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Antibacterial potency of *Vernonia amygdalina* on antibiotic-resistant non-typhoidal *salmonella* isolates from human and animal sources in southern Taraba, North-East, Nigeria

Edobor Peter Kenneth Imarenezor [1], Onolunosen Abel Abhadionmhen [1,*], Samuel Tamuno Cockeye Brown [1] and Zakari Isiaka Osheku [2]

[1] *Department of Microbiology, Federal University Wukari, Taraba State, Nigeria.*
[2] *Primary Health Care Initiative, Africa.*

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Abstract

Antibiotic discovery was considered a wonder of the world. Shortly after, many microorganisms began to exhibit resistance to prior antimicrobial actions of antibiotics. Notwithstanding, Quinones, Flavonoids, Flavones, Flavonols, Tannins, and Coumarins are major phytochemicals synthesized by plant parts and have shown decisive antimicrobial activities against a panel of microorganisms. In this research hot and cold extracts of fresh and dried Vernonia, amygdalina showed remarkable in vivo antimicrobial activity on antibiotic-resistant *Salmonella* isolates from various poultry farms and food vendors in Ibi, Donga, Takum, and Wukari. 5 g-10 ml, 10 g-10 ml, 15g-10ml, 20g-10ml, and 25g-10ml are arrays of *Vernonia Amygdalina* concentrations tested on the inocula. Significant light growth was observed for both menstruum extracts of dried and fresh leaves of *Vernonia Amygdalina* at higher concentrations levels of 20g-10ml, 25g-10ml, while and moderate growth was seen at lower concentrations levels of 5g-10ml, 10g-10ml. Also, heavy growth of *Salmonella* was detected in the control plates inoculated with the test microorganism but without the addition of any extracts. Weak political will in implementing regulation policies, funding of research, and perceived antimicrobial resistance to plant extracts as well as acceptability and cultural factors have threatened the extensive use of plant extract in the management of health. Improved universal health coverage and enhanced economic potentials are core benefits of the use of plant extracts for health management. Conclusively, plant extracts possess appreciable antimicrobial activities against Multi-Drug Resistance (MDR) microorganisms compared to currently prescribe synthetic drugs. Hence, further studies on clinical efficacy trials and the safety of more plants need to be intensified.

Keywords: Bitter leaf; Non-Typhoidal *Salmonella* (NTS); Phytochemicals; Plant Extract; *Salmonella*; *Vernonia amygdalina*

1. Introduction

Interestingly, plants have even before the onset of medieval history provided succor to mankind in improving medical conditions [1]. The recent clamor for the extensive use of antimicrobials of plant origin is deliberate, owing to the devastating menace of Antimicrobial resistance (AMR). Many researchers had suggested that plant extracts possess sophisticated and terrific antimicrobial properties suitable for inhibiting the growth of multidrug-resistant microorganisms based on their respective photochemical capacity [2]. Plant extracts contain phytochemicals that are not only affordable but are less likely to acquire antimicrobial resistance than manufactured compounds [3]. The antimicrobial qualities of plant extracts and essential oils from various plants have previously been evaluated [1]. Currently, more than 80% of the world's population uses medicinal herbs to cure a variety of illnesses [4]. The fresh

* Corresponding author: Onolunosen Abel Abhadionmhen
Department of Microbiology, Federal University Wukari, Taraba State, Nigeria.

discovery of medications is now based on the isolation and validation of potent antibacterial components from medicinal plants. Therefore, it is advantageous to research the therapeutic qualities of plants gathered from various sources [5]. There is no gainsaying that food is a major source of *Salmonella* transmission in humans. Hence, managing the infection using plant extracts could be more beneficial than conventional antibiotics. In fact, [6] has documented the use of plant extract as medicine to manage Salmonellosis in humans.

Vernonia Amygdalina, a member of the Asteraceae family is a shrub that grows in tropical Africa with a typical height of 2- 5m [1]. The green leaf is characterized by a bitter taste and contains lipids [7], and carbohydrates [8]. Bitter leaf as it is alternatively called has a moisture content of 82.0%; dry matter of 17.20%; protein of 0.30% and ash content of 0.50% [9]. *Vernonia Amygdalina* is claimed not only to exhibit anti-helminthic and anti-malaria properties [10] but antimicrobial properties as well [11]. In fact, it has been demonstrated by [1] to possess useful phytochemicals which are potential alternatives in treating urinary tract infections. Hence, this disciplined inquiry will focus on the *in-vitro* antimicrobial activity of *Vernonia Amygdalina* on invasive Non-Typhoidal *Salmonella* isolates from a human and animal source in Southern Taraba, North-East, Nigeria.

2. Methods

This *in-vitro* experimental study was conducted to investigate the antimicrobial efficacy of *Vernonia Amygdalina* on *Salmonella Species* isolated from poultry sources and humans.

2.1. Medicinal plants collection

In this study, leaves of the medicinal plant of interest *Vernonia Amygdalina* were collected from the Federal University Wukari School Garden and authenticated by a plant specialist in the Department of Crop Production and Protection, Federal University Wukari. The collected plant part was properly handled with strict adherence to plant collection and storage protocols and transported immediately to the laboratory for further analysis.

