**An Evaluation Of The Phytochemical And Antimicrobial Profiles Of Vernonia Amygdalina And Bark Of *Magnifera indica***

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**Abstract**: Since the emergence of tetracycline – resistant bacterium, *Shegella dysenteriae* in 1953, there has been a lot of research on the production of semi-synthetic drugs against several emerging drug-resistant bacteria. In this regard, herbal scientists have contributed very little. This study investigated the phytochemical compositions and antimicrobial effects of *Vernonia amygdalina* (E1) and the bark of *Magnifera indica* (E2) in combination with themselves and conventional drugs, Ampicillin (AmP) and Chloramphenicol (CPC), against *Salmonella* species isolated from poultry farms. Broth dilution and disc diffusion methods were respectively applied to determine the sensitivity of *Salmonella* species and the minimum inhibitory concentrations (MICs) of the plants affecting *Salmonella* species; the phytochemical analysis was carried out using standard methods. Results revealed that E1 possessed greater antimicrobial effect on *Salmonella* species (Zone of inhibition: 9.06+0.66 to 15.12+0.61mm) than E2 (Zone of inhibition: 0.0 to 12.10+0.20mm); while *Salmonella* was resistant to E2. The combination of E1 and E2 gave antagonistic results with E1 antagonizing E2. There was significant difference (p < 0.05) between E1 and E2, and the combination of each of the plants and antibiotics. The maximum zone of inhibition of E1 + AMP (21.66+0.97mm) indicateing better effectivity than E2 + AMP (13.77+0.86mm). Also, E1 + CPC has the same advantage over E2 + CPC. There was antagonism in 100% of the isolates when E1 and E2 were combined. However, E1 + AMP and E1 + CPC resulted in synergism in 93% and 100% of the isolates respectively, indicating a possible hope in the fight against antimicrobial resistance. Also, the MIC of E1 (3.12mg/ml) affected 38.46% of the isolates unlike that of E2 (6.25mg/ml) which affected 12.82% of the isolates, thus confirming E1 as having greater effectivity than E2. In conclusion, *Vernonia amygdalina* in combination with ampicillin and chloramphenicol could be drugs of choice against resistant *Salmonella* species.

[Matthew Egbobor Eja, Joseph Ubi Otu, Nsor Odo Alobi, Uno Agbo Uno, Magdalene Obi-Abang. **An Evaluation Of The Phytochemical And Antimicrobial Profiles Of Vernonia Amygdalina And Bark Of *Magnifera indica*.** *N Y Sci J* 2016;9(6):85-94]. ISSN 1554-0200 (print); ISSN 2375-723X (online). <http://www.sciencepub.net/newyork>. 14. doi:[10.7537/marsnys09061614](http://www.dx.doi.org/10.7537/marsnys09061614).

**Keywords**: Phytochemical profiles, antimicrobial effects, conventionaldrugs, *Vernonia amygdalina, Magnifera indica*.

**Introduction**

The emergence of resistant strains of pathogens to antibiotics has remained a global concern since the last five decades. It started with the discovery of tetracycle-resistant bacterium, *Shigella dysenteriae* in 1953, following the discovery of tetracycline in the 1940s (MMBR, 2001). The routine use of antibiotics in medicinal and agricultural practices has resulted in widespread antibiotic resistance and development of genetic mechanisms efficient in the dissemination of antibiotic resistant genes, especially among gram-negative organisms (Ackers *et al*., 2000).

The frequency of isolation of *Salmonella* strains resistant to one or more antibiotics has also risen all over the world. An example is a recent newcomer to the food safety pathogen list, *Salmonella* *typhimurium* phage type DT104, which possesses resistance to multiple antibiotics, including ampicillin, tetracycline and streptomycin (Jones, 2005).

Okeke *et al*. (2005) state that, in developing countries where household subsistence farming is common, a large proportion of the population has close contact with food animals (poultry) and, if resistant organisms are common in animals, the chance that they will be transmitted to human beings is more likely.

Therefore, research is still on-going on the production of synthetic resistance – free antibiotics. Herbal scientists are also researching on alternative sources of resistance-free drugs from medicinal plants, but their contribution is little. However, a few studies on the sensitivity of bacteria to some plants have recently been carried out. Eja *et al*. (2011) examined the antimicrobial synergy of garlic (*Allium sativum*) and utazi (*Gongronema latifolium*) on *Escherichia coli* and *Staphylococcus aureus*, and observed some synergy in the combination of garlic and ampicillin against *S. aureus* besides additive and antagonistic reactions between utazi and ciprofloxacin. Also, antimicrobial and phytochemical effects against *E. coli* and *S. aureus* have been observed by Enyi-Idoh *et al*. (2011). Atangwho *et al.* (2009) have worked on the comparative chemical composition of some antidiabetic medical plants, *Azadirachta indica, Vernonia amygdalina* and *Gongronema latifolium* and identified useful phytochemical components including alkaloids and a few other relatively antibacterial components. Andy *et al*. (2008) have observed some synergy when *Lansianthera africana* or *Heinsia crinata* in combination with chloramphenicol was tested against *Candida albicans*. These give hope of medicinal plants as alternative sources of resistance-free drugs.

