



## Investigation of Antioxidant and Antimicrobial Properties of Garlic Peel Extract (*Allium sativum*) and Its Use as Natural Food Additive in Cooked Beef

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### Authors' contributions

This work was carried out in collaboration between all authors. Author BOTI designed, supervised the study, and prepared the first draft of the manuscript, author EAF managed the analyses and the literature search, while author BTI contributed to the literature search and performed the statistical analysis. All authors read and approved the final manuscript.

Original Research Article

Received 1<sup>st</sup> July 2013  
Accepted 21<sup>st</sup> August 2013  
Published 18<sup>th</sup> January 2014

### ABSTRACT

**Aims:** To investigate the antioxidant and antimicrobial properties of garlic peel extract and its possible use as natural food additive in cooked beef

**Study Design:** Multifactorial Design

**Place and Duration of Study:** Department of Food Science and Technology, Federal University of Technology, Akure, Ondo State, Nigeria between August 2011-September 2012.

**Methodology:** Crude ethanolic extract from garlic peel was investigated for its total phenol content (TPC), according to Folin-Ciocalteu method and calculated as Gallic Acid Equivalent (GAE), while the flavonoid content was determined using the AlCl<sub>3</sub> colorimetric method and expressed as Quercetin Equivalent (QE). In addition, antioxidant test was carried out using 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay. The crude extract at different concentrations (2.7mg/ml, 5.4mg/ml and 10.8mg/ml) were incorporated into minced beef, cooked in the microwave, inoculated with selected bacteria

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(*Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Bacillus cereus*, and *Proteus vulgaris*) and stored at 4°C for 9 days. The effects of garlic peel crude extract on lipid peroxidation and microbial growth was then evaluated.

**Results:** The results obtained showed that garlic peel extract added to the beef was able to lower the pH of the beef significantly when compared with the control. Furthermore, garlic peel crude extract demonstrated antioxidant activity which lowered thiobarbituric acid-reactive substances (TBARS) (mg malonaldehyde/kg muscle) from 11.23 in control meat sample to 2.62 in sample treated with 10.8mg extract on day 9. Antibacterial activity of the extract against tested bacteria inoculated into the cooked beef revealed that at a concentration of 10.8mg of extract/100g of meat, the extract reduced the bacterial population (*Staphylococcus aureus*, *Escherichia coli* and *Proteus vulgaris*), by at least one log compared to control at 9 days of storage.

**Conclusion:** Garlic peel ethanol extract demonstrated both antioxidant and antibacterial activities in cooked beef.

**Keywords:** Antioxidant properties, antimicrobial activity, natural additive, garlic peel extract

## 1. INTRODUCTION

Garlic is widely used around the world for its pungent flavor as a seasoning or condiment. Garlic cloves are used for consumption (raw or cooked) or for medicinal purposes. Garlic contains three times greater levels of organosulphur compounds than onion [1]. Due to the health problems associated with synthetic antioxidants, application of garlic as an alternative antioxidant [2,3,4] and antimicrobial [5,6] agents in food system is reported. Garlic cloves are used as a remedy for infections (especially chest problems), digestive disorders, and fungal infections such as thrush [7,8].

In another study, the effect of garlic in improving cardiovascular health by lowering blood pressure and cholesterol levels, and inhibition of several steps in the inflammation process was reported [9]. Furthermore, several clinical reports and meta-analyses have revealed the cholesterol-lowering effects of raw garlic and some garlic supplements, such as garlic essential oil [10,11]. In addition, garlic supplementation significantly reduced aortic plaque deposits of cholesterol-fed rabbits [12]. Recent studies have demonstrated the effectiveness of aged garlic extract (AGE) to reduce the plasma concentration of homocysteine in rats with hyperhomocysteinemia induced by severe folic acid deficiency [13,14].

Garlic, onion and other fruits are used to prevent gastric cancers and countries where garlic is consumed in higher amounts, in traditional cuisine, have been found to have a lower prevalence of cancer [15]. In addition to the anticarcinogenic activity of garlic components studies, a number of researchers have recently focused on its antimutagenic activity, observing that certain sulphur compounds have an effect on DNA repair mechanisms [16]. It has been demonstrated that aged garlic extract exerted an anti-allergic [17] and antitumor effect [18].

