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Antimicrobial Potentials of (UDA) *Xylopia aethopica and Occimum gratissimum L.* on Some Pathogens of Man

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Abstract: Antimicrobial effect of uda *(Xylopia aethopica)* and nchuawu (*Occimum gratissimum*) on some pathogens: *Proteus mirabilis, Esherichial coli, Staphylococcus aureus and Candida albicans* were carried out. Different solvents used for the extraction of the plant were water (hot and cold) and ethanol. The antibacterial effect of *Xylopia aethopica* of its aqueous (cold) and (hot) showed inhibition on *Proteus mirabilis, Staphylococcus aureus* and *Candida albicans* with MIC of 0.0625 on *Proteus mirabilis* of aqueous cold and 0.25 of aqueous hot, 0.0625 on *Staphylococcus aureus* of aqueous cold and 0.0625 on aqueous hot, 0.25 on *Candida albicans* of aqueous cold extract and 0.5 on aqueous (hot) extract and no inhibition on *Esherichial coli. Occimum gratissimum* showed inhibition on *Proteus mirabilis*, *Esherichial coli* and *Staphylococcus aureus* of it's ethanolic and aqueous extracts. *Proteus mirabilis* on ethanolic extract with the MIC of 0.5, *Esherichia.coli* with 0.125 and *Staphylococcus aureus* with 0.25. the aqueous cold extract on, *Proteus mirabilis* with MIC of 0.125 on *Esherichial coli* with 0.5 and on *Staphylococcus aureus* 0.25 while aqueous hot with *Proteus mirabilis* with 0.125, *Esherichial coli* with 0.25 and *Staphylococcus aureus* with 0.25 and no inhibition on *Candida albicans.* This justifies the therapeutic uses of *Occimum gratissiumum* and *Xylopia aethopica*. Further investigation can combine the plants extracts for possible synergetic effects.

Key words: Antimicrobial, *Xylopia aethopica, Occimum gratissimum,* human bacteria

INTRODUCTION

Many indigenous plants are widely spread in the tropical forest in which rare and useful herbs from which important drugs could be prepared and may also serve as starting material drugs[1]. Indigenous plants are reservoirs of various metabolites and provide a limitless source of important chemicals that have diverse biological properties. People of all continents have long applied poultices and imbibed infusions of indigenous plants dating back to prehistory for health purposes[2]. Some of these forest fruits and seeds have potent antifungal, antibacterial and antiviral properties[3].

Some commonly encountered pathogens have been associated with some of the human diseases. *Lactobacillus* is gram- positive, catalase negative, which causes tooth decay, producing carious lesions[4]. *Candida albicans*, which is a common commensals of the gastrointestinal and urogenital tracts of human[4,5] is the cause of candiasis in woman[6]. Also, *Escherichia coli* a gram negative usually motile is an extremely versatile opportunistic pathogen[5] causes septice mias and can infect the gall bladder, meningers, surgical wound, skin lesions and the lungs especially in debilitate and immunodefiecient patients[4]. *Proteus mirabilis* which is also Gram-negative motile rod, causes urinary track infections in the elderly and young males often following catheterization or cystoscopy, wound infection also as often a secondary invader of ulcers, pressure sores, burns and damaged tissues and septicemia and occasionally meningitis and chest infections[5]. *Staphylococcus aureus* is a facultatively anaerobic, gram-positive[5] which causes food poisoning and usually grows on the nasal membranes and skin. It is also found in the gastrointestinal and urinary tracts of warm-blooded animals[5]. Also causes boils, abscesses, wound infection, Pneumonia, toxic shock syndrome and other disease[5].

