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Phytochemicals and antioxidant potential of ginger (*Zingiber officinale*) and garlic (*Allium sativum*) extracts

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Abstract

Matured fresh root of Ginger (*Zingiber officinale* R.) and bulb of Garlic (*Allium sativum* L.) and their binary mixture were evaluated for phytochemicals and antioxidant potential using standard methods. The results of preliminary phytochemical screening of aqueous and ethanol extract of Ginger and Garlic showed the presence of some phytochemicals such as tannin, saponin, flavonoids, alkaloids, terpenoids, steroids and glycosides at various levels. The antioxidant activities of aqueous and ethanol extracts of ginger and garlic showed a total phenolic content mg/g of Ginger (6.432 and 14.176) Garlic (7.172 and 2.146), DPPH content Ginger (58.817 and 86.915) % Garlic (37.582 and 26.451), OH radicals ginger (85.358 and 87.694) garlic (83.956 and 79.751), Fe2+ chelation of ginger (4.584 and 43.444) garlic (7.961 and 53.984), NO radicals of ginger (74.894 and 12.658) garlic (58.966 and 86.392). However the antioxidant contents of the binary mixture of both aqueous and ethanol extracts of ginger and garlic (70:30) showed increased in all antioxidant properties. These implies that both aqueous and ethanol extracts of ginger and garlic possessed good phytochemicals and antioxidant potential which indicates that consumption of these two spices either separately or as a mixture could be encouraged especially for those suffering from any form of cardiovascular diseases.

Keywords: Phytochemical; Antioxidant; Ginger; Garlic; Aqueous; Ethanol

1. Introduction

Phytochemicals (from the Greek word phyto, meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients [1]. They protect plants from disease and damage and contribute to the plant's color, aroma and flavor. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called phytochemicals [2; 3]. Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant. In wide-ranging dietary phytochemicals are found in fruits, vegetables, legumes, whole grains, nuts, seeds, fungi, herbs and spices [3]. Broccoli, cabbage, carrots, onions, garlic, whole wheat bread, tomatoes, grapes, cherries, strawberries, raspberries, beans, legumes, and soy foods are common sources [4; 5]. Phytochemicals accumulate in different parts of the plants, such as in the roots, stems, leaves, flowers, fruits or seeds [4; 6]. Many phytochemicals, particularly the pigment molecules, are often concentrated in the outer layers of the various plant tissues. Levels vary from plant to plant depending upon the variety, processing, cooking and growing conditions [7; 8]. Phytochemicals are also available in supplementary forms, but evidence is lacking that they provide the same health benefits as dietary phytochemicals [9].

In recent years, a growing interest in the food industry for the use of antioxidants from natural sources (e.g., polyphenolic compounds from plants, fruits and vegetables, whole grains) has been noticed. There is a growing body of

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evidence that the most commonly used synthetic antioxidants have potential to damage health. Antioxidants are our first line of defense against free radical damage, and are critical for maintaining optimum health and well-being. Regular consumption of anti-oxidative vegetables fruits and spices has been recognized as reducing the risk of chronic diseases [10]. Studies demonstrate that an antioxidant rich diet has a very positive health impact in the long run [11; 12].

Food producers, in order to protect consumers, their interests and their safety have drawn attention to production of natural antioxidants instead to synthetic one. Benefits of polyphenols intake are result of their antioxidant activity, the presence in the human diet and their influence in the prevention of various chronic diseases associated with oxidative stress [13; 14]. Several studies have described the antioxidant properties of medicinal plants, foods and beverages which are rich in phenolic compounds [15]. Natural antioxidant mainly come from plants in the form of phenolic compounds such as flavonoids, phenolic acids, tocopherols, etc. [16]. This study aimed at investigating the phytochemical constituents and antioxidant potential of two spices (ginger and garlic) so as to establish the scientific proof and application of their bioactive components.

