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# Possibilities of basic oils and plant removes as bio-antimicrobials on Gram-negative pointer bacterial microorganisms of poultry inception

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Abstract

**Increasing antibiotic resistance of veterinary importance is of global health significance and concerns, due to antibiotic-resistant bacteria originating from animals; therefore, easily obtainable and cheap alternatives to antibiotic use in prophylaxis and also as growth promoters are imperative. Antibiotic resistance of 73 and 51 gram-negative, indicator bacterial strains isolated from 68 crop and 51 gizzard contents of layer hens respectively, and identified as *Citrobacter*, *Enterobacter*, *Escherichia coli*, *Klebsiella*, *Proteus*, *Salmonella*, *Shigella* and *Vibrio* species were determined. The bacterial flora exhibited *in vitro* resistance to one or more antibiotics but the most generally resisted antibiotics (discs) were amoxycillin (66.7 to 100%; 71.4 to 100%), augmentin (64.3 to 100%; 50.0 to 100%), cotrimoxazole (42.6 to 100%; 42.1 to 100%), nitrofurantoin (32.1 to 66.7%; 25.0 to 100%) and tetracycline (36.4 to 60.0%; 42.1 to 100%) respectively; while 31 different antibiotic resistant patterns were recorded. Essential oils of *Eugenia aromatica* (90.9 to 100%), *Ageratum* (27.3 to 100%), *Chrysophyllum albidum* juice (22.2 to 100%), lactic acid (100%), acetic acid (72.7 to 100%) and carvone (33.3 to 100%) were however, inhibitory towards the multi-drug resistant Gram-negative bacteria. This study is the first to conclude that essential oils of *Ageratum, Eugenia, Anacardium,* as well as carvone, Chrysophyllum albidum juice, lactic and acetic acids can serve as easily-produced, natural bio-antibacterial agents of poultry importance instead of antibiotics, in Nigeria.**

**Keywords:** Antimicrobial resistance, avian health, essential oils, veterinary public health, zoonosis

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## INTRODUCTION

Antibiotic resistance in bacteria that cause diseases in man is an issue of major concern (Barton, 2000; Bywater, 2005; Soulsby, 2007; Hur et al., 2011; Lu et al., 2011), and although misuse of antibiotics in human medicine is the principal cause of the problem, antibiotic-resistant bacteria originating from animals are also contributory factors (Barton, 2000l; Ferens et al., 2011; Gousia et al., 2011; Oliver et al., 2011). For nearly fifty years, farmers have been adding low doses of antibiotics to their livestock's food because it improves their feeding efficiency leading to increasing amounts of antibiotics used prophylactically, and also as growth promoters, so that the animals need less food to reach marketable weight. However, there has been heated debates on whether these additives pose a real risk to animal and human health (Gustafson and Bowen, 1997; Phillips, 1999) and there also had been a cause for concern about the use of antibiotics in poultry and livestock production ever since their first usage. Even presently, there is a consumer and government outcry to eliminate this practice from poultry and livestock production because evidence has been accumulating to show that there is a link between risk of zoonotic diseases and growth-promoting antibiotic usage in livestock and poultry (Edens, 2003; Dibner and Richards, 2005; Thorsteinsdottir et al., 2010).

There was general ban or discontinued use of certain antibiotics in poultry in some countries (van den Bogaard, 1998; Klare et al., 1999; Pantosti et al., 1999; van den and Stobberingh, 2000) and a major concern that resulted in the EU's ban of growth-promoting antibiotics in poultry was that many of the multiple antibiotic resistant strains of bacteria are capable of passing resistance factors to unrelated bacteria (Edens, 2003; Soulsby, 2007). As genetic material can be transferred between bacteria, there is every reason to suspect that genes carrying antibiotic resistance could also be transferred; and since bacteria with resistance genes would have an obvious advantage over bacteria that are susceptible to antibiotics, intense selective pressure would cause resistance to spread throughout the bacterial populations. In theory, diseases could be contracted directly if such animal bacteria get into human food, so, antibiotic-resistant bacteria could then cause diseases in humans, which can be untreatable with conventional antibiotics. Recent evidence from scientists around the world also affirmed the link between the use of antibiotic growth-promoters in food animals and increasing antimicrobial resistance (Phillips, 1999; Caprioli et al., 2000; van den and Stobberingh, 2000; WHO, 2003; Oliver et al., 2011); therefore, the emer-gence of bacteria which are resistant to most of the commonly used antibiotics/ drugs is of considerable human and veterinary health / medical significance.

