Antimicrobial activity of *Moringa oleifera, Croton zambesicus* and *Ocimum gratissimum* Against Some Multidrug Resistant Bacterial Isolates

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Abstract
An antimicrobial study of extract from Moringa oleifera, Croton zambesicus and Ocimum gratissimum was carried out against some selected multi-drug resistant isolates in vitro. Water, ethanol and n-hexane were employed as the solvents for extraction process at a ratio of 8g (macerated plants) to 100mL (solvents). Staphylococcus spp. Klebsiella oxytoca, Klebsiella ornithinolytica, Escherichia coli, Pseudomonas aeruginosa, Enterobacter agglomerane, Budvicia aquatica, Staphylococcus aureus, Klebsiella terrigena, Escherichia hermamii, Burkhoderia cepacian, Citrobacter gilleric, Acinetobacter haemolyticus, Budvicia aquatic, Pseudo fluorescens, Enterobacter gergoriac, and coagulase -ve Staphylococcus sp. were isolated from urine samples from patients at different general hospitals at Ondo State, Nigeria. Antibacterial activity was carried out using agar diffusion method while agar dilution method was employed for determining the minimum inhibitory concentration (MIC) on Mueller-Hinton agar. The antibacterial activity of water, ethanol and n hexane leaf extracts of Moringa oleifera, Croton zambesicus and Ocimum gratissimum at concentrations of 100 mg/ml were analyzed on the selected MDR bacteria using agar disc diffusion method. The drug of choice for all isolates was ciprofloxacin based on its wide range of effectiveness. Water and ethanol extract for Ocimum gratissimum was observed to be the most effective in inhibiting the growth of multidrug resistant bacteria. In this study, Ocimum gratissimum was active against a great number of MDR bacteria (with an average of 9mm). Croton zambesicus extracted from water and ethanol was also active against most MDR isolates with an average of 7.5mm. However, all extracts from Moringa oleifera showed the lowest or no inhibitory value. Moringa oleifera water extract was only sensitive to Klebsiella ornithinolytica and Escherichia coli by 13mm and 12mm respectively while the n-hexane extract of Moringa oleifera was only active against Enterobacter agglomerane and Escherichia coli isolates by 13mm and 14mm; 11mm respectively. Zone of inhibition from Croton zambesicus for n-hexane showed

inhibitory effect to 10 MDR isolates. Findings from this study indicate that Ocimum gratissimum has a broad-spectrum antibacterial activity against all tested isolates and thus has a potential as a source of natural drugs. Furthermore, both Ocimum gratissimum and Croton zambesicus can be a potential source for the treatment of different infections caused by multiple drug resistant bacteria. However, in vivo studies are recommended.

Keywords: *Antimicrobial, Croton zambesicus, Medicinal plants, Moringa oleifera, Multidrug resistant bacteria, and Ocimum gratissimum*

INTRODUCTION

Medicinal plants have been given great attention recently because of its health impact to individuals and communities at large. Medicinal plants are embodied with bioactive substances capable of eliciting specific physiological actions in the body for an improved health system. Alkaloids, tannins, flavonoids and phenols are some of the bioactive compounds that medicinal plants possess. Medicinal plants-based drugs has gotten recognition because of its advantage as being simple, effective and capable of combating multidrug resistant with a broad spectrum activity (Le Jeune *et al.,* 2001, Gidey *et al.,* 2007 and Carlet *et al.,* 2012). This has turned researchers' attention towards the search for novel compounds from plants to tackle infections.

Moringa oleifera Lam (Moringaceae) (Somali *et al*., 1984, Morton, 1991 and Mughal *et al*., 1999) has gotten global attention and interest because of its numerous applications. The Moringa tree can be cultivated and used as vegetables, its roots can be used as spice, the seed can be used for cooking and cosmetic oil production and all the plants can be used as a medicine (Rebecca *et al*., 2006). It has an impressive range of medicinal uses with high nutritional value. Different parts of this plant contain different minerals and phytochemicals that are good source of protein, vitamins, β – carotene, amino acids and various phenols (Farooq *et al*., 2007). Cardiac related issues have been treated with this plant. Various parts of the plants possess anti-pyretic, anti-epileptic, anti-inflammatory, anti-ulcer and anti-tumor properties (Pal *et al*., 1995, Makonnen *et al.,* 1997). Other important medicinal properties of the plant include anti-spasmodic (Caceres *et al*., 1992), diuretic (Morton, 1991), anti-hypertensive (Dahot, 1988), cholesterol lowering (Mehta *et al*., 2003), anti-oxidant, anti-diabetic, hepatoprotective (Farooq *et al*., 2007), anti-bacterial and anti-fungal activities (Nickon *et al*., 2003; Farooq *et al*., 2007). In addition, *M. oleifera* seeds possess water purifying powers (Muyibi and Evison, 1995; Kawo, 2007) by flocculating Gram – positive and Gram – negative bacterial cells (Kawo, 2007*). M. oleifera* seeds can also be used as a less expensive bio-absorbent for the removal of heavy metals (Sharma *et al*. 2006).

