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* (E_1) and the bark of *Magnifera indica* (E_2) 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 E_1 possessed greater antimicrobial effect on *Salmonella* species (Zone of inhibition: 9.06±0.66 to 15.12±0.61mm) than E_2 (Zone of inhibition: 0.0 to 12.10±0.20mm); while *Salmonella* was resistant to E_2. The combination of E_1 and E_2 gave antagonistic results with E_1 antagonizing E_2. There was significant difference ($p < 0.05$) between E_1 and E_2, and the combination of each of the plants and antibiotics. The maximum zone of inhibition of E_1 + AMP (21.66±0.97mm) indicateing better effectivity than E_2 + AMP (13.77±0.86mm). Also, E_1 + CPC has the same advantage over E_2 + CPC. There was antagonism in 100% of the isolates when E_1 and E_2 were combined. However, E_1 + AMP and E_1 + 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 E_1 (3.12mg/ml) affected 38.46% of the isolates unlike that of E_2 (6.25mg/ml) which affected 12.82% of the isolates, thus confirming E_1 as having greater effectivity than E_2. In conclusion, *Vernonia amygdalina* in combination with ampicillin and chloramphenicol could be drugs of choice against resistant *Salmonella*species.

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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 (Onyeagba *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 37°C 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 10^{3} was the number of organisms in the standard inoculums used for the MIC test. In this technique Mueller-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 Mueller-Hinton broth to obtain final concentrations of 5000, 2500, 1250, up to 0mg/ml, and incubated at 37°C 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* (E_1) and the bark of *Magnifera indica* (E_2) and their combinations on *Salmonella* isolates are represented in Table 2. The table shows that E_1 possessed reasonable antibacterial effect (Zone of inhibition: 9.06±0.66 to 15.12±0.61mm) on *Salmonella*, unlike E_2 which had little or no effect (Zone of inhibition from 0.0 to 12.10±0.20mm) on *Salmonella* species. In the combination of E_1 and E_2, E_1 antagonised or interfered with E_2 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 E_1 and AMP was greater than the sum of effects of each of the extracts acting alone (Oko and Itah, 2014). Regarding the combined effect of E_1 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 E_2 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 E_1 and E_2 in 100% of the isolates tested, 93% for E_1 +

AMP (Synergism and 100% for E_1 + 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 E_2 was combined with Ampicillin and Chloramphenicol. The figure reveals 71% antagonism and 29% synergy for E_2 + AMP, and 100% antagonism for E_2 + 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 E_2.

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.	E_1^c	+++	+++	+++	+++	+++	+++	-	+++
2.	E_2^c	++	++	+++	++	+	+++	++	++

E_1 = Vernonia amygdalina; E_2 = 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* (E_1) and the bark of *Magnifera indica* (E_2) and their combination on *Salmonella* organisms

Isolate	**Mean zones of inhibition (mm)**		
	E_1	**E_2**	**$E_2 + E_2$**
[a]LL	10.83±0.35	00	00
[a]BIS	10.77±0.63	00	00
[a]LIS	10.08±0.50	00	6.0±0.25
[a]CS	15.12±0.61	3.55±0.10	10.12±0.66
[a]BL	9.06±0.66	00	8.16±0.66
[b]BIS	13.00±0.26	00	00
[b]LIS	12.06±0.50	00	8.0±0.50
[b]BL	9.00±0.50	00	8.0±0.50
[b]LL	12.16±0.50	00	8.0±0.50
[c]BL	9.66±0.14	00	7.0±0.50
[c]BIS	10.12±0.40	00	9.33±0.50
[c]LL	10.50±0.50	00	7.66±0.98
[c]CS	14.00±0.20	00	12.10±0.20
[c]LIS	10.50±0.50	00	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 E_2 or $E_1 + E_2$.

