ANTIBACTERIAL AND ANTIOXIDATIVE EFFECTS OF *ALOE BARBADENSIS* LEAF METHANOL EXTRACTS ON QUALITY AND SPERMATOZOA FERTILISING POTENTIAL OF EXTENDED RED SOKOTO BUCK SEMEN

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ABSTRACT

A study was conducted to investigate the antibacterial and antioxidative effects of Aloe barbadensis leaf methanolic extract (ABLME) on the quality and spermatozoa fertilising potential of extended goat semen. 100 g of fresh A. barbadensis leaves were washed, chopped, dried, ground and extracted with 95.0% methanol (ABLME). The ABLME was added to egg yolk + sodium citrate (EYSC) diluent at 0.0, 0.5, 1.0, 1.5 and 2.0 g/L and used to extend the goat semen. The extended semen samples were stored at 4°C and were evaluated at 0, 24, 48 and 72 hours post storage. All treatments were replicated thrice in a completely randomized design. Spermatozoa progressive motility (SPM), spermatozoa liveability (SPL), normal spermatozoa (NSP), acrosome integrity (ACI), plasma membrane integrity (PMI), secondary morphological abnormalities (SMA) and microbial load (CFU/mL) were evaluated and analyzed using descriptive statistics and ANOVA at α0.05. Significant reduction in microbial load was observed in samples with 2.0 g/L ABLME (6.83 ± 4.80%) compared to control (27.50 ± 3.20%) at 48 hours. Reduced SMA was recorded for 2.00 g/L ABLME (9.00 ± 0.90%) compared to control (13.67 ± 1.00%). Methanolic extract (2.00 g/L) of A. barbadensis included in egg yolk + sodium citrate diluent gave antibacterial activity to extended buck semen for up to 48 hours.

Keywords: Antibacterial, Anti-oxidant, Methanol Extract, *Aloe barbadensis,* Spermatozoa, Extended semen

INTRODUCTION

Small ruminants are important genetic resources and play a prominent role in the sustenance of the livelihoods of impoverished families, especially in the rural areas of tropical countries (Adamu *et al.*, 2020). The Red Sokoto goat (RSG) or Maradi is the most predominant breed and accounts for about 70% of Nigeria's total goat population (Ademosun, 1994; Adamu *et al.*, 2020). Therefore, the genetic resources of this important cold shock species need to be exploited and this can be achieved through assisted reproductive techniques such as artificial insemination (AI) associated with semen technology which allows the preserved male genetic material to be used in females that are isolated from the males (Morrell and Mayer, 2017; Santos and Silva, 2020). Meanwhile, the use of semen extenders has been adjudged to be necessary for semen preservation and storage (Alaba *et al.*, 2010). The composition of semen extenders varies considerably depending on the species and type of sperm preservation (i.e.,

cryopreserved or refrigerated). In general, the semen extender contains nutrients (mainly monosaccharides), cryoprotectant agents (CPAs) as protection against, buffers, antioxidants and antibiotics (Barbas and Mascarenhas, 2009). Irrespective of the extender and the storage conditions used, semen handling and preservation negatively affect sperm quality. Moreover, oxidative stress, which often arises during semen storage, significantly reduces sperm function and compromises sperm fertilising ability by inducing oxidative damage to proteins, lipids and nucleic acids (Ros-Santaell and Pintus, 2021). Ordinarily, semen from a healthy animal is expected to be free of pathogenic contaminants, but contamination still occurs during collection. The prepuce, foreskin and the environment are likely sources of contaminants. Semen processing in most cases takes place under normal laboratory conditions without access to sterile or contaminant-free air resulting in possible contamination from the laboratory atmospheric condition and equipment hence, antibiotics used in semen extenders inhibit microbial growth and prevent transmission of infections to the inseminated doe. The female genital tract is physiologically designed to destroy infections introduced during mating but the mechanism may be overwhelmed by a large number of pathogens in the semen (Morrell and Wallgren, 2011). Bacteria vary in their resistance to antibiotics. Gram-positive organisms are generally sensitive to penicillin because its mode of action is to attach to the bacterial cell wall and disintegrate the cell. Gram-negative bacteria have a protective layer of lipids on their cell surface and so are protected from the attachment of penicillin molecules. Streptomycin is more effective in inhibiting the growth or destruction of Gram-negative bacteria (Campbell *et al*., 2009). There are many resistance factors, such as the production of enzymes that destroy antibiotic molecules. The best known of these is penicillin or B-lactamase, the enzyme that hydrolyses the B-lactam ring structure of penicillin G rendering the antibiotic inactive. Most strains of *Staphylococcus aureus,* a bacterium that was generally sensitive to penicillin in the early days of its use, have now acquired the genes to produce these enzymes and have now become penicillin-resistant (Campbell *et al*., 2009). Exposure of bacteria to marginally lethal doses of antibiotics tend to kill many cells but permits the growth of a subset of the population that is resistant.

