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Anti-microbial Activities of Selected Ghanaian Medicinal Plants and Four Structurally Similar Anti-protozoan Compounds against Susceptible and Multi-drug Resistant Bacteria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors ANA, KBAO, MAB, NBW, F. Ayertey, LA, JA, TT, GID, SKB, F. Azerigyik, AA, NHT, TU, AAA, SI, YS, NO, BE and MO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ANA, KBAO, MAB, NBW and MO managed the analyses of the study. Authors ANA, KBAO, MAB, NBW, NHT, TU, YS, BE and MO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Antibacterial resistance is one of the fast rising health concerns globally. WHO emphasized the need for development of new drugs to combat antimicrobial resistance. Our group previously found several anti-protozoan compounds: ML-2-3, Molucidin and ML-F52 from a Ghanaian medicinal plant *Morinda lucida* and oregonin from a Japanese medicinal plant Alnus japonica, which share a similar aromatic ring structure. In this study, we investigated the antimicrobial activities of our compounds and some selected Ghanaian medicinal plants` extracts (n= 92) against five (5) Gram-negative (*Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 33495), *Shigella flexneri* (ATCC 12022), *Proteus mirabilis* (ATTC 35659)), two (2) Gram-positive bacteria, (*Staphylococcus epidermidis* (ATCC 12228) and *Staphylococcus aureus* (ATCC 29213)) and 28 Methicillin Resistant *Staphylococcus aureus* (MRSA) strains isolated from carriage and clinical infection in Ghana, in an in vitro colorimetric based assay. IC_{50}, Minimum inhibition concentration (MIC) and Minimum bactericidal concentration (MBC) were determined with ampicillin and ciprofloxacin as reference antibiotics. Oregonin had activity against both Gram-positives and negatives, while the remaining three compounds had activity only against Gram-positive bacteria. 12 out of 92 plant extracts tested showed significant activity against the standard bacteria strains. Oregonin was the most active compound against all 28 isolates of MRSA with a least MIC of 100 µM and a least MBC of 400 µM; 19 isolates had IC_{50} < 100 µM.

Keywords: Antibiotic resistance; MRSA; MIC; MBC; IC_{50}; oregonin; Molucidin.

1. INTRODUCTION

Antimicrobial resistance has occurred for every major class of antimicrobial agent [1]. The increasing occurrence of microbial resistance against clinical available drugs has made it imperative to discover effective and safe antibiotics in an era where emergence and spread of drug resistance bacteria is a major health problem across the world. The cost of antibiotic resistant bacteria to human health relates to the increasing number of nosocomial infections from opportunistic pathogens, increasing severity of infections and treatment failures [2]. This global crisis reflects the abuse of drugs worldwide and lack of development of new antibiotic agents by pharmaceutical companies to address the challenge. In order to help curb the problem of resistance, there should be a control on the availability, ease of use, and general low cost of antibiotics [3].

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major public health concern due to its resistance to a wide range of anti-microbial agents frequently used in clinical medicine. Information concerning its carriage and antimicrobial resistant patterns in Ghana and on the African continent is however limited due to the lack of adequate infrastructures for MRSA surveillance and control in this geographical setting [4,5]. In a recent Ghanaian study, a total of 30 MRSA strains isolated between 2011 to 2013 from carriage and clinical infection were investigated. Isolates were resistant to tetracycline (67%), norfloxacin (40%), moxifloxacin (37%), erythromycin (37%), clindamycin (33%), gentamicin (30%), kanamycin (30%) and ceftaroline (20%).

There have been reports on the emphasis of medicinal plants worldwide. Despite the major role of medicinal plants for the treatment of infectious diseases in Africa, scientific evidence of the medicinal properties of these plants have not been fully evaluated. The first part of this study therefore was to screen selected Ghanaian medicinal plants against 5 Gram-negative and 2 Gram-positive bacteria, *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 33495), *Shigella flexneri* (ATCC 12022), *Proteus mirabilis* (ATTC 35659), *Staphylococcus epidermidis* (ATCC 12228) and *Staphylococcus aureus* (ATCC 29213). We further focused on one of the most popular medicinal plants, *Morinda lucida* Benth. (Rubiaceae), an evergreen medium-sized tree with dark-shiny leaves on the upper surface, widely distributed in the whole African continent. *M. lucida* is known to be rich in anthraquinones like oruwacin, oruwal, 3-hydroxyanthraquinone-2-carboxyaldehyde, 1,3-dihydroxy-2 methylanthraquinone, 1,3-dihydroxyanthraquinone-2-carboxyaldehyde, and many others and used among traditional healers to treat fever, dysentery, abdominal colic, and intestinal worm infestation. Our group previously identified three novel tetracyclic irridoid compounds; Molucidin, ML-2-3 and ML-F52 (Fig. 1), from *M. lucida* leaves and found their

