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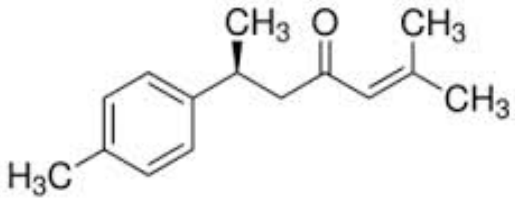
Ar-Turmerone



Ar-Turmerone

http://www.greenmedinfo.com/blog/how-whole-turmeric-heals-damaged-brain-1

How WHOLE Turmeric Heals The Damaged Brain

by Sayer Ji, Founder

...Now, an exciting new study published in the journal Stem Cell Research & Therapy provides additional support for the concept that curcumin alone is not enough to explain the healing power of turmeric as a whole plant. The study found that a little known, fat-soluble component within turmeric – Ar-tumerone – may make "a promising candidate to support regeneration in neurologic disease."

Titled, "Aromatic-turmerone induces neural stem cell proliferation in vitro and in vivo," German researchers evaluated the effects of this turmeric-derived compound on neural stem cells (NSCs) – the subgroup of brain cells capable of continuous self-renewal required for brain repair.

The study found that when brain cells were exposed to ar-tumerone, neural stem cells increased in number through enhanced proliferation. Moreover, these newly formed neural stem cells also increased the number of fully differentiated neuronal cells, indicating a healing effect was taking place. This effect was also observed in a live animal model, showing that rats injected with ar-tumerone into their brains experienced increases in neural stem cell proliferation and the creation of newly formed healthy brain cells...

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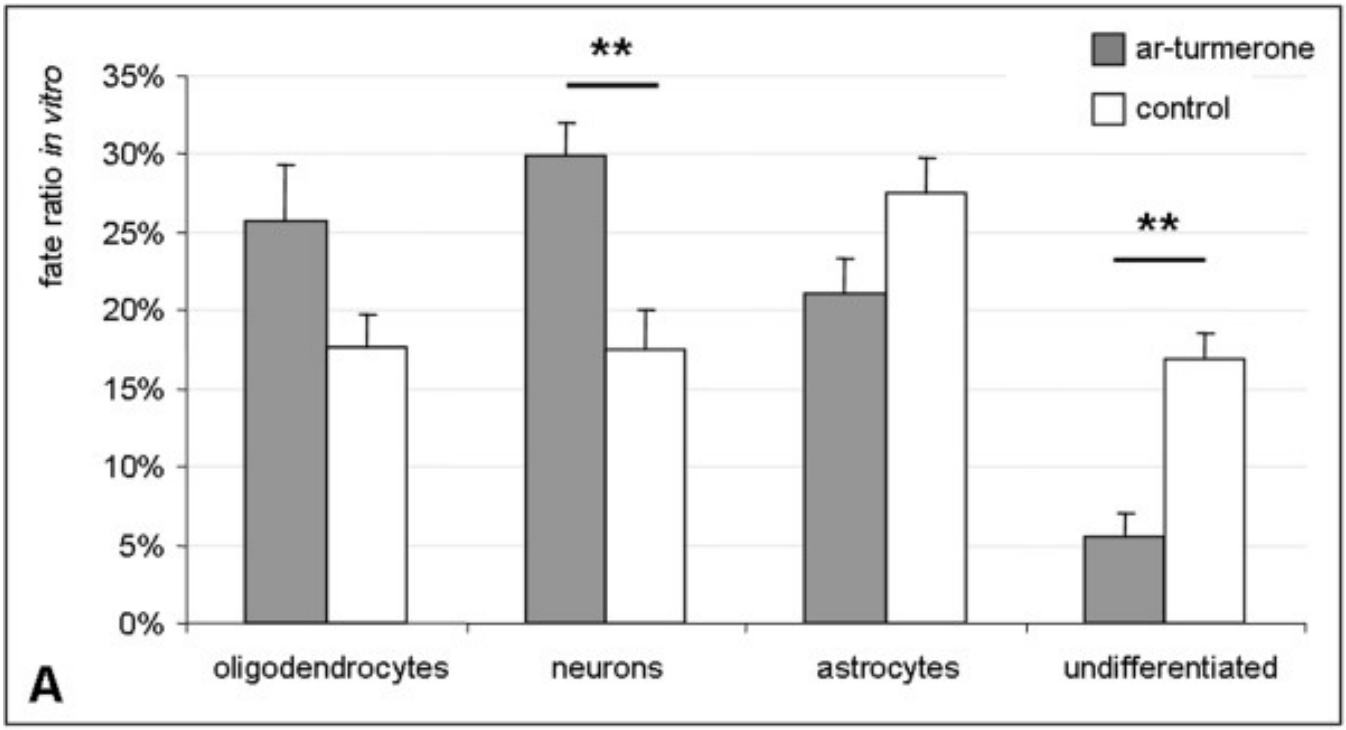
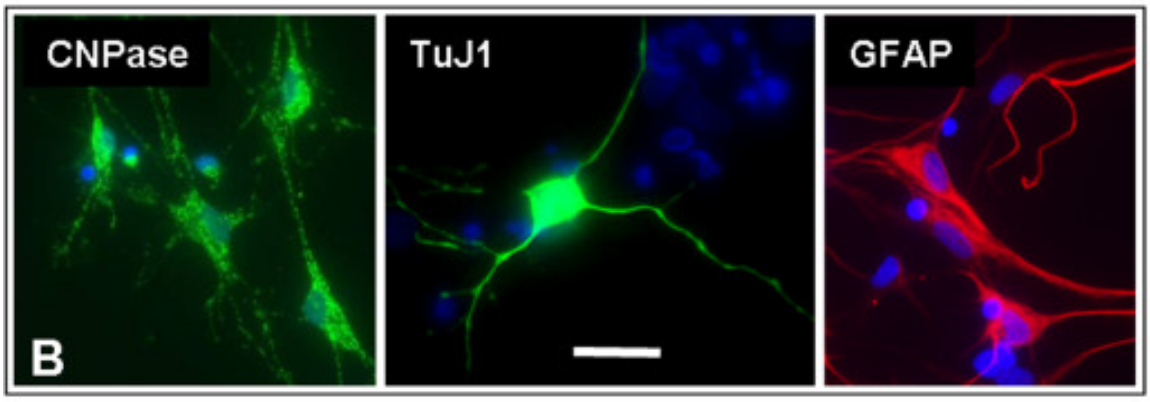
http://www.stemcellres.com/content/5/4/100
Stem Cell Research & Therapy 2014, 5:100
doi:10.1186/scrt500

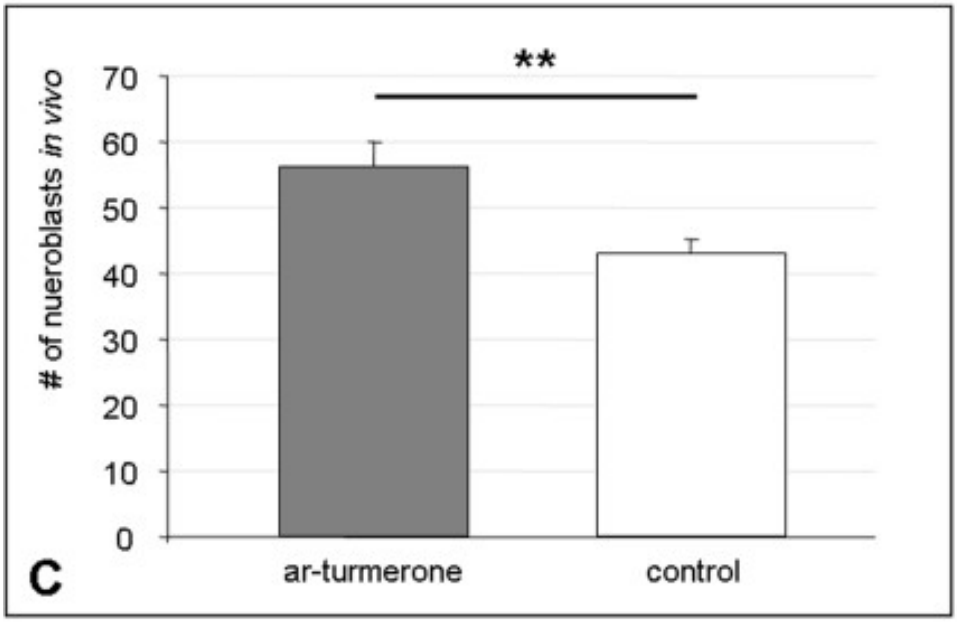
Aromatic-turmerone induces neural stem cell proliferation in vitro and in

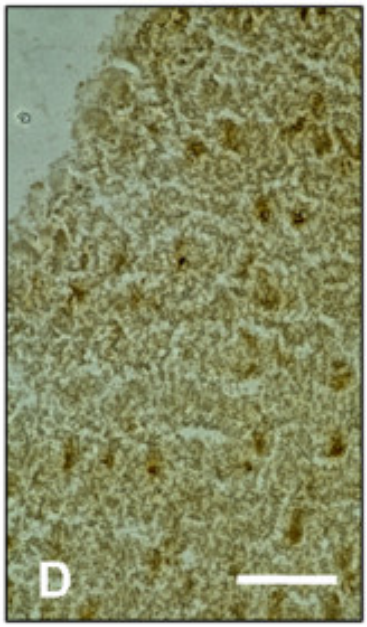
vivo

Joerg Hucklenbroich, Rebecca Klein, Bernd Neumaier, Rudolf Graf, Gereon Rudolf Fink, Michael Schroeter and Maria Adele Rueger









Abstract
Introduction

Aromatic (ar-) turmerone is a major bioactive compound of the herb Curcuma longa. It has been suggested that ar-turmerone inhibits microglia activation, a property that may be useful in treating neurodegenerative disease. Furthermore, the effects of ar-turmerone on neural stem cells (NSCs) remain to be investigated.

Methods

We exposed primary fetal rat NSCs to various concentrations of ar-turmerone. Thereafter, cell proliferation and differentiation potential were assessed. In vivo, naïve rats were treated with a single intracerebroventricular (i.c.v.) injection of ar-turmerone. Proliferative activity of endogenous NSCs was assessed in vivo, by using noninvasive positron emission tomography (PET) imaging and the tracer [18F]-fluoro-L-thymidine ([18F]FLT), as well as ex vivo.

Results

In vitro, ar-turmerone increased dose-dependently the number of cultured NSCs, because of an increase in NSC proliferation ($P < 0.01$). Proliferation data were supported by qPCR-data for Ki-67 mRNA. In vitro as well as in vivo, ar-turmerone promoted neuronal differentiation of NSCs. In vivo, after i.c.v. injection of ar-turmerone, proliferating NSCs were mobilized from the subventricular zone (SVZ) and the hippocampus of adult rats, as demonstrated by both [18F]FLT-PET and histology ($P < 0.05$).

Conclusions

Both in vitro and in vivo data suggest that ar-turmerone induces NSC proliferation. Ar-turmerone thus constitutes a promising candidate to support regeneration in neurologic disease…

Figure 2. Ar-turmerone induces neurogenesis in vitro and in vivo. (A) NSCs were allowed to differentiate in the absence (control) or presence of 6.25 μg/ml ar-turmerone. Immunocytochemistry 10 days after growth-factor discontinuation revealed fewer undifferentiated (SOX2+) NSCs in the turmerone-treated group, but more young neurons. The generation of astrocytes and oligodendrocytes was not affected by ar-turmerone (mean ± SEM; $^{**}P < 0.01$, compared with control). (B) Representative images of differentiated cells include CNPase-positive oligodendrocytes (left), TuJ1-positive young neurons (middle), and GFAP-positive astrocytes (right); bar represents 50 μm. (C) After i.c.v. injection of 3 mg (1 mg/μl) ar-turmerone, significantly more DCX-positive neuroblasts were observed in the SVZ compared with placebo-injected control animals (mean ± SEM; $^{**}P < 0.01$). (D) Representative staining of DCX-positive neuroblasts in the SVZ (bar represents 50 μm)...

Conclusions

In this study, we investigated the effects of ar-turmerone on NSCs in vitro and in vivo. Ar-turmerone increased the number of NSCs both in cell culture and in the adult rat brain in vivo. This increase resulted from enhanced NSC proliferation and led to promoted neurogenesis during differentiation. In vivo, ar-turmerone mobilized endogenous NSCs from both neurogenic niches, the SVZ and the hippocampus. We propose that ar-turmerone

constitutes a promising future drug candidate to support regeneration in neurologic disorders.

PATENTS

KR20150036936
A composition comprising an non-polar solvent soluble extract or ar-turmerone for preventing or treating a stress-involved disease

The present invention relates to a composition containing a nonpolar solvent soluble extract of Curcuma longa L. or aromatic turmerone isolated therefrom. According to the present invention, a cytotoxicity experiment using the cerebral cortex of an SD rat and a verification experiment of a neuronal damage-preventive effect have been performed on a nonpolar solvent soluble extract of Curcuma longa L. or aromatic turmerone isolated therefrom, wherein the neuronal damage-preventive effect refers to neuronal damage caused by corticosterone that is a stress hormone secreted due to psychological stress in cerebral cortex cells. As results of the experiments, it has been verified that the nonpolar solvent soluble extract of Curcuma longa L. or aromatic turmerone isolated therefrom has a strong neuronal damage-preventive effect. Accordingly, it has been verified that the composition is useful for a pharmaceutical composition or a health food for prevention and treatment of a stress-related disease.

CN104478686
Preparation method of ar-turmerone reference substance in turmeric volatile oil

The invention discloses a preparation method of an ar-turmerone reference substance in turmeric volatile oil. According to the invention, turmeric volatile oil is used as a raw material, silica gel column chromatography and preparative high performance liquid chromatography are used as separation methods, and petroleum ether-ethyl acetate and methanol-water are proportionally used as an elution system. It is determined that the prepared ar-turmerone pure product has a main chromatographic peak at different chromatographic columns and mobile phases through HPLC detection, and no anomaly peak appears when chromatographic columns and mobile phases are changed. By an area normalization method, purity of the reference substance is greater than 99%, thus meeting requirements of a traditional Chinese medicinal chemical reference substance in content determination.

TECHNICAL FIELD
[0002]

The present invention relates to a separation and purification technology, in particular a method for preparing aromatic turmeric volatile oil turmerone reference standard.
[0003]

Background technique

[0004]

Turmeric is Curcuma Genus (Curcuma) plant dry turmeric rhizomes.
Turmeric as a traditional Chinese medicine, only contained in the "Tang Materia Medica", with expelling gas line, pass through the pain of functions, modern medical research shows that turmeric has anti-inflammatory, antioxidant, free radical scavenging, anti-microbial and anti-tumor effect.
In recent years it has been used in the treatment of hyperlipidemia and has a hepatic toxicity.
[0005]

Aryl turmerone as an important component of turmeric, which belongs to terpenes, in medicine research involves inducing tumor cell apoptosis, anti-gram-positive bacteria and gram-negative bacteria, anti-fungal, anti-growth, anti-venom and other effects of clinical development to treat leukemia, malignant lymphoma, bacterial inflammation, fungal inflammation, and even diabetes, obesity and other metabolic diseases and birth control integrated broad prospects and other aspects.
[0006]

Xiaocuo liniment active ingredient is turmeric volatile oil, the main component of volatile oil is an aromatic ginger flavonoids.
In aromatic turmerone as a functional index measuring Xiaocuo liniment quality control system to further ensure the efficacy, enhance the quality of controllability is important.
How to obtain a high-purity aromatic volatile oil from turmeric turmerone reference is to be resolved.
[0007]

SUMMARY OF THE INVENTION
[0008]

Technical problems to be solved by the present invention is to provide a method for preparing turmeric volatile oil aromatic turmerone reference, you can get a high-purity separation of aromatic ginger flavonoids from turmeric volatile oil.
[0009]

The present invention is achieved: turmeric volatile oil aromatic turmerone reference preparation to turmeric volatile oil as raw material, the process comprising the steps of:
[0010]

(1) pressure normal phase silica gel column rough separator: the feedstock with silica gel by 1: 10-1: 15 ratio of mass to volume ratio of 30: 1-15: 1 petroleum ether - ethyl acetate as eluant gradient elution, pressurized column chromatography, thin-layer plate by point with petroleum ether - ethyl acetate to start, petroleum ether and ethyl acetate in a volume ratio of 15: 1-25: 1, observed under ultraviolet collection a mixture of similar polarity, isolated crude isolate;
[0011]

(2) pressure normal phase silica gel thin separation: The crude isolate was added to silica, the quality of the raw materials and the first added finely divided silica gel ratio of 1: 10-1: 15, in a volume ratio of 100: 1-50: a petroleum ether - ethyl acetate as eluent gradient elution, column chromatography pressurized, by spot TLC plate with petroleum ether - ethyl acetate

to expand, the volume ratio of petroleum ether and ethyl acetate 25: 1-15: 1, in the ultraviolet observation, and the mixture was collected polarity very close, fine isolate isolated;
[0012]

(3) aryl preparative high performance liquid chromatography to give turmerone reference: The added acetone fine isolate, isolate and acetone thin volume ratio of 1: 5-1: 10, methanol and water as the mobile phase, methanol the volume of water is 60: 40-90: 10, a flow rate of 5-10 mL / min, 15-17 minutes peak, 20-22 minutes to the end, was collected with a purity of 99% or more of the aromatic turmerone pure.
[0013]

To further verify the technical effect of the present invention, carried out the following experiment:
[0014]

Separating aromatic monomer compound turmerone
[0015]

1.1 Separation by silica gel column chromatography crude
[0016]

Weigh turmeric volatile oil 10 g, with petroleum ether was dissolved in porcelain evaporating dish, mixed with 12 g of silica gel-like, solvent evaporated to dryness, spare; 1:10 with 100 g of silica gel (300-400 mesh) with petroleum ether - acetic acid ethyl ester (15: 1) Wet the column; column of silica gel layer to be no longer down and then the sample, and petroleum ether - ethyl acetate (15: 1) elution pressurized, by spot TLC plate petroleum ether - ethyl acetate (15: 1) to expand, at 254nm under observation, collecting 0.7 Rf polarity similar mixture to give 7.3g.
[0017]

1.2 finely divided silica gel column chromatography
[0018]

Weigh crude mixture isolated 7.3 g, with petroleum ether was dissolved in porcelain evaporating dish, mixed with 10 g of silica gel-like, solvent evaporated to dryness, spare; with 110 g of silica gel (300-400 mesh) with petroleum ether - ethyl acetate Column: (1 50) on wet; no longer be a column of silica gel layer and then down on the sample, and with petroleum ether - ethyl acetate (50: 1) pressurized elution by point TLC plate, petroleum ether - ethyl acetate (25: 1) to expand, under observation at 254 nm, collected Rf 0.5 处 polarity very close to the mixture to give 5.2 g.
[0019]

The mixture was separated by HPLC detection.
HPLC conditions column Hedera C 18 (200 mm), the mobile phase of methanol - water (90:10), the column temperature was 25 ℃, the detection wavelength was 254 nm, the injection volume was 15 μL, measurement results are shown in Figure 2, found that the mixture of 3 components, from left to right as 1, 2, 3, respectively.
[0020]

1.3 Preparative High Performance Liquid Separation

[0021]

