

ANTIMICROBIAL AND ANTIOXIDANT PROPERTIES OF SELECTED LOCAL SPICES USED IN “KUNUN” BEVERAGE IN NIGERIA

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ABSTRACT

Background. *Kunun-zaki* is an indigenous non alcoholic beverage in Nigeria which has been reported to contain high nutritional values because of the raw materials from which it is made. Therefore, this research was carried out to investigate the antioxidant and antimicrobial activities of extracts of some of the spices used in the production of the beverage.

Material and method. The spices; *Xylopia aethiopica* (Eru), *Syzgium aromaticum* (Kanafuru), *Monodora myristica* (Ariwo), *Piper guineensis* (Iyere) and *Piper guineense* (Uziza leaf) were purchased from local market in Akure, Nigeria. Crude extracts were obtained from the spices using three solvents which include; ethanol, hexane and water. Twenty five g of each spice was soaked in 50 mls of solvent respectively. After evaporation filtrate of water extract and dry extracts of hexane and ethanol were obtained and stored at 4°C until use. The extracts from each of the spices were subjected to screening for their total phenolic content, antioxidant activity and antimicrobial assay using standard methods.

Results. *Syzgium aromaticum* exhibited the highest total phenol content for both the ethanolic (8.75 TAE/g) and hexanolic extract (10.73 TAE/g) while no phenol was detected from the water extract. The ability of the extracts to bleach the free radical 2,2'-diphenyl-1-picrylhydrazyl (DPPH) revealed that the water extracts of the spices possessed better antioxidant properties with values ranged from 58.17-77.53% compared to hexane extracts (44.79-73.87%) and ethanol extracts (23.10-47.05%). Contrastly, ethanolic extracts of the spices exhibited best antibacterial activities with inhibition zones of 10.0-27.0 mm and the antifungal activity (11.0-34.0 mm) against *Aspergillus niger* and *Aspergillus flavus* except for *M. myristica* that produced no inhibition against *A. flavus*.

Conclusion. It can be deduced from this study that water extracts of the spices investigated may be good antioxidant while ethanol extracts are better antimicrobial agent against certain food spoilage or foodborne microorganisms.

Key words: antimicrobial activity, antioxidant property, kunun spices extracts, total phenol content

INTRODUCTION

Since ancient times, spices have been used to improve flavours as well as antioxidant [Adegoke et al. 1999, Rey et al. 2005, Ifesan et al. 2009 a] and antimicrobial properties [Davidson 1997, Ahn et al. 2007,

Ifesan et al. 2009 b, Zaborowska et al. 2012] of different types of foods.

The tree of *Monodora myristica* (ariwo) is most prevalent in the Southern part of Nigeria and is

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commonly known as Jamaican or African nutmeg [Adegoke et al. 1970]. When ground to powder, the kernel is used to prepare pepper soup, as stimulants to relieve constipation and to control passive uterine haemorrhage in women immediately after child birth [Udeala 2000]. African nutmeg seeds possess potassium, calcium, phosphorus, and magnesium with average values of 8017.33 ppm, 4210.12 ppm, 1034.50 ppm and 923.40 ppm respectively [Burubai 2007]. *Piper guineense* (iyere) is a type of spice which contains 5-8% of the chemical piperine, 10% of myristicine, elemicine, saffrole and dillapiol. Important secondary metabolites found in pepper plants are piperine and chavicine [Okokon 2002]. *Piper guineense* (Uziza leaf) is obtained from the plant of *Piper guineense* and is processed and consumed as vegetables in meals [Onwuka and Nwosuagwu 2005].

Xylopiya aethiopicca (eru) seeds have an aromatic pungent taste and dried fruits are important as flavourings to prepare local soups in West Africa. Medicinally, the fruit is used to treat cough, stomachache, dizziness, amenorrhea, bronchitis and dysentery. The fruits mixed with roots are used in the treatment of rheumatism [Orwa et al. 2009]. *Syzygium aromaticum* (kanafuru) are the aromatic dried flower buds of a tree in the family Myrtaceae. The cloves can be put to several uses which include; dental care, treatment of infections, indigestion and in stomach related problems, in diabetes to control the blood sugar levels [Bensky et al. 2004].

Kunun-zaki, an indigenous non-alcoholic beverage widely consumed in Nigeria for its thirst quenching properties has been reported to contain high nutritional values because of the raw materials from which it is made. Spices are usually added in small quantities to improve taste and flavour of kunun [Elmahmood and Doughari 2007]. Therefore, this work carried out to investigate the antimicrobial and antioxidant properties of the selected spices employed in the beverage production.