2. Material and methods

2.1. Collection and preparation of samples

Ginger (Zingiber officinale R.) and Garlic (Allium sativum L.) were purchased directly from a farm at Mubi area of Adamawa State, Nigeria. The samples were identified and authenticated at the Department of Crop, Soil and Pest Management of the Federal University of Technology, Akure Ondo State, Nigeria. The samples were sorted to remove bad ones, pilled, thoroughly washed, chopped into smaller sizes and ground with electric grinder. The ground samples were air-dried at ambient temperature until fine powder was obtained, they were thereafter transferred into screw-caped air tight plastic rubber and refrigerated at 4 °C prior to analysis.

2.2. Preparation of Extracts

The powdered samples of Ginger and Garlic were divided into two equal half. One portion in each of the sample was extracted with water, while the second one was extracted with 95% ethanol. 500g each of the samples were extracted with 2 litres of water and ethanol separately using cold maceration for 24h at room temperature with occasional shaking. The extracts were filtered under vacuum through Whatman filter paper No. 1; then the solvent removed by evaporation using a Buchi rotary evaporator at 38°C before the crude samples were freeze-dried on a CHRIST alpha 1–4 LD plus (UK) freeze dryer.

2.3. Phytochemical screening

Phytochemical screenings for tannins, saponins, alkaloids, flavonoids, steroids, terpenoids, and anthraquinones were conducted in accordance with standard methods [17. 18].

Tannins, 0.02 g of extract was dissolved in 2 mL of Milli-Q water and filtered. A few drops of 1% ferric chloride solution were added to the filtrate. Formation of a blue colour indicated the presence of tannins;

Saponins, the frothing test was used. About 0.5 g of extract was mixed with 15 mL of Milli-Q water and shaken vigorously for 5 minutes. The formation of a stable froth indicated the presence of saponins;

Alkaloids, 0.02 g of extract was stirred with 2 mL of 1% HCl on a steam bath and then filtered. A few drops of Dragendorff's reagent was used to treat 1 mL of filtrate. An orange precipitate indicated the presence of alkaloids;

Flavonoids, 0.02 g of extract was dissolved in 1 mL of methanol. A chip of magnesium metal was added to the solution followed by the addition of a few drops of 11.6 M HCl. The occurrence of a magenta colour indicated the presence of flavonoids;

Steroids, 0.02 g of extract was dissolved in 2 mL of chloroform and filtered (using Whatman No. 1 filter paper). 98% H_2SO_4 was carefully added to the filtrate. A reddish brown colour at the interface indicated the presence of steroids;

Terpenoids, 0.02 g of extract was dissolved in 2 mL of methanol and filtered. Acetic anhydride (1 mL) was added to the filtrate and then 2 mL of concentrated H_2SO_4 was added carefully to the side of the tube. Formation of a reddish brown colour at the interface indicated the presence of terpenoids; and Anthraquinone glycosides were detected using the Borntrager's test after hydrolysis of the extract with 10% hydrochloric acid. Chloroform was added to the hydrolysate

and the contents were shaken and treated with 10% ammonia solution. The development of a pink colour indicated the presence of anthraquinone glycosides.

2.4. *In Vitro* Antioxidant Assays

2.4.1. Determination of total phenol content

The phenolic contents were determined using Follin-Ciocalteu reagent and expressed as Garlic Acid Equivalents (GAE) [19]. The extracts were diluted with methanol, by taking 3 ml of methanol and 1 ml of crude extract solution. To this sample solution, 1 ml of 5-fold diluted Folin Ciocalteu's reagent was added. The contents were mixed well, kept for 5 min at room temperature followed by the addition of 1 ml of 10 % aqueous sodium carbonate. After incubation at room temperature for one and half hour the absorbance of the developed blue colour was read at 760nm (Shimadzu UV-1650 PC Shimadzu Corporation, Kyoto, Japan) against reagent blank. Garlic acid (100-1000 mg/mL) was used to construct the calibration curve. Results were calculated as garlic acid equivalent (mg/g) of sample extracts. The determination was done in triplicates and concentrations of phenolic compounds were calculated from obtained standard garlic acid graph.

