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In Vitro Antioxidant Activity of Aqueous and Ethanol Extracts of Cucumis Sativus

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Abstract

The aim of this study was to evaluate the in vitro antioxidant activities of aqueous and ethanol extracts of Cucumis sativus. Shade-dried C. sativus fruits were extracted using distilled water or absolute ethanol and then subjected to in vitro antioxidant analyses using standard methods. The results showed that the 1, 1–diphenyl–2–picrylhydrazyl (DPPH) radical scavenging activity, nitric oxide (NO) scavenging capacity and thiobarbituric acid reactive substances (TBARS) of the ethanol extract were significantly higher than those of aqueous extract ($p < 0.05$). However, reducing power, ferric reducing antioxidant potential (FRAP) and total antioxidant capacity (TAC) of the aqueous extract were significantly higher than those of ethanol extract ($p < 0.05$). These results indicate that extracts of the medicinal plant are effective in scavenging free radicals caused by oxidative stress, and the potency depends on the solvent used for extraction.

Keywords: Antioxidants, Cucumis Sativus, Free Radical, Lipid Peroxidation, Membrane Damage.

Introduction

People in developing countries rely heavily on herbal products for the maintenance of their health [1-3]. The search for novel natural compounds with potent biological effects has remarkably increased over the years [4, 5]. Plant materials used as medicine (crude drug) have the added advantage of being readily available, effective, and offers a broad spectrum of biological and pharmacological activity. These plants are known to be reservoirs of numerous phytochemicals [6-9]. Many of the phytochemicals function as antioxidants [10]. Antioxidants help prevent tissue damage by neutralizing the deleterious effects of free radicals. They act as scavengers. Antioxidants of nutritional origin play key roles in complementing in vivo antioxidant enzymes and molecules in the fight against free radicals. They donate electron (hydrogen ion) to the highly unstable free radicals [11].

Cucumis sativus is a vegetable crop, belonging to the family Cucurbitaceae [12]. Cucumbers are botanically categorized as berries, which are available in many different sizes, shapes and colors. The parts of this medicinal plant which are traditionally used are leaves, flowers, seeds, fruits, and bark. These parts contain bioactive compounds responsible for particular pharmacological activity [13]. Cucumis sativus is used in traditional medicine for the treatment of various ailments [14, 13]. The aim of this study was to evaluate the in vitro antioxidant activities of aqueous and ethanol extracts of Cucumis sativus.

Materials and Methods

Chemicals

Ethanol, methanol, sodium acetate, sodium carbonate, ferric chloride (FeCl3), trichloroacetic acid (TCA) and hydrochloric acid (HCl) were bought from Bell, Sons & Co. (England). Sulphuric acid (H2SO4), sodium nitropruside, sodium phosphate, ammonium molybdate and ascorbic acid were obtained from British Drug House (BDH) Chemicals Ltd. (England), while Griess reagent, sodium dodecylsulphate (SDS), DPPH, potassium ferricyanide and ferric tripyridyl triazine (Fe3+-TPTZ) were products of Thermo Fisher Scientific Ltd. (USA). All the chemicals and solvents used in this study were of analytical grade.

Collection of Plant Material

The fruits of Cucumis sativus were obtained from a major market in Benin City, Nigeria and authenticated at the herbarium of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria, by Dr. Henry Akinnibosun.

Preparation of Plant Extract

The fruits were washed and shade-dried at room temperature for a period of two weeks and then pulverized using a blender. Aqueous and ethanol extracts of the plant were obtained using

cold maceration method as described in literature [15].

Determination of DPPH Radical Scavenging Activity of Plant Extracts

The free radical scavenging capacity of the plant extracts against DPPH free radical was determined by a slightly modified method of Brand-Williams et al. [16]. Briefly, 0.5 mL of 0.3 mM DPPH solution in methanol was added to 2 mL of various concentrations of the extracts (0.2 - 1.0 mg/mL). The test tubes were shaken and incubated in the dark for 15 min at room temperature, and the absorbance was read at 517 nm. All tests were performed in triplicate. Ascorbic acid (vitamin C) was used as control, with similar concentrations as the test samples. A blank containing 0.5 mL of 0.3 mM DPPH and 2 mL methanol was prepared and treated as the test samples. The radical scavenging activity was calculated as shown:

$$\text{DPPH Radical Scavenging Activity (\%)} = \frac{A_o - A_1}{A_o} \times 100$$

where Ao was the absorbance of DPPH radical + methanol; A1 was the absorbance of DPPH radical + sample extract or standard.

