Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

European Journal of Experimental Biology, 2013, 3(6):193-202

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Microbial profile, antibacterial and antioxidant activities of some imported spices in Nigeria

Oranusi S.*, Nwachukwu C., Adekeye B. Temitope, Dahunsi O. Samuel and Adeyemi A. Oladipupo

Department of Biological Sciences, Covenant University, Ota, Nigeria

---

ABSTRACT

Spices are commonly used in most Nigerian dishes as flavour and colour enhancer, nutrient supplements or to serve as preservatives, medicine and as part of cultural inclinations. They are naturally of plant parts and in recent times, certain synthetic creations of flavorists have found use in food as flavour and colour enhancers. The upsurge in quest for dishes of other cultures, and for food containing plant products deemed to have antioxidant properties have resulted in large import of different spices into the Nigerian market. This study was conducted to determine the microbial profile, antibacterial and antioxidant activities of some imported spices in Nigerian market. Fifteen each, of five different brands of imported spices packaged in polyethylene containers were purchased from supermarkets in different regions of Nigeria. The mean (cfu/g) total aerobic plate counts in the samples range from 1.8 x10^3 to 7.0x10^4, Coliform count was 1.1x10^2 to 4.1x10^3 and mean fungi count was 1.0x10^1 to 2.9x10^3. Microorganisms isolated from some of the spices include spp of Bacillus, Staphylococcus, Proteus, Enterobacter, Pseudomonas, Aspergillus, Rhizopus and Fusarium. Some of the spices had antimicrobial effects on the clinical isolates tested with MIC ranging from 6.25 to 25.0 mg/ml. The spices contain Phenolics and flavonoids and have DPPH, Hydrogen peroxide and Nitric oxide scavenging activities. Adequate HACCP evaluation and GMP in the processing of spices is advanced, further studies are necessary to harness the full antimicrobial and antioxidant activities of these spices for therapeutic purposes.

Key words: Spices; antimicrobial; antioxidant; Phenolics; flavonoids; scavenging activities.

INTRODUCTION

Spices are products from plants seeds, fruits, flowers, roots, leaves or bark that are added to food to improve flavour, taste, colour, or act to minimize the rate of rancidity and as preservatives that suppress microbial activities [1, 2]. Each spice has a unique aroma, flavour and antimicrobial activities which derive from phytochemicals. Concisely put, Spices are strongly flavored parts of plants usually rich in essential oils used in fresh or dry forms [3]. Some spices are reported to have microbicidal or microbistatic activities [4, 5]. The inhibitory effects of spices are mostly due to the volatile oils present in their composition [6]. More recently, the interest in herbs and spices has grown not only for their seasoning and flavoring properties, but also for their antioxidant potential [7, 8]. Consumption of herbs and spices has been implicated in the prevention of cardiovascular diseases, carcinogenesis, inflammation, atherosclerosis, reduced beta-amyloid and plaque burden in the brain, increasing cognitive function [9-13]. Natural antioxidants are known to protect cells from damage induced by oxidative stress, which is generally considered to be

a cause of aging and degenerative diseases [14-17].

Spices contain natural and contaminating micro flora and can be a source for food contamination. The growing concern about food safety has recently led to the development of natural antimicrobials to control food borne pathogens and spoilage bacteria [10, 18]. Spices are one of the most commonly used natural antimicrobial agents in foods, factors that determine the activity of spices are the concentration and composition of the spices, the amount of spices used, type of microorganisms, composition of the food, pH value, temperature of the environment and proteins, lipids, salts and phenolic substances present in the food [19]. Spice extractives, such as oleoresin of rosemary, can provide inhibition of oxidative rancidity and retard the development of "warmed-over" flavor in some products. Thus, some spices not only provide flavor and aroma to food and retard microbial growth, but are also beneficial in prevention of some off-flavor development. These attributes are useful in the development of snack foods and meat products [20].

Food habits are changing from demand for well cooked/processed to partially cooked or raw food. Thus there is very high possibility to the use of heavily contaminated/adulterated spices raw/partially cooked, and could be deleterious to consumers' health. Antimicrobial studies on plants have evolved from multi disciplinary investigations where researchers have used the antimicrobial data to complement the phytochemical study, to more focused studies pertaining to specific information about the plants antimicrobial activity. The compositions and activities of most local spices indigenous to Nigeria and Africa have been well documented [14, 21, 22]. The conditions of imported spice in Nigeria may not be free from microbial contamination and can be a source of contamination to food and food stuffs. The report of Chandarana *et al*. [23] suggests that the chemicals present in spices can be allergens, carcinogens, and mutagens. Knowledge of the chemical composition of imported spices is apt, more so with numerous chemical creations of flavorists coupled with porous border checks that allow importation of substandard goods into Nigeria, evaluation of products at the consumer level is thus necessary. This research is aimed at evaluating the microbial profile, antibacterial and antioxidant activity of some imported spices in Nigeria, with a view to educating the public on the need for food safety consciousness.

