Comparative Antibacterial Analysis and Synergistic Potency of the Leaf Extracts of *Ocimum gratissimum* Linn. and *Gongronema latifolium* Benth. on some Enteric Bacterial Isolates

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Abstract

The research was undertaken to investigate the comparative phytochemical and in-vitro antibacterial activity of the single and combined strengths of the leaf extracts of *Ocimum gratissimum* Linn and *Gongronema latifolium* Benth. on some enteric bacterial isolates. The sensitivity test and minimum inhibitory concentration (MIC) were carried out using a modified agar-well diffusion method. The enteric bacterial isolates tested included *Escherichia coli, Shigella dysenteriae, Salmonella* Typhi and *Enterobacter aerogenes*. Standard methods were applied to obtain the ethanol and aqueous leaf extracts. The ethanol extracts of *O. gratissimum* and *G. latifolium* produced highly significant inhibitory activity against all the enteric bacterial isolates tested. Comparatively, the ethanol plant extracts were more potent than the commercially available drug, Ciprofloxacin and the aqueous plant extracts. The isolates were sensitive at a minimum inhibitory concentration of 1.25 mg/ml for the ethanol extract but varied from 2.5 mg/ml - 5.0mg/ml in the aqueous extract. It was also observed that the synergistic antibacterial effect of the medicinal plant extracts was greater than the singular antibacterial effect of the individual plant extracts in both the ethanol and aqueous extracts. The potency of the individual extracts and the combined effect may be due to the presence of flavonoids, alkaloids, phenols, tannins and saponins in the leaves of the plants. This study partly validates the use of the plant extracts in the treatment of disease caused by the enteric bacterial isolates by multiple traditional medicine practitioners in Nigeria, however, strict adherence to dosage is recommended. The leaf extract is a potential source of the new drug if the components are purified and enhanced for treating infections caused by these enteric pathogens.

Keywords: *Ocimum gratissimum*, *Gongronema latifolium*, enteric bacterial isolates, antibacterial activity, plant extract

Highlights

- *O. gratissimum* and *G. latifolium* ethanol and aqueous leaf extracts possess antibacterial activity against *E. coli*, *S. dysenteriae*, *S.* Typhi and *E. aerogenes*
- The ethanol extracts produced higher inhibition than the aqueous extract and the control antibiotic, ciprofloxacin
- The combined extracts of the medicinal plants were more potent than the single extracts of each medicinal plants and the control antibiotic, Ciprofloxacin.
- The two plant extracts are rich in phytochemicals, which may account for the potency of the extracts

1. Introduction

The human gastrointestinal tract contains an innumerable amount of bacteria. The role of these enteric bacteria in the normal physiological function of tissues and organs is of considerable interest (Canny and McCormick, 2008). These enteric bacteria such as *Escherichia coli, Shigella dysenteriae*, *Salmonella* Typhi, and *Enterobacter aerogenes* enjoy a symbiotic relationship with the host but can have a deleterious effect on the system when imbalance arises. Enteric bacteria are a source of metabolic support to the host as they contribute in the synthesis of vitamins such as vitamin K, folate, biotin and B_{12} (Bentley and Meganathan, 1982). They also assist in combating invading pathogens, fermentation of non-digestible carbohydrates leading to the generation of useful short-chain fatty acids, and beneficial cellular communication with the host (Thursby and Juge, 2017). However, these gut organisms have the potential to cause plethora of diseases in the host when the symbiotic balance is lost (dysbiosis), primarily due to impaired physiological regulation of the existing biological relationship (Canny and McCormick, 2008).

Enteric bacteria can compete for essential nutrients leading to adverse effects in the host. Also, enteric bacteria-derived metabolites and enzymes, colonization of pathogenic bacteria in the intestinal tract, compromised local immunity, and poor diet are some drivers of a disease condition in the host (Batt *et al*., 1996). Enteric bacteria such as *E. coli* which forms part of the normal gut flora has been reported to cause several disorders including bladder infection (cystitis), urinary tract infection and gastrointestinal infection. Some strains of *E. coli* produce toxins that result in severe illness that can potentially lead to death, if untreated (Allocati *et al*., 2013). *E. aerogenes* also cause urinary tract infections, respiratory infections, skin and soft tissue infection, and adult meningitis (Davin-Regli and Pages, 2015). Similarly, *S. dysenteriae* causes dysentery characterized by scant stool containing mucous and blood while *S.* Typhi

causes enteric fever (Kaur *et al.*, 2018). These infectious enteric bacteria have remained a major public health concern, and the lack of adequate treatment due to poverty especially in developing countries and antibiotic resistance portends grave danger for global health.