MATERIAL AND METHODS

Sample collection

The five (5) spices namely: *Xylopiya aethiopicca* (Eru), *Syzygium aromaticum* (Kanafuru), *Monodora myristica* (Ariwo), *Piper guineense* (Iyere) and *Piper*

guineense (Uziza leaf) were purchased from a local market in Akure, Ondo state. They were then taken to the Department of Forestry, Federal University of Technology, Akure, Nigeria for identification.

Sample preparation

Ariwo was cracked to separate the nuts from the kernel and was sun dried. The Uziza leaf on the other hand was shredded, washed and dried in the sun. After this, each of the five spices was milled separately using an Attrition mill (locally fabricated milling machine). The powdered samples were weighed and stored at room temperature. Extracts were prepared as follows using the modified method of Ifesan et al. [2009 a]. Water extract was prepared by soaking 25 g of each of the five spices in 50 mls of hot water (at 80°C) for one hr, after which the contents were sieved with the aid of a muslin cloth. The filtrate was stored respectively, in sterile bottles at 4°C for further analyses. To prepare ethanol and hexane extracts, 25 g of each of the spices were respectively soaked in 50 mls of 95% ethanol and hexane respectively for 3 days, filtered with the muslin cloth and the filtrate was evaporated under reduced pressure in a rotary evaporator (BUCHI Rotavapor R-114, Switzerland) at 45°C until the extracts became completely dry. After evaporation, dry extracts of hexane and ethanol were obtained and stored at 4°C until use.

Determination of total phenolic content

Phenolics and polyphenol compounds constitute the main classes of natural antioxidants present in plants. The amount of total phenolics in the extracts obtained from the five spices were measured using Folin-Ciocalteu reagent method [Djeridane et al. 2006]. One hundred microlitres of each extract (2 mg/ml) was pipetted into different test tubes. To this solution 100 µl of distilled water was added and the tubes were shaken thoroughly. After 1 min, 2.0 ml of sodium carbonate solution (7.5%) was added and the mixture was incubated at 45°C for 40 mins. The absorbance was measured at 760 nm using UV-Vis spectrophotometer (JENWAY UV-visible spectrophotometer). The total phenolic content was expressed as tannic acid equivalent (mg of TAE/g sample) through the calibration curve of tannic acid. All tests were carried out in triplicate.

Free radical scavenging activity (DPPH) of the spices extracts

The hydrogen atom or electrons donating ability of the corresponding extract was measured from bleaching of purple colour methanol solution of DPPH (Sigma-Aldrich, St. Louis, Mo., U.S.A.). The spectrometric assay used stable radical 2, 2-diphenyl picrylhydrazyl (DPPH) reagent [Butris and Bucar 2000]. Six hundred microliter of extract concentration (using extract solvent) containing 0.2 mg of extract was added to 0.6 ml of 0.0185% methanoic solution of DPPH. After a 30 min incubation period at room temperature the absorbance was read against distilled water as blank at 517 nm. Controls with 1% solvent (ethanol, hexane and water) and without the extract were also set up under the same condition for all the experiments. The percentage antioxidant activity (AA%) = $(A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}}) \times 100\%$.

Antimicrobial activity of the crude spices extracts

The extracts were tested for activity against microorganisms using modified agar-well diffusion method procedures described by Clinical and Laboratory Standard Institute [Performance... 2006]. The ethanol, hexane and water extracts of the spices were individually tested against eight microorganisms. The microorganisms stock cultures which include *Acinetobacter* spp., *Bacillus cereus*, *Escherichia coli*, *Salmonella* spp., *Shigella dysenteriae*, *Staphylococcus aureus*, *Aspergillus flavus* and *Aspergillus niger* were provided by the Microbiology unit of Department of Food Science and Technology, Federal University of Technology, Akure. Five hr broth cultures of the test bacteria were adjusted to 10^8 cfu/ml and applied on the surface of Nutrient agar (HiMedia Laboratories Limited, Mumbai, India). A sterile flamed cork borer of 8 mm diameter size was used to punch four wells into each of the seeded plates and 0.5 ml of each extract was dispensed in each well. For fungi, the density of spore suspension was determined using haemocytometer and adjusted to 4×10^5 spores/ml. One millilitre of the suspension was added to 20 ml PDA, shaken gently and allowed to solidify before boring wells into the agar and applying the extract. Controls were set up by filling wells with 1% of various solvents used. The plates were then incubated at 35°C for 24 hr for bacteria and at 30°C for 48-72 hr for fungi. The experiments were performed in duplicate and the means of the diameters of the inhibition zones were calculated.

Statistical analysis

All determinations were carried out at least in triplicates. One way ANOVA was used to find statistical difference between the means of the values reported. The means were separated by using new Duncan multiple range technique with SPSS package (version 17.0).

