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Antimicrobial Effects of Spices Indigenous to South Eastern Nigeria on Some Test Pathogenic: Bacteria (E.coli, Salmonella typhi, Pseudomonas aeuroginosa and Bacillus sp.)

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Abstract:
The aqueous and ethanol extracts of four spices (Monodora myristica, Piper guineense, Detarium microcarpun, Tetrapleura tetraptera) were prepared and the antibacterial properties assessed using the agar diffusion method. The test organisms were E. coli, Salmonella typhi, Pseudomonas aeuroginosa and Bacillus sp. 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. 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.

Keywords: *Spices, antimicrobial activities, aqueous extract, susceptibility, phytochemical screening*

1. Introduction

Spices such as Detarium microcarpun, Tetrapleura tetraptera, piperguineense, Monodora myristica which include plant materials of medicinal importance, have been used for the treatment of human ailments as far back as prehistoric times [7]. These 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 [2] [3]. Spices are widely used for flavoring foods since the ancient times, and as natural medicines against a variety of human diseases. Most of the Asian countries are rich heritage of traditional medical system and rich biodiversity of the diverse range of spices used for treatments [30]. Spices could be a seed, fruit, root, bark, berry, bud or vegetable substance that is primarily used for flavoring, coloring or preserving food [31]. Due to the presence of different chemicals and metabolites in different amount in the plant body, many spices exhibit antimicrobial properties [29]. Spices are more commonly used in warmer climates, where infectious diseases are common [32]. Usage of spices is prominent in meat, which is particularly susceptible to spoiling by microbes[34] [35] [36]. Other than the medicinal usage, the spices are used for the production of cosmetics and perfume and sometimes they are directly consumed as fresh vegetables [33] [36].

The use of medicinal plants in traditional medicine has been recognized and widely practiced. According to the World Health Organization, 80% of the world's population relies on traditional medicines to meet their health regiments [2]. 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 [25]. It is estimated that today, plant materials are present in or have provided models for 50% of western drugs [25]. Plant derived medicines are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment [11].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 [27].

The African traditional medicine is the oldest medicinal system and often culturally referred to as the Cradle of Mankind [35]. Traditional herbal medicines have been used to treat infectious diseases for thousands of years in various parts of the world [4]. There has been a renewed interest in indigenous medicine worldwide because orthodox medicine is not widespread [16]. In poor countries, the health care has been sustained by other practices based on cultural alternatives [37]. In many developing countries, including Nigeria, 80% of patients use indigenous herbal remedies to treat infectious diseases [22]. Despite the availability of modern medicine in some communities, herbal medicines (medicinal plants) have continued to maintain popularity for historical and cultural reasons, in addition to their efficacy and cheaper cost [16]. They also represent sources of potentially important new pharmaceutical substances since all parts of a plant, from roots to seed heads and flowers, are employed in traditional remedies and can, therefore, act as sources of lead compounds [37]. Moreover, molecules from natural products have represented about 80% of drugs that have been put into the market [16]. The use of plant remedies has steadily increased worldwide in recent years as well as the search for new phytochemicals that potentially could be developed as useful drugs for the treatment of infectious diseases [37].

Medicinal plants constitute of primary and secondary metabolites which are of great therapeutic value [5] [6]. Medicinal plants play a major role in the traditional health care system of the rural population in developing Asian countries. People living in the remote areas of the developing countries in Asia and Africa depend on home care and traditional natural medicines for therapies. Most of these therapies are done using plant extracts. The plant extracts are used to treat wide range of diseases like asthma, urinary tract infection, intestinal disorder and recurrent fever [1]. Further, the natural herbal treatments are famous and effective for their cost effective and ecofriendly aspects, in spite of the introduction of a number of different antibiotics by the pharmacological industries time to time [2] [3]. Human body naturally develops resistance to some antibiotics in the long run, but the natural herbal or spices that are in practice have no such resistance. The development of resistance to conventional antimicrobials has been a serious issue in the invention of new medicines for both acute and chronic diseases. There has been more constant focus in the development of newer antimicrobial agents [7]. Hence there is an increasing trend of using plants or plant products as therapeutic agents for the control of antibiotic resistant microbes [18] [19].