Reducing Power Assay

The reducing power (RP) of extracts was determined according to the method described by Lai et al. [17]. Briefly, 1 mL of different concentrations of extracts (0.1- 1.0 mg/mL) in water was mixed with 2.5 mL of 0.2 M phosphate buffer, pH 6.6 and 2.5 mL of 1 % potassium ferricyanide. The mixture was incubated at 50oC for 20 min. Thereafter, 2.5 mL of trichloroacetic acid (10 %) was added to the mixture to stop the reaction. Distilled water (2.5 mL) and 0.5 mL of 0.1 % FeCl3 were then added and the absorbance was read at 700 nm. High absorbance values indicated higher reducing power. Ascorbic acid served as the control.

Ferric Reducing Antioxidant Power (FRAP) of the Extracts

A modified method of Benzie and Strain was used for the FRAP assay [18]. The principle behind this assay is the ability of the sample to reduce ferric tripyridyl triazine (Fe (III)- TPTZ) complex to ferrous tripyridyl triazine (Fe (II) - TPTZ), which at low pH produces an intense blue color that can be read at 593 nm. Briefly, 1.5 mL of freshly prepared FRAP solution (25 mL of 300 mM acetate buffer pH 3.6, 2.5 mL of 10 mM 2,4,6-tripyridylstriazine (TPTZ) in 40 mM HCl, and 2.5 mL of 20 mM ferric chloride ($FeCl_3 \cdot 6H_2O$) solution) was mixed with 1 mL of varied concentrations of the extracts (0.2 - 1.0 mg/mL). The reaction mixtures were incubated at 37oC for 30 min and the absorbance was read at 593 nm. Ascorbic acid served as the control, while $FeSO^4$ was used for calibration and values expressed as mmol FeSO4 equivalents per gram of sample.

Total Antioxidant Capacity (TAC) Determination

The TAC of the extracts was evaluated using the phosphomolybdenum method based on the procedure described by Prieto et al. [19]. The assay is based on the reduction of Mo (+6) to Mo (+5) by the extracts and subsequent formation of green phosphate Mo (+5) complex at acidic pH. Briefly, 0.3 mL of graded concentrations of extracts was mixed with 3 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The absorbance of the reaction mixture was measured at 695 nm using a spectrophotometer against a blank after cooling to room temperature. Methanol (0.3 mL) in the place of extract was used as the blank. The TAC was expressed as milligram equivalents of ascorbic acid and calculated as shown:

$$\text{TAC (mg AAE/g extract)} = \frac{C \times V}{m}$$

where c = concentration of ascorbic acid in mg/mL extrapolated from the standard calibration curve; V = volume of extract in mL; and m = weight of crude plant extract in grams.

Nitric Oxide Radical Scavenging Capacity

The method described by Makhija et al. was used [20]. Briefly, 1 mL of 10 mM sodium nitroprusside was mixed with 1 mL of extract prepared in phosphate buffer. The mixture was incubated at 25 °C for 150 min. To 1 mL of the incubated solution, 1 mL of Griess' reagent was added. Then, the absorbance was read at 546 nm.

$$\text{Nitric Oxide Scavenging Activity (\%)} = \frac{A_{control} - A_{extract}}{A_{control}} \times 100$$

Estimation of Thiobarbituric Acid Reactive Substances (TBARS)

Thiobarbituric acid reactive substances (TBARS) was estimated according to the method described by Ohkawa et al. [21]. Egg yolk homogenate (0.5 mL of 10 % v/v) and 0.1 mL of extract were mixed in a test tube, and made up to 1 mL with distilled water. Then, 50 µL of FeSO4 (0.07 M) was added to induce lipid peroxidation and incubated for 30 min. This was followed by the addition of 1.5 mL of 0.8 % TBA in 1.1 % sodium dodecyl sulphate (SDS) and 50 µL of 20 % TCA and vortexed. The resultant mixture was heated at 95 oC for 60 min. The absorbance of the sample was read at 532 nm. Inhibition of lipid peroxidation (%) was calculated as shown:

$$\text{Inhibition of lipid peroxidation (\%)} = \frac{A_{control} - A_{extract}}{A_{control}} \times 100$$

Statistical Analysis

Data are presented as mean ± SEM. Statistical analysis was performed using SPSS version 21.0. Statistical significance was assumed at $p < 0.05$.

Results

In Vitro Antioxidant Activities of Extracts of C. sativus

The DPPH radical scavenging activity, NO scavenging capacity and TBARS of the ethanol extract were significantly higher than those of aqueous extract ($p < 0.05$). However, reducing power, FRAP and total antioxidant capacity (TAC) of the aqueous extract were significantly higher than those of ethanol extract ($p < 0.05$). These results are shown in Tables 1 to 5 and Figure 1.

