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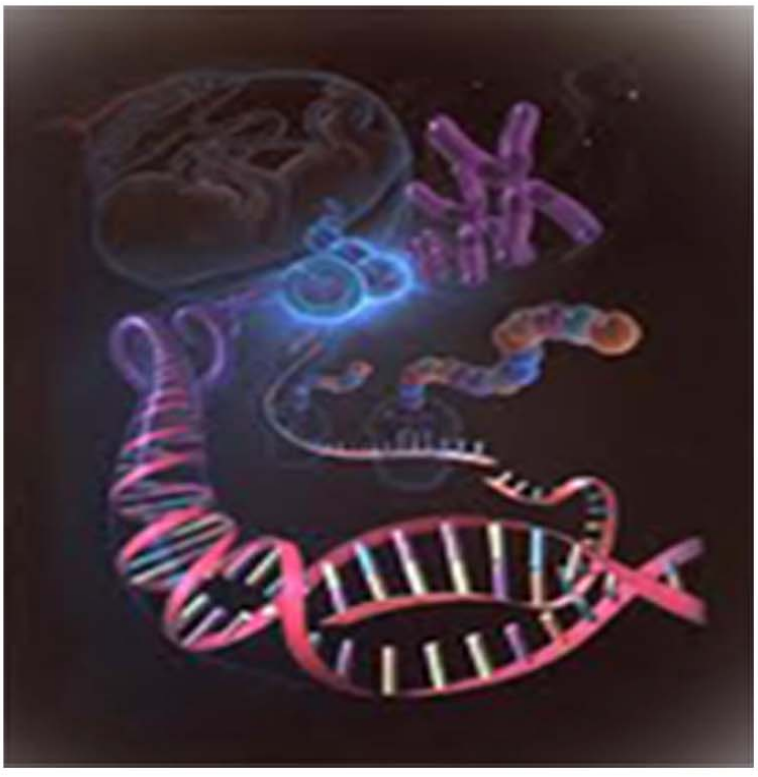

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Research Paper

ANTIMICROBIAL ACTIVITY OF *XYLOPIA AETHIOPICA* (UDA) ON *ESCHERICHIA COLI* AND *STAPHYLOCOCCUS AUREUS* ISOLATES FROM GASTROENTERITIC PATIENTS

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Xylopia aethiopica (Uda) is a slim, tall, evergreen aromatic medicinal plant of West Africa which produces a variety of complex chemical compounds. The dried fruits showed various degrees of activity against gram positive bacteria *Staphylococcus aureus* and gram negative bacterial *Escherichia coli* isolated from gastroenteritic patients. Anti-microbial screening test of (Uda) extract was carried out on the isolated organism using Agar diffusion and disc diffusion methods in various (Uda) extracts (aqueous, hot and ethanol extract) at varied concentrations. However, the results showed different zones of inhibition and Minimum Inhibitory Concentration (MIC). Aqueous extract of *X. aethopica* had MIC of 50µg/ml, hot extract had MIC of 25µg/ml while ethanol extract had MIC of 50µg/ml and 75µg/ml on the isolated organism. The findings suggest that the anti-microbial activity of *X. aethiopica* extract resides in their aqueous and hot fractions and also the results indicated that volatile compound (ethanol) should not be used in plant extraction as a result of the escape of active component of the plant extract.

Keywords: *Xylopia aethiopica*, Uda, *Escherichia coli*, *Staphylococcus aureus*, Gastroenteritic patients

INTRODUCTION

The global emergence of antimicrobial resistant bacterial strains is increasingly limiting the effectiveness of current drugs and significantly causing treatment failure of infections (Hancock, 2005). According to WHO (2002), the available antimicrobial drugs are costly and beyond the reach of the common man in many poor countries.

Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being. It was estimated by (Hancock, 2005) that more than two thirds of the world's population relied in plant derived drugs. It is also estimated that local communities have used about ten percent (10%) of all flowering plants on earth to treat various infections, although

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only one percent (1%) have gained recognition by modern scientists (Kafaru, 2002). Antimicrobial properties of medicinal plants are increasingly reported from different plants of the world (Ashish *et al*., 2011). These plant based systems will continue to play an essential role in health care especially in rural area around the world.

The generations of first plant drugs were usually simple botanicals employed in more or less crude form. Several effective medicines used in their natural state such as cinchona, opium, aloe were selected as therapeutics agents based on empirical evidence of their clinical applicant by traditional societies from different plants of the world (Iwu, 2003).

