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Synergistic Evaluation of *Moringa oleifera, Hunteria umbellate* and *Azadirachta indica* with Antibiotics Against Environmental MRSA Isolates: An *In-vitro* Study

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Abstract: Methicillin–Resistant *Staphylococcus aureus* (MRSA) is known to show resistance to beta-lactam class of antibiotics. MRSA is among the highest superbugs posing dangerous threats to humans. This study aimed at determining the *in-vitro* synergistic evaluation of *Moringa oleifera, Hunteria umbellate* and *Azadirachta indica* extracts with existing antibiotics (Azithromycin, Clindamycin and Vancomycin) on isolated MRSA from fomites. MRSA was isolated using the BBL™ Oxacillin agar screen test (Müller Hinton Agar with 6 µg/mL Oxacillin and 4% NaCl). The Minimum Bactericidal concentration (MBC) of the MRSA were determined by Agar well diffusion using antibiotics in solitary, plant extracts in solitary, combination of these antibiotics with plant extracts at different concentrations. The agar diffusion assay showed that *H. umbellate* extract-Azithromycin combination had the least zones of inhibition ≥21.00±1.92 mm in 75% of all isolates testedwhile *M. oleifera* extract-Azithromycin combination had the highest zones of inhibition ≥22.20 ±2.27 mm. Comparison of bactericidal activities of all plant extracts and antibiotics synergy shows Azithromycin to have a significant value of P>0.05. The agar well diffusion method showed synergistic effects between combination of antibiotics and all extracts with significant increase in the zones of inhibition of the test antibiotics against environmental strains of MRSA. The synergistic interactions indicated that the inhibitory potentials of the plant extracts increased hence, combining natural products derived from phytochemicals and antibiotics could be another way to mitigate and fight against resistant infectious bacteria.

Keywords: Antimicrobial Resistance, MRSA, Antibiotics, Plant Extracts, Synergism

1. Introduction

An escalating antibiotic resistance by the pathogenic bacteria has been observed since last decade and the adverse effects of conventional antibiotics calls for a friendly alternative. The search for new antibiotics is a matter of global importance, the world is facing a decline in the use of antibiotics [1, 2] which is due to the accelerating rate of antibiotic resistance amongst certain pathogenic bacteria. Many of these pathogens pose a risk to people who have undergone surgery or someone with a compromised immune system (Immuno-compromised). Various types of plants have been used for several centuries worldwide not only as dietary supplements but also as traditional treatments for many diseases [3].

Herbal medicine is one of the oldest forms of treatment for diverse ailments and it has enjoyed a relatively high subscription for obvious reasons like being cost effective, accessible, and it blends with socio-cultural life of the people around the world. W. H. O has stated that herbal or medicinal plants are the best source to obtain a variety of drugs, out of the 250,000 to 500,000 species of plants on earth [4]. Phytochemicals derived from *Moringa oleifera* and

Azadirachta indica is one of the 10% which have a profound potentials in pharmaceutical industry as a source of bioactive constituents for drug development and therapeutic uses [5, 6] while *Hunteria umbellate* is just finding its own potential use in the pharmaceutical companies.

Medicinal plants have greatest potential for benefitting people, especially those living in countries suffering from poverty, poor health, malnutrition, unemployment and isolation across different continents [6]. Some microorganisms may develop resistance to a single antimicrobial agent while others, named "Multidrug-resistant (MDR)" strains, are resistant to several antimicrobial agents. Infections caused by these strains often fail to respond to standard treatment and generate a greater risk of morbidity due to the spread of the resistance to other microorganisms [7, 8]. The failure of the existing antibiotics to control infections makes it crucial to find alternative agents with new mechanisms of action. One such novel therapeutic strategy involves the use of phytochemicals such as plant-derived products i.e. the consumption of herbal drink/extracts alone or in combination with antibiotics during the course of treatment [9].