2.4.2. Determination of total flavonoids content

Total flavonoids content (TFC) was determined spectrophotometrically using the method of [20] based on the formation of flavonoid-aluminium complex. An aliquot (0.5 ml) of the extract solution were mixed with 2ml double distilled water, followed by 0.15 ml of 5 % $NaNO_3$ solution. After 6 min, 2 ml of $AlCl_3$ (10 %) was added, followed by addition of 0.5ml of NaOH (1M) to the mixture. The mixture was diluted by adding 2.5ml of double distilled water immediately, and then mixed thoroughly. Absorbance of the mixture, pink in color, was determined at 510nm against reagent blank without extract. The absorbance of each blank consisting of some mixture in which $AlCl_3$ solution was substituted with double distilled water which was subtracted from the test absorbance. Rutin (0.04-2.5 µg/ml) was used as standard and TFCs from extracts were expressed as µg-rutin equivalent TR/g dry weight of fruit sample. The concentrations of the flavonoids were calculated from obtained standard rutin graph.

2.4.3. DPPH radical scavenging activity

Free radical scavenging activities of the extracts were determined using a stable 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) [21; 22]. DPPH is a free radical of violent colour. The antioxidants in the sample scavenge the free radicals and turn it into yellow colour from violet which was proportional to the radical scavenging activity. The assay contained 1 ml of 0.1mM DPPH in methanol and varying concentrations of extracts (50-1000 ug/ml) methanol and standards in the same solvent and made up to 3.5 ml with methanol. The contents were mixed immediately and then incubated for 30 min at 30 ^{o}C in water bath. The degree of reduction of absorbance was recorded in UV-Vis spectrophotometer at 517 nm. The percentage of scavenging activity was calculated as:

$$A\% = (Ac - As)/Ac \times 100$$

Where:

- Ac = Absorbance of control (without sample)
- As = Absorbance of sample

2.4.4. Determination of Fe^{2+} Chelation

The ability of the extract to chelate Fe^{2+} was determined using a modified method described by [23]. Briefly, 150mM $FeSO_4$ was added to a reaction mixture containing 168mL of 0.1M Tris-HCl pH 7.4, 218mL saline and extract and the volume is made up 1mL with distilled water. The reaction mixture will be incubated for 5min, before the additional of 13ml of 1, 10-phenantroline and the absorbance was read at 510nm.

2.4.5. Hydroxyl radical scavenging activity

The influence of extractw on the formation and stabilization of hydroxyl radicals was determined by adding investigated extracts in the Fenton reaction system at the range of concentrations 25-1500 µg/ml. Hydroxyl radicals are identified because of their ability to form nitroxide adducts (stable free radicals form) from the commonly used DMPO as the spin trap [24]. The Fenton reaction was conducted by mixing 200 µl of DMPO (112mM), 200 µL of DMF, 200 µL of H_2O_2 (2mM) and 200 µL of $FeCl_2$ (0.3 mM) (control). ESR spectra were recorded after 5 minutes, with the following spectrometer settings: field modulation 100 kHz, modulation amplitude 0.226 G, receiver gain 5 x10^5, time constant

80.72 ms, conversion time 327.68 ms, center field 3,440.00 G, sweep width 100.00 G, x-band frequency 9.64 GHz, power 20 mW, temperature 23°C. The SAOH value of the extract was defined as: SA●OH = 100 × (h0 – hx) / h0 [%]; where h0 and hx are the height of the second peak in the ESR spectrum of DMPO-OH spin adduct of the control and the probe, respectively.