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

	Mean zones of inhibition				
Isolate	**E_1**	**AMP**	**E_1+AMP**	**CPC**	**E_1+CPC**
[a]LL	10.83±0.35	3.16±0.78	13.99±0.20	18.00±0.50	21.66±0.35
[a]BIS	10.77±0.63	13.22±0.66	21.66±0.20	17.21±0.21	19.88±0.20
[a]LIS	10.08±0.50	10.66±0.62	21.66±0.97	15.09±0.85	17.74±0.50
[a]CS	15.12±0.61	14.98±0.66	17.77±0.57	22.88±0.60	31.55±0.94
[a]BL	9.06±0.66	13.66±0.56	13.74±0.30	18.55±0.25	22.66±0.93
[b]BIS	13.00±0.26	14.50±0.50	16.99±0.67[s]	18.44±0.30	21.16±0.61
[b]LIS	12.06±0.50	14.33±0.30	15.22±0.47[s]	14.66±0.46	17.11±0.57
[b]BL	9.00±0.50	19.67±0.57	13.66±0.61	16.66±0.50	20.22±0.30
[b]LL	12.16±0.50	9.44±0.40	15.33±0.61[s]	15.16±0.46	17.16±0.61
[c]BL	9.66±0.14	13.83±0.35	17.99±0.86[s]	14.16±0.61	18.50±0.50
[c]BIS	10.12±0.40	12.66±0.74	17.99±0.86[s]	16.46±0.46	17.49±0.45
[c]LL	10.50±0.50	11.50±0.50	14.49±0.20[s]	18.50±0.50	18.60±0.61
[c]CS	14.00±0.20	15.33±0.72	16.33±0.35[s]	19.83±0.35	20.50±0.50
[c]LIS	10.50±0.50	14.00±0.50	14.11±0.50[s]	16.44±0.60	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	**E_1**	**AMP**	**E_1+AMP[K]**	**CPC**	**E_1+CPC[K]**
[a]LL	0.00	3.16±0.78	13.99±0.20	18.00±0.50	21.66±0.35
[a]BIS	0.00	13.22±0.66	21.66±0.20	17.21±0.21	19.88±0.20
[a]LIS	0.00	10.66±0.62	21.66±0.97	15.09±0.85	17.74±0.50
[a]CS	3.55±0.10	14.98±0.66	17.77±0.57	22.88±0.60	31.55±0.94
[a]BL	0.00	13.66±0.56	13.74±0.30	18.55±0.25	22.66±0.93
[b]BIS	0.00	14.50±0.50	16.99±0.67[s]	18.44±0.30	21.16±0.61
[b]LIS	0.00	14.33±0.30	15.22±0.47[s]	14.66±0.46	17.11±0.57
[b]BL	0.00	19.67±0.57	13.66±0.61	16.66±0.50	20.22±0.30
[b]LL	0.00	9.44±0.40	15.33±0.61[s]	15.16±0.46	17.16±0.61
[c]BL	0.00	13.83±0.35	17.99±0.86[s]	14.16±0.16	18.50±0.50
[c]BIS	0.00	12.66±0.74	17.99±0.86[s]	16.46±0.46	17.49±0.45
[c]LL	0.00	11.50±0.50	14.49±0.20[s]	18.50±0.50	18.6±0.61
[c]CS	0.00	15.33±0.72	16.33±0.35[s]	19.83±0.35	20.50±0.50
[c]LIS	0.00	14.00±0.50	14.11±0.50[s]	16.44±0.60	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.

