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New approach for production of volatile and acephenanthrylene constituents (anti-breast cancer) using biotic and abiotic elicitors in cell cultures of *Pimpinella anisum* L.

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Dimpinella anisum L. (anise) is a worthy aromatic medicinal plant involving medical and pharmacological potentials. Γ Our study aimed to apply the biotechnological techniques using biotic (yeast) and abiotic (L.phenylalanine) elicitors for enhancement callus productivity of anise and enrichment their content of biological efficient phytochemical constituents to be applicable in medical and pharmacological purposes. Total 15 of volatile constituents and acephenanthrylene skeleton were detected comparatively in callus cultures of Pimpinella anisum L. and were identified by GC-MS. Cytotoxicity of the extracts for suppression breast cancer cell lines (MCF7) proliferation was evaluated. Leaf, stem and shoot tip *explants* of in vitro seedlings were examined for callus initiation, shoot tip was the best on medium containing 1mg/l IAA+1mg/l 2.4-D+2mg/l kin. Fresh weight, growth rate and increase value of shoot tip calli were maximum values (18.356±0.269, 2.407±0.038, and 11.237±0.179, respectively) after 4 weeks as the optimal period for sub-culture. Shoot tip calli were improved by culturing on medium containing either yeast at 0.5, 1.0, 1.5 g/l concentrations or L.phenylalanine at 2, 4, 6 mM/l concentrations. Treatments of yeast (0.5, 1.0, 1.5 g/l) and 2mM/l L.phenylalanine increased callus fresh weight (17.556±0.195, 21.864±0.428, 26.92±0.440 and 13.734±0.199, respectively) compared to calli grown on medium free elicitors (control) which recorded 12.996±0.284. Total volatile constituents content were boosted to be 32.38%, 40.96%, 43.51% in treatments of 2, 4, 6 mM/l L.phenylalanine, respectively and 45.45%, 31.09%, 26.01% in treatments of 0.5, 1.0, 1.5 g/l yeast, respectively, in comparison to the control (16.83%). 1,2-Diphenyl-5-(t-butyl) acephenanthrylene was detected in all treatments at different proportions. Anise callus extracts possessed high positive efficiency against breast cancer prevalence. Cytotoxic activity of callus extracts was between IC50 of 1.33 µg/ml to IC50 of 2.39 µg/ml. From the phytocomponents point of view, the percents of acephenanthrylene and volatile constituents in anise callus contributed to their anticancer influence.

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