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ANTI-MICROBIAL PROPERTIES OF SOME COMMONLY USED SPICES

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ABSTRACT

The anti-microbial properties of four commonly used spices were investigated. The spices were *Allium sativum* (garlic), *Allium cepa var.cepa* (onion), *Zingiber officinale* (ginger) and *Piper guineense* (Uziza-Ibo) seeds. Phytochemical screening of the spices revealed the presence of tannins, *saponins,* oxalates and cyanogenic glycosides. The crude aqueous and alcoholic extracts of the spices were found to inhibit a number of test microorganisms (5 bacterial species and a fungus), except *Neisseria gonorrheae*. *Shigella dysenteriae* was found to be resistant to both aqueous and alcoholic extracts of ginger and uziza. The alcoholic extracts of all the spices were found to be more effective compared to the aqueous extracts. Anti-fungal activity of the extracts of the spices was also observed. By this study, the usefulness of these spices for medicinal purposes has been verified, this being indicated by their anti-microbial activities. Also the present work supports the possible use of these spices as potential medicinal plants, at least for the treatment of those diseases caused by the organisms whose growths were inhibited by the extracts.

KEYWORDS: Anti-microbial properties, spices, extracts, phytochemical screening, medicinal use.

INTRODUCTION

The American Spice Trade Association (ASTA) defined spice as a product of plant origin which is used mainly for the purpose of seasoning foods (ASTA, 1970). This definition points to the fact that a spice must be of plant origin, and should be used as a result of its aromatic properties.

Plants are useful to man not only as food or as sources of raw materials for industrial purposes, but also sources of medicaments (Etang, 1999). Plants have provided man with real and supposed means of healing right from the earliest time. Plants, including their fruits, seeds, twigs, barks, roots and leaves, have been employed in the production of concoctions that proved effective in the treatment of various human diseases (Peng and Wang, 1981; Sofowora, 1984). Chinese primary healthcare depends on plants and they are as important as synthetic drugs, accounting for 30-50 percent of total consumption of medicaments (Xiao, 1981). In Africa, and in Nigeria in particular, traditional medicine healers or herbalists play a vital role in the healthcare delivery system (Sofowora, 1984; Itah, 1996).

Spices are known to have a very broad application in medicine. Many spices have both established and unestablished therapeutic applications. According to Iwuh (1989), some spices are employed in traditional medicine for the preparation of antiseptics and germicides, and as ingredients in medications associated with gynaecological problems and obstetrics.

Piper guineense is a commonly used spice in Nigeria. Garlic, ginger and onion are used internationally. Several spices including garlic are used extensively in the Indian system of medicine for correcting a variety of intestinal disorders (Krishnamurthy and Screenivasamurthy, 1956). Garlic is one plant that is world acclaimed for its usefulness in herbal/folk medicine. It is simply described as being good for every ailment (Duke, 1983).

Therapeutic compounds that are pharmacologically active include alkaloids, cyanogenic glycosides, phlobatannins, polyphenols, saponins and anthraquinones. These are known to be present in most medicinal plants (Sofowora, 1984; Madunagu and Ebana, 1991; Itah, 1996). The test spices were then screened for the presence of some of these bioactive agents.

A lot of wild claims have been made locally and internationally about the therapeutic or curative values of spices. This study will thus show if there is any justification for such claims, in respect of garlic, ginger, onion and *Piper guineense*.

MATERIALS AND METHODS

Sample collection and preparation

Allium sativum (garlic), *Allium cepa var cepa* (onion) and *Zingiber officinale* (ginger) were purchased from the Port Harcourt Garden Market along Kaduna Street, Port Harcourt, Nigeria. *Piper guineense* were purchased from Choba market Choba, Nigeria. All materials were native to Nigeria and were procured fresh in unprocessed state. The purchased ginger was thoroughly washed with tap water, the light outer skin scrapped off using a blunt knife and later cut into tiny pieces. The light scaly leaves on the onion bulbs and garlic cloves were removed and the naked bulbs and cloves washed in plain tap water before being chopped into tiny pieces. The *Piper guineense fruits* were washed with water. All samples so prepared were then dried gently in an air circulating oven in the laboratory and ground manually into fine powder, using a manual grinder (Corona, Landers and CIA. SA). The powder of each sample was sieved through mesh 300µm and stored in an air-tight cellophane bag as stock sample in a refrigerator until required for analyses.

