In vitro comparative analysis of the antibacterial activity of ethanolic extracts of *Ocimum gratissimum* (Fever Leaf) and Ciprofloxacin against *Salmonella typhi*

Mitchell Sarmie[1], Kajali Kangar[1], S. Archie Hne Toomey[1], Bode Ireti Shobayo[1,2]

1. Department of Biological Sciences, T. J. R. Faulkner College of Science and Technology; 2. National Public Health Institute of Liberia, Congo Town, Monrovia, Liberia
bodeishobayo@gmail.com, karngarka@ul.edu.lr

Abstract: The emergence of microbial resistance to currently used antibiotics is a serious global health issue. Obtaining scientific evidence on the use of medicinal plants as an alternative to conventional antibiotics in the treatment of microbial infections is therefore warranted. This study evaluated the efficacy of ethanolic extracts of *O. gratissimum* in comparison with a second-generation fluoroquinolone (ciprofloxacin) against *Salmonella typhi* isolate. Fresh plant leaves were collected from the Fendell Community, dried, and grounded into powder. 82.5g of the powder was extracted with 300ml of ethanol to determine the presence of phytochemical compounds of the powder. *S. typhi* isolates were obtained from the National Standards Laboratory and their susceptibility profile against the plant extracts and Ciprofloxacin was determined using the Agar Well Dilution Method. Phytochemical analysis revealed the presence of Tannins, Saponins, Alkaloids, Flavonoids, Glycosides, Phenols, Terpenoids, and Steroids in the extracts of *O. gratissimum.*, Comparatively, Ciprofloxacin showed 60.96mm zone of inhibition against the tested isolates compared to 5.08mm which was shown by the plant's extracts. The study revealed that Ciprofloxacin showed significantly higher degree of inhibition against the bacterial strain as compared to *O. gratissimum* extracts. Further studies are however needed to examine the antibacterial potential of other forms of extracts of the plant. The biologically active compounds of *O. gratissimum* should also be further investigated using Quantitative methods to determine efficiency of each secondary metabolite, in addition to determining the MIC and MBC of the plant extracts.
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1. Introduction

In several nations, plants are a potential source of antibacterial agents (1). In developing nations, between 60 and 90 percent of the population uses plant-based medicines (1,2). The antimicrobial activity of herbal plants remains an area of interest for researchers due to the development of microbial resistance to the available antibiotics and the need to identify alternative therapeutic sources for treatment of diseases caused by common pathogens (3). The World Health Organization (WHO) estimates that 80% of the world's population uses herbal medicine to treat a variety of illnesses (4). This reliance on herbal therapy is due to the cheaper cost, global availability, and ongoing evolution of antibiotic resistance in herbal plants. Resistance has, however, also been noted to a variety of natural antimicrobial medications. As a result, it becomes vital to research more herbal plants for possible phytochemicals with stronger antibacterial effects (5).

Different medicinal plants and herbs have been utilized by all cultures and faiths throughout history to treat the health of both people and animals. Additionally, the general public views these naturally derived goods favorably and views them as essential components of health care. Since they are sources of several compounds with antibacterial and radical scavenger characteristics, these products also frequently have success treating illnesses (6). Clove basil or *O. gratissimum* L, is a member of the Lamiaceae family and is often found in South America, Asia, and Africa (7). There have previously been reports of the use of *O. gratissimum* leaf infusion in traditional medicine to treat fever, the flu, and renal issues (8). The antibacterial capabilities of the plant have also been explored independently or in combination with conventional antibiotics to restore the sensitivity of multi-drug resistant bacteria (8–10). Due to their similar safety and efficacy, research into the antibacterial role of plants against resistant strains is reviving in response to the recent onset of antibiotic resistance and related toxicity problems that limit the use of antimicrobial treatments (11).

A significant public health issue is the prevalence of infectious diseases caused on by bacteria worldwide (12,13). Numerous human diseases are brought on by bacteria such *Salmonella, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis,* and *Proteus vulgaris* (14,15). Particularly, *Salmonella enterica* subspecies serovars *Typhi* and *Paratyphi* infection in the body is what causes typhoid and paratyphoid fevers, often known as enteric fever (16). Typhoid and paratyphoid infections primarily cause bacteraemic febrile illnesses with typical symptoms including prolonged high fever, headache, and malaise, in contrast to most non-typhoidal *Salmonella spp* infections which typically cause diarrheal illness and less frequently cause bloodstream infection. Finding new methods of treating typhoid infections therefore becomes important due to the rise of antibiotic resistance (17). Typhoid is prevalent in Liberia, where there are reportedly more than 7,400 cases per year. At least 7,401 typhoid cases (160 cases per 100,000 people), 84 typhoid fatalities, and 5,906 disability-adjusted life years lost to typhoid are predicted to have occurred in 2016 (18). This study compares the antibacterial activity of an ethanolic extract of *O. gratissimum* against *S. typhi* to ciprofloxacin, a commonly used second generation fluoroquinolone antibiotic. The results of this study are anticipated to add to the body of knowledge regarding the effectiveness of this plant in treating *S. typhi* infections as against conventional antibiotics.

