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RESEARCH ARTICLE

ANTIBACTERIAL POTENCY OF THE COMBINED EXTRACTS OF *OCIMUM GRATISSIMUM* AND ANTIBIOTICS AGAINST *SALMONELLA* TYPHI AND *SALMONELLA* PARATYPHI

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ABSTRACT

The study assessed the antibacterial effect of the extracts of *Ocimumgratissimum* and their combined effect with antibiotic drugs against *Salmonella* Typhi and *Salmonella Paratyphi* respectively. The extract of *Ocimumgratissimum* were prepared using Soxhlet apparatus for ethanol and petroleum ether extract, and hot water percolation for aqueous extracts. The antibacterial activities of the plant extracts, the inhibitory effect to some antibiotic drugs; Ciprofloxacin, Gentamycin, Rifampicin, Erythromycin, Chloramphenicol and Ampiclox as well as their combined effect with the antibiotics were evaluated using the disk diffusion method. The inhibitory zones were recorded in millimeters. Phytochemical analysis of the extract were as well carried using standard procedure. The result of this study showed that the hot water extract showed higher antibacterial activities against *S.* Typhi and *S.* Paratyphi respectively, followed by the ethanol extract. The petroleum extract however had the least antibacterial effect at the concentration used. All the antibiotic used had greater inhibitory activities on the test organisms except chloramphenicol that showed partial inhibition against *S.* Typhi. The result also showed that the combined effect of the plant extracts and antibiotics used showed additive interaction against the test isolates. The result of the phytochemical analysis revealed the presence of some secondary metabolites such as flavonoids, carbohydrates, tannins, alkaloids and steroids and terpenes. The findings of this study therefore validate the antibacterial properties of *Ocimumgratissimum* and its possession of some active compounds as well as its concomitant use with conventional antibiotics to treat many bacterial infections. It is recommended that the in vivo toxicological activity of the plant be evaluated to determine the real effectiveness and potential toxic effects.

INTRODUCTION

Ocimumgratissimum belongs to the group of plants known as spices. The plant is an erect small plumb with many barnacles usually not more than 1m high (Vierra and Simon, 2000). It is of the family Labiatea, genus *Ocimum* and *species gratissimum* (Nweze and Eze, 2009). The plant is found throughout the tropics and subtropics. It is widespread in Nigeria. Its greatest variability occurs in tropical Africa and India (Aruna and Sivaramakrishna, 1990). It is known by various names in different parts of the world. In India, it is known by its several vernacular names, the most commonly used is Vriddhutulsi (Sanskrit), Ram tulsi (Hindi), Nimmatulasi (Kannada) (Prabhu *et al.*, 2009). In the Southern part of Nigeria, the plant is called "Efinrin" by the Yoruba speaking tribe and "Nchaun" by the Igbos in the Eastern part of Nigeria. In the Northern part of Nigeria, the Hausas call it "Daidoya" (Effraim *et al.*, 2003). In traditional medicine, the leaves have been used as a general tonic and anti-diarrhoea agent and for the treatment of conjunctivitis by instilling directly into the eyes; the leaf oil when mixed with alcohol is applied as a lotion for skin infections, and taken internally for bronchitis. The dried leaves are snuffed to alleviate headaches and fever among other uses (Iwu, 1993). Irrespective of the fact that antibiotics and medicinal plants are used in the management of infectious diseases, still the condition has not been fully eradicated. Enteric fever still causes substantial illness and death in many parts of the world, especially in poorer nations. *S. enterica*serovar Typhi is believed to cause most enteric fever episodes, and a smaller portion are caused by *S.* Paratyphi (Herikstad*et al*., 2002; Crump *et al*., 2003). Typhoid fever is a life-threatening illness caused by the bacterium *Salmonella* Typhi. In the United States, it is estimated that approximately 5,700 cases occur annually.

