Comparative Study on the Antioxidant Capacity of Aqueous Extract of *Costus spicatus* (jacq.) Leaf and Rhizomes

RESEARCH ARTICLE

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ABSTRACT

Antioxidants play an important role in inhibiting and scavenging free radicals, thus providing protection to humans against infection and degenerative diseases. Modern research is directed towards "natural antioxidants" from herbal plants due to their safe therapeutic. *Costus spicatus* is an herbaceous plant whose parts- leaves, rhizome, stem and roots are used locally because of its medicinal properties. The specific objective of the study was to determine and compare the antioxidant capacity of aqueous extracts of *Costus spicatus* leaf and rhizome for their antioxidant activity and radical scavenging potential. The *in vitro* antioxidant status of the aqueous extract of *Costus spicatus* leaf and rhizome was compared using 2,2-diphenyl-1-picrylhydrazyl (DPPH), H_2O_2, Nitric Oxide (NO), 2,2-azinobis-(3-ethylbenzthiazoline-6-sulfonate) (ABTS), Total Antioxidant Capacity (TAC) and Ferric Ion Reducing Antioxidant Power Assay (FRAP). The total phenolics and flavonoid contents were also estimated quantitatively. The aqueous extract of *Costus spicatus* leaf and rhizome both have notable flavonoid content. However the rhizome had slightly higher phenolic content compared to the leaf. Both the leaf and rhizome of aqueous extract of *Costus spicatus* have notable antioxidant capacity when compared with the standard used. The aqueous extract of *Costus spicatus* rhizome showed higher antioxidant activity than the leaf in a concentration dependent manner. These results revealed that the aqueous extract of *Costus spicatus* leaf and rhizome has antioxidant capacity and scavenging potential, with the rhizome having the most notable capacity and therefore can be further explored in antioxidant research and in management of diseases related to oxidative stress.

Research Focus

Comparative analysis of antioxidant capacity between *Costus spicatus* leaf and rhizome aqueous extracts

Key Methods

DPPH, H_2O_2, NO, ABTS, TAC and FRAP assays with quantitative phenolic and flavonoid analysis

Main Finding

Rhizome extract showed superior antioxidant activity compared to leaf extract in concentration-dependent manner

Keywords: *Costus spicatus*, antioxidant, total phenolics, total flavonoids, radical scavenging potential

INTRODUCTION

Plants with an ethnobotanical history are known to harbor diverse group of chemicals, which constitute major natural sources of bioactive compounds with the chemical structures. The biosynthesis and metabolism of these substances as well as their natural distribution and their biological function (Femi-Olabisi *et al.,* 2021; Ajayi, 2019). Medicinal plants are plants which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis (Shankar, 2016).

When a plant is designated as medicinal, it is implied that the said plant is useful as a drug or therapeutic agent or an active ingredient of a medicinal preparation (Shankar, 2016). Medicinal plants are regarded as rich resources of traditional medicines and from these plants many of the modern medicines are produced (Dar *et al.,* 2017). For thousands of years medicinal plants have been used to treat health disorders, to add flavor and conserve food and to prevent diseases epidemics. The secondary metabolites produced by the plants are usually responsible for the biological characteristics of plant species used throughout the world (Dar *et al.,* 2017). Many herbal plants contain antioxidant compounds which protects cells against degenerative effects of Reactive Oxygen Species (ROS).

01

Traditional Medicine Heritage

Herbal medicine has been a common practice for thousands of years, but the search for active constituents in the medicinal plants started in the 19th century

02

Modern Research Focus

Currently, many pharmaceutical companies have expressed renewed interest in natural products as sources of new therapies

03

Antioxidant Applications

Recent studies have shown that diverse biological activities, such as antioxidant, antidiabetic and anti-inflammatory activities, are associated with the phenolic compound contents found in fruits and vegetables

Furthermore, many plant species are widely utilized as spices and condiments which have strong flavor, taste and smell as well as nutritional and medicinal importance.

Costus spicatus is a perennial herb known commonly as spiked spiral head ginger or Indian head ginger, or insulin plant in English, *Tete-egun* in Yoruba, *Okpete* in Igbo and *Kakizuwa* in Hausa, there are more than 100 species of the *Costus*. A few assortments with flowers and bracts look like conservative cones, while others are molded like pineapple or delicate crepe emerging from green cones (Femi-Olabisi *et al.,* 2021). *Costus spicatus* (Jacq.) Sw. (Costaceae) is used by the Amazonian population to treat inflammation, pain and other pathological manifestations (Picanco *et al.,* 2016). The genus *Costus spicatus* belongs to the family Costaceae. They are perennial tropical

plants. They are often distinguished from plants of the genus *Zingiber* by the spiraling growth of their stems. *Costus spicatus* presents alternating leaves, membranous, papyraceous provided with sheaths, smooth on the two sides, 25-40 cm long and 6-10 cm wide. It has strobili-shaped inflorescences in terminal spikes, with large showy red bracts, which shield the yellowish flowers. Multiply both by seeds and by rhizomes. *Costus spicatus* Sw. (Costaceae) is a conspicuous herb utilized by Dominicans in the Dominican Republic and the US for the treatment of diabetes, a developing scourge in the Hispanic community (David *et al.,* 2021).

Several studies have reported the association between the presence of the secondary metabolites as phenolic and flavonoids in medicinal plants, with potential antioxidant and antimicrobial activity (Liu *et al.,* 2010; Proestos *et al.,* 2006; Rauha *et al.,* 2000; Ravikumar *et al.,* 2010). Another in vivo study proposed an analgesic and anti-inflammatory activities of the methanol extract obtained from the leaves of *Costus spicatus* (Quintans Júnior *et al.,* 2010)

ANTIOXIDANTS AND FREE RADICALS

Antioxidants are complexes found in the food that can retard or deter oxidation by preventing the initiation and propagation of oxidizing chain reactions (Moriasi *et al*., 2020; Panche *et al*., 2016). They are man-made or natural substances that may prevent or delay some types of cell damage (Yadav *et al.,* 2016). Antioxidants are found in many foods, including fruits and vegetables. Although oxidation reactions are crucial for life, they can also be damaging; plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, vitamin A, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxides (Yadav *et al.,* 2016).

