Studies on the antimicrobial effects of *Acacia nilotica* and *Vitex doniana* on the thermophilic *Campylobacter* species

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ABSTRACT

This study investigated the *in vitro* activity of extracts of *Acacia nilotica* and *Vitex doniana* against *Campylobacter jejuni, C. coli,* and *C. laridis* isolated from sheep in Zaria and Kaduna. The emergence of *Campylobacter* strains resistant to most common antibiotics calls for the exploration of new methods for therapeutics against *Campylobacter* infections. The dried, powdered bark of *V. doniana* and leaves of *A. nilotica* were subjected to petroleum ether extraction to remove the fat content. The crude extracts were then dissolved in water and ethanol and later evaporated at low temperature. Water and ethanol crude extracts of *A. nilotica* and *V. doniana* were tested on the thermophilic *Campylobacter* species. The results showed that ethanol extract of *A. nilotica* had minimal inhibition concentration (MIC) of 80 mg ml^{-1}, while water extract of this same plant had MIC of 250 mg ml^{-1}. However, ethanol extract of *V. doniana* had no inhibitory effects on the *Campylobacter* species tested. *Acacia nilotica* and *V. doniana* were tested at concentrations ranging from 2 to 200 mg ml^{-1} of the extracts. The ethanol extract of *A. nilotica* at 200 and 20 mg ml^{-1} had inhibitory diameter zones of 6 and 4 mm, respectively. The water extract of the same plant at 200 and 20 mg ml^{-1} had diameters of only 2 and 1 mm, respectively. The *V. doniana* water and ethanol extracts had very little or no inhibitions. This study has indicated that extracts of *A. nilotica* show antibacterial activities against *Campylobacter* species isolated from sheep in Zaria and Kaduna.

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RÉSUMÉ

RAJI, M. A., ADEKEYE, J. O., KWAGA, J. K. P. & BALE, J. O. O.: *Etudes sur les effets antimicrobiens d'*Acacia nilotica *et* Vitex doniana *sur la* Campylobacter *species thermophile.* Cette étude s'est déroulée pour enquêter sur l'activité *in vitro* des extraits d'*Acacia nilotica* et *Vitex doniana* contre *Campylobacter jejuni, C. coli* et *C. laridis* isolés des moutons en Zaria et Kaduna. L'apparition des souches de *Campylobacter* résistantes aux antibiotiques les plus communs fait appel à l'exploration de nouvelles méthodes pour les thérapeutiques contre les infections de *Campylobacter.* L'écorce de *Vitex doniana* séchée, réduite en poudre et les feuilles d'*Acacia nilotica* étaient exposées à l'extraction d'éther de pétrole pour retirer le contenu de graisse. Ceci était donc suivi par la dissolution d'extraits bruts de toutes deux en eau et éthanol et plus tard évaporés à une température basse. L'eau et l'éthanol d'extraits bruts d'*Acacia nilotica* et *Vitex doniana* étaient testés sur *Campylobacter* species thermophile. Les résultats obtenus montrent que l'extrait d'éthanol de *Acacia nilotica* avait concentration d'inhibition minimal (CIM) de 80 mg ml^{-1} alors que l'extrait d'eau de la même plante donnait une CIM de 250 mg ml^{-1}. Cependant, les extraits d'éthanol de *Vitex doniana* n'avaient pas d'effets inhibiteurs sur *Campylobacter* species testées. *Acacia nilotica* et *V. doniana* étaient testés aux concentrations variant entre 2 et 200 mg ml^{-1} des extraits. Extrait d'éthanol de *A. nilotica* aux concentrations de 200 et 20 mg ml^{-1} avaient des zones de diamètres inhibiteurs respectives de 6 et 4 mm. Extrait d'eau de la même plante aux concentrations de 200 et 20 mg ml^{-1} avaient des diamètres respectifs de 2 et 1 mm seulement. Il y avait très petites ou nulle inhibitions avec *Vitex doniana* extraits d'eau et d'éthanol. Cette étude a démontré que les extraits d'*Acacia nilotica* montrent les activités antibactériennes contre *Campylobacter* species isolées des moutons en Zaria et Kaduna.

Introduction

Campylobacter enteritis caused by thermophilic species (*C. jejuni* and *C. coli*) is frequently a mild to moderate self-limiting illness (Pilar *et al.*, 1986). *Campylobacter* species have been reported among healthy and diseased farm animals in many countries (Prescott & Brum-Mosch, 1981; Olubunmi & Adeniran, 1986).

Jiwa & Ishengoma (1985) reported *C. jejuni* and *C. coli* in several farm animals including goats at the Sokoine University of Agriculture, Morogoro, Tanzania. Raji *et al.* (2000) isolated *C. jejuni, C. coli,* and *C. laridis* from sheep in Zaria and Kaduna, Nigeria.

