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Evaluation of Antibacterial Activity of Selected Ethnomedicinal Plants for Poultry in Masaka District, Uganda

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Abstract: The antibacterial activity of ethanol and ether extracts of 30 medicinal plants used against poultry diseases in Masaka district of Uganda was evaluated. The extracts were tested against gram positive (*Staphylococcus aureus*, *Streptococcus faecalis*) and gram negative bacteria (*Escherichia coli*, *Salmonella typhimurium*) using the agar well diffusion assay. Both ethanol and ether extracts showed activity (100 and 97%, respectively) against at least one bacterium. In general, gram positive bacteria were more susceptible than gram negative bacterial species. Of the four bacteria species, *Staphylococcus aureus* was the most susceptible to both extracts. These results therefore suggest that these plants can be used to provide lead compounds which can be used in the discovery of new antibacterials.

Key words: Ethnomedicinal plants, crude extracts, antibacterial activity, bacteria, poultry, Uganda

INTRODUCTION

The production of medicines and the pharmacological treatment of diseases began with the use of herbs (Tyler, 1997). Many of the drugs currently used to treat bacterial and other infections were 1st isolated from natural sources including ethnomedicinal plants (Coe and Anderson, 1996). Plant based antimicrobials represent a vast untapped source of medicines with enormous therapeutic source potential (Cowan, 1999). Herbs represent one of the 1st pharmacological interventions attempted by healers and even today, 25% of the conventional drugs are plant derived in a traditional format (SPORE, 1992).

High levels of antibiotics use, often clinically unnecessary has led to a steady increase in drug resistance and low-income countries, home to the majority of the world's population are particularly affected by this phenomenon (Radyowijati and Haak, 2003). Antibiotic resistant strains of bacteria are an increasing threat to animal and human health with resistance mechanisms having been identified and described for all known antimicrobials currently available for clinical use (McDermott *et al*., 2002). This therefore, necessitates a constant search for newer antibacterial substances.

The use of medicinal plants as traditional medicines is well known in rural areas of many developing countries (Sandhu and Heinrich, 2005; Gupta *et al*., 2005). Traditional healers claim that their medicine is cheaper and more effective than modern medicine. Many plants have been used because of their antimicrobial traits whcih are chiefly synthesized during secondary metabolism of the plant (Heinrich *et al*., 2004). The present study was aimed at screening *in vitro* antibacterial activity of plants used to treat poultry diseases against some major disease causing bacteria in Uganda.

MATERIALS AND METHODS

Study area: Plants were obtained from Kyanamukaka and Buwunga sub-counties of Masaka district. Thirty plants were considered for this study. Antibacterial screening tests were done at the Microbiology Laboratory of the School of Veterinary Medicine of Makerere University.

Plant collection and botanical identification: Thirty plants used by poultry farmers were collected and taken to the Botany Herbarium of Makerere University Kampala for species identification and taxonomic identity. The plants were then air dried under a shade at room temperature, i.e., 24°C for at least 2 weeks. The barks of the trees were further dried at 45°C in an oven overnight to completely remove residual moisture before milling into fine powder. The plant material from plant parts specified by the farmers was ground in a grinding machine. The powders were then sealed in air-tight polyethylene bags and stored in a cool dry place.

Extraction procedure: Extracts were prepared using the cold extraction methods. The plant samples were extracted using pure analytical solvents, i.e., a polar solvent (ethanol, BDH, UK) and a non polar solvent (diethyl ether,

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BDH, UK). Briefly, the grounded powder was weighed on Satorius balance type BA610 and soaked for 3 days at room temperature with intermittent shaking. Filtration through cotton wool was done to remove coarse particles and then through filter paper (Whatman No.1, England) in Buchner funnel to get a pure filtrate. Ether and ethanol extracts were obtained. The extracts were concentrated by evaporation using a rotary evaporator (Perkins, UK), weighed and reconstituted in dimethyl sulfoxide [DMSO] (BDH, UK) to a concentration of 1 g mL^{-1}. These samples were then stored in a refrigerator at 4°C and later used in the proceeding antibacterial tests.

Antibacterial assays: The antibacterial assay was carried out using agar well diffusion tests. The antimicrobial activity of the plant extracts was tested on four standard bacteria species namely; *Streptococcus feacalis* (wild strain), *Staphylococcus aureus* (ATCC 25923), *Eschericia coli* (ATCC25922) and *Salmonella typhimurium* (ATCC14028). These were standard laboratory cultures whose susceptibility on commonly used antibiotics was already established. *Streptococcus feacalis*, *Staphylococcus aureus* represented gram positive bacteria while *Eschericia coli* and *Salmonella typhimurium* represented gram negative bacteria. These bacteria species are also responsible for a cross-section of poultry disease conditions namely; staphylococcosis, colibacillosis, salmonellosis and secondary bacterial infections which become opportunistic during viral infections. A standardized bacterial suspension was prepared by picking a colony of respective bacteria using sterile wire loop and suspending it in 5 mL of Brain heart infusion liquid media (Mast diagnostics, UK). The dilutions formed the bacterial stock solutions for use in the agar well diffusion assays as outlined below.

