Antimicrobial Activities of Aframomum Melegueta (Alligator Pepper)

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Abstract

Antibacterial activity of *Aframomum melegueta* was tested on *salmonella spp, Escherichia coli, Shigella spp and klebsiella spp*. Ethanol and distilled water were used as solvents for the extraction of the plant. The ethanolic extract was found to be most effective at high concentration of 50mg/ml on all the isolates. The zones of inhibition of *klebsiella spp*, *salmonella spp*, E. coli and *Shigella spp* are 30mm,15mm,20mm,and 15mm respectively with ethanolic extract. The aqueous extract was found to be less effective when compared with ethanolic extract.

The phytochemcial analysis carried out on *Aframomum melegueta* revealed the presence of alkaloids, tannins, saponin, steroids, cardiacglycoside, flavonoid, terpenoids and phenol. The presence of these phytochemcials support the use of this plant as antimicrobial agent. *Aframomum melegueta* can therefore be used as antimicrobial agent against the groups of Enterobacteriaceae tested.

Keywords: Antimicrobial activity, *Aframomum,* Terpenoids, Phytochemical

1. Introduction

The use of traditional medicine is wide spread throughout the world. The term, traditional medicine is interchangeably used with herbal medicine and natural medicine (Hazan and Atta, 2005). Since antiquity, man has used plants to treat common infectious diseases and even long before mankind discovered the existence of microbes; the idea that certain plants had healing potential was well accepted (Rios and Recio, 2005).A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purpose or which are precursors for the synthesis of useful drugs. A number of plants have been used in traditional medicine for many years due to their antimicrobial properties(Sofowora, 1993). Specifically, the medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human or animal body (Edeoga et al., 2005). The most important of these bioactive constituents which are mainly secondary metabolites are alkaloids, flavonoids, tannins and phenolic compounds.These phytochemicals are toxic to microbial cells.

Aframomum melegueta is a tropical herbaceous perennial plant of the genus *Aframomum* belonging to the family zingiberaceae (ginger family) of the angiosperms in the Kingdom plantae. The seeds have pungent peppery taste due to aromatic ketones (Galal,1996, Tackie el. al,1975). It is a plant with both medicinal and nutritive values, found commonly in rain forest. It is widely spread across tropical Africa including Nigeria, Liberia, Sierra Leone, Ghana, Cameroon, Cote D' ivoire and Togo. The phytochemicals obtained from the seed of *Aframomum melegueta* has been used for years in the treatment of infectious diseases. The grains of *Aframomum melegueta*

possess active ingredients that may be exploited for local development of antimicrobials (Oyegade,1999). The presence of phenolic compounds in the seed of *Aframomum melegueta* indicates that this plant is an antimicrobial agents and this is because phenols and phenolic compounds have been extensively used in disinfections and remain the standard with which other bactericides are compared (Okwu, 2001b). Extracts from the seed of *Aframomum melegueta* with have potent antiseptic or bactericidal properties, have therefore been used in treating wounds and preventions of infections (Okwu, 2004). According to (Oladunmoye, 2007). *Aframomum melegueta* was tested for antimicrobial effects on five pathogenic bacteria which include *Bacillus cereus, Staphylococcus aureus, Escherictaia coli, Salmonella typhi and klebsiella pneumonia*. The plant extract of *Aframomum melegueta* was found inhibitory to the growth of *Klebsiella pneumonia and Salmonella typhi*. The findings revealed that extract from *Aframomum melegueta* contain phytochemicals which offer an enormous potential as bio control of these pathogens and source of antimicrobial agents of therapeutic importance. The objectives of this study is therefore to carry out phytochemical analysis on the seed extracts, determine the minimum inhibitory concentration (MIC) of the crude extracts of the seeds and to ascertain the antimicrobial property of the crude extract

2. Materials and methods

2.1 Collections of samples

The sample was bought from Oyingbo market in Ebute metta, Lagos, Nigeria. The variety was chosen because it is widely used in all parts of the country as spice, condiments and in soup making.

