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Comparative Antioxidant and in-vivo Toxicological Studies of Selected Spices as Antioxidant Supplements in Kaduna. Kaduna State, Nigeria.

Audu Hajara
Department of Biology,
Federal College of Education, Okene
hajaraanubeaudu@gmail.com

Abstract

Natural antioxidants are increasingly gaining attention as potential preventive and therapeutic agent. This research is aimed at comparing the individual antioxidant properties of six selected spices using three different standard methods. The fresh leafy one (Ocimum gratissimum will be collected from gardens in Barnawa area of Kaduna, fresh Parkia biglobosa, Allium cepa and Allium sativum will be purchased from the central market and sun-dried while the dry seeds of Cuminum cyminum and Xylopia aethiopica will be purchased and processed. Standard Folin-Ciocalteau method will be adopted to determine their Total Phenolic Content (TPC), 2, 2-diphenyl-1-picrylhydrazyl for the Radical Scavenging Activities (RSA) and Phosphomolybdate assay for Total Antioxidant Capacity (TAC) of all spices after extraction with aqueous methanol. Acute toxicity of all extracts will be carried out to determine the lethal dose of the spices according to Lorke's method. The results obtained from the antioxidant analysis will give rise to a spice-food supplement, rich in antioxidants to help combat the rising cases of degenerating disease while the toxicity study will serve as a guide for prescribed usage while benefitting from its medicinal properties.

Keywords: Spices, Free Radicals, Antioxidants, Toxicity, Supplement

Introduction

The rise in the development and diagnosis of diseases related to changes in the body caused by free radicals has sparked an ongoing search for natural ways to prevent or treat these conditions with few or no side effects. Free radicals are unstable and reactive molecules due to unpaired electrons in their atomic orbitals (Lobo *et al.*, 2010). These radicals can form internally in the body or come from our diet and environment. They consist of one or more unpaired electrons in their outer shell and arise from the breaking of chemical bonds, where each new fragment retains one electron, the breaking of a radical to create another radical, and through redox reactions. Common free radicals include hydroxyl (OH-), superoxide (O2-), nitric oxide (NO-), nitrogen dioxide (NO2-), peroxyl (ROO-), and lipid peroxyl (LOO-). Additionally, substances like hydrogen peroxide (H2O2), ozone (O3), singlet oxygen (O), hypochlorous acid (HOCl), nitrous acid (HNO2), peroxynitrite (ONOO-), dinitrogen trioxide (N2O3), and lipid peroxide (LOOH) are not classified as free radicals; they are generally referred to as oxidants but can easily contribute to free radical formation in living organisms (Sies, 2020).

Endogenous free radicals are generated from immune cell activation, inflammation, mental stress, excessive exercise, ischemia, infection, cancer and even aging while Exogenous Reactive Oxygen Species (ROS) and Reactive Nitrogen Specie (RNS) result from air and water pollution, cigarette smoke, alcohol, heavy or transition metals (Cd, Hg, Pb, Fe, As), certain drugs (cyclosporine, tacrolimus, gentamycin, bleomycin), industrial solvents, cooking (smoked meat, used oil, fat) and radiation (Qadr,2025) After penetration into the body by different routes, these exogenous compounds are decomposed or metabolized into free radicals. The body experiences oxidative stress when an excess of free radicals cannot be progressively eliminated. Chronic and degenerative diseases like cancer and autoimmune disorders are largely influenced by this process (Sies, 2020).

According to Altemimi *et al.*, 2017, antioxidants even when present in the body in very minute quantity can delay the oxidative process. This property provides them with a great deal impact or role in the body physiology. The research of Singh, (2021), gave an indication that among the compounds present in spices which offer them their antioxidant properties are poly phenols, flavonoid, terpenoids, lignin, saponins, coumarins, plant sterols and curcumins. This property of spices to inhibit oxidation is known as antioxidants. They are either able to repair the damages caused by oxidation or they are able to prevent the formation of free radicals, and are able to remove those radicals that are already formed. They also have the ability to remove damaged molecules.