MATERIALS AND METHODS

Sample collection

Fifteen each of five brands of imported spices in powdered form (jeera (cumin), curry, paprika, white pepper and ginger) were obtained from super stores/shopping malls/supermarkets/megastores in different regions of Nigeria: Lagos and Ota in the west, Onitsha and Port Harcourt in the east and south and Abuja in the north. The spices were all within expiry date and no physical damage to the packs were observed. The manufacture and expiry dates were recorded as well as batch number, manufacturer address and other information provided on the labels.

Sources of test organisms

The pure isolates of *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Salmonella typhi and Proteus mirabilis* used as test organisms were obtained from the department of microbiology, Covenant University. The purity and identification of the organisms was confirmed by using cultural, microscopic and biochemical methods [22].

Microbiological evaluation of spices

One gram of each of the samples was weighed aseptically and diluted 1:10 in sterile peptone water (Fluka, Germany). Further serial tenfold dilution was carried out to 10^{-4} in the same diluents. Aliquot 0.1ml of each dilution was plated out on Plate count agar, Macconkey agar, Potato Dextrose agar (All media from Fluka, Germany), for Total aerobic plate count, Total coliform count and Total fungi count respectively. Incubation of Plate count agar and Macconkey agar was at 37°C for 24h while Potato Dextrose agar was incubated at laboratory room temperature of 29±2 $^{\circ}$C for 3-5 days.

Enumeration and Characterization of Microbial Isolates

Enumerations of colonies at the expiration of incubation period were with digital colony counter (Gallenkamp, England) and total population expressed as colony forming units per gram (cfu/g). Pure culture of isolates obtained by repeated subculture on freshly prepared nutrient agar was stored on slants at 4°C refrigeration temperature for further identification. Identification of characteristic bacteria isolates was based on colonial morphology, microscopy

and biochemical tests [24]. Fungal isolates were identified based on morphological characteristics, pigmentation on media and microscopy with reference to standard atlas and manuals [25].

Coliform test

Presumptive Coliform Test: A gram samples of each of the spice was inoculated respectively into sterile lactose broth in McCartney bottle containing inverted Durham tubes. Incubation was at 37°C for 24- 48h. Positive samples with gas production and/or colour change of dye were used for confirmatory coliform test.

Confirmatory Coliform Test: Presumptive test positive samples where sub-cultured onto duplicate plates of Eosin Methylene Blue (EMB) agar (Micromaster, India) incubation was at 37ºC and 44ºC for 24h. Positive test was indicated by growth of characteristic colonies that is Gram negative non-spore forming rods.

Completed Test: Colonies on EMB agar (positive for confirmatory test) where sub-cultured into sterile lactose broth in McCartney bottle containing inverted Durham tubes, and onto Nutrient agar slants for further characterization. Incubation was at 37ºC for 24h. Gas production and/or colour change of dye in lactose broth was recorded for positive completed test and thus presence of coliforms. If and when growth at 44ºC is positive, feacal coliform is confirmed [24, 26].

Evaluation of spices for antimicrobial activity

Spice extraction

Twenty (20) g of each spice in powdered form was extracted in 200ml of methanol for 24h and using soxhlet apparatus. The extracts in solvent were placed in rotary evaporator (IKA HB10 basic) at 70°C for removal of the extraction solvent. The total weight of extract in grams was calculated and the solvent fee extracts was stored at 4°C until use.

Preparation of test organisms for susceptibility test

The test organisms on slants were purified by repeated sub-culture on Nutrient agar (Biomark, India). This sub-culturing also helped to check for viability. Pure cultures were inoculated into Nutrient broth (Fluka, Germany) for overnight incubation at 37 ºC and were standardized to 1.5x 10^8 cells/mL using 0.5 McFarland standards.

Determination of antimicrobial activity of methanolic crude extracts

The standard agar well diffusion method of CLSI [27] was adopted and following the description of [28]. Mueller Hinton agar (Oxoid, England) was uniformly seeded with standardized cultures of the test organisms. A sterile cork borer was used to cut uniform wells of 5mm diameter on the surface of the agar and the wells were filled with 0.2mL of 25.0, 12.5 and 6.25mg/mL crude extracts (concentrations were chosen based on results of preliminary investigations). Sterile distilled water and Gentamicin (10µg) were used as negative and positive controls. The plates were incubated at 37 ºC for 18-24h before observation for zones of inhibition and measurement of zone diameter. Results were recorded as sensitive (S) and resistance (R) following the interpretations of [27].

Determination of minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of methanolic crude extracts

MIC is defined as the lowest concentration of extract that can completely inhibit the growth of the organisms. Ten (10) mL of the different concentrations 25.0, 12.5, 6.25 and 3.125mg/mL of the extracts were inoculated with 1mL of standardized test organisms for 24h incubation at 37 ºC. The tubes were examined for growth depicted by turbidity of the medium. The lowest concentration that inhibited growth of the test organisms was recorded as MIC. Tubes showing no growth were plated out on Nutrient agar, the lowest concentration that yielded no growth of bacteria colonies after 24h incubation was recorded as MBC.

