Annual Research & Review in Biology

36(11): 93-102, 2021; Article no.ARRB.75642
ISSN: 2347-565X, NLM ID: 101632869

In-vitro Antimicrobial Activity of the Combined Effect of *Kalanchoe crenata* and *Vernonia amygdalina* on *Salmonella Species*

Emmanuel Effah-Yeboah [a*], Emmanuel Agyapong Asare [b], Gadafi Iddrisu Balali [c], Vincent Aboagye [d], Kofi Sarpong [b], Mohammed Taufiq [c] and Abdul-Malik Bawah [e]

[a]Department of Biological Science Education, Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development (AAMUSTED), Ghana.
[b]Department of Chemistry Education, Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development (AAMUSTED), Ghana.
[c] Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology, KNUST, Ghana.
[d] Department Physiology, Korle-bu, University of Ghana, Ghana.
[e]Department of Dietetics, Faculty of Applied Science and Technology, Tamale Technical University, Tamale, Ghana.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/arrb/2021/v36i1130455
Editor(s):
(1) Dr. Md. Torequl Islam, Federal University of Piaui, Brazil.
Reviewers:
(1) Guillermo Muñoz Zurita, Benemérita Universidad Autónoma de Puebla, Mexico.
(2) Claudious Gufe, Central Veterinary Laboratories, Zimbabwe.
Complete Peer review History, details of the editor(s), Reviewers and additional Reviewers are available here: https://www.sdiarticle5.com/review-history/75642

Original Research Article

Received 10 August 2021
Accepted 21 October 2021
Published 04 December 2021

ABSTRACT

Introduction: The breakthrough in the treatment of pathogenic diseases was the unearthing of naturally occurring antipathogenic agents or antibiotics. There have been upsurges in antibiotic-resistant strains of clinically important pathogens, which made way to the emergence of new-fangled bacterial strains that are multi-resistant. The major aim of scientists is to develop new antibiotics or other therapeutic strategies at a pace greater than that at which bacteria are developing resistance. The development of resistance to first-line antimicrobial therapies made way to recommendations for combination therapies for the treatment of some infections and some of this form of chemotherapy seems to be very successful.

**Corresponding author: E-mail: eeyeboah@uew.edu.gh;*

Objectives: This research was carried out to determine the effect of *Kalanchoe crenata* extracts on *Salmonella typhi* load. Also, to assess the potency of the extract of *Vernonia amygdalina* on *Salmonella typhi* and finally to ascertain the effect of the combined extract of *Kalanchoe crenata* and *Vernonia amygdalina* on *Salmonella typhi.*

Methods: In this research, *Salmonella typhi* was exposed to a crude extract of *Kalanchoe crenata* and *Vernonia amygdalina* and also the combination of the two extracts. Agar wells diffusion method was employed.

Results: The combined effect was not sensitive to the *Salmonella* strain. The *Salmonella* strain was resistant to *V. amygdalina* than to *K. crenata. K. cranata* had the strongest activity against *S. typhi* with its highest zone of growth inhibition of 20 mm and lowest zone of inhibition of 7 mm while *V. amygdalina* produced a consistent zone of growth inhibition of 5-6 mm; The combined effect produced a zone inhibition diameter only at the 100 mg/ml with a zone of inhibition value of 14 mm. The subsequent lower concentrations did not show any activity against the microbes. At P-value = 0.05 two-way ANOVA statistics exhibited significant differences amongst the effects produced by the different extracts, though there were no substantial differences in the effects produced by the various concentrations.

Conclusion: The *Salmonella* strain was resistant to *V. amygdalina* than to *K. crenata.* At P-value = 0.05 there was a substantial difference in the sensitivity of the bacteria to the different extracts.

Keywords: Antimicrobial; inhibition; sensitivity; Salmonella typhi; zones of inhibition and extracts.

1. INTRODUCTION

Microorganism normally referred to as microbes are a very important group of organisms in the environment. Various groups of these microbes exist; bacteria, viruses and fungi are examples of major groups of microbes that exist [1]. These individual groups have some peculiar characteristics and properties and hence they tend to undergo different life activities to survive [2]. However, individuals in the same group usually have similar life activities but with few variations among members in the group [3]. When these microorganisms get into the human body and can overwhelm the immune system and also the normal resident microflora then they cause infectious diseases [4]. These bacteria responsible for many of the current infectious diseases are therefore referred to as the infectious agent (pathogens) [5].

1.1 Typhoid Fever

Typhoid fever is a bacterial infection that affects people globally and is caused by the bacterium *Salmonella typhi*. The disease can be transmitted through the bacterial contamination of water, milk, food, fruits and vegetables. Healthy carriers of the infection and even contaminated food handlers can transmit the disease to healthy individuals. The bacteria can also be carried from faeces to food by flies [6]. The World Health Organization estimated an annual rate of about 12.6 million infections with about 600,000 possible deaths [7].

