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ISOLATION OF AN ANTIOXIDANT COMPOUND FROM *ABUTILON INDICUM* LINN LEAVES

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ABSTRACT

Abutilon indicum Linn *(A. indicum* L.) has several pharmacological properties including antioxidant activity. Recently we have shown that ethanol extract of *A. indicum* L. leaves of summer has maximum *in vitro* antioxidant activity. Aim of the present work was to isolate antioxidant compound from *A. indicum* L. leaves. Summer samples of *A. indicum* L. leaves were collected from the medicinal plant garden of the North Bengal University and identified by the taxonomist. Ethanol 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: *Abutilon indicum* Linn leaves, Isolation of antioxidant compound, In vitro antioxidant activity of the compound.

1. INTRODUCTION

In living system oxidation is a common process which generates free radicals.[1] Free radicals cause oxidative stress which could develop degenerative diseases like diabetes, ischemic heart disease, cancer, atherosclerosis, neurodegenerative diseases etc. in human body.[2] Antioxidants can break free radical chain reaction properties. Hence search for antioxidants is

going on and even extended to the field of medicinal plants. Many medicinal plants such as *Camellia sinensis sinensis, Curcuma longa, Ficus bengalensis, Ananas comosus, Amaranthus gangeticus, Artemisia absinthium, Hemidesmus indica, Ixora coccenia, Justicia adhatoda, Berberis integerrima, Berberis vulgaris, Bacopa monnieri, Coffea Arabica, Foenciculum vulgare, Moringa oleifera, Terminalia chebula, Vitex negundo, Mentha piperita, Melissa officinalis, Piper betle, Sida retusa, Salvia officinalis* etc. exert antioxidant activity.[3,4]

A. indicum L. *(*Family: *Malvaceae),* commonly known as Abutilon, is a medicinal plant. The plant is used in traditional medicine for treatment of bronchitis, diarrhoea, gonorrhoea, toothache, catarrhal bilious, and inflammation of bladder as well as in fever.[5] The plant contains various bioactive materials like luteolin 7-O-beta glucopyranoside, chrysoeriol 7-O-betaglucopyranoside, quercetin 3-Oalpha- rhamnopyranosyl (1 --> 6)-beta-glucopyranoside, flavonoids, amino acids, ketone, aldehyde, terpenes, hydrocarbon, fatty acids like stearic, palmitic linoleic, oleic acid, endesmol, α-pinene, caryophyllene, caryophyllene oxide, apigenin 7-O-beta-glucopyranoside, quercetin 3-O-beta-glucopyranoside, luteolin, chrysoeriol.[6] The plant exerts numerous pharmacological activities such as anti bacterial, anti fungal, anti-inflammatory, anti cancer, anti diabetic, antipyretic, anti oxidant, antifertility, anticholinestrase, antihelminthic, hepatopretective, hypolipidemic, adaptogenic activities etc.[7]

Recently we have observed that ethanol extract of summer sample of *A. indicum* L. 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 *A. indicum* L. leaves.

2. METHODOLOGY

2.1 Collection of plant materials

Leaves of *A. indicum* L. were collected from from the medicinal plants garden of the University of North Bengal, Siliguri ($26^0 41' 30.9984"$ N, $88^0 27' 4.5756"$ E, elevation, 410 ft), Dist. Darjeeling, West Bengal during summer (March – May). Leaves were authenticated by the experts of the department of Botany of the said University**.** A voucher specimen (No. SM-MB-012/19) 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.

***Abutilon indicum* Linn.**

2.2 Preparation of Test drug

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

2.3 Isolation work

Applying principles of standard isolation procedures of chemical compounds from plant sources[8,9], this was done by the following scheme.

2.4 Chemicals

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

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

Powdered leaves of *A. indicum* L. (100 g)

SOLVENT EXTRACTION

Extracted with 500 ml of ethanol for 10 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 50 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 ETHYL ACETATE

Treated with 60 ml ethyl acetate on a rotary shaker for 10 min. It was then centrifuged. Supernatant was evaporated to dryness.

Active brown mass

ALUMINA COLUMN CHROMATOGRAPHY

Extracted active brown mass with 20 ml of ethanol for 10 min. It was then filtered. With filtrate alumina column chromatography was performed. Elution was done by chloroform – ethanol mixture (50:50 v/v).

Second band was found active

POLYAMIDE COLUMN CHROMATOGRAPHY

Eluent of active second band was evaporated to dryness. The dry mass was extracted with 20 ml ethanol for 10 min. It was then filtered. With filtrate polyamide column chromatography was performed. Elution was done by chloroform : ethanol mixture (50:50 v/v).

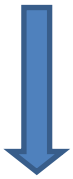
Third band was active

SILICA GEL G COLUMN CHROMATOGRAPHY

Eluent of active third band was evaporated to dryness. The dry mass was extracted with 20 ml ethanol 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 : ethanol mixture (50:50 v/v).