*V. amygdalina* (bitter leaf) is a member of the family, Asteraceceae. It is a small shrub that typically grows up to a height of 2-5cm tall in tropical Africa. Its bark is rough; and it is commonly called “bitter leaf” because of its bitter taste. The Nigerian common names are *ewuro* (Yoruba), *etidot* (Ibibio), *onugbu* (Igbo), *ityuna* (Tiv), *chusar-doki* (Hausa), etc. (Kokwaro, 2009). It is used locally for the treatment of intestinal infections, reduction in fever and diabetics and headache (Ejike, 2011).

*Magnifera indica*, commonly known as mango, belongs to the family, Anacerdiaeceae which consists of about sixty genera and six hundred species (Akinpelu and Onakoya, 2006). It is one of the most popular fruit-bearing trees in the world (Kabuki *et al*., 2000). It is used in native Africa for treating mouth – *Salmonella* – related infections in children such as diarrhoea, dysentery, typhoid and throat fever. The bark of mango has been found to possess anti-helminthic and anti-allergic properties (Campbell *et al*., 2003; Abdalla *et al*., 2007).

The aim of this study was to investigate the phytochemical and antimicrobial potency of two common plants, *Vernonia amygdalina* (bitter leaf) and the bark of *Magnifera indica* (mango) on *Salmonella* species isolated from poultry farms by staff of the Microbiology Laboratory of the Department of Biological Sciences, Cross River University of Technology, Calabar.

**2. Materials and Methods**

**2.1 Sources of test organisms and plants**

Fourteen *Salmonella* isolates from Unical, Almond and Sandra Poultry Farms were obtained from the Microbiology Laboratory of Cross River University of Technology (CRUTECH), and used for the sensitivity tests against *Vernonia amygdalina* and *Magnifera indica*. The two plants were obtained from the Botanical Garden of CRUTECH, Calabar.

**2.2 Preparation of the plants extracts**

The plant samples were thoroughly washed and then air-dried gently in an air circulating oven in the laboratory, and individually ground manually into fine powder, using a manual grinder (Corona, Landers and CIA, SA) (Nwinuka *et al*., 2006). The powder of each sample was sieved through mesh 300µm (Nwinuka *et al*., 2006). The powdered sample of each of the plant (50g) was transferred into a soxhlet apparatus for the complete extraction of the plant extracts, using absolute ethanol as the extraction solvent.

**2.3 Preparation of extract and conventional drug concentrations for sensitivity test**

The ethanolic extract (10mg) was dissolved in 1ml of dimethyl sulfoxide (DMSO) to obtain a concentration of 10mg/ml, marked solution 1. When 0.1ml of solution 1 was dissolved in 9.9ml of DMSO, a solution of concentration 1.0mg/ml was obtained, which was referred to as solution 2. Incorporation of 1ml from solution 2 into 9ml of DMSO gave solution 3 with a final concentration of 100µg/ml, which was used to impregnate the discs, or combined with conventional antibiotics (ampicillin and chloramphenicol in a volume ratio of 0.1:0.1

Chloramphenicol and ampicillin were selected to be tested in combination with the plants because of reported development of resistance by environmentally isolated *Salmonella* strains to these drugs (Prescott *et al*., 2005; Patterson, 2006). Ampicillin (500mg) was dissolved in deionized water and DMSO as a solubility agent and the volume made up to 50.0ml at room temperature (Mukhtar and Huda, 2005), giving a concentration of 10mg/ml. Further dilutions as with the extracts were made to obtain a solution with a concentration of 1mg/ml. By incorporating 1ml of the solution into 9ml of DMSO, a final concentration of 100µg/ml was obtained. Chloramphenicol (250mg) was dissolved in deionized water and DMSO and the volume was made up to 25.0ml at room temperature. This gave a concentration of 10mg/ml. Further dilutions as stated above were made to obtain 100µg/ml. To test the extract combined with ampicillin or chloramphenicol, equal volumes of extracts and ampicillin or chloramphenicol (0.1:0.1) were mixed and the mixture tested along with the individual extracts and the drugs separately.

**2.4 Testing for antimicrobial effects of extracts along with ampicillin and chloramphenicol**

A disc diffusion technique using the Kirby-Bauer method (Prescott *et al*., 2005; Eja *et al*., 2011) was applied in testing pure cultures of the *Salmonella* isolates for their antimicrobial sensitivities.

The discs used for the test were punched from Whatman No. 1 filter paper. The discs were 5mm in diameter. They were sterilized and then impregnated with the extracts separately (Onyeaba *et al*., 2004). Five agar plates for each test organism per plant were inoculated with 0.1ml broth culture of test organisms and spread with a glass rod shaped like a hockey stick, and incubated at 37oC for 24h. The antibiotics, ampicillin (AMP) and chloramphenicol (CPX) were used as controls for comparison with the extracts (Eja *et al*., 2011). After incubation, the plates were observed for zones of inhibition.