Poor food hygiene, poverty and inadequate supply of clean water has increased typhoid infections and acute gastroenteritis in African [8]. In recent years multidrug resistance to typhoid infection has increased worldwide [9-11].

Due to the increase in resistance to the treatment of typhoid fever by antibiotics, herbal preparations are gaining popularity in both rural and urban areas in Africa for the treatment of the disease. These medicinal herbal preparations have fewer side effects compared to chemical agents [12]. This work was carried out to investigate the combined effect of *kalanchoe crenata* and *Vernonia amygdalina* on *salmonella typhi*.

1.2 *Vernonia amygdalina*

V. amygdalina, which is commonly known as the bitter leaf, is one of the plants mostly exploited in West Africa [13]. Studies have proven many therapeutic properties of phytochemicals present in the plant [13-15]. The antimicrobial activities of *V. amygdalina* on *E. coli, Salmonella and Shigella sp* [16, 17] have experimented. The result revealed that the organisms were not as active except for *Shigella sp* which showed considerable sensitivity [17]. It was further proven that the growth of gram-positive bacterium *Staphylococcus aureus* and the gram-negative bacterium *E. coli* have been strongly inhibited by the aqueous extracts of the leaves [18].

The entirety of the plant is pharmacologically useful [19]. Both the roots and leaves are utilized in phytomedicine for the treatment of kidney diseases, fever, stomach discomfort and hiccups, among others [20]. Bukar *et al.*, (2013) affirmed that the sensitivity of *V. amygdalina* was more towards gram-positive bacteria compared to the of gram-negative bacteria; However, other researchers revealed that the activity of *V. amygdalina* on gram-negative bacteria was comparable to its effect towards the gram-positive species [18,21]. For example, methanol extract of *V. amygdalina* affected not only the growth of gram-positive bacteria such as *B. subtilis, B. cereus, M. kristinae, B. pumilus, S. aureus and E. cloacae* but exhibited potency against gram-negative bacteria which include *Proteus vulgaris, Klebsiella pneumoniae, Shigella dysenteriae, Pseudomonas aeruginosa, and E. coli* [22,23]. Extract of ethanol from the plant also exhibited an antibacterial effect on both gram-positive (*Clostridium sporogenes, Staphylococcus pyogenes and S. aureus*) as well as the gram-negative (*E. coli and Salmonella typhi*) bacteria [23-25]. Nevertheless, a contradiction occurred in the findings regarding the activity of the ethanol extract obtained from *V. amygdalina*. (Ogbulie *et al.*, 2007) showed that the best solvent and technique to give the optimal antibacterial effect of *V. amygdalina* are ethanol and Soxhlet extractions. On the other hand, despite the inhibitory effect of *V. amygdalina* on *S. aureus*, [26] showed that this extract could not inhibit the methicillin-resistant (MRSA UELSHB 102, UELSHB) and methicillin-sensitive (MRSA NCTC 6571) strains of the bacteria while chloroform, water and blended extract of *V. amygdalina* leave exhibited a low inhibitory effect on its growth.

1.3 *Kalanchoe Crenata*

The external applications of *K. crenata* are the same as those of *Bryophyllum pinnatum* [27]. The juice obtained by squeezing the leaves that have been passed over fire slightly is most commonly used for the treatment of headache, general debility, dysentery, smallpox and convulsion. One or two drops of the leaf juice are dropped into the ear for earaches. A poultice of the leaves is applied over wounds and sores. The leaves can be boiled in water and the extract is given as a sedative for asthma and palpitation [27]. Similarly, the leave extract mixed with honey and salt serves as a remedy for chronic cough. Also, dried leaves extract is applied to the infected wound [28].

The treatment of rheumatism, as well as stiff joints in East Africa, is done by slightly heating the leaves and rubbing them over the human body [29]. Solvent type as well as the preparation technique utilized strongly affect the antipathogenic (antimicrobial) potency of plants [26,30,31]. Based on this background, in-vitro antimicrobial activities of the extracts *B. pinnatum* and *K. crenata* from various solvents were tested against clinically important pathogens in the work done by [27].

