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Research Article

Stimulating Antimicrobial Activity in Aspirin with *Psidium guajava* and *Syzygium aromaticum* Extracts against Multi-drug Resistant *Salmonella Spp*: A Comparative Study of Multiple Combinations

Mathew Gideon[1*], Zakari Ladan[1], Emmanuel K. Duniya[1], Mamman A. James[1], Samuel Dennis[2]

[1]Department of Pure and Applied Chemistry, Faculty of Science Kaduna State University (KASU) Tafawa Balewa Way P.M.B 2339 Kaduna Nigeria
[2]Department of Chemical Pathology, Haematology and Microbiology Diagnostic Laboratory of Oxford Hospital Makera, Kakuri, RC KD0010980 Kaduna State Nigeria
E.mail: mathewace8@gmail.com

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Abstract: Factros such as gene mutation and transfer, some inappropriate uses and diagnostics, and the prescription of antibiotics to undiagnosed patients has significantly led to the emergence of drug resistance in bacteria, which is also hampering our ability to treat infections. As the declines of new antibiotics, scientists and researchers keep trying to develop or propose some synergistic combinations of antibiotics with medicinal plants extracts in an effort to boost their antimicrobial activities against drug resistant bacteria. This work aimed to develop a cocktail regimen, which will be highly susceptible to confirmed drug resistant clinical isolate of *Salmonella spp*, using Tetracycline, Co-trimoxazole, Aspirin, *Psidium guajava* and *Syzygium aromaticum* extract alongside minimizing toxicity potential. Twenty-four (24) different combinations were made from 10 µg/mL of Co-trimoxazole, Tetracycline and Aspirin with each clove extract and separately again with guava extract. Eighteen (18) of them were combined in three different stages (6 each) using concentrated Tetraoxosulphate (vi) acid (H_2SO_4) and 0.1 Molar sodium hydroxide (NaOH), while 31 antimicrobial disks were prepared and tested on *Salmonella spp*. The antimicrobial susceptibility test revealed that **Scl,** a stage 1 combination of Co-trimoxaazole with clove extract; **Ac2,** a stage 2 combination of Aspirin and clove extract; **Ag2,** a stage 2 combination of Aspirin and guava leave extract were all susceptible to the confirmed resistant isolate of *Salmonella spp*. Ag2 had the best zone of inhibition better than Ciprofloxacin inhibition zone at 20 µg/mL. Aspirin was the best precursor drug which favourably combines with both clove and guava extract to give a desired cocktail regimen with potential antimicrobial characteristics. Hence, it is needed to identify the compounds obtained in this combinations, isolates, and purify their active principles, and subjected to other pharmacological test to ascertain if they can be used to combat multi-drug resistant bacteria and other pathogenic organisms.

***Keywords*:** antibiotics, drug resistance, Aspirin, *Salmonella spp*, *Psidium guajava*, *Syzygium aromaticum*, inhibition

1. Introduction

Currently the development of new antibiotics is decreasing, the emergence of resistance bacteria due to factors such as Mutation, gene transfer, inappropriate uses and diagnostics, and prescription of antibiotics to persistent patients

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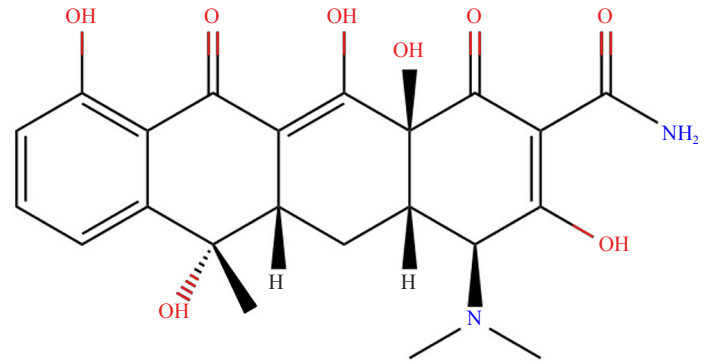
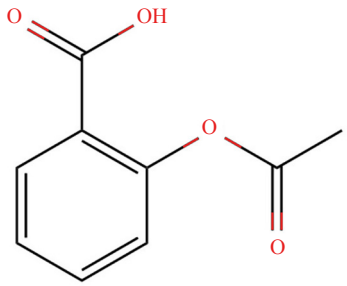
which are yet undiagnosed are significant ways in which antimicrobial resistance happens faster[1] and is gradually phasing out or limiting the use of some early commonly prescribe antibiotics in the treatment of infections and diseases.[2] The generation of drug-resistant tumors after continued therapy is still inevitable, and this eventually narrows down the treatment of these drug-resistant tumors. Therefore, it is imperative to keep looking for new drug combinations (i.e. compounds of natural and synthetic molecules) that can enhance or maintain efficacy, while minimizing toxicity and delaying the development of drug resistance.[3]