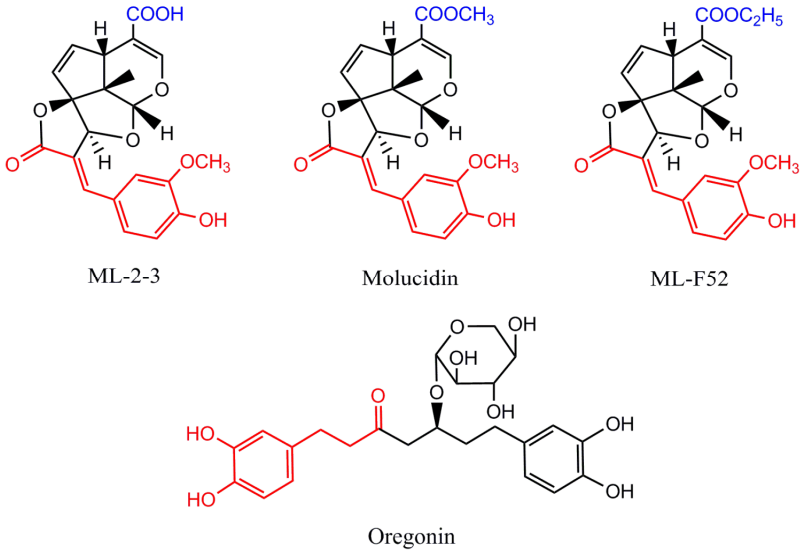

Fig. 1. Chemical structures of Molucidin, ML-2-3 and ML-F52 from *Morinda lucida* and Oregonin from *Alnus japonica*. Regions of similarity are shown in red

anti-trypanosoma, anti-leishmania and anti-malaria activities *in vitro* and *in vivo* [6,7]. The structural similarity of these compounds with oregonin purified from *Alnus japonica*, which possessed anti-inflammatory and anti-trypanosome activities has been reported [8-10]. Structure-activity relationship analysis revealed that they shared an aryl propanone moiety as well as similar aromatic rings as a part of the molecule (Fig. 1) [10]. Owing to the reports of anti-protozoan compounds having anti-bacterial activity [11], second part of this study was to determine the anti-bacterial activity of those compounds against both standard strains of Gram-negative and Gram-positive bacteria including MRSA strains isolated in Ghana.

2. MATERIALS AND METHODS

2.1 Plant Materials and Preparation of Crude Extracts

Based on the traditional knowledge of their medicinal use, extracts from different plant parts (leaves, stem bark, fruits, seeds or roots) of 73 plants were collected in Ghana by the Centre for Plant Medicine Research (CPMR), Mampong, Ghana during the period of October, 2010 to November, 2012. Authentication was done by one of the authors (Y.S.). Voucher specimens have been deposited in CPMR. The air dried and pulverized plant samples (200g) were extracted by 50% aqueous EtOH (2L) 3 times under room temperature. The accumulated solution was evaporated in vacuum at 40℃ to give the crude extract. The extracts were kept in sterile tubes and stored at 4°C until use. Prior to the antimicrobial assays, 10 mg/ml of stock concentrations of extracts were prepared with 50% EtOH and filter-sterilized. ML-2-3, Molucidin and ML-F52 used for this study was isolated form the leave of *M. lucida* as previously described [10]. Oregonin was isolated form the bark of *A. japonica* as previously described [10].

2.2 *In vitro* Antimicrobial Assay

Seven different standard bacteria, 5 Gram-negative, *Eschericha coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 33495) *Shigella flexneri* (ATCC12022) and *Proteus mirabilis* (ATTC 35659) and 2 Gram-positive, *Staphylococcus aureus* (ATCC29213) and *Staphylococcus epidermidis* (ATCC 12228), as well as 28 different MRSA isolates from Ghana [12] were used in this study. Each stocked standard bacteria species/strain was incubated overnight at 37ºC on a Mueller-Hinton agar (Park Scientific Limited) plate, while the stocked MRSA clones were plated on a Blood agar plate at 37ºC overnight just before the antimicrobial assay. Three individual colonies from the bacteria plate were selected, transferred into media and incubated at 37ºC overnight, for the bacteria to reach the log phase of growth. The log phase bacteria were diluted with sterile saline to achieve a turbidity of 0.5 McFarland standard, an approximate concentration of 2 x 10^8 CFU/ml.

