Original Research Article

Antimicrobial Activity of *Vernonia amygdalina*, *Ocimum gratissimum* and *Gongronema latifolium* Synergy on Common Foodborne Pathogenic Bacteria

ABSTRACT

The search for antimicrobial agents from natural sources such as diverse plant species against foodborne pathogens has less side effect than chemically synthesized compounds. In this study, antimicrobial activity of ethanolic extract, hot and cold aqueous extracts of *Vernonia amygdalina*, *Ocimum gratissimum* and *Gongronema latifolium* was tested against *Escherichia coli, Staphylococcus aureus* and *Listeria monocytogenes*. The leaf extracts of *Vernonia amygdalina* did not show antimicrobial activity against the test isolates. The diameter of zone of inhibition shown by ethanolic leaf extract of *Gongronema latifolium* and *Ocimum gratissimum* against *S. aureus* is 12.0mm and 10 mm, respectively. With regards to ethanolic leaf extract of *Gongronema latifolium* and *Ocimum gratissimum,* the diameter of zone of inhibition demonstrated against *L. monocytogenes* and *E. coli* is 18.0 mm and 6.0 mm, respectively. A synergistic ethanolic or aqueous extracts of the leaves did not show antimicrobial activity against the test isolates. However, ethanolic extracts of *Gongronema latifolium* and *Ocimum gratissimum* used separately is an effective antimicrobial agent the test isolates.

Keywords: Traditional medicine, bioactive compounds, medicinal plants, antimicrobial activity

1. INTRODUCTION

Traditional medicine is very popular in many developing countries till date. The historical and cultural antecedents of traditional medicine is responsible for its wide acceptability [1, 2]. The effectiveness of using herbal plants in the treatment of bacterial infections is a strong belief among residents in most rural communities in Nigeria [3]. According to World Health Organization (WHO), primary health care sought after by approximately 80 % of the world's population is traditional or herbal medicines. The practice of traditional medicine largely involves the use of plants [4, 5]. Interestingly, traditional medicine and modern pharmaceuticals make use of plant and plant products to treat various ailments [6].

Plants especially vegetables such as *Vernonia amygdalina* (bitter leaf), *Ocimum gratissimum* (scent leaf) and *Gongronema latifolium* (bush buck) contain physiologically active components which over the years, have been exploited in traditional medicine for the treatment of various ailments [7]. The three major tribes in Nigeria know bitter leaf as "Olugbu", "Ewuro'' and "Fetefete", respectively [8]. In the South-East and South-West, bush buck is known as "Utazi/Utasi and "Aroreke", respectively [9]. *Ocimum gratissimum* is known as 'Nchuanwu' by the Igbos in the South-East [10]. According to Omogbai *et al*. [11], 'utazi' contains pregnanes, saponins and essential oils. The use of *G. latifolium* in traditional folk medicine and also as a spice is well known. *Ocimum gratissimum* is well known because of its culinary and medicinal properties [12]. The common name 'scent leaf' is attributed to sweet scent an aroma associated with *Ocimum gratissimum* [13].

Saponins, glycosides, alkaloids, and tanins present in bitter leaf is associated with bitterness of *Vernonia amygdalina* [14]. *Ocimum gratissimum* contain large amount of oligosaccharides, phytates, alkaloid, tannins, flavonoids that qualifies it as a medicinal plant [15]. Scent leaf is useful in the treatment of skin diseases and many other diseases which include fever, headache, upper tract infection, pneumonia, tooth and gum disorders [12]. Different parts of bitter leaf plant is useful in traditional medicine as antihelminth, antimalarial, febrifuge, laxative, digestive tonic and appetizer. It also help in the treatment of diabetes [16].

Staphylococcus aureus, Escherichia coli and *Listeria monocytogenes* are common microorganisms associated with foodborne diseases [17]. The fact that these foodborne pathogens have demonstrated antimicrobial resistance to common antibiotics is a threat to healthcare system [18]. Consequently, there is need to explore other possible options to combat infections caused by these foodborne pathogens which include intensive research on medicinal plants, among others. Antimicrobial effects of bitter leaf and bush buck against common pathogens such as *S. aureus, Pseudomonas aeruginosa*, *E. coli*, and *Klebsiella* sp have been reported [19]. The antimicrobial activity of leaf extracts of *Gongronema latifolium* against *S. aureus* and *Lactobacillus fermentum* was reported by Omogbai *et al*. [11]. It has also been reported that leaf extract of *Ocimum gratissimum* (scent leaf) possess antimicrobial activity against microorganisms such as *E. coli, Streptococcus fecalis*, *S. aureus*, and *Lactobacilli* sp. [15].

