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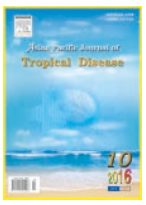

Review article doi: 10.1016/S2222-1808(16)61141-6

Conservation, genetic characterization, phytochemical and biological investigation of black calla lily: A wild endangered medicinal plant

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ARTICLE INFO

Article history:
Received 14 Jun 2016
Received in revised form 4 Jul, 2nd revised form 8 Aug, 3rd revised form 10 Aug 2016
Accepted 22 Aug 2016
Available online 25 Aug 2016

Keywords:
Arum palaestinum
Tissue culture
Phytochemical analysis
Antioxidant
Anticancer

ABSTRACT

Scientists continue to search for and conserve plants whose medicinal properties have become crucial in the fight against diseases. Moreover, lessons from folk medicine, indigenous knowledge and Chinese medicine on crude extracts points to possible findings of novel promising and strong pharmaceutically bioactive constituents. *Arum palaestinum*, commonly known as black calla lily, is one of the most important medicinal plants belonging to the family Araceae, which has not been well studied. Little is known about its pharmaceutically bioactive constituents and the effective conservation through the use of biotechnology. Thus, *Arum Palaestinum* is selected and reviewed for its phytochemical analysis and biological activities. Besides, the tissue culture and genetic characterization developed for effective conservation of the plant were also summarized.

1. Introduction

As a result of the unfavorable climatic conditions, salinity, drought, desertification and urbanization, medicinal plants are subjected to endangerment. For the majority of the world's population, medicinal plants are the main source of drugs that cure many diseases and save many lives. In this context, biotechnological techniques are very vital in selection, conservation and multiplication of the important genotypes of medicinal plants[1]. *In vitro*, regeneration plays an important role in the production of high-quality plant-based medicine.

Araceae, also called Arum family, belongs to monocotyledonous flowering plants, in which flowers are borne on a type of inflorescence called spadix. The spadix is usually accompanied by leaf-like bract. The members of Arum family are often known as aroids. This family consists of approximately 3 700 species of 107 genera distributed in the north temperate regions and the old and new world tropics[2]. Araceae species are often tuberous or rhizomatous and are often found to contain calcium oxalate crystals. In this family, many plants are so thermogenic that their flowers can reach up to 45 ℃. This unusually high temperature attracts insects to pollinate the plant and prevents tissue damage in cold regions[3]. The *Arum* genus has 31 species of plants that have been already identified in nature.

Black calla lily, the English name of *Arum palaestinum* Boiss. (*A. palaestinum*) is one of the most important plants in the family Araceae, which is native to Northern Africa, Europe and Western Asia with wide distribution in the Mediterranean region[4]. *A. palaestinum* is a typical cryptic species producing a smell of rotten fruit, since its appendix emits mainly ethyl acetate. In many countries, its aerial parts are edible after drying or soaking in salty water. *A. palaestinum* acts as a kind of ornamental plants and animal fodders and is used traditionally to cure several chronic diseases such as stomach acidity, atherosclerosis, diabetes and cancer[5]. It grows naturally in mountains and hills near water in rocky places, in the upper Jordan Valley and in many spots such as Irbid, Wadi Shua'ib, Jarash, Ajlun, Amman and Al Balqa[5,6]. The plant is endangered due to many factors like forestry practices, excessive

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Foundation Project: Support by Science and Technology Development Fund (Grant No. 4402).
The journal implements double-blind peer review practiced by specially invited international editorial board members.

collection, pollution, inappropriate agriculture, urbanization, habitat destruction, conflicts, climatic changes, desertification, degradation and seed germination difficulty[6]. Therefore, there is a need to establish a reliable strategy for conservation and multiplication of this plant germplasm[7].

2. Tissue culture on *Arum* plants

The term plant tissue culture broadly refers to the aseptic cultivation of any plant segments, single cell and tissue organs[8]. During the last few decades, plant cell and tissue culture have arisen as new techniques in plant biology for many purposes, among which genetic germplasm conservation and *in vitro* production of bioactive substances are used in pharmaceutical industries[9]. Herbal medicines are generally perceived as safe products[10] and some of these safe therapies are used to treat liver diseases, diabetes and jaundice[11]. In the search for alternatives to traditional agriculture in the large scale production of bioactive plant metabolites and biotechnological techniques specially, plant tissue culture is found to be very useful in the production of desirable medicinal compounds from plants[1].