Agar well diffusion assay: The agar well diffusion technique was the standard method used to determine the antibacterial activity of the bioactive compounds. Briefly in the method, the media, i.e., Mueller hinton agar (Becton Dicknson M.D, USA) was prepared and treated according to manufacturer's guidelines where 35 g of media was mixed with 1 L of distilled water and enclosed in a container and autoclaved at 121°C for 15 min. The media was later dispensed into 90 mm sterile agar plates (Oxoid, UK) and left to set.

The agar plates were incubated for 24 h at 37°C to confirm their sterility. Absence of growth after 24 h showed that the plates were sterile. The sterile Mueller Hinton agar plates were inoculated with the test culture by surface spreading using sterile wire loops and each bacterium evenly spread on the entire surface of the plate to obtain uniformity of the inoculum. The culture plate then had at most four wells of 6 mm diameter and 5 mm depth made into it using a sterile agar glass borer. Gentamycin was used as a positive control while normal saline was used as a negative control. Approximately, 0.2 mL of the bioactive test compound of concentration 1 g mL^{-1} was suspended in the wells and thereafter inoculated plates/culture were incubated for 24 h at 37°C.

The plates/cultures were examined for the presence of bacterial inhibition zones around each well. Antibacterial activity was determined by the presence of a zone of inhibition around the wells. Single readings were carried out. Non-active compounds did not show any inhibition zone. The zones of inhibition were measured using a ruler and a pair of dividers (Picfare, Uganda) and results were reported in millimetres (mm). All inhibition zone diameters were considered important, since the extracts from the plants were still crude. A zone size interpretive chart was then drawn to show the different plant extracts and their corresponding inhibition zone diameter to the nearest millimetre.

RESULTS AND DISCUSSION

Antibacterial screening tests: Thirty plants were screened for antibacterial activity. The antibacterial activities of the plant extracts varied according to the species of bacteria tested and the solvents used. For all the thirty plants tested at least one plant extract produced a zone of inhibition against at least one bacteria species. For both extracts, *Staph. aureus* was the most susceptible of the four organisms. *E. coli* was the least susceptible to ethanol plant extracts while *Strep. feacalis* was the least susceptible to ether plant extracts.

Antibacterial activity of ether extracts: For the ether extracts, 97% of the thirty tested plants had antibacterial activity against at least one of the four bacteria species as shown in Table 1. Of these plants (n = 29), 24% had activity on all the four bacteria species, 45% on only three bacteria species, 17% on only two bacteria species while 14% showed activity on only one bacteria species.

Of all the plants whose ether extracts (n = 29) showed antibacterial activity, 97% were active on gram positive bacteria while 76% were active on gram negative bacteria. All plants that had activity on gram positive bacteria were active on *Staph. aureus* while 52% were active on *Strep. feacalis*. Of all the plants that showed activity, 69% were active on *S. typhymurium* while 66% were active on *E. coli*.

Table 1: Antibacterial screening of the extracts

	Strep. faecalis		*Staph. aureus*		*E. coli*		*S. typhymurium*	
Plant extract	1	2	1	2	1	2	1	2
Datura stramonium	0.9	1.1	1.5	1.3	0.0	0.0	0.0	0.0
Moringa oleifera	1.1	4.2	2.8	1.3	0.0	1.1	0.0	0.0
Azadirachta indica	1.8	0.8	2.2	1.7	1.2	0.0	0.0	0.0
Senecio cydoniifolius	1.1	1.1	2.3	1.3	2.7	0.0	1.3	0.0
Carica papaya	1.3	1.0	1.9	1.6	0.0	0.0	1.8	1.4
Lantana trifolia	0.0	1.4	2.4	2.1	0.0	1.0	0.0	0.0
Sida cuneifolia	1.1	1.2	2.0	2.3	1.5	1.5	1.5	1.4
Vernonia cineria	1.3	1.1	3.2	2.3	2.1	0.0	0.0	0.0
Jatropha curcas	1.2	1.8	0.9	0.0	0.0	0.0	0.0	0.0
Nicotiana tobaccam	1.5	1.0	1.5	1.4	2.1	1.2	1.2	0.0
Tetredenia riparia	0.0	0.0	2.4	2.6	1.5	1.2	1.5	0.0
Capsicum annuum	0.0	0.0	2.9	1.9	2.2	1.0	1.4	0.0
Agave sisalana	0.0	0.0	2.3	1.2	1.1	0.0	2.0	1.6
Aloe vera	0.0	0.0	2.5	1.4	2.2	0.0	2.5	2.0
Justicia betonica	0.0	0.0	0.0	0.0	0.0	0.0	1.0	1.2
Chenopodium opulifolium	1.0	2.0	1.0	1.2	0.0	0.0	0.0	1.6
Desmodium salicifolium	1.6	1.0	1.5	1.3	2.3	1.3	2.6	2.1
Rubia cordifolia	0.0	0.0	1.4	1.2	1.6	0.0	1.9	0.0
Vernonia amygdalina	1.4	1.3	3.1	1.4	2.6	0.0	2.4	1.3
Persea americana	0.0	1.2	1.7	1.6	0.0	0.0	1.6	0.0
Aspilia africana	1.2	0.0	2.6	0.8	2.9	0.0	2.5	0.0
Syzygium cuminii	0.0	0.0	1.9	1.6	2.0	0.0	2.5	0.0
Solanum mauritianum	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0
Albizia coriaria	1.5	1.7	1.7	2.0	0.0	0.0	0.0	1.6
Momordica foetida	0.0	0.0	1.1	1.2	1.3	0.0	2.3	0.0
Conyza floribunda	0.0	1.0	1.9	1.3	2.1	1.5	2.7	2.0
Phaseolus lunatus	0.0	0.0	1.9	1.3	1.3	0.0	2.0	1.3
Leonotis nepetifolia	0.0	1.1	1.7	1.3	0.0	0.0	0.0	0.0
Garcinia buchananii	0.0	1.8	2.4	2.3	1.5	0.0	1.7	1.5
Bidens pilosa	1.4	0.0	3.5	1.3	3.4	0.0	3.6	0.0