Although the primary purpose of spices is to improve the taste and aroma of the food preparations, they are important due to its medicinal, antimicrobial and antioxidant properties [14] [15]. The spices have unique aroma and flavor due to the presence of different compounds known as photochemical or secondary metabolites [1] [20]. Numerous classes of phytochemicals including is o flavonoids, anthocyanins and flavonoids are found associated with the spices [28]. There is a growing interest in using natural compounds in food protection instead of chemical preservatives [1]. The objective of this study was to evaluate the antimicrobial effect of aqueous and ethanol extract of four common spices indigenous to South Eastern Nigeria namely Detarium microcarpum, Tetrapleura tetraptera,Piper guineese, and Monodora myristica on four test pathogenic bacteria, E.coli, Bacilus sp, P. aeruginosa and Salmonella typhi and to determine the antibiotic susceptibility pattern of these test strains

2. Materials and Methods

2.1. Sample Collection

Four spices Detarium microcarpun, Tetrapleura tetraptera,Piper guineense, Monodora myristica were purchased from Orsumoghu village in Ihiala local government area, Anambra state, Nigeria and were identified by the department of Botany,Nnamdi Azikiwe University, Awka, Nigeria.

2.2. Test Organisms

Pure cultures of E. coli, Bacilus sp, P. aeruginosa and Salmonella typhi were obtained from the department of microbiology, Chukwuemeka Odumegwu Ojukwu University (COOU) Uli. These test organisms were maintained on a nutrient agar slant at 4°C.

2.3. Extraction Of Spices

The extraction was carried out in accordance with the procedure of [23]. The four different spices Detarium microcarpun (Ofor), Tetra pleura tetraptera (Uhiokirihio), Piper guineense (Uziza seed), Monodora myristica (Ehuru) were cut into bits respectively, sun dried for 6 days and then pulverized into powder using manual grinder [23]. 10g of the pulverized Detarium microcarpun andTetrapleura tetraptera were weighed using a weighing balance and soaked in two beakers containing 100ml of distilled water and 70% ethanol respectively, while 5g of the pulverized Piper guineense seeds were weighed and suspended in two beakers containing 50ml of distilled water and 70% ethanol. Also, 5g of the Monodora myristica were weighed and suspended in two beakers containing 50ml of distilled water and 70% ethanol. The distilled water and ethanol preparations were allowed to stand for 7 days with interval shaking of 1hour (This is to prevent the growth of mold).

The four preparations were filtered inside a conical flask using What man filter paper no.1, pore size of 11μm and a thickness of 180μm.

2.4. Preparation of Bacterial Culture

The stock culture of the test organisms used were sub cultured at 37°C for 24hrs. The media used was prepared following the manufacturer's instruction as stated by [20] where by 11.5g of the nutrient agar were weighed and poured into a beaker containing 500ml distilled water. The agar is then sterilized at 121°C for 15mins using an autoclave after which sub culturing is done under aseptic conditions. The turbidity of the bacterial suspension was standardized using 0.5 McFarland's solution.

2.5. Assay for Antimicrobial Activity

Antimicrobial activity was determined using the agar well diffusion methods [27]. Wells were made using cork borers into previously seeded Mueller Hinton agar plates containing 108cfu/ml of the test organism. The wells made on the agar plates were filled with 0.2ml of each of the spice extracts using a micropipette and 70% ethanol with sterile distilled water were used as controls. The plates were then incubated at 37°C for 24hrs, all tests were performed in duplicates and the antimicrobial activity expressed as the mean diameter of the clear zone (mm) produced by the spice extracts. Minimum inhibition concentrations were determined for each of the spice extracts. The bacteriostatic and bactericidal effects were determined as the plates were further incubated for another 48hrs. The ability to maintain clear zone after 72hrs is then considered as bactericidal and the presence of very little colonies on the zone of inhibition was taken as bacteriostatic.

