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Potency of selected herbs on inhibition of ammonia producing bacteria

[1]Omidiwura, B. R. O., [1]Agboola, A. F. and [2] Adekambi, A. O.
[1]*Department of Animal Science, University of Ibadan, Ibadan, Nigeria*
[2] *Department of Microbiology, University of Ibadan, Ibadan, Nigeria*
Corresponding author: *richardwura@gmail.com ; +234-708-207-7886*

Abstract

In effort to combat environmental pollution, improve animal production and avoid drug residue, producers have resorted to the use of phytobiotics to inhibit ammonia producing microbes in the gut. Freshly harvested Azadirachta indica, Carica papaya, Saccharum officinarum, Chromolaena odorata, Eucalyptus camadulensis and Mangifera indica leaves were air dried, blended and extracted using five concentrations of solvent (100% water, 70% water + 30% methanol, 50% water + 50% methanol, 30% water + 70% methanol and 100% methanol). The antibacterial activities of the extracts and antibiotics (doxycycline) as control, were tested according to standard procedures against both gram positive (Bacillus subtilis, Staphylococcus aureus) and gram negative (Escherichia coli, Vibrio cholerae) ammonia producing bacteria. In most cases, it was observed that the inhibitory effect of methanolic extracts of Azadirachta indica, Carica papaya, Saccharum officinarum, Chromolaena odorata, Eucalyptus camadulensis and Mangifera indica leaves were not significantly different from that of synthetic antibiotics, doxycycline, when tested against the test bacteria, Bacillus subtilis, Staphylococcus aureus and Vibrio cholera. However, leave extracts showed significantly better inhibitory effect on Escherichia coli than the antibiotic drug. It can be concluded, therefore, that the leaf extracts of Azadirachta indica, Carica papaya, Saccharum officinarum, Chromolaena odorata, Eucalyptus camadulensis and Mangifera indica can serve as alternative to synthetic drugs in animal production due to their effective actions against microbial organisms.

Keywords: Ammonia producing microbes, Phytobiotics, Herbs, Antibiotics, Inhibition

Introduction

Poultry production is the largest animal industry in Nigeria and consists of a vertically integrated broiler chicken production and commercial egg production. In the industry, air pollution has been a major issue. Urease producing bacteria in poultry litter are mainly responsible for polluted environment of the sheds, because of high protein = in their diets. Chickens have no storage mechanism for amino acids consumed beyond the requirement for protein synthesis, so excess amino acids are deaminated and the derived nitrogen is excreted as uric acid (80%), ammonia (10%) and urea (5%) (Goldstein and Skadhauge, 2000). Poultry litter is the source of volatilized ammonia. If the litter is properly managed, the rate of ammonia emission will be reduced with resultant better health status of the birds. Metabolism of the protein produces uric acid and urea as waste. This waste is eliminated along with intestinal microorganisms in droppings on litter. In presence of high humidity and ambient temperature, urease producing bacteria grow and convert the urea into ammonia. This is why effort is now being made to control air pollution caused by nitrogen producing bacteria in poultry production. According to Gospodinov (2016), ammonia levels as low as 10 parts per million (ppm) has been implicated to affect birds' health and performance in laboratory and field studies. The bird's respiratory system can be damaged at ammonia levels above 25 ppm and allow infectious agents

to become established, leading to declining flock health and performance. Gospodinov (2016) observed that broilers in poultry house having between 25-50 ppm ammonia showed reduced body weight, immunosuppression and enhanced susceptibility to respiratory pathogens. Not only does indoor ammonia affect the chickens but also pose a risk to the health of agricultural workers in these facilities. This polluted environment is serious health hazard for poultry workers as depicted by Occupational Safety and Health Administration (OSHA) (Gay and Knowlton, 2005). Ammonia emission from poultry houses cause environmental problems such as acid precipitation, fine particulate matter formation (particulate matter with an aerodynamic diameter less than 10μm) and nitrogen deposition in aquatic systems (Choi and Moore, 2008). High levels of NH_3 emissions cause nitrogen decomposition in aquatic systems. This deposition is usually referred to as eutrophication, if it occurs on water bodies with high concentration of phosphates. Some methods developed to minimize nitrogen production and ammonia volatilization include ammonia gas absorbers, enzyme inhibitors, feed additives such as aluminum sulphate and sodium bisulphate (Choi and Moore, 2008). Research is needed to develop methods of reducing and or eradicating ammonia producing organisms other than the use of conventional drugs.