The most potent antioxidants work by stopping the chain reactions caused by free radicals. These substances add hydrogen ion radicals to the free radicals created throughout oxidation, turning into stabilized free radicals by the electron's resonance delocalization within the aromatic ring. According to Shahidi and Ambigaipalan (2015), phenolic chemicals are primarily responsible for the antioxidant qualities of spices and are indirectly related to their effectiveness. Antioxidants have a diversity of physiological roles in the body because they alter the oxidation process, even when present in very small concentrations. They serve as radical scavengers and aid in the alteration of radicals into species that are less reactive when they are present in plant materials (Pisoschi & Pop, 2015). The spice additives made at the end of this research will help to inhibit and scavenge oxidative free radicals which accumulates in the body and hence provide an enhanced health for the consumers.

Statement Problem/Justification

Oxidative stress resulting from the excessive production of free radicals has been implicated in the development of numerous chronic diseases, including cancer, cardiovascular disorders, diabetes mellitus, and neurodegenerative diseases. Consequently, there has been an increasing number of diagnoses and report of degenerating diseases in our communities and around the world which calls for concerns. The physiological changes which occur in the body have been reported to be linked to these diseases. At the same time, these physiological

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changes are caused by oxidation as a result of free radical accumulation in the body from food, environmental exposure and lifestyle. According to health reports on disease incidences by World Health Organization (WHO) in 2020, the prevalence and reports of these degenerating diseases is on a geometrical increase.

Interestingly, there has been a growing interest in the use of natural antioxidants derived from plants as alternatives to synthetic antioxidant supplements. In Nigeria, several spices are traditionally consumed not only as flavoring agents but also for their perceived medicinal and health-promoting properties, particularly their antioxidant potential. Therefore, to help manage the development of these diseases in the body is best achieved by the oral intake of antioxidants in form of spice supplements which can rescue man from diseases. They are also seen to be 10 times higher in herbs and spices when compared to fruits and vegetables. (Yashin, 2017, Karakol, 2021). From several research, the phytochemical composition like polyphenols, flavonoids and essential oils present in abundance in spices are responsible for their antioxidant property by way of supporting cellular health, neutralizing the effect of free radicals and reducing inflammation.

Despite the widespread use of these spices as dietary supplements and in traditional medicine, scientific information regarding their comparative antioxidant capacities remains limited. Most available studies focus on individual spices, making it difficult to determine which species possess superior antioxidant properties. Furthermore, while the beneficial effects of these spices are often emphasized, there is insufficient information on their safety profiles, especially when consumed in large quantities or over prolonged periods. The increasing popularity of natural antioxidant supplements necessitates a thorough evaluation of both their efficacy and potential toxicological effects. Without adequate toxicological assessment, consumers may be exposed to adverse health risks arising from the indiscriminate use of these plant products. Therefore, there is a need for a comprehensive comparative study to evaluate the antioxidant activities and in vivo toxicological effects of selected Nigerian spices.This study seeks to address these knowledge gaps by comparatively assessing the antioxidant potential of selected spices and determining their safety through in vivo toxicological investigations. The findings will provide scientific evidence for the safe and effective use of these spices as antioxidant supplements, contribute to the development of nutraceutical products, and inform public health recommendations regarding their consumption in Kaduna State in particular and Nigeria in general.

Objective(s) of the Study

1. To determine the spice with the highest phenolic content
2. To determine the spice with the highest Radical scavenging property
3. To determine the spice with the greatest antioxidant property.
4. To determine the safety dosage for the use of the spices as antioxidants.