According to the World Health Organization (WHO), about 80% of the populations in many developing countries still use traditional medicine for their primary health care. This has been attributed to poverty and lack of access to modern medicine (Hugo and Russell, 2003). Since about 80% of the estimated 7.5 billion people of the world live in developing countries, this implies that more than 4 billion people will likely use medicinal plants frequently (Sofowora, 2008). Therefore, there is a need to study medicinal plants for their efficacy, safety and quality in the treatment of diseases including enteric bacteria. This could potentially lead to the identification of invaluable medicinal material from which novel curative agents may be created for the benefit of all mankind (Omaye, 2004).

Most medicinal plants also serve the nutritional purpose. For instance, *Ocimum gratissimum* Linn. and *Gongronema latifolium* Benth serve as a spice which is sometimes added to food meant for pregnant and nursing mothers, for medicinal purposes (Okwu, 2005). These plants also have leaves that are consumed as a conventional leafy vegetable. The plant parts often used for traditional medicine include leaves, stems, roots, and bark (Edeoga and Eriata, 2001). The aforementioned plant's parts have several active components such as tannins, flavonoids, phenols, terpenoids, glycoside, vitamins, minerals and antibiotics which confer the plants with their medicinal property (Edeoga *et al.* 2003; 2005). The medicinal efficacy of these plants as claimed by local medicinal practitioners range from headache, asthma, cough, diarrhoea, malaria, diabetes, eye and ear problems, urinary tract infections, respiratory infections,

skin and soft tissue infection indigestion and constipation, nausea and vomiting, bleeding, healing of wounds, sores and tooth extraction (Ijeh *et al*., 2005).

O. gratissimum Linn (also known as African basil), is a medicinal plant which belongs to the family of Lamiaceae. In Nigeria, it is commonly known as "scent leaf." *O. gratissimum* is rich in the essential amino acids; leucine, valine and phenylalanine and essential fatty acids (linoleic acid, arachidonic acid, docosahexaenoic acid. It also has a bacteriostatic and bactericidal effect on some bacteria (Eleyinmi, 2007). It is widely used in folk medicine for the treatment of various ailments including fever, cough, and respiratory disorder, sore throat, kidney stones, epilepsy and dermatitis (Jimoh *et al.*, 2008).

G. latifolium Benth, commonly called 'utasi' (Igbo) and 'arokeke' (Yoruba) in the South Eastern and South Western parts of Nigeria, respectively is a tropical rain forest plant belonging to the family of Asclepiadaceae. It is primarily used as spice and vegetable in traditional folk medicine (Ugochukwu and Babady, 2002). Reports by various authors show that it contains secondary metabolites such as saponin, alkaloid, phylobatinnins and flavonoids (Eleyinmi, 2007; Morebise, 2015). The report also showed that aqueous and ethanolic *G. latifolium* extract has hypoglycemic and anti-inflammatory properties (Osuagwu and Nwosu, 2006).

Plethora of studies have been conducted assessing the phytochemical content and efficacy of *O. gratissimum* Linn. and *G. latifolium* Benth, however, to the best of our knowledge the synergistic effect of these two plants on enteric bacteria (*E. coli, S. dysenteriae*, *S.* Typhi, and *E. aerogenes*) have not been explored. Also, a comparative inhibition of these enteric bacteria by these plant extracts with the known antibiotic, Ciprofloxacin has not been studied. Thus, this study evaluated the phytochemical constituents, and comparative *in-vitro* antibacterial effect of the single

strength and synergistic effect of the leaf extracts of *O. gratissimum* and *G. latifolium* on *E. coli*, *S. dysenteriae*, *S.* Typhi, and *E. aerogenes*. Comparative inhibition of these enteric bacterial isolates by the plants extracts with the known antibiotic, Ciprofloxacin was also investigated. This study promises to provide invaluable information for the scientific community and with a view of providing valuable information to other scientists who may use these plants for other purposes.

2. Materials and Methods

2.1 Collection and identification of plant materials

Mature vegetative parts of Ocimum gratissimum and Gongronema latifolium were purchased from the local market in Yenagoa, Bayelsa State. Only healthy, fresh and succulent leaves of the plants were selected. The plant specimens were identified and authenticated at the Herbarium of the Department of Plant Science and Biotechnology, University of Port Harcourt, Rivers State, Nigeria. Herbarium specimens were also studied at the institution as well as making reference to the Flora of West Tropical Africa by Hutchinson and Dalziel (1963) and The Useful Plants of West Tropical Africa by Burkill (1994).

