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Influence of Polyphenolic Contents on the Antioxidant Properties of *Hibiscus sabdariffa* Extract (HSE), Aged Garlic Extract (AGE) and Garlic Tablet (GT) *in Vitro*

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Abstract

This study compared the antioxidant properties of *Hibiscus sabdariffa* extract (HSE), commercial aged garlic extract (AGE, Kyolic) and garlic tablet (GT). The results indicate that HSE, AGE and GT are effective antioxidant as xanthine/xanthine oxidase generated superoxide ions are significantly inhibited in the presence of 20% (v/v) diethyl ether extract of HSE, AGE and GT by 100%, 66.39% and 18.61% respectively, while uric acid production is not affected by AGE and GT. However, at 2.5% and 5.0% (v/v) reaction volumes, HSE significantly inhibits uric acid production by 9.5% and 33.0% respectively, and this suggests that components of HSE inhibit xanthine oxidase activity while AGE and GT scavenge superoxide. In addition, it is also found that the three extracts scavenge ABTS radical cations in dose-dependent manner. In all cell free assays, HSE is found to be more effective as an antioxidant when compared with AGE and GT under the same experimental conditions. Chromatographic and colorimetric analyses suggest that HSE has numerous different types of phenolic compounds with higher amount of phenolic compounds (14.9 mg/g) when compared with AGE (2.8 mg/g) and GT (3.6 mg/g) catechin equivalent respectively. Therefore, the effectiveness of antioxidant activities of these three extracts may be related to their phenolic content.

Keywords

***Hibiscus sabdariffa*, Aged Garlic Extract, Antioxidants, Phenolic Compounds**

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1. Introduction

Reactive oxygen and nitrogen species are generated both endogenously and in response to external factors, such as diet and lifestyle, which play a major role in the etiology of several degenerative diseases [1] [2]. The effect of reactive oxygen species (ROS) and reactive nitrogen species (RNS) is balanced by the action of antioxidants enzymes such as superoxide dismutase, glutathione peroxidase, catalase, antioxidant compounds such as, thioredoxin, lipoic acid and dietary antioxidant such as Vitamin C, Vitamin E, carotenoids, flavonoids [3] [4]. Antioxidant defences are extremely important as they represent the direct removal of free radicals, thus providing protection for biological sites [5]. However, as this protection may not be sufficient to entirely prevent the damage by ROS/RNS, consumption of food rich in dietary antioxidants which offers a supportive role in antioxidant defence system in removing excessive ROS/RNS becomes even more important in protecting cell biomolecules against oxidative damage.

This has triggered the growing market for nutraceutical and functional foods with emphasis on the study of natural sources of antioxidants, their potential as nutraceutical and functional foods [5]. Such plants that have attracted much attention over the years are roselle (*Hibiscus sabdariffa*) and garlic (*Allium sativum*), and many studies on these two plants, have focused on the antioxidant properties of their numerous preparation and constituents. Garlic powder extract and AGE have been demonstrated to scavenge hydroxyl and peroxyl radicals and prevent t-butyl hydroperoxide-induced lipid peroxidation of liver microsomes [6] [7]. A constituent of AGE, S-allylcysteine has also been shown to scavenge superoxide anion ($O_2^{\bullet -}$), hydroxyl radical ($OH^{\bullet}$), peroxynitrite anion ($ONOO^-$), singlet oxygen ($^1O_2$) and hypochlorous acid (HOCl) [8], while *Hibiscus sabdariffa* has been shown to scavenge 1, 1-diphenyl-2-picrylhydazyl free radical, hydroxyl radical and hydrogen peroxide in a cell free system [9]-[13]. The extract also scavenges t-butyl hydroperoxide radical and hence prevents oxidative damage in rat primary hepatocytes [9] [14]-[16].

