African Journal of Biotechnology Vol. 9 (21), pp. 3178-3182, 24 May, 2010
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Phytochemical constituents and antimicrobial activity of leaf extracts of three *Amaranthus* plant species

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Accepted 16 June, 2009

This study investigated the phytochemical constituents and antimicrobial activity of hexane, ethyl acetate, dichloromethane and methanol leave extracts of *Amaranthus hybridus, Amaranthus spinosus* and *Amaranthus caudatus.* The microorganisms assayed for antimicrobial activity were: the gram-positive *Staphylococcus aureus* and *Bacillus* spp, the gram-negative *Escherichia coli, Salmonella typhi, Pseudomonas aeruginosae, Proteus mirabillis* and *Klebsiella pneumoniae* and a pathogenic fungus *Candida albicans*. The leave extracts showed a broad spectrum anti-bacterial activity but resistance to the fungus. Commonly encountered phytochemical constituents in the leaf extracts of the three *Amaranthus* species included flavonoids, steroids, terpenoids and cardiac glycosides. The minimum inhibitory concentration (MIC) exhibited by *A. spinosus* extracts against the *Salm. typhi* was 129 mg/ml. The MIC exhibited by *A. hybridus* extracts against the tested organisms ranged between 200 and 755 mg/ml whereas that of *A. caudatus* was between 162.2 and 665 mg/ml. The antimicrobial properties of these plants which have been used by mankind for centuries without any signs of toxicity can be used in the traditional herbal medicines which play a very important role in primary care systems in the developing world and are becoming increasingly popular in the developed world.

Key words: *Amaranthus* spp., phytochemical constituents, antimicrobial activity.

INTRODUCTION

Many studies have been undertaken with the aim of determining the different antimicrobial and phytochemical constituents of medicinal plants and using them for the treatment of both topical and systemic microbial infections as possible alternatives to chemical synthetic drugs to which many infectious microorganisms have become resistant (Akinpelu and Onakoya, 2006; Chopra et al., 2007). Plants have provided a source of inspiration of novel drug compounds, as plant derived medicines have made large contributions to human health and well-being. Their role is two fold namely; they provide key chemical structure for the development of new antimicrobial drugs and also as a phytomedicine (Abukakar et al., 2008) to be used for the treatment of disease.

A wide variety of indigenous and minor crops have been utilized for daily consumption since ancient times. They are not only important ingredients of unique gastronomic dishes but also traditional functional food to maintain wellness (Kazuhiko et al., 2002). In order to elucidate such a phenomenon, as well as seek highly effective plants, a number of plant extracts and isolated compounds have been tested for their bioactivity by various *in vitro* model systems. Information on the biological functions and active constituents of each plant species may contribute to the improvement of food habits and public health in tropical countries. Furthermore, it is expected that the wide use and extension in the utilization of such local agricultural products would increase and stabilize the income of farmers in the rural areas (Kazuhiko et al., 2002).

Amaranthus spinosus is an annual weed that is widely distributed in the humid zone of the tropics including Kenya. The weed has been reported to have some pharmacological properties (Ayethan et al., 1996). Extracts of the leaf had also been used in the treatment of menstrual disorders in man (Ayethan et al., 1996). The plant is used as a sudorific and febrifuge

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and is recommended for eruptive fevers. The leaves are considered a good emollient, lactogogue and a specific treatment for colic (Ayethan et al., 1996). Externally, the bruised leaves are applied locally to treat eczema (Leyel, 1987). *A. caudatus* is domesticated mostly for its grain. It prefers well-drained fertile soil of light sandy, medium loamy and heavy clay type within a sunny environment. The plant prefers acid, neutral and alkaline soils (Faccila, 1990).