Literature Review

Antioxidant system plays a very important role in general health maintenance. Diet rich in antioxidants can function in one of the following ways: Reduce oxidative stress, prevent cancer, reduce radicalism, prevent heart diseases and other degenerating diseases and increase longevity. Studies have shown that regular consumption of fruits, vegetables, spices that are rich in antioxidants has a very positive long-term health effect. Antioxidants work as a team and primarily they reduce the level of oxidation of fats in the body (Pisoschi & Pop, 2015). They also control the formation of free radicals and reduce the devastating effects of already formed free radicals, and the toxic substances produced from their breakdown. Among the food ingredients that fight against prolonged diseases, scientific research in the field of plants have drawn attention to the capability of phytochemicals and plant-derived molecules with intact antioxidant power (Xiao *et al*.,2022) The cumulative and synergistic activities of these molecules found in plant foods have been proven to be responsible for their antioxidant properties (Megan, 2023). In 2011, Ragaa *et al. (2011)* investigated the Antioxidant and Anti-carcinogenic effects of Fennels seeds. They considered the methanol extract and the volatile oil for these activities and they found out that they may possess anti carcinogenic effect against liver and breast cancer. Their conclusion was based on the twin fact that the extract and oil exerted protective effect on gamma irradiation by restoration of glutathione content to its normal level after its induction to abnormal levels. Catalase activity and malondialdehyde levels were restored. The lipid peroxidation levels were also found to be modulated and varying significant percentages of flavonoids, terpenoids, alkaloid, sterol and phenols were present in the extract.

The treatment with aqueous extracts of *Ocimum gratissimum* and *Xylopia aethiopica* in increasing dose revealed that both plants had the ability to reduce MDA levels and increase antioxidant capacity and it was dose dependent. However, *Xylopia aethiopica* was found to have more significant effect on the antioxidant capacity more than *Ocimum* and the combination of both spices didn't yield any significantly better effect like their separate usage yielded (Chuks *et al*., 2020).

Bouhenni*et al*. (2021) determined the antioxidant potential of *Allium sativum* and *Allium cepa* cultivated in Algeria using HPLC to determine total phenolic content and DPPH for antioxidant capacity. The result revealed that cepa had a higher total phenolic content of 86±1.00mgGAE/100g while sativum with 45±1.00mgGAE/100g.

The antioxidant properties of *Cuminum cyminum* was reviewed by Gulzar, (2018). The methanolic extract of Cumin was found to exhibit more antioxidant capacity when compared to the aqueous extract. Also, the overall amount of phenol presents in three different species of cumin extracted with methanol was found to be high, ranging between 4.1 to 53.6 mg/g dry weight.

According to Abubakar *et al.* (2018) Scavenging capacity of Negro pepper extract using DPPH and FRAP assay gave IC_{50} values of 52.45±3.05 µg/mL and 73.45±3.89 µg/mL

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However, Tijjani, *et al.*, (2022) obtained a relatively lower values for the invitro antioxidant potential of aqueous extracts of negro pepper whole seed and the pod.

Methodology

The methodology that was adopted in this study is purely experimental where standard procedures and methods were adopted for different parameters. The chemical analysis of the extracts was carried out at the pharmacology laboratory, Ahmadu Bello University Zaria while the Toxicity Study was carried out at the animal house of the Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria.

Determination of Total Phenolic Content (TPC)

To determine the absolute amount of phenol in the spices, standard folin-ciocalteau method was adopted. According to Moresan,*et al*. (2012) and Kodama, *et al*. (2010) with slight modification. Exactly 0.25 methanolic extract of the spices was mixed with 0.12ml of distilled water. After 5 minutes at room temperature, 0.34ml of 7.5% sodium carbonate (Na_2Co3) solution was added and mixed for two hours. The absorbance was then measured using a spectrophotometer. All measurements were done in triplicate. Standard Gallic acid solution and concentration was used to perform a calibration curve and TPC was expressed as mgGAE/g of dried spice material.