2.2 Preparation and extraction of leaf materials

The methods of Chen *et al*. (2007) were applied in the preparation of the leaf extracts. Fresh leaves of *O. gratissimum* and *G. latifolium* were separated manually. The materials were cleaned with sterile distilled water, dried with hot air oven. Then, the dried materials were pulverized to a fine powder using an electric grinder and stored in air-tight bottles. 50 g of fine powder from each of the leaves were presented out in powder, wrapped in filter paper and transferred to the thimble of the Soxhlet chamber. Ethanol (400 ml) and warm water (400 ml) placed in separate

distillation flasks were used for the extraction. After extraction, the solvent was removed typically using a rotary evaporator, yielding the extracted compound. The non-soluble portion of the extracted solid remained in the thimble and was discarded. The extracted compounds were then stored in the refrigerator at 4^0C.

2.3 Phytochemical screening of the leaves

The aqueous extracts of *O. gratissimum* and *G. latifolium* were subjected to qualitative and quantitative screening for chemical constituents using standard procedures (Harborne, 1998; Trease and Evans, 2004).

2.4 Source of bacterial isolates

After the preparation of media, clinical isolates of *Escherichia coli, Salmonella* Typhi, *Shigella dysenteriae,* and *Enterobacter aerogenes* were collected from a stock culture in the Microbiology Laboratory, Federal University Otuoke, Bayelsa State. These isolates were then sub-cultured in a nutrient agar and incubated at 37^0C for 18 hours and identified by carrying out morphological and biochemical tests using Gram staining, catalase test, oxidase test, indole test, citrate utilization test, sugar fermentation test and methyl red for *E. coli, S.* Typhi, *S. dysenteriae* and *E. aerogenes* (Chessbrough, 2006).

2.5 Antibacterial sensitivity testing

The antibacterial sensitivity test was carried out using the methods modified by Atlas and Parks (1997). Twenty millilitres (20 ml) of molten sterile nutrient agar was poured into Petri dishes. After solidification overnight, broth cultures of bacteria were introduced into the surface of the sterile nutrient agar plate and a sterile glass spreader was used for even distribution. Holes were

made aseptically with a 5.0 mm diameter sterile cork borer and 0.1 ml of the test solution of different concentrations (ethanol and water extracts) were introduced into the well. The extract was allowed to diffuse into the medium for 1 hour. The bacteria plates were incubated for 24 hours at 37°C. The plate containing the control was also incubated. The plates were later examined for zones of inhibition, which indicated the degree of susceptibility of the test organisms. The diameter of the inhibition zones was measured with the aid of a transparent meter rule. The effect of the extract on bacterial isolates was compared with Ciprofloxacin (5 mg/ml).

2.6 Determination of minimum inhibition concentration (MIC)

Each reconstituted crude extracts were serially diluted in different concentration using double-fold method at 5, 2.50, 1.25 and 0.625 mg/ml and was stored in the refrigerator for anti-bacterial screening. The minimum inhibitory concentration of *O. gratissimum* and *G. latifolium* extracts were determined using agar-well techniques. Media plates containing varying concentrations of 0.625 mg/ml – 5 mg/ml of the water and ethanol extracts, respectively were incubated at 37°C for 24 hours. The lowest concentration of the various extracts causing complete inhibition of the bacterial growth was taken as the minimum inhibitory concentration (MIC) (Atlas and Parks, 1997).

2.7 Statistical analysis

Data are expressed as the mean ± SEM of three independent experiments. The data were analysed using the Statistical Package for Social Sciences (SPSS) version 20.0.

3 Results

The qualitative and quantitative analysis of the phytochemical screening of the aqueous leaf extract of *O. gratissimum* and *G. latifolium* (Table 1) shows that leaves of *O. gratissimum* have a high concentration (+++) of flavonoids and alkaloids. In the leaves of *G. latifolium*, alkaloids alone were moderately concentrated (++). Tannins, saponins, and phenols were in low concentration (+) in both plants. Comparatively, the leaves of the plants investigated contained appreciable amount of alkaloids, tannins, flavonoids, saponins and phenols ranging from (2.34% - 9.84%), (0.48% - 0.96%), (0.42% -9.15%), (0.05% - 0.79%) and (0.04% - 0.28%), respectively.