Animals are the commonest reservoir of *Campylobacter* infections, and they may serve as sources of human infection as a result of consumption of contaminated meat and meat products (Raji *et al.*, 1997). Herbs have long been used in treating animal and human diseases in modern medicine. Examples are cinhona bark for treating malaria and *Specacuantia* (Ipecac) for treating amoebic dysentery (Zaria, Akinniyi & Mshelia, 1995). Many plant extracts possess antimicrobial agents that are active against microorganisms *in vitro*. For example, Little, Thomas & Murray (1988) isolated a naturally occurring 2-methoxy, 1-4 naphthoquinone from *Impatiens balsamina* (Rose Balsam) which is active against several phytopathogenic organisms.

Some workers in Nigeria have reported that the sap and bark of *Pycnanthus angolensis* (African nutmeg) and extracts of *Cassia alata* (Dates Jaunes) inhibited the growth of various types of bacteria, including Gram negative and Gram positive bacteria; and also fungi such as *Trichophyton, Microsporium* and *Penicillium* species as well as *Aspergillus niger* (Sofowora, 1983; Babalola, 1988).

Some extracts of garlic, onion, green pepper, and raddish also inhibit the growth of *Escherichia coli, Salmonella typhosa, Shigella dysenteriae,* and *Staphylococcus aureus in vitro* (Sofowora, 1983; Thomas, 1983).

About 12,500 plants of *Acacia* found in the tropics have 134 species (representing 170 taxa) native to East Asia (FAO, 1983). *Acacia nilotica* is very useful in treating diarrhoea and cough in humans in Burkina Faso (Guinko, 1991). *Vitex doniana* is used to treat anaemia and the root is used for gonorrhoea (FAO, 1983). It is also used to improve fertility and to treat dysentery (Rulangaranga, 1989). Despite this known usefulness of *Acacia* species and *Vitex doniana,* relatively few have seemingly been investigated in Nigeria.

This study aimed at investigating *in vitro* activity of extracts of *A. nilotica* and *V. doniana* against *C. jejuni, C. coli,* and *C. laridis* isolated from sheep in abattoirs in Zaria and Kaduna.

Materials and methods

Extract preparation

Water (W. E) and ethanol (E. E) extracts of both *Acacia nilotica* and *Vitex doniana* were prepared by methods of Tijani-Eniola & Fawusi (1989) and Ibrahim *et al.* (1983). The dried, powdered bark of *V. doniana* and leaves of *A. nilotica* were subjected to petroleum ether extraction to remove the fat content. The crude extracts were then dissolved in water and ethanol and later evaporated at low temperature. The extracts were used for the study.

Microbiological assessment

An aliquot of 0.1 ml of 1% barium chloride was added to 9.9 ml of 1% tetraoxosulphate (VI) to give a McFarland turbidity standard suspension No. 1. This turbidity approximates bacterial density of 3×10^8 organisms per ml and was used to standardize bacterial inocula (Odama, Shock & Olurinola, 1986). About 0.2 ml of the standardized suspension of each test bacterial agent grown in nutrient broth was pipetted onto Muller Hinton agar plates and spread evenly with the aid of glass rods on the agar (Odama *et al.*, 1986).

The paper discs of various concentrations of ethanol and water extracts of *A. nilotica* and *V. doniana* were placed on agar. The concentrations

of 2, 20 and 200 mg ml^{-1} of the extracts were used.

The plates were incubated at 37 °C for 24 h and zones of inhibition were then measured to the nearest millimeter with a ruler (Ikenebomeh & Metitiri, 1988).

The MIC was determined by the agar incorporated method as described by Abdulrahman (1986). This was performed by using 0.2 ml of the standardized bacterial density of 3×10^8 organisms per ml. The inocula were pipetted onto the Muller Hinton agar incorporated with the extracts at various concentrations and incubated at 37 °C for 24 h. The growths of *Campylobacter* organisms on the agar plates with different concentrations of the extracts were then observed and the MIC calculated. The growths of *Bacillus subtilis* used as control organisms on the agar plates were also tested at different concentrations of the extracts and the MIC also calculated.

Results

Tables 1 and 2 show the effects of *A. nilotica* and *V. doniana* water and ethanol extracts at 2, 20 and 200 mg ml^{-1}, respectively, on the test *Campylobacter* organisms used in this study. The results show that *A. nilotica* ethanol extract strongly inhibited the isolates. No differences were observed between the inhibition effects of the ethanol extracts at different concentrations against the *Campylobacter* species tested. At 200 mg ml^{-1}, the diameter of zone of inhibition was 6 mm, while for the water extract of the same plant it was 4 mm. *Vitex doniana* ethanol and water extracts showed little or no inhibitory effects on the isolates (Table 2).

The MIC for all the isolates was 80 mg ml^{-1} for the ethanol extract of *A. nilotica*, while water extract of the same plant had MIC of 250 mg ml^{-1}. In the latter concentration, none of the tested organisms was able to grow.

