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EFFICACY OF *AZADIRACHTA INDICA* A. JUSS LEAF AQUEOUS EXTRACT AGAINST BACTERIA ISOLATED FROM THE GUTS OF IXODID TICKS IN MAIDUGURI, NIGERIA.

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Abstract: This study was conducted to determine the efficacy of *Azadirachta indica* leaf aqueous extract against the bacterial isolates from the gut of ixodid ticks of small ruminants viz *Hyalomma spp., Rhipicephalus spp.* and *Amblyomma spp.* in Maiduguri Metropolis, Nigeria. Four bacteria were isolated from these ticks comprising three Gram positive: *Streptococcus spp., Staphylococcus spp.* and *Corynebacterium spp.* and one Gram negative: *Proteus spp.* The efficacy of the leaf extract at various concentrations of 1000mg/ml, 800mg/ml, 600mg/ml, 400mg/ml and 200mg/ml, was compared with standard antibiotics using disk diffusion methods on Mueller – Hinton Agar (Oxoid®). In both tests, the diameter of the inhibition zones were measured in millimeters and recorded. No zone of inhibition was observed for all concentrations of the tested extract among the bacteria isolates. However, the antimicrobial sensitivity testing revealed highest inhibitory zones of 30mm each on ciprofloxacin by *Corynebacterium spp.*, and *Proteus spp.* and by *Streptococcus spp.* on augmentin Complete resistance was observed with Cotrimoxazole, Cloxacillin and Clindamycin and pockets of partial resistance with Erythromycin and Cefraxone for the Gram positive isolates. In conclusion, this study revealed that the aqueous extract of *A. indica* leaf was not effective against bacteria isolated from hard ticks of small ruminants in Maiduguri.

Keywords: *Azadirachta indica*, Aqueous Extract, Ixodid Ticks, Bacteria, Maiduguri, Nigeria

INTRODUCTION

Parasitic diseases are economically important livestock diseases and are responsible for huge economic losses to livestock farmers worldwide. They are responsible for reduced production, infertility, diseases and death (Rajput *et al.* 2006). This may result from infestations with ectoparasites such as ticks, fleas, mites, and midges which attach and feed on the body surface of their hosts or endoparasites that live inside the body tissues of the hosts (Soulsby, 1982). Among the ectoparasitic infestations, ticks remain the most important amongst livestock in tropical and sub-tropical Africa (Jongejan and Uilenberg, 2004). They are important vectors of tick-borne diseases (TBDs) such as cowdriosis, babesiosis, anaplasmosis and theileriosis (Norval *et al.* 1988). Ticks are economically important due to their blood sucking nature resulting to damages to hides and skin and this reduces their quality and market value. Due to their feeding nature, they create wounds, which may subsequently serve as portal of entry of disease agents and predispose animals to myiasis (Gates and Wescott, 2000; Mtshali *et al.* 2004). Production losses due to ticks and tick-borne diseases around the globe have been estimated at US$ 13.9 to US$ 18.7 billion annually leaving world's 80% cattle at risk (de Wall, 2000, de Castro, 1997 and Ghosh *et al.* 2007). Therefore, the control of tick infestations and transmission of TBDs remains an important challenge to the livestock industry in tropical and sub-tropical regions of the world. Chemical control through the use of acaricides was considered as one of the best methods to mitigate this menace worldwide. Until recently, the evolution of resistance of ticks to synthetic acaricides and antibiotic - resistant strains of some

pathogens has given rise to the need for new scientific investigations on alternative ways to control the disease they caused (Martine *et al.*, 1995; Ghosh *et al.* 2007; Kiss *et al.* 2012). In addition, their toxicity and expensive nature further limits their use (Martine *et al.* 1995). In this regard, various studies have been developed in an attempt to identify plant extracts with bactericidal and acaricidal properties. Of interest is neem tree (*Azadirachta indica*), which is a native of Indian subcontinent and grows in tropical and subtropical regions of the world including Northern Nigeria. The leaf, bark, roots, flowers, seed and fruit coat of *A. indica* possesses wide spectrum of biological activities ranging from antimalarial, anti – inflammatory, antihyperglyceamic, antiulcer, antifungal, antibacterial to anticarcinogenic properties (Siddique *et al.* 2004; Atawodi and Atawodi, 2009). Therefore, this study was designed to determine the efficacy of *A. indica* leaf aqueous extract against bacteria isolated from hard ticks of small ruminants in Maiduguri.

MATERIALS AND METHODS

Plant Collection, Identification and Processing

Fresh leaves of *A. indica* were collected from the neem orchard of the University of Maiduguri, Nigeria and authenticated by a botanist in the Department of Biological Sciences, Faculty of Sciences, University of Maiduguri, Nigeria. The leaves were then rinsed with distilled water and air dried under shade for 8 – 10 days in the Department of Veterinary Physiology and Pharmacology Laboratory. A 100g of dried leaf powder was placed in a flask and distilled water was added at 1:10 w/v in order to obtain clear leaf extracts. The mixture was incubated in a steaming water bath at 50°C for 48 hours and the leaf extract was filtered using Whatman® filter paper. The clear filtrate was stored in a deep freezer for drying at – 50°C 0.2m bar for 48 hours to obtain water – free extracts. The concentrated extracts obtained were then weighed and stored at 4°C in the laboratory for further use.

Isolation of Bacteria from the Guts of Ticks

Three Gram +ve bacteria, *Corynebacterium spp.*, *Streptococcus spp.* and *Staphylococcus spp.* and one Gram – ve, *Proteus spp.* were isolated from the gut of *Hyalomma spp.*, *Rhipicephalus spp.* and *Amblyomma spp.* in the Bacteriology Laboratory, Department of Veterinary Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Maiduguri, Nigeria.