Many studies have reported on tissue culture of the Araceae family such as the *in vitro* rapid propagation of *Acorus calamus, Aglaonema commutatum, Anthurium andreanum, Caladium bicolor, Homalomena aromatica, Homalomena pineodora*[12-18]. There are few records concerning tissue culture of *A. palaestinum*[19,20]. Shibli *et al.*[19] obtained somatic embryogenesis from black lily callus which induced from sterilized corm bud sprouts cultured on basal medium containing 5.4 μmol/L 1-naphthaleneacetic acid and 4.4 μmol/L 6-benzyladenine. The germinating embryos were developed into rooted plantlets, then grown in the greenhouse and adapted effectively with 95% survival rate. Furthermore, the same survival rate of acclimatized plantlets under *ex vitro* conditions was obtained from tissue culture of *A. palaestinum* seeds[20]. Murshage and Skoog medium supplemented with 5.0 mg/L benzyl adenine and 0.1 mg/L naphthalene acetic acid was the best medium for shoots proliferation. The Murshage and Skoog medium containing 1.0 mg/L naphthalene acetic acid and 2.0 g/L charcoal was the best medium in rooting of the regenerated shoots.

3. Biochemical analysis of black calla lily

Polyacrylamide gel electrophoresis (PAGE) is a technique widely used to separate biological macromolecules like nucleic acids or proteins according to their electrophoretic mobility which is a function of charge of the molecule, the length and conformation[21]. Sodium dodecyl sulfate (SDS) is an anionic detergent applied to protein sample to impart a negative charge to linearized proteins. This procedure is called SDS-PAGE. Isozymes are enzymes that catalyze the same chemical reaction, but differ in the amino acid sequence. There is only one report concerning protein analysis of *A. palaestinum*[20] and suggesting that a real genetic change might be occurred due to the difference in protein profiles in the tissue culture explants and mother plant. There are few studies reported on Araceae family, such as the analysis of the phylogenetic relationships and geographical differentiation of Indian taro, *Colocasia esculenta* (L.) by isozyme of seven enzyme systems[22] and also the study of taxonomic relationships, genetics and breeding in *Zantedeschia aethiopica* and *Zantedeschia elliottiana* leaf protein by the analysis of karyotype, SDS-PAGE and DNA content[23].

4. Molecular profiling using DNA markers

Advances in DNA technology in the last three decades have created a wealth of new data that revolutionized biological studies and the molecular markers generated by the new possibilities of genome genotyping. In the last two decades, several PCR-based molecular markers have been made available for biological research[24]. The most common methods are random amplification of polymorphic DNA, inter simple sequence repeats (ISSR) and amplified fragment length polymorphism (AFLP).

There are many reports concerning the molecular analysis to determine the genetic stability, differences and phylogenetic relationship using random amplification of polymorphic DNA, ISSR and AFLP molecular markers of many plants in Araceae family, such as *Acorus calamus, Amorphophallus albus, Amorphophallus rivieri, Anthurium andreanum, Colocasia esculenta, Syngonium podophyllum*[22,25-30].

The use of molecular markers to assess levels of genetic variation of *Arum* species was studied by Makhadmeh *et al.*[5]. Twenty one locations were surveyed to assess the genetic variation. Ten plants from each location were collected and measured directly in the field. In total, 210 plants were studied and three *Arum* species (*Arum palaestinum*, *Arum dioscoroides* and *Arum hygrophilum*) were found in these locations. AFLP technique was used to study the genetic variation between *Arum* species. Six combinations of selective primers generated a total of 2 882 reproducible and clearly scorable bands of different sizes. About 98.6% of them were polymorphic. The overall genetic distance between *Arum* species was 25%. The results showed that *Arum* populations of the same species or having a common genome were grouped in the same cluster regardless of the collection site. The wide range of genetic distance was represented by the high level of DNA polymorphism occurring among *Arum* species. The results attained in this study will be of great importance for further work aiming at establishing a conservation strategy for preserving and maintaining the germplasm of this plant using *in* and *ex situ* gene banks, while Farid *et al.*[20] studied the molecular variation of tissue culture explants and control plant of *A. palaestinum* and the obtained results of ISSR showed variations between the control plant and tissue culture regenerated plantlets.

