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The Effects of Extraction Methods of *Mangifera indica* and *Azadirachta indica* Bark on *in vitro* Antimicrobial Efficacy and Performance of Broiler Chickens

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ABSTRACT

This study investigated the *in vitro* efficacy of extracts of *Mangifera indica* and *Azadirachta indica* bark obtained by different extraction methods. Also, in an eight-week trial, the effect of these extracts on the performance of broiler chickens were evaluated. The barks were collected, air-dried and pulverized. The samples were extracted using maceration, infusion, and decoction methods. The extracts were screened for their activity against *Escherichia coli* and *Streptococcus aureus*. Two hundred and eighty-eight birds were divided into two groups (144 each) administered with *A. indica* or *M. indica*. Each group was subdivided into four subgroups, including control subgroup (no herb) and subgroups administered with bark extracted by one of the three extraction methods. The birds on herbal treatments were not given antibiotics. Results showed that the growth of *E. coli* was more inhibited by the various extracts irrespective of the methods of extraction. Weights were significantly influenced by the interaction between herb types and extraction methods at the starter phase. Infused herbs induced mortality at the finisher phase. In conclusion, *S. aureus* was more susceptible to the extracts compared to *E. coli*. However, decocted *A. indica* and *M. indica* bark, as well as macerated M. indica, showed antimicrobial potency against *E. coli*. It can be recommended that neem or mango bark extracted by maceration or decoction can be administered orally to broiler chickens especially at the starter phase, for improved performance and reduced mortality.

Key words: Antimicrobial assay, *Azadirachta indica*, Extraction, *Mangifera indica*, performance

INTRODUCTION

Poultry farmers are interested in raising their birds to gain market weight as early as possible within the shortest period. This has led to the use of antibiotics that can modify the intestinal microbiota and eliminate harmful bacteria, which in turn improves the growth of the birds. However, there are global concerns over the use of antibiotics for growth promotion or therapy purposes because despite rigorous withdrawal measures to prevent antibiotic residues in food some drugs enter the human food chain and lead to increased antibiotic resistance (Molbak, 2005). For these reasons, European countries have banned the use of antibiotics as growth promoters in animal feed (Castanon, 2007). Therefore, research attention has been shifted to using natural alternatives such as medicinal plants as natural feed additives in poultry diet to enhance production performance as well as to counter bacteria growth (Abaza et al., 2008).

Recent studies have tested the use of herbal extracts as alternatives to antibiotic growth promoters (Biswas et al., 2002; Landy et al., 2011; Sarker et al., 2014). The mechanism of action of bioactive components of these extracts is based on the alteration of the intestinal microbiota, increased enzyme secretion, histo-morphological maintenance of the gastrointestinal tract, and enhancement of immune system (Brugalli, 2003). Various research studies have demonstrated antimicrobial, antifungal, anthelmintic and antioxidant effects of plant extracts (Kamel, 2000). Allinson et al. (2013) reported that herbal extracts improve the performance and Feed Conversion Ratio (FCR) in poultry as well as decrease the bacterial and oocyst counts. Neem (*Azadirachta indica*) is one of the most prominent herbal medicines with different biologically active compounds such as azadirachtin, nimbin, salanin, meliacin, and triterpenoids (National Research Council, 1992; Ansari et al., 2012).

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Mangifera indica (mango) is another plant whose leaves, fruits and barks are known for their medicinal potential and are being explored. Khan et al. (1993) detected compounds such as terpenoidal saponins, polygalacturonase, fructose-1,6- diphosphatase, triterpenoid, 2- hyroxymangiferonic acid tetracyclic triterpenoid and pentacyclic triterpenoid in *Mangifera indica* extract. The bark infusion has been used as a gargle to treat mouth infections in children (Doughari and Manzara, 2008).

Methods of preparation of crude extracts and their purity greatly influence the inhibitory activity of some herbs against infectious organisms. Also, the extraction method, extraction solvent and the plant part used determines the quality of the extract. Hence, this study aimed to evaluate the performance of broiler chickens administered with neem or mango bark extract prepared by decoction, infusion and maceration techniques.

MATERIALS AND METHODS

Experimental Site

The research was carried out at the Poultry Unit of the Directorate of University Farms (DUFARMS), Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

Ethical approval

The present study was approved by the ethics and research committee of the College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta, Nigeria.

