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Antimicrobial and Synergistic Potentials of *Xylopia Aethiopica* (UDA) and *Occimum Gratissimum* (Nchanwu) Leaf Extracts

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Abstract:- Soxhlet ethanolic leaf extracts of *Xylopia aethiopica, Occimum gratissimum* and their combined extracts were analyzed for antimicrobial activities against clinical pathogenic bacteria (*Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Pseudomonas aeruginosa*) and a fungus (*Candida albicans*) using the agar well diffusion technique. The susceptibility with regards to their zones of inhibition, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) unraveled that the extracts exhibited antibacterial activities without effect on *Candida albicans.* Diameter zone of inhibition of the *Xylopia aethiopica* extract ranged from 15mm to 19mm without effect on *Escherichia coli*. *Occimum gratissimum* zone of inhibition ranged from 16mm to 34mm while the combined extracts zone of inhibition ranged from 19mm to 35mm. The MIC result revealed that both *X. aethiopica* and *O. gratissimum* extracts ranged from 200mg/ml to >250mg/ml. An improved MIC was observed on the activity of the combined extracts of *X. aethiopica* and *O. gratissimum* which ranged from 100mg/ml to 250mg/ml. The MBC ranged from 200mg/ml to >250mg/ml for the separate extracts of *X. aethiopica* and *O. gratissimum* but *X. aethiopica* exhibited bacteriostatic effect on *Escherichia coli*. Whereas the combined extracts had MBC of 100mg/ml and 200mg/ml on the test isolates. The combined extracts and the individual extracts could be described as antibacterial rather than antifungal. The combined extracts had synergistic effect on the test bacterial organisms and were more effective than the individual extract. The combined extracts can be incorporated into drugs for broad spectrum antibacterial activity.

Keyword:- *Antibacterial, Xylopia aethiopica, Ocimum gratissimum, isolates, synergy.*

I. INTRODUCTION

Antibiotics usage in the treatment of infections with microorganisms follows herbal medicine (Owoyale *et al.,* 2005) and so, herbal medicine cannot be neglected because it will continue to play an essential role in health care system especially in the remote areas where medical doctors are scarce (Saraf *et al.*, 2011; Sofowora, 1982). The antibacterial potentials of plant extracts have been attributed to the active phytochemical contents such as alkaloids, tannins, saponins, flavonoids, anthraquinone, steroids and glycosides (Edeoga & Eriata, 2001; Edeoga *et al.,* 2005; Emeh *et al.,* 2010; Emeh *et al.,* 2014; Okigbo *et al.,* 2005). These antimicrobial properties are of importance in therapeutic treatments, as a number of studies have been conducted in different countries to prove such efficiencies (Anyanwu & Okoye, 2017; Asekun & Adeniyi, 2004; Emeh et al., 2014; Fleischer et al., 2008; Nwachukwu and Osuji, 2008; Okigbo et al., 2005; Tatsadjieu *et al.,* 2003).

Xylopia aethiopica, an evergreen, aromatic tree, belonging to the Annonaceae family grows up to 20m high in rain forests. It is found in the moist fringe forests and lowland rainforest in the savanna zones of Africa (Erhirhie & Moke, 2014). *Xylopia aethiopica* is used extensively as spice in African cuisine and as herbal medicine. Ailments treated with *X. aethiopica* in traditional medicine include; helminthiasis, candidiasis, biliousness, cough, bronchitis, dysentery, boils, sores (Asekun & Adeniyi, 2004; Fall et al., 2003; Fleischer et al., 2008; Nwachukwu and Osuji, 2008; Okigbo et al., 2005; Tatsadjieu et al., 2003), stomach aches, rheumatism or used as a mouthwash to treat toothaches (Mshana et al., 2000; Okigbo et al., 2005). It is used as antiseptic and analgesic (Konnning *et al.,* 2004), anti-feedant activity on termite (Lajide *et al.,* 1995), insecticide (Adewoyin *et al.,* 2006; Ukeh *et al.,* 2012) and exhibits an antioxidant activity which defend and protect the body from adverse effects of irradiation (Asekun & Adeniyi, 2004).