Determination of Radical Scavenging Capacity Using DPPH Free Radical Assay

The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method adopted by Anesini, *et. al*.(2008) was used to determine the scavenging activities of the extracts on the reagent. DPPH is a reagent used to measure the reducing ability of antioxidants towards it. This assay was used to observe and measure the loss of DPPH colour (deep purple) at 515 nm after reaction with the antioxidant. The percentage of DPPH remaining was calculated and proportional to the antioxidant concentration that causes a decrease in the initial DPPH concentration.0.1ml methanolic extract was mixed with 0.9ml distilled water and 3.9ml methanolic DPPH solution. The mixture was then incubated for 30 minutes in darkness and the absorbance of each sample was measured at 515nm against a blank of methanol. The absorbance was used to calculate the percentage scavenging activity of the DPPH using the following equation:

$$\% \text{ of Inhibition} = [1 - As/Ao] \times 100$$

Where: As is the Sample absorbance and Ao represents DPPH absorbance

Determination of Total Antioxidant Capacity (TAC)

Phosphomolybdate assay was used to determine the Total Antioxidant Capacity (TAC) of each spice extract using a simple spectrophotometric method. The phosphomolybdate reagent was first prepared with 28mM of sodium phosphate, 0.6M of Sulphuric acid and 4mM of ammonium molybdate separately in equal volumes and then mixed in an airtight container and kept in the dark. A blank reagent which consists of the solvent used for extraction, the reagent and the standard antioxidant solution (Ascorbic acid + reagent) was also required.

pISSN 2536-6661, eISSN 2536-6653 Vol.14, No.1, April/June, 2026
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Experimental Animals

Wistar rats of certain weight range were procured from the Animal house of the Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The animals were then kept under standard conditions to acclimatize and fed with rodent diet and water. The study was approval from the Institutional Anticorruption and Transparency Committee constituted for the purpose of moral responsibility and compliance.

Acute Toxicity Test

To determine the acute toxicity of the spices, a total of 108 Wistar Rats was used. Lorke's method was adopted. 81 Animals was first divided into three groups of four animals each and oral administration of 10mg/kg, 100mg/kg and 1000mg/kg doses of each spice extract was given. The animals were then monitored for behaviour and mortality within 24 hours. After which another set of 27 animals were grouped into three groups of one animal each and administered higher doses of 1600, 2900 and 5000mg/kg of extracts and observed for behavioural change or possible mortality within 24 hours. The heart, kidney, liver, stomach walls were observed for possible ulceration, inflammation, or other side effects after sacrifice and the LD_{50} was determined (Lorke, 1983).

Result

Table 1: **Quantification of TPC, Radical Scavenging Activity and TAC of selected spicy plant extract**

S/N	Spice Samples	Total Phenolics Content (TPC) mgGAE/100g	Radical Scavenging Activity RSA(DPPH) (%)	Total Antioxidant Content (TAC) (%)
1	***Ocimum gratissimum***	**$6.85{\pm}0.01^{d}$**	$27.71{\pm}0.01^{c}$	$5.94{\pm}0.02^{b}$
2	***Allium cepa***	$5.62{\pm}0.12^{b}$	**$38.17{\pm}0.00^{e}$**	**$9.45{\pm}0.01^{e}$**
3	*Xylopia aethiopica*	$5.42{\pm}0.01^{a}$	$12.15{\pm}0.01^{a}$	$8.55{\pm}0.01^{c}$
4	***Allium sativum***	**$9.34{\pm}0.01^{f}$**	**$47.81{\pm}0.01^{f}$**	**$10.61{\pm}0.01^{f}$**
5	*Cuminum cyminum*	$5.92{\pm}0.01^{c}$	$23.44{\pm}0.01^{b}$	$4.85{\pm}0.01^{a}$
6	***Parkia biglobosa***	**$8.03{\pm}0.01^{e}$**	**$33.55{\pm}0.01^{d}$**	**$9.33{\pm}0.01^{d}$**

Mean values with different superscript on the same column are significantly different at ($P \leq 0.05$). Each value is a mean of three replicate ±SD using Duncan multiple tests.

The result obtained from the antioxidant activities of the selected spices using TPC, RSA and TAC as shown on Table 1 above reveals that *Allium sativum* had the highest TPC of 9.34mgGAE., *Parkia biglobosa* had 8.03mgGAE, *Ocimum gratissimum* had 6.85mgGAE, *Xylopia aethiopica showed* the lowest TPC of 5.42mgGAE. RSA for *Allium sativum*, *Allium cepa*, *Parkia biglobosa*, *Cuminum cyminum*, *Ocimum gratissimum*, to *Xylopia aethiopica* in descending order had, (47.81%,38.17%, 33.55%, 27.71%, 27.59%, 23.44% and 12.15%) respectively. *Allium sativum* showed the highest TAC of (10.61%), *Allium cepa* had (9.45%),

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Parkia biglobosa had (9.33%) *Xylopia aethiopica* had (8.55%, 5.94%) for *Ocimum gratissimum* and the spice with the least was *Cuminum cyminum* with (4.85%).

