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Comparative Studies on Antioxidant and Antifungal Potentials of Some Tropical Spices

Grace O Babarinde [a], Oluwasayo Esther Ogunjinmi [b*], Peter Ifeoluwa Adegbola [c], Oluwatooni Jumoke Adewole [a] and Ibukun Happiness Olatunbosun [a]

[a] Department of Food Science, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.
[b] Department of Industrial Chemistry, First Technical University, Ibadan, Nigeria.
[c] Department of Biochemistry and Forensic Science, First Technical University, Ibadan, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Authors GOB and OEO designed the study and wrote the protocol. Author PIA performed the statistical analysis and wrote the draft. Authors OJA and IHO managed the analyses of the study and literature search, wrote the draft. All authors read and approved the final manuscript.

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ABSTRACT

Spices have lots of health benefits due to their antioxidative properties. However, the antioxidative efficacy of these spices differs from one another and depends on the extracting solvents. This study, therefore aimed at determining the antioxidant properties and antifungal activities of eight tropical spices (*Aframomum danielli, Allium sativum, Piper guineense, Curcuma longa*, *Syzygium aromaticum, Tetrapleura tetraptera, Monodora myristica,* and *Xylopia aethiopica*) using two extracting solvents (water and ethanol). The extracts obtained were analysed for antioxidant and antifungal activities. The antioxidant activities of the extracts were determined using DPPH, FRAP,

**Corresponding author: E-mail: oluwasayo.ogunjinmi@tech-u.edu.ng;*

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nitric oxide, metal ion chelating activity. The IC_{50} values were calculated to identify the extract with the best antioxidant activity. Antifungal activities of the extracts were determined using the mycelia inhibition method. Spice ethanol extracts showed good antifungal properties with higher mycelial inhibition percentages. *M. myristica, X. aethiopica, S. aromaticum, and M. myristica* showed the highest mycelial inhibition percentage *against A. niger* (83.75%), *A. fumigatus* (93%), *A. flavus* (87.5%), *Candida albican* (89.6%) and *Fusarium solani* (88.6%) respectively. *Syzygium aromaticum* and *Tetrapleura tetraptera* showed the best DPPH radical scavenging activity in water and ethanol extracts with IC_{50} values of 0.67 and 0.64, respectively. *M. myristica* and *X. aethiopica* showed the best Ferric reducing antioxidant power for water and ethanol extracts with IC_{50} values of 0.40 and 0.69, respectively. The water extracts showed better metal ion chelating activity than the ethanol extracts. Overall observation showed that the selected food spices are good source of antioxidants, could protect against oxidative stress related diseases and toxicogenic fungi.

Keywords: Food spices; antioxidant; anti-fungal; oxidative stress.

1. INTRODUCTION

Spices are natural ingredients available as dried seeds, fruits, roots, rhizomes, barks, leaves and flowers. They play a major role in food processing mostly as additives in order to enhance colour, flavour or preserve food [1,2]. Apart from their importance in food, spices also serve as medicinal products when utilized over a long period, hence, prevent human diseases. In addition, their values in domestic cooking, pharmaceutical, perfume industries, in insect control, food preservation and safety cannot be overemphasized. It, therefore, becomes imperative to compare the antioxidants and antimicrobial potentials of Nigeria grown spices for culinary purposes. Food spoilage, food safety and food loss are major issues of concern in the developed and developing world [3]. Foodborne diseases are a major cause of human illness and death creating a barrier to socio-economic development in the world today [4]. This foodborne illness may result in acute toxicity that lasts for just a few days or inflicts serious long-term consequences that could include cancer [4,5]. Species from Aspergillus, Fusarium and Penicillium are particularly major food spoilage microorganisms and are highly ubiquitous [3]. Their presence in food could also bring off-flavour formation, production of allergic compounds and mycotoxins, invariably leading to qualitative food losses [6,7,8]. Furthermore, rancidity of food products is a result of oxidation during storage or food processing leading to structural degradation with the development of rancid flavours that reduces the organoleptic characteristics and formation of oxidation products consequently, reduction in shelf life, loss of taste, flavour, food quality and detrimental effect on human health [9,10,11]

The harmful effects associated with synthetic preservatives have limited the interest in their use and had led to the search for new alternatives in natural products [12,13]. Spices are potent tools that are used either whole, ground, or in the form of extracts (oils and oleoresins). In industrial applications, ground spices are used to provide flavour and visual appeal. Their extracts (essential oil and oleoresins) also serve as an alternative to whole and ground spices and provide the stability required in product formulation. They have been proven a potent preservative that may replace some synthetic antioxidants used in food processing [14].

