

**A process for producing Ginkgo extracts with a high content of
proanthocyanidins and food supplements and medicaments comprising
Ginkgo extracts**

The present invention describes an improved multistage process for producing an extract from leaves of Ginkgo biloba and the use thereof as medicaments or food supplements.

Extracts from the leaves of Ginkgo biloba have been used as medicaments for decades. The medicaments are currently used for the treatment of various types of dementia and the symptoms thereof and of cerebral and peripheral circulatory disturbances. Ingredients with which the activity is linked are terpene lactones (ginkgolides A, B, C and bilobalid) and glycosides of flavones (quercetin, kampferol and isorhamnetin).

Extraction processes relevant for Ginkgo biloba are described in EP 431536 B1 and in EP 360556 B1.

According to claim 1 of EP 431536 B1/DE 3940092 A1, the process claimed therein for producing an extract from Ginkgo biloba leaves is characterized in that

- (a) fresh or dried green leaves of Ginkgo biloba are extracted with water-containing acetone, a water-containing alkanol having 1 to 3 carbon atoms or anhydrous methanol at a temperature of about 40 to 100°C,
- (b) the majority of the organic solvent is separated from the extract to a content of at most 10% by weight, wherein water is added in the last distillation stages, if necessary,
- (c) the remaining concentrated aqueous solution is diluted with water to a solids content of 5 to 25% by weight, cooled with stirring to a temperature below 25°C, allowed to stand until a precipitate is formed and the precipitate formed, consisting of the lipophilic constituents sparingly soluble in water, is separated,

- (d) ammonium sulfate is added to the remaining aqueous solution and the solution formed is extracted with methyl ethyl ketone or a mixture of methyl ethyl ketone and acetone,
- (e) the extract obtained is concentrated to a solids content of 50 to 70% and the concentrate obtained is diluted with water to a solids content of 5 to 20%,
- (f) the solution thus obtained is extracted in multiple stages with a water-immiscible butanol or pentanol,
- (g) the butanol or pentanol phases are concentrated to a solids content of 50 to 70%,
- (h) the concentrate is diluted by addition of such amounts of water and ethanol that a solution of 5 to 20% by weight of dry extract in 20 to 60% by weight aqueous ethanol is obtained,
- (i) the aqueous-alcoholic solution is extracted with an aliphatic or cycloaliphatic solvent having a boiling point of 60 to 100°C for further separation of the alkylphenol compounds,
- (j) the aqueous phase is concentrated under reduced pressure and dried at a temperature of at most 60 to 80°C to form a dry extract having a water content of less than 5%.

Leaves of *Ginkgo biloba* also contain, in addition to the compounds mentioned, the so-called ginkgolic acids (anacardic acids). These compounds are 6-alkylsalicylic acids having n-C13 to n-C19 alkyl radicals having 0 to 3 double bonds; cf. J. L. Gellermann et al., *Phytochemistry*, Vol. 15 (1976), 1959-1961 and *Analytic. Chem.* Vol. 40 (1968), 739-743.

By decarboxylation of the ginkgolic acids, the "ginkgol", a phenol substituted by the corresponding alkyl radical, can be formed biogenetically or else in the industrial processing of the *Ginkgo biloba* leaves; cf. Kawamura, *Japan. J. Chem.* Vol. 3 (1928), 91-93.

The ginkgolic acids and ginkgols in *Ginkgo biloba* are accompanied by corresponding derivatives having a further phenolic hydroxyl group in the 4-position, the 6-

alkylresorcinolic acids or 5-alkylresorcinols; cf. J. Gellermann et al., *Phytochemistry*, Vol. 15 (1976), 1959-1961. These resorcinol derivatives are responsible for the toxic effects and in particular the severe allergies and contact dermatitis which are caused by plants of the genus *Toxicodendron*; cf. G. A. Hill et al., *J. Am. Chem. Soc.*, Vol. 56 (1934), 2736-2738.

The importance of the allergies caused by alkylphenol compounds from anacardiaceae and ginkgoaceae is discernible by the development, described in the patent literature (cf. U.S. 44 28 965), of substances and methods for desensitizing to the allergies caused by alkylphenol compounds.

Commercially available extracts from leaves of *Ginkgo biloba* also contain between 50 and 10 000 ppm of ginkgolic acids.

The extracts from leaves of *Ginkgo biloba* produced by the processes known from DE 17 67 098 and DE 21 17 429 are virtually free of alkylphenol compounds because the lipophilic extract constituents are removed by liquid-liquid extraction of the water-containing acetone extract with a lipophilic solvent that is practically immiscible with water, for example a chlorinated lower aliphatic hydrocarbon such as carbon tetrachloride. However, in the process stage, the therapeutically valuable ginkgolides and the bilobalide are also greatly reduced so that their content in the end product according to example 1 of DE 21 17 429 is at most 0.5% for the sum of the ginkgolides, A, B, C and J and about 0.3% for the bilobalide. By contrast, the sum of the flavone glycosides was greatly increased by this process, namely from 3 to 4% in the crude extract to about 24% in the end product.

The use of substances hazardous to health is naturally undesirable in order to provide medicaments or food supplements based on extracts.

DE 39 40 092 A1 describes, for example, a corresponding process in which no lead compounds are used in the removal of the undesirable polyphenol compounds having a tanning substance character (proanthocyanidins). Compounds of lead are highly undesirable because of the health risk of the persons working with them and furthermore cause considerable costs in their proper disposal.

In recent years, foods with ginkgo extracts have also increasingly come on the market. Foods are to be understood here as meaning, in particular, dietetic foods, food supplements and “medical foods” and “dietary supplements”.

In the industrialized countries, foods which are consumed daily by a large part of the population have been industrially produced on a large scale for decades. It has been known for a long time that, as a result of the industrial production and the associated technical procedures such as, for example, pasteurization, homogenization, mechanical comminution, separation, heating or extraction, a considerable part of their basic constituents is removed from the foods. This relates, in particular, to vitamins in the basic foods which are partially destroyed or ineffective by the technical process, for example as a result of heating or other methods. In this case, the various vitamins (vitamins B, C, D, A etc.) are essential for trouble-free metabolism.

Furthermore, the various additives such as preservatives, for example in the form of benzoic acid, antioxidants, artificial flavourings, sugars or enzymes, are a significant problem of today's foods. Many of these additives in conjunction with the technical processing of the foods are suspected of being responsible, inter alia, for the enormous increase in allergies in recent decades.

For the reasons listed above, food supplements have been offered for years which are intended to help supplement the today's diets, which are based for the most part on industrially produced foods, and to improve their deficiencies with regard to essential food constituents.

However, many of the food supplements offered today on the market also contain additives or fillers in the form of milk powder, maltodextrin, starch, gelatin, wheat grit, whey powder, fruit sugar, glycerol or sorbitol. Further additives relate to antioxidants; furthermore, calcium phosphate and flavorings (mostly in the form of fruit flavorings), artificial dyes or citrates are admixed. The compositions of the various preparations vary only to a very insignificant extent if they are vitamin preparations in tablet or powder form.

These agents known from the prior art are all mixtures of various vitamins, amino acids, minerals and/or proteins. What is common to most of these preparations is that

they are artificial compositions which are directed non-specifically against possible deficiency states such as vitamin or mineral deficiency or bring about a non-specific improvement in general well-being.

In addition, body cells are exposed to daily attacks by free radicals – that is to say aggressive or highly reactive substances with free electrons which arise in the course of metabolic processes or as a result of natural UV or radioactive radiation. If the physical repair mechanisms are no longer fully functional, these radicals are able to trigger unwanted reactions with the genetic substance or essential proteins or to damage these lastingly and thereby contribute to the formation of tumors. This process can be prevented or minimized by administrations of so-called antioxidants. The most effective antioxidants for the cells include certain vitamins such as, for example, vitamin C, vitamin E, vitamin A and various B vitamins, furthermore the trace element selenium and some phytoflavones.

