Antibacterial activity of *Ocimum gratissimum* AND *Gongronema latifolium* ON *Staphylococcus aureus* AND *Salmonella typhi*

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Abstract

Ocimum gratissimum and *Gongronema latifolium* have been used by herbal medicine practitioners in combination with other herbs in the treatment of Staphylococcal and Salmonellal infections. The aim of this study was to evaluate antibacterial activity of extracts of *Ocimum gratissimum* (*Efinrin*) and *Gongronema latifolium* (*Utazi*) on *Staphylococcus aureus* and *Salmonella* species. Extracts were obtained by aqueous and ethanolic methods with a concentration range of 25% to 75%. The diameter of inhibition zones by aqueous extract of *Ocimum gratissimum* was between 7-15 mm while that of the ethanolic extract was between 7 and 12 mm. The minimum inhibitory concentration (MIC) was 25% for the aqueous extract of *O. gratissimum* on *S aureus* with no effect on *Salmonella*, while that of the ethanolic extract was 75% for both organisms. *G. latifolium* showed MICs of 25% and 75% for the aqueous extract on *S. aureus* and *Salmonella* respectively while the ethanolic extracts showed MICs of 25% and 50% on *S. aureus* and *Salmonella* respectively. Minimum bactericidal concentration (MBC) for the aqueous extracts of *G. latifolium* were 50% and 75% for *S aureus* and *Salmonella* respectively while that of the ethanolic extract was 25% for *S aureus* and 75% for *Salmonella. O. gratissimum* showed MBC at 75% concentration only for the aqueous extract alone on *S aureus.* Therefore, the results obtained indicated that the extracts of *O. gratissimum* and *G. latifolium* possess antibacterial activities against *S. aureus* and *Salmonella* species.

Key words: Antibacterial activity, *Ocimum gratissimum, Gongronema latifolium* , *Staphylococcus aureus* , *Salmonella typhi*

Introduction

Spices have a long history of both culinary and medicinal uses (Tapsell and Swrahi, 2006). Some spices have been reported to have both bactericidal and bacteriostatic activities (Onwuliri and Wonang, 2005). They are generally recognized to be safe, either because of their traditional use without any documented detrimental impact or because of dedicated toxicological studies (Smid and Gorris, 1999). Some spices are highly medicinal and have been used to sustain health and cure illnesses (Osunwole, 1999). Some of the factors that determine their antimicrobial activities include concentration and composition of the spices, the type of microorganism, pH value, temperature and phenolic substances present in the food (Sagdic, 2003).

Ocimum gratissimum belongs to the family Leguminoceae commonly known as *Alfacava*. It is found in tropical and warm temperate regions such as India and Nigeria, particularly in the savannah and coastal areas (Okigbo and Ogbonnaya, 2006). It is generally asserted to possess various culinary and medicinal properties. The medicinal properties exert both bacteriostatic and bactericidal effects on some bacteria (Okigbo and Igwe, 2007). It has been used in the treatment of different diseases such as upper respiratory tract infection, diarrhoea, headache, conjunctivitis, skin disease, pneumonia, tooth and gum disorders, fever and as mosquito repellents (Onajobi, 1986; Ilori *et al*., 1996; Okigbo and Mneka, 2008) and active against several species of bacteria including *Listeria monocytogenes, Shigella, Salmonella* and *Proteus* (Nwosu and Okafor, 1995; Akinyemi *et al.*, 2005).

Gongronema latifolium, known as *utazi* in the south eastern part of Nigeria is a tropical rainforest plant which belongs to the family Ascepiadaceae (Ugochukwu and Babady, 2002; Akinnuga *et al.*, 2011). It has antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* and shows strong curative properties. It is thus used for the management of various diseases caused by these organisms (Akinyemi *et al.*, 2005).

Salmonella is a facultative anaerobe that causes foodborne disease that result to a condition known as Salmonellosis while *Staphylococcus aureus* on the other hand has been implicated in several illnesses and stomach upset (Mosset and Van, 1990). *Staphylococcus aureus* produces heat stable toxin which is a major public health threat, being one of the common cause of hospital and community acquired

infections (Aires-de-Soura *et al.*, 2006). This study was undertaken therefore, to investigate the antibacterial properties of the crude ethanolic and aqueous extracts of *Ocimum gratissimum* (*efinrin*) and *Gongronema latifolium* (*utazi*) against food-borne pathogens of *Staphylococcus aureus* and *Salmonella* Species.

