Regular Article

Laxative and antimicrobial activities of ethanolic extract of leaf and roots from *Amaranthus viridis* L. on wistar albino rats

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Abstract

The purpose of the present study was to evaluate the laxative and antimicrobial activities of ethanol extracts of leaf and root of *Amaranthus viridis* L. The laxative activity of ethanolic leaf extract of *A. viridis* was studied using six groups of wistar albino rats; Group I which served as the negative control received 0.5ml/kg of normal saline, Group II received 10mg/kg of Dulcolax and the rest of the groups (III-VI) received 400, 200, 100 and 50mg/kg of the extract respectively. The laxative activity of the ethanolic leaf extract was expressed as the mean of total weight of faecal output in each group. A significant ($p < 0.05$) dose dependent increase in the faecal output was observed at the 200mg/kg (3.00 ± 1.41 gm) and 400mg/kg (3.50 ± 2.12 gm) doses compared with the negative control. The antimicrobial activity was expressed as the diameter of the zone of inhibition hence the minimum inhibitory concentrations were determined. The antimicrobial activity of the *A. viridis* leaf and root extracts had dose dependent increases in all the tested organisms from their various minimum inhibitory concentrations (MIC). The result confirmed that the leaves and root of *A. viridis* possess laxative and antimicrobial activity.

Key words:

Amaranthus viridis, laxative, antimicrobial, albino rat, minimum inhibitory concentrations, Dulcolax

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Introduction

The phytochemicals from plant species are of current interest due to their potential effects on different ailments (Baris et al., 2006). The plants which can be used for medicinal purpose are the major bio-resource of drugs of ethnomedicine and modern medicines, which pave the way for invention of synthetic drugs (Hammer et al., 1999). Throughout the world the plant based medicine is gaining more interest, and most of the drugs are derived from the plants after following upon ethno medical use of the plants (Gibbons, 2003; Katiyar et al., 2012).

Amaranthus viridis Linn. Is grown as annual herb plant which grows up to 10 to 75 -100 cm

which mostly flowering in summer fall (Ali and Kaiser, 1995-2004). This plant has been listed as an important ethnomedicinal plant with many traditional medicinal uses by various studies Ahmad et al., 2007; Qureshi et al., 2007; Vanila et al., 2008). Even though there are reports on the many medicinal use of this plant, the studies related to laxative activities and antimicrobial activities from *A. viridis* is scanty. The four different classes of laxatives include: bulking agents, osmotic laxatives, irritant and stimulant laxatives and fecal softeners. The bulking agents have hydrophilic activity through retention of water in the intestinal tract, thereby expanding

and softening of faeces (Berardi et al., 2006). Microbes are microorganisms, especially pathogenic bacteria that can cause disease (Madigam and Martinko, 2006). An antimicrobial is related to any agent that kills microorganism or inhibits their growth (MWOD, 2009). In this study, we determined the laxative and antimicrobial activities of *Amaranthus viridis*.

Materials and methods

Collection of plant material

Amaranthus viridis L. leaves and roots used in this research was obtained from the surrounding of Michael Okpara University of Agriculture, Umudike. The plant was identified by Dr. M.A. Jiomoh from the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike.

Bacterial strains

Clinical strains of microorganism used for the antimicrobial study were obtained from the Microbiology Laboratory of Federal Medical Centre, Umuahia, Abia state. These microorganisms include: *Streptococcus pneumonia*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi* and *Candida albican*.

Experimental animal

Twenty four (24) albino rats were used to study the laxative activity of *Amaranthus viridis* leaf. These rats were gotten 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, College of Natural Sciences.

Preparation and concentration of ethanolic extracts

The leaves and the roots of *Amaranthus viridis* were sun dried, grinded and preserved in separate reagent bottles for use. 100g and 50g of the ground leaves and roots materials respectively were weighed out into separate bottles. Then 500ml and 250ml of ethanol were added into the respective bottles and allowed for 72 hours for adequate dissolution after which Whatman No 1 filter papers were used in each case to separate the residue from the filtrate and then the filtrate was heated in a water bath to allow for ethanol evaporation.

Acute toxicity studies (LD50)

A total of 16 Albino mice with an average weight of 18-22g were used. The animals were divided into four groups. The leaf extract was administered at the dose of 2000mg/kg, 1000mg/kg, 500mg/kg and 250mg/kg body weight respectively. The animals were then observed for a period of 24 hours respectively.

Laxative activity

The experimental method use in this study is in accordance with Institutional Animal Ethics Committee (688/2/C-PCSEA) method. Rats fasted for 18 hours before the experiment and they were placed in their respective cages lined with clean filter paper. The animals were divided into 6 groups. Group I which served as control received Saline (0.5ml/kg), Group II served as reference control received Dulcolax (10mg/kg), and Group III, IV, V and VI received 400, 200, 100 and 50mg/kg of ethanolic extract respectively through oral administration. The total weights of normal as well as wet faeces production in all the 6 groups were monitored for 16 hours.

