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Preliminary screening of the antimicrobial activities of some medicinal vegetables and spices indigenous to Abraka South-south Nigeria

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ABSTRACT

The phytochemical and antimicrobial activities of some indigenous Nigerian vegetables and spices against E.coli and C.albicans were determined using the susceptibility and inhibitory concentration technique. The vegetables Cucurbitacaea peltafum, Acalypha begonia and spices Mondora myristica and Aframomum sceptrum extracts showed susceptibility with zones of inhibition diameters of 10mm, 8mm,10mm and 7mm respectively after 24 hours of incubation while vegetables like V. amygdalina, Talinium triangulare, C. peltafum, A. begonia, Telferia occidentalis and the spice Monodora myristica extracts had zones of inhibition of 6mm,7mm,9mm,11mm,10mm and 10mm respectively against C. albicans. The phytochemical screening analysis showed that extracts with antimicrobial activity contains some quantity of phytochemicals like saponin, tannin, flavonoid and alkaloids. These results shows that this plant can be used for the treatment of diseases caused by these microorganisms. These antimicrobial properties exerted may be due to the presence of the phytochemicals.

Keywords: *Phytochemical, antimicrobial activity, spices, vegetables, saponin.*

INTRODUCTION

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans[5].Wide range of dietary phytochemicals are found in fruits, vegetables, legumes, whole grains, herbs and spices[8]. Phytochemicals accumulate in different parts of the plants, such as the roots, stem, leaves, flowers, fruits and seeds[3]. Plants have been used as medical agents from the earliest days of man's existence[1] and has made it necessary to study them in details in order to discriminate the kinds employed for different purposes[4]. The adverse effects of the drugs available today, necessitates the discovery of new harmless pharmacotherapeutic agents from medicinal plants[12]. A great number of antibacterial agents exist for various purposes; some of these are usually in the form of plants and vegetables[11]. The action of these plants on microorganisms have been found to be due to the presence of certain substances such as alkaloids, glycosides, volatile oil, gums, tannins, steroids, saponins, flavonoids and other secondary metabolites present in them[7,9].

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These medicinal plants, vegetables and spices can play a major role in the treatment of bacterial and fungal infections. The aim of this research was to investigate the phytochemicals in these indigenous vegetables and spices and their antimicrobial activities on *Escherichia coli* and *Candida albicans.*

MATERIALS AND METHODS

COLLECTION OF PLANT MATERIALS

Fresh *V. amygdalina, T.triangulare, C. Peltafum, E. Shaveolens, O. canum, G. acidas, A. ghiozzi, Manodora myristica* and *Aframomum sceptrum* were purchased from the local markets in Abraka. These plants were taken to the Botany Department of Delta State University, Abraka, Delta State for proper identification and authentication.

PREPARATION OF EXTRACTS

Aqueous extract: Five grams of leaves was measured into a conical flask and 20mL of sterile distilled water was added, covered with a cork, mixed together properly and left on the shaker at 100 revolutions per minute for 24 hours. The extract was filtered and squeezed through four layers of muslin cloth. The filtrate was centrifuged at 1,500 revolutions per minute for 10minutes after which it was decanted. The pellet was discarded and the supernatant was sterilized by using the membrane filtration unit with type HC filters. The filtrate obtained was stored in sterile McCartney bottles and kept in the refrigerator at 4^oC and later used for antimicrobial tests.

Ethanolic extract: Five grams of leaves was measured into a conical flask and 20mL of 95% ethanol was added, covered with a cork, mixed together and left on the shaker at 100 r.p.m for 24 hours after which the extract was filtered and squeezed through four layers of muslin cloth. The filtrate was centrifuged at 1,500 revolutions per minute for 10minutes after which it was decanted. The pellet was discarded and the supernatant was sterilized by using the membrane filtration unit with type HC filters. The filtrate obtained was stored in sterile McCartney bottles and kept in the refrigerator at 4^oC and later used for antimicrobial tests.

STERILITY TEST OF THE PLANT EXTRACTS

Each of the above extracts (ethanol and aqueous extract) were tested for growth or contaminants. This was carried out by inoculating 1ml of each of them on nutrient agar and incubated at 37^oC for 24hours. These plates were observed for microbial growth. None of the extracts showed growth and they were assessed for antimicrobial activity.

COLLECTION AND MAINTENANCE OF TEST ORGANISMS

The clinical isolates used were collected from Eku Baptist Hospital, Delta State Nigeria. These organisms were *Escherichia coli* and *Candida albicans*. *E. coli* was maintained on nutrient agar slant while *Candida albicans* was on Potato Dextrose Agar slant and stored in the refrigerator at temperature of 4^oC. Subcultures were made from it at regular intervals until it was used for the test.

PHYTOCHEMICAL SCREENING OF THE EXTRACTS

The methods described by Odebiyi and Sofowora (1978) were used to test for the presence of saponin, tannin and alkaloids while the method described by Herbore[6] was used to test for flavonoid.