On the other hand, Ocimum gratissimum L. commonly known as "Alpha or Nchanwu'' which belongs to the family of Lamiaceae is an important aromatic and medicinal plant that possesses water stress tolerance capacity. It is either cultivated or seen naturally wild in various tropical and subtropical regions of the world (Pandey, 2017). The plant contains essential oil with much chemical composition including; alcohols, aldehydes, ketones, ethers, esters, lactones, oxides, peroxides (Adesegun *et al.,* 2013). *Ocimum gratissimum* L. is naturally used in the treatment of different bacteria and fungi diseases such as upper respiratory tract infection, diarrhea, headache, fever, skin diseases and pneumonia (Joshi, 2013; Katara *et al.,* 2013; Matasyoh et al., 2008; Mbata & Saikia, 2007; Mith et al., 2016; Nguefack et al., 2007). Studies have shown that the phytochemical content of the plant are; tannins, flavonoids, saponins and anthroquinone (Alexander, 2016; Ijeh et al., 2005; Macdonald et al., 2010). In previous work documented by

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(Adebolu & Salau, 2005), pathogenic bacteria that cause diarrhea; *Staphylococcus aureus, Escherichia coli, Salmonella typhi and Salmonella typhimurium* were susceptible to the extracts of *Ocimum gratissimum.* (Nweze & Eze, 2009) stated that the regular consumption of *Ocimum gratissimum* as spice without affecting the activities of conventional antibiotics makes it a strong antimicrobial herbal product throughout the tropics.

Often times, a combination of two or more medicinal plant have achieved some interesting positive curation of ailments traditionally. This has necessitated and motivated this present study. Although, (Fall et al., 2003) and (Ogunkunle & Ladejobi, 2006) have documented that the combination of different parts of *X. aethiopica* or *X. aethiopica* with other plant types is used to achieve the desired effects, the necessity to challenge the conventional antibiotics resistant strains with the proper way of application of medicinal plants has not been exhausted. Furthermore, according to (Sofowora, 1982), the use of herbal medicine cannot be ignored in rural areas where medical doctors are scarce. The fact that research on synergy of medicinal plants is very limited and there is a tremendous pressure on these plants probing the proper way of application for most effective result. This study was therefore aimed at determining the antimicrobial properties of leaf extracts of *Xylopia aethiopica* and *Ocimum gratissimum* as well as evaluating the synergistic effect of the combined extracts on the test organisms.

II. MATERIALS AND METHODS

The fresh leaves of *Xylopia aethiopica* and *Occimum gratissimum* were purchased from Naze market, Owerri in the month of July, 2019. The plants were properly identified by Dr. C. M. Duru, a botanist of Federal University of Technology Owerri.

- *Preparation and Extraction of the Plant Materials*

The leaf samples were dried at room temperature and pulverized into powder using sterile manual blender. Soxhlet alcoholic extraction method as described by (Association of Analytical Chemist, 1980) was adopted for this study. 20g of each of the grinded leaf samples were transferred into different thimble and subjected to extraction in 200ml of 99% ethyl alcohol. The extracts were concentrated by evaporation using a rotary evaporator (Model type 349/1, Corning Limited). The separate concentrated extracts were labeled appropriately and stored at 4^{o}C in the refrigerator before use.

- *Preparation of Crude Extract*

1.5g of each of the plant concentrated extracts was mixed thoroughly in a dry sterile cork screw universal bottle to obtain 3g of the combined *Xylopia aethiopica* and *Occimum gratissimum* extracts and was properly labeled. 1g of each of the extracts was dissolved in 4ml of 30% dimethylsulphoxide (DMSO) to obtain concentrations of 250mg/ml in the test tubes. Then, 0.8g of each of the extracts was added as labeled respectively in 4ml of DMSO to obtain 200mg/ml concentration for each of the extracts. Subsequently, two fold serial dilutions of the three extracts (*Xylopia aethiopica*, *Occimum gratissimum*, and combined *Xylopia aethiopica* and *Occimum gratissimum*) were carried out from the 200mg/ml concentration by transferring 2ml of the 200mg/ml concentration to 2ml of DMSO and homogenized properly. Hence, the entire procedure provided concentrations of 250mg/ml, 200mg/ml, 100mg/ml and 50mg/ml of each of the extract (Akujobi *et al*., 2004). These were refrigerated at 15^{o}C until required.

- *The Test Microorganisms*

The test organisms were four clinical bacterial isolates (*Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Pseudomonas aeruginosa*) and a fungus (*Candida albicans*) collected in nutrient agar slants and Sabouraud dextrose agar slant respectively from the Microbiology department of the Federal Medical center, Owerri. They were then sub-cultured and purified appropriately on sterile Nutrient agar and Sabouraud dextrose agar plates and preserved in slants. The microbial pure cultures were identified using standard texts (Baker *et al*., 2001, Cheesbrough, 2006).