The human diet contains an array of different compounds that possess antioxidant activities and their ROS scavenging abilities have been suggested to be due to their structural properties [17]. Such compounds include vitamin E, vitamin C, carotenoids and phenolics (flavonoids and phenolic acids). These compounds are reported to play preventing role in the development of various pathological diseases [18]. A high flavonoid consumption has particularly been associated with a decreased risk of cardiovascular disease [19] and lower rates of stomach, pancreatic, lung and possibly breast cancer [20]. It has been identified that *Allium sativum* is a rich source of antioxidant flavonoids and phenolic acids such as quercetin, apigenin, myricetin, caffeic acid, vanillic acid, salicylic acid [21] [22], while *Hibiscus sabdariffa* is also rich in phenolic compounds such as quercetin, luteolin, chlorogenic acid, protocatechuic acid, catechin, epigallocatechin, epigallocatechingallate and caffeic acid [23] [24]. However, in spite of this varied phenolic content of *Allium sativum* and *Hibiscus sabdariffa*, the contributory role played by these phenolic compounds as an antioxidant is sparingly evaluated.

The aim of this study was to investigate the *in vitro* antioxidant potential of *Hibiscus sabdariffa* extract (HSE) by comparing its antioxidant activity with AGE (a proven extract of *Allium sativum* with antioxidant property) and Garlic Care Tablets (GT) (a commercial garlic tablet).

2. Materials and Methods

2.1. Chemicals

All chemical used were of analytical grade. Special reagents were cytochrome C, xanthine, xanthine oxidase (Grade III from bovine milk), ABTS (2,2-azino-bis-(3-ethylbenzthiazoline 6-sulfonic acid), Folin-ciocalteu's Phenol reagent, Trolox (6-hydroxy-2, 5, 7, 8,-tetramethyl-chroman-2-carboxylic acid) (C-stock-solution = 2.5 mmol/L), Gallic acid (C-stock-solution) = 0.568 mmol/L), catechin hydrate, salicyclic acid; ferulic acid; mangan (iv) oxide; potassium peroxodissulfate; quercetin; *p*-coumaric acid; caffeic acid; rutin hydrate; and all other chemicals were purchased from Sigma-Aldrich Company Limited, Dorsert, United Kingdom.

2.2. Materials

The following natural products were investigated in this study:

1) *Hibiscus sabdariffa* (Malvaceae): Flowers of this plant was bought at a market in Nigeria. The identification and authentication of the plant was done by Professor A.J. Ogunkunle at Department of Pure and Applied Biology, LadokeAkintola University of Technology, Ogbomoso.

2) Kyolic, aged garlic extract is a product of Wakunaga of America Company Ltd (Mission Viejo, CA, USA). The extract is formulated by soaking sliced raw garlic (*Allium sativum*) in 15% - 20% aqueous ethanol for up to 20 months at room temperature. The extract is filtered and concentrated under reduced pressure at low temperature.

3) Garlic Care Tablets—*Allium sativum* (standardized-300 mg), is a product of Quarshi Industries (PVT) Ltd, Hattar-Pakistan.

2.3. Preparation of *Hibiscus sabdariffa* Extract (HSE)

Hibiscus sabdariffa was dried at room temperature and grounded with mortar and pestle. Weighed samples of this powdered material (20 g) were loaded into extraction thimbles of Soxhlet extractor and were then extracted with methanol for 16 hours. The pooled methanolic solution of the extracts was thereafter concentrated in-vacuo by distillation at 50˚C to recover most of the methanol.

2.4. Preparation of a Diethyl Ether Extract of HSE, AGE and GT

Due to intense colouring of extracts employed in this study (HSE, AGE, GT), it was necessary to prepare a less intense extracts, which did not interfere with some of the spectrophotometric assays. One part of each extracts were gently mixed with two parts diethyl ether and left to stand at room temperature for 5 minutes (for AGE) or centrifuge at 2000 rpm for 10 minutes (for other extracts). The diethyl ether extract was removed and dried under a stream of oxygen-free nitrogen gas. The residue was then re-suspended in 0.01 M phosphate buffered saline (PBS) pH 7.2 to its original volume for all assays unless otherwise stated

2.5. Preparation of Aqueous Extracts of HSE, AGE and GT

1 g of each samples were mixed with 10 ml of phosphate buffered saline (PBS), this was centrifuged at 2000 rpm for 10 minute and the supernatant was collected for trolox equivalent antioxidant capacity (TEAC) analysis.

