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Comparative Studies on the Antimicobial Activity of Leaf Extract from *Aframomum melegueta* and Antagonistic Activity of Isolates from Refuse on Some Selected Pathogenic Bacteria

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Abstract: Ethanolic leaf extract from *Aframomum melegueta* was accessed for *in vitro* antimicrobial activity as well as phytochemical constituents. Refuse dump materials were screened for resident micro floral with antagonistic activities against the pathogens. Thirteen pure isolates were obtained in all including nine bacteria and four fungi. Five pathogenic bacteria; *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Klebsiella pneumoniae* were tested for their susceptibilities to the antimicrobial activity of the leaf extract as well as the antagonistic potential of the isolates refuse Standard antibiotics were also tested on the pathogens and their susceptibilities investigated. The effectiveness of the antimicrobial phytoconstituents from *Aframomum melegueta* and microbial antagonisms was compared with that of the standard antibiotics in which the former fairly compete with the latter. The plant extract of *Aframomum melegueta* was found inhibitory to the growth of *Salmonella typhi* and *Klebsiella pneumoniae while the refuse isolate*s were more effective than the bacteria as shown growth inhibitory indices. Seven of the nine isolates inhibited the growth of at least one of the pathogenic organisms; *Klebsiella pneumoniae* and *Staphylococcus aureus* being the most susceptible. The findings revealed that the extract from the plant and the refuse isolates offer an enormous potential as biocontrol of these pathogens and source of antimicrobial agent of therapeutic importance.

Key words: *Aframomum melegueta*, antimicrobial agents, phytoconstituents

INTRODUCTION

In recent years, acquired resistance to most commonly available standard antibiotics like chloramphenicol and ampicillin by most commonly encountered pathogenic microbes like *Shigella dysenteriae* and *Haemophilus influennzae* type b is a growing world wide problem (Monica, 2000). It has gotten to the extent that one could correctly predict the resistance of certain organisms to specific antimicrobials. Monica, 2000 has stated that Proteus species are generally resistant to nitrofurantoin and tetracycline, *Streptococcus pyogenes* to penicillin, *Klebsiella pneumoniae* to ampicillin and anaerobes to metronidazole. There have also been reported cases of drug hypersensitivity and many third generation antibiotics have prolific side effects on patients' normal flora. Protracted use of these refined drugs also results in immunosuppression.

It has therefore become expedient that an alternative therapeutic measure be adopted. This may include the use of medicinal plant extract and general traditional medicine. There is also a glimpse into biocontrol, which is the use of antagonistic microorganism to control and curtail these deleterious creatures.

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Traditional medicine is wide spread throughout the world. It is the total combination of knowledge and practices, whether explicable or not, used in diagnosing, preventing, or eliminating a physical, mental, or social disease. This may rely exclusively on past experience and observation handed down from generation to generation, verbally or in writing; while bearing in mind the original concept of nature which includes the material world, the sociological environment, whether living or dead and the metaphysical forces of the universe (Sofowora, 1993).

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 disease 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 (Sofowora, 1993).

The fact that plants have anti-microbial properties has been proven by many scholars. Specifically, the medical 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 majorly secondary metabolites are alkaloids flavonoids tannins and phenolic compounds These phytochemicals are toxic to microbial cells.

Aframomum melegueta, alligator pepper is a Tropical plant of the genus Aframomum, belonging to the family Zingiberaeae of the angiosperms in the kingdom planta. It is a perennial herb of about 3 ft, with narrow leaves. The fruits are ovate with reddish-brown irregular seed that is strongly aromatic and of pungent taste (Gordian, 2005). It is widely spread across west tropical Africa including Cameroon, Cote D' Ivoire, Ghana, Liberia, Nigeria, Sierra Leone and Togo; often used as food additives and flavouring agents apart from serving medicinal purposes.

This plant has been found to exert some physiological affect on human body. Gordian (2005) reported that it is being used in Nigeria as stimulant. Antimicrobial properties investigation carried out by Razaq *et al*. (2003) revealed that its extract is a broad spectrum antimicrobial agent.

The objectives of the present investigation are to isolate, characterize and identify microorganisms associated with refuse dump site and assay their antagonistic effect on some pathogens. Comparative evaluation of the antimicrobial activities of the crude extracts from *Aframomum melegueta* on the pathogenic organisms as well as screening for the phytochemicals of pharmacological importance was also determined.