Following the industrial revolution, generation of second plant based drugs emerged based on scientific processing of the plant extracts to isolates.

It is estimated that today, plant materials are present in, or have provided the models for 70% western drugs (Roberts, 2006). Many commercially proven drugs used in modern medicine were initially use in crude form in traditional or folk healing practices, or for other purposes that suggested potentially useful biological activity. The primary benefits of using plant derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatments (Akintonwa *et al*., 2009).

It is reported, that there are between 100 and 150 species of *Xylopia* distributed throughout the tropical regions of the world, particularly Africa, among them are *X. aethiopica, X. brasiliensis, X. frutenscens* and *X. grandiflora,* which have been studied more completely, than *X. aromatic*. The various extracts from *Xylopia spp*. have been shown to possess antiseptic and analgesic properties, and insecticidal activity against adult mosquitoes, several leaf-eating insects and houseflies. Various parts of the plants have been traditionally employed in different therapeutic preparations (Konning *et al.,* 2004). A fruit extract or decoction of the bark as well as of the fruit is useful in the treatment of bronchitis and dysenteric conditions. In Congo, it is used for the attacks of asthma, stomach and rheumatism (Burkill, 2006). *Xylopia aethiopica* has been reported to be recommended to women who have newly given birth as a tonic, it is taken also to encourage fertility and for ease of child birth (Burkill, 2006). Several studies have shown that *X. aethiopica* extract posseses antibacterial, antifungal and antiplasmodial activities (Ijeh *et al.,* 2004).

Xylopia aethiopica extract contains an antioxidant activity (Asekun and Adeniyi, 2004). It also increases antioxidant defense and protect rats from the adverse effects of irradiation (Asekun and Adeniyi, 2004). Sometimes, a combination of *X. aethiopica* with other plant types or a combination of different parts of *X. aethiopica* is used to achieve the desired effects (Fall *et al*., 2003; Ogunkunle and Ladejobi, 2006). Among the conditions treated with *X. aethiopica* in traditional medicine are cough (fruits and roots of the plant) bronchitis, dysentery and biliousness (fruits, stem and bark) (Ghana Herbal Pharmacopoeia, 2002; Mshana *et al*., 2000).

Although, some extracts of this plant have antioxidant properties, others have antimicrobial effect on a wild range of Gram positive and Gram negative bacterial (Adaramoye *et al.*, 2010). A recent study of various (Cameroonian species found that extract of Xylopia aethiopica had antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus* infections sufficient for

the plant to be considered a potential source of antimicrobial compounds (Kuete, 2010). According to the plant screening program of the national infection Institute (2005), it was confirmed that the antimicrobial activity of *X. aethiopica* extract against panel of infections line, identify phonelics and alkaloid as the main compound responsible for this antimicrobial effect. *X. aethipica* not only has antimicrobial activity but also have nutritive and energy values (Murray, 2005).

The need to search for an antimicrobial agent among natural sources becomes a thing of importance due to the increasing resistance of most microorganisms to antibiotics and the presence of chemical residue in foods. The aim of this research was evaluated the effects of various extracts *X. aethipica* on gastroenteretic *Escherichia coli* and *Staphylococcus aureus* isolates.

MATERIALS AND METHODS

Sterilization

Autoclavable materials such as agar were aseptically sterilized in an autoclave at 121ºC for 15 min. Properly washed petri-dishes, beakers, wash bottles, test tubes, pipettes, conical flasks, spatula, inoculating needles and forceps were sterilized using hot air oven at a temperature of 150ºC for 1 h. The wire loops were sterilized by heating in the blue flame of the Bunsen burner until red-hot and allowed area to cool before using 70% alcohol is use to swab the working bench area to prevent contamination.

Collection of Plant Material (Sample)

Fruits of *Xylopia aethiopica* (Uda) were bought from Ogbete main market Enugu State, Nigeria.

Preparation of Sample

The sample was ground with the aid of mortar, the ground sample was stored in a labeled container ready for extraction process.

Extraction Procedure

Extraction was done using soaking method. 32g of the powdered plant materials were soaked and macerated in 250ml of distilled water to prepare the aqueous extract. In 250ml of hot water to prepare the hot water extract and 250ml of ethanol to prepare the ethanol extract. It was allowed to stand for 24 h after which it was filtered using a whatman No. 1 filter paper. The extract was collected in a round bottom flask.