2. Methods and Materials

2.1 Plant material

The leaves of *O. gratissimum* L. were collected in October 2021 from wild populations growing in the Fendell community, Louisianain, Montserrado County, Liberia. The plant was identified as *Ocimum gratissimum* by a botanist in the Department of Biological Sciences, College of Science and Technology, University of Liberia.

2.2 Test organism

The bacteria strain used in this study was *S. typhi* which was obtained from the National Standards Laboratory, Monrovia, Liberia. Bacterial cultures were inoculated on Hektoen Enteric Agar for use in experiment. The media was prepared according to manufacturer's instructions.

2.3 Preparation of plant material and extraction

Fresh leaves were collected, washed, and rinsed in distilled water. The leaves were shade dried and grounded into powder using a mortar and pestle. Ethanol, 300ml, was added to 82.5g of the powder in a jar and the mixture was manually mixed and shaken consistently for four days. This process was done at the T. J. R Faulkner College of Science and Technology Laboratory.

2.4 Phytochemical Analysis

The qualitative identification of Saponins, Alkaloids, Flavonoids, Glycosides, Phenols, Terpenoids, Tennins and Steroids was qualitatively carried out using the recommended protocols (19).

Test for Tannins: To 1 ml of extract, 2 ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

Test for Saponins: To 2 ml of extract, 2 ml of distilled water were added and shaken in a graduated cylinder for 15 minutes lengthwise. It resulted in the formation of a 1 cm layer of foam that indicated the presence of saponins.

Test for Alkaloids: To 2 ml of extract, 2 ml of concentrated hydrochloric acid was added. Then a few drops of Mayer's reagent were added. Presence of green color or white precipitate indicates the presence of alkaloids.

Test for Flavonoids: To 2 ml of extract, 1 ml of 2N sodium hydroxide was added. Presence of yellow color indicates the presence of flavonoids.

Test for Glycosides: To 2 ml of extract, 3ml of chloroform and 10% ammonia solution was added. Formation of pink color indicates presence of glycosides.

Test for Phenols: 2 ml of distilled water followed by few drops of 10% ferric chloride was added to 1ml of the extract. Formation of blue or green color indicates presence of phenols.

Steroids: To 1 ml of the extract equal volume of chloroform is added and a few drops of concentrated sulfuric acid added. The appearance of a brown ring indicates the presence of steroids, and the appearance of a bluish brown ring indicates the presence of phytosterols.

Test for Terpenoids: 0.5 ml of the extract was treated with 2ml of chloroform and concentrated sulfuric acid. Formation of red brown color at the interface indicates the presence of terpenoids.

2.5 Antibacterial assay

Using the Agar Well Dilution Method, two petri dishes inoculated with the isolate of *S. typhi* were used to determine the antibacterial activity of both the ethanolic extract of *O. gratissimum* and Ciprofloxacin Hydrochloride. The petri dishes were divided into two plates: A and B. Plate A (Ciprofloxacin Hydrochloride): 500mg of Ciprofloxacin Hydrochloride powder was inoculated in the well and incubated at 37°C for 24 hours. This plate was also used as the control. Plate B (Extract of *O. gratissimum*): 500mg of B was inoculated in the well and incubated at 37°C for 24 hours. All antibacterial assays were performed at the National Standards Laboratory, Monrovia, Liberia.

Figure 1: *S. typhi* growth on Plate A

Figure 2: *S. typhi* growth on Plate B

3. Results

Phytochemical analysis carried out on the leaves of *O. gratissimum* using ethanol revealed the presence of major phytochemicals including flavonoids, alkaloids, glycosides and saponins as shown in Table 1.

