Research Article

Phytochemical screening and evaluation of antioxidant capacities of *Allium cepa, Allium sativum,* and *Monodora myristica* using in *vitro* and *in vivo* models

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Received: 15 August 2021, Revised: 08 September 2021, Published: 14 September 2021

ABSTRACT

Background: *Allium cepa, Allium sativum,* and *Monodora myristica* are commonly sourced food condiments in every household in Nigeria. In the present study, we investigated the phytochemical compositions, *in vitro* and *in vivo* antioxidant activity of these plants. **Methods:** The aqueous extracts from the *A. cepa, A. sativum,* and *M. myristica* were evaluated for phytochemical composition using standard protocols while the antioxidant activities were evaluated using the reducing power assay. Forty-five (45) Male Wistar rats (weighing 185±10 g) were divided into five groups (n=9) and were orally administered with 100 mg/kg BW each of *A. sativum*, *M. myristica, A. cepa*, and ascorbic acid while the control group received 0.5 mL/kg BW distilled water alone. Animals (n=3) from each group were sacrificed after the 20th, 25^{th,} and 30th days of oral administration. The blood and tissue samples were collected for the analysis of biochemical parameters. **Result:** Our results revealed the presence of flavonoids, alkaloids, tannins, saponins, and terpenes in the plant extracts. *A. sativum* had the highest reducing power capacity followed by *M. myristica* and then *A. cepa.* The *in vitro* antioxidants activities demonstrated by the plant extracts were higher than that of ascorbic acid but less than butylated hydroxytoluene. *In vivo* antioxidant studies showed a marked increase ($p<0.05$) in the level of catalase with a concurrent decrease ($p<0.05$) in the levels of MDA and H_2O_2 in the liver and kidney of rats administered with aqueous extracts of the condiments compared to the normal control and ascorbic acid in the following order control < ascorbic acid < *A. cepa* < *M. myristica* < *A. sativum.* **Conclusion:** Based on these findings, we infer that the aqueous extracts of *A. cepa, A. sativum,* and *M. myristica* are rich in antioxidants and as a result could serve as promising novel functional foods and nutraceuticals.

Keywords: *Allium cepa; Allium sativum; Monodora myristica;* antioxidant; phytochemicals.

Citation: Irozuru, C.E., Olugbodi, J.O., Arunsi, U.O., Ladeji, O. (2021). Phytochemical screening and evaluation of antioxidant capacities of *Allium cepa, Allium sativum,* and *Monodora myristica* using in *vitro* and *in vivo* models. AROC in Food and Nutrition, 1(1), 41-52, https://doi.org/10.53858/arocfn01014152

1.0 Introduction

Diatomic oxygen is an unavoidable product of aerobic metabolism which is formed from the incomplete reduction or oxidation of molecules during metabolism generating reactive oxygen species (ROS) [1]. ROS plays a dual role as both toxic and beneficial compounds. At moderate or low concentration, ROS mediates essential roles in the body, among other things: immune function, cell signaling, redox regulation, and apoptosis [2]. However, at high levels, they become harmful to cell [3]. Enhanced generation of ROS in the cell may induce oxidative stress, and this has been implicated in the pathogenesis of many chronic and degenerative diseases [4,5]. Antioxidant systems in living organisms are capable of scavenging and stabilizing ROS by donating additional electrons, delaying or inhibiting the oxidation of substrates [6]. They are produced in situ or sourced externally from food and supplements [7]. Studies have shown that the natural human antioxidant defenses are not always sufficient to maintain the proper ROS balance, and some normal biological processes can become detrimental when it persists long term [6]. This has led to the search for exogenous (synthetic) antioxidants by man to ameliorate oxidative stress in the body. Butylated hydroxytoluene (BHT) is a synthetic antioxidant allowed for use in fat and oils, heat treated foods, frying oil, frying fats, and in beef, poultry, and sheep fats. Inadvertent exposure to BHT in animals has been shown to induce renal and liver

toxicities, reduce humoral immune response in animals [8]