Determination of Moisture Content of Samples

The moisture content was determined by standard analytical method [29]. Samples were oven dried at 105°C for 2 h to constant weight.

Determination of antioxidant activity of methanolic crude extracts

Total Phenolic content

The total phenolic contents were estimated according to the Folin-Ciocalteu method, with modifications [30-32]. To 10µl of extract, 600µl of distilled water was added, followed by 50µl of Folin–Ciocalteu reagent. Aliquot 150µl of

7% $Na_2CO_3$was added and the mixture was put in a vortex for 2min. The sample was incubated for 8min at room temperature. Aliquot 190µl of distilled water was added. The sample was allowed to stand for 2h and the absorbance was read at 765nm using Spectrophotometer (Model 23D, Uniscope Surgifield, England). The values are presented as means of triplicate analysis. A blank was prepared by replacing 10µl extract with water.

Total Flavonoid Content

The method of Zhishen *et al.*[33] was adopted with modifications [15, 30,34]**.** To 490µl of distilled water, 10µl of extract was added followed by 30µl of 5% sodium nitrite and 30µl of 10% aluminum chloride. Incubation was at room temperature for 5min. Aliquot 200µl of 1M sodium hydroxide was added followed by 240µl of distilled water and vortex thoroughly. Absorbance was read at 570nm. Analysis was in triplicate and mean value recorded. Standard pyrocatechol was used for control.

DPPH (2, 2'-Diphenyl-1-picrylhydrazyl) radical-scavenging activity

The method of Manzorro *et al.* [35] was used with modifications [36, 37]. Different concentrations (25.0, 12.5, 6.25, and 3.125mg/mL) of extract were assayed. To 100µl of extract was added 1M 0.004% methanolic solution of DPPH and incubate for 30min in the dark. Absorbance was read at 517nm; results of triplicate analysis was averaged and recorded. The percentage of the DPPH radical scavenging was calculated according to the formula of Alothman *et al*. [34]. The same was done for varying concentration of standard.

Hydrogen peroxide Scavenging Activity

The extracts ability to scavenge hydrogen peroxide at different concentrations (25.0, 12.5, 6.25, and 3.125mg/mL) was determined according to the method of Ruch *et al*. [38] with slight modifications [16, 39]. To 100µl of extract, 600µl of hydrogen peroxide in a phosphate buffer, 0.1M pH 7.4 was added and incubated in the dark for 10min. A negative control was set up in parallel with entire reagent except extract or standard. Absorbance of hydrogen peroxide at 230 nm was determined against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging of extracts and standard compounds were calculated.

Nitric Oxide scavenging activity

The scavenging activity of NO was determined using the different concentrations of extracts and following the method of Alisi and Onyeze [40] with modification [15]. To 60µl of 10M sodium nitropruside was mixed 60µl of extract/ standard and incubated for 2.5h. A control was set up as above but the sample replaced with water. After incubation, 1.0ml of sulfanilic acid reagent (0.33% sulfanilic acid in 20% glacial acetic acid) was added to 0.5ml of the reaction. After 5min, 1ml 0.1% NEDD (naphthylethylenediamine dihydrochloride) was added for 30min at 25°C. Absorbance was read at 540nm.

Statistical analysis

The data analysis was made using SPSS 20.0 software for windows [41]. Analysis of variance (ANOVA) and Duncan's multiple range tests were applied to compare any significant differences ($P < 0.05$). All the analyses were carried out in triplicates.

RESULTS

The result of the mean microbial counts of the spices is shown in Table 1. The table reveals ginger (*Zingiber officinale*), curry (*Murraya koenigii*) and Jeera (*cuminum cyminum*) to be the most contaminated with total aerobic plate count, coliform and fungi counts ranging from $3.8x10^3$ to $7.0x10^4$; $1.7x10^3$ to $4.1x10^3$ and $1.9x10^3$ to $2.9x10^3$ cfu/g respectively.

Table 1: Mean microbial counts (cfu/g) of spice

Sample	TAPC	Coliform count	Fungi count
Ginger powder	$7.0x10^4$	$1.7X10^3$	$2.9X10^3$
Curry powder	$4.5x10^3$	$4.1X10^3$	$2.1X10^3$
Paprika powder	NG	NG	$1.0X10^1$
White pepper powder	$1.8X10^3$	$1.1X10^2$	$9.7X10^2$
Jeera powder	$3.8X10^3$	$3.9X10^3$	$1.9X10^3$

Table 2: Susceptibility test by agar well diffusion of spice extracts on test isolates.