Three components finely divided silica gel column chromatography to obtain type by using the Agilent 1260 preparative liquid to Agilent Technologies Agilent ZORBAX SB-C 18 (21.2 × 250 mm, 5 μm) column separation, taking a silica gel column chromatography fine isolated mixture 1 mL, was dissolved in 5 mL of methanol is diluted with the mobile phase were selected methanol - water: 95: 5,90: 10,85: 75,80: 20,75: 25, flow rate 2 mL were selected / min, 5 mL / min, 8 mL / min, 10 mL / min, 12 mL / min, from which to determine the optimal separation conditions.
Finally, to determine the optimum separation conditions mobile phase of methanol - water (80:20), flow rate 10 mL / min.
The three components were collected by a rotary evaporator and the solvent spin dry, give the purified product 1, 2, 3, by MS, 1 H-NMR, 13 C-NMR measurement.
[0022]

1.4 Conclusion
[0023]

By MS, 1 H-NMR, 13 C-NMR were determined to finalize an aryl turmerone.
MS measurement, and the library of the aromatic turmerone data matching was 98%; 1 H-NMR, 13 C-NMR measurement structure is correct, 1 H NMR (CDCl 3, 400 MHz) δ: 1.17 (d, J = 7.2 Hz, 3H), 1.78 (d, J = 1.2 Hz, 3H), 2.03 (d, J = 0.8 Hz, 3H), 2.23 (s, 3H), 2.50-2.56 (m, 1H), 2.61-2.66 (m , 1H), 3.19-3.24 (m, 1H), 5.95 (t, J = 1.2 Hz, 1H), 7.03 (d, J = 1.2 Hz, 4H); 13 C NMR (CDCl 3, 100 MHz) δ: 20.72 , 20.99, 22.00, 27.65, 35.30, 52.70, 124.11, 126.68, 129.12, 135.56, 143.71, 155.10, 199.89.
The results shown in Fig. 3, 4 and 5.
[0024]

2.1 High Performance Liquid Chromatography - purity test
[0025]

Using Agilent 1260 high performance liquid chromatograph (quaternary pump, autosampler, column oven, DAD UV detector, ChemStation chromatography workstation) to parti company Hedera C 18 (4.6 × 200 mm, 5 μm) chromatography column at 25 ℃, the mobile phase was methanol - water (80:20), DAD set wavelength range of 190 ~ 400 nm, setting five 204,220,254,280,310 nm detection wavelength, and at the same time observe Inspection of other wavelengths to study the aromatic turmerone purity.
[0026]

Take aryl turmerone amount, with methanol produced per 1 mL solution containing 1 mg as the test solution, take a blank solvent (methanol) and the test solution of 10 μL, were injected into the liquid chromatograph, results deduct blank After the solvent peaks generated, aromatic turmerone chromatographic peak area normalized content at each wavelength were more than than 99%, see Table 1, Fig. 6 and 7.
[0028]

2.2 mobile phase study
[0029]

Take aryl turmerone test solution, respectively, with acetonitrile - water (80:20), methanol -

water (80:20), methanol -0.05% phosphoric acid (80:20), methanol -0.1% phosphoric acid (80:20), methanol -0.3% phosphoric acid (80:20) as the mobile phase HPLC profiles obtained results show that under various mobile phases were abnormal peak to area normalization method for the determination of mobile phase conditions at each of the aromatic turmerone content of greater than 99%.
The results are shown in Table 2, Figure 8-12.
[0031]

2.3 pillars of study
[0032]

Take aryl turmerone test solution, respectively Hedera C 18 (4.6 × 200 mm, 5 μm) column, Agilent C 8 (4.6 × 150 mm, 5 μm) column, Waters C 18 (4.6 × 250 mm, 5 μm) column HPLC profiles obtained results showed no abnormal change column peak area normalization method to obtain an aromatic ginger measured flavonoids were more than 99%.
The results are shown in Table 3, Figure 13-15.
[0034]

As a result of the technical proposal, compared with the prior art, the present invention turmeric volatile oil as raw material, the silica gel chromatography and preparative HPLC to separation means, to a certain percentage of petroleum ether - ethyl acetate, methanol - water as elution system, aryl turmerone obtained pure product prepared by HPLC, columns and mobile phases in different measurement results are a main peak, change column and mobile phase measurement were abnormal peak, In the area of normalization reference Determination purity greater than 99%, in line with the traditional Chinese medicine chemical reference substance Determination of requirements.
[0035]

Brief Description
[0036]

HPLC test results of products embodiment 1 of the present invention;
[0037]

Figure 2 fine separation of the mixture by HPLC profiles of the present invention;
[0038]

Aryl turmerone EI mass spectra in Figure 3 of the present invention;
[0039]

Aryl turmerone 1 H-NMR Figure 4 of the present invention;
[0040]

Aryl turmerone 13 C-NMR Figure 5 of the present invention;
[0041]

Figure 6 is a HPLC solvent blank map 5 wavelengths;
[0042]

Figure 7 for the next five wavelength measurement of aromatic ginger Flavonoids HPLC

profiles;
[0043]

Figure 8 is acetonitrile - water 80:20 HPLC profiles;
[0044]

Figure 9 is a methanol - water 80:20 HPLC profiles;
[0045]

Figure 10 is an aqueous solution of methanol -0.05% phosphoric 80:20 HPLC profiles;
[0046]

Figure 11 is an aqueous solution of methanol and 0.1% phosphoric acid HPLC chromatogram 80:20;
[0047]

Figure 12 is an aqueous solution of methanol -0.3% phosphoric 80:20 HPLC profiles;
[0048]

13 is Hedera C 18 (4.6 × 200 mm, 5 μm) column;
[0049]

Figure 14 is Agilent C 8 (4.6 × 150 mm, 5 μm) column;
[0050]

Figure 15 is a Waters C 18 (4.6 × 250 mm, 5 μm) column.
[0051]

DETAILED DESCRIPTION
[0052]

Embodiments of the present invention: preparation of aromatic turmeric volatile oil turmerone reference to turmeric volatile oil as raw material, the process comprising the steps of:
[0053]

(1) pressure normal phase silica gel column rough separator: 10g of turmeric volatile oil plus 300-400 mesh silica gel 100g, in a volume ratio of 15: 1 petroleum ether - ethyl acetate as eluant, pressure column chromatography by spot TLC plate with petroleum ether - ethyl acetate (15: 1) to expand, at 254nm under observation, collecting 0.7 Rf mixture of similar polarity, i.e. the crude isolate, to 7.3g;
[0054]

(2) pressure normal phase silica gel thin separation: the plus 300-400 mesh silica gel 110g 7.3g crude isolates in a volume ratio of 50: 1 petroleum ether - ethyl acetate as eluant, pressure column chromatography Analysis by point TLC plate with petroleum ether - ethyl acetate (25: 1) to expand, at 254 nm observed collecting Rf 0.5 处 very close to the polar mixtures of finely divided matter ,, namely, to 5.2g;
[0055]

(3) aryl preparative high performance liquid chromatography to give turmerone reference: the addition of finely divided matter acetone, finely divided per 1ml 5ml of acetone was added to dilute each injection 0.4 mL, methanol and water as the mobile phase, volume of methanol and water 80:20, a flow rate of 10 mL / min, 16 mins peak, 21 minutes ended, once Tokuyoshi turmerone pure 30 mg.
[0056]

The obtained aromatic turmerone pure by HPLC detection of methanol and water as the mobile phase, the volume of methanol to water is 80:20, the column temperature is 25 ℃, the flow rate was 0.8ml / min, the detection wavelength was 242nm, column It is Hederac C 18 200mm, detection seen in FIG. 1, a purity of 99.95%.

TW200904465
Method of purifying turmerone in turmeric oil

This invention relates to a method of purifying turmerone in turmeric oil, comprising: extracting turmeric oil from turmeric, a raw material of Chinese herbal medicine, with the use of supercritical carbon dioxide; proceeding with a normal phase resin column purification; and eluting the column with an elution solution with five different ratios (volume ratio) containing hexane and ethyl acetate and so as to increase the purity of three different turmerones, namely ar-turmerone, alpha-turmerone, and beta-turmerone.

KR20100105162
APOPTOTIC EFFECT OF AR-TURMERONE IN HUMAN HISTIOCYTIC LYMPHOMA U 937 CELLS

PURPOSE: A composition containing ar-turmerone for preventing and treating human malignant lymphoma cells is provided to suppress cancer cell proliferation and to treat malignant lymphnoma. CONSTITUTION: A composition for treating or preventing human malignant lymphoma contains ar-turmerone. The ar-turmerone is derived from turmeric or Curcuma zedoaria. ar-turmerone has apoptosis efficiency to human malignant lymphoma cells(U937).

Aromatic Tew Melon of human malignant lymphoid tumor cell killing effect for {Apoptotic effect of ar-turmerone in human histiocytic lymphoma U 937 cells.

The ar-turmerone to prove this assignment 40-160 ug / ml during treatment, it was confirmed the DNA fragmentation (DNA fragmentation).

In addition, the amount of [3H] -thymidine incorporation is a DNA segment according to the segment rate when the concentration increased to make test was confirmed that the increase.

When measuring the state of cell division in the flow cytometer (flowcytometry) U937 cells in the same concentration, sub-diploid number of cells also exhibited increases in accordance with increase in the concentration of ar-turmerone.

By treatment 40-160 ug / ml of ar-turmerone to prove this assignment, DNA electrophoresis, [3H] -thymidine incorporation test, was used for flow cytometry technology (FACScan flowcytometry).

That is, cancer cells, not to ar-turmerone (human lymphoid tumor cells U937 malignant) cell proliferation inhibitory activity is proliferation is inhibited by cell death (necrosis), and demonstrated the growth is suppressed by cell death (apoptosis).

Treated 48 hours and ar-turmerone to 40-160 ug / ml concentrations to demonstrate the problem, it was confirmed a DNA fragmentation (DNA fragmentation).

In addition, the amount of [3H] -thymidine incorporation is a DNA segment according to the segment rate when the concentration increased to make test was confirmed that the increase.

When measuring the state of cell division in the flow cytometer (flowcytometry) U937 cells in the same concentration, sub-diploid number of cells also exhibited increases in accordance with increase in the concentration of ar-turmerone.

As a result, the cancer cells in the ar-turmerone (human malignant lymphoid tumor cell U937) death (apoptosis) has proven efficacy.

The challenge for ar-turmerone to prove 40-160 ug / ml 48 h treatment, 80 ug / ml was identified clearly by DNA fragmentation (DNA fragmentation) in more depth.

Also, when checking the degree of DNA fragmentation in [3H] -thymidine incorporation test, at 40 ug / ml or more concentrations (48 hr), the segment rate was markedly increased compared with the control group showed significant.

After a 48-hour treatment Ar-turmerone, U937 when measuring the state of cell division in a flow cytometer (flowcytometry) cells, 80 [mu] g / ml or more concentrations in the number of sub-diploid cells showed significantly increased.

US2014243420
ANTICONVULSANT ACTIVITY OF TURMERIC OIL AND BISABOLENE SESQUITERPENOIDS

The present invention relates to the anti-convulsant activity of turmeric oil and its volatile bisabolene sesquiterpenoids ar-turmerone, [alpha]-turmerone, [beta]-turmerone (curlone) and [alpha]-atlantone, as an anticonvulsant agent for the treatment of epilepsy and/or as therapeutic agents for the treatment of disorders of the central nervous system, including tremor, pain, mood disorders (including depression, bipolar disorder, attention deficit-hyperactivity disorder, and schizophrenia), and neurodegenerative diseases.

FIELD OF THE INVENTION

[0001] The present invention relates to the anticonvulsant activity of turmeric oil and its volatile bisabolene sesquiterpenoids ar-turmerone, α-turmerone, β-turmerone (curlone) and α-atlantone, as an anticonvulsant agent for the treatment of epilepsy and/or as a therapeutic

agent for the treatment of disorders of the central nervous system, including tremor, pain, mood disorders (including depression, bipolar disorder, attention deficit-hyperactivity disorder, and schizophrenia), and neurodegenerative diseases.

BACKGROUND OF THE INVENTION

[0002] Epilepsy is a widespread neurological disorder that affects approximately 50 million people worldwide (1). According to the World Health Organization (WHO), about 1% of the total burden of disease corresponds to various forms of epilepsy. Its pharmacologic treatment comprises a number of currently available antiepileptic drugs (AEDs) (2). The main problem concerning AEDs is the high incidence of side effects ranging from gastrointestinal distress, hepatotoxicity, depression, cognitive impairment and even refractory seizures (2) (3) (4) (5) (6). Moreover, about one third of patients suffering from epilepsy remain resistant to available treatments (1) (2). Hence, there is a clear need to continue to identify novel AEDs that control seizures with minimal adverse effects.

[0003] Medicinal plants and the chemical compounds contained therein represent a potential source of novel AEDs. Numerous studies on the use of ethnomedicinal plants for the treatment of seizures have been reported (7). Small molecule compounds and essential oils extracted from plants have been shown to exhibit anticonvulsant properties (18) (19) (20). One compound, losigamone, derived from the kava kava plant and originally used by traditional healers in the South Pacific as an anxiolytic, is now in early clinical development as a novel antiepileptic drug (8) (9). Another plant, Curcuma longa L., is a medicinal perennial herb of the Zingiberaceae family native to South Asia. It has been traditionally used as a carminative, laxative, anthelmintic and as a treatment for liver disorders. The powder of its rhizomes, turmeric, has been used not only as a condiment and color additive in food but also in traditional medicine against epilepsy (10). Its major active chemical constituents are the curcuminoids (3-5%) and the volatile turmeric oil (2-7%). Turmeric oil is mainly composed of bisabolene sesquiterpenoids: ar-, α-, β-, turmerone, α-atlantone and curlone, whereas the curcuminoids include curcumin, monodemethoxycurcumin and bisdemethoxycurcumin. Nearly all investigations on the medicinal properties of turmeric have been focused on curcumin, whose anticonvulsant activities have been demonstrated in several rodent models such as the iron-induced epileptogenesis (11), maximal electroshock (12), kainic acid-induced (13) and pentylenetetrazole-kindling (14) models. However, while a few studies on the neuroprotective activity of turmeric oil have been performed (15) (16) (17), a specific link between anticonvulsant activity and non-curcuminoid compounds such as volatile turmeric oil or bisabolene sesquiterpenoids has not been evaluated. Notably, previous studies on the volatile constituents of turmeric oil were limited due to the complex isolation steps involved.

[0004] Described herein is a practical method to isolate the main constituents of turmeric oil through RP-HPLC. The isolated compounds were individually evaluated in two vertebrate model systems: the zebrafish (Danio rerio) and the mouse (Mus musculus). Over the past decade, the zebrafish has emerged as a valuable model for genetic studies and drug screening. The strength of this in vivo model relies on its high genetic, physiologic and pharmacologic homology to humans. Their high fecundity and small size allow for the performance of tests in a medium- to high-throughput fashion using minute (microgram-scale) quantities of compound. The zebrafish also holds promise as an in vivo model for identifying novel neuroactive compounds since the dopaminergic, serotonergic, and GABAergic systems develop early during embryogenesis and are already functional in larvae (21). In addition,

their rapid development ex utero and optical transparency makes it possible to easily detect morphological and behavioral effects of test compounds on living embryos and larvae (22).

[0005] More recently, zebrafish have also proven useful for the primary screening of potential novel anticonvulsants (23) (24) (25). An acute zebrafish seizure model based on the proconvulsant pentylenetetrazole (PTZ) has been described (23). The exposure of zebrafish larvae to PTZ evoked a sequence of behavioral changes, which were classified into three phases: a notable increase in swimming activity (stage I); rapid “whirlpool-like” circular swimming motion (stage II), and clonic movements with subsequent loss of posture and loss of movement for 1-3 seconds followed by tonic contractions (stage III) (23). In addition, electrophysiological recordings confirmed that zebrafish larval brains treated with PTZ displayed a series of ictal and interictal discharges. A follow-up study validated this zebrafish chemoconvulsant model by showing that 13 out of 14 clinically used AEDs were capable of suppressing PTZ-induced seizure behaviors in zebrafish (24).

[0006] In the course of screening a series of medicinal plants for their potential anticonvulsant activities in the zebrafish PTZ chemoconvulsant model, we confirmed the reported anticonvulsant properties of curcumin. Surprisingly, however, further testing of turmeric oil and its chromatographic fractions revealed additional constituents capable of suppressing PTZ-induced seizure behaviors in larval zebrafish. Mass spectrometry and NMR analysis of these active purified fractions revealed them to belong to the bisabolene sesquiterpenoids ar-turmerone, α-, β-turmerone (curlone) and α-atlantone. The anticonvulsant activities identified using the zebrafish PTZ assay were then confirmed in the equivalent mouse PTZ-induced seizure model and the 6 Hz psychomotor seizure model of partial epilepsy. Additionally, an assessment on motor coordination and balance was performed jn mice using the elevated bridge after i.v. injection of ar-turmerone in order to determine any side effects leading to motor impairment.

[0007] There have been some publication providing turmeric extracts, for use in medicine, however, none of them provides the use thereof as an anticonvulsant agent in the treatment of disorders of the central nervous system. For example WO2007109210 and WO2010045577 provide extracts of curcuma plants, and methods of treating neurodegenerative disorders such as disorders associated with amyloid plaque aggregation or fibril formation (e.g. Alzheimer's disease), however, neither patent application discloses or suggests a potential use of curcuma extracts as anticonvulsant agents. WO2011080090 provides formulations of turmeric oil having anti-inflammatory, analgesic and/or anti-cancer activities, however, again it neither discloses nor suggests a potential use of curcuma extracts as anticonvulsant agents.

[0008] Even more, it is known that bisabolene-type sesquiterpenoids exhibit Acetylcholine esterase inhibitory activity (41), whereas it has also been shown that AChE blockers, in general induce seizures and may lead to status epilepticus, resulting in spontaneous seizures following a latent period (42). It was therefore surprising to find that bisabolene-type sesquiterpenoids are in fact capable of reducing the extent of epileptic seizures, rendering them suitable as anticonvulsant agents in the treatment of central nervous system disorders.

SUMMARY OF THE INVENTION

[0009] A first aspect of the present invention relates to a bisabolene sesquiterpenoid for use as an anticonvulsant agent in disorders of the central nervous system. In some embodiments, the present invention relates to a bisabolene sesquiterpenoid of turmeric oil for use as an

anticonvulsant agent in disorders of the central nervous system. The turmeric oil may be from a Curcuma genus in particular Curcuma longa L.

[0010] In a certain embodiment, the bisabolene sesquiterpenoid according to this invention is selected from the list comprising ar-turmerone, α-turmerone, β-turmerone and α-atlantone. In a further aspect, the present invention provides a liquid composition comprising one or more bisabolene sesquiterpenoids according to this invention; for use as an anticonvulsant agent in disorders of the central nervous system. In a preferred embodiment, the liquid composition according to this invention is turmeric oil from a Curcuma genus in particular Curcuma longa L.

[0011] In a further aspect, the invention relates to bisabolene sesquiterpenoids or a composition comprising one or more bisabolene sesquiterpenoid for use as a therapeutic agent for the treatment of disorders of the central nervous system; wherein said disorders are selected from the list comprising: epilepsy, tremor, pain, mood disorders and neurodegenerative diseases; in particular epilepsy. Said mood disorders can be depression, bipolar disorder, attention deficit-hyperactivity disorder, and schizophrenia. Said neurodegenerative disorders might not include Alzheimer's disease.

[0012] Preferably, said disorders of the central nervous system are not cerebrovascular disorders. With cerebrovascular disorders are meant the disorders indicated as cerebrovascular disorders in patent application WO03/051380 published on 26 Jun. 2003. Hence, with “cerebrovascular disorder” is meant a disorder selected from a group comprising ischaemia, stroke, post-stroke injury, hemorrhage, reperfusion injury, thrombosis, vasoconstriction, nitric oxide-induced free radical oxidative damage, infraction, inflammation, and Alzheimer's disease.

