International Journal of Agricultural and Applied Sciences, June 2024, 5(1): 1-5
https://www.agetds.com/ijaas
ISSN: 2582-8053
https://doi.org/10.52804/ijaas2024.511

Research Article

OPEN ACCESS

Comparative studies of antioxidant activity and profile of some spices

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(Received: 13/12/2023; Revised: 01/04/2024; Accepted: 05/05/2024; Published: 20/06/2024)

ABSTRACT
Compounds that prevent or slow down the oxidation of lipids or other molecules like free radicals are known as antioxidants. The Study evaluated the antioxidant capacity, hydrogen peroxide scavenging capacity, and total flavonoid and phenol contents of six spices. Dried samples of spices were extracted in distilled water and analyzed using spectrophotometric and titrimetric methods. TAC results ranged from 232.79 - 64.32 mg/100g, with clove and black cardamom exhibiting the highest and lowest antioxidant activity respectively. The H_2O_2 scavenging activities result had clove and rosemary with the highest scavenging activity. Rosemary had the highest total phenol (5060 mg/100g) and flavonoid (1339 mg/100g) contents while black cardamom had the lowest values in both. Spices could be substituted for synthetic antioxidants in the food industry and as dietary sources of natural antioxidants.

Keywords: spices, antioxidant capacity, star anise, cinnamon, black cardamom, and fenugreek.

INTRODUCTION

In all cultures around the world, spices and herbs are traditionally utilized to enhance food flavor, they are also used medicinally to treat various ailments (Shirazi *et al*., 2014). Many plants used in traditional medicine contain high levels of polyphenols and flavonoids. These substances are an important source of phytochemicals for human and animal health in addition to aiding in the regulation of plant growth (Garg *et al*., 2019). Spices have long been utilized as aromatic and pungent food components that are used in a variety of dishes to enhance their flavor. Many plant components are used to make them, including cinnamon bark, clove flower buds, ginger roots, pimento fruits, white pepper berries, and other dried, edible, aromatic plants (Marti´nez-Tome *et al*., 2001; Przygodzka *et al*., 2014). The majority of these spices have a wide range of potential medical benefits, including their favorable effects on lipid metabolism, effectiveness as anti-diabetics, capacity to stimulate digestion, and potential as anti-inflammatory, and antioxidant qualities (Strabbiol and Murcia, 2001). Herbs and spices are rich in phytochemicals like polyphenols, phenolic acids, tannins, flavonols, isoflavones, and curcuminoids, which act as antioxidants and provide various health benefits. (Shirazi *et al*., 2014).

Atmospheric oxygen reacts with food containing fats or oil leading to the formation of peroxides and hydroperoxides, which eventually produce carbonyl compounds as secondary oxidation products (Embuscado, 2015). This chemical reaction happens when fats, oils, or meals containing lipids are subjected to oxidation. As a result, many foods develop smells and odors that can give them a rancid taste, thus rendering these items unfit for ingestion. Food products that are susceptible to this kind of chemical change currently require the usage of antioxidants to prevent food from spoiling due to oxidation (Choe and Min, 2009). Natural antioxidants that are derived from herbs and spices have varied levels of effectiveness when used in various culinary contexts. The human diet includes not just certain antioxidant vitamins like pro-vitamin A, vitamin E, and vitamin C but also a sophisticated blend of many naturally occurring chemicals that can act as antioxidants. Studies have also been conducted on aromatic plants as sources of various natural antioxidant classes. (Bowser *et al.*, 2014; Ozcan *et al*., 2009).

Antioxidants are substances that inhibit oxidative chain reactions, by preventing lipids and other molecules from oxidizing. As a result, antioxidants can avert damage caused to the body's cells by oxygen due to the redox potential of phenolic moieties (Choudhary *et al*., 2017; Suhartono *et al*., 2012). They exhibit their mechanism by acting as reducing agents, scavenging free radicals, quenching singlet oxygen and potentially complexing pro-oxidant metals (Bowser *et al.*, 2014; Suhartono *et al*., 2012). Flavonoids and other phenolic compounds are secondary metabolites of plants that have an aromatic ring with a hydroxyl group or more (Ahmed *et al*. 2016). There are over 8,000 naturally occurring phenolic compounds found in plants, with half of them being flavonoids in various forms such as aglycones, glycosides, and methylated derivatives (Ahmed *et al*. 2016). One of the leading causes of food spoilage is lipid peroxidation, which can result in a loss of important nutrients and functional properties (Odukoya *et al*., 2005). The late 1940s saw the discovery that phenolic chemicals, or antioxidants, may reduce lipid peroxidation. This finding led to the development of chemical antioxidants such as tert-butylhydroquinone (TBHQ), Butylated hydroxyl anisole (BHA), butylated hydroxytoluene (BHT) and Propyl Gallate (PG) in the food processing industry, and have been dominant since their introduction. However, there are concerns by consumers about the use of synthetic food additives, hence the need for natural additives like herbs and spices, which are abundant in antioxidants (Saini *et al*., 2020; Choudhary *et al*., 2017; Odukoya *et al*., 2005).

