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In vitro antioxidant activity of *Amorphophallus paeoniifolius* tuber: Effect of extraction solvents

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ABSTRACT: Background: Since long different parts of *Amorphophallus paeoniifolius (A. paeoniifolius)* are being used in different systems of medicine. Tuber of *A. paeoniifolius*has possesses many pharmacological activities such as analgesic, antihelmintic, antioxidant, CNS depressant and immunomodulatory activities. **Aim:** Aim of the present study was to evaluate the effect of various solvents extracts on *in vitro* antioxidant activity of *A. paeoniifolius* tuber. **Method:** *A. paeoniifolius* tuber was collected from the local market and identified by the taxonomist. The tuber was shed dried and powdered. Extracts of the powder were prepared separately using ethanol, chloroform, acetone, methanol, benzene and petroleum ether. *In vitro* antioxidant activity of the extracts was checked by xanthine-xanthine oxidase, linoleic acid peroxidation and DPPH photometric assays. Ascorbic acid, flavonoids, total phenol and carotenoids contents of the extracts were also determined. **Results:** Methanol extract of *A. paeoniifolius* tuber showed maximum *in vitro* antioxidant activity in comparison to other solvent extracts which may be attributed to its higher phenolic content. **Conclusion:** Methanol extract of *A. paeoniifolius* tuber may be further investigated in search for natural antioxidant compounds.

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INTRODUCTIONS:

A. paeoniifolius (Family: *Areaceae*), a stout herbaceous plant, grows wild form in Malaysia, Philippines, Indonesia and other South East Asian countries. The plant has different names such as Ol (Bengali), Suran (Hindi), Elephant foot yam (English) and Suranah (Sanskrit) [1]. Different parts of the plant are used in traditional medicines as carminative, digestive, thermogenic, irritant, liver tonic, aphrodisiac, acrid, astringent, expectorant, appetizer and stomachic. The plant is also used in constipation, helminthiasis, tumors, inflammations, weakness, amenorrhea and anemia [2].

Phytochemical analysis of *A. paeoniifolius* revealed presence of flavonoids, alkaloids, steroids, carbohydrates, tannins and proteins [3]. *A. paeoniifolius*has exhibit many pharmacological activities likeanti-inflammatory, antidiarrhoeal, immunomodulatory, antitumour, antioxidant, antihelmintic, anticonvulsant, analgesic, CNS depressant and hepatoprotective activities [4]. The traditional information reported that *A. paeoniifolius* tuber is possessing antioxidant activity [5]. Aim of the present study was to see effect of extraction solvents on *in vitro* antioxidant activity of *A. paeoniifolius* tuber.

MATERIALS AND METHODS:

The solvents; benzene, chloroform, petroleum ether methanol, ethanol, and acetone were 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 Authentification:

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.

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

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 separately with 500 ml of benzene, chloroform, petroleum ether methanol, ethanol, and acetone in Soxhlet apparatus at 37 ^{0}C for 10 min. The extracts were filtered and the solvents were evaporated to dryness in vacuum with rotary evaporator at 40 to 50 ^{0}C. Brown masses obtained were used for antioxidants assays as well as for determination of total phenol, ascorbic acid, flavonoids and carotenoids contents.

Evaluation of Antioxidant activity:

In vitro antioxidant activity of different extracts of *A. paeoniifolius* tuber was assayed through superoxide anion generation by xanthine-xanthine oxidase [6], linoleic acid peroxidation [7] and by DPPH photometric assays [8].

Determination of Anti oxidant chemicals

Antioxidant chemicals such are flavonoids, total phenols, ascorbic acid and total carotenoids present in *A. paeoniifolius* tuber were determined by the methods of Chang *et al.* [9], McDonald *et al.* [10], Cakmak and Marschner [11] and Jensen [12] respectively.

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 [13].