BRIEF DESCRIPTION OF THE FIGURES

[0013] FIG. 1. Schematic diagram of the videotracking procedure in a 96-well plate using 7-dpf zebrafish larvae for the anticonvulsant activity evaluation (FIG. 1A). The plate was incubated in dark conditions inside the zebrabox with 100 μL vehicle or drug and one larvae per well. After 1 hour of incubation, 100 μL of vehicle of PTZ was added to the first and second wells respectively in order to monitor larval behavior in presence of the vehicle/compound and the proconvulsant for 30 minutes. FIG. 1B shows the zebrabox, core system, and larval behavior screen.

[0014] FIG. 2. Comparison of the anticonvulsant activity of A) curcuminoids (curcumin) and B) turmeric oil. Curcumin showed potent activity in inhibiting PTZ-induced seizures ($p<0.05$). Turmeric oil also displayed anticonvulsant activity ($p<0.05$).

[0015] Summary of the evaluation of the anticonvulsant activity of turmeric in the zebrafish PTZ seizure assay (C) Turmeric methanolic extract; (D) curcuminoids and (E) turmeric oil. Tested concentrations are indicated along the x-axis, and the total gross locomotor activity exhibited by zebrafish larvae within 30 min is displayed along the y-axis. Data are expressed as the mean±SD (n=10-12). Statistically significant differences between vehicle-treated and sample-treated (white bars) or PTZ-treated and sample plus PTZ-treated groups (gray bars) are labeled as * for $p<0.05$ and ** for $p<0.01$.

[0016] FIG. 3: Evaluation of the anticonvulsant effects of phenytoin (A) and diazepam (B),

which served as positive controls for the PTZ-induced zebrafish acute seizure model.

[0017] FIG. 4: HPLC chromatogram of turmeric oil and its major constituents. Peak 4 corresponds to A) ar-turmerone; peak 5 to B) α-turmerone and β-turmerone (curlone) and peak 6 to C) α-atlantone.

[0018] FIG. 5: Anticonvulsant activity evaluation of ar-turmerone: The x-axis represents the type of treatment. The y-axis indicates the total distance moved in 30 minutes. For PTZ group, statistical significance is identified as * for $p<0.05$ and ** for $p<0.01$; control group is indicated with s for $p<0.05$ and ss for $p<0.01$.

[0019] FIG. 6: Anticonvulsant activity evaluation of α-turmerone and β-turmerone (curlone). The x-axis represents the type of treatment. The y-axis indicates the total distance moved in 30 minutes. For PTZ group, statistical significance is identified as * for $p<0.05$ and ** for $p<0.01$; control group is indicated with s for $p<0.05$ and ss for $p<0.01$.

[0020] FIG. 7: Anticonvulsant activity evaluation of α-atlantone. (A) The x-axis represents the type of treatment. The y-axis indicates the total distance moved in 30 minutes. For PTZ group, statistical significance is identified as * for $p<0.05$ and ** for $p<0.01$; control group is indicated with s for $p<0.05$ and ss for $p<0.01$.

[0021] Summary of evaluation of the anticonvulsant activity of bisabolene sesquiterpenoids in the zebrafish PTZ seizure assay. (B) Ar-turmerone; (C) α,β-turmerone and (D) α-atlantone. The x-axis represents the tested concentration for each one of the sesquiterpenoids. The y-axis indicates the total gross locomotor activity exhibited by zebrafish larvae within 30 min. Data are expressed as the mean±SD (n=10-12). Statistically significant differences between vehicle-treated and sample-treated (white bars) or PTZ-treated and sample plus PTZ-treated groups (gray bars) are labeled as * for pb0.05 and ** for pb0.01.

[0022] FIG. 8: Anticonvulsant activity of turmeric oil and ar-turmerone in the mouse PTZ-induced seizure assay. Graphs depict the dose of PTZ required to evoke various seizure behaviors. The PTZ dose for control is set to 100% (inner heptagons in A and B) and results obtained with turmeric oil (outer heptagon in A) and ar-turmerone (outer heptagon in B) are depicted relative to control. Statistical significance between control and experimental PTZ doses required to induce each seizure behavior were calculated using the unpaired Student's t-test. Statistical significance vs. controls is labeled as (*) for $p<0.05$ and (**) for $p<0.01$.

[0023] FIG. 9. Evaluation of the anticonvulsant activity of turmeric oil in the mouse PTZ seizure model. Top panel: table listing PTZ dose/s required to elicit the indicated seizure behaviors after treatment with turmeric oil or vehicle only. Data are expressed as the mean ±SD (n=5). Graphical depiction of tabulated results from (A) turmeric oil at 50 mg/kg and (B) at 100 mg/kg. Results are expressed as relative values compared to control (set as 100%). Statistically significant differences between sample (dark gray) and control group (light gray) are labeled as * for pb0.05 and ** for pb0.01 (unpaired Student's t-test). For sake of clarity, SDs are not depicted in the graphs but are indicated in the tables. However, the coefficient of variation never exceeded 28% (unpaired Student's t-test).

[0024] FIG. 10. Evaluation of the anticonvulsant activities of α,β-turmerone and ar-turmerone in the mouse PTZ seizure model. Top panel: table listing PTZ dose/s required to elicit the indicated seizure behaviors after treatment with bisabolene sesquiterpenoid or vehicle only.

Graphical depiction of tabulated results from (A) α,β-turmerone at a dose of 100 mg/kg and (B) ar-turmerone at 50 mg/kg. 'Control A' column corresponds to vehicle-treated controls for α,β-turmerone; 'Control B' column corresponds to vehicle-treated controls for ar-turmerone. Data are expressed as the mean±SD (n=5). For sake of clarity, SDs are not depicted in the graphs but are indicated in the tables. Results are expressed as relative values compared to control (set as 100%). Statistically significant differences between sample (dark gray) and control group (light gray) are labeled as * for $p<0.05$ and ** for $p<0.01$ (unpaired Student's t-test). For the sake of clarity, SDs are not depicted. However, the coefficient of variation never exceeded 28% and 37% in the case of ar-turmerone and α,β-turmerone, respectively.

[0025] FIG. 11. Evaluation of the anticonvulsant activity of sodium valproate (positive control) in the zebrafish and mouse PTZ seizure assays. (A) Zebrafish PTZ assay. The x-axis represents the concentration of the sodium valproate evaluated. The y-axis indicates the total gross locomotor activity exhibited by zebrafish larvae within 30 min. Data are expressed as the mean±SD (n=10-12). Statistically significant differences between vehicle-treated and sample-treated (white bars) or PTZ-treated and sample plus PTZ-treated groups (gray bars) are labeled as * for $p<0.05$ and ** for $p<0.01$. (B) Mouse PTZ assay. Top panel: table listing PTZ dose/s required to elicit the indicated seizure behaviors after treatment with sodium valproate or vehicle only. Lower panel: graphical depiction of tabulated results from treatment with sodium valproate at a dose of 50 mg/kg. Results are expressed as relative values compared to control (set as 100%). Significant differences between sodium valproate (dark gray) and control group (light gray) are labeled as * for $p<0.05$ and ** for $p<0.01$ (unpaired Student's t-test). For the sake of clarity, SDs are not depicted. However, the coefficient of variation never exceeded 45%.

[0026] FIG. 12. Data set from the C57B1/6 male mice after i.v. injection of vehicle (negative control), diazepam 1 mg/kg (positive control), and ar-turmerone 50 mg/kg on the elevated bridge apparatus. Measures of number of footslips (A), number of falls (B) and total time on beam (C) are showed. Diazepam was selected as positive control due to its well-know motor impairment side effect after i.v/i.p administration in mice.

[0027] FIG. 13. Evaluation of the protective activity of ar-turmerone in the 6-Hz model. Vehicle (negative control) and valproic acid 300 mg/kg (positive control) were included in the assessment. Data points indicate the number of animals protected from seizures at the corresponding dose (n=6).

DETAILED DESCRIPTION OF THE INVENTION

[0028] A first aspect of the present invention relates to a bisabolene sesquiterpenoid for use as an anticonvulsant agent in disorders of the central nervous system. One or more bisabolene sesquiterpenoids may be used either alone or in combination. The bisabolene sesquiterpenoids may be a sesquiterpenoid of turmeric oil. In some embodiments, the bisabolene sesquiterpenoids are isolated from turmeric oil. The Curcuma longa L plant may be a source for turmeric oil and/or bisabolene sesquiterpenoids. Other sources for bisabolene sesquiterpenoids include, but are not limited to, essential oils from plants (for example Peltphorum dasyrachis Kurz ex Bakar (Yellow Batai)), insects, natural products produced by living organisms (for example, honeycomb extract), fungi, bacteria, and/or microorganisms. Bisabolene sesquiterpenoids may also be produced via chemical synthesis.

[0029] "Bisabolenes" are a group of closely related natural chemical compounds which are

classified as sesquiterpenes (a class of terpenes consisting of three isoprene units). Biochemical modifications such as oxidation or rearrangement produce the related sesquiterpenoids.

[0030] The term “turmeric” is also interchangeable with “curcuma” and includes plants, clones, variants and sports from the plant Zingiberaceae family. In particular, turmeric includes plants, clones, variants and sports from the plant genus Curcuma; more in particular Curcuma longa L. Therefore, in a preferred embodiment, the turmeric oil is from a Curcuma genus in particular Curcuma longa L. Turmeric, and in particular its rhizomes, contains about 3-5% of curcuminoids, such as curcumin and about 2-7% of turmeric oil. A “rhizome” is a stem of a plant which is usually found underground, often sending out roots and shoots from its nodes.

[0031] “Turmeric oil” can be obtained as detailed herein below in the examples, such as by hydro-distillation of dried rhizome powder of Curcuma. However, it may also be obtained via any other suitable way. Turmeric oil is mainly composed of bisabolene sesquiterpenoids: ar-turmerone, α-turmerone, β-turmerone and α-atlantone, and thus in a particular embodiment, the present invention provides ar-turmerone, α-turmerone, β-turmerone and/or α-atlantone for use as an anticonvulsant agent in disorders of the central nervous system. In certain embodiments, ar-turmerone, α-turmerone, β-turmerone, and α-atlantone are administered singly. In some embodiments, ar-turmerone is administered in combination with α-turmerone, β-turmerone, and/or α-atlantone. Ar-turmerone may also be adminstered with one or more of turmerone, β-turmerone, and/or α-atlantone. In certain embodiments, α-turmerone is administered in combination with ar-turmerone, β-turmerone and/or α-atlantone. α-turmerone may also be administered in combination with one or more of ar-turmerone, β-turmerone and/or α-atlantone. In some embodiments, β-turmerone is administered in combination with ar-turmerone, α-turmerone, and/or α-atlantone. β-turmerone may be administered in combination with one or more of ar-turmerone, α-turmerone, and/or α-atlantone. In certain embodiments, α-atlantone may be administered in combination with ar-turmerone, α-turmerone, and/or β-turmerone. α-atlantone may be administered in combination with one or more of ar-turmerone, α-turmerone, and/or β-turmerone. When two or more compounds are administered, the administration may be simultaneous or serial.

[0032] Turmeric oil and/or bisabolene sesquiterpenoids are lipophilic and cross the blood-brain barrier and other cell membranes, a quality which may enhance bioavailability of the compounds in the nervous system. Thus, the use of tumeric oil and/or bisabolene sesquiterpenoids confers advantages over the use of other compounds such as curcumin. Curcumin is also a component of the Curcuma longa L plant, and a compound to which the anticonvulsant activity of Curcuma has been attributed (11) (12) (32). However, certain formulations of curcumin are readily converted to water soluble metabolites in the intestines and excreted, so that little of the compound reaches the blood or the nervous system.

[0033] The term “anticonvulsant agent” as used herein is meant to include any compound suitable for the treatment of epileptic seizures, bipolar disorders, mood disorders and/or neuropathic pain. Epileptic seizures may result from any abnormal, excessive, or hypersynchronous neuronal activity in the brain. In some embodiments, epileptic seizures which require treatment with anticonvulsants are caused by infection, stroke, trauma, fever, tumors, drug use, damage to the blood-brain barrier, and/or neurodegenerative disease. In certain embodiments, epileptic seizures are triggered by emotional state, by response to light and/or sound, sleep, sleep deprivation, hormones, metabolic disorders, and/or congenital

defects. Epileptic seizures for which the anticonvulsants disclosed herein provide treatment may be classified as partial seizures, such as simple partial seizures and/or complex partial seizures, or they may be classified as generalized seizures, such as absence seizures, myoclonic seizures, clonic seizures, tonic seizures, tonic-clonic seizures, and/or atonic seizures, or a mixed seizure. The anticonvulsants described herein, such as turmeric oil, ar-turmerone, α-turmerone, β-turmerone and/or α-atlantone, may also provide treatment for therapy-resistant forms of seizure. Notably, the 6 Hz psychomotor seizure model of partial epilepsy has been used as a model therapy-resistant forms of seizures, including limbic seizures (40).

[0034] Patients suffering from epileptic seizures may be infants aged 0-6 months, 6-12 months, 12-18 months, 18-24 months. In certain embodiments, patients suffering from epileptic seizures are individuals aged 65-70, 75-80, 85-90, 95-100, 100-105, and older. Patients may also be children aged 2-12, adolescents aged 13-19, or adults aged 20-64.

[0035] Anticonvulsants may be used for the treatment of epileptic seizures, including treatment of symptoms associated with epileptic seizures and/or epilepsy. Anticonvulsants may also be used to treat epileptic seizures that result from central nervous system disorders such as cerebrovascular diseases and/or neurodegenerative diseases. One goal of an anticonvulsant agent (i.e., an "anticonvulsant") is to suppress the rapid and excessive firing of neurons that start a seizure. Another goal of an anticonvulsant is to prevent the spread of the seizure within the brain and offer protection against possible excitotoxic effects, that may result in brain damage. Anticonvulsants are also called antiepileptic drugs (abbreviated "AEDs"), and are sometimes referred to as antiseizure drugs. In epilepsy, an area of the brain and/or nervous system is typically hyper-irritable. Antiepileptic drugs function to help reduce this area of irritability and thus prevent epileptic seizures.

[0036] The term "central nervous system disorder" is meant to include any disease or disorder of the central nervous system (CNS) including epilepsy, tremor, pain, mood disorders (including depression, bipolar disorder, attention deficit-hyperactivity disorder, schizophrenia); infections of the CNS (e.g. encephalitis), neurodegenerative diseases (e.g. amyothrophic lateral sclerosis, Parkinson's Disease), autoimmune and inflammatory diseases (e.g. multiple sclerosis) and genetic disorders (e.g. Huntington's diseases); in particular epilepsy. In a particular embodiment, the neurodegenerative disorders of the present invention do not include Alzheimer's Disease. In some embodiments, Alzheimer's Disease and/or other neurodegenerative diseases lead to epileptic seizures, which may be treated using anticonvulsants as described herein.

[0037] The present invention further provides a liquid composition comprising one or more bisabolene sesquiterpenoids according to this invention; for use as an anticonvulsant agent in disorders of the central nervous system. In a particular embodiment, said liquid composition is turmeric oil from a Curcuma genus in particular Curcuma longa L.

[0038] The liquid composition according to this invention in particular comprises an effective amount of bisabolene sesquiterpenoids. As evident for a person skilled in the art, said effective amount may vary depending on the number and type of bisabolene sesquiterpenoids used. For example turmeric oil as a liquid composition may be used pure or further diluted to a concentration of about 1-50 μg/ml, more in particular about 2.5-20 μg/ml, in particular about 10 μg/ml. Ar-turmerone, α-turmerone, β-turmerone and α-atlantone either or not in combination with each other may for example be present at a concentration of about 11-46

μM, more in particular about 23-46 μM.

[0039] These liquid compositions may be formulated and administered systemically or locally. Techniques for formulation and administration may be found in the latest edition of "Remington's Pharmaceutical Sciences" (Mack Publishing Co. Easton Pa.). Suitable routes may, for example, include oral or transmucosal administration as well as parenteral delivery, including intramuscular, subcutaneous, intramedullary, intrathecal, intraventricular, intravenous, intraperitoneal, or intranasal administration.

[0040] In a further embodiment, the compositions may be in the form of nutritional or dietary supplements, including tablets, capsules, gels, pastes, emulsions, solutions, caplets, and the like.

EXAMPLES

[0041] Having provided a general disclosure, the following examples help to illustrate the general disclosure. These specific examples are included merely to illustrate certain aspects and embodiments of the disclosure, and they are not intended to be limiting in any respect.

[0042] Certain general principles described in the examples, however, may be generally applicable to other aspects or embodiments of the disclosure.

Example 1

Materials and methods

Chemicals and Reagents

[0043] Dimethyl sulphoxide (99.9%, spectroscopy grade) was procured from Acros Organics (Belgium); diethyl ether (99.9%, spectroscopy grade) from Aldrich Chemical; and acetonitrile (100%, HPLC grade) from Fisher Scientific (UK). Double-distilled water (ddH2O) was obtained from the Milli-Q purification system.

[0044] The curcuminoid mixture from turmeric (curcumin 98%, demethoxycurcumin and bisdemethoxycurcumin) and phenytoin was procured from Acros Organics. PTZ was obtained from Sigma-Aldrich (Germany) and diazepam from Roche.

Plant Material

[0045] Dried rhizome powder of Curcuma longa L. (turmeric) was acquired from a local supplier in Belgium with India as the source of origin. Microscopic authentication was completed by a research fellow: R. Ansalloni, Universidad de Cuenca, Cuenca, Ecuador (26).

[0046] Experimental Animals

[0047] All procedures for animal experiments were performed in accordance with the European and National Regulations and approved by the Animal Care and Use Committee of the Katholieke Universiteit Leuven.

[0000] Zebrafish (Danio rerio)

[0048] Adult zebrafish of the Tg (flu 1a: EGFP)yl strain were reared at 28.5° C. on a 14/10 hour light/dark cycle. Eggs were collected from natural breeding and fostered in embryo medium (17 mM NaCl, 2 mM KCl, 1.8 mM Ca(NO3)2, 0.12 mM MgSO4, 1.5 mM HEPES buffer pH 7.1-7.3 and 0.6 μM methylene blue) in an incubator at 28.5° C. Sorting of zebrafish embryos and larvae and medium refreshment were performed every day until 7 dpf. All larvae were sacrificed through administration of an overdose of anesthetic (tricaine).

[0000] Mice (Mus musculus)

[0049] Male C57B1/6 mice (20-30 g) from 8 weeks of age were housed in appropriate cages under 12/12 hour light/dark cycle at 28° C. in a quiet room. The animals were fed ad libitum with a pellet diet and water until they were 10 to 12 weeks old.

Example 2

Distillation of Turmeric Essential Oil

[0050] Volatile oil from turmeric was obtained by hydro-distillation using a Clevenger-type apparatus according to the European Pharmacopoeia. Turmeric sample (100 g) was extracted with 2 liters of ddH2O for 3 hours. Four hydro-distillations (400 g) were completed obtaining the pale yellowish and odoriferous oil (yield 2.14%). Turmeric oil was dried over anhydrous sodium sulphate and stored at 4° C. until used.