2.6. Determination of Minimum Inhibitory Concentration (MIC)

This method was done by takingthe spice extracts that exhibited considerable activity and diluting it double fold with nutrient broth in a series of six test tubes. An aliquot of 1ml of the bacterial suspension were inoculated into each tube. The control tubes were inoculated with same quantity of sterile distilled water and 70% ethanol. All the tubes were incubated at 37°C for 24hrs. After the incubation period, the tubes were brought out of the incubator and were compared with the control tubes to see for the lowest concentration that did not permit any visible growth and is then considered as the minimum inhibitory concentration. MacConkey agar is then prepared using the manufacturer's procedure and the tubes that showed no visible growth were cultured on it and stored in a refrigerator at 4°C prior to use. The minimum bactericidal concentration was considered as the lowest concentration that could produce a single colony [2].

2.7. Antibiotic Susceptibility Pattern

The following antibiotics: Nitroturaman (200µg), Gentamycin (10µg), Naldixic (30µg), Ofloxacin (200µg), Augumentin (30µg), Tetracycline (10µg), Amoxicillin (20µg), Cotrimoxazole (25µg), were purchased from Medicare laboratories, Lagos, Nigeria.The susceptibility of the test bacteria strains to various antibiotics was performed following National Committee for Clinical Laboratory Standard Recommendation [17]. McFarland's standard (0.5) was used to standardize bacteria suspension to 108CFU/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°C for 24hrs [17].

2.8. Phytochemical Analysis of the Spice Extracts

Qualitative phytochemical analysis of some of the spice extracts that had maximum antimicrobial activity was determined for the presence of alkaloids, flavonoids, glycosides, tannins, terpenoids and saponins.

3. Results

3.1. Assay for Antimicrobial Activity

The aqueous extracts showed antibacterial activities on the test organisms used. Zone of inhibition ranged from 10- 20mm with T.tetrapetra, 0-18mm with D. microcarpum , 12-23mm with P. guineense, and 0-10mm with M. mystrica. The ethanol extracts had minimal effect on the test organisms. (Tables 1 and 2).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- 4).

3.2. Determination of MIC

The aqueous extracts had antimicrobial activities on all test organisms used with MIC values of 30-60mg/ml. Some however were bactericidal while others were bacteriostatic (Table 3).

3.3. 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).

3.4. 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 5 and 6).

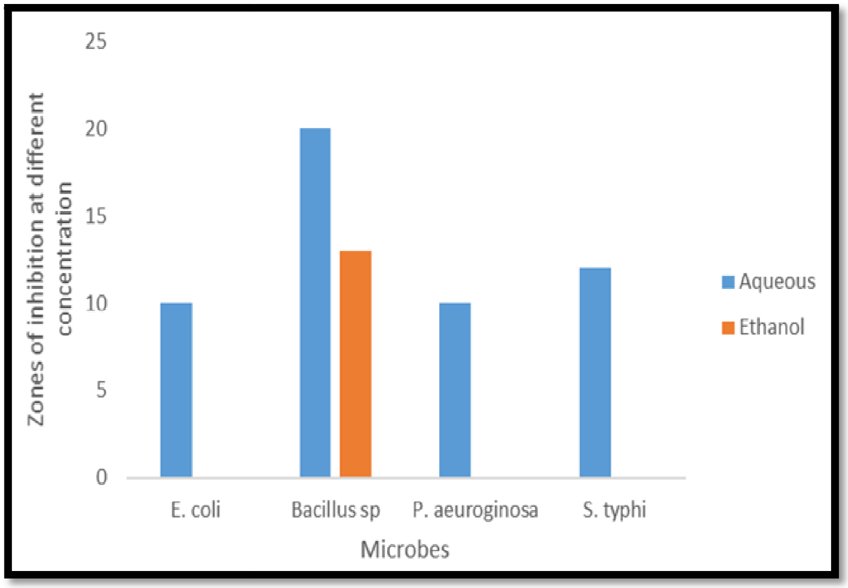

Figure 1: Graph Representing Zone of Inhibition (Mm) of Aqueous and Ethanol Extracts of T. Tetrapetra on Test Organisms