Chen, [10] in her studies on the synergistic effect of *M. oleifera* seeds and chitosan (an essential and abundant component of exoskeletons – the muco-adhesive polymer which is derived from chitin) on antibacterial activity against *Bacillus subtilis* and *Pseudomonas putida* found the individual samples to be more effective than the two combined. However, in another study to determine antimicrobial potential of different plant seed extracts against Multidrug Resistant Methicillin Resistant *Staphylococcus aureus* (MDR – MRSA), it was established that *Moringa oleifera* seeds had a synergistic potential to restore the effectiveness of B–lactam antibiotics against MRSA [11]. The importance of *Azadirachta indica* has been recognized by U.S. National Academy of Science, which published a report in 1992 entitled "Neem: a tree for solving global problems". More than 135 compounds have been isolated from different parts of the tree [12]. *A. indica*, commonly known as Neem, with the active compound named azadirachtin, is a multipurpose tree with variety of health benefits [13]. Neem leaves has antibacterial properties and could be used for controlling airborne bacterial contamination in residential premises [5, 6].

Hunteria umbellata (K. Schum.) is a medicinal plant with a long standing use in the treatment of infections, ailments and diseases in Nigeria and Ghana [14]. In addition, the use of the plant in herbal medicines has long been reported amongst the Yoruba and Benin in Southwestern Nigeria [15, 16], it is locally known as "Abeere". In African folklore medicine, various extracts prepared from different parts of the plant *Hunteria umbellata (K. Schum.)* have been employed in the treatment of various human diseases such as sexually transmitted infections, yaws, stomach ulcers, pains and swellings, diabetes mellitus, dysmenorrheal and to induce or augment birth labor [14, 17].

The use of Methicillin Resistance *Staphylococcus aureus* (MRSA) as the test organisms is because of its uniqueness; *S. aureus* becomes MRSA after acquiring genes that encode resistance to the broader class of β-lactam antibiotics. The specific gene taken up by *S. aureus* to make it resistant to Methicillin is called *mec*A. This gene is carried together with a larger set of genes, known as *SCCmec* (Staphylococcal cassette chromosome), which is mobile and capable of inserting into the DNA of other *S. aureus* bacteria [18] This multidrug resistance makes the infections caused by these *S. aureus* strains very difficult to treat; in addition to producing toxins and virulence factors, MRSA bacteria can generate biofilms, sticky layers and clumps of cells that enable the bacteria to adhere to medical devices such as catheters and implants [19] and also fomites [18].

In this study, we examined the synergistic activities of stem bark, leaves and root extracts (aqueous) of *M. oleifera, H. umbellata* and *A. indica* alone and in combination with antibiotics such as Azithromycin, Clindamycin and Vancomycin against environmental isolates of Methicillin Resistant *Staphylococcus aureus* which was isolated from door handles (fomites) of secondary schools in Abeokuta environs [20].

2. Material and Methods

2.1. Collection and Maintenance of Test Organisms

A total five consisting of environmental isolates of Methicillin Resistant *Staphylococcus aureus* which were obtained from fomites (door handles) in different community schools within Abeokuta environs [20] was used for this research purpose. Isolation, identification and verification of MRSA were carried out at Microbiology Laboratory of College of Veterinary School (COLVET), FUNAAB; the isolates were maintained on nutrient agar slants at 4°C until required for use.

2.2. Collection and Preparation of Plant Materials

Moringa oleifera, Hunteria umbellata and *Azadirachta indica* parts- leaves, roots bark and stem bark were collected in FUNAAB farmland and was authenticated by a botanist in the Department of Forestry and Wildlife; Plant identification number were obtained from university herbarium and issued out for *Moringa oleifera*-UAHA No 019/001, *Azadirachta indica* – UAHA No 019/002, *Hunteria umbellata*- UAHA No 019/003.