The bacteria were then diluted to the working concentration, which varied between bacteria.

Log phase of bacteria at a concentration range of 1 $x10^2$ to $1x10^6$ CFU/ml were incubated with different concentrations of extracts (400 µg/ml-0µg/ml), compounds (400 µM-0 µM) and 10% Alamar Blue® reagent at 37ºC for 6-8 hrs. Absorbance was read at 540 nm, reference 595 nm, using a spectrophotometer (TECAN Sunrise Wako). IC_{50} values of compounds were calculated by the plot of a growth curve. Ampicillin and Ciprofloxacin were used as positive controls.

2.3 Determination of MIC and MBC

In the determination of the bactericidal and bacteriostatic properties of the extracts and compounds, bacteria cells were seeded with different concentrations of extracts and compounds and 10 % Alamar Blue® as described above. The reducing power of cells which converts the Alamar Blue component resazurin to the pink resorufin was used to determine the Minimum Inhibitory Concentration (MIC) of both extracts and compounds. The least concentration of compounds with no observable colour change was noted as the MIC. In the determination of the Minimum Bactericidal Concentration (MBC), all concentrations of compounds where there was no observable colour change were streaked on a Mueller-Hinton agar plate and incubated at 37ºC overnight. The least concentration of compounds with no bacteria growth was noted as the MBC.

3. RESULTS AND DISCUSSION

3.1 Screening of Crude Extracts against Standard Bacteria Strains

The emergence of resistant strains of bacteria against current available drugs poses a great risk to humanity; this necessitates the continuous search of alternate drugs to combat the threat of bacterial infections. One aim of the study was to evaluate the antibacterial effect of crude plant extracts against the seven standard strains of bacteria. To determine the activity of extracts against the different bacteria strains, the 5 Gram-negative and 2 Gram-positive bacteria were challenged with different concentrations of 92 crude extracts from selected Ghanaian medicinal plants with the concentrations ranging from 0 to 400 µg/ml. The ability of the extracts to inhibit bacterial growth were tested based on their bacteriostatic (IC_{50} and MIC) and bactericidal (MBC) properties.

As shown in Table 1, Out of 92 crude extracts tested, 75 extracts (82%) showed some antibacterial activities with IC_{50} values less than 100 µg/ml. Among them, 33 extracts (36%) showed significant activity with IC_{50} less than 20 µg/ml on some particular strains. Only one extract from *Mitra gynainermis* (leaves), was active against all the seven bacteria tested. Among the bacteria we tested, *Escherichia coli* was the most susceptible bacteria with 22 extracts (24%) having high activity (IC_{50}< 20 µg/ml) while the Gram-positive bacteria were highly susceptible to a total of only 3 extracts.

MIC values were determined qualitatively by the change in colour of the dye. Concentrations of the extract that inhibited bacteria growth were marked by the retention of the blue colour of the dye. The least concentration amongst these for each extract was recorded as the MIC. Stem bark of *Parkia lappertoniana* (SB) showed the strongest activity against *Pseudomonas aeruginosa*, with IC_{50} of 1.21 µg/ml, while further testing for MIC and MBC showed moderate activities with values of 400 µg/ml and > 400 µg/ml, respectively. Stem/bark extract *of Cinnamomum zeylanicum* was active against *S. aureus* with 200 µg/ml of MIC and 400 µg/ml of MBC. Leaves extract of *Terminalia ivorensis* was the most active against *S. flexneri* with 50 µg/ml MIC and 200 µg/ml MBC. Stem/bark extract of *Anogeissus schimperi* was also very active against *P. mirabilis* with a bactericidal activity at 200 µg/ml. The MICs and MBCs of extracts against both *S. epidermidis* and *K. pneumoniae* were not recorded due to high MIC concentrations above highest limit of concentration range tested.

Comparatively, the extracts showed very good activity against the Gram-negative bacteria than the Gram-positive ones which is contraindicative to the general observation of Gram-positive bacteria being relatively more susceptible [13]. Regardless of which part of the plant the extract was obtained, *Anogeissus schimperi* was still potent, especially against *P. mirabilis*. Others include *Mitragyna inermis*, *Parkia clappertoniana*, *Cinnamomum zeylanicum*, and *Terminalia ivorensis* (Table 2).

