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Tissue and Cell Cultures of *Hypericum undulatum* for the Production of Acetylcholinesterase Inhibitors

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Hypericum undulatum Willd. (*Guttiferae*), general name wavy St. John's wort, is a medicinal plant. It is traditionally used for renal antispasmodic, hepatic protector, and the treatment of migraine, bladder and gall bladder ailments and intestinal-inflammatory. Alzheimer's disease (AD) is frequent in elderly people, being the leading cause of dementia among older people. An estimated 10% of the world's population over the age of 65 years is afflicted by AD. Acetylcholinesterase inhibitors (AcChEI) are currently the best available pharmacotherapy for AD patients. Presently, treating the symptoms of AD can only delay the progress of the disease. In addition, all the present medicines for AD have side effects. Therefore, it is of importance to screen for more powerful drugs from natural products to treat AD with fewer side effects. A recent work has demonstrated that *H. undulatum* plant has the AcChEI activity. In this work, cell suspension cultures of *H. undulatum* were established for the production of the AcChEI. Seeds were sterilized and aseptically germinated on MS medium solidified with agar without plant growth regulator. The germinated plants were maintained and used for *callus* induction. The best medium for *callus* induction and growth was MS plus 1 mg/l 2,4-D, 1 mg/l NAA and 0.2 mg/l 6-BA. Dispersed white *calli* were transferred to the same medium but without agar to establish cell suspension cultures. The suspension cultured cells turned dark and formed big cell blocks after subculture more than two months. Different cultivation parameters were tested to optimize the cell growth for a continuous culture. Kinetics of cell growth and sugar consumption was analyzed. The AcChEI activity of the plant cell extract was determined by capillary electrophoresis. The results shown that upon metabolic regulation by elicitors the suspension cultured plant cells had a higher AcChEI activity than that of the plants.

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Cloning of Pharmaceutical *Cannabis* through an Aeroponic Propagation System

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Cannabis sativa L. is an important pharmaceutical species because it is the only source for a whole series of chemically diverse bioactive compounds that are currently under intensive investigation. Cuttings of pistillate plants is the preferred propagation material for the pharmaceutical production to ensure continuous chemotype correspondence of clonal progenies. Aeroponic propagation gave satisfactory results on cloning of many economic important plant species and for that reason the aim of this study is to evaluate the feasibility of its use in the cloning phasis of *Cannabis* pharmaceutical production. Two experiments were conducted to evaluate the rooting capacity of three different stock plants ('mostly *sativa*', '*sativalindica* hybrid', 'mostly *indica*') and the rooting capacity of cuttings taken from three different positions (top, middle, bottom) of the stock plants used. Stock plants were selected from recreational strains on the basis of Δ9-THC yield per crop area unit and kept in vegetative stage in artificial growing conditions. The aeroponic propagation system resulted easy to use and efficient to observe root initiation as the cuttings remain suspended in the air. Significant differences on rooting capacity were found between the different stock plants used and the different positions from where the cuttings were taken. The highest percentage of rooted cuttings was obtained from a 'mostly *sativa*' biotype (80%), followed by a 'mostly *indica*' biotype (70%), and the lowest value was obtained from a 'hybrid *sativalindica*' (67%). Direct correlation was found between the percentages of rooted cuttings and the average of root lengths after 14 days from the edge of cuttings. Cuttings taken from the bottom position of stock plants had the highest rooting capacity. Cuttings were ready for transplantation in 14 days without the application of plant growth regulators. Despite the satisfactory results obtained, further research is needed to optimize the technique.

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In vitro Culture of *Coriandum sativum*

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Coriander (*Coriandrum sativum* L.) is a plant from the *Umbellifera* family. In Portugal, the use of coriander plants in gastronomy is very common, like in all the countries in the Mediterranean area. This plant species has also several other applications than as an aromatic plant, such as medicinal, being recommended for dyspeptic complaints, loss of appetite, convulsion, insomnia and anxiety. Moreover, the essential oils and various extracts from coriander have been shown to possess antibacterial, antioxidant, antidiabetic, anticancerous and antimutagenic activities among others, it has also been used as a flavoring agent in food products, perfumes and cosmetics. To study the potential use of this plant all over the year it is necessary to establish an *in vitro* system production and to evaluate the better conditions for its growth. *In vitro* coriander cultures were started from seeds of *Coriandum sativum* from a commercial origin. Seeds were inoculated in a MS medium containing different concentration of IBA and BAP. After 6 months of *in vitro* culture, the plants were separated in two lots named lot A and B differentiated by their pigmentation (clones with differentiation in flavonoids accumulation), being lot B the less pigmented and lot A the one who presented an higher purple coloration, under the same *in vitro* growth conditions (nutrition, temperature and light). The growth rates of both lots were determined through fresh and dried weights and evaluating how pigmentation affects these parameters. The medium with better growth rates was MS with 0.1mg/L IBA and 0.1mg/L BAP; The B lot grew better but have the stationary phase after 3 weeks while the A lot was still growing after 4 weeks but grew slower comparing wit lot B. These results will be used in further studies concerning the essential oils production in each lot.

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Determination of Antibacterial and Antiradical Activity of *Origanum vulgare* Clones Grown in Latvia

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Oreganum vulgare is widely used in pharmacies and folk medicine. *Origanum vulgare* clones grown in Latvia differ from their phytochemical content. The aim of the study was to determine the antimicrobial and antiradical activity of these clones. Antibacterial and antiradical activity of ethanol extracts prepared from 10 *Origanum vulgare* clones grown in Latvia was screened. The antibacterial activity was assessed against bacteria (*Escherichia coli, Pseudomonas aeruginosa, Staphylococcus epidermidis, Bacillus cereus*). A sensitivity tests were performed in the liquid nutrient media for bacteria. Plant ethanol extracts from leaves and flowers (25-40 g of fresh matter per L) were added to the growth media. Extract and media proportion was 1:20. Microorganisms growth were detected spectrophotometricaly at wavelength 550 nm after 24 and 72 hours of incubation at 28 °C. Plant extract antiradical activity was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH). *Oreganum vulgare* leaves and flowers showed different activity. The antimicrobial and antiradical activity depends on *O. vulgare* clone and sampling time. Different clones showed unlike activity on used microorganisms and it depends on oregano chemical content.