Scent leaf, *Ocimum gratissimum*, is a perennial plant which is widely distributed in the tropics of Africa and Asia. It belongs to the family Labiatae and it as the most abundant of the genus Ocimum. In the southern part of Nigeria, it is called "Efirin nla" by the Yoruba speaking tribe. "Nichonwu" in Igbo while in the northern part of Nigeria, it is called "Daidoga" (Effraim *et al*; 2003). Leaf extract of *Ocimum gratissimum* and Xylopia aethiopiea were analyzed against five pathogenic organisms. *Staphylococcus aureus, Escherichia coli, Streptococcus faecalis, Pseudomonas aeruginosa* and *Lactobacilli* (Ijeh *et al*, 2004) which indicated that the aqueous

fractions of both plants have more potential as antimicrobial agents than their ethanolic fractions (Ijeh *et al*., 2004)

It is commonly used in folk medicine to treat different diseases of upper respiratory tract, diarrhea, headache, skin disease, pneumonia, fever and conjunctivitis (Correa, 1932). Recent studies on *Ocimum gratissimum* proved it to be a useful medication for people living with Human Immune Deficiency Virus (HIV), and acquired Immune Deficiency Syndrome AIDS (Elujoba, 2000).

Croton zambesicus (family Euphorbiaceae) is an ornamental plant grown in Nigeria and widely spread in tropical Africa. It is a large shrub or small tree up to 16 to 25 ft. high (Joseph *et al*, 2011). The leaves are green, firmly membraneous and penninerved. Flowering usually occurs at the beginning of dry season. The plant is commonly known as koriba in Hausa and Ajekobale in Yoruba (Ofusori *et al,* 2012). Traditionally, the plant is used in the treatment of urinary infection, malaria and dysentery (Reuben *et al.*, 2008). Ethno-botanically, the leaf decoction is used in Benin as antihypertensive and urinary infections and in parts of Niger Delta region of Nigeria the plant is use as anti-diabetic and malarial remedy while the Yorubas of western Nigeria use it traditionally for the treatment of Cancer. The roots are used as anti-malarial, febrifuge and antidiabetic by the Ibibios of Niger Delta region of Nigeria. The ethanolic leaf extract has been reported to possess anti-plasmodial antidiabetic and hypolipidemic (Ofusori *et al*, 2012), anti-inflammatory, analgesic and antipyretic activities while the root extract has been reported to possess anti-malarial anticonvulsant antiulcer, anti-inflammatory, analgesic and antipyretic, and kidney-protective activities. The aim of this present study therefore is to determine the antimicrobial effects of the medicinal plant leaf extract of *Moringa oleifera, Croton zambesicus* and *Ocimum gratissimum* on some multidrug resistant pathogenic organisms.

METHODOLOGY

Sample Collection and Study/ Sampling

Fresh leaves of *Moringa oleifera, Croton zambesicus* and *Ocimum gratissimum* were collected locally from Akungba, Ondo state, Nigeria. The leaves were identified by a botanist (Dr A. O. Obembe and the vouchers deposited) in the Department of Plant Biotechnology, Faculty of Science, Adekunle Ajasin University, Akungba, Ondo State. The laboratory analysis was carried out at Centre for Infectious Disease Control and Drug Development (CIDCDD), Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria.

Preparation and Processing of Plant

The fresh leaves were harvested, properly washed, rinsed with sterile distilled water and left to dry for two (2) weeks. The dried leaves were micronized using an electric blender. The powdered sample was extracted using water, ethanol and n-hexane. The method described by Sule *et al*., 2008 was adopted for phytochemical extraction. The leaves were soaked with the different solvents (ethanol, water and n hexane) and were left to stand for 1week; the solutions were mechanically shaken every day at intervals and then filtered using cotton wool.

Antibiotic Sensitivity Testing

The disk diffusion method was employed for the antimicrobial susceptibility test and Muller Hinton Agar (Oxoid, UK CM0337) was used as the medium to cultivate the bacteria, in accordance with the CLSI guidelines, (2016). This method was adopted because it is suitable for organism that grows rapidly over night at 35°c – 37°c. The antibiotic (specific concentration) impregnated disc absorbs moisture from the agar and antibiotic diffuses in to the agar medium. The rate of extraction of the antibiotic from the disc is greater than the rate of diffusion. As the distance from the disc increases there is a logarithmic reduction in the antibiotic concentration. Zone of inhibition of bacterial growth around each disc is measured and the susceptibility is determined.

