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Original Research Article

Isolation of an Antioxidant Compound from Costus speciosus Leaves

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Abstract

Costus speciosus (*C. speciosus*) has several pharmacological properties including antioxidant activity. Recently we have shown that methanol extract of *C. speciosus* leaves of summer has maximum *in vitro* antioxidant activity. Aim of the present work was to isolate antioxidant compound from *C. speciosus* leaves. Summer sample of *C. speciosus* leaves were collected from the local market and identified by the taxonomist. Methanol extract of the leaves was prepared and processed for isolation of antioxidant compound. Acid hydrolysis, solvent treatment, chromatographic experiments followed by crystallization were done to isolate a compound. *In vitro* antioxidant activity of the isolated compound was measured by superoxide anion generation with the help of xanthine-xanthine oxidase assay, linoleic acid peroxidation assay as well as by DPPH photometric assay. Isolated compound showed significant *in vitro* antioxidant activity which was comparable to that of quercetin, a synthetic antioxidant. The isolated compound may, therefore, be used as natural antioxidant.

Keywords: *Costus speciosus* leaves, Isolation of antioxidant compound, In vitro antioxidant activity of the compound.

INTRODUCTION

Oxidative process is the most important route for producing free radicals in living system [1]. Free radicals cause oxidative stress which could develop many chronic and degenerative diseases including cancer, diabetes, ischemic heart disease, atherosclerosis, neurodegenerative diseases etc [2]. Antioxidants can break free radical chain reaction properties.

It is reported that many medicinal plants such as *Ananas comosus*, *Amaranthus gangeticus*, *Artemisia absinthium*, *Berberis integerrima*, *Berberis vulgaris Bacopa monnieri*, *Coffea Arabica*, *Camellia sinensis sinensis*, *Curcuma longa*, *Ficus bengalensis*, *Foenciculum vulgare*, *Hemidesmus indica*, *Ixora coccenia*, *Justicia adhatoda*, *Moringa oleifera*, *Mentha piperita*,,*Melissa officinalis*, *Piper betle*, *Sida retusa*, *Salvia officinalis*, *Terminalia chebula*, *Vitex negundo* etc. exert antioxidant activity [3, 4].

C. speciosus (family, Costaceae), commonly known as keu, found in tropical region of India along streams, roadsides, wastelands [5] as well as in moist tropical evergreen forests, up to an altitude of 1200 m [6], has long been medicinally used in different systems of medicine since long. Different parts of the plants are reported to possess anti diabetic, antipyretic, anti bacterial, anti fungal, antihelminthic, hepatopretective, anti-inflammatory, anti cancer, antioxidant, antifertility, anti cholinestrase, and hypolipidemic, adaptogenic activities etc [7, 8].

Recently we have observed that methanol extract of summer sample of *C. speciosus* leaves could exert maximum *in vitro* antioxidant activity (results are under communication). It was, therefore, thought worthwhile to isolate antioxidant compound from summer sample of *C. speciosus* leaves.

MATERIAL AND METHODS

Plant Material

Leaves of *C. speciosus* of summer (March – May) were collected from the local market and authenticated by the experts of the department of Botany of the University of North Bengal, Dist. Darjeeling, West Bengal, India. A voucher specimen was kept in the department of Medical Biotechnology, Sikkim Manipal Institute of Medical Sciences of the Sikkim Manipal University, Gangtok, Sikkim, India for future references.

***Costus speciosus* leaves**

Test Drug

C. speciosus leaves were washed thoroughly under tap followed by distilled water. Leaves were then shed dried and powered. The powder, used as test drug, was stored desiccated at 4 ^{0}C until further use.

Isolation Work

Applying principles of standard isolation procedures of chemical compounds from plant sources [9, 10], this was done by the following scheme.

Chemicals

Chemicals required for the study were purchased from Loba Chem. Lab, Himedia Lab, India and from Merck, Germany and Sigma Chemicals Co., USA.

Diagrammatic scheme for isolation of a compound from *C. speciosus* leaves.