Amaranthus hybridus named “Terere” by a majority of communities in Kenya is cultivated in several areas of the world including South America, Africa, India, China and the United States (He et al., 2002). In Kenya, their leaves are eaten as spinach or green vegetables. In Nigeria, *Amaranthus* leaves combined with condiments are used to prepare soup (Oke, 1983). These leaves boiled and mixed with a groundnut sauce are eaten as salad in Mozambique (Oliveira and De Carvalho, 1975) or pureed into a sauce and served over (farinaceous) vegetables in West Africa (Martin and Telek, 1979). The plant is used in the treatment of intestinal bleeding, diarrhoea and excessive menstruation (He et al., 2003). Nature has been a source of medicinal agents for thousands of years. Although advances have been made in pharmacology and synthetic organic chemistry, this reliance on natural products, particularly on plants, remains largely unchanged (Trevor, 2001). It is well established that some plants contain compounds able to inhibit microbial growth (Evarando et al., 2005). These plant compounds have different structures and different action when compared with antimicrobials conventionally used to control microbial growth and survival (Nascimento et al., 2000). The potential antimicrobial properties of plants are related to their ability to synthesize by secondary metabolism several chemical compounds of relatively complex structures with antimicrobial activity, including tannins, phlobatannins, alkaloids, coumarins, cardiac glycosides, terpenes, phenylpropanes, organic acids, flavonoids, isoflavonoids and saponins (Evarando et al., 2005; Matasyoh et al., 2009).

Because of the emerging development of drug resistance by pathogenic microorganism against synthetic antibiotics; attention has now shifted to extracts of biologically active components isolated from plant species used as herbal medicine. Medicinal plants may offer a new source of antibacterial, antifungal and antiviral activities. This study was aimed at determining and comparing the phytochemical constituents and *in vitro* evaluation of antimicrobial activity of the hexane, ethyl acetate, dichloromethane and methanolic extracts of *Amaranthus hybridus, A. spinosus* and *A. caudatus.*

MATERIALS AND METHODS

Plant material

Fresh leaves of *A. hybridus, A. spinosus* and *A. caudatus* were collected from Kakamega district in Kenya. Botanic identification was performed at the Egerton University, Botany Department. Voucher samples were prepared and deposited in the herbarium of the Botany Department of Egerton university. The leaves were air-dried to crispiness in the laboratory at room temperature of 25 - 26^{0}C. The specimen were then milled and the powdered sample were then stored in an air-tight container for further use.

Test microorganisms

The test microorganisms used for antimicrobial sensitivity testing included *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and clinical isolates of *Bacillus* spp, *Salmonella typhi*, *Klebsiella pneumoniae, Proteus mirabillis* and *Candida albicans*. The microorganisms were sourced from the Centre for Microbiology Research of the Kenya Medical Research Institute (KEMRI).

Preparation of solvent extracts

Powdered 500 g of each of the 3 plant species were separately and sequentially extracted in cold with 2 × 1L of distilled hexane, ethyl acetate, dichloromethane and methanol for 2 days in each of the solvent in order of polarity as hexane < ethyl acetate < dichloromethane < methanol. The mixtures of each solvent were then filtered through active charcoal and the filtrates were dried *in vacuo* using a rotatory evaporator. The filtrate of each solvent was evaporated to a residue in a drying cabinet. The percentage yield was 2.52, 8.16, 0.64 and 11.15% w/w for hexane, ethyl acetate, dichloromethane and methanol, respectively.

Phytochemical screening

The freshly collected extract fractions of *A. hybridus, A. spinosus* and *A. caudatus* were tested for the presence of phytochemical constituents. These were identified by characteristic colour changes using standard procedures (Trease and Evans, 1983).

Pharmacological screening

The antimicrobial activity of the extract fractions were tested according to the national committee of clinical laboratory standards protocol (CLSI, 2007). Freshly cultured microbial suspensions in Mueller Hinton broth were standardized to a cell density of $1.5x10^8$/ ml (McFarland No. 0.5). Antibacterial assay was carried out on Muller Hinton agar while antifungal activity was done using sabourauds dextrose agar. The active extract fractions were serially diluted in the respective solvent used for its extraction. The active extract fractions were diluted and used at concentrations of 25, 33, 50 and in undiluted 100% concentration. The positive antibacterial and antifungal activities were established by the measurable zones of inhibition after 24 h of incubation at 37^{0}C. Minimum inhibitory concentration (MIC) was defined as the lowest concentration that inhibited growth of the microorganism detected visually. Chloramphenicol and nystatin were used as positive standard control antibiotic and antifungal drugs, respectively. All tests were carried out in triplicate.