This study aimed to investigate the total antioxidant capacity and hydrogen peroxide scavenging activity of different spices (bay leaf, clove, star anise, cinnamon, black cardamom, and rosemary) sold in Ilorin, Kwara State, Nigeria.

MATERIALS AND METHODS

Sample Collection

The spices (Bay leaf, Clove, Star anise, Cinnamon, Black Cardamom, and Rosemary) were purchased from Yoruba Road market, Ilorin, Kwara State, Nigeria.

Sample preparation

The methods of Maduike and Anuna (2018) with slight modifications were used for the preparation of the samples. A high-speed blender (model 8011ESK) was used to grind the spices into a fine powder, which was then stored in airtight containers till further use. To prepare the samples, 20 grams from each of the spices were weighed individually and then put in different containers. To every container, 100 millilitres of distilled water was added and heated to 60 °C for 1hr. afterwards, the solutions were filtered and the resulting liquid was collected in amber bottles and sealed for future use.

Determination of Total antioxidant Capacity (TAC)

The level of antioxidants present in a substance was measured using the phosphomolybdenum assay through spectrophotometry. To conduct the test, 1 mL of each extract was combined with a 3 mL solution that contained 28 mM sodium phosphate, 4 mM ammonium molybdate and 0.6 M H_2SO_4. The blank sample, which served as the control, only contained 4 mL of the reagent solution. The mixture of samples and reagent solution was then incubated at 95 °C for 150 minutes and subsequently cooled down to ambient temperature. A set of varying concentrations of ascorbic acid as standard was prepared and treated as test samples. The absorbance of both test samples and standards was read at 695 nm. TAC was expressed as an Ascorbic Acid equivalent Prieto *et al.,* (1999).

Hydrogen peroxide scavenging assay

To determine the percentage of scavenging of H_2O_2, the replacement titrimetric method described by Zhang (2000) was used. One (1) mL of each extract was mixed with 2 mL of 1 mM H_2O_2, 2 drops of 10 mL of 0.2 M sulphuric acid, 3% ammonium molybdate, 7 mL of 1.8 mM potassium iodide, and 2 drops of starch indication were added separately to each mixture. The resulting reaction mixture was titrated against 0.5 mM sodium thiosulphate until the blue colour disappeared. It was determined what percentage of H2O2 was scavenged using the following formula:

$$\boldsymbol{Inibition}\ (\%) = \frac{\boldsymbol{Vo - V1}}{\boldsymbol{V1}}\boldsymbol{X100}$$

Where V_o and V_1 are the volume of thiosulphate used for blank and samples respectively.

Determination of Total phenol content (TPC)

According to Tambe and Bhambar's (2014) description, the spectrophotometric approach was employed to ascertain the phenolic content of every extract. In a 25 mL volumetric flask, 1 mL of the extract and 9 mL of distilled water were combined to create each reaction mixture. Following that, 1 mL of Folin-Ciocalteu phenol reagent was added to the mixture and thoroughly shaken. Ten millilitres of a 7% sodium carbonate solution were added to the mixture after five minutes. Then, distilled water was added to get the volume up to 25 mL. Using the previously mentioned procedure, a standard solution set of gallic acid was prepared. After this, the solution was incubated for 90 minutes at ambient temperature, the absorbance of the solution was measured at 550 nm concerning the against reagent blank using an ultraviolet/visible spectrophotometer

Determination of Total Flavonoid content (TFC)

Tambe and Bhambar (2014) described the aluminium chloride colourimetric technique used to evaluate the extracts' total flavonoid concentration. One ml of extract and 4 ml of distilled water were put in a 10 ml volumetric flask and combined to create the reaction mixture. After adding 0.30 ml of 5% sodium nitrite to the flask and letting it stand for five minutes, the mixture was then mixed with 0.3 ml of 10% aluminium chloride, and it was left for an additional five minutes. Two millilitres of 1M sodium hydroxide were added to the solutions, and then distilled water was used to dilute it to ten millilitres. Using the same procedure, a collection of quercetin concentrations was prepared as a reference standard. Determination of the absorbance of the solutions against the reagent blank at 510 nm was done using a UV/Visible spectrophotometer.

