Original Research Article

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

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 *Cymbopogan 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 concentration of 800 mg/ml, water extract of garlic has the highest zone of inhibition (14.00mm) against the tested isolates. Therefore garlic extract could be used for the formulation of drug and treatment of *Salmonella typhi* infection.

Key words: *Salmonella typhi, Cymbopogan citratus, Allium sativum,* Antimicrobial effects

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 (Agnieszka and Katarzyna, 2018). Typhoid fever still causes substantial illness and deaths in many parts of the world, especially in developing nations (Radhakrishnan *et al.,* 2018). 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 (Akinyemi *et al*., 2018). In 2008, it was reported that the incidence of typhoid fever is 451/100,000 per annum in Pakistan (Saima *et al.,* 2018). Multi drug resistant (MDR) *Salmonella typhi* showed resistance to all three first line drugs {Ampicillin (AMP), Chloramphenicol (C), and Trimethoprim-sulfamethoxazole (SXT)} (Rajesh *et al.,* 2018). MDR *S. typhi* emerged somewhere in the mid 1990s and are now reported in different regions of the world (Ward and Thelfall, 2001; Parry *et al.,* 2002). Under these circumstances, third generation cephalosporin namely, ceftriaxone (CRO) appears to be the most reliable choice for MDR and nalidixic acid resistant isolates of *S. typhi* (NARST) (Bandyopadhyay *et al.,* 2018; Bhutta, 2006). 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 (Archana and Abraham 2011; Umashanker and Shruti, 2011; Mahima *et al*., 2012; Midrarullah *et al.,* 2014; Rahal *et al*., 2014a). 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 (Rahal *et al.*, 2014b). 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 Graminae. 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 (Lawal *et al.,* 2017). 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, anti-purgative and sudorific to reduce fever (Venson *et al.*, 2018). Similarly pharmacological investigation on the essential oil of *C. citratus* revealed that it has a depressant effect on the Central Nervous System (Ayandele, 2007). 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 (Mostafa *et al.,* 2018) and (Parekh and Chanda, 2007). Gallstone dissolving preparations have been made of oil (Elastal *et al.,* 2005). 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 (Saima *et al.,* 2018; Iwalokun *et al.,* 2004). Recently, garlic has been found to be an effective agent for its application as anti-tumor, anti-oxidant, anti-viral, anti-fungal, anti-microbial, anti-thrombotic, anti-inflammatory, hypoglycemic, immune modulatory effects (Abraham *et al.,* 2018). Garlic holds up the highest concentration of sulfur compounds like allicin and others having biological activities. These are responsible for not only its pungent smell but also for its medicinal value (Sani *et al.,* 2018). The thiosulfate allicin has effective anti-microbial properties and other non-sulphur constituents like proteins, saponins and phenolic compounds may also contribute to its anti-microbial activity (Awan *et al*., 2018). Garlic is present in various forms namely, raw juice, garlic oil, garlic powder, and many types of extracts like, aged garlic extract, aqueous garlic extract (AGE), methanolic extract, ethanolic extract and many more (Sani *et al.,* 2018). Different garlic extract preparations demonstrated their *in-vitro* activity against Gram-negative and Gram-positive bacteria including species of *Escherichia*, *Staphylococcus*, *Streptococcus*, *Klebsiella*, *Proteus*, *Bacillus*, *Clostridium* and even acid fast bacilli (AFB) such as *Mycobacterium tuberculosis* (MTB). It is also effective against antibiotic resistant isolates like methicillin-resistant *Staphylococcus aureus* (MRSA) as well as other MDR enterotoxicogenic isolates of *Escherichia coli* (ETEC), *Salmonella goldcoast* and *Klebsiellae* (Lee *et al.,* 2015) and also MDR MTB (Sani *et al.,* 2018). Garlic having multiple biological properties; inspired to investigate local cultivated garlic (small cloves) for its anti typhoid effects against sensitive and MDR *S. typhi* isolates which might be helpful in combating this public health concern issue.

Materials and methods

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 isolates were 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 Fawole and Oso (2001). This was followed by characterization of the isolates using Holt *et al*. (1994). *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.

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 were separated from stems, washed in clean water and dried at room temperature. The dried grasses were milled to a fine powder, and stored in an airtight container at room temperature until when required.The cloves of garlic were separated and peeled to obtain the edible portion. 435g of the edible portion of garlic was chopped using pestle and mortal and was divided into three equal weights.

