Evaluation of the Laxative and Antibacterial Activities of Bitter Leaf (*Vernonia amygdalina* Del.)

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Abstract

Laxative and antibacterial activities of ethanol extracts of Vernonia amygdalina leaf was evaluated in this study. In the laxative study, fourteen (14) Wistar albino rats were randomly divided into seven groups of two (2) rats per group. Group I received 0.5 mg/kg of normal saline (negative control). Group II received 10 mg/kg of Dulcolax (positive control), groups III to VII received 2000, 1000, 500, 250, 125 mg/kg of the extract respectively. Agar well diffusion method was used to carry out the antimicrobial activity of ethanol leaf extract of V. amygdalina. The extract showed significant ($p<0.05$) laxative effects in a dose dependent manner with 2000 mg/kg extract having the highest laxative effect (0.55±0.71). There was also a significant ($P<0.05$) reduction in the faecal weight of the groups that received extracts of V. amygdalina when compared with the untreated group (Group 1). The antibacterial activity of the extract at different concentrations (1000, 500, 250, 125, 62.5 and 31.25 mg/mL) significantly ($P<0.05$) inhibited the growth of selected bacteria. The extract had reasonable antimicrobial activity at 1000 mg/mL on S. aureus (42.50±0.71). It moderately inhibited K. pneumoniae (33.00±0.71), P. aeruginosa (32.50±0.71), S. pneumoniae (30.50±0.71), S. typhi (30.50±0.71) and E. coli (22.50±0.71) compared to the standard antibiotic Ciprofloxacin 500 mg/mL (50.50±0.71). The result obtained showed that the leaves of V. amygdalina have moderate laxative effect and also could be used for the treatment of infections.

Key words: Antimicrobial agent, laxative, pathogenic organisms, *Vernonia amygdalina*

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Introduction

Bitter leaf *(Vernonia amygdalina* Del.) belongs to the family Asteraceae. It is commonly referred to as bitter leaf in English language and also has several local names peculiar to some Nigerian tribes. The plant is called 'Onugbu' in Igbo land, 'Ewuro' in Yoruba, 'Shuwaka' in Hausa and 'Oriwo' in Edo land. Bitter leaf plant is a shrub of 1–3 m in height found widely growing in the tropical regions of Africa as wild or cultivated plant all over sub-Saharan Africa (Bosch *et al*., 2005). Igile *et al*. (1995) described bitter leaf plant as having a petiole leaf of about 6 mm in diameter and elliptic in shape. All parts of the plant are pharmacologically useful as both the roots and leaves are used in phyto-medicine to treat fever, hiccups, kidney disease and stomach discomfort (Gill, 1992; Hamowia and Saffaf, 1994). It also possesses anthelmintic and antimalarial properties (Abosi and Raseroka, 2003).

Vernonia amygdalina is a widely used in Nigeria for food, therapeutic and its nutritional purposes (Oliver-Bever, 1986; Audu *et al*., 2018). It has a lot of bitter principles in all parts of the plant and the bitter taste is due to anti-nutritional compounds present in the plant like alkaloids, saponins, tannins and glycoside (Bonsi *et al.*, 1995).

The leaf of *V. amygdalina* has been shown to be useful as a remedy for gastrointestinal disorders and as a general tonic (Iwu, 1986), and has been reported to have blood sugar lowering effects (Uhuegbu and Ogbuchi, 2004). The plant has protective effects against the toxic effects of aflatoxin B_1 exposure (Ijeh and Obidoa, 2004) and may help in kidney clearance functions (Ijeh and Adedokun, 2006).

Laxatives or purgatives are foods or medications consumed or taken to either induce bowel movements or loosen the stool - generally taken when a person has constipation, a term used to describe the difficulties that patients experience with moving their bowels (Sodipo *et al*., 2020; Sathyanathan *et al*., 2013). However, the choice of laxative applied for treatment or prevention of

constipation depends on the etiology. Primary constipation may be characterized by normal or slow intestinal transit or may be caused by an anatomical issue (e.g. pelvic flow dysfunction). Secondary constipation is associated with endocrine or metabolic disorders (e.g. hypercalcemia, hypothyroidism, pregnancy, and diabetes mellitus), neurologic disorders (stroke, Parkinson disease, and multiple sclerosis), connective tissue disorders (scleroderma, amyloidosis) and eating disorders (Rose, 2014; Leung *et al.*, 2011). Therefore, medications for the treatment of constipation include bulk-forming agents, lubricant laxatives, stool softeners, stimulant laxatives, osmotic laxatives, saline laxatives and prokinetic agents (Rose, 2014; Thayalasekeran *et al.*, 2013; Leung *et al.*, 2011)

Also, synthetic laxatives are indicated for short-term use. Nonetheless, many people with chronic constipation use them routinely. Abuse of synthetic laxatives, including misuse in treatment of chronic constipation is a significant problem that has been mentioned in the medical literature while natural laxatives like *V. amygdalina* has no side effect.