Powdered leaves of *C. speciosus* (100 g)

↓

SOLVENT EXTRACTION

Extracted with 500 ml of methanol for 15 min at 37^{0}C in a Soxhlet apparatus. It was then centrifuged. Supernatant collected and evaporated to dryness.

Active brown mass

↓

ACID REFLUX

Refluxed with 30 ml of 1(N) HCL for 10 min on a water bath at 100 ^{0}C. It was then cooled and centrifuged. Supernatant was evaporated to dryness.

Active brown mass

↓

TREATMENT WITH BENZENE

Treated with 50 ml benzene on a rotary shaker for 15 min. It was then centrifuged. Supernatant was evaporated to dryness.

Active brown mass

↓

ALUMINA COLUMN CHROMATOGRAPHY

Extracted active brown mass with 25 ml of methanol for 10 min. It was then filtered. With filtrate alumina column chromatography was performed. Elution was done by chloroform – methanol mixture (40:60 v/v).

Third band was found active

↓

POLYAMIDE COLUMN CHROMATOGRAPHY

Eluent of active third band was evaporated to dryness. The dry mass was extracted with 25 ml methanol for 10 min. It was then filtered. With filtrate polyamide column chromatography was performed. Elution was done by chloroform : methanol mixture (40:60 v/v).

Fourth band was active

↓

SILICA GEL G COLUMN CHROMATOGRAPHY

Eluent of active fourth band was evaporated to dryness. The dry mass was extracted with 25 ml methanol for 10 min. It was then filtered and the filtrate was subjected to silica gel column chromatography using silica gel G as adsorbent. Elution was done by chloroform : methanol mixture (40:60 v/v).

Second band was found active

↓

CRYSTALLIZATION

Eluent of the active second band obtained from the above step was evaporated to dryness. Repeated crystallization was done from Benzene : ethyl acetate (60:40, v/v) mixture.

Crystals obtained (8.1 mg)

Antioxidant Assays

Antioxidant activity of the isolated compound was assayed by superoxide anion generation with the help of xanthine-/xanthine oxidase assay [11] linoleic acid peroxidation assay [12] and by DPPH photometric assay [13].

Statistical Analysis

All experiments were performed in triplicate. The results were expressed as mean ± SE. Statistical analyses were performed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. A p-value of <0.05 was considered statistically significant [14].