Spice extract/ concentration(mg/ml)	Test organisms				
	S. aureus	*E. coli*	*K. pneumoniae*	*S. typhi*	*P. mirabilis*
Ginger powder					
25.0	S	R	R	S	R
12.5	R	R	R	R	R
6.25	R	R	R	R	R
Curry powder					
25.0	R	R	R	R	R
12.5	R	R	R	R	R
6.25	R	R	R	R	R
Paprika powder					
25.0	S	S	S	S	S
12.5	S	R	S	S	S
6.25	S	R	R	R	R
White pepper powder					
25.0	R	R	R	R	R
12.5	R	R	R	R	R
6.25	R	R	R	R	R
Jeera powder					
25.0	R	R	R	R	R
12.5	R	R	R	R	R
6.25	R	R	R	R	R

Table 3: Minimum inhibitory concentration of spice extracts against test isolates

Spice extract/ concentration(mg/ml)	Test organisms				
	S. aureus	*E. coli*	*K. pneumoniae*	*S. typhi*	*P. mirabilis*
Ginger powder					
25.0	NG	NG	G	NG	G
12.5	NG	G	G	NG	G
6.25	G	G	G	G	G
3.12.5	G	G	G	G	G
Curry powder					
25.0	G	G	G	G	G
12.5	G	G	G	G	G
6.25	G	G	G	G	G
3.12.5	G	G	G	G	G
Paprika powder					
25.0	NG	NG	NG	NG	NG
12.5	NG	NG	NG	NG	NG
6.25	NG	G	G	NG	G
3.12.5	G	G	G	G	G
White pepper powder					
25.0	G	G	G	G	G
12.5	G	G	G	G	G
6.25	G	G	G	G	G
3.12.5	G	G	G	G	G
Jeera powder					
25.0	G	G	G	G	G
12.5	G	G	G	G	G
6.25	G	G	G	G	G
3.12.5	G	G	G	G	G

G= growth of test organism. NG= no growth of test organism

Table 4: Mean total phenol content of spices (mg gallic acid equivalent/mg extract)

Spice	Total phenol content
Curry	0.795±0.02
Ginger	0.897±0.20
Jeera	0.774±0.07
Paprika	0.798±0.02
White pepper	0.785±0.18

Table 2 shows the result of the antimicrobial activity of the methanolic extract of the spices. It reveals that paparika (*Capsicum annuum*) showed the broadest antibacterial activity as all the test organisms were susceptible to it at 25.0 and 12.5mg/ml. Ginger at 25.0mg/ml had effect on *S. aureus* and *S. typhi*. The MIC for Paparika was 12.5mg/ml for

the test organisms. Ginger had an MIC of 12.5mg/ml for *S. aureus* and *S. typhi* Table3. Plate 1 shows the sensitivity pattern of test organisms to Paparika spice extract. Table4 presents the total phenol content in milligram gallic acid equivalent/mg extract. There is no significant difference (P< 0.05) in the total phenol contents of the spices. Table5 shows the total flavonoid content in milligram pyrocathecol equivalent/mg extract, the table reveals a significantly high (white pepper (*Piper nigrum L.*) and low (paprika) flavonoid contents compared to the other spices. Table6 shows scavenging of DPPH radical by the extract and the scavenging values as inhibition (%). It reveals varying degrees of scavenging capacities. Curry and ginger extracts exhibited the highest (P< 0.05) radical scavenging effect which was higher than the other extracts. The lowest activity was shown by Jeera. The scavenging of hydrogen peroxide radical is shown in Table7. There appear to be no significant difference (P< 0.05) in radical scavenging capacities of the different spice extracts. A non-concentration dependent scavenging of nitrogen oxide values as inhibition (%) was found for all the spice extracts (Table 8). Curry however, tend to have exhibited stronger radical scavenging effect.

Table 5: Mean flavonoid content of spices (mg pyrocathecol equivalent/mg extract)

Spice	Flavonoid content
Curry	0.254±0.03
Ginger	0.147±0.02
Jeera	0.145±0.03
Paprika	0.058±0.01
White pepper	1.095±0.03

Table 6: Antioxidant activity of spices using the corresponding concentrations
(A=25.0 mg/ml, B=12.5 mg/ml, C=6.25 mg/ml and D=3.125mg/ml) measured by DPPH (% inhibition) method

Spice	Concentration (mg/ml)			
	A	B	C	D
Curry	62.651±0.46	30.694±3.71	18.589±1.79	11.360±1.20
Ginger	53.299±2.42	49.627±1.11	30.981±0.46	12.507±2.05
Jeera	22.375±1.64	15.892±2.84	8.778±0.52	8.491±1.05
Paprika	32.760±0.40	20.310±0.46	12.220±1.03	8.663±0.70
White pepper	30.178±1.86	21.170±0.46	12.909±1.07	8.893±0.72