The literature (Qu`dan et al. 2011, He et al. 2016, Cao et al. 2016, Xie et al., 2014, Yao et al. 2020) describes the antioxidant and neuroprotective properties of proanthocyanidins from Ginkgo leaves and various other sources.

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A combined spectrum of actions which can positively influence both deficiency states, general well-being, health and also illnesses is therefore desirable.

An increased content of proanthocyanidins in extracts from leaves of Ginkgo biloba is therefore desirable in order to reduce the daily attacks by free radicals.

The object of the present invention is therefore to develop a process in which a Ginkgo biloba extract is obtained which comprises an increased content of proanthocyanidins and is suitable for use as medicaments, food supplements or as foodstuffs. The extract preferably meets all the requirements of Pharm. Eur. and preferably has a high content of proanthocyanidins.

The object is therefore achieved by a process for producing an extract, preferably a dry extract, from Ginkgo biloba leaves comprising the following steps:

- (a) extraction of fresh or dried leaves of Ginkgo biloba with water-containing acetone, a water-containing alkanol having 1 to 3 carbon atoms or anhydrous methanol at a temperature of 40 to 100°C in order to obtain a crude extract solution,
- (b) separation by distillation of the acetone, alkanol having 1 to 3 carbon atoms or anhydrous methanol from the crude extract solution from step (a) to a content of at most 10% by weight thereof, based on the total weight of the crude extract solution, in order to obtain a concentrated aqueous solution, wherein, in the case of the use of anhydrous methanol in step (a), water is added one or more times at the end of the distillation,

- (c) dilution of the concentrated aqueous solution from step (b) with water to a solids content of 10 to 30% by weight, preferably 15 to 25% by weight, cooling to a temperature below 25°C, keeping cool until a precipitate is formed and separation of the precipitate formed in order to obtain an aqueous solution again,
 - (d) addition of ammonium sulfate to the aqueous solution obtained from step (c) and extraction of the ammonium sulfate-containing solution formed with methyl ethyl ketone/acetone in a ratio of 3/2 to 1/1 (m/m) and separation of the aqueous phase from the methyl ethyl ketone acetone phase in order to obtain a methyl ethyl ketone acetone phase,
- wherein the extract after step (d) after drying to form the dry extract has a content of 2.0% by weight to 5.0% by weight of bilobalid, 1.5% by weight to 5.0% by weight of ginkgolides A, B and C and 25-40% by weight, preferably 30-40% by weight, of proanthocyanidins.

The abbreviation (m/m) advantageously stands for the respective masses, i.e. for example 3 g of methyl ethyl ketone to 2 g of acetone.

The extraction in step (a) is preferably carried out with water-containing acetone, a water-containing alkanol having 1 to 3 carbon atoms or anhydrous methanol in a ratio of 5-12 (volume of extractant in liters) to 1 (mass of leaves of Ginkgo biloba in kg). The ratio of extractant/drug (volume in liters/mass in kg) is generally obtained by weighing the drug (leaves of Ginkgo biloba) and adding the solvent for the extraction in L (via a volume meter). The extraction can preferably be repeated several times in succession.

The process according to the invention preferably comprises the further steps

- (e) concentration of the methyl ethyl ketone acetone phase from step (d) to a dry extract content of 40 to 80% by weight in order to obtain a concentrate,
- (f) if necessary, dilution of the concentrate obtained from step (e) with water to a solids content of 25-50% by weight in order to obtain a solution (if the concentrate from step (e) has a dry extract content of 40-

50% by weight, i.e. a solids content in the range from 25-50% by weight, the concentrate from step (e) can be used directly in step (g)).

- (g) at least single-stage extraction of the solution obtained in step (f) with a water-immiscible butanol or pentanol in order to obtain a butanol or pentanol phase,
- (h) concentration of the butanol or pentanol phase from step (g) to a solids content of at least 50% by weight in order to obtain a concentrate,
- (i) dilution of the concentrate obtained in step (h) by addition of such amounts of water and ethanol, if necessary, that a solution of 5 to 20% by weight of dry extract in water or at most 60% by weight aqueous ethanol (synonymous with "water-containing ethanol with an ethanol content of 60% by weight") is obtained,
- (j) extraction of the aqueous or aqueous-ethanolic solution from step (i) with an aliphatic solvent having a boiling point of 60 to 100°C, and separation of the aqueous phase from the aliphatic solvent phase in order to obtain an aqueous phase,
- (k) concentration of the aqueous phase obtained in step (j) under reduced pressure and at a temperature of at most 60 to 80°C in order to obtain a dry extract having a water content of less than 5% by weight.

Preferably in step (d) in the process the volume of the methyl ethyl ketone/acetone used is in the range from 0.3 to 1.8 times the volume of the aqueous solution. More preferably in step (d) in the process the volume of the methyl ethyl ketone/acetone used is in the range from 0.6 to 1.8 times the volume of the aqueous solution, even more preferably in the range from 0.8 to 1.5 times the volume of the aqueous solution.

Particularly preferably in step (d) in the process the volume of the methyl ethyl ketone/acetone used is in the range from 0.3 to 0.4; 0.4 to 0.5; 0.5 to 0.6; 0.6 to 0.7; 0.7 to 0.8; 0.8 to 0.9; 0.9 to 1.0; 1.0 to 1.1; 1.1 to 1.2; 1.2 to 1.3; 1.3 to 1.4; 1.4 to 1.5; 1.5 to 1.6 or 1.6 to 1.7 times the volume of the aqueous phase (aqueous solution).

Preferably the extract after step (d) has a content of 10.0% by weight to 27.0% by weight of flavonoids, calculated as flavone glycosides, 2.0% by weight to 5.0% by weight of bilobalid, 1.5% by weight to 5.0% by weight of ginkgolides A, B and C and 30-40% by weight of proanthocyanidins. Here, the percentages are based on the dry extract.

Preferably the extract after step (d) has a content of 10.0% by weight to 27.0% by weight of flavonoids, calculated as flavone glycosides, 2.1% by weight to 5.0% by weight of bilobalid, 2.1% by weight to 5.0% by weight of ginkgolides A, B and C and 30-40% by weight of proanthocyanidins. Here, the percentages are based on the dry extract.

Preferably the extract after step (d) has a content of 10.0% by weight to 27.0% by weight of flavonoids, calculated as flavone glycosides, 2.1% by weight to 3.6% by weight of bilobalid, 2.1% by weight to 3.8% by weight of ginkgolides A, B and C and 30-40% by weight of proanthocyanidins. Here, the percentages are based on the dry extract.

Preferably the extract after step (d) has a content of 10.0% by weight to 27.0% by weight of flavonoids, calculated as flavone glycosides, 2.1% by weight to 3.2% by weight of bilobalid (particularly preferably 2.6% by weight to 3.2% by weight of bilobalid), 2.1% by weight to 3.4% by weight of ginkgolides A, B and C (particularly preferably 2.8% by weight to 3.4% by weight of ginkgolides A, B and C) and 30-40% by weight of proanthocyanidins. Here, the percentages are based on the dry extract.

Preferably the extract after step (g) has a content of 20.0% by weight to 27.0% by weight of flavonoids, calculated as flavone glycosides, 2.6% by weight to 5.0% by weight of bilobalid (preferably 2.6% by weight to 3.6% by weight of bilobalid), 3.0% by weight to 5.0% by weight of ginkgolides A, B and C (preferably 3.0% by weight to 3.8% by weight of ginkgolides A, B and C) and 5.5-25% by weight, preferably 5.5-20% by weight, more preferably 5.5-18% by weight, very particularly preferably 7-14% by weight, of proanthocyanidins. Here, the percentages are based on the dry extract.

Preferably the extract after step (g) has a content of 20.0% by weight to 27.0% by weight of flavonoids, calculated as flavone glycosides, 2.6% by weight to 3.2% by

weight of bilobalid, 2.8% by weight to 3.4% by weight of ginkgolides A, B and C and 5.5-18% by weight, preferably 7-14% by weight, of proanthocyanidins. Here, the percentages are based on the dry extract.