2.6. Superoxide and Xanthine Oxidase Activity

This was carried out as reported by Dillion *et al.*, [22] with slight modifications. Briefly superoxide production and xanthine oxidase activity were measured as cytochrome C reduction and uric acid production, respectively. Xanthine oxidase was prepared to a concentration of 53.5 mU/ml in Phosphate Buffered Saline (PBS), pH 7.2 and xanthine was prepared as a 0.8 mM solution also in PBS. Superoxide ions were generated in a reaction volume of 1 ml containing 80 µM xanthine and 0.625 mg cytochrome C. The reaction was initiated by the addition of 5.35 mU xanthine oxidase, and superoxide ion production was monitored at 550 nm [25]. In a series of separate experiments, xanthine oxidase activity was monitored as the production of uric acid at 284 nm. Generation of superoxide ions was confirmed by the addition of 50 U superoxide dismutase (SOD), which inhibited the reduction of cytochrome C without affecting xanthine oxidase activity. Extracts were added at 0% - 20% (v/v). Results for superoxide production are expressed as ΔA550 nm/minute whilst, result for uric acid production are expressed as ΔA284 nm/minute.

2.7. Trolox Equivalent Antioxidant Capacity (TEAC) with Manganese Dioxide

The assay was performed as previously described by Schelesier *et al.*, [18] with slight modifications. Briefly, the ABTS radical cation was prepared by filtering a solution of ABTS (in PBS) through manganese dioxide powder. Excess manganese dioxide was removed from the filtrate by passing it through a 0.2 µm syringe filter. This solution was diluted in 5 mM PBS pH 7.4, adjusted to an absorbance of 0.700 ± 0.020 at 734 mm and pre-incubated at room temperature prior to use for 2 hours. 1 ml of $ABTS^{\bullet+}$ solution and various concentrations of the extracts (diluted with water) were vortexed for 45 seconds in reaction tubes, and the absorbance (734 nm) was taken exactly 2 minutes after initiation of mixing. PBS blanks were run with each assay. The antioxidant activity of the extracts was calculated by determining the decrease in absorbance at different concentrations by using the following equation:

$$\%\ \text{antioxidant activity} = \left(E_{\left(\text{ABTS}^{\bullet+}\right)} - E_{(\text{Extracts})}\right) \Big/ \left(E_{\left(\text{ABTS}^{\bullet+}\right)}\right) \times 100 .$$

where E is the extinction.

2.8. Trolox Equivalent Antioxidant Capacity with Potassium Persulfate

The assay was performed as described by Re *et al.*, [26] with modifications. Briefly ABTS radical cation was produced by reacting 3.5 mM ABTS stock solution with 1.225 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature for 12 - 24 h before use. The $ABTS^{\bullet+}$ solution was diluted with water for the hydrophilic assay and with ethanol for the lipophilic assay and adjusted to an absorbance of 0.700 ± 0.020 at 734 nm. For the photometric assay, 1 ml of the $ABTS^{\bullet+}$ solution and various concentrations of the extracts were mixed for 45 seconds and measured immediately after 1 minute at 734 nm. The antioxidant activity of the extracts was calculated by determining the decrease in absorbance at different concentrations by using the following equation:

$$\%\text{ antioxidant activity} = \left(E_{\left(\text{ABTS}^{\bullet+}\right)} - E_{(\text{Extracts})}\right) \Big/ \left(E_{\left(\text{ABTS}^{\bullet+}\right)}\right) \times 100$$

2.9. Determination of Total Phenolic Compounds in HSE, AGE and GT

The amount of total phenolic was determined using Folin-Ciocalteu's reagent, as described by Ragazzi and Veronese, [27]. One ml of HSE, AGE and GT were added to 10.0 ml of distilled water and 2.0 ml of Folin-Ciocalteu phenol reagent. The mixture was allowed to stand at room temperature for 5 min, and then 2.0 ml Sodium carbonate was added to the mixture. The absorbance of the resulting blue complex was then measured at 680 nm. Catechin was used as a standard for the calibration curve. The phenolic compound contents were calibrated using the linear equation base on the calibration curve. The contents of phenolic compounds were expressed as mg catechin equivalent/g dry weight.