2.4.6. Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was carried out by following the method described by [25]. The FRAP reagent included 300 mM acetate buffer (3.1 g of CH_3COONa in 16 mL glacial acetic acid), 10 mM TPTZ solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution in the ratio of 10 : 1 : 1 (v/v). The solutions of extracts were prepared at a final concentration of 0.2 mg/mL in methanol. Sample solution (400 μL) was mixed with 3 mL of freshly prepared FRAP solution and the solution incubated at 37°C in a water bath for 30 min. The absorbance of the samples was then measured at 593 nm. Trolox was used as a standard solution to draw the calibration curve in a concentration range of 10–100 μg/mL. The FRAP results were calculated as mg of Trolox equivalent per gram extract. All experiments were done in triplicate.

2.4.7. Determination of NO (Nitrous oxide) Radical Scavenging Ability

Sodium Nitroprusside in aqueous solution at physiological pH spontaneously generates NO, which interacts with oxygen to produce nitrite ions that can be estimated by use of Greiss reagent. Scavengers of NO compete with oxygen, leading to reduced production of NO [26]. Briefly 5 mM sodium nitroprusside in phosphate- saline was mixed with the sample, before incubation at 25 ^{0}C for 150 min. Thereafter the reaction mixture was added to Greiss reagent. Before measuring the absorbance at 546 nm, relative to the absorbance of standard solution of potassium nitrate treated in the same way with Greiss reagent.

2.5. Statistical analysis

All experiments were carried out in 3 replicates and results presented as mean ±standard error of mean (S.E.M) using SPSS version 22.0

3. Results and discussion

3.1. Phytochemical composition

Table 1 Phytochemical Screening of Aqueous and Ethanol Extracts of Ginger and Garlic

Phytochemical screening	Ginger extract		Garlic extract	
	Aqueous	Ethanol	Aqueous	Ethanol
Tannin	+++	++	++	+
Saponin	+++	+	++	+
Alkaloid	++	+++	+++	++
Flavonoid	++	+++	+	-
Terpenoid	+	++	+++	+
Steroid	+	+++	+	+
Glycoside	+	++	++	++

Note: - = Absent + = Present ++ = Moderately Present; +++ = Abundantly Present

Table 1 showed the results of the preliminary phytochemical screening of the aqueous and ethanol extracts of ginger and garlic. It revealed the presence of some phytochemical constituents such as tannin, saponin, terpenoid, alkaloids, flavonoid and steroid and glycoside. Most of the phytochemicals were abundantly present in ethanol and aqueous extract of Ginger but moderately present that of Garlic. The Tannin content was abundantly present in aqueous extract of ginger and moderately present both the aqueous and ethanol extract ginger and garlic. Tannins, according to research, are known to have antibacterial [27], antitumor and antiviral activities [27]. They work by precipitating microbial protein thus making nutritional protein unavailable for them. Tannins and their derivatives are phenolic compounds considered to be primary antioxidants or free radical scavengers [28; 29; 30; 31]. The Saponin content was moderately present in the ethanol extract but was abundantly present in the aqueous extract of ginger. High presence of saponin in

sample have a beneficial effect of cholesterol lowering, deleterious properties, and exhibit structure dependent of medicinal activities [29]. Saponins are also bioactive constituent which involved in plant defense system because of their antimicrobial activity [28; 29]. Alkaloid was abundantly present in the ethanol and aqueous extract ginger and garlic but moderately present in the aqueous and ethanol extract of both. Alkaloids have been reported as powerful poison and many alkaloids derived from medicinal plants show biological activities like, anti-inflammatory [32] antimalarial [33], antimicrobial [34], cytotoxicity, antispasmodic and pharmacological effects (18; 35]. Flavonoid and Terpenoid were abundantly present in the aqueous extract of ginger and ethanol extract of garlic. Steroid was abundantly present in the ethanol extract ginger but present in ethanol and aqueous extract of garlic. Steroids derived from plants are known to have cardiotonic effect and also possess antibacterial and insecticidal properties [36]. They are very often used in medicines due to their well-known biological activities. Steroid was moderately present in ethanol extracts of both spices which makes it safe for human consumption. The Glycoside content was moderately present in both aqueous and ethanol extract of ginger and garlic. Glycosides have been used to treat congestive heart failure and cardiac arrhythmia [37]. Glycosides, whose presence in samples helps in supporting strength and in controlling the rate of heart contraction, are moderately present in the extracts which is an indication that the sample could be a good material for drug formulation. Phytochemical constituents in the plant samples are known to be biologically active compounds and they are responsible for different activities such as antioxidant, antimicrobial, antifungal, and anticancer. [38; 39]. All secondary metabolite components displayed antioxidant and antimicrobial properties through different biological mechanisms. Generally, both aqueous and ethanol extract of ginger and garlic showed the presence of some phytochemicals at various levels which could make them of great importance as anti-malaria, antioxidant, antifungal, anticancer, rheumatism, antibacterial, anti-inflammatory, anti-ulcer and anti-nociceptive.