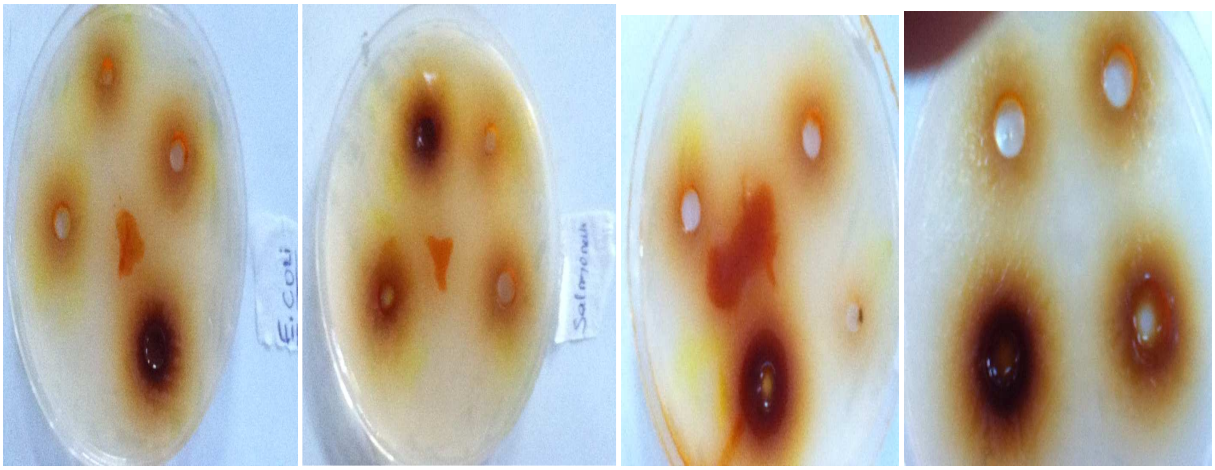

Plate1: Susceptibility pattern of test organisms to Paprika (*Capsicum annuum*) spice extract

Table 7: Antioxidant activity of spices using the corresponding concentrations
(A=25.0 mg/ml, B=12.5 mg/ml, C=6.25 mg/ml and D=3.125mg/ml) measured by hydrogen peroxide (% inhibition) method

Spice	Concentration (mg/ml)			
	A	B	C	D
Curry	73.826±0.65	71.546±6.67	73.142±0.16	72.139±1.11
Ginger	72.549±3.24	72.914±0.14	75.285±0.42	75.650±0.83
Jeera	75.057±2.65	75.331±2.95	73.735±0.27	74.601±2.80
Paprika	76.243±1.51	71.911±9.30	72.959±1.94	74.510±0.75
White pepper	75.650±3.34	73.233±0.08	75.559±1.23	74.282±0.14

Table 8: Antioxidant activity of spices using the corresponding concentrations
(A=25.0 mg/ml, B=12.5 mg/ml, C=6.25 mg/ml and D=3.125mg/ml) measured by nitrogen oxide (% inhibition) method

Spice	Concentration (mg/ml)			
	A	B	C	D
Curry	29.594±4.97	36.845±1.42	13.862±4.02	23.575±3.89
Ginger	18.787±1.92	32.148±5.69	31.874±1.66	22.845±1.37
Jeera	19.562±0.14	10.078±1.35	17.921±1.73	31.874±0.99
Paprika	18.742±0.72	15.550±7.67	19.927±1.42	28.044±0.49
White pepper	14.318±6.43	37.437±5.27	32.285±1.58	29.047±3.44

DISCUSSION

The microbial loads implicated in the spices are within acceptable standard specifications [42, 43]. The organisms isolated in this work have been implicated in earlier works on spices [44, 45]. They are common environmental contaminants and are known to be hardy. The Bacillus and moulds are spore bearers that can survive processing conditions. *S. aureus* is of human origin and may have been introduced via the personnel to the products. Though the microbial counts are within acceptable limit, the presence of coliforms is an indication of lapses in sanitary conditions, toxigenic strains of *S. aureus* have equally been implicated in food poisoning and species of Aspergillus and Fusarium are known to produce deleterious mycotoxins under favourable condition. The moisture content (30-45%) of these spices could encourage survival and growth of the contaminants specifically the moulds and in absence of proper storage. The presence of these organisms in spices must therefore not be treated with levity. More so some of these spices could be incorporated into food without further treatment or with minor processing before consumption.

Paprika (*Capsicum annuum*) showed the highest antibacterial activity to all the test organisms. Paprika has been reported to contain an antibacterial protein that has been found to naturally control (by limiting the growth of) certain bacteria such as *Salmonella* and *E. coli* [46]. The result of the present study shows that *S. aureus* and *S. typhi* were susceptible to ginger (*Zingiber officinale*), this is in agreement with the reports of earlier researches indicating sensitivity of these organisms to ginger [37, 47-49]. Jeera (cumin), curry and white pepper as observed in this report has no antimicrobial activity on the test organisms. This is contrary to the findings of inhibitory effect of cumin against *S. aureus, K. pneumonia,* P. *aeruginosa, Vibrio vulnificus and Micrococcus lutieus* [37]. Similarly Curry has been reported to have antimicrobial activity against diverse microorganisms [50-54]. White pepper contrary to this report has been observed to have antimicrobial activity [55, 56]. The report of Nanasombat and Lohasupthawee [57] however found no antimicrobial activity in white pepper and thus in accordance with the present study.