In traditional medicine practice in Nigeria, decoctions are the primary forms in which plants are administered, thus the ethanolic and aqueous leaf extracts of *O. gratissimum* and *G. latifolium* were tested in the undiluted form, and two-fold serial dilution of the extracts. Aliquots (100 µl) of each extract, the serial dilutions and the positive control (5 mg/ml) were tested against the four enteric bacterial isolates, *E. coli*, *S.* Typhi, *S. dysenteriae* and *E. aerogenes.* The ethanol leaf extract of *O. gratissimum* and *G. latifolium* (Figure 1) shows exceptional inhibitory activity against the enteric bacterial isolates. *O. gratissimum* leaf extract inhibited the growth of the isolates in this order: *S. dysenteriae* > *E. coli* > *S.* Typhi > *E. aerogenes*. In *G. latifolium* leaf extract treatment, *E. coli* was the most inhibited, followed by *S. dysenteriae*, *S.* Typhi and *E. aerogenes* as determined by the zones of inhibition. Interestingly, the combination of the leaf extract of both plants (synergistic effect) showed significantly higher ($p \leq 0.05$) inhibitory activity against the four enteric bacterial isolates compared to the positive control drug, Ciprofloxacin. Also, the individual treatment with *O. gratissimum* leaf extract significantly inhibited the growth of all the four enteric bacterial isolates while *G. latifolium* significantly inhibited ($p \leq 0.05$) the growth of only two isolates; *E. coli* and *S.* Typhi compared to their respective positive control drug, Ciprofloxacin.

Figure 2 shows the inhibitory activity of the aqueous leaf extracts of *O. gratissimum and G. latifolium. O. gratissimum* leaf extract inhibited the growth of the isolates in this order: *E. coli* > *S. dysenteriae* > *S.* Typhi > *E. aerogenes. G. latifolium* leaf extracts inhibitory activity was in this order: *E. coli, S.* Typhi *and S. dysenteriae*, while the inhibition zone of *E. aerogenes* (8.3 mm) is less than 10 mm and thus not considered active against *E. aerogenes* (Usman *et al*., 2005). The inhibitory effect of both plant extracts as determined by their respective zones of inhibition was lower compared to the positive control drug, Ciprofloxacin. However, the combination of both plant extracts shows a greater inhibitory activity towards the enteric bacterial isolates compared to the individual plant extracts, although this effect was still lower compared to the positive control drug, Ciprofloxacin. The efficacy of the ethanolic leaf extracts of both plants is stronger than the aqueous leaf extract. As seen in Figure 3, the synergistic effect of the ethanolic leaf extracts showed significantly higher ($p \leq 0.05$) inhibitory activity against all four bacterial isolates compared to the aqueous leaf extracts and the positive control drug, Ciprofloxacin.

Table 2 displays the minimum inhibitory concentration (MIC) for the aqueous and ethanolic leaf extract of *O. gratissimum* and *G. latifolium* and their combinations (synergy). The ethanolic leaf extract of both extracts and the synergy were effective at inhibiting the growth of the four enteric bacterial isolates at low concentrations, with MIC value at 1.25 mg/ml. In contrast, the aqueous extracts inhibited bacterial growth at higher concentrations; *O. gratissimum* MIC value against *E. coli, S.* Typhi and *E. aerogenes* was at 2.50 mg/mL, while *G. latifolium*, MIC against *E. coli* was at 2.50 mg/mL but showed higher MIC against *S.* Typhi , *S. dysenteriae* and *E. aerogenes* at 5.00 mg/ml. However, the plant extracts synergy exhibited a uniform MIC against the bacterial isolates at 2.50 mg/ml except for *S. dysenteriae* at 5.00 mg/ml.

4. Discussion

The application of traditional medicine and medicinal plants as a normative basis for the maintenance of good health has been widely observed (UNESCO, 1996). Since ancient times, natural products such as herbs have been used for curing diverse human diseases in most African countries and around the world. Due to the enormous biodiversity of the Nigerian flora, new plants are always identified for their potency in curing, managing or sustaining good health. There is constant need to assess the efficacy of these plants in the scientific community, vis a vis their acclaimed use by traditional medicine practitioners in the treatment of diseases. Thus, the current study shows that the leaf extracts of *O. gratissimum* and *G. latifolium* are potent against the growth of some enteric bacterial isolates *E. coli, S. dysenteriae, S.* Typhi, and *E. aerogenes* as demonstrated by their zones of inhibition at reasonably low MIC.

