

METHOD FOR ISOLATING MICROVESICLES FROM PLANTS OF
THE AMARANTHACEAE FAMILY

A method for isolating microvesicles from plants of the Amaranthaceae family.

5 The invention relates to methods for isolating biologically active substances from raw plant matter, in particular to methods of obtaining microvesicles (50–1200 nm in diameter) from plants of the Amaranthaceae family. The claimed method can be used in the medical, cosmetics and food industries.

10 From the state of the art a method is known for isolating microvesicles from a culture medium, including centrifugation, successive ultracentrifugation, dissolving the precipitate obtained in FSB and repeated centrifugation; see p. 25, first 2 paragraphs from the reference [https://www.researchgate.net/publication/323269152_Isolation of extracellular microvesicles from cell culture medium_Comparative_evaluation_of_methods](https://www.researchgate.net/publication/323269152_Isolation_of_extracellular_microvesicles_from_cell_culture_medium_Comparative_evaluation_of_methods).

15 The main disadvantage of the method listed under paragraph 1 on p. 25 is an inadequate effect, since this method is not able to separate microvesicles according to size from the remaining particles in the precipitate obtained. Another disadvantage is the use of a cell culture medium as a source material for isolation of exosomes, while obtaining the medium requires the consumption of certain
20 reagents (nutrient medium, serum, L-glutamine, sodium pyruvate, antibiotics, solutions for washing such as Hank's solution, ordering and obtaining of registered cell lines, handling of the cell lines or obtaining a cell culture with the subsequent need to describe this cell culture. These stages require some expenditure of materials and time, which makes this method for obtaining microvesicles time-
25 consuming and expensive.

 The technical result of the method for isolating microvesicles that we have proposed is the possibility of isolating microvesicles from plants of the Amaranthaceae family.

30 The above-mentioned technical result is achieved with the use of a method of isolating microvesicles in which plants of the Amaranthaceae are collected and ground to obtain a homogenized mixture, from which by means of a coarse filter the liquid phase is then separated and successively centrifuged for 30 minutes at 3000 g and for 60 minutes at 10 000 g respectively to remove large particles – cell

conglomerates and individual cells – from the mixture obtained. The extracted supernatant then undergoes ultracentrifugation for 90 minutes at 150 000 g, and the precipitate obtained is then suspended in a sodium phosphate buffer in the ratio 1:10 and again undergoes ultracentrifugation for 90 minutes at 150 000 g. The
5 ultracentrifugation stages are required for removal of apoptotic bodies, protein conglomerates and proteins >30 kDa from the mixture. The precipitate obtained is then again suspended in sodium phosphate buffer in the ratio 1:10, which is followed by filtration using a filter with a pore diameter of 1.2 µm, allowing microvesicles of the required size – up to 1200 nm – to be separated from the
10 remaining particles in the precipitate obtained. Furthermore, we have established experimentally that with this sequence of operations and systems the claimed method enables microvesicles from plants of the Amaranthaceae family to be isolated in the most effective way.

Example of performing the claimed method:

15 To an automatic grinder are added 10 g of leaves, flowers and roots of plants of *Amaranthus blitoides* S. Wats (any proportions can be used; achievement of the claimed technical result is not affected, since the microvesicles are contained in the different parts of the plants in approximately equal quantities), with grinding to obtain a homogeneous (uniform) mixture. The liquid phase is then separated by
20 filtration through a screen with a pore diameter of 1–2 mm. Successive centrifugation is then carried out for 30 minutes at 3000 g and for 60 minutes at 10 000 g respectively. The precipitate obtained is then suspended in a sodium phosphate buffer in the ratio 1:10 and ultracentrifugation is again carried out for 90 minutes at 150 000 g. The precipitate obtained is again suspended in a sodium
25 phosphate buffer in the ratio 1:10, following by filtration using a filter with a pore diameter of 1.2 µm.

Then 500 µl of the solution obtained is analysed by means of photon correlation spectroscopy (Nanophox Sympatec GmbH, Germany), where sizes in the region of 50–1200 nm are detected, indicating the presence in the solution of
30 microvesicles of the claimed size.

CLAIMS

1. A method for isolating microvesicles in which plants of the Amaranthaceae family are collected and ground to obtain a homogenized mixture, from which by means of a coarse filter the liquid phase is separated and
5 successively centrifuged for 30 minutes at 3000 g and for 60 minutes at 10 000 g respectively. Ultracentrifugation of the extracted supernatant is then carried out for 90 minutes at 150 000 g, and the precipitate obtained is then suspended in a sodium phosphate buffer in the ratio 1:10 and
10 ultracentrifugation is again carried out for 90 minutes at 150 000 g; the precipitate obtained is again suspended in a sodium phosphate buffer in the ratio 1:10, followed by filtration using a filter with a pore diameter of 1.2 μm .