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Most cases (up to 75%) are acquired while travelling internationally while in the developing world, it affects about 21.5 million persons each year (CDC, 2014).School age children especially those from resource poor settings with inadequate water and sanitation systems are disproportionately affected. According to the World Health Organization, over 16million people worldwide are affected with typhoid fever each year, 500 000 to 600 000 fatal cases and prevalence rate among children between the ages of 5-19 years (WHO, 2009). An increase in resistance to available antimicrobial drugs including fluoroquinolones may foretell dramatic increase in case fatality rates (CDC, 2014). Without therapy, the illness may last for 3 to 4 weeks and number of deaths range between 12 to 30% (CDC, 2014). There is a need to focus on the alternative sources of the antibiotics as the pathogenic microbes are gaining resistance against standard antibiotics (Alo *et al*., 2012). Recently, the exploitation of wild plants for medicinal purposes has gained more acceptances in many countries of the world. This may be because traditional medicine has long been practiced even before the orthodox medical practice appeared (Okafor *et al.,* 2001).Even though, conventional antibiotics have been very useful in orthodox medicine, it has been argued by many that its concomitant use with herbal extracts is not desirable as one normally antagonizes the activity of the other (Nweze and Eze, 2009). *Ocimumgratissimum* is used in most local dishes or foods to achieve a variety of purposes, it is against this backdrop the study intends to ascertain the combined effect of the extracts of *Ocimumgratissimum* with conventional antibiotics.

MATERIALS AND METHODS

Collection and Identification of Plant

Careful selection of fresh leaves of *Ocimumgratissimum* free of blotting and infections were obtained from the National Museum, Jos North local Government Area of Plateau State. The plant was authenticated at the department of Plant Science and Technology, University of Jos, Nigeria where the voucher specimen were deposited.

Collection, Identification and Preparation of Test Organisms

Clinical isolates of S*almonella* Typhi and *Salmonella* Paratyphi were obtained from the Federal College of Veterinary and Medical Laboratory Technology, Vom, Jos East Local Government Area of Plateau State, Nigeria. The test organisms were confirmed using standard microbiological procedures. A 24h culture of the bacterial culture isolate was diluted with physiological saline solution and the turbidity corrected by adding sterile physiological saline until a McFarland turbidity standard of 0.5 (106 CFU/ ml) was obtained (Cheesbrough, 2006).

Culture Media, Antibiotics and Solvents

Different types of media were required in the study, it includes, blood agar, Nutrient agar and Mueller-Hinton agar. Also ethanol and petroleum ether were used for extraction processes. Antibiotics used included, Ciproflox (CPX 30µg), Gentamycin (CN 10µg), Rifampicin (RD 30µg), Erithromycin (E 15µg), Chloramphenicol (CH 30µg), and Ampiclox (APX 30µg). These media and solvents were purchased from Medicom Laboratory Nig. limited, Jos, Plateau State.

Preparation of Plant Extracts

The leaves of *Ocimumgratissimum* were allowed to shade dry at room temperature for four weeks. The air-dried leaves were pounded into fine powder. Fifty (50g) of the powdered materials was weighed and percolated with 500ml of respective solvents(ethanol, Water and Petroleum ether) for 8 hours in Soxhlet apparatus and then the extracts was filtered using Whatman N0 1 filter paper. Each extract was evaporated to dryness in an oven at 45°C. They were then stored in refrigerator until required for use (Shihabudeen *et al.,* 2010).

Determination of Phytochemicals

Qualitative phytochemical analysis of the extracts of the plant was determined by the methods used by Edeoga *et al.,* (2005); Jigna and Sumitra, (2007).

Preparation of Plant extract concentrations

One gram (1g) of each aqueous, ethanol and pet. ether extracts pre-prepared (each separately) was taken and the aqueous extract was dissolved in 10ml sterile distilled water, while the ethanol and pet ether extracts were dissolved in 10ml of DiMethylSulphoxide (DMSO). Thus 100 mg / ml of stock was obtained as a standard concentration of aqueous, ethanol and pet.ether extracts respectively.

Antibiotic Activity Assay

Disc diffusion method of Bauer (1996) was used for this assay. Antibiotic discs to be used were placed on the surface of a Mueller-Hinton agar that has been inoculated with test microorganisms using a sterile swab. The inoculated plates were then incubated at 37°C ±1 for 24 hours. The resulting zones of inhibition were measured in millimeters (mm) using a transparent ruler and values were tabulated.