Several products of garlic are available in the international market, which include; garlic essential oil, garlic oil macerate, garlic powder as garlicin and aged garlic extract [19], however, there has been no report on the garlic peel. Garlic peel has been treated as waste and may constitute nuisance, therefore, this study was carried out to investigate the

antioxidant and antimicrobial properties of garlic peel and its application as preservative in cooked beef.

## **2. MATERIALS AND METHODS**

### **2.1 Collection and Preparation of Sample**

Bulbs of garlic were purchased from Sabo market in Ile-Ife, Osun State Nigeria. The bulbs were sorted and the peels were manually removed, dried and milled into powder using attrition mill. Samples were then packaged, labeled, sealed and stored for further analysis.

### **2.2 Chemicals and Reagents**

Ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), 2-deoxyribose, and tannic acid were obtained from Sigma-Aldrich (St. Louis, Mo., U.S.A.). Ethylenediaminetetraacetic acid (EDTA), Folin–Ciocalteu reagent, trichloroacetic acid (TCA), hydrogen peroxide, ferric chloride, sodium carbonate, gallic acid, and thiobarbituric acid (TBA) were obtained from Merck (Merck, Darmstadt, Germany). Tryptic soy agar (TSA; Merck, Darmstadt, Germany), Brain heart infusion broth (BHI), buffered peptone water (BPW), plate count agar (PCA) were obtained from Difco labs (Difco Labs, Div. of Becton Dickinson and Co., Sparks, Md., U.S.A.). All other reagents used were of analytical grade.

### **2.3 Determination of Proximate Composition**

Garlic peel was analyzed for moisture, protein, fat, carbohydrate, fibre and ash in triplicate using standard methods [20].

### **2.4 Preparation of Garlic Peel Ethanolic Extracts**

Crude extracts of garlic peel was prepared in the laboratory following the modified method [21]. Twenty grams of the powdered samples was extracted with 200ml of 80% ethanol for 24hours with magnetic stirrer. The extract was filtered by a Whatman filter paper 125 mm (No 1) under vacuum at room temperature. The filtrate was evaporated under reduced pressure in a rotary evaporator at 45°C until the extracts became completely dry. After evaporation, the extract was stored at 4°C until use.

### **2.5 Determination of Total Phenol**

Total phenol of sample were determined using the modified method [22]. The calibration curve of aqueous gallic acid solutions of known concentrations were prepared. Folin–Ciocalteu's phenol reagent (5 ml) and 20% sodium carbonate solution (15 ml) were added to each 1 ml of gallic acid standard solution. The solutions were kept at room temperature for 90 min before measuring their absorbance at 760 nm by UVspectrophotometer. About 0.1 ml aliquot of the extract was prepared and mixed with Folin–Ciocalteu's phenol reagent and 20% sodium carbonate. The mixtures were kept at room temperature for 90 min before measuring their absorbance at 760 nm. For blank, the distilled water was added to replace the standard solution. The total phenolic content in the extracts were calculated from the calibration curve and determined as gallic acid equivalent (micromol gallic acid equivalent per milligram dried weight of crude extract).

## 2.6 Determination of Total Flavonoid

Flavonoid content of the peel was determined based on the aluminium chloride calorimetric assay method as described by [23]. Approximately 1 ml of extract was mixed 1 ml of 2%  $\text{AlCl}_3$  in methanol. The mixture was then diluted with methanol to 25ml. The absorbance was then read at 415nm. Blank samples were prepared from 1ml of peel extract and one drop of acetic acid and diluted to 25ml. The absorbance of rutin solutions was prepared from 0.05g (50mg). The amount of flavonoids in peel extract in rutin equivalents was calculated by the following formula;

$$\text{Antioxidant capacity} = (A_{\text{final}} / \text{slope})(V_f / V_s) \times r \times (V_{\text{cup}} / m)$$

