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ANTIMICROBIAL EFFECTS OF THE LEAVES EXTRACTS FROM *AMARANTHUS VIRIDIS* L.

K.V. SABLE AND R.R. SASWADE*
Radhabai Kale Mahila Mahavidhyalaya, Ahmednagar
* Jijamata College of Science and Arts, Bhende, Tal. Newasa, Dist. Ahmednagar

ABSTRACT

The emergence of drug-resistant bacteria strains encouraged the study of natural products from plants with potential promise for clinical use. The purpose of this study was to investigate *in vitro* the antibacterial and antifungal effects of aqueous, ethanol and chloroform extracts from different parts of *Amaranthus viridis* L. The antimicrobial activity was evaluated using the agar diffusion method. Each extract exhibited antimicrobial activity against strains (*Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa)*. In addition, the chloroform extracts from *A. viridis* dry leaves had significant antibacterial and antifungal activity. The results of the combined action of the different antibiotics and chloroform extract did not display synergism. This study indicates that different amaranth extracts are antibacterial and mostly antifungal and may represent a future therapeutic strategy.

Keywords: *Amaranthus viridis*, antifungal and antibacterial activity.

INTRODUCTION

Plants have been exploited for their food value and for their therapeutic effect in the traditional systems of medicine. Some plants have the potential to be useful antibacterial and antifungal agents. The development of resistance among Gram-positive and Gram-negative species to many antimicrobial agents can seriously compromise the effectiveness of antibiotic treatment and there is clearly an urgent need for the discovery of new synthetic or plant-derived drugs. Thus, a number of bioactive molecules from various plants have been exploited, due to their therapeutic effects. Over 150.000 of novel natural molecules derived from various herbs and microorganisms are mentioned in the literature.

Amaranthus viridis (*Amaranthaceae* family) is an annual herb with a shallow taproot, stem up to 2 m high, simple or branched, and often hairy in the upper part. Leaves are alternate, long-stalked, ovate to rhombic-ovate and sparsely hairy. Amaranthus leaves have high nutrition value due to elevated concentrations of proteins, carbohydrates, lipids and minerals.

Nonetheless, *Amaranthus viridis* can be used as vegetable, forage, grain crop and medicinal plant and for the phytoremediation of contaminated sites. Moreover, weeds are highly resistant to plant pathogens so it is possible that some plant metabolites are also active against human pathogens.

Previous studies (Zhanh et al, 2013, Tharun et al, 2012, Jin et al, 2013, Maiyo et al, 2010) reported the antimicrobial activity of the *Amaranthus* species. To the best of our knowledge, just one paper focused on the antimicrobial activity of *A. viridis* seeds on phytopathogenic fungi. Considering the potential application in medicine, the present study evaluates the activity of different extracts from roots, stems, leaves and seeds of *A. viridis* against human pathogens. In the ongoing search for novel antimicrobial agents from Romanian plants, we report the evaluation of antimicrobial activity of leaves extracts from *A.viridis*.

MATERIALS AND METHODS

Plant material and preparation of extracts

The plants were collected surroundings of Ahmednagar city. The leaves, and the seeds were pulverized and extracted (1:10) with water, 80% ethanol and chloroform by mixing for 3 hours on a magnetic stirrer at room temperature, then filtrated. The chloroform extract was evaporated to dryness and the residue dissolved in DMSO before the antimicrobial test.

Susceptibility testing

The antimicrobial activity of leaves parts of *A. viridis* was tested by the disc diffusion method in Mueller-Hinton agar, according to the guidelines recommended by the National Committee of Clinical Laboratory Standards (NCCLS, 2000).

The *in vitro* antimicrobial profile of *A. viridis* was determined against three reference strains: *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* were used for microbial growth and susceptibility testing.

The plate bioassay technique was used. A standard suspension of each strain was prepared from fresh overnight culture and was mixed with 15 ml portions of molten Mueller-Hinton agar, in a sterile petri plates resulting in a final concentration. When the plates were solid, metal cylinders (6 mm diameter) were placed on the medium surface and the samples (0.2 ml) were pipetted into each well. A standard commercially disks of Ampicillin (10µg) and Chloramphenicol (30µg). The size of the zone of inhibition around the wells was measured after incubation at 37°C for 24 h. The values of diameter of the inhibition zones are expressed as mean of triplicates ± SD.

Test of combined antibacterial action

Antimicrobial synergy and antagonism were demonstrated in agar diffusion tests that offer a visually method todetect antimicrobial interactions. In the “two disc method” antimicrobial agents are placed on the surface of leaves plates, at distances from each other that are predicted upon optimum demonstration of antagonistic or synergistic action (Acar,1981).Results of the “two disc method” were correlated with another experiment designed to determine coactions, described by Natarajan et al. (2008). In order to detect combined antimicrobial activity in this type of test, the samples, at defined concentrations, are added into the molten agar and after the solidification, different antibiotic discs are placed on the plate surfaces. If the inhibition zone remained unchanged after incubation at 37^0 C for 24 h, there was no synergic effect.

RESULTS AND DISCUSSION

The agar disk diffusion procedure was one of the first methods for determining the *in vitro* microbial susceptibility to antimicrobial agents. In this microbiological assay the antimicrobial agent placed in a reservoir (sterile paper discs, cylinders) diffuses directly against seeded bacteria. We used the cylinder technique that is more sensitive by comparison with the method using paper discs. We tested the ability of different extracts from *A. viridis* to inhibit microbial growth and the results are shown in table 1.