Most tropical forest plants are used in treating tropical diseases. *Occimum gratissimum L.* (*Labiatae*) is widely distributed in tropical and warm temperature regions. The plant is commonly used in folk medicine to treat different diseases, e.g. upper respiratory tract infections, diarrhea, headache, ophthalmaic, skin disease,

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pneumonia and also as a treatment for cough, fever and conjunctivitis[7]. Previous studies showed that the essential oils (EO) of four ocimum species grown in Rwanda. *i.e. O. canum, O. gratissimum, O. trichodon* and *O. urticifolium,* display antimicrobial activity[8]. It has been reported that the volatile oil of this plant contains mostly phenols particularly thymol[9,10] and that these are probably responsible for its reported antimicrobial action. The essential oil of *Ocimum gratissimum* has antimicrobial effect on proteus, *Escherichia coli*, *Klebsiella, Salmonella*, *Staphylococcus* and *Shigella* species[8].

Xylopia aethiopica which is largely located in West Southern African has its medicinal uses as a carminative, as a cough remedy and a post partum tonic and also in stomachache, bronchitis, biliousness and dysentery and in lemon grass for female hygiene and high in copper, manganese and zinc[11,12]. Also in the preparation of soups that exhibit hot and spicy taste, which are consumed during cold season or added to food, meant for pregnant and nursing mothers as medicinal spices[13]. It is claimed that spices and herbs assist in the contraction of the uterus among the postnatal women[13]. This plant has been shown to be active as an antimicrobial against gram positive and negative bacteria such as *Staphylococcus aureus*, *Esherial coli*, *Proteus mirabilis* and demonstrated activity against *Candida albicanus*[16].

In light of the recent emergence of bacteria which are resistant to multiple antimicrobial drugs posing a challenge for the treatment of infections, the need to discover new antimicrobial substances for use in combating such micro-organism becomes pertinent. Resistant bacteria represents a challenge in the treatments of infections, which are well known, necessitated the need to find new substances with antimicrobial properties to be used in the combat to these micro-organisms.

Considering the importance of some herbs, which are commonly used as spices in flavouring food it became necessary to investigate the antimicrobial effect and drugs derived from these indigenous plants. As some microbial diseases remain intractable to orthodox drugs, this therefore, necessitated the need to examine some of these local medicinal plants for antimicrobial activities of some plant extracts on human pathogens or microbial organisms. In this work *Occimum gratissimum* L. and *Xylopia aethiopica* (Dunal) A. Rich were used on *Staphylococcus aureus*, *Esherichia coli*, *Proteus mirabilis* and *Candida albicans* as traditional antibiotics.

MATERIALS AND METHODS

Collection and Identification of Plant Materials: The leaves of Nchuawu (*Occimum gratissimum*) were collected from the premises of Michael Okpara University of Agriculture, Umudike and Uda (*Xylopia aethiopica*) was obtained from of Amato Avuvu, Ikeduru, Nigeria. Mr. G.G.E. Osuagwu, of the Department of Biological Sciences of Michael Okpara University of Agriculture, Umudike, Nigeria, identified the plants. These were deposited in the herbarium of the Department.

Extraction Of Plant Materials: Leaves or seeds of each of the plant samples were thoroughly washed with tap water and weighed after which was dried in room temperature of about 20-27°C for seven days. These were ground into powder using National Blender (MX-110PN, Japan) and clean mortar and pestle and sieved after which was weighed and stored in some containers.

Extraction Technique

Ethanol Extraction: Fifty grams of the 2 samples, *Occimum gratissimum* and *Xylopia aethopica* were weighed out and each of the plant extracts was soaked separately into 200 mL of ethanol in a conical flask with rubber corks and left for 24 h undisturbed, they were filtered off with sterile filter paper (Whattman No1 filter paper) into a clean conical flask and the filtrate was transferred into the sample holder of the rotary vacuum evaporator[18] where the ethanol solvent was evaporated at its boiling temperature of 70°C . The standard extracts obtained were then stored in refrigerator at 4°C until when required for use.