5. Phytochemical analysis of *A. palaestinum*

The genera of the family Araceae contain various classes of secondary metabolites including flavones, flavonols, C-glycosyl flavone, polyphenols, alkaloids and proanthocyanidins[31,32]. Moreover, the phytochemical screening of *A. palaestinum* extracts revealed the presence of terpenoids, flavonoids and phenolic acids[33]. Its phenolic constituents were investigated using high performance liquid chromatography analysis leading to the

identification of eight flavonoids and two phenolic acids (Table 1) [20].

Various flavonoid compounds were isolated and identified from successive extracts of *A. palaestinum* (Table 1). One alkaloid, (S)-3,4,5-trihy-droxy-lH-pyrrol-2(5H)-one, was isolated from the ethyl acetate extract (EAE) in addition to six phenolics including caffeic acid, luteolin, 3,6,8-trimethoxy-5,7,3',4'-tetrahydroxy flavone, isovitexin, isoorientin and vicenin II[4,34,36].

Three flavonoids including luteolin, chrysoeriol and isoorientin were isolated from the DEE and also identified in the total plant extract[34]. The gas chromatography–mass spectrometry analysis of the same extract led to the identification of 15 compounds representing 95.01% of the extract belonging to different groups of chemical compounds (Table 2)[34]. Moreover, an alkylated piperazine (3α, 5α-diisobutyryl-6α-isopropyl-piperazine-2-one) was isolated from the *n*-butanol leaves extract[37].

Furthermore, the evaluation of the phenolic and flavonoid contents of the methanol, ethyl acetate and chloroform extracts of *A. palaestinum* showed that the highest phenolic (mg gallic acid equiv./g dry extract) and flavonoid (mg quercetin equiv./g dry extract) contents were in the methanol (30.9, 14.4), followed by chloroform (13.7, 11.9) then ethyl acetate (5.3, 8.5) extracts, respectively[38]. Additionally, the phytochemical profiling of *A. palaestinum* leaves via liquid chromatography–tandem mass spectrometry led to the characterization of several metabolites including flavonoids, phenolic acids, terpenoids, iridoids and amino acids[35].

Table 2

Compounds identified by gas chromatography–mass spectrometry from the DEE of *A. palaestinum*.

Compounds	MW (g/mol)	RT (min)	Relative areas (%)	Molecular formula
Butylated hydroxytoluene	220	20.42	1.610	$C_{15}H_{24}O$
Dihydroactinidiolide	180	20.79	0.76	$C_{11}H_{16}O_2$
3-Buten-2-one,4-(4-OH-2,2,6 tri-OCH3-7oxabicyclo[4.1.0]hept-1-yl)	224	22.84	1.41	$C_{13}H_{20}O_3$
(-)-Loliolide	196	23.90	0.79	$C_{11}H_{16}O_3$
Pluchidiol	208	24.12	1.03	$C_{13}H_{20}O_2$
Hexahydrofarnesyl acetone	268	24.56	4.50	$C_{18}H_{36}O$
Hexadecanoic acid, methyl ester	270	25.42	21.89	$C_{17}H_{34}O_2$
Linoleic acid, methyl ester	294	27.10	6.54	$C_{19}H_{34}O_2$
Linolenic acid, methyl ester	292	27.18	22.37	$C_{19}H_{32}O_2$
Phytol	296	27.29	25.29	$C_{20}H_{40}O$
Phytol acetate	338	28.21	5.05	$C_{22}H_{42}O_2$
1-Monolinolenin	352	29.28	1.39	$C_{21}H_{36}O_4$
Cholestan-3-one,cyclic-1,2-ethanediyl aetal,(5á)	430	29.46	0.68	$C_{29}H_{50}O_2$
Bis (2-ethylhexyl) phthalate	390	31.06	1.27	$C_{24}H_{38}O_4$
Quercetin 7,3',4'-tri-OCH3	344	40.58	0.43	$C_{18}1H_{16}O_7$

MW: Molecular weight; RT: Retention time.