Medicinal plants contain organic compound which provide definite physiological action on the animal body and these bioactive substances includes; Tannins, Alkaloids, Carbohydrates, Triterpenoids, steroids and Flavonoids. (Edogo *et al.,* 2005). These compounds are synthesized by primary or rather secondary metabolism of living organism. Secondary metabolism is chemically and taxonomically extremely diverse compound with obscure function. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas. (Vasu *et al.,* 2009). A large number of phytochemicals belonging to several chemical classes have been shown to have inhibitory effects on all types of microorganisms (Cowan, 1999). Hence, the need to research into the inhibitory effect of some of the indigenous herbs, such as *Carica papaya*, *Azadirachta indica, Saccharum officinarum. Chromolaena odorata, Eucalyptus camadulensis* and *Magnifera indica* on ammonia-producing bacteria

These medicinal herbs and other plants contain numerous biologically active compounds, many of which have antimicrobial activity (Cowan, 1999). Numerous studies have been conducted with the extracts of various plants, screening antimicrobial activity as well as for the discovery of new antimicrobial compounds (Chah *et al*., 2006; Nair and Chanda, 2006; Parekh and Chanda, 2007; Omidiwura, 2017). Extraction of bioactive compounds from medicinal plants permits the demonstration of their physiological activity. It also facilitates pharmacology studies leading to synthesis of a more potent drug with reduced toxicity. Furthermore, the active components of herbal remedies have the advantages of being combined with many other substances that appear to be inactive. However, these complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components.

Materials and methods

The experimental work was carried out at the Agricultural Biochemistry laboratory of Department of Animal Science and Microbiology laboratory of Department of Microbiology, University of Ibadan, Nigeria.

Fresh leaf samples of selected plants (*Carica papaya*, *Azadirachta indica,*

Saccharum officinarum. Chromolaena odorata, Eucalyptus camadulensis and *Mangifera indica*) were collected (hand plucked) within the University. The plants were sorted, cleaned, cut into smaller sizes and air dried at room temperature (20-27^{0}C) for 3 weeks. The leaves were authenticated at the Botanical garden and Department of Forestry of the University. The air-dried leaves were blended using a pre-cleaned domestic blender. Powdered samples were stored in airtight plastic bottles, protected from sunlight for subsequent extraction and further analysis. Five concentrations of solvent (100% water; 70% water, 30% methanol; 50% water, 50% methanol; 30% water, 70% methanol and 100% methanol) were prepared for the extraction. 50 grams of the powdered leaf samples were soaked in 500mL of solvent each in a sterile glass jar. The samples were soaked for 48 hours at room temperature (20-27^{0}C). The soaked samples were periodically shaken (agitated) to ensure complete extraction. The extracts were then filtered through a muslin cloth, and the filtrate concentrates were oven dried at 40^{0}C. Dried samples (0.3g) were dissolved into 3mL of its initial solvent and used for microbial analysis.

The test bacteria used; *Staphylococcus aureus, Vibrio cholera, Escherichia coli* and *Bacillus subtilis* were obtained from a personal culture collection at the Environmental Microbiology and Biotechnology Laboratory, University of Ibadan, Nigeria.

The work bench was sterilized using 70% ethanol and inoculating loops were sterilized by direct flaming using a spirit lamp. All experiments were carried out under aseptic conditions.

The test bacteria which were initially stored on glycerol stock were resuscitated on nutrient agar. They were incubated at 35±2^{0}C for 24 hours. This was carried out before employing them in the antibacterial study.

The medium used for the antibacterial set up was nutrient agar. It was prepared following the manufacturer's instruction and sterilized at 121^{0}C for 15 minutes in an autoclave. The medium was allowed to cool to around 42-45^{0}C and poured into sterile disposable petri dishes and allowed to solidify prior to use.

