ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 8, Issue 6, 2015

ISSN - 0974-2441

Research Article

ANTIOXIDANT AND CYTOTOXIC EFFECTS OF METHANOL EXTRACTS OF *AMORPHOPHALLUS PAEONIIFOLIUS* NI

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Received: 03 July 2015, Revised and Accepted: 22 August 2015

ABSTRACT

Objectives: To identify the bioactive compounds present in *Amorphophallus paeoniifolius* and to evaluate the antioxidant and cytotoxic property of the plant extract.

Methods: Preliminary phytochemical analysis was carried out to determine its major chemical constituents such as alkaloids, sterols, flavonoids, terpenoids, phenolic compounds, saponins, carbohydrates, glycosides, and tannins. Bioactive compounds were identified by gas chromatography - mass spectrometry (GC/MS) analysis. Antioxidant activity of the methanol extract were determined by two different methods *viz*., 2,2-diphenyl-2-picryl hydrazyl hydrate radical scavenging assay and reducing power assay. Furthermore, the extracts were tested for their ability to kill the proliferative cells (MCF-7) by (3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyltetrazolium bromide) (MTT) assay.

Results: Ten pharmacology important compounds were identified by GC/MS analysis. The higher antioxidant potential of the extracts was observed in both assays. The appreciable free radical scavenging activity of the extracts might be attributed to the secondary metabolites such as alkaloids, flavonoids, tannins, phenols, and sterols present in the extract. The results of MTT assay indicated a significant growth inhibition at a low concentration of IC_{50} values (51.07 µg/ml).

Conclusion: The present study is an evidence to support the usage of the plant which possesses several antioxidant principles for medicinal uses.

Keywords: *Amorphophallus paeoniifolius,* 2,2-diphenyl-2-picryl hydrazyl hydrate, Reducing power, MCF-7 cells.

INTRODUCTION

Medicinal plants typically contain mixtures of different chemical compounds that may act individually, additively or in synergy to improve health. The main objective of this study is, to search for a plant with a strong antioxidant activity which could serve as a good candidate for the development of standardized phytomedicine.

The plant *Amorphophallus paeoniifolius* (Dennst.) Nicolson (Araceae) commonly known as elephant foot yam is a tropical tuber crop that offers excellent scope for adoption in the tropical countries as a cash crop due to its production potential and popularity as a vegetable in various delicious cuisines. It is a crop of south-east Asian origin and grows in the wild form in the Philippines, Malaysia, Indonesia, and south-east Asian countries [1]. Many indigenous Ayurvedic and Unani medicinal preparations are made using its tubers. The tubers are believed to have blood purifying characteristics and are used in medicines for the treatment of piles, asthma, dysentery, and other abdominal disorders [2].

Now-a-day, the role of free radicals in many ailments and diseases has been widely established. Natural antioxidants tend to be safer, and they also possess antiviral, anti-inflammatory, anticancer, antitumor, and hepatoprotective properties [3]. Significant antioxidant properties have been recorded with phytochemicals that are necessary for the reduction in the occurrence of many diseases. Plants contain a wide variety of free radical scavenging molecules such as phenols, flavonoids, vitamins, and terpenoids that are rich in antioxidant activity. In view of the above, the antioxidant activity of methanol extract of corm of *A. paeoniifolius* was evaluated.

The utility of cell lines acquired from tumors allows the investigation of tumor cells in a simplified and controlled environment. Cytotoxicity assay is an appropriate method for screening new substances within a short time to determine the cell killing property on cancer cells [4]. Usually, in oncology research and clinical practices, *in vitro* testing is preferred prior to *in vivo* testing. 3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay has been described as a rapid, simple, and reproducible method, widely used in the screening of anticancer drugs and to measure the cytotoxic properties. Hence in the current study, the cytotoxic properties of methanol extract of *A. paeoniifolius* were evaluated.

METHODS

Collection of the sample

The tubers of *A. paeoniifolius* were collected from the local vegetable market of Coimbatore district, Tamil Nadu (India). Initially, the tubers were washed with tap water to remove the surface contamination and then sliced into small pieces. The pieces were shade dried and were powdered using an electrical grinder.

Soxhlet extraction

10 g of the powder was weighed using an electrical balance (Denver 210) and made into 8 packets using xerohaze filter paper (10 A grade SD's). Petroleum ether, chloroform, methanol, and water were used as solvents for soxhlet extraction in the increasing order of polarity. The distillation process was carried out at a low temperature of 40°C. After evaporation of solvents, corresponding residues were obtained and stored in the refrigerator for further use.