Recent research studies reveal the need to replace synthetic antioxidants such as BHT with natural antioxidants such as plants and herbs [9]. In this direction, natural antioxidants have been used in place of synthetic ones because of their broad spectrum of applications in the prevention and treatment of diseases and broad safety margin; and this has influenced food manufacturers, pharmaceutical industries, and many investigators [10-12]. *A. cepa* (onions) is the most widely cultivated species of the genus Allium. Externally, fresh onion juice is used to prevent bacterial and fungal infections [13], remove warts [14], stimulate hair growth [15], and reduce unwanted skin blemishes [15-17]. Onion contains a rich blend of S-methyl cysteine sulfoxide, flavonoids such as quercetin and organosulfur compounds such as allyl propyl disulphide [18]. Experimental reports have shown that these phytochemicals found in onions have antioxidant [19,20], anti-inflammatory, hypolipidemic [21], and antidiabetic effects [22]. *M. myristica* (African nutmeg) is an easily sourced condiment used to prepare different delicacies because of its aromatic flavour [23]. Traditionally, it has been used to heal sores caused by guinea worms [24], constipation [25], stomach ache [26], headache [27,28] as well as to stop intra-uterine bleeding in women after childbirth [29]. The oil from the seed is used in relieving the discomfort of gas in the digestive tract, for making perfume and soap scents [27,30]. The bark is used in the treatment of stomach aches, febrile pains [31], eye diseases [32], while the root is munched to mitigate toothaches, arthritis [33,34]and also in the management of anaemia [27,33], haemorrhoids as well as sexual weakness [30,32]. Studies have revealed that *M. myristica* possesses antioxidant [24,28], hypolipidemic [33], anti-sickling, antimicrobial, [25,26] as well as anthelmintic activities [27]. *A. sativum* (garlic) is the most widely consumed bulb after onion [35]. Traditionally, it has been used as a flavouring agent [13], seasoning in cooking [36], and in medicine to manage fever [37], headache [38], cholera, and dysentery [35]. A component of garlic, S-methyl cysteine sulfoxide, has been shown to reduce both blood cholesterol and the severity of atherosclerosis [39], stroke [40], coronary thrombosis, platelet aggregation, as well as infections and vascular disorder [17]. The study aimed to investigate the phytochemical contents and antioxidant capacity of *A. cepa, A. sativum,* and *M. myristica* through *in vitro* and *in vivo* methods. The outcome of this study will serve as a blueprint for the branding of these condiments as natural antioxidants in the food industry.

2.0 Material and methods

2.1 Chemicals

Trichloroacetic acid (TCA), thiobarbituric acid (TBA)**,** butylated hydroxyl toluene (BHT), hydrogen peroxide, dichromate, xylenol orange, sorbitol, were purchased from Sigma-Aldrich Co. (St Louis, MO, USA). All other reagents were of analytical grade.

2.2 Plant collection and preparation

A. cepa (bulb)*, A. sativum* (bulb), and *M. myristica* (seed) were purchased from Nyanya market in Abuja, Nigeria. They were peeled, washed, and allowed to air dry. The dried samples were coarsely minced and blended to a fine powder. Exactly 300 g of each of the samples were soaked in 700 ml of distilled water for 48 hours and then filtered using Whatman filter paper No1. The filtrate was evaporated to dryness in a water bath at 100°C and the preparations served as stock and were stored at 4°C.

2.3 Phytochemical screening

Phytochemical screening was carried out with the aqueous extract of *A. cepa, A. sativum,* and *M. myristica* for the detection of various phytochemicals. The extracts were tested for the presence of tannins, saponins, glycosides, alkaloids, flavonoids and phenolic compounds using the standard procedures described by Trease and Evans [41].

2.4 Reducing power assay

The reducing power of the extracts were determined by a method described by Oyaizu [42]. Different concentrations of extracts in 1 mL of distilled water were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferrocyanide. The mixtures were incubated at 50°C for 20 min. Aliquots 2.5 mL of 10% trichloroacetic acid were added to the mixtures and centrifuged at 3000 rpm for 10 min, the supernatant of the

solution (2.5 mL) was added to 2.5 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$. The absorbance was read at 700 nm by UV-Spectrophotometer.