In this study, there was evident inhibition of the growth of *E. coli, S. dysenteriae, S.* Typhi, and *E. aerogenes* by the ethanolic leaf extract of *O. gratissimum*. This result confirms the potential of *O. gratissimum* for the treatment of bacterial diseases and partly validates its usage in traditional medicinal practice. The antibacterial activity of this plant may be due to the high concentration of flavonoids and alkaloids, as well as the presence of other phytochemicals such as tannins, phenols and saponins. This observation corroborates the works of Akinmoladun *et al*. (2007) and Nweze and Eze (2009) which reported the rich phytochemical content of *O. gratissimum*. Talabi and Makanjuola (2017) previously reported the phytochemical constituents and antibacterial activity of *O. gratissimum* against *E. coli*. Interestingly, the current work has shown that *O. gratissimum* ethanolic leaf extract shows significant antibacterial activity against *E. coli, S. dysenteriae, S.* Typhi, and *E. aerogenes* (MIC 1.25 mg/ml) compared to the commercially available antibiotic, Ciprofloxacin. Similarly, the aqueous extract of *O.*

gratissimum is active against all four bacterial isolates, although at higher MIC (2.50 mg/ml for *E. coli, S.* Typhi and *E. aerogenes,* and *S. dysenteriae* at 5 mg/ml), but comparatively less active than the drug and the ethanol extract, suggesting a more effective extraction procedure with ethanol.

The ethanolic leaf extract of *G. latifolium* demonstrated antibacterial activity against *E. coli, S. dysenteriae, S.* Typhi, and *E. aerogenes* (MIC 1.25 mg/ml) as demonstrated by their zones of inhibition. Like *O. gratissimum*, the ethanolic leaf extract of *G. latifolium* showed greater inhibitory activity than Ciprofloxacin to *E.coli* and *S.* Typhi, but was comparatively less effective to *S. dysenteriae* and *E. aerogenes*. *G. latifolium* leaf is moderately rich in alkaloids, but also has low concentrations of tannins, flavonoids, saponins and phenols. This finding complements the previous work of Eleyinmi (2007) showing the antibacterial activity of *G. latifolium* against *Staphylococcus aureus*, although the author used methanol extract at a higher MIC (5 mg/mL). The author also reported the presence of essential amino acids and fatty acids in the leaf of *G. latifolium*. Previous works on *G. latifolium* showed it has the anti-inflammatory property (Morebise *et al*., 2002) and rich in phytochemicals (Adeleye *et al*., 2011; Morebise, 2015). The observed antibacterial activity of *G. latifolium* could be attributed to the presence of the phytochemicals. The presence of phytochemicals such as flavonoids and alkaloids in plants have been shown to contribute to their antibacterial, antiviral, antitoxin, antioxidant, anti-inflammatory and anti-carcinogenic activities (Farombi, 2003; Osuagwu and Nwosu, 2006; Macready *et al.*, 2014; Udochukwu *et al*., 2015; Sakharkar and Chauhan, 2017).

In typical African traditional health practice, decoctions of several plants are used to treat diseases as treatment is believed to be more potent with a combination of plants. In the current study, we have also described the synergistic effect of the extracts of *O. gratissimum* and *G.*

latifolium when used in combination against the four enteric bacterial isolates. The combined ethanolic extract of *O. gratissimum* and *G. latifolium* showed significantly greater antibacterial activity on the four bacterial isolates compared to Ciprofloxacin suggesting that the combined strength of the plant extracts is greater than or comparable to the single strength of each extract (Bonjar, 2004; Usoh and Akpan, 2015). Synergistic activities resulting from the interaction of antibiotics and plant extract have been studied by a limited number of scientists (Taria *et al*., 2014). However, it is noteworthy, that the combined effect does not always result in a greater effect as seen in the antibacterial activity of *O. gratissimum* alone (20 mm inhibition zone) and the combined extract of both plants (20 mm inhibition zone), suggesting the need for caution in the combination of these extracts.

The leaf extracts of *O. gratissimum* and *G. latifolium* are active against the tested enteric bacterial isolates. The ethanol extract is more potent than the aqueous extract. The individual extract components responsible for the antimicrobial potential of the plant extracts were not identified in the current study. However, the current study provides partial scientific bases for local usage of these plants in the cure of various diseases that are being caused by the enteric bacterial isolates. The plant extract should be used with caution to avoid the elimination of beneficial microorganisms in the intestinal flora. The need for adequate dosage is strongly recommended to avoid toxicity.

5. Conclusion

The search for substances with antimicrobial activity is continuous and medicinal plants are considered veritable sources for researchers. This work has shown that the leaf extracts of *O.*

gratissimum and *G. latifolium* have antibacterial property against the tested enteric bacterial isolates. The combined use of both extracts showed greater inhibitory effect in most cases. The ethanolic extract is more potent and requires a lower dosage to achieve inhibition. The observed antibacterial activity may be due to the presence of alkaloids, tannins, flavonoids, saponins, and phenols in the plant extracts. The traditional medicinal use of the leaves of these plant extract is encouraged but traditional health practitioners must adhere to a dosage to avoid toxicity. However, the full potential of these plants is dependent on the characterization of the biologically active components.