Long-term use of a single drug, may, however, favour colonization with the more resistant bacteria. Complicating the issue is the potential for transmission of resistance gene, R-factor, from one bacterium to another. The resistance gene does not have to exist in a pathogenic cell to be passed to a drug-sensitive pathogen. It can be contaminated with any bacterium capable of transferring genes with a pathogen. Fortunately, passage from the cell of another is generally to bacteria of similar genetic makeup. These resistance genes may become incorporated into the chromosomal DNA or may reside in the extrachromosomal DNA known as plasmid DNA (Campbell *et al*., 2009). Another factor to consider is the potential for bacteria to mutate. Bacteria grow rapidly, sometimes duplicating themselves in as little as 10 minutes at the more usual generation time of 30 minutes, a single cell can grow to one million within 10 hours and to one billion within 15 hours. if the rate of non-lethal mutation is 10^{-7}, 100 mutants would be produced among these cells. Whether resistance is natural, transferred, or because of mutations, the presence of antibiotics that kill much of the flora provides opportunities for resistant cells to grow far beyond their normal capacity: the fittest survive and rise to prominence (Campbell *et al*., 2009).

Antibiotic resistance as well as the emergence of new diseases and the resuscitation of previously eradicated diseases are problems associated with the excessive use of drugs in the livestock industry posing a great threat to global human health (El-Mahmood *et al.,* 2008; Zaman *et al.,* 2017). The majority of these antimicrobial agents simply induce bacteriostatic antibodies. Some are very toxic to vital organs of the body (Tatli and Akdemir, 2005). Thus, it is critical to tackle emerging and re-emerging infectious diseases by focusing on the discovery of novel medicines with a superior therapeutic profile to combat disease outbreaks that pose a new danger to global health security (Mohanta *et al*., 2017).

Antibiotic-resistant microorganisms inform the quest for new and effective antibacterial substances to which these bacteria have not developed resistance (El-Mahmood *et al.*, 2008). Researchers are looking to natural chemicals from plants as sources of antimicrobial and antioxidative bioactive compounds to supplement or replace the existing synthetic antibiotics, which are becoming less effective against some diseases (Nitta *et al.*, 2002).