V amygdalina is used traditionally to promote wound healing (Sodipo *et al*., 2020; Iwu, 1986) and to treat microbial infections (Yeap *et al*., 2010). The current study was designed to evaluate the laxative and antibacterial potential of *V. amygdalina*.

Materials and Methods

Collection of plant material

V. amygdalina leaves used for this research were obtained from the farm land within the vicinity of Michael Okpara University of Agriculture, Umudike (MOUAU) in Abia State. The plant materials were botanically identified by Dr. G. Omosun of the Department of Plant Science and Biotechnology, MOUAU before taken to the laboratory for analyses.

Source of test bacterial isolates

Clinical strains of bacterial isolates used for the antibacterial study were obtained from the Microbiology laboratory unit of Federal Medical Centre, Umuahia, Abia State. These microorganisms include *Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli, Klebsiella spp., Salmonella typhi, Pseudomonas spp.*

Experimental animals

Fourteen (14) albino rats allocated to seven (7) groups of 2 animals per each group were used for the study of the laxative activities of *V. amygdalina* leaf. These rats were obtained from the Department of Veterinary Medicine in the University of Nigeria, Nsukka. The rats were acclimatized for 14 days in the animal section of the Biochemistry unit of Michael Okpara University of Agriculture, Umudike in the College of Natural Sciences. During the period of acclimatization, the rats were fed with grower's feed and given water ad libitum.

Preparation of ethanol extract of bitter leaf

The leaves of *V. amygdalina* were air dried at room temperature, ground and preserved in bottles for use. 100 g of the ground leaves was macerated in ethanol for 72 hours and then filtered with Whatman No. 1 filter paper, afterwards heated in a water bath to allow for ethanol evaporation in other to obtain the ethanol extract (Omodamiro and Ajah, 2017)

Preparation of stock and standard of ethanol extract of bitter leaf

The ethanol extract of *V. amygdalina* was dissolved in distilled water to obtain concentration of 31.25, 62.5, 125, 250, 500 and 1000 mg/mL according to the method described by Omodamiro *et al.* (2020).

Laxative activity of ethanolic extract of bitter leaf

All the seven groups (2 animals per group) of animals were fasted for 18 hours before the experiment. The animals were placed in individual cages lined with clean filter paper. Group 1 served as negative control and was administered 0.5 ml/kg of normal saline orally. Group II was administered with Dulcolax (10 mg/kg) which served as the positive control. Group III was administered 2000 mg/kg Body Weight (BW) of extract, group IV was administered 1000 mg/kg BW extract, group V was administered 500 mg/kg BW extract, group VI was administered 250 mg/kg BW extract, while group VII was administered of 125 mg/kg BW extract. Each animal was placed in an individual cage and the floor was lined with A4 white paper. The faecal matter was then observed for 3 hours (Audu *et al*., 2018)

Antimicrobial susceptibility testing

Agar well diffusion method was applied for sensitivity testing as described by Lino and Deogracious, (2006). Similarly, the Muller Hinton agar plate surface was inoculated by spread plating an aliquot of the microbial inoculum over the entire agar surface. In each of these plates, six wells (5mm diameter) were punched into the agar by using sterile cork borer for different concentrations of the extract on each of the plates containing cultures of the different test organisms. 0.1 ml of each of the concentration of the extract was introduced into the wells appropriately using a sterile Pasteur pipette. A standard antibiotic disc was then placed at the middle of the media to serve as the positive control (Ciprofloxacin, 500 mg/mL). The culture plates were allowed on working bench for 30 minutes for pre-diffusion and were incubated at 37 ˚C for 24 hours after which antimicrobial activity was determined by the measurement of diameter zones of inhibition (mm) against organisms around each of the holes and the antibiotics disc.

Statistical analysis

The results were statistically analyzed for significance using SPSS version 22.0 software. Duncan's Multiple Range Test was employed for comparison of means. All values were expressed as mean ± SD and $p<0.05$ values were considered significant.

Results

The results of the laxative activity of ethanolic extract of *V. amygdalina* on test albino rats are presented in Table 1. The result revealed significant difference ($p<0.05$) for positive control, where increase in the faecal weight of the group that received standard drug (10 mg/kg Dulcolax) was observed, suggesting induction of stooling and bowel emptying. However, the groups that received different concentration of *V. amygdalina extract* showed significant ($p<0.05$) reduction in the stool compared to that of the negative control, though not in a dose dependent manner (Table 1).