A sterile swab stick was used to take inoculums from a solution of the test organism. The entire surface of the Mueller Hinton agar (MHA) plates were uniformly swabbed with the inoculums of test organisms and allowed to dry for 3-5 minutes. The plates were incubated over night at 37°C in an inverted position and the zones of inhibition were measured and interpreted using the standard recommendation of the Clinical Laboratory Standard Institute guidelines, 2016.

Preparation of wells

With the aid of a sterile Cork borer of 8 mm diameter, equidistant wells were bored into the seeded agar and the wells labeled accordingly after the plates had been allowed to dry. Using a micro pipette different concentrations of the extracts were dropped into each well which filled them respectively to fullness. Sterile distilled water was used as controls in each case. The plates were allowed to stand for one hour on the bench to allow for proper diffusion of extracts into the medium and then incubated uprightly at 37°C for 24 hours. Zones of inhibition were measured to nearest millimeter (mm).

Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentrations (MIC) were determined by standard agar dilution method (Lawal *et al*., 2018) in a series of doubling extract concentrations.

RESULT

Table 1, Shows the antibacterial susceptibility test of the water and ethanol extracts against the test organisms. From the results, the diameter of zone of inhibition among the test organisms by various extracts ranges from 9 – 24 mm.

Table 1: Water and Ethanol extracts of selected medicinal plants against clinical bacterial isolates

Lab. Labelling	Site labelling	Bacterial species (Isolates)	*Ocimum gratissimum* (Water extract)	*Ocimum gratissimum* (Ethanol extract)	*Croton zambesicus* (Water extract)	*Croton zambesicus* (Ethanol extract)	*Moringa oleifera* (water extract	*Moringa oleifera* (Ethanol extract
1	Ok 26	*Staphylococcus* spp.						
2	Tr 23	*Klebsiella oxytoca*	15	13	22	24	-	-
3	Tr 30	*Klebsiella oxytoca*					-	-
4	Ok 18	*Klebsiella ornithinolytica*	12	12	-	11	-	13
5	Owo 28	*Escherichia coli*					-	12
6	Ik 24	*Escherichia coli*	-	13	-	10	-	-
7	Ow 5	*Pseudomonas aeruginosa*					-	-
8	Ik 9	*Escherichia coli*					-	-
9	Ak 18	*Enterobacter agglomerans*	15	-	-	-	-	-
10	Tr 12	*Budvicia aquatica*	15	20	9	-	-	-
11	Ak 16	*Escherichia coli*	-	-	-	10	-	-
12	Ok I 5	*Staphylococcus aureus*	-	10	9	13	-	-
13	Ak 17	*Escherichia coli*	17	11	12	11	-	-
14	Tr 28	*Escherichia coli*	-	18	-	12	-	-
15	Tr 29	*Escherichia coli*					-	-
16	Tr 18	*Klebsiella oxytoca*	13	10	14	-	-	-
17	Ow 9	*Actinobacillus sp*					-	12
18	Ow 16	*Escherichia hermannii*	13	15	-	20	-	-
19	Ow 13	*Burkhoderia cepacia*	10	10	12	10	-	-
20	Ow 6	*Klebsiella terrigena*	-	-	-	-	-	-
21	Tr 11	*Citrobacter gillenii*	-	12	10	13	-	-
22	Tr 32	*Escherichia coli*	15	-	-	17	-	-
23	Ow 15	*Pseudomonas aeruginosa*	-	11	-	12	-	-
24	Tr 7	*Escherichia coli*	15	13	-	-	-	-
25	Ow 21 B	*Staphylococcus aureus*					-	-
26	Ik 14	*Escherichia coli*	-	-	-	11	-	-
27	Ok 10	*Acinetobacter haemolyticus*					-	-
28	Ik 13	*Klebsiella ornithinolytica*					-	-
29	Ik 27	*Budvicia aquatic*	19	13	19	10	-	-
30	Ow 22	*Pseudomonas fluorescens*					-	-
31	Ow 30	*Enterobacter gergoviae*	-	12	-	-	-	-
32	Ak 7	*Staphylococcus sp (coag –ve)*					-	-

Table 2: n-hexane extracts of selected medicinal plants against clinical isolates