- *Determination of the Antimicrobial Potency of the Plant Extracts*

Each of the bacterial pure isolate was diluted with peptone water to obtain a suspension containing 2.0 x 10^{5}cells/ml. A loopfull of *Candida albicans* was emulsified in 5ml of sterile water. The agar well diffusion technique as described by (Osadebe & Ukwueze, 2004) was employed in this study to evaluate the antibacterial activities of the extracts comprising of *Xylopia aethiopica*, *Occimum gratissimum*, and combined *Xylopia aethiopica* and *Occimum gratissimum*. 15ml of sterile molten nutrient agar was poured into separate sterile petridishes and plates of Sabouraud dextrose agar (SDA) were also prepared and allowed to solidify. Using the spread plate method, 0.1ml of each of the bacterial test isolate suspension was aseptically inoculated on the nutrient agar plates while *Candida albicans* was inoculated onto the SDA plates. Six holes (agar wells) measuring 5.0 mm each in diameter were bored equidistant to each other in each of the inoculated plates using a standard sterile cork borer. One drop of molten agar was used to seal the base of each of the wells to prevent diffusion of the extract beneath the agar. Using a sterile Pasteur pipette, 0.2ml of each of the prepared extract was appropriately added to wells 1 to 4 of each inoculated plate representing the different test microorganisms. The 5th and 6th wells contain ciprofloxacin and dimethylsulphoxide (DMSO) acting as the positive and negative controls respectively while fluconazole was the positive control for *Candida albicans* (Emeh et al., 2010). The bacterial and fungal plates were allowed to stand for one hour in order to allow pre-diffusion of the extracts to take place and then incubated for 24hours and 48hours respectively. The developed diameters of zones of inhibition were measured in millimeter (mm) using a meter rule. The average of the four readings in each plate containing the specified extract

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was calculated and taken as the zone of inhibition of the extract on the particular test organism.

- *Minimum Inhibitory Concentrations (MIC) of the Plant Extracts*

The minimum inhibitory concentrations **(**MIC) of the extracts were determined using the micro-broth dilution technique (Emeh et al., 2010). Series of sterile tubes containing dilutions of the specified leaf extract were inoculated with 2.0 x 10^5cells/ml suspension of the test organisms and incubated for 24 hours at 37^{o}C. The lowest concentration of each of the extracts that inhibited the growth of the test organism was recorded as the minimum inhibitory concentration **(**MIC).

- *Minimum Bactericidal Concentrations (MBC)*

Tubes showing no visible growth from the minimum inhibitory concentrations (MIC) test were selected out carefully and a loopfull from each tube sub-cultured onto sterile nutrient agar plates and incubated at 37^{0}C for 24 hours. The lowest concentration of the extracts yielding no growth was recorded as the Minimum Bactericidal Concentration (Tilton & Howard, 1987).

III. RESULTS

The results of the antimicrobial activities of the extracts of *Xylopia aethiopica* and *O. gratissimum* revealed that the extracts of *X. aethiopica* exhibited zone of inhibition that ranged from 15mm to 19mm in diameter against the test organism with the exception of *Escherichia coli* and *Candida albicans*. *O. gratissimum* extract had diameter zone of inhibition on the bacterial test organisms ranging from 16mm to 34mm. All the bacterial test isolates were sensitive to the combined extracts ranging from 19mm to 35mm. There was no sensitivity recorded for *Candida albicans* (Table 1).

The results of the minimum inhibitory concentration of the *Xylopia aethiopica* and *Occimum gratissimum* on the bacterial test isolates unraveled that both extracts had MIC on the bacterial test organisms ranging from 200mg/ml to >250mg/ml. While that of the combined extracts ranged from 100mg/ml to 250mg/ml (Table 2). In table 3 the result of the minimum bactericidal activities of *Xylopia aethiopica* and *O. gratissimum* showed that *Xylopia aethiopica* extract had MBC effect of 250mg/dl on *S. aureus*, 200mg/ml on *Streptococcus pyogenes* and >250mg/ml minimum bactericidal concentration effect on *P. aeruginosa*, while it had bacteriostatic effect on *Escherichia coli*. On the other hand, leaf extract of *O. gratissimum* had 250mg/ml MBC on all the bacterial test organisms. While the combined extracts of *Xylopia aethiopica* and *Occimum gratissimum* exhibited MBC effect of 100mg/ml on gram positive *S. aureus* and *Streptococcus pyogenes* but had MBC effect of 200mg/ml on the gram negative *P. aeruginosa* and *E. coli*.