**2.5 Testing for minimum inhibitory concentration of extracts**

In the determination of minimum inhibitory concentration (MIC), a standard inoculums was first prepared. This involved transferring a portion of pure culture of each isolate into tryptone soya broth (oxoid CM129) and incubating at room temperature overnight (Eja *et al*., 2011). The overnight broth culture (0.1ml) was diluted with 1ml of distilled water in the ratio of 1:1000 to give a final dilution of 10-3 of the standard inoculums (Adoum *et al*., 1997) following which the dilution susceptibility technique (Cheesbrough, 2000) was applied. The reciprocal of 10-3 equivalent to 103 was the number of organisms in the standard inoculums used for the MIC test. In this technique Muella-Hinton broth containing various concentrations of the plant extracts was prepared. In the preparation, 1ml from the different dilutions of the extracts was added to 10 labelled test tubes containing 9ml Muella-Hinton broth to obtain final concentrations of 5000, 2500, 1250, up to 0mg/ml, and incubated at 37oC for 16-20h. The presence or absence of growth for each concentration was recorded at the end of incubation. The MIC was taken as the lowest concentration of the extracts resulting in no growth after 16-20h of incubation.

**2.6 Synergy test**

The plant extracts (0.1:0.1) were combined with each other, and separately combined with antibiotics (ampicillin and chloramphenicol).

**2.7 Phytochemical screening of the plant extracts**

A qualitative analysis of the plant extracts was carried out using the methods of Cuilei (1982), Sofowora (1984) and Gundiza (1985).

**2.8 Statistical analysis**

Differences, if any, between the two plants with respect to their MIC, and in combination with each other and with the antibiotics, using statistical analysis of variance (ANOVA) (Bailey, 1981; Miller and Miller, 1986), was carried out.

**3. Results**

**3.1 Phytochemical screening**

The result of the phytochemical screening of the two plants is shown in Table 1 which shows that both plants possess varying concentrations of glycosides, flavonoid, polyphenols, saponins, alkaloids, tannins, phlabotinnins and steroids. However, the levels of most of these bioactive components appeared to be higher in *V. amygdalina* than *M. indica*. Tannins and polyphenols were observed to be present at the same levels in both plants.

**3.2 Testing for antibacterial effects of extracts on *Salmonella* species**

The effects of ethanolic leaf extracts of *V. amygdalina* (E1) and the bark of *Magnifera indica* (E2) and their combinations on *Salmonella* isolates are represented in Table 2. The table shows that E1 possessed reasonable antibacterial effect (Zone of inhibition: 9.06+0.66 to 15.12+0.61mm) on *Salmonella*, unlike E2 which had little or no effect (Zone of inhibition from 0.0 to 12.10*+*0.20mm) on *Salmonella* species. In the combination of E1 and E2, E1 antagonised or interfered with E2 in all the tests against *Salmonella* species. That means that the combined effect is less than that of a more potent extract acting alone (Oko and Itah, 2014).

**3.3 Testing for antibacterial effects of extract of *V. amygdalina* in combination with Ampicillin (AMP) and Chloramphenicol (CPC) on *Salmonella* species**

The effects of the extract in combination with ampicillin and chloramphenicol are shown in Table 3. The table shows that all the combinations against *Salmonella* isolates from broilers, layers, soil impacted litters and control soil, exhibited synergistic effect. That is, the joint effect of E1 and AMP was greater than the sum of effects of each of the extracts acting alone (Ok and Itah, 2014). Regarding the combined effect of E1 and CPC on *Salmonella* species, there was synergism in almost all the tests.

**3.4 Testing for antibacterial effect of extract of *M. indica* in combination with Ampicillin (AMP) and Chloramphenicol (CPC) on *Salmonella* species**

The effects of the extract of *M. indica* in combination with AMP and CPC are represented in Table 4. All the combinations of *M. indica* extract with AMP revealed antagonistic effect on *Salmonella* species with the exception of layers litters from all the farms and litter impacted soil from the University Poultry Farm which showed synergism. Also, with the exception of isolates of layers litters from University and Almond Farms, and broilers litter impacted soil from University Farm, besides broilers litters from Sandra and Almond Farms, other combinations of E2 with CPC revealed antagonistic effects.

**3.5 Percentage representation of *Salmonella* isolates under the effect of ethanolic extract of *V. amygdalina* in combination with Ampicillin and Chloramphenicol**

Figure 1 represents the percentage of *Salmonella* isolates affected by ethanolic extract of *V. amygdalina* in combination with *M. indica*, AMP and CPC. The figure revealed that there was antagonism between E1 and E2 in 100% of the isolates tested, 93% for E1 + AMP (Synergism and 100% for E1 + CPC antagonism).

**3.6 Percentage representation of *Salmonella* isolates under the effect of ethnaolic extract of the bark of *Magnifera indica* in combination with Ampicillin and Chloramphenicol**

Figure 2 represents the percentage of *Salmonella* isolates affected when E2 was combined with Ampicillin and Chloramphenicol. The figure reveals 71% antagonism and 29% synergy for E2 + AMP, and 100% antagonism for E2 + CPC.

**3.7 Percentage representation of *Salmonella* isolates inhibited by various concentrations of *Vernonia amygdalina***

Figure 3 represents the percentage of *Salmonella* isolates inhibited by various concentrations of *V. amygdalina*. The figure shows that 38.46% test organisms were inhibited at 3.12mg/ml, 30.77% at 6.25mg/ml, 15.38% at 12.50mg/ml, 20.51% at 25mg/ml and 10.25% at 50mg/ml, indicating the effectiveness of the plant.

