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Antimicrobial activities of some Nigerian spices on some pathogens

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ABSTRACT

The aqueous and ethanol extracts of four spices (*Monodora myristica, Piper guineense, Xylopia aethiopica, Tetrapleura tetraptera*) were prepared and the antibacterial properties assessed using the agar diffusion method. The test organisms were Enterohaemorrhagic *E.coli, Shigella, Salmonella, Klebsiella, Pseudomonas, Klebsiella pnemonium, Staphylococcus aureus, Staphylococcus aureus (ATCC 25923), Bacillus* sp. and *Enterococcus faecalis*. The susceptibility of the test bacteria strains to various antibiotics was performed. The aqueous extracts had antimicrobial activities on all test organisms used (MIC values of 30-60mg/ml and a range of inhibition, 10-25mm). The ethanol extracts were less sensitive (3.3-26mg/ml on *E. feacalis*). The phytochemical screening of the potent extracts revealed the presence of terpenoids, flavonoid and glycosides. The test organisms showed susceptibility to majority of the antibiotics used ranging from an average of 10mm-37mm. The aqueous extracts can be used as an alternative therapy to the use of antibiotics as the zones of inhibition exhibited by the test strains to both were comparable.

Key words: Antimicrobial, plant extract, phytochemical, Inhibitory and agar diffusion

INTRODUCTION

Spices which include plant materials of medicinal importance have been used for the treatment of human ailments as far back as prehistoric times (Cowan, 1999). Spices are used as condiments and ingredients in foods. In Nigeria, some are used for the preparation of certain type of soups which are delicacies and also recommended for fast relief of ailments such as malaria fever (Sofowora, 1993). The use of medicinal plants in traditional medicine has been recognised and widely practised. According to the World Health Organization, 80% of the world's population rely on traditional medicines to meet their health regiments (Maffi, 1999). Many commercially proven drugs used in modern medicine were initially used in crude form in traditional or folk healing practices or for other purposes that suggested potentially useful biological activity. Medicinal plants generally contain a number of compounds which may be potential natural antibacterial for the treatment of common bacterial infections (Ratnasooriya *et al.,* 2005). It is estimated that today, plant materials are present in or have provided models for 50% of western drugs (Robbers, 1996). Plant derived medicines are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment (Kareem *et al.,* 2010, Raskin *et al.,* 2002). Therefore there is urgent and continuous need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases (Rojas *et al.,* 2003).

MATERIALS AND METHODS

Sample Collection: The spices *Xylopia aethiopica, Monodora myristica, Tetrapleura tetraptera* and *Piper guineense* were purchased and identification confirmed by a plant taxonomist at the Department of Botany, University of Lagos, Akoka.

Bacterial strains: The test organisms were obtained from the Department of Microbiology and Parasitology, Lagos University Teaching Hospital, (LUTH). Cultures of these test organisms were maintained on Nutrient agar slants at 4ºC. The test organisms were:-

- **Gram-negative:** Enterohaemorrhagic *E.coli (EHEC), Shigella, Salmonella, Klebsiella, Pseudomonas* and *Klebsiella pneumonia*.
- **Gram-positive**: *Staphylococcus aureus, Staphylococcus aureus (ATCC 25923), Bacillus sp* and *Enterococcus faecalis.*

Extraction of Spices: Approximately 250gm of each of the pulverised spices was weighed, suspended in 100ml of distilled water and 70% v/v ethanol and left to stand in the refrigerator for four (4) days. The extracts were filtered and dried in the oven at 60ºC. Each of the dried extract was reconstituted and stored in the refrigerator at 4ºC prior to use.

Preparation of bacterial culture: The stock culture of each of the bacteria used was subcultured at 37^0C for 24 hours. The culture was emulsified in 3ml sterile saline and adjusted using 0.5 McFarland's standard.

