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Research Article
Synergistic Effect of the Extracts of *Vernonia amygdalina* and *Solenostemon monostachyus* on Gram-negative Bacteria

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Abstract

Background and Objective: Herbal medicines are getting more importance in the treatment of infections ailments because most of the synthetic drugs have side effects. A large proportion of the developing countries populations depend on herbal remedy for their physical and psychological health needs depend. Hence, the anti-bacterial activity of the mixture of the extracts of *Solenostemon monostachyus* and *Vernonia amygdalina*, common medicinal plants use in Africa, Asia and Europe on some Gram-negative bacteria was investigated. **Materials and Methods:** The plants that were cultivated in a well-drained soil and the bacteria, *Salmonella typhii*, *Escherichi coli* and *Enterobacter aerogenes* freshly isolated from clinical samples were used for this study. The plant leaves were grounded separately into a powder and analyzed quantitatively for phytochemical composition and extracted using acetone. Similarly, equal volume of the two plants was homogenized. The sensitivity of the bacteria isolates was performed using disk diffusion method and the antimicrobial activity was determined by measuring the diameter zones of inhibition and for the sensitive measurement (inhibitory zones $\geq$20) and resistant measurement (inhibitory zones $\leq$17). **Results:** Analysis of the plants revealed their phytochemical composition. The plant's extracts had high diameter zones of inhibition at the higher concentrations and the concoction treatment was more sensitive. In *V. amygdalina*, the zone of inhibition varied significantly between *S. typhi* and *E. aerogenes* ($p<0.05$, $F = 7.2$) and between *E. coli* and *E. aerogenes* ($p<0.05$, $F = 6.7$), while in *S. monostachyus,* it varid significantly between *S. typhi* and *E. coli* ($p<0.05$, $F = 9.2$) and between *E. coli* and *E. aerogenes* ($p<0.05$, $F = 5.8$). **Conclusion:** These plants were readily available at no cost therefore, they could be exploited to provide novel compounds that may be used as starting materials for the production of drug that can obliterate resistance bacteria.

Key words: *Solenoste monmonostachyus*, *Vernonia amygdalina*, *Salmonella typhii*, *Escherichi coli*, *Enterobacter aerogenes*, phytochemical composition, concoction treatment

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The use of plant extracts and phytochemicals, both with known antimicrobial properties can be of great significance in therapeutic treatments. Recently, a number of researches have been conducted in different countries to show such efficiency. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant[1]. It has become more popular in the treatment of minor ailments and also on account of the increasing costs of personal health maintenance[2]. The rural population for instance, that do not have access to primary health care, either as a result of non-availability or inability to afford it depends solely on plant remedies for their health problems[3]. It has been proven that majority of Africa populations rely on conventional medicine for treatment and for the past decades, result in the rising trend in the exploitation of herbal medication[4].

It is likely that the deep understanding of herbal mixture in customary cultures, developed through trial and error over many centuries, along with the most important cures was carefully passed on verbally from one generation to another without proper documentation[5]. Without a doubt, modern allopathic medicine has its basis in this ancient medicine and it's likely that many important new remedies will be developed and commercialized in the future from the African biodiversity as it has been till now, by following the preamble put in place by traditional knowledge and conception[6]. The continuous reliance on traditional medicine in Africa has been linked to cultural and economic reasons. Hence, the WHO[7] is in support of African member states to encourage and incorporate conventional medical practices in their health system.

Plants usually contain secondary metabolites that my act individually, additively or in synergy to improve health. Certainly, medicinal plants, unlike pharmacological drugs, commonly have several chemicals working together catalytically and synergistically to produce a collective effect that surpasses the total activity of the individual constituents[4]. The combined actions of these substances tend to increase the activity of the main medicinal constituent by speeding up or slowing down its assimilation in the body. Secondary metabolites from plant's origins might increase the stability of the active compound (s) or phytochemicals, minimize the rate of undesired adverse side effects and have an additive, potentiating or antagonistic effect[7].

Some believed that isolation of phytochemicals and their use as single chemical entities as a better option forming a concussion of different plants extract. However, nowadays a view that there may be some advantages of the medical use of crude and/or standardized extracts as opposed to isolated single compound is gaining much momentum in the scientific research[5].