Lab. Labelling	Site labelling	Bacterial species (Isolates)	*Croton zambesicus* (n-hexane extract)	*Moringa oleifera* (n-hexane extract
1	Ok 26	*Staphylococcus* spp.	-	-
2	Tr 23	*Klebsiella oxytoca*	-	-
3	Tr 30	*Klebsiella oxytoca*	-	-
4	Ok 18	*Klebsiella ornithinolytica*	22	-
5	Ow 28	*Escherichia coli*	-	-
6	Ik 24	*Escherichia coli*	-	-
7	Ow 5	*Pseudomonas aeruginosa*	-	-
8	Ik 9	*Escherichia coli*	28	-
9	Ak 18	*Enterobacter agglomerans*	10	13
10	Tr 12	*Budvicia aquatica*	26	-
11	Ak 16	*Escherichia coli*	10	-
12	Ok 5	*Staphylococcus aureus*	-	-
13	Ak 17	*Escherichia coli*	-	14
14	Tr 28	*Escherichia coli*	-	-
15	Tr 29	*Escherichia coli*	-	-
16	Tr 18	*Klebsiella oxytoca*	-	-
17	Ow 9	*Klebsiella terrigena*	-	-
18	Ow 16	*Escherichia hermannii*	16	-
19	Ow 13	*Burkhoderia cepacia*	25	-
20	Ow 6	*Klebsiella terrigena*	-	-
21	Tr 11	*Citrobacter gillenii*	-	-
22	Tr 32	*Escherichia coli*	10	-
23	Ow 15	*Pseudomonas aeruginosa*	-	-
24	Tr 7	*Escherichia coli*	-	-
25	Ow 21 B	*Staphylococcus aureus*	-	-
26	Ik 14	*Escherichia coli*	10	11
27	Ok 10	*Acinetobacter haemolyticus*	-	-
28	Ik 13	*Klebsiella ornithinolytica*	-	-
29	Ik 27	*Budvicia aquatic*	-	-
30	Ow 22	*Pseudo fluorescens*	-	-
31	Ow 30	*Enterobacter gergoviae*	20	-
32	Ak 7	*Staphylococcus sp (coag –ve)*	-	-

DISCUSSION

Recently, there are growing interests in ethno-medicine which might be as a result of the increase in demand for more potent drugs. Also there is a belief that plant-derived drugs and green medicine remains safe, dependable and cost effective than the synthetic drugs of which many have adverse side effects (Agbafor *et al*., 2011).

In this study there are several determinant factors of the antimicrobial susceptibility pattern of the plant extracts used. Some of which could be; environmental factors, choice of solvent, source of the organisms, biochemistry, physiology, metabolism and adaptation strategies of the microbes, plant species, biochemistry, age and parts, concentration of the plant extract and period of extraction.

In contrast to positive results gotten by Obi and Onuoha (2000) for *Moringa* plant, the water extract of *Moringa oleifera* showed no observed inhibitory effect to all MDR isolates as seen in Table 1. The resistance of all the test organisms to water extract of *Moringa oleifera* may be due to insufficient release of the volatile oil of *Moringa* during extraction. For *Moringa oleifera* ethanol extracts, *Escherichia coli* had the lowest inhibition zone of 12mm while *Enterobacter agglomerans* had the highest zone of 13mm.

N hexane extract of *Moringa oleifera* was only reactive to *Enterobacter agglomerans* (13mm), and two isolates of *Escherichia coli* (14mm and 11mm). This low sensitivity can be justified by Amadioha and Obi (1999), Okigbo and Ajale,(2005), Okigbo *et al*. (2005) whom have reported that inactivity of plant extracts may be due to age of plant and its physiology, solvent used for extraction, method of extraction and time of harvesting of plant materials.

On a brighter note, the leaves of *Ocimum gratissimum* exhibited a wide range of antibacterial activity which corroborates its ethno-botanical importance as therapeutics. 50% of the test organisms were susceptible to both water and ethanol extracts. However, strains of *E coli, Klebsiella oxytoca, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella terrigena, Citrobacter gillenii* and *Enterobacter gergoviae* were resistant to these extracts.

For both water and ethanol extracts for *Ocimum gratissimum*, strains from *Budvicia aquatica* was most susceptible with 20mm and 19 mm as zone of inhibition. For all test organisms, the highest zone of inhibition ranges from 10mm – 28 mm. The least susceptible of all test organisms to all extracts are with zone of inhibition ranging from 0mm - 9mm.

This research has shown that the water and ethanol extracts of the leaves of *O. gratissimum* possesses potential factor which confers measurable in-vitro antibacterial activity on the test organisms. This is because water and ethanol are better extractants of the volatile oil of the leaves of *O. gratissimum*. Orafidiya *et al*., (2000) who showed that the volatile oil of the leaves of *O. gratissimum* was active against entero-aggregative *E. coli* corroborates the results from this study. According to Sofowora (1982), thymol is one of the active component which impacts antibacterial activity on *O. gratissimum* and this compound has sufficient explanation for the antibacterial activity of *O. gratissimum*. Nakaruma *et al*., 1999 showed that Eugenol has both antibacterial and

anti-helminthic activities which could have impacted the antimicrobial property on *O. gratissimum* in a study carried out by Pessoa *et al*., 2002.

