PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF ALIGATOR PEPPER(AFRAMOMUM MELEGUTA) IN CLINICAL ISOLATES

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ABSTRACT

The purpose of this study is to examine the phytochemical composition and antimicrobial activity of Alligator pepper (Aframamum melegueta) extract in clinical isolates. The analysis was carried out using standard method. The Phytochemical analysis of the ethanoluic extracts of aframamum melegueta showed the presence in high concentration of terpenoid while glycosides and flavonoids are present in moderate concentration. Saponins, phenols, alkaloids, and tannins were slightly present. The antimicrobial activity was carried out using agar disk diffusion method. The test showed that the ethanolic extract of aframamum melegueta had high inhibition on Escherichia coli (25mm), Staphylococcus spp. (20mm), and Salmonella spp. (20mm) while moderately ,it show inhibition on pseudomonas aeruginosa (15mm), Streptococcus spp. (16mm) and Bacillus substilis (18mm), diameter zone of inhibition. This suggests that ethanolic extract of Aframum Melegueta showed good antimicrobials properties. Therefore, Aframamum Melegueta can further be investigated on, in order to explore the possibility of producing drugs that can be used in the treatment of bacterial infections.

Keywords: Bacteria Infection, Anti-bacteria, Alligator pepper, treatment

INTRODUCTION

Herbal medicine is the oldest and still most widely used system of medicine in the world today. Medicine made exclusively from plant is used in all societies and is common to all cultures.

Plants have mainly been the basis for medicinal treatments in human history. Researchers in Africa are paying attention to plants as source of new therapeutic leads for reasons which includes; Africa's rich flora which make the continent a hub for plants with medical potentials and continuing dependence of a sizeable proportion of the African populace on the herbs for the treatment of the common infectious disease (Ekor, 2014).

According to the world health organization (WHO), they defined herbal medicines as herb materials, final product and finished herbal product that's bioactivity is attributed to plant or its part (sahoo and Hamed, 2010). Traditional medicine is "the knowledge, skills and practices based on the theories, belief and indigenous to different cultures, used in the maintenance of health and in the prevention, diagnosis, improvement or treatment of physical and mental illness".

Regardless of why people uses it, traditional medicine provides an important health care services whether people have physical or financial access to allopathic medicine (Science-base), and it is a flourishing global commercial enterprise (Engebretson, 2002).

Alligator pepper (Afaromomum melegueta) is a West Africa spice to season foods. This herb is botanically in the same family as ginger (zingberacera) and shares many bioactive. It has been traditional used mostly for digestive and intestinal health with some other sporadic uses not related to food. The spice is used in West Africa for the purpose of alleviating stomach ache and diarrhea as well as hypertension, it is also used as a remedy for snake bite and scorpion stings (Lans, 2001).

Alligator pepper is widely used by many cultures in Nigeria for various purposes. It is used by religious rites by diviners for invoking spirits. It is a common ingredient in pepper soap. Concoctions made from alligator pepper are often used by traditional doctor as medications for various ailments. The constituents of essential oil extract from the seed of Aframomum melegueta by hydro distillation include two sesquiterpene hydrocarbons (humelene and caryosphyllene), their oxides are few non terpennnoid (Ajaiyeoba and Ekundayo, 2009).

Afaromomum melegueta is a member of zingiberacea which is popular for its distinct attribute as a spice used wide world. It is variously known locally as ose oji in Igbo, Ataare in Yoruba and Citaa in Hausa language in Nigeria (Chinaka *et al.*, 2014).

Internationally known as grain of paradise, melegueta pepper, Alligator pepper and guinea pepper. Alligator pepper has immersed medicinal importance. Its seed extract heal wounds and invigorates the immune system against diseases (Ntonifor *et al.*, 2010). Studies have shown that its seed contain important phytochemicals namely, alkaloids, glycosides, tannins, flavonoids, sterols, trite pens and oils; some of which are responsible for its pestical and antimicrobial properties (Doherty *et al.*, 2010).

Alligator pepper is often included in anti-allergy, anti-flammation, anti-toxin and anti-ulcer herbal remedies for liver problems and tumours and its potency can be attributed also to some of the photochemical (iwu, 2003).