Materials and methods

Sample collection

The plants used in this study, *Ocimum gratissimum* (Efinrin) and *Gongronema latifolium* (Utazi) were purchased from the vegetable section of Badagry local market in Badagry Local Government Area, Lagos State, Nigeria. The plants were taxonomically identified at the Department of Botany, Lagos State University, Ojo. The plant parts used are the stalk and leaves.

Growth habit of the plants

Ocimum gratissimum is an annual herb which grows in several regions all over the world (Sajjadi, 2006, Simon et al., 1990). It grows in the cool moist and tropical rain forest zones in annual temperatures between 6 - 24°C and receiving 500 - 8000 mm annual precipitation (Grayer et al., 1996).

Gongronema latifolium (Asclapiadaceae) is a perennial edible plant with soft and pliable stem. It is harvested from forest in southeastern states of Nigeria and some other parts of Sub-Saharan Africa (Okafor, 1995).

Microorganisms

The test microorganisms, *Staphylococcus aureus* and *Salmonella* species were obtained from the Department of Microbiology, Lagos State University, Ojo, Nigeria. All the cultures were obtained in their pure form.

Preparation of plant extracts

The fresh spices were separated, cleaned and washed in sterile distilled water, dried, weighed and powdered finely using electric blender. After obtaining a fine powder, the spices were weighed. 25g of air dried material was shaken in 250ml of 96% (w/v) ethanol at room temperature for the ethanolic extraction and in 250ml of ssterile distilled water for the aqueous extraction. The mixtures were allowed to stand for 24 hours after which they were filtered using a fine mesh cloth. The solvents were evaporated to dryness at a temperature of 40^0C using water bath and the extracts were then reconstituted in sterile distilled water to make concentrations of 25%, 50% and 75%.

Antibacterial sensitivity testing using filter paper method

Filter paper discs of 6mm were prepared from Whatman No.1 filter paper and sterilized. Using ethanol dipped and flamed forceps, the discs were inserted into the various concentrations of the extracts and placed aseptically over the nutrient agar and salmonella-shigella agar plates seeded with the test microorganisms. A total of five discs were placed on each plate, with three for the various spice concentrations, one for chloramphenicol which served as positive control and the last disc for distilled water which served as the negative control. The inoculated plates were incubated at room temperature for 24 hours. The antibacterial activity was evaluated by measuring the zones of inhibition, which is the clear zone around the various discs in millimetres.

Dilution method for inhibitory effect and bactericidal effect (qualitative test)

Ten millilitre of each spice extract was inoculated with 0.1ml of pathogenic microbial culture and mixed well. Two hundred microliters of this mixture was pipetted into the microarray plates. After incubation at 37^0C for 24 hours, the wells were checked for turbidity, spots, dots or pellets formed at the base of the wells. Any growth at a particular concentration represented minimum inhibitory concentration. Samples from the microarray plates that did not show any growth or turbidity were used to inoculate fresh sterile broth that contained no spice extracts. 100ul of this culture was reintroduced into fresh 100ul nutrient broth. The lowest concentration of the spice extract that yielded no turbidity or spots or dots of growth following this second inoculation or sub-culturing showed the minimum bactericidal concentration. Sterile distilled water inoculated with bacterial strains without spices was used as control.

Results

Table 1 shows the minimum inhibitory concentration (MIC) of the aqueous and ethanolic extract of *Gongronema latifolium* and *Ocimum gratissimum* against *S. aureus* and *Salmonella typhi.* At all the concentrations tested, aqueous and ethanolic extracts of *G. latifolium* inhibited the growth of *S. aureus*. However, it only inhibited the growth of *S. typhi* at high concentrations. Aqueous extract *O. gratissimum* inhibited the growth of *S. aureus* at 50 and 75% concentration while it only inhibited *S. typhi* at 75% concentration.

The Minimum Bactericidal concentration (MBC) of aqueous and ethanolic extract of *Gongronema latifolium* and *Ocimum gratissimum* against *S. aureus* and *Salmonella typhi* is depicted in table 2. Aqueous and ethanolic extracts of *G. latifolium* inhibited *S. aureus* at all concentrations except 25% aqueous extract while *O. gratissimum* extracts had no effect except at 75% aqueous extract. As for *S. typhi*, extracts of *G. latifolium* only inhibited the micro-organism at the highest concentration while *O. gratissimum* had no effect.