Example 3

RP-HPLC Analysis of Turmeric Oil and Isolation of its Constituents

[0051] Sample of turmeric oil (334 mg) was dissolved in 10 ml of acetonitrile. The injection volume was 300 μl. RP-HPLC analysis of turmeric oil and the subsequent isolation of its constituents were adapted from the original work of He and colleagues (27). RP-HPLC analysis was performed on a high performance liquid chromatographer (LaChrom Elite HPLC System, VWR Hitachi) equipped with diode array detection (DAD) system. RP-HPLC separation of turmeric oil constituents on a preparative scale was achieved using Econosphere 10 μm C18 (250 mm×10 mm) reversed phase column (Grace Davison Discovery Sciences, Belgium) attached to an Econosphere 10 μm C18 (33 mm×7 mm) guard column (Grace Davison Discovery Sciences, Belgium). The column operated at a flow rate of 5 ml/min at room temperature. The profile of the gradient elution was: double-distilled water (ddH2O) (A) and acetonitrile (B); 0-15 min, 40-60% B; 15-20 min, 60-100% B; 20-25 min, 100% B; 25-30 min, 100-40% B. The analytes were monitored with DAD at 260 nm. Eight fractions were individually collected (FIG. 4). Solvents from the collected fractions were removed by separation between diethyl ether and ddH2O.

[0052] The ether phase was dried over anhydrous sodium sulphate and the solvent was removed by passing a slow stream of nitrogen over the sample at room temperature. The concentrated samples were stored at 4° C. until analyzed.

Example 4

Chemical Structure Elucidation of Bisabolene Sesquiterpenes

Nuclear Magnetic Resonance (NMR) Analysis

[0053] <1>H and <13>C NMR-spectra of fractions 4, 5 and 6 were obtained from Bruker 300 Avance and Bruker 600 Avance II<+> equipment using deuterated chloroform as solvent and tetramethylsilane (TMS) as internal standard.

Mass Spectroscopy (MS) Analysis

[0054] The LC-MS analysis was performed on an Agilent 1100 system equipped with degasser, quaternary pump, autosampler, UV-DAD detector and thermostatised column module coupled to Agilent 6110 single-quadrupole MS. Data acquisition and quantification were obtained from Agilent LC/MSD Chemstation software. Fractions 4, 5 and 6 were analyzed on a Grace Prevail RP-C18 column 3 μm (150 mm×2.1 mm) at a flow rate of 0.2 ml/min. The LC gradient comprised two solvents: double-distilled water (ddH2O)+0.1% formic acid (A) and acetonitrile (B); 0-17 min, 40-60% B; 17-32 min, 60-100% B; 32-55 min, 100% B.

[0055] The ESI-MS analysis was completed in a Thermo Electron LCQ Advantage apparatus with Agilent 1100 pump and injection system coupled to Xcalibur data analyzing software.

Example 5

Toxicological Evaluation in Zebrafish Model

[0056] The aim of this assay was to determine the range of appropriate concentrations to be tested in zebrafish for the anticonvulsant activity evaluation. Seven-dpf zebrafish were placed into a 24-well plate (tissue culture plate, flat bottom, FALCON®, USA), six larvae per well. They were incubated with different concentrations of a test compound dissolved in 1 ml of embryo medium (1% DMSO). The larvae were examined each hour during the period of 6 hours, and compared to control group to detect the following signs of toxicity: the absence of startle response to plate taps, changes in heart rate or circulation, presence of edema, paralysis and death. Thus, the maximum tolerated concentration (MTC) was defined as the highest concentration at which no signs of toxicity were observed in 6 out of 6 zebrafish larvae within 6 hours of exposure to a test compound.

[0057] In addition, the larvae were examined during a period of 24 h in sample and compared to control group to detect toxicity. Thus, the maximum tolerated concentration (MTC) was also defined as the highest concentration at which no signs of toxicity were observed in 6 out of 6 zebrafish larvae within 24 h of exposure to sample.

Example 6

Anticonvulsant Activity Evaluation in Zebrafish PTZ Model

[0058] Zebrafish larvae from 7-dpf were tracked using the ViewPoint VideoTrack System for Zebrafish™ (Version 2.3.1.0, ViewPoint, France). The system consists of an infrared light source, a high-resolution digital videocamera to capture larval movements within a defined time period (30 minutes in our experimental set-up) and the software to analyze larval locomotor activity (FIG. 1).

[0059] The highest concentration tested corresponds to the previously determined MTC. Zebrafish larvae were placed in a 96-well plate (tissue culture plate, flat bottom, FALCON®, USA); one larva per well. Each row of the plate (12 wells) comprised different treatment groups. Two adjacent rows contain the same compound but received two different treatments: a) first row, embryo medium (DMSO 1%), and b) second row, PTZ 20 mM. The first two rows of the plate (vehicle control group, where vehicle was embryo medium) contained a volume of 100 μl of embryo medium (1% DMSO) per well. The following three test groups (two rows each) contained 100 μl of different concentrations of test compound in embryo medium. The larvae thus treated were incubated at room temperature in dark and quiet conditions for 1 hour. Embryo medium (100 μl) was added to the first rows of each one of the four groups. Likewise, 100 μl of PTZ 40 mM were added to the second rows of each treatment group (final concentration of PTZ: 20 mM). Thus, the movement pattern of the exposed zebrafish larvae was video-tracked and assessed in presence of embryo medium (1% DMSO) and PTZ 20 mM. Videotracking of larval movements was started 5 minutes after addition of embryo medium or PTZ to the wells and was recorded for 30 minutes. A total of 8 wells in each plate were left without larvae (medium only) as a negative control, so that each experimental parameter consisted of an average of 10 to 12 larvae. The tracker software measured three periods of 10 minutes of larvae movement. Results were registered as the average value of the total time of larvae movement during 30 minutes. The figures shown are representative of a series of two similar experiments.

[0060] The anticonvulsant properties of curcuminoids were assessed through video tracking analysis of seizure-like movements of zebrafish larvae. The higher tested concentrations correspond to MTC, thus in any case larvae did not display any sign of toxicity at these dose. MTC for curcuminoids corresponds to 10 μg/mL. Curcuminoids showed significant anticonvulsant activity ($p<0.05$) at 2.5 μg/mL and at 5 and 10 μg/mL ($p<0.001$) (FIG. 2). This finding is in line with the anticonvulsant properties of curcumin revealed in rodent models (11) (12) (13) (14). On the other hand, further analysis uncovered the anticonvulsant activity for the turmeric oil. The larvae showed significant decrease ($p<0.001$) of PTZ-induced seizures after exposure to turmeric oil (10 μg/ml) (FIG. 2).

[0061] The anticonvulsant activity of curcuminoids and turmeric oil was compared to phenytoin and diazepam, two widely used drugs for the treatment of epilepsy. Higher tested concentrations correspond to MTC. Phenytoin showed significant activity at 75 μg/ml ($p<0.05$) and 252.26 μg/ml ($p<0.001$). Diazepam decreased PTZ-induced movements in larvae at the concentrations of 1.42 μg/ml and 14.23 μg/ml ($p<0.001$) (FIG. 3). Curcumin and turmeric oil displayed interesting activity to delay seizure generation at significantly lower concentrations than phenytoin and at equivalent ones of diazepam.

[0062] RP-HPLC analysis of turmeric oil revealed eight peaks (FIG. 4). The peaks were individually collected to evaluate the anticonvulsant activity and find the active constituents. Fractions 2 and 7 were not tested in zebrafish model since the collected amounts were not enough for the assay performance. Significant decrease of the seizures triggered by PTZ was observed with fraction 4 ($p<0.05$) at 10 μg/ml, fraction 5 ($p<0.001$) at 5 μg/ml and fraction 6 at concentrations of 5 μg/ml ($p<0.001$) and 10 μg/ml ($p<0.05$) (FIG. 5, FIG. 6, FIG. 7A). The bisabolene sesquiterpenoids exhibited anticonvulsant properties at lower concentrations compared to phenytoin. Fraction 4 and 6 displayed positive response at similar concentrations than diazepam. Fraction 5 was effective at lower concentration than diazepam and phenytoin.

[0063] Fractions 4, 5 and 6 that showed positive activity in zebrafish PTZ model were further

analyzed for chemical structure elucidation. Retention time, MW and UVmax of fraction 4 are consistent with the product proposed in FIG. 4. <1>H- and <13>C-NMR spectra of this fraction are in agreement with reported values for ar-turmerone (29), possibly a mixture of enantiomers. NMR analysis indicates that Fraction 5 is a 1:1 mixture of two isomeric structures, possibly mixture of enantiomers. Compounds of this fraction were identified by 1D- and 2D-NMR analysis as α-turmerone and β-turmerone (curlone) (30). Isomerisation to the aromatic analogue ar-turmerone was not observed by NMR after one week. Structure of Fraction 6 was identified as α-atlantone (probably the E-isomer) based on MW, 1D- and 2D-NMR spectra (29) (31) (FIG. 4; Table 1).

[0000]

TABLE 1

UV and MS data of bisabolene sesquiterpenoids from turmeric oil.
The obtained data from analysis is compared with values from the analysis of X.He and colleagues (24) referred between brackets [ ].

Fraction	Rt (lit.) (min.)	UVmax (lit.) (nm)	Mass Peak	Suggested MW
4	24 [25.3]	238 [238]	217	216
5	27.2 [28.1]	229 [/]; 238 [238]	219	218
6	29.3 [/]	195 [/]; 269 [/]	219	218

Fraction 4: ar-turmerone;
fraction 5: α,β-turmerone (curlone);
fraction 6: α-atlantone

[0064] The analysis of the methanolic extract of turmeric (C. longa rhizome powder) revealed anticonvulsant activity in the zebrafish larval PTZ assay. In order to identify the active constituents present in the methanolic extract of turmeric, the anticonvulsant properties of curcuminoids and turmeric oil were also assessed through videotracking analysis. Curcuminoids showed anticonvulsant activity at 2.5 μg/ml ($p<0.05$) and at 5 and 10 μg/ml ($p<0.01$) in our larval PTZ assay. Further analysis uncovered an additional anticonvulsant activity for turmeric oil. The larvae showed a decrease ($p<0.01$) of PTZ-induced convulsions after exposure to turmeric oil (10 μg/ml) (FIG. 2C-2E). Notably, exposure of zebrafish larvae to curcuminoids or turmeric oil alone (i.e. in the absence of proconvulsant) also resulted in a slight increase in locomotor activity compared to vehicle-treated controls. However, no obvious signs of toxicity (as measured by change in heart rate, loss of posture, lack or delay in response to tactile stimuli, or death were observed in these larvae.

[0065] The anticonvulsant properties of the bisabolene sesquiterpenoids were also assessed through video tracking analysis of seizure-like movements of zebrafish larvae. The higher tested concentrations correspond to MTC, thus in any case larvae did not display any sign of toxicity at these dose. Significant decrease in the convulsions triggered by PTZ was observed for fractions ar-turmerone, α,β-turmerone and α-atlantone. Ar-turmerone showed anticonvulsant activity at 46 μM ($p<0.05$), α-,β-turmerone at 23 μM ($p<0.01$) and α-atlantone at concentrations of 23 μM (pb0.05) and 46 μM ($p<0.01$) (FIG. 7B-7D).

Example 7

Generation of PTZ-Induced Seizures in Mice

[0066] Mice were randomly divided into groups of five animals (vehicle (where vehicle was polyethylene glycol 200 (PEG200):water 1:1) and sample). The animals were pre-warmed under an infrared lamp for 10 minutes to dilate the tail veins. They were then placed in a restrainer and the lateral tail vein was catheterized with 1-cm long, 29-gauge needle. The needle was secured to the tail with surgical tape after confirming a correct placement. The needle was attached to a 0.7-m long polyethylene tubing connected to two 2.5-ml glass syringes containing: a) sample (control vehicle or test compounds) and b) PTZ (7.5 mg/ml ddH2O). These syringes were mounted on an infusion pump (ALADOIN-1000 11 VDC, 0.75 Å, World Precision Instruments). Thus, 100 μL of control vehicle (PEG 200: ddH2O 1:1) or test compounds (turmeric oil and ar-turmerone) were IV infused at the rate of 50 μl/min for 2 minutes. Ten minutes later, mice were released from the restrainer and placed in a transparent plastic cage for observation.

[0067] PTZ was constantly infused at the rate of 150 μl/min. Seizure manifestation stages in mice were scored according to the time between the start of PTZ infusion and the following behavioral events: ear, tail and myoclonic twitch, forelimb clonus, falling, tonic hindlimb extension and death (28). Behavior was observed up to 5 minutes of PTZ infusion. In case of any surviving mice, they were sacrificed.

[0068] PTZ doses were calculated according to the formula: PTZ dose [mg/kg]=(PTZ concentration [mg/ml]×infusion rate [ml/s]×infusion duration [s]×1000)/mouse weight [g]). All work solutions contained heparine (20 μl/ml).

[0069] Further evaluation of turmeric oil to control generation of PTZ-induced seizures in mice showed a delay on the onset of seizure parameters in mouse PTZ assay. Mice treated with turmeric oil (50 mg/kg) showed a significant increase in PTZ doses required to trigger all behavioral endpoints: tail twitch ($p<0.001$), ear twitch, myoclonic twitch, forelimb clonus, falling, tonic hind limb extension and death ($p<0.05$) compared to control group (FIG. 8A). Interestingly, ar-turmerone at a dose of 200 mg/kg also showed in mice a significant PTZ dose increase for generating ear, tail and myoclonic twitch, tonic hind limb extension and death ($p<0.05$) as compared to control (FIG. 8B).

[0070] When the vehicle PEG200:DMSO 1:1 was used, mice treated with turmeric oil (50 mg/kg) showed a significant increase in PTZ doses required to trigger all behavioral endpoints: forelimb clonus, falling and tonic hindlimb extension ($p<0.05$) and ear, myoclonic, tail twitch, and death ($p<0.01$), compared to control group (FIG. 9A).

[0071] Moreover, a dose of 100-mg/kg turmeric oil in the mouse PTZ assay exhibited significant activity in delaying seizure generation for all seizure parameters and death as compared to control ($p<0.01$) (FIG. 9B). Regarding the active bisabolene sesquiterpenoids, ar-turmerone and α,β-turmerone were assessed using the mouse PTZ seizure model (FIG. 10). Mice infused with a dose of 50 mg/kg of ar-turmerone exhibited significant resistance to the generation of seizures leading to an increase in the required dose of PTZ to trigger all assessed events: tonic hindlimb extension ($p<0.05$) and ear, myoclonic and tail twitch, forelimb clonus, falling and death ($p<0.01$). Likewise, the anticonvulsant activity of α,β-turmerone was evaluated, and positive results were also found with a dose of 100 mg/kg for all seizure parameters: forelimb clonus, falling, ear and tail twitch ($p<0.05$) and myoclonic twitch, tonic hindlimb extension and death ($p<0.01$). α-Atlantone was not tested in the mouse

model since the collected amount was not sufficient to carry out the assay.

[0072] Sodium valproate was included as positive control in our PTZ tail infusion method for AED screening in mice (FIG. 11). Using this assay, sodium valproate (50 mg/kg) was capable of delaying tonic hindlimb extension ($p<0.01$) and death ($p<0.05$). Sodium valproate was also able to control seizures generation in zebrafish larvae where it was also used as positive control (FIG. 11).

Example 8

Statistical Analysis

[0073] All statistical analyses were performed using GraphPad Prism 5 software (GraphPad Software, Inc.). Values were presented as means±standard deviation (SD). The locomotor activity of zebrafish larvae was analyzed using one-way ANOVA followed by Dunnett's multiple comparison test. Statistically significant differences ($p<0.05$) between a treated group and the equivalent control groups (vehicle or PTZ) were considered indicative of decrease or increase in locomotor activity of zebrafish larvae. For mouse experiments, significant differences between estimated time intervals prior to above-mentioned seizure stages were calculated using the unpaired Student's t-test.

Example 9

Anti-Convulsant Activity Evaluation of Ar-Tumerone in the 6 Hz Psychomotor Seizure Model of Partial Epilepsy

[0074] For assessing the anti-convulsant activity of ar-Tumerone, the 6 Hz psychomotor seizure model of partial epilepsy (Barton M. E. et al., 2001) was used, thereby applying the following stimulation parameters: 6 Hz, 0.2 ms rectangular pulse width, 3 s duration.

[0075] Every mouse (male NMRI+/−30 g) was administered the compound (ar-tumerone 50 mg/kg, 20 mg/kg, 1 mg/kg and 100 μg/kg) or vehicle (PEG 200: DMSO 1:1) via i.p. injection. After 30 minutes of incubation, seizures were induced via corneal stimulation using the Ugo-Basil device. Prior to the placement of corneal electrodes, a drop of 0.5% xylocaine was applied to the eyes of the animal. Animals were restrained manually and released immediately in a transparent plastic cage following the stimulation. Then, the animal was observed. The seizure was characterized by stun, forelimb clonus, twitching of vibrissae, straub-tail for at least 45 s. Protection was defined as the absence of a seizure. A minimum of six animals per dose was used. Six out of six mice showed protection with concentrations of 100 μg/kg, and 1, 20, 50 mg/kg ar-turmerone (FIG. 13). Negative control (only vehicle) and positive control (valproic acid 300 mg/kg) were included as well. As expected, 6 out of 6 mice treated with only vehicle were not protected and 6 out of 6 mice treated with valproic acid were protected.

Example 10

Motor Coordination and Balance on the Elevated Bridge

[0076] In this example, the motor coordination and balance of mice using the elevated bridge was observed as described in (Brooks et al., 2012).

[0077] The elevated bridge measures the ability of a mouse (male C57B1/6+/−25 g) to traverse the beam without losing its balance (measured as footslips). Every mouse was trained until proficient at running the beam without pausing during the traverse. Two areas of the beam are designated the ‘start’ and ‘stop’ areas to allow the operator to start and stop the timing of the animal when running the beam. Following training, every mouse was administered the compound or vehicle via i.v. injection. After 10 minutes, the mouse was placed on the tip of the beam in the star facing towards the beam. The operator timed from the start line until the mouse reaches the stop line. The number of footslips (FIG. 12A), falls (FIG. 12B) and total time (FIG. 12C) on the beam (from ‘start’ to ‘stop’ areas) were counted. In this test, 5 out of 5 mice treated with ar-turmerone 50 mg/kg showed a behaviour comparable to the control group (treated with vehicle). Thus, from the obtained results it can be suggested that ar-turmerone does not cause motor or balance impairment as side effect of its anticonvulsant activity. Mice treated with diazepam were included due to the well-know side effect of this AED to cause motor and balance alterations in mice after i.v./i.p. administration.

DISCUSSION

[0078] The zebrafish PTZ-induced seizure model (24) was validated using first-line AEDs: phenytoin and diazepam. Additional validation of this screening system was achieved by identification of the known anticonvulsant properties of curcuminoids. Curcumin has often been cited as the main active substance responsible for the anticonvulsant properties of turmeric (11) (12) (32). Although its medicinal properties have been demonstrated, Phase I clinical trials have revealed important pharmacokinetic limitations for curcumin. When administrated p.o., the small amount of curcumin absorbed through the gut is mostly converted to water-soluble metabolites and excreted. Thus, the amount of curcumin reaching the circulation is very low. Therefore bioavailability issues have notably limited its therapeutical applications. Thus, several formulation studies have been performed to enhance curcumin bioavailability (33).

[0079] On the other hand, neuroprotective studies in rodent models have shown that turmeric oil and its main bisabolene sesquiterpenoids easily cross the blood-brain barrier likely due to their lipophilic nature which allows them to pass through cell membranes (18). Since turmeric oil and its constituents present better bioavailability and cross biomembranes with less difficulty when compared to curcumin (15) (16) (33), our finding that turmeric oil also displays anticonvulsant properties is indeed interesting. Moreover, turmeric safety is supported by the fact that it has been widely used as a food condiment predominantly in India for centuries and its use has been approved for human consumption. Furthermore, toxicity studies performed in human healthy patients (34) and in silico analysis (35) have predicted ar-turmerone as a safe potential candidate for further drug development.