Furthermore, medicinal plants are rich in phytochemicals, some of which act as antioxidants (Williams *et al.*, 2004). Natural antioxidants increase the antioxidant capacity of plasma and reduce the risk of cancer, heart disease and stroke (Prior and Cao, 2000). Free radical scavengers include the secondary metabolites; phenolic and flavonoids from plants. They can be found in the leaves, fruits, seeds and roots of the plant. Synthetic antioxidants are commonly used and are believed to have some detrimental consequences including the risk of liver damage and carcinogenesis in laboratory animals (Lourenço *et al.,* 2019). As a result, more effective, less harmful and cost-efficient antioxidants are required. Medicinal herbs are preferred because they provide these desired benefits (Namiki, 1990). A possible alternative to adding antibiotics to the semen may be adding plant-based substances with a known antibacterial and antioxidative effect on different bacterial genera and reactive oxygen species. Various plant extracts showed some antimicrobial and antioxidant properties by increasing the activity of several antioxidative enzymes. *Aloe vera* appears to be a more sanitary substitute as it contains some biologically active substances that can also act as conventional cryoprotectants (Boudreau and Beland, 2006). Previous studies (Gutierrez *et al*., 2006; Aguiar *et al*., 2012; Souza *et al*., 2016) have already shown that *A. vera* is a natural product that can be used as a protectant in the preservation of sperm for land animals. *A. vera* has been classified as medicinal plant with specific bio-active ingredients such as alkaloids, saponins, flavonoids, proteins, lipids, amino acids, vitamins C, B (1, 2 and 6), A, E, enzymes, organic and inorganic compounds and mineral salts such as sodium, calcium, iron, potassium, chloride, manganese, copper and zinc (Hamman, 2008; Darzi *et al.*, 2021). It has been used as a natural antioxidant with a high potential to reduce fat oxidation and oxidative stresses (Vinson *et al*., 2005). Raw gels of *Aloe barbadensis* Miller, 1768 (Asparagales: Asphodelaceae) have been reported to maintain plasma membrane integrity of spermatozoa in extended buck semen above 60% after 48 hours of preservation (Agbaye *et al*., 2023). Also, pharmacological effects of *A. vera* viz: anti-inflammatory, anti-arthritic and antimicrobial have been documented (Newall *et al*., 1996) but very few studies on the effects of the leaf extracts on spermatozoa characteristics have been reported. This study appeared to be one of the few to document the research related to *A. barbadensis* as an alternative to antibiotics and antioxidants for the preservation of buck semen.

MATERIALS and METHODS

The Soxhlet extraction process was used for methanol extracts of the leaf. Fresh *A. barbadensis* leaves were harvested from the Botanical Garden of the University of Ibadan, and identification and authentication were carried out at the Department of Botany of the same institution, with the herbarium number UIH-23009. The leaves were washed and cut into pieces and 50 g was weighed into a Soxhlet reflux apparatus containing 300 mL of absolute methanol. The electronic hot plate was set according to the boiling point of the solvent. The extraction process took about 8 hours until the refluxing solvent became clear. The extract was concentrated by evaporating the solvent using a rotary evaporator. This product is now referred to as ABLME, the extracts were refrigerated (-1 °C) pending usage. The physical properties of gels of *A. barbadensis* leaf and glycerol are presented in Table 1.

Semen Collection: Three matured and healthy Red Sokoto bucks aged 2 – 3 years, weighing 41.5 ± 2.0 kg were used for the experiment. They were ejaculated weekly using the electro-ejaculation method as described by Oyeyemi *et al.* (2000).

Table 1: Comparison of physical properties of gels of *Aloe barbadensis* leaf and glycerol

Characteristic	Aloe *barbadensis* Leaf Gel	Glycerol†
Density (g/cm³)	1.20 ± 0.10	1.26
Molecular weight (g)	267.27 ± 0.57	92.09
pH	4.85 ± 0.88	6.70 - 7.50
Viscosity at 32°C (mPas)	6.29 ± 0.66	1.41
Solubility in water	Fairly miscible	Miscible
Boiling point (°C)	442.5 ± 0.45	290.00
Freezing point (°C)	NA	- 46.50
Melting point (°C)	NA	17.90

† = Source: CHEMSRC (2021), NA = Not Available

During the collection process, the buck was restrained in a standing position by two attendants, the prepuce was cleansed and the rectal probe of the ejaculator was lubricated before inserting gently into the rectum of the buck. The electro-ejaculator was adjusted to supply 10 to 15 volts to create stimulation; the process was repeated with a gradual increase in the voltage. By regulating the voltage, the nerves responsible for erection were stimulated, enabling erection and ejaculation. Ejaculates were collected into calibrated tubes using a funnel.

Preparation of Extender and Experimental Treatments Layout: The tris-fructose-citrate extender (TFCE) was used for this study. The compositions of the extender are presented in Table 2 and the experimental layout is presented in Table 3.