The antibacterial activities of aqueous extract of *G. latifolium* with respect to the inhibition diameters (mm) on the test organisms are shown in figure 1. The inhibition diameter of *S. aureus* at 25 and 50% are not significantly different ($p > 0.05$) from each other.

However, the inhibition diameters at all the concentrations of the extract were significantly different from the control ($p < 0.05$).

At all the concentrations tested, the ethanolic extract of *G. latifolium* were significantly different ($p < 0.05$) from one another and that of the control (figure 2).

Figures 3 and 4 showed the antibacterial activities of aqueous and ethanolic extracts of *O. gratissimum* on both *S. aureus* and *S. typhi*. At all the concentrations tested, the inhibition diameters of the extracts were significantly different ($p < 0.05$) from one another and to the control. However at 25% concentration, aqueous extract of *O. gratissimum* had no effect on *S. typhi* (figure 3).

Table 1: Minimum inhibitory concentration (MIC) of aqueous and ethanolic extract of *Gongronema latifolium* and *Ocimum gratissimum* against *S. aureus* and *Salmonella typhi*

Samples	Concentration	*S. aureus*	*Salmonella typhi*
G. latifolium	25%	++	-
Aqueous Extract	50%	++	-
	75%	++	++
	Control	-	-
G. latifolium	25%	++	-
Ethanolic Extract	50%	++	++
	75%	++	++
	Control	++	-
O. gratissimum	25%	-	-
Aqueous Extract	50%	++	-
	75%	++	-
	Control	-	-
O. gratissimum	25%	-	-
Ethanolic Extract	50%	-	-
	75%	++	++
	Control	-	-

- No growth inhibition; ++ Complete growth inhibition

Table 2: Minimum Bactericidal concentration (MBC) of aqueous and ethanolic extract of *Gongronema latifolium* and *Ocimum gratissimum* against *S aureus* and *Salmonella typhi*

Samples	Concentration	*S aureus*	*Salmonella*
G. latifolium	25%	-	-
Aqueous Extract	50%	++	-
	75%	++	++
	Control	-	-
G. latifolium	25%	++	-
Ethanolic Extract	50%	++	-
	75%	++	++
	Control	-	-
O. gratissimum	25%	-	-
Aqueous Extract	50%	-	-
	75%	++	-
	Control	-	-
O. gratissimum	25%	-	-
Ethanolic Extract	50%	-	-
	75%	-	-
	Control	-	-

- No growth inhibition; ++ Complete growth inhibition.

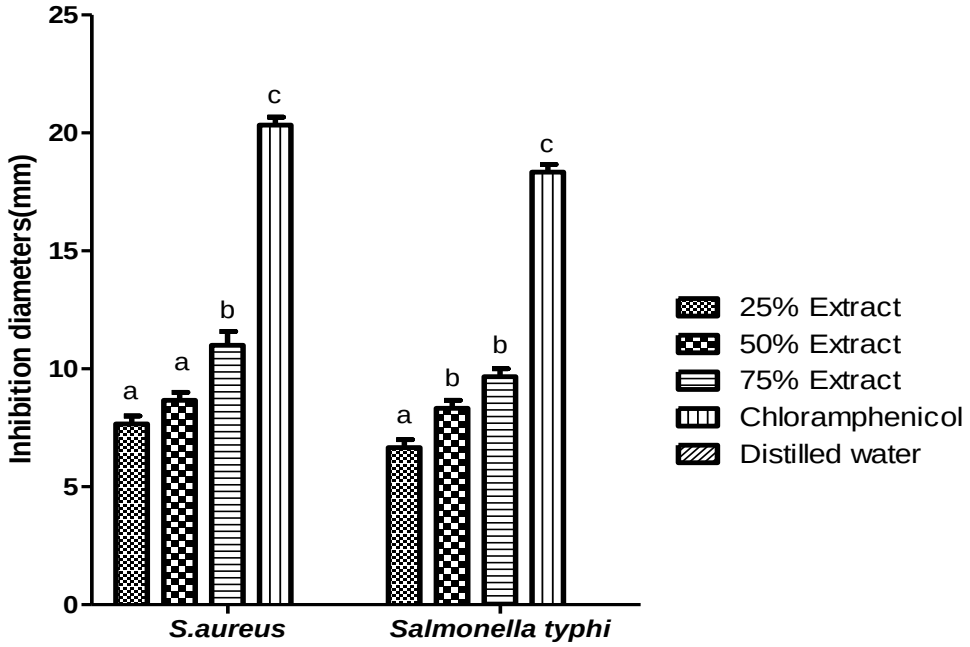

Figure 1: Antibacterial activity of aqueous extract of *Gongronema latifolium*.
Groups with the same letters are not significantly different at (p < 0.05)