RESULTS

Total phenolic content

Hexane and ethanol extracts of the five spices were tested positive for total phenolic content while no phenol was obtained from water extract (Fig. 1). The total phenol of the hexane extract ranged from 0.5 mg TAE/g to 10.7 mg TAE/g (mg tannic acid equivalent) and ethanolic extracts (0.8 mg TAE/g – 8.8 mg TAE/g)

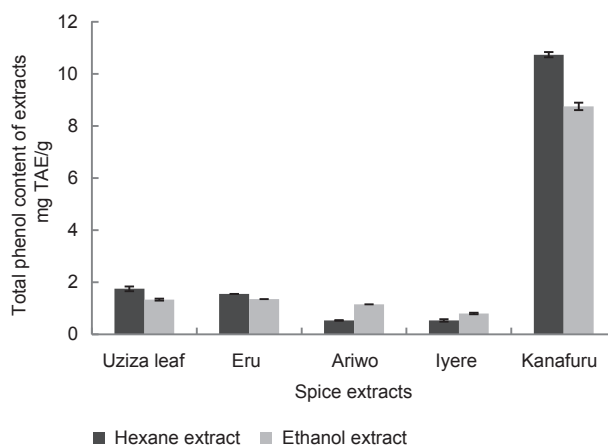


Fig. 1. Total phenolic content of ethanol and hexane extracts from spices

with the highest value from *S. aromaticum* (10.73 mg TAE/g and 8.75 mg TAE/g respectively). *M. myristica* had the least TPC yield in the hexane extract (0.53 mg TAE/g) while *P. guineense* exhibited the lowest TPC in ethanolic extracts (0.80 mgTAE/g).

Antioxidant property

The abilities of the extracts to scavenge DPPH chemical, a stable free radical with characteristic absorption at 517 nm, and stable at room temperature is shown on Figure 2. Water extracts (58-73%) of the five spices yielded the highest DPPH scavenging activity,

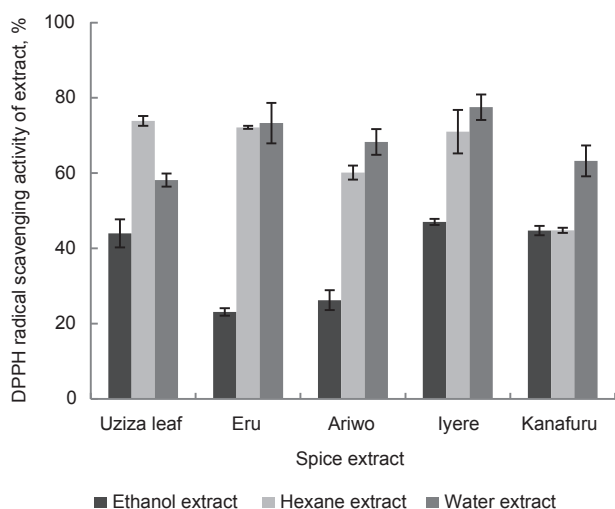


Fig. 2. Free radical scavenging activity (DPPH) of ethanol, hexane and water extracts from spices

followed by the hexane extracts (44.5-74%) and ethanol extracts (23-47%). The radical scavenging activities demonstrated by water extract of spices include; *P. guineense* (58%), *X. aethiopica* (73%), *M. myristica* (68%), *P. guineensis* (77.5%) and *S. aromaticum* (63%).

Antimicrobial activity

The antibacterial properties of the water extract (Table 1) and ethanolic extract (Table 2) of the spices against some bacteria used in this study are shown. Water extract of *X. aethiopica* and *S. aromaticum* produced mild antibacterial activity against all the test microorganisms except *E. coli*. The antibacterial activity of ethanol extracts of *X. aethiopica* (20-27 mm) against all bacteria except *S. aureus* was the highest followed by *S. aromaticum* (13-20 mm) and *Piper guineense* (10-20 mm). Only the ethanolic extracts of the five spices were tested against *Aspergillus niger*

Table 1. Antibacterial activity of the water extracts from the spices, mm

| Bacteria tested | <i>Piper guineense</i> | <i>Xylopia aethiopica</i> | <i>Monodora myristica</i> | <i>Piper guineensis</i> | <i>Syzygium aromaticum</i> | Control |
|------------------------------|------------------------|---------------------------|---------------------------|-------------------------|----------------------------|---------|
| <i>Bacillus cereus</i> | – | 10 | – | – | 8 | – |
| <i>Shigella dysenteriae</i> | – | 8 | – | – | 8 | – |
| <i>Escherichia coli</i> | – | – | – | – | – | – |
| <i>Salmonella</i> spp. | – | 10 | – | – | 10 | – |
| <i>Acinetobacter</i> spp. | – | 10 | – | – | 10 | – |
| <i>Staphylococcus aureus</i> | – | – | – | – | – | – |

Control – sterile water.
“–” – no inhibition.