Free radicals react (ROS) and reactive nitrogen species (RNS) are generated by our body by different endogenous systems, exposure to conditions or pathological states. A balance between free radicals and antioxidants is necessary for proper physiological action. If free radicals overwhelm the body's ability to regulate them, a condition known as oxidative stress ensues (Mohammed *et al*., 2015). Free radicals consequently adversely change lipids, proteins, and DNA and trigger a number of human diseases. Hence application of external source of antioxidants can assist in coping with oxidative stress. Plants have antioxidant properties and are believed to play a very important role in the body defense system against reactive oxygen species. Medicinal plants have been used for centuries by man to manage diseases and have a host of antioxidant complexes. Antioxidant compounds like phenolic acids, polyphenols and flavonoids are commonly found in plants have been reported to have multiple biological effects, including antioxidant activity. Thus, the search for effective, nontoxic natural compounds with antioxidant activity has been intensified in recent years.

Antioxidant Defense

Natural substances that prevent or delay cell damage by scavenging free radicals and inhibiting oxidation

Phenolic Compounds

Key bioactive compounds in plants responsible for antioxidant activity and therapeutic properties

Oxidative Balance

Maintaining equilibrium between free radical production and antioxidant defense systems

Over the years, there has been debate to the activity and effectiveness in treatment of illness and pathogens between the leaves and rhizome of medicinal plants such as *Costus spicatus* among various practitioners of traditional medicine. Therefore, it is imperative to search for affordable natural alternatives in medicinal plant parts with high antioxidant activity with a reduced or complete absence of side effects and the study also aims at a comparative analysis on the antioxidant activity and scavenging potential of the *C. spicatus* leaves and rhizome. This is to infer scientifically which of the plants part will be more active and effective in traditional medicinal purposes.

MATERIALS AND METHODS

Plant Material

Fresh leaves and rhizomes of the *Costus spicatus* plant were collected at the Mountain Top University Campus premises, Prayer City, Ibafo, Ogun State, Nigeria. The plant was identified at the University of Lagos Botany department, where a voucher specimen (Number 8571) was prepared and deposited.

Chemicals and Reagents

2,2-diphenyl-1-picrylhydrazyl (DPPH), Phosphate buffer ferricyanide, trichloroacetic acid, ferric chloride tetraoxosulphate (VI) acid, sodium phosphate, ammonium molybdate, $FeSO_4$, hydrogen peroxide, sodium salicylate, sodium nitroprusside (SNP), ascorbic acid, Griess reagent (1% sulphanilamide, 0.1% napphthylethylenediamine dihydrochloride in 5% phosphoric acid), ABTS, potassium persulfate and other reagents used are of analytical grade and product of Sigma Aldrich, Steinheim, Germany.

Preparation of aqueous extract of *Costus spicatus* leaf and rhizome

The identified samples, leaf and rhizome of *Costus spicatus* were thoroughly rinsed under running

water to remove contaminants, oven dried at 40 degree Celsius to a constant weight and pulverized to powder using Rex 500 stainless steel Bajaj Mixer Grinder, the rhizome and leaf (250g) powder were weighed, using a weigh balance and soaked separately in 2500ml of distilled water for 48 hours at room temperature with constant shaking using orbit shaker, after which the extract was filtered using Whatman No.1 filter paper into a conical flask. The rhizome and leaf filtrate were collected in beakers and lyophilized to yields of 10.90g and 7.29g (4.36 % and 2.92 % yield) respectively

Extract Preparation

- Oven dried at 40°C to constant weight
- Pulverized to powder form
- Soaked in distilled water for 48 hours
- Filtered and lyophilized

Yield Results

Rhizome: 10.90g (4.36% yield)
Leaf: 7.29g (2.92% yield)

Analytical Methods

Total phenolics, flavonoids, DPPH, ABTS, FRAP, H_2O_2, NO, and TAC assays

Determination of Total Flavonoid and Phenolic Content

The total phenolics content in aqueous extracts of the *C. spicatus* leaf and rhizome were determined according to the Folin-Ciocalteu procedure (Singleton et al., 1999). The total flavonoid content in the aqueous extracts of the *C. spicatus* leaf and rhizome was determined by aluminum chloride colorimetric method based on the method indicated by Ordonez et al. (2006).

in vitro Antioxidant Studies

The total antioxidant capacity of aqueous extract of *C. spicatus* leaf and rhizome was evaluated using the phosphomolybdenum assay described by Prieto *et al.* (1999). The Ferric Ion Reducing Antioxidant Power Assay (FRAP) (Oyaizu 1986; and Nam *et al.* 2017); 2, 2 Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay (Sun and Wang 2010); Hydrogen radical-scavenging activity (Smirnoff and Cumbes 1989); Nitric Oxide (NO) scavenging activity (Fiorentino *et al.* 2008) and 2,2'-azinobiz (3-eyhylthiazoline-6-sulfonic acid) diammonium salt (ABTS) scavenging activity (Re *et al.*, 1999).

Data Analysis

Experimental data were expressed as the mean ± SEM, n = 3. Statistical evaluation of data was performed with SPSS version 20.0.