Where r = dilution factor,  $V_{\text{cup}}$  = Volume used for the extraction, m = Mass of initial sample  
 $V_f$  = Final reaction volume,  $V_s$  = Sample volume,  $A_{\text{final}}$  = Final absorbance of sample

## 2.7 Determination of 2,2-diphenyl-1-picrylhydrazyl Free Radical Scavenging Ability (DPPH)

The free radical scavenging ability of the extract against DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical was evaluated as described by [24]. One milliliter of the extracts was mixed with 1 ml of 0.4 mM methanolic solution containing DPPH solution, the mixture was left in the dark for 30 min and the absorbance was taken at 516 nm in the JENWAY UV–visible spectrophotometer. Radical scavenging ability of samples was calculated as the percentage of DPPH free radicals inhibited by samples in comparison to radical inhibition in the negative water control used. Control with 1% ethanol and without the extract was also set up under the same condition for the experiment. The percentage antioxidant activity

$$(\text{AA} \%) = \frac{\text{absorbance of control} - \text{absorbance of extract}}{\text{absorbance of control}} \times 100$$

The 50% inhibition concentration ( $\text{IC}_{50}$ ) was then obtained from a linear regression plot of percentage inhibition against concentration of the extract using graph software package (Sigma plot(R) version 7.0).

## 2.8 Preparation of Beef Samples

Fresh lean beef was bought from an abattoir, and was transported to the laboratory immediately within 30 min of purchase. The meat was washed with sterile water and then manually and aseptically minced to pieces. Ten grams of meat was put into sterile test tubes containing 10ml of garlic peel extract at various concentrations (2.7mg, 5.4mg and 10.8mg). The various treatments were thoroughly mixed with sterile spatula for uniform distribution of the added extract. The control was without extract (80% ethanol). Each portion was cooked in the microwave until the internal temperature reached 80°C and was held for 2min after which they were allowed to cool down at room temperature. They were then divided and packaged separately into plastic bags, sealed and stored at 4°C for chemical and microbiological analyses to be carried out on 0, 3, 6, and 9 days of storage [25].

## 2.9 Determination of pH and Thiobarbituric Acid-Reactive Substances (TBARS)

The pH (Beckman pH Meter) of cooked beef was determined by homogenizing 10 g of sample with 50 ml distilled water in an Ultra Turrex T25 tissue homogenizer for 1 min.

Modified TBARS of meat samples were carried out following the method described by [4]. One gram of sample was mixed with 4 ml of TBA reagent (0.375% thiobarbituric acid, 15% trichloroacetic acid, and 0.25N HCl) in screw cap test tubes. The mixture was heated in boiling water for 10 min and then cooled under running tap water. The mixture was centrifuged (Hitachi, Tokyo, Japan) at 3600 g for 20 min. The supernatant was removed and absorbance was read at 532 nm using a UV-160 spectrophotometer. TBARS were calculated from standard curve of malonaldehyde and expressed as milligram malonaldehyde per kilogram sample.

## 2.10 Bacterial Strain and Preparation of Inoculums

Typed cultures of *Staphylococcus aureus* (NCIB 8588), *Escherichia coli* (NCIB 86), *Bacillus subtilis* (3610), *Bacillus cereus* (NCIB 6349), and *Proteus vulgaris* (NCIB 67) used for this study were supplied by the Pharmaceutical Microbiology Laboratory of the Obafemi Awolowo University Ile Ife Osun State, Nigeria. The strains were subcultured in overnight buffered peptone water and incubated at 37°C for 6hours. The inocula were finally adjusted to 10<sup>8</sup>CFU/ml using the McFarland standard.

## 2.11 Microbiological Analysis

About 0.2ml of the overnight broth culture of bacteria matched with the McFarland standard solution was added to the already cooled mixture of beef and extract while the control was without extract. Exactly 0.1ml of the mixture was then spread on surface of dried nutrient agar plates in duplicate on day 0 and left on the bench for 1hour to allow for absorption. The plates were then incubated at 37°C for 24hours, after which the numbers of organisms found on each plate was then counted.