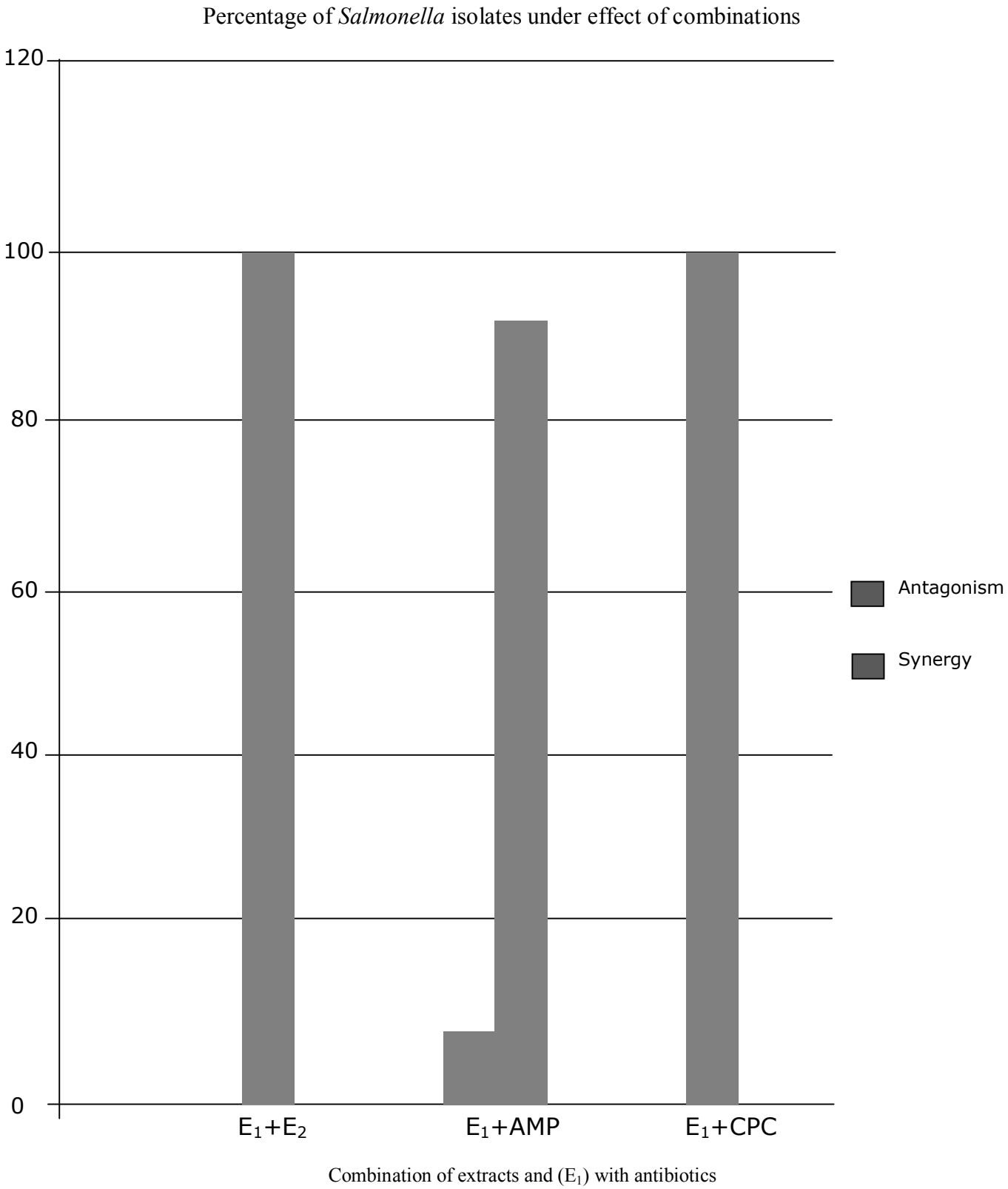

Figure 1: Percentage representation of the effect of ethanolic extract of *V. amygdalina* in combination with Ampicillin and Chloramphenicol on *Salmonella* isolates