It was also observed that ethanol extract for *Ocimum gratissimum* was more sensitive than its water extract which further shows that the organic extracts (ethanol) is more active than the aqueous extracts. This may be due to the better solubility of the active components in organic solvents and their polarity (de Boer *et al*., 2005). It may also be that ethanol is a better extractant of the bioactive components of the leaves of *O. gratissimum* which speaks volume to its antimicrobial activity.

Ethanol extracts of *O. gratissimum* showed more antibacterial activity against *S. aureus* than *E. coli*. This result is in agreement to Agatemor (2009) where it was reported that gram negative bacteria are more resistant than gram positive bacteria to the essential oil which are antimicrobial agents. Nweze *et al*. (2004) reported phytochemical screening of *O. gratissimum* as having the presence of alkaloids, tannins, glycoside, saponin, resins, cardiac glycoside, steroidal terpenes and flavonoids.

Flavonoids are reported to exhibit antioxidant activity (Kumar and Pandey, 2013) and are effective scavengers of superoxide anions. Thus, this can significantly affect the cell wall of *S. aureus* which invariably may lead to the collapse of the cell wall and overall, affect the entire mechanism of the organism. *E. coli* a gram-negative organism contains a high level of lipid materials. These materials are thought to make a substantial contribution to the mechanism whereby injurious chemicals are prevented from reaching their sites of action within the cell. This result agrees to Obafemi *et al*. (2006) where sesquiterpene was tested against *S. aureus*. From this study, it was observed that ethanol extracts exhibited high inhibitory activity on the test organisms.

This can be deduced to the ability of ethanol to extract more of the essential oils and secondary plant metabolites which are believed to exert antibacterial activity on some of the test organisms. This study however can justify the use of these leaves in traditional medicine practice as a therapeutic agent and can explain the long history use of these plants. The results obtained from this study showed that the ethanolic extract of the plants inhibited the growth of the test isolates at varying concentrations. This is similar to the findings of Obi and Onuoha (2000), who reported alcohol to be best solvent for the extraction of most plant active principles of medical importance

In addition, N hexane extract of *Croton zambesicus* showed remarkable activity with zones of inhibitions 20mm, 22mm, 25mm, 26mm and 28mm against *Enterobacter gergoviae, Klebsiella ornithinolytica, Burkhoderia cepacia, Budvicia aquatica* and *Escherichia coli.* Its highest inhibitory activity, suggests that the active component of this plant may be a highly polar compound. This is similar to the findings of Ijeh *et al*. (2004), but in contrast to the report of Obi and Onuoha (2000), who reported alcohol to be the best solvent for the extraction of most plants' active principles of medical importance. The susceptibility of *E. coli* to extracts of fresh and dried leaf, confirms the antimicrobial activity reported by (Sofowora, 1982) using both fresh and dried leaf of the plant, but the none inhibitory activity of the dried leaf extract, suggest that active principle of the plant may be heat labile, and likely lost during drying. It is not unusual to observed antimicrobial activity of plant extract to have been contributed by solvents of extraction, but in

previous study solvents used to reconstitute extracts were observed to possess a decrease in antibacterial effect for some extracts.

CONCLUSION

The molecular principle behind antibacterial activities of these plants would need more screening. Molecular assessment for antibacterial activity of *Moringa* and its clinical implication needs to be explained. Since many plant antimicrobial property contain totally different purposeful elements in their structure, their antimicrobial activity can be linked to multiple mechanisms. Therefore, not like antibiotics, the potential for pathogens to develop resistance to plants is comparatively smaller. This fact if tested and utilized within the management of infections can be a springboard to avoid multiple bacteria drug resistance evoked by the recurrent use of antibiotics.

The ethanol, methanol and water extract of Ocimum gratissimum show activity against the test organisms. Staphylococcus aureus was more susceptible to the extract with mean zone of inhibition of 24.5±0.7mm and 22.5±2.1mm in 100mg/mL respectively. Escherichia coli 17.5±0.7mm, and Klebsiella spp 15±0.0mm, and no activity was recorded in water extract ofOcimum gratissimum against Pseudomonas aeruginosa in 25mg/mL. The result of antibacterial activity of ethanol, methanolic and water extract of Ocimum gratissimumon the test organismsrevealed decreased in mean zone of inhibition with decreased concentrations of the extract. As the concentrations decreases, the antibacterial activity decreases. This conforms with the work of Agholor et al., [28], who observed a decrease in antibacterial activity with decrease in concentration of the extract as the concentration decreases from 0.20-0.025mg/mL. The present study shows that ethanol extract of Ocimum gratissimum was a better extraction solvent than water and methanol. This also corroborate with the study conducted by Amjad, [29] who recorded a high mean zone of inhibition using ethanol extract of Ocimum gratissimum. A similar result was obtained by Adebolu and Oladineji [30] against Staphylococcusaureus and Escherichia coli.

