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Callus formation and isolation of cyclodecane from tissue culture of *Apium graveolens* L.

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ABSTRACT

Nowadays, isolation of useful natural products from plant callus culture has been used as a modern biotechnological technique to obtain those useful chemicals. *Apium graveolens* (Apiaceae) is a well known edible herb was known to produce a variety of plant secondary metabolites such as flavonoids and coumarins. In this study, we focused on induction of callus tissue of the plant seedling that may be used as a source of different metabolites. The seeds of the plants after sterilling were cultured in a petri dishes line with MS medium. After emergence of seedling, epicotyl segments were transferred to another MS cultures with contain different combination of plant hormones, *kin* and *2,4-D*. The petri dished incubated in a growth chamber at 25ºC and certain photoperiod Weight of produced callus were measured for all treatments. On the other hand, n-hexan extract of dried callus was obtained by a soxhelet apparatus and were analyzed using thin layer chromatography technique (TLC) to afford a colorless oily substance. The structure of isolated compound was elucidated by spectroscopic methods such as IR, UV, Mass and ^{13}C and ^{1}H NMR.

Our results indicated that although callus induction take place in *MS* medium without phyto hormones, maximum callus production induced at MS medium with 2, 4-D (4 mg/L) and kinetin (2mg/L). The structure of isolated natural product from the callus was determined as cyclodecane. It was be concluded that callus tissue of *Apium graveolens* can be source for production of cyclodecane.

KEYWORDS

Apium graveolens;
Callus;
Cyclodecane.

INTRODUCTION

During the last decades, plant tissue culture is regarded as new technique to plant propagation, breeding programs or regeneration of transgenic plants. This modern technique has also been used for production of plant secondary metabolites, in turn, may be utilized in medicine or pharmaceutical industries. Hence plant tissue culture has now direct commercial applications as well as value in basic research into cell biology, genetics, biochemistry and pharmaceutical sciences[1].

Callus formation is the first event in plant tissue culture process. Callus is a mass of unorganized parenchyma. It derived from explants of mature plant tissue on a gel medium enriched with agar. Macronutrients, micronutrients, vitamins and phytohormones. It has been well documented that calli may be source to production of plant secondary metabolites[2].

Apium graveolens L., Celery, is a biennial herb of *Apiaceae* family. It has extensively cultivated in all over

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of the world and has been used as a common vegetable and a spice in different countries. Wild celery has a wide distribution extending from north Europe to Africa and Asia. The wild plant is bitter and was probably used for medicinal propose before gaining popularity as a herb and a vegetable[3]. Both the roots and seeds of celery were used medicinally, especially in the treatment of fevers, jaundice, pains in the chest and diarrhea. It is ascribed that the celery seeds exhibit antiseptic, antiinflamatory, abortifacient and sedative activities[4].

In the present work, we focused on induction of callus tissue of celery and isolation of a chemical from it.

EXPERIMENTAL

Plant culture and callus induction

The seeds of *A. graveolens* were purchased from Pakanbazr Esfahan Company. The seeds were surface sterilized with sodium hypochloride(1%) and ethanol 70% and then washed three times by distillated water. The steriled seeds put in pteri dishes lined with wathman no1 papers. After seed germination, epicothyl of seedlinfs were isolated and transfer to solid MS media supplemented with vitamins, sucrose (3%) and different concentration phytohormones. Four concentration of 2,4-D and kinetin (0,0; 2,1; 3,1; 4,2 mg/ml) were used in different cultures. Tree replication of any culture were used. The pH of the mediums was adjust to 5.7 with NaOH or HCl before autoclaving at 120 ºC for 15 min. After then, Isolated radicles segments were incubated under photoperiod course of 8/12 h dark/light with 1200 lux of light regime at 25±1ºC. The obtained callus tissue from each of cultures were sub cultured onto fresh medium every tree weeks. The fresh weight of produced callus was measured for any treatment after 12 weeks.

Analytical methods

Dried callus of *A. graveolens* (30g) was soxhlet extracted successively, with n-hexane, dichloromethane and methanol. The hexane extract (1g) were purified by preparative silica TLC using $(CH_3)_2CO$ - $CHCl_3$, 99:1 as the mobile phase. Vanillin-sulfuric acidwas used as reagent for detection process. This procedure conduce to afford a liquidly substance (21mg)with R_f value of 0.45. The compounds were identified by comparing their UV, ^1H NMR, ^{13}C NMR, IR amd Mass spectral data with those of published data[5].

NMR spectra were recorded in $CDCl_3$ on a DRX-500 Avance instrument (500MHz for ^1H and 125MHz for ^{13}C) using the residual solvent peak (δ 3.31 ppm) as internal standard. Preparative TLC was performed on RP-18 GF_{254} plates (20×20 cm, Merck).

Statistical analysis

In thetreatment assays, SPSS 11.5 software was used for statistical analysis. Analysis of variance (ANOVA) followed by Duncan test was used to see the different amongst various groups. The significance level was set at $p<0.05$.