The leaves were air dried at room temperature (29°C±1°C) until dryness. The root bark, stem bark, seed and leaves were washed with clean running tap water to remove soil. Plant materials were then pulverized separately using mortar & pestle to obtain powders for use during extraction [21]

2.3. Extraction

The extraction of plant parts was carried out according to the method suggested by Fatope *et al*., [22] with minor modification; sequential extractions were done using distilled water; 10g of each powdered plant part were soaked in 100ml of appropriate distilled water for 24h. This was allowed to stand for 7 days to permit full extraction of the active ingredient, after which the extracts were filtered off and concentrated using rotary evaporator at room temperature for 24 h at 100 rpm. A 2.0g/l solution of each extract was then prepared and fractionated into 0.4g/l,0.2g/l and 0.1g/l concentration needed for the bioassay.

2.4. Phytochemicals Screening of the Extracts

Phytochemicals are non-nutritive chemical present in plants that contribute to the medicinal properties of that plant, which have disease preventive properties [3, 23]. Phytochemicals screening of root, stem, seeds and leaves of *Moringa oleifera, Hunteria umbellata* and *Azadirachta indica* was carried out according to the methods of Ayoola *et al*., [24]. The extracts prepared were screened for glycosides, flavonoids, saponins, tannins, and phenols respectively.

2.4.1. Test for Flavonoids

In 0.5 ml of filtrate of each of the plant parts extracts, 5 ml of dilute ammonia was added, followed by addition of 1 ml of concentrated sulfuric acid to it. The presence of flavonoids was detected by yellow coloration of the solution that disappears on standing [25].

2.4.2. Test for Saponins

To 0.5 g of each plant extracts, 5 ml of distilled water was added in test tubes. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3–4 drops of olive oil and again shaken vigorously after that it was observed for the formation of emulsions.

2.4.3. Test for Tannins

About 0.5 g of each plant extracts were boiled in 10 ml of water in each test-tube and then filtered. Then few drops of 0.1% ferric chloride were added and observed for brownish green or a blue black color formation, which indicate the presence of tannins [26].

2.4.4. Test for Phenols (Ferric Chloride Test)

Small quantities of alcoholic and aqueous extracts were dissolved in 2 ml of distilled water separately, and into it, few drops of 10% aqueous ferric chloride solution were added. A blue or green color was produced which indicating the presences of phenols.

2.5. Antibiotics and Preparation of Stock Solution

Antimicrobials were purchased from their respective manufacturers, such as Azithromycin and Vancomycin from Ciron drugs & pharmaceutical PVT, Ltd (N-118/119, M. I. D. C, Tarapur, Bolsar; India) and whereas Clindamycin were purchased from Arco Life sciences PVT, Ltd (C-86, M. I. D. C., Hingna, Nagpur-16, India). The stock solutions from the dry powders were prepared at a concentration of 5120 mg/L for the antibiotics according to Clinical and Laboratory Standards Institute (CLSI) recommendations [27-29]. They were filtered for sterilization and stored frozen at -40 C for up to six months.

2.6. Antibacterial Assay Using Diffusion Method

This was done using the diffusion method as described by Kirby-Bauer *et al*., [30]. Mueller Hinton agar medium was poured into sterile Petri dishes and allowed to solidify. Ten (10) ml of 0.5 McFarland standards of test organisms was poured on the solidified agar and was spread all over the surface of the agar. Discs of 6mm in diameter were punched out using a cork borer and placed in a sterile glass Petri dish, and then sterilized in an oven at 40°C for about 30 minutes after which they were allowed to cool.

One (1.0) ml of the diluted extracts concentration (*M. oleifera, H. umbellata* and *A. indica*) and antibiotics were then inoculated into each well. The plates were incubated for 24 hours at 37°C. The zone of clearance was measured using a meter rule. Controls were set up with wells soaked with different solvents namely; 100% Extracts, 50% Extracts, 25% Extracts, 100% Extracts + Antibiotics, 50% Extracts + Antibiotics, 25% Extracts + Antibiotics, 100% Antibiotics, Distilled Water with no plant extract.

