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# DEVELOPMENT OF TECHNOLOGY FOR OBTAINING DRY EXTRACT FROM MULBERRY BRANCHES

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# ABSTRACT

Natural product compounds have recently received significant attention from the scientific community due to their potent effects on inflammatory diseases, including cancer. A significant number of studies, including preclinical, clinical and epidemiological studies, have shown that consumption of polyphenols, found in large quantities in grains, legumes, vegetables and fruits, can prevent the development of a number of diseases, including cancer.

#### **KEYWORDS**

A significant number of studies, including preclinical, clinical, an auxiliary, not vital role.

#### **INTRODUCTION**

Currently, more than 8,000 phenolic compounds have been discovered, more than half of which belong to the flavonoid family, making it the most important family of polyphenols, tannins, lignans, as well as stilbenes. Polyphenols are very widespread molecules in the plant kingdom. These are secondary metabolites that will play an auxiliary, not vital role. It is these phenolic compounds that will be responsible for the taste and nutritional value of fruits and vegetables. Due to the presence of hydroxyl chemical groups associated with benzene rings (or phenols), plant

polyphenols (also called phytophenols) have important antioxidant properties. Living cells naturally produce oxidative compounds such as free radicals, which are highly reactive singlet electrons (example: superoxide anions, hydrogen peroxide, etc.). These free radicals play a dual role: one protects the body by having a bactericidal or antiviral effect (produced by macrophages), and the other has a harmful effect by altering the basic macromolecules of life: DNA breaks, lipid peroxidation or even protein oxidation. These free radicals are mainly produced by the mitochondria, where oxygen produced during respiration is

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converted into superoxide anion. Their toxic effect underlies the transformation of healthy cells into cancer cells, as well as cellular aging. Thus, polyphenols will capture singlet electrons, making them mobile within the polyphenol molecule and therefore much less reactive towards neighboring molecules.

The results showed that the content of resveratrol in currants was only 1.9 mg/kg, mulberry roots were 32.45 and 3.15  $\mu$ g/g, respectively, which is significantly higher than in mulberry fruits (0.48 and 0.0020  $\mu$ g /g) and mulberry branches (5.70 and 0.33  $\mu$ g/g).

Other sources of resveratrol include: Cocoa beans and therefore dark chocolate, containing 0.4 mg/kg; Cranberry: juice contains 0.27 mg/kg; Raw peanuts contain up to 0.147 mg/kg (after roasting, peanuts lose most of their resveratrol content); Rhubarb, pomegranate and blackberries also contain small amounts of resveratrol.

Resveratrol exists in nature in two forms: cisresveratrol and trans-resveratrol. It is in the second form that it is most active, since its antioxidant activity is 7 times higher than that of cis-resveratrol.

Extracts obtained from mulberry branches do not contain emodin or other irritating active ingredients and do not require the use of advanced purification methods.

For this purpose, the proposed process is an extraction process of resveratrol from mulberry branches.

To extract resveratrol, mulberry branches were airdried for at least two months; branches air-dried for three months gave very good results. Preferably the branches used have a moisture level of less than 20%, preferably less than 5%.

The invention also proposes to extract resveratrol using a process in which, after the drying step of the mulberry branches, an extraction step is carried out using a solvent selected from a set comprising products having a carbonyl radical, such as, for example, esters, ketones and alcohols are provided. method, the extraction is preferably carried out in the absence of water. This is contrary to all known processes of the prior art, where the solvent used for the extraction of resveratrol is always an aqueous solvent. The solvent used to carry out this extraction may be, for example, ethanol or acetone, both of which give very good results, but acetone is the preferred solvent.

To increase the extraction yield, several sequential contacts can be carried out, as well as continuously with countercurrent circulation of crushed branches and extraction solvent.

Preferably, the method according to the invention includes, after the extraction stage, a primary purification stage, in which an aqueous-alcohol mixture is used as the primary purification solvent. The solvent used is, for example, a mixture of water and ethanol.