[27] have reported that *K. crenata* extracts have unequal effects on tested organisms which includes Gram-negative *E. coli* ATCC 25922, *E. coli*, *Pseudomonas aeruginosa*, *Salmonella paratyphi*, *Klebsiella pneumoniae*, *Shigella flexneri*, *Citrobacter* spp, Gram-positive organisms *S. aureus* ATCC 25213, *Bacillus subtilis, S. aureus*, *Enterococcus faecalis*, and a fungus *Candida albicans* excluding “Omidun” extract of *K. crenata* which revealed no substantial activity. It was further suggested by Aibinu et al., that extracts of *K. crenata* showed a broad spectrum in their activities and that extracts from the squeezed leaves of *K. crenata* proved to be the most active; it exhibited improved antimicrobial activity compared to leaves from *B. pinnatum* prepared using the same method. The effect of *K. Crenata* has manifested against the Gram-positive as well as the Gram-negative organisms with its activity greatly manifested on the Gram-negative organisms [27]. It was also reported that palm wine extracted from *K. crenata* showed higher activity on the *E. coli* organisms tested, *Citrobacter* spp and *Salmonella paratyphi* and further explain that utilization of palm-wine as a solvent release some amount of active elements which shows high activity against enteric organisms. The different antimicrobial potency exhibited by the plant extract dissolved in different solvents as revealed by various researchers, confirms the traditional use of the plant in the treatment of microbial infections such as sore, dysentery, ear infections, abscesses as well as wound infections. The work reported by [27] stated that aqueous extracts, as well as methanol extraction of dried leaves of K. crenata, exhibited moderate antibacterial activities.

1.4 Microbial Resistance

The development and widespread use of antibiotics must rank as the most remarkable of all medical advances made in the 20th century. Overconfident assertions that infectious diseases

would soon be a thing of the past, however, the threat of resistant strains casts a shadow over all the past achievements of antibiotics [32]. Recently, there is an upsurge of antibiotic-resistant strains of important clinical pathogens, which led to the development of new bacterial species that are multi-resistant [33-35]. Mortality and morbidity have suddenly increased, owing to the high cost coupled with the non-availability of new generation antibiotics [36]. There is, therefore, the need to unravel other sources with proven antimicrobial potency. This paved way for researchers to delve into more effective antimicrobial agents of plant origin, with much emphasis to discover active ingredients with a potential effect on infectious microbes to synthesize new antimicrobial drugs [37,38]. The major aim of scientists now must be to develop new antibiotics or other therapeutic strategies at a pace greater than that at which bacteria are developing resistance [39]. In 2000, the Food and Drugs Administration approved a new synthetic agent shown to be effective against both MRSA and vancomycin-resistant *Enterococcus faecalis.* Linezolid (Zyvox), which works by blocking the initiation of protein synthesis, belongs to a new class of antibiotics called *oxazolidinones* [39]. It is the first new anti-MRSA compound to be introduced in more than 40 years. Another approach to countering resistant forms is to identify and target the mechanism by which the bacteria combat antibiotic therapy [39]. A team at Rockefeller University in New York have identified two genes that enable resistant forms to rebuild their cell walls after antibiotic treatment. Therefore targeting these genes, they hope to restore the potency of a cell wall inhibitor such as penicillin [39].

[40] has reported *S. typhi* resistance to many antibiotics on the market. Antimicrobial susceptibility testing was performed on all *serovar typhi* isolates by using the Kirby-Bauer disk diffusion method for ampicillin, chloramphenicol, tetracycline, trimethoprim, gentamicin, amoxicillin, ciprofloxacin and ceftriaxone. It was found that *S.typhi* was resistant to chloramphenicol (73%), trimethoprim (71%), ampicillin /amoxicillin (70%) tetracycline (64%), gentamicin (46%) and amoxicillin/clavulanic acid (24%) but susceptible to ciprofloxacin and ceftriaxone.

[41] also collected *S. typhi* samples from the University College Hospital, Ibadan, Nigeria and exposed them to ten standard different antibiotics and also to crude extract of *Phyllanthus amarus* and *Paraquetina nigrescent*. Ethanolic extracts of *P. amarus* had the strongest activity against *S. typhi* with an 8.0mm zone of growth inhibition followed by hot water (4.7mm) and cold water (3.8mm) with statistically significant at P= 0.05 when compared with hot and cold water extracts. Amongst the commercial antibiotics examined, it was concluded that ciprofloxacin had the highest zone of growth inhibition of 9.0mm; Ofloxacin (6.0mm) Amoxicillin, (4.0mm) while other antibiotics did not affect test organism. Unfortunately, the resistance of *S. typhi* strains to all of these antibiotics is becoming more common globally. As such, appropriate treatment varies with the geographic distribution of resistant strains [41].

[42] evaluated the current fluoroquinolones susceptibility criteria and a nalidixic acid screening test in *Salmonella enterica* serovar Typhi and Paratyphi A. All the isolates were found susceptible to ciprofloxacin and ofloxacin. However, some research has proven that the combined effects of two drugs have shown more antimicrobial potency than the effects of the two when applied separately. That is, an antibiotic that has not much effect when combined with another will produce an effect greater than the separate effects of each drug.