Vernonia amygdalina is an edible plant that its leaves are consumed as vegetable and condiments, after macerating and washing thoroughly to remove the bitterness. The bitter taste of *V. amygdalina* is due to anti-nutritional factors such as alkaloids, saponins, tannins and glycosides. Anthelmintic, antimalarial, antitumourigenic, hypoglycemic and hypolipidaemic properties of *V. amgdalina* have been reported[8,9]. Both the roots and leaves are used in phyto-medicine to treat fever, hiccups, kidney disease and stomach discomfort[10].

Solenostemon monostachyusis an important herb that is native to West and Central Africa, Asia and Europe. The leaves have been traditionally used for various medicinal purposes however the scientific basis for these effects is rare[11]. The plant is commonly known as coleus or sometimes known as chocolate mint is a genus of flowering plants in the family lamiaceae. It occurs as an annual weed in anthropogenic habitants and rocky savannahs. It is slightly succulent, aromatic and grows up to 100 cm tall[12]. The aerial parts of the plant are used in various decoctions traditionally by the ibibios of the Niger Delta of Nigeria to treat stomach ulcer, fever/malaria[13]. The decoction of the plant is also used as a diuretic as well as to treat hypertension[14]. The leaf essential oil of *S. monostachyus* has been reported to contain : β-pineue, oct-1-en-3-ol, β-caryophyllene, octan-3-ol and (E,E)-α-farnesene[12] reported biological activities of the plant include, chitioxidant[15], antihypertensive[16] antimicrobial activities[17] and antiulcer[18]. The health promoting properties of plants are ascribed to the possession of various phytochemicals especially phenolic and this beneficial activity is related to their antioxidant activity[19]. Many disorders especially cardiovascular disorders, diabetes, cataract, cancer, ageing are partly caused by reaction oxygen species (ROS) and reactive nitrogen species (RNS)[20].

The available research addressed only antibacterial activities of either *Solenostemon monostachyus* or *Vernonia amygdalina*, none had addressed the effects of the mixture, hence this research was undertaken in order to assess the synergistic effects of the extracts of *Solenostemon monostachyus* and *Vernonia amygdalina*, common plants used by native folks in Africa and Asia on some Gram-negative bacteria.

MATERIALS AND METHODS

Chemicals/equipment: Acetone (95.8% purity), Whatman No. 1, magnesium metal strip, bromine water were obtained from chemical Service. Others were analytical grades. The main equipment used are weighing balance, heating mantle, water reflux and nutrient agar.

Cultivation of *V. amygdalina* and *S. monostachyus*: The stem of *V. amygdalina* and seeds of *S. monostachyus* were collected from a botanical garden in Otuoke Bayelsa state, Nigeria and planted on a fertile soil within Federal University Otuoke premises.

Crude extraction: The fresh leaves of *V. amygdalina* and *S. monostachyus* were harvested from the garden and dried separately at room temperature (22±0.15)°C for 30 days. The plants leaves were grounded separately into a powder form using pestle and mortar. Similarly, equal volume of the two plants was homogenized. The powder was filtered through a 40-mesh screen and extracted for 5 h using the Soxhlet apparatus as described by Obi and Onuoha[21] and Khandelwal[22] with little modification. Briefly, 100 g of the powder was extracted using acetone in a flat bottom flask. About 500 g of acetone was measured using a volumetric flask and was dispensed into the flat bottom flask containing the plants powder and subjected to a vigorous shaking in a sonication bath for 3 h. The mixture was filtered Whatman No. 1 filter paper into pre-weighed beakers and solvent separated. The solvent was evaporated and concentrated in vacuum at 30°C until a constant dry weight was obtained and preserved aseptically at 4°C until analysis.

Collection of test organisms: The bacteria species used for this investigation (*S. typhii*, *E. coli* and *E. aerogenes*) were freshly isolated from clinical samples were obtained from the Department of Microbiology, Federal Medical Centre, Otuoke Annexe Bayelsa State, Nigeria. The bacterial strains were grown and maintained on Muller-Hinton Agar medium, slants at 4°C in incubator.

Antimicrobial screening: The sensitivity of the bacteria isolated from the clinical samples was performed using disk diffusion method as described by Ahmed *et al.*[23] and ofloxacin was used as a sensitivity drug. In brief, 1 mL of each bacterium isolates were seeded into each of the Petri dishes containing Mueller-Hinton agar (MHA) and were allowed to stand for 45 min to allow the pre-diffusion of the inoculated organisms. The disc that has ofloxacin was placed on the surfaces of the Muller-Hinton agar plates with a sterilized forceps and lightly pressed to allow even contact and these were then incubated for 24 h at 38°C. The antimicrobial activity was determined by measuring the diameter of zones of inhibition (mm) produced. For the sensitive measurement (Inhibitory zones ≥18) and resistant measurement (Inhibitory zones ≤15) was determined following the manufacturer's standard zone size manual.