Although each country has its peculiarity in poultry practices but most wrong poultry practices like prophylactic antibiotic usage in poultry are usually not made known to regulating bodies; while high estimates of antibiotics entering into the human system through animal sources have also been largely unreported or under-reported. It is therefore, impossible to document antibiotic resistance in food animals like poultry and livestock in Nigeria, meanwhile, this has indirect veterinary and human public health significance, especially with regards to zoonotic diseases. So, just as it has been done in some advanced countries, it is quite necessary to develop alternative antimicrobial agents in cases of poultry bacterial infections (Edens, 2003; Garrido et al., 2004; Prabuseenivasa et al., 2006) because food safety is probably the biggest issue facing animal production systems today. This current research therefore, tries to isolate and determine the antibiotics' resistance rates of bacteria from poultry, and the possibility of using some essential oils and organic acids as readily obtainable bio-antimicrobials, as adjuncts or alternatives to antibiotics in poultry health.

## MATERIAL AND METHODS

### Sampling and bacterial isolation

Bacterial isolates were obtained from crop and gizzard contents of layer hens between the ages of 28 and 40 weeks, and which were reared under intensive management system with automated cages, open feeders and nipple drinking system, at two very large poultry farms in western-Nigeria. Each crop and gizzard was aseptically opened with sterile scalpels and some of the contents dispensed into sterile unbuferred peptone water in sterile McCartney bottles, which were transported to the laboratory within 6 h of isolation for microbiological analyses. The cultured peptone water samples were incubated overnight and later pour-plated on plate count agar, cystein lactose electrolyte deficient agar, blood agar, MacConkey agar, eosin methylene blue agar, thiosulphate citrate bile sucrose agar (TCBS), *Salmonella-Shigella* agar and mannitol salt agar (MSA), all from Lab M, Basingstoke, England The isolated bacterial strains were phenotypically identified using standard taxonomic tools (Summanen et al., 1993; Crichton, 1996).

### Antibiotic susceptibility test

Antibiotic susceptibility and resistance profiles and patterns of the isolated Gram-negative bacterial strains were determined using the agar disc-diffusion method of Piddock (1990). The Gram-negative bacterial isolates were screened for *in vitro* antibiotic susceptibility to routinely used antibiotic discs in Nigeria– gentamicin (GEN 10 μg) tetracycline (TET 30 μg), amoxycillin (AMX 25 μg) cotrimoxazole (COT 25μg) nitrofurantoin (200μg), nalidixic acid (30 μg), ofloxacin (30 μg) and augmentin (30 μg) obtained from ABTEK biologicals, England, on Mueller–Hinton agar (Lab M, England). The entire surface of each sterile Mueller-Hinton agar plate was seeded with each bacterial isolate using sterile swab sticks. The plates were left for about 15 min before aseptically placing the antibiotic discs on the agar surfaces using sterile forceps, followed by incubation at 35°C for 24 h. Zones of inhibition surrounding the discs after incubation were noted and recorded in milimetres diameter, while the discs with no inhibition zones or zones less than 10.0 mm in diameter were recorded as resistant (Bauer et al., 1966; NCCLS, 2003).

### Determination of antimicrobial activities of essential oils and organic acids

Essential oils of *Ageratum* and *Eugenia aromatica* and carvone used in this study were distilled / extracted at the organic unit of the Department of Chemistry, Faculty of Science, University of Ibadan, Nigeria. Purity of the essential oils was primarily determined by sensory evaluation (colour, appearance and aroma) and skin patch test. In the skin patch test, a drop of each essential oil was applied to the skin to determine skin reactions like itching and redness of the skin after 12 h application of the essential oils.

The essential oils, *Chrysophyllum albidium* juice and two chemical grade organic acids were screened for *in vitro* inhibitory activities against 124 Gram-negative bacteria isolates from the crop and gizzard contents of layer hens, using a modified agar well-diffusion methods of Tagg and McGiven (1971) on Mueller–Hinton agar (Lab M, England). Test pure Gram-negative indicator bacterial strains were suspended in sterile peptone water and incubated at 35 to 37°C for 24 to 36 h. Holes, 6.0 mm in diameter were bored into the sterile agar plates, followed by seeding (streaking of the entire agar surface) with 500 μl of $10^{-3}$ cfu $ml^{-1}$ inoculum size of each active cultures of Gram-negative bacterial strain in sterile peptone water. Modification of the agar well-diffusion method as prepared in this study was by incorporating 500-1000 μl of each of the essential oils (50.0 mg $ml^{-1}$), organic acids and *Chrysophyllum albidium* juice into sterile semi-solid agar (45°C) before being dispensed into the agar wells to prevent spreading of the essential oils, organic acids and *Chrysophyllum albidum* juice on the agar surfaces. Culture plates were left at room temperature for 15 min before incubation at 35°C for 24 to 48 h, and zones of inhibition surrounding the agar wells after incubation were noted and recorded