2.5 *In vivo* antioxidant assay

A total of 45 male Wistar rats (185±10 g) raised in the animal house of Bingham University were used for the experiment. Animals were housed in polypropylene cages and kept at standard laboratory condition (at 24 ± 2 °C under 12:12 h of light and dark cycle). The research was carried out in accordance to the ethical rules on animal experimentation approved by the ethical committee of Bingham University. The animals had free access to a standard pellet diet and water *ad libitum*. After that, the animals were divided into five groups of 9 rats each, as described below:

Group I rats were administered with 0.5 mL/kg BW distilled water alone

Group II rats were administered with 100 mg/kg BW ascorbic acid

Group III rats were administered with 100 mg/kg BW *A. cepa*

Group IV rats were administered with 100 mg/kg BW *M. myristica*

Group V rats were administered with 100 mg/kg BW *A. sativum*

Three rats from each group were sacrificed on the 20th, 25^{th,} and 30th days of oral administration through cervical dislocation. The animals were fed once daily with commercially formulated rat feed, and water was given *ad libitum*. The research was carried out in accordance to the ethical rules on animal experimentation approved by the ethical committee of Bingham University.

2.6 Preparation of liver and kidney homogenate

The liver and kidney were excised from the sacrificed Wistar rats the organs were weighed, kept in precold 0.1M phosphate buffer, pH 7.4 and 10% homogenate were prepared by homogenizing 10 g of each organ in the cold phosphate buffer at 4°C. The homogenate was centrifuged at 3000 rpm for 15 min, and the clear cell-free supernatant obtained was used for the study.

2.7 Determination of oxidative stress biomarkers

The supernatants of the liver and kidney tissues of control, ascorbic acid, *A. cepa, A. sativum* and *M. myristica* treated rats were collected for the estimation of catalase (CAT) activity using hydrogen peroxide as a substrate according to the method of Clairborne [43]. Hydrogen peroxide generation was assessed by the method of Wolff [44]. Lipid peroxidation was quantified as the amount of malondialdehyde (MDA) produced according to the method described by Varshney and Kale [45].

2.8 Statistical analysis

The experiments were conducted on the 20th ,25th and 30th day of administration, all determinations were performed in triplicates, and results were expressed as Mean ± SEM. Statistical analysis was performed by one-way analysis of variance (ANOVA) with GraphPad Prism statistical software package, version 9. Statistically significant differences were set at values of $p<0.05$.

3.0 Results

3.1 Phytochemical composition

The phytochemical constituents of aqueous extracts of the selected condiments consisting of *A. cepa, A. sativum,* and *M. myristica,* were analyzed qualitatively, as shown in Table 1. The spices are replete in the following phytochemicals at varying intensity: flavonoid, tannin, saponin, cardiac glycoside, terpene, steroid, and phlobatannin. Alkaloid was present in *A. cepa* and *A. sativum* only.

Table 1. Phytochemical composition of aqueous extract of selected food condiments

Phytochemicals	*A. cepa*	*M. myristica*	*A. sativum*
Alkaloid	++	-	+
Flavonoid	+	+++	++
Tannin	+	+	+++
Saponin	+	+	+++
Cardiac glycoside	++	+	+++
Terpene	++	++	++
Steroid	+	+	++
Phlobatannin	+	+	+

-; Absent +; Mildly present, ++; Moderately present, +++; Abundantly present

3.2 *In vitro* antioxidant capacity

To investigate the antioxidant capacity of the crude extract of selected food condiments (*A. cepa, A. sativum* and *M. myristica*), the reducing capacity of these extracts were measured using reducing power (%) at various concentrations (50 –1000 µ/mg) and compared it with synthetic antioxidants; butylated hydroxytoluene and ascorbic acid (Figure1). The result revealed that the percentage reducing power slightly increased with concentrations, i.e., from 50 to 1000 µ/mg. BHT recorded the highest percentage reducing power at all levels. This is followed by *A. sativum, M. myristica, A. cepa,* and ascorbic acid. There is a significant difference ($p<0.05$) in the percentage reducing power between BHT and the selected condiments at various concentrations. However, there is no statistical difference in the percentage reducing capacity when compared to ascorbic acid. The results further revealed that at higher concentrations of 800 and 1000 µ/mg, the percentage reducing power of *M. myristica* and *A. sativum* increased significantly when compared to BHT and ascorbic acid.