6. Biological investigation of *A. palaestinum*

6.1. Antioxidant activity

The antioxidant activity of the EAE of *A. palaestinum* was characterized by its strong 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging activity (half maximal scavenging concentration 3.1 μg/mL)[4]. Both of the aqueous and ethanol

Table 1

Flavonoid compounds isolated from different fractions of *A. palaestinum*.

Compounds	Fractions	Organs	References
Isoorientin	EAE, DEE, TE	Leaves, seeds, aerial parts	[4,20,34]
Vicenin II	EAE, TE	Leaves, seeds	[4,20,35]
3,6,8-trimethoxy, 5,7,3',4'-tetrahydroxy flavones	EAE	Leaves	[4]
Isovitexin	EAE, TE	Leaves, seeds, aerial parts	[4,20,34,35]
Luteolin	DEE, EAE, TE	Leaves, aerial parts	[4,34,35]
Chrysoriol	DEE, TE	Leaves, aerial parts	[34,35]
Orientin	TE	Leaves, seeds	[20,35]
Vitexin	TE	Leaves, seeds	[20,35]
Kaempferol-sophoroside-glucoside	TE	Leaves	[35]
Naringenin-6,8-di-*C*-glucoside	TE	Leaves	[35]
Diosmetin-6,8-di-*C*-hexose	TE	Leaves	[35]
Kaempferol rutinoside	TE	Leaves	[35]
Di-*C*-glucosylluteolin I	TE	Leaves	[35]
Vitexin-*O*-glucoside	TE	Leaves	[35]
Di-*C*-glucosylluteolin II	TE	Leaves	[35]
Kaempferol 3-*O*-arabinosylgalactoside	TE	Leaves	[35]
Kaempferol sophoroside-rhamnoside	TE	Leaves	[35]
Kaempferol trihexoside	TE	Leaves	[35]
Kaempferol-3-*O*-β-(6-*O*-E-pcoumaroylglucoside)-7-*O*-β-hexoside	TE	Leaves	[35]
Kaempferol-3-*O*-[(6-*O*-E-feruloyl)-β-D-glucopyranosyl]-(1→2)-β-D-galactopyranoside	TE	Leaves	[35]
Apigenin trihexoside	TE	Leaves	[35]
Kaempferol-3-*O*-2-(6′-p-coumaroyl) glucosyl rhamnoside	TE	Leaves	[35]
Diosmetin-neohesperidoside	TE	Leaves	[35]
Chrysoeriol dihexoside	TE	Leaves	[35]
Quercetin-3-*O*-β-D-(6-*O*-(E)-p-coumaryl) glucopyranoside	TE	Leaves	[35]
Chrysoeriol-7-β-D-glucoside	TE	Leaves	[35]
Kaempferol-3-*O*-(6-*O*-p-coumaroyl) glucoside	TE	Leaves	[35]
Apigenin	TE	Seeds	[20]
Luteolin 7-glucoside	TE	Seeds	[20]
Quercetin	TE	Seeds	[20]

TE: Total extract; DEE: Diethyl ether extract.

extracts had remarkable antioxidant activities (43.1%, 12.7%), respectively[39]. Recently, the antioxidant activity of the ethanol extracts from different parts of plants including *A. palaestinum* was evaluated and the results showed that *Teucrium creticum* extract is an efficient scavenger of O_2 with IC_{50} = 83 μg/mL followed by *Majorana syriaca*, *Coridothymus capitatus* (*C. capitatus*), *Teucrium capitatum*, *A. palaestinum* and *Urtica pilulifera* (*U. pilulifera*)[40].

In addition, 21 species including *A. palaestinum* were collected from different Jordanian localities and evaluated for their antioxidant activities and showed that the DPPH- 6-hydroxyl-2,5,7,8 tetramethyl chroman-2 carboxylic acid (Trolox) equivalent antioxidant capacity of the methanol extract of the studied taxa were varied from 4.1 to 365.0 mg/g of plant dry weight *vs.* 0.6 to 267.0 mg/g in aqueous extracts. *A. palaestinum* leaves gave moderate DPPH-Trolox equivalent antioxidant capacity activity ranged from 20 to 80 mg/g and showed that the potential role in radical scavenging of these plants' extracts and their antioxidant activities agreed with their uses as traditional anti-diabetic agents by the Jordanian population[41].

