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Potentials of *Veronica amygdalina* Extracts as an Alternative Anti-Microbial Feed Additive in Poultry

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ABSTRACT

Due to the problem of antibiotic resistance, the use of synthetic antibiotics has been restricted in animal farming, which has spurred research into the development of alternatives. This study was designed to assess the *in vitro* antibiotic activity of the water and ethanolic extract of bitter leaf against *Salmonella* sp, *Shigella* sp, *Staphylococcus* sp, *Escherichia coli* and *Pseudomonas aeruginosa.* Antimicrobial susceptibility test was done using Kirby-Bauer disc diffusion method. Results obtained showed that none of the organism tested was sensitive at 20% of the water extract. Sensitivity increased from 40% to 100% of the water extract. The highest zone of inhibition (ZOI) of 17.00±1.00 mm was obtained for *Staphylococcus* sp, followed by 16.33±1.15 mm for *P. aeruginosa*. The highest ZOI for the ethanolic extract of bitter leaf (27.67±2.52 mm) was recorded for *Staphylococcus* sp followed by 25.00±2.00 mm for *E. coli* both at ethanol extract concentration of 300g /ml. We therefore conclude that bitter leaf can be considered as potential alternative to synthetic antibiotic growth promoters in poultry.

Key Words: Alternative to antibiotics, Bacteria, Poultry, feed additive, *Vernonia amygdalina*

INTRODUCTION

Bitter leaf, *Vernonia amygdalina* is a shrub that is commonly used in the preparation of various traditional African soups and foods. The plant has several medicinal properties including hypoglycemic (Uchenna *et al.,* 2008), anti-parasitic including antimalarial (Abosi and Raserika, 2003), and anticancer properties (Izevbigie *et al.,* 2004). Okigbo and Mmeka (2008) reported that bitter leaf is used for treatment of constipation, fever, high blood pressure and many infectious diseases. Adetunji *et al.* (2013) reported that the extract of bitter leaf exhibit antibiotic properties against drug resistant microorganisms and possess antioxidant, anti-cancer, anti-viral, anti-helminthic and anti-inflammatory activities. Uzoigwe and Agwa (2011) reported the antibacterial properties of bitter leaf against selected bacteria implicated in urinary tract infection including *Klebsiella* sp, *E. coli* and *Staphylococcus* sp. Kigigha and Onyema (2015) demonstrated the antibiotic activity of bitter leaf soup on *Staphylococcus aureus* and *Escherichia coli*.

Due to the problems of antibiotic resistance in humans and animals, several countries have restricted the use of infeed antibiotics in animal farming. Typically, antibiotic growth promoters (AGP) are added to the feed to prevent microbial infection. With the restrictions on the use of AGP, poultry farmers are at loss on how to handle microbial infections. This challenge has spurred the scientific community to research on alternatives to AGP. Some of the promising alternatives to AGP include prebiotics (Ohimain and Ofongo, 2012), probiotics (Ohimain and Ofongo, 2012), synbiotics (Awad *et al.,* 2008); enzymes (Ohimain and Ofongo, 2013; Ofongo *et al.,* 2016), mushrooms (Willis *et al.,* 2011) and plants (Ogbe and Affiku, 2012). The use of plant or its extracts, which have been variously described as phytobiotics or phytogenics is becoming more common in poultry ration. We had tested the antibiotic potentials of some plant species including *Azadirachta* indica (Ofongo and Ohimain, 2019), *Ocimum gratissimum* (Ohimain *et al.,* 2015), *Zingiber officinale* (Ofongo-Abule and Ohimain, 2015). Hence,

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in this study, we assessed the antibiotic properties of bitter leaf to selected pathogens of importance in poultry farming with an aim to develop an alternative to antibiotic growth promoters in poultry feed.

MATERIALS AND METHODS

Plant samples of bitter leaf (*Vernonia amygdalina*) were collected freshly from the teaching and research farm of the Faculty of Agriculture, Niger Delta University on the 14th of November 2019. The plant materials were washed lightly with distilled water and sun dried for two days until a constant weight was attained. The dried leaves were milled with electric grinder to powder form.

About 100 g of the dried powder was mixed with 1000 ml of sterile distilled water and stirred. The mixture was heated for 2 hours, then allowed to stand for 24 hours. The aqueous extract was obtained by passing the mixture through Whatman No. 1 filter paper and the filtrate was collected. Four different concentrations (20%, 40%, 80%, 100%) of the aqueous extract was used for the experiment.