A study carried out by Opara *et al.* [20] evaluated the antibacterial activity of *Ocimum gratissimum* and *Vernonia amygdalin against E. coli, S. aureus, P. aeruginosa* and *S. pyogenes*. Findings from the study revealed that ethanolic extract of the dried leaves demonstrated zones of inhibition (> 5mm diameter) against all the isolates. There is dearth of information on synergistic antimicrobial activity of *Ocimum gratissimum, Vernonia amygdalin* and *Gongronema latifolium* against common foodborne pathogens. In addition to evaluating the antimicrobial activity of the leaf extracts, this study is aimed at reporting the antimicrobial activity of a mixture of leaf extracts of *Ocimum gratissimum, Vernonia amygdalin* and *Gongronema latifolium* against *Staphylococcus aureus, Escherichia coli* and *Listeria monocytogenes*.

2. MATERIALS AND METHODS

Fresh leaves of *Vernonia amygdalina* (bitter leaf), *Ocimum gratissium* (scent leaf), and *Gongronema latifolium* (utazi) plant were bought from Choba market, Rivers State, Nigeria using sterile polythene bags. The leaves used in the study was authenticated and identified in the Department of Plant Science and Biotechnology, University of Port Harcourt. All the materials were transported to the Food and Industrial Microbiology Laboratory, University of Port Harcourt in less than 12 hours from the time of purchase.

2.1 Plant extraction

Aqueous (hot and cold water) and ethanol extracts of the plant were prepared using the method described by Madunagu *et al*. [21]. Thirty grams (30 g) of each of the vegetables were washed and ground using a clean mortar and pestle.

2.2 Aqueous extract

Twenty grams (20 g) of the ground leaves were measured into different sterile conical flasks containing 50 ml hot and sterile distilled water. The conical flask was covered with a cork and manually shaken for proper mixing. The content of the flask was kept for 48 hours. The extracts were filtered using Whatman No. 1 filter paper.

2.3 Ethanol extract

Twenty grams (20 g) each of the ground leaves were put in different conical flasks and 50 ml of 100 % ethanol was added to each sample. The content of conical flasks was covered with a cork and manually shaken for proper mixing. The content of the flask was kept for 48 hours. The extracts were filtered using Whatman No. 1 filter paper and concentrated with the aid of a rotary evaporator. The concentrated extract was stored in the refrigerator at 4ºC prior to use.

2.4 Sterility test of the plant extracts

Each of the extracts (aqueous and ethanol extract) was tested for the presence of microbial contaminants. The test involved inoculating 1 ml each of the extracts on nutrient agar and incubated at 37 ˚C for 24 hours. The plates were observed for microbial growth. The absence of microbial growth on the culture plates inoculated with the

plant extracts after incubation indicated that the extracts were sterile. The plant extracts were then assayed for antimicrobial activity.

2.5 Source of test organism

Escherichia coli, *Staphylococcus aureus* and *Listeria monocytogenes* were selected as test organisms. The bacterial isolates were obtained from Medical laboratory, Department of Microbiology, University of Port Harcourt. The bacteria were maintained on nutrient agar slants and stored in the refrigerator at 4 ^{o}C. The isolates were reconfirmed prior to use for this work, in the laboratory, following the conventional method of identification, such as morphological features, Grams staining reaction, motility and biochemical characteristics.

2.6 Preparation and standardization of bacterial inoculum

Preparation and standardization of each bacterial inoculum was done by picking test organism growing as a pure culture on solid media and then transferred into sterile normal saline and left for two hours to produce a growth of the same turbidity with McFarland standard. A sterile swab stick was used to inoculate each test organism into the Muller-Hinton Agar.

2.7 Identification of isolates

The colonial morphology of the bacterial isolates in the Petri dishes were observed and noted. Gram and endospore staining of the bacterial isolates were carried out, followed by biochemical reactions which include oxidase, catalase, indole, methyl red, motility, Voges Proskauer, triple sugar iron agar (TSIA) and sugar fermentation tests.

2.8 Determination of antimicrobial activity

The antimicrobial activity of the plant extract of *Vernonia amygdalina, Ocimum gratissimum, and Gongronema latifolium* were determined using agar well diffusion method. Muller-Hinton Agar was prepared and allowed to solidify. Afterwards, the agar was inoculated with the test organism. A sterile cork borer of 10 mm diameter was used to bore uniform wells on the surface of the agar. Exactly 0.1 ml of the extract was introduced into the well.

