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Antioxidant,chelating and phytotoxic potentials of ethanolic seeds extract of *Aframomum melegueta*

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ABSTRACT
The chelating ability of ethanolic extract of Aframomum melegueta was assessed in vitro. This showed a dose dependent increase in chelating ability in vitro The values of chelating ability for graded doses 5%, 10%, 15%, 20% and 25% (v/v) were 13.96 %, 15.24%, 19.02%, 50.98% and 68.36% respectively. The dose 25% had the highest chelating ability of 68.36%. Antioxidant activity of the extract ranged from 21.60% to 77.25%. At 10% concentration, a strong positive inhibition was observed, which was higher than the inhibition percent of the standard gallic acid used as the control. Phytotoxic activity of the extract inhibited the germination of maize and pea seeds and the growth of shoots and roots of the seedlings. The inhibition for graded doses 2%, 1%, 0.5% and 0.25% (w/v) was concentration dependent. In conclusion, the ethanolic extract of A.melegueta could be used as antioxidant, in the treatment of iron – overload disorders due to its high chelating ability and for phytoremediation

KeywordsAframomum melegueta, Chelating, Antioxidant, Phytotoxic, Allelopathic.

I. INTRODUCTION

Antioxidants are known to protect food quqlity by delaying or inhibiting free radical oxidation of fats and oils and the resulting off-odour and flavor [1]. Although, synthetic antioxidants like buthylated hydroxytoluene, and buthylated hydroxyanisole are currently being used in food industries, concern about the possible adverse effects of these antioxidants and others have been documented [2].Increasing consumers' concern over adverse effects of synthetic antioxidants have therefore stimulated research for alternative antioxidants [2]. Aframomum melegueta is a non-commercial spice which belongs to the family Zingiberaceae. In Nigeria and some other parts of west Africa, the seeds are used as a spicy and have a wide range of folkloric uses in traditional medicine. They are used as a remedy for treating stomach ache; diarrhea and snake bite [5].

Chelating of metal ions and quench of singlet oxygen are the major characteristics of antioxidant activity [3]. A major disorder associated with iron overload is thalassemia which usually results in under production of normal globin proteins, often through mutations in regulatory genes [4], [5]. Phytotoxicity is a delay of seed germination, inhibition of plant growth caused by specific substances (phytotoxins) [6]. The present study aimed at establishing the in vitro antioxidant, chelating and phytotoxic potentials of the ethanolic seed extract of Aframomum melegueta

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II. MATERIALS AND METHODS

The seeds of Aframomum melegueta was collected from a farm at Ile –Oluji, Ondo State , South-west, Nigeira. This was identified in Pure and Applied Biology Department of Ladoke Akintola University of Technology, Ogbomoso, Nigeria. 500g of pulverized seeds of Aframomum melegueta was packed and soaked into 2litres capacity flask with absolute ethanol for two weeks. This was filtered with whatman No2 filter paper. The filterate obtained was concentrated using Rotary evaporator. the extract obtained was used for subsequent preparation of test solution.

Preparation of DPPH solution (stock): 0.3mM was prepared by dissolving 0.03g of DPPH in the solvent and the volume was added up to 250ml.

Preparation of gallic acid solution (Stock): 0.2g of gallic was dissolved in 20ml of deionized water. The solution was incubated in water bath at 380C for 5mins because gallic acid was not completely soluble in water. Different concentration (grade doses) 500 – 300ug/ml of gallic acid were prepared from the stock gallic acid (10mg/ml).

Preparation of Curcumin Solution (Stock): 0.2g of curcumin was dissolved in 20ml of deionized water. The
solution was incubated in water bath at 380C for 5mins because curcumin was not completely soluble in water. Different concentrations (grade doses) (500-300ug/ml) of curcumin were prepared from the stock curcumin (10 mg/ml).

Preparation of extract solution (stock) : Test solutions were prepared by serial dilution. 2g of extract was dissolved in 100ml of distilled water to get 2% solution. 1% solution was prepared by taking 50ml of 2% and make up to 100ml with distilled water. 0.25% was prepared by taking 50ml of the 0.5% solution into 100ml of volumetric flask making it to the mark with distilled water.

Biochemical Assay: Antioxidant activity determination: The antioxidant activity of the ethanolic extract were determined according to the method of Blois (1958). 1ml of 0.3min of DPPH solution was added to 1ml each of the test solution and this was allowed to react at room temperature in the dark for 30min. the absorbance of the solution was read at 517nm in a UV/visible spectrophotometer against blank (distilled water).Gallic acid was used as standard antioxidant.

The antioxidant activity of the extract was determined as follow; % inhibition = $A_{control}$ - A_{sample} /$A_{control}$ * 100

Ao was the absorbance of control and Ai was the absorbance of the extracts [9].