The total phenolic and flavonoids contents of spices expressed in the present study with curry, ginger and jeera (cumin) having higher phenolic contents compared to paprika and white pepper and higher flavonoids in white pepper, corroborate the report of Kim *et al*.[58]. It is also in agreement with the findings that spices are rich sources of phenolics and flavonoids known to be strong antioxidants [14, 15, 30, 31, 34, 59, 60]. Flavonoids are also known to have anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activity and provide health benefits against heart diseases [9, 11, 13].

The antioxidant activity by DPPH assay were in the order of curry>ginger>paprika>white pepper>jeera (cumin). Curry (*Murraya koenigii*) has been reported to have strong antioxidant activity [60]. Asimi *et al*. [37] reported to the contrary for ginger (*Zingiber officinale*). The DPPH radical scavenging activity of spices could be mediated by individual phenolic acids, if in high percentage as main constituents, but also to the presence of other constituents in small quantities or to synergy among them [37, 58]. DPPH acts as a stable free radical in methanol solution that easily accepts an electron or hydride radical and converted to a stable diamagnetic molecule. By reacting with suitable reducing agents DPPH radicals formed into the corresponding hydrazine [15]. In other words the DPPH assay measures the ability of the extract to donate hydrogen to the DPPH radical, resulting in bleaching of the DPPH solution [37]. It has been reported that phenolic compounds contribute significantly to the DPPH radical scavenging capacity of spice plants [59]. The finding of these earlier observations are on a par with the present study considering the higher contents of total phenol in curry, ginger and paprika and flavonoids in curry and ginger.

All the spice extracts showed strong hydrogen peroxide scavenging activity that is not concentration dependent and not significantly varied among the different spices ($P< 0.05$). Yen and Chen [39,61] reported concentration dependent hydrogen peroxide scavenging in tea extracts, similarly Keser *et al*.[16] reported that water and ethanol extracts of *C. monogyna* were capable of scavenging hydrogen peroxide in an amount dependent manner. Curry and

jeera (cumin) have been reported to have appreciable hydrogen peroxide scavenging activity [58, 60].Yen and Chen [39] observed that typical application of the polyphenol fraction of green tea inhibited TPA-induced hydrogen peroxide formation in mouse epidermis; this factor may be important for the inhibitory effect of the polyphenol fraction of green tea on TPA-induced inflammation and tumor promotion. Though hydrogen peroxide is not very reactive, its removal from cell is important in cell defense because it may give rise to toxic hydroxyl radical in the cells [16, 63].

The percentage nitric oxide radical inhibition for all five samples in this study range from 37.44-10.08 mg/ml. Ginger, curry and white pepper has better activity compared to paprika and jeera (cumin). Ghosh *et al*. [64] reported a NO scavenging activity of *B. monnieri* with IC_{50} value of 29.17 ug/mL while Hasan *et al*. [15] and Haque *et al*.[65] estimated values of 7.29 ug/mL and 11.23 ug/mL for NO scavenging activity and peroxynitrite (ONOO) scavenging activity of the methanolic extract of *B. monnieri.* Excessive production of free radicals has been noted to cause damage to biological material leading to several physiological and pathological abnormalities an essential event in the etiopathogenesis of various diseases [15, 16, 30, 34]. Verónica *et al*. [66] has shown inactivation and nitration of human superoxide dismutase (SOD) by fluxes of nitric oxide radicals. Nitrosative stress results from the overproduction of reactive nitrogen species that may occur when the generation of reactive nitrogen species in a system exceeds the system's ability to neutralize and eliminate them [67].

CONCLUSION

Spices can be contaminated by natural microflora via handling and packaging. It could contain pathogens thus effective hazard analysis critical control point (HACCP) and good manufacturing practice (GMP) must be employed in their processing and consumption. Paprika had a good antibacterial action on all test organisms, further investigation is necessary for possible use of the active component in chemotherapy. Most of the spices studied in this report had no antibacterial activity contrary to earlier reports, this could be attributed to use of industrially processed products already polished and mixed with other additives compared to natural materials in most other works, similarly differences in use concentrations could account for disparity in report findings because the antimicrobial properties of spices differ depending on the form of spices added, such as fresh, dried, or extracted forms. The results in this study using three different methods to evaluate the antioxidant activity (DPPH, H_2O_2 and NO) showed that the spice extracts can be considered good sources of natural compounds with significant antioxidant activity. Consumption of microbiologically safe spices should be encouraged for their rich antioxidants and possible health benefits.

Acknowledgment
We thank Dr. S. Rotimi of Biochemistry unit, Biological Sciences Department, Covenant University, Nigeria, for his technical assistance.