3. RESULTS

Table 1 shows the biochemical characteristics of the test organisms to reconfirm the isolates. They include *Escherichia coli, Staphylococcus aureus* and *Listeria monocytogene*. Antimicrobial activity of bitter leaf extract against these isolates is presented in Table 2. The result shows that hot, cold water and ethanol extract from bitter leaf did not demonstrate antimicrobial activity against the three bacterial isolates. Presented in Table 3 is the antimicrobial activity of utazi leaf against the three bacterial isolates. Ethanol extract of utazi showed antimicrobial activity against *E. coli* and *L. monocytogene.* Table 4 shows the antimicrobial activity of scent leaf against the three isolates. The results obtained showed that ethanol extract of scent leaf possess antimicrobial activity against *E. coli* and *S. aureus.* Table 5 shows the effect of combination of the three leaf extracts on the three isolates. The result shows that hot, cold water and ethanol extract from scent leaf, utazi and bitter leaf did not demonstrate antimicrobial activity against the three isolates.

Table 1. Biochemical characteristics of the test organisms

Isolate code	Shape	Gram reaction	Endospores	Oxidase	Catalase	Indole	Methyl Red	Voges Proskaeur	Citrate	Motility	Slant/Butt	H_2S	Gas	Glucose	Lactose	Sucrose	Organism
PIE	Rod	-	-	-	+	+	+	-	-	+	A/A	-	+	+		+	*Escherichia coli*
PIS	Cocci	+	-	-	+	-	+	+	+	-	A/A	-	-	+	+	+	*Staphylococcus aureus*
PIL	Rod	+	-	-	+	-	+	+	+	+	A/A	-	-	+	+	+	*Listeria monocytogene*

Key: A - Acid; + Positive; - Negative

Table 2. Antimicrobial activity of *Vernonia amygdalina* (bitter leaf) against the test isolates

Extract	*Staphylococcus aureus*	*Escherichia coli*	*Listeria monocytogene*
HEB	-	-	-
CEB	-	-	-
EEB	-	-	-

HEB - Hot extract of bitter leaf; CEB - Cold extract of bitter leaf; EEB - Ethanol extract of bitter leaf

Table 3. Antimicrobial activity of *Gongronema latifolium* (utazi) against the test isolates

Extract	*Staphylococcus aureus*	*Escherichia coli*	*Listeria monocytogene*
HEU	_	_	_
CEU	_	_	_
EEU	12 mm	_	18 mm

HEB - Hot extract of utazi leaf; CEB - Cold extract of utazi leaf; EEB - Ethanol extract of utazi leaf

Table 4. Antimicrobial activity of *Ocimum gratissimum* (scent leaf) against the test isolates

Extract	*Staphylococcus aureus*	*Escherichia coli*	*Listeria monocytogene*
HES	_	_	_
CES	_	_	_
EES	10 mm	6 mm	_

HEB - Hot extract of scent leaf; CEB - Cold extract of scent leaf; EEB - Ethanol extract of scent leaf

Table 5. Antimicrobial activities of a combination of leaf extracts of *Vernonia amygdalina, Ocimum gratissimum and Gongronema latifolium* against the test isolates

Extract	*Staphylococcus aureus*	*Escherichia coli*	*Listeria monocytogene*
CHE	_	_	_
CCE	_	_	_
CEE	_	_	_

CHE-Combination of hot Extract; CCE-Combination of cold extract; CEE-Combination of ethanol extract

4.0 DISCUSSION

The result obtained from this study has shown that aqueous extract (hot and cold) and ethanolic extract of *Vernonia amygdalina* did not show antimicrobial activity against *Escherichia coli, Staphylococcus aureus* and *Listeria monocytogenes*. This result is not in agreement with the findings of Unegbu *et al.* [14] which reported that hot ethanolic extract of *Vernonia amygdalina* showed antimicrobial activity against *S. aureus* and *E. coli* based on minimum inhibitory diameter of 8.0-19.0 mm and 7.0-20.00 mm, respectively. A recent study carried out by Femi *et al*. [22] reported the presence of alkaloids, cardiac glycosides, flavonoids, saponins, steroids, tannins and glycosides in ethanol and cold water *Vernonia amygdalina* leaf extract. Going by the result obtained in this study, it could be that strains of *E. coli, S. aureus* and *L. monocytogenes* have developed resistance to the bioactive compounds in *Vernonia amygdalina*. Findings from a recent study carried out by Abike *et al*. [23] reported that 100 mg/ml and 50 mg/ml leaf extract of *Vernonia amygdalina* showed antimicrobial activity against *Staphylococcus* sp and *Escherichia coli* except for 50 mg/ml of the extract against *E. coli*. To a large extent, the report is not in agreement with the findings from this study and could be attributed to the solvent (methanol) used in extracting bioactive compounds in *Vernonia amygdalina*.