To obtain an even more concentrated product, the process may include an additional secondary purification step in which a water-alcohol mixture having a lower alcohol content than the alcohol content of the primary purification solvent is used as the secondary purification solvent. just be water

After the extraction step and/or after the primary purification step, the solvent used in this step is, for

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example, evaporated. An embodiment provides that this solvent is distilled in the presence of water to precipitate a solid.

The preferred extraction process from branches dried for three months in the open air is described below, with some numerical values obtained.

To carry out the extraction of resveratrol, the rate of solvent by weight, which must be sufficient to impregnate the entire solid and allow stirring of the mixture, is usually from 5 to 15. Contact is carried out at a temperature between room temperature and the boiling point of the solvent. The contact time between the plant material coming from the mulberry branches and the extraction solvent ranges from a few hours to 24 hours.

The solvent and plant material from the mulberry branches are then separated by filtration and the solvent is removed by evaporation. The resulting crude extract has a resveratrol content of 1 to 5% and has a greenish resinous texture.

The crude extract is then purified to produce a less colored powder product. Primary purification is done by dissolution in a solvent, which is a water-alcohol mixture. An alcohol is an alcohol that is miscible with water, such as ethanol. The alcohol content ranges from 10 to 80%. Dissolution is carried out at a temperature from 20 to 40°C. Impurities insoluble in this solvent are separated from the supernatant by centrifugation.

The solid obtained after removing the solvent from the supernatant by evaporation is a light brown powder with a resveratrol content of 7 to 20%. The ratio of the mass content of resveratrol to the mass content of  $\varepsilon$ -vinifera in the purified extract is of the order of 1

This extract is then purified a second time by dissolving certain impurities in a secondary purification solvent. This solvent is water or a hydroalcoholic solvent with a lower alcohol content than the primary cleaning solvent. Dissolution is carried out at temperatures from 20 to 40°C. The supernatant is separated from the insoluble portion by centrifugation. The solid obtained after removing the solvent from the supernatant by evaporation is a light brown powder containing 1 to 3% resveratrol. the ratio between resveratrol content and  $\epsilon$ -vinifene content is between 0.25 and 1. The purified extract obtained after drying the insoluble part is light brown in color and in the form of a powder. The resveratrol content ranges from 10 to 50%. The present invention also relates to a natural extract of mulberry branches obtained by the method described above. This natural extract of mulberry branches has, for example, a resveratrol content of more than 1%, preferably more than 10%.

One kilogram of mulberry branches of the variety Morus alba L, air-dried for four months and having a moisture level of 4%, is crushed to a particle size of less than 4 mm. The resulting ground solid is mixed with 7 L of ethanol. Extraction is carried out for 20 hours at a temperature of 30°C, with moderate stirring.

The solid and supernatant are separated by filtration. After evaporating the ethanol, 28 grams of crude extract is obtained, which has a greenish color, sticky appearance and a resveratrol content of 3%.

The crude extract is treated with 225 milliliters of a mixture of water and ethanol at a concentration of 50 vol.%. The mixture is kept at 25°C with stirring for one hour, then the undissolved solid and supernatant are separated by centrifugation.



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After removing the solvent from the supernatant by evaporation, 8 grams of purified extract containing 10% resveratrol are obtained. The purified extract is light brown in color and is available in powder form.

The purified extract is dissolved in 225 milliliters of water. The mixture is kept at a temperature of 25°C with stirring for one hour. The undissolved solid and supernatant are separated by centrifugation. After drying the undissolved solid, 2.5 grams of extract containing 28% resveratrol is obtained. The purified extract is light brown in color and is available in powder form. After removing the water from the supernatant, 5.5 grams of extract containing 2% resveratrol is obtained. The purified extract is light brown in color and is available in powder form. After removing the water from the supernatant, 5.5 grams of extract containing 2% resveratrol is obtained. The purified extract is a light brown powder.

#### Example two

One kilogram of mulberry branches of the variety Morus alba L, air-dried for three months and having a moisture level of 12%, is crushed to a particle size of less than 4 mm. The resulting crushed material is mixed with 7 liters of acetone

Extraction is carried out for 20 hours at a temperature of  $25^{\circ}$ C, with moderate stirring.