One or two columns of 18-24 hours old culture of the test bacteria were inoculated in normal saline and the turbidity was adjusted to 0.5 MacFarland standard. The suspension of the test bacteria (already adjusted to 0.5 MacFarland standard) was inoculated onto prepared nutrient agar plates using sterile swab sticks. After inoculation, a 5mm cork borer was used to make wells in the plates to allow the introduction of the extracts. Commercially available antibiotics, doxycycline was used as the control. The set up was incubated at 35±2^{0}C for 18-24 hours. Zones of inhibition around the wells were measured after the incubation period with a graduated ruler and recorded. The data were analyzed using One-Way ANOVA and the mean differences in inhibitory activities of extracts and antibiotics were compared using Duncan's post hoc test. The statistical program SPSS 20.0 (Statistical Package for the Social Sciences) and 2007 version (Microsoft) were used. The results were presented as the means ± standard deviation. Significance level for the differences was set at $p<0.05$.

Results and discussion

Antibacterial effect of Azadirachta indica on ammonia producing bacteria

As reported in Table 1, there was no significant difference ($P>0.05$) between the antibacterial effect of 100%water and 70% water: 30% methanol-extracted *Azadirachta indica* leaf on *Staphylococcus aureus, Vibrio cholerae, Escherichia coli* and *Bacillus subtilace* as no inhibition (0.00mm) was observed all through.

However, the antibacterial effect of 50% water: 50% methanol, 30%water:70%methanol and 100% methanol-extracted *Azadirachta indica* leaf and doxycycline on test bacteria was significantly different ($P<0.05$). The inhibitory effect of doxycycline (16mm) on *Staphylococcus aureus* was significantly better than that of 50% water: 50% methanol (10mm) and 100%methanol (10mm) - extracted *Azadirachta indica* leaf. The antimicrobial effects of doxycycline (13mm), 50% water: 50% methanol (14mm), 30% water: 70% methanol (13mm) and 100%methanol (13mm) on *Vibrio cholerae* were similar statistically in their inhibitory action. Against *Escherichia coli*, 50% water: 50% methanol (10mm) and 30%water:70%methanol (12mm)-extracted *Azadirachta indica* leaf were more effective than others (0.00mm). Also, the inhibitory effect of doxycycline (20mm) on *Bacillus subtilace* was significantly better than that of 50% water: 50% methanol (14mm), 30% water: 70% methanol (10mm) and 100%methanol (10mm)-extracted *Azadirachta indica* leaf. Several studies (Maragathavalli *et al.*, 2011; Aslam *et al.*, 2009; Subapriya and Nagini, 2005) had been carried out to investigate the antimicrobial activity of *Azadirachta indica* leaf extract and the results reported were similar to what was obtained in this study where methanolic extracts of *Azadirachta indica* leaf exhibited high antibacterial activity against all tested organisms (*staphylococcus aureus, Vibrio cholera, Escherichia coli* and *Bacillus subtilace,*) especially at 50% water: 50% methanol similar to synthetic antibiotics, doxycycline, against *Vibrio cholerae* but better than it against *Escherichia coli*. This is in consonance with the report of Okemo *et al* .(2001) who stated that crude extract of *Azadirachta indica* plant was very effective against *Staphylococcus aureus* and *Escherichia coli.* They found that an extract concentration of 0.5 mg/mL significantly reduced *Staphylococcus aureus* inoculum after 24 hours, while extracts with increasing concentrations completely wiped out viable bacteria in a lesser time. Furthermore, Maragathavalli *et al* .(2011) studied the antimicrobial activities of ethanolic extracts of *Azadirachta indica* leaves in various concentrations against pathogenic bacteria and compared that to gentamycin. They found that the ethanol extract showed maximum inhibition on *Bacillus pumillus*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* in an ascending order. On the other hand, Aslam *et al* .(2009) were able to check the action of extract on three bacterial strains: *Staphylococcus aureus, Corynebacterium bovi* and *Escherichia coli*; and concluded that 75 mg/mL concentration was very effective. Also, Raja *et al*, (2013) compared the antimicrobial efficacy of aqueous extracts of leaf of *Azadirachta indica* against human pathogenic bacteria *(Staphylococcus aureus, Enterococcus faecalis, Proteus mirabilis* and *Pseudomonas aeruginosa*). They found that the leaf extract exhibited strong antimicrobial activity against these bacteria at all the concentrations tested (500, 1000 and 2000µg/ml). As concluded by Subapriya and Nagini (2005), the presence of high concentrations of azadirachtins, quercetin and β-sitosterol in *Azadirachta indica* leaves might be responsible for strong antibacterial and antifungal activity.