2.7. Determination of Minimum Bacterial Concentration of the Extracts

The concentrations of the extract that showed no visible growth after incubation were inoculated on Mueller-Hinton agar plates and incubated at 37°C for 24 hours. The lowest concentration of the extracts that showed no growth on the plate after 24 hours of incubation was taken as the Minimum bactericidal concentration [31-33].

2.8. Evaluation of Synergy

Minimum Bactericidal Concentration of *Moringa oleifera, Hunteria umbellata* and *Azadirachta indica* (aqueous) such as leaves, root, stem bark extracts alone or in combination was determined by Microdilution technique in accordance to CLSI standards [28].

2.9. Statistical Analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS) version 17.0 for Windows (SPSS, Chicago IL, and U.S.A). The mean Standard Deviation (SD), Standard Error (SE), median and ranges was calculated for continuous variables whereas proportions and frequency tables were used to summarize categorical variables. The levels of significance were considered, data obtained were inputted using SPSS (version 17.0). Significant differences between means ($p < 0.05$) were separated using Duncan multiple range test.

3. Results

3.1. Phytochemicals Screening of Plant Extracts

The qualitative phytochemical screening of crude extracts of plant contained alkaloids and Saponins, while all except *M. oleifera* also possess phenols. Steroids were present in *M. oleifera* but absent in *A. indica* and *H. umbellata*. However only four types of secondary metabolites (glycosides, Alkaloids, flavonoids and saponins) were identified in almost all the plant extracts as shown in Table 1; suggesting their implication in the exerted antibacterial activity. Mean values and standard deviations for the quantitative analysis of the plant extracts is shown in Table 2, generally Saponins had the highest value in the plant extracts.

Table 1. *Qualitative Phytochemical analysis of Hunteria umbellate, Moringa oleifera and Azadirachta indica various part.*

S/N	PLANT EXTRACTS	Alkaloids	Flavonoids	Glycosides	Phenols	Saponins	Steroids	Tannins
1	*Azadirachta indica (leaves)*	+	+	+	+	+	-	+
2	*Azadirachta indica (root part)*	+	+	+	+	+	-	+
3	*Azadirachta indica (stem bark)*	+	+	+	+	+	-	+
4	*Hunteria umbellate (leaves)*	+	-	+	+	++	-	+
5	*Hunteria umbellate (seed)*	+	+	+	++	++	-	-
6	*Hunteria umbellate (stem bark)*	++	+	+	+	++	-	+
7	*Moringa oleifera (stem bark)*	+	+	+	-	+	+	+
8	*Moringa oleifera (leaves)*	+	+	-	-	+	+	-
9	*Moringa oleifera (root)*	+	+	-	-	+	+	+

KEYS: + = constituent is present, ++ = constituent present in copious amount, - = constituent absent

Table 2. *Mean value of the Quantitative Phytochemical analysis of H. umbellate, M. oleifera and A. indica various part.*