2.2. Preparation of plant extracts

The extraction method used in this research is cold and hot water maceration technique for wet and dried leaves. The collected plant leaves were thoroughly washed under running tap water and rinsed in distilled water and air-dried at room temperature for 7 days in the case of the dried leaf extraction. In the case of the fresh leaf extraction, the collected plant was not air-dried but immediately processed. Both dried and fresh leaves were independently ground using an electric blender and were successively and separately macerated for 3 days. Random shaking of the concentrates was done to facilitate the release of the bioactive components of the plant. The extracts were separated from the marc and further filtered using Whatman filter paper. Thereafter evaporation was done and different concentrations of both menstruum ranging from 5 g-10 ml, 10 g-10 ml, 15 g-10 ml, 20 g-10 ml, 25 g-10 ml was obtained.

2.2.1. Sterility test of the plant extracts

The hot and cold aqueous extracts were tested for microbial contamination and growth. This was carried out by inoculating 0.5 ml of each of the concentrates on prepared sterile Sabouraud Dextrose Agar and incubating at 37^0c, for 18–24 hrs. The plates were observed for growth. The absence of growth in the media after incubation indicates sterility and is evaluated for antimicrobial activity.

2.3. Test microorganisms

A stratified sampling technique was used to select an isolate from stock cultures of antibiotic-resistant *Salmonella* isolated from various poultry farms and food vendors in Ibi, Donga, Takum, and Wukari. The isolates were subjected to microscopy and biochemical analysis for confirmation before introduction into peptone water contained in a universal container which was shaken to get homogenous inocula.

2.4. Antimicrobial activity of plant extracts on isolates using Agar well diffusion method

Using the agar well diffusion method, 1ml of each of the inocula previously mixed in peptone water was spread uniformly on the surface of prepared media plates representing each concentration as well as menstruum used and labeled appropriately. Thereafter, a hole with a diameter of 6 to 8 mm was punched aseptically with a sterile cork borer, and 1ml of the *Vernonia Amygdalina* at various concentrations was introduced into the well. Then, agar plates were incubated for 24 -48 hours at 37°c before observing for growth inhibition. During incubation, the antimicrobial agent diffused in the agar medium and inhibited the growth of the *Salmonella*. A control measure for this experiment was

taken. Culture plates of the test organisms were prepared without the addition of concentrations of cold and hot water extracts of the plant.

3. Results

Bioactive contents of dried and fresh leaves of *Vernonia Amygdalina* were extracted with hot water and cold water and screened for their antibacterial activities against gastroenteric pathogens isolated from poultry droppings and human sources. Both extracts had shown promising repelling effects against *Salmonella* isolates.

The morphological and biochemical characteristics of isolated and identified bacterial pathogens are presented in Table 1. The respective indication of antimicrobial effectiveness of the various concentrations of extracts of fresh and dried *Vernonia Amygdalina* on the test organism is shown in Tables 2 and 3 respectively.

Table 1 Identification of *Salmonella Species* isolated from poultry droppings

Isolate	Morphological Characteristic	Gram stain reaction	Biochemical test						Organism
			CAT	INDO	MR	LAC	SUC	GLU	
A	Smooth, low convex, greyish white, and translucent	Negative bacilli	+	-	+	-	-	+	*Salmonella*

Key: +; Positive, -; Negative. CAT; Catalase, INDO; Indole. MR; Methyl Red, LAC; Lactose, SUC; Sucrose, GLU; Glucose

Table 2 Effects of hot and cold water extract of fresh *Vernonia Amygdalina* leaves on *Salmonella* spp

Concentration	*Salmonella* isolates		
	Cold water extraction	Hot water extraction	No extracts
5 g-10 ml	Moderate growth	Moderate growth	Heavy growth
10 g-10 ml	Moderate growth	Moderate growth	Heavy growth
15 g-10 ml	Moderate growth	Light growth	Heavy growth
20 g-10 ml	Light growth	Light growth	Heavy growth
25 g-10 ml	Light growth	Light growth	Heavy growth

Table 3 Effects of hot and cold water extract of dried *Vernonia Amygdalina* leaves on *Salmonella* spp

Concentration	*Salmonella* isolates		
	Cold water extraction	Hot water extraction	No extracts
5 g-10 ml	Moderate growth	Moderate growth	Heavy growth
10 g-10 ml	Moderate growth	Light growth	Heavy growth
15 g-10 ml	Moderate growth	Light growth	Heavy growth
20 g-10 ml	Light growth	Light growth	Heavy growth
25 g-10 ml	Light growth	Light growth	Heavy growth