2.10. Thin Layer Chromatography (TLC) Analysis of HSE, AGE and GT

Diethyl ether extracts of HSE, AGE and GT were used for TLC analysis. Standard compounds were dissolved in methanol to a concentration of 1 mg/ml. Diluted 30 µl of extracts and 10 µl of standards were loaded onto silica gel 60 F_{254} TLC plates and left to dry. The plates were run for ~1 h in an ethylacetate: methanol: water (10:2:1, v/v/v) solvent system. The plates were dried for 15 minutes at 115˚C, left to cool, and then visualized using UV light and iodine vapour. In all instances, the distance moved by the sample/standard was divided by the distance moved by the solvent front to obtain the R_f value.

2.11. High Performance Liquid Chromatography (HPLC) Analysis of HSE

The HPLC method employed a 5 µ RP-18 column, *Hibiscus sabdariffa* extract (HSE) and various polyphenolic standards were filtered through a 0.45 µm filter disc and 20 µl were injected onto the column. The chromatography was monitored at 280 nm. The mobile phase contained two solvents (A, 0.1% formic acid; B, 100% methanol) run by bi-gradient method at room temperature as follows: 15% B to 50% B for 20 minute and remaining at 50% B for the next 20 minute. The flow rate was 1 ml/minute.

2.12. Statistical Analysis

Results are expressed as means ± SEM. Statistical analyses were performed using Student's t test; a $p < 0.05$ was considered statistically significant.

3. Results

3.1. Superoxide Scavenging Ability of HSE, AGE and GT

Superoxide production by xanthine-xanthine oxidase gave a reaction rate of 0.108 ± 0.001 ΔA550 nm/min while xanthine oxidase activity gave a reaction rate of 0.106 ± 0.001 ΔA284 nm/minutes. At 20% (v/v) of the reaction volume, diethyl ether extract of HSE, AGE and GT significantly inhibited superoxide production *i.e.* the reduction of cytochrome C by 100%, 64.81% and 16.67% respectively (**Table 1**). Superoxide production was inversely related to the concentrations of diethyl ether extract of HSE, AGE and GT. Uric acid production was not

Table 1. The effect of SOD, diethyl ether extracts of HSE, AGE and GT on superoxide and uric acid production by xanthine/xanthine oxidase.

Extract/Agent	Superoxide Production (ΔA550 nm/min)	Uric Acid Production (ΔA295 nm/min)
None[1]	0.108 ± 0.001	0.106 ± 0.001
50U SOD	0.002 ± 0.001*	0.101 ± 0.001
2.5% HSE	0.103 ± 0.001	0.095 ± 0.002*
5.0% HSE	0.100 ± 0.002*	0.071 ± 0.001*
10.0% HSE	0.053 ± 0.001*	N.D.
10.0% AGE	0.066 ± 0.002*	0.103 ± 0.001
10.0% GT	0.112 ± 0.002	0.105 ± 0.001
20% HSE	0.000 ± 0.000*	N.D
20% AGE	0.038 ± 0.001*	0.102 ± 0.001
20% GT	0.090 ± 0.001*	0.110 ± 0.002

N.D: not determined. Values are means of three experiments ± SEM, each experiment comprised of three observations (n = 9). Significant differences ($p < 0.05$) from control[1] are indicated by *.

significantly affected in the presence of diethyl ether extracts of AGE and GT, however diethyl ether extract of HSE significantly inhibited uric acid production by 9.5% and 33.0% when present at 2.5% and 5.0% (v/v) reaction volumes respectively indicating that the components of HSE inhibit xanthine oxidase.