Aside from food preservative potentials, the benefits of these spices include antimicrobial properties, cholesterol and sugar-lowering effects, and anti-inflammatory properties [2,15, 16,17,18]. In Nigeria, natural food ingredients are being used for culinary purposes due to their availability, especially among the rural and urban populations. Spices that have been utilized across the globe include red and green chilli pepper, garlic, onion, cinnamon, ginger, curry, rosemary and nutmeg [1]. These spices have been globally recognized for their phytochemical and anti-oxidative constituents [19], and health-promoting agents [20,21] due to their total phenol contents. Antioxidants are substances that retard deterioration, rancidity, and discolouration due to oxidation [22]. As an important source of antioxidants, herbs and spices are considered to have great potential as food preservatives [17] consequently enhancing flavour and extending shelf life. Some active compounds isolated from spices have been used to inhibit the growth of pathogenic microbes because of their antimicrobial potential [23].

Some prominent Nigerian spices such as *Aframomum danielli, Allium sativum, Piper guineense, Curcuma longa*, *Syzygium aromaticum, Tetrapleura tetraptera, Monodora myristica* and *Xylopia aethiopica* have been used in many traditional cuisines. Considering the rapid utilization of these spices, it becomes necessary to evaluate and compare their antioxidant activities and antifungal properties for adequate use in food processing. Comparing the efficacy of these spices will further aid the recommendation of appropriate spices to be used for the antioxidant and antimicrobial purpose in food processing and preservation, especially in developing countries where spices are in abundance. Since the efficacy of the extract depends on the extracting solvents, there is a need to also critically evaluate the anti-oxidative and antimicrobial potency of different extracts using different extract media. This study, therefore, evaluated the antioxidant and antifungal activities of eight grown Nigeria spices and the effect of extracting solvent on their activities.

2. MATERIALS AND METHODS

2.1 Plant Materials

Eight tropical spices (*Aframomum danielli, Allium sativum, Piper guineense, Curcuma longa*, *Syzygium aromaticum, Tetrapleura tetraptera, Monodora myristica* and *Xylopia aethiopica*) were procured from Bode market in Ibadan, Nigeria. The extraction, antioxidant activity and antifungal properties of the spices were carried out in both Ibrahim Owodunni Food Processing Laboratory, Food Science and Engineering Department and Science Laboratory Technology Department LAUTECH, Ogbomoso, Nigeria.

2.2 Reagents

DPPH (2,2-diphenyl-1-picryhydrazyl), phosphate buffer, potassium ferricyanide, trichloroacetic acid, supernatant, ferric (11) chloride, phosphate buffer saline, sodium nitroprusside, Griess reagents mL sulphanilamide, glacial acetic acid, 1-naphthylenediamin were products of Sigma.

2.3 Preparation of Spices

Samples of spices were washed and air-dried wholly at ambient temperature 28± 2ºC at 76 ± 5% relative humidity for 4 days. The spices were milled separately into a fine powder. They were packed into air-tight glass bottles and stored at 4°C until needed.

2.4 Preparation of Spice Extracts

2.4.1 Water extract

The milled samples (100 g each) were macerated separately in distilled water (1 L). Each mixture was constantly shaken through the maceration period of 72 h in a refrigerator at 4°C to prevent the growth of microbes. Spices soaked in water were filtered through a Whatman No 6 filter paper. The water filtrate of each spice was concentrated using a freeze drier and stored at 4°C until used for further analyses.

2.4.2 Ethanol extract

Dried samples, 100 g each, were extracted with 1000 ml of 80% ethanol in sterile glass bottles. The ethanol mixture was kept at room temperature for 72 h and occasionally shaken vigorously. The ethanol mixture of each spice was evaporated to dryness to obtain the extract. The ethanol extract was kept at 4°C until used for further analyses.

2.4.3 Determination of antioxidant properties of spices

The extracts were analyzed using four different complementary assays, for their radical scavenging activities against 2, 2-diphenyl-1-picryhydrazyl (DPPH), nitric oxide (NO) radicals, ferric reducing antioxidant power and metal ion chelation capacity [24]

2.4.4 Determination of free radical-scavenging activity (2, 2-diphenyl-1-picryhydrazyl (DPPH)

The modified method of Brand-Williams et al. [25] was used to study the free radical activity of the extract using DPPH (2, 2-diphenyl-1-picrylhydrazyl, Sigma-Aldrich, Germany) assay. 1 mL of each extract was dispensed in 4 mL of a methanolic solution of 0.1 mM DPPH. The mixture was shaken and left in a dark box to stand for 30 min at room temperature. The blank was also prepared with 1 mL of absolute ethanol mixed with 4 mL of 0.1 mM methanolic DPPH. The absorbance was read at 517 nm on UV-VIS Spectrophotometer. The inhibition% of the DPPH was calculated**.**

$$\text{DPPH scavenging (\%)} = \frac{Abscontrol - Abssample}{Abscontrol} \times 100$$

Where Abs_{sample} is the absorbance of the sample, $Abs_{control}$ is the absorbance of the blank