**3.8 Percentage representation of *Salmonella* isolates inhibited by various concentrations of *Magnifera indica***

Figure 4 represents the percentage of *Salmonella* isolates inhibited by various concentrations of the bark of *M. indica*. The figure shows that 12.52% test organisms were inhibited at 6.25mg/ml, 23.08% at 12.50%, 23.08% at 25mg/ml, 25.3% at 50mg/ml and 15.38% at 100mg/ml, indicating less effectivity than E2.

**Table 1:** Results of phytochemical screening from ethanolic leaf extract of *Vernonia amygdalina* and bark of *Mangifera indica*

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| S/N | Name of sample (plants) | Alkaloids | Flavonoids | Tannins | Glycosides | Saponins | Polyphenols | Phlabotinnins | Steroids |
| 1. | E1c | +++ | +++ | +++ | +++ | +++ | +++ | - | +++ |
| 2. | E2c | ++ | ++ | +++ | ++ | + | +++ | ++ | ++ |

E1 = Vernonia amygdalina; E2 = Mangifera indica; e = Ethanolic; + = Low concentration of bioactive substances; ++ = Moderate concentration of bioactive substances; +++ = High concentration of bioactive substances; - = Absence

**Table 2:** Effect of ethanolic leaf extract of *Vernonia amygdalina* (E1) and the bark of *Magnifera indica* (E2) and their combination on *Salmonella* organisms

|  |  |  |  |
| --- | --- | --- | --- |
| **Isolate** | **Mean zones of inhibition (mm)** | | |
|  | **E1** | **E2** | **E2 + E2** |
| aLL  aBIS  aLIS  aCS  aBL  bBIS  bLIS  bBL  bLL  cBL  cBIS  cLL  cCS  cLIS | 10.83+0.35  10.77+0.63  10.08+0.50  15.12+0.61  9.06+0.66  13.00+0.26  12.06+0.50  9.00+0.50  12.16+0.50  9.66+0.14  10.12+0.40  10.50+0.50  14.00+0.20  10.50+0.50 | 00\*  00\*  00\*  3.55+0.10  00\*  00\*  00\*  00\*  00\*  00\*  00\*  00\*  00\*  00\* | 00\*  00\*  6.0+0.25  10.12+0.66  8.16+0.66  00  8.0+0.50  8.0+0.50  8.0+0.50  7.0+0.50  9.33+0.50  7.66+0.98  12.10+0.20  9.0+0.70 |

LL = Layers litter; BL = Broilers litter; BIS = Broilers litter impacted soil; LIS = Layers litter impacted soil; CS = Control soil; a = Unical Poultry farm; b = Sandra poultry farm; c = Almond poultry farm; \* = *Salmonella* was resistant to E2 or E1 + E2.

Table 3: Effect of ethanolic leaf extract of *Vernonia amygdalina* in combination with ampicillin and chloramphenicol on *Salmonella* organisms

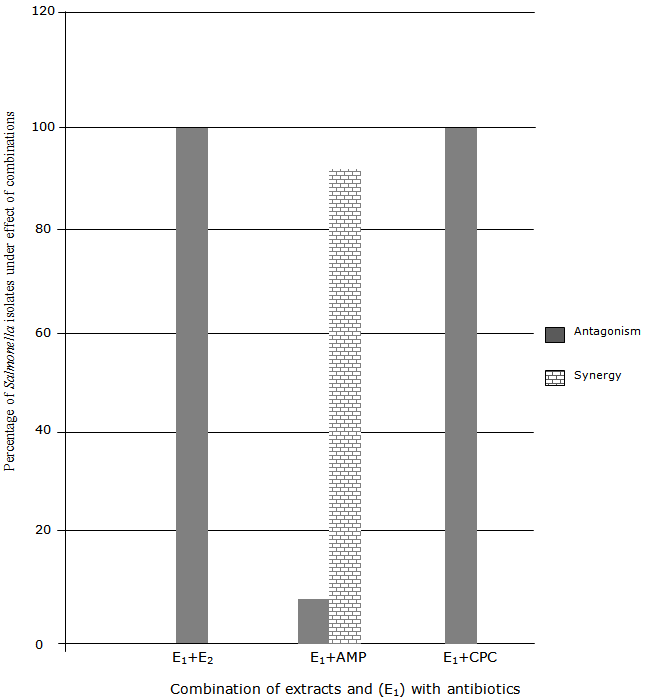
|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Mean zones of inhibition** | | | | |
| **Isolate** | **E1** | **AMP** | **E1+AMP** | **CPC** | **E1+CPC** |
| aLL  aBIS  aLIS  aCS  aBL  bBIS  bLIS  bBL  bLL  cBL  cBIS  cLL  cCS  cLIS | 10.83+0.35  10.77+0.63  10.08+0.50  15.12+0.61  9.06+0.66  13.00+0.26  12.06+0.50  9.00+0.50  12.16+0.50  9.66+0.14  10.12+0.40  10.50+0.50  14.00+0.20  10.50+0.50 | 3.16+0.78  13.22+0.66  10.66+0.62  14.98+0.66  13.66+0.56  14.50+0.50  14.33+0.30  19.67+0.57  9.44+0.40  13.83+0.35  12.66+0.74  11.50+0.50  15.33+0.72  14.00+0.50 | 13.99+0.20  21.66+0.20  21.66+0.97  17.77+0.57  13.74+0.30  16.99+0.67s  15.22+0.47s  13.66+0.61  15.33+0.61s  17.99+0.86s  17.99+0.86s  14.49+0.20s  16.33+0.35s  14.11+0.50s | 18.00+0.50  17.21+0.21  15.09+0.85  22.88+0.60  18.55+0.25  18.44+0.30  14.66+0.46  16.66+0.50  15.16+0.46  14.16+0.61  16.46+0.46  18.50+0.50  19.83+0.35  16.44+0.60 | 21.66+0.35  19.88+0.20  17.74+0.50  31.55+0.94  22.66+0.93  21.16+0.61  17.11+0.57  20.22+0.30  17.16+0.61  18.50+0.50  17.49+0.45  18.60+0.61  20.50+0.50  20.50+0.50 |