[0080] Previous studies on the volatile constituents of turmeric oil were limited due to their complex isolation. Our work presents a practical method to isolate the main constituents of turmeric oil through RP-HPLC. The isolated compounds were individually evaluated in the zebrafish PTZ epilepsy model (data not shown for peaks 1, 3, 8). This model revealed significant activity for turmeric oil and the major bisabolene sesquiterpenoids: ar-; α,β-turmerone (curlone) and α-atlantone. Moreover, the anticonvulsant properties of turmeric oil (50 and 100 mg/kg), ar-turmerone (200 mg/kg), and α-,β-turmerone (100 mg/kg) were successfully corroborated in mice PTZ model and 6 Hz psychomotor seizure model of partial epilepsy. Regarding to the activity of turmeric oil vs ar-turmerone, it seems to be an additive

activity since it is necessary higher dose of the isolated bisabolene sesquiterpenoid to observe anticonvusant properties in this model. Nevertheless, these findings reveal the major bisabolene sesquiterpenoids, especially ar-turmerone, as potential anticonvulsant drug candidates to be investigated further.

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US8859020
Formulation of Curcumin with Enhanced Bioavailability of Curcumin and method of preparation and treatment thereof

Disclosure provides a formulation of curcuminoid with essential oil of turmeric to enhance the bioavailability of curcumin and to augment the biological activity of curcumin, wherein curcumin is the main constituent of curcuminoid and wherein Ar-turmerone is the main constituent of the essential oil of turmeric. An application of curcuminoid with essential oil of turmeric to enhance the bioavailability of curcumin for oral supplementation against a variety of diseases and method of doing the same is provided.

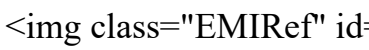
OBJECTIVE OF THE INVENTION

[0002] The following specification describes an invention which relates to a formulation of curcuminoid with essential oil of turmeric to enhance the bioavailability of curcumin and to augment the biological activity of curcumin, wherein curcumin is the main constituent of curcuminoid and wherein Ar-turmerone is the main constituent of the essential oil of turmeric. Such enhanced bioavailability of curcumin has been demonstrated in human volunteers. The present invention also relates to an application of curcuminoid with essential oil of turmeric to enhance the bioavailability of curcumin for oral supplementation against a variety of diseases and method of doing the same. In particular the present invention relates to oral supplementation of curcuminoid with essential oil of turmeric to enhance the bioavailability of curcumin for the prophylaxis, treatment, maintenance therapy and as add on therapy for disease conditions such as cancer, heart diseases, diabetes, rheumatoid arthritis, osteoarthritis, alzheimer's disease, inflammatory bowel diseases, liver fibrosis and cirrhosis, abdominal aortic aneurysms, HIV, pancreatitis, drug-resistant malaria, psoriasis, cystic fibrosis, epilepsy, wound healing, diseases of the central nervous system, chronic degenerative diseases and potentially many other diseases where better delivery of curcumin from the supplement to the blood and tissues is critical for the enhanced therapeutic benefit and an improved method of delivering curcumin and ensuring bioavailability in humans.

BACKGROUND OF THE INVENTION

Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione]

[0003]



is the major yellow pigment of turmeric, a commonly used spice, derived from the rhizome of the herb Curcima longa Linn. In the Indian subcontinent and Southeast Asia, turmeric has traditionally been used as a treatment for inflammation, skin wounds, and tumors. Clinical activity of curcumin is yet to be confirmed; however, in preclinical animal models, curcumin has shown cancer chemo preventive, antineoplastic and anti-inflammatory properties<1>. Especially interesting is its ability to prevent the formation of carcinogen-induced intestinal premalignant lesions and malignancies in rat<2, 3 >and in the multiple neoplasia (Min/+) mouse<4>, a genetic model of the human disease familial adenomatous polyposis. Curcumin acts as a scavenger of oxygen species such as hydroxyl radical, superoxide anion and singlet oxygen<5,6,7 >and interferes with lipid peroxidation<8,9>. Curcumin suppresses a number of key elements in cellular signal induction pathways pertinent to growth, differentiation and malignant transformations. Among signaling events inhibited by curcumin are protein kinases<10>, c-Jun/AP-1 activation<11>, prostaglandin biosynthesis<12 >and activity and expression of the enzyme cyclooxygenase-2<13,14>. This latter property is probably mediated by the ability of curcumin to block activation of the transcription factor NF-κB at the level of the NF-κB inducing kinase/IKKα/β signalling complex<15>.

[0004] Curcumin directly inhibits cyclooxygenase-2 and also inhibits the transcription of the gene responsible for its production. Cyclooxygenases (COX) catalyze the synthesis of prostaglandins (PGs) from arachidonic acid. There are two isoforms of COX, designated COX-1 and COX-2. COX-1 is expressed constitutively in most tissues and appears to be responsible for housekeeping functions<16 >while COX-2 is not detectable in most normal tissues but is induced by oncogenes, growth factors, carcinogens and tumor promoters<17,18,19>. Several different mechanisms account for the link between COX-2 activity and carcinogenesis.

[0005] Curcumin is not simply an alternative to non-steroidal anti-inflammatory drugs (NSAIDS), which also have anti-inflammatory and cancer chemopreventive properties. This is so because COX is a bifunctional enzyme with cyclooxygenase and peroxidase activities. Aside from being important for PG synthesis, the peroxidase function contributes to the activation of procarcinogens. Therefore, the failure of NSAIDS to inhibit the peroxidase function of COX potentially limits their effectiveness as anticancer agents. Curcumin, in contrast, down-regulates levels of COX-2 and thereby decreases both the cyclooxygenase and peroxidase activities of the enzyme.

[0006] Curcumin is among the few agents to block both the COX and LOX (lipoxygenase) pathways of inflammation and carcinogenesis by directly modulating arachidonic acid metabolism. In a study to evaluate the effect of curcumin on the metabolism and action of arachidonic acid in mouse epidermis, it was found that topical application of curcumin inhibited arachidonic acid-induced ear inflammation in mice<20>. Curcumin (10 μM) inhibited the conversion of arachidonic acid to 5- and 8-hydroxyeicosatetraenoic acid by 60% and 51%, respectively (LOX pathway) and the metabolism to PGE2, PGF2α and PGD2 by 70%, 64% and 73%, respectively (COX pathway). In another study, dietary administration of 0.2% curcumin to rats inhibited azoxymethane-induced colon carcinogenesis and decreased colonic and tumor phospholipase A2, phospholipase Cγ1, and PGE2 levels<21>. In this study, dietary curcumin also decreased enzyme activity in the colonic mucosa and tumors for the formation of PGE2, PGF2α, POD2, 6-keto-PGF2α and thromboxane B2 via the COX system and production of 5(S)-, 8(S)-, 12(S)-, and 15(S)-hydroxy-eicosatetraenoic acid via

the LOX pathway was also inhibited.

[0007] Despite this impressive array of beneficial bioactivities, the bioavailability of curcumin in animals and man remains low. In rodents, curcumin demonstrates poor systemic bioavailability after p.o. dosing<22 >which may be related to its inadequate absorption and fast metabolism. Curcumin bioavailability may also be poor in humans as seen from the results of a recent pilot study of a standardized turmeric extract in colorectal cancer patients<23>. Indirect evidence suggests that curcumin is metabolized in the intestinal tract. Curcumin undergoes metabolic O-conjugation to curcumin glucuronide and curcumin sulfate and bioreduction to tetrahydrocurcumin, hexahydrocurcumin and hexahydrocurcuminol in rats and mice in vivo<24,25 >in suspensions of human and rat hepatocytes<26 >and in human and rat intestine<27>. Metabolic conjugation and reduction of curcumin was more in human than in rat intestinal tissue. It has been suggested that the intestinal tract plays an important role in the metabolic disposition of curcumin. This is based predominantly on experiments in which [<3>H] labeled curcumin was incubated with inverted rat gut sacs<28>. This was later confirmed in intestinal fractions from humans and rats. Intestinal mucosa, as well as liver and kidney tissue from the rat, can glucurodinate and sulfate curcumin, as judged by the analysis of differential amounts of curcumin present before and after treatment of tissue extracts with conjugate-hydrolyzing enzymes<29>. Thus, gut metabolism contributes substantially to the overall metabolic yield generated from curcumin in vivo. In human intestinal fractions, conjugation with activated sulfuric or glucuronic acids was much more abundant, whereas conjugation in human hepatic tissues was less extensive, than in the rat tissues<30>.

[0008] Although p.o. administered curcumin has poor bioavailability and only low or non-measurable blood levels were observed<31>, this route of administration inhibits chemically induced skin and liver carcinogenesis<32, 33>. Oral administration of curcumin also inhibits the initiation of radiation-induced mammary and pituitary tumors<34>. Similarly, in a study to assess the curcumin levels in the colorectum, a daily dose of 3.6 g curcumin achieves pharmacologically effective levels in the colorectum with negligible distribution of curcumin outside the gut<35>.

[0009] Earlier Shobha et al<36 >had observed that administering piperine along with curcumin enhances the bioavailability of curcumin. However, the level of enhancement was only modest and no curcumin could be detected after 3 hours even when supplemented with piperine.

[0010] Although some questions remain unanswered regarding the pharmacokinetics of curcumin in humans, there is no denying the fact that considerable proportion of ingested curcumin is excreted through feces and at least about one-half of absorbed curcumin is metabolized. The quantity of curcumin that reaches tissues outside the gut is probably pharmacologically insignificant. Several studies have failed to demonstrate the positive in vitro results with curcumin in in vivo animal and human studies due to lack of absorption of curcumin after oral administration. To provide the clinical benefits, curcumin must be absorbed from its oral route of administration at a suitable rate, be distributed in adequate concentration in the blood and remain in the system for a sufficient period at an effective concentration level.

SUMMARY

[0011] Some embodiments provide a composition of a curcuminoid mixture and added essential oil of turmeric. In some embodiments, the weight ratio of the curcuminoid mixture to the added essential oil of turmeric ranges from about 1:3 to about 99:1. In some embodiments, the curcuminoid mixture includes curcumin, demethoxycurcumin and bisdemethoxycurcumin. In some embodiments, the essential oil of turmeric includes ar-turmerone. In some embodiments, the essential oil of turmeric includes about 40-50% ar-turmerone.

[0012] Some embodiments provide a method of treating rheumatoid arthritis by administering a composition having a curcuminoid mixture and added essential oil of turmeric.

[0013] Some embodiments provide a method of reducing visual analogue scale for pain by administering a composition having curcuminoid mixture and added essential oil of turmeric.

[0014] Some embodiments provide a method of decreasing disease activity score by administering a composition having the curcuminoid mixture and added essential oil of turmeric. Some embodiments provide a method of improving patient response to ACR criteria by administering composition of a curcuminoid mixture and added essential oil of turmeric. Some embodiments provide a method of reducing C-reactive protein levels by administering a composition of a curcuminoid mixture and added essential oil of turmeric. Some embodiments provide a method of reducing rheumatoid Arthritis Factor by administering a composition of a curcuminoid mixture and added essential oil of turmeric. Some embodiments provide a method of decreasing joint pain by administering a composition of a curcuminoid mixture and added essential oil of turmeric. Some embodiments provide a method of improving walking distance scores by administering a composition of a curcuminoid mixture and added essential oil of turmeric. Some embodiments provide a method of treating osteoarthritis by administering a composition of a curcuminoid mixture and added essential oil of turmeric. Some embodiments provide a method of treating Alzheimer's disease by administering a composition of a curcuminoid mixture and added essential oil of turmeric. Some embodiments provide a method of improving mini mental state exam scores by administering a composition of a curcuminoid mixture and added essential oil of turmeric. Some embodiments provide a method of increasing Vitamin E levels by administering a composition of a curcuminoid mixture and added essential oil of turmeric. Some embodiments provide a method of increasing serum amyloid beta levels by administering a composition of a curcuminoid mixture and added essential oil of turmeric. Some embodiments provide a method of disaggregating amyloid beta by administering a composition of a curcuminoid mixture and added essential oil of turmeric. Some embodiments provide a method of lowering plasma isoprostane levels by administering a composition of a curcuminoid mixture and added essential oil of turmeric. Some embodiments provide a method of treating depression by administering a composition of a curcuminoid mixture and added essential oil of turmeric. Some embodiments provide a method of improving response rate on Hamilton Depression rate scale by administering a composition of a curcuminoid mixture and added essential oil of turmeric. Some embodiments provide a method if improving clinical global impression by Global Severity comprising administering a composition of a curcuminoid mixture and added essential oil of turmeric. Some embodiments provide a method of improving clinical global impression by Global Change scale by administering a composition of a curcuminoid mixture and added essential oil of turmeric.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] The above objectives and advantages of the disclosed teachings will become more apparent by describing in detail preferred embodiments thereof with reference to the attached drawings in which:

[0016] FIG. 1 provides a graph showing the bioavailability of curcumin in humans upon administration of (1) gelatin capsules, which were prepared by admixing curcuminoid isolated from turmeric with essential oil of turmeric, and, (2) gelatin capsules of curcuminoid alone, which were prepared without adding essential oil of turmeric to the curcuminoid isolated from turmeric. The x-axis shows time in hours following administration of the gelatin capsules. The y-axis shows the concentration of curcumin (ng/g) in blood

[0017] FIG. 2 provides a graph showing the bioavailability of curcumin in human upon administration of 1) gelatin capsule, which were prepared by admixing curcuminoid with added essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio, 2) gelatin capsules of curcuminoid alone, which were prepared without adding essential oil of turmeric to the curcuminoid isolated from turmeric, 3) gelatin capsules of raw turmeric powder alone, 4) gelatin capsules of Essential oil of turmeric with 45% Ar-turmerone alone, 5) gelatin capsules of essential oil of turmeric with 10-15% Ar-turmerone alone, 6) gelatin capsule, which were prepared by admixing curcuminoid with added essential oil of turmeric with 45% Ar-turmerone in 12:1 ratio, The x-axis shows time in hours and y-axis shows the concentration of curcumin (ng/g) in blood

[0018] FIG. 3 provides a comparison of the bioavailability of curcumin from the curcuminoid mixture without added essential oil of turmeric group and the curcuminoid mixture with added essential oil of turmeric with 45% Ar-turmerone in a weight ratio ranging from about 1:3 to 99:1. The x-axis shows the ratio of curcumin to essential oil of turmeric and y-axis shows the AUC value of curcumin

[0019] FIG. 4 provides a comparison of curcumin bioavailability from 10:1 and 1:10 weight ratios of 1) curcuminoid (454.55 mg) with added essential oil of turmeric (45.45 mg) with 45% Ar-turmerone in 10:1 ratio, 2) curcuminoid (20 mg) with added essential oil of turmeric (2 mg) with 45% Ar-turmerone in 10:1 ratio, 3) curcuminoid (20 mg) with added essential oil of turmeric (200 mg) with 45% Ar-turmerone in 1:10 ratio, 4) curcuminoid (20 mg) with added essential oil of turmeric (200 mg) with 10-15% Ar-turmerone in 1:10 ratio, 5) curcuminoid alone (454.55 mg), 6) curcuminoid alone (20 mg), 7) Essential oil of turmeric with 45% Ar-turmerone alone (45.45 mg), 8) Essential oil of turmeric with 10-15% Ar-turmerone alone (200 mg). The x-axis shows time in hours and y-axis shows the concentration of curcumin (ng/g) in blood

[0020] FIG. 5 provides Method of preparation of Essential oil of turmeric with varying concentration of Ar-turmerone.

[0021] FIG. 6 provides Table 9 (ACR response of different groups)

[0022] FIG. 7 provides Table 12 (Joint pain measurements and % response of patients in each group over 3 months)

[0023] FIG. 8 provides Table 13 (Joint line tenderness and % response of patients in each

group over 3 months)

[0024] FIG. 9 provides Table 14 (Walking distance scores and % response of patients in each group over 3 months)

DETAILED DESCRIPTION

[0025] The disclosure relates to a product to enhance the bioavailability of curcumin by mixing a suitable portion of the volatile oil obtained from turmeric with the curcuminoids isolated from turmeric.

[0026] As disclosed herein the term “curcuminoid” is a mixture of curcumin, demethoxycurcumin and bisdemethoxycurcumin. In some embodiments, curcumin is the major component of the curcuminoid mixture. In some embodiments, demethoxycurcumin and bisdemethoxycurcumin are minor components of the curcuminoid mixture. In some embodiments, 95% of the crystals having curcuminoid mixture are composed of curcumin, demethoxycurcumin and bisdemethoxycurcumin.

[0027] The term “essential oil” or “essential oil of turmeric” is also referred to as “volatile oil” or “volatile oil of turmeric.” The essential oil of turmeric is a mixture of oils. Essential oil is obtained as a by-product during the extraction of curcumin or curcuminoids from turmeric. In some embodiments, Ar-turmerone, which is also referred to as turmerone, is the main constituent of essential oil. In some embodiments, Ar-turmerone constitutes about 40-50% of the essential oil of turmeric. In some embodiments, Ar-turmerone comprises about 45% of the essential oil of turmeric.

[0028] As stated herein, the term “a” or “an” refers to one or more.

[0029] As stated herein, the terms “isolated” and “purified” are referred to interchangeably.

[0030] The volatile oil of turmeric was isolated by conventional methods of steam distillation to isolate essential oils and is well known in the art.

[0031] Curcumin is isolated from the de-oiled turmeric by solvent extraction. Suitable solvents for this purpose include acetone, hexane, ethyl acetate, dichloroethane, chloroform, etc. The extraction is conveniently carried out at moderate temperatures (40-55° C.) and the solvent is partially removed to yield a concentrate containing 30-60% solids. This solution is cooled to obtain crystals of curcuminoid which are isolated by any suitable method such as filtration or centrifugation. Analysis of this product, which is composed of the isolated crystals of curcumoid mixture, showed that, in some embodiments, 95% of the product was composed of curcumin, demethoxycurcumin and bisdemethoxycurcumin.

[0032] The disclosure provides a composition having curcuminoid and an essential oil of turmeric. Curcumin and the volatile oils of curcumin are mixed and blended to get a uniform product. If small percentages (˜5%) of the essential oil of turmeric are added to the curcuminoid, then the bioavailability of curcumin is significantly enhanced. Accordingly, a composition of curcuminoid admixed with a suitable proportion of Ar-turmerone (the main component of the turmeric essential oil) is provided.

[0033] In some embodiments, the weight ratio of the curcuminoid to the essential oil of

turmeric ranges from about 1:1 to about 90:1. In some embodiments, the weight ratio of the curcuminoid to the essential oil of turmeric ranges from about 1:1 to about 3:1. The weight ratio of the curcuminoid to the essential oil of turmeric can be varied from about 3:1 to about 99:1. In some embodiments, the weight ratio of the curcuminoid to the essential oil of turmeric ranges from about 1:1 to about 70:1. In some embodiments, the weight ratio of the curcuminoid to the essential oil of turmeric ranges from about 1:1 to about 45:1. In some embodiments, the weight ratio of the curcuminoid to the essential oil of turmeric ranges from about 3:1 to about 50:1. In some embodiments, the weight ratio of the curcuminoid to the essential oil of turmeric ranges from about 8:1 to about 25:1. In some embodiments, the weight ratio of the curcuminoid to the essential oil of turmeric is about 90:7. In some embodiments, the weight ratio of the curcuminoid to the essential oil of turmeric is about 90:8. In some embodiments, the weight ratio of the curcuminoid to the essential oil of turmeric is about 90:9. In some embodiments, the weight ratio of the curcuminoid to the essential oil of turmeric is about 89:9. In some embodiments, the weight ratio of the curcuminoid to the essential oil of turmeric is about 89:8. In one embodiment, the ratio is about 85:15. In another embodiment, the ratio is about 92:8. In another embodiment, the ratio is about 95:5. In another embodiment the weight ratio is about 10:1. In some embodiments, the weight ratio is about 12:1. In some embodiments, the weight ratio of the curcuminoid to the essential oil of turmeric is about 1:2. In some embodiments, the weight ratio of the curcuminoid to the essential oil of turmeric is about 2:1. In some embodiments, the weight ratio of the curcuminoid to the essential oil of turmeric ranges from about 1:3 to about 99:1.