Preparation of Culture Media

The media of culturing were aseptically prepared when needed according to the manufacturer's instructions, and autoclaved at 121ºC for 15 min.

Preparation of the Innocula

Microorganism (*Staphylococcus aureus* and *Escherichia coli*) used in this work were obtained from the stock culture of the microbiology laboratory of the University of Nigeria Teaching Hospital (UNTH), Enugu, Enugu State, Nigeria. Viability test of each isolates were carried out by re-suscitation of the organisms in buffered peptone broth and there, after sub-cultured into nutrient agar media and incubated at 37 °C for 24 h, then subjected to gram staining and biochemical test for proper identifications.

Gram Staining for Identification of Organism

The slide containing the heat fixed smear was laid across a staining rack and placed over a sink. The smear was flooded with 0.5% aqueous crystal violet for 30 s, excess stains were washed

off with excess of lugols iodine and allow to react for 30 s, while the slide was laid across the staining rack, the iodine was carefully rinsed out with distilled water. Then the smear was rinsed briefly for 3 s with 50% acetone alcohol until blue color eases to come out, this is a declourizing step which if prolonged will interfere with the result, thus the side was quickly rinsed out with distilled water to avoid excess decolouration. The slide was dried between fold of blotting paper, the slid was again laid across the staining rack and flooded with 1% aqueous safranin for 60 s after which the stain was washed off with distilled water. The slide was air dried and placed with a drop of immersion oil and examine under oil immersion objectives of the microscope. Bacterial stained blue-black are said to be Gram positive while those stained red are Gram negative.

Biochemical Tests for Identification of Organism

Catalase Test: This test is used to differentiate those bacteria that produce the enzyme catalase such as *Staphylococcus* from non-catalase producing bacteria such as *Streptococcus.*

A colony of cultured organism was picked from agar slopes, using a clean sterile platinum wire loop. This was inserted in drops of H_2O_2 on a clean microscopic slide. The production of gas bubbles indicates a positive reaction.

Coagulase Test: This test is used to identify *Staphylococcus aureus* which produces the enzymes coagulase.

A drop of saline was placed on a clean slide. About one or two colonies of the test organism were picked with a sterile wire loop and emulsified in the drop of saline to form a smooth milky suspension. The autoaggultinable strains which could not form smooth suspension were discarded. Coarse clumpsing becoming visible to the naked eye within 5-10 s indicated a positive result.

Indole Test: This test is used to detect the production of indole by bacteria grooving on media.

Peptone water media was prepared and incubated for 48 h at 37ºC. About one or two colonies of the test organism were inoculated into a test tubes and were allowed to stay for 48 h in the incubator for the accumulation of indole. After this period, 0.5ml kovas' reagent was added separately to each tube and shaked gently. The appearance of red colour in the alcohol layer indicates a positive reaction.

Citrate Utilization Test: This is used to test whether the organism can use the compound citrate as its only source of carbon and energy.

Using Simon's citrate agar, the sterile medium was inoculated from a saline suspension of the test organism and incubated for 96 hours at 37ºC. A blue color and streak of growth indicate a positive reaction, while the original color and no growth were indicative of a negative reaction.

Motility Test: This is used to test whether the organisms are motile, i.e., the presence of flagella for movement.

About 2-5 drops of peptone water with growth of the organism was placed on a clean slide with a loop. The cover slip was placed over the slide. The slide was left for sometimes and then examined microscopically with the high power objective. Motile organisms would be seen swimming around.

Sugar Fermentation Test: This is done to know whether the organism can ferment sugar and produce acid.

Fermentation test were carried out using the following sugars: glucose, mannitol, lactose, sucrose and maltose. To each, 10ml of peptone water in test tube, 1.5g of each sugar was separately dissolved into it and labeled, and 3 drops of 0.01% phenol red was added. Durham tubes were plugged with non-absorbent cotton wool and sealed with aluminium foil before being sterilized in an autoclave at 121ºC for 15 min. After sterilization, any trace of air in the Durham tubes were removed by inverting the test tubes. The tubes were then aseptically inoculated with small bacterial colonies using sterile wire loop. The tubes were incubated for 24 h at 37°C and un-inoculated tubes served as controls.