Preparation of water, ethanol and methanol extracts of lemon Grass and garlic

Dried lemon grasses were extracted by weighing samples of 264g 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

Iwalokun, *et al*. (2004), 435g 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 700mls 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 (Oluduro *et al*., 2010). The extraction efficiency was quantified by determining and comparing the weight of each of the extracts yield.

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 Cheesbrough (2014).The standardized inoculum was used for antibacterial assay.

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 (Bauer *et al.,* 1966). The following clinical antibiotics, with their concentrations given in parentheses were used as recommended by Committee for Clinical Laboratory Standards (2014); Tarivid Ofloxacin (30µg), Gentamicin (20µg), Chloramphenicol (30µg), Augmentin (30µg), Ciprofloxacin (10µg), Amoxacillin (30µg), Streptomycin (10 µg), Sparfloxacin (10µg), Septrin (30µg), and Pefloxacin (10µg). The zone of inhibition was noted and recorded in millimeter.

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 Abdelbasset and Djamila (2008).The recovered plant extracts were being reconstituted using 30% v/v Dimethyl sulfoxide and sterilized (by filtration) using autoclavable sterile injection filters of 0.22µm 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 DMSOwhile Ciprofloxacin (50 mg/ml) was used as the positive control. All the plates were incubated at 37^{o}C for 24 hours after which the zone of inhibition was measured.

Data analysis

Data were statistically analyzed using SPSS (Statistical Package for Social Science) version 20, mean zones of inhibitions were separated using new Duncan's Multiple Range Test and significant differences were value at $\rho \leq 0.05$.

Results

Morphological, biochemical characterization and identification of the clinical isolates

All bacterial isolates showed different biochemical reactions and were characterized and identified. Thirty eight (38) isolates were identified as *Salmonella* species in which twelve (12) are *Salmonella typhi* and twenty six (26) were identified as other *Salmonella* species.

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 ($p \leq 0.05$) 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 ($p \leq 0.05$) higher than the yield from methanol and ethanol respectively in both plants.

Table 1: effects of extraction solvent on percentage yield of plant extract

Solvents	Garlic (%)	Lemon grass (%)
Methanol	16.48 ± 0.01^{a}	4.15 ± 0.08^{b}
Ethanol	16.31 ± 0.57^{a}	3.69 ± 0.12^{a}
Water	21.83 ± 0.58^{b}	6.67 ± 0.11^{c}

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 $p \leq 0.05$

Key: SE- Standard Error, **%:** Percentage

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 (Figure 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.

Antimicrobial activity of plant extracts on *Salmonella typhi*

The result revealed that there was significant difference ($p \leq 0.05$) 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.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of plants extracts on *Salmonella typhi*

The result revealed that there were significant differences ($p \leq 0.05$) in the MIC and MBC of the plants extracts from various solvents against *Salmonella typhi* (Table 3 and 4). The MIC of the methanol, ethanol and water extracts of garlic for *Salmonella typhi* isolate I and K were (450.00mg/ml, 550.00mg/ml and 150.00mg/ml) and (600.00mg/ml, 500.00mg/ml and 150.00mg/ml) respectively while the MBC were (500.00mg/ml, 600mg/ml and 200mg/ml) and (650.00mg/ml, 550mg/ml and 200.00mg/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.

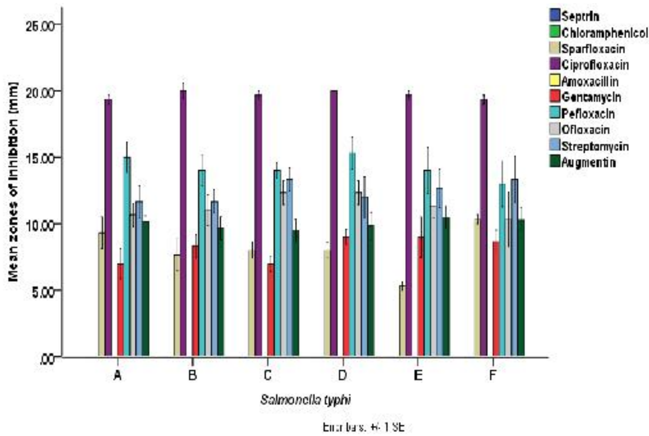

Figure 1: Antibiotics susceptibility test of clinical isolates *Salmonella typhi* (isolatesA-F)