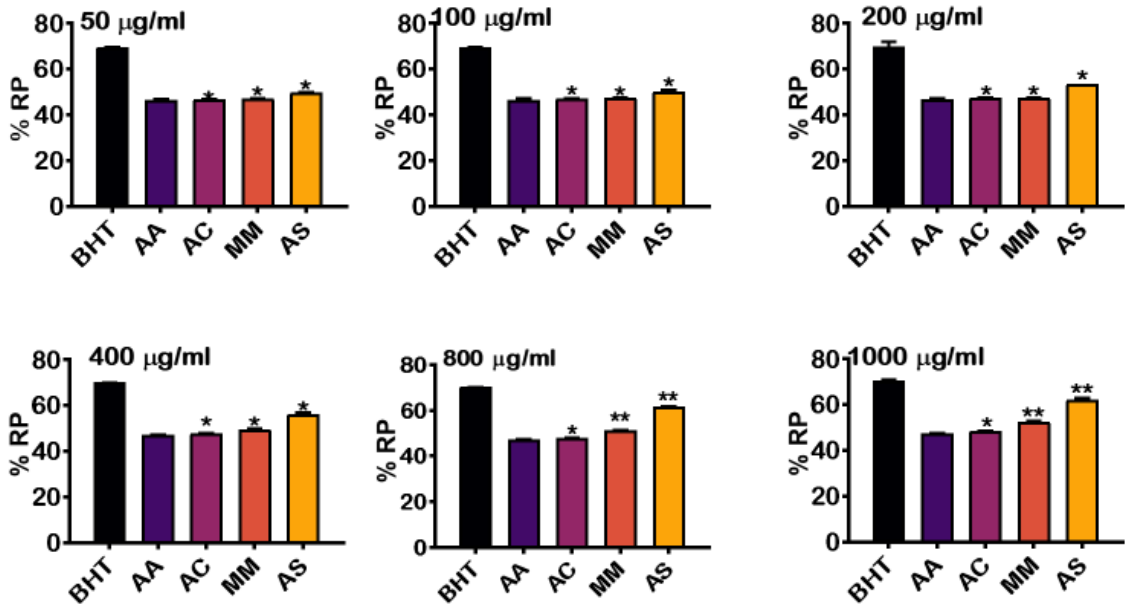

Figure 1. *in vitro* (Reducing power) activity of BHT, AA and selected food condiments. *P<0.05 versus BHT; **P<0.05 versus AA, BHT, Butylated hydroxytoluene, AA, Ascorbic acid, AC; *Allium cepa*; MM, *Monodora myristica; AS, Allium sativum;* RP, Reducing power.

3.3 Effect of selected food condiments on oxidative stress biomarkers

Figures 2 and 3 showed the tissue levels of MDA and H_2O_2 respectively on the 20th,25th and 30th day of extract administration. The level of LPO decreased steadily with *A. sativum* having the least LPO level, followed by *M. myristica, A. cepa,* ascorbic acid and then control. Interestingly, there was marked significant difference in the free radical scavenging activity in the liver of rats treated with aqueous extract *A. sativum* and *M, myristica* when compared to the synthetic antioxidant supplement, ascorbic acid. In the kidney of animals treated with the aqueous extract of the selected food condiments, there was no significant difference in the level of LPO compared to ascorbic acid (Figure 2). Also, the level of H_2O_2 in the liver and kidney tissues was significantly decreased in days 20, 25 and 30 (Figure 3).

The H_2O_2 levels in the liver was reduced considerably by A. *cepa, M. myristica*, and *A. sativum* in days 20, 25, and 30 when compared with the normal control and ascorbic acid treated groups. At day 20 and 25, A. *cepa* recorded the highest free radical scavenging activity, whereas, on day 30, the level of H_2O_2 was lowered by A. *sativum.* In the kidney, a similar observation was made. However, the levels of H_2O_2 in days 20, 25, and 30 decreased significantly in the groups treated with A. *sativum* followed by A. *cepa* and then M. *myristica*.