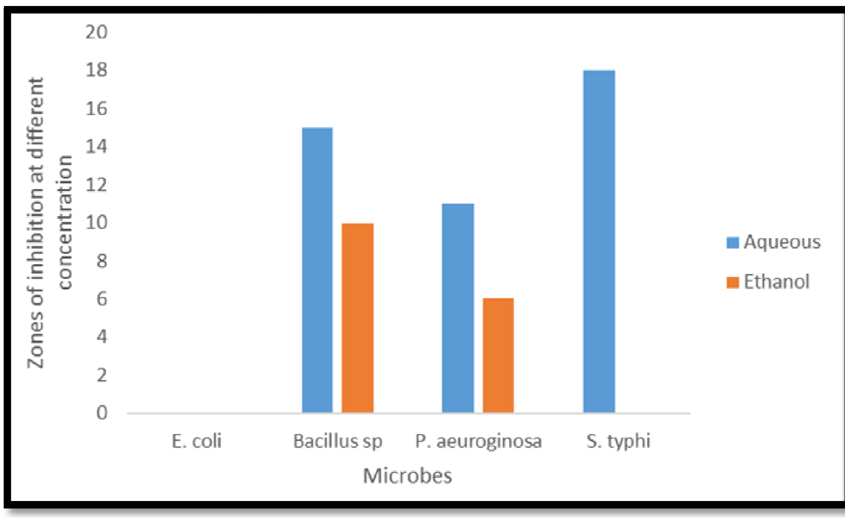

Figure 2: Graph Representing Zone of Inhibition (Mm) of Aqueous and Ethanolic Extract of D.Microcarpun on Test Organisms

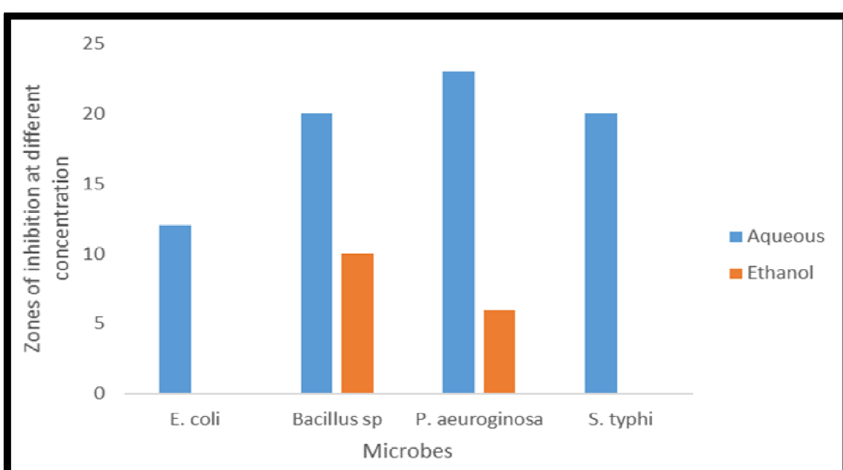

Figure 3: Graph Representing Zone of Inhibition (Mm) of Aqueous and Ethanol Extracts of P. Guineense on the Test Organisms

