ANTIBACTERIAL EFFECT OF METHANOLIC EXTRACT OF *Moringa oleifera* LEAF ON THE RAW SEMEN OF DUROC BOARS

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ABSTRACT

The study was carried out to determine the antibacterial activities of methanolic extract of *Moringa oleifera* leaf on the microorganisms found in raw Duroc semen. Qualitative screening for phytochemicals was carried out and Disc diffusion method was used to determine the antimicrobial activities ofthe plant extract which was compared with standard antibiotics (Penicillin and Streptomycin). The extract showed the presence of alkaloids, saponin and phenols in appreciable amount, tannin, phlobatannins, steroids, terpenes and cardiac glycosides were present in moderate amount, while cardenolides and chalcones were completely absent. There was the presence of microorganisms in the boar semen but not higher than the allowed value. Methanolic extract of *Moringa oleifera* leaf showed zones of inhibition against *Esherichia coli, Proteus mirabilis, Enterobacter* sp *and Staphylococcus aureus* while it did not inhibit the growth of *Klebsiella* sp.The control, penicillin and streptomycin had zones of inhibition against all the isolates.The results obtained in the present study indicate that the leaves of *Moringa oleifera*are a potential source of natural antibacterial.

Keywords: *Moringa oleifera*, antibacterial, phytochemicals, Duroc semen

INTRODUCTION

High quality semen is critical for the reproductive performance of commercial swine herds because the semen of one single boar can be used to inseminate many females. Semen collection is a process that often presents bacterial contamination during semen collection and processingmainly by Gram negative bacteria from the *Enterobacteriace* family (Maes *et al.*, 2008). It may occur during complete semen processing starting from semen collection untilsealing of semen tubes or bags. Althouse and Lu (2005), among the several microorganisms detected *Escherichia coli* (*E. coli*), *Staphylococcus aureus (S. aureus)* as contaminants of boar semen. The spermicidal effect of *E. coli*, when in high concentrations (>2 x10CFU/mL), decreases sperm motility and increases the sperm agglutination in human seminal plasma (Diemer *et al.*, 1996).The presence of *S. aureus* can lead to decreasein the number of spermatozoa, the suppression of their motility, changes in their morphology and fertilizing capacity (Emokpae *et al.*, 2012). Semen contamination may impair female's performance, causing return to estrus and vulvar discharges, and reduce the number of piglets born alive (Althouse *et al.*, 2000). According to the International Office of Epizootics (OIE, 2001) the maximum number of colonyforming unities (CFU) allowed to be present in semen samples without negative effects in boar semen quality is 5 $x10^3$ per ml. Sources of contamination could be from the animal's origin, the environment or from human during semen collection and processing. Antibiotics are types of antimicrobials used in the treatment and prevention of bacterial infection. An antibacterial is a compound or substance that kills or slows down the growth of bacteria. They could be natural or synthetic. Plants have been reported to contain large varieties of chemical substances that possess important preventive and curative therapies (Nascimento *et al.*, 2000).Plant extracts and phytochemicals with antimicrobial properties are of great significance in therapeutic treatments. *Moringa oleifera* is a highly valued plant, distributed in many countries of the tropics and subtropics. It has an impressive range of. medicinal uses with high nutritional value. Various parts of the plant such as the leaves, roots, seed, bark, fruit, flowers and immature

pods are being employed for the treatment of different ailments in the indigenous system of medicine, particularly in South Asia (Farooq *et al.*, 2007).

The objectives of the study is todetermine the phytochemical properties of methanolic extract of *Moringa oleifera* leaf (MEML),the microbial load and identification of isolates found in the raw semen and the antibacterial activities of the extracts to the microorganisms found in raw semen of Duroc pigs.