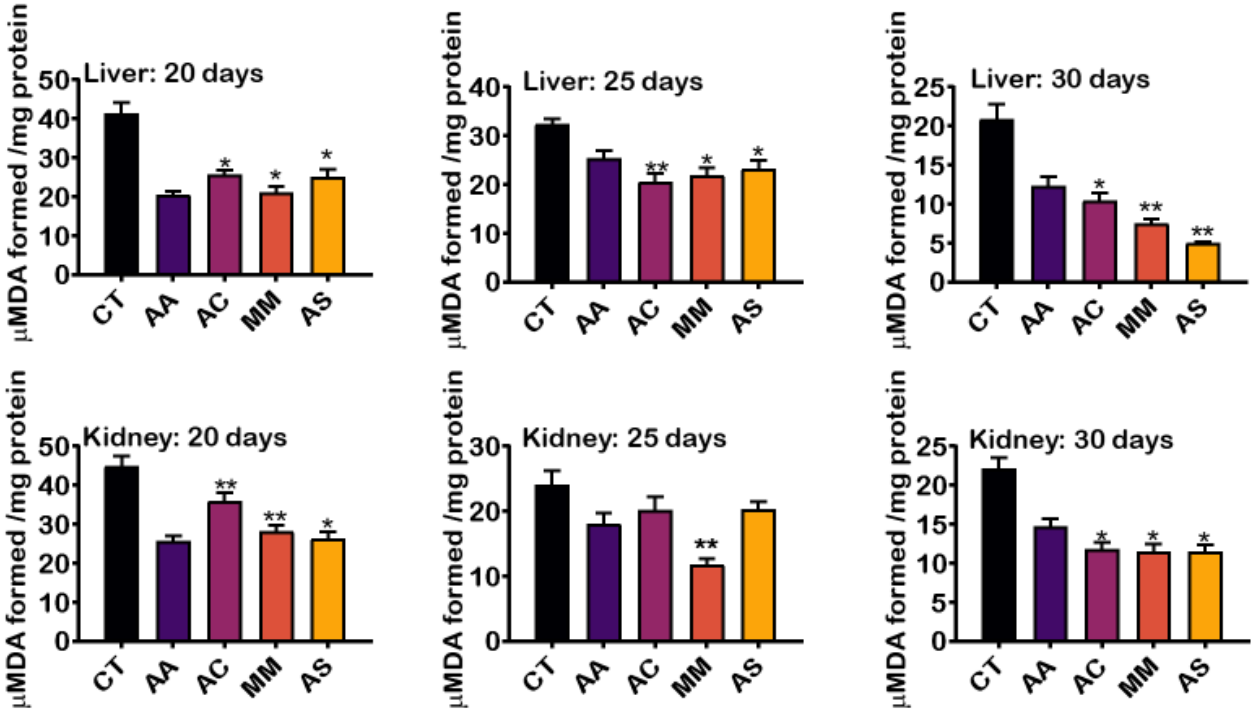

Figure 2. Effect of oral administration of ascorbic acid and some selected food condiments on the level of LPO in the liver and kidney. *P<0.05 versus Control; **P<0.05 versus AA, CT; control, AA; Ascorbic acid, AC; *Allium cepa*; MM; *Monodora myristica;* AS; *Allium sativum;* LPO, Lipid peroxidation.