Table 2: Composition of tris-fructose-citrate spermatozoa extender

Component (g)	Quantity
Tris Buffer	2.40
Fructose	1.00
Citrate	1.40
Penicillin	0.01
Streptomycin	0.10
Distilled Water (mL)	95.09

Semen Processing: Semen samples were diluted in part A of the extender at 37°C (part A is the portion without cytoprotectant). Diluted semen and part B of the extender (the portion with cytoprotectant) were gradually cooled to 4°C in the refrigerator before the addition of part B extender. The addition of part B extender was carried out in three steps at 30-minute intervals for equilibration at this temperature before freezing to -22°C for evaluation at 0, 24, 48 and 72 hours.

Post-Thaw Semen Quality and Spermatozoa Fertilising Potential Evaluation

Semen quality assessment: Spermatozoa progressive motility (SPM), Spermatozoa liveability (SPL), Normal spermatozoa (NSP), pH and spermatozoa fertilising potential assessment; plasma membrane integrity (PMI) and acrosome integrity (ACI) were carried out at 0, 24, 48 and 72 hours. SPM was assessed with a phase contrast microscope at x400 (37 °C). NSP and SPL were assessed from two hundred spermatozoa stained with nigrosine-eosin stain per sample reading (Rege *et al.*, 2000). The ACI percentage was estimated from smears stained with nigrosine-eosin examined under a phase contrast microscope at x1000 magnification under an oil immersion objective and bright-field (Yildiz *et al.*, 2000). A total of 200 spermatozoa in four microscopic fields were counted. The PMI was assessed by a hypo-osmotic swelling test (Buckett *et al.*, 1997).

Table 3: Experimental layout on the effects of *Aloe barbadensis* leaf methanolic extract (ABLME) on the quality and spermatozoa fertilising potentials

Treatment (ABLME, mg/L)	Composition
T1	TFCE-A + 0.0 ABLME
T2	TFCE-B + 0.5 ABLME
T3	TFCE-B + 1.0 ABLME
T4	TFCE-B + 1.5 ABLME
T5	TFCE-B + 2.0 ABLME

TFCE = tris- fructose-citrate extender

Bacterial count: To determine the viable bacterial count, the medium was prepared by dissolving 23 g of nutrient Agar in 1000 mL of distilled water and heated to boiling point. The solution was autoclaved at 121°C under 103.42 kPa for 20 minutes and was allowed to cool to about 35°C. Salmonella-Shigella (SS) agar and Eosin Methylene Blue (EMB) agar media were used for the isolation of microorganisms and were prepared according to the manufacturer's instructions. Serial dilutions were carried out to

produce the bacterial load in the samples. Distilled water (9 mL) was pipetted into a clean test tube covered with cotton wool and foil and was autoclaved at 121°C. 1 mL of each sample was dispensed into the test tubes containing 9 mL of sterile distilled water and were serially diluted. 1 mL of the last dilution factor for each treatment with replicates was inoculated into well-labelled empty sterile Petri dishes. Nutrient Agar was added to each sample in the Petri-dishes and incubated at 37°C for 0, 24, 48 and 72 hours. Manual counting was performed with the aid of magnification under uniform and properly controlled artificial illumination (El-Tayeb *et al*., 2007).

Lipid peroxidation: This test is based on a Malondialdehyde (MDA) reaction with Thiobarbituric acid (TBA). 2 mL of semen, 2 mL of TBA (0.7) reagent and 1 mL of Trichloroacetic acid (TCA) were added. The solution was thoroughly mixed before being heated in a water bath at 80°C for 20 minutes. It was then chilled and centrifuged for 10 minutes at 400 rpm. After centrifuging for another 10 minutes, the absorbance of the supernatant was evaluated at a wavelength of 540 nm. The concentration of MDA in the serum was then calculated as follows: (Absorbance of the test at 532 nm x Total Volume of the reaction mixture x 1000) / (56 X 10^5 $m^{-1}cm^{-1}$) x (Volume of semen x 1 cm).

Statistical Analyses: Data collected were subjected to descriptive statistics and one-way Analysis of variance procedure of SAS (2011) and means were compared using Duncan's multiple range test of the same software.