Metal chelating activity: The iron chelating activity of the spice extracts was determined by method of Minotti (Minotti and Aust, 1987) with slight modification by Puntel (Puntel et al., 2005). To 0.2ml of each extracts was added 0.5ml of freshly prepared 500uM FeSO4. 0.3ml of 0.1M Tris –HC l PH 7.4) and 0.4ml of saline was added. The mixture was incubated for 5min, followed by the addition of 3 drops of0.25% 1,10-Phenanthroline (w/v) .The absorbance was measure at 510nm with spectrophotometer. The iron chelating ability was subsequently calculated with respect to the control by using the
formula: Chelating activity (%) = Acontrol - Asample /Acontrol * 100

Phytotoxic assay: In order to show phytotoxic activities of the extract solution, different concentrations of the examined solutions were prepared and applied to check germination of both root and shoot of the germinating seeds. Seeds of maize (Zea may) and pea (Pisum sativum) were collected from local market. Phytotoxicities of the extract solutions were determined on the healthy seeds of maize and pea purchased from Wazobia Market, Sabo, Ogbomoso, Southwest, Nigeria. The assay seeds were shorted for uniformity of size and all damaged seeds were discarded. The bioassay seeds were washed with tap water and the surface were sterilized using NaCl (10% v/v) for 10min, followed, by several washes in sterile distilled water [7],[6], [8]. For testing the phytotoxicity dehydrated ethanol was used as control. Bioassays were carried out using petridishes (90 mm diameter) containing cotton wool as support. Test solutions (5 ml) was added to the cotton wool in the petridish and dried completely in vacuo at 40oC. Five seeds from each category were placed on the cotton wool and incubated for 7 days at 250C in the dark using prepared solutions of 0.25%, 0.5%, 1%and 2% respectively. The effects of the examined solutions were determined by measuring the elongation of roots and shoots averaged for each concentration.. The treated seeds of maize and pea were allowed to germinate on a mat of moist cotton wool by addition of 5ml of distilled water daily for seven days. Each experiment was based on 80 seeds of each variety of plants. After seven days of incubation the length of roots and shoots of germinated seeds were measured. Treated experimental sets were compared with that of control sets where no examined solutions were used to treat the seeds. Each experiment was repeated in triplicate. All

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apparatus and materials used were sterilized where necessary.

III. RESULT

Table 1: DPPH Free Radical Scavenging Activity of A. melegueta

Concentration (mg/ml)	Inhibition of Extract (%)	Inhibition of Gallic acid (%)
5	21.60± 0.18	79.94± 0.26
10	55.63± 0.11	49.78± 0.21
15	50.30± 0.09	48.54± 0.98
20	48.30±0.94	39.56± 0.94
25	69.30±0.89	19.54± 0.12

Table 2: Chelating activity of ethanolic extract of A. melegueta.

Concentration (mg/ml)	Inhibition of Extract (%)	Inhibition of Curcumin (%)
5	13.96± 0.19	24.97 ±0.88
10	15.24 ±0.89	29.92 ±0.92
15	19.02±0.74	62.17 ±0.74
20	50.98± 0.44	77.84 ±0.68
25	68.36 ±0.09	83.26 ±0.88

Table 3: Growth Profile of Seed Culture treated with ethanolic seeds extract of A.melegueta

Extract solution (%)	Maize		Pea	
	Shoot (cm)	Root (cm)	Shoot (cm)	Root (cm)
0.25	11.10±0.94	10.00±0.07	12.13±0.10	8.93±0.79
0.50	10.03±0.55	9.00±0.07	9.25±0.04	7.27±0.70
1.00	7.46±0.26	7.70±0.34	9.13±0.10	6.96±0.80
2.00	5.97±0.27	5.32±0.33	9.82±0.32	6.22±0.37
Control	**12.82±0.07**	**15.55±0.03**	**15.37±0.96**	**5.42±0.15**

Values are expressed as Mean+ Standard deviation

Table 4: Percentage Growth Profile of Seeds Cultures treated with ethanolic seeds extract of A.melegueta

Extract solution (%)	Maize (%)		Pea (%)	
	Shoot	Root	Shoot	Root
0.25	86.58	70.74	46.19	76.17
0.50	77.04	56.14	21.08	60.97
1.00	58.19	49.52	13.66	59.39
2.00	46.57	34.21	15.09	48.69

Percentage Growth = GT/GC *100. GT = Average growth length of Root and Shoot in the examined medium, GC = Average growth length of root and shoot in the control index.

Table 5: Percentage Growth Inhibition of Seeds Cultures treated with ethanolic seeds extract of A.melegueta

Extract solution (%)	Maize (%)		Pea (%)	
	Shoot	Root	Shoot	Root
0.25	13.42	29.26	53.81	43.09
0.50	13.96	43.86	78.92	39.83
1.00	41.81	50.48	86.34	23.61
2.00	53.43	65.79	84.91	20.31

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Percentage Growth Inhibition was expressed as: 100 – GT/GC * 100.

Table 1: shows the dose dependency of DPPH radical scavenging activity of the ethanolic extract of A. melegueta with standard antioxidant. The ethanolic extract had the highest activity of 69.3% at highest concentration of 25mg/ml which is higher and better than that of the control index (gallic acid).