2.4.5 Determination of ferric reducing antioxidant power (FRAP) assay

The antioxidant property was evaluated using the Oyaizu [26] method. One ml of each concentration of the extract was added to 250 µL of phosphate buffer (pH 6.6) and 2.5 mL of potassium ferricyanide. The solution was incubated at 50ºC for 20 min. After cooling, 150 µl of trichloroacetic acid was added and centrifuged for 10 min at 500 rpm. The supernatant was mixed thoroughly with distilled water (250 µL) and ferric (II) chloride (500 µL). The absorbance reading was taken at 700 nm. A blank was equally prepared and FRAP was calculated in percentage.

$$\text{Increase in reducing power (\%)} = \frac{Atest - Ablank}{Ablank} \times 100$$

Where A_{test} is the absorbance of test solution and A_{blank} is the absorbance of blank

2.4.6 Determination of nitric oxide (NO) scavenging activity

Samples' inhibitory abilities against NO radicals were determined using a modified method of Idris [27]. 10 mM phosphate buffer saline (pH 7.4) was dissolved in 10 mM sodium nitroprusside. The solution (2 mL) was added to 0.5 mL of extract at varying concentrations. After an incubation period of 2.5 h at 27°C, 0.5 mL of Griess reagent mL sulphanilamide (0.33% dissolved in 20% glacial acetic acid) was added to the earlier mixed extract. One millilitre of 0.1% w/v 1-naphthylethylenediamin was also added and the entire mixture was incubated at room temperature for 30 mins. At 540 nm, the absorbance of the sample was read against the blank (methanol) and the number of nitric oxide radicals inhibited by the extract was determined.

$$\text{Nitric oxide scavenged (\%)} = \frac{Acontrol - Atest}{Acontrol} \times 100$$

where $A_{control}$ = absorbance of the control sample, A_{test} = absorbance in the presence of the samples of extracts

2.4.7 Determination of metal (Ferrous ion) chelating activity

The ferrous ion chelating was determined by the method of Ebrahimzadeh et al. [28]. Here, the ability of the extracts to chelate ferrous ion (Fe^{2+}) was estimated. One millilitre each prepared from different concentrations of the extract was thoroughly mixed with 1 mL of $FeSO_4$ (0.125 M) and 1 mL of ferrozine (0.3125 Mm). After incubating at room temperature (10 min), the mixture solution was measured using a spectrophotometer at 562 nm against a blank prepared using the same procedures for spice extract. Sodium EDTA (Na_2EDTA) was used as a control. The percentage inhibitions of ferrozine (Fe^{2+}) was calculated.

$$\%\text{ inhibition of ferrozine } (Fe^{2+}) = \frac{Ao - As}{As} \times 100$$

Where A_o is the absorbance of the control and A_s is the absorbance of the extracts

2.4.8 Determination of antimicrobial properties of the spice extracts

Antifungal Studies: Stock solution (1 mg/ml) of the extract was initially prepared in distilled water; 2.5 mL of each of the spice extract was carefully dispensed into 7.5 mL of potato dextrose agar (PDA) plates, which was then inoculated with 6 mm of 72 h old cultures of *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Fusarium solani* and (yeast) *Candida albicans,* separately. The control plates with no spice extracts were also set up. All the plates were incubated at an ambient temperature of 25±2°C for 72 h. The diameters of fungal growths in all were measured and the values were used to determine the percentage growth inhibition of fungi. The antifungal activities of spice extracts were measured using mycelia inhibition assay [29].

$$\%\text{ inhibition} = \frac{Dcontrol - Dtest}{Dcontrol} \times 100$$

Where D is the diameter of fungal growth on the PDA plates and control represent plate without incorporation of the spice extracts

3. RESULTS AND DISCUSSION

3.1 DPPH Radical Scavenging Activity by the Spices

Antioxidant compounds exert their effects through different mechanisms such as inhibiting