RESULTS

Effect of ABLME on Quality and Spermatozoa Fertilising Potential of Extended (4°C) Buck Semen at 0 Hour: Table 4 showed that semen samples preserved in 2.00 g/L ABLME had 84.67 ± 4.57% SPL, while 0.00 g/L ABLME treatment recorded 92.33 ± 2.67% SPL which implies about 7.66% reduction in value of SPL in the treatment compare to the control. However, samples preserved with other concentrations of ABLME were statistically similar ($p>0.05$) to the control. Normal spermatozoa were significantly greater ($p<0.05$) in the control than in all of the ABLME-extended samples. Semen pH in 0.00 g/L ABLME was significantly more acidic compared to samples preserved in ABLME extenders. There were no statistical variations ($p>0.05$) in values obtained for ACI and PMI in all treatments at hour.

Effect of ABLME on Quality and Spermatozoa Fertilising Potential in Extended (4°C) Buck Semen at 24 Hours: Table 5 revealed that there were no significant differences ($p>0.05$) in results obtained for all semen samples extended with 0.00 g/L ABLME and extenders containing varying concentrations of ABLME for SPL and PMI at 24 hours of storage. However, samples extended with 0.00 g/L ABLME had significantly higher ($p<0.05$) values for semen pH, NSP and ACI compared to those extenders containing ABLME.

Effect of ABLME on Quality and Spermatozoa Fertilising Potential of Extended (4°C) Buck Semen at 48 Hours: At 48 hours (Table 6), SPL decreased significantly ($p<0.05$) as the concentration of ABLME in the extenders increased. There was an increase of about 14.34% SPL in semen extended with 0.00 g/L ABLME with an SPL value of 87.67 ± 1.12% compared to semen samples extended with 2.00 g/L ABLME with an SPL value of 73.33 ± 3.58%. The same relationship was also seen in semen pH with samples preserved in 0.00 g/L ABLME being significantly less ($p<0.05$) acidic than all samples preserved with varying concentrations of ABLME in the extender. NSP was also significantly greater ($p<0.05$) in all semen extended with ABLME extenders compared to being semen in 0.00 g/L ABLME extenders. Increases of about 12.34% and 10.00% respectively were recorded for NSP in samples extended with extenders containing 0.50 g/L with the value of 79.67 ± 2.33% NSP and extenders containing 2.00 g/L ABLME with NSP value of 77.33 ± 1.54% respectively compared to that of 0.00 g/L ABLME. ACI for samples in 0.00 g/L ABLME was similar to all samples in extenders containing varying amounts of ABLME.

Table 4: Effect of *Aloe barbadensis* leaf methanolic extract (ABLME) on quality, and spermatozoa fertilising potential of extended (4°C) buck semen at 0 hour

Parameter	ABLME (g/L)				
	0.00	0.50	1.00	1.50	2.00
Spermatozoa Liveability (%)	92.33 ± 2.67^{c}	89.00 ± 0.47^{b}	90.67 ± 2.27^{b}	91.33 ± 1.94^{b}	84.67 ± 4.57^{a}
Normal Spermatozoa (%)	92.67 ± 2.85^{b}	92.67 ± 2.85^{b}	86.33 ± 3.05^{a}	87.00 ± 3.05^{a}	84.00 ± 3.06^{a}
pH	6.63 ± 0.24^{a}	6.89 ± 0.01^{b}	6.93 ± 0.01^{bc}	6.96 ± 0.01^{c}	6.91 ± 0.02^{b}
Acrosome Integrity (%)	89.33 ± 6.69	85.33 ± 3.08	89.33 ± 3.08	84.67 ± 2.75	85.33 ± 2.76
Plasma Membrane Integrity (%)	85.67 ± 0.00	88.33 ± 0.15	84.33 ± 2.00	89.00 ± 0.01	90.67 ± 0.03

^{abc}= means in a row having different letter superscripts differ statistically ($p<0.05$)

Table 5: Effect of *Aloe barbadensis* leaf methanolic extract (ABLME) on quality, and spermatozoa fertilising potential in extended (4°C) buck semen at 24 hours