3.2. Antioxidant Activities of Extracts

Table 2 Antioxidant properties of aqueous and ethanol extracts of ginger and garlic

Antioxidant	Ginger extract		Garlic extract	
	Aqueous	Ethanol	Aqueous	Ethanol
Total Phenol (mg/g)	6.432 ± 0.21	14.176 ± 0.05	7.172 ± 0.15	2.193 ± 0.42
Flavonoids (mg/g)	0.328 ± 0.01	1.183 ± 0.02	0.096 ± 0.01	0.241 ± 0.03
DPPH (%)	58.817 ± 0.15	86.915 ± 0.33	37.882 ± 0.63	26.451 ± 0.41
OH Radicals (%)	85.358 ± 1.19	87.694 ± 1.26	83.956 ± 1.41	79.751 ± 1.33
FRAP (mg/g)	19.023 ± 0.36	29.124 ± 0.11	9.828 ± 0.06	5.617 ± 0.28
Fe^{2+} Chelation (%)	4.884 ± 0.13	43.444 ± 1.03	7.961 ± 0.46	53.984 ± 1.19
NO Radical (%)	74.894 ± 2.32	12.658 ± 0.05	58.966 ± 1.13	86.392 ± 2.51

Mean ± Standard Error of triplicate determinations

Table 3 Antioxidant properties of aqueous and ethanol extracts of ginger and garlic mixture

Antioxidant	Aqueous extract	Ethanol extract
Total Phenol (mg/g)	11.438 ± 0.42	19.015 ± 1.03
Flavonoids (mg/g)	1.062 ± 0.01	1.872 ±0.09
DPPH (%)	74.513 ± 1.08	92.416 ± 2.14
OH Radicals (%)	88.761 ± 1.13	86.384 ± 1.06
FRAP (mg/g)	32.684 ± 0.05	41.539 ± 0.21
Fe^{2+} Chelation (%)	17.216 ± 0.03	62.238 ± 1.09
NO Radical (%)	87.568 ± 2.04	96.642 ± 2.13

Mean ± Standard Error of triplicate determinations

Table 2 showed the antioxidant constituents of aqueous and ethanol extracts of ginger and garlic. It revealed total phenolic content mg/g of ginger (6.432 and 14.176) and garlic (7.172 and 2.146), which indicates that phenolic content