Antibacterial Sensitivity Testing using Extract - Impregnated Filter Paper Method

Isolated colonies of the four bacteria were cultured into tubes containing 10ml of sterile nutrient broth (Oxoid®) and incubated at room temperature till the cultures turbidity reached 0.5 McFarland and were aseptically swabbed on the surface of sterile Mueller – Hinton Agar plates (Oxoid®). Filter paper discs of 6mm diameter were prepared and sterilized using an ethanol dipped and flamed forceps. These discs were aseptically placed over Mueller – Hilton Agar (Oxoid®) plates seeded with the respective test microorganisms. Twenty-five microliters of 200, 400, 800 and 1000mg/ml concentrations of the extract were aseptically transferred to the discs. The plates were incubated in an upright position at 37°C for 24 hours. The diameter of inhibition zones were measured in mm and the results recorded. Diameters of < 12mm, between 12 and 16mm and those > 16mm were considered as having no antibacterial activity, moderately active and highly active respectively (Indu *et al.* 2006).

Antibiotic Sensitivity Testing

Antibiotic susceptibility pattern of the bacterial isolates to selected antibiotics was carried out using disk diffusion method on Mueller-Hinton Agar (Oxoid®) according to NCCLS (1999) standards. The antibiotics were tetracycline (30 µg), gentamycin (10 µg), chloramphenicol (30 µg), Ciprofloxacin (15 µg), augmentin (30 µg), Cephalothrin (30 µg) and erythromycin (10 µg).

RESULTS

Table 1 shows that none of the concentrations of 1000mg/ml, 800mg/ml, 600mg/ml, 400mg/ml and 200mg/ml of the aqueous leaf extract was effective against bacteria isolated from the guts of *Hyalomma spp.*, *Rhipicephalus spp.*, and *Amblyomma spp.* Also the zones of inhibitions with standard antibiotics tested against the bacterial isolates from the gut of the ticks showed both resistance and susceptibility levels. The highest inhibitory zones of 30 mm on Ciprofloxacin was recorded for *Corynebacterium spp.* and *Proteus spp.* and on Augmentin by *Streptococcus spp.* Complete resistance was recorded oncotrimoxazole, cloxacillin and clindamycin and partial resistance on cefraxone and erythromycin.

Table 1: Bacteriogram of *Azadirachta Indica* Leaf Aqueous extract and Antibiotic Sensitivity Testing

	Concentration	Width (mm) Zone of inhibition by			
		Coryne.	*Strep.*	*Staph.*	*Proteus*
Neem extract(mg/ml):	200	R	R	R	R
	400	R	R	R	R
	600	R	R	R	R
	800	R	R	R	R
	1000	R	R	R	R
Antibiotics (µg):					
Ciprofloxacin	5	30	28	15	30
Gentamycin	10	20	15	8	15
Cephalexin	30	25	25	10	-
Cotrimoxazole	50	R	R	R	-
Cefraxone	30	R	20	R	-
Cloxacillin	30	R	R	R	-
Clindamycin	10	R	R	R	-
Augmentin	30	10	30	R	-
Ofloxacillin	5	25	20	20	-
Erythromycin	10	20	R	R	-
Norfloxacillin	10	-	-	-	20
Amoxycillin	30	-	-	-	R
Chloramphenicol	10	-	-	-	10
Tetracycline	10	-	-	-	28
Cefuroxime	50	-	-	-	R
Ampicillin	30	-	-	-	R
Nitrofurantoin	10	-	-	-	15

R = Resistance; S = Sensitive; - = Antibiotic is not common to Gram +ve or – ve bacteria.

DISCUSSION

Herbal medicine is now forming an alternative and cheaper therapy throughout the world due to the growing resistance of pathogens to conventional antibiotics (Srivastava *et al.* 2000). The results of several studies proved medicinal plants might be potential sources of new antibacterial agents and even against antibiotic-resistant strains of disease causing agents (Indu *et al.*, 2006). This study has revealed the prevalence of *Corynebacterium spp.*, *Staphylococcus*

spp., *Streptococcus spp.* and *Proteus spp.* in the gut of ixodid ticks in Maiduguri, Nigeria. The various concentrations of the neem leaf aqueous extract did not show any antimicrobial activity. This is not in agreement with other studies on the leaves and bark extracts of *A. indica* which showed antibacterial potential against both Gram positive and Gram negative bacterial isolates (Singh and Bhat, 2003) However, the antibacterial potential of neem plants has been attributed to the type of the extraction solvents used (Yusha'u *et al.* 2010). Orhue *et al.,* (2004) observed that leaf and bark petroleum ether extracts were more potent compared with water, ethanol, 1% HCL and acetone extracts. The present study did not compare the antibacterial potentials of the leaf extract of *A. indica* using various extraction solvents. Also the phytochemicals of *A. indica* have been reported to have medicinal uses (Ojo *et al.*, 2006), especially the saponins which has antimicrobial effects and could therefore, serve as precursors of steroidal substances with a wide range of physiological activities (Madusolomuo *et al.*, 1999). Antimicrobial sensitivity testing in this study has revealed that both the Gram positive and negative bacteria isolated from the guts of ixodid ticks of small ruminants were resistant to antibiotics. Antimicrobial resistance is now an emerging public health issue in both human and veterinary medicine (Biu *et al.* 2013), and many programs have been set up targeting mainly at human pathogens, agents of zoonoses and indicator bacteria of the normal intestinal flora from animals (Habrun *et al.* 2010).

In conclusion, the results of the present study suggests that the aqueous extract of *A. indica* leaf does not possess compounds that inhibit bacterial isolates from the gut of ixodid ticks of small ruminants.

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