Parameter	ABLME (g/L)				
	0.00	0.50	1.00	1.50	2.00
Spermatozoa Livability (%)	85.00 ± 2.16	85.33 ± 1.99	85.33 ± 3.60	83.67 ± 4.73	83.33 ± 6.24
Normal Spermatozoa (%)	94.00 ± 1.63^{c}	76.33 ± 4.48^{a}	85.00 ± 2.36^{b}	84.00 ± 2.16^{b}	76.67 ± 2.72^{a}
pH	6.73 ± 3.89^{a}	6.61 ± 3.81^{b}	6.57 ± 3.8^{bc}	6.54 ± 3.76^{c}	6.51 ± 0.02^{c}
Acrosome Integrity (%)	95.00 ± 0.47^{c}	79.33 ± 3.88^{a}	88.33 ± 1.45^{b}	89.00 ± 0.47^{b}	89.00 ± 0.58^{b}
Plasma Membrane Integrity (%)	70.67 ± 0.39	70.00 ± 0.58	71.00 ± 1.25	70.33 ± 1.35	75.00 ± 2.22

^{abc}= means in a row having different letter superscripts differ statistically ($p<0.05$)

Table 6: Effect of *Aloe barbadensis* leaf methanolic extract (ABLME) on quality, and spermatozoa fertilising potential of extended (4°C) buck semen at 48 hours

Parameter	ABLME (g/L)				
	0.00	0.50	1.00	1.50	2.00
Spermatozoa Liveability (%)	87.67 ± 1.12^{c}	84.33 ± 7.12^{bc}	80.00 ± 3.77^{b}	77.67 ± 5.31^{ab}	73.33 ± 3.58^{a}
Normal Spermatozoa (%)	67.33 ± 1.18^{a}	79.67 ± 2.33^{c}	72.33 ± 5.99^{b}	75.67 ± 1.07^{bc}	77.33 ± 1.54^{bc}
pH	6.75 ± 0.01^{c}	6.65 ± 0.01^{b}	6.61 ± 0.01^{b}	6.58 ± 0.01^{bc}	6.54 ± 0.00^{a}
Acrosome Integrity (%)	76.00 ± 2.90^{ab}	81.00 ± 6.66^{c}	72.00 ± 6.00^{ab}	68.00 ± 3.84^{a}	70.33 ± 2.13^{b}
Plasma Membrane Integrity (%)	71.00 ± 1.73^{c}	69.33 ± 1.95^{bc}	66.33 ± 1.18^{b}	72.33 ± 3.85^{c}	61.33 ± 1.76^{a}

^{abc}= means in a row having different letter superscripts differ statistically ($p<0.05$)

Effect of ABLME on Quality and Spermatozoa Fertilising Potential of Extended (4°C) Buck Semen at 72 Hours: In Table 7 it was observed that above 1.00 g/L ABLME, there was a substantial decrease in the proportion of SPL, NSP and spermatozoa with intact acrosomes compared to those exposed to 0.00 g/L ABLME for 72 hours. Also, it was clear that semen samples extended in 0.00 g/L ABLME are less acidic than others containing different amounts of ABLME.

Types of abnormal Spermatozoa in Buck Semen Extended with 2.00 g/L ABLME: Table 8 shows the classification of abnormal spermatozoa in samples extended with 2.00 g/L ABLME. At 0 and 72 hours, 0.20 ± 0.20 and 0.30 ± 2.70% of the rudimentary tail (RDT) respectively were the only primary abnormality observed. Occurrence of secondary abnormalities viz: bent tail (BNT), looped tail (LPT), coiled tail (CLT), curved mid-piece (CMP) and detached tail (DTT) increased gradually with duration of storage regardless of the concentration of the ABLME in the extender.