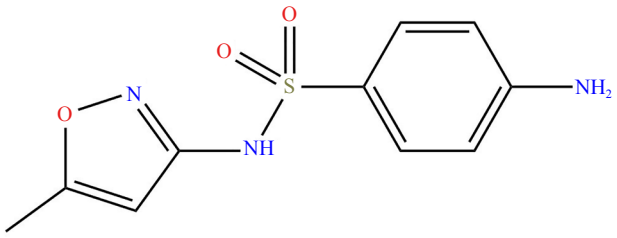
Typhoidal and non-typhoidal *Salmonella* infections are of growing concern in developing countries. New therapeutic approaches are required for the treatment of infections caused by these pathogenic bacteria, their resistance to available and commonly prescribe antibiotics poses serious health threat to humans and animals,[4,5] *Salmonella* infections cause an estimate of 1.3557 million infections, 27,120 hospitalization, and 420 deaths in the United States each year, with $400 million medical cost approximately.[6] *Salmonella typhi* were shown to be resistant to commonly prescribe antibiotics in some part of Nigeria, Pakistan, and Ethopia.[4,5] Infections caused by gram negative bacteria are of serious health threats, since 1962 only few major classes of antibiotics gain approval to treat common and deadly gram negative bacteria.[2]

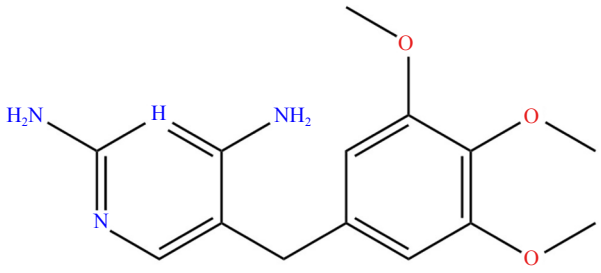


Tetracycline



Aspirin





Cotrimoxazole

Figure 1. Two antimicrobial and one non-antimicrobial drug[7,8,10]

Tetracycline as shown in Figure 1 is a broad-spectrum polyketide antibiotic, while it has roles as an antimicrobial agent, an antibacterial drug, an antiprotozoal drug, a protein synthesis inhibitor and an *Escherichia coli* metabolite.[7] Co-trimoxazole as shown in Figure 1 is a synergistic synthetic combination of two antimicrobial agents, trimethoprim and sulfamethoxazole, while it is a broad-spectrum antimicrobial agent and its effective in the treatment of many infections

including pneumocystis pneumonia in AIDS.[8]

Acetylsalicylic acid (ASA) also known as Aspirin as shown in Figure 1 belongs to the groups of medications called *analgesics* (pain relievers), *antipyretics* (fever reducers), *anti-inflammatories* (inflammation reducers), and *platelet aggregation inhibitors* (anticlotting agents). Acetylsalicylic acid (ASA) is not considered an antibiotic but it is used to relieve pain, fever, and inflammation in various conditions such as lower back and neck pain, the flu, common cold, burns, menstrual pain, headache, migraines, osteoarthritis, rheumatoid arthritis, sprains and strains, nerve pain, toothache, muscle pain, *bursitis*, and following surgical and dental procedures,[9] also used in long-term low-doses to prevent heart attack and cancer.[10]

For millennia, extracts from plants have been in use for the treatment of various infections and diseases. In developed countries, active component extracted from plant sources, isolated and purified were estimated at one fourth of all prescribe drugs,[11] Herbal medicines are known to have numerous benefits, including lower toxicity and fewer side effects than traditional chemotherapeutic drugs.[12]

Another important issue is the idea of synergistic interactions between drugs and chemicals in the biomedical world for over a century. Some complex diseases, especially cancer, are being treated with various drug cocktails, understanding the interactions among these drugs is increasingly vital to ensuring successful treatment regimens.[13]

Drug repurposing is a tactic or trend which uses a known compound or an existing drug (i.e., gives a known molecule a second or new opportunity) to treat a challenging, rare, resistant, or difficult-to-treat disease.[14] Two potent bioactive classes of compounds in medicinal chemistry, with known diverse pharmacological activities in humans, with prominent antimicrobial/antiviral activities, the key functional structural moieties of both of them meet into the same compounds to a new opportunity to treat an existing recalcitrant infection/disease.[15] An example is the expected potent clinical inhibitory effects of riboprine and forodesine against SARS-CoV-2 replication principally attributed to the triple synergistic inhibitory activities against the three enzymes RdRp, ExoN, and adenosine kinase (ADK).[16]

Psidium guajava and *Syzygium aromaticum* are important medicinal plants in the tropics. They are used in the treatment of diarrhoea, stomach ache, diabetes of bronchitis, asthma attacks, sexually transmitted infections and for the treatment of dysentery.[17] Water extract of *Syzygium aromaticum* (clove) were effective against *Salmonella typhi* at 62.5-250 µg/mL,[18] crude herbal extract of *Syzygium aromaticum* (clove) shown a minimum inhibitory concentration (MIC) of 1,560 µg/mL against *Salmonella typhimurium,*[2] water extract and ethanolic extract of *Psidium quajava* (guava) were effective against *Salmonella typhi* (Typhoidal), *Salmonella Paratyphi A.* (Typhoidal) and *Salmonella typhimurium* (Non-Typhoidal) 25,000 µg/mL.[19]

The existing knowledge of the pharmacological activities of medicinal plants and prescribe drugs can be explore to develop a cocktail regimen with new or enhance antimicrobial activities against drug-resistant pathogens. Medicinal plants are characterized by the presence of various phytocompounds such as Alkaloid, Glycosidic Flavonoids, Sterol, Tanins, Terpenoids, Saponins, combined Anthraquinones, Cardiac glycosides, etc.[20] These phytocompounds are also responsible for the difference in pharmacological activities of medical plants on a given pathogen, as in the case of crude herbal extract of *A. nilotica, T. arjuna, and S. aromaticum showing an MIC* of 9.75 µg/mL, 0.0 µg/mL and 1,560 µg/mL *on S. typhimurium* ATCC-13311.[2] Although some extracting solvents are often associated with toxicity and safety of the phytocompounds extracted from medicinal plants, it is desirable if the solvent is non-toxic or not flammable but unfortunately on few meet this criteria.[21]