S/N	Plant Extracts	*Hunteria umbellate (seed)*	*Hunteria umbellate (stem bark)*	*Hunteria umbellate (leaves)*	*Moringa oleifera (leaves)*	*Moringa oleifera (stem bark*	*Moringa oleifera (root)*	*Azadirachta indica (leaves)*	*Azadirachta indica (root)*	*Azadirachta indica (stem bark)*
1	Alkaloids	0.013±0.005	1.01±0.004	0.08±0.0017	0.143±0.0003	0.103±0.002	0.01±0.003	0.05±0.0002	0.007±0.0005	0.302±0.003
2	Flavonoids	0.008±0.0007	0.036±0.001	0.00±0.00	0.030±0.0001	0.056±0.007	0.021±0.005	0.073±0.003	0.02±0.004	0.325±0.0007
3	Glycosides	0.003±0.001	0.046±0.0002	0.084±0.002	0.00±0.00	0.063±0.007	0.00±0.00	0.074±0.0007	0.004±0.0008	0.008±0.0005
4	Phenols	0.302±0.003	0.023±0.00013	0.074±0.004	0.00±0.00	0.00±0.00	0.00±0.00	0.094±0.005	0.025±0.0001	0.152±0.0002
5	Saponins	1.023±0.016	1.24±0.021	1.08±0.012	0.142±0.002	0.17±0.006	0.013±0.007	0.076±0.0001	0.018±0.0002	0.082±0.0069
6	Steroids	0.00±0.00	0.00±0.00	0.00±0.00	0.0138±0.0008	0.012±0.005	0.011±0.003	0.00±0.00	0.00±0.00	0.00±0.00
7	Tannins	0.00±0.00	0.159±0.0007	0.04±0.0001	0.00±0.00	0.15±0.0003	0.010±0.001	0.03±0.0008	0.152±0.0002	0.03±0.0001

3.2. Antibacterial Activity of Aqueous Extracts of Plant Parts Alone, Antibiotics Alone and Combination Using Agar Well Diffusion Method

Tables 3-6 shows the inhibition pattern of MRSA isolates to various aqueous plant extracts alone, antibiotics alone and combination of both. The agar diffusion assay showed that *H. Umbellata* had the lowest inhibition (mm) on all the tested MRSA isolates. The combination of the aqueous plant extracts and the three antibiotics shows different zones of inhibition pattern; *H. umbellata* extract-Azithromycin combination had zones of inhibition ≥21.00±1.92 mm in about 75% MRSA tested, while *H. umbellata* extract-clindamycin had ≥ 21.20±2.58 mm (80%), *H. umbellata* extract-vancomycin combination had ≥ 22.80±1.77 mmin about 75% MRSA tested; *A. indica* extract-Azithromycin combination had ≥ 22.38±1.87 mm (100%), *A. indica* extract-Clindamycin combination had zones of inhibition ≥ 22.88±1.98 mm in about 100% MRSA tested, *A. indica* extract-Vancomycin combination had ≥ 22.38±1.63 mm (100%), *M. oleifera* extract-Azithromycin combination had ≥ 22.20±2.27 mm (100%), *M. oleifera* extract-Clindamycin combination had ≥ 21.20±1.16 mm (95%), *M. oleifera* extract-Vancomycin combination had ≥ 21.20±2.43 mm (100%).

The inhibition produced by the antibacterial combinations, however, varied in size and were mostly wider than those obtained from either the extracts or the antibiotics (Table 3). *A. indica* extract-vancomycin combination exhibited highest antibacterial activity with the highest zones of inhibition sizes, followed by *M. oleifera* extract-azithromycin combination, while other combinations exhibited various degree of antibiotics activity, based on the percentage of the number of bacteria with zones of inhibition equal to or greater than 20 ± 1.0 mm, the effect of combining the extract with the different antibiotics showed varying levels of inhibition.

Table 3. *Antibacterial activity of aqueous extracts of plant parts and antibiotics using agar well diffusion method.*