Preparation of plant extracts

The *A. indica* and *M. indica* barks were air-dried and pulverized. Three methods of extraction used; maceration, infusion, and decoction. Maceration was performed by soaking of 100 g of dried barks of each sample in 1 L of cool water in a covered plastic for 72 hours at room temperature, and then the mixture was strained. The infusion process involved soaking of 100 g of either *A. indica* or *M. indica* dried barks in 1 L of hot water for 12 hours, then was filtered to obtain the extract. Decoction method performed by boiling 100 g dried barks in 1 L of water for 1 hour. After cooling, the extract was obtained by decantation.

Assessment of antimicrobial activity of plant extracts

The agar well diffusion method was used for the antimicrobial susceptibility test. Mueller Hilton agar was prepared according to the manufacturer's specifications. The media were autoclaved and dispensed into sterile Petri-dishes and allowed to gel. Standardized inocula of *Escherichia coli* and *Staphylococcus aureus* were streaked on the agar plate. Six wells of 6 mm each were made in each plate with a central well for positive control using a sterile cork borer. The wells were filled with 0.1 ml of different extracts of two herbs prepared by different methods (infusion, maceration, and decoction). In addition, 0.1 ml of ciprofloxacin were used in separate plates to serve as positive control while sterile distilled water was used as a negative control on separate plates. The plates were allowed to stand for 15 minutes to allow free diffusion of the extracts. After 24 hours of incubation at 37 $^{\circ}$C, a transparent plastic meter rule was used to measure the diameters of zone of inhibition, according to Dahiru et al. (2013).

Growth response trial

A total of 288 day-old broiler chicks was divided into two groups (144 birds each group) administered with *A. indica* or *M. indica*. Each group was subdivided into four subgroups (36 birds each) including control (no herb administered) and subgroups administered with herbal extracts obtained from different extraction methods: maceration, infusion, and decoction. Hence, the birds were arranged in a 2 × 4 experimental layout. Brooding was done for two weeks. Commercial broiler starter was given for the first four weeks, while commercial broiler finisher was given from four weeks to eight weeks (Table 1). The groups were given necessary medications (antibiotics, coccidiostats, and vitamins) and vaccinations (Gumboro vaccine on 7th and 15th day, and Lasota at 4 weeks of age). Birds treated with medicinal herbs were not given antibiotics. Herbs were supplied in drinking water (the extracts were added at a dosage of 150 ml to 1 L water) for three consecutive days per week for six weeks.

Table 1. Nutrient composition of feed

Parameter	Starter diet	Finisher diet
Crude protein (%)	21.00	18.00
Fat (%)	6.00	6.00
Crude fiber (5%)	5.00	5.00
Calcium (%)	1.00	1.00
Available phosphorus (%)	0.45	0.40
Lysine (%)	1.00	0.85
Methionine (%)	0.50	0.35
Salt (%)	0.30	0.30
Metabolizable energy (Kcal/kg)	2900	2800

Data collection

Feed intake

The amount of feed given to the birds and the leftover were measured weekly to determine the feed intake according to the following equation:

Feed intake = Feed given − Feed leftover

Body weight and weight gain

The birds were weighed on a replicate basis at the commencement of the experiment and subsequently every week.

Body weight (g) = Total weight of birds (g) / Total number of birds
Total weight gain (g) = Final weight (g) − Initial weight (g)
Daily weight gain = (Final weight – Initial weight) / Number of days

Feed conversion ratio

The FCR was calculated as total feed intake divided by weight gain.

FCR = Total feed intake(g) / Total weight gain(g)

Mortality rate

The mortality rate was calculated as the total number of dead birds divided by the total number of birds and expressed in percentage.

Statistical analysis

Data obtained on antibacterial assay were subjected to one-way analysis of variance while those on performance were subjected to one way of analysis of variance in a 2×4 factorial experimental layout using SPSS software (version 23.0) Significant means were separated using Duncan's multiple range test at 5% level of significance ($p<0.05$).

RESULTS

Antimicrobial assay of differently extracted *Mangifera indica* and *Azadirachta indica* bark on selected bacteria

Figure 1 shows the bar chart representation of the antimicrobial assay of differently extracted *M. indica* and *A. indica* bark on the Gram-negative organism *(Escherichia coli)* and gram-positive (*Staphylococcus aureus*) bacteria. Significant differences ($p<0.05$) were observed among the distance of inhibition zone of *E. coli* and *S. aureus* with respect to the type of herbs and extraction methods. The highest zone of inhibition of the two bacteria (33.00 mm and 29.00 mm for *E. coli* and *S. aureus*, respectively) was recorded for positive control samples (ciprofloxacin) ($p<0.05$). However, it was observed that the extracts of *A. indica* obtained by infusion and maceration methods and *M. indica* obtained by infusion did not inhibit the growth of *E. coli* while others had similar values. growth of *S. aureus* was inhibited by the extracts from the various methods of extraction except for extract of *M. indica* obtained by decoction.