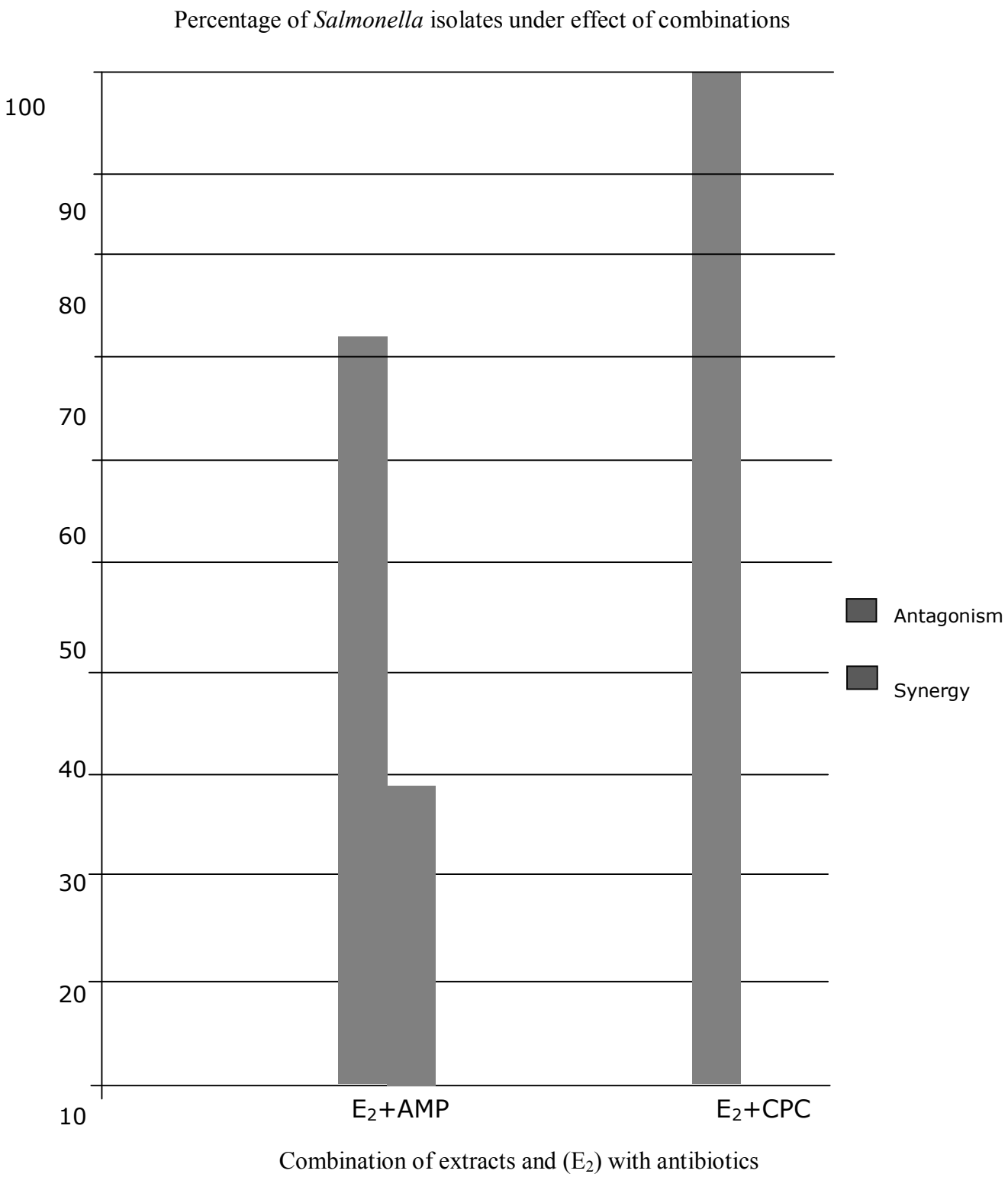

Figure 2: Percentage representation of the effect of ethanolic extract of the bark of *Mangifera indica* in combination with Ampicillin and Chloramphenicol on *Salmonella* isolates