Statistical treatment of the results

The results were expressed as means ± standard error (SE). Significance of differences compared to the control groups was determined using students t-test.

Table 1. Phytochemical constituents of leaf extract of *A. hybridus, A. spinosus* and *A. caudatus.*

	Hexane			Ethyl acetate			Dichloromethane			Methanol		
Phytochemical constituent	***A. hybridus***	***A. caudatus***	***A. spinosus***	***A. hybridus***	***A. caudatus***	***A. spinosus***	***A. hybridus***	***A. caudatus***	***A. spinosus***	***A. hybridus***	***A. caudatus***	***A. spinosus***
Tannins	-	-	-	-	-	-	-	-	-	-	+	-
Phlobatannins	-	-	-	-	-	-	-	-	-	-	+	-
Saponins	-	-	-	-	-	-	-	-	-	-	-	+
Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+
Steroids	+	+	+	+	+	+	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	-	+	+	-	+	+	+
Cardiac glycosides	+	+	+	+	+	-	+	+	-	+	+	+

+ Represents presence of the phytoconstituent; - represents absence of the phytoconstituent.

RESULTS AND DISCUSSION

Medicinal plants play a central role not only as traditional medicines but also as commercial commodities meeting the demand of distant markets. To compete with the growing market, there is need to expeditiously utilize and scientifically validate more medicinally useful plants. Because of the appearance of drug resistance to antimicrobial agents, more effort is being made to find alternative antimicrobial components. It had been suggested that natural products are a preferable option to synthetic ones.

Literature indicates that medicinal plants are the backbone of traditional medicine (Fransworth, 1994) and the antibacterial activity of plant extract is due to different chemical agent in the extract with antimicrobial compounds (Rojas et al., 1992). In plants, these secondary metabolites function to attract beneficial and repel harmful organisms, serve as phytoprotectants and respond to environmental changes. In humans, however the compounds have beneficial effects including antioxidant, anti-inflammatory effects, modulation of detoxification enzymes, stimulation of the immune system, modulation of steroid metabolism and antibacterial and antiviral effects (Johanna, 2003). Results from the current study indicate that *A. hybridus*, *A. spinosus* and *A. caudatus* leave extract contained varied types of pharmacologically active compounds with antimicrobial activity. The commonly identified components in the 3 species included flavanoids, steroids, terpenoids and cardiac glycosides. In addition tannins and phlobatanins were also present in *A. caudat*a while saponins were present in *A. spinosus* (Table 1).

The various leave extracts demonstrated varied antimicrobial activity to the test organism which was species and concentration dependent. *A. hybridus* extracts were active against *E. coli*, *Salm. typhi*, *K. pneumoniae* and *P. aeruginosae* with minimum inhibitory concentration ranging between 200 and 755 mg/ml (Table 2). A. caudatus was active against *E. coli*, *Salm. typhi*, *P. mirabilis, Staph. aureus and Bacillus* sp. However *A. spinosus* extracts were only active against *Salm. typhi.* All species extracts were however ineffective against *C. albican* test fungi.

Flavanoids (Mendosa et al., 1997), terpenoids (Aurelli et al., 1992; Cowan, 1999), tannins and phlobatanins (Stern et al., 1996) are phytochemicals that have been demonstrated to have antimicrobial activity.

The currents results support findings of Broekaert et al. (1992) who demonstrated the presence of antimicrobial activity in seeds of *A. caudatus*. Tannins have been found to form irreversible complexes with proline-rich proteins (Akinpelu and Onakoya, 2006) resulting in the inhibition of the cell protein synthesis. Medicinally, this is important for the treatment of inflamed or ulcerated tissues (Akinpelu and Onakoya, 2006). Indeed herbs that have tannins as their main component are astringent in nature and are used for treating intestinal disorders such as diarrhoea and dysentery (Dharmananda, 2003). This is the basis for the antimicrobial use of such plants in the treatment of intestinal bleeding, diarrhoea and excessive menstruation (He et al., 2003).