Statistical analysis

The experiments were conducted in triplicate to ensure accuracy and precision. The results were expressed as mean±SE (standard error) and analyzed using SPSS statistical software. The Duncan test was used to check the significance of the difference, and $P<0.05$ was considered significant.

RESULTS AND DISCUSSION

One of the primary mechanisms of phenolic phytochemicals' antioxidant effect is believed to be the scavenging of various forms of reactive oxygen species, primarily free radicals (Shan *et al*., 2005). The ability to provide hydrogen atoms and scavenge free radicals is reflected in anti-oxidation activity. Greater capacity is indicated by higher anti-oxidation action. (Odukoya *et al*., 2005). The result of TAC and H_2O_2 scavenging activity, as presented in Table 1 indicated a variation between the highest and lowest total antioxidant capacity. Clove exhibited the strongest total antioxidant capacity (437.12 mg/100g), which was significantly higher than rosemary (434.99 mg/100g). Shan *et al*. (2005) also reported high levels of total equivalent antioxidant capacity in Clove (168.7 mmol/100g of dry weight) where the total equivalent antioxidant capacity and total phenolic content of 26 spices were compared. Cinnamon and Bayleaf also had high total antioxidant capacity (232.79 and 210.30 mg/100g respectively) though with significant differences. Lu *et al*., (2011) also reported a strong total antioxidant activity for cinnamon. However Black cardamom recorded the lowest value (64.32 mg/100g) while Star anise had a value of 122.46 mg/100g.

According to Shan et al. (2005), clove has a high total antioxidant capacity due to its potent hydrogen-donating potential, metal-chelating capabilities, and efficacy as superoxide, hydrogen peroxide, and free radical scavengers According to Hossain *et al*. (2008), cinnamon's potent antioxidant action may be attributable to both its high eugenol content and its high cinnamaldehyde level. The spice extracts had remarkable H_2O_2 scavenging activity except Star anise and black cardamom whose inhibitory effects were below 50%. The hydrogen peroxide scavenging activity was highest in clove (96.73% inhibition) and lowest in star anise (48.59% inhibition).

Plant materials possess antioxidant properties because of the phenolic chemicals that are present in them (Soobrattee *et al.* 2005). Being among the most abundant and varied classes of natural chemicals, flavonoids are arguably the most significant natural phenolics. According to Panche *et al*., (2016), these substances possess a broad spectrum of chemical and biological actions, including the ability to scavenge radicals. According to Il-Suk *et al*. (2011), the antioxidant characteristics of flavonoids and phenolic compounds stem from their redox characteristics, capacity to chelate metals and singlet oxygen quenching ability. TPC and TFC levels in cloves were reported by Ali *et al*., (2021) to be 21510 mg GAE/100g and 560 mg quercetin equivalents (QE)/100g, respectively. Frohlich *et al*. (2022) extracted bioactive chemicals using an ultrasound-assisted extraction method and 70% aqueous ethanol at 60°C for 20 minutes as a solvent. They discovered a TPC of 38790 mg GAE/100g. Using boiling water (95°C) for three hours, Suantawee *et al*., (2015) extracted clove buds, yielding an extract with TPC and TFC values of 23960 mg GAE/100g extract and 6570 mg CE/100g extract, respectively. Ahmed et al. reported high levels of total flavonoid and phenolic content in their study in 2022. However, a new study has found lower levels of TPC and TFC, recording them to be 5036.11 mg/100g and 299.90 mg/100g respectively. The difference could be due to the variant of the species used in each study area and location. Two studies were conducted to determine the TPC and TFC of Rosemary. The first study by Megateli et al. (2018) found the TPC to be 12,787 ± 2.1 mg GAE/g dw and TFC to be 14.48 ± 1.5 mg QE/g dw using a plant material-to-liquid ratio of 1:2 (w/v). In contrast, the second study found significantly higher TPC and TFC levels, measuring at 5025.34 mg/100g and 1330.27 mg/100g, respectively. The TPC (2069.28 mg/100g) and TFC (330.82 mg/100g) levels of bay leaves in this investigation were likewise higher than those reported by Amal *et al.* (2018) (494.86±3.62 mg equivalent to gallic acid/g DM).