Table 2. Antibacterial activity of ethanol extracts from the spices, mm

| Bacteria tested | <i>Piper guineense</i> | <i>Xylopia aethiopica</i> | <i>Monodora myristica</i> | <i>Piper guineensis</i> | <i>Syzygium aromaticum</i> | Control |
|------------------------------|------------------------|---------------------------|---------------------------|-------------------------|----------------------------|---------|
| <i>Bacillus cereus</i> | 10 | 27 | 15 | 10 | 10 | – |
| <i>Shigella dysenteriae</i> | 12 | 27 | 25 | 13 | 12 | – |
| <i>Escherichia coli</i> | 15 | 20 | 12 | 15 | 15 | – |
| <i>Salmonella</i> spp. | 15 | 20 | 16 | 15 | 20 | – |
| <i>Acinetobacter</i> spp. | 20 | 20 | – | – | 10 | – |
| <i>Staphylococcus aureus</i> | 18 | – | – | – | 20 | – |

Control – 95% ethanol.
“–” – no inhibition.

Table 3. Antifungal activity of ethanolic extracts from the spices, mm

| Spices extract | <i>Aspergillus flavus</i> | <i>Aspergillus niger</i> |
|----------------------------|---------------------------|--------------------------|
| <i>Piper guineense</i> | 25 | 20 |
| <i>Xylopi aethiopica</i> | 11 | 15 |
| <i>Monodora myristica</i> | – | 20 |
| <i>Piper guineensis</i> | 20 | 20 |
| <i>Syzygium aromaticum</i> | 25 | 20 |
| Control | – | – |

“–” – no inhibition.

and *Aspergillus flavus* (Table 3). All the five spices showed inhibition against *A. niger* (15-34 mm) while all except *M. myristica* inhibited *A. flavus* within the range of 11 mm to 30 mm.

DISCUSSION

The result from total phenol content could be an indication that different solvents demonstrated varying extraction capacities for the various spices. As one of the most important antioxidant plant component, phenolic compounds are widely investigated in many medicinal plants and vegetables [Djeridane et al. 2006]. A direct relationship has been established between the phenolic content and antioxidant capacity of plants [Al-Mamary et al. 2002]. Plant extract or their essential oils that possess very strong antibacterial properties against pathogens have been reported to contain a high percentage of phenolic compounds [Lambert et al. 2001]. In addition, phenolic compounds contribute to quality and nutritional value in terms of modifying colour, taste, aroma and flavour and also in providing health beneficial effects.

As antioxidants donate protons to the radicals, the absorption decreases. The degree of discolouration of the solution reveals the scavenging efficiency of the added extracts [Turkoglu et al. 2007]. Water extract from the spices exhibited highest free radical scavenging property though water extract possessed no phenol content. This result may be an indication that the antioxidant capacity in water extract may be due to the presence of some other phytochemicals and pigments, as well as synergistic effects among them. Furthermore,

antioxidant property recorded from the spices could be attributed to the bioactive compounds they possess. *Piper guineensis* was reported to contain 5-8% piperine, 10% myristicine, elemicine, safrole and dillapiol [Osuala and Anyadoh 2006]. The content of essential oil in cloves is dominated by eugenol (72-90%), eugenol acetate (15%), beta-caryophyllene (5-12%) and 2% of the triterpene olenolic acid [Balch and Balch 2000]. In addition, *X. aethiopica* contained β -pinene, P-cymene, α -cadinol, trans-pinacaveol, α -pinene and 1,8-cinaole [Keita et al. 2003]. Polyphenolic compounds are primarily responsible for the antioxidant activity of natural extract due to their redox properties and chemical structures [Sun et al. 2007].

Action of essential oils present in plants against food borne pathogens reported that they are slightly more active against the Gram positive than Gram negative [Lambert et al. 2001]. Hence, the antimicrobial activity of *S. aromaticum* may be due to the high concentration of caryophyllene, since there is a relationship between the chemical structure of most abundant oils and their antimicrobial activities. Essential oils with high levels of eugenol, cinnamamic aldehyde and citral are usually strong antimicrobials [Davidson and Naidu 2000]. Plant extracts are also known to possess antifungal activity [Soliman and Badeaa 2002], and generally they are more active against fungi than Gram-positive bacteria.

Kunun-zaki is widely believed to be of immense social, economic and medicinal importance to its numerous consumers [Akoma et al. 2006]. It may be concluded that the medicinal property demonstrated by kunun beverage is contributed by the spices added to it. Results from this study revealed that water extracts of the spices investigated may be a good antioxidant while ethanol extracts are better antimicrobial agents against certain food spoilage organisms.

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