STATEMENT OF PROBLEM

Microbial pathogenicity and other infectious diseases have been controlled by the use of drugs. For the past years, the increasing reliance on drug from natural source has lead to the extraction and development of several drugs and chemotherapeutic agents from traditional herbs.

Tremendous use of antibiotic has developed multiple drug resistance (MDR) (Davies and Davies, 2010). In many bacterial pathogens, the increasing drug resistance is the main hindrance in successful treatment of infectious diseases and the control of microbial pathogenicity.

Objectives Of The Study

1. To carry out extraction of the active ingredients of Aframomum melegueta(Alligator pepper) seed using water and ethanol.
2. To determine the type of phytochemical present in Alligator pepper seed.
3. To determine the antibacterial activity on the extracts on some clinical isolates.

RELATED LITERATURE

Medicinal plants have been integral to traditional medicine systems for centuries, offering a vast array of bioactive compounds with therapeutic potential. Alligator pepper (Aframomum melegueta), a member of the ginger family, has gained attention for its diverse phytochemical composition and potential health benefits. This literature review aims to explore the phytochemical screening and antibacterial activity of Alligator pepper, specifically focusing on its effects on clinical isolates.

Importance of Phytochemicals

Phytochemicals are natural compounds found in plants, known for their diverse biological activities. Alligator pepper is rich in phytochemicals such as alkaloids, flavonoids, tannins, saponins, and essential oils. These compounds have demonstrated various pharmacological properties, including antimicrobial, anti-inflammatory, and antioxidant effects (Ajaiyeoba and Ekundayo, 2009).

Phytochemical Screening of Alligator Pepper

Alkaloids

Alkaloids are nitrogen-containing compounds often associated with antimicrobial properties. Studies have identified alkaloids in Alligator pepper, suggesting potential antibacterial effects against clinical isolates (Ajaiyeoba and Ekundayo, 2009).

Flavonoids

Flavonoids are known for their antioxidant and antibacterial activities. Alligator pepper contains flavonoids, which may contribute to its therapeutic potential against bacterial infections (Ntonifor *et al.*, 2010).

Tannins

Tannins are polyphenolic compounds with antimicrobial properties. Alligator pepper has been found to contain tannins, suggesting a role in inhibiting bacterial growth and promoting health.

Saponins

Saponins, possessing surfactant properties, are found in Alligator pepper. These compounds may contribute to the plant's antibacterial activity, disrupting bacterial cell membranes.

Essential Oils

Alligator pepper essential oils are rich in bioactive compounds. The volatile nature of these oils may play a role in inhibiting bacterial growth and influencing clinical isolates (Ntonifor *et al.*, 2010).

Antibacterial Activity of Alligator Pepper

Several in vitro studies have investigated the antibacterial activity of Alligator pepper extracts against clinical isolates. These studies demonstrate promising results, indicating the potential of Alligator pepper in combating bacterial infections.

The antibacterial mechanisms of Alligator pepper are diverse and may involve disruption of bacterial cell membranes, interference with cell wall synthesis, and inhibition of essential bacterial enzymes. Understanding these mechanisms is crucial for elucidating the plant's therapeutic potential.

Synergistic effects between Alligator pepper and conventional antibiotics have been explored. Combinations of plant extracts with antibiotics may enhance antibacterial efficacy and reduce the risk of antibiotic resistance.

METHODOLOGY

Sample Collection

The alligator pepper was bought from Eke OKo market in Anambra State, Nigeria. The seeds dried in a shade for two weeks. The seeds were grounded into fine powder and were stored in a clean airtight container.

Ethanolic Extraction

94.6 Gram of the sample was extracted with 200ml of ethanol in 250cm conical flask and was allowed to stay overnight, the extract was separated using a sterile muslin cloth and filtered with a sterile filter paper.

Phytochemical screening of the extract:

The ethanol leave extracts was analyzed for the presence of Alkaloids, Saponins, Tannins, Cyanogenic Glycoside, Phenol, Terpenoid, Flavonoids using the procedure described by (AOAC,2002).