Zone of inhibition in (mm) for different MRSA species							
Extracts	**Concentration**	**MRSA1**	**MRSA2**	**MRSA3**	**MRSA4**	**MRSA5**	**Control mrsa**
Azadirachta indica (leaves)	50%	16.63±3.74	14±2.13	14.94±3.72	10±1.79	13.21±3.2	11.13±2.7
Azadirachta indica (root part)	50%	15.70±2.47	14.21±1.98	14.11±1.78	13.45±2.07	11.89±1.50	11.02±2.24
Azadirachta indica (stem bark)	50%	19.03±2.62	19.39±2.0	17.45±3.2	15.26±1.79	15.38±0.9	14.88±2.0
Hunteria umbellate (leaves)	50%	10±1.3	7.6±0.5	0.0±0.0	0.80±0.30	7.50±1.0	10.05±1.7
Hunteria umbellate (seed)	50%	6.30±1.87	4.8±1.6	0.0±0.0	1.50±0.70	6.40±0.60	5.30±1.80
Hunteria umbellate (stem bark)	50%	14.25±1.9	8.6±0.6	0.0±0.0	1.70±0.40	9.70±1.20	7.50±0.70
Moringa oleifera (stem bark)	25%	18.32±1.91	17.20±2.60	17.50±1.70	16.34±0.98	15.21±1.5	14.54±1.70
Moringa oleifera (leaves)	25%	15.08±0.5	14.20±0.60	13.68±1.9	13.75±2.30	12.97±0.60	12.53±0.97
Moringa oleifera (root)	25%	19.04±1.32	18.02±1.98	18.05±0.66	17.25±1.99	17.01±0.68	16.50±1.07
Azithromycin	25mg	22.35±1.70	20.47±1.98	23±2.08	24.43±0.98	27.07±0.66	25.78±0.75
Clindamycin	12.5mg	19.35±0.70	17.47±1.90	17.24±1.08	20.21±0.95	20.19±1.60	22.95±0.75
Vancomycin	25mg	20.32±1.00	18.46±1.00	19.20±1.08	20.78±0.96	21.10±1.62	21.95±1.00

Values presented are Mean ±S.E in mm.

S.E= Standard Error, MRSA = Methicillin Resistant *Staphylococcus aureus,* Control organisms: MRSA (ATCC 33591)

Table 4. *Mean Zone of Inhibition of combination of aqueous extract of Hunteria umbellata and antibiotics using agar diffusion.*

EXTRACTS	ANTIBIOTICS	MRSA1	MRSA2	MRSA3	MRSA4	MRSA5	CONTROL ORGANISM
Zone of inhibition in (mm) for different MRSA species							
Hunteria Stem bark50%	Azithromycin	3.20±2.18	0.00±0.00	0.00±0.00	8.00±4.93	20.00±1.05	5.10±1.86
	Clindamycin	5.00±1.61	0.80±0.80	0.00±0.00	14.60±3.90	17.80±0.73	4.00±0.80
	Vancomycin	2.00±2.00	0.00±0.00	0.00±0.00	4.80±4.80	18.20±0.734	4.00±0.56
Hunteria Seed50%	Azithromycin	5.00±2.24	1.24±0.84	0.00±0.00	15.00±1.14	16.80±1.16	4.80±2.06
	Clindamycin	5.00±2.14	0.40±0.40	0.00±0.00	16.20±4.65	14.80±2.08	3.00±1.89
	Vancomycin	6.60±0.93	1.60±0.98	0.00±0.00	19.20±2.29	14.20±1.24	5.00±2.32
Hunteria Leaves 50%	Azithromycin	5.60±2.31	0.00±0.00	0.00±0.00	21.00±1.92	17.80±1.53	7.60±2.25
	Clindamycin	3.80±1.71	0.00±0.00	0.00±0.00	21.20±2.58	15.60±1.86	6.60±2.71
	Vancomycin	6.20±1.85	0.60±0.60	0.00±0.00	22.80±1.77	17.00±0.55	9.20±2.59

Values presented are Mean ±S. E in mm.
S. E= Standard Error, MRSA = Methicillin and oxacillin Resistant *Staphylococcus aureus,* Control organisms: MRSA (ATCC 33591)

Table 5. *Mean Zone of Inhibition of combination of aqueous extract of Azadirachta indica and antibiotics using Agar well diffusion Method.*