The *V. doniana* extracts did not show any inhibitory effect on the isolates even at 500 mg ml^{-1}. *Bacillus subtilis* ATTC strain used as control showed MIC at 65 and 125 mg ml^{-1} for ethanol and water extracts of *A. nilotica,* respectively. The zones of inhibition observed for ethanol extract of *A. nilotica* had diameters of 8 and 5 mm at 200 and 20 mg ml^{-1}, respectively (Table 1). The water extract of *A. nilotica* had zones of inhibition of 4 and 2 mm at 200 and 20 mg ml^{-1}, respectively (Table 1). The *V. doniana* water extract had no

TABLE 1

Effect of Acacia nilotica *Water and Ethanol Extracts at Various Concentrations on* Campylobacter *Species Isolated from Sheep and Zone of Inhibition*

Campylobacter species	Biotype number	Number of isolates	Zone of inhibition (mm)					
			Concentration of ethanol extract			Concentration of water extract		
			2mg	20mg	200mg	2mg	20mg	200mg
C. jejuni	Biotype I	16	6	4	2	2	1	0
	Biotype II	8	6	4	2	2	1	0
	Biotype III	4	6	4	2	2	1	0
	Biotype IV	3	6	4	2	2	1	0
C. coli	Biotype I	3	6	4	2	2	1	0
	Biotype II	2	6	4	2	2	1	0
C. laridis	Biotype I	2	6	4	2	2	1	0
	Biotype II	1	6	4	2	2	1	0
B. subtilis	ATTC	1	8	6	4	4	2	1

TABLE 2

Effect of Vitex doniana *Water and Ethanol Extracts at Various Concentrations on* Campylobacter *Species Isolated from Sheep and Zone of Inhibition*

Campylobacter *species*	*Biotype number*	*Number of isolates*	*Zone of inhibition (mm)*					
			Concentration of ethanol extract			*Concentration of water extract*		
			2mg	*20mg*	*200mg*	*2mg*	*20mg*	*200mg*
C. jejuni	Biotype I	16	1	0	0	0	0	0
	Biotype II	8	1	0	0	0	0	0
	Biotype III	4	1	0	0	0	0	0
	Biotype IV	3	1	0	0	0	0	0
C. coli	Biotype I	3	1	0	0	0	0	0
	Biotype II	2	1	0	0	0	0	0
C. laridis	Biotype I	2	1	0	0	0	0	0
	Biotype II	1	1	0	0	0	0	0
B. subtilis	ATTC	1	2	1	0	0	0	0

measurable inhibitory effect on the isolates tested (Table 2). However, the ethanol extract of *V. doniana* had zones of inhibition of 2 and 1mm. The water extract of *V. doniana* had no effect on the isolates (Table 2).

Discussion

The uses of *A. nilotica* for *Campylobacter* organisms have not been supported by any clinical studies, although the successful treatment of venereal diseases and diabetes, or use as an aphrodisiac have been documented by the rural populates (Brown, 1977). The active ingredients present may be efficacious against infectious diseases. Gum, for example, has an emollient activity, resulting in a softening, soothing action on the skin or on irritated internal surfaces. The astringent activity of tannins causes a contraction of mucous surfaces, coagulates proteins, and is useful in stopping bleeding of small wounds and other discharges (Brown, 1977). Acetic acid, alcohol and water extracts of the fruits of *A. dudgeoni, A. nilotica* subspecies *adstringens* and subspecies *nilotica* have molluscocidal activity. The planting of the latter subspecies along waterways could prove beneficial in the control of schistosomiasis (Ayoub, 1982; Ayoub, Michael & Yankov, 1985; Kloos & McCullough, 1987).

The medicinal uses of *V. doniana* are numerous. The fruits are used to treat anaemia and the root is used for treating gonorrhoea (FAO, 1983). It also improves fertility and is used to treat jaundice and dysentery (Watt & Breyer-Brandwyk, 1982).

Based on the observation that ethanol and water extracts of *V. doniana* had no inhibitory effect on the *Campylobacter* isolates tested at MIC value of 500 mg ml^{-1}, it is unlikely that this plant would have any therapeutic effects on *Campylobacter* infections.

The inhibitory effects of the ethanol and water extracts of leaves of *A. nilotica* on *Campylobacter* species may be related to its antibacterial effect. This plant has saponin and lipid (Abdulrahman, 1986). The finding that the extracts of the plant showed inhibitory effect at 80 mg ml^{-1} against *Campylobacter* species indicates its potential in treating *Campylobacter* infections.

The susceptibility of *Campylobacter* organisms to the extract of *A. nilotica* is interesting, considering the worldwide phenomena of antibiotic resistance of the organisms (Coker

& Adefesor, 1994). Traditionally, the plant material is used as a crude extract and treatment does not aim at using the pure isolate. Since the objective of this study was to broadly establish a scientific basis, if any, for the use of this plant material in therapies, the work did not investigate which of the chemical constituents in the plant material could be responsible for the antimicrobial activity.

However, this study has clearly shown the *in vitro* antimicrobial activity of the crude extract of *A. nilotica* and its possible use in treating human diarrhoea. Further investigation is required to identify the plant's active components. There is also the need to establish standard dosages for *A. nilotica* preparations and to investigate its toxicity in medical use.

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