3.2 Activity of Compounds against Standard Bacteria Strains

This study also screened 4 compounds previously described to have anti-protozoan activity for their anti-bacterial activity. The

compounds were Molucidin, ML-2-3 and ML-F52, tetracyclic irridoid compounds isolated from *Morinda lucida*, and Oregonin which was isolated from *Alnus japonica*. The four compounds did not only share the anti-protozoan activity but also shared an aryl propanone moiety as well as similar aromatic rings. In order to determine the activity of the compounds; Molucidin, ML-2-3, ML-F52 and Oregonin, against the different bacterial species, 5 Gram-negative and 2 Gram-positive standard strains of bacteria were challenged with different concentrations of each compound. Each compound was also analyzed for its bacteriostatic (IC_{50} and MIC) properties and bactericidal (MBC) properties. With respect to the Gram-negative bacteria, only Oregonin and ML-2-3 were observed to have bacteriostatic and bactericidal properties (Tables 3 and 4). Oregonin had activity against all the Gram-negative bacteria except P. aeruginosa, IC_{50} > 1000µM, (Table 3). ML-2-3 had activity against only *P. mirabilis*, IC_{50} of 253.1 µM. Oregonin had strongest activity against *E. coli*, IC_{50} value of 25.2 µM and the least against *S. flexneri* with IC_{50} value of 90.4 µM (Table 3). The least MIC of Oregonin against all the Gram-negative bacteria was 100 µM while the MBC of ML-2-3 against *P. mirabilis* was greater than the concentration range tested. Only Oregonin was observed to have bactericidal activity with an MBC of 200 µM against *P. mirabilis*. The MBC against the remaining Gram-negative bacteria were not in the concentration range tested (Table 4).

The activity of these compounds against the standard bacteria strains were however distinctively different; with the tetracyclic irridoid compounds showing activity against only the Gram-positive bacteria while Oregonin showed activity against both the Gram-positive and Gram-negative bacteria. The mode of anti-bacterial activity was also different in that while the three tetracyclic irridoid compounds showed bacteriostatic activity, Oregonin showed some bactericidal activity. There is however no distinction between the function of bacteriostatic and bactericidal agents *in vivo* except in the intensity of activity, in that although bacteriostatic agents result in bacteria death, they do not kill enough bacteria to be considered bactericidal [14]. In terms of anti-Gram-positive activity, there is no difference in clinical efficiency of bactericidal and bacteriostatic agents with bacteriostatic agents like chloramphenicol, clindamycin and linezolid successfully used to treat infections such as endocarditis, meningitis and osteomyelitis which were previously considered to be controlled with only bactericidal agents [14]. All compounds were observed to have varying degrees of bacteriostatic activity against the Gram-positive bacteria (Table 4). *S. aureus* was the most susceptible to the compounds with Oregonin as the most active compound. Oregonin also had the highest bactericidal activity against the two Gram-positive bacteria with MBC of 100 µM against both *S. aureus* and *S. epidermidis* (Table 4).

3.3 Activity of Compounds against MRSA

Although the emergence of MRSA remains a public health concern, information of the presence and diversity in Ghana, Africa, remained limited until a study by Egyir et al. in 2015 gave an overview of the presence and diversity of MRSA in Ghana. Although their study was performed in the Southern part of Ghana, their results provided an overview of the strains circulating the country as most of their samples were collected from the main referral hospital, Korle bu Teaching hospital, in Ghana.

The low prevalence but high diversity of MRSA lineages in Ghana relative to developed countries prompted the screening of the narrow spectrum tetracyclic irridoid compounds and the broad spectrum Oregonin against 28 of the MRSA isolates from the Egyir et al. study in 2015. In the determination of bacteriostatic and bactericidal activity of the compounds against MRSA isolated from Ghana, 28 field isolates were challenged with varying concentrations of Molucidin, ML-2-3, ML-F52 and Oregonin. Of the four compounds, Oregonin had activity against most field isolates, 17 out of 28, followed by ML-2-3, 3 out of 28, and then ML-F52 and Molucidin, both with 2 out of 28, (Table 5). Out of the 28 field isolates challenged, only isolate 4812, originating from a wound infection, was susceptible to all the compounds (Table 5). Although the compounds, especially Oregonin, had bacteriostatic efficacy at 400µM or less for majority of the isolates, only one isolate, WB, recorded bactericidal activity at an Oregonin concentration of 400 µM. The MBC of all the compounds for the remaining bacteria were not in the concentration range tested (Table 6). Although all four compounds had activity against some of the MRSA isolates, Oregonin had the most activity, 17 out 28 isolates. The activity of the compounds were however mainly bacteriostatic.