LL = Layers litter; BL = Broilers litter; BIS = Broilers litter impacted soil; LIS = Layers litter impacted soil; CS = Control soil; a = Unical Poultry farm; b = Sandra poultry farm; c = Almond poultry farm; S = Synergistic effect exhibited; + = All the combinations exhibiting synergistic effect.

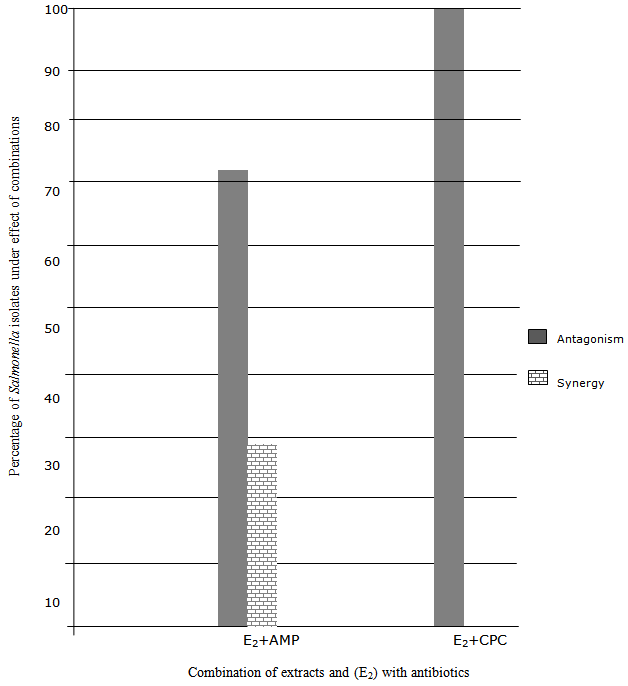
Table 4: Effect of ethanolic extract of *Magnifera indica* in combination with Ampicillin and Chloramphenicol on *Salmonella* isolates

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Mean zones of inhibition** | | | | |
| **Isolate** | **E1** | **AMP** | **E1+AMPK** | **CPC** | **E1+CPCK** |
| aLL  aBIS  aLIS  aCS  aBL  bBIS  bLIS  bBL  bLL  cBL  cBIS  cLL  cCS  cLIS | 0.00  0.00  0.00  3.55+0.10  0.00  0.00  0.00  0.00  0.00  0.00  0.00  0.00  0.00  0.00 | 3.16+0.78  13.22+0.66  10.66+0.62  14.98+0.66  13.66+0.56  14.50+0.50  14.33+0.30  19.67+0.57  9.44+0.40  13.83+0.35  12.66+0.74  11.50+0.50  15.33+0.72  14.00+0.50 | 13.99+0.20  21.66+0.20  21.66+0.97  17.77+0.57  13.74+0.30  16.99+0.67s  15.22+0.47s  13.66+0.61  15.33+0.61s  17.99+0.86s  17.99+0.86s  14.49+0.20s  16.33+0.35s  14.11+0.50s | 18.00+0.50  17.21+0.21  15.09+0.85  22.88+0.60  18.55+0.25  18.44+0.30  14.66+0.46  16.66+0.50  15.16+0.46  14.16+0.16  16.46+0.46  18.50+0.50  19.83+0.35  16.44+0.60 | 21.66+0.35  19.88+0.20  17.74+0.50  31.55+0.94  22.66+0.93  21.16+0.61  17.11+0.57  20.22+0.30  17.16+0.61  18.50+0.50  17.49+0.45  18.6+0.61  20.50+0.50  20.50+0.50 |

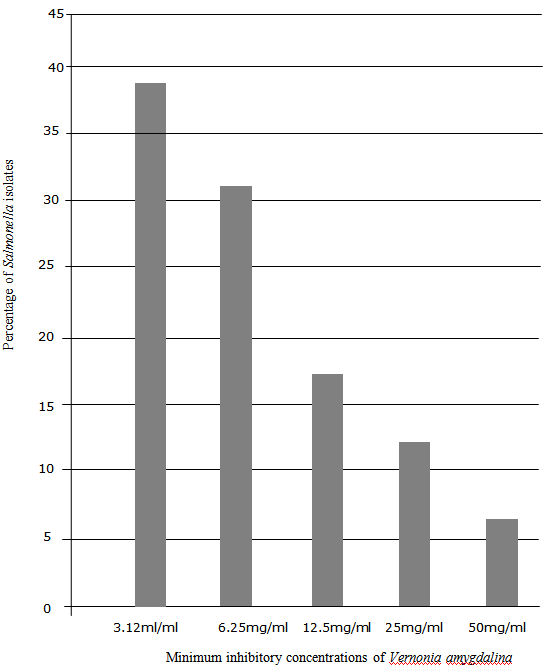
LL = Layers litter; BL = Broilers litter; BIS = Broilers litter impacted soil; LIS = Layers litter impacted soil; CS = Control soil; a = Unical Poultry farm; b = Sandra poultry farm; c = Almond poultry farm; k = over 71% antagonistic.