## 3. RESULTS AND DISCUSSION

### 3.1 Proximate Composition

The results of the proximate composition of garlic peel is shown on Table 1. Result showed that the peel is very high in carbohydrate (93.26%), and the moisture content was 5.50%. The protein content of garlic peel (0.57%) is lower than that reported for garlic clove (7.87%) in previous research [26]. Also, the value obtained for the crude fat content of the peel (0.05%) is much lower compared to 0.52% recorded from garlic bulb. Lower fat content observable in garlic peel may be due to the volatility of the essential oil.

**Table 1. Proximate composition of garlic peel**

Characteristics (%)	Garlic peel
Ash	0.49 ± 0.04
Carbohydrate	93.26 ± 0.04
Crude fibre	0.13 ± 0.01
Fat	0.05 ± 0.01
Moisture content	5.50 ± 0.00
Protein	0.57 ± 0.02

Table 2 revealed the total phenolic (355.50µg/ml GAE) and flavonoid content (33.27µg/ml QUE) of garlic peel crude extracts. It has been reported that flavonoid is one of the most diverse widely spread group of natural products and probably the most important natural phenolic compound in spices. Research findings have shown that garlic bulb contained flavonoids (allixin), essential micronutrients (selenium) and macronutrients such as lectins, that has been shown to exhibit antiperoxide properties in the liver, kidney and heart of rats [27]. Phenolic compounds with strong antioxidant properties are prominent components of many food plants, including aromatic plants such as onion and garlic which are used to enhance the sensory quality of foods [28].

**Table 2. Total phenol and flavonoid content of ethanolic garlic peel extract**

Assays	Garlic peel extract (500µg/ml)
Total phenolic content(gallic acid equivalent (µg/ml)	355.50 ± 2.17
Total flavonoid content (µg QUE/ml)	33.27 ± 2.95

The scavenging effect of the crude extract of garlic peel on DPPH radical is expressed on Table 3. The scavenging activity demonstrated by garlic peel crude extracts at IC<sub>50</sub> is 79.07µg/ml. The strong antioxidant activity demonstrated by extract from garlic peels against 1,1-diphenyl-2-picrylhydrazyl was attributed to presence of certain compounds identified as phenylpropanoids [29]. Another recently identified antioxidant compounds of garlic, sliced and soaked, in a water or ethanol mixture, for longer than 10 months (aged garlic extract) are N-fructosyl glutamate and N-fructosyl arginine, whose antioxidant activity is comparable to that of ascorbic acid [30]. Furthermore, allicin has been shown to act as an antioxidant by scavenging reactive oxygen species and preventing lipid oxidation and production of pro-inflammatory messengers [31].

**Table 3. Antioxidant assay of crude ethanolic extract from garlic peel**

Sample	DPPH Radical Scavenging Assay (IC <sub>50</sub> µg/ml)
Garlic peel extract	79.07 ± 2.69
Vitamin C	4.09 ± 0.04

The effect of the garlic crude extract on the pH of the beef sample is given on Table 4. The pH of the garlic peel crude extract was 6.74 and found to be stable when subjected to heat treatment. At day 0, the pH of the samples treated with different concentrations of garlic peel crude extract were significantly lower than the control sample. It was found from the result of the pH determination of the cooked beef treated with different concentrations of garlic peel crude extract that the pH reduced consistently from day 3 to day 9. The result

obtained is similar to the previous finding where chicken sausage was treated with fresh garlic, garlic powder and garlic oil [4]. The decrease in the pH of the treated cooked beef samples over day 3 to 9 storage period may be an evidence of the potential microbial inhibitory capacity of the garlic peel crude extract.