Test for Saponins

Saponins were detected using the forth test. 1ml of the ethanol extract was boiled with 5ml of distilled water for 5 minutes and filtered while still hot. The filtrate was used for the fronting test. 1ml of the filtrate was diluted with 4ml of distilled water in a test tube and the test tube was shaken vigorously and observed on standing for stable froth which was an evidence for the presence of saponin.

Test for Alkaloid:

1ml of extract was shaken with 5ml of 2% HCL on a steam bottle and filtered; 1ml of (filtered) extract was measured into a test tube and 3 drops of Wagner's reagent was added. Alkaloids are

indicated by reddish brown precipitate which was present.

Test for Tannins

1ml of extract was measure with equal volume of bromine water and a greenish to red precipitate observed show the evidence for the presence of tannins

Test for Cardiac Glycoside:

1ml of the extract was added to 2ml of glacial acetic and few drops of 50% ferric chloride ($Fecl_2$). 2ml of concentrated sulphuric acid (H_2SO_4), was carefully added by the side of the test tube, formation of brown at interface indicated the presence of glucoside.

Test for Phenol:

1ml of the extract was added to 2ml of distilled water followed by few drops of 10% ferric chloride. The formation of blue or green color indicated the presence of phenols.

Test for Terponoids

5ml of the extract with 2ml of chloroform and 3ml of concentrated H_2SO_4 was added into a test tube. The acid is carefully added by the side of the test tube to form two layers. The formation of reddish brown color at the interface indicated the presence of terpenes.

Test for Flavonoids

1ml of 10% ferric chloride was added to 1ml of extract. A greenish brown precipitate was observed.

Test Organism:

The microorganisms used were *Eschericha coli*, *Streptococcus* species, *Staphylococcus aureus*, *Bacillus subtilis*, *Sacchraromyce cerevisae*, *Salmonella* species.

They were isolated from Microbiology Laboratory Unit of federal polytechnic Oko, Anambra State, Nigeria

Antimicrobial Assay

The antimicrobial activity was performed by disc diffusion method. The bacterial strains were grown in nutrient broth. Muller Hinton agar was the media used to study the bacterial susceptibility. The broth culture were grown for 24 hours and serially diluted to 10-3 in the same broth (sterilized at 121^0c for 15 minutes)

The 24 hours broth culture contains approximately 1.7 x 10^7 cfu/ml 2.8 x 10^6 cfu/ml, 3.2 x 10^6cuf/mu, 2.6 x 10^6 cfu /ml and 5.0 x 10^7 cfu/mu for *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* species, *Bacillus subtillis*, *Streptococcus* species respectively are determined by agar disc diffusion method. Sterile swab stick was used to inoculate the media by dipping it in the diluted culture and spreading all the surface of the agar plate. Sterile plate disc about 10mm diameter was soaked with the extract and allowed to dry for some minutes. This was placed on the surface of inoculated agar plats. Another disc was soaking in a solution containing 100ug/1ml of chloramphenicol antibiotics and placed by the side of the extract disc to serve as a positive control. The plate were then incubated for 24 hours at 37oC. After incubation, the diameter zone of inhibition was measured using millimeter rule.

RESULTS AND FINDINGS

The result of the phytochemical screening of *Aframomum melegueta* seed extracts showed that all the parameters tested for were present as shown in table 1.

Table 1: Phytochemical screening of Afranomum meleguta

Parameter	Intensity
Saponins	+
Terpenoids	+ +
Tannins	+
Alkalnoids	+
Glucoside	++

Flavonoids	+++
Phenols	+

Keys

+++ = Present in high concentration

++ = Present in moderate concentration

+ = Slightly or sparingly present

The result of the antimicrobial screening of the plant extract showed that ethanolic inhibited all the test organisms as shown in table 2

Table 2: Antimicrobial activity of the plant extracts Aframomum melegueta seed

Names of Organisms	Control chloramphenicol	Ethanolic Extract of Aframomum melegueta seed
Escherichia coli	35	30
Staphlococcus aureus	21	22
Pseudomonas aeruginosa	30	20
Streptococcus Species	29	26
Bacillus subtilis	30	15
Samonella Species	45	24