Antimicrobial Screening Test

The sensitivity of selected organisms to the aqueous, hot and ethanol extracts of *Xylopia aethiopica* (Uda) were evaluated by the use of Agar diffusion and Disc diffusion methods.

Agar Diffusion Method

This is the direct use of extract on the agar containing the test organism. Nutrient agar was poured in sterile Petri-dishes and was allowed to solidify. 1ml of the test colony was dropped on the solidified agar and the colony was spread all over the surface of the agar using a hward stick. Wells of approximately 5mm in diameter were made on the surface of the agar medium using a sterile cork borer. The plates were turned upside down and well labeled with a marker. Each well was filled with 0.2ml of the extract, tetracycline discs was used as control for the cultures. The plates were incubated aerobically at 37ºC for 24 h. Sensitivity of the organisms to the extracts was recorded.

Disc Diffusion Methods

This is the application of locally prepared antibiotic disc in carrying out antimicrobial screening test. The locally prepared sterile discs were soaked in the extracts for some hours and nutrients agar media was poured in sterile. Petri dishes were allowed to solidified. 1ml of the test organisms was placed on the solidified agar and was spread over the surface of the agar. The soaked discs was picked using sterile forceps and it was dropped on surface of the agar. The plates were incubated at 37ºC for 24 h. Sensitivity of the organisms was recorded.

The result presented in Table 1 above shows the isolation of *Escherichia coli* and *Staphylococcus aureus* from gastroenteritic patients. According to the morphological characteristics gotten from the cultured sample, the colonies showed smooth pink/red and cream round colonies in cluster, these were evidence of *Escherichia coli* and *Staphylococcus aureus.* The biochemical reactions also showed that the organisms identified are *Escherichia coli* and *Staphylococcus aureus*.

Result presented in Table 2 above shows the antimicrobial activity of aqueous extract of *Xylopia aethiopica* (Uda) against *Escherichia coli* and *Staphylococcus aureus* with varied concentrations of the plant extract. Analysis of *Escherichia coli*, using varied *Xylopia aethiopica* (Uda) Extract concentrations of (100µg/ml, 75µg/ml, 50µg/ml and 25µg/ml) shows that zone of inhibition differs.

Therefore, 100µg/ml gave a zone of inhibition of 19mm which shows high effect of the extract

Table 1: Showing The Characteristics, Gram Reaction and Biochemical Test Results Carried Out on the Isolated Test Organisms

Morphological Characteristics	Gram Reaction	Catalase	Coagulase	Indole	Citrate	Motility	Glucose	Lactose	Sucrose	Maltose	Mannitol	Probable Organism
Smooth pink/red colour	(–) Negative	–	–	+	+	+	Ag	Ag	Ag	Ag	Ag	*Escherichiacoli*
Cream round colony in Cluster	(+) Positive cocci	+	+	–	–	–	Ag	Ag	Ag	Ag	Ag	*Staphylococcus aureus*

KEYS: – Organisms that are not reactive to the biochemical test carried out; + Organisms that are reactive to each of the biochemical test carried out; Ag – Acid gas producing organism.

Table 2: Anti-Microbial Effects of Aqeous Extract of *Xylopia aethiopica* (Uda) Against *Escherichia coli* and *Staphylococcus aureus*

Organism	Zone of Inhibition (mm)				
	100µg/ml of Extract	75µg/ml of Extract	50µg/ml of Extract	25µg/ml of Extract	MIC (µg) ml
Escherichia coli	19.0mm	8.0mm	5.0mm	0.0	50
Staphylococcus aureus	15.0mm	10.0mm	7.0mm	0.0	50

on the organism. Then concentrations of 75µg/ml, 50µg/ml and 25µg/ml showed a decreasing zone of inhibitions (8mm, 5mm and 0.0). A similar effect of plant extract is also seen in *Staphylococcus aureus*. The least concentration at which the plant extract exert its minimum inhibitory effect was at 50µg/ml each.

The results in the Table 3 above shows the anti microbial effect of hot extract of *Xylopia aethiopica* (uda) on microbes. According to the analysis, hot extract of the plant gave an active effect on the organisms. On *Escherichia coli* varied concentration of 100µg/ml, 60µg/ml, 50µg/ml and 25ug/ml gave zone of clearance of 23mm, 20mm, 12mm and 0mm respectively. The minimum inhibitory concentration (MIC) of the plant extract which exert a minimum zone of clearance 10mm was 25ug/ml.