Table 1: Laxative activity of *V. amygdalina* on albino rat's faecal average weight (g)

Animal Group	Treatment	Faecal weight (g)
Group 1	Normal saline (0.5ml)	0.60±0.14**
Group 2	Std. drug Dulcolax (10mg/kg)	1.00±0.14*
Group 3	Extract (2000mg/kg)	0.55±0.07*
Group 4	Extract (1000mg/kg)	0.40±0.14*
Group 5	Extract (500mg/kg)	0.50±0.14*
Group 6	Extract (250mg/kg)	0.30±0.14*
Group 7	Extract (125mg/kg)	0.35±0.07*

1. Values are written as mean ± SD
2. Mean values with single asterisks (*) are significantly ($p\leq0.05$) different from control while mean with double asterisk (**) are non-significantly different from the extracts.

The results of the antimicrobial activity of the ethanolic extract of *V. amygdalina* on the selected test organisms (*Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli, Klebsiella spp., Salmonella typhi, Pseudomonas spp.*) are presented in Table 2. These organisms were tested using 1000 mg/mL ethanolic leaf extract of *V. amygdalina* and standard antibiotic disc ciprofloxacin (500 mg/mL). Table 2 showed that the extract at 1000 mg/mL had the highest antimicrobial activity against *S. typhi* though the activity was significantly ($p<0.05$) lower compared to ciprofloxacin suggesting that the extract at this concentration could not significantly inhibit *S. typhi* growth. It was evident that the extract of Bitter leaf at 1000 mg/mL inhibited the test organisms lower than that of ciprofloxacin at 500 mg/mL with the exception of *P. aeruginosa* where the extract produced higher inhibition at 1000 mg/mL (32.50±0.71 mm) than ciprofloxacin at 500 mg/mL (28.50±0.70 mm) as depicted in Table 2.

Table 2: Anti-microbial activity of *V. amygdalina* against the test organisms

Organism	*V. amygdalina* Extract (1000 mg/mL)	Standard Ciprofloxacin (500 mg/mL)
S. typhi	30.50±0.71**	62.50±0.71*
E. coli	22.50±0.71**	50.50±0.71*
P. aeruginosa	32.50±0.71**	28.50±0.70*
S. aureus	42.50±0.71**	48.50±0.71*
K. pneumoniae	33.00±0.71**	40.50 ±0.71*
S. pneumoniae	30.50±0.71**	38.50±0.71*

1. Values are written as mean ± SD. **(**Diameter Zone of inhibition measured in millimeters)

2. Mean values with single asterisks (*) are significantly ($p\leq0.05$) different from control while mean with double asterisk (**) are non-significantly different from control.

The minimum inhibitory concentration (MIC) results for the ethanol extract of *V. amygdalina* are shown in Table 3. The MIC for *S. typhi* was 250 mg/mL, *E. coli* (62.5 mg/mL), *P. aeruginosa* (125 mg/mL), *S. aureus* (125 mg/mL), *K. pneumoniae* (250 mg/mL) and *S. pneumoniae* (125 mg/mL). The result showed that *E. coli* was the most sensitive organism to the ethanolic extract of *V. amygdalina* with MIC of 62.5 mg/mL, whereas *S. typhi* and *K. pneumoniae* were least sensitive to the extract with MIC of 250 mg/mL.

Table 3: Minimum inhibitory concentration (MIC) of ethanolic extract of bitter leaf against test organisms

Organism	1000 mg/mL	500mg/mL	250 mg/mL	125mg/mL	62.5mg/mL	31.25 mg/mL	MIC (mg/mL)
S. typhi	30.50±0.71**	15.50±0.71*	6.50 ±5.51*	0.00±0.00	0.00±0.00	0.00±0.00	250
E. coli	22.50±0.71**	12.00±1.41*	7.00±1.41*	4.50±0.71*	2.50±2.08*	0.00±0.00	62.5
P. aeruginosa	32.50±0.71**	16.50±0.71*	7.50±0.71*	4.50±0.71*	0.00±0.00	0.00±0.00	125
S. aureus	42.5±0.71**	24.50±1.41*	10.5±0.71*	5.50±0.71*	0.00±0.00	0.00±0.00	125
K. pneumonia	33.00±0.71**	15.5±0.71*	2.50±0.71*	0.00±0.00	0.00±0.00	0.00±0.00	250
S. pneumonia	30.5±0.71**	16.56±0.71*	6.50±0.71*	2.50±0.71*	0.00±0.00	0.00±0.00	125