RESULTS

Total Phenolic and Flavonoid Content

In aqueous extract of *Costus spicatus* leaf and rhizome, the total phenolic content was found to be 77.06µg/100mg and 90.5µg/100mg respectively in terms of gallic acid equivalent (Figure 1).The total flavonoid content of aqueous extract of *Costus spicatus* leaf and rhizome was determined to be 35.76µg/100mg and 37.165µg/100mg respectively in terms of quercetin equivalent (Figure 2). The total phenolic content of the aqueous extract of *Costus spicatus* leaf and rhizome shows that the rhizome has higher phenolic content as to the leaf. The total flavonoid content of the aqueous extract of *Costus spicatus* rhizome and leaf shows both leaf and rhizome has notable flavonoid content with no significant difference in comparism.

90.5µg

Rhizome Phenolics

Gallic acid equivalent per 100mg extract

77.06µg

Leaf Phenolics

Gallic acid equivalent per 100mg extract

37.16µg

Rhizome Flavonoids

Quercetin equivalent per 100mg extract

Antioxidant Assay Results

Aqueous extract of *Costus spicatus* rhizome and leaf produced a concentration dependent scavenging effect on DPPH radicals when compared with reference compound BHT at concentrations 7.8125µg/ml, 15.625µg/ml and 31.25µg/ml. Although the scavenging effect of DPPH radical of aqueous extract of leaf and rhizome was decreased at 250, 500 and 1000 compared to the standard, there was a significant difference in the scavenging effect of aqueous extract of *Costus spicatus* rhizome when compared with aqueous extract of *Costus spicatus* leaf at concentrations 62.5µg/ml, 125µg/ml and 250µg/ml (Figure 3).

Aqueous extract of *Costus spicatus* rhizome and leaf did not produce a concentration dependent scavenging effect of ABTS radical scavenging activity when compared with reference compound BHT at concentrations 7.8125ug/ml to 1000ug/ml (Figure 4). Although the leaf has a higher ABTS inhibition at 7.812µg/ml and 15.625µg/ml compared to rhizome, but at 62.5ug/ml, 125µg/ml, 250µg/ml and 500µg/ml the ABTS inhibition of the rhizome was higher, but at concentration of 1000µg/ml, both the leaf and rhizome aqueous extract of *Costus spicatus* had significant increase in a concentration dependent manner (Figure 4).

At low concentrations the FRAP scavenging activity of *Costus spicatus* leaf and rhizome shows a minimum antioxidant scavenging activity that is relatively lower than that of the standard. However, the *Costus spicatus* rhizome extract has reducing effect higher than that of the leaf extract (Figure 5).

Aqueous extract of *Costus spicatus* rhizome and leaf produced a concentration dependent scavenging effect on H_2O_2 radical at 31.25ug/ml and 62.5ug/ml which was significantly higher than the reference compound BHT. Both the leaf and rhizome aqueous extract of *Costus spicatus* had significant increase in a concentration dependent manner at concentrations 250µg/ml, 500ug/ml, and 1000ug/ml. Aqueous extract of *Costus spicatus* rhizome and leaf produced a concentration dependent scavenging effect on NO radical at 7.81265µg/ml, 15.625µg/ml and 31.255µg/ml with no significant difference from that of the reference compound, BHT. The scavenging effect of the rhizome was significantly higher than the leaf and BHT at 62.5µg/ml, 125µg/ml, 250µg/ml, in a concentration dependent manner.

The Aqueous extract of *Costus spicatus* rhizome and leaf shows a minimum TAC radical scavenging activity that is relatively lower than that of the standard BHT, however in comparison to the leaf and rhizome, the TAC of the rhizome is higher than the leaf at 10.8µg/ml