Plant Extract Activity Assay

Disc diffusion method of Bauer (1996) was used for this assay. A top layer of Mueller Hinton agar was inoculated with 0.1mls of the of the test organisms by the spread plate technique. The dried sterile discs (5mm diameter) impregnated with 20µl plant extract (concentration 100mg/ml) was mounted on the spread test organisms. The plates were then subsequently incubated at 37^0C for 24 hours. The resulting zones of inhibition were measured in millimeters (mm) using a transparent ruler and values were tabulated.

Combined effects of Plant extracts and Antibiotics

The combined effects between plant extract and antibiotic drugs was determined by paper disc diffusion assay as described by Jouda (2013). The tests organisms were grown in Mueller Hinton broth at 37^0C. After 4 hrs of growth, each bacteria was inoculated on the surface of Mueller Hinton agar plates. Subsequently, the antibiotic discs (diameter 5mm) was placed on the surface of each inoculated plate and then 20µl of the plant extracts (at a concentration of 100mg/ml) was added to the antibiotic discs to determine the combined antibacterial effects between the plant extracts and antibiotics. The plates

were incubated at 37^0C for 24h. The resulting zones of inhibition were measured in millimeters (mm) using a transparent ruler and values were tabulated.

RESULTS

Table 1 shows the phytochemical results of extracts of *Ocimumgratissimum*. The result shows the presence of some secondary metabolites such as flavonoids, carbohydrates, alkaloids, steroids and terpenes as they were evident in all the extract tested. Tannins and saponins were however absent in petroleum ether extracts and hot water extracts, alongside cardiac glycoside absent in ethanolic extracts.

Table 1. Phytochemical Constituents of *Ocimumgratissimum*

Secondary metabolites	HWE	EE	PEE
Flavonoids	+ +	+ +	+
Saponins	_	+	_
Carbohydrates	+ +	+	+
Tannins	+	+ +	_
Alkaloids	+ +	+ +	+
Cardiac glycoside	+ +	_	+
Steroids and Terpens	+ +	+	+
Anthraquinons	_	_	_

Key: HWE = Hot water Extract, EE = Ethanol Extract, PEE = Petroleum ether extract; (+) = Present in small amount; (++) = Present in moderate amount; (+++) = Present in high amount; (-) = Absent

Anthraquinons was absent in all the extracts tested. The result also shows that petroleum ether extracts showed the least presence of active compounds compared to ethanol and hot water extracts respectively. Table 2 depicts the susceptibility of the test organisms to the various antibiotics used. Generally, all the antibiotics exhibit strong antibacterial activity against the test organisms. Gentamycin and Erythromycin gave the highest activities against *Salmonella* Typhi and *Salmonella* Paratyphi as indicated by 28mm inhibition zones at the concentration used respectively.

Table 2. Antibacterial activities of *S.* Typhi and *S.* Paratyphi to Antibiotics

Antibiotics	*Salmonella* Typhi	*Salmonella* Paratyphi
Cyproflox	28mm	23mm
Gentamycin	28mm	26mm
Rifampicin	20mm	23mm
Erythromycin	24mm	28mm
Chloramphenicol	3mm	25mm
Ampiclox	26mm	24mm

Table 3. Antibacterial activities of the extracts of *Ocimumgratissimum* against *S.* Typhi and *S.* Paratyphi

Extracts (100mg/ml)	*Salmonella* Typhi	*Salmonella* Paratyphi
Hot water	22mm	18mm
Ethanol	12mm	20mm
Petroleum ether	8mm	9mm

All other antibiotic shows higher activities in which case, the inhibition zones range between (20mm-26mm) respectively. Chloramphenicol however had the least activity against *Salmonella* Typhi as indicated by 3mm zone of inhibition at the concentration used. The result of the antibacterial activity of the extracts of *Ocimumgratissimum*is represented in table 3. The result shows that the hot water extract was the most potent against *S.* Typhi followed by the ethanol extract. The ethanol extract however gave the highest activity against *S.* Paratyphi, followed by Hot water extract. Pet. ether extract gave the least activity against *S.* Typhi and *S.* Paratyphi respectively. The result of the invitro combined effect between extracts (aqueous, ethanol and petroleum ether) and antibiotic drugs used against *Salmonella* Typhi and Paratyphi is presented in table 4 and 5. The result showed that the plant extracts exhibit significant additive effect with all the antibiotic used against the test organisms respectively.