[0034] In some embodiments of the composition having curcuminoid and added essential oil of turmeric, the curcuminoid ranges, by weight, from about 24% to about 96%. In some embodiments of the composition having curcuminoid and added essential oil of turmeric, the curcuminoid ranges, by weight, from about 30% to about 96%. In some embodiments of the composition of curcuminoid and added essential oil of turmeric, the curcuminoid ranges, by weight, from about 40% to about 75%. In some embodiments of the composition having curcuminoid and added essential oil of turmeric, the curcuminoid ranges, by weight, from about 50% to about 60%.

[0035] In some embodiments of the composition having curcuminoid and added essential oil of turmeric, the demethoxycurcumin ranges, by weight, from about 5% to about 25%. In some embodiments of the composition having curcuminoid and added essential oil of turmeric, the demethoxycurcumin ranges, by weight, from about 10% to about 20%.

[0036] In some embodiments of the enhanced curcumin bioavailability composition having curcuminoid and added essential oil of turmeric, the bisdemethoxycurcumin ranges, by weight, from about 2% to about 7%.

[0037] In some embodiments of the enhanced curcumin bioavailability composition having curcuminoid and added essential oil of turmeric, the essential oil of turmeric ranges, by weight, from about 4% to about 50%. In some embodiments, of the composition of curcuminoid and added essential oil having turmeric, the essential oil of turmeric ranges, by weight, from about 15% to about 50%. In some embodiments of the composition having curcuminoid and added essential oil of turmeric, the essential oil of turmeric ranges, by weight, from about 20% to about 50%. In some embodiments of the composition having curcuminoid and added essential oil of turmeric, the essential oil of turmeric ranges, by weight, from about 25% to about 40%.

[0038] Some embodiments include a composition having a curcuminoid and an added amount of essential oil of turmeric, wherein the essential oil is present in an amount sufficient to cause an enhancement of bioavailability of the curcumin when administered to a human as compared to the bioavailability of curcumin upon administration of a composition prepared using curcuminoid alone without adding essential oil. Curcumin levels in blood samples is greater following administration of a composition having curcuminoid and added essential oil of turmeric as compared to a composition of curcuminoid alone. In some embodiments, the enhancement of bioavailability of curcumin following administration of a composition of curcuminoid and added essential oil of turmeric ranges from about 5-fold to about 16-fold. Enhancement of bioavailability of curcumin from a composition prepared by mixing curcuminoid and essential oil of turmeric is provided in FIG. 1 and Example 1.

[0039] In some embodiments, a composition of a curcuminoid and added essential oil of turmeric is orally administered to a human.

[0040] A method of extraction of curcuminoids includes treating dried and powdered rhizomes of turmeric with a solvent, followed by solvent stripping, and steam distilling to obtain an essential-oil free extract. The essential oil-free extract is cooled to about 4° C. to allow the curcuminoids to crystallize. The curcuminoids are then separated by filtration, centrifugation or any other method of solid-liquid separation well-known in the art. In some embodiments, 95% of the separated crystals are composed of curcumin, demethoxycurcumin and bisdemethoxycurcumin.

[0041] Curcumin is isolated from the de-oiled turmeric by solvent extraction. Suitable solvents for this purpose include acetone, hexane, ethyl acetate, dichloroethane, chloroform, etc. The extraction is conveniently carried out at moderate temperatures (about 40° C. to about 55° C.) and the solvent is partially removed to yield a concentrate containing 30-60% solids. This solution is cooled to obtain crystals having curcuminoid mixture which are isolated by any suitable method such as filtration or centrifugation. 95% of this product (crystals) was composed of the curcuminoid mixture. The remaining may contain traces of essential oil plus other constituents such as carbohydrates, etc, which were not characterized.

[0042] The disclosure provides a method of extracting a curcuminoid from turmeric including:

drying rhizomes of turmeric to form a dried turmeric;
powdering the dried turmeric to form a powdered turmeric;
treating the powdered turmeric with a solvent selected from the group consisting of ethyl acetate, acetone, hexane, ethylene dichloride, ethyl alcohol, and combinations thereof to form a solution;
stripping the solvent from the solution to form an extract;
cooling the extract to about 4° C. to form crystals and a liquid, wherein the liquid comprises the essential oil of turmeric and a resin; and
separating the crystals from the liquid to obtain the curcuminoid crystals.

[0043] In some embodiments, curcumin, demethoxycurcumin and bisdemethoxycurcumin comprise 95% of the curcuminoid crystals.

[0044] Some embodiments include a method of extracting a curcuminoid from turmeric by drying rhizomes of turmeric to form dried turmeric. The dried turmeric is powdered to form

powdered turmeric. The powdered turmeric is treated with a solvent selected from the group consisting of ethyl acetate, acetone, hexane, and combinations thereof to form a solution. The solvent is stripped from the solution to form an extract. The extract is cooled to about 4° C. to form crystals having curcuminoid mixture, and, a liquid. The liquid comprises the essential oil of turmeric and a resin. The crystals having the curcuminoid mixture are separated from the liquid. In some embodiments, 95% of the crystals having the curcuminoid mixture is composed of the curcuminoid mixture, namely, curcumin, demethoxycurcumin and bisdemethoxycurcumin.

[0045] The volatile oil of turmeric was isolated by conventional methods of steam distillation to isolate essential oils and is well known in the art.

[0046] Curcuminoid and the essential oil are blended in a suitable proportion by a process including, suspending the curcuminoid in about 3 to 5 times its quantity of water, mixing in the essential oil, pulverizing in a colloidal mill into fine slurry, and stripping the slurry off water under heat and vacuum to obtain a uniform blend. Five hundred milligram capsules are made from this blend for human consumption.

[0047] The disclosure provides a method of preparing a composition including a curcuminoid and an essential oil of turmeric including:

suspending the curcuminoid in water to form a suspension:
adding the essential oil to the suspension to form a mixture;
homogenizing the mixture to obtain a fine slurry; and
drying the fine slurry under heat and vacuum to form a uniform blend of a composition including the curcuminoid and the essential oil of turmeric. Drying of the fine slurry under heat and vacuum can be performed using a vaccumized desolventiser with a stirrer.

[0048] A composition of curcuminoid and added essential oil of turmeric can be prepared by suspending the curcuminoid in water to form a suspension. Essential oil is added to the suspension to form a mixture. The mixture is homogenized to form fine slurry. The fine slurry is dried under heat and vacuum to form a uniform blend of a composition of curcuminoid and an essential oil of turmeric. The fine slurry can be dried under heat and vacuum using, for example, a vaccumized desolventiser having a stirrer.

[0049] In one embodiment, a homogeneous mixture of curcuminoid and water is prepared by suspending the curcuminoid in water to form a suspension. The suspension is homogenized to obtain fine slurry. The fine slurry is dried under heat and vacuum to form a composition having a homogeneous mixture of the curcuminoid and water.

[0050] The disclosure provides a method of preparing a homogeneous mixture having a curcuminoid and water by,

suspending a curcuminoid in water to form a suspension;
homogenizing the suspension to obtain a fine slurry; and
drying the suspension under heat and vacuum to form a composition including a homogeneous mixture of the curcuminoid and water.

[0051] Hard gelatin capsules, which contain about 500 mg of a blend of curcuminoid and essential oil of turmeric, are prepared. A 500 mg capsule for enhanced bioavailability of

curcumin, having the curcuminoid mixture and essential oil of turmeric in a weight ratio of about 95:5 is expected to contain about 460 mg of curcuminoid and about 40 mg of essential oil. The curcuminoid mixture is composed of curcumin, demethoxycurcumin and bisdemethoxycurcumin. In terms of active constituents, the respective figures would be about 437 mg of curcumin and about 18 mg of Ar-turmerone. In some embodiments, the gelatin capsules have about 300 mg to about 460 mg of curcuminoid and about 40 mg to about 375 mg of essential oil of turmeric. In some embodiments of the composition having curcumin and added essential oil of turmeric, wherein the gelatin capsule comprises 500 mg of a blend including the curcuminoid and the essential oil, the curcuminoid in the blend ranges from about 300 mg to about 485 mg, and the Ar-turmerone in the blend ranges from about 5 mg to about 200 mg.

[0052] Gelatin capsules with curcuminoid alone but without added essential oil were similarly prepared to study the comparative efficacies of the capsule containing added essential oil versus the capsule prepared without adding essential oil.

[0053] The disclosure provides a method of preparing a gelatin capsule having a curcuminoid and an essential oil of turmeric by suspending a curcuminoid in water to form a suspension. Then adding an essential oil to the suspension to form a mixture. Then homogenizing the mixture to obtain a fine slurry. Then drying the slurry under heat and vacuum to form a uniform blend of a composition having the curcuminoid and the essential oil of turmeric. Then compressing the blend into the hard gelatin capsule.

[0054] Hard gelatin capsules of a composition having a curcuminoid and an added essential oil of turmeric can be prepared by compressing a uniform blend of the composition into a capsule. Gelatin capsules are prepared by standard methods using instrument such as a capsule tilling machine manufactured by Pam Pharmaceuticals, Mumbai, India.

[0055] The disclosed compositions can be administered to a human for treating conditions including various human cancers such as colon cancer, prostate cancer, breast cancer, lung cancer, oral cancers, leukemias, etc, diabetes, depression, epilepsy, and various chronic inflammatory diseases such as rheumatoid arthritis, Alzheimer's disease, inflammatory bowel diseases (Crohn's disease, ulcerative colitis), coronary artery diseases, fibrosis and cirrhosis of liver, pancreatitis, abdominal aortic aneurysms, drug-resistant malaria, psoriasis, cystic fibrosis, HIV, wound healing, central nervous system disorders and potentially many other diseases. Another embodiment of the present invention provides for an application of a formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio for oral supplementation against rheumatoid arthritis and an improved method of delivering curcumin in human blood and tissues and ensuring better bioavailability in humans for the prophylaxis and treatment for active rheumatoid arthritis patients, maintenance therapy for preventing flare up of symptoms and as add on therapy with antiarthritic medications. In some embodiments, the ratio of curcuminoid mixture to essential oil of turmeric is 12:1 ratio for oral supplementation against rheumatoid arthritis and an improved method of delivering curcumin in human blood and tissues and ensuring better bioavailability in humans for the prophylaxis and treatment for active rheumatoid arthritis patients, maintenance therapy for preventing flare up of symptoms and as add on therapy with antiarthritic medications. Raw turmeric powder, essential oil of turmeric with 45% Ar-turmerone, essential oil of turmeric with 10-15% Ar-turmerone, curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 1:10 ratio, curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 1:1 ratio, curcuminoid 24% with essential oil of turmeric

with 45% Ar-turmerone in 10:1 ratio, curcuminoid with essential oil of turmeric with 10-15% Ar-turmerone in 10:1 ratio, formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio, curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 12:1 ratio or curcuminoids 95% were given to patients with active rheumatoid arthritis for 2 months duration in a dose of 500 mg capsules twice daily. Formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 and 12:1 ratios were able to significantly decrease disease activity score, total number of swollen and painful joints and erythrocyte sedimentation rate. The patients administered formulation of Curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 and 12:1 ratios, also showed significant improvement when assessed according to the American College of Rheumatology criteria, functional status and pain score. The inflammatory marker C reactive protein (CRP), anti streptolysin O (ASO) values and rheumatoid arthritis factor (RA) also drastically decreased in patients taking formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 and 12:1 ratios. Similar benefits were not evidenced in any of the patients given curcuminoids 95% alone in similar dose. The patients who were given maintenance therapy of formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 and 12:1 ratios alone after 2 months continued to be asymptomatic during the follow up phase of 4 more months.

[0056] Another embodiment of the present invention provides for application of a formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio for oral supplementation against osteoarthritis and an improved method of delivering curcumin in the human blood and tissues and ensuring bioavailability in humans for the prophylaxis and treatment for osteoarthritic patients, maintenance therapy for preventing flare up of symptoms and as add on therapy with antiarthritic medications. In some embodiments, the ratio of curcuminoid mixture to essential oil of turmeric is 12:1 ratio for oral supplementation against osteoarthritis and an improved method of delivering curcumin in the human blood and tissues and ensuring bioavailability in humans for the prophylaxis and treatment for osteoarthritic patients, maintenance therapy for preventing flare up of symptoms and as add on therapy with antiarthritic medications. Osteoarthritic patients were given Raw turmeric powder, essential oil of turmeric with 45% Ar-turmerone, essential oil of turmeric with 10-15% Ar-turmerone, curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 1:10 ratio, curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 1:1 ratio, curcuminoid 24% with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio, curcuminoid with essential oil of turmeric with 10-15% Ar-turmerone in 10:1 ratio, formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio, Curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 12:1 ratio, and curcuminoids 95% in a dose of 500 mg twice daily for 3 months. Almost all patients in formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 and 12:1 ratio group had significant improvement in the joint tenderness, crepitus, joint swelling, range of movements and gait. In the group given curcuminoids 95%, majority of patients remained symptomatic throughout the study and had to be started on analgesic drugs and antiarthritic medications before the end of the study.

[0057] Another embodiment of the present invention provides for application of formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio for oral supplementation in patients with Alzheimers disease and an improved method of delivering curcumin in the human blood and tissues and ensuring bioavailability in humans to delay the onset of neurodegenerative diseases like Alzheimers disease, for treatment and symptomatic improvement in patients with Alzheimers disease. In some embodiments, the ratio of

curcuminoid mixture to essential oil of turmeric is 12:1 ratio for oral supplementation in patients with Alzheimers disease and an improved method of delivering curcumin in the human blood and tissues and ensuring bioavailability in humans to delay the onset of neurodegenerative diseases like Alzheimers disease, for treatment and symptomatic improvement in patients with Alzheimers disease. Alzheimers disease patients were given raw turmeric powder, essential oil of turmeric with 45% Ar-turmerone, essential oil of turmeric with 10-15% Ar-turmerone, curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 1:10 ratio, curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 1:1 ratio, curcuminoid 24% with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio, curcuminoid with essential oil of turmeric with 10-15% Ar-turmerone in 10:1 ratio, formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio, Curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 12:1 ratio or curcuminoids 95% in a dose of 3 gm/day. The patients on curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 and 12:1 ratio formulations significantly benefitted cognitive performance, functional impairment, behavior and global function compared with commercial curcumin formulation in the same dose. The serum level of Amyloid beta increased significantly in group taking the formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 and 12:1 ratios reflecting the ability of formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 and 12:1 ratios to disaggregate Amyloid beta deposits in the brain compared to curcuminoids 95%. It was also associated with an increase in the Vitamin E content between curcuminoids 95% and formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 and 12:1 ratios, the values being significantly higher for the formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 and 12:1 ratio groups. Another human study which supplemented formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 and 12:1 ratios to patients with mild cognitive impairment over a period of 2 years showed that the risk of development of dementia and Alzheimers disease is reduced drastically in all patients on formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 and 12:1 ratios therapy while majority of the patients in curcuminoids 95% progressed to dementia and 50% to Alzheimers disease within 2 years.

[0058] Another embodiment of the present invention provides for application of a formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 and 12:1 ratios for oral supplementation in patients with depression and an improved method of delivering curcumin in the human blood and tissues and ensuring bioavailability in humans for treatment of patients with depression. Patients with depression were given raw turmeric powder, essential oil of turmeric with 45% Ar-turmerone, essential oil of turmeric with 10-15% Ar-turmerone, curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 1:10 ratio, curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 1:1 ratio, curcuminoid 24% with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio, curcuminoid with essential oil of turmeric with 10-15% Ar-turmerone in 10:1 ratio, formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio, curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 12:1 ratio and curcuminoids 95% in a dose of 500 mg twice daily for 8 weeks. Almost all patients in formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 and 12:1 ratio groups had significant reduction in the severity of depression as assessed by the Hamilton depression scale and showed significant reduction in severity of illness and improvement and response to treatment as assessed by the clinical global impression scale.

[0059] The inventive compositions have the additional benefit that the essential oil components are themselves bioactive (for example, see Yue, A et al, Int. J. Mol. Med., 2002, 9:481-84; Jayaprakasha, G. K. et al, Z. Naturforsch., 2002, 57:828-35) and thus are expected to synergistically enhance the bioactivity of curcumin.

[0060] It will be readily understood by the skilled artisan that numerous alterations may be made to the examples and instructions given herein. These and other objects and features of present invention will be made apparent from the following examples. The following examples as described are not intended to be construed as limiting the scope of the present invention.

EXAMPLES

Example 1

[0061] Nine healthy human volunteers aged between 25 and 45 years of age were selected for the study. They were given capsules of curcuminoid mixture alone and capsules of enhanced curcumin capsules at the dosage of 50 mg curcuminoid/kg body weight. Enhanced curcumin is a composition having curcuminoid and added essential oil of turmeric. In the enhanced curcumin capsules the weight ratio of curcuminoid to essential oil of turmeric was 10:1. The subjects were advised to take curcuminoid capsules first. Blood samples were collected at zero hour and periodically at one-hour or half-hour intervals for 8 hours. After a washout period of one week, the same protocol was repeated with enhanced curcumin bioavailability capsules. The whole blood was extracted exhaustively with ethyl acetate to recover curcumin. The ethyl acetate extract was analyzed by HPLC on a RP-C18 column (25×4.5 mm) using tetrahydrofuran (THF) as solvent and UV detection at 420 nm. The eluant flow rate was 1 ml/min. Efficiency of the extraction procedure for recovering curcumin from blood samples was determined by measuring recovery of curcumin upon extraction of normal blood samples. Normal blood samples were collected by adding curcumin to normal blood (of persons not consuming curcumin or enhanced curcumin capsules). Curcumin was extracted from the normal blood samples by the above procedure. The efficiency of recovery of curcumin by the above extraction procedure was estimated to range between 80.12% and 86.49%.

[0062] A Typical Result is Given in Table 1.

TABLE 1

Curcumin content in blood (ng/g)
Enhanced curcumin
Curcumin bioavailability
Time (h) composition composition
0.0 0.0 0
0.5 3.17 7.85
1.0 7.57 6.23
1.5 4.42 4.84
2.0 13.81 11.95
2.5 9.61 19.22
3.0 5.67 92.59
4.0 8.2 24.33

6.0 1.62 8.43
8.0 1.11 5.09

[0063] The results are also graphically represented in FIG. 1. Following administration of capsules having a 10:1 weight ratio of curcuminoid to essential oil of turmeric, the peak absorption of curcumin occurred at 3 hr. Furthermore, curcumin persisted in small amounts in the blood till 8 hr beyond which measurements were not made. At peak absorption the enhancement of bioavailability ranged, among the 9 persons, between 5 and 16-fold with a mean value of 10.62.

Example 2

[0064] Human subjects were administered capsule (4×500 mg) prepared with curcuminoids and without added essential oil of turmeric (curcuminoids group in Table 2). Blood was drawn at different intervals (one hour) and tested for curcumin content. After two weeks the same groups were administered an enhanced curcumin bioavailability composition (4×500 mg). The varying ratios of curcuminoids and added essential oil of turmeric are as provided in Table 2. Blood from the enhanced curcumin group was drawn at different intervals and tested for curcumin content. As seen in Table 2, bioavailability of curcumin was greater when enhanced curcumin capsules were administered as compared to administration of capsule containing curcuminoids without added essential oil of turmeric.