**Figure 1: Percentage representation of the effect of ethanolic extract of *V. amygdalina* in combination with Ampicillin and Chloramphenicol on *Salmonella* isolates**

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**Figure 2: Percentage representation of the effect of ethanolic extract of the bark of *Mangifera indica* in combination with Ampicillin and Chloramphenicol on *Salmonella* isolates**

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**Figure 3: Percentage inhibition of concentrations of leaf extract of *Vernonia amygdalina* on *Salmonella* isolates**

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**Figure 4: Percentage inhibition of concentrations of *M. indica* on*Salmonella* isolates**

**Discussion**

The phytochemical screening of the leaf of *V. amygdalina* and the bark of *M. indica* revealed varying proportions of bioactive substances such as alkaloids, saponins, glycosides, polyphenol, tannins, flavonoids and steroids. These bioactive substances reported by several researchers, are indicative of the potential medicinal values of the plants in which they appear (Enyi-Idoh *et al*., 2011; Alobi *et al*., 2012; Alobi *et al*., 2015). Also, Madunagu *et al*. (1990) have demonstrated the occurrence of different concentrations of phlobatinnins, glycosides, saponins, alkaloids, polyphenol, tannins and flavonoids in the bark of *M. indica*. The result revealed that, although *M. indica* contained phlabatinnins which was not present in *V. amygdalina, V. amygdalina* contained more concentrations of bioactive substances, e.g., alkaloids, flavonoids, glycosides, saponins, polyphenols and steroids, indicating greater medicinal potential than *M. indica*. The variation in the phytochemical composition and concentration in both plants may explain why the extracts of the two plants had different effects and minimum inhibitory actions on *Salmonella* species. Ahmad *et al*. (1998) and Eloff (1998) report that ethanolic extracts of some medicinal plants lack antimicrobial activities, thus confirming the poor effect of *M. indica* on the test organism.

In this study, however, *V. amygdalina* (E1) and *M. indica* (E2) individually revealed some levels of antimicrobial effect on *Salmonella* species, although E1 (zone of inhibition: 9.06+0.66 to 15.12+0.61mm) showed greater effect than E2 (zone of inhibition: 0.0 to 12.10+0.20mm). In effect, *Salmonella* species was resistant to E2. This agrees with other findings (Kabuki *et al*., 2000; Mboto *et al*., 2009; Olamide and Agu, 2013; Hodul *et al.*, 2013). However, a combination of E1 and E2 resulted in antagonism, i.e., the combined effect of E1 and E2 was less than that of a more potent drug (or plant) acting alone (Oko and Itah, 2014). A combination of E1 and CPC tested against *Salmonella* species revealed synergism in all the isolates, while E2 + AmP revealed antagonism except when tested against *Salmonella* isolates from layers from all the farms and impacted soil, which showed synergism, i.e., the joint effect was greater than the sum effects of each plant extract acting alone (Oko and Itah, 2014). However, antagonism was most prevalent in the combination of E2 and CPC against the isolates. There was a significant difference (p < 0.05) between the extracts and their combinations, and between their combinations with AMP and CPC with respect to their effectivity. There was also significant difference (p < 0.05) between *Salmonella* isolates with respect to their sensitivities to extracts singly, or in combination. It was observed that E1 had a greater effect on *Salmonella* isolates from control soil (zone of inhibition: 14.00+0.20 to 15.12+0.61mm) than E2 (zone of inhibition: 0.00 to 3.55+0.10mm) (Table 4); the effects of E1 or E2 on *Salmonella* species from control soil were highest among the effects on *Salmonella* species from other sources. This indicates that isolates from control soil were more susceptible to both plant extracts than isolates from poultry litter and poultry impacted soil, which are reported to be resistant to conventional antibiotics resulting from the incorporation of antibiotics into poultry feed formulations (Smith, 2005; Arikpo *et al*., 2006; Eja *et al*., 2012). These results indicate that some medicinal plants in combination with some conventional drugs can help solve the problem of antibiotic resistance experienced globally today. The combined effect of *Allium sativum* and ciprofloxazone or ampicillin, has been demonstrated by Eja *et al*. (2011). Elsewhere *Lasianthera africana* or *Heinsia crinata* in combination with CPC against *Staphylococcus aureus*, *Salmonella typhi* and *Candida albicans*, has revealed similar results (Andy *et al*., 2008).