MATERIALS AND METHODS

Fresh *Moringa oleifera* leaves were sourced from the Institute of Agriculture Research and Training (IAR&T) envionment. The plant materials were air-dried in laboratory for 7 days and blended. Powdered plant material was soaked methanol at ratio1: 4 for 24 hours, then sieved and filtered using Whatman filter paper. The filtrate was then concentrated to dry form using water bath at 60 ^{o}C to get a thick residue.The extracts obtained were then stored in a refrigerator. Semen was collected and pooled from 4 proven boar using the gloved hand technique. Qualitative screening for phytochemicals was carried out according to the methods of Harborne (1973) and Trease and Evans (1989). The total viable count of the semen samples were carried out using nutrient agar as described by Ngu *et al.* (2013). The antibacterial sensitivity test was carried out using disc diffusion method as described by (Kirby-Bauer, 2009).

RESULTS AND DISCUSSION

The result from Table 1showedthe quantitative screening of the phytochemicals from the MEMLThe extract showed the presence of alkaloids, saponin and phenols in appreciable amount, tanin, phlobatannins, steroids, terpenes and cardiac glycosides were present in moderate amount, while cardenolides and chalcones were completely absent.This results correspond with the study of Kasolo *et al* (2010) who found similar phytochemicals from Moringa extract. However, Oluduro (2012) reported absence of steroids and cardiac glycoside. This could be due to the fact that different solvents have different extraction capabilities and spectrum of solubility for the phytochemicals. El Astal *et al* (2005) reported that these bioactive components are known to be bactericidal, pesticidal or fungicidal in nature. From the result, phenol was present in appreciable amount and it has been found to be applied in the control of human pathogenic infections (Doughari, 2009). Tanins was also present in in moderate amount and they have been known to be astringents, which help in wound healing, treatment intestinal disorders such as diarrhoea and dysentery (Dharmananda, 2003). Usman and Osuji (2007) also reported the curative activity of these metabolites against several pathogens. The presence of these metabolites suggests great potential MEML as a source of antibiotics useful in phyto-medicine. Table 2 showed the microbial loadfrom the raw semen of the Duroc boars. It showed thatthe semen was contaminated with bacteria. Total viable counts TVC increased with time from $2.48 \times 10^{2} \pm 0.02$cfu/mlat 24hrs and to $3.63 \times 10^{2} \pm 0.04$cfu/ml at 48hrs.The International office of epizootics (OIE 2001) reported that the maximum number of colony forming units (cfu) allowed to be present in semenwithoutnegative effect in boar semen quality is 5×10^{3} / ml. The Total Viable Counts (TVC) from the result showed that it was not higher than the requirement which makes the semen still be in good condition. The microorganisms isolated from the semen include; *Esherichiacoli, Klebsiella*sp, *Enterobacter* sp, *Proteus mirabilis*and *Staphylococcus aureus.*

The species of microorganisms isolated from the raw semen samples from the study agrees with the work of (Fernandez *et al.*, 2001). Althouse and Lu (2005) also amongst others also identified *Esherichia coli* and *Staphylococcus aureus* as contaminants of boar semen. Majority of the isolates (*Esherichia coli., Klebsiella*sp , *Staphylococcus aureus , Enterobacter* sp, *and Proteus mirabilis)*are gram negative which agrees to the work of Maes (2008), that semen collected from boar semen commonly contains bacteria mostly being gram negative from the *Enterobacteriaceae* family. The prevalence of the microorganisms found in the semen was *E.coli* (38%), the most prevalent followed by *Staphylococcus aureus* (22%) *Klebsiella*sp(16%), *Enterobacter*sp(13%) and *Proteus mirabilis* (11%). The result support the work of Althouse and Lu (2005) who identified *E.coli* and *Staphylococcus aureus* more often.