Cytotoxicity studies are useful in determining the potential toxicity of a test substance, including plant extracts or biologically active compounds isolated from plants. Minimal to no toxicity is essential for the successful development of a pharmaceutical in this regard.[22] Toxicity in medicinal plants and in chemically synthesize drugs may results from the type of extracting solvent, chemicals and reagents used in their preparations or when administered in high doses, likewise, an increase in antimicrobial concentration eventually may lead to an increase in bacteria inhibition alongside possible toxicity, an example is the observed zone of inhibition of *Psidium quajava* (guava) extract which was 4 mm at 25 mg/mL increased significantly to 17 mm when it concentration was raised to 400 mg/mL against *Salmonella typhi* (Typhoidal). [19]

Concentrated tetraoxosulphate (vi) acid, and Sodium hydroxide, has always been used to initiate or catalyse certain reactions; examples includes the formation of esters, ethers, alcohols, etc.Their combined activity can be used to alter or induced antimicrobial activity in a mixture of antibiotic or acetylsalicylic acid with plant extract(s) at lower concentrations. Several literatures detailed some synergistic combinations of antibiotic with medicinal plants extracts in

an effort to boost their antimicrobial activities against drug resistant bacteria.[23-25] Yet, no literature was able to suggest a stepwise strategic acid and alkaline enhance combinatory method on the use of antimicrobial and non-antimicrobial drugs like acetylsalicylic acid to combat multi-drug resistant bacteria.

In this work, we aimed to develop a method for which the antimicrobial activity of ineffective commonly prescribe antibiotics for the treatment of *Salmonella spp* can be enhance at lower concentration and also to induce antimicrobial activity on a non-antimicrobial drug, in this method, our objectives is to use water to extract some of the phytocompounds present in clove and quava plants in a highly diluted form which when tested on *Salmonella spp* no inhibition will be observed, and also to prepare the drug solutions at extremely low concentrations below the concentration which they were able to inhibit resistant *Salmonella spp*, our goal is to have a potent antimicrobial candidate that will show a slight sign of bacteria inhibition on *Salmonella spp* thus minimizing toxicity potential (hence the use of water and lower drug concentration), and finally make comparison in MIC values with Ciprofloxacin in inhibition of resistant *Salmonella spp*.

2. Methods

2.1 *Sampling*

Tablets of Ciprofloxacin 500 mg Jopan pharmaceuticals Nigeria Limited, Lagos Nigeria and Asprin 75 mg Exus pharmaceutical Limited Lagos Nigeria were purchased from pharmaceutical vendors within the Kaduna metropolis Kaduna North Kaduna, Nigeria. Dry *Syzygium aromaticum* (clove) were purchased at Television market Kaduna South, *Psidium guajava* (guava) leaves were harvested in a Florist garden along Television market, Kaduna South, and *Salmonella spp* were collected at Chemical Pathology, Haematology and Microbiology Diagnostic Laboratory of Oxford Hospital Makera, Kakuri, Kaduna State Nigeria.

2.2 *Experimental*

2.2.1 *Preparation of plant extracts*

Psidium guajava leaves weighing 10 g was washed with distilled water, cut to average size pieces with a knife to fit into a 500 mL beaker containing 100 mL of distilled water and was heated to boil on a hot plate for 10 minutes, it was then filtered using a Whatman no. 1 filter paper and the extract was labelled **Gu** (Guava extract). 10 g of dry *Syzygium aromaticum* was washed with distilled water, transferred into a 500 mL beaker containing 100 mL of distilled water and was heated to boil on a hot plate for 10 minutes, it was then filtered using a Whatman no. 1 filter paper and the extract was labelled **Cl** (Clove extract).

2.2.2 *Preparation of antibiotic samples and combinatorial process*

A volumetric flask containing 1000 mL distilled water was heated to 65 °C and was used to prepare 10 µg/mL solution of Ciprofloxacin, Co-trimoxazole, Tetracycline, Aspirin and 20 µg/mL of Ciprofloxacin. The 10 and 20 µg/mL of Ciprofloxacin solution were labelled **C10** and **C20**, **T10** for Tetracycline, **A10** Aspirin and **S10** for Co-trimoxazole respectively. 0.1 M solution NaOH was prepared, and conc. H_2SO_4 was used for the combinatorial processes.

The methods adopted by[26] were used with few modifications, Figure 2 also shows a comprehensive combinatorial processes of the samples in stages. 1 mL of **Gu** was added to 1 mL of **T10** in a 5 mL test-tube and was labelled **Tgu** (Guava Tetracycline mixture), it was repeated for Co-trimoxazole and labelled **Sgu** (Guava Co-trimoxazole mixture), and for Aspirin **Agu** (Guava Aspirin mixture).