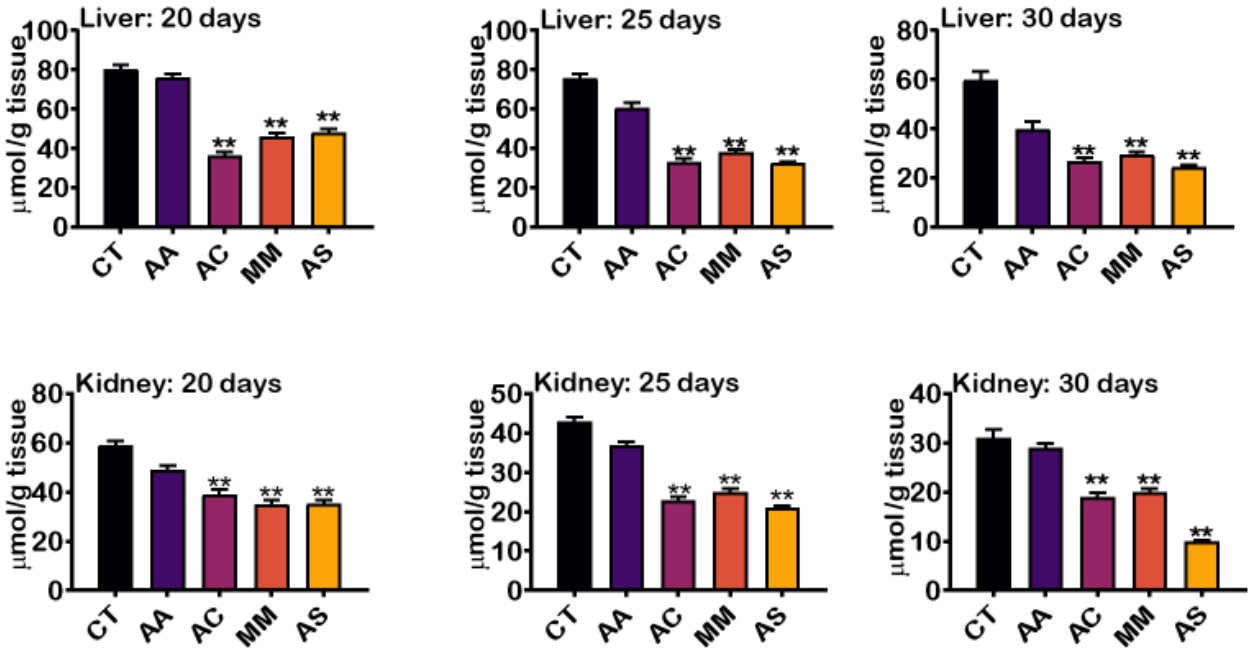

Figure 3. Effect of oral administration of ascorbic acid and some selected food condiments on the level of H_2O_2 in the liver and kidney. *P<0.05 versus Control; **P<0.05 versus AA, CT, control, AA; Ascorbic acid, AC; *Allium cepa*; MM; *Monodora myristica;* AS*; Allium sativum*; H_2O_2 Hydrogen peroxide.

3.5 Effect of some selected food condiments on enzymatic Antioxidants.

Activity of catalase was evaluated in the liver and kidney across the different groups, and the result is presented in Figure 4. The result revealed an increase in the activity of catalase in the liver and kidney catalase on the 20th, 25th, and 30th day. The liver catalase level at day 20 did not differ significantly when compared with the control and ascorbic acid. However, at days 25 and 30, the level of catalase significantly increased in rats treated with *A. cepa, M. myristica,* and *A. sativum* when compared to ascorbic acid and control groups. The kidney catalase levels at day 20 and 25 were, however, lower in groups treated with *A. cepa, M. myristica,* and *A. sativum* when compared to those treated with ascorbic acid. At day 30, catalase level in groups that received food condiments peaked significantly compared to the ascorbic acid and control groups.