hydrogen abstraction, binding transition metal ions, radical scavenging, and disintegrating peroxides [30]. The DPPH radical reaction has an advantage in that the reaction process is not interrupted by certain side reactions, such as metal-ion chelation and enzyme inhibition, brought about by various additives. DPPH test is also important because it recognizes free-radical scavenging effects and not pro-oxidant activity [31]. The method is based on the ability of antioxidants to donate protons thus neutralize the free radical character of the DPPH and produce non-radical DPPH [32]. The scavenging potentials of the extracts of the eight spices against DPPH radical are shown in Tables 1 and 2. The radical scavenging activities of both the water and ethanol extracts increased in concentration-dependent manner. The IC_{50} value which indicates the concentration of the extracts needed to reduce the initial DPPH radical by 50% was reported for all the plant extract. Generally, a stronger radical quenching agent has a lower IC_{50} value. From our result, the IC_{50} value recorded for the ethanol extract of *A. danielli* was the lowest (0.45 mg/ml) and therefore had the highest antioxidant activity while *S. aromaticum* had the least activity. On the other hand, the water extract of *S. aromaticum* ($IC_{50,}$ 0.67 mg/ml) had the strongest antioxidant activity while the least activity was observed in *C. longa* ($IC_{50,}$ 2.19 mg/ml). The extract of *P. guineense* inhibited DPPH radical by 48.88 % and 37.42 % at the highest concentrations with an IC_{50} of 0.75 mg/ml and 0.82 mg/ml for the ethanol and water extracts respectively. The result of this study is in close agreement with the observation of Fajobi et al. [33] who reported that the defatted methanolic extract of *P. guineense* has potential as an antioxidant agent. The IC_{50} of the eight spices, regardless of the type of solvent used, fall within a very close range of 0.45 to 2.19 mg/ml. The ethanol extracts of *T. tetraptera* showed better antioxidant activity than the water extracts. The order of potency against DPPH radical for the ethanol extract is *A. danielli*> M. myristica>*T. tetraptera>P. guineense>C. longa*> *S. aromaticum>A. sativum>X. aethiopica,* while the water extracts follow this order *Syzygium aromaticum,*> *Tetrapleura tetraptera*> M. myristica> A. danielli> *A. sativum>P. guineense*> *Xylopia aethiopica C. longa*> (Table 1).

Favourably to the report of Joel et al. [34], the water extract of *T. tetrapteura* exhibited better activity than the ethanol extract. Similarly, ethanol extract of *C. longa* demonstrated better activity than the aqueous extract of *C. longa* indicating the influence of extracting solvent on antioxidant properties. This observation is also in tandem with Qader et al. [35]. In addition, *C, longa had an* IC_{50} value of 21.25 µg/ml when Muhamed et al. [36] investigated its DPPH scavenging potential. Both the ethanol and hydroethanolic extracts of *M. myristica* leave and stem bark were potential DPPH radical scavengers with an inhibition range of 25.00±0.55 and 85.52±1.46%. The seed extract caused a decline and slowed down the oxidation rate of soya bean oil when it was used to preserve the oil. The antioxidative activity of the extract was attributed to the phenolic diterpene constituent of the plant [37]. *Xylopia aethiopica* was also reported to be effective in the inhibition of DPPH radical with both the hydroethanolic and ethanol extracts having IC_{50} values of 184.60±1.47 and 201.22±3.59, respectively. Dongmo et al. [38] reported a higher scavenging efficacy of *A. danielli* seed. Essien *et al.* [39] suggested that 1,8-cinoeole and other oxygenated components may be responsible for the prominent scavenging effect of *A. danielli*.

3.2 Ferric Reducing Antioxidant Power (FRAP) Assay

The interaction of antioxidants with ferric tripyridyltriazine complex favours the reduction of the complex to produce ferrous tripyridyltriazine with intense blue colour [40]. The reducing power is generally associated with the presence of reductones which exerts antioxidants actions by breaking the free radical chain by donating hydrogen atoms [41]. The ferric reducing antioxidant power (FRAP) assay is widely used in the evaluation of the antioxidant component in dietary polyphenols [42].

The result of the Ferric reducing antioxidant power of the eight spices reported in Tables 1 and 2 showed that antioxidant activity decreases with an increase in concentration. Higher reducing antioxidant activity was recorded in the water extract than ethanol extract. Water extract from *M. myristica* had the lowest IC_{50} indicating the most efficient reducing power while *P. guineense* had the highest values for both water and ethanol extracts (Table 1). The order of activity for the water extract is *M. myristica >C. longa > T. tetraptera > S. aromaticum > A. sativum* >A. danielli > *X. aethiopica > P. guineense.* Our observation is consistent with previous reports Joel et al. [34] reported the potent reducing power of *T. tetraptera*, however,

Table 1. Antioxidant activity (mean±SD) of *Aframomum danielli, A. sativum, Piper guineense* and *C. longa* Ethanol extracts