1 mL of **Cl** was added to 1 mL of **T10** in a 5 mL test-tube and was labelled **TCl** (Clove Tetracycline mixture), it was repeated for Co-trimoxazole and labelled **SCl** (Clove Co-trimoxazole mixture) and for aspirin **ACl** (Clove Aspirin mixture). Other combinations were carried out as follows:

1 mL of A10 was added to 1 mL of Gu in a 5 mL test-tube, 0.1 mL of 0.1 M NaOH was added, the solution was heated to boil at 110 °C for 10 minutes, 0.1 mL Conc. H_2SO_4 was added to the boiling solution in the test-tube and was allowed to continue boiling for another 10 minutes, the total combination was labelled **Ag1.** The procedure was repeated for T10 and was labelled **Tg1,** also for S10 and labelled **Sg1.**

1 mL A10 was added to 1 mL Cl in a 5 mL test-tube, 0.1 mL of 0.1 M NaOH was added, the solution was heated to boil at 110 °C for 10 minutes, 0.1 mL Conc. H_2SO_4 was added to the boiling solution in the test-tube and was allow to continue boiling for another 10 minutes, the total combination was labelled **Ac1.** The procedure was repeated for T10 and was labelled **Tc1,** and for S10 labelled **Sc1.**

1 mL A10 was added to 1 mL Gu in a 5 mL test-tube, 0.1 mL of 0.1 M NaOH was added, the solution was heated to boil at 110 °C for 10 minutes, a fresh 0.5 mL portion of Gu was added to the boiling solution in the test-tube followed by addition of 0.1 mL of 0.1 M NaOH, and was allowed to continue boiling for another 10 minutes, 0.1 mL Conc. H_2SO_4 was added to the boiling solution in the test-tube and was also allowed to continue boiling for another 10 minutes, the total combination was labelled **Ag2.** The procedure was repeated for T10 and was labelled **Tg2,** and for S10 labelled **Sg2.**

1 mL A10 was added to 1 mL Cl in a 5 mL test-tube, 0.1 mL of 0.1 M NaOH was added, the solution was heated to boil at 110 °C for 10 minutes, a fresh 0.5 mL portion of Cl was added to the boiling solution in the test-tube followed by addition of 0.1 mL of the 0.1 M NaOH and was allowed to continue boiling for another 10 minutes, 0.1 mL Conc. H_2SO_4 was added to the boiling solution in the test-tube and was also allowed to continue boiling for another 10 minutes, the total combination was labelled **Ac2.** The procedure was repeated for T10 and was labelled **Tc2,** and for S10 labelled **Sc2.**

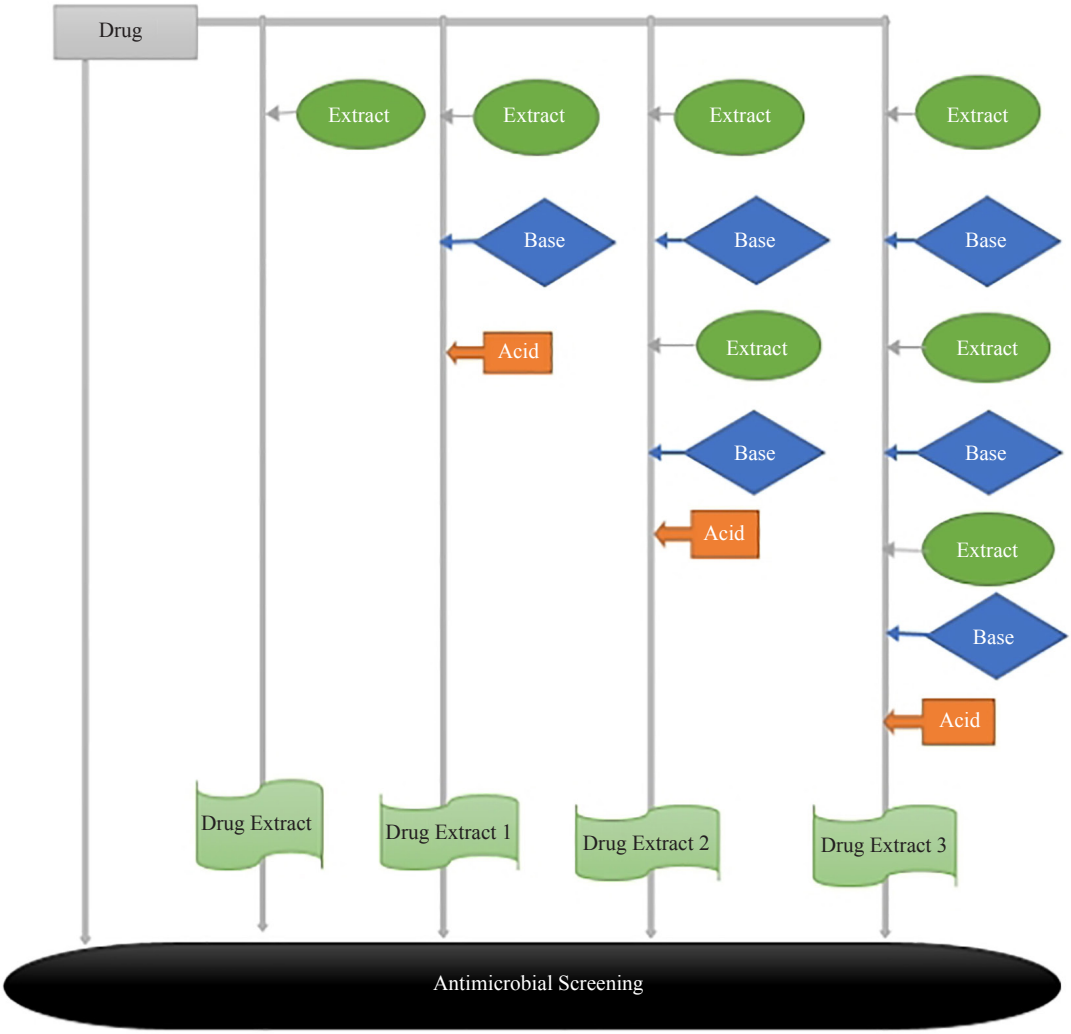


Figure 2. Schematic representation of the strategy used in the combinatorial process