REFERENCES

[1] Thomas V, Medicinal Importance of Spices, **2007**, http://ezinearticles.com/?Medicinal-Importance-Of-Spices&id=602577 Retrieved October 10, **2013**.
[2] Teye GA, Mustapha FB, Abu A, Teye M, *Scientific Journal of Animal Science*, **2013**, 2(2), 41-46.
[3] Iwu MM, Handbook of African Medicinal Plants, CkC Press, Bocca Raton, **1993**, pp 435.
[4] Onwuliri FC, Wonang DL, *Nigerian Journal of Botany*, **2005**, 18, 224-228.
[5] Witkowska AM, Hickey DK, Gomez MA, Wilkinson M, *Journal of Food Research*, **2013**, 2(4), 37-54.
[6] Arora D, Keur J, *International Journal of Antimicrobial Agents*, **1999**, 12, 257-262.
[7] Mukul Sharma, Avneesh Kumar, Babita Sharma, Akshita and Naina Dwivedi, European Journal of Experimental Biology, **2013**, 3(5):432-436.
[8] Anita Dua1, Gaurav Garg, Ritu Mahajan, European Journal of Experimental Biology, **2013**, 3(4), 203-208.
[9] Yamamoto Y, Gaynor RB, *Journal of clinical Investigation*, **2001**, 107(2), 135-142.
[10] Lai PK, Roy J, *Current Medical Chemistry*, **2004**, 11(11), 1451-1460.
[11] Cushnie TPT, Lamb AJ, *International Journal of Antimicrobial Agents*, **2005**, 26(5), 343-356.
[12] Ng Tze-Pin, Peak-Chiang, Theresa Lee, Hong-Choon Chua, Leslie Lim, Ee-Heok Kua, *American Journal of Epidemiology*, **2006**, 164(9), 898-906.
[13] Ververidis F, Trantas E, Douglas C, Vollmer G, Kretzschmar G, Panopoulos N, *Biotechnology Journal*, **2007**, **2**(10), 1214-1218.
[14] Odukoya AO, Ilori OO, Sofidiya MO, Aniunoh OA, Lawal BM, Tade IO, *EJEAFChe*, **2005**, 4 (6), 1086-1093.

