of *Salmonella typhi*. The inhibitory activities of the chloroform lemon grass extract on the test organisms indicate that the plant possess high active ingredients which may be chloroform soluble [34]. The result revealed that there was significant difference (p≤0.05) in the MIC and MBC of the two plants extracts from various solvents against *Salmonella typhi*. The MIC and MBC of the Methanol, ethanol and water extracts of lemon grass for *Salmonella typhi* isolates I and K were on the high side. This may be due to low antimicrobial properties present in water, ethanol and methanol extracts of lemon grass [34].

5. CONCLUSION

This study has shown that water extract of garlic could be incorporated to established treatment of *Salmonella typhi* infection. However, further research should be carried out on garlic for its effectiveness.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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