DISCUSSION

The result of the work showed that the seed extract of Aframomum Meleyueta inhibited the growth all the bacteria tested (Table 2), This suggests that the plant extract is broad spectrum in activity. Higher antimicrobial activity of the extracts was observed on *Escherichia coli, staphylococcus aureus* and *salmonella* ssp. at high concentration, while moderately, it showed inhibition on *Pseudomonas aeruginosa. Streptococcus* species,

and *Bacillus subtilis.* This is similar to the earlier result obtained by (Oyagade,2009), (Akpulu, 2004) and (Oladummoye, 2007) as they reported that Aframomum melegueta ethanolic extract had inhibition on *Escherichia coli*, *Staphylococcus* spp. *Salmonella* spp. *Pseudomonas Aeruginosa*, *Streptococcus* species and *Bacillus* spp, The Antimicrobial effect of Aframomus melegueta is due to the Phylochemical constituents present in it. Afromonum meleyueta Seeds are rich in Phytonutrient such as Flavonoids, Phenolic compoud Tannins, Saponins, Terpernoids, Cardiac glycosides and Alkaloids.

The biological function of flavonoids includes protection against allergies, inflammation, free radicals, platelet aggregation,microbes, ulcer, hepatoxins, viruses and tumors (Okwu,2004).This may be the reason behind the use of the extracts of this plant in the treatment of intestinal upset in herbal medicine (Okwu,2004). The presence of phenolic compounds in the seed of Afromonum meleyueta indicates that this plant might be an Antimicrobial agent. This is because phenols and phenolic compounds have been extensively used in disinfection and remain the standard with which other bactericides are compared. Phenolic compounds as electron donors are readily oxidized to form phenolate ion or quinine, an electron acceptor. This gave rise to practical uses. Protonated phenol is used as cleaning agent. Extracts from Aframonum melegueta therefore have potent antiseptic or bactericidal properties (Okwu, 2004). This findings

Supports the use of extracts from Aframonum melegueta in treating wounds that not only heals fast but also prevents the formation of infections (Okwu, 2004). The presence of phenol further indicated that the seed of this plant could act as an anti-inflammatory, anti-clotting, antioxidant, immune enhancers and hormone modulations. This plant also has quality of saponin content. Some of the general characteristics of saponin include formation of foam in aqueous solutions, hemolytic activity cholesterol binding, properties and bitterness (Okwu, 2004). Apart from saponin, other metabolite constituents of Aframonum meleguata detected include the alkaloids and tannin. Alkaloids ranked the most efficient therapeutically significant plant substance. Pure isolated plant alkaloids and their synthetic derivative are used for physiological activity when administered to animals. The high tannin content could be partly responsible for the hot, bitter and pungenty taste of Aframonum meleguata seed. Tannin has a stringent property; it hastens the

healing of wounds and inflamed mucous membrane (Okwu and Okwu, 2004). The presence of tannin in this plant strongly supports its use in treating wounds, burns and hemorrhoids in herbal medicine. This observation is very significant because of the possibility of developing, therapeutic substances that will be active against multi-drug resistance pathogens.

CONCLUSION

In conclusion, the result of this study showed that extract of Aframonum meleguata has antimicrobial effect on gram positive and gram negative bacteria. The presence of photochemical in the extract may have been responsible for the activity possessed by the plant extracts.

RECOMMENDATIONS

Since (Aframonum meyeguata) contains many valuable phytochemicals which indicates possible preventive and curative properties and also possesses same degree of antimicrobial activity.

2. It is recommended to use for pharmaceutical products and should also be used for traditional medicines.

3. Aframonum meleguata can also be of immense help in phytomedicine and health care delivery system in developing economy due to the presence of the phytochemicals present in it.

4. The antibacterial properties discovered shows thst Aframonum meleguata will be helpful for developing valuable medications that can be incorporated into products for sustainable treatment of skin and intestinal etiology diseases whose causative agent originated or are related to *staphylococcus aureus*, *streptococcus* and other clinical bacteria.

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