A hundred (100 g) gram of the dried powder of *V. amygdalina* was weighed and extracted with 1000 ml of 100 % ethanol. The mixture was vigorously stirred intermittently, then allowed to stand for 72 hours after which it was filtered through a Whatman No. 1 filter paper-lined funnel into a conical flask. The solvent from the filtrate was recovered with the aid of a rotary evaporator under vacuum at 40°C. The extract was further concentrated and dried using a water bath at 40° C for 48 hours. Three different concentrations (75 g/ml, 150 g/ml and 300 g/ml) were used for the study.

The antimicrobial properties of *V. amygdalina* extracts were investigated using five bacterial isolates obtained from poultry litter and confirmed after identification with the stock culture collection of the Department of Microbiology, Faculty of Science, Niger Delta University, Wilberforce Island. The test isolates were *Salmonella* sp, *Shigella* sp, S*taphylococcus* sp, *Escherichia coli,* and *Pseudomonas aeruginosa.* The isolates were confirmed using morphological features and biochemical tests, which were performed according to the procedures outlined in Cheesbrough (2006) and the organisms were confirmed using Bergeys Manual of Determinative Bacteriology (Bergey and Holt, 1993). The antimicrobial susceptibility test was done using Kirby-Bauer disc diffusion method as outlined in Cheesbrough (2006).

Statistical analysis including descriptive statistics (mean and standard deviation), analysis of variance (ANOVA) and posthoc to detect the level of significance was done using software package of SPSS version 21 (IBM SPSS Inc, Chicago).

RESULTS AND DISCUSSION

The result showed that the mean ZOI generally increased as the concentration of *V. amygdalina* extracts increased, but higher values were obtained for the ethanol extract than the aqueous extract (Tables 2 and 3). None of the organism tested was sensitive at 20% of the aqueous extract. Sensitivity increased from 40% to 100%. The mean ZOI was 6.33±0.058 mm, 11.00±1.00 mm and 13.00±1.00 mm; respectively for *Salmonella* sp. Values recorded for *Shigella* sp was 9.33±2.52 mm, 11.00±1.00 mm and 13.00±1.00 mm. A value of 10.33±0.58 mm, 11.67±1.15 mm and 17.00±1.00 mm was obtained for *Staphylococcus* sp. Antibacterial activity of *V. amygdalina* extract against E. coli was 7.33±0.58 mm, 7.67±0.58 mm and 11.67±1.53 mm. a value of 10.67±0.58 mm, 13.67±0.58 mm and 16.33±1.15 mm was obtained against *P. aeruginosa* at 40%, 80% and100% aqueous *V. amygdalina* extract; respectively. The differences in the mean ZOI were significant in all the test organisms ($P<0.05$) except *Shigella* sp that was not significant ($P>0.05$). The highest ZOI of 17.00±1.00 mm was obtained against *Staphylococcus* sp, followed by 16.33±1.15 mm for *P. aeruginosa* both at 100%.

The results obtained for the ethanolic extract of *V. amygdalina* indicate that mean diameter (mm) of ZOI was 10.00±2.00, 13.67±2.31 and 19.00±1.73; respectively for *Salmonella* sp. A value of 12.67±1.16, 19.33±1.16 and 24.00±2.00 was recorded against *Shigella* sp. The value recorded for *Staphylococcus* sp was 13.67±1.16, 17.33±1.53 and 27.67±2.52;

Table 1. Biochemical tests for the characterization of bacteria isolated from poultry litter

Tentative Bacteria	*Salmonella* Sp	*Shigella* Sp	*Staphylococcus* Sp	*Escherichiacoli*	*Pseudomonas* Sp
Gram stain	-ve rod	-ve rod	+ve cocci	-ve rod	-ve rod
Catalase	-	+	+	+	+
Oxidase	-	-	-	-	+
Indole	-	-	-	+	-
Glucose in KIA	+	+	+	+	-
Lactose in KIA	-	-	+	+	-
Gas in KIA	-	+	+	+	-
H_2S in KIA	+	-	-	-	-
Citrate	-	-	+	-	+
Pigmentation	-	-	+	-	+

KIA, Kligler iron agar; Key: +/- (positive result/negative results)

respectively. The value recorded against *E. coli* was 13.67±1.16, 18.00± 2.65 and 25.00±2.00. While a value of 10.67±0.56, 17.33±0.56 and 23.67±1.16 for *P. aeruginosa* at 75 g/ml, 150 g/ml and 300 g/ml ethanol extract; respectively (P<0.05). The highest zone of inhibition of 27.67±2.52 mm was recorded for *Staphylococcus* sp followed by 25.00±2.00 mm for *E. coli* both at ethanol extract concentration of 300 g/ml.