Aqueous Extracts (cold water): Fifty grams of the two samples *Occimum gratissimum* and *Xylopia aethopica* were weighed out and soaked separately in 200 mL cold water into a conical flask for 24 h undisturbed the were filtered off with sterile filter paper (Whatman No I) filter paper, into a clean conical flask and the filtered was transferred into the sample holder of the rotary vacuum evaporator, where the aqueous solvent was evaporated art at its boiling temperature 0f 100° C. The standard extracts obtained were then stored in refrigerator at 4°C until when require for use [19].

Aqueous Extracts (hot water): 50g of the two samples *Occimum gratissimum* and *Xylopia aethopica* were weighted out and soaked separately in 200 mL of hot water which was boiled for thirty 30 min[19] into a conical

flask for 24 h undisturbed, they were filtered off with sterile filter paper (Whattman No1 filter paper) into a clean conical flask and the filtrate was transferred into the sample holder of the rotary vacuum evaporator, where the aqueous solvent was evaporated out at its boiling temperature of 100°C. The standard extracts obtained were when stored in refrigerator at 4°C until require for use[14].

Test For Purity: The dried extracts were exposed to u. v. rays for 24 h and checked for sterility by streaking on nutrient agar plates and sabourand dextrose agar plates [5].

Media Preparation: All the materials used were sterilized according to Cheesbrough[5]. The glasswares were washed with detergents and rinsed severally with distilled water. They were placed in racks to dry and then packed into the hot air oven for sterilization at 170^{0}C for 15 min. They were cooled in the oven before use[5].

The media used for culturing the organisms were nutrients agar for *S.aureus*, *Esherical coli* and *Proteus mirabilis* and Sabourand Dextrose Agar (SDA) for *Candida albicans*[5]. Nutrient agar media was prepared by dissolving 28 g of nutrient agar in one litter of sterile water and autoclaved at 121°C for 15 min and allowed to cool to body temperature, the medium was dispersed into Petri dishes, it was flamed to remove air bubbles.

Sabourand dextrose agar medium was prepared by dissolving 65g of sabourand dextrose agar in one litter of sterile water. It was autoclaved for 15 mins at 121° C and cooled to body temperature before dispersing into Petri dishes

Bacterial Strain Confirmation and test for purity: The bacterial stock cultures of *E.coli, S. aureus*, *Proteus mirabilis* and fungi *Candidia albicans* were obtained from the Microbiology Laboratory of the Federal Medical Center, Umuahia, Abia State, Nigeria. The organisms were confirmed by carrying out Gram staining procedures and some biochemical tests and by re-isolating in nutrient agar medium and subcultured into nutrient agar slants while the yeast *Candida albicans* was re-isolated in Sabouraud Dextrose Agar (SDA) medium and sub-cultured into SDA slants. The slants were kept in the refrigerator at 4°C [5].

Preparation of different concentration of the plants extract: The paper disc method was used. Disc measuring 6 mm in diameter were punched from Whattman No I filter paper using a cork borer of 6mm diameter. The discs were labeled according to the plant extract and sterilized by autoclaving in different bottles at 15lbs pressure for 15 min. In this method, different concentrations used for the test were 2^0, 2^{-1}, 2^{-2}, 2^{-3}, 2^{-4}, 2^{-5}, (2 fold serial) dilution method and were dried in an incubator at 37°C and stored in the refrigerator before use. Also some antibiotic were used for the control of the extract (Gentamycin for the bacteria, *E. coli, Proteus* and *S. aureus* and Nystatin for *Candidia albicans*, because the content has been known and dimethylsulpoxide (DMSO) for the negative control because (DMSO) does not contain any antimicrobial effect[20].

Screening the extracts for antibacterial activity: The extracts were spot checked for their antimicrobial activity by using disc method. Nutrient agar media was used for testing bacterial *E. coli*, *Proteus* and *S. aureus* and Sabouraud Dextrose Agar (SDA) for *Candidia albicans*[5]. Triplicate plates of media for each of the organisms were inoculated with standardized inoculum of each test organisms and spread on the plants. The plates were allowed to dry. The discs were carefully placed on the media of different concentration of the different plant extracts on the different organisms with negative and positive control of drugs.