3.2. ABTS Radical Cation Scavenging Ability of HSE, AGE and GT

In the three versions of TEAC assay, TEAC II and TEAC III (hydrophilic and lipophilic version) the inhibition of $ABTS^{\bullet+}$ radical cation was directly related to the concentration of the extracts and the TEAC value of Trolox is 1.00 (**Figures 1-3** & **Table 2**). HSE responded in all assays as the strongest antioxidant. HSE and GT showed comparable antioxidant activity in TEAC II and TEAC III (hydrophilic and lipophilic versions). The TEAC values of AGE did not differ in TEAC III hydrophilic and lipophilic version but the TEAC value analyzed in TEAC II is much lower when compared with the two versions of TEAC III (**Table 2**).

3.3. The Phenolic Nature of HSE, AGE and GT

The phenolic content of diethyl ether extracts of HSE, AGE and GT was determined using acolorimetric assay, namely Folin-Ciocalteu assay and by constructing a standard curve using catechin as the standard. The total amount of phenolic compounds presents in HSE was found to be 14.9 ± 0.08 mg/g dry weight; both GT and AGE had much lower phenolic compounds which were found to be 3.83 ± 0.04 mg/g dry weight and 2.80 ± 0.06 mg/g respectively (**Table 3**).

3.4. Analysis of Phenolic Compounds in HSE, AGE and GT by TLC

Thin-layer chromatography (TLC) analysis was performed to compare the properties of the phenolic compounds present in HSE, AGE and GT with a battery of known standard phenolic compounds. For a comparative TLC analysis diethyl ether extracts of HSE, AGE and GT were employed. Silica gel 60 plates and an organic solvent system (ethyl acetate: methanol: water (10:2:1, v/v/v)) were employed for TLC analysis and phenolic compounds were visualized with UV light and iodine vapour. Using this system the diethyl ether extracts of HSE, AGE and GT were found to contain six, four and two well-resolved blue, fluorescent bands respectively under UV light (**Table 4**). The fluorescence bands obtained with these compounds further indicate they were phenolic in nature. When iodine vapour was used to visualized the plates, more bands were detected. Nine, seven and five bands were observed with diethyl ether extracts of HSE, AGE and GT respectively (**Table 5**).

3.5. Analysis of Phenolic Compounds in HSE by HPLC

High performance liquid chromatography (HPLC) analysis is a useful technique for investigating complex mix-

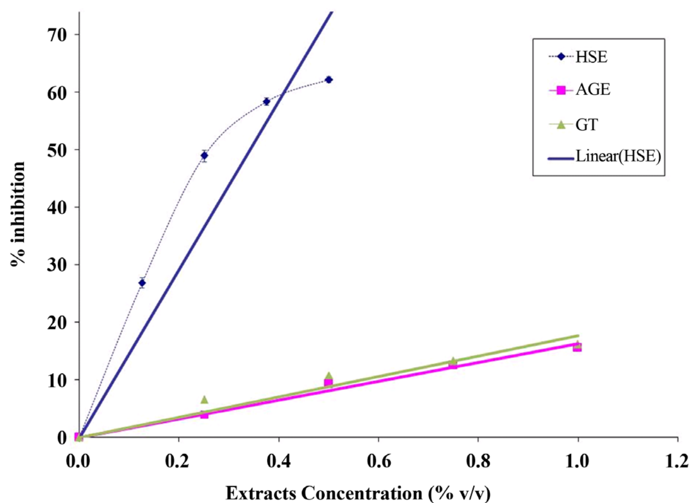

Figure 1. The effects of different concentrations of HSE, AGE and GT on the inhibition of the ABTS radical in TEAC II. Values are the means of three experiments ± SEM.