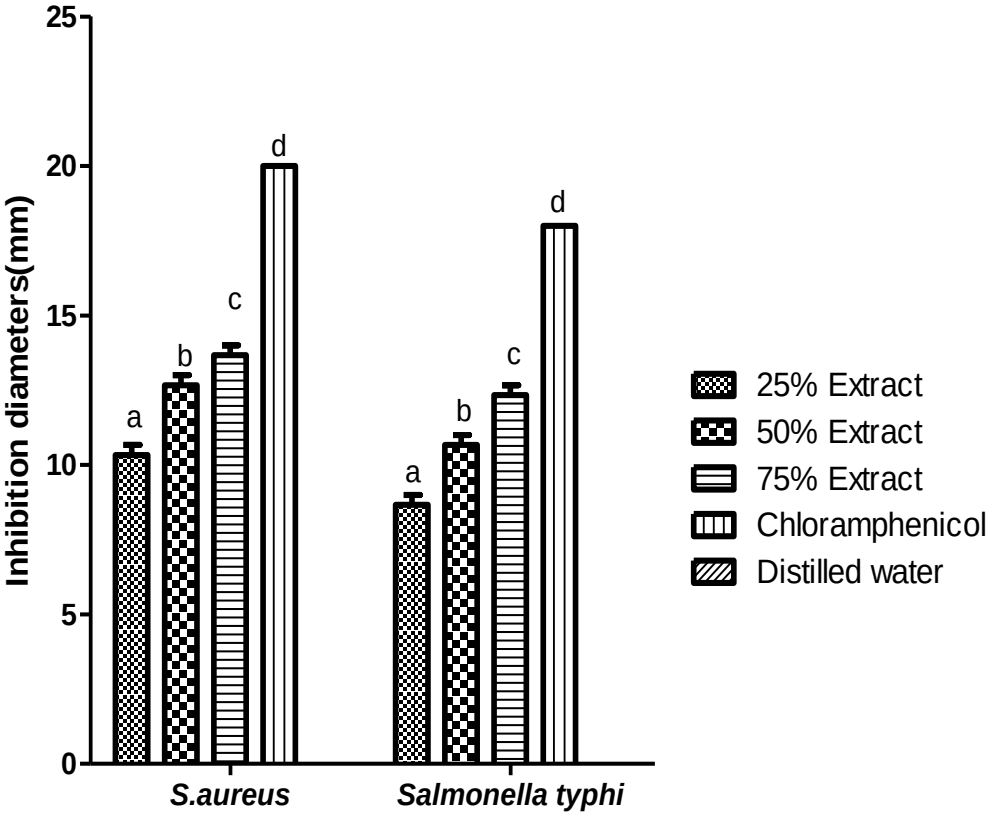

Figure 2: Antibacterial activity of ethanol extract of *Gongronema latifolium*.
Groups with the same letters are not significantly different at (p < 0.05)