Antibacterial effect of Carica papaya on ammonia producing bacteria

It was observed, as indicated in Table 2, that the synthetic antibiotics, doxycycline had inhibitory effect on all test bacteria except against *Escherichia coli* where doxycycline had zero inhibition. The methanolic extracts of *Carica papaya* leaf at all levels had no effect on all test bacteria except *Vibrio cholera* (10mm) and *Escherichia coli* (13mm) at 100% Methanol extraction.

Table 1 Antibacterial effect of *Azadirachta indica* on ammonia producing bacteria

Treatment (mm)	*Staphylococcus aureus*	*Vibrio cholera*	*Escherichia coli*	*Bacillus subtilace*
100% water	0±0.00^{c}	0±0.00^{b}	0±0.00^{b}	0±0.00^{c}
70%water:30%methanol	0±0.00^{c}	0±0.00^{b}	0±0.00^{b}	0±0.00^{c}
50%water:50%methanol	10±1.00^{b}	14±3.61^{a}	10±1.00^{a}	14±3.46^{b}
30%water:70%methanol	0±0.00^{c}	13±1.00^{a}	12±2.65^{a}	10±2.00^{b}
100% methanol	10±2.65^{b}	13±1.73^{a}	0±0.00^{b}	10±2.65^{b}
Doxycycline	16±4.36^{a}	13±1.73^{a}	0±0.00^{b}	20±265^{a}
SEM	1.90	1.77	2.10	2.25

SEM: Standard Error of Mean.
^{abc} Means with different superscript are significantly different from each other.

From this study 100% methanol extract of *Carica papaya* leaf showed zone of inhibition on *Vibrio cholerae and Escherichia coli,* with decrease in volume of methanol of the solvent used. This is agreement with the report of Omidiwura (2017), who concluded that the inhibitory effect methanol extract of pawpaw leaf was observed to be significantly better than ethanol and n-hexane extracts including control against *Aspergillus niger and Escherichia coli* . This negate the result of research conducted by Chandra *et al* . (2011) using Aqueous, n-hexane and ethanol extract of *Carica papaya* leaves for antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis, Escherichia coli,* and *Pseudomonas auruginasa* where it was observed that the three extracts inhibited all the bacteria tested. Also, Mangalanayaki and Nirosha (2013) examined the antibacterial activity of the leaves of the *Carica papaya* using solvents ethanol and ethyl acetate against *Staphylococcus aureus, Streptococcus pneumonia, Bacillus cereus, Salmonella typhi, Escherichia coli* and *Pseudomon*as *aeruginosa* by well diffusion method. The extract demonstrated higher activities against all the Gram-negative bacteria than Gram positive bacteria tested, with the highest activity (16 mm zone of inhibition) demonstrated against *Salmonella typhi*. The disparity observed in this study as compared against other ones on *Carica papaya* leaf may be due to the solvent used for extraction.

Table 2: Antibacterial effect of *Carica papaya* on ammonia producing bacteria

Treatment	*Staphylococcus aureus*	*Vibrio cholera*	*Escherichia coli*	*Bacillus subtilace*
100% water	0±0.00^{b}	0±0.00^{c}	0±0.00^{b}	0±0.00^{b}
70%water:30%methanol	0±0.00^{b}	0±0.00^{c}	0±0.00^{b}	0±0.00^{b}
50%water:50%methanol	0±0.00^{b}	0±0.00^{c}	0±0.00^{b}	0±0.00^{b}
30%water:70%methanol	0±0.00^{b}	0±0.00^{c}	0±0.00^{b}	0±0.00^{b}
100% methanol	0±0.00^{b}	10±1.00^{b}	13±2.00^{a}	0±0.00^{b}
Doxycycline	16±4.36^{a}	13±1.70^{a}	0±0.00^{b}	20±2.65^{a}
SEM	2.13	2.05	2.18	2.70

SEM: Standard Error of Mean.
^{abc} Means with different superscript are significantly different from each other.