RESULTS AND DISCUSSION:

Results of *in vitro* antioxidant activity of different extracts of *A. paeoniifolius* tuber through superoxide anion generation by xanthine- xanthine oxidase, linoleic acid peroxidation and by DPPH photometric assays are given in Table 1. Results showed that all extracts (Acetone, methanol, ethanol, chloroform, benzene and petroleum ether) of *A. paeoniifolius* tuber had *in vitro* antioxidant activity but maximum activity was exhibited by the methanol extract. Inhibitions in xanthine oxidase, linoleic acid peroxidation and DPPH in methanol extract were found 90, 79 and 77 % respectively. Results were statistically significant in comparison to that of other extracts. Results were also comparable with that of standard drug quercetin, a known antioxidant, where inhibition in xanthine oxidase, linoleic acid and DPPH came 100, 88 and 90 % respectively.

Table 1. Inhibition in xanthine oxidation, linoleic acid peroxidation and DPPH scavenging capacity by the different solvent extracts of *A. Paeoniifolius* tuber.

PEAPT	XO (PI)	LAP (PI)	DPPH (PI)
Pet. ether	10±0.2	12±0.4	17±0.3
Benzene	18±0.3	21±0.4	23±0.4
Ethanol	37±0.5	37±0.5	32±0.4
methanol	90±0.9*	79±0.7*	77±0.5*
Acetone	40±0.5	39±0.6	39±0.5
Chloroform	39±0.4	32±0.5	29±0.3
Quercetin	100±0.02	88±0.02	90±0.01

PEAP - Powder obtained from extract of *A. paeoniifolius* tuber. All solvents extract are used at concentration of 100 µg/ml . All data are presented as Mean ± SEM (n = 3).*Significant. XO – Xanthine oxidase, LAP - Linoleic acid peroxidation and PI – Percentage Ingibition.

The Table 2 showed that total phenols content of the methanol extract of *A. paeoniifolius* tuber was 67±0.5 mg/mg dry weight which was maximum in comparison to that of petroleum ether extract (15±0.2 mg/mg dry weightt), benzene extract (19±0.3 mg/mg dry weight), ethanol extract (20±0.5 mg/mg dry weight), acetone extract (27±0.4 mg/mg dry weight) and chloroform extract (25± 0.5 mg/mg dry weight). The results were statistically significant. Ascorbic acid, flavonoids and carotenoid contents of different solvent extracts of *A. paeoniifolius* tuber apparently showed different values but the results were not statistically significant.

Table 2. Total phenol, flavonoids, ascorbic acid and carotenoid contents of different solvent extracts of *A. paeoniifolius* tuber.

LAPT	TPC	TFC	AAC	CC
Pet. ether	15±0.2	18±0.9	20±0.9	3.7±0.8
Benzene	19±0.3	20±0.8	19±1.7	4.0±0.9
Ethanol	20±0.5	19±0.9	22±1.8	4.1±0.8
Methanol	67±0.5*	21±1.9	21±0.9	4.8±1.7
Acetone	27±0.4	19±0.9	19±1.6	4.2±0.9
Chloroform	25±0.5	17±0.9	20±1.5	3.8±0.8

LAPT - Leaves of *A. paeoniifolius* tuber, TPC – Total Phenol Content, TFC - Total flavonoids content, AAC - Ascorbic acid content and CC - Carotenoids content. All content are expressed as mg/g of dry weight. All results are presented as mean ± SEM (n=3).*Significant.

Maximum inhibitory effects of methanol extract of *A. paeoniifolius* tuber in xanthine oxidation and linoleic acid peroxidation as well as scavenging capacity of DPPH were comparable to that of quercetin, a standard antioxidant compound (Fig 11). Ansil *et al.* also showed antioxidant activity of methanol extract of *A. paeoniifolius* tuber for its free radical scavenging activity [5]. The present results, therefore, are in agreement with that of Ansil *et al.*

It is reported that total phenol, flavonoids, ascorbic acid and carotenoids present in a plant are responsible for antioxidant activity of the plant [18]. In the present study methanol extract of *A. paeoniifolius* tuber showed presence of maximum amount of total phenols in comparison to that of ethanol, acetone, chloroform, petroleum ether and benzene extracts. Flavonoids, ascorbic acid and carotenoids contents of all the extracts of *A. paeoniifolius* tuber, however, did not show any significant change (Fig 2). *In vitro* antioxidant activity of *A. paeoniifolius* tuber was therefore due to presence of high amount of total phenols in the tuber.