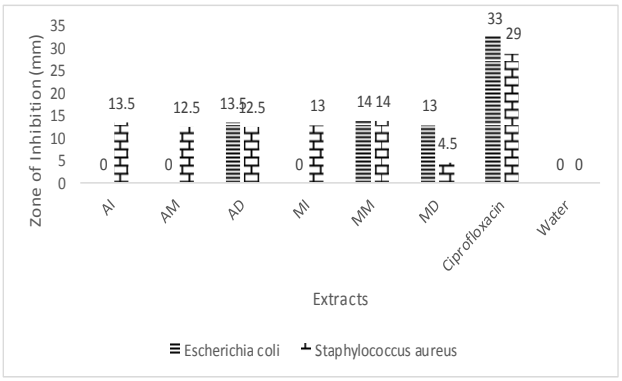

Figure 1. Antimicrobial assay of differently extracted *Mangifera indica* and *Azadirachta indica* bark on *Escherichia coli* and *Staphylococcus aureus.* AI: *Azadirachta indica* extracted by infusion; AM: *Azadirachta indica* extracted by maceration method; AD: *Azadirachta indica* extracted by decoction; MI: *Mangifera indica* extracted by infusion; MM: *Mangifera indica* extracted by maceration method; MD: *Mangifera indica* extracted by decoction.

Effect of herb types and extraction methods on the performance of broiler chickens at the starter phase

The performance traits of birds were not significantly ($p>0.05$) affected by herbs and different extraction methods at the starter phase (Table 2). The interaction effect of herbs and extraction methods on the performance of broiler chickens at the starter phase is presented in table 3. Final weight and total weight gain were highest ($p<0.05$) in the control group of birds administered with *M. indica* extract while the lowest were recorded for birds administered with *A. indica* prepared by the infusion method.

Effect of herbs and extraction methods on the performance of broiler chickens at the finisher phase

The main effect of herbs and extraction methods on the performance of broiler chickens at the finisher phase is presented in table 4. Herb type had no influence ($p>0.05$) on all parameters measured. However, the highest ($p<0.05$) mortality (1.67%) was observed in birds administered herb extract prepared by infusion. The effects of interaction between herbs and extraction methods on the performance of broiler chickens at the finisher phase are shown in table 5. All performance traits evaluated were similar ($p>0.05$) except the mortality. Mortality was highest for birds administered with *A. indica* extract prepared by infusion and the lowest was recorded for control birds and birds administered with *M. indica* and *A. indica* extracts prepared by maceration.

Table 2. Main effect of different herbs and different extraction methods on the performance of broiler chickens at starter phase

	Effect of Herbs		Effect of Extraction Methods			
Parameters	**MI**	**AI**	**Control**	**Maceration**	**Infusion**	**Decoction**
Initial weight (g/bird)	41.01±0.71	41.14±2.89	42.06±3.86	40.89±0.65	40.71±0.83	40.64±1.37
Final weight (g/bird)	760.32±41.12	738.02±50.67	764.52±61.13	770.48±47.96	722.22±40.02	739.44±22.62
Total Weight gain (g)	719.31±41.12	698.88±51.90	722.46±63.41	729.59±48.28	681.51±40.29	698.81±22.80
Weight gain/day (g/ bird /day)	25.69±1.47	24.89±1.85	25.80±2.26	26.06±1.72	24.34±1.44	24.96±0.81
Total feed (g/bird)	1580.55±220.69	1603.37±89.59	1605.78±55.33	1532.72±262.85	1538.37±70.88	1690.97±169.13
Total feed/day (g/ bird /day)	56.45±7.88	57.26±3.20	57.35±1.98	54.74±9.39	54.94±2.53	60.39±6.04
Total water intake (ml/ bird)	3588.46±264.41	3418.44±146.14	3604.62±238.75	3555.65±203.04	3417.06±167.53	3436.47±281.60
Water intake/day (ml/ bird /day)	128.16±9.44	122.09±5.22	128.74±8.53	126.99±7.25	122.04±5.98	122.73±10.06
Mortality (%)	0.50±0.80	0.25±0.62	0.17±0.41	0.50±0.84	0.17±0.41	0.67±1.03
FCR	2.20±0.33	2.31±0.22	2.24±0.26	2.10±0.38	2.26±0.16	2.42±0.27