The minimum inhibitory concentration of Ocimum gratissimum against the test organisms in ethanol, methanol and water extract ranges between 0.39-50mg/mL, 0.78-50mg/mL, and 12.5-50mg/mL respectively. The low minimum inhibitory

concentration values in the present study suggest the antibacterial efficacy of the plant. Staphylococcus aureus had the least MIC value of 0.39mg/mL followed by *Escherichia coli* andSalmonella spp with MIC values of 1.56mg/mL respectively. While Klebsiella spp, Proteus andPseudomonas aeruginosa had the highest MIC values of 50mg/mL respectively. This work supports the traditional use of the leaves of Ocimum gratissimum (Scent leaves) for the treatment of water related diseases.

The present study shows the effectiveness of the leaves extract ofOcimum gratissimum against the test organisms, suggesting that the plant can be used as an agent against bacterial infections. The antibacterial The ethanol, methanol and water extract of Ocimum gratissimum show activity against the test organisms. Staphylococcus aureus was more susceptible to the extract with mean zone of inhibition of 24.5±0.7mm and 22.5±2.1mm in 100mg/mL respectively. Escherichia coli 17.5±0.7mm, and Klebsiella spp 15±0.0mm, and no activity was recorded in water extract ofOcimum gratissimum against Pseudomonas aeruginosa in 25mg/mL. The result of antibacterial activity of ethanol, methanolic and water extract of Ocimum gratissimumon the test organismsrevealed decreased in mean zone of inhibition with decreased concentrations of the extract. As the concentrations decreases, the antibacterial activity decreases. This conforms with the work of Agholor et al., [28], who observed a decrease in antibacterial activity with decrease in concentration of the extract as the concentration decreases from 0.20-0.025mg/mL. The present study shows that ethanol extract of Ocimum gratissimum was a better extraction solvent than water and methanol. This also corroborate with the study conducted by Amjad, [29] who recorded a high mean zone of inhibition using ethanol extract of Ocimum gratissimum. A similar result was obtained by Adebolu and Oladineji [30] against Staphylococcusaureus and Escherichia coli.

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The present study shows the effectiveness of the leaves extract ofOcimum gratissimum against the test organisms, suggesting that the plant can be used as an agent against bacterial infections. The antibacterial The ethanol, methanol and water extract of Ocimum gratissimum show activity against the test organisms. Staphylococcus aureus was more susceptible to the extract with mean zone of inhibition of 24.5±0.7mm and 22.5±2.1mm in 100mg/mL respectively. Escherichia coli 17.5±0.7mm, and Klebsiella spp 15±0.0mm, and no activity was recorded in water extract ofOcimum gratissimum against Pseudomonas aeruginosa in 25mg/mL. The result of antibacterial activity of ethanol, methanolic and water extract of Ocimum gratissimumon the test organismsrevealed decreased in mean zone of inhibition with decreased concentrations of the extract. As the concentrations decreases, the antibacterial activity decreases. This conforms with the work of Agholor et al., [28], who observed a decrease in antibacterial activity with decrease in concentration of the extract as the concentration decreases from 0.20-0.025mg/mL. The present study shows that ethanol extract of Ocimum gratissimum was a better extraction solvent than water and methanol. This also corroborate with the study conducted by Amjad, [29] who recorded a high mean zone of inhibition using ethanol extract of Ocimum

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The fact that *Croton zambesicus* and *Ocimum gratissimum* extracts were active against both gram negative and gram-positive bacteria tested may indicate a broad spectrum of activity. This observation is very significant because of the possibility of developing therapeutic substances that will be active against multi-drug resistant organisms.

The observed antimicrobial effects of *Croton zambesicus* and *O. gratissimum* leaf on the bacterial isolates used, though in vitro appear interesting and promising. This implies that the plant extracts may indeed be effective in management of pneumonia, urinary tract infection, septicaemia, meningitis, diarrhea and other opportunistic infections, supporting its ethno-medicinal use; thus these plants may be presented as potential source of novel and broad spectrum antimicrobial drugs.

Further pharmacological evaluations, toxicological studies and possible mechanism of action processes are the future challenges that require further research.

The weak activities of the
ethanolic crude extract, chloroformic and n-hexane
fractions are understood and probably could have resulted
from antagonistic interactions of compounds from
different groups in them.
activity against these organisms. However, the
antibacterial activity of the root and that reported of the
stembark extract (Abo *et al*., 1999; Reuben *et al*., 2008)
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REFERENCES

Le Jeune J.T.; Besser T.E.; Hancock D.D., Cattle water troughs as reservoirs of *Escherichia coli* O157. *Appl. Environ. Microbiol*. 2001; 67.

Gidey M; Teklehaymanot T; Animut A; Mekonnen Y; Medicinal plants of the Shinasha, Agew and Amhara Peoples in the Northwest Ethiopia. *J. Ethnopharm*. 2007; 110:20-22.