Assay for antimicrobial activity: Antimicrobial activity was determined using the agar diffusion method (Rojas *et al.,* 2003). Wells made into previously seeded Mueller Hinton agar plates containing 10^8cfu/ml (0.5 McFarland's standard) of each of the test organism were filled with 0.2ml of each extract. Ethanol (70% v/v) and sterile distilled water were used as controls. The plates were incubated at 37^0C for 24hrs. All tests were performed in duplicates and antimicrobial activity expressed as the mean diameter of the clear zone (mm) produced by the plant extracts. Varying concentrations of the extracts were prepared and the above procedure repeated. Minimum inhibition concentrations were determined for each extract. The bacteriostatic and bacteriocidal effects were determined as the plates were further incubated for another 48hrs. The ability to maintain clear zone after 72hrs was considered as bactericidal and the presence of tiny colonies on the zone of inhibition was taken as bacteriostatic.

Determination of Minimum inhibitory concentration (MIC): The extracts that exhibited considerable activity were diluted double fold (2:2) with nutrient broth in a series of six test tubes. An aliquot of 1ml of the bacterial suspension (1 X 10^8) was inoculated into each tube. The control tubes were inoculated with same quantity of sterile distilled water and 75% ethanol. All tubes were incubated at 37^0C for 24h. The lowest concentration that did not permit any visible growth when compared with the control was considered as the minimum inhibitory concentration. The contents of all tubes that showed no visible growth were cultured on MacConkey agar plates and incubated at 37^0C for 24hrs. The minimum bacteriocidal concentration was considered as the lowest concentration that could produce a single bacterial colony.

Antibiotic susceptibility pattern of test strains: The following antibiotics: Nitroturaman (200µg), Gentamycin (10µg), Naldixic (30µg), Ofloxacin (200µg), Augmentin (30µg), Tetracycline (10µg), Amoxicillin (200µg), Cotrimoxazole (25µg), Chloramphenicol (10µg), Cloxacillin (5µg), and Erythromycin (5µg), were purchased from ABTEK laboratories, Liverpool, UK. The susceptibility of the test bacteria strains to various antibiotics was performed following National Committee for Clinical Laboratory Standard Recommendation (NCCLS, 2006a). McFarland's standard (0.5) was used to standardize bacteria suspension to 10^8CFU/ml. Sterile swabs were dipped into the standardized bacterial suspensions and then streaked in three directions over the surface of the agar plate and allowed to dry for 5mins before the antibiotics were applied. The zones of inhibition were recorded after incubation at 37^0C for 24h.

Phytochemical analysis of plant extracts: Qualitative phytochemical analysis **(**Harbone, 1998) of some of the extracts that had maximum antibacterial activity was determined for the presence of alkaloids, flavonoids, glycosides, tannins, terpenoids and saponins.

RESULTS

Assay for antimicrobial activity: The aqueous extracts showed antibacterial activities on the test organisms used. Zone of inhibition ranged from 12-25mm with *Piper guineense* and 10-18mm with *Xylopia aethiopica*. The ethanol extracts had minimal effect on the test organisms. (Tables 1 and 2).

Table 1: Zones of inhibition of aqueous extracts of the spices in mm

Test organism	*Piper guineense*	*Mondora myristica*	*Tetrapleura tetraptera.*	*Xylopia aethiopica*	Control
Shigella	20	0	8	0	0
S.aureus	25	0	10	18	0
ATCC *25923*	20	0	15	18	0
K. pneumonia	15	0	10	0	0
Klebsiella	20	0	15	11	0
E. feacalis	20	10	10	15	0
Bacillus	20	10	11	0	0
Salmonella	20	0	12	0	0
Pseudomonas	23	0	10	10	0
EHEC	12	0	10	0	0

Table 2: Zones of inhibition of ethanol extracts of the spices in mm

Test organism	*Piper guineense*	*Mondora myristica*	*Tetrapleura tetraptera*	*Xylopia aethiopica*	Control
Shigella	10	0	0	10	0
S.aureus	15	0	10	10	0
ATCC *25923*	15	0	10	15	0
K. pneumonia	0	0	0	0	0
Klebsiella	13	10	0	13	0
E. feacalis	0	0	0	0	0
Bacillus	10	9	13	10	0
Salmonella	0	0	0	0	0
Pseudomonas	6	0	0	6	0
EHEC	0	0	0	0	0

Effect of concentration on antimicrobial activity showed that the trend was similar for all extracts as higher concentrations produced wider zone of inhibition. (Figures 1- 7).