All the extracts obtained from different plant material showed antimicrobial activity. These plant extracts showed a moderate antibacterial activity against *Staphylococcus aureus*when compared to the positive control ampicillin and chloramphenicol. In contrast, bacteria were not inhibited by either aqueous or ethanol extracts. This can be explained by the presence of the permeability barrier – the outer membrane which acts as a barrier to the penetration of antimicrobial molecules and the periplasmic space which contains enzymes that destroy the molecules introduced from outside.

Unlike the other *A. viridis* tested samples, the chloroform extract from leaves exhibited at concentration of 16 mg/ml, excellent activity against bacteria. The results demonstrate the similar potential between the chloroform extract from seeds and ampicillin, or chloramphenicol against standard strains of *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*.

Table 1. Antimicrobial activity of different extracts of *A. viridis* plant material

Sr. No.	**Sample** (0.2 ml volume)	**S. aureus**	**E. coli**	**P. aeruginosa**
1.	Leaves - aqueous extract	10±0.1	0	0
2.	Leaves -ethanol extract (80 %)	11±0.4	0	0
3.	Leaves- chloroform extract	15±0.4	18±0.3	18±0.4
4.	Ampicillin (10 µg)	25±0.6	20±0.5	18±0.5
5.	Chloramphenicol (30 µg)	23±0.3	21±0.3	16±0.2

The possibility that chloroform extract from *A. viridis* leaves may have synergistic or antagonistic interaction with different therapeutic antimicrobial agents has been explored by the *in vitro* susceptibility test methods. Analysis of our data did not demonstrate a significant relationship between these samples and different antimicrobial agents (Table 2). The enhancement of antibacterial activity of chloroform extract from *A. viridis* leaves by the addition of ampicillin, chloramphenicol, tetracycline and ciprofloxacin to form a potent combination in inhibiting both Gram-positive and Gram-negative bacteria, is not relevant.

The combined antibacterial effect may be greater than that which each agent alone could achieve and indicates antimicrobial synergy. Together these two compounds may potentiate each other activity at a biochemical level or one may assist the other to penetrate into the microbial cell or may protect from destruction or the agents may act separately against the microorganism. When the effect of one agent is reduced by the presence of another, the combination is antagonistic. In our case, these two agents, the chloroform extract of seeds and ampicillin, chloramphenicol, tetracycline or ciprofloxacin tested together demonstrated indifference, because the combined action was no greater than that of the individual compounds.

As show in Table 1, *A. viridis* extracts exhibited antimicrobial abilities. All extract types were clearly less effective than the chloroform extract from the plant seeds, which was the most active against all tested strains, with the largest inhibition zones.

Table 2. *In vitro* combined effect of chloroform extract from leaves of *A. viridis* and some antimicrobial agents

Organism	Diameter of inhibition zone (mm)			
	Antimicrobial agents alone		The combined effect	
	A	C	A+Lce	C+Lce
Staphylococcus aureus	27.0	25.5	26.5	26.0
Escherichia coli	21.0	24.0	21.0	24.0
Pseudomanos aeruginosa	19.0	18.0	19.5	18.0

A- ampicillin (10µg); C-chloramphenicol(30µg); T- tetracycline (30µg); Cip – ciprofloxacin (30µg); Lce – leaves chloroform extract

The literature data show that from the different extracts of *A. viridis* tested in our study showed antimicrobial activity due probably to the presence of active constituents like antimicrobial peptides. Our results indicate that the active compounds from leaves of *A. viridis* have also the abilityto inhibit *in vitro* Gram-negative strains (*Escherichia coli* and *Pseudomanos aeruginosa*). At concentrations, the leaves chloroform extract had a good antibacterial activity.

The results are in agreement with other reports which showed that bioactive principles of *Amaranthus* species manifest antimicrobial activity. It has been shown that *A. tricolor* leaf extracts and the whole grass extracts from *A. viridis* have significant antimicrobial activity (Tharun et al, 2012, Jin et al, 2013). The ethyl acetate extracts (40-100 mg/ml) from the leaves and stems of *A. mangostanum* inhibited the growth of *Pseudomonas solanacearum, Acidovoraxavenae, Rhizoctonia solani, Collelotrichum capsici, Pseudomonas aeruginosa, Bacillus cereus* and *Escherichia coli* (Zhang et al, 2013).

Mayo et al. (2010) report that different extracts from the leaves of *A. hybridus, A. spinosus* and *A. caudatus* showed a broad spectrum of antibacterial activity against Gram-positive and Gram-negative bacteria. According to these authors, the minimum inhibitory concentrations (MICs) of amaranth extracts against species (*Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Proteus mirabilis* and *Klebsiella pneumoniae*) ranged between 129-755 mg/ml (Mayo et al, 2010). In our experiments the chloroform extract obtained from the leaves of *A. viridis* is active against tested strains at significant lower concentrations.

CONCLUSIONS

In summary, the main result of the present study indicates that all extract types from *A. viridis* exhibited antimicrobial activity against bacteria. The agar diffusion method indicated the highest antifungal activity for the chloroform extract from laves of *A. viridis*, which was also the most active against bacteria species. These data, in combination with other studies on amaranth suggests that *A.viridis* antimicrobial compounds, probably peptides, may be a useful source for the discovery of new antimicrobial agents.

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