2.2 Preparation of sample

400 grammes of the sample were dried at room temperature for one week. After drying, 350 grammes were obtained. The sample was ground and put into a container and it was labeled and stored in preparation for the extraction process.

2.3 Sterilization

Autoclavable materials such as agar and broth were aseptically sterilized in an autoclave at 121^0C for 15 minutes.Petri dishes, beakers, McCartney bottles, pipette, test tubes, filter papers and other metal apparatus such as spatula and forceps were sterilized using hot air oven at a temperature of 160^0C for 1 hour. The wire loops were sterilized by heating in the blue flame of the bunsen burner until red-hot and allowed to cool before using 70% alcohol to swab the work bench area to prevent contamination. The process was carried out aseptically.

2.4 Extraction procedure

The extraction was done using soaking method. The ground sample was extracted using water and ethanol as solvent.100g of the powdered sample was extracted with 1000ml of distilled water while 100g of the sample was extracted with 1000ml of 70% ethanol. The sample was soaked overnight for 24 hours. After 24 hours, the sample was filtered ten times with muslin cloth and the extract was collected in a round bottom flask, filtered and concentrated using a rotary evaporator and then oven dried at 70%.

2.5 Preparation of culture media

All culture media were prepared according to manufacturers' instructions and autoclaved at 121^0C for 15 mins.

2.6 Preparation of organisms

Serial dilution was carried out on the isolates collected from Microbiology Department of Lagos State University Teaching Hospital, Idi-Araba Lagos, Nigeria and 10^{-4} of the serial dilution was used for the sensitivity testing.

2.7 Reconstitution of extract

The dried extracts were reconstituted by dissolving 5g each of the extract in 50ml ethanol and 50ml distilled water. The solution was filtered using the sterile whatman no1 filter paper. The stock solution was sterilized by filtration through filter paper to remove impurities and other contaminants. The stock solution was further dissolved at different concentration and it was then stored in sterile universal bottles and refrigerated for further analysis.

2.8 Antimicrobial screening for reconstituted extract

Two methods were employed for the antimicrobial testing which are the, Agar diffusion method and Disc diffusion method.

2.8.1 Agar diffusion method

The antimicrobial screening of the ethanolic extract was done as described by Lino and Deogracious (2006).

Nutrient agar was poured in sterile Petri dishes and was allowed to solidify. 1ml of the test culture was dropped on the solidified agar and the organism was spread all over the surface of the agar using a spreader. 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 the wells labeled with a marker. Each well was filled with 0.2ml of the extract. Streptomycin disc was used as control for the cultures. The plates were incubated aerobically at 37^0c for 24 hours. Sensitivity of the organisms to the extract was recorded

2.8.2 Disc diffussion method

The locally prepared sterile discs were soaked in the water extract for some hours and nutrient agar medium was poured in sterile Petri Dishes and it was allowed to solidified.1ml of the test organisms was placed on the solidified agar and it was spread all over the surface of the agar. The soaked disc was picked using sterile forceps and it was dropped on the surface of the agar. The plates were incubated at 37^0C for 24 hours. Sensitivity of the organisms was recorded.

2.9 Phytochemical analysis

Phytochemcial screening was carried out on the obtained plant extracts, according to Okwu, (2005).

2.9.1 Qualitative analysis of the constituents. Test for tannins

About 0.5g of the dried powdered sample was boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue black colouration.

2.9.2 Test for phlobatannin

An aqueous extract of the plant sample was boiled with 1% aqueous hydrochloric acid and deposition of a red precipitate was taken as evidence for the presence of phlobatannins.

2.9.3 Test for saponins

About 2g of the powdered sample was boiled in 20ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil, shaked vigorously and then observed for the formation of emulsion.

