Original Research Article

JOURNAL OF BASIC PHARMACOLOGY AND TOXICOLOGY

Antibacterial and synergistic potency of methanolic leaf extracts of *Vernonia amygdalina* L. and *Ocimum gratissimum* L.

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Abstract

Vernonia amygdalina and *Ocimum gratissimum* are common perennial shrubs that are used as vegetables and spices. Studies have indicated that both plants have several medicinal properties. This study investigated the antibacterial and synergistic potency of methanolic leaf extracts of *Vernonia amygdalina* and *Ocimum gratissimum*. The leaf samples were shade dried, extracted using methanol and tested against some bacterial isolates using agar well diffusion method. The mean zone of inhibition against *E. coli*, *Staphylococcus aureus* and *Bacillus* sp. was 13.67 mm, 13.33 mm and 13.00 mm, respectively by *V. amygdalina*, 13.00 mm, 12.67 mm and 13.00 mm, respectively by *O. gratissimum,* and 15.00 mm, 13.67 mm, 13.33 mm, respectively for synergy of *V. amygdalina* and *O. gratissimum* at 100% concentration. There was no significant variation ($p > 0.05$) among the various isolates, and plants and its combinations at 100% and 85% concentrations, but significant difference ($p < 0.05$) exists at 95% and 90% extracts. The findings showed that the combination had slight superior potency against the tested isolates.

Keywords: Antimicrobial activity, Medicinal plants, *Vernonia amygdalina*, *Ocimum gratissimum*, Bacterial pathogens

1. Introduction

Medicinal plants emerged as essential starting materials for the development of drug [1]. Research into search of new source of raw materials for the development of new drugs is due to microbial resistance, emerging and re-emerging diseases [2]. Research have been widely carried out with regard to medicinal plant in both developed and developing nations especially in Africa where about 80% of the inhabitants of the area depends on medicinal plants for health care delivery [1].

The use of medicinal plants is associated to the presence of several bioactive chemicals and phytochemical constituents of plants [3 – 9]. Since ancient times, plants are used in the treatment of some human diseases especially in rural areas in many African communities. Bukar et al. [10] attributed the use of medicinal plants to poverty and poor hygiene standards due to possibility of infection diseases. Lack of access to modern medical facility is also another factor leading to dependency on medicinal plants in many rural areas. Furthermore, Bukar et al. [10] reported that herbal medicine is the first line of treatment of about 60% of the children with high fevers in some countries such as Ghana, Mali, Nigeria and Zambia.

Several parts of plants (root, stem-bark, root, leaves, fruit, flower, juice/latex) that have therapeutic properties are used for the treatment of disease. Several medicinal plants are also used as food condiments. Some common medicinal plants that are used as food include *Vernonia amygdalina* (bitter leaf), *Ocimum gratissimum* (scent leaf), *Aframomum melegueta* (alligator pepper), *Piper nigrum* (climbing pepper) among others. Specifically, *O. gratissimum* and *V. amygdalina* play essential role in the diets of several Nigerian ethnic groups and are traditional ingredients in some African cuisine [11]. Furthermore, Ohadoma et al. [12] have reported that both plants have therapeutic properties.

V. amygdalina which belongs to *Asteraceae* family is a common shrub or small tree that grows in tropical Africa [10, 13] such as Nigeria. The plant is also distributed in Asia, and is commonly found along drainage lines and in natural forest or commercial plantation [13]. The plant can grow up to 1 – 5 meter high [10, 14]. The leaves of *V. amygdalina* are used as vegetable and/ or as spices [10, 14] and traditional medicine [10].

O. gratissimum which belongs to *Lamiaceae* family is a shrub, and it is commonly known as Scent leaf or Clove basil or lemon basil [15, 16]. *O. gratissimum* is a perennial plant that is widely distributed in the tropic of Africa and Asia [17]. *O. gratissimum* is an aromatic medicinal plant [18] with wide range of culinary applications [17, 19]. Similar to *V. amygdalina*, *O. gratissimum* is also a popular medicinal plant with several therapeutic potency. This could be due to the presence of bioactive and phytochemical constituents. For instance, previous studies reported that *V.*

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amygdalina and *O. gratissimum* contain oxalate, phytate, tannins, saponins, flavonoid, terpenoids, cyanogenic glycoside, alkaloids, anthraquinone, steroid and phenol [3, 13, 18, 20, 21]. *O. gratissimum* has several medicinal properties including antidiarrhoeal [22, 23], antioxidative [24 – 31], antimicrobial [14, 15, 20, 21, 27, 30, 32–34], antidiabetic [31, 35], antiurolithiatic [36], larvicidal, pupicidal and adulticidal against insects such as mosquito [37]. In a similar manner, *V. amygdalina* have been widely reported to have anticancer properties [1, 3, 38, 39], antiparasitic [40], hypoglycaemic activity [41], antidiabetic activity [42], antimalarial activity [43, 44], analgesic activity [44, 45], antipyretic activity [45] and antimicrobial activity [14, 20, 21].