Minimum Inhibitory Concentration (MIC) Determination: It was taken as the concentration giving the lowest possible zone of inhibition. The paper disc of different concentration were placed at different portion and were labeled as 2^0, 2^{-1}, 2^{-2}, 2^{-3}, 2^{-4}, 2^{-5}, as two fold serial dilution[17]. The zones of inhibition were measured as the diameter of the clearing and recorded as the MIC.

Statistical analysis: Analysis Of Variance (ANOVA) was used to compare the ethanol and aqueous yield of the plant extracts. The mean activities of the extracts were also compared[21].

RESULTS

Yield Of Plant Extracts: After drying of the plant leaves of *Occimum gratissimum,* it weighed 350 g, after which was grounded and sieved to get 280g. For *Xylopia aethopica,* it weighed 210 g, after grounding and sieving the weight was 192 g. The yield and percentages yield of the plant extracts were higher with ethanol (Table 1).

Antimicrobial Sensitivity Assay Of The Different Extracts: The antimicrobial screening of the plant extract of *Occimum gratissimum* and *Xylopia aethopica* on *Proteus mirabilis*, *E. coli, S. aureus* and *Candida albicans* revealed the effect of the agent (Table 2a, b). There was inhibition of *Occimum gratissimum* on the

Table 1: Yield of extracts of plants with respect to solvent.

Plant	Solvents	Yield(g)	Yield(%)
O. gratissimum	Ethanol	5.6g	11.2
O. gratissimum	Aqueous (cold)	4.3g	9
O. gratissimum	Aqueous (hot)	3.9g	7.81
X. aethopica	Ethanol	8.9g	17.8
X. aethopica	Aqueous (cold)	45g	9
X. aethopica	Aqueous (hot)	4.1g	8.21

Where *O. gratissimum* is *Occimum gratissimum, X. aethopica* is *Xylopia aethiopica*

The percent yield was calculated against the 56g product of the plant material subjected to each extraction method

three bacterial *E.coli, Proteus mirabilis* and *S. aureus* but none on *Candidia albicans* when ethanolic, aqueous (cold) and aqueous (hot) extracts were applied (Table 2a, b).

The ethanolic extract of *Occimum gratissimum* showed zone of inhibition on *S. aureus* and *Proteus mirabilis*, than *E. coli* ranging about 3 mm while the aqueous (cold) extract showed zones of inhibition ranging about 2-3 mm on *S. aureus* and Proteus mirabilis

Table 2a.: Antimicrobial activities of *Ocimum gratissimum* and *Xylopia aethopica* extracts on *Escherichia coli* and *Staphylococcus aureus*

	Escherichia coli				*Staphylococcus aureus*			
	Ethanolic	Aqueous (cold)	Aqueous (hot)	DMSO	Ethanolic	Aqueous	Aqueous (Cold)	DMSO (Hot)
O.gratissimum	+ +	+	-		+	+	+	-
X. aethiopica	- -	-	-		-	+	+	-

Key: + = Inhibition
- = No inhibition

Table 2b: Antimicrobial activities of *Occimum gratissimum and Xylopia aethiopica* extracts on *Proteus mirabilis* and *Candida albicans.*

	Proteus mirabilis				*Candida albicans*			
	Ethanolic	Aqueous (cold)	Aqueous (hot)	DMSO	Ethanolic	Aqueous	Aqueous (Cold)	DMSO (Hot)
O.gratissimum	+	+	+	-	-	-	-	-
X. aethiopica	-	+	+	-	-	+	+	-

Key: + = Inhibition
- = No inhibition

Table 3: Mean zone of inhibition (mm) of the different extract (mg/ml) on *Occimum gratissimum* on the test organism with postive and negative control (gentamycin, nysta and dimenthy/sulphoxide