MATERIALS AND METHODS

Test Organisms

The pure culture of *Escherichia coli*, *Samonella typhi*, *Klebsiella pneumoniae*, *Staphylococcus aurerus* and *Bacillus ceraus*. were collected from the Medical Microbiology Laboratory of the University College Hospital (UCH), Ibadan, Oyo State, Nigeria.

Plant Sample: Source, Extraction and Fractionation

The leaves of plant, Aframomum melegueta were bought from central market, Akure, Ondo State. The leaves were air dried and grinded into fine powder. About 500 g of the pulverized plant sample was extracted with 60% ethanol. The sample was soaked for 72 h after which the resultant mixture sieved with muslin cloth. The sieved extract was concentrated to dryness using a rotary evaporator The extract was dissolved in 0.1M Tris-HCL buffer (pH 7.0, 5 mL) and applied to a column (5×85 cm) of Sephacryl S-300 HR, pre-equilibrated and developed with the same buffer. Fractions corresponding

to the peak were pooled together concentrated and freeze dried. The powder was dissolved in water and applied to a Sephadex G-25, column (1.5×50 cm), then eluted with water and fractions were collected. The eluate obtained was concentrated and lyophilized.

Refuse Dump Sample Collection and Isolation

Both decomposing and decomposed refuse materials were separately collected in sterile MacCarney bottles. The samples were collected from three different dump sites in Akure, Ondo State, Nigeria. Microorganisms associated with the refuse was isolated and identified using standard methods (Prescott *et al*., 2002).

Detection of Antagonistic Activity

The antagonistic activities of the refuse isolates against the pathogenic organisms were determined using the modified agar diffusions assay described by Schillinger and Lucke (1989). Old culture of the refuse bacterial (isolates on slant) was inoculated asceptically into the tubes of fresh nutrient broth and incubated at 37°C for 24 h. After 24 h incubation, the culture supernatant of broth was obtained by configuration.

Wells were made with an 8 mm cork borer in plates containing solidified nutrients agar already seeded with 0.1 mL of the pathogenic isolates from 24-h-old culture. Then, few drops of culture supernatant of actively growing refuse isolates were introduced into the wells with the aid of sterile syringes. The plates were incubated at 37°C for 24 h after which zones of inhibition around each well were examined.

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).

Phytochemical Screening

The bioactive molecules were screened for using the methods of Harbone (1984).

Antibiotic Sensitivity Tests

To determine the antimicrobial activities of the standard antibiotics, the disc diffusion method described by Brock and Madigan (1988) was employed.

RESULTS AND DISCUSSION

The total number of distinct bacterial isolates obtained from the refuse samples based on their colonial, morphological and biochemical characteristics were nine (Table 1) and four fungi species (Table 2). It shows that refuse dump is inhabited by myriad of biota as reported by Koestler (2006).

The results of the antimicrobial activities of the extract are presented on Table 3. It was unable to inhibit the growth of all the organisms at lower concentration of 100 and 200 mg mL^{-1}. This suggests that the inhibitory potential of the extract is concentration dependant. Similar findings had been reported by Cowan (1999). However, at 300 mg mL^{-1} zones of inhibition of 5.0 mm and 6.5 mm were observed with *Salmonella typhi* and *Kebsiella pneumoniae* conforming to the result obtained by Razaq *et al*. (2003).

The antagonistic activities of the refuse isolates on the pathogens are presented on Table 4. All the isolates were found to posses' antagonistic activity. Microorganisms associated with biodegradation had been reported to produce toxic metabolite to other competing micro floral (Koestler, 2006). *Staphylococcus aureus* was the most susceptible of the pathogens. *Escherichia coli* were

resistant to all the isolates. The variation in the susceptibility and resistivity may be due to some inherent genetic factors in the organisms which may be chromosomal or plasmid coded. It may also be as a result of their gram reaction owing to difference in chemical composition that determines the structural integrity of each organism. All the fungi inhibited the growth of all the selected pathogens (Table 5). *Aspergillus repens* was the most effective having zones of inhibition ranging from 11 mm to 13 mm. Fungi had been shown to be an excellent reservoir for antibiotics (Prescott *et al*., 2002)

The sensitivity of the organisms to the standard antibiotics varies. Susceptibility of the gram negative ones was higher (Table 6). None of the organisms however, is susceptible to Cefunxine. Only Salmonella was susceptible to Ofloxacin while Ciprofloxacin was effective against all the organisms. The reason for the observed trend may be similar to those ascribed for the refuse isolates. The potency of the antibiotics was found to be higher than that of the extract and the antagonist in some cases. This can be due to high purification and concentration of active antimicrobial ingredients in the antibiotics.