Samples	Local Name	Concentration (mg/ml)	DPPH Radical Scavenging (%)	DPPH IC_{50} (mg/ml)	FRAP (%)	FRAP IC_{50} (mg/ml)	Nitric Oxide (%)	NO IC_{50} (mg/ml)	Metal ion Chelating Activity (%)	Metal ion Chelation IC_{50} (mg/ml)
A. danielli		0.2	13.95±2.36	0.45	92.68±2.82	0.75	37.68±0.84	0.35	86.11±4.81	0.55
		0.4	29.59±1.02		85.37±2.82		46.38±0.84		69.44±4.81	
		0.6	52.72±1.56		63.41±7.45		79.71±2..21		47.22±12.73	
A. sativum		0.2	8.44±2.12	0.84	92.68±4.88	0.87	43.03±2.21	0.34	88.89±4.81	0.55
		0.4	11.57±0.59		87.80±2.82		48.55±2.20		63.89±4.81	
		0.6	36.74±7.14		68.29±7.45		74.64±1.54		44.44±4.81	
P. guineense		0.2	10.20±1.77	0.70	92.68±2.82	1.34	36.67±0.59	0.51	88.89±4.81	0.72
		0.4	21.09±2.12		82.93±2.82		43.04±1.67		72.22±4.81	
		0.6	48.88±2.70		76.61±2.82		55.07±1.67		52.78±9.62	
C. longa		0.2	21.49±1.77	0.74	78.05±2.82	0.71	44.93±2.21	0.38	88.89±4.81	0.55
		0.4	33.67±1.02		68.29±4.88		50.72±0.84		61.11±4.81	
		0.6	42.18±2.36		56.10±4.88		56.52±2.51		47.22±4.81	
S. aromaticum		0.2	22.11±2.12	0.76	87.80±2.82	1.09	40.58±2.21	0.37	86.11±4.81	0.60
		0.4	33.33±2.36		75.61±4.88		50.72±0.84		72.22±4.81	
		0.6	41.84±0.00		63.41±2.82		76.81±0.84		47.22±9.62	
M. myristica		0.2	18.37±1.02	0.68	87.80±4.88	0.78	42.03±4.43	0.35	80.56±4.81	0.63
		0.4	24.15±1.56		75.61±2.82		51.74±0.25		63.89±4.81	
		0.6	46.94±2.04		63.41±4.88		63.77±0.84		50.00±8.33	
Xylopia aethiopica		0.2	14.63±2.12	0.84	70.73±5.63	0.56	14.39±1.22	2.15	83.33±7.22	0.43
		0.4	22.45±1.77		60.98±5.63		19.47±0.53		54.17±7.22	
		0.6	53.40±1.18		48.78±2.82		21.58±0.53		20.83±7.22	
Tetrapleura tetrapteura		0.2	11.22±1.77	0.71	73.17±2.82	0.51	16.84±0.53	1.93	79.17±7.22	0.40
		0.4	20.07±2.124		63.41±2.82		19.82±0.30		54.17±7.22	
		0.6	43.54±1.559		39.02±4.88		24.56±0.30		20.83±7.22	

Table 2. Antioxidant activity (mean±SD) of *A. danielli, A. sativum, P. guineense* and *C. longa water extract*

Samples	Concentration (mg/ml)	DPPH Radical Scavenging (%)	DPPH IC_{50} (mg/ml)	FRAP (%)	FRAP IC_{50} (mg/ml)	Nitric Oxide (%)	NO IC_{50} (mg/ml)	Metal ion Chelating Activity (%)	Metal ion Chelation IC_{50} (mg/ml)
A. danielli	0.2	13.27±1.02		82.93±2.82		12.81±0.61		83.33±7.22	
	0.4	22.11±1.56	0.76	65.85±5.63	0.55	17.37±0.53	1.412	58.33±7.22	0.43
	0.6	40.48±2.12		43.90±2.82		25.26±1.05		25.00±12.50	
A. sativum	0.2	5.78±1.18		82.93±2.82		10.35±1.10		75.00±12.50	
	0.4	11.57±2.57	0.78	68.29±2.28	0.52	13.51±0.30	1.381	45.83±7.22	0.38
	0.6	40.14±2.57		39.02±2.82		24.21±1.39		25.00±12.50	
P. guineense	0.2	8.16±1.77		82.93±2.28		17.89±0.53		83.33±7.22	
	0.4	11.91±1.18	0.82	68.29±2.28	0.70	20.35±0.30	2.088	54.17±7.22	0.41
	0.6	37.42±10.47		58.54±2.82		24.74±0.53		25.00±12.5	
C. longa	0.2	7.82±1.18		85.37±2.82		15.96±0.80		83.33±7.22	
	0.4	11.57±0.59	2.19	63.41±2.82	0.45	17.54±0.30	1.764	54.17±7.22	0.41
	0.6	16.33±1.02		34.96±2.82		24.91±0.30		20.83±7.22	
Syzygium	0.2	9.184±2.041	0.67	87.80±2.82	0.52	11.05±1.05	2.07	83.33±7.22	0.40
Aromaticum	0.4	20.748±1.18		68.29±2.82		19.65±0.61		16.67±7.22	
	0.6	19.39±0.00		65.85±2.82		16.84±0.53		83.33±7.22	
Monodora	0.2	19.39±0.00	0.75	65.85±2.82	0.40	16.84±0.53	2.17	83.33±7.22	0.41
	0.4	27.89±1.56		53.66±7.45		20.88±0.61		41.67±7.22	
	0.6	42.52±1.56		34.15±4.88		23.51±0.30		20.83±7.22	
Xylopia	0.2	10.88±1.56	0.66	92.68±2.82	0.69	44.93±0.84		83.33±8.33	0.52
Aethiopica	0.4	43.88±3.54		75.61±5.63		55.07±6.54	0.29	63.89±4.81	
	0.6			58.54±2.82		68.12±2.21		41.67±8.33	
Tetrapleura	0.2	11.57±2.57	0.64	90.24±4.88	1.42	30.72±1.74	1.16	86.11±4.81	0.58
Tetrapteura	0.4	29.93±1.18		82.93±2.82		36.38±0.80		72.22±4.81	
	0.6	45.91±5.58		75.61±2.82		38.55±0.17		44.44±4.81	