The presence of these micoorganisms in porcine semen have significant deleterious effect on the semen quality, Althouse and Lu (2008). *E.coli*, when in high concentrations decreases sperm motility and increases the sperm agglutination in the seminal plasma (Diemer *et al.*; 1996). The presence of *S. aureus* can lead to decrease in the number of spermatozoa, the suppression of their motility, changes in their morphology and fertilizing capacity (Emokpae *et al.*, 2009). Wolff *et al.*,(1993) reported that *Escherichia coli* adheres to sperm surface via mannose binding sites and Huwe *et al.* (1998) reported that isolates *Escherichia coli* lowered sperm motility *in vitro.*Microbial contamination can also have an indirect effect by producing toxins (Morrell, 2006) which is a possible source of endotoxins capable of damaging the fertilizing capacity of spermatozoa. Semen contamination may impair female's performance, causing return to oestrus and vulvar discharges, and reduce the number of piglets born alive (Althouse*et al.*, 2000).Table 3 showed the diameter zones of inhibition of the methaolic extract of *Moringa oleifera* leaves. It had inhibitory effect on *E.coli, Proteus mirabilis, Enterobacter* spand *Staphylococcus aureus.* However, there was no observable activity against *Klebsiella* sp. The extract was able to inhibit the growth of *E.coli* to the lowest concentration of 0.125g/ml, *Proteus mirabilis* to 0.5g/ml, *Enterobacter* sp 0.25g/ml and Staphylococcus aureus to 0.125g/ml. The antibacterial activity of *Moringa oleifera* may be due to the presence of an array of phytochemicals. Bukar *et al.*, (2010) identified most importantly, the presence of a short poly-peptide named 4 ά– L – rhamnosyloxy) benzyl-isothiocy-anate in *M. oleifera*. They argued that the peptide may act directly on microorganisms and result in growth inhibition by disrupting cell membrane synthesis or synthesis of essential enzymes. The result obtained was similar to that of Napoleon *et al* (2009) who reported that *Enterobacter* sp, *Staphylococcus aureus* and *Esherichia coli* to be sensitive to *Moringa oleifera*at similar concentrations. The strong activities of this leaf suggests that it may be used for treatment of infections caused by these organisms except *Klebsiella*sp which was found to be resistant to the activity of the methanolic leaf extract. The control Penicillin plus Streptomycin had the highest zone of inhibition (25mm for *E.coli,* 9mm, for *Klebsiella* sp, 25mm for *Proteus mirabilis*, 26mm for *Enterobacter* sp and 15mm for *Staphylococcus aureus* respectively) on the test organisms. The antibiotics was more effective in inhibiting the test organisms than the plant extracts. This may be due to the purity level of commercial antibiotics which is in line with the findings of Doughari *et al* (2007) who reported that antibiotics are in a refined state while the plant extracts are still in the crude state.

CONCLUSION

It can be deduced that the methanolic extract of *Moringa oleifera* leaf has the potential to treat any ailment associated with these bacterial pathogen effectively. The study indicates that *Moringa oleifera* plant contains antimicrobial compound that can be further developed as phytomedicine for the therapy of infection.

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Table 1: Phytochemical Screening of Methanolic Extract of *MoringaOleifera* Leaf

Phytochemical	Observation	Remark
Alkaloids	+++	Appreciable Amount
Tannin	++	Moderate Amount
Phlobatannin	++	Moderate Amount
Saponin	+++	Appreciable Amount
Flavonoids	+	Trace Amount
Anthraquinones	+	Trace Amount
Steroids	++	Moderate Amount
Terpenes	++	Moderate Amount
Cardenolides	-	Completely Absent
Phenols	+++	Appreciable Amount
Chalcones	-	Completely Absent
Cardiac glycosides	++	Moderate Amount

Table 2: Microbial Load of Raw Semen sample of Duroc boar

Time (hrs)	Total viable counts (cfu/ml)
24	$2.48 \times 10^2 \pm 0.03$
48	$3.63 \times 10^2 \pm 0.02$

Table 3: Diameter zones of inhibition of *Moringaoleifera leaf* on isolates from semen

Isolates	Concentration (g/ml)						
	PS	1	0.5	0.25	0.125	0.625	0.0315
E.Coli	25mm	17mm	8mm	8mm	4mm	-	-
Klebsiella	9mm	-	-	-	-	-	-
Proteus mirabllis	25mm	5mm	2mm	-	-	-	-
Enterobacter sp	26mm	9mm	4mm	2mm	-	-	-
Staphylococcus aureus	15mm	10mm	7mm	5mm	3mm	-	-

PS- penicillin –streptomycin; mm- millimeters