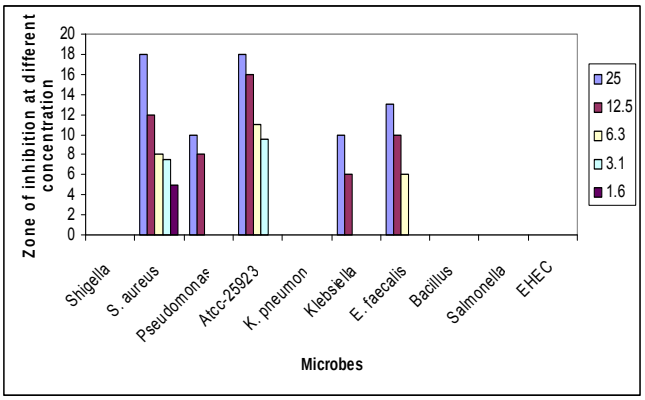

Fig 1: Graph showing zone of inhibition (mm) of aqueous extract of X. aethiopica at different concentration (mg/ml) on the test organisms.

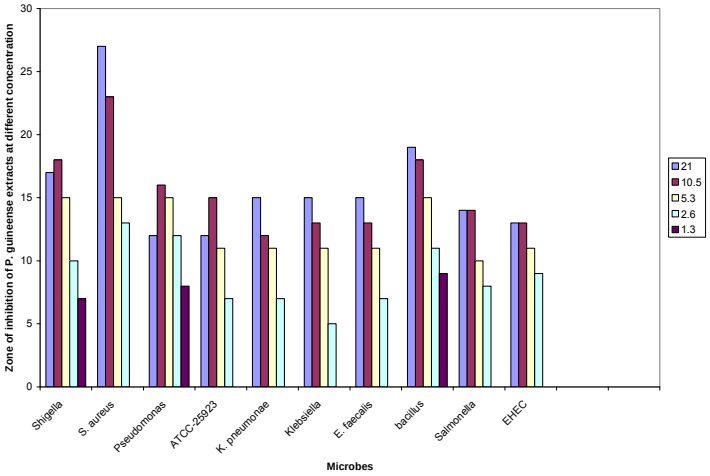

Fig 2: Graph showing zone of inhibition (mm) of aqueous extract of *P. guineense* at different concentration (mg/ml) on the test organisms.

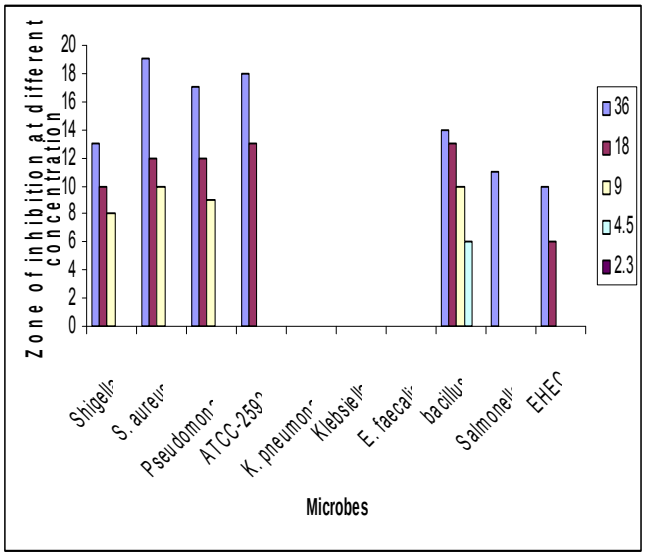

Fig 3: Graph showing zone of inhibition (mm) of aqueous extract of *T. tetraptera* at different concentration in mg/ml on the test organisms.