The solid and the supernatant are separated by filtration. After evaporating the acetone, 25 g of crude extract is obtained, which has a greenish color, sticky appearance and a resveratrol content of 3%.

The crude extract is treated with 250 milliliters of a mixture of water and ethanol at a concentration of 50 vol. The mixture is kept at 25°C with stirring for one hour, then the undissolved solid and the supernatant are separated by centrifugation.

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After removing the solvent from the supernatant by evaporation, 7 g of purified extract with a resveratrol content of 11% is obtained. The purified extract is light brown in color and is in the form of a powder.

The purified extract is dissolved in 250 milliliters of water. The mixture is kept at a temperature of 25°C with stirring for one hour. The undissolved solid and supernatant are separated by centrifugation. After drying the undissolved solid, 2.5 grams are obtained. extract containing 25% resveratrol. The purified extract is light brown in color and is in powder form. After removing the water from the supernatant, 6.5 grams of extract is obtained. extract containing 4% resveratrol. The purified extract is light brown in color and is in powder form and is in powder form.

### Example three

Tests were carried out on the following three mulberry varieties: Mórus álba, Morus nigra L, Mórus rúbra,. For each of these mulberry varieties, resveratrol extraction was carried out according to the same operating protocol. Tests were carried out on A) fresh branches, cut less than fifteen days ago and with a moisture level of about 50%, B) fresh branches, cut less than fifteen days ago, chopped and dried in an oven at a temperature of 40 ° C to a moisture level of less than 3%, and C) branches dried naturally in the open air for three months and having a humidity level of 3 to 12%.

A total of twelve separate extractions were performed. The extraction process is described below for 500 g of mulberry branches.

The branches are first crushed to a particle size of less than 4 mm. Of course, the branches that were chopped and dried in the oven were not chopped a second time.



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Shredding fresh, steamed branches is the same as chopping fresh and dried branches.

Then the chopped branches are macerated in 3 liters of acetone at 30°C for 12 hours. The mixture thus obtained is then filtered and the resulting solid is evaporated to dryness with acetone.

The resulting crude extract is then dissolved in 150 ml of a mixture of water and ethanol with a concentration of 50 vol.% ethanol. This mixture is kept at 25°C for one hour and stirred. The undissolved solid and supernatant are then separated by centrifugation.

By evaporating the solvent, a solid containing resveratrol is obtained.

The resulting solid was then ground for analysis. The results of this analysis are summarized in the table below. The table below shows that the highest yields are obtained from treatments using dry branches. They are significantly higher even if the branches were dried in the open air for three months.

# **Example four**

This option does not involve evaporation to dryness after the extraction and first purification phase, but rather distillation of the solvent in the presence of water to precipitate the solid.

After concentrating the extraction solvent, water is added to the mixture, and solvent removal is continued by distillation so that at the end of the operation, the solvent content in the mixture is less than 5% and the volume of the mixture is from 25 to 500 milliliters per kilogram (ml/kg) of mulberry branches. An equivalent volume of cleaning solvent is then added to the mixture. The mixture is continued to stir for one hour, then the undissolved solid is separated from the mixture by filtration or centrifugation.

The purification solvent is then removed from the supernatant by distillation, during distillation water is added to the mixture so that at the end of the operation the solvent content of the mixture is less than 5% and the volume is between 25 and 500 milliliters per kilogram of mulberry branches. The precipitated solid is separated from the mixture by filtration. An aqueous phase and a wet solid are obtained. After drying and grinding the solid, a brown powder with a resveratrol content of 15 to 50% is isolated, with a resveratrol content of 15 to 50%. the ratio between the content of resveratrol and ε-vinifera is from 0.25 to 1. After evaporation of water from the liquid mixture, a brown powder is obtained with a resveratrol content of 1 to 3% with a ratio between resveratrol. Many options can be considered depending on the circumstances of use, taking into account operating parameters of various stages of the process.

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