1. Values are written as mean ± SD. (zones of inhibition are measured in millimeters, mm.)
2. Mean values with single asterisk (*) are significantly ($p \leq 0.05$) different from control with double asterisks (**)

Discussion

The faecal weight of the animals was estimated and the result showed that there was a significant ($p<0.05$) increase in the faecal weight of the group that received standard drug (Dulcolax) suggesting induction of stooling and bowel emptying. However, the groups that received different extracts of *V. amygdalina* showed significant ($p<0.05$) reduction in the stool compared to that of the negative control though not in a dose dependent manner. This observation could be explained by drug occupancy theory. Drugs must bind with receptors so as to produce pharmaceutical response. Although in normal animals, increase in concentration of drug is directly proportional to increase in pharmacological effects. Hence further increase in drugs may not lead to further increase in pharmacological effects since the receptor site is fully occupied. However, a different observation was reported by Owu *et al.* (2008) where aqueous extracts of *V. amygdalina* was found to induce gastric secretion and dose dependent contraction of the ileum in guinea pigs. The induction of stooling in the animals agrees with the work of Igile *et al*. (1994) who reported that in traditional medicine, practitioners use the plant as an antimalarial and as a laxative. Others use the plant as a digestive tonic and for tropical treatment of wounds (Iwu, 1986).

The antimicrobial activity of *V. amygdalina* extract has been carried out by many researchers. Ijeh *et al*. (1996) revealed the sap of the leaves of *V. amygdalina* was inhibitory against *Staphylococcus epidermidis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas auroginosa*. Also, 60 % of methanolic extract of the leaves gave antimicrobial activity against *Bacillus subtilis, Klebsiella pneumoniae, P. aeruginosa, Proteus vulgaris, Shigella dysentariae* and *S. aureus* (Akinpelu, 1999). Studies have also shown that extracts from *V. amygdalina* are effective in the management of fungal infections and parasitic diseases (Wedge *et al*., 2000). These botanicals may be very useful in the management/control of fungal diseases, especially since they are

effective, cheap and environmentally non-hazardous (Kuruchev *et al.*, 1997).

In this present study, the ethanolic leaves extract of *V. amygdalina* was investigated to ascertain its antimicrobial activity against *S. typhi, E. coli, P. aeruginosa, S. aureus, K. pneumoniae* and *S. pneumoniae* at 1000 mg/mL, 500 mg/mL, 250 mg/mL, 125 mg/mL, 62.5 mg/mL and 31.25 mg/mL. The extract at 1000 mg/mL showed the highest antimicrobial activity against all the test organisms. However, the antibacterial activity of Bitter leaf extract against the test organisms was found to be lower than the standard drug ciprofloxacin at 500 mg/kg, except for *P. aeruginosa* with DZI higher than that of the standard drug (Table 2).

Erasto *et al*. (2006) reported that the presence of vernolide, vernodalol and other phytochemicals such as steroids and alkaloids in the leaves of *V. amygdalina* is largely responsible for the antimicrobial properties inherent in the plant. Therefore, the different zones of inhibition recorded at varied concentration could be attributed in one hand to sensitivity of the organisms to active ingredients present in the extract.

The findings of this study is in conformity with the earlier works of Erasto *et al*. (2006) and Udochukwu *et al*. (2015) who reported that ethanol extracts of *V. amygdalina* had antibacterial activity on both gram positive and some gram negative bacteria.

The minimum inhibitory concentration (MIC) for *S. typhi* was 250 mg/ml, *E. coli* 62.5 mg/ml, *P. aeruginosa* 125 mg/ml, *S. aureus* 125 mg/ml, *K. pneumonia* 250 mg/ml and *S. pneumonia* 125 mg/ml. The result revealed that *E. coli* was the most sensitive organism to the *V. amygdalina* ethanolic extract with MIC of 62.5 mg/mL while *S. typhi* and *K. pneumoniae* were the least sensitive with MIC of 250 mg/mL.

The potency of an antibacterial agent is an inverse measurement of its MIC as plant extract or drugs that have low MIC against bacteria are said to be very potent. In this study it was observed that the ethanol extract of *V. amygdalina* was more potent

in inhibiting *E. coli* with recorded MIC of 62.5 mg/mL when compared with other bacterial isolates investigated. This observation is consonance with the findings of Evbuomwan *et al*. (2018) in which *E. coli* was the most sensitive organism to ethanolic extract of *V. amygdalina* with an MIC of 25 mg/mL.

Conclusion

The study has shown that ethanol extract of *V. amygdalina* to some extent has laxative and antimicrobial activities. This has further validated the reason why the plant is usually recommended by traditional herbalists for treatment of many ailments locally.

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