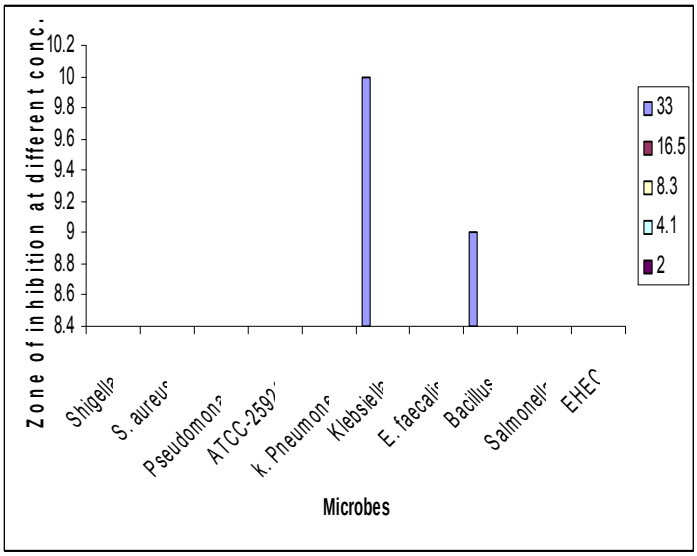

Fig 5: Graph showing zone of inhibition (mm) of ethanol extract of *M. myristica* at different concentration in mg/ml on the test organisms.

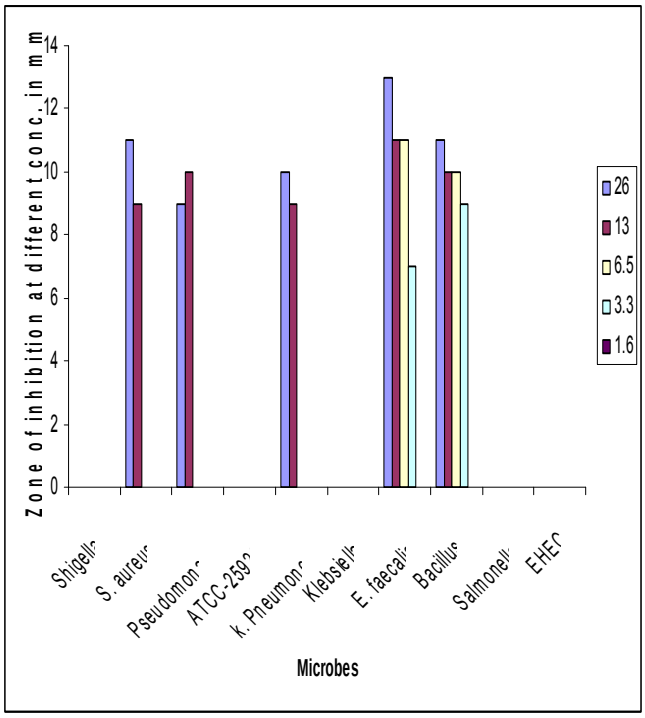

Fig 4: Graph showing zone of inhibition (mm) of ethanol extract of *X. aethiopica* at different concentration in mg/ml on the test organisms.

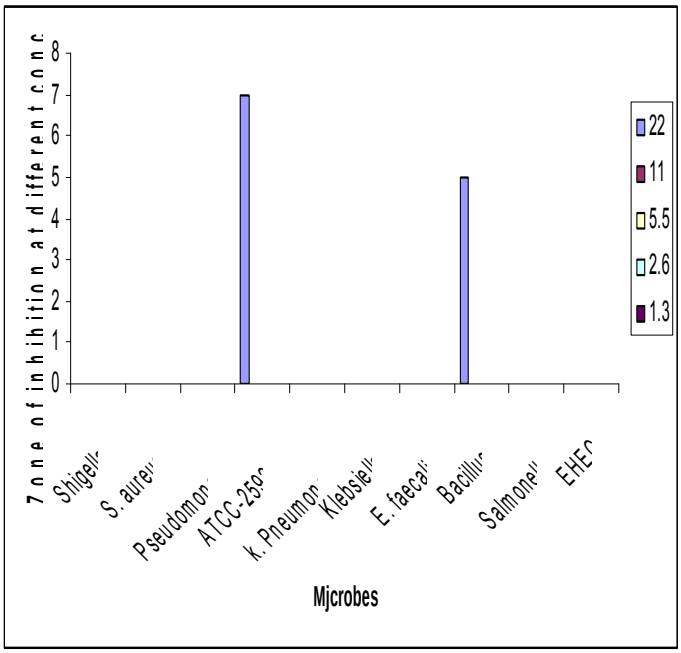

Fig 6: Graph showing zone of inhibition (mm) of ethanol extract of *P. guineense* at different concentration in mg/ml on the test organisms.