the water extract exhibited greater efficacy than the ethanol extract. Moukette et al. [43] similarly reported the potent reducing power of the ethanol fruit extract. The FRAP values reported by Tanvir et al. [44] for the ethanol and aqueous extract of *C. longa* indicates robust antioxidant properties of the spice. As reported by Adegoke and Golapa [45], the polar extracts of *A. danielli* exhibited more effective antioxidant activity than the non-polar extracts. Interim identification of the antioxidant components of the extracts revealed phenolics with reducing potentials. From the report of Moukette et al. [43] hydroethanolic extracts of *X. aethiopica* was more effective as a ferric reducing agent. Aazza et al. [46] reported that the ferric reducing ability of *P. guineense* could be linked to the α-pipene and 1, 8-cineole content, which possesses reductive potentials. The result of this study shows that all the spices contain effective FRAP scavenging activity with the water extract being most efficient. The antioxidant compounds were responsible for the reduction of ferric (Fe^{3+}) form to (Fe^{2+}) form. Nitric Oxide radical scavenging activity.

Nitric oxide is an unstable free radical involved in many biological processes which is associated with several diseases. Persistence increase in the concentration of NO radicals in the vascular endothelium causes direct toxicity to tissues and contributes to the vascular collapse associated with septic shock. Overexpression of the radical is also associated with inflammatory conditions including arthritis, multiple sclerosis and ulcerative colitis [47]. Incubation of solutions of sodium nitroprusside in phosphate buffer saline resulted in the generation of NO in which the extracts acted as a scavenger of the radical. At the lowest concentration, the ethanolic extract of *C. longa* scavenged the highest percentage of nitric oxide radical (44.93%), while *X. aethiopica* had the lowest nitric oxide scavenging activity (14.39 %). The order of nitric oxide radical inhibition of the ethanol extracts as indicated by the IC_{50} values is *A. sativum> A. danielli> C. longa > T. tetraptera> S. aromaticum >P. guineense > X. aethiopica >M. myristica.* The aqueous extract of the spices also exhibited good NO scavenging, however, better efficacy was observed in the ethanol extracts of the spices with IC_{50} values ranging from 0.34-0.51 mg/ml. The ethanolic extract of *A. danielli* scavenged the highest nitric oxide radical with a percentage of 79.71% at 0.6 mg/ml and an IC_{50} value of 0.35 mg/ml. The observed activity could be attributed to the presence of phenolic compounds in the plant which are reported to be the major group of compounds that act as a primary antioxidant or free radical scavengers [48]. In another study, the seed extract of *P. guineense* was found to rapidly scavenge nitric oxide in vitro at different intervals [49]. The result of this nitric oxide scavenging activity is in line with the findings of Ejele et al. [50] who reported that the phenolic compounds (tannins polyphenols sterols) of the spice extracts could be responsible for the antioxidants activities. Our observation throughout this study is consistent with the previous report [34,51,52].

3.3 Metal Ion Chelating Activity

Metal ion chelating capacity is an important mechanism of antioxidant activity that can prevent the catalysis of hydroperoxide decomposition [53]. Transition metals especially ferrous ions are potent catalysts in initiating lipid peroxidation through the Fenton reaction in the cellular membrane [54]. Also, the ferrous ion can accelerate peroxidation by the decomposition of lipid hydroperoxides into alkoxyl radicals that are capable of abstracting hydrogen and initiate the chain reaction of lipid peroxidation [55]. Chelation of metal ions is a key strategy to avoid the generation of free radicals that are associated with redox-active metal catalysis [54]. Among the various transition metals, iron is known as the most important lipid oxidation pro-oxidant because of its high reactivity [22,56]. Effective metal ion chelators prevent oxidative stress-based diseases by binding Fe^{2+} ions. The result of the metal ion chelation capacity of the ethanol and water extracts of the eight spices are revealed in Tables 1 and 2 respectively. As observed in the study, water extracts of the spices demonstrated similar capacity with ethanolic extracts for metal chelation with an inverse relationship between the concentration and the scavenging activity. Maximum chelation was observed in ethanol extracts of *C. longa* and *A. sativum* at 88.89% with an IC_{50} value of 0.55 mg/ml at 0.2 mg/ml (Table 1). The lowest activity was observed for the water extract of *A. sativum* (IC_{50} 0.38 mg/ml) (Table 2). Chakree *et al.* [57] reported that turmeric and garlic have chelating activity when extracted with both ethanol and water. The previous study on metal chelating activities of some medicinal plants [28] showed that the antioxidant activity of a plant is principally due to the presence of phenols and flavonoids. Also, Balasundram et al. [58] reported that phenolic compounds exhibited redox

properties such as metal reducing agents, hydrogen donators and singlet oxygen quenchers and were responsible for Fe-chelating activity. In this study, these aforementioned compounds might have contributed to the chelation capacity of the spices.