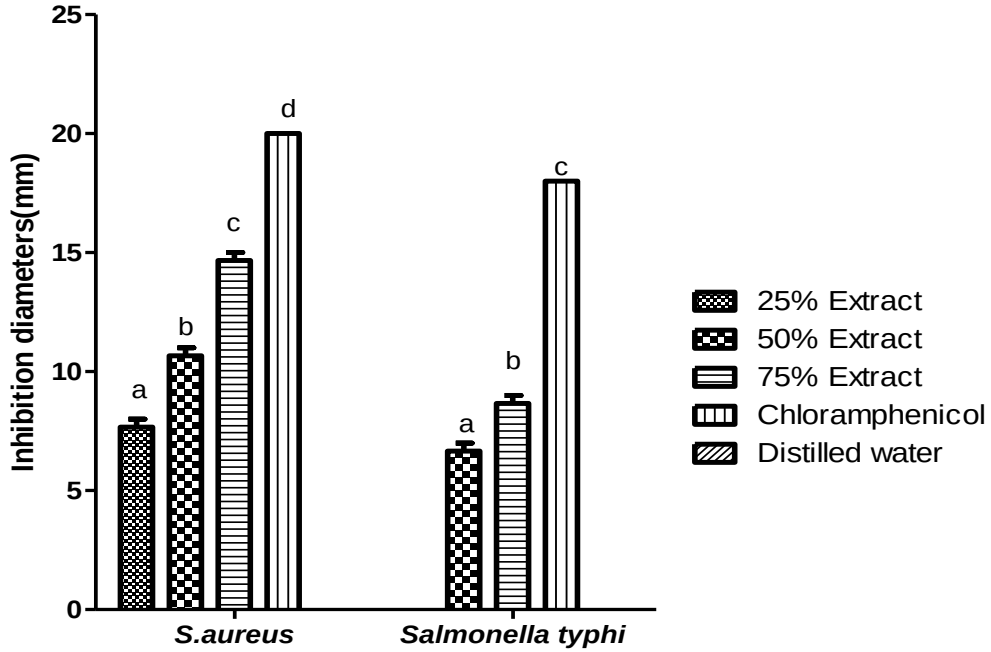

Figure 3: Antibacterial activity of aqueous extract of *Ocimum gratissimum*.
Groups with the same letters are not significantly different at (p < 0.05)

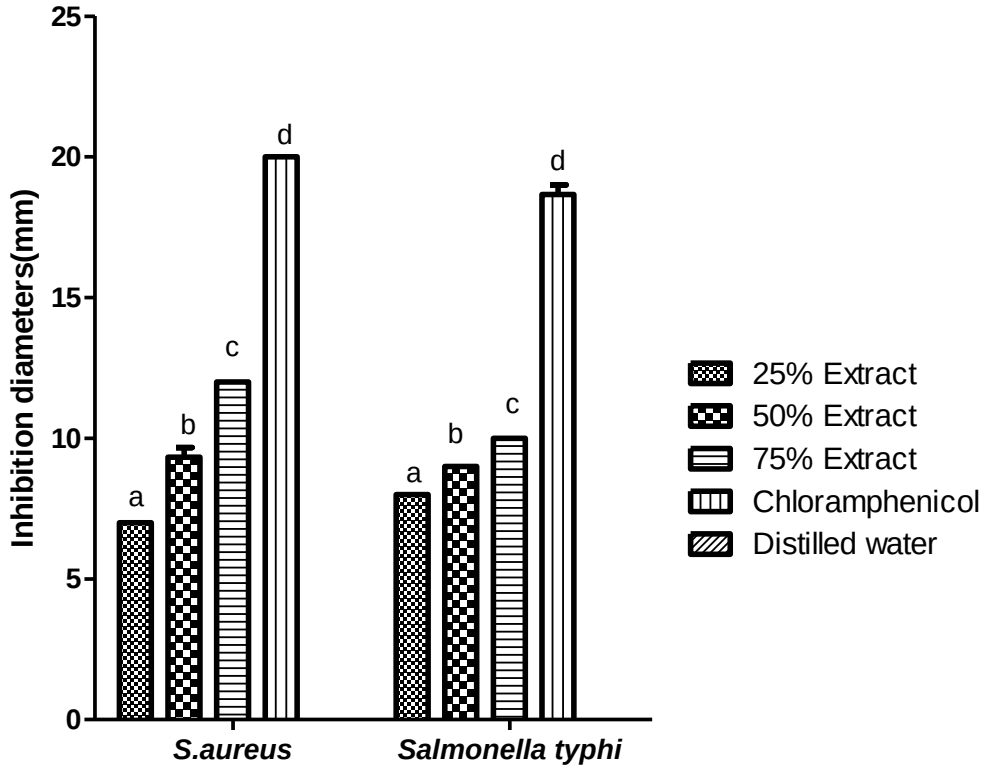

Figure 4: Antibacterial activity of ethanol extract of *Ocimum gratissimum*.
Groups with the same letters are not significantly different at (p < 0.05)

DISCUSSION

Antimicrobial activities of aqueous and ethanolic extracts of *O. gratissimum* and *G. latifolium* were examined for the presence or absence of bacterial growth, zone of inhibition and turbidity.