2.9.4 Test for flavonoids

5ml of 10% dilute ammonia solution was added to a portion of the aqueous filtrate of the plant extract, followed by addition of concentrated H_2SO_4. A yellow coloration observed in the extract indicated the presence of flavonoid.

2.9.5 Test for Cardiac Glycosides

5ml of the extract was treated with 2ml of glacial acetic acid containing I drop of ferric chloride solution (0.1%). This was underlayed with 1ml of concentrated H_2SO_4. A brown ring of the interface indicated a deoxysugar characteristics of cardenolides. A violet ring may appear below the brown ring, while in the acetic layer, a greenish ring may form just gradually throughout thin layer.

2.9.6 Determination of total phenols by spectrophotometer methods

The fat free sample was boiled with 50ml of ether for the extraction of the phenolic component for 15minutes. 5ml of the extract was pipetted into a 50ml flask and then 10ml of distilled water was added. 2ml of ammonium hydroxide solution and 5ml of concentrated amylachohol were made up to mark and left to react for 30min. Colour development was measured at 505nm.

2.9.7 Alkaloid determination using Harborne (1973) method

5g of the sample was weighed into a 250ml beaker and 200ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

2.9.8 Tannin determination by Van-Burden and Robinson method (1981)

500mg of the sample was weighed into a 50ml and shaken for 1 hour in a mechanical shaker. This was filtered into a 50ml volumetric flask and made up to the mark. Then 5ml of the filtered was pipetted out into a test tube and mixed with 2ml of 0.1M $Fecl_3$ in 0.IM HCl and 0.008m potassium Ferrocyanide. The absorbance was measured at 120nm within 10min.

2.9.9 Saponin determination

The method used was that of Obadoni and Ochuko (2001). Twenty grammes of ground samples was put into a conical flask and 100ml of 20% aqueous ethanol was added. The samples were heated over a hot water bath for 4 hours with continuous stirring at about 55^0C. The mixture was filtered and the residue re-extracted with another 200ml 20% ethanol. The combined extracts were reduced to 40ml over water bath at about 90^0C. The concentrate was transferred into a 250ml separatory funnel and 20ml of diethyl ether was added and shaken vigorously.

The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. Sixty millilitre(60ml)of n-butanol was added to the extracts and washed twice with 10ml of 55 aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the extracts were dried in the oven to a constant weight, and percentage saponin content determined.

2.9.10 Flavonoid determination by the method of Boham and Kocipaiabyazan (1974)

Ten grammes(10g) of the plant sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No. 42 (125mm). The filtrate was later transferred into a crucible and evaporated into dryness over water bath and weighed to a constant weight.

3. Result

The phytochemical analysis revealed the presence of Tannin, saponin, flavonoid, steroid, terpernoids, cardiac glycoside, alkaloid and phenol as shown in Table 1. The percentage composition of the phytochemcial constituents of *Aframomum melegueta* is shown in Table 2. The results showed that the extracts from *Aframomum melegueta* have antimicrobial activity against all the isolates tested even at lower concentration.

At 5mg/ml concentrations of both the ethanolic and aqueous extract, there are zones of inhibition, but the ethanolic extract showed greater antimicrobial activity than the aqueous extract as indicated by zones of inhibition in (Table 3) and (Table 4).

The largest zones of inhibition occurred with *Escherichia coli, Salmonella spp* and *Klebsiella spp* which indicated that, they were more sensitive to the effect of the extract than the other organisms.

Echerichia coli & *Klebsiella spp* showed highest zones of inhibition at high concentration of 50mg/mg. At 5mg/ml concentration, the ethanolic extract is effective on both *salmonella spp* and *shigella spp* but at 50mg/ml, the ethanolic extract is effective on all the isolates. This indicates that the antimicrobial activity of this plant is concentration dependent. Ethanolic extract showed high inhibitory zones than aqueous extracts. This indicates that ethanol is a better extract than distilled water.