Of the fourteen isolates tested, the combination of E1 and E2 exhibited antagonism in 100%; E1 + AMP exhibited synergism in 93% of the isolates, and antagonism in 7% of the isolate, while E1 + CPC exhibited antagonism in 100% of the isolates. This indicates that *V. amygdalina* in combination with ampicillin can be effective against antibiotic resistant *Salmonella* species. However, E2 + AMP resulted in synergism in only 29% of the isolates in which case E1 + AMP was better. Elsewhere, the reactions of medicinal plants in combination with conventional drugs have been demonstrated (Andy *et al*., 2008; Eja *et al*., 2011).

Accordingly, the percentage of isolates under the effect of the minimum inhibitory concentration (3.12mg/ml) of E1 on *Salmonella* isolates was 38.46%, whereas the percentage of isolates under the effect of the minimum inhibitory concentration of E2 (6.25mg/ml) was 12.82% which still proves that E1 possesses greater antimicrobial potential than E2. Even at a concentration of 50mg/ml, E2 gave 12.82% of isolates inhibited, as against 20.51% inhibited by 25mg/ml of E1.

**5. Conclusion**

The demonstration of antimicrobial activity of *Vernonia amygdalina* and the bark of *Magnifera indica* in this study indicates that the two plants are antimicrobial at various concentrations. *V. amygdalina* is more effective especially when combined with ampicillin. However, the bacterial isolates varied in their degree of susceptibility to the plant extracts. Thus, this study has demonstrated that it is feasible to use *Vernonia amygdalina* especially, in treating *Salmonella* infection under the current increasing development of resistance to conventional drugs by microorganisms.

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**References**

1. Abdalla, A. E., Darwish, S. M., Ayad, E. H., El-Hamahmy, R. M. Egyptian mango product 2: Antioxidant and antimicrobial activities of extracts and oil from mango seed kernel. Food Chem. 2007; 103:1141-1152.

2. Abdul, H., Samra, A., Tahir, N., Muhammad, I. U., Ijaz, A., Syeda, A., Shabbir, H. Antimicrobial effect of *Magnifera indica* leaf extract against antibiotic sensitive and multi-drug resistant *Salmonella typhi*. Pakistan J. Pharmacol. Sc. 2013; 24(4):715-719.

3. Ackers, M., Puhr, N. D., Tauxe, R. V., Mintz, E. D. Laboratory-base surveillance of *Salmonella* serotype Typhi infections in the United States. J. Clin. Microbiol. 2000; 283:2668-2763.

4. Ahmad, I. D., Memood, E. Z., Mohammad, F. Screening of some Indian medicinal plants for their antimicrobial properties. J. Ethopharmacol. 1998; 63:183-193.

5. Alobi, N. O., Ikpeme, E. M., Okoi, A. I., Etim, K. D., Eja, M. E. Phytochemical and nutritional profiles of *Lasianthera africana, Heinsia crinata* and *Gongronema latifolium*. N.Y. Sc. J. 2012; 5(3):45-48.

6. Alobi, N.O., Eja, M. E., Okoi, A. I., Uno, U. A., Bassey, G. A. Comparative evaluation of the nutritional composition of *Andrographis paniculata* and *Gongronema latifolium*. N.Y. Sc. J. 2015; 8(12):16-20.

7. Andy, I. E., Eja, M. E., Mboto, C. I. An evaluation of the antimicrobial potency of *Lasianthera* *africana* (BEAUV) and *Heinsia crinata* (G. Taylor) on *Escherichia coli, Salmonella typhi, Staphylococcus aureus* and *Candida albicans*. 2008; 4(1):25-29.

8. Arikpo, G. E., Eja, M. E., Ikpeme, E. M., Ofon, U. A., Enyi-Idoh, K. H., Udosen, G. Antimicrobial resistance patterns in a Calabar Poultry, Cross River State, Nigeria. Nig. J. Microbiol. 2006; 20(3):1244-1251.

9. Atangwho, I. J., Ebong, P. E., Eyong, E. U., Williams, I. O., Eteng, M. U., Egbung, G. E. Comparative chemical composition of some antidiabetic medicinal plants: *Azadirachta indica, Vernonia amygdalina* and *Gongronema latifolium*. Afr. J. Biotech. 2009; 8:4685-4689.

10. Atinpelu, D. A., Onakoya, T. M. Antimicrobial activities of medicinal plants used in folklore remedies in South-Western Nigeria. Afr. J. Biotech. 2006; 7:1078-1081.

11. Bailey, N. T. J. Statistical method in biology. 2nd edn. Hodder and Stoughton, London. 1981; pp. 216.

12. Campbell, R. J., Ledesma, N. H., Campbell, C. W. Tropical mangos, “How to grow the world’s most delicious fruit”: 1st ed. Fairchild Tropical Garden, Miami, Florida. 2002; pp. 222-507.

13. Cheesbrough, M. District laboratory practices in tropical countries. Part II. Cambridge University Press, United Kingdom. 2000; pp. 182-187.

14. Cuilei, J. Methodology of analysis of vegetable drugs: Practical manuals on the industrial utilization of medicinal and aromatic plants. Centr. Blvd. 1982; pp. 66-67.

15. Eja, M. E., Arikpo, G. E., Enyi-Idoh, K. H., Ikpeme, E. M. An evaluation of the antimicrobial synergy of Garlic (*Allium sativum*) and Utazi (*Gongronema latifolium*) on *Escherichia coli* and *Staphylococcus aureus*. Malaysian J. Microbiol. 2011; 7(1):49-53.