EXTRACTS	ANTIBIOTICS	MRSA1	MRSA2	MRSA3	MRSA4	MRSA5	CONTROL ORGANISMS
Zone of inhibition in (mm) for different MRSA species							
Neem Stem Bark 50%	Azithromycin	17.25±3.72	16.63±4.36	15.88±3.03	15.38±3.58	14.94±2.13	16.00±3.18
	Clindamycin	16.13±3.44	16.38±3.09	14.38±1.79	16.63±2.97	15.13±2.79	15.25±2.27
	Vancomycin	15.70±3.39	14.00±2.85	15.63±2.38	14.00±2.15	14.63±1.88	13.38±1.78
Neem Root 50%	Azithromycin	15.87±2.47	17.00±4.64	13.36±1.96	15.38±3.55	15.38±2.29	14.25±2.84
	Clindamycin	16.50±3.36	14.38±2.48	15.19±2.15	13.50±1.91	14.38±2.09	22.88±1.98
	Vancomycin	17.40±3.23	21.50±1.50	22.38±1.87	22.38±1.63	15.44±2.21	13.38±1.78
Neem Leaves 50%	Azithromycin	15.44±2.49	22.38±1.87	16.13±2.41	23.63±1.98	13.88±1.95	15.00±2.06
	Clindamycin	10.38±2.69	14.38±2.15	14.00±2.08	18.80±2.60	14.50±2.29	13.88±2.62
	Vancomycin	19.38±3.26	15.25±2.88	15.38±2.82	14.88±2.57	13.69±2.02	14.25±2.47

Values presented are Mean ±S. E in mm
SE= Standard Error, MRSA = Methicillin and oxacillin Resistant *Staphylococcus aureus,* Control organisms: MRSA (ATCC 33591)

Table 6. *Mean Zone of Inhibition of combination of aqueous extract of Moringa oleifera and antibiotics using Agar diffusion method.*

EXTRACTS	ANTIBIOTICS	MRSA1	MRSA2	MRSA3	MRSA4	MRSA5	CONTROL ORGANISMS
Zone of inhibition in (mm) for different MRSA species							
Moringa Stem 25%	Azithromycin	18.40 ±1.63	15.34 ±0.67	19.80 ±3.02	10.20 ±1.66	22.20 ±2.27	12.80 ±1.07
	Clindamycin	18.00±0.89	14.10±1.29	21.20±1.16	15.00 ±1.30	9.80 ±2.08	9.80 ±0.66
	Vancomycin	19.80±0.86	14.50±0.92	21.20±2.43	14.20±1.83	15.20±1.93	10.60±1.91
Moringa Leaves 25%	Azithromycin	11.00±1.08	19.60±0.74	11.60±0.93	8.80±1.59	19.40±2.60	14.00±0.60
	Clindamycin	10.40±0.68	19.00±1.79	11.00±1.09	10.00±0.87	20.80±1.66	16.00±0.73
	Vancomycin	9.60±0.51	12.48±0.80	22.00±2.07	11.60±1.29	8.80±0.97	19.20±1.82
Moringa Root 25%	Azithromycin	9.40±0.51	11.38±1.11	20.40±2.57	10.00±1.34	7.20±0.97	17.20±1.15
	Clindamycin	9.20±0.66	0.00±0.00	17.80±2.08	9.20±0.37	21.20±1.76	11.20±0.68.
	Vancomycin	7.60±1.96	10.60±1.03	18.60±0.93	9.60±0.51	19.20±0.97	9.50±0.60

Values presented are Mean ±S. E in mm.
S. E= Standard Error, MRSA = Methicillin and oxacillin Resistant *Staphylococcus aureus,* Control organisms: MRSA (ATCC 33591)

Figure 1. *Microbial plate showing zones of inhibition of the plants extracts and antibiotics at known concentration.*

KEYS: 1- 100% Extracts, 2- 50% Extracts, 3- 25% Extracts, 4- 100% Extracts + Antibiotics, 5- 50% Extracts + Antibiotics, 6- 25% Extracts + Antibiotics, 7- 100% Antibiotics, 8- Distilled Water

4. Discussion

In this study, synergism effect resulting from the combination of antibiotic agents with crude plant extracts was verified for all plant extracts (*Moringa oleifera*, *Hunteria umbellata* and *Azadirachta indica*). The results show synergistic effects with significant reduction in the MIC of the antibiotics due to combination of different antimicrobial agents with different crude plant extracts against MRSA strains [34-39]. In general, the zones of inhibition in antibiotic/plant extract plates were in the range of 20 ± 1.0 mm wider than the zones of inhibition in the control plates (MRSA: ATCC 33591) and environmental isolates of MRSA [18] depending on the tested bacteria. Enlargement of inhibition zone over 5 mm was considered significant.