1.5 Combination Chemotherapy

The development of resistance to first-line antimicrobial therapies has led to recommendations for combination therapies for the treatment of some infections [43]. The accomplishment of combination therapies in recent times has been revealed by researchers, owing to their application in the treatment of ailment. However, few reports have been given on the *in vitro* actions that result from various combinations of these drugs. For example, an assessment of the *in vitro* action of azithromycin combined with gentamicin showed growth inhibition which warranted a clinical trial of this combination in treating gonorrhoea infections [43].

Base on this idea some bacteria that used to be monoresistant to certain antibiotics are now being susceptible to this same antibiotic combined with another [43].

In this experiment, the separate effect produced by *V. amygdalina* and *K. crenata* is being experimented and then compared to the

combined effect produced by the two extracts with an initial hypothesis made that bacteria strain would be sensitive to the various extracts.

2. MATERIALS AND METHODS

2.1 Plant Materials

The leaves of *V. amygdalina* were collected from the UCC Science Botanic Gardens, University of Cape Coast, Ghana and were authenticated at the Cape Coast University's herbarium.

2.2 Preparation of Extracts

The method (Mother Tincture) devised by Jean-Michael (1994) was employed for the extraction. The leaves of the plant were air-dried for 2 days and then finally dried in an oven at 45 ^{o}C for 3 days as described by [44]. The dried leaves were then blended using an electric blender and the powder was stored in a sterile bottle at room temperature [45]. Fifty grams (50) g of the powdered plant leaves were weighed and dissolved in 450 mL 70% ethanol. The mixture was then held in an airtight container, kept in a cool dark place for 3 days and then filtered using sterile filter paper. The filtrate retrieved was concentrated by evaporation using a water bath at 97 ^{o}C and then stored in sterile bottles until it is needed.

2.3 Bacteria Species

Strains of *Salmonella* species were acquired from the Microbiology Laboratory of Centre for Plant Medicine Research, Mampong in the Eastern Region of Ghana.

Consent was sought from the lab technician, that the strains were not going to be used on animals or humans for the experimental research but was going to be an in-vitro experiment, therefore no ethical clearance was needed.

2.4 Preparation of Extract Concentrations

A thousand milligrams (1000 mg) each of the *V. amygdalina* and *K. crenata* extracts were weighed using a weighing balance and together dissolved into 20 mL of 2% DMSO in a clean and well-dried container. Another 2000 mg each of the extracts was weighed and dissolved in 20 mL each of 2 % DMSO in separate containers. For each of the stocks prepared (100 mg/ml), half of the volume (10 mL) was taken and serially diluted to get concentrations of 50 mg/mL, 25 mg/ml, and 12.25 mg/mL into separate containers. The containers with their contents were covered and stored in the refrigerator until they were needed for use [46].

2.5 Phytochemical Screening

The phytochemical constituents of both *V. amygdalina* and *K. crenata* ethanolic extracts were determined using methods described by [47]. The specific phytochemical tested for are Reducing sugars, Saponins, Flavonoids, Anthraquinones, Triterpenoids, Glycosides, Alkaloids and Tannins. The various methods used to test for the presence of these phytochemicals are briefly described below.

2.5.1 Reducing sugars

In carrying out the test for the presence of reducing sugars in the plant extracts, Fehling's test was conducted. Each sample was diluted in water and subjected heat source for warming until a total dissolution is observed. The Fehling's solution was then added coupled with stirring. The presence of reducing sugars were indicated based on the colour change. A colour change to red/rust precipitate indicates the presence of reducing sugars. Absence is indicated by no colour change and remaining blue or green colour.

2.5.2 Saponins

To find the presence of saponins, an amount of 0.5 ml of the plant extracts were liquefied in about 5 ml disinfected water in a test tube. This was then shaken robustly and detected for a stable tenacious foam with a honeycomb edifice showing the presence of saponins [35].

2.5.3 Flavonoids

In testing for the availability of flavonoids in the plant extracts, a sample of the plant extract was liquefied in a 10 percent HCl and limited zinc powder was then added. The presence of effervescences with pink colour shows the availability of flavonoids [36].

2.5.4 Anthraquinones

Born ranger's test was used for the test of presence of anthraquinones following procedures described by [33]. Briefly, plant extracts were cooked with H_2SO_4. After, the resultant liquid was allowed to cool and was then filtered. The

filtrates were then extracted with the help of chloroform and dilute ammonia added. A change in colour from a pink colour to red shows the presence of an anthraquinone derivative.

2.5.5 Triterpenoids

The presence of Triterpenoids was tested for using the Salkowski test. In this experiment, methods are described by [33]. In a brief, plant extracts were treated using chloroform with a few drops of concentrated H_2SO_4. They were then subjected to vigorous shaking mechanically and then allow to stay for a few minutes. The observation of yellow colour formed at the base layer indicated the presence of triterpenoids.

