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# Isolation of a compound from *Amorphophallus paeoniifolius* Tuber and studies on its *in vitro* antioxidant activity

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**ABSTRACT: Background:** *Amorphophallus paeoniifolius (A. paeoniifolius)* tuber has several Pharmacological properties including antioxidant activity. Recently we have shown that methanol extract of *A. paeoniifolius* tuber has maximum *in vitro* antioxidant activity. **Aim:** Aim of the present work was to isolate chemical compound from *A. paeoniifolius* tuber responsible for antioxidant activity. **Method:** *A. paeoniifolius* tuber was collected from the local market and identified by the taxonomist. The tuber was extracted by using Methanol as solvent and the methanol extract was processed for isolation of antioxidant compound. Acid hydrolysis, solvent treatment, chromatographic experiments followed by crystallization was 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, linoleic acid peroxidation and by DPPH photometric assays. **Results:** Isolated compound showed significant *in vitro* antioxidant activity as compared with standard antioxidant drug quercetin. **Conclusion:** The isolated compound may be responsible for exhibiting antioxidant activity, therefore, be used as natural antioxidant.

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## INTRODUCTIONS:

Oxygen is indispensable for life but under certain situations could exert deleterious effects on the human body. Oxygen can form number of chemical compounds, known as reactive oxygen species (ROS). Hydrogen peroxide, ozone, hypochlorous acid etc. are in the list of ROS.

Reactive species are free radicals. Due to presence of surplus free floating electrons free radicals can easily donate or accept electrons from other sources. They are, therefore, unstable and highly reactive. Hydroperoxyl radical, active oxygen, superoxide radical and triplet oxygen are the types of free radicals. Internal source

(enzymatic reactions), external source (Non enzymatic reactions, ozone, radiations, ultraviolet light, drugs, pesticides, cigarette smoke, environmental pollutant etc.) and physiological factors (stress, emotion and disease) are responsible for formation of free radicals. Free radicals cause oxidative stress which could develop many chronic and degenerative diseases including diabetes, cancer, atherosclerosis, ischemic heart disease and neurodegenerative diseases [1].

Antioxidants, on the other hand, are the synthetic chemicals or substances present in different dietary sources like fruits, vegetables, tea, etc. which in low concentration could delay or inhibit oxidation of the substance. They can also break free radical chain reaction properties thereby save the living system from deleterious effects of free radicals [2]. Hence search for antioxidants was going on from different sources and even extended to the field of medicinal plants.

It is known that many medicinal plants such as, *Amaranthus gangeticus, Artemisia absinthium, Berberis vulgaris, Bacopa monnieri, Coffea Arabica, Curcuma longa, Ficus bengalensis, Hemides musindica, Ixora coccenia, Moringaoleifera, Melissa officinalis, Piper betle, Salvia officinalis, Terminaliachebula* and *Vitex negundo* could exert antioxidant activities [3] .

*A. paeoniifolius* (family, *Areaceae*), also known as Ol (Bengali), Suran (Hindi), Elephant foot yam (English) andSuranah (Sanskrit).This planthas many pharmacological activities such as anti-diarrhoeal, immunomodulatory, hepatoprotective, analgesic,anti-inflammatory, anti tumour, antioxidant, antihelmintic, anticonvulsant and CNS depressant activities [4].

Tuber of *A. paeoniifolius* is reported to have antioxidant activity [5]. Recently we have observed that methanol extract of *A. paeoniifolius* tuber could exert maximum *in vitro* antioxidant activity [6].

**Fig 1. *A. paeoniifolius* tuber in dried form.**

Aim of the present study was to isolate antioxidant compound from *A. paeoniifolius* tuber.

## MATERIALS AND METHODS:

The solvent methanol was purchased from Himedia Lab and Loba Chem., India. The DPPH was purchased from Sigma Chemicals Co., USA; Merck, Germany. All other chemicals and reagents used in this Research study were of analytical grade and procured from an authorized dealer.

### Collection of plant and Authentication:

The plant, *A. paeoniifolius* tuber was collected from the local market and authenticated by the Taxonomist of the Department of Botany of the University of North Bengal, Siliguri. A voucher specimen was kept in the department of Medical Biotechnology, Sikkim Manipal Institute of Medical Sciences of Sikkim Manipal University, Gangtok, Sikkim, India, for future references.

### Processing of Plant (Test drug):

*A. paeoniifolius* tuber was washed thoroughly under tap followed by distilled water. The tuber was then cut into small pieces, shed dried and powered. The powder, used as test drug, was stored desiccated at 4 $^0$C until further use.

### Extraction of *A. paeoniifolius* tuber by solvents:

The test drug (75 g) was extracted with 500 ml of methanol in Soxhlet apparatus (Borosil GS Grade) at 37 $^0$C for 10 min. The extracts were filtered and the solvents were evaporated to dryness in vacuum with rotary evaporator (RV 10 digital V Rotary Evaporator, Raut Scientific & General Traders, Pune) at 40 to 50 $^0$C. Brown masses obtained were used for antioxidants assays as well as the extract was processed for isolation of chemical compound.

### Isolation of Tuber:

Applying principles of standard isolation procedures of chemical compounds from plant sources [7,8], this was done by the following scheme.