Carlet J.; Jarlier V; Harbarth S; Voss A; Goossens H; Pittet D; Ready for a world without antibiotics? The pensieres antibiotic resistance calls to action. *Antimicrob. Resist Infect Control. 2012; 1: 11.*

Somali, M.A; Bajnedi, M.A; Al-Faimani, S.S: Chemical composition and characteristics of *M. peregrine* seeds and seed oil. *J. Am. Chem. Sco.* 1984; 61:85 – 86.

Morton, J. F: The horse radish tree: *M. pterigosperma* (Moringacea). A boon to arid lands. *Economic Botany*.1991; 45:318–322.

Mughal, M.H.; Ali, G; Srivasta, P.S; Iqbal, M; Improvement of drumstick (*M. pterygosperma* Gaertn) – a unique source of food and medicine through tissue culture. *Harmdad Med*. 1999; 42:37 –42.

Rebecca, H.S.U; Sharon, M; Arbainsyah, A; Lucienne, D; *Moringa oleifera*: medicinal and socio-economic uses. International Course on Economic Botany. National Herbarium Leiden, Netherlands.2006; Pp. 2 – 6.

Farooq, A; Sajid, L; Muhammad, A; Anwarul Hassan, G; *Moringa oleifera*: a food plant with multiple medicinal uses. *Phytotherapy Research*; 2007; 21:17 – 25.

Pal, S.K; Mukherjee, P.K; Saha, B.P; Studies on the antiulcer activity of M. oleifera leaf extract on gastric ulcer models in rats. *Phytother. Res*.1995; 9:463–465.

Makonnen, E.; Hunde, A; Damecha, G. Hypoglycaemic effect of *M. stenopetala* aqueous extract in rabbits. *Phytother. Res.* 1997; 11:147 – 148.

Caceres, A; Saravia, A.; Rizzo, S.; Zabala, L.; Leon, E.D; Nave, F.; Pharmacological properties of *Moringa oleifera* 2: Screening for antispasmodic, anti-inflammatory and diuretic activity. *J. Ethnopharmacol* 1992; 36:233 – 237.

Dahot, M.U: Vitamin contents of flowers and seeds of *M. oleifera*. *Pak. J. Biochem*. 1988; 21:1 – 24.

Mehta, L.K.; Balaraman, R.; Amin, A.H; Baffa, P.A; Gulati, O.D; Effects of fruits of *M. oleifera* on the lipid profile of normal and hypercholesterolaemic rabbits. *J. Ethnopharmacol.* 2003; 86:191 – 195.

Nickon, F; Saud, Z; Rehman, M.H; Haque, M.E; In vitro antimicrobial activity of the compound isolated from chloroform extract of *M. oleifera* Lam. *Pak. J. Biol. Sci.* 2003; 22:1888 – 1890

Muyibi,, S.A. & Evison, L.M: Optimizing physical parameters affecting coagulation of turbid water with *M. oleifera* seeds. *Water Res*.1995; 29:2689 – 2695.

Kawo, A.H. (2007): Water purification potentials and *in-vivo* toxicity evaluation of the aqueous and petroleum ether extracts of *Calotropis procera* (Ait.F) Ait.F. latex and *Moringa oleifera* Lam seed powder. PhD thesis, Microbiology Unit, Department of Biological Sciences, Bayero University, Kano. 184pp.

Sharma, P; Kumari, P; Srivastava, M. M; Srivastava, S: Removal of cadmium from aqueous system by shelled *M. oleifera* Lam. seed powder. *Bioresource Technology* 2006; 97:299 – 305.

Effraim, K.D; Jacks, T.W; Sopio, O.A; Histopathological Studies on the Toxicity of *Ocimum gratissimum* Leaves Extracts on Some Organs of Rabbits: *African Journal of Biomedical Research* 2003; 6: 21-25.

Ijeh, I.I; Njoku, O.U; Ekenza, E.C; Medicinal Evaluation of Xylopia aethiopica and *Ocimum gratissimum*. *Journal of Medicial Aromatis Science* 2004; 26(1): 44-4

Correa, M.P; Dicimario Das, Plants Utesis Brasil, IBDF Ministeroida Agricultura, Riode Janesio 1932; 63pp.

Elujoba, A.A. (2000). Studies on the antidiarrhoea activity of *Ocimum gratissium* guavu. University of Ife Press, Ife. 160pp.

Joseph L; George M; Kaur L; Abhi V.; An overview- *Croton zambesicus* Muell. *Int J Pharm Res Dev* 2011; 3(4):187-192.

Ofusori D.A; Komolafe O.A; Adewole O.S; Obuotor E.M; Fakunle J. B; Effect of ethanolic leaf extract of *Croton zambesicus* (Müll. Arg.) On lipid profile in streptozotocin-induced diabetic rats. *Diabetologia Croatia* 2012; 4(1/2):69-75.