2.5.6 Glycosides

Using cold concentrated H_2SO_4, an amount of 250 µl extract was added to a similar volume of sulphuric acid (concentrated). An observation of the development of deep black/green/blue/red colour designates the presence of glycosides [35].

2.5.7 Alkaloids

The presence of alkaloids was tested by taking a sample of plant extracts was treated using an aqueous solution of HCL and an amount of 0.5ml Mayer's reagent was then added. An observation of white precipitate indicates the presence of alkaloids [34].

2.5.8 Tannins

Few drops of 0.1 percent of Ferric chloride was added to the sample of plant extracts and the observation of brownish-green colour shows the presence of tannins [35].

2.6 Preparation of Inoculum

The pure culture of the organisms from the cotton swab was plated out on Salmonella-Shigella agar and incubated at 37 ^{0}C for 24 hours. After incubation, the colony of the organisms was taken and inoculated into 7 mL of peptone water in test tubes and shook vigorously to obtain homogeneity of the solution as described by [17].

2.7 Microbial Sensitivity Test

Agar well diffusion method as described by [44] was employed using Salmonella-Shigella agar. Agar plates were prepared according to the manufacturer's specification aseptically to a thickness of 5-6 mm. The agar was then left to solidify and the plates were then upturned to prevent condensate from coming into contact with the agar surface. To ensure sterility, the plates were incubated at 37^{o}C for 24 hours. The prepared inoculum of the bacteria species was inoculated onto the prepared media by dipping the cotton swab into the inoculum and wiping it on the surface of the media. The inoculated agar plates with the lids covered were allowed to dry at room temperature. Thereafter, sterile pipette tips (5.0mm diameter) were used to punch wells in the seeded Salmonella-Shigella agar. The agar plugs were removed with a flamed and cooled inoculating loop. Into the separate well was poured different concentrations of the various plant's extracts and the solvent blank (2% DMSO). Standard antibiotic (Ciprofloxacin) which *salmonella* is known to be sensitive to was used as a positive control [42]. The experiment for each extract and organism was repeated in triplicates so all 12 plates were prepared. The samples were incubated at 37^{o}C for 24 hours. The diameter of zones of inhibition on the plates was measured using a transparent meter rule and recorded. The measured zones of inhibition of the different extracts were compared. The zones of inhibition created by the various quantities (concentrations) of the extracts also were compared [17].

3. RESULTS

3.1 Results Showing the Phytochemical Screening of *Vernonia amygdalina*

Table 1 shows the phytochemical constituent of the leaves of *Vernonia amygdalina.* The results obtained from the phytochemicals analysis showed the presence of some secondary metabolites like tannins, saponins, terpenoids, flavonoids, glycosides, alkaloids, anthraquinones. All tested positive except Anthraquinones which tested negative.

Table 1.

Test	Results
Reducing sugar	+
Saponins	+
Flavonoids	+
Anthraquinones	-
Terpenoids	+
Glycosides	+
Alkaloids	+
Tannins	+

+ indicates positive results; - indicates a negative result

3.2 Results Showing the Phytochemical Screening of *Kalanchoe crenata*

Table 2.

Test	Results
Alkaloids	+
Glycosides	+
Phenolics	+
Flavonoids	-
Triterpenes	+
Anthraquinones	+
Steroids	+
Saponins	+
Tannins	+
Terpenoids	+

+ indicates positive results; - indicates a negative result

Antimicrobial actions of the extracts acquired from *V. amygdalina as well as K. crenata* leaves and a mixture of the two plant extracts, using 70% ethanol on the test organisms are listed in Table 2. The screening of antimicrobial activity of extracts was assayed in vitro by the agar diffusion method and using Ciprofloxacin as a positive control drug in all samples.

The antimicrobial effect was determined by taking the diameter of the zone of inhibition recorded. The average and standard error mean (SEM) of the zone of inhibition for each concentration from each of the triplicates were calculated and recorded in Table 3.

The *K. crenata* extracts were found to be the most potent antimicrobial agent with its highest ZOI of 20 mm at 100 mg/ml and lowest ZOI of 7 mm at 12.25 mg/ml; comparing with the *V. amygdalina* extract and the combined extracts it is found the *K. crenata* is still the extract that produced the highest ZOI at their lowest concentrations.

4. DISCUSSION

The antimicrobial action was determined by taking the diameter of the zone of inhibition and recorded. The *K. crenata* extracts were found to be the most potent antimicrobial agent with its highest zone of inhibition of 20 mm at 100 mg/ml and lowest zone of inhibition of 7 mm at 12.25 mg/ml (Table 2); compared with the *V. amygdalina* extract and the combined extracts it is found the *K. crenata* is still the extract that produced the highest zone of inhibition even at their lowest concentrations (Table 1). The *V. amygdalina*, on the other hand, produced a consistent zone of inhibition with values of 5 and 6 mm. The combined effect of the two extracts did not produce any antimicrobial effect at all the concentrations prepared except for the 100 mg/ml which revealed an inhibition zone of 14 mm in diameter. The results for *V. amygdalina* are per some previous studies done on this aspect. [48], reported a zone of inhibition ranging from 4 – 8 mm for *Salmonella* species; the results of *V. amygdalina* from this research 5 mm and 6 mm falls within this range reported by [48]. The susceptibility of the organisms to *V. amygdalina* extracts elucidates their use for therapeutic purposes in Nigeria for the cure of infections such as dysentery.