Preferably the extract after step (k) or (j) has a content of 21.0% by weight to 27.0% by weight of flavonoids, calculated as flavone glycosides, 2.6% by weight to 5.0% by weight of bilobalid, 2.8% by weight to 5.0% by weight of ginkgolides A, B and C and at most 5 ppm of ginkgolic acids and 5.5-25% by weight, very particularly preferably 6.5-20% by weight, of proanthocyanidins. Here, the percentages are based on the dry extract.

Preferably the extract after step (k) or (j) has a content of 21.0% by weight to 27.0% by weight of flavonoids, calculated as flavone glycosides, 2.6% by weight to 3.6% by weight of bilobalid (preferably 2.6% by weight to 3.2% by weight of bilobalid), 2.8% by weight to 3.6% by weight of ginkgolides A, B and C (preferably 2.8% by weight to 3.4% by weight of ginkgolides A, B and C) and at most 5 ppm of ginkgolic acids and 6.5-20% by weight of proanthocyanidins. Here, the percentages are based on the dry extract.

Preferably the extract after step (k) or (j) has a content of proanthocyanidins of 7.0 to 8.0; 8.0 to 9.0; 9.0 to 10.0; 10.0 to 11.0; 11.0 to 12.0; 12.0 to 13.0; 13.0 to 14.0; 14.0 to 15.0, 15.0 to 16.0, 16.0 to 17.0, 17.0 to 18.0 or 18.0 to 19.0% by weight.

Particularly preferably the extract after step (k) or (j) has a content of proanthocyanidins of 7.0 to 10% by weight. Here, the percentages are based on the dry extract.

The process according to the invention preferably comprises the further steps

- (e1) concentration of the methyl ethyl ketone acetone phase from step (d) to a solids content of at least 40% by weight, preferably 45 to 95% by weight, in order to obtain a concentrate,
- (f1) dilution of the concentrate obtained in step (e1) by addition of such amounts of water and ethanol, if necessary, that a solution of 5 to 20% by weight of dry extract in water or at most 60% by weight aqueous ethanol is obtained,

- (g1) extraction of the aqueous or aqueous-ethanolic solution from step (f1) with an aliphatic solvent having a boiling point of 60 to 100°C and separation of the aqueous phase from the aliphatic solvent phase in order to obtain an aqueous phase,
- (h1) concentration of the aqueous phase obtained in step (g1) under reduced pressure and at a temperature of at most 60 to 80°C in order to obtain a dry extract having a water content of less than 5% by weight,

wherein the dry extract after step (h1) has a content of 2.0% by weight to 5.0% by weight of bilobalid (preferably 2.0% by weight to 3.6% by weight, more preferably 2.6% by weight to 3.6% by weight, particularly preferably 2.6% by weight to 3.2% by weight of bilobalid), 2.0% by weight to 5.0% by weight of ginkgolides A, B and C (preferably 2.0% by weight to 3.6% by weight, more preferably 2.8% by weight to 3.6% by weight, particularly preferably 2.8% by weight to 3.4% by weight of ginkgolides A, B and C) and at most 5 ppm of ginkgolic acids and 25-40% by weight, preferably 30-40% by weight, of proanthocyanidins. The content of flavonoids, calculated as flavone glycosides, is preferably 21.0% by weight to 27.0% by weight, particularly preferably 22.0% by weight to 27.0% by weight.

Steps (e1) to (h1) are to be understood as alternative method steps to steps (e) to (k).

Very particular preference is given to a process for producing an extract, preferably a dry extract, from *Ginkgo biloba* leaves comprising the following steps:

- (a) extraction of fresh or dried leaves of *Ginkgo biloba* with water-containing acetone, a water-containing alkanol having 1 to 3 carbon atoms or anhydrous methanol at a temperature of 40 to 100°C in order to obtain a crude extract solution,
- (b) separation by distillation of the acetone, alkanol having 1 to 3 carbon atoms or anhydrous methanol from the crude extract solution from step (a) to a content of at most 10% by weight thereof, based on the total weight of the crude extract solution, in order to obtain a concentrated aqueous solution, wherein,

in the case of the use of anhydrous methanol in step (a), water is added one or more times at the end of the distillation,

- (c) dilution of the concentrated aqueous solution from step (b) with water to a solids content of 10 to 30% by weight, preferably 15 to 25% by weight, cooling to a temperature below 25°C, keeping cool until a precipitate is formed and separation of the precipitate formed in order to obtain an aqueous solution again,
- (d) addition of ammonium sulfate to the aqueous solution obtained from step (c) and extraction of the ammonium sulfate-containing solution formed with methyl ethyl ketone/acetone in a ratio of 3/2 to 1/1 (m/m) and separation of the aqueous phase from the methyl ethyl ketone acetone phase in order to obtain a methyl ethyl ketone acetone phase,

wherein the extract preferably after step (d) has a content of 10.0% by weight to 27.0% by weight of flavonoids, calculated as flavone glycosides, 2.0% by weight to 5.0% by weight of bilobalid, 1.5% by weight to 5.0% by weight of ginkgolides A, B and C, 30-40% by weight of proanthocyanidins and the percentages are based on the dry extract,

- e) concentration of the methyl ethyl ketone acetone phase from step (d) to a dry extract content of 40 to 80% by weight in order to obtain a concentrate,
- (f) if necessary, dilution of the concentrate obtained from step (e) with water to a solids content of 25-50% by weight in order to obtain a solution,
- (g) at least single-stage extraction of the solution obtained in step (f) with a water-immiscible butanol or pentanol in order to obtain a butanol or pentanol phase, wherein the aqueous solution is preferably extracted in 1 stage in step (g).
- (h) concentration of the butanol or pentanol phase from step (g) to a solids content of at least 50% by weight in order to obtain a concentrate,
- (i) dilution of the concentrate obtained in step (h) by addition of such amounts of water and ethanol, if necessary, that a solution of 5 to 20% by weight of dry extract in water or at most 60% by weight aqueous ethanol (synonymous with

"water-containing ethanol with an ethanol content of 60% by weight") is obtained,

- (j) extraction of the aqueous or aqueous-ethanolic solution from step (i) with an aliphatic solvent having a boiling point of 60 to 100°C, and separation of the aqueous phase from the aliphatic solvent phase in order to obtain an aqueous phase,
- (k) concentration of the aqueous phase obtained in step (j) under reduced pressure and at a temperature of at most 60 to 80°C in order to obtain a dry extract having a water content of less than 5% by weight,

wherein the extract after step (k) or (j) has a content of 21.0% by weight to 27.0% by weight of flavonoids, calculated as flavone glycosides, 2.6% by weight to 5.0% by weight of bilobalid, 2.8% by weight to 5.0% by weight of ginkgolides A, B and C and at most 5 ppm of ginkgolic acids and 5.5-25% by weight, very particularly preferably 6.5-20% by weight, of proanthocyanidins, wherein the percentages are based on the dry extract.

Preferably the extract after step (d) has a content of 10.0% by weight to 27.0% by weight of flavonoids, calculated as flavone glycosides, 2.1% by weight to 3.6% by weight of bilobalid (particularly preferably 2.1% by weight to 3.2% by weight of bilobalid), 2.1% by weight to 3.8% by weight of ginkgolides A, B and C (particularly preferably 2.1% by weight to 3.4% by weight of ginkgolides A, B and C), 30-40% by weight of proanthocyanidins, wherein the percentages are based on the dry extract.

Preferably the extract after step (k) or (j) has a content of 21.0% by weight to 27.0% by weight of flavonoids, calculated as flavone glycosides, 2.6% by weight to 3.6% by weight of bilobalid, 2.8% by weight to 3.6% by weight of ginkgolides A, B and C and at most 5 ppm of ginkgolic acids and 5.5-25% by weight, very particularly preferably 6.5-20% by weight, of proanthocyanidins, wherein the percentages are based on the dry extract.

Preferably the extraction solvent used in step (a) is aqueous acetone having an acetone content of about 50 to 70% by weight, particularly preferably having an acetone content of about 60% by weight.