3.4 The sensitivity of Fungi and Yeast Isolates toward the Inhibitory Activity of Aqueous and Ethanol Extracts of the Spices

The tropical spices investigated in this study not only demonstrate antioxidant activity but showed efficacy in the inhibition of some toxigenic fungi and yeast. This is essentially valuable as it culminates possible use of the spices not only as antifungal agents but as food preservatives. For instance, Aguda et al. [59]**;** Al-bahtiti, [60]**;** Gul and Bakht, [61]; Ogbonna et al. [62]**,** Sokamte et al. [63] have highlighted the potentials of the understudied food spices in food preservation. The mycelia inhibition of aqueous and ethanolic extracts of the spices on the 5 fungi and yeast isolates *(Aspergillus niger, Aspergillus fumigatus, Candida albican, Fusarium solani and Aspergillus flavus)* were investigated and the result showed that each spice contains compounds that hindered the growth of fungi (Table 3). *Aframomum danielli* inhibited the growth of *Aspergillus niger, Aspergillus fumigatus, Candida albican, Fusarium solani and Aspergillus flavus* in both the ethanol and the aqueous extracts. The antifungal activities of the ethanol extract of *A. danielli* against these test organisms show that it possessed potent antifungal effects than the aqueous extract. The previous report on the antimicrobial properties of seed extract of *A. danielli* revealed that *A. danielli* contains good antifungal properties [64]). Okigbo and Ogbonnaya [65] ascribed the antimicrobial effect of *A. danielli* to the presence of alkaloids, tannins, steroids and flavonoids constituents. The order of inhibition by the ethanolic extract of *A. sativum* was *A. niger>A. fumigatus>C. albicans >F. solani> A. flavus* while the order of inhibition by the aqueous extract was *A. fumigatus>A. niger>F. solani>A. flavus>C. albicans* as shown in Tables 3 and 4, respectively. Our observation indicates strong antimicrobial activities of the extract and the activity may be related to allicin which has been said to curb the performance of some enzymes that are important to fungi [66]. The antifungal potential of garlic against *Penicillin expansum, Botrytris cinerea* and *Neofabrea alba* had been investigated and the ethanol extracts inhibited the growth of all tested fungi, particularly *Neofabrea alba* which cause bull's eye rot in apples [67]. This is an indication that garlic has a broad inhibitory spectrum against fungi and is highly effective against the test organism and therefore could be used in food preservation. Garlic is more effective with the least side effects as compared to commercial antibiotics; as a result, they are used as an alternative remedy for the treatment of various infections [68]. Other ethanol extracts with a higher percentage of mycelia inhibition against *A. niger* and *A. fumigatus* are *A. danielli, P. guineense* and *C. longa*.

The activity of *P. guineense* on *C. albican* demonstrates that its extracts could be effectively utilized in the treatment of infections caused by *Candida* strains in antifungal therapy [69]. Among all these fungal strains selected, *C. albican* is known to be the most significant human pathogenic species of yeast that can cause serious fungal diseases in human [70]. In the present study, the aqueous extract of *P. guineense* showed similar antifungal activity to the ethanol extract against the isolates. The order of fungal and yeast inhibition by *C. longa* was *A. fumigatus>A. niger>C. albican>F. solani>A. flavus.* Similar inhibitory potentials were demonstrated by the aqueous extracts of *C. longa* and *A. sativum* against *A. flavus*. A previous study showed that methanol extract of *A. sativum* has strong inhibitory activity against *C. albicans* [71] which is consistent with findings of Omodamiro and Ekeleme [52]. Our study, therefore, validated the broad spectrum of *C. longa* against a range of fungi. Pictorial representation of mycelia inhibition by the extracts is revealed in Plate 1.

As previously reported, ethanol extracts of *Syzygium aromaticum* significantly inhibited mycelial growth of *Aspergillus niger* [72]. Conversely, aqueous extracts of *X. aethiopica* exhibited higher inhibitory potential than the ethanol extract, indicating the more polar compounds of the plant to be responsible for the observed activity. Important phytochemicals viz, flavonoids, alkaloids, saponins, phenols and tannins were implicated in the antifungal properties of some already reported spices [73]. For instance, the strong inhibitory effect of *T. tetraptera* against Aspergillus species was attributed to the presence of phenolic compounds in the seeds reported by Yadav and Agarwala, [74]. The mycelia inhibition by the tropical spices may be due to interruption in the mycelium [75,76].