The microbes showed a slight high degree of resistance against the *V. amygdalina* more than against the *K. crenata*; From Table 1 it can be seen that at different concentrations *V. amygdalina* had a lower zone of inhibition than *K. crenata*.

The combined effect produced a zone inhibition diameter only at 100 mg/ml with a zone of inhibition value of 14 mm. The subsequent lower concentrations did not show any activity against the microbes even though their corresponding concentrations for *K. crenata* showed activity with decreasing effect directly proportional to the decrease in concentration. This shows that *V. amygdalina* and *K. crenata* together may produce an antagonistic effect. At lower concentrations (< 100 mg/ml based on this research) there was no activity. However, at higher concentrations (≥ 100 mg/ml) there was an activity with a 14 mm inhibition zone. This result may be because the two plants extract when combined produce an antagonistic effect (especially at lower doses of each extract in the mixture). Therefore the activity showed at the 100 mg/ml concentration maybe since one of the plants is more potent than the other so as there is an increase in concentrations of the combined and as such, the relative increase in the individual concentrations the more potent moiety (in this case *K. crenata*) of the mixture produced its effect but with a lesser activity; in this research *K. crenata* is the one that contributed more of the effect in the combined extract (zone of inhibition: 14 mm) since at all concentrations it had higher activity than *V. amygdalina* in their separate forms; therefore when comparing the zone of inhibition of *K. crenata* and ‘‘*K. crenata* in mixture’’ (combined extract) it is seen that at 100 mg/ml the zone of inhibition produced by *K. crenata* was reduced from 20 mm to 14 mm (Table 1) in the effect produced by ‘‘*K. crenata* in

Table 3. Zone of inhibition of extracts and the combined effect on *Salmonella typhi*

Concentration of extracts (mg/ml)	Zone of Inhibition (mm)			Control
	V. amygdalina	*K. crenata*	Combined effect	Ciprofloxacin (5 µg/ml)
100.00	5±5.000	20±0.882	14±4.667	26±1.333
50.00	5±5.000	12±6.245	0±0.000	
25.00	6±5.667	11±7.311	0±0.000	
12.25	5±5.000	7±7.333	0±0.000	

The values are mean from ZOI of three replicates ± standard error mean

mixture'' (combined extract). Note that *K. crenata* at the same 100 mg/ml concentration produced more activity singly than when it was combined with *V. amygdalina*.

The standard antibiotic (5 µg Ciprofloxacin) produced a zone of inhibition of 26 mm. This result is in line with the standard zone of inhibition range provided by the Clinical Laboratory Standard Institute (CLSI). According to CLSI reference (ZOI ≤ 15 mm is resistance, ZOI ≥ 21 mm implies sensitivity to Cipro). [40] also reported a zone of inhibition of 25.8125±1.875 mm for *Salmonella typhi* sensitive to Ciprofloxacin. However [41]*, reported that* ciprofloxacin had a zone of growth inhibition of 9.0mm for *S. typhi,* which implies resistance but the finding was reported as sensitivity.

The phytochemical screening of *K. crenata* extract revealed the presence of anthraquinones which was absent in *V. amygdalina.*

Statistical analysis from the two-way ANOVA at a P-value of 0.05 exhibited a significant difference between the effects produced by the different extracts, though there were no significant differences in the effects produced by the concentrations**.** For the concentrations, the calculated F-ratio, F (3,6) = 3.194 and P-value = 0.1052. The P-value was observed to be greater than 0.05 after calculation, which implies there is no significant difference among the effects produced by the different concentrations.

For the extracts the calculated F-ratio, F (2,6) = 5.995 and P-value = 0.0371. The P-value obtained after calculation was less than 0.05 which implies there exist a significant difference among the effects produced by the different extracts.

5. CONCLUSION

The combined effect was not sensitive to the *Salmonella* strain. However, *K. cranata* was sensitive and it is the extract that produced the maximum effects at all the concentrations prepared whereas *V. amygdalina* was also sensitive but with an almost constant zone of inhibition at all concentrations. It can also be concluded that the salmonella strain was resistant to *V. amygdalina* than to *K. crenata.* At P-value = 0.05 there was a substantial difference in the sensitivity of the bacteria to the different extracts.