**Table 1.** Overall antibiotic susceptibility and multiple antibiotic resistance profiles of Gram-negative bacterial strains from crop and gizzard contents of layer hens

| Bacterial isolate | Antibiotics (μg ml$^{-1}$) | | | | | | | | % MAR |
|---|---|---|---|---|---|---|---|---|---|
| | AMX | AUG | COT | NAL | NIT | TET | GEN | OFL | |
| *Citrobacter aerogenes* CR [9] | 88.9 | 88.9 | 88.9 | 66.7 | 33.3 | 55.6 | 22.2 | 11.1 | 37.5-100 |
| *Citrobacter aerogenes* GZ [4] | 100 | 75.0 | 100 | 75.0 | 50.0 | 75.0 | 25.0 | 25.0 | 25.0-100 |
| *Enterobacter aerogenes* CR [11] | 100 | 90.0 | 72.7 | 45.5 | 36.4 | 54.5 | 9.1 | 9.1 | 25.0-87.5 |
| *Enterobacter* aerogenes GZ [11] | 100 | 100 | 90.0 | 63.6 | 54.5 | 72.7 | 18.1 | 0.0 | 25.0-87.5 |
| *Enterobacter cloacae* CR [6] | 66.7 | 66.7 | 66.7 | 33.3 | 66.7 | 50.0 | 0.0 | 0.0 | 25.0-75.0 |
| *Enterobacter* cloacae GZ [7] | 71.4 | 71.4 | 57.1 | 57.1 | 57.1 | 71.4 | 42.9 | 42.9 | 75.0-100 |
| *Enterobacter* sp. CR [1] | 100 | 100 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 25.0 |
| *Escherichia coli* CR [28] | 82.1 | 64.3 | 42.6 | 39.3 | 32.1 | 46.4 | 17.9 | 17.9 | 25.0-100 |
| *Escherichia coli* GZ [19] | 94.7 | 89.5 | 42.1 | 47.4 | 36.8 | 42.1 | 21.0 | 15.8 | 25.0-100 |
| *Klebsiella pneumoniae* CR [11] | 100 | 100 | 72.7 | 90.0 | 45.5 | 36.4 | 9.1 | 0.0 | 25.0-75.0 |
| *Klebsiella pneumoniae* GZ [4] | 100 | 100 | 50.0 | 50.0 | 25.0 | 75.0 | 0.0 | 0.0 | 37.5-62.5 |
| *Proteus mirabilis* CR [5] | 100 | 100 | 60.0 | 60.0 | 60.0 | 60.0 | 40.0 | 40.0 | 75.0-100 |
| *Proteus mirabilis* GZ [2] | 100 | 50.0 | 50.0 | 50.0 | 0.0 | 50.0 | 50.0 | 50.0 | 25.0-62.5 |
| *Salmonella typhimurium* CR [1] | 100 | 100 | 100 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 37.5 |
| *Salmonella paratyphi* GZ [1] | 100 | 100 | 100 | 100 | 100 | 100 | 0.0 | 0.0 | 75.0 |
| *Shigella dysentariae* GZ [3] | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| *Vibrio parahaemolyticus* CR [1] | 100 | 100 | 100 | 0.0 | 0.0 | 100 | 0.0 | 0.0 | 50.0 |

CR = crop; GZ = gizzard; AMX = amoxicillin; AUG =augmentin; COT = cotrimoxazole; NAL = nalidixic acid; NIT = nitrofurnantoin; TET = tetracycline; GEN = gentamicin; OFL = ofloxacilin.

in millimetres diameter, while wells with no inhibition zones or zones of inhibition less than 10.0 mm in diameter were recorded as resistant.

## RESULTS

A total of 73 bacterial isolates from crop contents were *Citrobacter aerogenes* 9 (7.3%), *Enterobacter aerogenes* 11 (8.9%), *Enterobacter cloacae* 6 (4.8%), *Enterobacter* sp. 1 (0.8%), *Escherichia coli* 28 (22.6%), *Klebsiella pneumoniae* 11 (8.9%), *Proteus mirabilis* 5 (4.0%), *Salmonella enterica* serovar *Typhimurium* 1 (0.8%), and *Vibrio parahaemolyticus* 1 (0.8%); while the 51 bacterial isolates from 56 gizzard contents were *Citrobacter aerogenes* 4 (3.2%), *E. aerogenes* 11 (8.9%), *E. cloacae* 7 (5.6%), *E. coli* 19 (15.3.0%), *Klebsiella pneumoniae* 4 (3.2%), *Proteus mirabilis* 2 (1.6%), *Salmonella Enterica* serovar Paratyphi 1 (0.8%) and *Shigella dysentariae* 3 (2.4%) (Table 1, Figure 1).