Test organisms	Leaf extracts			
	X	O	XO	C
	Mean zone diameter of inhibition (mm)			
Staphylococcus aureus	15	34	35	35
Streptococcus pyogenes	19	20	22	23
Escherichia coli,	NI	19	23	24
P. aeruginosa	18	16	19	25
Candida albicans	NI	NI	NI	38*

Table 1:- Antimicrobial activity of leaf extract of *Xylopia aethiopica* and *O. gratissimum.*

Key: mm = millimeter; NI = no inhibition; C = bacteria positive control (10mg/ml ciprofloxacin)
*C = *Candida albicans* positive control (fluconazole); X = *Xylopia aethiopica* leaf extract
O = *O. gratissimum* extract; XO = combined extracts of *X. aethiopica* and *O. gratissimum*

Test organisms	Minimum Inhibitory Concentration (mg/ml)		
	X	O	XO
Staphylococcus aureus	250	200	200
Streptococcus pyogenes	200	200	100
Escherichia coli,	>250	>250	250
Pseudomonas aeruginosa	>250	250	200

Table 2:- Minimum Inhibitory Concentration (MIC) of the leaf extracts of *Xylopia aethiopica* and *O. gratissimum.*

Key: mg/ml =milligram per millimeter; X = *X. aethiopica* leaf extract; O = *O. gratissimum* leaf extract; XO = combined extracts of *Xylopia aethiopica* and *Ocimum gratissimum*

Test organisms	Minimum Bactericidal Concentration (MBC) (mg/ml)		
	X	O	XO
S. aureus	250	250	200
Streptococcus spp	200	250	100
Escherichia coli,	BS	250	200
P. aeruginosa	>250	250	200

Table 3:- Minimum Bactericidal Concentration (MBC) of the extract of *O. gratissimum* and *X. aethiopica*

Key: mg/ml =milligram per millimeter; BS= bacteriostatic; X = *X. aethiopica* extract; O = *O. gratissimum* extract; XO = combined extracts of *X. aethiopica* and O. gratissimum;

IV. DISCUSSION

The results realized in this research study unraveled that the soxhlet ethanolic extracts of *X. aethiopica* and *O. gratissimum* had varying degrees of antibacterial potentials on the test bacterial isolates but not on the fungal test isolate, *Candida albicans.* Table 1 revealed that the extracts of *X. aethiopica* exhibited zone of inhibition ranging from 15mm to 19mm. Its activity showed zone of inhibition in decreasing order as *Streptococcus pyogenes* (19mm) was most inhibited (19mm), followed by *P. aeruginosa* (18mm) and *Staphylococcus aureus* (15mm) while it had no effect on *Escherichia coli* and *Candida albicans.* This result supports the previous work of (Tatsadjieu et al., 2003), (Asekun & Adeniyi, 2004) and (Okigbo et al., 2005) except that it failed to inhibit the growth of *Escherichia coli* which these authors reported that the extract inhibited in their study. In addition, the resistance of *Candida albicans* to *X. aethiopica* extract in this study contradicts their inclusion of *Candida albicans* as being susceptible to *X. aethiopica* extract. In addition, (Fleischer et al., 2008) documented that the antibacterial properties of the extracts of *X. aethiopica* on *Staphylococcus aureus* and *Pseudomonas aeruginosa* gave zone of inhibition of 24.17mm and 16.17mm respectively which is similar to the results of this study. This present study also agrees with the work of (Fleischer et al., 2008) as regarding antibacterial activity which recorded that *Escherichia coli* was resistant but disagrees on the susceptibility of *Candida albicans* to *X. aethiopica* extract in their work. This slight discrepancy can be attributed to the fact that the species used may be of different strains. The extracts of *Occimum gratissimum* had zone of inhibition ranging from 16mm to 34mm. *Staphylococcus aureus* was most inhibited while *P. aeruginosa* was least inhibited. *Staphylococcus aureus* have zone of inhibition of 34mm, and in decreasing order; *Streptococcus pyogenes* (20mm), *Escherichia coli* (19mm) and *P. aeruginosa* (16mm) but *Candida albicans* was resistant to the extract. In this study, both the gram positive and gram negative bacteria test organisms employed were all susceptible to the *O. gratissimum* leaf extract and this in line with the work of (Pandey, 2017) who documented same. Furthermore, in line with the report obtained in this study, *Escherichia coli and S. aureus* were susceptible to the extracts of *O. gratissimum* which concurs with the findings of (Adebolu & Salau, 2005) who reported that *Escherichia coli and S. aureus* as pathogenic bacteria that cause diarrhea were susceptible to the extracts of *O. gratissimum.*