Data are expressed as mean ± standard deviation. MI: *Mangifera indica* AI: *Azadirachta indica* FCR: Feed conversion ratio

Table 3. Interaction effects of between herbs and extraction methods on the performance of broiler chickens at starter phase

	Mangifera indica				***Azadirachta indica***			
Parameters	**Control**	**Maceration**	**Infusion**	**Decoction**	**Control**	**Maceration**	**Infusion**	**Decoction**
Initial weight (g/bird)	40.64±0.84	41.31±0.63	40.92±1.02	41.17±0.44	43.47±5.12	40.47±0.38	40.5±0.73	40.11±1.91
Final weight (g/bird)	$801.26±56.45^{a}$	$753.33±10.14^{ab}$	$738.89±41.94^{ab}$	$747.78±27.15^{ab}$	$727.78±45.90^{ab}$	$787.63±69.03^{ab}$	$705.56±37.58^{b}$	$731.11±18.28^{ab}$
Total weight gain (g)	$760.62±55.75^{a}$	$712.03±9.51^{ab}$	$697.97±42.24^{ab}$	$706.61±27.40^{ab}$	$684.31±50.75^{ab}$	$747.15±69.37^{ab}$	$665.06±38.23^{b}$	$691.00±19.13^{ab}$
Daily weight gain (g/bird)	$27.17±1.99^{a}$	$25.43±0.34^{ab}$	$24.93±1.51^{ab}$	$25.24±0.98^{ab}$	$24.44±1.82^{ab}$	$26.68±2.48^{ab}$	$23.75±1.37^{b}$	$24.68±0.68^{ab}$
Total feed intake (g/bird)	1571.29±25.26	1438.01±379.28	1561.47±90.52	1751.44±200.56	1640.28±58.70	1627.42±44.34	1515.28±52.61	1630.50±142.52
Daily feed intake (g/bird)	56.12±0.90	51.36±13.55	55.77±3.23	62.55±7.16	58.58±2.10	58.12±1.58	54.12±1.88	58.23±5.10
Total water intake (ml/bird)	3730.11±230.67	3710.28±148.90	3449.48±192.17	3463.97±409.44	3479.14±205.08	3401.01±95.71	3384.64±173.44	3408.97±168.33
Water intake/day (ml/bird/day)	133.22±8.24	132.51±5.32	123.20±6.86	123.71±14.62	124.26±7.32	121.46±3.42	120.88±6.19	121.75±6.01
Mortality (%)	0.33±0.58	0.67±1.15	0.33±0.58	0.67±1.15	0±0	0.33±0.58	0±0	0.67±1.15
FCR	2.07±0.12	2.02±0.51	2.24±0.20	2.48±0.32	2.41±0.25	2.19±0.27	2.28±0.15	2.36±0.28

Data are expressed as mean ± standard deviation. Different superscript letters in the same row indicate significant differences ($p<0.05$). FCR: Feed conversion ratio

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Table 4. Main effect of herbs and extraction methods on the performance of broiler chickens at finisher phase

	Effect of Herbs		Effect of Extraction Methods			
Parameters	**MI**	**AI**	**Control**	**Maceration**	**Infusion**	**Decoction**
Initial weight (g/bird)	760.32±41.12	738.02±50.67	764.52±61.13	770.48±47.96	722.22±40.02	739.44±22.62
Final weight (g/bird)	1847.70±77.95	1825.62±90.63	1846.09±121.54	1853.33±48.32	1836.15±39.17	1811.07±110.25
Total weight gain (g)	1087.38±52.34	1087.60±87.17	1081.57±83.27	1082.85±47.62	1113.92±38.06	1071.63±104.33
Daily weight gain (g/bird)	38.84±1.87	38.84±3.11	38.63±2.98	38.67±1.70	38.78±1.36	38.27±3.73
Total feed intake (g/bird)	3349.67±221.60	3319.51±172.89	3245.46±116.22	3334.21±217.66	3354.95±121.10	3403.74±288.70
Daily feed intake (g/bird)	119.63±7.91	118.55±6.17	115.91±4.15	119.08±7.77	119.82±4.36	121.56±10.31
Total water intake (ml/bird)	9398.34±744.80	9279.79±463.35	9424.79±517.85	9466.10±775.48	9368.98±495.06	9096.40±703.59
Water intake/day (ml/bird/day)	335.66±26.60	331.42±16.55	336.60±18.49	338.08±27.70	334.61±17.68	324.87±25.13
Mortality (%)	0.92±0.67	1.08±0.79	0.67±0.52^{b}	0.67±0.52^{b}	1.67±0.52^{a}	1.00±0.89^{ab}
FCR	3.08±0.22	3.07±0.24	3.01±0.17	3.08±0.07	3.01±0.12	3.20±0.40