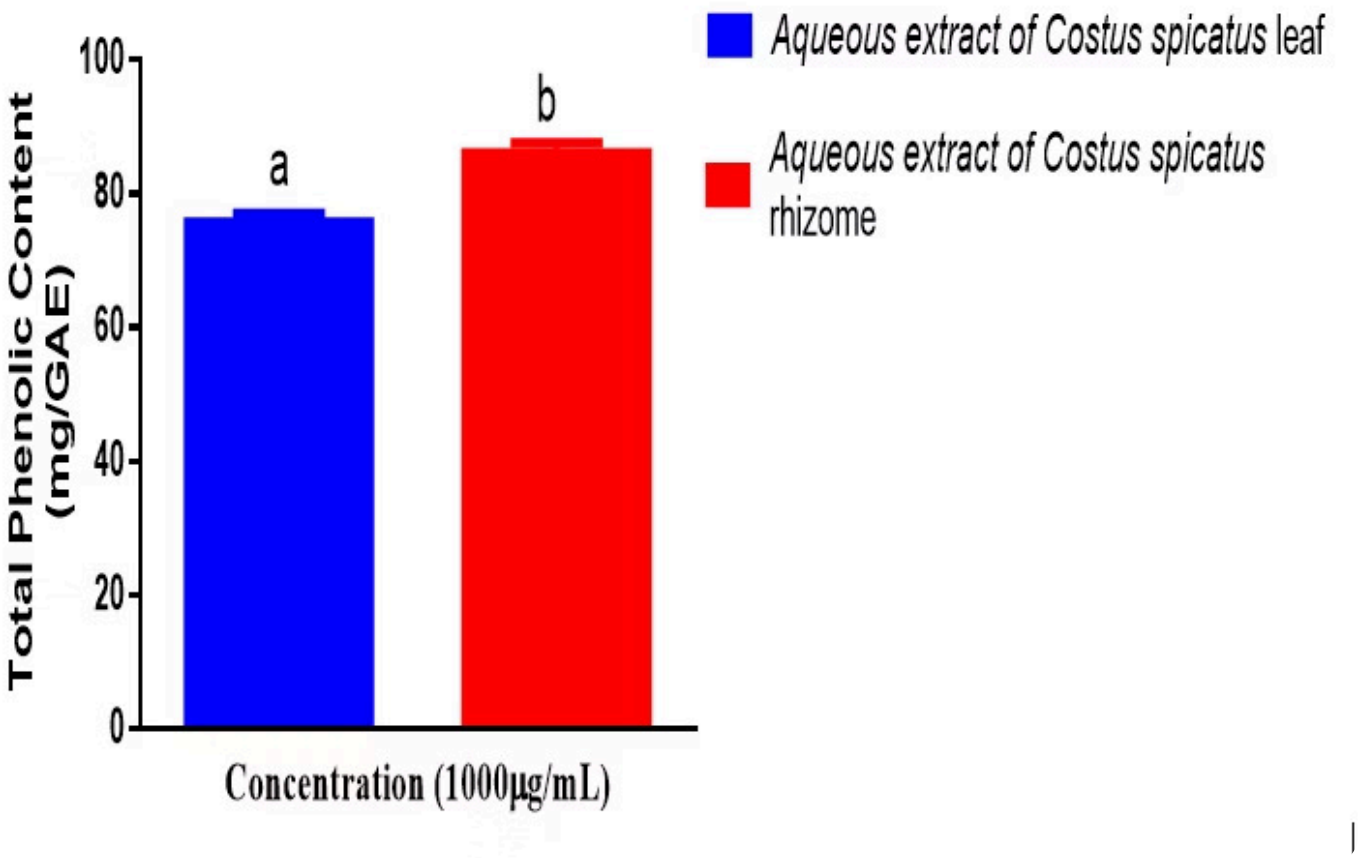

Figure 1: Total phenolics content of aqueous extract of *Costus spicatus* leaf and rhizome

Values were Means ± SEM of 3 determinations

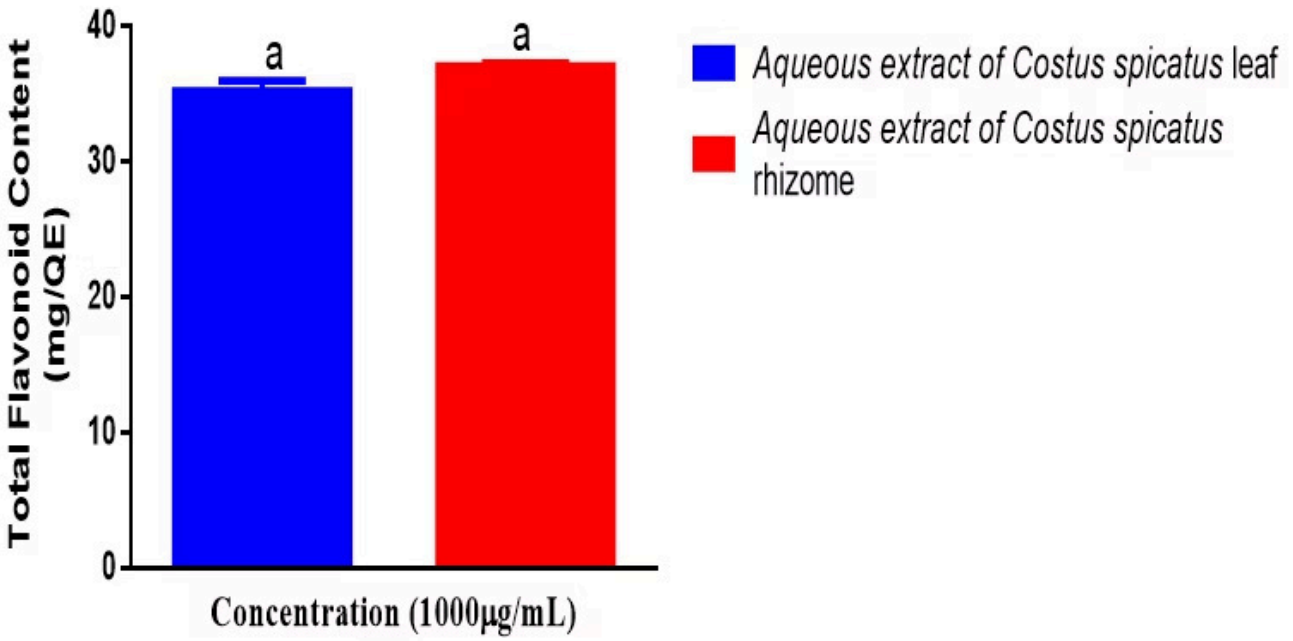

Figure 2: Total flavonoids content of aqueous extract of *Costus spicatus* leaf and rhizome