6.2. Anticancer activity

There are an increasing number of medical studies suggested that there is a relationship between the dose of flavonoid intake and the risk of cancer disease[42].

Many researchers have carried out *in vitro* studies on the potential anticancer activity of flavonoids in diverse cell systems[43,44].

The effect of ethyl acetate fraction on different human cancer cell lines showed that both lymphoblastic leukemia cells 1301 and human breast adenocarcinoma cell line (MCF7) with IC_{50} of 53.10 and 59.09 μg/mL, respectively have dose-dependent inhibition in cancer proliferation. However, it was found to have no effect on the growth of hepatocellular carcinoma cells (HepG2)[4]. Moreover, the leaves extract of *A. palaestinum* showed a significant cytotoxicity using the sulforhodamine B method against cultured tumor cell lines *in vitro* such as A549 non-small cell lung, SK-OV-3; ovary, SK-MEL-2; melanoma, HCT-15 colon with ED_{50} 4.26, 1.38, 0.51 and 2.47 μmol/L, respectively[37].

Furthermore, *A. palaestinum* with other 75 ethanolic extracts of medicinal plants from the Jordanian flora was estimated for their antiproliferative activities on MCF7 and indicated the possible potential use of medicinal plants from the Jordanian flora as antineoplastic agents[33]. The aqueous extract of *A. palaestinum* gave high anticancer activity more than the ethanolic extract against HepG2 cells[39]. Besides, the antiproliferative effect of the extracts against T-cell lymphoblastic leukemia, Jurkat cells at 24 and 48 h showed a dose dependent reduction in cell proliferation with more significances at 48 h. The IC_{50} values were (17.5 ± 2.1) μg/mL, (19.7 ± 2.8) μg/mL and (23.3 ± 2.8) μg/mL for ethyl acetate, methanol and chloroform extracts, respectively at non-cytotoxic concentrations[38]. Moreover, Zaid *et al.*[45] invented methods for the treatment of human cancers. The daily dosage forms for cancer patients contain from about 10 to 6000 mg of each of isovanillin, β-sitosterol and linolenic acid and the dosage forms are formulated by first creating an aqueous decoction of *A. palaestinum* followed by fortification of the decoction with additional quantities of isovanillin, β-sitosterol and linolenic acid.

Recently, the ethanol extracts from different parts of six plants including *A. palaestinum, C. capitatus, Majorana syriaca, Teucrium capitatum, Teucrium creticum and U. pilulifera* were investigated for their antitumor activities[40]. *U. pilulifera* showed a maximum cytotoxic activity with IC_{50} of 63 μg/mL followed by *C. capitatus* and *A. palaestinum* and also showed the highest amount of total phenolics and the second highest total flavonoids.

Moreover, the cytotoxic activity of fractionated extracts and four isolated flavonoid compounds including luteolin, chrysoeriol, isovitexin and isoorientin from *A. palaestinum* against MCF7, HepG2, epidermal carcinoma of larynx and human cervical carcinoma cell lines were evaluated and the results showed moderate antitumor activity in comparison with the isolated fractions[34]. Also, the effects of isoorientin in rat isolated aorta, trachea, ileum and uterus and in the uterus of guinea-pig were investigated and showed that isoorientin (10^{-7} mol/L – 6×10^{-4} mol/L) did not affect the isolated aorta, trachea or ileum but caused concentration-dependent inhibition of the amplitude and the frequency of the phasic contractions of the uterus[36].

The antitumor efficacy of synthetic formulations of *A. palaestinum* extract (GZ17-S, -05.00 and -06.02) in preclinical models of head and neck squamous cell carcinoma and the preliminary data demonstrates that these formulations have antitumor efficacy in several tumor types including head and neck squamous cell carcinoma, lung and ovarian cancer cells and hypothesized and GZ17-06.02 inhibits phosphorylation of key molecules inducing the apoptotic cell death[46].

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

Financial support of Science and Technology Development Fund (US-Egypt Joint Fund) (Grant No. 4402) is highly appreciated.

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