Collection of Test Organisms

The test of bacteria and the only fungus were collected from the University of Port Harcourt Teaching Hospital and the Department of Microbiology, University of Port Harcourt. The bacteria and fungus were maintained as described by Malik (1989). All test organisms were stored at 5°C in refrigerators till required for use. The test bacteria include *Staphylococcus aureus, Shigella dysenteriae, Neisseria gonorrheae, Klebsiella pneumoniae, Escherichia* coli (E coli). The only fungus tested was *Candida albicans*

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Phytochemical Screening

Extracts of the spices were screened for the presence of some active components using standards procedures described by Sofowora (1984), Trease and Evans (1989) and Culei (1982).

Preparation of Aqueous and Alcoholic Extracts of Test Spices: For aqueous extraction, 100g each of dry powered samples of ginger, garlic, onion and *Piper guineense* was soaked in 250 cm^3 of distilled water for 8 hours, with regular stirring at intervals of 3-5 minutes. The mixtures were then heated to boil on a hot plate and then filtered through a Whatman No. 1 filter paper (W and R. Balston Ltd., England). The filtrates were sterilized using a membrane filter of pore size 0.45cm diameter (Millipore Corp., England). Excess water was removed by concentration using rotary evaporator. The volume was reduced to 50cm^3 and then stored in the refrigerator at 5°C until required for use.

The alcohol extraction (ethanol extraction) was carried out as described by Babalola (1988) and Ekpe et al (1990). The powder from each of the samples was prepared in absolute ethanol (BDH Chemicals, Ltd, England) in a ratio of 2:1 (w/v). The extraction was then carried out in an orbital shaker (Stuart Scientific, U.K.) at room temperature for 72 hours. The extracts were then filtered using Whatman No. 1 filter paper and concentrated by evaporation in a water bath. The concentrated extracts were then filter sterilized by membrane filtration technique and stored at 5°C in the refrigerator until required for use.

Anti-Microbial Test

The extracts of the samples (garlic, ginger, onion and *Piper guineense* were used for anti-microbial testing by the disk diffusion method as described by Bauer *et al* (1966). The spreading method of Cruickshank, *et al* (1980) was used. A colony of each test organism was subcultured on nutrient broth and incubated at 37°C for 6 hours. A loopful of such culture was uniformly spread over the surface of a sterile Mueller Hinton Agar (Oxoid OM 337) plates. Sterilized filter paper disk (12mm in diameter each) soaked into the different spice extracts were placed on the different plates with the different organisms. The plates were incubated at 37°C for 24 hours. After incubation the zones of clearance were observed. *Neisseria gonorrheae* was cultured on chocolate agar and tested on modified Mueller Hinton agar to which heated blood (80°C) had been added. The disk diffusion method was also used for the only fungus, *Candida albicans*.

Controls

Filter paper disks of same diameter soaked in the solvents, distilled water and absolute ethanol, were similarly treated as in the samples and the zones of inhibition measured.

Measurement and Interpretation of Zones of Inhibition

The diameters of the zones of inhibition (including the 12mm diameter of the disk) were measured by the use of calipers and then transferred to a millimeter ruler for the value. Clear zones of inhibition indicate susceptibility of the organism, while absence of such zones indicate resistance. Resistant strains were also reported if the diameter of zones was not more than 16mm (that is, at least 2mm on each side of the 1mm disk). Zones of inhibition with diameters of 16mm and above were reported as sensitive.