The better synergistic capacity showed Azithromycin and vancomycin was more than Clindamycin. Distinguishing synergistic from antagonistic interactions is of major importance for the development of improved strategies for the management of microbial infections. Screening for such activities in crude extracts is the first step in identifying leads for isolation of such compounds and some plants have provided good indications of these potentials for use in combination with antimicrobial therapy. Further separation and purification of the crude extracts might show an increase in bioactivity than the crude extracts.

This may be due to numerous compounds within the crude extracts that may have interfered with the actions of one another. Once they were separated by various purification methods however, the inhibiting effect of one on the other had reduced significantly [40]. *In-vitro* combination of plant extracts with different antibiotics investigated against MRSA in several studies [35-36; 39, 41-42] showed that there are varied interactions between plant extracts and antibiotics.

In agreement with these studies, our study demonstrated synergism, additivity/indifference and antagonism between aqueous extract of *Moringa oleifera*, *Hunteria umbellata* and *Azadirachta indica* and different classes of glycopeptides and macrolide antibiotics.

The synergistic effects indicated that the antibacterial combinations were more effective than the activity of the individual agents. The increase in the sizes of the zones of inhibition resulting from the plant extracts and antibiotic combinations indicated the improved bactericidal and inhibitory potentials of the extract and the antibiotics as combined antibacterial agents. These variations was adjudged by Pei *et al*., [43] to have resulted from differences in mechanisms of action and variation of combined antibacterial action in the tested bacteria.

Although the extracts combined with antibiotics, having different target sites, exhibit varied degree of antibacterial activity, the ability of these antibacterial combinations to inhibit resistant bacteria showed that they have either attacked different target sites singly or combined to overcome inherent resistant mechanisms in the isolates. One of the phytoconstituents, such as flavonoids or the phenols, may have interacted with the antibiotics to enhance its mechanisms of action at the target sites for which the antibiotic was designed.

Since Cushnie and Lamb, [44] indicated synergy between flavonoids and chemotherapeutics and Sato *et al*., [45] reported antimicrobial resistance modulating potentials of flavonoids and phenols, combining the aqueous extract with the antibiotics could have altered the inherent resistant properties in the bacteria to be more effective [45-47]. In addition, the initial role of each phytoconstituent in antimicrobial activity and in their combination with antibiotics may not be underestimated.

While Zhao *et al*., [48] reported that some phytochemicals can improve the *in-vitro* activity of some peptidoglycan inhibiting antibiotics by attacking the same site in the cell wall, Esinome *et al*., [37] showed that polyphenols couple with β-lactams could enhance antibacterial activity to disrupt transpeptidation of the cell membrane. While these may account for the varied degree of antibacterial activities of the different combinations, it could account for the differences in the rate at which the antibiotics get to their target sites.

5. Conclusion

The combinations of antibacterial agents demonstrating *in-vitro* synergism against infectious agents are most likely to be a means of achieving pragmatic and effective treatment for bacterial infection especially in patients with infections difficult to treat. Since the development of new classes of antibacterial agents is of paramount importance.

The crude aqueous extract of *Moringa oleifera*, *Hunteria umbellata* and *Azadirachta indica* showed potential synergy on being combined with some antibiotics against resistant bacteria of clinical importance and suggested that varied degree of effective therapy will be more attained with the antibacterial combinations.

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