The antibiotic susceptibility / resistance patterns of 124 Gram-negative bacterial isolates from 68 crop and 56 gizzard contents of layer hens were determined in this study. Overall antibiotic susceptibility/resistance patterns of the Gram-negative bacteria obtained from crop and gizzard contents of layer hens in this study indicated that the most generally resisted antibiotics (discs) were amoxycillin (66.7 to 100%; 71.4 to 100%), augmentin (64.3 to 100%; 50.0 to 100%), cotrimoxazole (42.6 to 100%; 42.1 to 100%), nitrofurantoin (32.1 to 66.7%; 25.0 to 100%) and tetracycline (36.4 to 60.0%; 42.1 to 100%) respectively. Multiple antibiotic resistance (MAR) also varied between 25.0 and 100% respectively (Table 1). Only four (3.2%) out of the 124 bacterial strains were totally (100%) susceptible to the eight test antibiotics, while 31 different antibiotic resistance patterns and 27 different multiple antibiotic resistance patterns were recorded among the remaining 120 (96.8%) Gram-negative bacterial strains isolated from crop and gizzard contents of layer hens (Table 2).

Nine most prevalent multiple antibiotic resistance patterns recorded in this study were amoxicillin–augmentin–tetracycline 5 (4.2%), amoxicillin–augmentin–cotrimoxazole-nalidixic acid-nitrofurantoin 5 (4.2%), amoxicillin–augmentin–cotrimoxazole 6 (5.0%), amoxicillin–augmentin–cotrimoxazole-nalidixic acid-nitrofurantoin–tetracycline 6 (5.0%), amoxicillin-augmentin-nalidixic acid 8 (6.7%), amoxicillin–augmentin–cotrimoxazole–tetracycline 8 (6.7%), amoxicillin–augmentin 14 (11.7%), amoxicillin–augmentin–cotrimoxazole-nalidixic acid-nitrofurantoin–tetracycline 16 (13.3%), amoxicillin–augmentin–cotrimoxazole-nalidixic acid–nitrofurantoin–tetracycline–gentamicin–ofloxacilin 18 (15.0%), (Table 3).

In this study, it was found that the degree of susceptibility of the bacteria towards the essential oils and organic acids (18.0 to 100%) was quite higher and more inhibitory when compared with susceptibility patterns towards antibiotics. The essential oils and organic acids had inhibitory antibacterial activities against one or more of the test bacterial strains but the essential

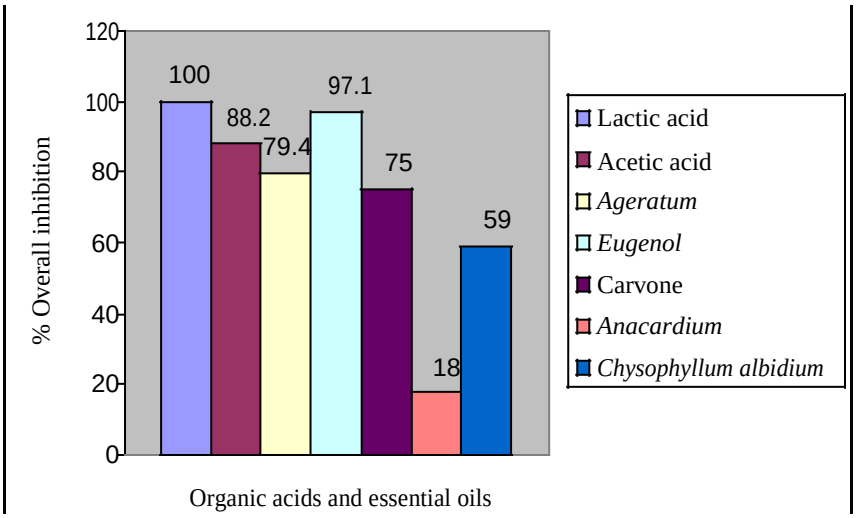


**Figure 1.** Percentage overall *in vitro* antimicrobial activities of organic acids and essential oils on bacterial species from crop contents.

