

SCREENING OF ANTIOXIDANT ACTIVITY AND PHENOLIC CONTENT IN *CUCUMIS SATIVUS* L. SEEDS

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Summary

Antioxidants are vital substances which possess the ability to protect the body from damages caused by free radical-induced oxidative stress. A variety of free radical scavenging antioxidants is found in dietary sources like fruits, vegetables and tea. The purpose of this study was to evaluate the antioxidant activity of methanolic extracts of cucumber seeds (*Cucumis sativus* L., *Cucurbitaceae*), which are used by common Indian people as folk remedies and/or food supplements. The antioxidant activity was evaluated against linoleic acid peroxidation using 1,3-diethyl-2-thiobarbituric acid as reagent. At the same time the phenolic content of the extracts was determined using Folin-Ciocalteu reagent to evaluate their contribution to total antioxidant activity. The antioxidant activity (expressed as IC₅₀) was in the concentration of 1.25 µg/ml and phenolic contents was 27.79 (mg/100 gm dry). The results of this study showed that there is no significant correlation between antioxidant activity and phenolic content of the studied plant material and phenolic content could not be a good indicator of antioxidant capacity.

Keywords: Antioxidant, *Cucumis sativus* L, Phenolic contents.

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Introduction

It has been established that oxidative stress is among the major causative factors in induction of many chronic and degenerative diseases including atherosclerosis, diabetes mellitus, cancer, Parkinson's disease and immune dysfunction and is involved in aging¹⁻³. Antioxidants, both exogenous and endogenous, whether synthetic or natural, can be effective in prevention of the free radical formation by scavenging or promotion of their decomposition and suppression of such disorders^{1,4}. There is growing interest toward natural antioxidants from herbal sources⁵⁻⁷. Epidemiological and *in vitro* studies on medicinal plants and vegetables strongly have supported the idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems⁸⁻¹⁰. Phenolic compounds with antioxidant activity, which are widely distributed in many fruits, vegetables, and tea are believed to account mainly for the antioxidant capacity of many plants¹¹⁻¹³. Therefore, the objective of this study was to evaluate the antioxidant activity of methanolic extracts of cucumber seeds (*Cucumis sativus L.*, *Cucurbitaceae*), which are used by common Indian people as folk remedies and/or food supplements. At the same time, phenolic content of the same plant was determined to evaluate their probable contribution to the total antioxidant capacity.

Materials And Methods

Plant material

Cucumber (*Cucumis sativus L.*, *Cucurbitaceae*), were purchased from the local herbal market in Bangalore. The plant materials (seeds) were cleaned, washed, dried and carefully powdered. All samples were kept in tightened light-protected containers.

Chemicals

Linoleic acid, gallic acid and Folin-Ciocalteu reagent were obtained from Merck (Darmstadt, Germany). 1, 3-Diethy-2-thiobarbituric acid (DETBA) was purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). Alpha-tocopherol, sodium dodecyl sulfate (SDS) and butylated hydroxytoluene (BHT) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and solvents were analytical grades.

Extraction

A quantity (50 g) of each powdered plant material (seeds) was soaked in 150 mL of methanol at room temperature overnight. The solvents were decanted and residues macerated two more days with the same solvent. The pooled solvents were combined and filtered. The filtrates were concentrated under reduced pressure and yields of extract were calculated.

Determination of antioxidant activity

The antioxidant activity of Cucumber (*Cucumis sativus L.*, *Cucurbitaceae*), extracts against peroxidation of linoleic acid was determined by the reported method¹⁶. Alpha-tocopherol was used as reference compound. For a typical assay an aliquot of 20 μ l of three dilutions of each extract in ethanol (0.002, 0.02 and 0.2 mg/ml) and 20 μ l of 2 mg/ml linoleic acid in ethanol were used. A spectrofluorimeter (Model RF-5000, Shimadzu, Kyoto, Japan) at an excitation wavelength of 515 nm and an emission wavelength of 555 nm was used for measurements and the antioxidant activity was calculated as the percent of peroxidation inhibition. All extracts and reference substance were assayed in triplicates and averages of results were calculated. A percent inhibition versus log concentration curve was plotted and the concentration of sample required for 50 % inhibition was determined and expressed as IC50 value.

Determination of phenolic content

The phenolic contents were determined according to the described method¹⁷, using the Folin-Ciocalteu reagent and a Shimadzu spectrophotometer (Model UV-160A, Kyoto, Japan) at 725 nm. Aliquots of 100 µl of each diluted extract (20 mg/ml in ethanol) were used for measurements. Phenolic contents of the samples were calculated on the basis of the standard curve for gallic acid. The results were expressed as milligrams of gallic acid equivalents per 100 g of the dry weight of the plant materials (seeds).

Results***Antioxidant activity***

The characteristics of the used cucumber and the inhibitory effects of methanolic extracts on linoleic acid peroxidation, expressed as IC₅₀. Considering the large variation of IC₅₀ values, 1.25 µg/ml was found in cucumber. The potential of antioxidant activity of cucumber was divided into 3 groups: high (IC₅₀<20 µg/ml), moderate (20 µg/ml <IC₅₀<75 µg/ml) and low (IC₅₀>75 µg/ml).

Phenolic content

The phenolic contents were also categorized into three groups: high (> 300 mg), moderate (100-300 mg) and low (< 100 mg).

Relationship between phenolic content and antioxidant activity

Attempts to correlate the level of phenolic content of cucumber seeds with their antioxidant activity were not successful. No significant correlation (R²=0.04) was observed between phenolic content and IC₅₀ values when it was included in the calculation.

Discussion

In this study cucumber seeds, which are used traditionally in India for various disorders were studied for their antioxidant activity and phenolic content. Low antioxidant activity is reported for cucumber⁸, the cucumber seed showed an exceptional antioxidant activity (IC₅₀= 1.25 µg/ml), which was about ten times higher than α-tocopherol (IC₅₀=15.00 µg/ml). The seed of this plant has been used as a favorite nutritive, emollient and as infusion for typhoid in folk remedies in India due to its cold temperament¹⁸. Findings of this study showed that no reasonable relationship could be found between antioxidant activity and phenolic content. The exceptional high antioxidant activity of specimens like cucumber with low phenolic content may be attributed to some individual phenolic units with special high antioxidant activity or some other constituents. Non phenolic compounds of the plants such as trace elements may also decrease the antioxidant activity of the phenolic compounds¹². Thus the measurement of phenolic content could not be a good indicator of the antioxidant capacity.

In conclusion, the findings of this study support this view that cucumber seeds were promising sources of potential antioxidants and may be efficient as preventive agents in the pathogenesis of some diseases. However, the strength of the existing data is not enough to suggest a reasonable mode of action for antioxidant effects. The data of this study may just enrich the existing comprehensive data of antioxidant activity of cucumber seeds.

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