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Antimicrobial effect and phytochemical analysis of *Aframomum melegueta* on some selected bacteria and fungi

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Abstract
Antibiotic toxicity and multidrug resistant pathogens are the two greatest challenges being faced by today's medical world. In this present study, the antimicrobial activity of spices has been investigated as an alternative to antibiotics in order to tackle these dangers. The choices of spice as an alternative is based on two basic reasons: firstly, plants have been the model sources of medicine since ancient times and secondly the increasing acceptance of herbal medicines by general population as therapeutic against many pathological infection. In this present study, the antimicrobial activity of alligator pepper leave has been investigated against local clinical bacterial and fungal isolates. The methanol extract has been assayed separately against drug resistance, *Staphylococcus aureus, Pseudomonas aeruginosa, Proteus* sp., *Salmonella* sp., *Bacillus* sp., *Escherichia coli, Klebsiella* sp., *Saccharomyces* sp., *Aspergillus* and *Candida.*The antibacterial and antifungal activities were determined by disc diffusion method. The result shows that the zone of inhibition of methanol extract for 48hours was more effective compare to that of 24hours of which *klebsiella* sp. has the highest zone of inhibition for the methanol extract. The water extract at 48hours was discovered to possess a higher inhibition zone on *Staphylococccus aureus, Pseudomonas* sp., *Proteus* sp., *Salmonella* sp., *Baccillus* sp., *Klebsiella* sp., *Aspergillus* sp., *Candida* sp. *and Saccharomyces* sp. with the following inhibition zone respectively: 40mm, 40mm, 45mm, 45mm, 45mm, 50mm, 45mm, 45mm and 40mm. *Escherichia coli* has the lowest inhibition zone both the methanol extract and water extract with the inhibition zone of 25mm after 24hours and 35mm after 48hours for water extract while the methanol extract has the inhibition zone of 40mm for 24hours and 55mm for 48hours. In the light of several socioeconomic factors of Nigeria mainly poverty and poor hygienic condition, present study encourages the use of spice (alligator pepper seed) as supplementary medicine to reduce the burden of high cost, side effects and progressively increasing drug resistance of pathogen.

Keywords: *Aframomum melegueta*, Microbial isolates, plant extracts, phytochemical properties

Introduction
Aframomum melegueta known as alligator pepper have been described as grains of paradise in Africa because of its vast use as food flavor and in cold days chewed to warm the body, in traditional occasions as well as during naming ceremony. *Aframomum melegueta* has also been used in West Africa for the purposes of alleviating stomachache and diarrhea as well as hypertension with some limited reports on it being used for Tuberculosis and a remedy for snakebites and scorpion stings. The seeds have been discovered to posses anti-microbial properties similar to many spices, and has some molluscidal and repellant properties as well. It is one of many pungent herbs said to aid in sexuality and aphrodisiac. *Aframomum melegueta* have been found to contain constitutuents that contributes to its antimicrobial properties and these are; Gingerol, Methyl-6-Gingero, Shogaol, Paradol (30.5% of ethanolic, Rac-6-Dihydroparadol, Gingeredione and {2-(5-butylfuran-2-yl)ethyl}-2-methoxyphenol *Aframomum melegueta* appears to also have polyphenolic contents which are comparatively high to other African spices tested although low relative to other herbs. The aim of this investigation is to process extract of alligator pepper leaf using the water and methanol and further carryout sensitivity test using known bacterial and fungal clinical isolates. Microbial pathogenicity and other infectious diseases have been controlled by the use of commercially available antimicrobial drugs since last many years the increasing reliance on drug from natural source has led to the extracts and development of several drugs and chemotherapeutic agents from traditional herbs, plants and crops which are present in abundance in tropic (Falodun, et al., 2006). Tremendous use of antibiotics has developed multiple drugs resistance (MDR) in many bacterial pathogens the increasing drug resistance is the main hindrance in

successful treatment of infectious disease and to the control of microbial pathogenicity. In vitro studies have shown that active constituents of ginger inhibit growth of fungi and bacteria. Extracts from the seeds of *Aframomum melegueta* have potent antiseptic, fungicidal and bactericidal properties, and have, therefore, been used in preventions of infections and treating wounds (Okwu, 2005). In studies or research, *Aframomum meleguata* is known to have antibacterial, antifungal antivirus activity and has been used extensively in traditional Chinese medicine for their warming and in food from knowing the benefit and antibacterial properties. Leaf extract of A. melegueta contains phytochemicals which offer an enormous potential as biocontrol agent of pathogens and a source of antimicrobial agents of therapeutic importance.