Table 2: Lethal Doses/ Toxicity of the six spice plant extracts

Dosage(mg/kg)	Parameter	A	B	C	D	E	F
10	Weight	**114**	**132**	**151**	**126**	**140**	**178**
	Fatality	**0**	**0**	**0**	**0**	**0**	**0**
	Behaviour	**Normal**	**Normal**	**Normal**	**Normal**	**Normal**	**Normal**
100	Weight	**130**	**147**	**168**	**145**	**123**	**177**
	Fatality	**0**	**0**	**0**	**0**	**0**	**0**
	Behaviour	**Normal**	**Normal**	**Normal**	**Normal**	**Normal**	**Normal**
1000	Weight	**117**	**128**	**144**	**153**	**126**	**136**
	Fatality	**0**	**0**	**0**	**0**	**0**	**0**
	Behaviour	**Normal**	**Normal**	**Normal**	**Normal**	**Normal**	**Normal**

Key: Group A-Scent leave, B-Onion, C- Cumin, D-Locust Bean, E- Garlic, F- Negro pepper.

The result from the first phase for the toxicity study as represented on Table 2 above and second phase as represented in table 3.1 -3.6 below showed that the Wistar rats exhibited normal behaviour throughout the study, there was no loss no weight, no fatality in both first and second phase of the toxicity study as such the LD50 of all the single and combination extract was above 500mg/kg.

Results of Second Phase of Toxicity Studies for six selected spice extracts

Table 3.1: LD_{50} for Scent leave *(Ocimum gratissimum)*

Group	No of animal	Dose(mg/kg)	BW before(g)	BW After(g)	Fatality	LD_{50}
A1	1	1600	112	112	Nil	Above 5000mg/kg
A2	1	2900	112	112	Nil	
A3	1	5000mg	190	190	Nil	

Table 3.2: LD_{50} for Onions *(Allium cepa)*

Group	No of animal	Dose (mg/kg)	Weight before(g)	Weight After(g)	Fatality	LD_{50}
B1	1	1600	104	104	Nil	Above 5000mg/kg
B2	1	2900	117	117	Nil	
B3	1	5000mg	187	187	Nil	

Table 3.3: LD50 for Cumin *(Cuminum cyminum)*

Group	No of animal	Dose (mg/kg)	Weight Before (g)	Weight After (g)	Fatality	LD_{50}
C1	1	1600	102	102	Nil	Above
C2	1	2900	101	101	Nil	5000mg/kg
C3	1	5000mg	191	191	Nil	

Table 3.4: LD50 for Locust bean *(Parkia biglobosa)*

Group	No of animal	Dose (mg/kg)	Weight Before (g)	Weight After (g)	Fatality	LD_{50}
D1	1	1600	131	131	Nil	Above
D2	1	2900	138	138	Nil	5000mg/kg
D3	1	5000mg	162	162	Nil	

Table 3.5: LD50 for Garlic *(Allium sativum)*

Group	No of animal	Dose (mg/kg)	Weight Before (g)	Weight After (g)	Fatality	LD_{50}
E1	1	1600	122	122	Nil	Above
E2	1	2900	112	112	Nil	5000mg/kg
E3	1	5000mg	157	157	Nil	

Table 3.6: LD50 for Negro pepper *(Xylopia aethiopica)*

Group	No of animal	Dose (mg/kg)	Weight Before (g)	Weight After (g)	Fatality	LD_{50}
F1	1	1600	116	116	Nil	Above
F2	1	2900	104	104	Nil	5000mg/kg
F3	1	5000mg	165	165	Nil	