The results showed that the extracts have a concentration dependent inhibitory effect on the test organisms. The aqueous extract of *Ocimum gratissimum* showed higher activity than the ethanolic fraction. This finding justifies the ethno- medical use of *O. gratissimum* leaves as a plaster to cover wound surfaces and baby's cord after it is soaked in water. This study also supports the application of *O. gratissimum* in dermatological creams against wound infection caused by *S. aureus* in accordance with the work of Papachan *et al.,* (1994). Although, much research has not been carried out on the antimicrobial activity of *G. latifolium* which has been used for ages by the people of West Africa particularly Nigerians for dietary and medicinal purposes (Eja *et al.,* 2011), antimicrobial activity revealed in this study agrees with the work of Nwinyi *et al.,* (2009) who reported the antimicrobial activities of *G. latifolium* against *S. aureus.* The ethanolic extracts of *G. latifolium* however were found to be more effective than the aqueous extract with significant differences ($p < 0.05$) between the various concentrations. This finding shows ethanol to be a better solvent than water in the extraction of the active principle of this plant. Okigbo and Ogbonnaya (2006) stated that the observed differences between the plant extracts may be due to insolubility of active compounds in water as in the case of *G. latifolium* or the presence of inhibitors to the antimicrobial components.

These results suggest that the concentration of *O. gratissimum* was not sufficient for the test microorganisms unlike the *G. latifolium* which showed MBC values for both the aqueous and ethanolic fractions on both organisms. This inactivity may be due to age of plant, extracting solvent, method of extraction and time of harvesting of plant materials and also that variations may also be due to the different active substances present in these plants and the ability of the different active substances to effectively extract these substances (Amadoiha and Obi, 1999; Okigbo and Ajale, 2005; Okigbo and Ogbonnaya, 2006).

Previous report revealed that *O. gratissimum* is rich in phytochemicals like

alkaloids, tannins, glycosides, steroidal terpenes and flavonoids (Nweze *et al.*, 2004). Flavonoids have been reported to have antioxidant activity and are effective scavengers of superoxide anions thus they can significantly affect the cell wall of the microorganisms which may invariably lead to the collapse of the cell wall and overall, affect the entire mechanism of the microbial cell (Nwinyi *et al.*, 2009). *G. latifolium* has been reported to consist of flavonoids, tannins, saponins, polyphenols, alkaloids and hydrogen cyanide (Atanghwo *et al.*, 2009). The presence of tannins, alkaloids, flavonoids, saponins and polyphenols may be responsible for the antibacterial activity of *G. latifolium* (Ibrahim *et al.*, 2006; Andy *et al.*, 2008; Eja *et al.*, 2011). Farombi (2003) noted that the active components of some Nigerian medicinal plants reside in the phytochemical constituents mentioned above. What has not been resolved is the separation of the specific bioactive components against specific organisms and this has been noted to affect quality and safety of herbal medicines (Eja *et al.*, 2011).

CONCLUSION

This study showed that the two plants, *efinrin* and *utazi* possess varying degrees of antimicrobial activity. These spices act through their natural inhibitory mechanisms, either by inhibiting or killing the pathogens. This study has provided the basis for the use of these two plants in the treatment of ailments caused by *S. aureus* and *Salmonella typhi.* Since the solvents used had varying effects on the active principles of these plants, further studies need to be done in determining the best strategy to be adopted in their extraction and administration.

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Legends to figures

Figure 1: Antimicrobial activity of the aqueous extracts of *G latifolium* against *S. aureus* and *Salmonella typhi* at concentrations of 25%, 50% and 75% with chloramphenicol and distilled water serving as positive and negative controls respectively.

Figure 2: Antimicrobial activity of the ethanolic extracts of *G latifolium* against *S aureus* and *Salmonella typhi* at concentrations of 25%, 50% and 75% with chloramphenicol and distilled water serving as positive and negative controls respectively.

Figure 3: Antimicrobial activity of the aqueous extracts of *O. gratissimum* against *S. aureus* and *Salmonella typhi* at concentrations of 25%, 50% and 75% with chloramphenicol and distilled water serving as positive and negative controls respectively.

Figure 4: Antimicrobial activity of the ethanolic extracts of *O gratissimum* against *S. aureus* and *Salmonella typhi* at concentrations of 25%, 50% and 75% with chloramphenicol and distilled water serving as positive and negative controls respectively.