Bioassay procedure: The screening test of antimicrobial activity to the different concentrations of the extracts was done using by using the disk diffusion method[24]. An inoculums suspension was swabbed evenly, solidified in 20 mL MHA and allowed to dry for 4 min. Sterile cork borer was used to make holes of 8 mm in diameter in the seeded agar. Aliquot of 50 µL from each graded extract concentrations (20, 40, 80, 160 and 320 mg mL^{-1}) were added into each well on the seeded medium and allowed to stand on the bench for 2 h for proper diffusion and thereafter incubated at 37°C for 24 h. The antimicrobial activity was determined by measuring the diameter of zones of inhibition (mm) produced. All the test were triplicated and were carried out within a week.

Activity index (A.I) of the crude plant's extract: The activity index of the extract was calculated as the ratio of the mean of zone of inhibition of the extract to the mean of zone of inhibition of ofloxacin (a standard antibiotic drug)[23]:

$$\text{A.I.} = \frac{\text{Mean of zone of inhibition of the extract}}{\text{Mean of zone of inhibition of standard antibiotic drug}}$$

Statistical analysis: The results of the antibacterial activity of different concentrations of the plants leaves extract treatments to various bacteria strains were expressed as Mean±Standard Error (SE). The difference between the control and the various treatments and within the treatments were analyses using the student's t-test at 95% confidence level [25] and one-way analysis of variance SPSS (14.0 version), SPSS Inc, Chicago, USA, p-values of 0.05 or less were considered statistically significant.

RESULTS

The phytochemical composition of the leaves extracts of the investigated plants showed the presence of anthraquinones, deoxy-sugar, flavonoids, saponins,

Table 1: Phytochemical compositions of the leaves extracts of *V. amygdalina* and *S. monostachyus*

Phytochemicals	*Vernonia amygdalina*	*Solenoste monostachyus*
Anthraquinones	**	*
Deoxy-sugar	*	*
Flavonoids	**	**
Saponins	*	*
Alkaloid	**	**
Phlobatanins	≠	**
Terpenes	*	**
Tannin	**	≠

*Present in moderately concentration, **Present in high concentrations, ≠Not detected

Table 2: Antibacterial activities of the leaves of *V. amygdalina* extracted at different concentrations on some Gram-negative bacteria species (Mean diameter zone of inhibition (mm)±SE)

Extract (mg mL^{-1})	*Salmonella typhii*	*Escherichi coli*	*Enterobacter aerogenes*
20	7.20±0.11^{a}	8.10±0.22^{a}	11.70±0.02^{b}
40	7.90±0.03^{a}	8.30±0.16^{a}	11.70±0.04^{b}
80	12.20±0.15^{a}	12.10±0.21^{a}	14.10±0.24^{b}
160	15.50±0.23^{a}	13.40±0.23^{a}	15.50±0.27^{a}
320	18.20±0.22^{a}	16.00±0.25^{a}	21.80±0.12^{a}
Ofloxacin	27.00±0.20^{a}	26.50±0.15^{a}	24.00±0.10^{a}

Mean with different superscript in the row are significantly different *($p<0.05$)

Table 3: Antibacterial activities of the leaves of *S. monostachyus* extracted at different concentrations on some Gram-negative bacteria species (Mean diameter zone of inhibition (mm)±SE)

Extract (mg mL^{-1})	*Salmonella typhii*	*Escherichi coli*	*Enterobacter aerogenes*
20	4.60±0.01^{a}	2.50±0.05^{b}	4.10±0.14^{a}
40	5.40±0.13^{a}	3.70±0.12^{a}	4.80±0.12^{a}
80	6.10±0.22^{a}	5.20±0.32^{a}	6.11±0.20^{a}
160	12.50±0.20^{a}	17.10±0.26^{b}	13.20±0.23^{a}
320	18.50±0.28^{a}	20.10±0.20^{a}	15.40±0.24^{b}
Ofloxacin	27.00±0.20^{a}	26.50±0.15^{a}	24.00±0.10^{a}

Mean with different superscript in the row are significantly different *($p<0.05$)