The work of Doherty et al. (2010) revealed that its ethanolic extract has greater antimicrobial activity than its aqueous extract as indicated by the zones of inhibition. The efficacy of *Azadirachta indica* and *Aframomum melegueta* on fungi isolated from diseased cassava tubers was tested by Okigbo et al. (2009). These extracts which showed no significant difference in the yield of cassava were found to be fungitoxic to *Fusarium oxalicum*, *Aspergillus niger* and *Botryodiplodia theobromae*. Ethanolic extract of *A. melegueta* inhibited A. niger most. The major methods employed in prevention of the spread of these pathogens are application of fungicides, solarisation, and the use of antagonistic micro organisms among others. The use of synthetic fungicides is environmentally hazardous, and therefore, has been recently banned (Okereke and Wokocha, 2007). There is, therefore, the need to search for cheaper environmentally friendly and readily available alternatives such as plant extracts for the control of these pathogens. In view of the fore mentioned hazards the search for alternative means for combating fungal diseases in plants was embarked upon in this work. Hence the fungitoxic potentials of *A. melegueta* was assessed and phytochemical analyses of their extracts ascertained.

Materials and Methods

The alligator pepper leaves sample used in the present study was gotten from the farm land of Lagos State Polytechnic, Ikorodu, Lagos Metropolis Lagos state Nigeria.

Bacteria test strains

Pure bacterial isolates for the antimicrobial testing were obtained from Nigeria Institute of Medical Research Yaba.Lagos State Nigeria. The isolates were: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus* sp, *Salmonella* sp, *Bacillus* sp, *Escherichia coli*, and *Klebsiella* sp.

Fungal test strains

Pure fungal and yeast isolate for the antifungal testing were obtained from the Biochemistry department of Lagos State Polytechnics which are *Aspergillus* sp, *Saccharomyces* sp and *Candida* sp.

Sampling and sample preparation

The plant part was washed with tap water and air dried. The dried part was chopped into pieces, milled into fine powder by pounding manually using sterile pestle and mortar. The powdered sample was collected into sterile cellophane bags and labeled. The sample was kept in cool dry place till further use. The powdered sample was used for the extraction.

Extraction procedure

The extraction process used was the soaking method as reported by Doherty et al. (2010). One hundred grams (100g) of each powdered sample was soaked in 1000ml of 70% methanol and 1000ml of water for 24hours and 48hours simultaneously. The samples were each filtered twice through cheese cloth and collected in a round bottom flask. They were later concentrated using a rotary evaporator.

Phytochemical analysis of plant extracts

A modified method of Harbone. (1984) was carried out to determine the presence of phytochemical compounds present in the plant extracts.

Antimicrobial Screening of the Plant Extract

The antimicrobial study of the plant extract was carried using the agar well diffusion method described by Cowan (1999).

Results

Table: 1 Phytochemical result of *Aframomum melegueta* leaves for Alkaloids test

Test	Observation	Inferences
2mls of extract A + 2ml of Dragendorff Reagent	Dark orange-red coloured	Alkaloid confirmed
2mls of extract B + 2ml of Dragendorff reagent	light orange turbid colored	Alkaloid confirmed
2mls of extract A + 2ml of MAYERS Reagent	Dark yellowish precipitate	Alkaloid confirmed
2mls of extract B + 2ml of MAYERS Reagent	Dark yellow precipitate	Alkaloid confirmed
2ml of extract A + 2ml of WAGNERS Reagent	Dark turbid brown precipitate obtained	Alkaloid confirmed
2ml of extract B+ 2ml of WAGNERS Reagent	Dark turbid brown precipitate obtained	Alkaloid confirmed

Key: A => Aframomum alcohol extraction, B=> Aframomum water extraction

Table: 2 phytochemical result of *Aframomum melegueta* leaves for Flavonoid test

Test	Observation	Inferences
2ml of extract A + 2ml of feCl3	wooly brownish precipitate obtained	Flavonoids inferences
2ml of extract B + 2ml of feCl3	Wooly brownish Precipitate obtained	Flavonoids inferences
2mls of extract A + 2ml of 10% lead ACETATE	Yellowish green Precipitate obtained	Flavonoids confirmed
2mls of extract B + 2ml of 10% lead ACETATE	Light yellowish green precipitate	Flavonoids confirmed
2mls of extract A + 2ml of Dilute NaOH	Golden Reddish colour	Flavonoids precipitate
2mls of extract B + 2ml of Dilute NaOH	Reddish golden colour	Flavonoids precipitate

Table: 3 Zone of inhibition (mm) of methanolic and water extracts of *Aframomum melegueta.*

Organism	Water Extract		Alcohol Extract	
	24 hrs	**48hrs**	**24hrs**	**48hrs**
Staphylococcus aureus	30mm	40mm	42mm	50mm
Pseudomonas aeruginosa	22mm	40mm	30mm	40mm
***Proteus* sp**	37mm	37mm	37mm	50mm
***Salmonella* sp**	39mm	45mm	57mm	60mm
***Bacillus* sp**	40mm	45mm	50mm	60mm
E. Coli	25mm	35mm	50mm	60mm
***Klebsiella* sp**	30mm	50mm	60mm	75mm