oils of *Eugenia* (90.9 to 100%), carvone (33.3 to 100%), *Ageratum* (27.3 to 100%), lactic acid (100%), acetic acid (72.7 to 100%) and *Chrysophyllum albidum* juice (22.2 to 100%) were the most inhibitory against the multi-drug resistant, Gram-negative bacteria from crop and gizzard contents of layer hens respectively. Minimum zone of inhibition in the bioassay screening was 10.0 mm in diameter, while highest maximum zones of inhibition of 40.0 to 45.0 mm in diametre were recorded among the essential oils and organic acids, while the maximum zones towards the antibiotics were 32.0 to 35.0 mm in diametre. Overall percentage inhibition rates of the essential oils and organic acids against the crop (18.0 to 100%) and gizzard (23.2 to 100%) bacterial strains are as shown in Figure 2.

## DISCUSSION

Crop and gizzard serve as storage compartments for consumed particles that are not finely grounded but if full of feed and poor quality water is added, then there is an increased risk of the development of harmful bacteria and moulds that could impact the rest of the digestive tract, like the ileum, caecum and colon, as well as contaminate the carcass (Mead, 1997; Smith and Berrang, 2006); however, the Gram-negative bacterial isolates obtained from crop and gizzard contents of layer hens in this study indicated members of the family Enterobacteriaceae, and these are bacteria commonly associated with diseases like acute gastroenteritis and crop stasis (Porter, 1998; Ritzman, 2005; Cox and Pavic, 2009). There is therefore, the possibility of poultry disease transmissibility among poultry birds and also to humans; more so, since it has been widely reported that most emerging infectious diseases are caused by zoonotic pathogens (Jones et al., 2008; Wassenaar and Silley, 2008; Pavlin et al., 2009). So, it is of necessity to promptly treat poultry infections caused by such bacteria, which is usually by antibiotic therapy.

Resistant bacteria have been known virtually since modern antibacterial chemotherapy became an accepted medical practice but antimicrobial resistance has reached a crisis stage in human cases (Shea, 2003; Zhang et al., 2006; Ogunshe and Bakare, 2009); while the increasing prevalence of antimicrobial drug-resistant bacteria is also a major concern to veterinary medicine (Aarestrup, 2005; Gyles, 2008; Gousia et al., 2011). In this study, bacterial flora isolated from crop and gizzard contents of layer hens exhibited between moderate and very high antibiotic resistance rates, especially towards four commonly used test antibiotics (discs) in Nigeria (amoxycillin, augmentin, cotrimoxazole and tetracycline), which confirmed earlier reported dangers of increased antibiotic resistance, particularly, with regards to multiple antibiotic resistance patterns exhibited by most food-borne or food-related bacterial species from poultry sources (Piddock, 2002; Vugia et al., 2009; Lu et al., 2011). These significant resistance rates can also be transferred to hatched chicks, leading to vertical antibiotic resistance along many offspring.

Multiple antibiotic resistance (MAR = 25.0-100%) were also reported among the Gram-negative bacterial species isolated from poultry sources in the present study, and it is even much more alarming to record up to 27 different multiple antibiotic resistance patterns, especially amoxicillin–augmentin–cotrimoxazole-nalidixic acid–nitrofurantoin–tetracycline–gentamicin–ofloxacilin MAR. The implication is that such food-borne or food-related multiple antibiotic-resistant bacteria can enter the food chain from poultry (Gousia et al., 2011) and cause diseases in humans that can be untreatable with conventional antibiotics, even in cases of combined antibiotic therapy. Like in many other countries, there is

**Table 2.** Antibiotic resistance patterns of Gram-negative bacterial strains from crop and gizzard contents of layer hens.