Values were Means ± SEM of 3 determinations

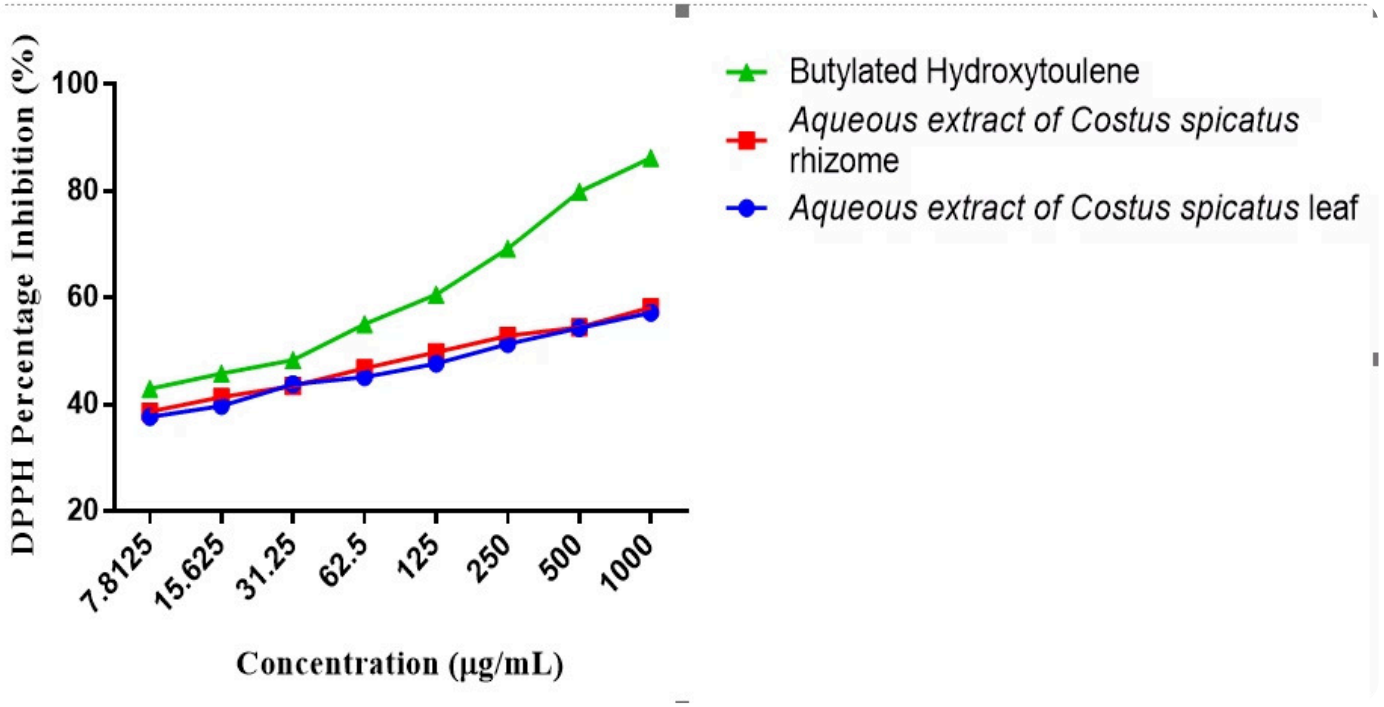

Figure 3: DPPH radical scavenging activity of aqueous extract of *Costus spicatus* leaf and rhizome

Values were Means ± SEM of 3 determinations

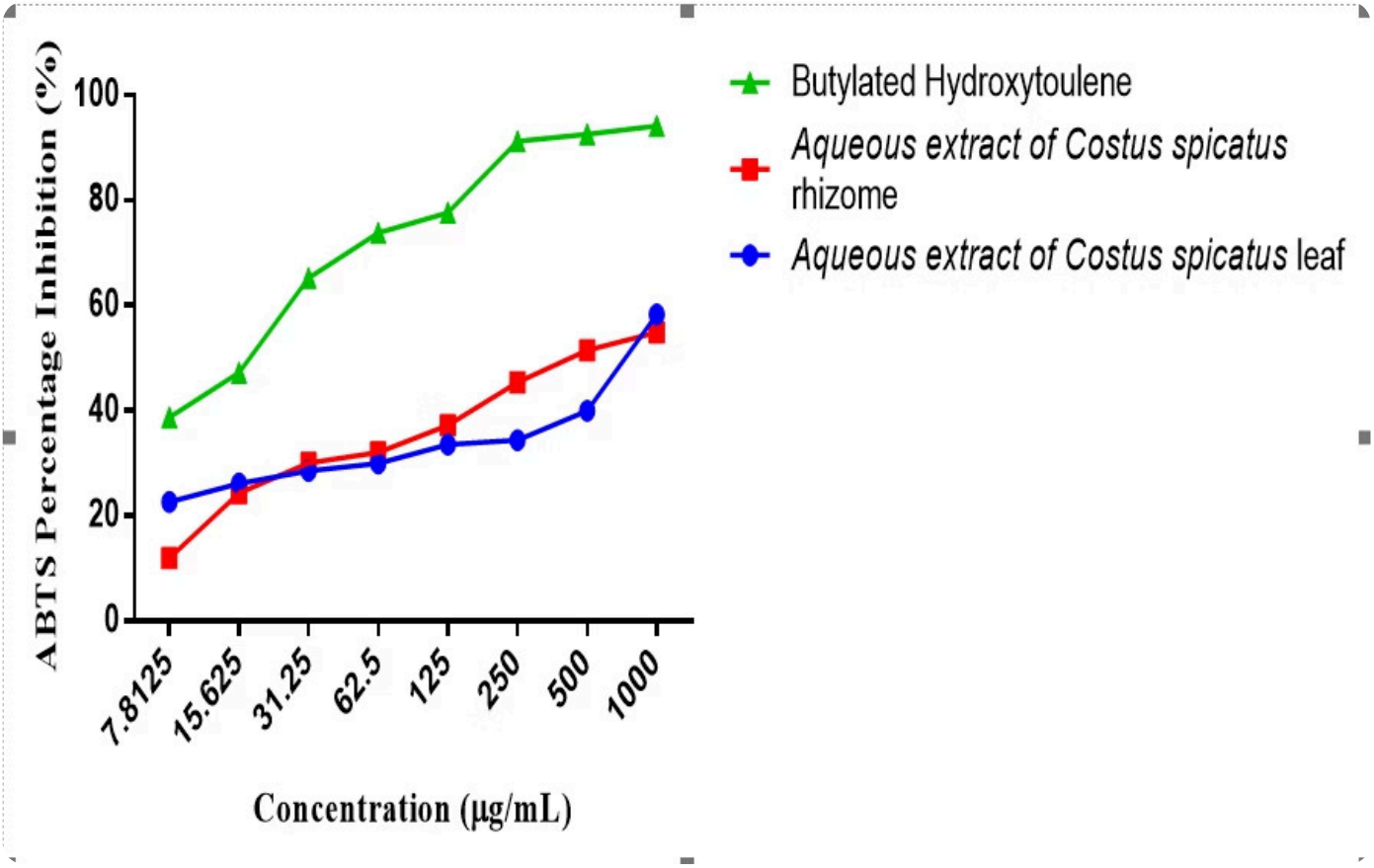

Figure 4: ABTS scavenging activity of aqueous extract of *Costus spicatus* leaf and rhizome