Table 1. IC_{50} values of active extracts

Extract	IC_{50} (µg/ml)						
	E. coli	***Shigella***	***Klebsiella***	***Pseudomonas***	***Proteus***	***S. aureus***	***S. epidermidis***
Moringa oleifera (L)	37.90	>100	20.10	>100	10.10	>100	>100
Cleistopholis patens (L)	14.90	23.70	44.70	>100	>100	>100	>100
Magnifera indica (SB)	14.90	17.30	57.20	>100	60.28	>100	>100
Ceiba pentandra (SB)	16.60	11.25	94.18	17.77	21.34	>100	37.10
Cola caricifolia (SB)	16.30	9.31	95.95	75.46	64.86	>100	35.40
Annona senegalensis (L)	18.02	11.42	>100	44.29	49.33	>100	21.20
Clausena anisata (R)	23.98	24.47	>100	17.83	36.05	30.02	22.70
Mitragyna inermis (L)	16.30	35.83	99.73	19.14	29.53	20.94	29.57
Mitragyna inermis (SB)	24.99	31.56	>100	16.42	53.40	36.98	33.39
Bridellia ferruginea (L)	12.10	>100	4.03	22.44	>100	>100	>100
Khaya grandifoliola (SB)	24.17	21.61	5.46	7.40	85.09	>100	>100
Baphia nitida (SB)	>100	>100	9.22	>100	>100	>100	>100
Heliotropium indicum (WP)	4.89	41.02	>100	48.18	>100	>100	>100
Gossypium arboretum (L)	>100	>100	>100	>100	>100	>100	16.50
Cymbopogon citrates (WP)	>100	42.70	66.79	44.23	17.72	52.08	>100
Terminalia ivorensis (L)	>100	10.82	26.82	>100	26.96	39.97	>100
Terminalia ivorensis (SBL)	11.84	7.00	29.81	>100	9.68	26.56	>100
Cola acuminate (LSB)	7.85	27.12	43.56	>100	49.03	>100	>100
Parkia clappertoniana (SBL)	16.84	>100	>100	1.21	44.31	>100	>100
Ximenia americana (L)	5.59	20.80	88.70	>100	23.14	>100	>100
Anogeissus schimperi (L)	7.70	7.90	>100	>100	18.02	37.01	84.10
Anogeissus schimperi (R)	17.14	10.84	48.34	>100	15.14	>100	53.19
Anogeissus schimperi (SB)	11.18	>100	81.97	>100	10.29	28.07	>100
Piliostigma thonningii (SC)	11.40	>100	>100	>100	>100	>100	>100
Securidaca longepedunculata (L)	9.85	>100	70.31	>100	>100	>100	>100
Carapa procera (SB)	14.90	>100	>100	>100	>100	>100	>100
Piper guineense (S)	98.10	>100	>100	16.60	>100	27.98	44.20