Chemical Analyses

Cyanogenic glycoside was estimated by the alkaline titration method of AOAC (1984). Oxalate was determined using the method described by Munro and Bassir (1969), while Tannin was estimated by the method of Burns (1971). Saponin determination was done using the method of AOAC (1984).

RESULT

Table 1 shows the results of phytochemical screenings of the spices, garlic, ginger, onion and *Piper guineense*. This revealed the presence of tannins, saponins, oxalates and cyanogenic glycosides.

The levels of these antinutrients in the spices studied are given in table 2. Tannin level in the four spices was 0.01g/100g. Onion had the highest concentration of saponin (48.35%) while the least concentration of 3.99% occurred in ginger. The samples had oxalate contents ranging between 0.03g/100g in onion and 0.23g/100g in ginger. The level of cyanogenic glycosides varied from 0.03g/100g in ginger and *Piper guineense* to 0.06g/100g in onion.

Table 3 shows the anti-microbial effects of aqueous and alcoholic extracts of garlic, ginger, onion and *Piper guineense*. Aqueous and alcoholic extracts of garlic inhibited the growth of test organisms except *E. coli*. Aqueous and alcoholic ginger extracts did not inhibit the growth of *Shigella dysenteriae* and *Neisseria gonorrheae* but effectively inhibited the growth of *Staphylococcus aureus, Klebsiella, pneumoniae,*

Table 1: Phytochemical Screening of the Spices, garlic, ginger, onion and *Piper guineense* (Uziza)

Phytochemical compounds	Spices			
	garlic	ginger	onion	*Piper guineense*
Tannins	+	+	+	+
Phlobatannins	-	-	-	-
Saponins	+	+	+	+
Oxalates	+	+	+	+
Cynogenic glycosides	+	+	+	+

\+ = Present

\- = Absent

Table 2: Concentration* of some Anti-nutrients in garlic, ginger, onion and uziza.

Sample	Tannin (g/100g)	Saponin (%)	Oxalate (g/100g)	Cyanogenic glycosides (g/100g)
Garlic	0.01±0.00	13.93±0.01	0.13±0.01	0.04±0.02
Ginger	0.01±0.00	3.99±0.00	0.23±0.00	0.03±0.00
Onion	0.01±0.01	48.35±0.05	0.03±0.00	0.06±0.03
Piper guineense	0.01±0.00	9.92±0.00	0.08±0.01	0.03±0.01

*Values are mean ± s.d. of three determinations

Table 3: Antimicrobial Effect* of Aqueous and Alcoholic Extracts of garlic, ginger, onion and uziza

	Extracts of Samples							
	Aqueous				Alcoholic			
Test Organisms	**Garlic**	**Ginger**	**Onions**	**Uziza**	**Garlic**	**Ginger**	**Onion**	**Uziza**
Staphylococcus aureus	16.00±0.01	16.00±0.02	-	18.00±0.10	26.00±0.20	26.00±0.10	-	**28.00±0.20**
Shigella dysenteriae	16.00±0.10	-	17.00±0.20	-	16.00±0.30	-	**18.00±0.20**	-
Klebsiella pneumoniae	38.00±0.10	33.00±0.10	38.00±0.20	-	36.00±0.01	31.00±0.02	36.00±0.04	-
Nesseria gonorrheae	18.00±0.01	-	-	-	31.00±0.02	-	-	-
E.coli		20.00±0.02	26.00±0.10	28.00±0.30	-	31.00±0.01	36.00±0.03	**38.00±0.12**
Candida albicans+	20.00±0.10	20.00±0.03	18.00±0.02	18.00±0.01	40.00±0.25	40.00±0.10	28.00±0.04	**28.00±0.03**

Values are mean ± standard deviation of three zones of inhibition (mm)

- = No inhibition (Resistance)

+ = A fungus

E. coli and the only fungus used, *Candida albicans*. *Staphylococcus aureus* and *Neisseria gonorrheae* were found to be resistant to aqueous and alcoholic extracts of onions, while the rest of the organisms including the only fungus used were inhibited. Aqueous and alcoholic extracts of *Piper guineense* inhibited the growth of *Staphylococcus aureus, E. coli* and the fungus, *Candida, albicans,* but were not effective against *Shigella dysenteriae, Klebsiella pneumoniae* and *Neisseria gonorrheae*.