Effect of ABLME on Microbial Load (10^5 CFU/mL) of Extended (4°C) Buck Semen: All semen samples preserved in ABLME extenders showed lower microbial loads compared to the control at 48 hours of storage (Table 9), In addition, regardless of the concentration of the extract, microbial population increased with the storage duration. The lowest population (0.00 ± 0.00 x 10^5 CFU/mL) was recorded for samples extended with 0.5 g/L ABLME after 24 hours of storage, while the highest population (90.50 ± 7.94 x 10^5 CFU/mL) was obtained for samples stored with 2.0 g/L ABLME after 72 hours.

DISCUSSION

From Zero to 48 hours of storage over 70% of life and normal spermatozoa with intact plasma membrane integrity were recorded for all semen samples stored with ABLME extender. Variation in concentration shows no difference in SPL, NSP and ACI but there were significant differences in the values obtained for PMI and pH at 72 hours. This result was in agreement with the findings of Yaniz *et al.* (2008) that the slight decrease in these parameters was due to the production of reactive oxygen species (ROS) and the susceptibility of the plasma membrane to peroxidative damage caused by changes in the pH.

Contaminant compromise of plasma membrane integrity causes spermatozoa progressive motility and induces a reduction in fertility and fertility declines. The forms of spermatozoa abnormalities observed were similar. Morphological abnormalities and defects are crucial because a deformed or immature sperm cell has a reduced or no chance of fertilizing an oocyte. RDT, BNT, CLT and CMP were observed in samples extended with varying concentrations of ABLME 72 hours. Different researchers have come to different conclusions about the most common morphological defects and the overall percentage of morphological abnormalities in a typical male. Tibary and Vaughan (2006) concluded that head abnormalities were the most common defect (Tibary and Vaughan, 2006) why Tail deformities were shown to be the most common in a trial at the University of Massachusetts by Bravo (Szymkowicz, 2012). A major factor detrimental to the quality of semen for AI is microbial contamination. can occur because of urinary tract infection or during semen collection (Wu *et al.*, 2019). From this study, it is observed that the longer the storage time, the higher the population of microbes irrespective of the concentration of ABLME. Between 0 to 24 hours, the microbial load declined with time in the control and samples extended with 0.5 g/L ABLME but there was a significant increase in the microbial load in samples extended with 1.0, 1.5 and 2.0 g/L ABLME, respectively. Also, a progressive increase in the microbial loads with time in all samples after 24 hours of storage up to 72 hours was observed. However, all samples preserved in extenders containing ABLME had a lower microbial population ranging from 6.8 to 20.7 × 10^5 CFU/mL compared to 27.5 × 10^5 CFU/mL in the control containing conventional antibiotics after 48 hours post storage.

Table 7: Effect of *Aloe barbadensis* leaf methanolic extract (ABLME) on quality, and spermatozoa fertilising potential of extended (4°C) buck semen at 72 hours

Parameter	ABLME (g/L)				
	0.00	0.50	1.00	1.50	2.00
Spermatozoa Liveability (%)	72.67 ± 4.1^{ab}	75.67 ± 2.37^{c}	69.33 ± 5.47^{bc}	63.00 ± 4.04^{a}	67.67 ± 6.31^{b}
Normal Spermatozoa (%)	74.33 ± 7.02^{c}	70.67 ± 2.25^{bc}	76.67 ± 4.65^{c}	60.67 ± 9.68^{b}	65.00 ± 2.89^{a}
pH	6.74 ± 0.00^{c}	6.66 ± 0.00^{b}	6.64 ± 0.00^{b}	6.57 ± 0.00^{a}	6.55 ± 0.00^{a}
Acrosome Integrity (%)	72.00 ± 3.51^{c}	67.67 ± 1.56^{bc}	62.67 ± 3.94^{b}	57.67 ± 4.47^{a}	63.33 ± 4.9^{b}
Plasma Membrane Integrity (%)	67.67 ± 1.46^{c}	67.67 ± 1.14^{c}	65.67 ± 1.14^{b}	65.33 ± 0.92^{b}	60.67 ± 1.24^{a}

^{abc}= Values in a row having different letter superscripts differ statistically ($p<0.05$)

Table 8: Types of abnormal spermatozoa in buck semen extended with 2.00 g/L *Aloe barbadensis* leaf methanolic extract (ABLME)