First band was found active

CRYSTALLIZATION



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

Crystals obtained (8.7 mg)

2.6 Antioxidant assays

Antioxidant activity of the isolated compound was assayed by superoxide anion generation with the help of xanthine-/xanthine oxidase assay[10] linoleic acid peroxidation assay[11] and by DPPH photometric assay.[12]

2.7 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.[13]

3. RESULTS

3.1 Isolation of compound

One compound was isolated from *A. indicum* L. leaves.

3.2 Anti oxidant activity of the isolated compound

In vitro antioxidant activity of the isolated compound from *A. indicum* L. 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.

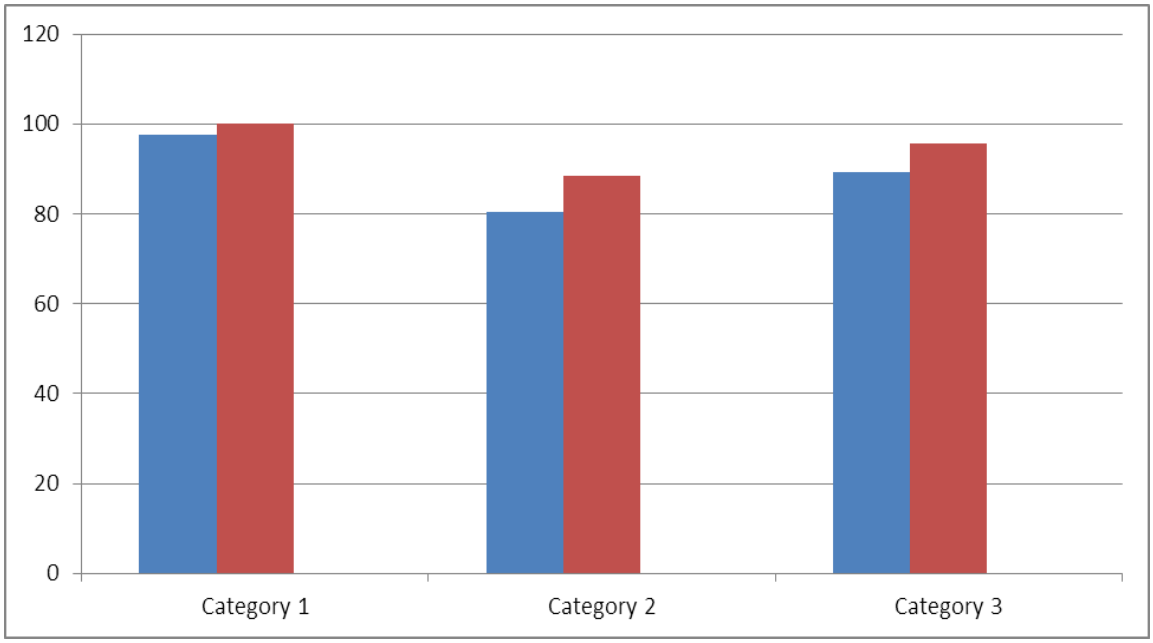


Figure 1: *In vitro* antioxidant activity of the compound, isolated from *A. indicum* L. leaves, through superoxide anion generation by xanthine-/xanthine oxidase assay, linoleic acid peroxidation assay and by DPPH photometric assay.

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.

Results showed that compound isolated from *A. indicum* L. leaves could inhibit superoxide anion generations by xanthine-/xanthine oxidase assay, linoleic acid peroxidation assay and by DPPH photometric assay by 97.5%, 80.3% and 89.2% 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%, 88.4% and 95.6% respectively.

4. DISCUSSION

Life process requires oxygen. Accordingly human uses oxygen for respiration. Reaction with oxygen is known as oxidation. Oxidation produces free radicals in organisms. Free radicals produce in human body damage body cells thereby produce various health problems like 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. Enzymes like super oxide dismutase, catalase etc. and vitamins like vitamin-E, vitamin-C etc. are involved in this antioxidant defense mechanism. Still exogenous antioxidant compounds are required.[14] Synthetic antioxidants such as butylated hydroxyl anisole and butylated hydroxyl toluene are available. But report says that uses of theses synthetic antioxidants are not good for humans, they can cause carcinoma in human body.[15]

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 lignans, phenolic acids, flavonoids, anthocyanins, stilbenes as well as xanthophylls, carotenes etc. were found present in extracts of medicinal plants.[16]

In the present study one antioxidant compound was isolated from *A. indicum* L. leaves. Antioxidant activity of the compound was confirmed by inhibition in superoxide anion generations by xanthine-/xanthine oxidase, linoleic acid peroxidation and by DPPH photometric assays. Antioxidant activity 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.

5. CONCLUSION

Compound isolated from *A. indicum* L. leaves may be used as natural antioxidant.

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Conflict of interest: There is no conflict of interest.

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