| Patterns antibiotics resisted | Resistant gram-negative bacterial strains |
|---|---|
| 1 [1]* Amx | [2] *E. coli* (1) (1)] |
| 2 [1]* Aug | [1] *E. coli* (1)] |
| 3 [1]* Cot | [1] *Citr. aerogenes* (1)] |
| 4 [1]* Tet | [2] *E. coli* (1), *Ent. cloacae* (1)] |
| 5 [2] Amx Aug | [14] *E. coli* (3) (4), *Ent. aerogenes* (1), *Ent. cloacae* (1) (1), *Enterobacter* sp. (1), *Pr. mirabilis* (2), *Kleb. pneumoniae* (1) |
| 6 [2] Amx Cot | [3] *Citr. aerogenes* (1), *E. coli* (1), *Ent. aerogenes* (1)] |
| 7 [2] Amx Tet | [3] *E. coli* (3) |
| 8 [2] Cot Nit | [1] *Ent. cloacae* (1) |
| 9 [3] Amx Aug Cot | [6] *E. coli* (1) (1), *Ent. aerogenes* (2), *Citr. aerogenes* (1), *Salmonella typhimurium* (1) |
| 10 [3] Amx Aug Nal | [8] *Citr. aerogenes* (1), *E. coli* (2) (3), *Ent. aerogenes* (1), *Kleb. pneumoniae* (1) |
| 11 [3] Amx Aug Nit | [1] *E. coli* (1) |
| 12 [3] Amx Aug Tet | [5] *E. coli* (1) (1), *Ent. aerogenes* (1), *Pr. mirabilis* (1), *Kleb. pneumoniae* (1) |
| 13 [3] Amx Cot Tet | [1] *E. coli* (1) |
| 14 [4] Amx Aug Cot Nal | [3] *Citr. aerogenes* (1), *E. coli* (1), *Kleb. pneumoniae* (1) |
| 15 [4] Amx Aug Cot Nit | [1] *Ent. aerogenes* (1) |
| 16 [4] Amx Aug Cot Tet | [6] *Ent. aerogenes* (1) (2), *Citr. aerogenes* (1), *Kleb. pneumoniae* (1), *V. parahaemolyticus* (1) |
| 17 [4] Amx Aug Nal Nit | [2] *E. coli* (1), *Kleb. pneumoniae* (1) |
| 18 [4] Amx Aug Nal Tet | [2] *Ent. aerogenes* (1), *Kleb. pneumoniae* (1) |
| 19 [4] Amx Aug Nit Tet | [2] *E. coli* (1) (1) |
| 20 [4] Amx Cot Nit Tet | [1] *E. coli* (1) |
| 21 [5] Amx Aug Cot Nal Nit | [5] *Ent. aerogenes* (1), *Kleb. pneumoniae* (3), (1) |
| 22 [5] Amx Aug Cot Nal Gen | [1] *Kleb. pneumoniae* (1) |
| 23 [5] Amx Aug Cot Nal Tet | [6] *Citr. aerogenes* (1), *E. coli* (2), *Ent. aerogenes* (1), *Citr. aerogenes* (1), *Kleb. pneumoniae* (1) |
| 24 [5] Amx Aug Cot Nit Tet | [2] *Ent. aerogenes* (1), *Ent. cloacae* (1) |
| 25 [5] Amx Cot Nal Gen Ofl | [1] *Pr. mirabilis* (1) |
| 26 [6] Amx Aug Cot Nal Nit Tet | [16] *Citr. aerogenes* (1)(1), *E. coli* (2)(1), *Ent. aerogenes* (2)(3), *Ent. cloacae* (2), *Kleb. pneumoniae* (2), *Pr. mirabilis* (1), *Salmonella paratyphi* (1)] |
| 27 [6] Amx Aug Cot Nal Nit Gen | [1] *E. coli* (1) |
| 28 [6] Amx Aug Cot Nal Tet Gen | [1] *Ent. aerogenes* (1) |
| 29 [7] Amx Aug Cot Nal Nit Tet Gen | [2] *Citr. aerogenes* (1), *Ent. aerogenes* (1) |
| 30 [7] Amx Aug Cot Nal Nit Gen Ofl | [2] *Ent. aerogenes* (1), *Citr. aerogenes* (1) |
| 31 [8] Amx Aug Cot Nal Nit Tet Gen Ofl | [18] *Citr. aerogenes* (1), *E. coli* (6)(4), *Ent. cloacae* (2), *Pr. mirabilis* (2), *Sh. dysentariae* (3) |

Amx = amoxicillin; Aug = augmentin; Cot = cotrimoxazole; Nal = nalidixic acid; Nit = nitrofurantoin; Tet = tetracycline; Gen = gentamicin; Ofl = ofloxacilin () = crop isolates; (_) = gizzard isolates; * = mono resistance.

no clear and unbiased estimate of antibiotic use in poultry and livestock industries in Nigeria. Since the 1960s however, public health officials and scientists worldwide have tried to quantify the risks of resistance arising from antibiotic growth promoters and frame appropriate responses, such as bans of some antibiotics (Klare et al., 1999; van den and Stobberingh, 2000; Dibner and Richards, 2005).