Values were Means ± SEM of 3 determinations

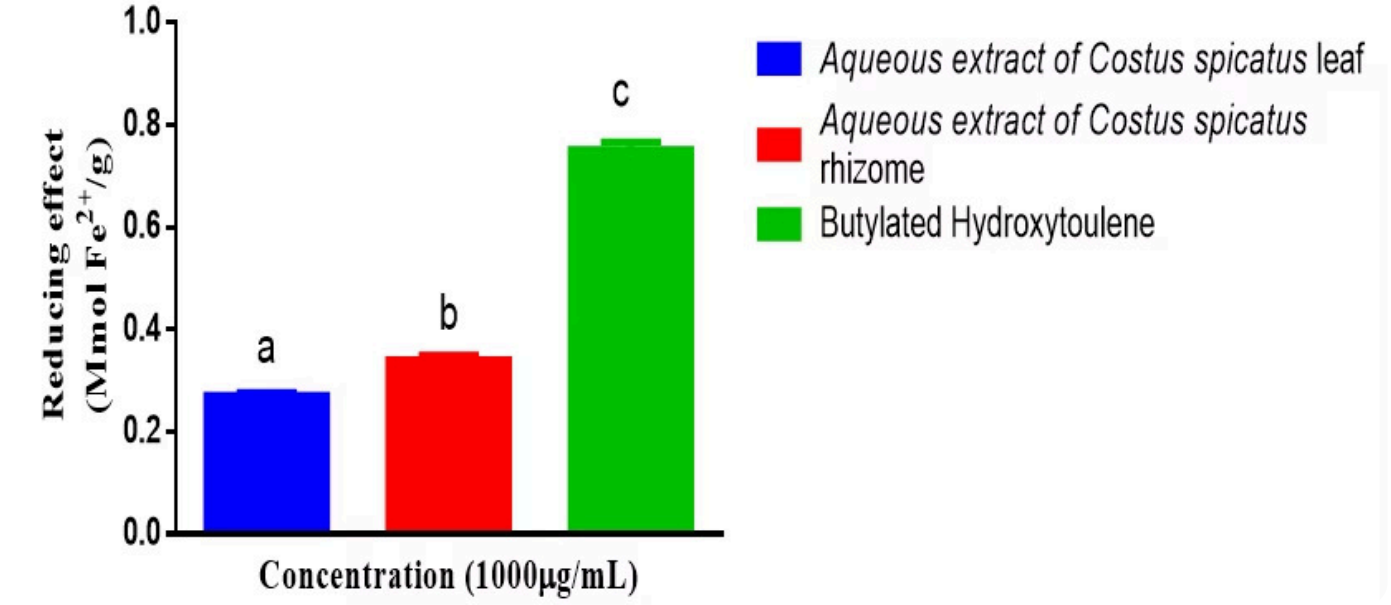

Figure 5: Ferric reducing antioxidant power activity of aqueous extract of *Costus spicatus* leaf and rhizome