Testing for saponins: Each extract (0.5g) was mixed with water in test tube. Foaming which persisted on warming was taken as an evidence for the presence of saponins.

Testing for tannins: Each extract (0.5g) was separately stirred with 10mL of distilled water and then filtered. Few drops of 5% $Fecl_3$ reagent was added to the filtrate. Blue-green or blue black colouration or precipitation is an indication of presence of tannins.

Testing for alkaloids:Each extract (0.5g) was stirred with 5mL of 1% HCl on a steam bath. The solution obtained was filtered and 1mL of the filtrate was treated with a few drops of Mayer's reagent. The turbidity of the extract on addition of the reagent was taken as evidence of the presence of alkaloids in the extracts.

Testing for flavonoids: 0.5g of the extract was mixed with 5ml of dilute ammonia solution and then concentrated H_2SO_4 was added. Yellow colourations observed indicates the presence of flavonoids[6].

ANTIMICROBIAL ACTIVITIES OF THE EXTRACTS

Antibacterial test

This was determined using the agar diffusion method[2]. Twenty four hour old broth culture of test organism (standard inocula) were swabbed on sterile Mueller Hinton Agar in Petri dishes using sterile cotton swabs. Sterile stainless steel cork borers (12 mm diameter) was used to make wells on the plates. The plates were filled with 0.1mL, 0.5mL and 1.0mL of the extracts, labeled appropriately and incubated for 24 hours at 37^{o}C.The results were read by measuring the zones of inhibition. Control experiments were equally carried out by filling the holes with sterile distilled water.

Antifungal test

Agar well diffusion method was used to determine the antifungal action of the leaves against *C. albicans* and 0.1mL, 0.5mL and 1.0mL of the extracts were incorporated into the wells in the Sabouraud's Dextrose Agar plates containing the organism. The wells were made using 12mm diameter sterile cork borers. Control experiments were still carried out by filling the agar wells in the Petri dish with sterile distilled water. The plates were incubated at 30^{o}C for 48 hours and the zones of inhibition were recorded.

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC) OF THE EXTRACTS

This was determined against bacteria after the antibacterial test had been performed. Nutrient agar was used and agar diffusion method was employed for those microbes whose growth could be inhibited at lower concentration of the extracts, while pour plate method was used for those bacteria whose growths could be inhibited at higher concentrations, that is, those that showed no inhibition at 0.1mL to 1.0mL of the extracts. Agar diffusion was used for bacteria whose growths were inhibited at 0.5mL of the extracts but not 0.1mL and 0.3mL, while 0.05mL of the extracts were tested against those bacteria that were sensitive to 0.1mL extracts. Holes were made on the plates using 12mm sterile cork borers after seeding it with the desired bacterial strain, left for 1hour at room temperature and incubated for 24 hours at 37^{o}C. The above procedure was used for *C. albicans* but Sabouraud's Dextrose Agar was used instead of nutrient agar and it was incubated at 30^{o}C for 72 hours.

RESULTS

Table 1: Qualitative analysis of phytochemicals in ethanol extract of vegetables and spices

Plants	Tannin	Saponin	Flavonoid	Alkaloids
Vernonia *amygdalina*	+	+	+	+
Talinium triangulare	+	+	+	+
Telferia occidentalis	-	+	-	+
Ertyphlenum shaveolens	-	+	-	-
Cucurbitaceae peltafum	+	+	+	+
Monodora myristica	+	+	+	+
Occimium canum	-	+	-	-
Gladiolus acidas	-	-	-	-
Astragalus ghiozzi	-	+	-	-
Afranomum sceptrum	+		+	+
Acalypha begonia	+	+	+	+
Ipomea batata	+	-	+	-

+ = Presence of constituent, - = Absence of constituent.

Table 2: Qualitative analysis of phytochemicals in aqueous extract of vegetables and spices

Plants	Tannin	Saponin	Flavonoid	Alkaloids
Vernonia amygdalina	+	+	+	+
Talinium triangulare	+	+	+	-
Telferia occidentalis	-	+	+	+
Ertyphlenum shaveolens	+	+	+	-
Cucurbitaceae peltafum	+	+	+	+
Monodora myristica	+	+	+	-
Occimium canum	+	+	-	-
Gladiolus acidas	-	-	-	-

Astragalus ghiozzi	-	+	-	-
Afranomum sceptrum	+	+	+	-
Acalypha begonia	+	+	+	+
Ipomea batata	+	-	-	-

+ = presence of constituents

- = Absence of constituents

Table 3: Diameter (mm) of zones of inhibition of ethanol and aqueous extracts on *E. coli*