Discussion of Findings

The determination of antioxidant capacity of the six selected spice plants which was carried out using three different spectrophotometric methods: Total Phenolic Content (TPC), Radical Scavenging Activity using DPPH and Total Antioxidant Capacity (TAC) as shown in table 1 indicates that *A. sativum* produced the highest phenolic concentration of 9.34mgGAE/100g, the next was *biglobosa* with 8.02 mgGAE/100g followed by *O. gratissimum* with 6.85mgGAE/100g. In descending order of concentration, the remaining spices were C. *cyminum* (5.92), A. cepa (5.62) and *X. aethiopica* (5.42) mgGAE/100g. These values all had significant differences from each other. The variation in the concentration of Total Phenolic in different spice plants could be due to variation in cultivation. Phenolic compounds are link to several health benefits from anticancer, antioxidant, anti-inflammatory, antalgic, antihypertensive, anti-arthritis, antimicrobial and cardio protective (Bhuyan & Basu. (2017).

For Radical Scavenging Activity (RSA) using DPPH, *Allium sativum* showed the highest percentage scavenging activity of 47.81%. The next was *Allium cepa* with 38.17% while third on the chart was *biglobosa* with 33.52%. This result is similar to that of Benkeblia, (2005) who reported that *Allium sativum* exhibited higher scavenging activity than some selected species of *Allium cepa*. He linked this property to the higher phenolic content in *sativum* when compared to the other species of *Allium*. Others in the same order were, *gratissimum*, *cyminum* and the one with the least activity was *aethiopica* with 12.15%. *Allium sativum* indicated the highest value of 10.61 mg/g for the TAC again then *A. cepa* and *biglobosa* revealed values of 9.45 mg/g and 9.34 mg/g which had no statically significant difference between them. Next was *X. aethiopica* with 8.55mg/g, *O. gratissimum* with 5.95mg/g and the least with TAC of 4.85mg/g was *C. cyminum*. Several authors including Hussein, (2017) had reported the mechanism of radical scavenging activity to either be the termination of the radical chemical chain reactions by the donation of hydrogen or the neutralization of the oxidative process by electron transfer. Another important factor in the peroxidation process is the structure of the phenolic compound and other antioxidant potential inherent.

The result obtained from the acute toxicity test using Lorke's method as shown in Tables 3.1- 3.6 revealed normal behaviour of all Wistar rats administered the six extracts. In the first stage of the test, the animals were administered single doses of 10mg/kg, 100mg/kg and 1000mg/kg while in the second stage, they were administered 1600mg/kg, 2900mg/kg and 5000mg/kg as the highest dose. It was observed that the animals had a stable body weight, normal behaviour, normal feed habit and zero mortality even after administering the highest doses of all extracts. This implies that the lethal doses for all 6 extracts are above 5000mg/kg as such it is an indication of its promised effectiveness and safety of using the spice plants. The result is in agreement with numerous researchers from old to recent times like (Shah, 1998) **(**Bahareh, 2018**)** (Ouolouho *et al*.2018) **(**Oso *et al*., 2019**)** who demonstrated that the use of spices plants even in very high use had no toxic effect on body weight, organ biochemical and histograms. These findings suggest the safe use of spices for culinary and medicinal purpose without fear of any major body physiological changes.

Conclusion

The antioxidant potential of the spices studied shown varying significant degree of TPC, TAC and RSA. The finding is suggestive that the antioxidant properties of the spices are linked to their phytochemical constituents, which contributes to their ability to neutralize free radicals. The spice with the highest phenolic concentrations exhibited higher radical activity and Total antioxidant capacity indicating a connection between phenolic content and antioxidant capacity. This ranking also provides a clear understanding of their antioxidant potentials thereby offering insight into their relative pharmacological applications and therapeutic use of the spices. The In vivo, toxicological study revealed that all spices studied were safe at the doses administered as adopted. This suggests their suitability as dietary antioxidant supplement. The overall study provides scientific evidences supporting a safe nutraceutical and therapeutic supplements for every health-conscious individual since exposure to free radical has a limited control.

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