Table 1. Total Antioxidant Capacity and H_2O_2 Scavenging activity of selected Spices

Spices	Total Antioxidant capacity (mg/100g)	H_2O_2 scavenging activity (% inhibition)
Bay leaf	210.30c±0.08	88.95d±0.06
Clove	437.12f±0.03	96.73f±0.12
Star anise	122.46b±0.00	48.59b±0.16
Cinnamon	232.79d±0.05	85.76c±0.10
Black cardamom	64.32a±0.02	40.41a±0.05
Rosemary	434.99e±1.10	90.48e±0.14

The result shows the standard error of triplicate values. Means on the same column with unshared superscripts are significantly different (p<0.05)

Table 2: Total phenol content (mg/100g) of selected spices

Spices	Total Phenol (mg/100g)
Bay leaf	$2069.28^{c}\pm0.14$
Clove	$5036.09^{e}\pm0.16$
Star anise	$1000.69^{b}\pm0.07$
Cinnamon	$3866.95^{d}\pm0.31$
Black cardamom	$410.37^{a}\pm0.42$
Rosemary	$5060.23^{f}\pm0.20$

The result shows the standard error of triplicate values. Means on the same column with unshared superscripts are significantly different (p<0.05)

Table 3: Total flavonoid content (mg/100g) of selected spices

Spices	Flavonoids (mg/100g)
Bay leaf	$330.83^{d}\pm0.02$
Clove	$299.91^{c}\pm0.14$
Star anise	$144.02^{b}\pm0.19$
Cinnamon	$709.89^{e}\pm0.19$
Black cardamom	$58.39^{a}\pm0.04$
Rosemary	$1339.51^{f}\pm0.08$

The result shows the standard error of triplicate values. Means on the same column with unshared superscripts are significantly different (p<0.05)

Yang *et al*., (2012) discovered in their investigation on cinnamon that the total phenolic concentration (8.854 g/100 g DW) in 95% ethanol extract was twice as high as the Total flavonoid content (3.348 g/100 g DW). Cinnamon leaves from Ceylon were found to have a higher level of TPC (44.57 mg/g) and a lower content of TFC (12.00 mg/g) when extracted with 95% ethanol in a prior work by Wang et al. (2023). The TPC and TFC for cinnamon bark were 33.43 and 3.07 mg/g, respectively. However, these values were found to be much lower compared to the values obtained in the Yang *et al*., (2012) study, which reported TPC and TFC values of 3866.91 and 709.88 mg/100g respectively. Black cardamom (410.37 mg/100g, 8.39 mg/100g) and Star anise (1000.69 mg/100g, 144.02mg/100g) for TPC were the least recorded values for this study. As stated by Shan *et al*., (2005), the presence of phenolic compounds in plants and plant parts and their redox properties accounts for their antioxidant effect. This could be a justification for the scavenging strength of the spice extracts analyzed.

CONCLUSION

The study confirms that phenolic phytochemicals contribute to the antioxidant effect of spices by scavenging free radicals. Most of the substances present in natural extracts with demonstrated antioxidant action contain phenolic moiety. The majority of the spices in the study were shown to have significant levels of Phenol and flavonoid contents, H_2O_2 scavenging activity and total antioxidant capacity. These natural spices have a lot of potential to replace the artificial ones being processed and used in the food Industry.

ACKNOWLEDGEMENT

The authors wish to acknowledge the central laboratory service of the Nigerian stored products research institute for the use of the facility in this study

CONFLICT OF INTEREST

The author here by declare that there is no conflict of interest in the publication of this article.

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Citation: Onyegbula, A.F.; Ahmed, T.; Olorunfemi, A.P.; Lawal, I.O. and Akande, E.J. 2024. Comparative studies of antioxidant activity and profile of some spices. *International Journal of Agricultural and Applied Sciences*, 5(1): 1-5. https://doi.org/10.52804/ijaas2024.511