Preferably the extraction solvent used in step (a) is an aqueous alkanol selected from the group consisting of methanol, ethanol, 1-propanol, 2-propanol or mixtures thereof, having an alkanol content of 50% by weight to 70% by weight, particularly preferably aqueous ethanol having an ethanol content of 60% by weight.

Preferably the aqueous solution diluted in step (c) is cooled to a temperature below 12°C, in particular to 0°C to 11°C (physical standard conditions).

Preferably at least 30% by weight of ammonium sulfate, particularly preferably 30 to 50% by weight of ammonium sulfate, based on the aqueous solution from step (c), is added in step (d).

Preferably the methyl ethyl ketone acetone phase from step (d) is concentrated in step (e) to a dry extract content of 45 to 75% by weight in order to obtain a concentrate.

Preferably, if necessary, the concentrate obtained in step (e) is diluted with water in step (f) to a solids content of 30-45% by weight in order to obtain a solution,

Preferably extraction with 1-butanol is carried out in step (g), wherein extraction is preferably carried out once or once to three times.

Preferably the aqueous solution is extracted in 1 stage in step (g) and extracted with heptane, particularly preferably with n-heptane or a heptane isomer mixture having a proportion of more than 25% by weight of n-heptane, in a further step (j).

After step (c) the content of PACs is preferably 8.5 to 12% by weight, based on the dry extract.

After step (g) the content of PACs is preferably 5.5 to 20% by weight, particularly preferably 5.5 to 18% by weight, based on the dry extract.

In addition, in a further embodiment an extract of Ginkgo biloba is provided which is obtainable by the process according to the invention.

To produce medicaments, the Ginkgo biloba extract according to the invention can be processed in the customary manner, for example to give solutions, coated tablets,

tablets or injection preparations. The medicaments of the invention are preferably used for the treatment of peripheral and cerebral arterial circulatory disturbances.

Therefore, in a further embodiment a medicament or food supplement comprising the extract according to the invention is provided.

The food supplement preferably comprises vital substances selected from the group consisting of vitamins, minerals, trace elements, plant dyes, coenzymes, amino acids, peptides or plant extracts.

The food supplement preferably comprises:

- (i) at least one vitamin selected from the group consisting of vitamin A (retinol), vitamin B1 (thiamine), vitamin B2 (riboflavin), vitamin B3 (niacin), vitamin B4 (adenine, choline), vitamin B5 (pantothenic acid), vitamin B6 (pyridoxine), vitamin B7 (biotin), vitamin B8 (adenosine phosphate), vitamin B9 (folic acid), vitamin B10 (para-aminobenzoic acid, mixture of B vitamins), vitamin B11 (folic acid), vitamin B12 (cobalamin), vitamin B13 (orotic acid), vitamin B14 (mixture of vitamins B10 and B11), vitamin B15 (pangamic acid), vitamin B16 (pyridoxine), vitamin B17 (amygdalin), vitamin B22 , vitamin BT (carnithine), vitamin BX (para-aminobenzoic acid), vitamin C (ascorbic acid), vitamin D (cholecalciferol), vitamin E (tocopherol), vitamin H (biotin), vitamin K (phyloquinin, menaquinone), vitamin Q (ubiquinone, coenzyme Q10) and derivatives thereof; and
- (ii) optionally a mineral component comprising at least one component selected from the group consisting of calcium, iron, potassium, copper, magnesium, molybdenum, selenium, silicon, zinc and salts thereof,
- (iii) optionally an added natural substance or natural substance mixture comprising at least one natural substance or natural substance mixture selected from the group consisting of carotenoids, in particular lutein, lycopene, lipids, in particular medium-chain triglycerides, phospholipids and omega-3 fatty acids and omega-6 fatty acids, resveratrol and curcumin,

- (iv) a plant extract component comprising at least two different plant extracts, which are each an extract from a plant or plant parts selected independently of one another from the group consisting of the Ginkgo extract according to the invention and additionally ginseng, green tea, *Rhodiola rosea*, lavender oil (essential oil), saffron, melissa, valerian, withania or combinations thereof,
- (v) optionally at least one probiotic selected from the group consisting of *Streptococcus thermophilus*, *Lactobacillus* strains (e.g. *Lactobacillus plantarum* and *Lactobacillus helveticus*), *Bifidobacterium* strains (e.g. *Bifidobacterium breve* strain A1), *Bacillus subtilis*, *Anaerostipes* strains, *Oscillibacter* strains, *Lachnospiraceae* strains, *Akkermansia* strains, *Christensenellaceae* strains, *Firmicutes* strains, *Ruminococcus* strains, *Clostridium* strains (e.g. *Clostridium butyricum*), *Eubacterium* strains (e.g. *Eubacterium Hallii*, *Eubacterium Rectale* and *Eubacterium plexicaudatum*), *Coprococcus* strains, *Faecalibacterium* strains, *Butyrivibrio* strains, *Actinomycetes* strains, *Bacteroidetes* strains, *Proteobacteria* strains, *Spirochetes* strains, *Blautia* strains (e.g. *Blautia hansenii*) and *Muribaculum* strains (e.g. *Muribaculum intestinale*).

“*Extraction*” in the sense of the present invention is to be understood as meaning a separation method in which one or more components is/are dissolved out of a substance mixture consisting of solid, liquid or gaseous individual substances with the aid of an extractant or mixtures thereof. The extractant used in the process according to the invention is an extraction solvent, for example aqueous acetone, a water-containing alkanol having 1 to 3 carbon atoms (preferably methanol, ethanol, 1-propanol, 2-propanol or mixtures thereof), anhydrous methanol, a methyl ethyl ketone acetone mixture (in the ratio of 3/2 to 1/1 (m/m) in step (d)), water-immiscible butanol or pentanol or aliphatic solvents (preferably having a boiling point of 60 to 100°C).

Water-immiscible butanol comprises *n*-butanol, *iso*-butanol and *sec*-butanol. Water-immiscible pentanol comprises pentan-1-ol, pentan-2-ol, pentan-3-ol, 2-methylbutan-1-ol, 2-methylbutan-2-ol, 3-methylbutan-1-ol, 3-methylbutan-2-ol and 2,2-dimethylpropan-1-ol.

A preferred aliphatic solvent is heptane, particularly preferably n-heptane or a heptane isomer mixture having a proportion of more than 25% by weight of n-heptane.

“*Extract*” in the sense of the present invention is to be understood as meaning the one or more components which is/are extracted from a substance mixture during the extraction. The extract is preferably a dry extract.

“*Dried leaves of Ginkgo biloba*” are obtained by means of a process known to the person skilled in the art. They are preferably obtained by drying in a drum oven at up to several hundred °C, preferably at temperatures greater than 60°C, and drying times of 10 min to several hours. Furthermore, “air drying” (without technical aids) is possible or the use of other technical drying processes or combinations of air drying and technical drying.

According to the current European Pharmacopoeia (version 11.0; monograph 04/2008: 1827 “Ginkgo dry extract, refined and quantified”), which is binding on all Ginkgo extracts which can be approved as medicaments within the scope of the Pharmacopoeia, Ginkgo extract comprises 22.0% to 27.0% flavonoids, calculated as flavone glycosides, 2.6% to 3.2% bilobalide, 2.8% to 3.4% ginkgolides A, B and C and not more than 5 ppm Ginkgol acids. The production is described in the „Bekanntmachung über die Zulassung und Registrierung von Arzneimitteln (Aufbereitungsmonographien für den humanmedizinischen Bereich) – Ginkgo folium (Ginkgo-biloba-Blätter)“ (Notice on the approval and registration of medicaments (preparation monographs for the human medicine sector)—Ginkgo folium (Ginkgo biloba leaves) (Bundesanzeiger 46, (133), 7360-7361 (1994)) as follows: “A dry extract produced from the dried leaves of Ginkgo biloba LINNE with acetone water and subsequent purification steps without admixing concentrates or isolated ingredients”.

The ginkgolides A, B, C and bilobalid are known compounds from the group of terpene lactones. The ginkgolides A, B, C are hexacyclic diterpenes and bilobalid is a tetracyclic sesquiterpene.