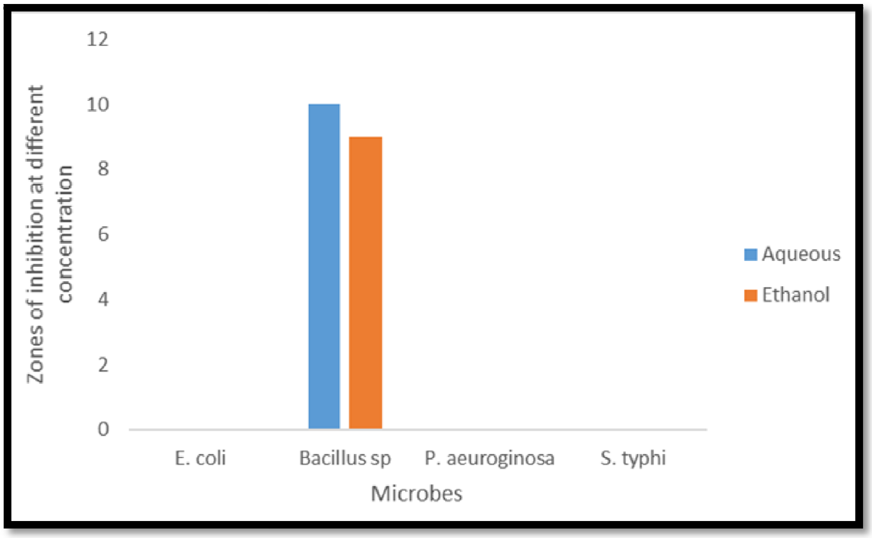

Figure 4: Graph Representing Zone of Inhibition (Mm) of Aqueous and Ethanolic Extracts of M. Mystrica on Test Organisms

Test Organism	T.Tetrapetra	D.Microcarpun	P. Guineense	M. Mystrica	Control
E. coli	10	0	12	0	0
Bacillus sp	20	15	20	10	0
P. aeuroginosa	10	11	23	0	0
S. typhi	12	18	20	0	0

Table 1: Zones of Inhibition of Aqueous Extracts of the Spices in Mm

Test organism	T.tetrapetra	D.microcarpun	P. guineense	M. mystrica	Control
E. coli	0	0	0	0	0
Bacillus sp	13	10	10	9	0
P. aeuroginosa	0	6	6	0	0
S. typhi	0	0	0	0	0

Table 2: Zones of Inhibition of Ethanolic Extracts of the Spices in Mm

Organisms	T.Tetrapetra		D.Microcarpun		P. Guineense		M. Mystrica	
	Aq	a/c	aq	a/c	aq	a/c	aq	a/c
E. coli	Bc	-	-	Bc	Bl	-	Bc	-
Bacillus sp	-	Bl	Bl	Bl	Bl	Bc	Bc	Bl
P. aeuroginosa	Bl	-	Bc	Bc	Bl	-	Bc	-
S. typhi	-	-	Bl	Bc	Bl	-	Bc	-

Table 3: Result of Bacteriostatic and Bactericidal Effect after 72 Hours of Incubation
Bl----Bactericidal
Bc---Bacteriostatic

Organisms	Antibiotics							
	Gen	Nit	Nal	Ofl	Aug	Tet	Amx	Cot
E. coli	29	30	0	37	0	0	0	15.5
Bacillus sp	25	0	0	0	11.5	23	11.5	0
P.aeuroginosa	18.5	0	0	19	0	5	0	0
S. typhi	17.5	18	20	27	0	14.5	0	19.5

Table 4: Antibiotics Susceptibility Pattern of the Test Organisms in Mm
Gen - Gentamycin
Nit - Nitroturaman
Nal - Naldixic
Ofl - Ofloxacin
Aug-Augumentin
Tet- Tetracycline
Amx- Amoxicillin
Cot - Cotrimoxazole

Phytochemicals	T.tetrapetra	D.microcarpun	P. guineense	M. mystrica
Alkaloids	-	-	-	+
Flavonoids	+	+	+	+
Glycosides	+	+	+	-
Tannins	-	-	+	+
Terpenoids	+	-	+	+
Saponins	+	+	+	+

Table 5: Result of Phytochemical Test for Aqueous Extracts of the Spices

Phytochemicals	T.tetrapetra	D.microcarpun	P. guineense	M. mystrica
Alkaloids	-	-	-	+
Flavonoids	+	+	+	+
Glycosides	-	+	-	-
Tannins	+	-	-	+
Terpenoids	+	-	+	+
Saponins	+	+	-	+

Table 6: Result of Phytochemical Test for the Ethanol Extracts of the Spices

4. 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 [30]. A large number of constitutive plant components have been reported to have antimicrobial activity. 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 [30].

The antimicrobial properties of substances are desirable tools in the control of infections and in food spoilage [2]. 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 most of the spices. 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 phenolic which are toxic to microbial pathogens [3]. 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 [1]. Plant based antimicrobials have enormous therapeutic potentials as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials.

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 [2]. 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 [18]. Also,the issue of resistance to these extracts cannot arise as is found with antibiotics [11]. 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.

5. 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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