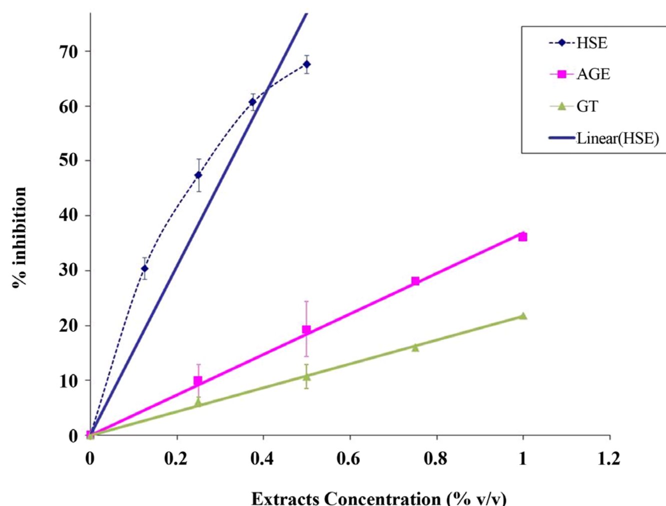

Figure 2. The effects of different concentrations of HSE, AGE and GT on the inhibition of the ABTS radical in TEAC III hydrophilic. Values are the means of three experiments ± SEM.

Table 2. Trolox equivalent antioxidant capacities (TEAC) (mmol/L) of trolox, HSE, AGE and GT.

Assay/Antioxidant	Trolox	HSE	AGE	GT
TEAC II	1.00	1.36 ± 0.01	0.11 ± 0.02	0.18 ± 0.04
TEAC III hydrophilic	1.00	1.20 ± 0.05	0.25 ± 0.03	0.15 ± 0.03
TEAC III lipophilic	1.00	1.37 ± 0.04	0.22 ± 0.04	0.16 ± 0.01

Values are means of three experiments ± SEM.

Table 3. The total phenolic content of HSE, AGE and GT in mg catechin equivalent/g dry weight. Values are the means of three experiments ± SEM.

Plant extracts	Total phenolic compounds mg/g plant extract (CE)
HSE	14.9 ± 0.08
AGE	2.80 ± 0.06
GT	3.83 ± 0.04

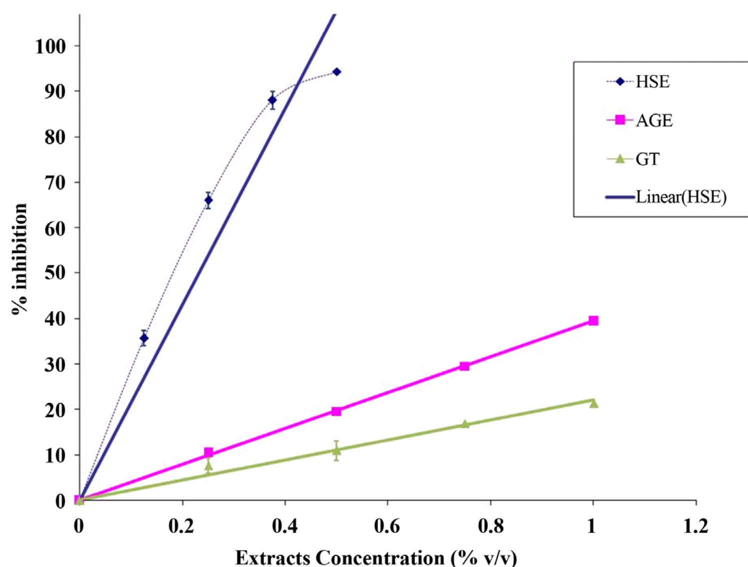

Figure 3. The effects of different concentrations of HSE, AGE and GT on the inhibition of the ABTS radical in TEAC III lipophilic. Values are the means of three experiments ± SEM.

Table 4. The R_f values for standard phenolic compounds and Diethyl Ether Extract of HSE, GT and AGE samples visualized with UV light. All experiments were repeated three times and values show a typical of the results obtained.

Phenolic standard	R_f values (in millimetres)			
	Standard	HSE	AGE	GT
Caffeic acid (white)	0.68	0.36	0.50	0.63
Catechin (dull)	0.72	0.52	0.55	0.77
Chlorogenic acid (dull)	0.12	0.60	0.62	
Coumaric acid (blue)	0.71	0.65	0.77	
EGCG (dull)	0.55	0.77		
Ferulic acid (blue)	0.70	0.83		
Gallic acid (blue)	0.63			
Naringenin (dull)	0.80			
Procatechuic acid (blue)	0.66			
Quercetin (dull)	0.75			
Rutin (dull)	0.32			
Salicyclic acid (white)	0.42			