Antibacterial effect of Saccharum officinarum on ammonia producing bacteria

As shown in Table 3, 100 %water, 70% water: 30% methanol and 50% water: 50% methanol -extracted *Saccharum officinarum* leaf had no significant inhibitory effect (0.00mm) on *Staphylococcus aureus, Vibrio cholerae, Escherichia coli* and *Bacillus*. However,

the antibacterial effect of 30%water:70%methanol and 100% methanol-extracted *Saccharum officinarum* leaf as compared with doxycycline on test bacteria was significantly different. The inhibitory effect of doxycycline (16mm) on *Staphylococcus aureus* was significantly better than that of 100% methanol (12mm) which is similar to the effect of 30% water: 70% methanol (9mm)-extracted *Saccharum officinarum* leaf. The antimicrobial effects of 100%methanol (14mm) on *Vibrio cholerae* was similar doxycycline (13mm) but significantly more effective in their inhibitory action than 30% water: 70% methanol (10mm). The 100% methanol (16mm)-extracted *Saccharum officinarum* leaf had the best inhibitory effect against *Escherichia coli.* Also, the inhibitory effect of the synthetic antibiotics, doxycycline (20mm) on *Bacillus subtilace* was significantly better than that of 30% water: 70% methanol (14mm)-extracted *Saccharum officinarum* leaf. In this study, 30% water: 70% methanol and 100% methanol extracts of *Saccharum officinarium* had the strongest inhibitory effect on *Escherichia.coli* ($p<0.05$) compare to the antibiotic drugs, Doxycycline which had zero inhibition. At the same time, it was observed that the extracts of *Saccharum officinarium* leaf using solvent with high concentration of water had zero inhibition. This explained the inability of water to extract the phytochemicals in the leaf. As the methanol content of the leaf extract increases, the inhibitory effect of the extract on ammonia producing bacteria increases. The high concentration of methanol extract was significantly better than high concentration of water extract. Isela *et al*. (2014) reported that the presence or absence of metabolites may be due to differences in polarity of the solvents used for the extraction.

Table 3: Antibacterial effect of *Saccharum officinarum* on ammonia producing bacteria

Treatment	Microbial zone of Inhibition (mm)			
	Staphylococcus aureus	*Vibrio cholera*	*Escherichia coli*	*Bacillus subtilace*
100% water	0.0±0.0 [c]	0.0±0.0 [c]	0.0±0.0 [c]	0.0±0.0 [c]
70%water:30%methanol	0.0±0.0 [c]	0.0±0.0 [c]	0.0±0.0 [c]	0.0±0.0 [c]
50%water:50%methanol	0.0±0.0 [c]	0.0±0.0 [c]	0.0±0.0 [c]	0.0±0.0 [c]
30%water:70%methanol	9.0±2.0 [b]	10.0±1.0 [b]	12.0±1.7 [b]	0.0±0.0 [c]
100% methanol	12.0±1.0 [b]	14.0±1.0 [a]	16.0±3.6 [a]	16.0±2.0 [b]
Doxycycline	16.0±4.4 [a]	13.0±1.7 [a]	0.0±0.0 [c]	20.0[c]±2.7 [a]
SEM	1.92	1.89	1.97	2.40

SEM: Standard Error of Mean.
[abc] Means with different superscript are significantly different from each other.