6. RECOMMENDATION

K. crenata has shown more potency for antimicrobial activity than *V. amygdalina* however further works must be done on the *V. amygdalina* and *K. crenata* to test for the actual phytochemicals that are producing the antimicrobial effects. The test should be repeated using gram-positive microbes especially to compare the combined effect to that of this research and also using higher concentrations of the combined effect.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mahon CR, Lehman DC, Manuselis G. Textbook of diagnostic microbiology-e-book. Elsevier Health Sciences;2018.
2. Batoni G, Maisetta G, Esin S. Antimicrobial peptides and their interaction with biofilms of medically relevant bacteria. Biochimica et Biophysica Acta (BBA)-Biomembranes. 2016;1858(5):1044-1060.
3. Turker G, et al. Changes in microbial community structures due to varying operating conditions in the anaerobic digestion of oxytetracycline-medicated cow manure. Applied Microbiology and Biotechnology. 2016;100(14):6469-6479.
4. Finlay BB, Finlay JM. The Whole-Body Microbiome: How to Harness Microbes—Inside and Out—for Lifelong Health. The Experiment;2019.

5. Nash AA, Dalziel RG, Fitzgerald JR. Mims' pathogenesis of the infectious disease. Academic Press;2015.
6. De Jong B, Andersson Y., Ekdahl K. Effect of regulation and education on reptile-associated salmonellosis. Emerging infectious diseases. 2005;11(3): 398.
7. Organization WH. Antimicrobial resistance and primary health care: brief. World Health Organization;2018.
8. Brown J, Cairncross S, Ensink JH. Water, sanitation, hygiene and enteric infections in children. Archives of disease in childhood. 2013;98(8):629-634.
9. Zaki SA, Karande S. Multidrug-resistant typhoid fever: a review. The Journal of Infection in Developing Countries. 2011;5(05):324-337.
10. Akram J, et al., Extensively Drug-Resistant (XDR) Typhoid: Evolution, Prevention, and Its Management. BioMed Research International;2020.
11. Acheampong, G., et al., Chromosomal and plasmid-mediated fluoroquinolone resistance in human Salmonella enterica infection in Ghana. BMC infectious diseases. 2019;19(1):1-10.
12. Maghrani M, et al. Acute diuretic effect of aqueous extract of Retama raetam in normal rats. Journal of Ethnopharmacology. 2005;99(1):31-35.
13. Mbatchou VC, Nabayire KO, Akuoko Y. Vernonia amygdalina Leaf: Unveiling its antacid and carminative properties *In vitro*. Current Science. 2017;3(3):148-155.
14. Ademosun AO, et al. Modulation of cholinergic, monoaminergic, and purinergic enzymes of the brain functions by bitter (*Vernonia amygdalina*) and water bitter (*Struchium sparganophora*) leaves extracts: comparison of phenolic constituents versus nootropic potentials. Comparative Clinical Pathology. 2017;26(6):1267-1272.
15. Oshim IO, et al. Kinetics of minimum inhibitory concentration, minimum bactericidal concentration and minimum fungicidal concentration of *Vernonia amygdalina* (Bitter leaf) on microorganisms isolated from wound infections. International Journal of Surgical Research. 2016;5(1):8-14.
16. Agbankpé A, et al. *In vitro* antibacterial effects of Crateva adansonii, Vernonia amygdalina and Sesamum radiatum used for the treatment of infectious diarrhoeas in Benin. Journal of Infectious Diseases & Therapy; 2016.
17. Olamide SO, Agu G. The Assessment of the antimicrobial activities of Ocimum gratissimum (wild basil) and Vernonia amygdalina (Bitter leaf) on some enteric pathogen causing dysentery or diarrhoea in patients. Int J Eng Sci. 2013;2(9):83-96.
18. Adetunji C, Olaniyi O, Ogunkunle A. Bacterial activity of crude extracts of Vernonia amygdalina on clinical isolates. Journal of Microbiology and Antimicrobials. 2013;5(6):60-64.
19. Usunomena U, Ngozi OP. Phytochemical analysis and proximate composition of Vernonia amygdalina;2016.
20. Usunobun U, Okolie N. Phytochemical, trace and mineral composition of Vernonia amygdalina leave. International Journal of Biological and Pharmaceutical Research. 2015;6(5):393-399.
21. Bukar AM, et al. Antibacterial activity of aqueous and ethanolic leaf extracts of vernonia amygdalina on selected species of gram-positive and gram-negative bacteria. International Journal of Environment. 2013;2(1):147-152.
22. Chukwuemeka NO, et al. Antibacterial assay and reversion of carbon tetrachloride-induced liver damage on Wistar mice by Vernonia amygdalina. Delile. Pakistan Journal of Pharmaceutical Sciences. 2018;31(4).
23. Yeap SK, et al. Vernonia amygdalina, an ethnoveterinary and ethnomedical used green vegetable with multiple bio-activities. Journal of Medicinal Plants Research. 2010;4(25):2787-2812.
24. Asuzu CU. Bitter herbs of eastern Nigeria (Gongronema latifolium, Vernonia amygdalina and Vitex doniana): A review. African Journal of Traditional, Complementary and Alternative Medicines. 2018;15(3):47-56.
25. Ogbulie T, Ogbulie J, Njoku H. Comparative study on the microbiology and shelf-life stability of palm wine from Elaeis guineensis and Raphia hookeri obtained from Okigwe, Nigeria. African Journal of Biotechnology. 2007;6(7).
26. Pesewu GA, Cutler RR, Humber DP. Antibacterial activity of plants used in traditional medicines of Ghana with particular reference to MRSA. Journal of Ethnopharmacology. 2008;116(1):102-111.
27. Odugbemi TO, et al. Medicinal plants useful for malaria therapy in Okeigbo,