Phytochemical studies

Preliminary phytochemical analysis was carried out according to the standard method of Yadav and Agarwala [5] for the following chemical compounds such as alkaloids, terpenoids, phenols, tannins, carbohydrates, saponins, flavonoids, quinones, proteins, and sterols.

Analysis of bioactive compounds

A Thermo GC-Trace Ultra Ver: 5.0 gas chromatography-mass spectrometry (GC-MS) system used for this study consisted of a model

Thermo MS DSQ II gas chromatograph. A fused-DB35-MS capillary standard non-polar column dimension (30 minutes, ID: 0.25 mm, FILM: 0.25 µm) was also used. The GC temperature program was as follows: Initial temperature was 100°C, held for 1 minute, increased to 130°C at a rate of 2°C/minute, then to 200°C at a rate of 3°C/minute, and finally to 280°C at a rate of 6°C/minute and held for 10 minutes. The split ratio was 1:12, injection temperature was 250°C, transfer line temperature was 270°C, The mass spectrometer was operated at 70 eV in run time 40.51 (minute).

Antioxidant activity

2,2-diphenyl-2-picryl hydrazyl hydrate (DPPH) radical scavenging assay [6]

The radical scavenging activities of the plant extract against DPPH radical were determined by ultraviolet spectrophotometry at 517 nm. The following concentrations of the extract were prepared (10, 20, 40, 60, 80, and 100 µg/ml) in methanol. Vitamins C was used as the antioxidant standard at concentrations of 10, 20, 40, 60, 80, and 100 µg/ml. 1 ml of the extract was placed in a test tube, and 3 ml of methanol was added followed by 0.5 ml of 1 mM DPPH in methanol. A blank solution was prepared to contain the same amount of methanol and DPPH. The radical scavenging activity was calculated using the following formula:

$$([Ab - Aa] / Ab) \times 100$$

Where, Ab is the absorption of the blank sample and Aa is the absorption of the extract.

Reducing power determination [7]

Different concentrations of an extract (10-100 µg/ml in methanol) were mixed with phosphate buffer (2.5 ml) and 1% of potassium ferricyanide (2.5 ml).The mixture was incubated at 50°C for 20 minutes. A portion of 10% trichloroacetic acid (2.5 ml) was added to the mixture, which was then centrifuged 5000 rpm for 10 minutes. The supernatant (2.5 ml) was mixed with distilled water (2.0 ml) and $FeCl_3$ (0.5 ml), and the absorbance was measured at 700 nm. Ascorbic acid served as control. Increased absorbance of the reaction mixture indicates increased reducing power.

Cytotoxicity studies

Cytotoxicity was evaluated using MTT assay. Sub confluent monolayer culture of MCF-7 cells was trypsinized, and the cells were collected in the growth medium. The suspension was centrifuged at 1400 rpm for 5 minutes, and the cell pellet was resuspended in growth medium. Viable cells were quantitated using the tryphan blue dye exclusion assay. The cells were diluted to 5×10^4 cells/ml and made up to 20 ml of cell suspension per microtiter plate. 200 µl of cell suspension to each wells including control were plated at a cell density of 1×10^4 cells/well and was incubated in the 5% CO_2 incubator for 24 hrs to enable them to adhere properly to 96 well polystyrene micro plates. After 24 hrs, media was removed. Extracts were prepared at 1 mg/ml concentration and mixed with fresh medium to achieve the final working concentration (10-100 µg/ml). Each concentration of extracts was repeated in 3 wells. After incubation for 72 hrs at 37˚C in a humidified incubator, fresh media was added. 20 µl of MTT (5 mg/ml in phosphate buffered saline) was added to each well and incubated for 4 hrs at 37°C. After this, viability was assessed by the ability of cells to convert the soluble salt of MTT into an insoluble formazan precipitate which was quantitated spectrophotometrically following solubilization in dimethyl sulfoxide. The absorbance was recorded on a microtiter plate recorder (Bio Rad Co.) at the wavelength of 570 nm and with the reference wavelength at 630 nm. The % of inhibition of each concentration was calculated by the following formula:

$$\% \text{ of inhibition} = \frac{\text{Control OD} - \text{ose OD}}{\text{Control OD}} \times 100$$

Inhibition concentration (IC_{50}) was evaluated by plotting a graph with concentration (µg/ml) of plant extract at X axis and % of inhibition at Y axis.

Statistical analysis

The experiments were carried out in triplicate. The results are given as mean ± standard deviation. Nonlinear regression graph was plotted between % Cell inhibition and Log_{10} concentration and IC_{50} were determined using Graph Pad Prism software.

RESULTS

Phytochemical analysis

In soxhlet extraction, among the four solvents, the methanol extracts of *A. paeoniifolius* showed highest extractive value of 66% followed by petroleum ether (46%), water (40%), and chloroform (26%). Qualitative chemical analysis of the plant extract provided the information regarding various types of phytoconstituents such as alkaloids, terpenoid, steroids, carbohydrate, phenols and flavonoids (Table 1).