Previous studies reported the synergistic efficacy of combination of *V. amygdalina* and *O. gratissimum* leaf extracts prepared by using ethanol [3] and water [18] as solvents. But information about the synergy of methanolic leaf extracts of *V. amygdalina* and *O. gratissimum* appears scanty in literature. Therefore, this study was carried out.

Ocimum gratissimum

Vernonia amygdalina

Figure 1. *V. amygdalina* and *O. gratissimum plants*

2. Materials and Methods

2.1. Samples collection, preparations and extraction

The leaves of *V. amygdalina* and *O. gratissimum* (Figure 1) were obtained from Ndemili in Ndokwa West Local Government of Delta state. The plant was identified based on the description made by Nyananyo [46]. The plant leaf samples were shade dried. The dried samples were blended to powdered form using Binatone blender (Model BLG – 451). Then 40 g of each of powered extract was soaked in 100 mL of methanol for 48 hr. Furthermore, 20 g of each of the powered leaves were weighed and added together making it 40 g, and it was soaked in methanol for 48 hr as well. Then, it was filtered using muslin cloth. The resultant filtrate was re-filtered using Whatman filter paper. The solvent were allowed to evaporate in a water bath.

2.2. Dilution of the extracts

Dilutions of the extracts were made following the methods previously applied by Kigigha et al. [7]. The original stock of the leaf extracts was considered as 100% concentration, and then it was further diluted into 95%, 90% and 85% of the original volume.

2.3. Source and preparation of organisms

The *Staphylococcs aureus, E.coli,* and *Bacillus* species used for the sensitivity testing was obtained from Medical Microbiology units, Federal Medical Centre, Yenagoa, Bayelsa state, Nigeria. The purity and characteristics of the bacterial isolates were checked using the guide provided by Cheesbrough [47]. Each of the microorganisms used were inoculated into sterile peptone water and incubated at room temperature for 24 hr, before it was used.

2.4. Antimicrobial screening of the extract

Agar well diffusion method previously described by Lino and Deogracious [48] cited in Doherty *et al.* [49] with slight modification by Agu and Thomas [50], Kigigha et al. [6, 7], Epidi et al. [4, 5] was used for this study. About 20 mL of prepared nutrient agar was poured onto sterile Petri dish and allowed to solidify. Approximately, 0.3 mL of the incoculum was spread over the agar surface using a spreader. Four holes of 6 mm in diameter were made in each agar plate. Three mL of the various concentrations of the extract was dispensed into the agar wells. The agar plates were properly labeled. The plates were incubated for 24 hr. The resultant zones of inhibition were measured using metre rule.

2.5. Statistical analysis

Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS) software version 20. The data were expressed as mean ± standard error, and one-way analysis of variance was carried out at $\alpha = 0.05$. Duncan multiple range test was used to ascertain the source of the variation.

3. Results and discussion

Table 1 presents the zone of inhibition of methanolic leaves extract of *V. amygdalina* and *O. gratissimum* at varying concentrations. The mean zone of inhibition exhibited by *E. coli*, *Staphylococcus aureus* and *Bacillus* sp. was 13.67 mm, 13.33 mm and 13.00 mm, respectively for *V. amygdalina*, 13.00 mm, 12.67 mm and 13.00 mm, respectively for *O. gratissimum,* and 15.00 mm, 13.67 mm, 13.33 mm, respectively for combination of *V. amygdalina* and *O. gratissimum* at 100% concentration. There was no significant variation ($p > 0.05$) among the various isolates, and plant extract and its combinations at 100% concentration. The mean zone of inhibition exhibited by *E. coli*, *Staphylococcus aureus* and *Bacillus* sp. was 11.33 mm, 10.67 mm and 9.67 mm, respectively for *V. amygdalina*, 8.33 mm, 10.67 mm and 12.00 mm, respectively for *O. gratissimum,* and 13.33 mm, 9.67 mm, 13.33 mm, respectively for combination of *V. amygdalina* and *O. gratissimum* at 95% concentration; and 9.00 mm, 8.67 mm and 7.67 mm, respectively for *V. amygdalina*, 7.67 mm, 9.33 mm and 9.33 mm, respectively for *O. gratissimum,* and 9.67 mm, 11.33 mm, 7.67 mm, respectively for synergy of *V. amygdalina* and *O. gratissimum* at 90% concentration. There was significant variation ($p < 0.05$) among the various isolates, and plant and its combinations at 95% and 90% concentrations. Furthermore, at 85%, there was no significant difference ($p > 0.05$) among the various isolates and plant combinations. The zone of inhibition of the plants showed that the synergy interaction has apparently superior efficacy compared to the individual plants for most of the isolates. As the extract is being diluted, the efficacy decreases.