Table 1: Characterization and identifinat of refuse bacterial isolates sugar fermentation

Isolates	Cultural characteristics	Morphology	Gram reaction	Spores staining	Coagulase	Catalase	Glu	Dex	Gal	Lac	Ara	GP	Rate of fermentation	Identity
1	Greenish colour on MacConkey agar with raise but dry surface and spreading growth pattern	Short rods	-ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	Early fermenter	*Pseudomonas* sp.
2	Sharp pinkish colonies with round margins and raised surface	Tiny rods	-ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	-ve	Late fermenter	*Acinetobacter* sp.
3	Light pinkish flat-surfaced colonies with spreading growth pattern	Tiny rods	+ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	Early fermenter	*Corynebacterium* sp.
4	Colonies that have entire edge, orange colour and raised elevation	Tiny rods	+ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	Early fermenter	*Actinomycets* sp.
5	Creamy white colonies with entire edge and flat surface	Long rods	+ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	Early fermenter	*Clostridium* sp.
6	Creamy appearance with flat, not elevated surface. The colonies have entire margins	Rods in clusters	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	Early fermenter	*Bacillus* sp.
7	Which colonies with flat surface spreading pattern of growth	Long rods	-ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve	Early fermenter	*Shigella* sp.
8	Creamy, flat-surfaced, spreading colonies	Long rods in clustered	-ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	Early fermenter	*Proteus* sp.
9	Colonies have raised surface, entire edges and are creamy	Long rods	+ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	Early fermenter	*Enterobacter* sp.

Table 2: Cultural and microscopic characterization of fungi isolates

No.	Isolates morphology on plate	Morphology under microscope	Isolate
1.	Fluffy with dark colour that covered the plate.	An upright conidiophore that terminate in a clavate phialides at the apex or surface conidia are one- celled and globose.	*Aspergillus niger*
2.	Fluffy with green colour. that covered plate.	An upright conidiphore that terminates in a cleavate phialides at the apex or radiating from the entire.	*Aspergillus flavus*
3.	Deep green mycelium that covered the plate.	Separated mycelium bearing single conidiophores, which are branched near the apex ending in phialides that carried the conidia.	*Penicillium* sp.
4.	Fluffy with dark brown colour that covered the plate.	An upright conidiophore that terminates in a cleavate phialides at the apex or radiating from the entire surface.	*Aspergillus repens*

Table 3: Antimicrobial activties of leaf extract of *Aframomum melegurta*

	Zones of inhibition (inmm)		
Isolates	100 mg mL^{-1}	200 mg mL^{-1}	300 mg mL^{-1}
Bacillus cereus	NI	NI	NI
Escherichia coli	NI	NI	NI
Klebsiella pneumoniae	NI	NI	5.0
Salmonella typhi	NI	NI	6.5
Staphylococcus aureus	NI	NI	NI

NI = Inhibition

Table 4: Antagonistic effects of refuse bacterial isolates on pathogenic isolates

	Zone of inhibition of pathogenic bacteria				
Refuse bacteria	*Bacillus coccus*	*Escherichia coli*	*Salmonella typhi*	*Klebsiella pneumoniae*	*Staphylococcus aureus*
Psuedomonas sp.	NI	NI	NI	NI	NI
Acinetobacter sp.	NI	NI	NI	NI	5.5
Corynebacterium sp.	NI	NI	NI	8.5	0.4
Actinomyces sp.	NI	NI	NI	7.0	6.5
Clostridium sp.	NI	NI	NI	NI	NI
Bacillus sp.	NI	NI	3.5	4.0	NI
Shigella sp.	0.9	NI	NI	11.0	19.0
Proteus sp.	NI	NI	NI	5.0	6.0
Clostridium sp.	NI	NI	NI	16.0	6.5