The content of flavonoids is calculated as the content of flavone glycosides. The flavone glycosides preferably comprise the glycosides of the flavones (also called flavonoids) quercetin, kampferol and isorhamnetin, which are known compounds.

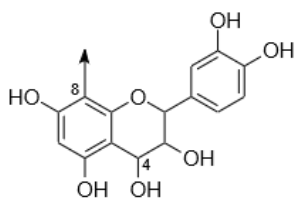
“Plant extract” in the sense of the present invention is to be understood as meaning an extract which has been obtained from a plant or from plant parts as starting material. The plant extract is preferably a dry extract.

“Dry extract” in the sense of the present invention is to be understood as meaning the residue which is obtained when the extractant has been removed after an extraction. The solid residue thus obtained is preferably additionally subjected to a drying step. Dry extracts are typically characterized by the ratio of drug to extract (DEV). Dry extracts preferably have a water content of at most 5% by weight, preferably at most 4% by weight, at most 3% by weight, at most 2% by weight or at most 1% by weight.

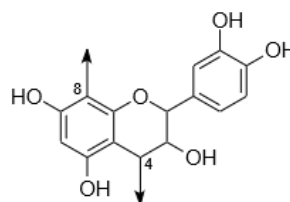
“Crude extract solution” in the sense of the present invention is to be understood as meaning the mixture of extract and extraction solvent obtained during an extraction.

In step (b), *“water is added one or more times at the end of the distillation”* is preferably understood as meaning the addition of water as soon as the dry extract content of the concentrated extract solution has reached at least 20% by weight, which can be effected by adding 25-150% by weight of water, based on the mass of the concentrated extract solution, one or more times, and further distillation, as a result of which the methanol content in the concentrated extract solution can be reduced.

“Proanthocyanidins” (PAC) in the sense of the present invention are to be understood as meaning secondary plant substances from the group of the tannins which, on heating with dilute mineral acids, give a color reaction, the so-called proanthocyanidin reaction. Proanthocyanidins are preferably polymerization products of flavan-3-ols and flavan-3,4-diols. Frequent proanthocyanidins comprise the condensation products of flavan-3,4-diols which condense via 4→8 or 4→6 bonds to form di-, tri- and oligomers, and the condensation products of flavan-3-ol units which are connected to one another via the positions 4 and 8. Proanthocyanidins preferably comprise dimeric proanthocyanidins, oligomeric proanthocyanidins (OPC) and polymeric proanthocyanidins (PPC).



Flavan-3,4-diol unit



Flavan-3-ol unit

“*Plant or plant parts*” in the sense of the present invention is to be understood as meaning an entire plant (for example Ginkgo, ginseng, green tea) or a part of this plant, for example one or more from the group selected from root, stem, leaf, flower, fruit, bud, shoot or seed.

“*Fresh or dried leaves of Ginkgo biloba*” in the sense of the present invention preferably means the green leaves as fresh leaves of the tree and, if these are dried gently, the dried leaves are formed therefrom.

“*Ginkgo*” in the sense of the present invention is to be understood as meaning the plant *Ginkgo biloba*, a plant species from the order of the *Ginkgoales*.

“*Ginkgolides*” in the sense of the present invention are preferably understood as meaning the ginkgolides A, B and C.

The term “*aqueous*” (e.g. aqueous acetone, aqueous alkanol) in the sense of the present invention is to be understood as meaning that a chemical compound, preferably a solvent, comprises a minimum amount of water, typically at least 1% by weight, preferably 10-90% by weight, more preferably 20-80% by weight, more preferably 30-80% by weight, more preferably 40-80% by weight, more preferably 50-70% by weight. For example, aqueous acetone preferably has an acetone content of about 50 to 70% by weight, particularly preferably about 60% by weight. For example, aqueous alkanol preferably has an alkanol content of about 50 to 70% by weight, particularly preferably about 60% by weight.

The term “*anhydrous*” in the sense of the present invention is to be understood as meaning that a chemical compound, preferably a solvent, comprises virtually no water. Commercially available anhydrous methanol which can be used in the present

invention comprises, for example, not more than 0.8% by weight, 0.2% by weight, 0.1% by weight, 0.02% by weight, 0.01% by weight or 0.002% by weight of water.

“Concentration” in the sense of the present invention is to be understood as meaning the partial or complete removal of a solvent (extractant) or of a solvent mixture from a substance mixture by means of distillation.

“Dry extract fraction” in the sense of the present invention is to be understood as meaning the nonvolatile fraction of an extract solution. As a rule, this is stated in % by weight. The term *“solids content”* is used synonymously.

“Extract solution” in the sense of the present invention is to be understood as meaning a solution of the constituents, soluble in a solvent, of the fresh or dried leaves of Ginkgo biloba. This term is also used for the further processed solutions of the intermediate stages.

“Mineral component” in the sense of the present invention is to be understood as meaning both macroelements and trace elements. These are essential for the human organism. Macroelements are present at a concentration of at least 50 mg/kg of body weight. Trace elements are present at a concentration of less than 50 mg/kg of body weight.

Examples:

Overview of Analysis Methods

Photometric Determination of Proanthocyanidins

The content of proanthocyanidins is determined photometrically against a proanthocyanidin fraction obtained from the Ginkgo biloba extract as calibration substance by means of 1-point calibration.

50 mg of extract, weighed exactly, are made up to 25 ml with a mixture of isopropanol/3 M HCl [5:1 (v/v)] and dissolved. 5.0 ml of this solution are transferred into a closable vessel and hydrolyzed in a water bath at 100° C. for 45 min.

The cooled sample is made up to 10 ml with the isopropanol/HCl mixture and measured on a photometer (wavelength: 556 nm; layer thickness: 1 cm).

A suitable amount of proanthocyanidin fraction is measured analogously.

The sample weights are adjusted such that the extinction is between 0.2 and 1.

HPLC determination of terpene lactones (ginkgolides A, B, C and bilobalid)

The terpene lactones are determined in accordance with the method described in Pharm. Eur. 11 for the determination of terpene lactones in Ginkgo dry extract.

HPLC determination of flavonoids

The flavonoids are determined in accordance with the method described in Pharm. Eur. 11 for the determination of flavonoids in Ginkgo dry extract.

HPLC determination of ginkgolic acids

The ginkgolic acids are determined in accordance with the method described in Pharm. Eur. 11 for the determination of ginkgolic acids in Ginkgo dry extract.

Corresponding determination methods can be found in “European Pharmacopoeia 11.0” under the item “Ginkgo dry extract, refined and quantified”.

Optimization of the parameters of process step (c)

Preparation of the starting solution for optimization of the parameters of process step (c)

(corresponding to process step (a); see also under “preparation of the starting solution”)

600 g of dried and comminuted leaves of Ginkgo biloba were extracted with 4.5 kg of 60% by weight of acetone at 60°C for 30 min. The plant material was filtered off, extracted again with 4.5 kg of 60% by weight of acetone at 60°C for 30 min and filtered off again. The two extract solutions thus obtained were combined, homogenized and then aliquots were used for the individual experiments.

Reference Example 1: Cold precipitation at DE (dry extract fraction)=5%

(corresponding to process steps (b) and (c))

0.68 kg of the above starting solution were concentrated to 41.7 g and adjusted to 291 g (dry extract fraction: 5%) with water. The mixture was cooled to 12°C for 1 h. The precipitate was filtered off and the crude extract solution was evaporated and dried (analytical sample KF-05%).

Example 2: Cold precipitation at DE=15%

(corresponding to process steps (b) and (c))

6.78 kg of the above starting solution were concentrated to 412.5 g and adjusted to 962 g (dry extract fraction: 15%) with water. The mixture was cooled to 12°C for 1 h. The precipitate was filtered off and 10 g of the crude extract solution were evaporated and dried (analytical sample KF-15%).