Reuben K.D., Akan J..C, Usman H., Sodipo O.A., Egwu G.O; Phytochemical screening and in vitro antimicrobial investigation of the methanolic extract of *Croton zambesicus* Muell Arg. stem bark. *Eur J Sci Res* 2008; 23(1):134-140.

Clinical and Laboratory Standards Institute (CLSI, 2016) Performance Standards for Antimicrobial Susceptibility Testing. 26th ed. CLSI supplement M100S (ISBN 1-56238-923-8]; ISBN 1-56238-924-6. 2016. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087, USA.

Lawal T.O; Ogudo B.U; Oyedemi T.O; Bamidele T.A; Adeniyi B. A; Proximate composition and phytochemical constituents of leaves of some *Acalypha* species. *Pak. J. Nutr*. 2014; 8:256-258.

Dalziel, J.M: A Hausa Botanical Vocabulary. T. Fisher Unwin Ltd, London.1916; Pp. 5 – 167.

Sofowora, A. (1982). Medicinal plants and traditional medicine in Africa. John Wiley and Chichester Pp 71 - 92.

Agatemor, C. (2009). Antimicrobial activity of aqueous and ethanol extracts of nine Nigerian spices against four food borne bacteria. *Elec. J. Environ. Agric. Food Chem*. 8(3): 195-200.

Agbafor, K.N; Akubuguo, E.I; Ogbashi, M.E; Ajah, P.M; Ukwandu, C.C; Chemical and antimicrobial properties of leaf extracts of *Zapoteca portoriceusis*. *Res. J. Med. Plant*. 2001; 5: 605–612.

Obi V.I; Onuoha C; Extraction and Characterization Methods of Plants and Plant Products. In: Biological and Agricultural Techniques. Ogbulie, J.N. and O.J. Ojiako (eds). Websmedia Publications, Owerri 2000; pp. 271-286.

Amadioha A.C; Obi V.I; Control of anthracnose disease of cowpea by *Cymbopogon citratus* and *Ocimum gratissimum*. *Acta. Phytopathol. Entomol. Hungerica* 1999; 34 (1-2): 85-89

Okigbo R.N; & Ajale A.N; Inhibition of some human pathogens with the tropical plant extracts *Chromolineena odorata* and *Citrus aurantifolia* and some antibiotics. *Int. J. Mol. Med. Adu Sci* 2005; 1:34-40.

Okigbo R.N; Mbajaka C; Njoku C.O; Antimicrobial potential of (UDA) *Xylopia aethopica* and *Ocimum gratissimum* on some pathogens of man. *Int J. Mol. Med. Ad. Sci. Pakistan* 2005; 1 (4): 392-394.

Orafidiya, O.O; Eluyoba, A.A; Iwalewa, F. O; Okeke, I. N; Evaluation of antidiarrhoea properties of *Ocimum gratissimum* volatile oil and its activity against enteroaggregative *Escherichia coli*. *Pharm. Pharmacol. Lett* 2000; 10: 9-12.

de Boer, H.J., Kool, A., Broberg, A., Mziray, W.R., Hedberg, I. and Levenfors, J.J. (2005). Anti-fungal and anti-bacterial activity of some herbal remedies from Tanzania. *Journal of Ethnopharmacology* 96: 461–469.

Kumar S. and Pandey A.K.(2013). Chemistry and Biological activities of Flavonoids. *The Scientific World Journal*. Article ID162750. https:doi.org/10.1155/2013162750. Accessed on 26th March, 2022

Nakaruma, C.V., Nakaruma, T.U., Bando, E., Melo, A.E., Cortez, D.A. and Diaz-Filho, B.P. (1999). Antibacterial activity of *Ocimum gratissimum* L. essential oil. Mem. Inst. Oswardo Cruz. 94: 675 678.

Nweze E.I., Okafor J.I, Njoku O. (2004). Antimicrobial activities of Methanolic Extracts of Trema guineensis (Schumm and Thorn) and Morinda lucida Benth used in Nigerian Herbal Medicinal Practice. *J. Biol. Res. Biotechnol*. 2(1):39-46

Obafemi C.A., Sulaimon T.A., Akinpelu D.A. and Olugbade T.A. (2006). Antimicrobial activity of extracts and a germacranolide-type of sesquiterpene lactone from Tithonia diversifolia leaf extract. *Afri. J. Biotechnol*. (12): 1254-1258

Raheela, J., Pessoa, L.M., Morais, S.M., Bevilaqua, C.M.L. and Luciano, J.H.S. (2002). Antihelmintic activity of essesntial oil of *Ocimum gratissimum* Linn and Eugenol against Haemonchuscontotus. *Vet. Parasitol*. 109: 59 - 63..