1 mL A10 was added to 1 mL Gu in a 5 mL test-tube, 0.1 mL of 0.1 M NaOH was added, the solution was heated to boil at 110 °C for 10 minutes, a fresh 0.3 mL portion of Gu was added to the boiling solution in the test-tube followed by addition of 0.1 mL of the 0.1 M NaOH and was allowed to continue boiling for another 10 minutes, another fresh 0.3 mL portion of Gu was added to the boiling solution in the test-tube followed by addition of 0.1 mL of 0.1 M NaOH and was allowed to continue boiling for another 10 minutes, 0.1 mL of Conc. H_2SO_4 was added to the boiling solution in the test-tube and was also allowed to continue boiling for another 10 minutes, the total combination was labelled **Ag3.** The procedure was repeated for T10 and was labelled **Tg3,** and for S10 labelled **Sg3.**

1 mL A10 was added to 1 mL Cl in a 5 mL test-tube, 0.1 mL of 0.1 M NaOH was added, the solution was heated to boil at 110 °C for 10 minutes, a fresh 0.3 mL portion of Cl was added to the boiling solution in the test-tube followed by addition of 0.1 mL of the 0.1 M NaOH and was allowed to continue boiling for another 10 minutes, another fresh 0.3 mL portion of Cl was added to the boiling solution in the test-tube followed by addition of 0.1 mL of 0.1 M NaOH and was allowed to continue boiling for another 10 minutes, 0.1 mL of Conc. H_2SO_4 was added to the boiling solution in the test-tube and was also allowed to continue boiling for another 10 minutes, the total combination was labelled **Ac3.** The procedure was repeated for T10 and was labelled **Tc3,** and for S10 labelled **Sc3.**

2.3 *Antimicrobial screening*

- Mueller-Hinton agar
- Antibiotic discs
- Cotton swabs
- Petri dishes
- 0.5 McFarland Turbidity standard
- Inoculum
- Forceps
- Metric rule

Salmonella spp (Stool), was isolated, characterized, and identified. High profile negative 10 tipped multiple susceptibility antibiotic discs was used for screening of resistant *Salmonella spp*, the prepared samples susceptibility test was carried out on the confirmed resistant isolate of *Salmonella spp.*

The patterns for antimicrobial susceptibility of confirmed clinical isolate of Salmonella *spp*, was done by Kirby-Bauer disk diffusion test using Mueller-Hinton Agar (MHA).[25] The area was Sterilize with disinfectant and open burner, a sterile cotton swab was dipped into the inoculum and excess medium was removed by pressing the swab onto the wall of the tube, the surface area of the plate was swab completely by rotating the plate. The plates were allowed to dry for 5 minutes so that the medium will absorb the inoculum properly, alcohol sterilize forceps were used in picking up the antibiotic discs and were lightly touched with the forceps to ensure that it is in good contact to avoid misplacement. The plates were then incubated upside down for 24 hours at 37 °C. After 24 hours of incubation, the Zone of Inhibition (ZOI) was observed and recorded as either positive or negative.

3. Results and discussions

Table 1, and Figure 3 shows the antimicrobial susceptibility test for the thirty one (31) prepared disk, Gu and Cl showed a negative inhibition for *Salmonella spp*, this is due to the lower concentration of the extract in the extraction process, plant extracts often need to be in a bit higher concentration before they can show a positive inhibition on bacteria and far more higher for resistant bacteria.[2,17-19] Ciprofloxacin at 10 µg/mL and 20 µg/mL showed positive inhibition against *Salmonella spp*, although the prepared concentration is not within the susceptibility threshold of 0.06 µg/mL for Ciprofloxacin against *Salmonella spp* but because of the resistant nature of the bacteria and also to have a uniform prepared drug concentration for ease of samples comparison.[27]

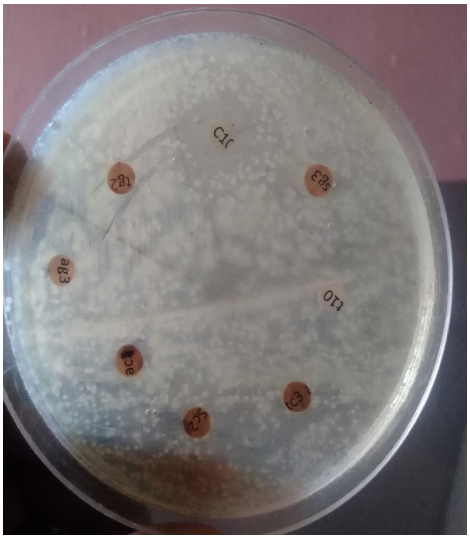
T10, and S10 showed a negative inhibition on salmonella spp, this is because the prepared concentration were three times lesser compared to 30 µg/mL of Tetracycline and Co-trimoxazole used for the antimicrobial screening of resistant *Salmonella spp*, definitely no inhibition of *Salmonella spp* is expected from A10 because it is not an antibiotic.[9,10,27]