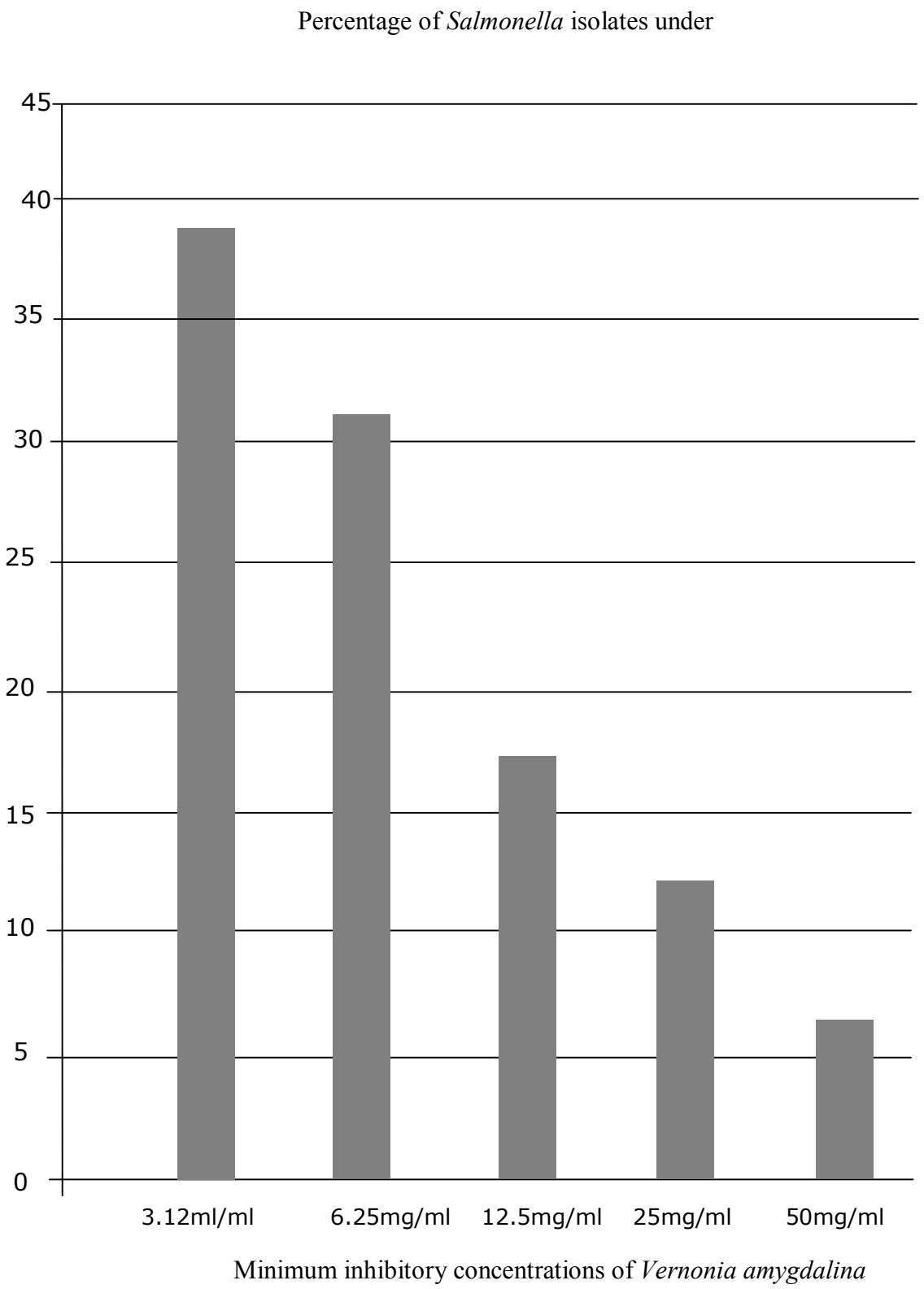

Figure 3: Percentage inhibition of concentrations of leaf extract of *Vernonia amygdalina* on *Salmonella* isolates