1 = ether; 2 = ethanol

Antibacterial activity of Ethanol extracts: For the ethanol extracts, 100% of the thirty tested plants had antibacterial activity against at least one of the four bacteria species as shown in Table 1. Table 1 shows the size of the inhibition zones obtained for the ether and ethanol extracts of the test plants against the four test bacteria. The gram positive bacteria were more susceptible than the gram negative bacterial species. Of these plants, 10% had activity on all the four bacteria species, 27% on only three bacteria species, 37% on only two bacteria species while 27% showed activity on only one bacteria species.

Of all the plants whose ethanol extracts (n = 30) showed antibacterial activity, 97% were active on gram positive bacteria while 53% were active on gram negative bacteria. About 93% of the extracts were active on *Staph. aureus*, 60% were active on *Strep. feacalis*, 40% showed activity on *S. typhymurium* while 23% were active on *E. coli*.

The results from this study showed that the ether and ethanol extracts showed differences in the sizes of inhibition zones for the different test bacteria. All the plant extracts which showed any zones of inhibition of growth were considered to have activity and those with no zones of growth inhibition were considered to have no activity. Thus in this study, gram positive bacteria were found to have more susceptibility as compared to gram negative bacteria species. This is in line with earlier studies which attribute the observed differences to the variation in chemical composition and structure of cell wall of both types of microorganisms (Yaghoubi *et al*., 2007; Nair and Chanda, 2007).

For the gram negative bacteria, ether extracts exhibited greater antibacterial activity compared to ethanol extracts, probably due to the differences in the phytochemicals extracted. This is important, since gram negative bacteria have been shown to be less susceptible to many antibacterial substances owing to their structure. For example in >1/3 of the salmonella-poisoning cases in 1997, the bacteria were resistant to antibiotics used to treat the disease according to Centers for Disease Control and Prevention.

In the study, carried out by Moniri and Dastehgoli (2007), it was found that the prevalence rate of *E. coli* resistant to ciprofloxacin and erythromycin in the samples from chickens with colibacillosis was higher than in health controls. The transfer of resistant *E. coli* from chickens to humans is a common event, as has been demonstrated by several groups of researchers (Moniri and Dastehgoli, 2007). Compounds from ether extracts of promising plants may be of value in the discovery of new lead compounds which can be used as antibacterials.

The ether extracts of several plants, i.e., *Sida cuneifolia*, *Senecio cydoniifolius*, *Nicotiana tobaccum*, *Vernonia amygdalina*, *Aspilia africana*, *Bidens pilosa* and *Desmodium salicifolium* reacted on all four bacteria species and this may indicate presence of compounds with broad spectrum activity. It has been reported that the crude extracts of *Vernonia amygdalina* have antimicrobial properties against gingivitis and toothache (Tella, 1976). Several of the compounds of *Vernonia amygdalina* are active against gram positive *Bacillus subtilis* and *Micrococcus lutea* (Kaufman *et al*., 1999). In a survey of antibacterial activity of South African herbs, *Bidens* sp. showed high activity against gram positive bacteria (Rabe and Van Staden, 1997). The ethanol extracts of *Sida cuneifolia*, *Cornyza floribunda* and *Desmodium salicifolium* showed activity on all four bacteria species and this may indicate presence of broad spectrum activity. Previous studies have shown that *Sida cuneifolia* has a good antibacterial activity on both gram negative and gram positive bacteria (Van-Vuuren and Viljoen, 2006) and phytochemical studies have shown that this plant contains alkaloids salts which may contribute to the antibacterial activity observed (Shelton, 1991).

CONCLUSION

In this study, there is considerable evidence that extracts from several plants used in this study have the potential to be developed into agents that can be used as preventative or treatment therapies for bacterial poultry diseases.

RECOMMENDATIONS

It is recommended that clinical trials on poultry are carried out to establish whether these plants offer therapeutic benefits either alone or in combination with other plants. This can help to reduce the overall burden of poultry diseases using cheaper methods especially among rural poor people worldwide. However while results of the ether extracts are promising, farmers are not advised to use volatile solvents to make extracts. Nevertheless, these plants may be of value to pharmaceutical companies in the search for newer drugs.

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