	Ethanol									Aqueous (cold)									Aqueous (hot)								
	2^0	2^{-1}	2^{-2}	2^{-3}	2^{-4}	2^{-5}	Mic	$cont^+$	$cont^-$	2^0	2^{-1}	2^{-2}	2^{-3}	2^{-4}	2^{-5}	Mic	con^+	con^-	2^0	2^{-1}	2^{-2}	2^{-3}	2^{-4}	2^{-5}	Mic	con^+	con^-
P. mirabilis	3±1	2±1	1±1	-	-	-	0.25	10±2	-	1±0	1±0	-	-	-	-	0.5	10±0	-	7±1	5±1	2±1	2±0	-	-	0.125	11±1	-
E. coli	2±1	2±1	2±1	1±1	-	-	0.125	2±2	-	2±1	1±0	-	-	-	-	0.5	11±1	-	2±1	2±1	-	-	-	-	0.25	12±0	-
S.aureus	3±1	2±1	1±0	-	-	-	0.25	13±1	-	3±1.4	2±1	1±0	-	-	-	0.25	13±1	-	2±0	1±1	-	-	-	-	0.25	12±1	-
C. albicans	-	-	-	-	-	-	-	4±1	-	-	-	-	-	-	-	-	5±0	-	-	-	-	-	-	-	-	4±1	-

Where:
P. mirabilis = *Proteus mirabilis*
E. coli = *Esherichial. coli*
S. aureus = *Staphylococcus aureus*
C .albicans = *Candida albicans*
Con^+ = Positive control
Con^- = Negative control
Each value is an average of replication with its standard deviation

Table 4: Mean zone of inhibition (mm) of the different exttract (mg/ml) on *Xylopia aethiopica* on the test organism with positive and negative control (gentanmycin, nystain and dimethy/sulphoxide

	Ethanol								Aqueous (cold)									Aqueous (hot)								
	2^0	2^{-1}	2^{-2}	2^{-3}	2^{-4}	2^{-5}	$cont^+$	$cont^-$	2^0	2^{-1}	2^{-2}	2^{-3}	2^{-4}	2^{-5}	mic	con^+	con^-	2^0	2^{-1}	2^{-2}	2^{-3}	2^{-4}	2^{-5}	mic	con^+	con^-
P. mirabilis	-	-	-	-	-	-	10±1	-	2±0	2±0	7±0	5±1.4	2±1	-	0.0625	10±0	-	2±1	2±1	4±1	2±0	-	-	0.125	10±1	-
E. coli	-	-	-	-	-	-	11±1	-	-	-	-	-	-	-	12±0	-	-	-	-	-	-	-	-	-	11±1	-
S.aureus	-	-	-	-	-	-	13±1	-	2±1	2±1.45±1	4±0	2±1	-	0.0625	13±0	-	2±1	2±1	4±1	3±1	1±1	-	0.062	13±0	-
C. albicans	-	-	-	-	-	-	5±1	-	4±1	3±1	2±1	-	-	-	0.25	5±1.4	-	4±0	3±0	-	-	-	-	0.5	5±14	-

Where:
P. mirabilis = *Proteus mirabilis*
E. coli = *Esherichial. coli*
S. aureus = *Staphylococcus aureus*
C .albicans = *Candida albicans*
Con^+ = Positive control
Con^- = Negative control
Each value is an average of replication with its standard deviation

but a better zone of inhibition on *proteus mirabilis* ranging about 7 mm of the aqueous (hot) extract on 2°dilution, which is the highest zone of inhibition (Table 3). There were zones of inhibition of *Xylopia aethopica* on the organisms, *Proteus mirabilis, S. aureus* and *Candida albicans* aqueous (cold) and aqueous (hot) but none on ethanolic extract (Table 4). *Proteus mirabilis* showed the highest zone of inhibition of 7 mm on 2^{-2} dilutions of the aqueous (cold) and *S. aureus* about 5 mm on 2^{-2} dilution of aqueous (cold) and *Candida* about 4 mm on 2^{0},dilution of *Xylopia aethopica* (Table 4). The aqueous (hot) showed zone of inhibition on *Candida* ranging about 4mm on 2^{0}, dilution and of *Proteus mirabilis* and *S. aureus* about 4mm on 2^{-2} which was about the highest zone of inhibition, but no traces of inhibition on *E. coli*. It was observed that there is no significant difference in the difference solvent used and also no significant difference amount the two plants used.