From Table 2, the chelating activity showed a dose dependent relationship. At highest concentration of 2% of the examined solution the extract had chelating activity of 68.36% which is less than the control index (curcumin). This result shows that ethanolic extract has a higher metal chelating activity than curcumin used as the control.The phytotoxic results in Tables 3, 4, and 5 showed that ethanolic seeds extract of A.melegueta inhibited the growth of shoot and root length of both maize and bean seedlings.This suggests that ethnolic seed extract of A. melegueta was significantly phytotoxic.

IV. DISCUSSION

This study reports antioxidant activities of Aframomum melegueta. Spices have been acknowledged not only to have properties that make food more pleasant but also important preservative and antioxidant properties[11].The relationship between total phenol contents and antioxidant activity has been widely studied in different food stuffs [12] . Antioxidant activity of food stuff significantly increases with the presence of high concentration of total phenol and flavonoid contents.

The observed scavenging ability of the extracts of A. melegueta against stable DPPH followed a dose-dependent pattern, with the highest activity observed at the highest concentration. The n- hexane extract has the highest antioxidant and chelating activities of the three extracts examined. This suggests that the extract possessed high content of polyphenol and flavonoid. This is an indication that A. melegueta is a potential source of dietary antioxidant that can be used in the prevention and management of various ROS-related ailments such as Parkinson's and Alzheimer's diseases.

Chelation property may afford protection against oxidative damage and iron - overload [13]. Chelating ability of the extract provide a strategy to avoid free - radical generation and iron - overload by chelation of the metal ion [11]. A. melegueta ethanolic extracts was a good chelator for iron removal at in vitro condition. The phytotoxic activity of the extract is in no doubt due to phytochemical constituents present in it. Aframomum melegueta seeds are rich in phytonutrient such as flavonoids, phenols, tannins, saponins, terpenoids, cardiac glycosides and alkaloids [8] [6]. Saponins are known to have inhibitory effect on plant growth hormones like auxins and gibberellins, which have also contributed to the inhibitory effectiveness of A. melegueta seeds [7], [12].

REFERENCES

[1]. Blois, M.S (1958). Antioxidant Determination by the use of a stable free radical. Nature 26: 199-120.

[2]. Fasoyiro, S.B, Adegoke, G.O and Idowu, O . O (2006). Characterisation and Partial Purification of Antioxidant Componemt of ethereal fraction of Aframomum danielli. Wolrd Journal of Chemistry 1(1):1-5

[3]. Maggio , D, Amico G, Morabito A, Capra M, Ciacco C. Cianciulli P.Gregorio FD, Garozzo G, Malizia R, Magnapo C, Mangiagli A, Quarta G, Rizzzo M, D'Asiola DG, Rizzo A, Midiri,(2002).Deferifone versus deferoxamine in patients with thalassemia major: a randomized clinical trial. Blood Cells Mols Dis 281(2), 196-208.

[4]. Ebrahimzadeh, M.A, Navavi, .S.M (2009). Correlation between the in vitro Iron chelating activity and Polyphenol and Flavonoid contents of some Medicinal Plants. J. Biological Science. 12(12), 934-938

[5]. Tajuddin, Z., S.S. Shaukat and I.A. Siddiqui (2002). Allelopathic potential solanum forshaki Dunal: A Tropical Weed. Pak. J. Biol. Sci., 5: 866-863.

[6]. Shaukat, S,S and Siddiqui, I.A. (2002). Allelopathic and antifungal potential of Lantana camara root leachates in soil. Pak.J. Biol .Sci., 5: 51-53

[7]. Rajbanshi, S.S and K. Inubushi, (1997). Chemical and biochemical changes during laboratory scale compostingof allelopathic plant leaves (Eupatorium adenophorum and Lantana camara). Weed Sci., 26: 66.

[8]. Mirzaei, A.and Raheleh Khatami (2013). Antioxidant and Iron Chelating Activity of Coriander sativum and Petroselinum crispum,, Bull.Env.Pharmacol.Life Sci., 2 (7), 27-31

[9]. Bakchiche Boulanouar, Gherib Abdelaziz, Small Aazza , Custodia Gago , M. Graca Miguel (2013).Antioxidant activities of eight Algerian Plant extracts and two essential Oils. Industrial Crops and Products 46: 85-96.

[10]. Robak, J, Marankiewiez, R (1995). Scavenging of reactive oxygen species as the mechanism of drug action. Polish J of Pharmacol 47: 89-98

[11]. Siddiqui, I .A., S.S. Shaukat, G.H. Khan and M.J. Zaki (2002). Evaluation of Argemone mexicana for the suppression of root-investing fungi in tomato. J. Phytopath., 150: 321-329. .

[12]. Minnoti G, Aust S.D, (1987). An investigation into the mechanism of citrate-Fe^{2+}dependent lipid peroxidation Free Rad .Biol Med 3(3), 79-387.