[15] Hasan M, Alam MN, Wahed TB, Sultana F, Jamiuddin A, *Journal of Pharmaceutical Sciences*, **2012**, 9(3), 285-292.
[16] Keser S, Celik S, Turkoglu S, Yilmaz O, Turkoglu I, *Chemistry Journal*, **2012**, 02(01), 9-12.
[17] Abdul Qaiyum Ansari, Syed Abrar Ahmed, Waheed MA, Sayyed Juned A, European Journal of Experimental Biology, **2013**, 3(5):502-507.
[18] Oranusi S, Braide W, Oguoma OI, *Global Advanced Research Journal of Food Science and Technology*, **2013a**, 2(1), 001-006.
[19] Sagdic O, Ozcan MM, Ozkan G, *Journal of Medicinal Food*, **2006**, 9(3), 418-21.
[20] Giese J, *Food Technology*, **1994**, 48(4), 87-98.
[21] Okeke EC, Eneobong HN, Uzuegbunam AO, Ozioko AO, Kuhnlein H, *Pakistan Journal of Nutrition*, **2008**, 7 (2), 365-376.
[22] Onimawo I, Nigerian traditional food system and nutrition security, International Scientific Symposium Biodiversity and Sustainable Diets United Against Hunger. 3-5 November **2010**.
[23] Chandarana H, Baluja S,.Chanda SV, *Turkish Journal of Biol*ogy, **2005**, 29, 83- 97.
[24] Speck ML, Compendium of methods for microbiological examination of foods, American Public Health Association, Washington DC, **1976**, pp 277 –328.
[25] Tsuneo W, Pictorial atlas of soil and seed fungi: Morphologies of cultural fungi and Key to Species. 3rd edition, **2010**.
[26] Oranusi S, Onyeike E, Galadima M, Umoh VJ, *Nig. J. Microbiol*., **2004**, 18(1-2), 346- 362.
[27] Clinical and Laboratory Standards Institute (CLSI), Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Eleventh Edition M02-A11, **2012**, Vol. 32 No. 1.
[28] Oranusi US, Akande VA, Dahunsi SO, *Journal of Biological and Chemical Research*, **2013b**, 30 (2), 570- 580.
[29] AOAC, Official Method of Analysis 15th edition, The Association of Analytical Chemists, **1999**.
[30] Sakanaka S, Tachibana Y, Okada Y, *Food Chemistry*, **2005**, 89, 569-575.
[31] Swamy NR, Samatha T, Rudroju S, Srinivas P, *Asian Journal of Pharmaceutical and Clinical Research*, **2012**, 5(4), 177-179.
[32] Blainski A, Lopes GC, Palazzo de Mello, João C, *Molecules*, **2013**, *18*, 6852-6865.
[33] Zhishen J, Mengcheng T, Jianming W, *Food Chemistry*, **1999**, 64, 555–559.
[34] Alothman M, Bhat R, Karim AA, *Food Chemistry*, **2009**, 115(3), 785-788.
[35] Manzorro L, Anese M, Nicoli MC, *Lebens-mittel-Wissenschaft Und-Technologie*, **1998**, 31(7-8), 694-698.
[36] Nagai T, Inoue R, Inoue H, Suzuki N, *Food Chemistry*, **2003**, 80, 29–33.
[37] Asimi OA, Sahu NP, Pal AK, *International Journal of Scientific and Research Publications*, **2013**, 3(3), 1-8.
[38] Ruch RJ, Cheng SJ, Klaunig JF, *Carcinogenesis*, **1989**, 10, 1003–1008.
[39] Yen Gow-Chin, Chen Hui-Yin, *J. Agric. Food Chemistry*, **1995**, *43*, 27-32.
[40] Alisi CS, Onyeze GOC, Afr. J. Biol. Res., **2008**, 2(7), 145-150.
[41] SPSS. IBM SPSS software for windows version 20.0, SPSS Inc., Chicago, IL; 2011.
[42] International Commission on Microbiological Specifications for Foods, *Microorganisms in Foods* 5: *Microbiological Specifications of Pathogens*, **1996**.
[43] Microbiological Guidelines for Ready-to-eat Food, **2007**.
[44] Ahene RE, Odamtten GT, Owusu E, *African Journal of Environmental Science and Technology*, **2011**, 5(9), 633-640.
[45] Khan MR, Saha ML, Khan FI, *International Journal of Microbiological Research*, **2012**, 3(1), 53-58.
[46] Health benefits of Paprika, *moiponetsoka.hubpages.com*, **2012**, *accessed 03/10/2013*)
[47] Onyeagba RA, Ugbogu OC, Okeke CU, Iroakasi O, *Afr. J. Biotechnol.*, **2004**, 3(10), 552-554.
[48] Yusha M, Garba L, Shamsuddeen U,. *International Journal of Biomedical and Health Sciences*, **2008**, 4(2), 57-60.
[49] Joe MM, Jayachitra J, Vijayapriya M, *Journal of Medicinal Plants Research*, **2009**, 3(11), 1134-1136.
[50] Radha CR, Menaka M, Krishnaveni R, Raadha CK, E-*Journal of Life Sciences* (EJLS), **2012**, 1 (4), 145-152.
[51] Malwal M,Sarin R, *Indian Journal of Natural Products and Resources*, **2011**, 2(1), 48-51.
[52] Ningappa MB, Dhananjaya BL, Dinesha R, Harsha R, Srinivas L, *Food Chemistry*, **2010**, 118(3), 747-750.
[53] Jayaprakash A, Ebenezer P, *J. Acad. Indus. Res.*, **2012**, 1(3), 124- 126.
[54] Das BN, Biswas BK, *International Journal of Life Sciences Biotechnology and Pharma Research*, **2012**, 1(3), 59-63.
[55] White pepper (*Piper nigrum*), MOM's Organic market, www.naturalstandard.com, **2013**, accessed 10/10/2013.
[56] SidartaY O, Prasetyaningrum N, Fitriani D, Prawiro SR, *IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)*, **2013**, 4(6), 25-29.

[57] Nanasombat S, Lohasupthawee P, *KMITL Science Technology Journal*, **2005**, 5, 527- 538.
[58] Kim Il-Suk, Yang Mi-Ra, Lee Ok-Hwan**,** Kang Suk-Nam**,** *International Journal of Molecular Science*, **2011a**, 12(6), 4120–4131.
[59] Kim Il-Suk, Yang M, Goo Tae-Hwa, Jo C, Ahn Dong-Uk, Park Jung-Hyun, Lee Ok-Hwan, Kang Suk-Nam, *International Journal of Food Sciences and Nutrition*, **2011b** , Early Online, 1–7.
[60] Settharaksa S, Jongjareonrak A, Hmadhlu P, Chansuwan W, Siripongvutikorn S, *International Food Research Journal*, **2012**, 19(4), 1581-1587.
[61] Das AK, Rajkumar V, Dwivedi DK, *International Food Research Journal*, **2011**, 18, 563-569.
[62] Hemalatha A, Girija K, Parthiban C, Saranya C, Anantharaman P, *Advances in Applied Science Research*, **2013**, 4(5), 151-157.
[63] Halliwell B, *American Journal of Medicine*, **1991**, 91, pp. 14-22.
[64] Ghosh T, Maity TK, Bose A, Das GK, Das M, Int. J. Pharm. Tech., **2007**, 6, 77-85.
[65] Haque ME, Alam MB, Hossain MS, Asadujjaman M, Islam MM, Mazumder MEH, *Int. J. Pharm. Sci. Res.*, **2010**, 1(10), 78-83.
[66] Verónica D, Celia Q, Beatriz A, Raphael R, *Bio. Med.*, **2007**, 42, 1359–1368.
[67] Klatt P, Lamas S, *European Journal of Biochemistry*, **2000**, 267, 4928-4944.