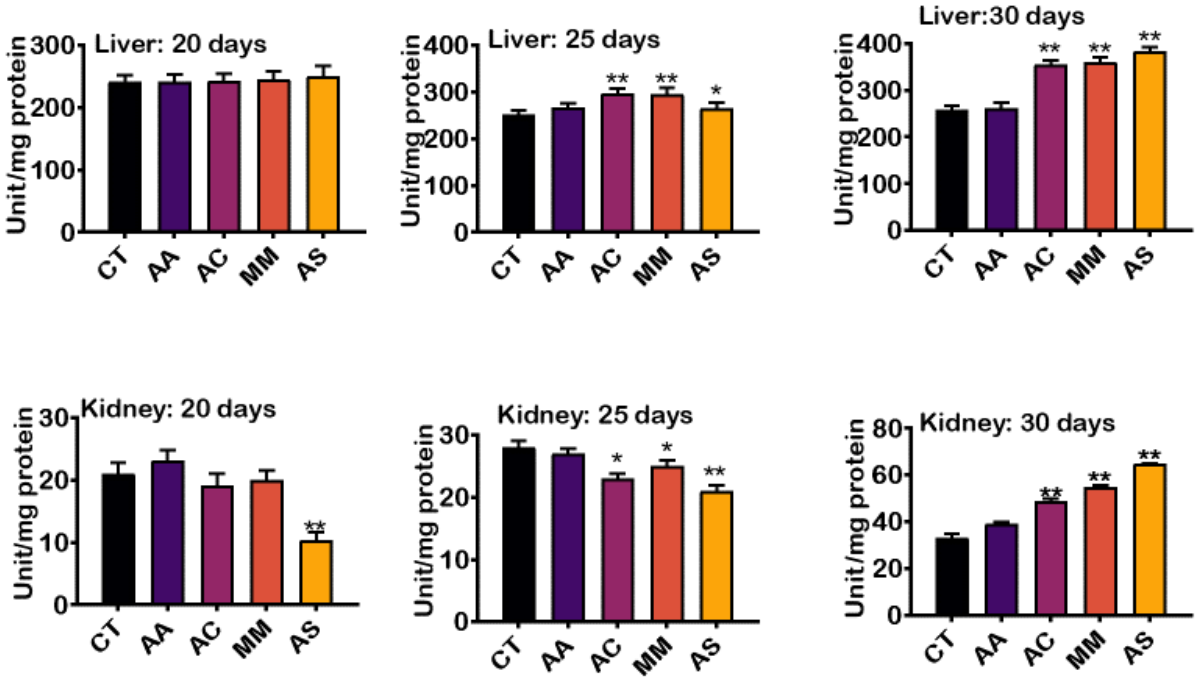

Figure 4. Effect of oral administration of ascorbic acid and some selected food condiments on the activity of catalase in the liver and kidney. *P<0.05 versus Control; **P<0.05 versus AA, CT, control, AA; Ascorbic acid, AC; *Allium cepa*; MM; *Monodora myristica;* AS*; Allium sativum*.

4.0 Discussion

Reactive oxygen species (ROS), such as hydroxyl radicals, superoxide radicals and hydrogen peroxide, are generated in living systems under favorable conditions [46]. Excess production of ROS can alter the homeostatic balance thereby initiating or exacerbating degenerative diseases in animals. However, endogenous antioxidants including superoxide dismutase (SOD), catalase, glutathione peroxidase, glutathione, and vitamins can prevent the onset of diseases by scavenging the excess ROS in the body [47]. Studies have shown that, our body does not need only endogenous antioxidants to fight against free radicals but also plant-derived antioxidants such as. vitamins A, C, E, polyphenols, flavonoids to effectively reduce the harmful effects of ROS [11]. To add to existing knowledge in the pharmacopeia, we investigated the in vitro and phytochemical constituents of selected food condiments (*A. cepa, A. sativium,* and *M. myristica*) as well as in vivo antioxidant activity in the liver and kidney of rats. Virtually all plants have one or more phytochemical residents in their leaves, stems, roots, fruits, and flowers [48]. From our present findings, the aqueous extracts of the selected condiments contain phytochemicals that contributed to their antioxidant capacity [18] and the pharmacological and biochemical actions of these phytochemicals have long been established. For instances, alkaloids possess analgesic, antispasmodic, antibacterial and antineoplastic effects [17]; flavonoids are

potent antioxidants that protect the human body against free radicals and have potent anticancer activity [49]; saponins have been found to possess hypocholesterolemic, antitumor, antioxidant, antimutagenic and antibiotic properties [50] while tannins possess among other functions, antioxidant, anti-inflammatory, antiulcer and anticancer effects [32]. The phytoprotective activity of one of the condiments (*M. myristica*) against sodium arsenite-induced clastogenicity in Wistar rats has long been investigated [51], thus validating our findings that these phytochemicals may confer health benefits.