Extract	IC_{50} (µg/ml)						
	E. coli	***Shigella***	***Klebsiella***	***Pseudomonas***	***Proteus***	***S. aureus***	***S. epidermidis***
Carica papaya (S)	5.35	>100	>100	>100	>100	>100	>100
Acacia nilotica (SB)	9.86	27.93	>100	>100	29.32	>100	>100
Cinnamomum zeylanicum (L)	97.70	>100	>100	>100	74.90	30.87	>100
Cinnamomum zeylanicum (SB)	14.90	85.47	>100	>100	76.04	16.85	95.55
Khaya senegalensis (SB)	>100	>100	>100	>100	24.72	14.53	>100
Tridaxs procumbent (WP)	13.40	>100	>100	>100	>100	>100	>100
Lippia multiflora (L)	33.40	57.20	85.81	>100	>100	>100	>100
Lippia multiflora (R)	30.30	98.40	>100	>100	>100	>100	>100
Lippia multiflora (SB)	74.09	>100	>100	>100	>100	>100	>100
Nauclea latifolia (L)	45.99	64.10	>100	>100	>100	>100	>100
Nauclea latifolia (R)	52.30	>100	>100	>100	>100	>100	>100
Nauclea latifolia (SB)	33.03	>100	>100	>100	>100	>100	>100
Thoningia sanguinea (WP)	97.20	>100	43.95	>100	>100	26.51	>100
Alstonia boonei (L)	52.53	>100	>100	>100	>100	>100	>100
Alstonia boonei (SB)	49.80	67.11	67.50	>100	>100	>100	>100
Magnifera indica (L)	45.95	34.80	54.98	>100	>100	>100	>100
Cleistopholis patens (SB)	21.67	22.54	98.90	>100	>100	>100	>100
Cassia siamea (SB)	31.59	24.14	>100	>100	>100	>100	>100
Psidium guajava (L)	>100	90.90	79.80	>100	>100	>100	>100
Spondias monbin (L)	26.70	24.6	>100	>100	>100	>100	>100
Citrus aurantifolia (F)	>100	24.09	>100	>100	>100	>100	>100
Citrus aurantifolia (L)	>100	29.77	>100	>100	34.85	>100	>100
Alchonia cordiflora (L)	>100	>100	40.12	>100	>100	40.13	44.00
Cassia sieberiana (R)	>100	>100	>100	>100	>100	>100	61.40
Garcinia kola (L)	>100	81.27	44.44	>100	>100	>100	>100
Garcinia kola (SB)	62.53	>100	33.44	75.35	21.50	94.26	>100
Pycnanthus angolensis (L)	>100	>100	42.12	>100	>100	>100	>100
Pycnanthus angolensis (LB)	>100	67.87	>100	>100	98.18	>100	>100
Picralima nitida (L)	>100	34.48	92.90	>100	38.09	>100	>100

Extract	IC_{50} (µg/ml)						
	E. coli	***Shigella***	***Klebsiella***	***Pseudomonas***	***Proteus***	***S. aureus***	***S. epidermidis***
Picralima nitida (SB)	>100	>100	50.53	>100	>100	>100	>100
Solanum torvum (L)	>100	49.68	53.72	>100	80.96	>100	>100
Solanum torvum (SB)	>100	>100	>100	>100	66.30	>100	>100
Anthocleista nobilis (L)	>100	30.33	>100	>100	71.30	>100	>100
Anthocleista nobilis (R)	>100	20.32	>100	>100	>100	>100	>100
Treculia africana (SB)	54.70	>100	39.08	>100	>100	>100	>100
Annona senegalensis (SC)	>100	>100	58.32	>100	>100	34.67	>100
Afzelia africana (SB)	>100	20.32	>100	54.51	>100	>100	>100
Parkia clappertoniana (L)	25.40	>100	32.73	>100	23.30	77.81	>100
Piliostigma thonningii (L)	>100	>100	80.00	>100	>100	>100	>100
Pseudocedreal kotschyi (SB)	56.82	58.80	>100	>100	99.88	>100	>100
Afaomomum melegueta (S)	21.40	>100	>100	>100	>100	28.64	>100
Piper guineense (L)	47.30	>100	>100	>100	>100	>100	>100
Zanthoxylum xanthoxyloides (L)	27.49	65.29	>100	>100	>100	>100	>100
Zanthoxylum xanthoxyloides (R)	>100	>100	>100	>100	>100	34.32	>100
Tabernaemontana crassa (R)	>100	>100	>100	>100	50.00	>100	>100
Tamarindus indica (L)	>100	>100	>100	>100	83.78	>100	>100
Tamarindus indica (SB)	>100	>100	>100	>100	>100	95.05	>100
Paullinia pinnata (R)	73.40	>100	30.23	>100	>100	>100	>100