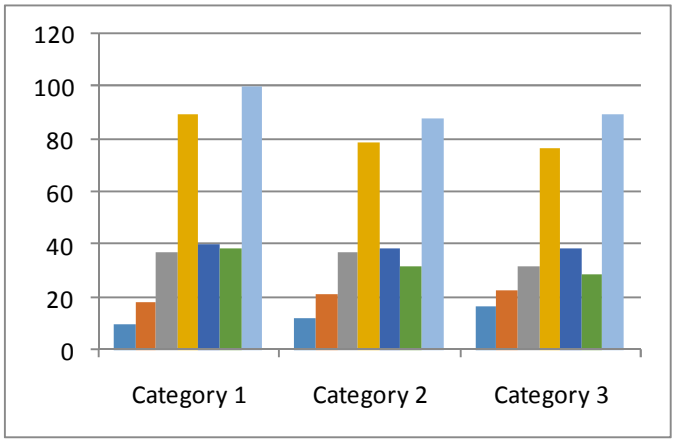

Fig 1. Showing percentage inhibition of xanthine oxidation, linoleic acid peroxidation and scavenging capacity of DPPH by different solvent extract of *A. paeoniifolius* tuber

Category 1: Xanthine oxidase (% inhibition), Category 2: Linoleic acid peroxidation (% inhibition) and Category 3: DPPH (% inhibition). Petroleum ether Benzene Ethanol Methanol Acetone Chloroform Quercetin.

Total phenols, flavonoids, ascorbic acid and carotenoids are the secondary metabolite of plant responsible for different biological activity. It is known that season has significant effect on the synthesis of secondary metabolites in plants thereby changing their biologic activity [19,20]. We are now studying seasonal effect on amounts of total phenol, flavonoids, ascorbic acid and

carotenoids in *A. paeoniifolius* tuber vis-à-vis it's *in vitro* antioxidant activity.

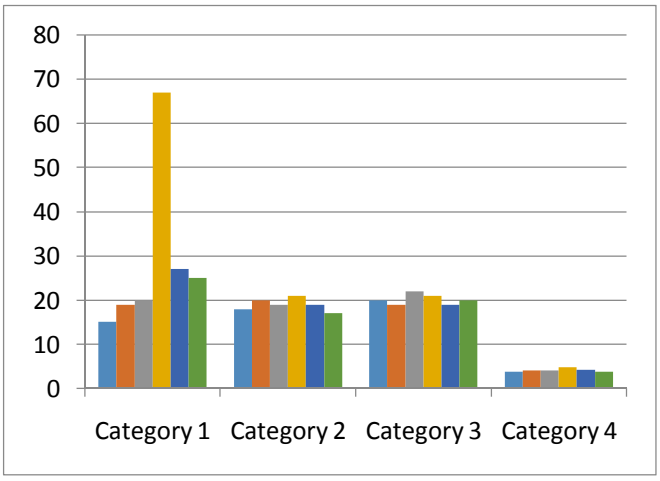

Fig 2. Showing amounts (mg/g dry weight) of total phenols, flavonoids, ascorbic acids and carotenoids indifferent solvent extracts of *A. paeoniifolius* tuber.
Category 1: Total phenol, Category 2: Total flavonoids, Category 3: Ascorbic acid and Category 4: Carotenoids content. Petroleum ether Benzene Ethanol Methanol Acetone Chloroform.

CONCLUSION:

It could be concluded that the methanol extract of *A. paeoniifolius* tuber exhibited significant antioxidant activity, which prove its Folklore Medicinal use. The research study also proved that there is definitely solvent affect the extraction of particular chemical compound, which might be responsible for exhibiting antioxidant activity. Methanol extract of *A. paeoniifolius* tuber may be further investigated in search for natural antioxidant compounds.

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