Values were Means ± SEM of 3 determinations

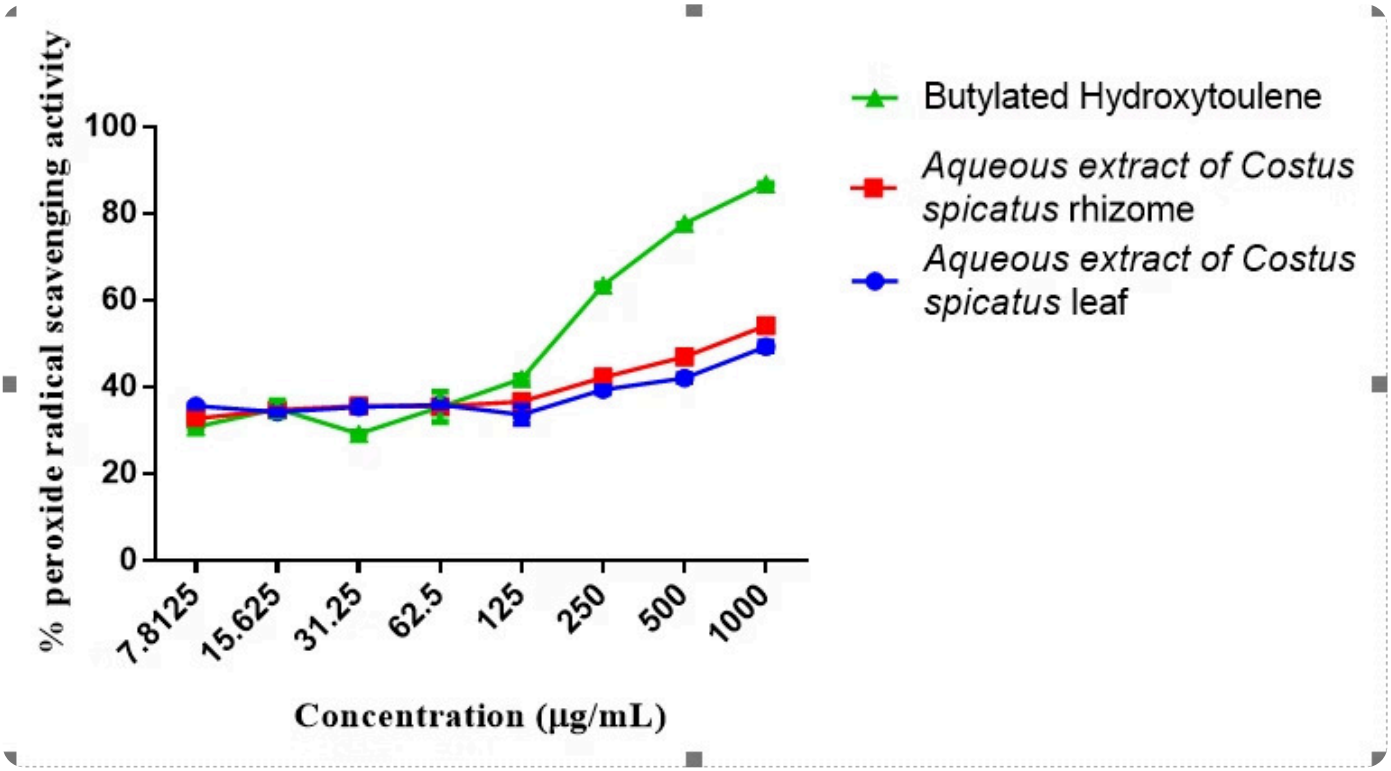

Figure 6: Peroxide radical scavenging activity of aqueous extract of *Costus spicatus* leaf and rhizome

Values were Means ± SEM of 3 determinations

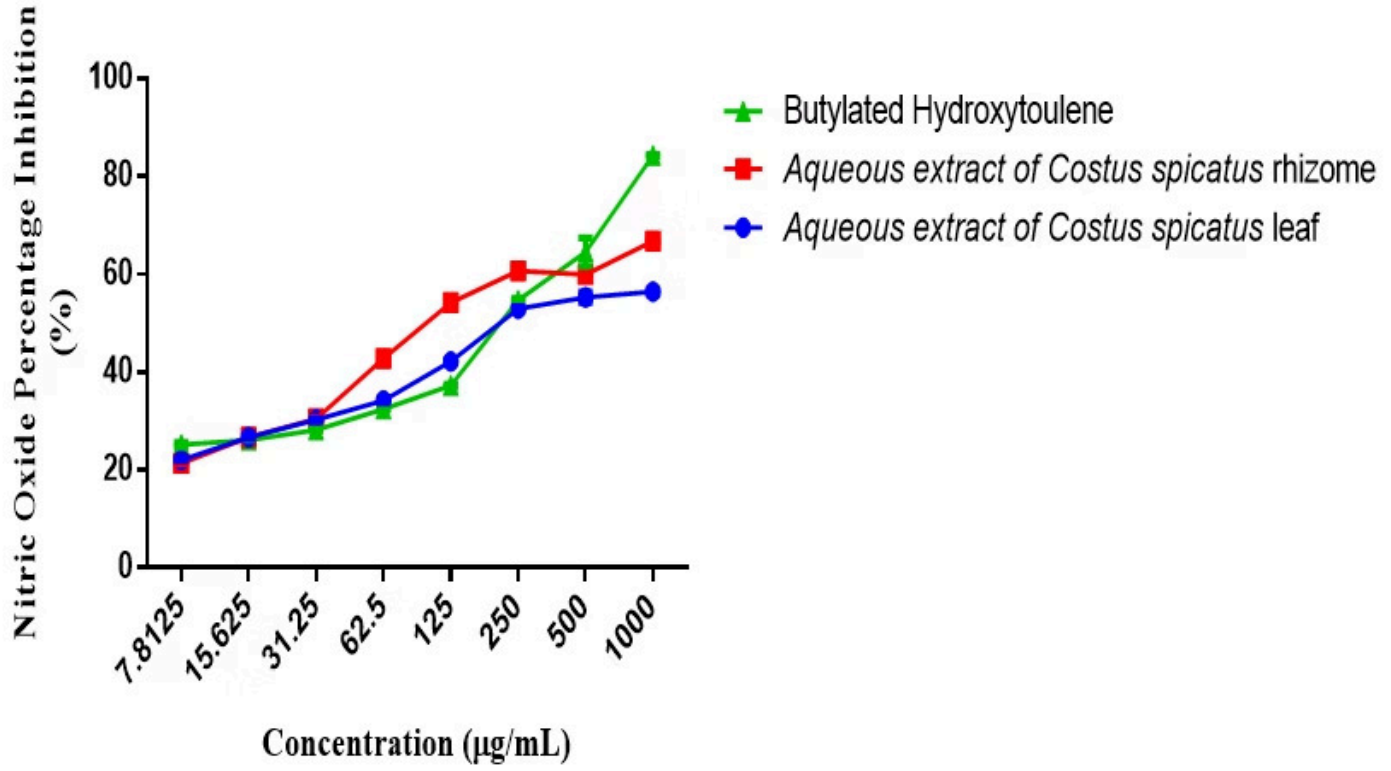

Figure 7: Nitric Oxide radical scavenging activity of aqueous extract of *Costus spicatus* leaf and rhizome

Values were Means ± SEM of 3 determinations

Figure 8: Total antioxidant capacity of aqueous extract of *Costus spicatus* leaf and rhizome

Values were Means ± SEM of 3 determinations

DISCUSSION

Although the utilization of herbs for medicinal purposes has diminished recently as a result of growing knowledge and civilization, the fact still remains that they are still used in other parts of the world. In Nigeria and other third world countries, a number of people still practice traditional medicine as a result of their perception, standard of living, and cultural background amongst other factors (Olutayo et al., 2021).

It has been affirmed scientifically that different plant parts such as roots, stems, rhizome and leaves contain phytochemicals which are the bioactive non-nutritive chemical components with a potential to reduce the risk of chronic diseases, and serve certain nutritional purposes (Sandra et al., 2018; EIke and Emanuele, 2013). Richard et al. (2021) reported that plant's mechanism of defence against micro and macro predation results from the plant's ability to synthesize to a limitless ability, aromatic compounds which are mostly secondary metabolites.