RESULTS

Our results showed that although callus induction of *Apium graveolens* epicotyl took place in MS medium without phytohormones, callus growth increased in the presence of 2,4-D and kinetin. The callus growth occurred in a dose dependent manner of phytohormones. The maximum growth of the callus was observed at 4 mg/mL of 2,4-D and 2 mg/mL of kinetin (Figures 1,2).

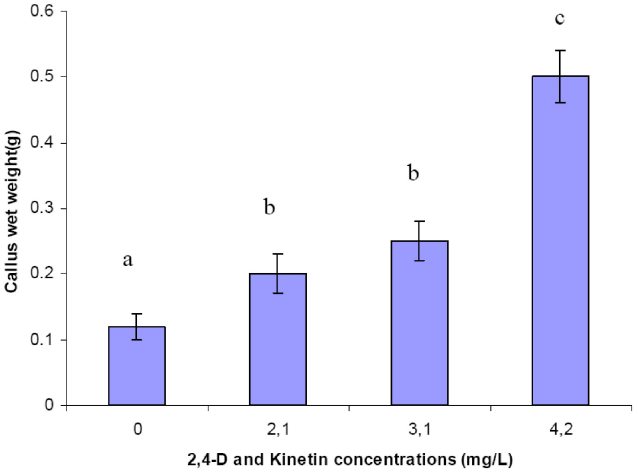

Figure 1 : Effects of different concentrations of 2,4-D and kinetin in production of callus tissue of *Apium graveolens*. Mean values with same latters are not significantly different at the 0.05 level according to the Duncan test

The results of analytical process on callus n-hexan extract deduced to introduce a cycloalkane derivative, cyclodecane, (Figure 3). The spectral data of the isolated compound revealed two singlet peaks at 1.25 and 28.7 ppm in HNMR and CNMR spectra, respectively. These peaks represent –CH2 groups of cycloalkanes

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(Figure 3). In the IR spectrum, peaks at 2850 and 2910 cm^{-1} is consistent with –CH2 groups. On the other hand, the peaks at 57, 71,85,99, 127 and 141m/z are related to fragments of a cycloalkane derivative. Ion molecule of 141m/z indicate that the compound has structurally 10 atom carbone. The structure of this isolated compound was confirmed with comparison of its spectral data with dose of literature[5].

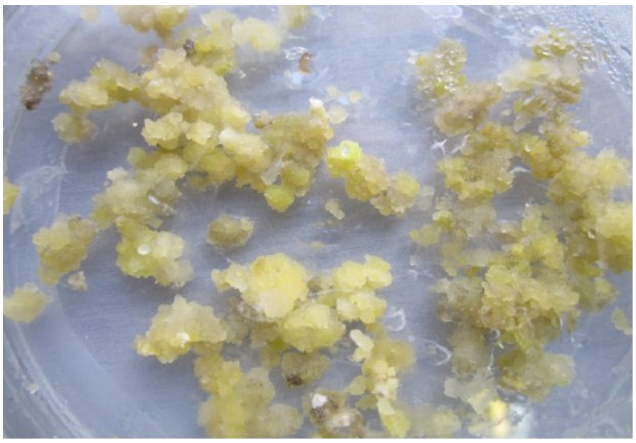

Figure 2 : Callus tissue of *Apium graveolens*

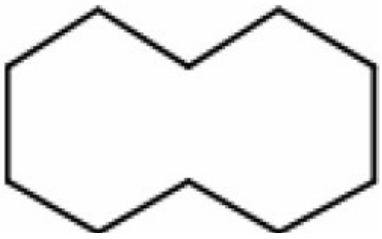

Figure 3 : Structure of cyclodecane

DISCUSSION

A literature survey showed that callus formation was previously induced from *Apium graveolens* anthers on B5 medium supplemented with 2,4-D and BA hormones[6]. To our knowledge, our work is the first report on callus formation from hypocotyl segments of *Apium graveolens*. It was previously shown that 2,4-D and kinetin are the most commonly used hormones for formation of callus tissue in different plant species[7]. Our results indicated that callus production of *Apium graveolens* increased by increasing both hormones together in the media. Extension of one of the hormones in the media without another one did not indice significant effect on callus growth.

It was well known that *Apium graveolens* contains a number of secondary metabolites such as coumarins, furanocoumarines and flavonoides in aerial parts and roots[4]. Hence, the plant parts exhibit various biological and pharmacological activities[3]. The aerial parts of the plant produce essential oil enriched with aliphatic hydrocarbons and alkanes with antioxidant and insecticidal effects[8]. Our results indicated that the callus obtained from epicotyls of *Apium graveolens* can be a source for biosynthesis of cycloalkane derivatives. It was also previously pointed out that in vitro culture of *Apium graveolens* can be origin of the production of flavors and different metabolites[9,10]. This is the first report of the isolation of a cycloalkane derivative from celery callus. However, some cycloalkanic compounds were isolated previously from liquid cultures of some plants or fungi using GC-MS technique[11,12].

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