Ethanolic extract of *Gongronema latifolium* leaves showed antimicrobial activity against *Staphylococcus aureus* and *Listeria monocytogenes*. The diameter of zone of inhibition of ethanolic extract of leaves of *G. latifolium* against *Staphylococcus aureus* and *Listeria monocytogenes* is 12.0 mm and 18.0 mm, respectively. However, hot and cold aqueous extract of *G. latifolium* did not show antimicrobial activity against these bacteria. The implication of the result is that ethanolic extraction of bioactive compounds in *Gongronema latifolium* leaves is more effective than aqueous extraction (hot and cold). In a related study, Bankole *et al*. [12] reported that 25 %, 50 % and 75 % *G. latifolium* aqueous and ethanolic extract showed complete growth inhibition of *S. aureus*. This result is in agreement with the findings from this study. According to the results reported by Omogbai *et al.* [11], the diameter of zone of inhibition of ethanolic leaf extracts of *G. latifolium* against *Escherichia coli* and *S. aureus* is 18 mm and 28 mm, respectively. Findings from this study shows that ethanolic extract and aqueous extract (hot and cold) of *G. latifolium* leaf did not show antimicrobial activity against *E. coli.* This result is in agreement with the report of Akani *et al.* [24].

The diameter of zone of inhibition of ethanolic extract of *Ocimum gratissimum* leaves against *Staphylococcus aureus* and *Escherichia coli* is 10.0 mm and 6.0 mm, respectively. Antimicrobial activity of ethanolic extract of *Ocimum gratissimum* against the test isolates reported in this study is in agreement with the report of Amengialue *et al*. [13]. They reported that diameter of zone of inhibition of ethanolic extract of *Ocimum gratissimum* against *S. auerus* and *Escherichia coli* is 11.0 mm and 9.0 mm, respectively. In this study, ethanolic extract and aqueous extract (hot and cold) of *Ocimum gratissimum* did not show antimicrobial activity against *Listeria monocytogenes*. This result is not in agreement with the findings of Adeshina *et al*. [25] which involved testing the antimicrobial activities of ethyl acetate and diethyl ether extract of *Ocimum gratissimum* against *Listeria monocytogenes*. The researchers discontinued the use of ethanolic extract of *O. gratissimum* in the course of the study because the solvent (ethanol) was unable to extract eugenol identified as the active ingredient in the leaves associated with antibacterial activities.

A striking result in this study is that a combination of ethanolic extract of *Ocimum gratissimum, Vernonia amygdalin* and *Gongronema latifolium* did not show antimicrobial activity against *S. aureus*, *E. coli* and *L. monocytogenes*. The same result was obtained after testing the antimicrobial activity of aqueous extract (hot and cold) of *O. gratissimum, V. amygdalin* and *G. latifolium* against the three bacterial isolates. This result could be attributed to chemical reaction between individual bioactive compounds in the leaf extracts which led to the formation compound(s) lacking in antimicrobial activity against the three test isolates. In a related study, Awomukwu *et al*. [26] reported that a combination of leaf extracts of *Ocimum gratissimum* Linn and *Gongronema latifolium* Benth had greater inhibitory effect against *Escherichia coli*, *Salmonella typhi, Shigella dysenteriae* and *Enterobacter aerogenes* compared to each of the plant extracts. The report is not in agreement with the findings from this study.

CONCLUSION

Aqueous extract (hot and cold) and ethanolic leaf extract of *Vernonia amygdalina* did not show antimicrobial activities against the test isolates (*Staphylococcus aureus*, *Escherichia coli* and *Listeria monocytogenes*). Antimicrobial activities was shown by ethanolic leaf extract of *Gongronema latifolium* and *Ocimum gratissimum* against *S. aureus.* Ethanolic leaf extract of *Gongronema latifolium* and *Ocimum gratissimum* demonstrated antimicrobial activity against *L. monocytogenes* and *E. coli*, respectively. A combination of leaf extract of *Ocimum gratissimum, Vernonia amygdalin* and *Gongronema latifolium* obtained using ethanol or aqueous solution (hot and cold) did not show antimicrobial activity against the test isolates.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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