Commercially, poultry will never be raised in the absence of infectious pathogens, in spite of biosecurity efforts to minimise pathogen exposure. Commercial poultry housing is not, and likely will never be a sterile environment and together, these issues dictate that poultry birds will be exposed to infectious and toxic

**Table 3.** Antimicrobial activities of organic acids and essential oils on strains from crop and gizzard contents of layer hens.

| Bacterial species [no. of strain] | Organic acid | | Essential oil (mg ml$^{-1}$) | | | | |
|---|---|---|---|---|---|---|---|
| | LA | AC | AG | EU | CA | AN | CH |
| *Escherichia coli* CR [28] | 100 | 85.7 | 78.6 | 92.9 | 78.6 | 28.6 | 53.8 |
| *Escherichia coli* GZ [19] | 100 | 94.7 | 84.2 | 100 | 73.7 | 26.3 | 57.9 |
| *Enterobacter aerogenes* CR [11] | 100 | 72.7 | 27.3 | 100 | 81.8 | 0.0 | 81.8 |
| *Enterobacter aerogenes* GZ [11] | 100 | 81.8 | 90.9 | 90.9 | 90.9 | 9.1 | 45.5 |
| *Enterobacter cloacae* CR [6] | 100 | 100 | 33.3 | 100 | 66.7 | 1.7 | 50.0 |
| *Enterobacter cloacae* GZ [8] | 100 | 87.5 | 87.5 | 100 | 100 | 25.0 | 87.5 |
| *Proteus mirabilis* CR [5] | 100 | 80.0 | 100 | 100 | 80.0 | 40.0 | 60.0 |
| *Proteus mirabilis* GZ [2] | 100 | 100 | 50.0 | 100 | 100 | 0.0 | 50.0 |
| *Citrobacter aerogenes* CR [9] | 100 | 88.9 | 55.6 | 100 | 66.7 | 11.1 | 22.2 |
| *Citrobacter aerogenes* GZ [4] | 100 | 100 | 100 | 100 | 100 | 75.0 | 100 |
| *Klebsiella pneumoniae* CR [11] | 100 | 81.8 | 63.6 | 100 | 72.7 | 0.0 | 63.6 |
| *Klebsiella pneumoniae* GZ [4] | 100 | 75.0 | 75.0 | 100 | 100 | 25.0 | 100 |
| *Salmonella typhimurium* CR [1] | 100 | 100 | 100 | 100 | 100 | 0.0 | 0.0 |
| *Salmonella paratyphi* GZ [1] | 100 | 100 | 100 | 100 | 0.0 | 0.0 | 100 |
| *Shigella dysentariae* GZ [3] | 100 | 100 | 100 | 100 | 33.3 | 33.3 | 33.3 |
| *Vibrio parahaemolyticus* CR [1] | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

CR = crop; GZ = gizzard; LA = lactic acid; AC = acetic acid; AG=Ageratum (Raw); EU = Eugenia; CA = Carvone; AN = Anacardium; CH = *Chrysophyllum albidium* juice.

agents through feeds and environment. Actions to reduce pathogen exposure must therefore, meet health safety, as well as biological and economic justifications (Hoerr, 2010), especially with due considerations for poultry farmers in developing countries like Nigeria, where regulations and regulating bodies in agriculture are grossly lacking.

Essential oils (also called volatile oils) are volatile, aromatic natural oily liquid products extracted from plant materials (flowers, buds, seeds, leaves, twigs, bark, herbs wood, fruits, roots and spices) by expression, fermentation, extraction, steam distillation methods (Prabuseenivasa et al., 2006). There are at least 2,600 reported essential oils, many of which can be produced synthetically, and which also possess antibacterial, antifungal, antiviral, insecticidal and antioxidant properties (Cimanga et al., 2002; Burt, 2004). Numerous modes of action for their bactericidal effects have been identified but the most important ones are associated with facilitation of increased bacterial cell-wall permeability and inactivation of enzyme systems. Carvones are also used in food and flavor industries, and like many essential oils, oils containing carvones are used in aromatherapy and alternative medicine (De Carvalho and Da Fonseca, 2006).

As earlier suggested by Ferket (2004), in response to consumer demands and government regulations, today's intensive animal agricultural industry must adapt to producing animals in a world without antibiotic growth-promoters. *In vitro* systems offers a reliable method of investigating alteration in the crop and gizzard microbiota in response to different bio-conditions; thereby, providing a useful approach to the screening of organic acids to be subsequently tested *in vivo*. Plant-based antimicrobial compounds, which function fundamentally similar to antibiotics, can therefore, be used to replace antibiotic growth-promoters, especially in developing countries where the amount of antibiotic use in animals and the hazzards due to this abuse are generally undocumented. The antimicrobial activities of plant oils and extracts have been recognised for many years (Hammer et al., 1999; Ho, 2010), so if they are properly utilised in combination with organic acids, they can serve as effective bio-antimicrobials in suppressing growth of pathogenic bacteria in poultry (Edens, 2003).