***Plant parts are indicated as follows, L-Leaves, R-Roots, S-Stem, B-Bark, SC- Stem cutting, WP-Whole plant*

Table 2. IC_{50}, MIC and MBC values of extracts against bacteria

Bacteria	Extract	IC_{50} (µg/ml)	MIC (µg/ml)	MBC (µg/ml)	Ampicillin	
					IC_{50} (µg/ml)	MIC (µg/ml)
Escherichia coli	*Cola acuminate* (LSB)	7.85	100	>400	2.27	>17.47
	Ximenia Americana (L)	5.59	400	>40		
	Anogeissu sschimperi (L)	11.18	100	>400		
	Securidaca longipedunculata (L)	7.7	400	>400		
	Terminalia ivorensis (SBL)	11.84	200	>400		
	Acacia nilotica (SB)	9.86	400	>400		
	Anogeissus schimperi (R)	17.14	100	400		
	Cinnamomum zeylanicum (SB)	14.9	400	>400		
	Parkia clappertoniana (SBL)	16.84	400	>400		
	Anogeissus schimperi (SB)	11.18	400	>400		
	Annona senegalensis (L)	18.02	400	>400		
	Mitragyna inermis (L)	16.3	400	>400		
Pseudomonas aureginosa	*Mitragyna inermis* (L)	19.14	400	>400	2.87	17.47
	Parkia clappertoniana (SB)	1.21	400	>400		
Shigella flexneri	*Terminalia ivorensis* (L)	7.0	50	200	1.54	>8.74
	Anogeissus schimperi (R)	10.82	50	400		
	Terminalia ivorensis (SBL)	10.84	100	400		
Proteus mirabilis	*Terminalia ivorensis* (SBL)	9.68	100	400	0.16	>34.9
	Moringa oleifera (L)	10.1	50	>400		
	Anogeissus schimperi (SB)	10.29	100	200		
	Anogeissus schimperi (R)	15.14	100	400		
	Anogeissus schimperi (L)	18.02	100	>400		
Klebsiella pneumoniae		-	-	-	13.76	>139.76
Staphylococcus epidermidis	*Gossypium arboretum* (L)	16.5	100	>400	0.42	>34.9
Staphylococcus aureus	*Cinnamomum zeylanicum* (SB)	16.85	200	400	0.17	8.74

Table 3. IC_{50} (µM) of compounds against standard bacteria strains

Compounds	*E. coli*	*S. flexneri*	*K. pneumoniae*	*P. aeruginosa*	*P. mirabilis*	*S. epidermidis*	*S. aureus*
Molucidin	>1000	>1000	>1000	>1000	>1000	**195.9**	**33.4**
ML-2-3	>1000	>1000	>1000	>1000	**253.1**	**273.9**	**61.2**
ML-F52	>1000	>1000	>1000	>1000	>1000	**40.6**	**24.7**
Oregonin	**25.2**	**90.4**	**27.04**	>1000	**30.2**	**23.9**	**8.5**
Ampicillin	6.49	4.4	39.35	8.2	0.47	1.2	0.5

Table 4. MIC and MBC (µM) of compounds against standard bacteria strains

Compounds	*E. coli*		*S. flexneri*		*P. mirabilis*		*K. pneumonia*		*S. aureus*		*S. epidermidis*	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Molucidin	>400	-	>400	-	>400	-	>400	-	**200**	**400**	**200**	>400
ML-2-3	>400	-	>400	-	>400	-	>400	-	**200**	>400	>400	-
ML-F52	>400	-	>400	-	>400	-	>400	-	**400**	>400	**400**	>400
Oregonin	**100**	>400	**100**	>400	**100**	**200**	**100**	>400	**50**	**100**	**100**	**100**
Ampicillin	>100	-	>100	-	>100	-	>100	-	25	100	>100	-

Table 5. IC_{50} (µM) of compounds against MRSA isolates

MRSA isolates	Origin	Molucidin	ML-2-3	ML-F52	Oregonin	Ciprofloxacin
44	Unknown clinical infection	>1000	>1000	>1000	**113.68**	0.02
1321	Eye infection	541.62	**91.13**	**167.97**	**31**	0.02
4782	Eye infection	437.63	**97.68**	>1000	**81.92**	0.02
4812	Wound infection	**187.99**	**196.35**	**151.67**	**91.59**	0.02
SDFU 001/D1	Nasal swab	>1000	>1000	>1000	**302.19**	0.02
CHFU 101/48	Nasal swab	>1000	>1000	>1000	**329.92**	0.02
258	Wound	>1000	>1000	>1000	>1000	0.02
744	Blood	>1000	>1000	>1000	**113.24**	0.02
2224	Wound	>1000	>1000	>1000	**134.87**	0.02
B2244	Blood	>1000	>1000	>1000	**146.24**	0.02
DT	Wound	>1000	>1000	>1000	**50.85**	0.02
ME	Wound	>1000	>1000	>1000	**116.16**	0.02
81	Unknown clinical infection	>1000	>1000	>1000	711.43	0.02
3464	Blood	>1000	>1000	>1000	>1000	0.02
5038	Wound infection	>1000	>1000	>1000	>1000	0.02
8231	Blood	>1000	>1000	>1000	>1000	0.02
KG195	Nasal swab	>1000	>1000	>1000	>1000	0.02
KG272	Nasal swab	>1000	>1000	>1000	>1000	0.02
CHFU 156/D1	Nasal swab	>1000	>1000	>1000	**53.53**	0.02
SD112	Nasal swab	>1000	>1000	>1000	**69.33**	0.02
WB	Wound infection	**93.07**	>1000	>1000	**45.31**	0.02
2207	Wound	>1000	>1000	>1000	**62.42**	0.02
5016	Soft tissue infection	>1000	>1000	>1000	**226.78**	0.02
1834	Nasal swab	>1000	>1000	>1000	>1000	0.02
5039	Wound infection	>1000	>1000	>1000	**157.51**	0.02
11087	Urinary tract infection	>1000	>1000	>1000	>1000	0.02
SDFU 019/D1	Nasal swab	>1000	>1000	>1000	>1000	0.02
42189	Unknown clinical infection	>1000	>1000	>1000	>1000	0.02