in ethanol extract of ginger was higher than that of aqueous extract, but it was reversed in the case of garlic phenolic content was high in aqueous extract. The total phenol contents obtained in this study were higher than (0.71 mg/g and 0.78mg/g) respectively, as reported by [40] for aqueous extract of Onion and Garlic, but lower than 598.47 reported by [41] for aqueous extract of Canerium fruit, [42] also reported values of 15.49% and 20.35% for red and green pepper fruit. The antioxidant activities of phenolic content of spices is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides [43]. Phenolics are the largest group of phytochemicals and have been touted as accounting for most of the antioxidant activity of plants and spices. The percentage (Nitrous oxide) NO radicals of ginger was (74.894 and 12.658) garlic (58.966 and 86.392), this showed a higher percentage inhibition in both spices but lower in ethanol extract of ginger. Flavonoids represent the most common and widely distributed groups of plant spices phenolics. They are potent water soluble super antioxidants that function in scavenging free radicals, inhibition of peroxidants and chelating transition metals [44]. Flavonoid content mg/g in ginger was (0.328 and 1.183) garlic (0.096 and 0.241) which showed that amount of flavonoid in ginger and garlic was generally low but a bit higher in ethanol extract of ginger. DPPH content of ginger (58.817 and 86.915) % garlic (37.582 and 26.451) % indicates that amount of DPPH scavenging ability was higher ethanol extract of ginger and aqueous extract of garlic. DPPH value of 75.17 and 22.63 was reported by [40] for Onion and garlic respectively, [41] also reported DPPH concentration of 17.28 for *Canarium schweinfurthii* fruit which was lower than the values obtained for two spices investigated. DPPH radical scavenging assay provides easy, rapid and convenient method to evaluate antioxidants and radical scavengers [45]. DPPH is a free radical donor that accepts an electron or hydrogen to become a stable diamagnetic molecule [46]. The tendencies of electron or hydrogen donation are critical factors in characterizing antioxidant activity that involves free radical scavenging [47; 48]]. This is based on the ability of 1,1-diphenyl-2-picryl-hydrazyl (DPPH) which is a stable free radical, to decolorize in the presence of antioxidants. DPPH radical contains an odd electron, which is responsible for the absorbance at 515nm and also responsible for the visible deep purple colour. The percentage (Hydroxyl) OH radicals of ginger was (85.358 and 87.694) garlic (83.956 and 79.751), this indicates the high scavenging ability of hydroxyl radicals in both aqueous and ethanol extracts. Fe^{2+} chelation of ginger was (4.584 and 43.444) garlic (7.961 and 53.984), this showed that Fe^{2+} chelation ability was higher in ethanol extracts of both ginger and garlic than aqueous extract. The presence of antioxidants in the samples would result in the reduction of Fe^{3+} to its lower valence state, Fe^{2+} by donating an electron. Amount of Fe^{2+} complex can then be monitored by measuring the formation of Perl's Prussian blue at 700nm. Increasing absorbance at 700nm indicates an increase in reductive ability [49; 50]. The results obtained for ethanol extract of ginger and garlic confirmed that Fe^{3+} - Fe^{2+} transformation occurred in the presence of the extracts, thereby confirming their antioxidant potentials. However, results of the binary mixture (70:30) of aqueous and ethanol extracts of ginger and garlic is shown in Table 3. It generally revealed an increased in all antioxidant activities than individual spice. This implies that consumption of the spices mixture could enhance the health status of consumers especially when extracted with ethanol.

4. Conclusion

The phytochemical and antioxidant potential of aqueous and ethanol extracts of ginger and garlic were investigated. The preliminary phytochemical screening revealed the presence of some phytochemicals constituents such as tannin, saponin, terpenoid, alkaloids, flavonoid, steroid and glycoside. Most of the phytochemicals were abundantly present in ethanol and aqueous extract of Ginger but moderately present in that of Garlic. The results of antioxidant properties of ginger and garlic also revealed a good antioxidative potentials of the extracts in total phenolic content, total flavonoid, DPPH radical scavenging, OH and NO radicals, Ferric reducing power and Fe^{2+} chelation ability at various concentrations. The results of the binary mixture of aqueous and ethanol extract of ginger and garlic also revealed an increased in all antioxidant activities than individual spice. The presence of these phytochemicals and antioxidant contents in both aqueous and ethanol extracts of ginger and garlic suggest their application as food or food supplement to be taken by male and female adults and especially those suffering from cancer, diabetic, hypertension and other cardiovascular diseases.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declared no conflict of interest.

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