Table 1: DPPH Radical Scavenging Activity of Extracts of C. sativus

Concentration Of Extract (mg/mL)	Inhibition (%)		
	Aqueous	Ethanol	Ascorbic Acid
0.20	65.48 ± 1.35	91.63 ± 0.35	88.80 ± 3.72
0.40	46.66 ± 5.47	91.70 ± 0.36	88.57 ± 3.84
0.60	31.28 ± 2.26	91.94 ± 0.20	93.20 ± 0.18
0.80	20.83 ± 2.05	88.19 ± 1.84	90.58 ± 1.17
1.00	16.58 ± 1.50	89.22 ± 0.56	93.54 ± 1.33

Data are DPPH radical scavenging activity and are expressed as mean ± SEM (n = 3).

Table 2: NO Radical Scavenging Activity of Extracts of C. sativus

Concentration Of Extract (mg/mL)	NO Scavenged (%)		
	Aqueous	Ethanol	Ascorbic Acid
0.20	48.03 ± 0.00	87.90 ± 0.85	71.67 ± 0.50
0.40	43.53 ± 0.00	83.12 ± 1.51	-
0.60	57.13 ± 13.98	75.86 ± 2.38	75.42 ± 0.94
0.80	59.50 ± 0.00	67.29 ± 1.82	-
1.00	55.35 ± 0.94	55.82 ± 0.00	73.92 ± 1.73

Data are percentage NO scavenged in vitro and are expressed as mean ± SEM (n = 3).

Table 3: Effect of Extracts of C. sativus on TBARS

Concentration Of Extract (mg/mL)	Inhibition of Lipid Peroxidation (%)		
	Aqueous	Ethanol	Ascorbic Acid
0.10	22.34 ± 0.00	49.14 ± 10.65	43.47 ± 2.92
0.50	18.90 ± 7.56	43.30 ± 7.90	36.77 ± 1.03
1.00	16.67 ± 2.92	40.55 ± 0.00	40.43 ± 13.76

Data are percentage inhibition of lipid peroxidation and are expressed as mean ± SEM (n = 3).

Table 4: Reducing Power of Extracts of C. sativus

Concentration Of Extract (mg/mL)	Absorbance at 700 nm		
	Aqueous	Ethanol	Ascorbic Acid
0.20	0.605 ± 0.145	0.305 ± 0.005	0.823 ± 0.266
0.40	0.729 ± 0.196	0.355 ± 0.095	1.047 ± 0.391
0.60	0.873 ± 0.191	0.355 ± 0.085	1.210 ± 0.263
0.80	0.900 ± 0.105	0.340 ± 0.000	1.220 ± 0.010
1.00	1.287 ± 0.210	0.310 ± 0.000	1.115 ± 0.315

Data are reducing power of extracts of C. sativus and are expressed as mean ± SEM (n = 3).

Table 5: Ferric Reducing Antioxidant Potential (FRAP) of Extracts of C. sativus

Concentration Of Extract (mg/mL)	Absorbance at 593 nm		
	Aqueous	Ethanol	Ascorbic Acid
0.20	0.613 ± 0.032	0.157 ± 0.000	0.420 ± 0.000
0.40	1.057 ± 0.039	0.170 ± 0.030	0.610 ± 0.000
0.60	1.360 ± 0.029	0.195 ± 0.045	0.500 ± 0.000
0.80	1.530 ± 0.050	0.250 ± 0.020	0.560 ± 0.000
1.00	1.607 ± 0.037	0.320 ± 0.030	0.470 ± 0.000

Data are FRAP of extracts of C. sativus and are expressed as mean ± SEM (n = 3).