TABLE 2

Analysis of curcumin content in blood.

Ratio of curcuminoids to added essential oil of turmeric	Curcumin content in blood (AUC) Curcuminoid mixture alone group	Enhanced curcumin group
90:4	725	5147.5
90:5	820	5904
90:6	750	5475
90:7	900	6300.0
90:8	752	5367.6
90.9	782	5552.2
89.9	696	5080.8
90:10	760	5320
80:9	726	5227.2
80:20	754	5315.7
90:20	765	5469.75
70:20	810	5147.5

[0065] The ratios of curcuminoids to added essential oil of turmeric in the enhanced curcumin bioavailability composition provided in Table 2 can also be represented as shown in Table 3. The unit of curcumin content in blood is provided as area under the curve (AUC).

TABLE 3

Ratio of curcuminoids to added essential oil in compositions for enhanced curcumin bioavailability

Ratio of	essential oil of turmeric	essential oil of turmeric

Ratio of Curcuminoids to added curcuminoids to added
90:4 22.5:1
90:5 18:1
90:6 15:1
90:7 12.9:1
90:8 11.25:1
90:9 10:1
90:10 9:1
80:9 8.9:1
80:20 4:1
90:20 4.5:1
70:20 3.5:1

Example 3

[0066] Bioavailability of curcumin from essential oil of turmeric alone, raw turmeric powder, curcuminoid alone, curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio and curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 12:1 ratio etc.

[0067] Nine healthy human volunteers were given capsules containing 475 mg of curcuminoid mixture without added essential oil of turmeric (the capsule was made up to 500 mg by addition of rice powder) at a dosage of 50 mg curcuminoid/kg body weight. Blood was drawn from the subjects at baseline, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6 and 8 hours post drug. The same subjects after a washout period of one week were given 500 mg capsule having 454.55 mg curcuminoid mixture with 45.45 mg essential oil of turmeric, wherein the essential oil of turmeric had about 45% Ar-turmerone (the weight ratio of curcuminoid mixture to added essential oil of turmeric was 10:1) at a dosage of 50 mg curcuminoid/kg body weight of the subject. Blood was drawn from the subjects at baseline, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6 and 8 hours post drug. Table 4 provides the amount of curcumin in nanograms per gram of blood for the subjects, which was averaged for each time point.

[0068] The above protocol was repeated with the following three formulations:

(1) A capsule having 500 mg of essential oil of turmeric, wherein the essential oil of turmeric had 10-15% Ar-turmerone, was administered at a dosage of 50 mg of essential oil of turmeric per kg body weight of the human subject:
(2) A capsule having 500 mg of essential oil of turmeric, wherein the essential oil of turmeric had 45% Ar-turmerone, administered at a dosage of 50 mg of essential oil of turmeric per kg body weight of the human subject; and
(3) A capsule having 500 mg of raw turmeric powder was administered at a dosage of 50 mg of raw turmeric powder/kg body weight of the human subject.
(4) A capsule having 500 mg of 461.5 mg curcuminoid mixture with 38.45 mg essential oil of turmeric, wherein the essential oil of turmeric had about 45% Ar-turmerone (the weight ratio of curcuminoid mixture to added essential oil of turmeric was 12:1)

[0069] Whole blood drawn from the subjects was extracted exhaustively with ethyl acetate to recover curcumin. The ethyl acetate extract was analyzed by HPLC on a RP-C18 column (25×4.5 mm) using tetrahydrofuran (THF) as solvent and UV detection at 420 nm. The eluent flow rate was 1 ml/min. As seen in Table 4 and FIG. 2, curcumin bioavailability in human

subjects following administration of raw turmeric was low. Curcumin bioavailability following administration of negative controls, namely, essential oil fractions having 10-15% or 45% Ar-turmerone was not detectable (referred to as Nd in Table 4). Whereas, curcumin was detectable in human subjects following administration of curcuminoid mixture without added essential oil of turmeric, the bioavailability of curcumin was enhanced by 6.7 fold upon administration of a composition having curcuminoid mixture and essential oil of turmeric with 45% Ar-t in 10:1 ratio and the bioavailability of curcumin was enhanced by 8.3 fold upon administration of a composition having curcuminoid mixture and essential oil of turmeric with 45% Ar-t in 12:1 ratio.

[0070] As seen in FIG. 2, the maximum concentration of curcumin in blood (Cmax of curcumin) was 13.81 ng/g upon administration of the negative control capsule having curcuminoid mixture without the added essential oil of turmeric, whereas, the Cmax of curcumin was 92.59 ng/g upon administration of the positive control capsule having curcuminoid mixture and added essential oil of turmeric with 45% Ar-t in 10:1 ratio. The Cmax of curcumin was 114.59 ng/g upon administration of the positive control capsule having curcuminoid mixture and added essential oil of turmeric with 45% Ar-t in 12:1 ratio. Therefore, comparison of the Cmax values shows that bioavailability of curcumin upon oral administration of the claimed composition having curcuminoid mixture and added essential oil of turmeric with 45% Ar-t in 10:1 was 6.7 times greater than bioavailability of curcumin upon oral administration of curcuminoid mixture without the added essential oil of turmeric. Bioavailability of curcumin upon oral administration of the claimed composition having curcuminoid mixture and added essential oil of turmeric with 45% Ar-t in 12:1 ratio was 8.3 times greater than bioavailability of curcumin upon oral administration of curcuminoid mixture without the added essential oil of turmeric.

TABLE 4

Negative and Positive Control experiments
Curcumin content in blood (ng/g)

Time hours	Raw turmeric powder	Curcuminoid mixture with Essential oil of turmeric (45% Ar-turmerone)	Curcuminoid mixture without Essential oil of turmeric (45% Ar-turmerone)	Curcuminoid added essential oil of turmeric (45% Ar-turmerone)	Curcuminoid added essential oil of turmeric 10:1	Curcuminoid added essential oil of turmeric 12:1
0	0	0	0	0	0	0
0.5	Nd	Nd	Nd	3.17	7.85	15.2
1	1.05	Nd	Nd	7.57	6.23	23.4
1.5	Nd	Nd	Nd	4.42	4.84	32.8
2	2.1	Nd	Nd	13.81	11.95	69.8
2.5	Nd	Nd	Nd	9.61	19.22	114.59
3	Nd	Nd	Nd	5.67	92.59	88.5
4	Nd	Nd	Nd	8.2	24.33	49.4
6	Nd	Nd	Nd	1.62	8.43	20.74
8	Nd	Nd	Nd	1.11	5.09	10.8

Example 4

[0071] Bioavailability of curcumin from capsules having a weight ratio of curcuminoid mixture to essential oil of turmeric ranging from about 1:3 to 99:1

[0072] Human volunteers aged between 25 and 45 years were randomized into separate groups having 3 subjects each (Groups A through W). For control experiment, at the initial time point, subjects in all the groups were four 500 mg capsules of C without added E having about 475 mg of curcuminoid mixture. Then blood was drawn from the subjects at different time periods (0.5, 1, 1.5, 2, 2.5, 3, 4, 6 and 8 hours post drug) and the amount of curcumin in blood (in nanograms per gram of blood) was determined. The average values of curcumin in blood at each time period was plotted in separate graphs for each of the groups (A to W). For each of the groups, the area under the curve (AUC) of curcumin was calculated from the figure. In Table 5 and FIG. 3, AUC is provided as nanograms of curcumin per gram of blood.

[0073] After a wash out period of 2 weeks, subjects in groups A through W were given four 500 mg capsules each, wherein set of 4 capsules had varying ratios of curcuminoid mixture to added essential oil of turmeric (referred to as C with added E capsule in Table 5), and wherein the essential oil of turmeric in the capsules had 45% Ar-turmerone. The ratio of curcuminoid mixture to essential oil of turmeric in the capsules ranged from about 99:1 to about 1:3. Some of the could be expressed as more than one type of ratio, for example, as 95:5 or 19:1; 90:4 or 22.5:1; 90:5 or 18:1; 90:6 or 15:1; 90:7 or 12.9:1; 90:8 or 11.3:1; 90:9 or 10:1; 90:10 or 9:1; 90:20 or 4.5:1; 89:9 or 9.8:1; 80:9 or 8.8:1; 80:20 or 4:1; 70:20 or 3.5:1; 75:25 or 3:1; 60:30 or 2:1; 50:50 or 1:1, 30:60 or 1:2 and 25:75 or 1:3 and therefore the ratios are referred to accordingly in Table 5.

[0074] As shown in Table 5, each of the groups was administered a capsule having a different weight ratio of curcuminoid mixture to essential oil of turmeric (referred to as C:E). Blood was drawn from the subjects and the AUC was calculated as described above. The curcumin content in the blood for each group was expressed as AUC, which was used to compare the bioavailability of curcumin from the different treatment groups.

[0075] Table 5 and FIG. 3 provide a comparison of the bioavailability of curcumin from the curcuminoid mixture without added essential oil of turmeric as the control group and the curcuminoid mixture with added essential oil of turmeric with 45% Ar-turmerone.

[0076] As seen in Table 5 and FIG. 3, curcumin bioavailability upon administration of capsules having curcuminoid mixture with added essential oil of turmeric with 45% Ar-turmerone resulted in an enhancement of bioavailability ranging from 1.8 to 7.3 fold over the curcumin bioavailability that was observed when negative control capsules having curcuminoid mixture without added essential oil of turmeric were administered. The results in Table 5 further show that the enhancement of bioavailability was observed over the entire claimed range of the ratio about 1:3 to about 99:1 of curcuminoid mixture to essential oil of turmeric.

TABLE 5

Bioavailability of curcumin from compositions having weight ratios of curcuminoid mixture to added essential oil of turmeric ranging from 1:3 to 99:1

C without added E	C with added E
C (ng)	C (ng)

Group	Ratio of C:E	Dosage 4 caps	C (mg) each capsule	per gm of capsule (AUC)	C (mg) per gm of blood	E (mg) per gm of blood	per gm of capsule (AUC)
A	99:1	500 mg	475	771	495	5	3855
B	95:5 or 19:1	500 mg	475	786	475	25	5515
C	90:4 or 22.5:1	500 mg	475	725	478.72	21.28	5147.5
D	90:5 or 18:1	500 mg	475	820	473.68	26.32	5904
E	90:6 or 15:1	500 mg	475	750	468.75	31.25	5475
F	90:7 or 12.9:1	500 mg	475	900	463.77	36.23	6300
G	90:8 or 11.3:1	500 mg	475	752	459.35	40.65	5367.6
H	90:9 or 10:1	500 mg	475	782	454.55	45.45	5552.2
I	90:10 or 9:1	500 mg	475	760	450	50	5320
J	90:20 or 4.5:1	500 mg	475	765	409.1	90.9	5469.75
K	89:9 or 9.8:1	500 mg	475	696	453.7	46.3	5080.8
L	80:9 or 8.8:1	500 mg	475	726	448.98	51.02	5227.2
M	80:20 or 4:1	500 mg	475	754	400	100	5315.7
N	70:20 or 3.5:1	500 mg	475	810	388.89	111.11	5147.5
O	70:1	500 mg	475	769	493	7	5124
P	60:1	500 mg	475	725	491.8	8.2	5200
Q	50:1	500 mg	475	749	490.2	9.8	5284
R	40:1	500 mg	475	737	487.8	12.2	5310
S	75:25 or 3:1	500 mg	475	756	375	125	4158
T	60:30 or 2:1	500 mg	475	742	333.3	166.6	3635.8
U	50:50 or 1:1	500 mg	475	788	250	250	2537
V	30:60 or 1:2	500 mg	475	715	166.6	333.3	1651
W	25:75 or 1:3	500 mg	475	726	125	375	1276

Example 5

[0077] Comparison of curcumin bioavailability from 10:1 and 1:10 weight ratios of curcuminoid mixture to essential oil of turmeric.

[0078] Nine healthy human volunteers were given four 500 mg capsules having 20 mg curcuminoid mixture without added essential oil of turmeric (referred to as 20 mg C in Table 6). Blood was drawn from the subjects at baseline, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6 and 8 hours post drug. Following one week washout period, the same nine subjects were given four 500 mg capsules having 200 mg of essential oil of turmeric having 10 to 15% Ar-turmerone. Blood was drawn from the subjects at baseline, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6 and 8 hours post drug.

[0079] With one week washout period between treatments, the subjects were tested for the following treatments, wherein four of 500 mg capsules were administered to each subject. If any of the capsules had less than 500 mg of the test component such as curcuminoid mixture or essential oil or the combination of curcuminoid mixture and essential oil, then the capsules were made up to 500 mg by addition of a placebo, e.g., rice powder. In one treatment, each capsule had a 1:10 ratio of curcuminoid mixture to added essential oil of turmeric. Each capsule contained 20 mg curcuminoid and 200 mg essential oil of turmeric, wherein the essential oil of turmeric had 10 to 15% Ar-turmerone (referred to as Ar-t in Table 6).

[0080] In another treatment, each capsule had a 1:10 ratio of curcuminoid mixture to added essential oil of turmeric, wherein the essential oil had 45% Ar-turmerone. Each capsule

contained 20 mg curcuminoid and 200 mg essential oil of turmeric. The capsule is referred to as 20 mg C: 200 mg E=1:10 (E had 10-15% Ar-t) in Table 6.

[0081] In another treatment, the capsule had a 10:1 ratio of curcuminoid mixture to added essential oil of turmeric, wherein the essential oil had 45% Ar-turmerone. Each capsule contained 20 mg curcuminoid and 2 mg essential oil of turmeric. The capsule is referred to as 20 mg C: 2 mg E=10:1 (E had 45% Ar-t) in Table 6.

[0082] In another treatment, each capsule had curcuminoid mixture without the added essential oil of turmeric. Each capsule contained 454.55 mg curcuminoids. The capsule is referred to as 454.55 mg C without added E in Table 6.

[0083] In another treatment, each capsule had essential oil of turmeric having 45% Ar-turmerone. Each capsule contained 45.45 mg essential oil of turmeric. The capsule is referred to as 45.45 mg E (45% Ar-t) in Table 6.

[0084] In another treatment, each capsule had curcuminoid mixture along with added essential oil of turmeric with 45% Ar-turmerone at a 10:1 ratio. Each capsule contained 454.55 mg curcuminoids and 45.45 mg of essential oil of turmeric. The essential oil of turmeric had 45% Ar-turmerone. The capsule is referred to as 454.55 mg C: 45.45 mg E=10:1 (E had 45% Ar-t) in Table 6.

[0085] Whole blood from the subjects was extracted exhaustively with ethyl acetate to recover curcumin. The ethyl acetate extract was analyzed by HPLC on a RP-C18 column (25×4.5 mm) using tetrahydrofuran (THF) as solvent and UV detection at 420 nm. The eluent flow rate was 1 ml/min. Curcumin content in the blood was determined for each group at each time point and the average value of curcumin in blood (in nanogram per gram of blood) was calculated. The average value of curcumin at each time point for various the treatment protocols is provided in Table 6 and in FIG. 4.

[0086] As seen in Table 6, low bioavailability of curcumin of about 1.05 ng curcumin per gm of blood was observed from the negative control having 20 mg of curcuminoid mixture without added essential oil of turmeric. In the negative controls having essential oil of turmeric alone, with either 10-15% Ar-turmerone or 45% Ar-turmerone, the bioavailability of curcumin was not detectable (referred to as Nd in Table 6). Further, bioavailability of curcumin from the capsule prepared and having a 1:10 ratio of curcuminoid mixture to essential oil of turmeric, wherein the essential oil had either a 10-15% Ar-turmerone content or 45% Ar-turmerone content, showed poor bioavailability of curcumin.

[0087] An experimental capsule prepared at the ratio of 10:1 of curcuminoid mixture to essential oil of turmeric, wherein the essential oil had a 45% Ar-turmerone content, having 20 mg curcuminoid mixture and 2 mg essential oil of turmeric showed greater than 2-fold enhanced bioavailability over the negative control of 20 mg curcuminoid mixture without the added essential oil of turmeric. On the other hand the positive control having 454.55 mg curcuminoid mixture and 45.55 mg essential oil of turmeric, wherein the essential oil of turmeric had a 45% Ar-turmerone content, i.e., a 10:1 ratio of curcuminoid mixture to essential oil of turmeric, showed a 6.97 fold enhancement of bioavailability of curcumin as compared to the bioavailability of curcumin from the negative control capsule having 454.55 mg curcuminoid mixture without the added essential oil of turmeric.

TABLE 6

Comparison of curcumin bioavailability from 10:1 and 1:10 weight ratios of curcuminod mixture to essential oil of turmeric

Nanograms of curcumin per gram of blood

Time (h)	20 mg C alone	(10-15% Ar-t)	20 mg C:200 mg E had Ar-t)	(10-15% Ar-t)	20 mg C:2 mg E = 200 mg E 1:10 had Ar-t)	45% Ar-t)	454.55 mg C:45.45 mg E = 10:1 added Ar-t)	(45% Ar-t)
0	0	0	0	0	0	0	0	0
0.5	Nd	Nd	Nd	Nd	1.1	3.02	Nd	7.45
1	Nd	Nd	Nd	Nd	1	7.27	Nd	5.81
1.5	Nd	Nd	Nd	Nd	1.05	4.11	Nd	4.52
2	1.05	Nd	1.1	1.3	1.3	13.18	Nd	11.46
2.5	Nd	Nd	Nd	1.1	1.7	9.17	Nd	15.66
3	Nd	Nd	Nd	Nd	2.67	5.21	Nd	91.9
4	Nd	Nd	Nd	Nd	1.34	7.82	Nd	22.44
6	Nd	Nd	Nd	Nd	1.1	1.54	Nd	8.01
8	Nd	Nd	Nd	Nd	1.05	1.05	Nd	6.18

Example 6

Method of Preparation of Curcuminoid Mixture with 95% Curcuminoids

[0088] The rhizomes of turmeric (300 Kg) were dried. The dried turmeric rhizomes were powdered to form powdered turmeric. The powdered turmeric was treated with ethyl acetate (900 L) to form a solution. The extraction was carried out at 78° C. temperature for 1 hr. After initial extraction, the extraction process was repeated 4 more times and the resultant solution was filtered and the solvent was stripped from the filtered solution to form an extract. This extract was cooled to about 4° C. to obtain crystals of curcuminoid (12 Kg) and a liquid. The crystals of curcuminoid were isolated from the liquid by filtration. The crystals were powdered to form powdered curcuminoid mixture with 95% curcuminoids.

Example 7

Method of Preparation of Curcuminoid Mixture with 24% Curcuminoids

[0089] The rhizomes of turmeric (50 Kg) were dried and flaked into required size. The flakes of turmeric was filled in the soxhlet apparatus and extracted with ethylene dichloride (EDC). The extraction was carried out for 5 his at a temperature of about 83° C. After the completion of extraction, the solvent was filtered. The solvent was removed by distillation and mild vacuum was applied to get an oleoresin which contains essential oil of turmeric and curcuminoid. The oleoresin was steam distilled to get essential oil and a residue. The residue was dried under vacuum to form a powder (10 Kg) with 24% curcuminoid content.

E**xample 8**

Method of Analysis of Total Curcuminoids by HPLC Method

[0090] From 500 mg capsule, 25 mg was accurately weighed and transferred into a 50 ml standard flask and made up to a 50 ml solution with methanol. From this pipette out 2 ml into 50 ml standard flask and made up to a 50 ml solution with methanol. Filter through 0.2 μm membrane filter before injection. Standard was prepared by weighing accurately 25 mg standard [Curcumin Standard: —99% Total Curcuminoids (Sigma)] and transferred into a 50 ml standard flask and made up to a 50 ml solution with methanol. From this pipette out 2 ml into 50 ml standard flask and made up to a 50 ml solution with methanol. Filter through 0.2 μm membrane filter before injection.