Antimicrobial activity

In-vitro antibacterial activities of the leaf and root extract of *Amaranthus viridis* and standard or antibiotic drugs were determined by disc diffusion method (Kohner et al., 1994).

Statistical analysis

All results were expressed as mean $\pm$ standard error. The data was analyzed using one-way analysis of variance (ANOVA). The statistical significance of the difference of the mean was evaluated.

Results and Discussion

The laxative activity of *Amaranthus viridis* was measured by the faecal output of the tested rats (Table 1). The results show that an oral administration of the leaf extract of *Amaranthus viridis* produced significant ($P \leq 0.05$) difference in faecal outputs of rats administered with the extract at a dose of 200mg/kg and 400mg/kg with the values (3.00 ± 1.41) and (3.50 ± 2.12) respectively compared with the Negative (2.00 ± 1.41) and positive (5.00 ± 1.41) control. The difference was a dose dependent increase. The faecal output of the rats that received lower doses (i.e. 100mg/kg and 50mg/kg) had no significant difference with those that received Normal saline (Negative control)

while the highest faecal output (5.00 ± 1.41^a) was experienced in rats that received the standard drug (10mg/kg Dulcolax). The laxative properties of *A. viridis* at higher doses of 200mg/kg and 400mg/kg, could be that the leave stimulates muscles in the walls of the small intestine and promotes evacuation of the colon to generate an increased bowel movement.

Table 1. Laxative activity of *Amaranthus viridis* leaf extract in rats.

Groups	Drug Treatment	Faeces output (gm)
I	Control Saline (0.5ml/kg)	2.00 ± 1.41^d
II	Dulcolax (10mg/kg)	5.00 ± 1.41^a
III	Extract (400mg/kg)	3.50 ± 2.12^b
IV	Extract (200mg/kg)	3.00 ± 1.41^c
V	Extract (100mg/kg)	2.00 ± 1.41^d
VI	Extract (50mg/kg)	1.35 ± 0.92^d

There is significant difference at $p < 0.05$

The antimicrobial activity of *Amaranthus viridis* was measured using the diameter at the zone of inhibition around each of the disc (Table 2-5). The microbial inhibition was higher in the leaf extract when compared with the root extract. Ethanolic extract at different concentration (2000, 1000, 500, 250, 125mg/ml) exhibited antibacterial activity against test organisms. In a dose dependent manner, zone of inhibition was found to decrease with decreasing concentration.

For the leaf extract, inhibition on tested organisms decreased dose dependently at their various minimum inhibitory concentrations (MIC). The highest inhibition of the leaf extract was experience on *S. aureus* (7.30 ± 0.10 mm) at 2000mg/ml, followed by *S. typhi* (5.60 ± 0.10 mm), *P. aeruginosa* (5.10 ± 0.10 mm) and *E. coli* (2.30 ± 0.10) respectively all at the same extract concentration of 2000mg/ml. The leaves extract indicates that *S. typhi* and *S. aureus* had the least MIC (125mg/ml), followed by *P. aeruginosa* and *E. coli* (250mg/ml) while *S. pneumonia* and *C. albican* had the highest MIC (500mg/ml).

For the root extract, inhibition on tested organisms also decreased dose dependently at their various minimum inhibitory concentrations (MIC). The highest inhibition of root extract was experienced on *S. pneumonia* (3.80 ± 0.10 mm), followed by *S. aureus* (2.80 ± 0.10 mm), *E. coli* (2.40 ± 0.10 mm) and *S. typhi* (0.60 ± 0.10 mm) respectively and at the same extract concentration of 2000mg/ml. *E. coli* and *P. aeruginosa* had the least MIC (250mg/ml), followed by *S. aureus* (500mg/ml) while *S. typhi*, *S. pneumonia* and *C. albican* had the highest MIC (1000mg/ml).

The antibacterial activities of ethanolic extract of *A. viridis* leaf and root were compared to that of ciprofloxacin, a broad spectrum antibiotic used as standard. The leaf and root extracts used in this study inhibited both gram positive and gram negative bacteria. This indicates that it has broad spectrum antimicrobial activity. *Staphylococcus aureus* and *E. coli* are organism implicated in nosocomial infection (Prescott et al., 2005); hence, the antagonistic activity of these extracts to such organisms may be vital in the clinical management of nosocomial infections. This natural herb is therefore effective in controlling microbial growth by selectively inhibiting growth, protein synthesis, cell wall and membrane and nucleic acid synthesis and the essential metabolic pathways that exist in the bacteria.

Table 2. Antimicrobial activity of *Amaranthus viridis* leaf extract and ciprofloxacin.