Ondo State, Southwest Nigeria. African Journal of Traditional, Complementary and Alternative Medicines. 2007;4(2):191-198.
28. Adetutu A, Morgan WA, Corcoran O. Ethnopharmacological survey and in vitro evaluation of wound-healing plants used in South-western Nigeria. Journal of Ethnopharmacology. 2011;137(1):50-56.
29. Iwu MM. Pharmacognostical profile of selected medicinal plants; 2014.
30. Uzoigwe C, Agwa O. Antimicrobial activity of Vernonia amygdalina on selected urinary tract pathogens. African Journal of Microbiology Research. 2011;5(12):1467-1472.
31. Alo M, et al. Antibacterial activity of water, ethanol and methanol extracts of Ocimum gratissimum, Vernonia amygdalina and Aframomum melegueta. Adv. Appl. Sci. Res. 2012;3(2):844-848.
32. Todd K. The promising viral threat to bacterial resistance: The uncertain patentability of phage therapeutics and the necessity of alternative incentives. Duke LJ. 2018;68:767.
33. Wellington EM, et al. The role of the natural environment in the emergence of antibiotic resistance in Gram-negative bacteria. The Lancet infectious diseases. 2013;13(2):155-165.
34. Warnke PH, et al. The battle against multi-resistant strains: Renaissance of antimicrobial essential oils as a promising force to fight hospital-acquired infections. Journal of Cranio-Maxillofacial Surgery. 2009;37(7):392-397.
35. WHO, Antimicrobial resistance and primary health care: brief. 2018, World Health Organization.
36. Mondal C. A Study to Evaluate Anti-microbial properties of an Indian medicinal plant-with particular reference to multidrug-resistant phenotypes;2012.
37. Pretorius J, et al. Purification and identification of antibacterial compounds from Euclea crispa subsp. crispa (Ebenaceae) leaves. South African journal of botany, 2003;69(4):579-586.
38. Suarez, M., et al., Structure-function characterization and optimization of a plant-derived antibacterial peptide. Antimicrobial Agents and Chemotherapy, 2005;49(9):3847-3857.
39. Hogg S. Essential microbiology. John Wiley & Sons;2013.
40. Agyekum A. Prevalence of Bacteria Causing Bacteraemia in Children Under Five Years in Agogo, Asante-Akyem and Their Antimicrobial Susceptibility Patterns;2010.
41. Oluwafemi, F. and F. Debiri, Antimicrobial effect of Phyllanthus amarus and Parquetina nigrescens on Salmonella typhi. African Journal of Biomedical Research, 2008. 11(2).
42. Acharya D, et al. Current fluoroquinolone susceptibility criteria for Salmonella needs re-evaluation. Kathmandu University Medical Journal. 2012;10(1):66-71.
43. Pettus K, Sharpe S, Papp JR. *In vitro* assessment of dual drug combinations to inhibit the growth of Neisseria gonorrhoeae. Antimicrobial agents and chemotherapy, 2015;59(4):2443-2445.
44. Agu G, et al. Prevalence of diarrhoea agents and the in-vitro effects of three plant extracts on the growth of the isolates;2013.
45. Akinsulire OR, et al. *In vitro* antimicrobial activity of crude extracts from plants Bryophyllum pinnatum and Kalanchoe crenata. African Journal of Traditional, Complementary and Alternative Medicines, 2007;4(3):338-344.
46. Anibijuwon I, et al. Antimicrobial activities of Vernonia amygdalina against oral microbes;2012.
47. Ayoola G, et al. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. Tropical Journal of Pharmaceutical Research. 2008;7(3):1019-1024.
48. Isa M, et al. Prevalence of salmonella agglutinins among patients attending hospitals in Biu, Borno State, Nigeria. Archives of Applied Science Research. 2013;5(1):83-87.

© 2021 Effah-Yeboah et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/75642