Ferric reducing antioxidant power is a protective antioxidant mechanism used to evaluate metal ions binding ability [52]. The reducing power is commonly related to the presence of efficacious molecules known as reductones that exhibits their antioxidant capacity by disrupting the free radical chain and donating hydrogen atom [53]. Ferric reducing assay evaluates the ability of antioxidant components to reduce Fe^{2+} / ferricyanide complex to its ferrous form, this reaction has a deep blue color [54]. From the present result, it was observed that the reducing property was highest in *A. sativum,* followed by *A. cepa,* and then *M. myristica*. The reducing power of these extracts increased with increase in the concentrations of plant extracts. This observation is line with earlier postulation that the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} was found to increase as the concentration increased [54]. The reducing power of selected food condiments suggests that the extracts may have antioxidant capacity.

Reactive oxygen species react with all biological substances [40]; however, the most vulnerable ones are polyunsaturated fatty acids [55]. Free radicals are unpaired electrons that bounce around and destroy healthy cells, attacking the cell membranes, leading to DNA damage and mutation. They react with the cellular membrane lipids, causing peroxidation of polyunsaturated fatty acids, and causing further generation of free radicals [56]. Due to the electron-donating and antioxidant capacity of plant extracts, they can mop out excess free radicals and reduce the generation of malondialdehyde (a marker of lipid peroxidation) in the body, thereby restoring the redox rheostat [57]. In this investigation, the level of malondialdehyde is used as a marker of lipid peroxidation [58]. The time-dependent decrease in the level of malondialdehyde by the different food condiments suggests that they are richly available in endogenous antioxidants [35]. *A. sativum* depicted the highest level of reduction due to the active ingredients such as phenols and saponins, which have a direct effect on the metabolism of cytochrome P450 and glutathione S-transferase. This result is consistent with studies done by Kang [59] and also Park [60] which showed that the mechanism of antioxidative action of *A. sativum* might be involved with the enhancement of antioxidant enzyme activities.

Hydrogen peroxide is widely regarded as a cytotoxic agent whose level must be minimized by the action of antioxidants [19]. The result showed a time-dependents decrease in the hydrogen peroxide generation of the selected food condiments when compared to the control indicating that the spices can protect cells from oxidative damage occasioned by exposure to toxic hydrogen peroxide [61]. *A*. *sativum* showed the highest level of reduction, and this is because of the bioactive compounds in *A. sativum,* which are crucial in the protection of the liver and kidney. Allicin is a bioactive compound in *A. sativum* and has been shown to mediate anti-oxidative activity [62].

Catalase is an enzymatic antioxidant widely distributed in all animal tissue [63]. It carries out the detoxification of H_2O_2 into molecular oxygen and protects the tissues from highly reactive hydroxyl radicals [64]. Catalase is an inducible enzyme whose production can be stimulated. Therefore, the elevated activity of catalase may suggest that there is an induction of the enzyme by the extracts in the rats, which corresponds with earlier reports that catalase can be induced in experimental animals [40].

The time-dependent increase in the activity of catalase enzyme in this study could also mean the enhanced antioxidative capacity of the animals, which leads to the inactivation of lipid peroxides, hence a decreased level of malondialdehyde. *A. sativum* showed the highest increase in this study because it contains mainly diallyl disulfide and other sulfur compounds that protect the cells through influencing peroxide forms and oxidation-reduction.

5.0 Conclusion

The antioxidant activity of *A. sativum, M. myristica* and *A. cepa* were investigated in this study, and the outcomes showed that these food condiments are rich in antioxidants as they were able to reduce the level of ROS as well as increase the level of endogenous antioxidants. Interestingly, the in vitro antioxidant activity of the three food condiments was higher than that of ascorbic acid – a standard antioxidant agent. This suggests that the inclusion of these food condiments in our diet during food preparation may ameliorate oxidative stress and reduce the incidence of degenerative disease in animals. Although results from animal studies cannot be extrapolated directly to humans, further studies need to be done on the standard of dosage to minimize any adverse side effects.

Acknowledgments

All authors would like to thank the Laboratory staff of the Department of Biochemistry, Bingham University, Karu, Nasarawa State, Nigeria for their technical assistance.

Disclosure statement: The Authors declares that there is no conflict of interest.

Author Contributions: Project administration and Supervision: Prof Janet Olubodi; Conceptualization: Olusola Ladeji. Data curation, analysis, investigation: Chioma Irozuru; Writing, review, and editing: Chioma Irozuru and Uche Okuu Arunsi.

Funding statement: This research was done without a specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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