The tuber extract active brown ma was refluxed with 30 ml of 1N HCl for 10 min on a water bath at 100 $^\circ$C. It was then cooled and centrifuged. The supernatant was evaporated to dryness. An active brown mass was formed. The active brown mass was treated with 50 ml benzene on a rotary shaker for 15 min. It was then centrifuged. The supernatant was evaporated to dryness. The active brown mass was extracted with 25 ml of

methanol for 10 min. It was then filtered. The filtrate was passed through alumina column for column chromatography (Borosil GS grade, Royal Scientific, New Delhi). The elution was done by chloroform and methanol mixture (40:60 v/v). The third band was found to be active and prominent. 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. The filtrate was again passed through polyamide column for column chromatography. The elution was done by chloroform: methanol mixture (40:60 v/v). The forth band was found to be active and prominent. The 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. The elution was done by chloroform: methanol mixture (40:60 v/v). The second band was found to be active and prominent. The eluent of the active second band obtained from the above step was evaporated to dryness. The process was repeated and finally crystallization was done from Benzene: ethyl acetate (60:40, v/v) mixture. The crystals were obtained with yield value of 5.1 mg.

**Evaluation of Antioxidant activity:**

*In vitro* antioxidant activity of the isolated chemical compound of *A. paeoniifolius* tuber was assayed through superoxide anion generation by xanthine-xanthine oxidase [9], linoleic acid peroxidation [10] and by DPPH photometric assays [11].

**Statistical analysis:**

All experiments were performed triplicate. The results were expressed as mean ± Standard Error of mean. 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 [12].

**RESULTS AND DISCUSSION:**

The isolation study revealed that one compound was isolated from tuber methanol extract with yield value of 5.1 mg. The i*n vitro* antioxidant activity of the isolated compound from *A. paeoniifolius* tuber was measured by superoxide anion generation by xanthine-/xanthine oxidase, linoleic acid peroxidation and by DPPH photometric assays. The result of antioxidant assay is presented in Fig 1. Results showed that compound isolated from *A. paeoniifolius* tuber could inhibit superoxide anion generations by xanthine-/xanthine oxidase, linoleic acid peroxidation and by DPPH photometric assays were found to be 97, 80 and 89 % respectively. Quercetin, a known antioxidant, under the same condition could inhibit superoxide anion generations by the said assays were found to be 100, 88 and 95 % respectively.

There is antioxidant defense mechanism in human body. Still there is demand for exogenous antioxidant compounds [13]. The demand is being fulfilled by synthetic antioxidants such as butylated hydroxyl anisole and butylated hydroxyl toluene. But the use of theses synthetic antioxidants are not good for humans, they can cause many diseases including carcinoma in human body [14]. 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. Presence of antioxidant compounds such as anthocyanins, lignans, phenolic acids, flavonoids, stilbenes as well as xanthophylls and carotenes were found in extracts of many medicinal plants [15].

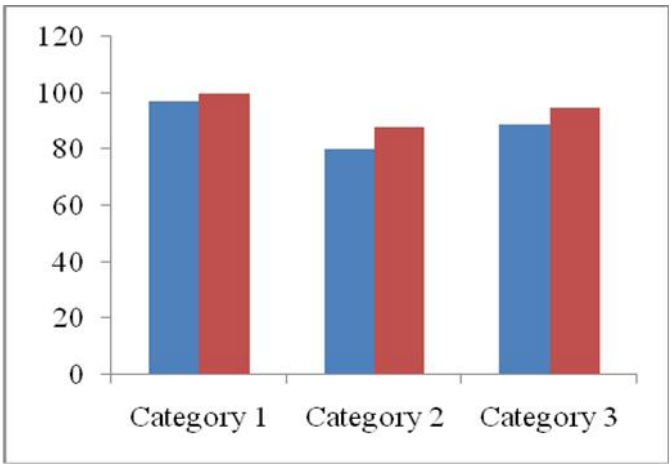


**Fig 1. *In vitro* antioxidant activity of the compound, isolated from *A. paeoniifolius* tuber, through superoxide anion generation by xanthine - xanthine oxidase, linoleic acid peroxidation and by DPPH photometric assays.**

**Category 1: xanthine - xanthine oxidase assay, Category 2: linoleic acid peroxidation assay and Category 3: DPPH photometric assay. The data are expressed as percentage inhibition.**

**■ Isolated compound from *A. paeoniifolius* tuber, 100 µg/ml. ■ Quercetin, 100 µg/ml. Results are expressed as mean ± Standard error of mean (n = 3).**

In the present study one antioxidant compound was isolated from *A. paeoniifolius* tuber. Antioxidant activity of the isolated compound, confirmedbyinhibition in superoxide anion generations by xanthine-/xanthine

oxidase, linoleic acid peroxidation and by DPPH photometric assays, was found well comparable to that of standard synthetic antioxidant drug quercetin. Isolated compound needs characterization. Work in this direction is presently going on in our laboratory.

## CONCLUSION:

It could be concluded that the chemical compound was successfully isolated from methanol extract of *A. paeoniifolius* tuber. The isolated compound exhibited significant antioxidant activity, which proves its Folklore Medicinal use. Thus the compound isolated from *A. paeoniifolius* tuber may be used as natural antioxidant. Further study has to be done for identification of isolated chemical compound with structural elucidation.

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