Example 3: Cold precipitation at DE=25%

(corresponding to process steps (b) and (c))

0.68 kg of the above starting solution were concentrated to 37.4 g and adjusted to 58 g (dry extract fraction: 25%) with water. The mixture was cooled to 12°C for 1 h. The precipitate was filtered off and the crude extract solution was evaporated and dried (analytical sample KF-25%).

Analytical results: process step (c) "Cold precipitation"

Analytical sample	in %	PAC [in %]	flavones [in %]	bilobalid [in %]	ginkgolides [in %]
KF-05%	Cold precipitation at a DE=5%	8	3.37	0.552	0.61
KF-15%	Cold precipitation at a DE=15%	9.1	3.39	0.492	0.529
KF-25%	Cold precipitation at a DE=25%	9.8	3.49	0.487	0.534

*all percentages in percent by weight

Optimization of the parameters of process step (d)

Optimization of the methyl ethyl ketone-acetone ratio

Preparation of the starting solution

(corresponding to process steps (a) to (c))

600 g of dried and comminuted leaves of Ginkgo biloba were extracted with 4.5 kg of 60% by weight of acetone at 60°C for 30 min. The plant material was filtered off, extracted again with 4.5 kg of 60% by weight of acetone at 60°C for 30 min and filtered off again. The two extract solutions thus obtained were combined and homogenized.

6.78 kg of the above extract solution were concentrated to 412.5 g and adjusted to 962 g (dry extract fraction: 15%) with water. The mixture was cooled to 12°C for 1 h. The precipitate was filtered off and the crude extract solution was aliquoted for the further experiments.

Reference Example 4: MEK : acetone distribution 0.75/1 (m/m)

(corresponding to process step (d))

100 g of filtrate (crude extract solution from “Preparation of the starting solution”) were admixed with 30 g of ammonium sulfate and this was dissolved. This solution was extracted twice with each 50 mL of methyl ethyl ketone/acetone 0.75/1 (m/m). The methyl ethyl ketone acetone phases were combined, homogenized, evaporated and dried (analytical sample: MEK : acetone 0.75/1).

Example 5: MEK : acetone distribution 6/5 (m/m)

(corresponding to process step (d))

656 g of filtrate were admixed with 197 g of ammonium sulfate and this was dissolved. This solution was extracted twice with each 330 mL of methyl ethyl ketone/acetone 6/5 (m/m). 100 g of the combined and homogenized methyl ethyl ketone acetone phases were evaporated and dried (analytical sample: MEK : acetone 6/5).

Reference Example 6: MEK : acetone distribution 2.25/1 (m/m)

(corresponding to process step (d))

52 g of filtrate were admixed with 16 g of ammonium sulfate and this was dissolved. This solution was extracted twice with each 25 mL of methyl ethyl ketone/acetone 2.25/1 (*m/m*). The methyl ethyl ketone acetone phases were combined, homogenized, evaporated and dried (analytical sample: MEK : acetone 2.25/1).

Analytical results:

Process step (d) "Optimization of the methyl ethyl ketone-acetone ratio"

Analytical sample		PAC [in %]	flavones [in %]	bilobalid [in %]	ginkgolides [in %]
MEK : acetone 0.75/1 (reference ex. 4)	ratio MEK : acetone (0.75:1 [m/m]) extraction with 1 times the volume of the water phase	30.6	11.93	1.956	2.132
MEK : acetone 6/5 (Example 5)	ratio MEK : acetone (6:5 [m/m]); extraction with 1 times the volume of the water phase	32.3	14.55	2.265	2.247
MEK : acetone 2.25/1 (reference ex. 6)	ratio MEK : acetone (2.25:1 [m/m]) extraction with 1 times the volume of the water phase	29.6	18.86	2.988	3.142

Optimization of the volume ratio of the upper phase to the lower phase

Here, the upper phase is the upper phase formed in the separating funnel (methyl ethyl ketone – acetone - phase). The lower phase is the lower phase formed in the separating funnel (aqueous phase).

Preparation of the starting solution

(corresponding to process steps (a) to (c))

Analogously to the general experimental procedure for preparation of the starting solution, however, the cold precipitation was carried out at a DE=25%. This value corresponds to the preferred process.

Reference Example 7: MEK : acetone distribution 0.25 times the volume of upper phase to lower phase

(corresponding to process step (d))

92 g of filtrate were admixed with 28 g of ammonium sulfate and this was dissolved. This solution was admixed twice with each 11.5 mL of methyl ethyl ketone/acetone 6/5 (*m/m*). After shaking, a 3-phase mixture forms (analytical sample: MEK : acetone 0.25).

Example 8: MEK : acetone distribution 0.5 times the volume of upper phase to lower phase

(corresponding to process step (d))

52 g of filtrate were admixed with 16 g of ammonium sulfate and this was dissolved. This solution was extracted twice with each 12.5 mL of methyl ethyl ketone/acetone 6/5 (*m/m*). The combined and homogenized methyl ethyl ketone acetone phases were evaporated and dried (analytical sample: MEK : acetone 0.5).

Reference Example 9: MEK : acetone distribution 2 times the volume of upper phase to lower phase

(corresponding to process step (d))

52 g of filtrate were admixed with 16 g of ammonium sulfate and this was dissolved. This solution was extracted twice with each 50 mL of methyl ethyl ketone/acetone 6/5 (*m/m*). The combined and homogenized methyl ethyl ketone acetone phases were evaporated and dried (analytical sample: MEK : acetone 2).

Analytical results:

Process step (d) "Optimization of the volume ratio of the upper phase to the lower phase"

Analytical sample		PAC [in %]	flavones [in %]	bilobalid [in %]	ginkgolides [in %]
MEK : acetone 0.25 (reference ex. 7)	ratio MEK : acetone (6:5 [m/m]) extraction with 0.25 times the volume of the water phase	formation of a 3rd phase (no longer feasible on an industrial scale)			
MEK : acetone 0.5 (Example 8)	ratio MEK : acetone (6:5 [m/m]) extraction with 0.5 times the volume of the water phase	37	18.37	2.984	1.589
MEK : acetone 6/5 (Example 5)	ratio MEK : acetone (6:5 [m/m]); extraction with 1 times the volume of the water phase	32.3	14.55	2.265	2.247
MEK : acetone 2 (reference ex. 9)	ratio MEK : acetone (6:5 [m/m]) extraction with 2 times the volume of the water phase	26.7	12.04	1.655	1.784

*all percentages in percent by weight

Optimization of the parameters of process step (g)

Preparation of the starting dry extract

(corresponding to process steps (a) to (e))

1500 g of dried and comminuted leaves of Ginkgo biloba were extracted with 11.25 kg of 60% by weight of acetone at 60°C for 1 h. The plant material was filtered off, extracted again with 11.25 kg of 60% by weight of acetone at 60°C for 1 h and filtered off again. The two extract solutions thus obtained were combined and homogenized.

The starting solution (21.85 kg) was concentrated to 1.2 kg (dry extract fraction 35%) and adjusted to 2545 g (dry extract fraction 15%) with water. The mixture was cooled to <12°C for 16 h. The precipitate was filtered off.

2402 g of filtrate were admixed with 721 g of ammonium sulfate and this was dissolved. This solution was extracted twice with each 2066 g of methyl ethyl ketone/acetone 5.4/6 (m/m). The methyl ethyl ketone acetone phases were combined, homogenized and dried.

Example 10: 1-butanol distribution 3-fold distribution

(corresponding to process steps (f) and (g))

15 g of dry extract were adjusted to a dry extract fraction of 40% with water. This phase was extracted by shaking 3× with 42 mL of 1-butanol. The 1-butanol phases were combined and dried (analytical sample: butanol 3×)

Example 11: 1-butanol distribution 1-fold distribution

(corresponding to process steps (f) and (g))

5 g of dry extract were adjusted to a dry extract fraction of 40% with water. This phase was extracted by shaking 1× with 70 mL of 1-butanol. The 1-butanol phase was dried (analytical sample: butanol 1×)

Analytical results: process step (g) “1-butanol distribution”

Analytical sample		PAC [in % by weight]	flavones [in % by weight]	bilobalid [in % by weight]	ginkgolides [in % by weight]
butanol 3×	aqueous phase adjusted to DE=40%; extracted by shaking 3× with 1-butanol	4.04	17.29	2.05	2.51
butanol 1×	aqueous phase adjusted to DE=40%; extracted by shaking 1× with 1-butanol	5.48	16.72	2.11	2.64

*all percentages in percent by weight

The process according to the invention is illustrated and described in more detail with reference to comparative example 1 according to EP 431 536 B1 and example 1 according to the invention.