Time (Hours)	Spermatozoa Abnormalities Classification (%)					
	RT	BT	LT	CT	CMP	DT
0	0.20 ± 0.2^{b}	0.93 ± 0.6^{a}	2.60 ± 1.0^{a}	0.73 ± 0.3^{a}	0.60 ± 0.4^{a}	3.33 ± 0.6^{a}
24	0.00 ± 0.0^{a}	1.00 ± 4.6^{b}	2.80 ± 0.9^{b}	1.30 ± 2.3^{b}	1.13 ± 1.2^{b}	3.93 ± 2.3^{a}
48	0.00 ± 0.0^{a}	2.70 ± 4.0^{c}	2.93 ± 0.6^{bc}	2.60 ± 0.7^{c}	2.13 ± 1.6^{c}	6.27 ± 1.3^{b}
72	0.30 ± 2.7^{c}	5.13 ± 2.6^{d}	3.20 ± 1.1^{c}	3.03 ± 1.7^{d}	2.63 ± 1.2d	9.20 ± 3.3^{c}

RDT = Rudimentary tail; BNT = Bent tail; LPT = Looped tail; CLT = Coiled tail; CMP = Curved mid-piece; DTT= Detached tail;
^{abc}= Values in a column having different letter superscript differ statistically ($p<0.05$)

Table 9: Effect of *Aloe barbadensis* leaf methanolic extract (ABLME) on microbial load (10^5 CFU/mL) of extended (4°C) buck semen

Time (Hours)	ABLME (g/L)				
	0.0	0.5	1.0	1.5	2.0
0	0.83 ± 0.27^{d}	0.50 ± 0.00^{c}	2.67 ± 0.49^{e}	0.00 ± 0.00^{a}	0.17 ± 0.14^{b}
24	0.67 ± 0.27^{b}	0.00 ± 0.00^{a}	7.00 ± 3.56^{d}	0.67 ± 0.36^{b}	1.00 ± 0.47^{c}
48	27.50 ± 12.32^{c}	16.83 ± 8.85^{b}	9.33 ± 0.60^{a}	20.67 ± 0.76^{b}	6.83 ± 1.16^{a}
72	38.12 ± 1.16^{a}	34.50 ± 1.31^{a}	41.12 ± 2.00^{a}	69.00 ± 5.67^{b}	90.50 ± 7.94^{c}

^{abc}= Values in a row having different letter superscripts differ statistically ($p<0.05$)

The reduction in the microbial population in samples extended in ABLME could be attributed to the secondary metabolites or bioactive ingredients in the ABLME However, the increase in the microbial loads after 24 hours of storage could be because of depletion in the concentration of these metabolites with time. This finding is consistent with the findings of several authors, including Shihabudeen *et al.* (2010), who reported that phytoconstituents in extender, such as alkaloids, flavonoids, tannins, phenols, saponins and a variety of other aromatic compounds, act as a defence mechanism against a variety of microbes. *Streptococcus pyogenes* and *Streptococcus faecalis* are microorganisms that have been reported to be inhibited by *A. vera* gel (Cera *et al*., 1981; Robson *et al.,* 1982). According to Saoo *et al.* (1996), lectins in a portion of *A. vera* gel inhibit cytomegalovirus (CMV) proliferation in cell culture by affecting protein synthesis. Many of the medicinal effects of *A. vera* leaf extracts have been attributed to the polysaccharides found in the inner leaf parenchymatous tissue (Ni *et al*., 2004). The antibacterial activity of the extract can also be linked to anthraquinones and saponins present in the extract (Boateng, 2000; García-Sosa *et al.,* 2006; Qun *et al.,* 2023).

Conclusion: Methanol extract (2.0 g/L) of *A. barbadensis* inclusion in egg yolk + sodium citrate diluent gave enhanced antibacterial activity to extend buck semen for up to 48 hours. Since the methanol extract of *A. barbadensis* leaf inhibits microbial growth better than conventional antibiotics, up to 48 hours of storage, the extract is suitable as a natural antibacterial agent in short time buck semen extension, not beyond 48 hours.

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