tures of phenolic compounds. The methanol/water gradient system used allowed separation of phenolic compounds present in *Hibiscus sabdariffa* by polarity where the most polar compounds eluted first. A Shimadzu detection system was employed, eight peaks were obtained when HSE extract was analyzed and their retention times were compared to retention times of standard plant phenolic compounds (**Table 6**). The result suggest the possible presence of protocatechuic acid, chlorogenic acid, *p*-coumaric and caffeic acid in HSE. These results in conjunction with the results from the Folin-Ciocalteu assay and TLC analysis suggest that *Hibiscus sabdariffa* is a rich source of phenolic compounds with numerous different types of phenolic compounds present.

4. Discussion

Oxidative stress occurs when there is imbalance between free radical generating and scavenging systems. It has

Table 5. The R_f values (in millimetres) for standard phenolic compounds and Diethyl Ether Extract of HSE, GT and AGE samples visualized with Iodine Vapour. All experiments were repeated three times and values show a typical of the results obtained.

Phenolic standard	R_f values (in millimetres)			
	Standard	HSE	AGE	GT
Caffeic acid (brown)	0.66	0.23	0.20	0.45
Catechin (brown)	0.69	0.27	0.27	0.56
Chlorogenic acid (brown)	0.13	0.36	0.40	0.63
Coumaric acid (brown)	0.68	0.52	0.50	0.77
EGCG (brown)	0.56	0.60	0.55	0.83
Ferulic acid (brown)	0.67	0.66	0.62	
Gallic acid (brown)	0.62	0.77	0.77	
Naringenin (brown)	0.81	0.83		
Procatechuicacid (brown)	0.66	0.90		
Quercetin (brown)	0.75			
Rutin (brown)	0.28			
Salicyclic acid (brown)	0.45			

Table 6. The retention times for standard phenolic compounds and HSE extract in HPLC. All experiments were repeated three times and values shown are typical of the results obtained.

Phenolic Standard	Retention Time (Minutes)	
	Standard	HSE
Gallic acid	2.56	1.42
Protocatechuic acid	2.99	2.70
Chlorogenic acid	5.28	4.97
Catechin hydrate	5.60	8.38
EGCG	6.54	10.28
Caffeic acid	7.94	11.83
p-Coumaric	10.51	13.81
Salicyclic acid	11.24	16.64
Ferulic acid	12.20	20.93
Naringenin	17.73	
Rutin	18.51	
Quercetin	23.15	

been implicated in the pathogenesis of wide range of disorders, which include neurodegerative disorders, cardiovascular diseases, cancer, and ageing [28]. This is based on evidence obtained from diseased tissues such as increased levels of free radical and, free radical-induced products of DNA, lipids or proteins and decreased levels of antioxidants [4] [29]. Several epidemiological studies suggest the importance of a high consumption of secondary plant products widely distributed in fruit and vegetables in ameliorating the effects of oxidative stress [18]. This has been attributed to the antioxidant properties of these substances which include scavenging of free radical such as superoxide anion, hydroxyl and nitric oxide radicals [30].

In cellular oxidation reactions, superoxide are normally formed first, and their effects can be magnified be-

cause they produce other kinds of cell-damaging free radicals and oxidizing agents [3]. Xanthine oxidase is one of the main enzymatic sources of ROS *in vivo*. It-mediated the breakdown of hypoxanthine to xanthine and then to uric acid which is a key source of the ROS superoxide and hydrogen peroxide. Evidence exists to suggest that ROS generated through this source may participate in fatty acid peroxidation and pathophysiology of a variety of diseases [31] [32]. In experiments reported here, the effects of diethyl ether extracts of *Hibiscus sabdariffa*, AGE and Garlic tablet on superoxide ions generated from this source were investigated and compared. The results suggest that both diethyl ether extracts of AGE and Garlic tablet may contain components that actively scavenge superoxide (**Table 1**). This result is in support of an earlier study, which demonstrated that AGE possesses superoxide scavenging ability [22]. On the other hand, diethyl ether extract of *Hibiscus sabdariffa* was more efficient in inhibiting the reduction of cytochrome C by superoxide (**Table 1**). The same extract also inhibit the uric acid production by xanthine oxidase (**Table 1**) suggesting that components of this extract may inhibit xanthine oxidase and may also scavenge superoxide ion. These results support works of Tseng *et al.*, (15), which observed similar inhibitory effect on xanthine oxidase activity by *Hibiscus sabdariffa* extract.