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Conflict of interest statement

The authors declare that they have no competing interests.

Ethical Declaration

Ethical approval was not required.

Author contribution Conceptualization and study design:

The study was planned and designed by DAA and AJO. Experiments were conducted by AJO and DAA. CKA provided materials for the experiments. Data analysis and interpretation of data:

AJO. Original draft preparation was by DAA and CKA. Critical review and editing: AJO. All authors read and approved the final manuscript.

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Table 1. Qualitative and quantitative screening of the phytochemical constituents of the leaves of *O. gratissimum* and *G. latifolium* using aqueous extract.

Phytochemical	***O. gratissimum***	***G. latifolium***

	Concentration	Composition (%)	Concentration	Composition (%)
Alkaloids	+++	9.84±0.11	++	2.34±0.16
Tannins	+	0.96±0.00	+	0.48±0.00
Flavonoids	+++	9.15±0.34	+	0.42±0.02
Saponins	+	0.05±0.01	+	0.79±0.14
Phenols	+	0.04±0.00	+	0.28±0.01

Results are mean ± SEM of three independent experiments

KEY: + = Low concentration; ++ = Moderate concentration; +++ = High concentration

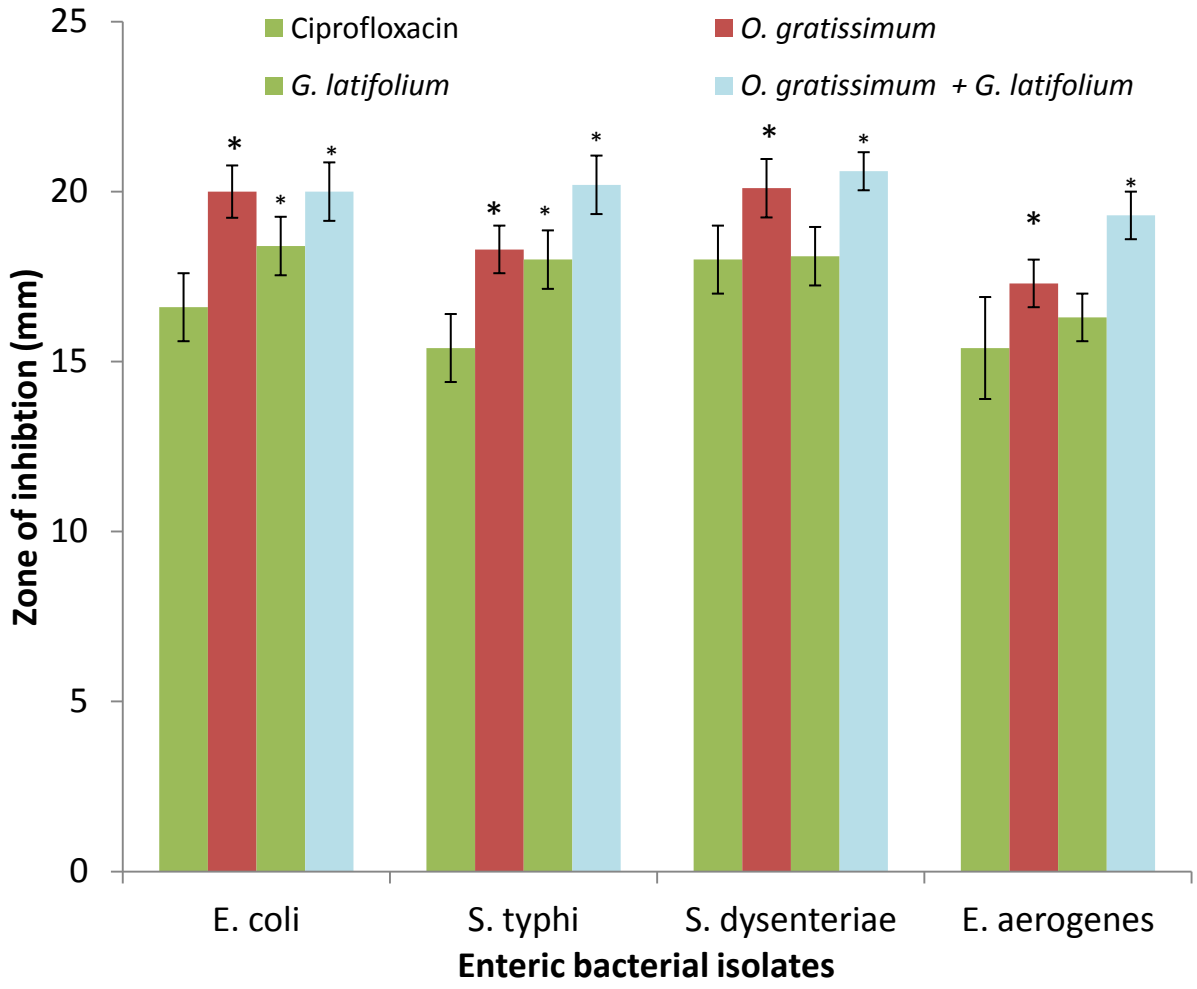

Figure 1. Antibacterial activity of ethanolic leaf extracts of O. *gratissimum* and *G. latifolium* measured as zones of inhibition (mm) against enteric bacterial isolates.

***** Significantly different to Ciprofloxacin (positive control) at ($p \leq 0.05$)

Results are mean ± SEM of three independent experiments

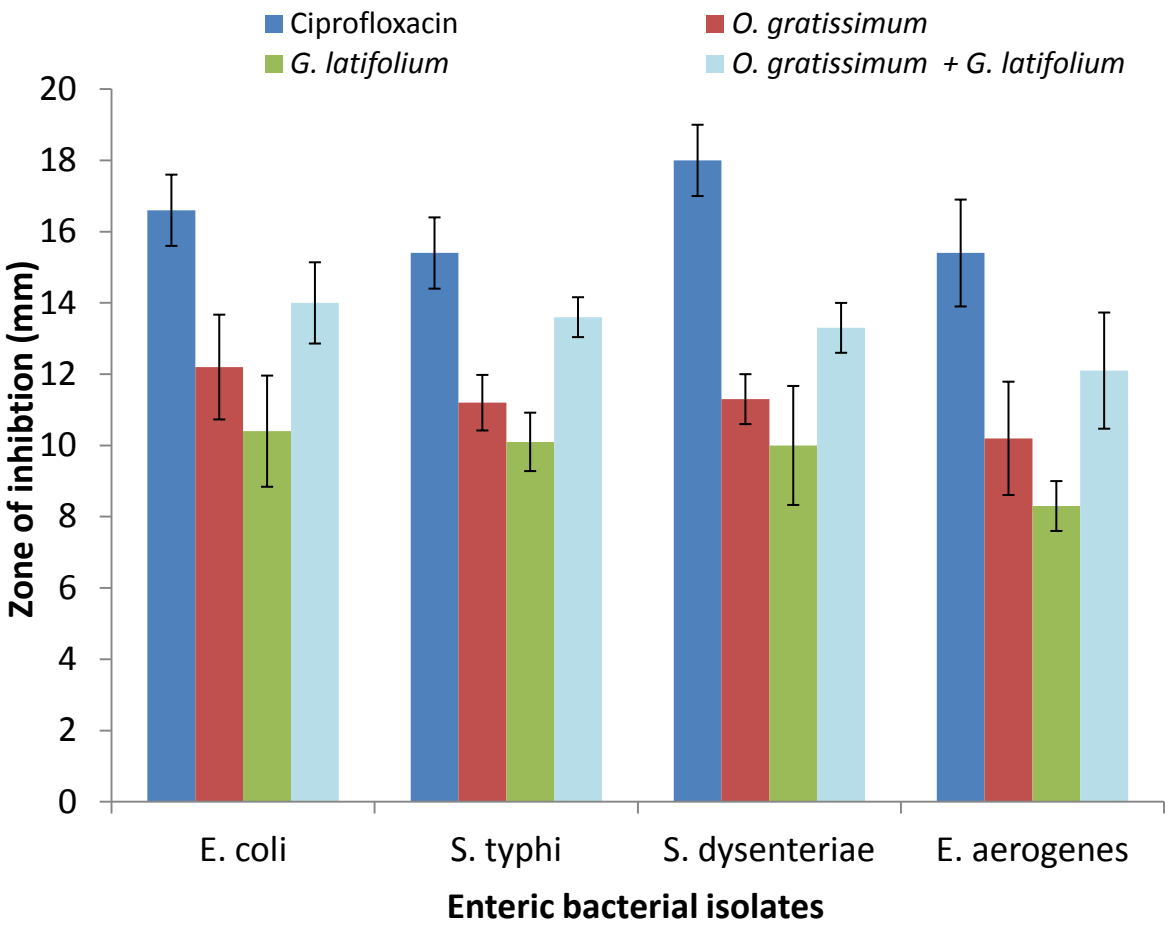

Figure 2. Antibacterial activity of aqueous leaf extracts of O. *gratissimum* and *G. latifolium* measured as zones of inhibition (mm) against enteric bacterial isolates.