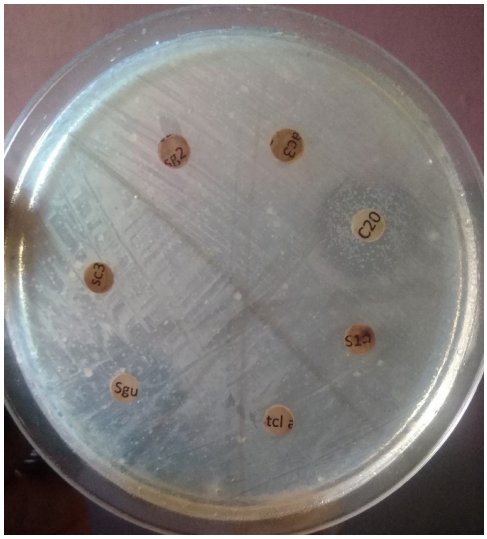
Table 1. Showing susceptibility test for 31 prepared antimicrobial disk

Samples						
Plant extracts	Gu	Cl				
	-	-				
Antibiotics	**C10**	**C20**	A10	T10	S10	
	+	+	-	-	-	
Antibiotics combined with plant extracts	Agu	Tgu	Sgu	**Acl**	Tcl	Scl
	-	-	-	+	-	-
One step combination	Ag1	Tg1	Sg1	Ac1	Tc1	Sc1
	-	-	-	-	-	-
two step combination	**Ag2**	Tg2	Sg2	**Ac2**	Tc2	Sc2
	+	-	-	+	-	-
three step combination	Ag3	Tg3	Sg3	Ac3	Tc3	Sc3
	-	-	-	-	-	-

(+) positive inhibition, (-) no inhibition

When *Syzygium aromaticum* (clove) and *Psidium quajava* (guava) extract were combined with the A10, T10 and S10 without the addition of the acid or base nor heat, only Acl showed a positive inhibition on *Salmonella spp*, with a fairly clear zone of inhibition, this possibly may be due to the nature of the phytocompounds present in clove and that of Aspirin led to a synergism, and also, clove extract has been inhibiting *Salmonella spp* at lower concentrations compared to guava extract.[17-19]





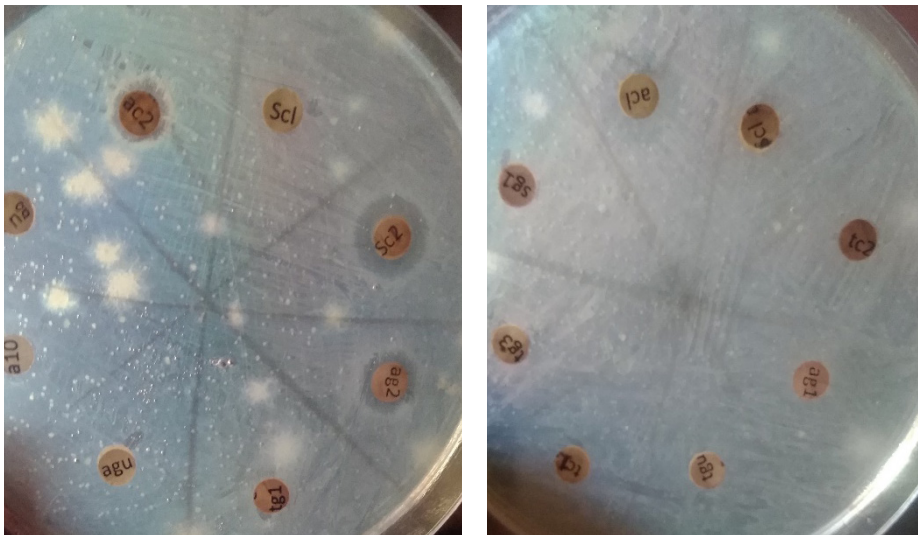


Figure 3. Showing antimicrobial susceptibility test for 31 prepared disk against *Salmonella spp*

All the one step combinatorial method Ag1, Tg1, Sg1, Ac1, Tc1 and Sc1 showed a negative inhibition for *Salmonella spp* despite the positive inhibition shown by Acl, this may be due to the lower concentration of initially prepared antibiotic/drug solution, and possibly the addition of acids and base which might have initiated certain chemical reactions between the available active sites present in the reacting species, or a functional group(s) inter-conversion might have occurred, or any other secondary reaction(s) which may not have favoured the formation of new active sites or preventing the initial active site present in the reacting specie from reacting[28,29] as shown in Figures 4 and 5, the colour RED was used as a sign for the active site while the blue little ball shape is a representation of the non-active sites of each reacting specie.

In the second stage combinatorial method for Ag2, Tg2, Sg2, Ac2, Tc2 and Sc2, only Aspirin/guava Ag2 and Aspirin/clove Ac2 showed positive inhibition for *Salmonella spp*, this may be due to the additional step which a fresh portion of the plant extract and sodium hydroxide were added before acidification, this might have actually generated new active sites either through conjugation, ring opening and closing, or a functional group(s) inter-conversion which might have acted in synergy with the active site initially present in the fresh portion of the plant extract that possibly did not react with the mixture obtain in stage 1 as shown in Figure 6, depicting there is no available active site for antibiotic combination as compared to the two available active sites (RED colure balls) in Aspirin combination.[28,29] also, the zone of inhibition for clove extract in this case was not clear compared to guava extract.[2,17-19]