MIC Test Result Of The Plant Extracts: The MIC, which is the concentration giving to the last inhibitory activity and below which there is no further inhibition. It was taken as the concentration giving the lowest possible zone of inhibition. The test result of ethanolic extract on *Occimum gratissimum* MIC on *Proteus* and *S. aureus* of the 2-fold dilution method was 0.25 ranging about 1mm and *E. coli* on 2^{-3} (0.125). The aqueous (cold extract) showed its MIC on *Proteus* and *E. coli* on 2^{-1} (0.5) of 1mm while *S. aureus* on 2^{-2} (0.25) of 1mm (Table 3). The aqueous (hot extract) showed its MIC on *E. coli* and *S. aureus* on 2^{-2} (0.25) of 1mm and on *Proteus* on 2^{-3} (0.125) of about 2mm (Table 3). *Xylopia aethopica* showed it's MIC on *S. aureus* and *Proteus* on 2^{-4} (0.0625) dilution with zone diameter of 2mm on aqueous (cold extract) while *Candida albicans* on 2^{-2} (0.25) dilution of 2mm zone diameter (Table 4). The aqueous (hot extract) showed its MIC on *Candida* ranging about 3mm than those of the bacteria ranging 2mm on *Proteus mirabilis* on 2^{-3} (0.125) dilution and on *S. aureus* of about 1mm on 2^{-4} (0.0625) dilution (Table 4).

DISCUSSION

The antimicrobial activities of the plants varied greatly with solvents because there are many factors that influence the active principles present in plant which include the age of plants, extracting solvent, method of extraction and time of harvesting plant materials[12,15,21,22]. The investigation revealed that there was antimicrobial activity against *S. aureus* and *Proteus mirabilis* from ethanolic extract of *Xylopia aethopica*. This probably indicates that there were bioactive ingredients that were inhibitory to the growth of this common pathogen[15,23]. The phytochemical analysis on the aqueous extract of the plants revealed the presence of saponins, tannins, steroids, flavonoids, Alkaloids, cyanogenic glycosides, phytic acid[1]. Most of these compounds have been shown to display physiological activity against most microorganisms[24,25].

This study revealed also that *Occimum gratissimum* inhibited the growth of *E.coli*, *S. aureus* and *Proteus mirabilis*. It has been reported that the volatile oil of this plant contains mostly phenols, particularly thymol[1]and that these are probably responsible for its reported antimicrobial action. The inability of the extracts to inhibit the growth of *Candida albicans* in the experiment showed that this plant drug cannot be used in treatment of fungal infection, like eczema, candidiasis which is caused by *Candida albicans*[26]. The effectiveness of *Xylopia aethopica* on *Candida albicans* indicates that the extract can be used in the treatment of fungal infections since *Candidia* is a fungi infection[16]revealed that *Xylopia aethopica* has demonstrated activity against *Candida albicans*.

This work has highlighted the antimicrobial effects of leaf *Xylopia aethiopica* and *Occimum gratissimum* on the known pathogens. Some antibiotics have become almost obsolete because of the problem of drug resistance[27] and that the consequence of drug resistance implies that new drugs must be sought for and to treat diseases for which known drugs are no longer useful. The result of this includes true improvement of diseases condition after herbal treatment and the non-available of other form of treatment[28].

The results obtained in this study justify the therapeutic uses of some *Occimum gratissimum* and *Xylopia aethopica*. The large volume administered in treatment of herbal diseases as well as the use of alcohol in extraction of herbal preparations. Further investigations can combine the plants extracts for possible synergetic effects. Also the plant extracts can be tested on other human pathogens to elucidate and ascertain their uses.

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