16. Eja, M. E., Arikpo, G. E., Enyi-Idoh, K. H., Ikpeme, E. M. An evaluation of the antimicrobial synergy of Garlic (*Allium sativum*) and Utazi (*Gongronema latifolium*) on *Escherichia coli* and *Staphylococcus aureus*. Mal. J. Microbiol. 2011; 7(1):49-53.

17. Eja, M. E., Udoekong, N. S., Ikpeme, E. M., Enyi-Idoh, K. H., Lennox, J. A., Etim, K. D. Antibiogram studies and extended beta-lactamase activity profile of *Salmonella*-like species isolated from poultry soil of the University of Uyo, Nigeria. Malaysian J. Microbiol. 2012; 8(4):280-284.

18. Ejike, H. C. Current perspective on the medicinal potential of *Vernonia amygdalina*. J. Med. Plant Res. 2011; 5(7):1051-1061.

19. Eloff, J. N. Which extractant should be used for the screening and isolation of antimicrobial components from plants? J. Ethnopharmacol. 1998; 60:1-8.

20. Enyi-Idoh, K. H., Utsalo, S. J., Epoke, J., Eja, M. E., Arikpo, G. E., Etim, S. E., Oruche, A. N. Antibacterial and phytochemical studies of *Allium sativum*. N.Y. Sc. J. 2011; 4(4):123-128.

21. Gundiza, M. Phytochemical screening of Zimbabwean medicinal plants. The Cente for Afr. J. Med. 1985; 31:328.

22. Jones, B. D. *Salmonella* gene invasion regulation: A story of environmental awareness. The J. Microbiol. 2005; 43:110-117.

23. Kabuki, T., Hakajima, H., Arai, M., Ueda, S., Kuwabara, Y., Dosako, S. Characterization of novel antimicrobial compounds from Mango (*Magnifera indica* L.) Kernel seeds. Food Chem. 2000; 71:61-66.

24. Kokwaro, J. Medicinal plants in East Africa (3rd ed.), Nairobi, Kenya: University of Nairobi Press. 2009.

25. Madunagu, B. E., Ebana, R. U., Ekpe, E. D. Antimicrobial and antifungal activity of some medicinal plants of Akwa Ibom State. West Afr. J. Biotech. Appl. Chem. 1990; 35:25-30.

26. Mboto, C. I., Eja, M. E., Adegoke, A. A., Iwatt, G. D., Asikong, B. E., Takon, I., Udo, S. M., Akeh, M. Phytochemical properties and antimicrobial activities of combined effect of extracts of the leaves of *Garcinia kola*, *Vernonia amygdalina* and honey on some medically important microorganisms. Afr. J. Microbiol. Res. 2009; 3(9):57-559.

27. Miller, J. C. and Miller, J. N. Statistics for analytical chemistry. Ellis Horwood, Chichester. 1986; pp. 202.

28. MMBR. Tetracycline antibiotics: Mode of action, application, molecular biology, and epidemiology of bacterial resistance. Microbial. Molecul. Biol. Rev. 2001; 65(2):232-260.

29. Murkhtar, M. D. and Huda, M. Prevention of tinea capitis in primary school and sensitivity of etiologic agents to *Pistia stratiotes* extracts. Nig. J. Microbiol. 2005; 19(1-2):412-419.

30. Nwinuka, N. M., Ibeh, G. O., Ekeke, G. I. Antimicrobial properties of some commonly used spices. Global J. Pure. Appl. Sc. 2006; 12(1):73-77.

31. Okeke, I. N., Laximinarayan, R., Butha, Z. A., Duse, A. G., Jenkins, P., O’Brian, T. F., Pablos-Mendez, A. Antimicrobial resistance in developing countries. Part I: Recent Trends and Current status. Lan. Inf. Dis. 2005; 5:481-493.

32. Oko, J. O., Itah, A. Y. Basic pharmaceutical microbiology. Ahmadu Bello University Press Limited, Zaria. 2014; pp. 28-39.

33. Olamide, S. O., Agu, G. C. The assessment of the antimicrobial activities of *Ocimum gratissimum* (Wild Basil) and *Vernonia amygdalina* (Bitter leaf) on some enteric pathogens causing dysentery or diarrhea in patients. Int. J. Engr. Sc. 2013; 2(9):83-96.

34. Onyeaba, R. A., Ugbogu, O. C., Okeke, C. U., Iroakasi, O. Studies on the antimicrobial effects of garlic (*Allium sativum* Linn), Ginger (*Zingiber officinale* Roscoe) and lime (*Citrus aurantifolia* Linn). Afr. J. Biotech. 2004; 3:552-554.

35. Patterson, D. L. Resistance in Gram-negative bacteria: Enterobacteriaceae. Am. J. Med. 2006; 119, 20-28.

36. Prescott, L. M., Hardley, J. P., Klein, D. A. Microbiology. 6th edn. McGraw-Hill, Boston. 2005; pp. 992.

37. Smith, W. T. Feed chicken properly cooperative extension. Extension Series of Mississippi State Universsit. 2005; pp. 58-60.

38. Sofowora, E. A. Medicinal plants and traditional medicine in Africa. 4th edition. John Willey and Sons, New York. 1984; pp. 26-105.

6/18/2016