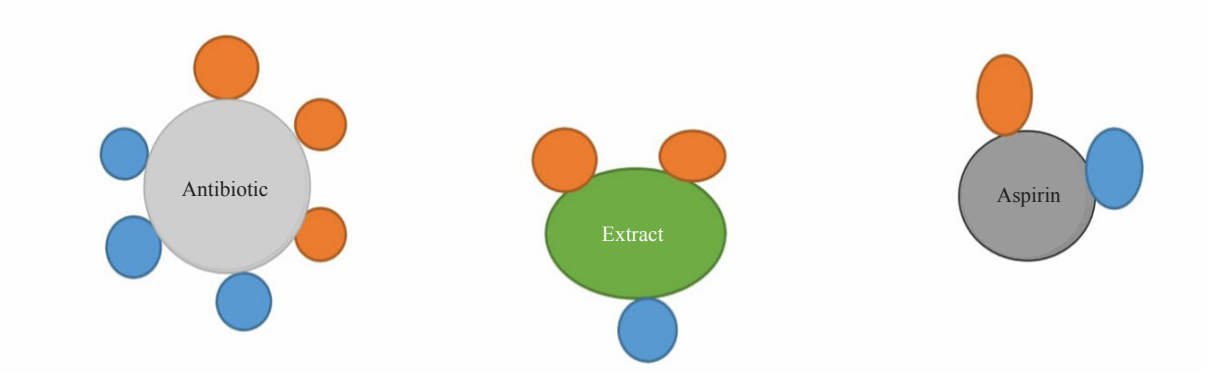


Figure 4. A simple illustration of samples with their active and non-active sites

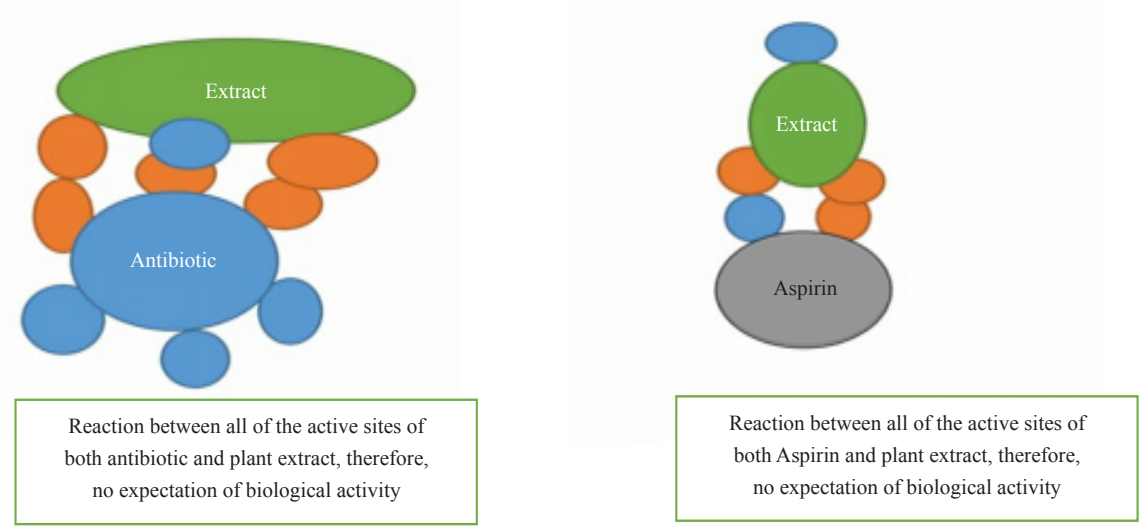


Figure 5. A simple illustration of one of many possible ways the samples might have reacted in stage 1

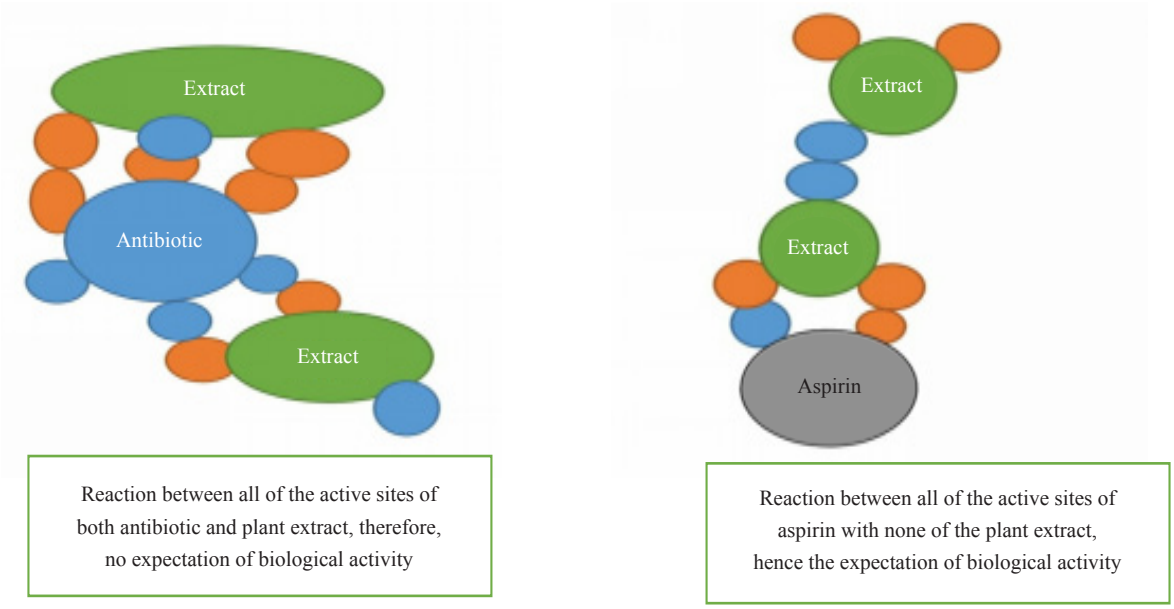


Figure 6. A simple illustration of one of many possible ways the samples might have reacted in stage 2