NI = No Inhibition

Table 5: Antagonistic effects of refuse fungi on pathogenic bacteria

Refuse fungi	*Bacillus cereus*	*Escherichia coli*	*Klebsiella pneumoniae*	*Salmonella typhi*	*Staphylococcus aureus*
Aspergillus niger	11.0	2.0	3.0	3.0	4.0
Aspergillus flavus	3.0	3.0	2.0	4.0	4.0
Aspergillus repens	4.0	3.0	2.0	2.0	4.0
Penicillium sp.	11.0	11.0	12.0	12.0	13.0

Phytochemical screening revealed that Aframomum melegueta contains antinutritional constituents like Saponins and Tannins, Cardiac glycosides, flavonoids and terpenoids. However, alkaloids, anthraquinonones and Phlobatannins were absent (Table 7). The presence of these bio-molecules may be responsible for the antimicrobial activities of the extract while the absence of some may be responsible for its inactivity at low concentration.

Table 6: Antibiotic sensitivity of pathogenic bacteria isolates

		Zone of inhibition (in mm)									
	Pathogens	OF	C	CF	AM	GN	N	CIP	TE	NF	AX
Gram negative	*Escherichia coli*	17.0	9.0	NI	NI	4.5	2.0	17.0	7.0	12.0	2.0
	Klebsiella pneumoniae	NI	NI	NI	NI	NI	NI	5.0	NI	NI	NI
	Salmonella typhi	16.0	10.0	NI	4.0	4.0	2.0	18.0	8.0	12.0	5.0
		FX	AU	OF	E	CIP	CD	GN	CX	CI	AP
Gram positive	*Bacillus cereus*	NI	NI	14.0	23.5	23.5	4.0	NI	NI	NI	NI
	Staphylococcus aureus	NI	NI	25.0	NI	NI	NI	NI	NI	NI	NI

OF = Ofloxacin, C = Chloranphenicol, CF = Cefunoxine, AM = Ampicillin, N = Nitrofurantione, CIP = Ciprofloxacin, TE = Tetracycline, NF = Narfloxacin, TE = Tetracyclin, AX = Amoxycillin, FX = Floxapen, AU = Augumentin, E = Erythromycin, CD = Clidamycin, GN = Gentamycin, Cx = Cephaplexin, CO = Cotrimoazole, AP = Cloxacilin NI = No Inhibition

Table 7: Phytochemical constituents of *Aframomum melegueta*

Chemical constituents	Results
Saponins	+
Tannins	+
Phlobatannins	-
Anthraquinones	-
Alkaloids	-
Cardiac glycosides	
i. Salkowski test (terpenoids)	+
ii. Keller killani test	+
iii. Legal test	+

+ = Present, - = Not present

Although the absence of Alkaloids, Anthraquinones and Phlobatanniins lowered its microbial activity, other Phytochemical Constituents preset justified their medicinal used as stated by Gordian, (2005). Alkaloids were mentioned alongside with other phytochemicals like tannins, flavonoids and phenolic compounds as most important bioactive constituents of leaf extracts (Edeoga, *et al.* (2005) and these compounds have *in vitro* antimicrobial properties according to Cowan (1999).

Almost all the refuse isolates were active against at least one of the pathogens.It therefore mean that they possess broad spectrum of activity more than the antibiotics. Krasil (1997) stated that the properties of antagonism is characteristic of all species of microorganisms. The results obtained here were in conformity with the report of Brock and Madigan, (1988) that antibiotics-producing microorganism occur very frequently in natural habitats However, better consistency was observed with the use of standard antibiotic discs.

CONCLUSIONS

In general, microbe-microbe interactions have not yet been extensively explored. The present study offers further knowledge on the subject matter of microbe interaction and specifically, antagonism. With further improvements, there is possibility of refuse isolates being used as biocontrol for pathogenic organisms.

The need to study medicinal plants cannot be overemphasized for a vista of reasons including *inter-alia* widespread use of plants in folk's medicine, rescuing traditional plants and knowledge about them from imminent loss as well as the need for health for all. The plant Aframomum *melegueta* can be of immense use in phytomedicine and can then be included in health care delivery system particularly in the developing economy. Further studies on more effective method of extracting only the necessary constituents and standard reconstitution means as well as other better processing, refining and purification measures using HPLC, GC, NMR and mass spectroscopy for structural elucidation of the biologically active molecules would be necessary.

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