Table 1: Qualitative phytochemical analyses of the extracts of *O. gratissimum*

Phytochemical	Indication
Steroids	+
Tannins	+
Saponins	+
Alkaloids	+
Flavonoids	+
Glycosides	+
Phenols	+
Terpenoids	+

(+) indicates presence while (–) indicates the absence of the components

As shown in Table 2, the two plates, A and B, were observed for the presence zones of inhibition. Both Plate A and Plate B produced zones of inhibition on the inoculated agar plates. According to the Clinical and Laboratory Standards Institute, a disc containing 5µg concentration of ciprofloxacin zone of inhibition is measure by the diameter of the zone in the following range: resistant, ≤15mm; intermediate, 16-20mm; and susceptible, ≥21mm (20). Using this standard, the zone of inhibition was determined for the 500mg ($5x10^5$µg) of both the ciprofloxacin hydrochloride powder and the *O. gratissimum* extract.

Table 2: Inhibition of *S. typhi* to ethanolic extracts of *O. gratissimum* and ciprofloxacin

Plate	Concentration	*S. typhi* inhibition	Zone measurement (mm)
A	500mg	+	S (60.96)
B	500mg	+	R (5.08)

4. Discussion

Phytochemical compounds present in leave extracts were determined and its efficacy against *S. typhi* was investigated. Tannins, Saponin, Alkaloids, Flavonoids, Glycosides, Phenols, Terpenoid, and Steroids were detected in the aqueous extract of *Ocimum gratissimum* leaf. Despite the presence of phytochemicals compounds in the plant extracts, ciprofloxacin showed significant inhibition against the tested bacteria. Although inhibition by *Ocimum gratissimum* ethanolic extract was comparatively low, there is still some indication that the plant has pharmacological significance. The result agrees with that of Olawale *et al*., (21) which revealed that Acetone extracts of *O. gratissimum* inhibited *S. typhi* ATCC 14024 at 6.25 mg/ml. Also, the MIC of Ciprofloxacin against *S. typhi* was $3.13×10^{-3}$ mg/ml. However, this study used ethanol instead of acetone for extraction because of ethanol miscibility with both polar and nonpolar substances. Difference in solvents used for extraction and source of bacterial isolate may also influence the effect on the bacteria.

Synergizing a particular secondary metabolite from *O. gratissimum* with ciprofloxacin also showed profound antimicrobial effects against *S. typhi*. According to a previous study, the effects of Alkaloids extracted from *O. gratissimum*on the activity of Ciprofloxacin against *S. tyhpi*has zones of inhibition of 27.33mm (22), however, the extraction of phytochemical constituents for analysis of the chemical composition of each metabolite was not done during this study.

5. Conclusion

The findings of this investigation demonstrated that the extract of *O. gratissimum* contained phytochemical substances. These substances may be effective in combating *S. typhi* and other human infections. Further studies are needed to identify the secondary metabolites quantitatively and evaluate the efficiency of each metabolite against *S* using synergistic approach with Ciprofloxacin and determine the MIC and MBC of the plant against S. typhi**.**