The results indicate that both the ethanolic and water extract of bitter leaf exhibited antibacterial activity *in vitro* in a dose-dependent manner. Other researchers have similarly observed this pattern. Okigbo and Mmeka (2008) at a concentration of 20 mg/ml reported a zone of inhibition of 15 mm and 11 mm of ethanol extract and water extract; respectively against *Staphylococcus aureus* and 10 mm and 8 mm; respectively against *E. coli*. The results, which is in agreement with our study shows that the zone of inhibition was generally higher in the ethanolic extract and higher zone of inhibition was obtained for *Staphylococcus* than *E. coli*. Oboh and Masodje (2009) using water extract of bitter leaf at a concentration of 10 % reported a zone of inhibition of 8 mm for *Staphylococcus aureus* and *Escherichia coli*. Udochukwu *et al.* (2015), using a concentration of 10 g/100 ml extract reported a zone of inhibition of 7 mm, 11.5 mm and 7.5 mm for ethanol extracts, and 7.0 mm, 5.0 mm and 6.0 mm for *E. coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*; respectively.

Adetunji *et al.* (2013) demonstrated increasing zone of inhibition of three bacteria species as the concentration of bitter leaf extract increased from 25 to 200 mg/ml, with the ethanolic extract generally being more potent than the water extract. Using a concentration of 100 mg/ml, Ghamba *et al.* (2014) reported the zone of inhibition of water and ethanol

Table 2. Antibacterial activity of aqueous extract of *V. amygdalina* against assay organisms

Test organisms	Mean inhibition zone diameter (mm)			
	20%	40%	80%	100%
Salmonella sp	0.00±0.00[d]	6.33±0.058[c]	11.00±1.00[b]	13.00±1.00[a]
Shigella sp	0.00±0.00[b]	9.33±2.52[a]	9.33±0.58[a]	11.33±0.058[a]
Staphylococcus sp	0.00±0.00[c]	10.33±0.58[b]	11.67±1.15[b]	17.00±1.00[a]
E. coli	0.00±0.00[c]	7.33±0.58[b]	7.67±0.58[b]	11.67±1.53[a]
P. aeruginosa	0.00±0.00[d]	10.67±0.58[c]	13.67±0.58[b]	16.33±1.15[a]

Means (n=3, ± standard deviation) with different alphabets are significantly different (P<0.05)

Table 3 Antibacterial activity of ethanolic extract of *V. amygdalina* against assay organisms.

Test organisms	Mean inhibition zone diameter (mm)		
	75 g/ml	150 g/ml	300 g/ml
Salmonella sp	10.00±2.00[b]	13.67±2.31[b]	19.00±1.73[a]
Shigella sp	12.67±1.16[c]	19.33±1.16[b]	24.00±2.00[a]
Staphylococcus sp	13.67±1.16[b]	17.33±1.53[b]	27.67±2.52[a]
E. coli	13.67±1.16[b]	18.00±2.65[b]	25.00±2.00[a]
P. aeruginosa	10.67±0.56[c]	17.33±0.56[b]	23.67±1.16[a]

Means (n=3, ± standard deviation) with different alphabets are significantly different (P<0.05)

extracts of bitter leaf on selected microbes. Unlike, other results including our study, they recorded higher zones of inhibition in water extracts than ethanol extracts. The zone of inhibition of the ethanol extract were 11.3 mm against *E. coli,* 10.8 mm against *Pseudomonas aeruginosa* and 11.4 mm for *Staphylococcus aureus*, but for the water extracts values were 12.5 mm, 12.2 mm and 11.4 mm; respectively. Generally, the different zones of inhibition recorded is due to the different concentrations of the plant extract used in the different studies.

CONCLUSION

The *in vitro* antibacterial properties of the water and ethanolic extracts of bitter leaf was investigated. In agreement with other studies, it was observed that the zone of inhibition of *Salmonella* sp, *Shigella* sp, *Staphylococcus* sp, *E. coli* and *P. aeruginosa* was dose dependent, with the ethanol extracts being generally showing stronger anti-microbial activity than the water extract. It is concluded that bitter leaf can be used as potential alternative to synthetic AGP in poultry feed.

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