The extracts of *O. gratissimum* exhibited higher antibacterial efficacy than the extract of *X. aethiopica* in this study. For instance, it was observed that *X. aethiopica* had no antibacterial effect on *Escherichia coli* whereas *O.*

gratissimum exhibited zone of inhibition of 19mm on the organism. It was remarkably obvious that the entire bacterial test isolates exhibited improved susceptibility to the combined extracts than to the separate extracts and exhibited diameter zone of inhibition ranging from 19mm to 35mm. Hence, the combined extracts could be said to have synergistic effect against the test organisms as a result of synergy of activity between the two extracts although *Candida albicans* was still resistant to it. The combined extracts as well as the individual extracts could be described as antibacterial rather than antifungal. This is desirable and suggests that both extracts together could be used to treat diseases caused by these microorganisms.

Table 2 indicated the results of the minimum inhibitory concentration of the *Xylopia aethiopica* and *Occimum gratissimum* which unraveled that both extracts had MIC ranging from 200mg/ml to >250mg/ml on the bacterial test organisms. While that of the combined extracts of *X. aethiopica* and *O. gratissimum* ranged from 100mg/ml to 250mg/ml. Though both extracts have the same range of MIC, the extracts of *Occimum gratissimum* exhibited greater antibacterial activity than *Xylopia aethiopica* extracts. The MIC of both extracts tallied on *Streptococcus pyogenes* (200mg/ml) and on *Escherichia coli* (>250mg/ml), but *Xylopia aethiopica* had varied MIC of 250mg/ml on *Staphylococcus aureus* while *Occimum gratissimum*'s leaf extract had 200mg/ml on the same organism. In the same vein, it was observed that *X. aethiopica* had MIC of >250mg/ml on *Pseudomonas aeruginosa* whereas *O. gratissimum*'s extract had on it 250mg/ml. Interestingly, an improved minimum inhibitory concentration (MIC) was observed on the activity of the combined extracts of *X. aethiopica* and *O. gratissimum* as this study recorded 100mg/ml on *Streptococcus pyogenes*, 200mg/ml on *Staphylococcus aureus* and *P. aeruginosa* and MIC of 250mg/ml on *Escherichia coli*. The minimum bactericidal concentration (MBC) ranged from 200mg/ml to >250mg/ml for *X. aethiopica* but had bacteriostatic effect on *Escherichia coli*. In the case of *O. gratissimum*, MBC of 250mg/ml was recorded on the entire bacterial test organism. Then, that of the combined extracts was 100mg/ml on gram positive *Streptococcus pyogenes* and *Staphylococcus aureus* while 200mg/ml MBC was observed on gram negative *P. aeruginosa* and *Escherichia coli*. This study confirmed the statement of (Fall et al., 2003) and (Ogunkunle & Ladejobi, 2006) who documented that the combination of *X. aethiopica* with other plant types is used to achieve the desired effects.

This research showed that the combined extracts of *X. aethiopica* and *O. gratissimum* were more effective on antibacterial basis than when applied separately. Therefore, the combined extracts of *X. aethiopica* and *O. gratissimum* will provide curative effect on diseases caused by any or combination of two or more of the bacterial test organisms used in this study.

V. CONCLUSION

This work has shown that the combined extracts were more effective than the individual efficacy of the extracts. This further strengthens the fact that the combination of the extracts exhibited synergistic activity against the test organisms used in this study. Conclusively, the extracts can be used to treat the disease caused by these test organisms.

RECOMMENDATION

- The combined extracts of *Xylopia aethiopica* and *Ocimum gratissimum* can be incorporated into drugs for more effective broad spectrum antibacterial activity which can be used to treat diseases or infections caused by the test organisms used in this study.
- Researchers in this field should be encouraged financially to enhance and expand the scope of work on plant remedies for various ailments caused by bacteria, fungi and even viruses.

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