In the examples below, the term "heptane" is to be understood as meaning a saturated aliphatic hydrocarbon mixture having a boiling range of 94-100°C and an n-heptane proportion of more than 25% by weight.

Starting solution for example 1 and comparative example 1

(corresponding to process step (a) of EP 431 536 B1 and the process according to the invention)

750 g of dried and comminuted leaves of Ginkgo biloba were extracted with 5.63 kg of 60% by weight of acetone at 60°C for 1 h. The plant material was filtered off, extracted again with 5.63 kg of 60% by weight of acetone at 60°C for 1 h and filtered off again. The two extract solutions thus obtained were combined, homogenized and then divided.

Comparative example 1 according to EP 431536 B1

(corresponding to process steps (b) to (k))

Half of the above starting solution (5162 g) was concentrated to 300 g (dry extract fraction 35%) and adjusted to 716 g (dry extract fraction 15%) with water. The mixture was cooled to 12°C for 1 h. The precipitate was filtered off.

An analytical sample was taken from the solution, concentrated and dried (analytical sample V 1, sample after step (c)).

680 g of filtrate were admixed with 204 g of ammonium sulfate and this was dissolved. This solution was extracted twice with each 340 mL of methyl ethyl ketone/acetone 0.75/1 (*m/m*). The methyl ethyl ketone acetone phases were combined and homogenized.

An analytical sample was taken from the solution, concentrated and dried (analytical sample V 2, sample after step (d)).

584 g of the combined methyl ethyl ketone acetone phase were concentrated and adjusted to a dry extract fraction of 10% with water. This phase was extracted by shaking three times with 55 mL of 1-butanol. The 1-butanol phases were combined and homogenized.

An analytical sample was taken from the solution, concentrated and dried (analytical sample V 3, sample after step (g)).

96 g of 1-butanol phase were freed from 1-butanol and adjusted to a dry extract fraction of 10% and an ethanol fraction of 30% with water and ethanol. This solution was extracted three times with 25 mL of heptane, concentrated and dried: 5.1 g (analytical sample V 4, sample after step (j), comparable to step (k) in the process according to the invention).

	PAC	flavones	bilobalid	ginkgolides
Analytical sample V 1	7.78%	3.32%	0.455%	0.504%
Analytical sample V 2	33.5%	12.84%	1.834%	1.990%
Analytical sample V 3	5.34%	26.11%	4.191%	4.295%
Analytical sample V 4	5.50%	26.39%	4.386%	4.573%

*all percentages in percent by weight

The content of ginkgolic acids in analytical sample V 4 was determined to be less than 5 ppm.

Inventive Example 1

(corresponding to process steps (b) to (k) according to the invention)

The second half of the above starting solution (5161 g) was concentrated to 300 g (dry extract fraction 35%) and adjusted to 430 g (dry extract fraction 25%) with water. The mixture was cooled to 12°C for 1 h. The precipitate was filtered off.

An analytical sample was taken from the solution, concentrated and dried (analytical sample E 1, sample after step (c)).

393 g of filtrate were diluted to 665 g with water, 200 g of ammonium sulfate were added and this was dissolved. This solution was extracted twice with each 330 mL of methyl ethyl ketone/acetone 6/5 (m/m). The methyl ethyl ketone acetone phases were combined and homogenized.

An analytical sample was taken from the solution, concentrated and dried (analytical sample E 2, sample after step (d)).

495 g of the combined methyl ethyl ketone acetone phase were concentrated and adjusted to a dry extract fraction of 30% with water. This phase was extracted by shaking once with 130 mL of 1-butanol. The 1-butanol phase was homogenized.

An analytical sample was taken from the solution, concentrated and dried (analytical sample E 3, sample after step (g)).

89 g of 1-butanol phase were freed from 1-butanol and adjusted to a dry extract fraction of 10% and an ethanol fraction of 30% with water and ethanol. This solution was extracted three times with 27 mL of heptane, concentrated and dried: 5.8 g (analytical sample E 4, sample after step (j)).

	PAC	flavones	bilobalid	ginkgolides
Analytical sample E 1	9.54%	3.33%	0.447%	0.492%
Analytical sample E 2	31.0%	15.30%	2.201%	2.366%
Analytical sample E 3	11.1%	21.47%	3.339%	3.533%
Analytical sample E 4	8.43%	22.17%	3.332%	3.540%

*all percentages in percent by weight

The content of ginkgolic acids in analytical sample E 4 was determined to be less than 5 ppm.

In the extract according to example 1 according to the invention (analytical sample E 4) a proanthocyanidin (PAC) content of 8.43% by weight was determined, whereas in the extract according to comparative example 1 only 5.50% by weight of PAC was found, which corresponds to an enrichment by a factor of 1.53.

FIG. 1: Inhibition of the production of reactive oxygen species by various ingredient groups isolated from EGb 761

The pharmacological studies show an increased antioxidant potential of extracts with a proanthocyanidin content of 6.5-9.5% by weight compared with proanthocyanidin-free extracts.

FIG. 1 shows the antioxidant properties of the PACs (proanthocyanidins). These were studied in comparison with other known ingredients of Ginkgo biloba extract (flavones and terpene lactones) in a cellular system. For this purpose the spontaneous production of reactive oxygen species in the rat neuroblastoma cell line RN46A was determined by means of dihydrofluorescein conversion. The addition of increasing concentrations of Ginkgo biloba ingredients led to a concentration-dependent reduction of the fluorescence signal, which indicates inhibition of the production of reactive oxygen species. Here, the PAC fraction was the most active ingredient group.

FIG. 2: Inhibition of the production of reactive oxygen species by EGb 761 in comparison with a PAC-depleted Ginkgo biloba extract

FIG. 2 describes the results of an investigation of the antioxidant properties of EGb 761 with a PAC content of 8% by weight in comparison with a Ginkgo biloba extract (with comparable production and specification), but with an additional process step for preparative depletion of the PACs (PAC content <1% by weight). The antioxidant effect of the PAC-depleted extract was reduced by a factor of 2.6 in comparison with the PAC-rich EGb 761.

CLAIMS

1. A process for the preparation of an extract of Ginkgo biloba leaves comprising the following steps:
 - (a) extraction of fresh or dried leaves of Ginkgo biloba with water-containing acetone, a water-containing alkanol having 1 to 3 carbon atoms or anhydrous methanol at a temperature of 40 to 100°C in order to obtain a crude extract solution,
 - (b) separation by distillation of the acetone, alkanol having 1 to 3 carbon atoms or anhydrous methanol from the crude extract solution from step (a) to a content of at most 10% by weight thereof, based on the total weight of the crude extract solution, in order to obtain a concentrated aqueous solution, wherein, in the case of the use of anhydrous methanol in step (a), water is added one or more times at the end of the distillation,
 - (c) dilution of the concentrated aqueous solution from step (b) with water to a solids content of 10 to 30% by weight, preferably 15 to 25% by weight, cooling to a temperature below 25°C, keeping cool until a precipitate is formed and separation of the precipitate formed in order to obtain an aqueous solution again,
 - (d) addition of ammonium sulfate to the aqueous solution obtained from step (c) and extraction of the ammonium sulfate-containing solution formed with methyl ethyl ketone/acetone in a ratio of 3/2 to 1/1 (m/m) and separation of the aqueous phase from the methyl ethyl ketone acetone phase in order to obtain a methyl ethyl ketone acetone phase,wherein the extract after step (d) after drying to form the dry extract has a content of 2.0% by weight to 5.0% by weight of bilobalid, 1.5% by weight to 5.0% by weight of ginkgolides A, B and C and 25-40% by weight of proanthocyanidins.