This study validates the finding of previous work that *O. gratissimum* and *V. amygdalina* has antimicrobial activities. Ogodo et al. [17] reported that aqueous and ethanol extracts of leaf and stem bark, and synergy of leaf and stem bark of *O. gratissimum* is potent against some foodborne pathogens such as *Escherichia coli, Staphylococcus aureus, Pseudomonas* species*, Salmonella* species and *Shigella* species. Bukar et al. [10] reported that ethanolic, methanolic and aqueous leaf extract of *V. amygdalina* have effect against *Staphylococcus aureus, E. coli, Pseudomonas species, Salmonella species* and *Proteus species.* Ghamba et al. [51] reported that aqueous leave extract of *V. amygdalina* have antimicrobial activities against *Escherichia coli* (12.5 mm), *Pseudomonas aeruginosa* (12.2 mm), *Klebsiella* sp. (11.8 mm), *Staphylococcus aureus* (11.4 mm), *Streptococcus* sp. (0.00 mm), and *Candida albicans* (11.8 mm). Hassan et al. [3] reported the zone of inhibition for *E.coli* and *S. aureus* as 17 mm and 19 mm, respectively for ethanolic *O. gratissimum*, 12 mm and 5 mm, respectively for ethanolic *V. amygdalina* and 0 mm and 22 mm, respectively for ethanolic *O. gratissimum* + *Vernonia amygdalina.* Akinjogunla et al. [18] reported that aqueous leaf extract of *V. amygdalina* and *O. gratissimum* is potent against *E. coli*. The authors further reported the source of isolate affect the sensitivity of the plants.

Table 1. Zone of inhibition (mm) of methanolic leaves extract of *V. amygdalina* and *Ocimum gratissimum*

Plant extracts	Isolates	100%	95%	90%	85%
Vernonia amygdalina	*E.coli*	13.67±0.33ab	11.33±0.33c	9.00±0.58ab	7.67±0.67b
	Staphylococcus aureus	13.33±0.33ab	10.67±0.33bc	8.67±0.33ab	7.33±0.33b
	Bacillus sp.	13.00±0.58a	9.67±0.33ab	7.67±0.33a	4.67±0.67ab
Ocimum gratissimum	*E.coli*	12.67±0.33a	8.33±0.33a	7.67±0.33a	0.00±0.00a
	Staphylococcus aureus	13.00±0.58a	10.67±0.67ab	9.33±0.67ab	5.00±2.52ab
	Bacillus sp.	12.33±0.33a	12.00±0.00cd	9.33±0.33ab	2.67±2.67ab
Combination of *V. amygdalina* and *Ocimum gratissimum*	*E.coli*	15.00±0.58a	12.00±0.58cd	9.67±0.88bc	4.67±2.33ab
	Staphylococcus aureus	13.67±0.33ab	13.33±0.33d	11.33±0.33c	7.67±0.33b
	Bacillus sp.	13.33±0.88ab	9.67±0.33ab	7.67±0.33a	2.33±2.33ab

Different letters along the column indicate significant variation ($p < 0.05$) according to Duncan statistics

The antimicrobial potentials alongside with other medicinal ability of both plants could be associated to the presence of bioactive ingredients. Salawu et al. [14] reported the presence of vicenin-2, caffeic acid, rutin, kaempferol 4' O-rutinoside, rosmarinic acid, cichoric acid, cirsimaritin and nevadensin in *O. gratissimum*, and caffeoyl quinic acid, chlorogenic acid, luteolin 7-O-glucoside, luteolin 7-O-glucuronide, 1,5 dicaffeoyl quinic acid, dicaffeoyl quinic acid, dicaffeoyl quinic acid, apigenin-O-glucuronide, luteolin and some unidentified flavonoids in *V. amygdalina.*

The variation that exists in the zone of inhibition for the various bacteria could be due to differences in cell wall of the organisms [52], physiology, metabolism, nutrition, genetic composition and biochemistry of the bacteria [4-8]. The age of the plants, environmental conditions under which it was cultivated, solvent used, method of extraction, time of harvesting are some of the factors that influence the sensitivity of plant extracts [2, 17, 53].

4. Conclusion

Research into the antimicrobial potentials of plant has increased in the last few decades. This study investigated the antibacterial and synergistic potency of methanolic leaves extract of *O. gratissimum* and *V. amygdalina*. The results showed that both *O. gratissimum* and *V. amygdalina* have antibacterial efficacy with regard to *S. aureus*, *E. coli* and *Bacillus* sp. Synergetic result showed slightly superior activities. The efficacy of the plants against both gram positive (*S. aureus* and *Bacillus* sp.) and gram negative (*E. coli*) organisms suggests that both extracts can be used as broad spectrum antibiotics.

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