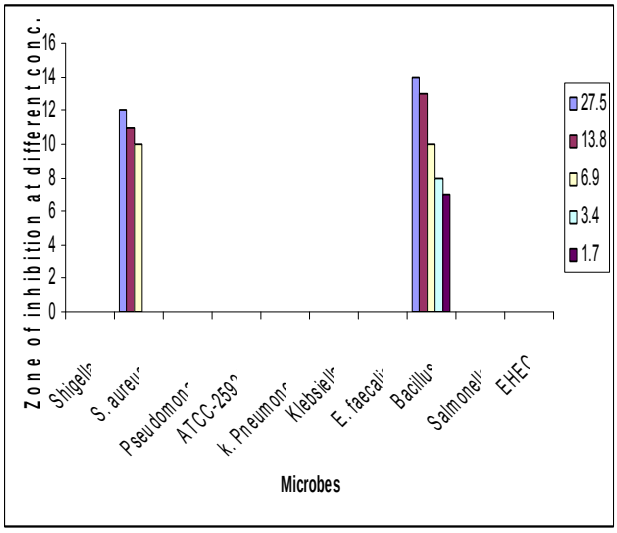

Fig 7: Graph showing zone of inhibition (mm) of ethanol extract of *T. tetraptera* at different concentration in mg/ml on the test organisms.

Determination of MIC: The aqueous extracts had antimicrobial activities on all test organisms used with MIC values of 30-60mg/ml. Some however were bacteriocidal while others were bacteriostatic (Table 3). The ethanol extracts were less sensitive (3.3-26mg/ml on *E. feacalis*).

Antibiotic susceptibility pattern of test strains

The bacterial isolates showed susceptibility to majority of the antibiotics used ranging from an average of 10mm-35mm. They all showed resistance to at least one of the antibiotics. None showed total resistance to all the antibiotics (Tables 4 and 5)

Table 3: Result of bactericidal and bactericidal effect after 72 hours of incubation

Organism	*Piper guineense*		*Monodora myristica*		*Xylopia aethopica*		*Terapleura tetraptera*	
	aq	a/c	Aq	a/c	Aq	a/c	aq	a/c
Shigella	Bl	Bc	Bc	-	-	-	Bc	-
S.aureus	Bl	-	Bc	Bl	Bl	Bc	Bc	Bc
Pseudomoniae	Bl	-	Bc	-	Bc	Bc	Bl	-
EHEC	Bl	-	Bc	-	-	Bc	Bc	-
ATCC 25923	Bl	Bc	Bc	-	Bl	Bc	Bl	Bc
K. pneumonia	Bl	-	Bc	-	Bc	Bl	Bl	-
Klebsiella	Bl	-	Bc	Bc	-	Bl	-	-
E. feacalis	Bc	-	Bc	-	-	Bc	-	-
Bacillus	Bl	Bc	Bc	Bl	Bl	Bl	-	Bl
Salmonella	Bl	-	Bc	-	Bl	Bc	-	-

Bl----Bactericidal
Bc---Bacteriostatic

Table 4: Antibiotics Susceptibility pattern of Gram Positive strains in mm

Bacteria	Antibiotics							
	Cxc	Gen	Cot	Chl	Aug	Amx	Ery	Tet
Enterococcus faecalis	0	14.5	19	14	28	15.5	0	0
Staphylococcus aureus	12	10	0	26	25	16	16	0
Bacillus sp.	0	25	0	24	14	11.5	11	23
Staphylococcus	22	30	33	22	35	34	0	24

Table 5 Antibiotics Susceptibility pattern of Gram Negative strains in mm

Bacteria	Antibiotics							
	Nit	Gen	Nal	Ofl	Aug	Tet	Amx	Cot
Klebsiella pneumonia	21	15.5	20.5	30	0	15.5	0	21
Pseudomonas sp.	0	18.5	0	19	0	5	0	0
Escherichia coli	30	29	0	37	0	0	0	15.5
Salmonella sp.	18	17.5	20	27	0	14.5	0	19.5