Plants	Zones of inhibition diameter (mm) after 24 hrs	
	Ethanol	**Water**
Vernonia amygdalina	9	8
Talinium triangulare	10	8
Telferia occidentalis	-	-
Ertyphlenum shaveolens	-	-
Cucurbitaceae peltafum	10	-
Monodora myristica	10	-
Occimium canum	-	-
Gladiolus acidas	-	-
Astragalus ghiozzi	-	-
Afranomum sceptrum	7	-
Acalypha begonia	8	8
Ipomea *batata*	-	-

- = no inhibition

Table 4: Diameters (mm) of zones of inhibition of ethanol and aqueous extracts on *Candida* albicans

Plants	Zones of inhibition diameter (mm)	
	After 72 hrs	**After 72 hrs**
Vernonia amygdalina	6	7
Talinium triangulare	7	8
Telferia occidentalis	10	-
Ertyphlenum shaveolens	-	-
Cucurbitaceae peltafum	9	-
Monodora myristica	10	-
Occimium canum	-	-
Gladiolus acidas	-	-
Astragalus ghiozzi	-	-
Afranomum sceptrum	-	-
Acalypha begonia	11	9
Ipomea batata	-	-

- = no inhibition

Table 5: Minimum inhibitory concentration (MIC) exhibited by different dilutions of extract on *E. coli*

	DILUTIONS						
Plants	10^{0}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Vernonia amygdalina	S	R	R	R	R	R	R
Talinium triangulare	R	R	R	R	R	R	R
Telferia occidentalis	R	R	R	R	R	R	R
Cucurbitaceae peltafum	S	R	R	R	R	R	R
Monodora myristica	S	R	R	R	R	R	R
Afranomum sceptrum	S	R	R	R	R	R	R
Acalypha begonia	S	R	R	R	R	R	R

R = Resistance

S = Sensitive

Table 6: Minimum inhibitory concentration (MIC) exhibited by different dilutions of extract on *Candida albicans*

	DILUTION						
Plants	10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Vernonia amygdalina	S	S	S	R	R	R	R
Talinium triangulare	S	S	S	R	R	R	R
Telferia occidentalis	S	S	R	R	R	R	R
Cucurbitaceae peltafum	S	S	R	R	R	R	R
Monodora myristica	S	R	R	R	R	R	R
Afranomum sceptrum	S	S	R	R	R	R	R
Acalypha begonia	S	S	R	R	R	R	R

R = Resistance
S = Sensitive

DISCUSSION

All the extracts were rich in steroids which are the common constituents of plants. Alkaloids and tannins were present in six of the extracts, which was in agreement with the report by Rahila *et al.,*[10] who reported that plants contained components which were active against microorganisms. All the ethanol extracts were richer in these metabolites than aqueous extracts which may be due to its ability to extract more components. *T. triangulare* showed the highest zone of inhibition of 10 mm and 8 mm for ethanol and water extracts on *E. coli* respectively. These values were followed by that of *V. amygdalina* with 9mm and 8mm zones of inhibition for water and ethanol extracts (Table 3). Ethanol and aqueous extracts of *Acalypha begonia* had the highest zones of inhibition of 11mm and 9mm on *Candida albicans*. Ethanol extract of *Telferia occidentalis* had a zone of inhibition of 10mm while that of the water extract showed no zone of inhibition. This confirms the fact that ethanol extracts the vital phytochemical constituents of plants. Table 5 and 6 showed the minimal inhibitory concentrations of dilutions of various extract which had antimicrobial properties against *E. coli* and *C. albicans.* Table 5 showed that some plant extract of 10^0 which had higher concentrations were sensitive to *E. coli* (that is suppressed the growth of *E. coli*) but that of *T. triagulare, C. peltafum* and other serially diluted extracts of 10^{-1}, 10^{-2} to 10^{-6} were not sensitive (resistant). This showed that high therapeutic dose of the plant extracts were needed for it to be bactericidal on the microorganism. Table 6 also showed that plant extracts needed for antimicrobial activity against *C. albicans* is in low concentration. That is it is bactericidal at low therapeutic dose of the extracts.

CONCLUSION

The result of this research therefore offers a scientific basis for the traditional use of the vegetable plants *V. amygdalina, T. triangulare, C. peltafum, A. begonia* and spices *M. myristica* and *A. sceptrum* in the treatment of *E. coli* related diseases like traveler's diarrhea and dysentery. Also, they can be used to treat ailments caused by *C. albicans.* It also provides the baseline data for researchers to investigate more into the potential use of these indigenous vegetables and spices in chemotherapy and the relevance of their consumption in our daily foods. But in vivo studies on these medicinal plants are necessary and should seek to determine toxicity of the active constituents, their side effects, serum attainable levels, pharmacokinetic properties and diffusion in different sites. The antimicrobial activities could be enhanced if the active components are purified and adequate dosage determined for proper administration. This will go a long way in curbing the administration of inappropriate concentration which is a common practice among many traditional medical practitioners in Nigeria.

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