Pathogen	Zone of inhibition of <i>A. viridis</i> (2000mg/ml)	Zone of inhibition of ciprofloxacin (500mg/ml)
<i>S. typhi</i>	5.60 ± 0.10^b	9.80 ± 0.10^a
<i>S. aureus</i>	7.30 ± 0.10^b	8.30 ± 0.10^a
<i>P. aeruginosa</i>	5.10 ± 0.10^b	6.80 ± 0.10^a
<i>S. pneumonia</i>	2.77 ± 0.15^b	8.30 ± 0.10^a
<i>C. albican</i>	2.37 ± 0.15^b	6.50 ± 0.10^a
<i>E. coli</i>	2.30 ± 0.10^b	6.83 ± 0.57^a

There is significant ($p < 0.05$) change between ciprofloxacin and the 2000mg/ml concentration.

Table 3. Antimicrobial activity of *Amaranthus viridis* leaves at various concentrations.

Pathogen	2000mg/ml	1000mg/ml	500mg/ml	250mg/ml	125mg/ml	MIC mg/ml
<i>S. typhi</i>	5.60 ± 0.10^a	4.30 ± 0.10^b	3.83 ± 0.05^c	2.10 ± 0.10^d	0.80 ± 0.10^e	125
<i>S. aureus</i>	7.30 ± 0.10^a	5.76 ± 0.15^b	3.23 ± 0.15^c	2.33 ± 0.15^d	0.63 ± 0.05^e	125
<i>P. aeruginosa</i>	5.10 ± 0.10^a	3.70 ± 0.10^b	2.30 ± 0.10^c	0.50 ± 0.10^d	0.00 ± 0.00^e	250
<i>S. pneumonia</i>	2.77 ± 0.15^a	1.20 ± 0.10^b	0.50 ± 0.10^c	0.00 ± 0.00^d	0.00 ± 0.00^e	500
<i>C. albican</i>	2.37 ± 0.15^a	0.70 ± 0.10^b	0.13 ± 0.05^c	0.00 ± 0.00^c	0.00 ± 0.00^e	500
<i>E. coli</i>	2.30 ± 0.10^a	1.10 ± 0.10^b	0.50 ± 0.10^c	0.23 ± 0.05^d	0.00 ± 0.00^e	250

The antimicrobial activity of the leaf extract significantly ($p < 0.05$) increased as the concentration increase.

Table 4. Antimicrobial activity of *Amaranthus viridis* roots extract and ciprofloxacin.

Pathogen	Zone of inhibition of <i>A. viridis</i> (2000mg/ml)	Zone of inhibition of ciprofloxacin (500mg/ml)
<i>S. typhi</i>	0.60 ± 0.10 ^b	8.10 ± 0.10 ^a
<i>S. aureus</i>	2.80 ± 0.10 ^b	6.76 ± 0.15 ^a
<i>P. aeruginosa</i>	1.76 ± 0.15 ^b	5.30 ± 0.10 ^a
<i>S. pneumonia</i>	3.80 ± 0.10 ^b	4.80 ± 0.10 ^a
<i>C. albican</i>	1.40 ± 0.10 ^b	5.70 ± 0.10 ^a
<i>E. coli</i>	2.40 ± 0.10 ^b	6.10 ± 0.10 ^a

There is a significant ($p < 0.05$) difference between ciprofloxacin and the 2000mg/ml concentration.

Table 5. Antimicrobial activity of *Amaranthus viridis* roots at different concentration.

Pathogen	2000mg/ml	1000mg/ml	500mg/ml	250mg/ml	125mg/ml	MIC mg/ml
<i>S. typhi</i>	0.60 ± 0.10 ^a	0.30 ± 0.10 ^b	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c	1000
<i>S. aureus</i>	2.80 ± 0.10 ^a	2.20 ± 0.10 ^b	0.30 ± 0.10 ^c	0.00 ± 0.00 ^d	0.00 ± 0.00 ^d	500
<i>P. aeruginosa</i>	1.76 ± 0.15 ^a	1.30 ± 0.10 ^b	0.30 ± 0.10 ^c	0.20 ± 0.10 ^d	0.00 ± 0.00 ^e	250
<i>S. pneumonia</i>	3.80 ± 0.10 ^a	2.20 ± 0.10 ^b	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c	1000
<i>C. albican</i>	1.40 ± 0.10 ^a	0.70 ± 0.10 ^b	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c	1000
<i>E. coli</i>	2.40 ± 0.10 ^a	2.10 ± 0.10 ^b	0.90 ± 0.00 ^c	0.70 ± 0.10 ^d	0.00 ± 0.00 ^d	250

There is a significant ($p < 0.05$) difference at concentration 2000mg/ml and 1000mg/ml but there is no significant ($p > 0.05$) difference from 500mg/ml below

Conclusion

Amaranthus viridis is of great pharmacological relevance due to the presence of laxative as well as antimicrobial effect possessed by the plant in addition to the various physiological effects earlier reported by other researchers. The results of this study justify the use of the leaves of *Amaranthus viridis* as laxative in traditional medicine.

Authors' contributions

This work was carried out in collaboration between both authors. Author ODO designed the study, wrote the protocol and wrote the first draft of the manuscript. Author AO managed the literature searches, analyses of the study performed the analysis and managed the experimental process Both authors read and approved the final manuscript.

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