Table 4: Antibacterial activities of the equal mixture of the leaves extract of *V. amygdalina* and S. *monostachyus* at different concentrations on some bacteria species (Mean diameter zone of inhibition (mm)±SE)

Extract (mg mL^{-1})	*Salmonella typhii*	*Escherichi coli*	*Enterobacter aerogenes*
20	11.20±0.28^{a}	10.30±0.07^{a}	10.20±0.25^{a}
40	17.10±0.03^{a}	18.30±0.10^{a}	17.70±0.13^{a}
80	22.00±0.10^{a}	22.60±0.25^{a}	24.20±0.20^{a}
160	25.20±0.20^{a}	24.70±0.21^{a}	25.10±0.11^{a}
320	27.30±0.30^{a}	26.30±0.20^{a}	26.80±0.15^{a}
Ofloxacin	27.00±0.20^{a}	26.50±0.15^{a}	24.00±0.10^{a}

Mean with different superscript in the row are significantly different *($p<0.05$)

alkaloids, phlobatannins, terpenes and tannin. The extent of concentrations of the chemicals in these plant leaves extract differed as denoted with representation (Table 1).

The mean diameter zone of inhibition of the individual plants extract on the investigated gram negative bacteria revealed that the higher the concentrations of the extract, the better the zone of inhibition (Table 2, 3) and the synergy treatment exhibited better inhibition zone when treated individually (Table 4).

In this study, the inhibitory or of bacteria to the extract was plants and species dependent. For *V. amygdalina*, the diameter zone of inhibition was highest in *E. aerogenes* (21.80±0.12) and least in *E. coli* (16.0±0.25), irrespective of the extract concentrations (Table 2). The diameter zone of inhibition varied significantly between *S. typhi* and *E. aerogenes* ($p<0.05$, $F = 7.2$) and between *E. coli* and *E. aerogenes* ($p<0.05$, $F = 6.7$) at the extract concentrations of 20, 40 and 80 mg mL^{-1}. No significant difference was observed at the other treatments. Similarly, the activity index revealed that *E. aerogenes* was more susceptible to *V. amygdalina* than other bacterial species, with *S. typhi* the least and was significant at higher concentrations of 160 and 320 mg mL^{-1} (Fig. 1).

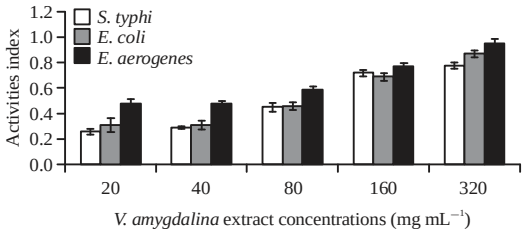

Fig. 1: Activities index (A.I) of the leaves of *V. amygdalina* extracted with acetone at different concentrations in a freshly bacteria isolates (Ofloxacin as the standard antibiotic drug)

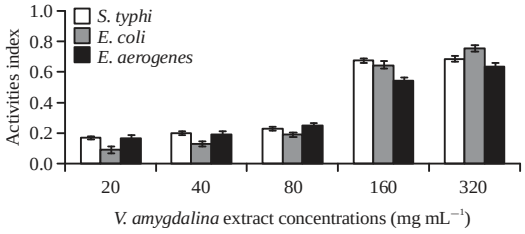

Fig. 2: Activities index (A.I) of the leaves of *S. monostachyus* extracted with acetone at different concentrations in a freshly bacteria isolates (Ofloxacin as the standard antibiotic drug)

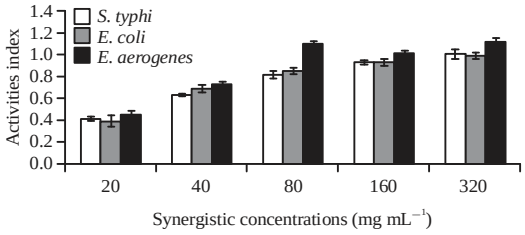

Fig. 3: Activities index (A.I) of the leaves of *V. amygdalina* extracted with acetone at different concentrations in a freshly bacteria isolates (Ofloxacin as the standard antibiotic drug)

In *S. monostachyus,* the diameter zone of inhibition varied significantly between *S. typhi* and *E. coli* ($p<0.05$, $F = 9.2$) and between *E. coli* and *E. aerogenes* ($p<0.05$, $F = 5.8$). Other treatment showed no significancy (Table 3). The A.I. showed that *E. coli* was most predisposed to the plant's extract than any other studied bacterial species, while *E. aerogenes* was the least (Fig. 2). At the lower concentrations of 20, 40 and 80 mg mL^{-1}, the responses of the plant's extract to the bacteria was feeble, but boosted and was significant as the concentrations of the extract was increased to 160 and 360 mg mL^{-1} (Fig. 2).