Although the human system performs antioxidants of mopping up free radicals naturally, these reactive species can also be mopped by exposure of the cell to chemical agents. Due to the presence of unpaired valence electrons, generated free radicals are very reactive and unstable, enabling them to destroy the membrane structure of cell and its constituents, thus causing diseases (Somade *et al.,* 2020).

Phytochemical Basis

Plants rich in secondary metabolites like phenolics and flavonoids exhibit antioxidant efficacy due to their redox properties and chemical structures (Naczk, 2006).

Phenolic Compounds

Abundant phenolic compounds in plants protect against UV radiation, microbial attacks, and predators (Naczk, 2006).

Antioxidant Mechanism

Phenolic compounds effectively scavenge free radicals due to their hydroxyl groups (Soobrattee *et al.*, 2005).

Polyphenols are key plant compounds with high antioxidant activity, absorbing, neutralizing, and quenching free radicals (Oyedemi *et al.,* 2010). Their ability as free radical scavengers is also attributed to their redox properties, conjugated ring structures, and carboxylic groups, which inhibit lipid peroxidation (Oyedemi *et al.,* 2010). This study compared the antioxidant activity and scavenging potential of aqueous extract of *Costus spicatus* rhizome.

Phenolic and flavonoid compounds are vital plant constituents with redox properties responsible for antioxidant activity. They achieve this by donating hydrogen atoms to free radicals or binding to transition metal ions, leading to more stable forms (Kumar and Sandhir, 2014).

Polyphenols demonstrate physiological activities like protection against neurodegenerative and cardiovascular diseases, and cancer, due to their high antioxidant capacity (WoottonBeard and Moran, 2011).

Reducing power is a significant indicator of antioxidant activity in medicinal plants (Awala and Oyetayo, 2016). The increased reducing power of ethanol extract of *S. pallescens* leaf suggests its ability to reduce iron (Fe^{2+}) to iron (Fe^{3+}), conferring antioxidant activity. However, in this study, the antioxidant capacity (reducing power) of the extract was not as high as the reference drug.

2,2'-diphenyl-1-picrylhydrazyl (DPPH), peroxide radical, ferric reducing ability, and total antioxidant capacity are widely used models for assessing *in vitro* antioxidant activity in plant extracts (Oboh and Rocha, 2007; Awala and Oyetayo, 2016). This study found that the aqueous extract of *C. spicatus* leaf and rhizome scavenged DPPH radicals, indicating proton donation and potential as free radical inhibitors or scavengers (acting as primary antioxidants). The rhizome extract of *C. spicatus* was also more effective at scavenging peroxide radicals than the leaf extract (Figure 3).

Total antioxidant capacity has also been reported to scavenge reactive oxygen specie (Pandit and Setty, 2015). The increase in the total antioxidant capacity of aqueous extract of *Costus spicatus* rhizome suggests its ability to scavenge agent of oxidative stress (Figure 8).

These *in vitro* antioxidant properties of aqueous extract of *Costus spicatus* rhizome may be due to the functional groups of phytoconstituents present in the extract as phenolics compounds have been indicated to have a synergistic effect on antioxidant capacity when they are together (Aparadh *et al*., 2012; Ibrahim *et al*., 2012; Liang and Kitts, 2014; Medini *et al*., 2014). These results suggest a probable paramount role that the polyphenol constituents of the extracts might play in free radical neutralization and lipid peroxidation inhibition (Khan *et al.,* 2013).

From the findings of Femi-Olabisi *et al*. (2021), aqueous extract of *Costus spicatus* leaves has similar phytochemical constituent to its rhizome except for the absence of phenols, terpenoid and saponin. These phenolic compounds, terpenoids and saponins possess antioxidant and antimicrobial properties (El-Aziz *et al.,* 2019; Brahmkshatriya and Brahmkshatriya, 2013). Similarly, Himajia *et al.,* 2010 reported that the rhizome of *Curcuma zedoaria* showed potential antioxidant property compared to other region of the plant as it contained terpenoids, saponins and flavonoids. This is in line with the results from this study which infers that the rhizome of aqueous extract of *Costus spicatus* may be more effective in counteracting free radical and as it shows more potential anti-oxidant activity than the leaf of aqueous extract of *C. spicatus*

1

Phytochemical Composition

Rhizome contains phenols, terpenoids and saponins absent in leaves, contributing to superior antioxidant capacity

2

Concentration-Dependent Activity

Both leaf and rhizome showed concentration-dependent antioxidant activity, with rhizome consistently outperforming leaf extracts

CONCLUSION

Both *Costus spicatus* leaf and rhizome aqueous extracts exhibit antioxidant and radical scavenging properties, protecting cells from oxidative stress. The rhizome extract demonstrates superior antioxidant capacity, suggesting its potential for phytomedicinal research and drug discovery.

01

Superior Rhizome Activity

Rhizome extract showed higher antioxidant capacity than leaf extract.

02

Concentration-Dependent Effects

Both extracts exhibited concentration-dependent antioxidant activity.

03

Phytochemical Basis

Higher phenolic content in rhizome correlated with enhanced antioxidant activity.

04

Research Applications

Further research on *C. spicatus* rhizome for antioxidant applications is warranted.

RECOMMENDATION

Further *in vivo* studies are needed to assess the antioxidant capacity and toxicity of *Costus spicatus* leaf and rhizome aqueous extracts for safe clinical application. Additionally, identification of specific bioactive constituents and *in silico* mechanistic studies are recommended to understand their role in alleviating reported diseases.

CONFLICT OF INTERESTS

There were no conflict of interests in the course of the research work.

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