The results pertaining to the GC/MS analysis leads to the identification of 10 pharmacologically important compounds from the methanolic extract of *A. paeoniifolius.* The major compounds were octadecanoic acid, nonadecenoic acid, diethylene glycol monododecyl ether, hexadecanoic acid, 1-pentadecanol, sucrose, tetradecane, naphthalene, undecane, and phenol (Fig. 1).

DPPH radical scavenging activity

The results of the assay are expressed in the scavenging ability of DPPH free radical. The ability of radical scavenging activity of the extract at different concentrations increases with increasing concentrations (Table 2).

Table 1: Phytochemical screening of methanol extract of *A. paeoniifolius*

Phytochemicals	Reagents used	Methanol extract
Alkaloids	Mayer's test	+++
	Dragendroff's test	+++
	Hager's test	+++
	Wagner's test	+-+
Carbohydrates	Molish's test	+++
	Fehling solution	+-+
	Benedict's test	+++
Steroids and terpenoids	Lieberman's and Burchard's test	+--
	Salkowski's test	+-+
Flavonoids	Ferric chloride test	+++
	Shinod's test	+++
Phenolic compounds	5% ferric chloride	+++
	10% lead acetate	+++
Saponins	Foam test	-
Proteins and amino acids	Ninhydrin test	+++

-: Absent, +: Present, *A. paeoniifolius: Amorphophallus paeoniifolius*

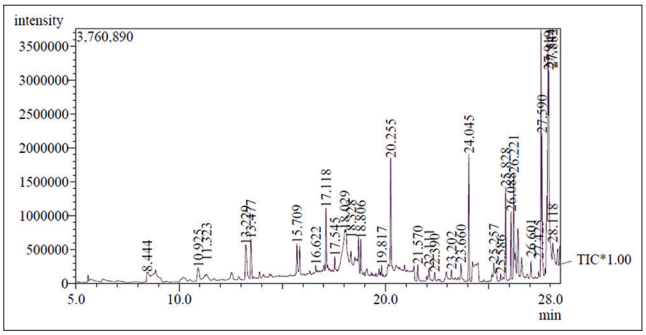

Fig. 1: Gas chromatography-mass spectrometry chromatogram of methanol extract of *Amorphophallus paeoniifolius*

Reducing power activity

The reducing capability of a compound may serve as a significant indicator of its potential antioxidant ability. In the present study, the reducing power of extracts increased with increasing concentration. The reducing capability of *A. paeoniifolius* compared to standard was given in Table 3.

Cytotoxic activity

The cytotoxic activity of the methanolic extract of *A. paeoniifolius* showed a dose-dependent MTT reduction in MCF-7 cell lines. The 50% inhibitory concentration was found to be at 51.07 µg/ml (Fig. 2).

DISCUSSION

Plant products have been part of phytomedicines since time immemorial. Secondary metabolites are chemically and taxonomically diverse compounds with obscure function. They are widely used in the human therapy, veterinary, agriculture, scientific research, and countless other areas [8].

Table 2: DPPH radical scavenging activity of methanolic extract of *A. paeoniifolius*

Concentrations (µg/ml)	% inhibition	
	Ascorbic acid	Methanol extract
10	58.7±0.27	17.80±1.20
20	65.3±0.69	24.82±0.50
40	72.4±0.41	28.71±0.40
60	77.0±0.56	35.31±0.61
80	81.03±0.20	41.07±0.40
100	89.7±0.56	49.3±0.51

Values are given as mean±SD, *A. paeoniifolius: Amorphophallus paeoniifolius*, SD: Standard deviation

Table 3: Reducing power activity of methanol extract of *A. paeoniifolius*

Concentrations (µg/ml)	Absorbance (nm)	
	Ascorbic acid	Methanol extract
10	0.51±0.03	0.06±0.01
20	0.81±0.06	0.15±0.02
40	1.53±0.07	0.30±0.01
60	1.69±0.02	0.37±0.04
80	3.42±0.02	0.44±0.06
100	3.60±0.04	0.85±0.02

Values are given as mean±SD, *A. paeoniifolius: Amorphophallus paeoniifolius*, SD: Standard deviation