The synergistic treatments revealed that all the investigated isolates, *E. aerogenes*, *S. typhi* and *E. coli* were highly sensitive to the plants's extract mixture with high diameter zone of inhibition and were species sensitive and concentrations dependent. However, there was no significant difference among the three isolates irrespective of the treatments and the concentrations of the extract ($p>0.05$, $F = 5.6$). In this treatment, A.I. was above 1.0 in some of the isolates, In *E. aerogenes*, it was higher than 1.0 at 80, 160 and 320 mg mL^{-1} treatments. In *S. typhi*, A.I. was above 1.0 only at 320 mg mL^{-1} treatment, while it was less than 1.0 in *E. coli* in all the treatments (Fig. 3).

DISCUSSION

In this investigation, all the investigated bacteria showed sensitivity to the plant's extract and followed the same pattern as the concentrations of the extracts increases, the higher the bacterial isolates sensitivities, the higher the increased in size of the bacterial growth inhibition zones and this was in agreement with Akinjogunla *et al.*[26], Jagtap and Karkera[27] and Okigbo *et al.*[28]. The sensitivity of the investigated Gram-negative bacteria to *V. amygdalina* and *S. monostachyus* may be attributed to the potency of their phytochemical composition especially flavonoids that exhibited antimicrobial, antioxidant, anti-inflammatory, anti-allergic and cytostatic properties[29]. The antibacterial activity of these plants corroborated the findings of Akinjogunla *et al.*[26]. However, the sensitivity of the bacterial isolates to plants's extracts differed. The order of sensitivity revealed that in *V. amygdalina*, *E. aerogenes*> *E. coli*> *S. typhii*, while in *S. monostachyus, E. coli*> *S. typhii*> *E. aerogenes*. This may be attributed to the fact that some bacteria were more resistant to the action of antimicrobial compounds than others due to differences complexity of their cell wall[30]. Similarly, it may also be due to the lipid content of the membranes of the different groups of the microorganisms.

The average sensitivity of the bacteria isolates to the plant extract showed that *V. amygdalina* had higher activity index than *S. monostachyus*. This may be on account of the differences in the active phytochemicals concentrations and their permeability rate[31]. The synergistic effects of the plants extract was higher than when they were exposed individually. The activity index of the test substance above 0.5 was considered as significant activity[32] hence, the A.I. in both plants extracts were significant at the highest concentrations of the extract. The sensitivity of the concoction may be as a

result of complex combination of different phytochemicals that emanated from the different plants extracts. Similar observation was reported by Chukwura and Iheukwumere[33] when they studied the synergistic effect and Activity index of *S. monostachyus* and *O. gratissimum* on selected bacteria. Similarly studies[24] proved the potency of the synergistic treatment of *Staphylococcus aureus* using the combination of Salvadorapersica extracts, tetracycline and penicillin.

Recommendation the extracts of these plants should be used with care as abuse could be detrimental. For specification an expert should be consulted. Further research can be conducted using laboratory animals to test the efficacy of the extracts *in vivo* and to address the limiting issue of dosage.

The extracts should be tested on other micro-organisms to ascertain their activity on other disease-causing agents. Research on the efficacy of other parts of the plant as the roots or flowers should be given utmost attention.

CONCLUSION

In this investigation the individual extract of *V. amygdalina*, *S. monostachyus* and their synergistic had shown to inhibit bacterial activities. The presence of bioactive compounds especially flavonoids, in both plants supports the traditional use of these plants by the native folks in Africa for their use as antimicrobial therapy.

These plants are readily available at no cost, thus they could be exploited to provide novel compounds that may be used as starting material for the production of drug that can obliterate resistance bacteria.

SIGNIFICANCE STATEMENT

This study revealed the significance of the synergistic of two plants. The plants extract mixture had shown to exhibit broad-spectrum antibacterial activities that can be beneficial for the treatment of resistance microbes. This study will help the researcher to uncover the critical areas of phyto-medicine that many researchers were not able to explore. Thus a new theory on exploitation of untapped knowledge on medicinal plants may be arrived at.

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