Table 4. Combined effect of Plant extracts and Antibiotics against S. Typhi

Antib.	Antibiotic alone(mm)	Hot water extract Zones of inhibition (mm)		Ethanol extract Zones of inhibition (mm)		Petroleum ether extract Zones of inhibition (mm)	
		Ex. alone	Ex+Anti	Ex. alone	Ex+Anti	Ex. alone	Ex+Anti
CP	28		33		36		35
GN	28		21		35		26
RF	20		20		29		23
ER	24	22	27	12	37	8	24
CL	3		31		35		31
AM	26		23		32		21

Key: CP = Ciprofloxacin, GN = Gentamycin, RF = Rifampicin, ER = Erythromycin, CL = Chloramphenicol, AM = Ampiclox, Ex = Extract, Anti = Antibiotics.

Table 5. Combined effect of Plant extracts and Antibiotics against S. Paratyhi

Antib.	Antibiotic alone(mm)	Hot water extract Zones of inhibition (mm)		Ethanol extract Zones of inhibition (mm)		Petroleum ether extract Zones of inhibition (mm)	
		Ex. alone	Ex+Anti	Ex. alone	Ex+Anti	Ex. alone	Ex+Anti
CP	23		27		26		30
GN	26		20		22		22
RF	23		19		19		16
ER	28	18	28	20	35	9	33
CL	25		22		22		29
AM	24		16		16		8

Key: CP = Ciprofloxacin, GN = Gentamycin, RF = Rifampicin, ER = Erythromycin, CL = Chloramphenicol, AM = Ampiclox, Ex = Extract, Anti = Antibiotics.

DISCUSSION

The phytochemical analysis of *Ocimumgratissimum* revealed the presence of secondary metabolites such as Flavonoids, carbohydrates, alkaloids, tannins, steroids and terpenes, cardiac glycosides and saponins. This study has shown that *Ocimumgratissimum* plant is used in the treatment of many infectious diseases and in particular enteric fever. These plants demonstrates varying activities in terms of their inhibitory effects on *Salmonella*Typhi and Paratyphi. The extracts that showed stronger activity were aqueous extract and ethanol extract with zones of inhibition greater than 10mm against the test organisms. Akinjogunla *et al*. (2011) had reported the antibacterial activity of crude extracts of *Ocimumgratissimum* leaves on *Escherichia coli* and *Salmonella* Typhi. They attributed the inhibitory activity of the crude extract of the plant to the presence of saponins, steroids, tannins, terpenes, anthraqiunones and carbohydrates. In a similar work by Koche*et al*. (2012), ethanolic extracts of *Ocimumgratissimum* was shown to have antibacterial activity against *E. coli* and *Listeria monocytogenes*. They also reported bioactive components of the extracts as; alkaloids, phenolics, tannins, steroids, glycosides and resins. The findings of the study also show that all the antibiotic used had greater inhibitory effects on tested organisms. *Salmonella* Typhi was however resistant to chloramphenicol. The observed resistance of *Salmonella*Typhi to chloramphenicol may be attributed to the growing reported resistance of *S.* Typhito chloramphenicol (White, 2010). The result of the combined effects of the extract of *Ocimumgratissimum*and antibioticshas shown that the plant exhibit significant additive effect on the test isolates. The extracts that showed stronger activity was ethanol extract with zones of inhibition greater than 19mm and 21mm against *Salmonella* Typhi and Paratyphi respectively. Plants antimicrobials have been found to be additive enhancers in that though they may not have any antimicrobial properties alone, but when they are taken concurrently with standard drugs they enhance the effect of that drug (Chanda and Rakholiya, 2011). The result obtained in this study is in contrast to what was obtainable in the report of Jouda (2013) who reported the synergistic effect of *Ocimumgratissimum* among medicinal plants tested with antibiotics and non-antibiotics respectively. He attributed the synergistic effect of the plant and the antibiotic tested to combined interaction of the phytochemical components and active components of the drugs.

Conclusion

The findings of study has proved that available Plants like *Ocimmgratissimum* have antibacterial properties as seen with presence of some active compounds. The antibiotics and plant extracts both inhibited the growth of *S.* Typhiand *S.* Paratyphi. Their combined effects showed additive interaction with antibiotics tested. Drug synergism between known antibiotics and bioactive plant extracts is a novel concept and could be a potential source of antibacterial compound.

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