Terpenoids on the other hand have been demonstrated to be active against bacteria, fungi, viruses and protozoa (Tassou et al., 1995; Cowan, 1999), which has enabled food scientists to use terpenoids present in essential oils of plants to control *Listeria monocytogenes* (Aurelli et al., 1992). The mechanism of action of terpenes is by lipophilic membrane disruption. Indeed, Mendoza et al. (1997) found that increasing the hydrophilicity of kaurene diterpenoids by addition of a methyl group drastically reduces their antimicrobial activity. Flavanoids on the other hand are known to be produced in plants in response to microbial infections (Dixon et al., 1983). *In vitro* they have been shown be

Table 2. Phytochemical constituents of leaf extract of *A. hybridus*, *A. spinosus* and *A. caudatus.*

Test organism	Inhibition zone (mm)							MIC (mg/ml)	
	Extract concentration (%, w/w)					+ve control[a]	-ve control[b]		
	100	50	33	25	20			Extract	+ve control[a]
A. hybridus									
Hexane									
E. coli	17.5 ± 0.7*	15 ± 1.4	11 ± 1.4	7.5 ± 0.7	0	48.3 ± 1.7	0	453	25
Salm. typhi	11.0 ± 1.4*	9.0 ± 1.4	0	0	0	33.7 ± 0.9	0	755	25
K. pneumoniae	15.0 ± 1.4*	13.0 ± 1.4	10.5 ± 0.9	0	0	37.7 ± 1.5	0	566	22.5
P. aeruginosa	13.0 ± 1.4*	11.0 ± 1.4	0	0	0	24.3 ± 2.3	0	755	-
Ethyl acetate									
Salm. typhi	11.0 ± 1.7*	9.0 ± 1.4	0	0	0	33.7 ± 0.9	0	200	25
Dichloromethane									
Salm. typhi	9.5 ± 1.7*	7.5 ± 3.3	0	0	0	33.7 ± 0.9	0	344	25
K. pneumoniae	17.5 ± 2.4*	15.0 ± 1.4	9.5 ± 1.7	0	0	37.7 ± 1.5	0	258	22.5
A. caudatus									
Ethyl acetate									
E. coli	11.0 ± 1.7*	0	0	0	0	48.3 ± 1.7	0	162.2	25
Dichloromethane									
Salm. typhi	11.0 ± 1.7*	9.0 ± 1.4	0	0	0	33.7 ± 0.9	0	665	25
P. mirabilis	13.5 ± 1.5*	11.0 ± 1.7	9.5 ± 1.5	0	0	34.3 ± 2.3	0	449	25
Methanol									
Salm. typhi	17.5±2.0*	15.0±1.4	0	0	0	33.7 ± 0.9	0	259.3	25
S. aureus	15.0 ± 1.2*	13.5±1.5	11.0 ± 1.7	7.5±3.3	0	37.7 ± 1.5	0	155.6	31.3
Bacillus spp[c]	13.5 ± 1.5*	9.5±1.5	7.5 ± 3.3	0	0	32.7 ± 1.5	0	194.5	26.3
A. spinosus									
Hexane									
Salm. typhi	13.0 ± 1.2*	11.0±1.7	0	0	0	33.7 ± 0.9	0	129	25

Results = mean ± standard error.
*Significantly lower compared with chloramphenicol.
[a]Positive control (chloramphenicol), [b]Negative control.

effective antimicrobial agents through complexing with extra-cellular and soluble proteins and also with bacterial cells (Tsuchiya et al., 1996).

Conclusion

The three species of *Amaranthus* leaves contain various pharmacologically active compounds. The leave extracts demonstrated antimicrobial activity that was plant species, extraction fraction and also concentration dependent. Results from the current study indicate that these plants are of ethno-pharmacological importance further confirming the pharmacological basis in the use of the said plant in traditional medicine for the treatment of infections and consumption. It is also hoped therefore that this study will contribute to the improvement of food habits and public health in Kenya and other tropical countries.

ACKNOWLEDGEMENT

We wish to acknowledge Dr. Kariuki S. T. of biological sciences department, Egerton University, Kenya for identifying the plants used in this study.

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