Table 3. Antifungal activity of the ethanol extract of the spice on fungi and yeast isolates

Spices	Percentage mycelia inhibition of fungi and yeast (%)				
	A. niger	***A. fumigatus***	***C. albican***	***F. solani***	***A. flavus***
A. danielli	90	92.5	88.54	87.5	85
A. sativum	93.75	91.25	87.5	86.36	85
P. guineense	91.25	91.89	83.33	86.36	87.5
C. longa	91.25	92.5	88.54	86.36	85
Syzygium aromaticum	80	92.5	87.5	85.4	88.6
Monodora myristica	83.75	93	83.75	85.4	88.6
Xylopia aethiopica	75	93	85	89.6	84
Tetrapleura tetraptera	78.75	85	82.5	87.5	87.5

Table 4. Antifungal activity of aqueous extracts of the spice on fungi and yeast isolates

Spices	Percentage mycelia inhibition of fungi and yeast (%)				
	A. niger	***A. fumigatus***	***C. albican***	***F. solani***	***A. flavus***
A. danielli	83.75	91.89	86.46	84.09	82.5
A. sativum	88.75	91.89	83.33	85.23	83.75
P. guineense	90	90	83.33	84.09	82.5
C. longa	93.13	86.25	89.58	81.82	85
Syzygium aromaticum	37.5	-	71.25	-	52
Monodora myristica	83.7	88	82.5	79	66
Xylopia aethiopica	83.8	86.25	85	23.9	52
Tetrapleura tetraptera	73	91	72.5	47.9	59

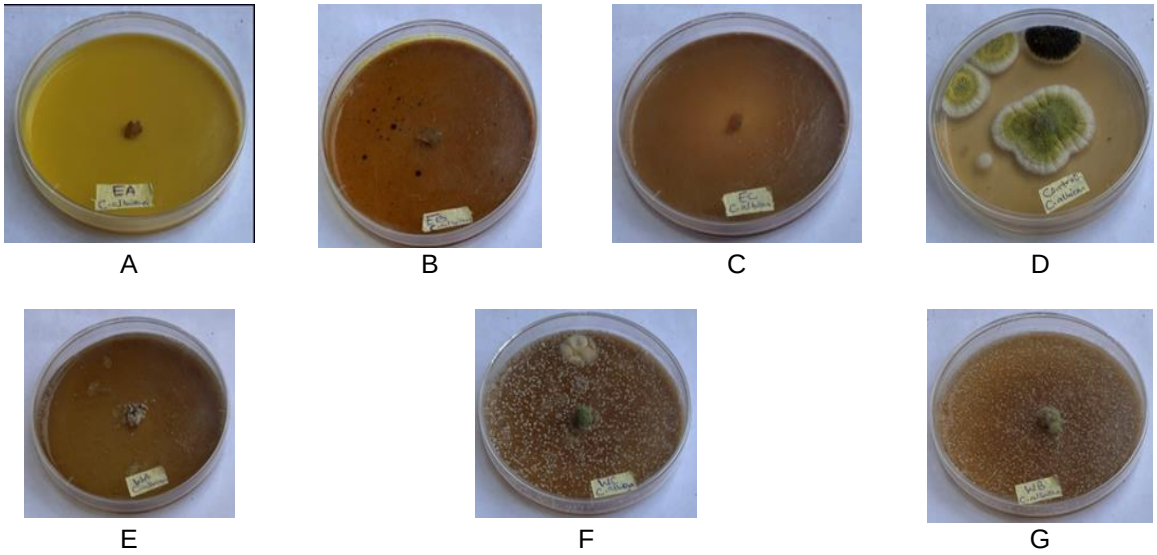

Plate 1. Pictorial view of *Candida abicans* treated with water and ethanol spice extracts

KEY: A: Ethanol extract of Xylopia aethiopica with the Yeast isolate Candida albican, B: Ethanol extracts of Monodora myristica with yeast isolate Candida albican, C: Ethanol extracts of Tetrapleura tetraptera with the yeast isolate Candida albican, D: Control of Candia albicans, E: Water extract of Xylopia aethiopica with the Yeast isolate Candida albican, F: Water extracts of Monodora myristica with yeast isolate Candida albican, G: Water extracts of Tetrapleura tetraptera with the yeast isolate Candida albican

4. CONCLUSION

The spice extracts possessed varying levels of antioxidants activity. The result also revealed a considerable efficacy against five importation opportunistic fungi and yeast. Ethanol was the best extracting solvent than water. The antioxidant and antimicrobial activities of garlic was a competitor to the other spices. In conclusion, these spices are better choices as

promising natural antioxidants and antifungal agents. Their applications in food processing and preservation should be adequately explored for functional and preservative properties benefits. Future research should extract the bioactive compounds of these spices and further investigate their potency in foods as antioxidant and antifungal agents.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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