**Table 4. pH values of beef cooked with crude garlic extract peel during storage at 4°C**  
(Extract concentration (10g of meat cooked with 10ml of extract))

Storage days	Control	2.7 mg/g	5.4 mg/g	10.8mg/g
0	7.20±0.010 <sup>a</sup>	6.93±0.030 <sup>b</sup>	7.05±0.000 <sup>c</sup>	6.71±0.065 <sup>d</sup>
3	7.28±0.020 <sup>a</sup>	7.05±0.015 <sup>b</sup>	7.12±0.000 <sup>c</sup>	7.05±0.005 <sup>b</sup>
6	7.44±0.025 <sup>a</sup>	6.95±0.015 <sup>b</sup>	7.00±0.020 <sup>c</sup>	6.93±0.035 <sup>b</sup>
9	6.87±0.020 <sup>a</sup>	6.70±0.025 <sup>b</sup>	6.72±0.035 <sup>b</sup>	6.75±0.045 <sup>b</sup>

Values are means ± SD from duplicate determinations, different superscripts in the same row are significantly different ( $P \leq 0.05$ ).

The result of TBARS showed that garlic peel crude extract could delay the lipid oxidation process in the treated samples (Table 5) over 9 days of refrigerated storage. At day 0, TBARS in control sample was significantly higher than those in the treated samples and as the storage days increased the lipid peroxidation in the control sample increased. Ability of garlic to delay production of TBARs may be attributed to its high content of organosulphur compounds. Antioxidant phytochemicals (flavonoids, carotenoids and thiols) are known to slow down, stop or reverse oxidation of proteins and lipids by scavenging oxidizing agents [32]. The application of natural equivalent of synthetic antioxidants is important for human health because some synthetic antioxidants have been reported to demonstrate carcinogenic activity [33].

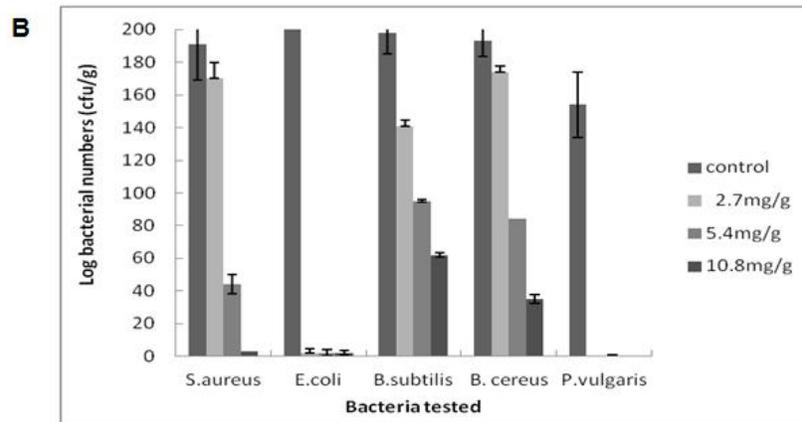
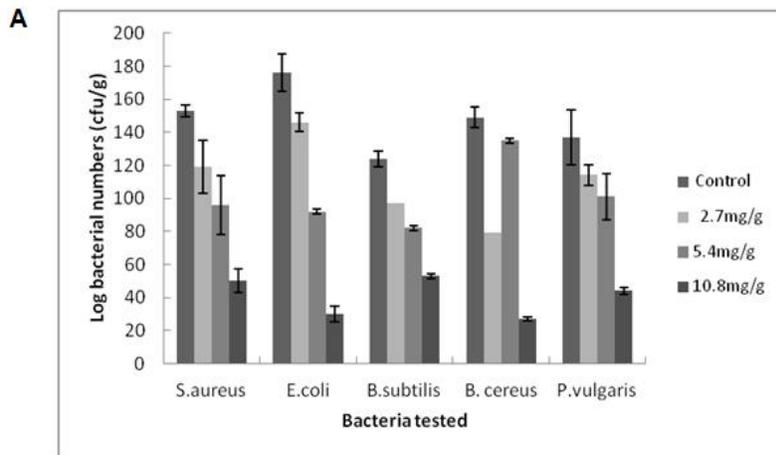
**Table 5. TBARS values of beef cooked with garlic peel crude extract during storage at 4°C**  
Treatments (cooked meat + extract mg/100 g meat)