Table 6 Result of Phytochemical test

Phytochemicals	*P.gunineense* (ethanol extract)	*T. tetraptera* (aqueous extract)	*P.gunineense* (aqueous extract)	*X. aethiopica (*ethanol extract*)*
Alkaloids	_	_	_	_
Flavonoids	+	+	+	_
Glycosides	_	+	+	+
Tannins	_	_	+	_
Terpenoids	_	+	+	+
Saponins	_	+	+	+

Phytochemical properties of plant extracts: The aqueous extract of *Piper guineense* contained terpenoids, tannins, saponins, flavonoids and glycosides except alkaloids while its ethanol extract contained only flavonoids and terpenoids (Table 6).

DISCUSSION:

The activity of plant extracts against bacteria has been studied for years but in a more intensified way during the last three decades. During this period numerous antimicrobial screening evaluations have been published based on the traditional use of Chinese, African and Asia plant-based drugs (Suffrendini *et al.,*2004). A large number of constitutive plant components have been reported to

have antimicrobial activity. Well known examples include phenols, unsaturated lactones, saponins, cyanogenic glycosides and glucosinolates (Adejumobi *et al.,* 2008*)*. The inhibitory effect of four plant extracts (spices) on some test organisms was investigated in vitro. The results obtained in this study revealed the antibacterial potential of these extracts especially the aqueous extract of *Piper guineense*. This is not surprising as the antimicrobial nature of many edible plant extracts such as cranberry, lime and lemon juices have been demonstrated (Mara *et al.,* 2003). The influence of solvent for extraction on the inhibitory capacity of the extract on the test organism has been reported by Al-Bayati and Sulaiman (2008).

The antimicrobial properties of substances are desirable tools in the control of infections and in food spoilage (Aboaba, *et al.,* 2005). The high level of sensitivity observed in the aqueous extracts towards the bacterial pathogens showed that the active components were soluble in water. This property is very desirable as these spices are used as condiments in food preparation. This supports the extensive use of these spices for treatment of ailments by traditional African medical practitioners. It is believed that the *Piper guineense* stimulates the production of hydrochloric acid in the stomach and promotes the health of the digestive tract.

Phytochemical screening showed the presence of terpenoids and the absence of alkaloids in all the spices. This is not surprising as alkaloids readily decompose with time. Aqueous extract of *P. guineense* indicated the presence of flavonoids while *Tetrapleura tetraptera* and *P. guineense* aqueous extracts contain glycosides. The glycosides detected are non-toxic but can get hydrolyzed to release phenolics which are toxic to microbial pathogens (Aboaba and Efuwape, 2001). Several phenolic compounds like tannins present in the cells of plants are potent inhibitors of many hydrolytic enzymes such as pectolytic macerating enzymes used by plant pathogens (Abd El Rahman *et al.,* 2003, Osbourn, 1996). Plant based antimicrobials have enormous therapeutic potentials as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials (Iwu *et al.,* 1999).

The active components of these extracts usually interfere with the growth and metabolism of microorganisms in a negative manner and are quantified by determining the minimum inhibitory concentration and the minimum bactericidal activity. These values are used as guide for the treatment of most infections (Aboaba, *et al.,* 2005).

Comparing the sensitivity of the bacterial strains to both the plant extracts and to synthetic antibiotics, the result showed that the plant extracts can be used as an alternative to the antibiotics as the zones on inhibition shown were very comparable and the extracts have lesser side effects which are often associated with the use of antibiotics (Marchese and Shito, 2001; Poole, 2001). Also the issue of resistance to these extracts cannot arise as is found with antibiotics (Kareem *et al.,* 2010). The results obtained support the fact that further work needs to be done to determine and identify, purify and quantify the antibacterial compound within these plants and also to determine their full spectrum of efficacy.

CONCLUSION

The aqueous extracts show promise and form a primary platform for further phytochemical and pharmacological studies for use as alternative therapy.

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