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References

1. Alviano DS, Alviano CS. Plant extracts: search for new alternatives to treat microbial diseases. Curr Pharm Biotechnol. 2009 Jan;10(1):106–21.
2. Meldred Baguio, Mariam Dumato, Mindanao State University-Maguindanao. Antimicrobial Activity of the Ethanol Extract of Caesalpinia Pulcherrima (Linn.) Sw. Leaves by Meldred Baguio, Mariam Dumato :: SSRN [Internet]. [cited 2023 Feb 20]. Available from: https://papers.ssrn.com/sol3/papers.cfm?abstract_id=3409260
3. Gupta PD, Birdi TJ. Development of botanicals to combat antibiotic resistance. J Ayurveda Integr Med. 2017;8(4):266–75.
4. Eve A, Aliero AA, Nalubiri D, Adeyemo RO, Akinola SA, Pius T, et al. In Vitro Antibacterial Activity of Crude Extracts of Artocarpus heterophyllus Seeds against Selected Diarrhoea-Causing Superbug Bacteria. ScientificWorldJournal. 2020 Sep 7;2020:9813970.
5. Álvarez-Martínez FJ, Barrajón-Catalán E, Micol V. Tackling Antibiotic Resistance with Compounds of Natural Origin: A Comprehensive Review. Biomedicines. 2020 Oct 11;8(10):405.
6. Parham S, Kharazi AZ, Bakhsheshi-Rad HR, Nur H, Ismail AF, Sharif S, et al. Antioxidant, Antimicrobial and Antiviral Properties of Herbal Materials. Antioxidants (Basel). 2020 Dec 21;9(12):1309.
7. Mohr FBM, Lermen C, Gazim ZC, Gonçalves JE, Alberton O. Antifungal activity, yield, and composition of Ocimum gratissimum essential oil. Genet Mol Res. 2017 Mar 16;16(1).
8. Penido AB, Morais SM de, Ribeiro AB, Silva AZ. Ethnobotanical study of medicinal plants in Imperatriz, State of Maranhão, Northeastern Brazil. Acta Amazonica. 2016;46(4):345.
9. Melo RS, Albuquerque Azevedo ÁM, Gomes Pereira AM, Rocha RR, Bastos Cavalcante RM, Carneiro Matos MN, et al. Chemical Composition and Antimicrobial Effectiveness of Ocimum gratissimum L. Essential Oil Against Multidrug-Resistant Isolates of Staphylococcus aureus and Escherichia coli. Molecules. 2019 Jan;24(21):3864.
10. Intorasoot A, Chornchoem P, Sookkhee S, Intorasoot S. Bactericidal activity of herbal volatile oil extracts against multidrug-resistant Acinetobacter baumannii. J Intercult Ethnopharmacol. 2017;6(2):218–22.
11. Eggleston K, Zhang R, Zeckhauser RJ. The Global Challenge of Antimicrobial Resistance: Insights from Economic Analysis. Int J Environ Res Public Health. 2010 Aug;7(8):3141–9.
12. Zhang SX, Yang CL, Gu WP, Ai L, Serrano E, Yang P, et al. Case–control study of diarrheal disease etiology in individuals over 5 years in southwest China. Gut Pathog. 2016 Nov 16;8:58.
13. Paterson DL. Impact of antibiotic resistance in gram-negative bacilli on empirical and definitive antibiotic therapy. Clin Infect Dis. 2008 Sep 15;47 Suppl 1:S14-20.
14. Meltzer AS. Medical Laboratory Manual for Tropical Countries. Vol. II. CMAJ: Canadian Medical Association Journal. 1986 Jun 6;134(12):1378.
15. Pitout JDD. Multiresistant Enterobacteriaceae: new threat of an old problem. Expert Rev Anti Infect Ther. 2008 Oct;6(5):657–69.
16. Kim JH, Mogasale V, Im J, Ramani E, Marks F. Updated estimates of typhoid fever burden in sub-

Saharan Africa. Lancet Glob Health. 2017 Oct;5(10):e969.

17. Stanaway JD, Reiner RC, Blacker BF, Goldberg EM, Khalil IA, Troeger CE, et al. The global burden of typhoid and paratyphoid fevers: a systematic analysis for the Global Burden of Disease Study 2017. The Lancet Infectious Diseases. 2019 Apr 1;19(4):369–81.
18. Typhoid Vaccine, Acceleration Consortium. Liberia_typhoid-infographic_7-2018.pdf [Internet]. [cited 2023 Feb 20]. Available from: https://www.coalitionagainsttyphoid.org/wp-content/uploads/2018/07/Liberia_typhoid-infographic_7-2018.pdf
19. R. Roghini and K. Vijayalakshmi *. PHYTOCHEMICAL SCREENING, QUANTITATIVE ANALYSIS OF FLAVONOIDS AND MINERALS IN ETHANOLIC EXTRACT OF CITRUS PARADISI | INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH [Internet]. 2018 [cited 2023 Feb 20]. Available from: https://ijpsr.com/bft-article/phytochemical-screening-quantitative-analysis-of-flavonoids-and-minerals-in-ethanolic-extract-of-citrus-paradisi/
20. Ali SQ, Zehra A, Naqvi BS, Shah S, Bushra R. Resistance Pattern of Ciprofloxacin Against Different Pathogens. Oman Med J. 2010 Oct;25(4):294–8.
21. Olawale OE, Olusola-Makinde O, Oladunmoye MK. Synergistic Antibacterial Potentials of Ocimum gratissimum, Honey and Ciprofloxacin against some Multiple Antibiotic-Resistant Bacteria Isolated from Stool Samples. Walailak Journal of Science and Technology (WJST). 2021 Apr 20;18(9):9192 (15 pages)-9192 (15 pages).
22. Amadi RE, Ikechukwu HI, Bright CU. EFFECTS OF CRUDE ALKALOID EXTRACTED FROM OCIMUM GRATISSIMUM ON THE ACTIVITY OF CIPROFLOXACIN AGAINST SALMONELLA ENTERICA SEROVAR TYPHI. Advances in Life Science and Technology. 2017;58(0):43.

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