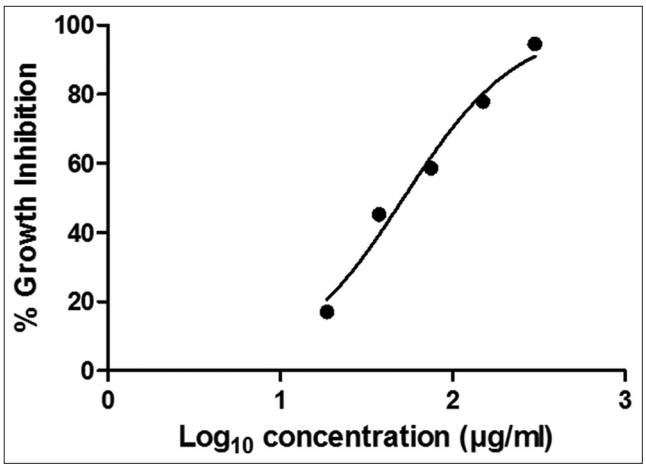

Fig. 2: *In vitro* cytotoxic activity of *Amorphophallus paeoniifolius* on MCF-7 cell lines

The phytochemical examination has been making rapid progress, and herbal products are becoming popular as sources of plausible anticancer compounds. In the present work, qualitative analysis of the plant extracts revealed the presence of phytochemicals such as alkaloids, carbohydrates, sterols, terpenoids, flavonoids, phenolic compounds, proteins, and amino acids. Edoga *et al.* [9] reported that the medicinal value of plants lies in these chemical substances (usually secondary metabolites), that produce a definite physiological action on the human body. The most important of these bioactive compounds are alkaloids, flavonoids, tannins, and phenolics.

Knowledge of the chemical constituents of plants is desirable for the synthesis of complex chemical substances and for discovering the actual significance of folkloric remedies. 10 pharmacologically important compounds were identified in methanol extracts of *A. paeoniifolius* by GC/MS analysis. These components possess diverse medicinal value. Similar studies on *Amorphophallus campanulatus* tuber by GC/MS analysis reported tetradecene, 1-pentadecanol, hexadecanoic acid, and nonadecanoic acid with antioxidant and cancer preventive activities [10].

The efficacies of antioxidants are often associated with their ability to scavenge stable free radicals [11]. DPPH assay is a valid accurate, easy and economic method to measure the ability of compounds to act as free radical scavengers or hydrogen donors. This method is unique in carrying out the reaction of the sample with DPPH in methanol/water, which facilitates the extraction of antioxidant compounds from the sample [12].

In the present study, the methanol extract of the plant *A. paeonfolius* used to find the reactivity of the stable free radical exhibited high radical scavenging ability. This study is supported by the observation of Jenecius and Mohan [13] on the *in vitro* antioxidant activity of the same plant. The activity in different extracts has been tested using various antioxidant model systems revealed that methanol extract of corm showed potent *in vitro* antioxidant activities.

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. In this assay, the yellow of the test solution changes to various shades of green and blue depending on the reducing power of each compound. The presence of reducers in the methanol extract causes the conversion of the Fe^{3+}/ferricyanide complex to the ferrous form. The reducing power of different concentrations of *A. paeonfolius* was found to be remarkable, and the absorbance of each concentration was found to rise as the concentration gradually increases.

Cytotoxicity determination is a common method of evaluating the biological activity of a natural product. It is useful in confirming whether the plant extracts have potential antiproliferative properties. For this study, we used MCF-7 cell lines treated with various concentrations of methanolic extract of *A. paeoniifolius* ranging from 18.75 to 300 µg/ml at 72 hrs. After 24 hrs of treatment, higher concentrations (150 and 300 µg/ml) killed more than 50% of cells.

A. paeoniifolius treatment on MCF-7 cell lines showed a significant decrease in growth rate compared with control. On the other hand, the percentage of non-viable cells increased with increasing period of treatment. It was reported that the IC_{50} of the extract on cell line <100 µg/ml is categorized as a potential cytotoxic substance [14].

These results corroborate with the findings of Sreejaya and Santhy[15] who studied the cytotoxic effect of *Acorus calamus* toward human breast cancer cells. Cytotoxic property of different solvent extracts of *A. paeoniifolius* tuber using HEp-2 cell line has already reported. The magnitude of cytotoxicity was predominant in petroleum ether and ethanolic extract and displayed a dose-dependent antiproliferative activity [16].

CONCLUSION

Qualitative chemical examination showed the presence of various quantities of therapeutically important phytochemicals. DPPH scavenging activity of the extract was measured in terms of hydrogen donating, or radical scavenging ability using the stable radical DPPH showed considerable *in vitro* antioxidant activity. The higher absorbance of the reaction mixture indicates higher reductive potential. These activities are attributable to the presence of bioactive compounds present in the extract analyzed by GC/MS. In screening result, *A. paeoniifolius* has showed broad spectrum cytotoxicity on MCF-7 cells with an IC_{50} value of 51.07 µg/ml.

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