Results are mean ± SEM of three independent experiments

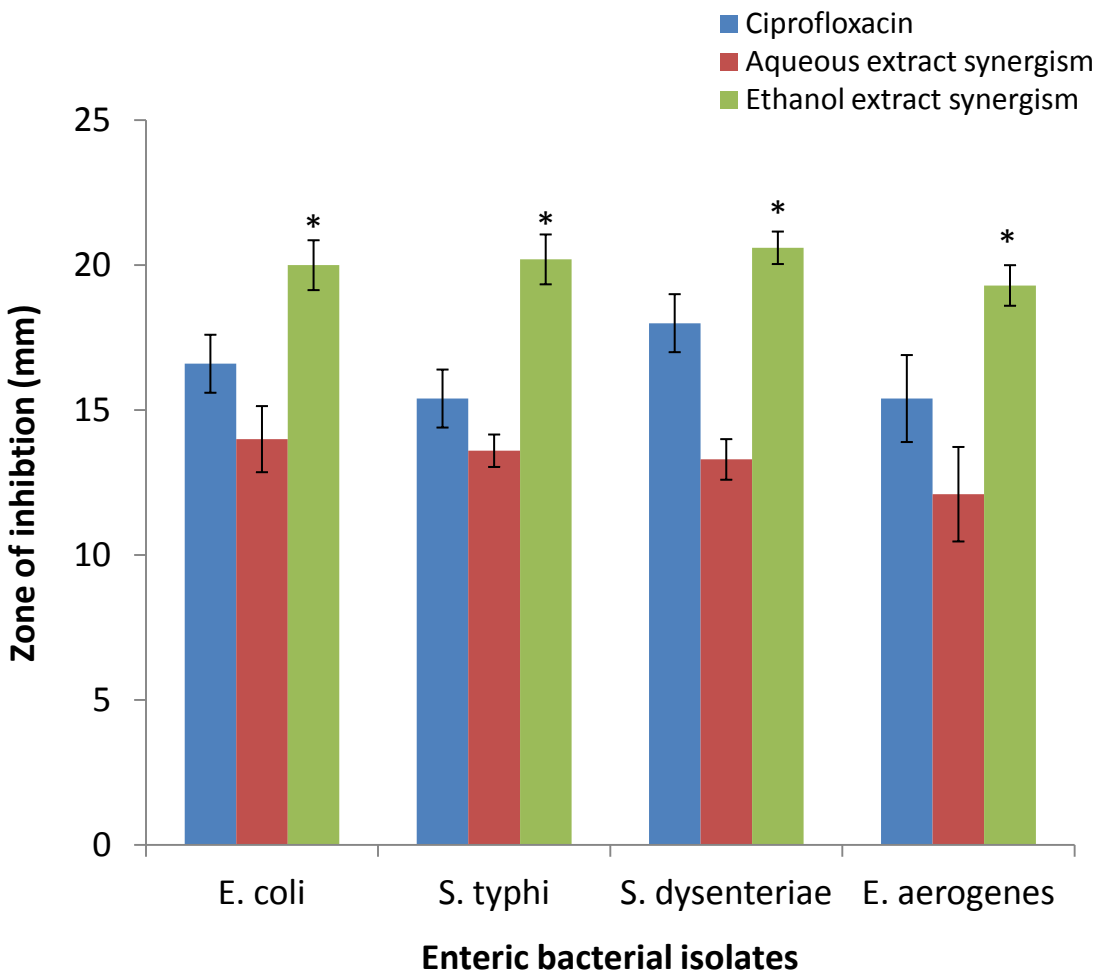

Figure 3. Comparative antibacterial activity of synergistic ethanolic and aqueous extracts and Ciprofloxacin

***** Significantly different to ciprofloxacin and aqueous extract synergistic effect at ($p \leq 0.05$)

Results are mean ± SEM of three independent experiments

Table 2. Minimum inhibitory concentrations (mg/mL) of ethanolic, aqueous and synergistic combination of *O. gratissimum* and *G. latifolium* against enteric bacterial isolates.

Isolates	**Ethanol extract (mg/mL)**			**Aqueous extract (mg/mL)**		
	O. gratissimum	***G. latifolium***	**Synergy**	***O. gratissimum***	***G. latifolium***	**Synergy**
E. coli	1.25	1.25	1.25	2.50	2.50	2.50
S. Typhi	1.25	1.25	1.25	2.50	5.00	2.50
S. dysenteriae	1.25	1.25	1.25	5.00	5.00	2.50
E. aerogenes	1.25	1.25	1.25	2.50	5.00	2.50