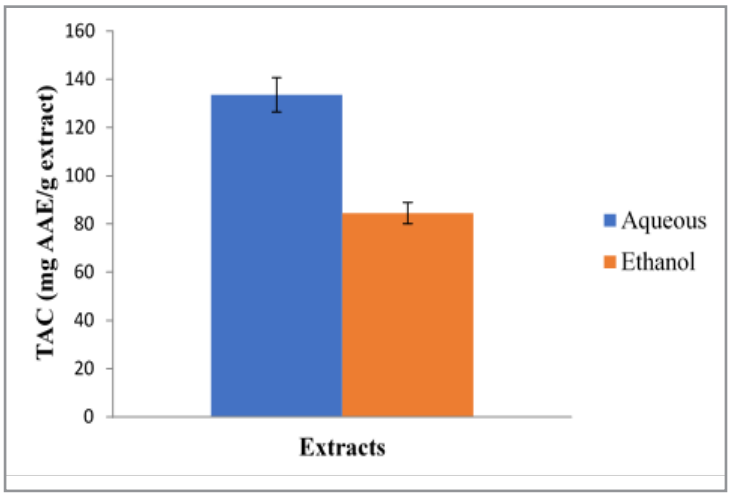

Figure 1: Total Antioxidant Capacity of Extracts of C. sativus

Discussion

Free radicals are constantly formed in living cells and removed by antioxidant defenses. Antioxidant enzymes are the main line of defense against free radicals in animal and plant cells [8, 9]. When cells are exposed to oxidative stress a defense system ensures the expression and regulation of antioxidant enzymes as a defense mechanism to protect them from the damaging effect of free radicals. Antioxidant enzymes are capable of stabilizing, or deactivating free radicals before they attack cellular components [22]. They act by reducing the energy of the free radical or by giving up some of their electrons for its use, thereby causing it to become stable. In addition, they may also interrupt the oxidizing chain reaction to minimize the damage caused by free radicals. It has been reported that a substantial link exist between free radicals and more than sixty different health conditions, including aging, cancer, diabetes mellitus, Alzheimer's disease, strokes, heart attacks and atherosclerosis. By reducing exposure to free radicals and increasing the intake of antioxidant enzyme-rich foods or antioxidant enzyme supplements, the body's potential to reduce the risk of free radical-related health problems is made more palpable [23].

Synthetic free radical scavengers such as butylhydroxyanisole (BHA) and butylhydroxytoulene (BHT) exist, but concerns over possible side effects necessitate the continued screening of natural plant parts for potential antioxidant properties. Antioxidants help prevent tissue damage by neutralizing the deleterious effects of free radicals. They act as scavengers. Antioxidants of nutritional origin play crucial roles in complementing in vivo antioxidant enzymes and molecules in the fight against free radicals. The DPPH radical can accept an electron or hydrogen ion to become a stable molecule [23]. Scavenging of DPPH radical is a widely used method for assessing the free radical scavenging ability of plant or chemical materials [24].

The DPPH method is rapid, sensitive, and reproducible and requires simple conventional laboratory equipment for the evaluation of antioxidant activity of samples [23]. Phenols and flavonoids represent phytochemicals which relative abundance in plant extracts has been linked to antioxidant effect [25, 26]. Reactive nitrogen species (RNS) are free radicals derived from the interaction of NO with oxygen or reactive oxygen species (ROS) [27]. Nitric oxide is classified as a free radical because of its unpaired electron and displays important reactivity with certain types of proteins and other free radicals such as superoxide anion [28]. It is synthesized by three isoforms of the enzyme nitric oxide synthase (NOS): endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS). Nitric oxide (NO) is generated from amino acid L-arginine by enzymes in the vascular endothelial cells, certain neuronal cells, and phagocytes [29]. Low concentrations of NO are sufficient in most cases to effect the physiological functions of the radical. It is a diffusible free radical that plays many roles as an effector molecule in diverse biological systems including neuronal messenger, vasodilatation, and antimicrobial and antitumor activities [30]. Chronic exposure to NO radical is associated with various carcinomas and inflammatory conditions such as juvenile diabetes, multiple sclerosis, arthritis, and ulcerative colitis. The toxicity of NO increases greatly when it reacts with superoxide radical, forming the highly reactive peroxynitrite anion ($ONOO^-$) [28]. Nitric oxide has been shown to be directly scavenged by flavonoids [31]. Due to the reactivity of thiobarbituric acid (TBA) with several reactive substances in a biological sample, a more widely accepted terminology called TBARS is now commonly used [32]. Thiobarbituric acid reactive substances (TBARS) is now considered as a standard marker for lipid peroxidation-induced oxidative stress [33].

Phenolic compounds are antioxidant agents which act as free radical terminators. The antioxidant potential of phenols is believed to be conferred on them by their hydroxyl group (-OH), which is bonded directly to an aromatic hydrocarbon (phenyl) ring. This makes them donate electrons easily to electron-seeking free radicals, thus down-regulating their menace in living cells [34]. Studies have revealed a direct relationship between total phenol content and antioxidant effect in different plants. High phenol-containing plant materials have high radical scavenging abilities [25, 35, 36]. Half-maximum inhibitory concentration (IC50) is the amount of antioxidant required to reduce the concentration of DPPH radical by 50 %. It is inversely proportional to antioxidant potential and hence a lower IC50 corresponds to higher antioxidant potential [37]. The results of this study indicate that the DPPH radical scavenging activity, NO scavenging capacity and TBARS of the ethanol extract were

significantly higher than those of aqueous extract. However, reducing power, FRAP and TAC of the aqueous extract were significantly higher than those of ethanol extract. These results are consistent with those of previous studies [38-40]. In a study carried out by Osama et al., it was reported that aqueous extracts from three sea cucumber species showed higher antioxidant effects than organic extracts, and it was concluded that most of the antioxidant compounds were hydrophilic [40]. In one study, it was suggested that the presence of flavonoids and tannins in C. sativus extract was responsible for free radical scavenging and analgesic effects of the extract [41]. Plants rich in polyphenols have been demonstrated to have high in vitro antioxidant activity [4, 42-52].

Conclusion

The results of this study indicate that extracts of C. sativus are effective in scavenging free radicals caused by oxidative stress, and the potency depends on the solvent used for extraction.

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