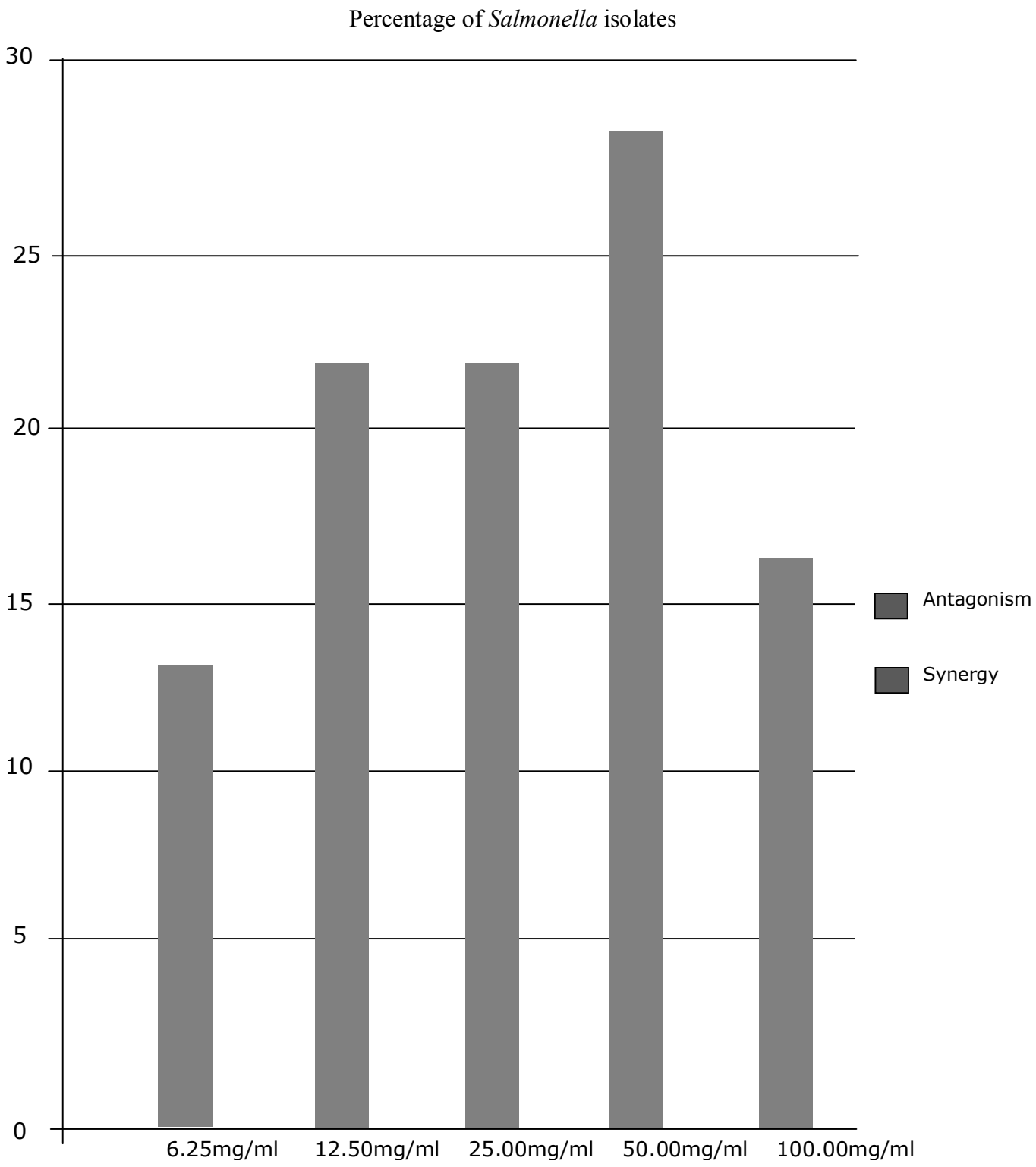

Figure 4: Percentage inhibition of concentrations of *M. indica* on*Salmonella* isolates

4. 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* (E_1) and *M. indica* (E_2) individually revealed some levels of antimicrobial effect on *Salmonella* species, although E_1 (zone of inhibition: 9.06±0.66 to 15.12±0.61mm) showed greater effect than E_2 (zone of inhibition: 0.0 to 12.10±0.20mm). In effect, *Salmonella* species was resistant to E_2. This agrees with other findings (Kabuki *et al*., 2000; Mboto *et al*., 2009; Olamide and Agu, 2013). However, a combination of E_1 and E_2 resulted in antagonism, i.e., the combined effect of E_1 and E_2 was less than that of a more potent drug (or plant) acting alone (Oko and Itah, 2014). A combination of E_1 and CPC tested against *Salmonella* species revealed synergism in all the isolates, while E_2 + 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 E_2 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 E_1 had a greater effect on *Salmonella* isolates from control soil (zone of inhibition: 14.00±0.20 to 15.12±0.61mm) than E_2 (zone of inhibition: 0.00 to 3.55±0.10mm) (Table 4); the effects of E_1 or E_2 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 E_1 and E_2 exhibited antagonism in 100%; E_1 + AMP exhibited synergism in 93% of the isolates, and antagonism in 7% of the isolate, while E_1 + 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, E_2 + AMP resulted in synergism in only 29% of the isolates in which case E_1 + 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 E_1 on *Salmonella* isolates was 38.46%, whereas the percentage of isolates under the effect of the minimum inhibitory concentration of E_2 (6.25mg/ml) was 12.82% which still proves that E_1 possesses greater antimicrobial potential than E_2. Even at a concentration of 50mg/ml, E_2 gave 12.82% of isolates inhibited, as against 20.51% inhibited by 25mg/ml of E_1.

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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