Also staphylococcus aureus had a decreasing zone of inhibition on application of plant extract

Table 3: Anti-Microbial Effect of Hot Extracts of *Xylopia aethiopica*

Organism	Zone of Inhibition (mm)					
	100µg/ml of Extract	60µg/ml of Extract	50µg/ml of Extract	25µg/ml of Extract	15µg/ml of Extract	MIC (µg) ml
Escherichia coli	23.0mm	20.0mm	12.0mm	10.0mm	0.0	25
Staphylococcus aureus	20.0mm	18.0mm	10.0mm	9.0mm	0.0	25

Table 4: Anti-Microbial Effect of Ethanol Extract of *Xylopia aethiopica*

Organism	Zone of Inhibition (mm)				
	100µg/ml of Extract	75µg/ml of Extract	50µg/ml of Extract	25µg/ml of Extract	MIC (µg) ml
Escherichia coli	9.0mm	3.0mm	1.0mm	0.0	50
Staphylococcus aureus	8.0mm	4.0mm	0.0	0.0	75

giving a minimum inhibitory concentration of 25ug/ml.

Result presented in Table 4 above shows the effect of ethanol extract of *Xylopia aethiopica* on microbes.

According to the analysis varied concentration of 100µg/ml, 75µg/ml, 50µg/ml and 25µg/ml of extracts were used. Zone of inhabition were proportional to the concentration and above all ethanol extract had a low antimicrobial effect on the micro organism.

DISCUSSION, CONCLUSION AND RECOMMENDATION

The result of the inhibitory effect of *Xylopia aethiopica* (uda) extract on micro-organisms according to the analysis above, shows that a high concentration of the plant extract be it in aqueous extract, hot extract or ethanol extract, could cause a high zone of inhibition while low concentration results in low zone of inhibition. That is to say, the higher the concentration of the extract, the more effective the extract is on the microorganisms. The component used in extraction of these plants (water and ethanol) affects the activity of the extract on the microorganism. Thus, this shows that hot extract of *Xylopia aethiopica* (uda) has high effect on the microorganisms as seen in Table 2, then ethanol prepared extract shows a low effect on the microorganisms as a result of the escape of active component of these extracts by the acid components (ethanolic acid).

Then, the Minimum Inhibitory Concentration (MIC) for all extract prepared (aqueous, hot and ethanol extract) shows the minimum concentration of those extract at which there would be least inhibition of micro-organism. The minimum inhibitory concentration was 50µg/ml for aqueous extract, 25µg/ml for hot extract and 50µg/ml and 75ug/ml for ethanol extract.

These shows that the MIC depends on the effectiveness/potency of the extract, it is low in active compounds and high in less active compound (ethanol and water). Finally, the concentration of extract has low minimum inhibitory concentration. Thus, the minimum concentration is high for less active extract preparation (ethanolic) and active extract preparation (aqueous extract and hot extract) has low inhibitory concentration. This is similar to the result obtained by (Oyagade, 2009, Akpulu, 2002 and Oladunmoye, 2007).

CONCLUSION

The result of this study indicated that the extract of *Xylopia aethiopica* (uda) has shown potential antimicrobial properties for the treatment of diseases as claimed by traditional healers. But the major factors that determine activities of the extracts are the concentration of plant extract when prepared and also the components/

substances used in the preparation. These show that high concentration of the extract has a high effect on the microorganisms, but this assumption thereby becomes false when solvent like ethanol are used in the preparation. This component tends to be very active and volatile and as a result decreases the effect of plant extract thereby making it to have a high minimum inhibitory concentration.

RECOMMENDATION

Having known the antimicrobial effect of *Xylopia aethiopica* (uda), it is therefore recommended that people should use these plants as a bactericidal agent. There should be need for more analysis of the plant extract which will help to know the concentration of this extract that would not be toxic to human cells.

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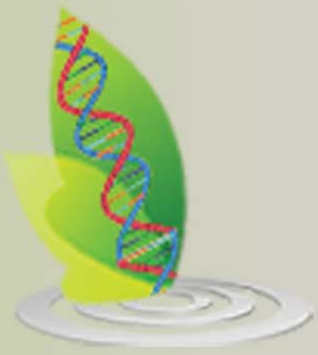

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