***Saccharomyces* sp**	33mm	40mm	33mm	40mm
***Aspergillus* sp**	40mm	45mm	45mm	47mm
***Candida* sp**	39mm	45mm	46mm	48mm

Table: 4 phytochemical screening chemical tests of methanolic and water extract of *Aframomum melegueta.*

Chemical Component	Methanolic extract	Water Extract
Alkaloid	+++	+++
Flavonoid	+++	+++
Tannin	+++	+++
Cardiac glycosides	+++	+++
Anthraquinone glycoside	+++	+++
Saponins	+++	+++
Anthocynanosides	- - -	- - -
Cyanogenic glycosides	+++	+++
Reducing sugar	- - -	- - -
Terpenoids	+++	+++
Steroids	+++	+++
Phenols	+++	+++

Key: + = Present, - = Absent

Results and Discussion

The table 1 showed test for alkaloid compound present in the *Aframomum melegueta* extracts. From the table, results showed the presence of the compound in the sample. Table 2 showed results for presence of flavonoid compound in the sample. Microbial susceptibility to the sample extracts were determined in Table 3. From the table, zones of inhibition were measured after 24hr and 48hr incubation respectively using water and alcohol extracts. The results obtained showed that the tests strains were more susceptible to alcohol extract than the water extract. Klebsiella sp had the highest zone of inhibition in diameter with 75mm after 48hr but gave 60mm in diameter after 24hr as the highest value. *Klebsiella* sp was alo recorded as having the highest of 50mm in diameter after 48hr with water extract but had 30mm in diameter after 24hr with water extract. The results obtained from Table 3 was able to determine the effect alcohol has on the sample extract compared to water exctract. Table 4 showed the screening of phytochemical compounds present in the Aframomum melegueta using water and methanolic extracts. The table showed that only anthocynanosides and reducing sugars were absent from both extracts. This showed that water is also a good source for extraction of phytochemical compounds from plant source. In the present study, antibacterial effect of *Aframomum melegueta* extract against *Staphylococcus aureus, Pseudomonas aeruginosa, Proteus, Salmonella, Bacillus, E. coli, Klebsiella, Saccharomyces, Aspergillus, Candida* show that ethanol extract possess a remarkable therapeutic action in the treatment of gastrointestinal infection, Nausea, respiratory problems, cold, fever, allergies, urinary tract infection, fungi infections, reduces fat and also use to relieve painful arthritis when *Aframomum melegueta* oil is used in massaging. The presence of flavonoid indicate that can be used to prevent coronary heart disease. It can be used in promoting sex hormone also treatment of syphilis and other veneral diseases due to the presence of steroid, alkaloid and saponin which indicate the class of compound for effective treatment. The antimicrobial activity of these extract in the study agree with the findings of previous researchers. The phytochemical analysis carried out on *Aframomum melegueta* revealed the presence of alkaloids, tannis saponin, steroids, cardiacglycoside, flavonoid, terpenoids and phenol. The presence of these phytochemicals supports the use of this plant as antimicrobial agent Adefegha and oboh, (2012). *Aframomum melegueta* can therefore be used as antimicrobial agent against the groups of *Enterobacteriaceae* and some tested fungus both the methanol and water extract shows few presence of reducing sugar and anthocyanosides.

Traditionally, it has been best known for its anti flamatory properties because of the content of 6-paradol, an active flavour constituent that effectively act as an antioxidant and anti-tumor agent due to this it makes it a good alternative to commercially available medicine aspirin. It is a plant that has several natural values which are in natural forms (Ajaiyieoba and Ekundayo, 1999). As a plant of several high values, which can be used as cusine, spice of its warming and digestive properties. It is a medicinal plant that possess series of chemical combination such as phenolic compounds that makes it antimicrobial agent which is extensively use in the treatment of infectious diseases.The pungent, peppery taste of the seeds is caused by aromatic ketones such as 6 – paradol, which only occur in traces. Plants have not only nutritional value but also, in the eyes of the local people, they have medicinal and ritual or magical values (Sofowora, 1993). Plants have been a major source of medicine for human kind. According to available information, a total of at least 35000 plants species are widely used for medicinal purposes. The demand for traditional herbs is increasing very rapidly, mainly because of the harmful effects of synthetic chemical drugs. The global clamor for more herbal ingredients creates possibilities for the local cultivation of medicinal and aromatic crops as well as for the regulated and sustainable harvest of wild plants. Additionally studies have shown this plant to have bactericidal activity while clinical study have shown extract of the plant to be effective in parasitemia. It is used for urinary tract infections especially *candida* and other inflammatory conditions, malaria, hypertension, microbial infections and stomach ache. The use of *Aframomum melegueta* as a natural supplement is considered healthy choice of the treatment of diabetes, cardiovascular diseases, hypertension, inflammation, thrombosis and even cancer with the increasing awareness of population toward natural therapies spices can be considered as obvious alternate medication. Based on the result of findings in this work, *Aframomum melegueta is recommended for use* as a supplement if not to replace the chemical antibiotics because it is not found to have side effect.

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