2. The process according to claim 1, comprising the further steps
- (e) concentration of the methyl ethyl ketone acetone phase from step (d) to a dry extract content of 40 to 80% by weight in order to obtain a concentrate,
 - (f) if necessary, dilution of the concentrate obtained from step (e) with water to a solids content of 25-50% by weight in order to obtain a solution,
 - (g) at least single-stage extraction of the solution obtained in step (f) with a water-immiscible butanol or pentanol in order to obtain a butanol or pentanol phase,
 - (h) concentration of the butanol or pentanol phase from step (g) to a solids content of at least 50% by weight in order to obtain a concentrate,
 - (i) dilution of the concentrate obtained in step (h) by addition of such amounts of water and ethanol, if necessary, that a solution of 5 to 20% by weight of dry extract in water or at most 60% by weight aqueous ethanol is obtained,
 - (j) extraction of the aqueous or aqueous-ethanolic solution from step (i) with an aliphatic solvent having a boiling point of 60 to 100°C, and separation of the aqueous phase from the aliphatic solvent phase in order to obtain an aqueous phase,
 - (k) concentration of the aqueous phase obtained in step (j) under reduced pressure and at a temperature of at most 60 to 80°C in order to obtain a dry extract having a water content of less than 5% by weight.
3. The process according to claim 1, comprising the further steps
- (e1) concentration of the methyl ethyl ketone acetone phase from step (d) to a solids content of at least 40% by weight in order to obtain a concentrate,

- (f1) dilution of the concentrate obtained in step (e1) by addition of such amounts of water and ethanol, if necessary, that a solution of 5 to 20% by weight of dry extract in water or at most 60% by weight aqueous ethanol is obtained,
 - (g1) extraction of the aqueous or aqueous-ethanolic solution from step (f1) with an aliphatic solvent having a boiling point of 60 to 100°C and separation of the aqueous phase from the aliphatic solvent phase in order to obtain an aqueous phase,
 - (h1) concentration of the aqueous phase obtained in step (g1) under reduced pressure and at a temperature of at most 60 to 80°C in order to obtain a dry extract having a water content of less than 5% by weight,

wherein the dry extract after step (h1) has a content of 2.0% by weight to 5.0% by weight of bilobalid, preferably 2.0% by weight to 3.6% by weight of bilobalid, 2.0% by weight to 5.0% by weight of ginkgolides A, B and C, preferably 2.0% by weight to 3.6% by weight of ginkgolides A, B and C, and at most 5 ppm of ginkgolic acids and 25-40% by weight of proanthocyanidins.
4. The process according to any of claims 1 to 3, wherein in step (d) the volume of the methyl ethyl ketone/acetone used is in the range from 0.3 to 1.8 times the volume of the aqueous solution.
 5. The process according to claim 2, wherein the extract after step (j) or (k) has a content of 21.0% by weight to 27.0% by weight of flavonoids, calculated as flavone glycosides, 2.6% by weight to 5.0% by weight of bilobalid, 2.8% by weight to 5.0% by weight of ginkgolides A, B and C and at most 5 ppm of ginkgolic acids and 6.5-20% by weight of proanthocyanidins.
 6. The process according to claim 5, wherein the extract after step (j) or (k) has a content of 22.0% by weight to 27.0% by weight of flavonoids, calculated as flavone glycosides, 2.6% by weight to 3.2% by weight of bilobalid, 2.8% by weight to 3.4% by weight of ginkgolides A, B and C and at most 5 ppm of ginkgolic acids and 6.5-20% by weight of proanthocyanidins.

7. The process according to any of claims 1 to 6, wherein the extraction solvent used in step (a) is aqueous acetone having an acetone content of about 50 to 70% by weight, particularly preferably having an acetone content of about 60% by weight.
8. The process according to any of claims 1 to 7, wherein the extraction solvent used in step (a) is an aqueous alkanol selected from the group consisting of methanol, ethanol, 1-propanol, 2-propanol or mixtures thereof, having an alkanol content of 50% by weight to 70% by weight, particularly preferably aqueous ethanol having an ethanol content of 60% by weight.
9. The process according to any of claims 1 to 8, wherein the aqueous solution diluted in step (c) is cooled to a temperature below 12°C.
10. The process according to claims 1 to 9, wherein at least 30% by weight of ammonium sulfate, particularly preferably 30 to 50% by weight of ammonium sulfate, based on the aqueous solution from step (c), is added in step (d).
11. The process according to claims 1 to 10, wherein extraction with 1-butanol is carried out in step (g).
12. The process according to claims 1 to 11, wherein the aqueous solution is extracted in 1 stage in step (g) and/or is extracted with heptane, particularly preferably with n-heptane or a heptane isomer mixture having a proportion of more than 25% by weight of n-heptane, in step (j).
13. An extract of Ginkgo biloba obtainable by the process according to any of claims 1 to 12.
14. A medicament or food supplement comprising an extract according to claim 13.
15. The food supplement according to claim 14, wherein the food supplement comprises vital substances selected from the group consisting of vitamins, minerals, trace elements, plant dyes, coenzymes, amino acids, peptides or plant extracts.
16. The food supplement according to claim 15, comprising:

- (i) at least one vitamin selected from the group consisting of vitamin A (retinol), vitamin B1 (thiamine), vitamin B2 (riboflavin), vitamin B3 (niacin), vitamin B4 (adenine, choline), vitamin B5 (pantothenic acid), vitamin B6 (pyridoxine), vitamin B7 (biotin), vitamin B8 (adenosine phosphate), vitamin B9 (folic acid), vitamin B10 (para-aminobenzoic acid, mixture of B vitamins), vitamin B11 (folic acid), vitamin B12 (cobalamin), vitamin B13 (orotic acid), vitamin B14 (mixture of vitamins B10 and B11), vitamin B15 (pangamic acid), vitamin B16 (pyridoxine), vitamin B17 (amygdalin), vitamin B22, vitamin BT (carnithine), vitamin BX (para-aminobenzoic acid), vitamin C (ascorbic acid), vitamin D (cholecalciferol), vitamin E (tocopherol), vitamin H (biotin), vitamin K (phylloquinin, menaquinone), vitamin Q (ubiquinone, coenzyme Q10) and derivatives thereof; and
- (ii) optionally a mineral component comprising at least one component selected from the group consisting of calcium, iron, potassium, copper, magnesium, molybdenum, selenium, silicon, zinc and salts thereof,
- (iii) optionally an added natural substance or natural substance mixture comprising at least one natural substance or natural substance mixture selected from the group consisting of carotenoids, in particular lutein, lycopene, lipids, in particular medium-chain triglycerides, phospholipids and omega-3 fatty acids and omega-6 fatty acids, resveratrol and curcumin,
- (iv) a plant extract component comprising at least two different plant extracts, which are each an extract from a plant or plant parts selected independently of one another from the group consisting of the ginkgo extract according to claim 13 and additionally ginseng, green tea, *Rhodiola rosea*, lavender oil (essential oil), saffron, melissa, valerian, withania or combinations thereof,
- (v) optionally at least one probiotic selected from the group consisting of *Streptococcus thermophilus*, *Lactobacillus* strains (e.g. *Lactobacillus plantarum* and *Lactobacillus helveticus*), *Bifidobacterium* strains (e.g. *Bifidobacterium breve* strain A1), *Bacillus subtilis*, *Anaerostipes* strains,

Oscillibacter strains, Lachnospiraceae strains, Akkermansia strains, Christensenellaceae strains, Firmicutes strains, Ruminococcus strains, Clostridium strains (e.g. Clostridium butyricum), Eubacterium strains (e.g. Eubacterium Hallii, Eubacterium Rectale and Eubacterium plexicaudatum), Coprococcus strains, Faecalibacterium strains, Butyrivibrio strains, Actinomycetes strains, Bacteroidetes strains, Proteobacteria strains, Spirochetes strains, Blautia strains (e.g. Blautia hansenii) and Muribaculum strains (e.g. Muribaculum intestinale).