Antibacterial effect of Chromolaena odorata on ammonia producing bacteria

As reported in Table 4, *Chromolaena odorata* leaf extracts, especially those extracted with solvents containing methanol had inhibitory effects on the test bacteria. Against Staphylococcus, *Chromolaena odorata* leaf extracted with 100% methanol (18mm) inhibited significantly better than others. When tested for inhibition of *Vibrio cholerae,* Doxycycline (13mm) was better than others but similar to the inhibitory action of 100% methanol (12mm)-extracted *Chromolaena odorata* leaf. The synthetic antibiotics, Doxycycline (0mm) did not inhibit the growth of *Escherichia coli*, however, 100% methanol (13mm)-extracted *Chromolaena odorata* leaf inhibited the test bacterium significantly but similar to the effect of 50% water to 50% methanol (11mm) and 30% water to 70% methanol (12mm) on it. 100%

methanol had zero inhibition. Similar to the result on the antibacterial activities of *Saccharum officinarum* leaf when extracted with high water quantity, *Chromolaena odorata* leaf did not inhibit the test bacteria at high water. This may be as concluded by Isela *et al*. (2014) that the presence or absence of metabolites may be due to differences in polarity of the solvents used for the extraction.

Table 4: Antibacterial effect of *Chromolaena odorata* on ammonia producing bacteria

Treatments	Microbial zone of Inhibition (mm)			
	Staphylococcus aureus	*Vibrio cholerae*	*Escherichia coli*	*Bacillus subtilace*
100% water	0.0±0.0 [d]	0.0±0.0[c]	0.0±0.0 [b]	0.0±0.0 [d]
70%water:30%methanol	10.0±2.0 [c]	0.0±0.0 [c]	0.0±0.0 [b]	0.0±0.0 [d]
50%water:50%methanol	11.0±1.0 [c]	11.0±2.7 [b]	11.0±1.0 [a]	8.0±2.7 [c]
30%water:70%methanol	13.0±2.7[c]	11.0±1.0 [b]	12.0±2.0 [a]	15.0±1.0 [b]
100% methanol	18.0±2.0 [a]	12.0±2.0[a]	13.0±2.0 [a]	0.0±0.0 [d]
Doxycycline	16.0±4.4 [b]	13.0±1.7 [a]	0.0±0.0 [b]	20.0±2.7 [a]
SEM	1.69	1.74	1.82	2.26

SEM: Standard Error of Mean.
[abcd] Means with different superscript are significantly different from each other.

Antibacterial effect of *Eucalyptus camadulensis* on ammonia producing bacteria

The antibacterial activity of *Eucalyptus camadulensis* on the four ammonia producing bacteria is shown on Table 5. The *Eucalyptus camadulensis* leaf extracts inhibited the growth of all the test bacteria regardless of the solvent. The inhibitory effect of the synthetic antibiotics, doxycycline (16mm) on *Staphylococcus aureus* was observed to be significantly higher than the effect of *Eucalyptus camadulensis* leaf extracted with 70% water: 30% methanol (14mm) but similar (P>0.05) to those extracted with 50% water: 50% methanol (15mm), 30% water: 70% methanol (16mm) and 100% methanol (16mm) shows the 100% aqueous leaf extract showed the least inhibitory activity (10mm). While doxycycline showed the least inhibitory effect (13mm) on *Vibrio cholera*, leaf extracts of 70% water: 30% methanol (22mm), 30% water: 70% methanol (20mm) and 100% methanol (20mm) exhibited similar (P>0.05) antibacterial effect but higher (P<0.05) than the effects of 50% water: 50% methanol (16mm) and 100% water (17mm). For *Escherichia coli*, aqueous leaf extract of 100% and doxycycline showed no inhibitory activity. There was no significant difference in inhibitory activity in leaf extracts from 70% water: 30% methanol (10mm), 50% water: 50% methanol (10mm), 30% water: 70% methanol (11mm). However, activities of these extract were higher than the effect of 100% methanol (8mm) on *Escherichia coli*. The inhibitory activity of leaf extracts of 100% water (20mm), 70% water: 30% methanol (21mm), 50% water: 50% methanol (20mm), 30% water: 70% methanol (21mm) and doxycycline (20mm) on *Bacillus subtilis* were similar (P>0.05) but higher (P<0.05) than the effect of 100% Methanol (18mm). This study shows that methanol extract of eucalyptus leaf exhibited a broad-spectrum activity against all test microorganisms which is in consonance with observations of Ayepola and Adeniyi (2008) and Pranay *et al*. (2010). In this study, Doxycycline has an inhibitory effect on *Staphylococcus aureus* but no inhibitory effect on *Escherichia coli*. This is not in agreement with the findings of Bachir and Benali (2008; 2014) who concluded in their studies that Doxycycline