4. Discussion

Findings from the in vitro antibacterial experimentation of dried and fresh bitter leaf extracts on *Salmonella* confirmed growth inhibition of the organism but at varied spectrums of different concentrations levels of 5 g-10 ml, 10 g-10 ml, 15 g-10 ml, 20 g-10 ml, 25 g-10 ml. Significant light and moderate growth were observed for both menstruum extracts of

dried and fresh leaves of *Vernonia Amygdalina* at higher concentrations levels of 20 g-10 ml, 25 g-10 ml, and at lower concentrations levels of 5 g-10 ml, 10g-10 ml respectively. Heavy growth of *Salmonella* was seen in the control plates inoculated with the test microorganism but without the addition of any extracts. This finding is consistent with that of [12] which depicts that the bioactive components of plants which ordinarily should protect plants from severe environmental conditions possesses antimicrobial potentials useful to man in combatting diseases and achieving wellness. According to [13], phytochemical contents of Lipia Adonis have shown decisive inhibitory potentials against critical multidrug-resistant bacterial such as Methicillin-resistant *Staphylococcus aureus* (MRSA), *Streptococcus Pyogene, Escherichia coli*, as well as *Klebsiella pneumonia*. A significant concern from this study is the unprecedented effectiveness of the extraction solvent employed. On the one hand, there was no difference in the growth pattern of the test organism in the separate media containing the cold water extraction of fresh and dried leaves, as moderate and light growths were significant in lower and higher concentrations of the extracts respectively. On the other hand, a different growth pattern was seen in the case of hot water extracts of *Vernonia Amygdalina* as a significant antimicrobial potency (light growth) was observed for dried leaves at a lower concentration of 10 g-10 ml as opposed to the moderate growth seen in hot aqueous extracts of fresh leaves at same low concentration of 10 g-10 ml. This finding is not unconnected to that of [14] which established that the phytochemical contents of plants are best extracted with water at a higher degree. The efficiency of hot water in extracting concentrates of *Vernonia Amygdalina* leaves as shown in this current study is contrary to the findings of [1] that gave that the ethanolic solution is more potent than the aqueous solution in extracting components of *Vernonia Amygdalina*. The peculiarity of this current study is that the aqueous solution used for extraction in this aspect is heated to a high temperature. This particularity is consistent with that of [15] who discovered that pressurized hot water extracted a higher yield of phytochemical concentrate than methanol. Arguably, aqueous extraction is considered one of the common extraction methods used for a wide range of polar compounds including phytochemicals [16]. Water is the most common solvent used by traditional healers in extracting bioactive constituents of a wide range of potential medicinal substances including plants [17]. This is because of its cheap and innocuous qualities. However, a major disadvantage of water extraction is that it encourages microbial contamination, and may denature one or several chemical bonds of phytochemicals [18]. Also, the significant light growth observed in lower concentrations (10 g-10 ml) of dried leaves is based on the earlier discovery of [19] who gave that naturally air-dried leaf extracts possess reduced concentrations of toxic non-nutrients than fresh leaf extracts. Furthermore, the moisture content in fresh leaves is a potential media itself that supports the growth of microorganisms [20]

Combination therapy of synthetic drugs or polyherbal formulations is a proven strategy that can overcome the overwhelming resistance mechanisms of microorganisms [21]. The use of natural products together with antibiotics to enhance treatment efficacy is an innovative tactic developed to overcome the problem of antibiotic resistance [22]. The presence of many bioactive compounds in a medicinal plant provides a synergistic therapeutic effect when used simultaneously with standard drugs. They often improve the activity of the drug and will confront problems of toxicity and overdose [23]. An example is a combination of *Helichrysum pedunculated* leaf extracts with antibiotics [24] to treat wounds associated with a bacterial infection which resulted in about 60% synergistic effect. Also, co-administration of *Carica papaya* and *Vernonia Amygdalina* plants in ameliorating *Plasmodium* infection in mice showed synergistic effects with significant percentage suppression of parasite load within three days of clinical therapy [25]. If opportunities for traditional medicine using plant extracts are intensified, the activities of multidrug-resistant microorganisms will be suppressed leading to better and affordable health coverage. Currently, 0ver 90% of individuals in India rely on traditional medicine for their primary healthcare needs [26].

Weak political will towards the regulation of the traditional use of herbs as well as cultural and acceptability factors has impeded the opportunity for continuous research and extensive use of herbal regimens for medical purposes. Ultimately, the lingering mutual disparagement between allopathic and traditional medicine practitioners in Africa has unremittingly hindered the process of integration of antibiotic use and crude herbal remedies [27]. Another fundamental challenge to herbal use is the widely reported cases of fake and substandard commercially available herbal remedies [28].

5. Conclusion

As demonstrated in this research, plant extracts have continued to show appreciable antimicrobial activities more than antibiotics against MDR microorganisms. Hence, further studies on clinical efficacy trials, safety, toxicity, and affordability analyses of more plants need to be given due attention. Doing so will undoubtedly ensure a prompt pharmacognostic approach of seamless synthesis of more precursor molecules for new effective antimicrobials.

Compliance with ethical standards

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Disclosure of conflict of interest

The researcher declares no conflict of interests in this review.

Statement of ethical approval

The study was approved by the Review Board of Tropical disease Unit, Microbiology Department, Federal University Wukari. This research was conducted considering all ethical issues such as Justice, Beneficence, Non-Maleficence and Autonomy were considered when conducting the research.

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