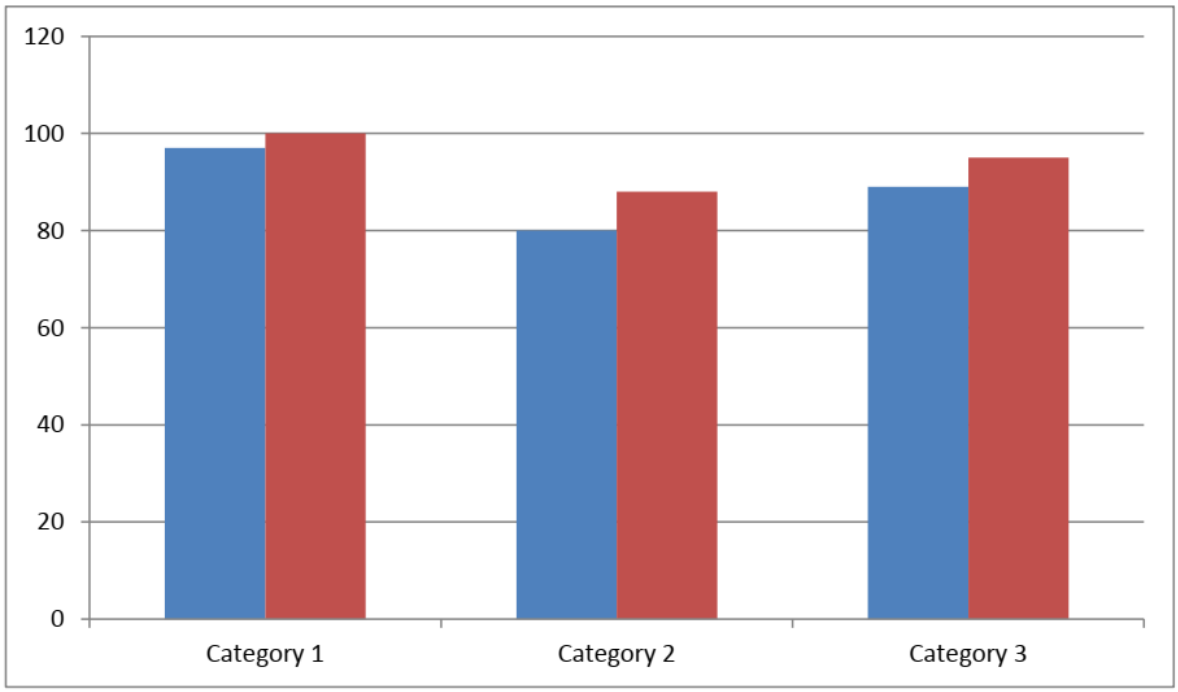
RESULTS

Isolation of Compound

One compound was isolated from *C. speciosus* leaves.

Anti oxidant activity of the isolated compound

In vitro antioxidant activity of the isolated compound from *C. speciosus* leaves was measured by superoxide anion generation by xanthine-/xanthine oxidase, linoleic acid peroxidation and by DPPH photometric assays. Results were given in Figure-1.



Category 1: xanthine- xanthine oxidase assay, Category 2: linoleic acid peroxidation assay
Category 3: DPPH photometric assay
■ Isolated compound from *M. koenigii* L. leaves, 100 µg / ml. ■ Quercetin, 100 µg / ml Results were a mean of triplicate experiments ± SE

Fig-1: *In vitro* antioxidant activity of the compound, isolated from *C. speciosus* leaves, through superoxide anion generation by xanthine-/xanthine oxidase assay, linoleic acid peroxidation assay and by DPPH photometric assay

Results showed that compound isolated from *C. speciosus* leaves could inhibit superoxide anion generations by xanthine-/xanthine oxidase assay, linoleic acid peroxidation assay and by DPPH photometric assay by 97%, 80% and 89 % respectively. Quercetin, a known antioxidant compound, under the same condition could inhibit superoxide anion generations by xanthine-xanthine oxidase, linoleic acid peroxidation and by DPPH photometric assays by 100%, 88% and 95 % respectively

DISCUSSION

Oxidation is a chemical reaction that produces free radicals in organisms. Due to regular use of oxygen there is oxidation in human body which constantly produces free radicals. Free radicals may damage body cells thereby produce various kinds of health problems, such as heart diseases, diabetes, macular degeneration, cancer etc. Antioxidants, on the other hand, are the free radical scavengers and heavy metal ion chelators which help in preventing and repairing the cell damage caused by these radicals. There is antioxidant defense mechanism in human body. Still there is demand for exogenous antioxidant compounds [15]. This demand is being fulfilled by synthetic antioxidants such as butylated hydroxyl anisole and butylated hydroxyl toluene. But report says that the uses of theses synthetic antioxidants are not good for humans, they can cause carcinoma in human body [16].

Under the circumstances search is going on for natural antioxidants which are considered safe for human body. Many sources were utilized, medicinal plants were one of them. Many antioxidant compounds such as anthocyanins, lignans, phenolic acids, flavonoids, stilbenes as well as xanthophylls, carotenes etc. were found present in extracts of medicinal plants [17].

In the present study one antioxidant compound was isolated from *C. speciosus* leaves. Antioxidant activity of the compound, confirmed by inhibition in superoxide anion generations by xanthine-/xanthine oxidase, linoleic acid peroxidation and by DPPH photometric assays, was found comparable to that of quercetin, a known synthetic antioxidant compound. Isolated compound now needs characterization. Work in this direction is presently going on in our laboratory.

CONCLUSION

Compound isolated from *C. speciosus* leaves may be used as natural antioxidant.

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Identification of *C. speciosus* leaves by the taxonomists of the department of Botany, University of North Bengal, Siliguri, Dist. Darjeeling, West Bengal is gratefully acknowledged.

Conflict of Interest

There is no conflict of interest.

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