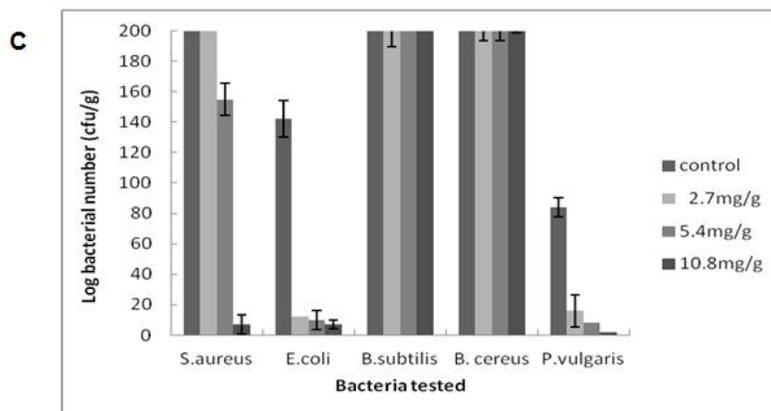
Storage days	Control	2.7 mg	5.4 mg	10.8mg
0	6.91±0.030 <sup>d</sup>	5.10±0.040 <sup>c</sup>	3.76±0.055 <sup>b</sup>	2.55±0.120 <sup>a</sup>
3	8.795±0.035 <sup>d</sup>	6.265±0.025 <sup>c</sup>	4.130±0.040 <sup>b</sup>	2.900±0.030 <sup>a</sup>
6	9.510±0.080 <sup>d</sup>	7.805±0.135 <sup>c</sup>	4.410±0.320 <sup>b</sup>	3.410±0.330 <sup>a</sup>
9	11.235±0.075 <sup>d</sup>	8.230±0.220 <sup>c</sup>	4.395±0.085 <sup>b</sup>	2.620±0.140 <sup>a</sup>

Values are means ±SD from duplicate determinations, different superscripts in the same row are significantly different ( $P \leq 0.05$ ).

Fig. 1 showed the antibacterial activity of the garlic peel extract in the cooked beef sample stored for 9 days at 4°C. At day 0, (Fig. 1A) there was a significant decrease in population of the bacterial cells as concentration of garlic crude extract increased from control sample to 10.8mg/g of cooked beef for all the test bacteria. Of all these test bacteria, *E. coli* showed highest resistance to the garlic crude extract at 2.7mg/g crude extract concentration with 146.0cfu/g, whereas garlic crude extract at the same concentration retarded the growth of *B. cereus*. Fig. 1B showed that the antibacterial activity of the peel extract was maintained

till day 3. The extract inhibited the growth of *E.coli* and *P. vulgaricus* at all concentrations contrary to what was observed with *B. cereus*, *B. subtilis* and *S. aureus*. It was observed from the result of day 6 (Fig.1C) that garlic peel crude extract demonstrated bacteriostatic activity in the beef sample. The major active antibacterial components in garlic are the allicin-derived organo-sulphur compounds isolated from oil-macerated garlic [34]. The antibacterial effect of garlic resulted from interaction of sulphur compounds, allicin, with sulphur (thiol) groups of microbial enzymes (trypsin and other proteases), leading to an inhibition of microbial growth [35]. We may conclude that garlic peel extract exhibited antibacterial activity similar to garlic bulb which may be explained that the bioactive compounds present in the garlic bulb are likely to be available in the peel.





**Fig. 1. Antibacterial effect of crude extract from garlic peel on bacteria inoculated into cooked beef and stored at 4°C. The day 0 (A), day 3 (B) and day 9 (C)**

#### 4. CONCLUSION

Ethanollic extract of garlic peel demonstrated both antioxidant and antibacterial activities in cooked beef. We may conclude that the peel extract should not be used alone as a preservative in food system but can be combined with other hurdles to obtain an effective preservation. This study has also opened new possible use of garlic peel after proper cleaning. However, further studies should be carried out to determine the bioactive compounds responsible for the radical scavenging and antibacterial activities exhibited by garlic peel.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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