Generation of the ABTS (2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical cation forms the basis of one of the spectrophotometric methods that have been applied to the measurement of the total antioxidant activity of solutions of pure substances [26] [33], aqueous mixtures and beverages [18] [34]. The abilities of antioxidants to scavenge the pre-formed $ABTS^{\bullet+}$ radical cation measured in TEAC value are influenced by the presence of functional groups and number of conjugated double bonds in carotenoids [33], as well as polyphenols and ascorbic acid content in the beverages [18]. To further evaluate the antioxidant potential of HSE, AGE and GT, the antioxidant activities of these three extracts were also determined by the ABTS radical-scavenging method. It was found that HSE responded in all TEAC assays as strongest antioxidants (**Table 2**). The result also shows that inhibition of the $ABTS^{\bullet+}$ radical by all the antioxidants is concentration dependant (**Figures 1-3**). A more comparative look at all the cell-free antioxidant assays used in this study showed HSE to respond as strongest antioxidants in all the assays (**Table 1**, **Table 2** & **Figures 1-3**).

Phenolic compounds are commonly found in the plant kingdom, and they have been reported to have multiple biological effects, including antioxidant activity [35] [36]. *Hibiscus sabdariffa* and Garlic are rich source of plant polyphenols such as flavonoids and phenolic acids [21]-[24]. Using numerous methods of analysis the current study, examined the content of phenolic compounds for the HSE, AGE and GT. The results presented showed that the HSE contained higher amount of phenolics than AGE and GT based on Folin-Ciocalteu assays (**Table 3**), with many different types of polyphenols present in diethyl ether extracts of HSE, AGE and GT based on TLC analysis (**Table 4** & **Table 5**). Further analysis of phenolic compounds in HSE with HPLC suggest the possible presence of protocatechuic acid, chlorogenic acid, *p*-Coumaric and caffeic acid (**Table 6**). The results from antioxidant assays also showed that, overall, the antioxidant activity of the HSE extract is more effective than that of the AGE and GT extract. These results suggested that the effectiveness of the antioxidant activity of these three plant extracts may be associated with phenolic content. It is proposed that the phenolic compounds of the HSE, AGE and GT may play an important role in the observed antioxidant activities of the extracts.

The beneficial health properties of garlic (*Allium sativum* L.), and its antioxidant activities, are accredited to the biologically active lipophilic sulfur-bearing compounds like allicin, S-allylycysteine (SAC), diallyl-di-sulfide (DADS) and diallylsulfide (DAS) [37]-[39], although the contributory role play by phenolic content observed in this study to the health benefit of garlic may be highly significant. However, since polyphenols are by far the major antioxidant constituents of *Hibiscus sabdariffa*, this class of compounds appears to be of major relevance for the observed antioxidant properties of this plant, although other antioxidant compounds such as ascorbic acid may also contribute.

5. Conclusion

The result of this study indicates that garlic extracts (AGE and GT) contain components that actively scavenge superoxide ions while *Hibiscus sabdariffa* extract (HSE) is more efficient in inhibiting superoxide ion production. The three extracts (HSE, AGE and GT) also scavenge ABTS radical cations and these abilities are found to be dose-dependent. In all antioxidant assays, HSE is found to be more effective as an antioxidant when compared with antioxidant ability displayed by AGE and GT. The same extract also has higher amount of phenolic compounds than AGE and GT suggesting that antioxidant activities of these three extracts may be related to

their phenolic content. Therefore, the strong antioxidant properties and the presence of phenolic compounds in Garlic and *Hibiscus sabdariffa* may justify their popular consumption and usage in herbal medicine. Further research that will examine other pharmacological properties of the plants as well as their mechanism of action is urgently needed.

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