Surprisingly, the three step combinatorial process in all samples Ag3, Tg3, Sg3, Ac3, Tc3 and Sc3 did not show any inhibition against *Salmonella spp* despite the positive results show by both aspirin combination in step 2, this clearly shows that the added step which a fresh portion of the plant extract and sodium hydroxide were added before acidification, might have exhausted the active site generate by step 2 in Aspirin combinations and creating none in the antibiotic combination, thereby limiting the expectation of antimicrobial properties in the combination as shown in Figure 7.[28,29]

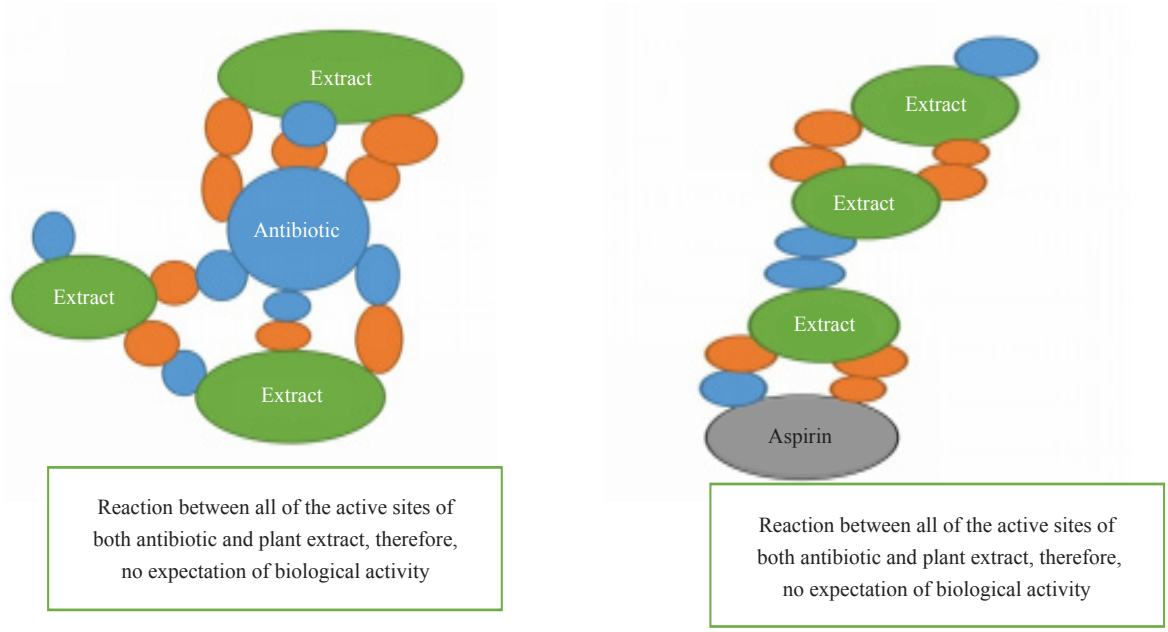


Figure 7. A simple illustration of one of many possible ways the samples might have reacted in stage 2

3.1 *Comparison with ciprofloxacin*

Although C10, C20, Ag2 and Ac2 both show positive inhibition against *Salmonella spp* as shown in Figure 3, the zones of inhibition for Ciprofloxacin at both concentrations were broad compared to that of Aspirin but, the zones were not clear zones because some specs of *Salmonella spp* were observed within the zones, indicating that C10 and C20 were more bacteriostatic at that concentrations. Ag2 showed good inhibition of *Salmonella spp* and no spec of the bacteria was observed within the zone thus, is fair to infer that its bactericidal, and the clarity of it zone could be effectively compared to the referenced drug Ciprofloxacin. The inhibition of *Salmonella spp* shown by Ag2 was better than twice the concentration of Ciprofloxacin C20, Ciprofloxacin has shown to be three (3) times better and effective in *Salmonella spp* inhibition compared to Tetracycline and Co-trimoxazole,[2,17-19,27] this can be further confirmed by their concentrations in purchased high profile negative antibiotic disk,[26] and also from the result obtained in this work.

4. Conclusion

Synergism in drug combination for *Salmonella spp* inhibition was observed in **Scl**, a one-step combination of Co-trimoxaazole with clove extract, **Ac2**, a two-step combination of aspirin and clove extract, **Ag2**, a two-step combination of Aspirin and guava leave extract. Ac2 and Ag2 showed a bactericidal activity on *Salmonella spp* as compared to twice the concentration of ciprofloxacin. Aspirin a non-antimicrobial drug was the best reacting specie with the plant extract give a predicted cocktail regimen thus, showing that a simple poly-functional drug or a simple poly-functional organic compound with known pharmacological characteristic can be used to develop a potent cocktail regimen for pathogen inhibition instead of ineffective antibiotics. Further analysis is required/recommended to identify the compounds obtained in this combinations, isolates, and purify their active principles, and subjected to other pharmacological test to ascertain if they can be used to combat multi-drug resistant bacteria and also to be explored in antifungal, antiviral and anti-cancer studies.

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Conflicts of Interest

The authors declare no competing financial interest.

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