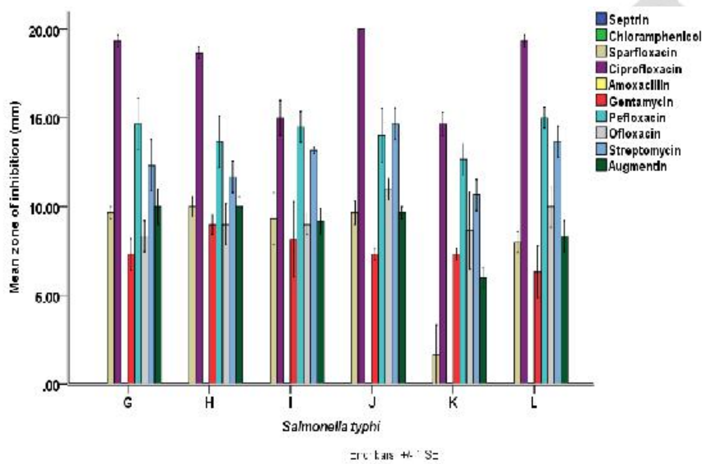

Figure 2: Antibiotics susceptibility test of clinical isolates *Salmonella typhi* (isolates G-L)

Table 2: Antimicrobial susceptibility test of the plants extracts

	Zones of inhibition (mm)						
	Ciprofloxacin	GM	GE	GW	LM	LE	LW
Salmonella isolates	(50mg/ml)	(800mg/ml)	(800mg/ml)	(800mg/ml)	(800mg/ml)	(800mg/ml)	(800mg/ml)
I	7.00 ± 0.17^{d}	4.50 ± 0.18^{c}	2.50 ± 0.00^{b}	7.00 ± 0.63^{d}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
K	7.00 ± 0.57^{c}	0.00 ± 0.00^{a}	1.00 ± 0.58^{b}	10.00 ± 0.05^{d}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

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 $p\leq0.05$

KEY: GM- Methanol extract of Garlic, GE- Ethanol extract of Garlic, GW- Water extract of Garlic, LM- Methanol extract of Lemon grass, LE- Ethanol extract of Lemon grass, LW- Water extract of Lemon grass and SE-Standard Error.

Table 3: Minimum Inhibitory Concentration (MIC) of plants extracts on clinical isolate of *Salmonella typhi*

	MIC (mg/ml)						
***Salmonella* isolates**	**Ciprofloxacin**	**GM**	**GE**	**GW**	**LM**	**LE**	**LW**
I	50.00	450.00	550.00	150.00	1300	1300	1400
K	50.00	600.00	500.00	150.00	1300	1350	1450

KEY: GM- Methanol extract of Garlic, GE- Ethanol extract of Garlic, GW- Water extract of Garlic, LM- Methanol extract of Lemon grass, LE- Ethanol extract of Lemon grass, LW- Water extract of Lemon grass.

Table 4: Minimum Bactericidal Concentration (MBC) of plants extracts on clinical isolate of *Salmonella typhi*

***Salmonella* isolates**	**MBC (mg/ml)**						
	Ciprofloxacin	**GM**	**GE**	**GW**	**LM**	**LE**	**LW**
I	100.00	500.00	600.00	200.00	1350	1400	1500
K	100.00	650.00	550.00	200.00	1400	1400	1500

KEY: GM- Methanol extract of Garlic, GE- Ethanol extract of Garlic, GW- Water extract of Garlic, LM- Methanol extract of Lemon grass, LE- Ethanol extract of Lemon grass, LW- Water extract of Lemon grass

Discussion

The results obtained in this study have shown that is possible to overcome antibiotic resistant *Salmonella typhi* through the use of natural products. Garlic and lemon grass were extracted with methanol, ethanol and water. Percentage yield from water was significantly ($p \leq 0.05$) higher than the yield from methanol and ethanol, which it is in accordance with the findings of Mona and Safinaz (2015). 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 Gaherwal *et al.,* (2014) 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 Adebolu *et al.* (2011) 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 800mg/ml. Methanol, ethanol and water extracts of lemon grass shows no antimicrobial activity. This is in line with the study carried out by Akiyama *et al*. (2001), who also reported that ethanol extract of lemon grass had no antimicrobial activity on the test organisms. However, the findings of Ewansiha *et al*. (2012), 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 (Ewansiha *et al.,* 2012). The result revealed that there was significant difference ($p \leq 0.05$) 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 (Ewansiha *et al.,* 2012).

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.

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