Table 6. MIC and MBC (µM) of compounds against MRSA isolates

MRSA isolates	Molucidin		ML-2-3		ML-F52		Oregonin		Ciproflaxin	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC (nM)	MBC (nM)
44	>400	-	>400	-	>400	-	>400	-	15.63	15.63
1321	>400	-	**400**	>400	**400**	>400	**200**	>400	15.63	15.63
4782	>400	-	**400**	>400	**400**	>400	**100**	>400	31.25	31.25
4812	>400	-	>400	-	>400	-	**200**	>400	15.63	15.63
SDFU 001/D1	>400	-	>400	-	>400	-	**200**	>400	15.63	31.25
101/48	>400	-	>400	-	>400	-	**200**	>400	15.63	15.63
258	>400	-	>400	-	>400	-	>400	-	15.63	15.63
744	>400	-	>400	-	>400	-	**200**	>400	15.63	15.63
2224	>400	-	>400	-	>400	-	**400**	>400	15.625	15.625
B2244	>400	-	>400	-	>400	-	>400	-	15.625	15.625
DF	>400	-	>400	-	>400	-	**200**	>400	15.625	15.625
ME	>400	-	>400	-	>400	-	**200**	>400	15.625	15.625
81	>40	-	>400	-	>400	-	>400	-	15.625	15.625
3464	>400	-	>400	-	>400	-	>400	-	62.5	125
5038	>400	-	>400	-	>400	-	>400	-	125	250
8231	>400	-	>400	-	>400	-	**400**	>400	15.625	15.625
KG195	>400	-	>400	-	>400	-	>400	-	15.625	15.625
KG272	>400	-	>400	-	>400	-	>400	-	15.625	15.625
CH156	>400	-	>400	-	>400	-	>400	-	15.625	15.625
SD112	>400	-	>400	-	>400	-	>400	-	15.625	15.625
WB	>400	-	>400	-	>400	-	**200**	**400**	15.625	15.625
2207	>400	-	>400	-	>400	-	>400	-	15.625	15.625
5016	>400	-	>400	-	>400	-	**400**	>400	15.625	15.625
1834	>400	-	>400	-	>400	-	>400	-	15.625	15.625
5039	>400	-	>400	-	>400	-	>400	-	15.625	15.625
11087	>400	-	>400	-	>400	-	>400	-	125	500
SDFU 019/D1	>400	-	>400	-	>400	-	**400**	>400	15.625	15.625
42189	>400	-	>400	-	>400	-	>400	-	15.625	15.625

4. CONCLUSION

This study provides information on Ghanaian medicinal plants with anti-bacterial activity from which active compounds may be developed or may themselves be used for the development of alternate anti-bacterial drugs. Also this study showed the narrow spectrum of activity for three tetracyclic irridoid compounds and the broad spectrum of activity of Oregonin which can be used in the development of first line and second line treatment therapies, respectively. The study also provides information that may be used to develop chemotherapy that will be relatively more suited to the African sub region since screening was done on both standard strains and field isolates from Ghana.

CONSENT

All authors declare that written informed consent was obtained from the patients for publication of this paper.

ETHICAL APPROVAL

Archived Clinical isolates from previous study were used. Ethical approval was obtained from the University of Ghana Medical School Ethical and Protocol Review Board (Accra, Ghana) [reference no. MS-EI/M.9 – P.3.212010-11].

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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