Data are expressed as mean ± standard deviation. Different superscript letters in the same row indicate significant differences ($p<0.05$). MI: *Mangifera indica.* AI: *Azadirachta indica.* FCR: Feed conversion ratio

Table 5. Effects of interaction between herbs and extraction methods on the performance of broiler chickens at finisher phase

	Mangifera indica				***Azadirachta indica***			
Parameters	**Control**	**Maceration**	**Infusion**	**Decoction**	**Control**	**Maceration**	**Infusion**	**Decoction**
Initial weight (g/bird)	801.26±56.45^{a}	753.33±10.14^{ab}	738.89±41.94^{ab}	747.78±27.15^{ab}	727.78±45.90^{ab}	787.63±69.03^{ab}	705.56±37.58^{b}	731.11±18.28^{ab}
Final weight (g/bird)	1922.73±105.92	1855.15±60.58	1828.96±6.89	1783.97±57.77	1769.44±89.95	1851.52±46.43	1843.33±60.28	1838.18±157.63
Total weight gain (g)	1121.47±52.92	1101.82±59.19	1090.07±37.38	1036.19±37.01	1041.67±98.79	1063.89±32.96	1137.78±22.75	1107.07±148.56
Daily weight gain (g/bird)	40.05±1.89	39.35±2.11	38.93±1.33	37.01±1.32	37.20±3.53	37.10±1.18	40.63±0.81	39.54±5.31
Total feed intake (g/bird)	3277.53±96.02	3402.51±283.09	3332.37±148.16	3386.27±379.88	3213.38±146.49	3265.91±155.90	3377.53±117.16	3421.21±251.30
Daily feed intake (g/bird)	117.05±3.43	121.52±10.11	119.01±5.29	120.94±13.57	114.76±5.23	116.64±5.57	120.63±4.18	122.19±8.97
Total water intake (ml/bird)	9853.93±328.54	9792.97±1067.47	9130.47±408.60	8816.00±689.49	8995.66±100.11	9139.24±208.38	9607.49±524.51	9376.79±725.49
Water intake/day (ml/bird/day)	351.93±11.73	349.75±38.12	326.09±14.59	314.86±24.62	321.27±3.58	326.40±7.44	343.12±18.73	334.89±25.91
Mortality (%)	0.67±0.58^{b}	0.67±0.58^{b}	1.33±0.58^{ab}	1.00±1.00^{ab}	0.67±0.58^{b}	0.67±0.58^{b}	2.00±0^{a}	1.00±1.00^{ab}
FCR	2.92±0.10	3.08±0.09	3.06±0.09	3.27±0.37	3.10±0.20	3.07±0.05	2.97±0.14	3.13±0.49

Data are expressed as mean ± standard deviation. Different superscript letters in the same row indicate significant differences ($p<0.05$). FCR: Feed conversion ratio

DISCUSSIONS

Failure of bark extracts of infused *A. indica*, infused *M. indica* and macerated *A. indica* to inhibit *E. coli* growth (no inhibition zone diameter observed) indicates the resistance of the organism to those extracts. The appearance of the zone of inhibition by herbs prepared by decoction may indicate that this method can lead to a better release of phytochemicals and active ingredients effective in inhibiting *E. coli* activity. Since infusion and maceration techniques involve soaking in hot and cold water, respectively, thus the antimicrobial efficacy could be less potent due to reduced quality and quantity of phyto-components released, resulting in the bacteria resistant. Azwanida (2015) stated that decoction is the most effective method for extracting hard plant materials and heat-stable compounds.

It has been documented that *E. coli* can rapidly change their genetic makeup as gram-negative bacteria, this enables them to develop resistance to antibiotics (Uwimbabazi et al. 2015). This can be attributed to the lower potency of the extracts on *E. coli* compared to *S. aureus*. Resistant bacteria change their cell walls lightly, so the antibiotics cannot attach, or they produce enzymes to disable the antibiotics. Hence, the result of this study is inconsistent with findings of Gajendrasinh et al. (2012) who reported that aqueous and ethanol extracts of *A. indica* leaves were most effective against *E. coli.* The variation observed in the result could be attributed to the differences in solvent types and plant parts used.