Antibacterial potentials / bactericidal effects of organic acids as recorded in the present study are in support of other previous studies with respect to the efficiency of acidification, through the use of organic acids to reduce the infection of chickens by pathogenic bacteria (Waldroup et al., 1995; Yegani and Korver, 2008). As an example, lactic acid has been found to acidify crop contents, making them less conducive to bacterial growth. Just as previously reported by Hinton et al. (2000) that the environment of the crop with respect to microbial composition and pH seems to be very important in relation to the resistance of pathogens, the bio-antimicrobial organic acids (lactic and acetic) assayed for in this study had pH of 4.0, which probably accounted for their high inhibitory activities against the Gram-negative bacterial isolates.

The result findings of this study confirmed that except for *Anarcadium* which had lower inhibitory activities, essential oils of *Eugenia* and *Ageratum*, as well as lactic

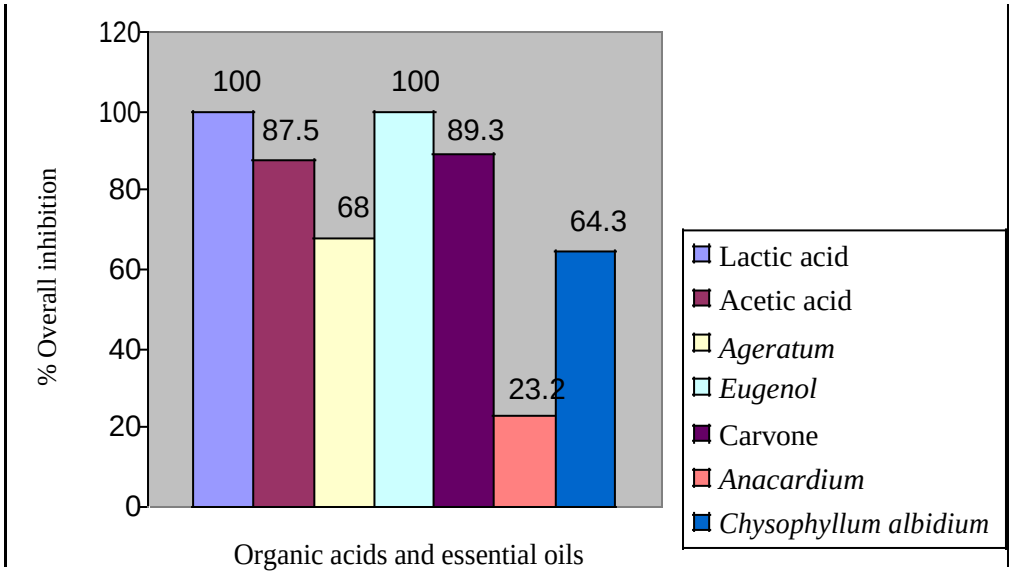


**Figure 2.** Percentage overall *in vitro* antimicrobial activities of organic acids and essential, oils on bacterial species from gizzard contents.

and acetic acids had very high *in vitro* antibacterial activities against the gram-negative, bacterial indicator isolates from crop and gizzard contents of layer hens, thereby indicating the possibility of developmental efforts to provide the poultry industry with effective and safe bio-antimicrobial alternatives, which can consist of a blend of essential oils from plant sources and natural food-based organic acids. The pH of *Chrysophyllum albidum* juice has been reported to be around 3.3, while magnesium and iron have been found to be the main minerals in the juice (Jayeoba et al., 2007).

This further indicates that these bioantimicrobials can also serve as natural mineral additives in poultry practices, and can be easily produced at minimal costs, even by an average poultry farmer. Recovery of the multi-resistant drugs bacterial flora from crop and gizzard contents in this study also strongly suggests prophylactic addition of doses of antibiotics to poultry feeds, as growth promoters because it improves feeding efficiency.

Result findings of this study can serve as a template for research-based data for advocacy on general ban or discontinued use of certain antibiotics in poultry by the Nigerian government. It is therefore, concluded from this study that most of the Gram-negative bacterial isolates from crop and gizzard contents of layer hens exhibited multiple antibiotic resistance but were mostly susceptible to essential oils of *Ageratum*, *Eugenia*, carvone, *Anacardium*, *Chrysophyllum albidum* juice, aswell as lactic and acetic acids, all of which can serve as commonly available, good sources of natural bio-antibacterial agents of poultry importance. Further studies on essential oils, organic acids and probiotic use in poultry, as alternative to antibiotic therapy are on-going in our laboratories.


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