has no inhibitory effect on *Staphylococcus aureus* but had an inhibitory activity on *Escherichia coli*. Inhibitory activity against Staphylococcus increased as the methanol content of leaf extracts increases. For Bacillus, inhibitory activity decreased as the methanol content in leaf extract increases. In both ways, eucalyptus leaf extract had effect on the test bacteria which further confirms the broad-spectrum activity of eucalyptus.

Table 5: Antibacterial effect of *Eucalyptus camaldulensis* on ammonia producing bacteria

Treatment	Microbial zone of Inhibition (mm)			
	Staphylococcus aureus	*Vibrio cholerae*	*Escherichia coli*	*Bacillus subtilace*
100% water	10.00±1.00^{c}	17.00±1.00^{b}	0.00±0.00^{c}	20.67±4.04^{a}
70%water:30%methanol	14.00±2.00^{b}	22.00±6.25^{a}	10.00±2.65^{a}	21.00±1.73^{a}
50%water:50%methanol	15.00±2.00^{a}	16.00±2.65^{b}	10.00±1.00^{a}	20.00±1.73^{a}
30%water:70%methanol	16.00±5.30^{a}	20.00±6.25^{a}	11.33±3.51^{a}	21.00±5.29^{a}
100% methanol	16.00±3.61^{a}	20.00±1.00^{a}	8.00±2.65^{b}	18.00±2.65^{b}
Doxycycline	16.00±4.36^{a}	13.00±1.73^{c}	0.00±0.00^{c}	20.00±2.65^{a}
SEM	1.06	1.09	1.71	1.07

SEM: Standard Error of Mean.
^{abc} Means with different superscript are significantly different from each other.

Antibacterial effect of Mangifera indica on ammonia producing bacteria

Table 6 shows the antibacterial activity of *Mangifera indica* on some gram positive (*Bacillus subtilis, Staphylococcus aureus*) and gram negative (*Escherichia coli, Vibrio cholerae*) bacteria. *Mangifera indica* leaf extract by 100% Methanol showed no inhibitory activity on *Staphylococcus aureus*. Similarly, there was zero inhibition of *Escherichia coli* when Doxycycline and leaves extracted by 50% water: 50% methanol, 100% water and 100% methanol were used. Against *Staphylococcus aureus,* the inhibitory action of Doxycycline (16mm) was similar statistically to that of 30% water: 70% methanol-extracted *Mangifera indica* leaf (15mm) but higher than others. while on inhibition of *Vibrio cholera, Mangifera indica* leaf extract by 100% water (20mm) was more effective than the antibiotics but similar to other leaf extracts. Furthermore, 30% water and 70% methanol-extracted *Mangifera indica* leaf (12mm) showed better inhibitory activity on *Escherichia coli* but similar with the effect of 50% water: 50% methanol (10mm). The inhibitory activity of *Mangifera indica* leaf by 100% water (20mm) was higher than that of 100% Methanol but similar to others in its action on *Bacillus subtilis*. The results of antibacterial activity of the extracts of *Mangifera indica* from this study were in conformity with several studies conducted by many researchers on antibacterial activity of *Mangifera indica (Ayepola and Adeniyi, 2008; Sahrawat et al., 2013; Chidozie et al., 2014).* This work is also in agreement with the conclusion of Vega-vega *et al*. (2013) who found the antibacterial ability of extract against *Salmonella enterica*, *Listeria monocytogenes* and *Escherichia coli*. Sahrawat *et al*. (2013) reported antibacterial activities of methanol, ethanol and benzene extracts of *Mangifera indica* leaf using on some bacteria such as *Proteus vulgaris, Pseudomonas fluorescens*, *Shigella flexneri*, *Klebsiella pneumonia* and *Salmonella typhi* at 100µl/ml concentration. It is thought that the