M. indica showed a slightly stronger potency on both bacteria when extracted by the maceration method compared to infused and macerated *A. indica* and infused *M. indica* This finding indicated the role of different methods of extraction in influencing the potency of the extract.

The similar performance in birds administered with *A. indica* and *M. indica* at the starter and finisher phase is an indication that both herbs induced similar growth response in the birds. Meanwhile, in a similar study by Sarker et al. (2014), it was reported that body weight and weekly weight gain in broilers were improved with oral supplementation of 1% aqueous neem leaf extract compared to control group.

The fairly poor performance recorded for birds administered with infused *A. indica* bark indicates that growth performance decreased at the starter phase. This finding may indicate that the birds cannot well tolerate infused extract or that the potency of the herb prepared through this method is lower compared to other methods. Infusion is generally used for softer parts including leaves and flowers, thus this method may not effectively release the beneficial bioactive components in plant parts such as bark that was used in this study. Also, the temperature might not be adequate to destroy or reduce the quantity of antinutritional components of the extract which could impair feed utilization and hence suppress the growth. Tannin is a known antinutritional factor present in both herbs used and can be degraded at high temperatures. However, the temperature at infusion might not be sufficient to degrade it. Tannins in diet decrease palatability, reduce feed intake, suppress growth rate, impair net metabolizable energy and protein digestibility resulting in poor feed efficiency in animals. Tannins can also inhibit cellular protein synthesis by forming irreversible complexes with proline-rich proteins (Adejuwon et al., 2011). Contrarily, similar feed intake and FCR in the birds administered with infused bark extract with other groups in this study could be due to the fact that the herbs were administered orally and not incorporated into the diet.

The insignificant effect of the interaction of herbs and extraction methods on all performance parameters with the exception of mortality at the finisher phase corroborates the statement reported by Ayoola et al. (2015) that neem leaf had no significant effect on broiler performance at the finisher phase. Some authors found no effect of these additives on growth, feed consumption or FCR in broilers (Cross et al., 2007; Ocak, et al., 2008).

The similar effect of water intake throughout the study is in agreement with Durrani et al. (2007), who reported the non-significant effect of medicinal herbs on water intake of birds.

The highest mortality recorded in birds administered with the extract obtained by the infusion method at the finisher phase could be attributed to the accumulation of tannin which eventually became toxic and induce mortality among the birds. Also, temperature in the infusion method may not be enough to destroy toxic components present, unlike decoction which involves higher temperature and longer heating time. Thermal treatment of plant materials reduces the tannin content. (Rakic, 2004). Levels of tannin above 5% are often lethal, and it was reported that neem bark contains about 14% tannin (NRC, 1992). It is thought that the mortality rate was higher due to inability of the infusion method to properly reduce the tannin content. It can be also related to the length of time because too long infusion can cause high tannin content and tannins at high levels can result in mortality. According to Calislar (2017), poultry develops

bone problems and necrotic organs (crop, gizzard, and duodenum) resulting from liver and kidney poisoning due to excess tannin consumption. Smulikowska et al. (2001) also reported that inclusion of feed ingredients containing tannins resulted in undesirable physiological and biochemical effects including growth inhibition and negative nitrogen balances.

CONCLUSIONS

This study concludes that gram-positive (*Staphylococcus aureus*) bacteria in comparison to gram-negative *(Escherichia coli)* bacteria are more susceptible to antimicrobial effect of extracts of *Mangifera indica* and *Azadirachta indica*, regardless of the extraction methods. *Mangifera indica* and *Azadirachta indica* extracts had similar effects on the growth performance of broiler chickens at starter and finisher phases. Administration of infused neem bark decreased weight gain at the starter phase and increased mortality at the finisher phase. Hence, it can be recommended that neem or mango bark extracted by maceration or decoction can be administered orally to broiler chickens especially at the starter phase to improve performance and reduce mortality.

DECLARATIONS

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Competing interest

The authors have declared that no competing interest exists.

Authors' contribution

Ayoola A. A. conceptualized and designed the experiment, collected data and wrote the manuscript draft. Ekunseitan D. A. analyzed the data and interpreted the results. Muhammad S. B. and Oguntoye M. A. read and revised the first and second drafts and Adejola Y. A. assisted in manuscript writing.

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