It is worthy to note that the aqueous and alcoholic extracts of all four spices studied were effective against the growth of the only fungus studied (*Candida albicans*). The highest inhibition zones observed were those of alcoholic extracts of ginger and garlic against *Candida albicans*. For the test organisms, where they were sensitive, the alcoholic extracts were consistently more effective or potent with greater zones of inhibition than the aqueous extracts for the corresponding sample, except in the case of *Klebsiella pneumoniae* where the reverse was the case. Inhibition was observed in test organisms by their failure to grow on the medium to which the extracts were applied. It is pertinent to point out here that the degree of inhibition varied. The degree of inhibition was indicated by the diameter (mm) of the zones of inhibition. The higher the value of the zone of inhibition, the more effective is the extract.

DISCUSSION

Phytochemical screening revealed that all four spices had tannin, saponin, oxalate, and cyanogenic glycosides. The presence of these compounds has been associated with anti-microbial properties and other physiological activity (Bloom and Ullyat, 1971; Benjamin and Lamikanra, 1981; Clause, *et al*, 1974; Sofowora, 1980). The presence of these compounds in the spices possibly accounts for part of their usefulness as medicinal spices.

The action of the aqueous and alcoholic extracts of garlic, ginger, onion and *Piper guineense* indicated their inhibitory activity against the growth of various pathogenic micro-organisms, both bacteria and a fungus studied. This showed that the extracts of these spices have broad spectrum antibiotic activity. The anti-microbial property of the extracts resides in the *phytochemical* drug bases contained in the spices (Sofowora, 1984; Ekpe *et al* 1990). The anti-microbial and anti-fungal properties of many other plants have been previously reported (Benjamin, 1979; Emeruwa, 1982; Ebana, *et al*, 1995; Etang 1999). Malik and Chulglitai (1979) reported the anti-microbial activity of the alcoholic extracts of the leaves of *Calotrepis proters* against various pathogenic microorganisms including *Treponema pallidum*.

The results of the anti-microbial properties of garlic and ginger obtained in this work are in agreement with earlier works reported by other investigators (Ahmed, 1966; Skapska, 1996; Delaha and Garagusi, 1985; Watt 1986). Thus alliin and allicin found in garlic are known to have antibiotic properties (Mochizuki *et al* 1977). Garlic juice has also been reported to significantly inhibit fungal growth (Abdel Hafez and El-said, 1997). Aqueous and alcoholic extracts of ginger and garlic have been reported by Ejechi, *et al* (1987) to inhibit the growth of the fungi *Botryodiploda theobromae, Aspergillus niger, Aspergillus flavus, mucor sp., Rhizopus stolonifer, Penicillium sp.* and *Fusarium sp.*, isolated from deteriorating Okra. They concluded from their work that spices may be economically important in the application of hurdle technology for the preservation of fruits.

The present work supports the possible use of these spices as potential medicinal plants, at least for the treatment of those diseases caused by the organisms which were inhibited by the extracts. The problem of drug resistance to antibiotics has led to a decline in the therapeutic value of most antibiotics in existence. This problem is very common in hospital strains (Siebet and William, 1975; Grumberge, 1980). The preponderance of multiple resistant organisms has elicited an unending search for alternative anti-microbial drugs. The results from the anti-microbial tests have proved the extracts of these spices to be veritable sources from which a good number of new anti-microbial substances may be obtained.

CONCLUSION

The present study has provided some basic information on the usefulness of these spices for medicinal purposes. This medical usage is indicated by the anti-microbial property of the extracts of the spices. This study therefore justifies the use of these spices as potential medicinal ingredients, at least for the treatment of those diseases caused by the organisms whose growths were inhibited by their extracts.

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