4. Discussion

The result of this work showed that the seed extract of Aframomum melegueta inhibited the growth of all the bacteria tested (Table 3 & 4). This suggests that the plant extract is broad spectrum in activity. Higher antimicrobial activity of the extracts was observed on *E.coli, Klebsiella spp and Salmonella spp* at high concentration, this is similar to the earlier result obtained by (Oyagade, 1999), (Akpulu, 1994) and (Oladunmoye, 2007). The antimicrobial effect of A*framomum melegueta* is due to the phytochemical constituents present in it. *Aframomum melegueta* seeds are rich in phytonutrient such as flavonoids, phenolic compound tannins, saponin, terpernoids, cardiac glycosides and alkaloids.

The biological function of flavonoids includes protection against allergies, inflammation, free radicals, platelet aggregation, microbes, ulcers, 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 troubles in herbal medicine. (Okwu, 2004). The presence of phenolic compounds in the seed of *Aframomum melegueta* 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 gives rise to practical uses. Protonated phenol is used as cleaning agent. Extracts from Aframomum melegueta therefore have potent antiseptic or bactericidal properties. (Okwu, 2004). This finding supports the use of extracts from *Aframomum 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 anti inflammatory, anti clotting, antioxidant, immune enhancers and hormone modulators. This plant also has quantity 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 *Aframomum melegueta* detected include the alkaloids and tannin. Alkaloids ranked the most efficient therapeutically significant plant substance. Pure isolated plant alkaloids and their synthetic derivatives are used

as basic medicinal agents for their analgesic, antispasmodic and bactericidal effects. They exhibit marked physiological activity when administered to animals. The high tannin content could be partly responsible for the hot, bitter and pungenty taste of *Aframomum melegueta* 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. The aqueous extract was found to be bacteriostatic. While ethanolic extract was bactericidal at higher concentrations. The presence of phytochemcial supports its uses as antimicrobial agent

5. Conclusion

The plant *Aframomum melegueta* can be of immense use in phytomedicine and can be included in health care delivery system particularly in the developing economies. Further studies on more effective method of extracting only the necessary constituents and standard reconstitution means as well as other processing, refining and purification measures would be necessary. It can be concluded from this study that the extracts from the seed of *Aframomum melegueta* showed antimicrobial activity against the tested isolates at high concentration of 50mg/ml.This probably justifies its use as an antimicrobial agent.

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Table 1. Phytochemical constituents of the seeds of *Aframomum melegueta*

Constituents	Results
Tannin	+
Saponin	+
Flavonoid	+
Steroid	+
Terpernoids	+
Cardiac glycoside	+
Alkaloid	+
Phlobatannis	-

+ = present, - = Not present

Table 2. Percentage composition of the Phytochemical constituents of seeds of *Aframomum melegueta*

Constituents	Results
Phenol	0.83
Alkanoids	1.14
Tannins	17.21
Flavonoids	0.54
Saponin	1.40

Table 3. Zones of inhibition of ethanolic extracts of the seed of *Aframomum melegueta*

	Mean Zones of inhibition (mm)				
Isolates	50mg/ml	30mg/ml	20mg/ml	10mg/ml	5mg/ml
Salmonella spp	15	15	12	9	9
Escherichia coli	20	10	8	NIL	NIL
Klebsiella spp	30	15	14	NIL	NIL
Shigella spp	12	12	10	10	9

Mean Zones of inhibition in millimeters,
NIL =No inhibition

Table 4. Zones of inhibition of aqueous extracts of the seed of Aframomum melegueta

	Mean Zones of inhibition (mm)				
Isolates	50mg/ml	30mg/ml	20mg/ml	10mg/ml	5mg/ml
Salmonella spp	10.5	9.0	9.0	8.0	7.0
Escherichia coli	10.0	9.0	9.0	8.0	8.0
Klebsiella spp	13.0	9.0	8.0	8.0	8.0
Shigella spp	12.0	9.0	9.0	9.0	8.0