antibacterial activity of mango extract is due to the presence of tannin and mangiferin. In the study conducted by Majourie (1999), the result showed that different extracts of *Mangifera indica leaf* had different compounds with several antibacterial activities. This suggests that the antibacterial activity could be due to different classes of compounds present in the extract. Some of the classes of compounds identified in the crude extract, such as alkaloids and terpenoid have been reported to possess antibacterial activity (Majourie, 1999). The study conducted by Doughari and Manzara (2008), revealed that the active components of leaves of *Mangifera. indica* L. which were extracted using cold water and organic solvents (acetone and methanol) and were tested against *Staphylococcus aureus, Streptococcus pyogenese, Streptococcus pneumoniae, Bacillus cereus, Escherichia coli, Pseudomonas aerugenosa, Proteus mirabilis, Salmonella typhi* and *Shigella flexnerri* using the agar well (cup plate) diffusion method. Both the acetone and methanol extracts inhibited the growth of gram-positive bacteria, with acetone extract exerting more activities on all the gram-positive bacteria with zone of inhibition between 15 - 16 mm, and a gram negative bacterium *S. typhi* (14 mm) at 250 mg/ml. Whereas, water extract was not active on any of the bacterial pathogens tested at any of the concentration of the extract used.

Table 6: Antibacterial effect of *Mangifera indica* on ammonia producing bacteria

	Microbial zone of Inhibition (mm)			
Extract	*Staphylococcus aureus*	*Vibrio cholerae*	*Escherichia coli*	*Bacillus subtilace*
100% water	10.00±1.73^{c}	21.00±1.00^{a}	00.00±0.00^{b}	20.00±1.73^{a}
70%water:30%methanol	14.00±2.00^{b}	20.00±2.65^{a}	10.00±2.00^{a}	20.00±2.00^{a}
50%water:50%methanol	14.00±2.65^{b}	17.00±1.73^{ab}	0.00±0.00^{b}	18.00±5.29^{ab}
30%water:70%methanol	15.00±2.00^{a}	19.00±2.65^{a}	12.00±2.65^{a}	18.00±2.65^{ab}
100% methanol	0.00±0.00^{d}	19.00±1.00^{a}	0.00±0.00^{b}	17.00±2.00^{b}
Doxycycline	16.00±4.36^{a}	13.00±1.73^{b}	0.00±0.00^{b}	20.00±2.65^{a}
SEM	1.44	0.96	1.92	1.15

SEM: Standard Error of Mean.
^{abcd} Means with different superscript are significantly different from each other.

Conclusion and recommendation

It can be concluded, therefore, that the leaf extracts of *Azadirachta indica*, *Carica papaya*, *Saccharum officinarum*, *Chromolaena odorata, Eucalyptus camadulensis* and *Mangifera indica* can serve as an alternative to synthetic drugs in animals due to its effective action against microbial organisms. The result of this research also justified the traditional usage of these plants as health remedy. Extracts of leaves could be used as components of new antimicrobial agents with a broad spectrum of antimicrobial effect on ammonia releasing bacteria. The use of these leaf extracts in feed formulation will be more economical to the livestock farmer compared to synthetic drugs. Disease resistance and residual effects of synthetic drugs in animal products will be reduced if not eliminated. It is recommended that further investigations should be done on toxicological studies and purification of active components with the view to using the plants in novel drug development. Since aqueous and methanol extract of the leaves of *Azadirachta indica*, *Carica papaya*, *Saccharum officinarum*, *Chromolaena odorata, Eucalyptus camadulensis* and *Mangifera indica* have proved to be effective against the test bacteria, their effects on fungi and viruses should also be

investigated. There should also be an *in vivo* study of the leaves in animals, to ascertain their effects on animal health. Such study will ascertain the potency of the leaf extracts in the gut, product and by-product of animals. It is necessary to conduct further research to evaluate changes in the productive performance and the mechanisms of action by which these compounds exert their effects to optimize their use, especially in terms of appropriate doses and exposure periods. However, care should be taken on the quantity of extracts used to prevent resistance by disease causing agents.

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