[0091] The total Curcuminoids was analyzed by high performance liquid chromatography (HPLC) on a C18 column ((250×4.6 mm Shimadzu Co., Japan.) using tetrahydrofuran (THF) as the mobile phase and UV detection at 420 nm. The eluent flow rate was 1 ml/min.

[0092] By comparing the area of standard and sample, the percentage of total curcuminoids was calculated using the formula

[mathematical formula]

Example 9

Method of Preparation of Essential Oil of Turmeric with Varying Concentration of Ar-Turmerone

[0093] The rhizomes of turmeric (500 Kg) were dried. The dried turmeric rhizomes were powdered to form powdered turmeric. The powdered turmeric was treated with ethyl acetate (1500 L) to form a solution. The extraction was carried out at 78° C. temperature for 1 hr. After initial extraction, the extraction process was repeated 4 more times and the resultant solution was filtered and the solvent was stripped from the filtered solution to form an extract. This extract was cooled to about 4° C. to obtain crystals of curcuminoid (20 Kg) and a liquid. The crystals of curcuminoid were isolated from the liquid by filtration.

[0094] The remaining liquid comprises the essential oil of turmeric and a resin. The liquid was then steam distilled to isolate essential oil of turmeric with 10-15% Ar turmerone (25 Kg). After fractionating this oil, essential oil with 45% Ar turmerone (7.5 Kg) was obtained as fraction 3, essential oil of turmeric with 4-5% Ar turmerone (8.3) was obtained as fraction 2 and essential oil of turmeric with 2-3% Ar turmerone (9.3 Kg) was obtained as fraction 1. (FIG. 5)

Example 10

Method of Preparation of Combination of Curcuminoids and Essential Oil of Turmeric with 45% Ar Turmerone in 10:1 Ratio

[0095] The curcuminoid powder prepared as per Example 6 (2.7 Kg) was suspended in water (12 L) to form a suspension. Fraction of essential oil containing 45% Ar-turmerone prepared as per Example 9 (0.27 Kg) was added to the suspension in 10:1 ratio. The mixture is pulverized in a colloidal mill to form fine slurry. Water is stripped from the slurry under heat and vacuum to form a uniform blend. (3 Kg).

[0096] A 500 mg capsule containing 454.55 mg of curcuminoid and 45.45 mg of Essential oil

with 45% Ar-turmerone in a weight ratio of about 90:9 (10:1) was prepared by encapsulating the above blended extract powder in hard gelatin capsules done in an air-conditioned at 21° C. and de-humidified room. 3 kg of extract powder was charged into the hopper of a semi-automatic capsule filling machine. '0' size hard gelatin capsule shell was loaded to the tray and the blended extract powder was filled into the shell. The filled weight of capsules were checked simultaneously and these capsules were sorted by a sorting machine and polished with the help of a polishing machine to give 6000 capsules of 500 mg each.

Example 11

Method of Preparation of Combination of Curcuminoids and Essential Oil of Turmeric with 45% Ar-Turmerone in 1:10 Ratio

[0097] The powdered curcuminoid mixture prepared as per Example 6 (0.27 Kg) was suspended in water (1 L) to form a suspension. Fraction of essential oil of turmeric containing 45% Ar-turmerone prepared as per Example 9 (2.7 Kg) was added to the suspension in 1:10 ratio. The mixture is pulverized in a colloidal mill to form fine slurry. Water was stripped from the slurry under heat and vacuum to form a uniform blend (3 Kg).

[0098] Capsule containing curcuminoid and Essential oil of turmeric with 45% Ar-turmerone in a weight ratio of about 1:10 was prepared by encapsulating the above blended extract powder in soft gelatin capsules done in an air-conditioned at 21° C. and de-humidified room. 3 kg of extract powder was charged into the hopper of a semi-automatic capsule tilling machine. '0' size soft gelatin capsule shell was loaded to the tray and the blended extract powder was filled into the shell. The filled weights of capsules were checked simultaneously and these capsules were sorted by a sorting machine and polished with the help of a polishing machine.

Example 12

Method of Preparation of Combination of Curcuminoids and Essential Oil of Turmeric with 45% Ar Turmerone in 1:1 Ratio

[0099] The powdered curcuminoid mixture prepared as per Example 6 (1.5 Kg) was suspended in water (6 L) to form a suspension. Fraction of essential oil of turmeric containing 45% Ar-turmerone prepared as per Example 9 (1.5 Kg) was added to the suspension in 1:1 ratio. The mixture was pulverized in a colloidal mill to form fine slurry. Water was stripped from the slurry under heat and vacuum to form a uniform blend. (3 Kg).

[0100] A 500 mg capsule containing 250 mg of curcuminoid and 250 mg of Essential oil of turmeric with 45% Ar-turmerone in a weight ratio of about 1:1 was prepared by encapsulating the above blended extract powder in hard gelatin capsules done in an air-conditioned at 21° C. and de-humidified room. 3 kg of extract powder was charged into the hopper of a semi-automatic capsule filling machine. '0' size hard gelatin capsule shell was loaded to the tray and the blended extract powder was filled into the shell. The filled weight of capsules were checked simultaneously and these capsules were sorted by a sorting machine and polished with the help of a polishing machine to give 6000 capsules of 500 mg each.

Example 13

Method of Preparation of Combination of Curcuminoids and Essential Oil of Turmeric with 10-15% Ar Turmerone in 10:1 Ratio

[0101] The powdered curcuminoid mixture prepared as per Example 6 (2.7 Kg) was suspended in water (12 L) to form a suspension. Fraction of essential oil of turmeric containing 10-15% Ar-turmerone prepared as per Example 9 (0.27 Kg) was added to the suspension in 10:1 ratio. The mixture was pulverized in a colloidal mill to form fine slurry. Water was stripped from the slurry under heat and vacuum to form a uniform blend (3 Kg).

[0102] A 500 mg capsule containing 454.55 mg of curcuminoid and 45.45 mg of Essential oil of turmeric with 10-15% Ar-turmerone in a weight ratio of about 90:9 (10:1) was prepared by encapsulating the above blended extract powder in hard gelatin capsules done in an air-conditioned at 21° C. and de-humidified room. 3 kg of extract powder was charged into the hopper of a semi-automatic capsule filling machine. '0' size hard gelatin capsule shell was loaded to the tray and the blended extract powder was filled into the shell. The filled weight of capsules were checked simultaneously and these capsules were sorted by a sorting machine and polished with the help of a polishing machine to give 6000 capsules of 500 mg each.

Example 14

Method of Preparation of Capsules Containing Essential Oil of Turmeric with 45% Ar-Turmerone

[0103] A 500 mg capsule with essential oil of turmeric containing 45% Ar-turmerone was prepared by encapsulating the essential oil of turmeric with 45% Ar-turmerone prepared as per example 9 (2.5 kg) in soft gelatin capsules done in an air-conditioned at 21° C. and de-humidified room. 2.5 kg essential oil of turmeric with 45% Ar-turmerone was charged into the hopper of a semi-automatic capsule filling machine. '0' size soft gelatin capsule shell was loaded to the tray and the blended extract powder was filled into the shell. The filled weight of capsules were checked simultaneously and these capsules were sorted by a sorting machine and polished with the help of a polishing machine to give 5000 capsules of 500 mg each.

Example 15

Method of Preparation of Capsules Containing Essential Oil of Turmeric with 10-15% Ar-Turmerone

[0104] A 500 mg capsule with essential oil of turmeric containing 10-15% Ar-turmerone was prepared by encapsulating the essential oil with 10-15% Ar-turmerone prepared as per example 9 (2.5 kg) in soft gelatin capsules done in an air-conditioned at 21° C. and de-humidified room. 2.5 kg essential oil of turmeric with 10-15% Ar-turmerone was charged into the hopper of a semi-automatic capsule filling machine. '0' size soft gelatin capsule shell was loaded to the tray and the blended extract powder was filled into the shell. The filled weight of capsules were checked simultaneously and these capsules were sorted by a sorting machine and polished with the help of a polishing machine to give 5000 capsules of 500 mg each.

Example 16

Method of Preparation of Capsules Containing Curcuminoids 95%

[0105] A 500 mg capsule containing curcuminoids 95% was prepared by encapsulating the curcuminoid powder with 95% curcuminoids in hard gelatin capsules done in an air-conditioned at 21° C. and de-humidified room. 3 kg of powder was charged into the hopper of a semi-automatic capsule filling machine. '0' size hard gelatin capsule shell was loaded to the tray and the powder was filled into the shell. The filled weight of capsules were checked simultaneously and these capsules were sorted by a sorting machine and polished with the help of a polishing machine to give 6000 capsules of 500 mg each.

Example 17

Method of Preparation of Combination of Curcuminoids with 24% Curcuminoids and Essential Oil of Turmeric with 45% Ar-Turmerone in 10:1 Ratio

[0106] The powdered curcuminoid prepared as per Example 7 (2.7 Kg) was suspended in water (12 L) to form a suspension. Fraction of essential oil containing 45% Ar-turmerone prepared as per Example 9 (0.27 Kg) was added to the suspension in 10:1 ratio. The mixture was pulverized in a colloidal mill to form fine slurry. Water was stripped from the slurry under heat and vacuum to form a uniform blend (3 Kg).

[0107] A 500 mg capsule containing 454.55 mg of curcuminoid and 45.45 mg of Essential oil with 45% Ar-turmerone in a weight ratio of about 90:9 (10:1) was prepared by encapsulating the above blended extract powder in hard gelatin capsules done in an air-conditioned at 21° C. and de-humidified room. 3 kg of extract powder was charged into the hopper of a semi-automatic capsule filling machine. '0' size hard gelatin capsule shell was loaded to the tray and the blended extract powder was filled into the shell. The filled weight of capsules were checked simultaneously and these capsules were sorted by a sorting machine and polished with the help of a polishing machine to give 6000 capsules of 500 mg each.

Example 18

Method of Preparation of Raw Turmeric Powder

[0108] The raw turmeric rhizomes (10 Kg) were collected and cleaned. The rhizomes were dried and pulverized to get turmeric powder (2.5 Kg). The turmeric powder was sieved through 20 meshes. A 500 mg capsule with raw turmeric powder (curcuminoids 5%) was prepared by encapsulating the powder in hard gelatin capsules done in an air-conditioned at 21° C. and de-humidified room. 2.5 kg raw turmeric powder is charged into the hopper of a semi-automatic capsule filling machine. '0' size hard gelatin capsule shell was loaded to the tray and the blended extract powder was filled into the shell. The filled weight of capsules were checked simultaneously and these capsules were sorted by a sorting machine and polished with the help of a polishing machine to give 5000 capsules of 500 mg each.

Example 19

Method of Preparation of Combination of Curcuminoids and Essential Oil of Turmeric with 45% Art Turmerone in 12:1 Ratio

[0109] The curcuminoid powder prepared as per Example 6 (3.5 Kg) was suspended in water (15 L) to form a suspension. Fraction of essential oil containing 45% Ar-turmerone prepared as per Example 9 (0.29 Kg) was added to the suspension in 12:1 ratio. The mixture is

pulverized in a colloidal mill to form fine slurry. Water is stripped from the slurry under heat and vacuum to form a uniform blend. (3.8 Kg).

[0110] A 500 mg capsule containing 461.5 mg of curcuminoid and 38.45 mg of Essential oil with 45% Ar-turmerone in a weight ratio of about 12:1 (curcumin 69.5%, demethoxy curcumin 17% and bisdemethoxy curcumin 4% and Essential oil of turmeric 7.5%) was prepared by encapsulating the above blended extract powder in hard gelatin capsules done in an air-conditioned at 21° C. and de-humidified room. 3 kg of extract powder was charged into the hopper of a semi-automatic capsule filling machine. '0' size hard gelatin capsule shell was loaded to the tray and the blended extract powder was filled into the shell. The filled weight of capsules were checked simultaneously and these capsules were sorted by a sorting machine and polished with the help of a polishing machine to give 6000 capsules of 500 mg each.

Example 20

Human Clinical Study of Different Turmeric Extracts in Patients with Rheumatoid Arthritis

[0111] In a human clinical study to assess the efficacy of formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio compared to raw turmeric powder, essential oil of turmeric with 45% Ar-turmerone, essential oil of turmeric with 10-15% Ar-turmerone, curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 1:10 ratio, curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 1:1 ratio, curcuminoid 24% with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio, curcuminoid with essential oil of turmeric with 10-15% Ar-turmerone in 10:1 ratio, Curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 12:1 ratio, and curcuminoids 95% in patients with rheumatoid arthritis, 50 patients diagnosed with rheumatoid arthritis were randomized into 10 groups viz.,

Group1: Subjects receiving raw turmeric powder 500 mg capsules prepared as described in Example 18 twice daily
Group2: Subjects receiving essential oil of turmeric with 45% Ar-turmerone (EOT with 45% Ar-t) 500 mg capsules prepared as described in Example 14 twice daily
Group3: Subjects receiving essential oil of turmeric with 10-15% Ar-turmerone (EOT with 10-15% Ar-t) 500 mg capsules prepared as described in Example 15 twice daily
Group4: Subjects receiving curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 1:10 ratio (C+E with 45% Ar-t in 1:10 ratio), 500 mg capsules prepared as described in Example 11 twice daily.
Group5: Subjects receiving curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 1:1 ratio (C+E with 45% Ar-t in 1:1 ratio), 500 mg capsules prepared as described in Example 12 twice daily
Group6: Subjects receiving curcuminoid 24% with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio (C 24%+E with 45% Ar-t in 10:1 ratio), 500 mg capsules prepared as described in Example 17 twice daily.
Group7: Subjects receiving curcuminoid with essential oil of turmeric with 10-15% Ar-turmerone in 10:1 ratio (C+E with 10-15% Ar-t in 10:1 ratio), 500 mg capsules prepared as described in Example 13 twice daily
Group8: Subjects receiving curcuminoids 95% 500 mg capsules prepared as described in Example 16, twice daily.

Group9: Subjects receiving formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio (C+E with 45% Ar-t in 10:1 ratio) 500 mg capsules prepared as described in Example 10, twice daily dose after food with water for a period of 8 weeks. Group10: Subjects receiving formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 12:1 ratio (C+E with 45% Ar-t in 12:1 ratio) 500 mg capsules prepared as described in Example 19, twice daily dose after food with water for a period of 8 weeks.

[0112] Subjects aged 18-65 years of either sex diagnosed to have rheumatoid arthritis (RA) according to the revised 1987 ACR criteria for the classification of rheumatoid arthritis Class I or II, with Disease Activity Score (DAS)>5, receiving treatment on an outpatient basis were included in the study. Patients with inflammatory joint disease other than RA and having concurrent treatment with any NSAID, DMARD or any anti-TNF-α therapy or other anti arthritic therapy were excluded. The study examinations included general and clinical examination, evaluation of disease, recording of vital signs, X-ray AP view of chest/hands/wrist/foot, ECG, Haematology. Blood chemistry and Urine Pregnancy Test for women of child bearing potential.

[0113] Efficacy and safety evaluations were performed at biweekly intervals. Patients were assessed for the primary efficacy endpoints disease activity score (DAS) 28 and ACR criteria. DAS is the numerical sum of four outcome parameters: tender and swollen joint count (28-joint assessment), patient's global assessment of disease on a visual analog scale (VAS; 0, no pain and 100, severe pain); and erythrocyte sedimentation rate. The ACR criteria are indicated as ACR 20, ACR 50, and ACR 70. ACR criteria measures improvement in tender or swollen joint counts and improvement in three of the following five parameters: patient global assessment-global assessment of disease activity on a 0-100 scale (0, best; 100, worst); physician assessment-global assessment of disease activity on a 0-100 scale (0, best; 100, worst); pain scale disability-visual analogue scale for pain (VAS; 0, no pain and 100, severe pain); functional questionnaire-HAQ (Health Assessment Questionnaire) includes four categories: dressing and grooming, arising, eating, and walking, on a 0-3 scale (0, best; 3, worst); acute phase reactant (such as sedimentation rate). ACR20 is defined as a reduction in tender and swollen joint counts of 20%, ACR 50 of 50% and ACR 70 of 70%, from baseline. Monitoring of vital signs, physical examinations, laboratory parameters (hematology, blood chemistry, C-reactive protein (CRP), antistreptolysin-O (ASO), rheumatoid factor and blood sugar) were performed biweekly for safety evaluation. The occurrence of adverse events was the primary safety variable.

[0114] Treatment with formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 12:1 ratio showed decrease in disease activity score from 6.5 at baseline to 3 at the end of treatment.

[0115] Treatment with formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio showed decrease in disease activity score from 6.5 at baseline to 3.5 at the end of treatment. The results are summarized in Table 7. Mean VAS scores for pain in all the groups were comparable at baseline, and formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio group showed significant reduction (65%) in VAS score from 79 mm at baseline to 27.5 mm at the end of treatment. Mean VAS scores for pain in the group with formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 12:1 ratio group also showed significant reduction (69%) in VAS score from 78 mm at baseline to 24 mm at the end of treatment. The results are summarized in Table 8. All components of ACR response criteria viz., Total Painful Joints, Total Swollen

Joints, Patient's GA, Physician's GA, Disability Index and HAQ showed a significant reduction from baseline to end of study in the formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 12:1 and 10:1 ratios. The results are summarized in Table 9 (FIG. 6). Treatment with formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 12:1 ratio group showed decreased C reactive protein from 12 mg/L at baseline to 5.3 mg/L at the end of treatment and formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio showed decreased C reactive protein from 12 mg/L at baseline to 5.7 mg/L at the end of treatment. The results are summarized in Table 10. Treatment with formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 12:1 ratio group showed decrease in Rheumatoid Arthritis factor from 23 IU/L at baseline to 13 IU/L at the end of treatment. Formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio showed decrease in Rheumatoid Arthritis factor from 24 IU/L at baseline to 15 IU/L at the end of treatment. The results are summarized in Table 11. The study shows that formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 and 12:1 ratios can provide significant improvement in treatment efficacy in active RA. All the patients who were given raw turmeric powder, essential oil of turmeric with 45% Ar-turmerone, essential oil of turmeric with 10-15% Ar-turmerone, curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 1:10 ratio, curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 1:1 ratio, curcuminoid 24% with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio, curcuminoid with essential oil of turmeric with 10-15% Ar-turmerone in 10:1 ratio or curcuminoids 95% capsules showed no significant improvement with treatment and some of the patients even showed worsening of their symptoms with time even with treatment.

TABLE 7

Treatment efficacy results - Disease Activity Score

Group	Baseline	End of Treatment	% Change
Raw turmeric	Mean	6	6.5	8%
EOT with 45% Ar-t	Mean	6.5	6.5	0
EOT with 10-15% Ar-t	Mean	6.5	7	7%
C + E with 45% Ar-t in 1:10 ratio	Mean	7	7.5	6.6%
C + E with 45% Ar-t in 1:1 ratio	Mean	7.5	7.5	0
C 24% + E with 45% Ar-t in 10:1 ratio	Mean	7	7	0
C + E with 10-15% Ar-t in 10:1 ratio	Mean	6	6	0
C + E with 45% Ar-t in 10:1 ratio	Mean	6.5	3.5	46%
Curcuminoids	Mean	6.5	6	8%

95%
C + E with Mean 6.5 3 54%
45% Ar-t in
12:1 ratio

TABLE 8
Treatment efficacy results - VAS
End of Treatment
Group Baseline (mm) (mm) % Change
Raw turmeric Mean 80 78 2.5%
EOT with 45% Ar-t Mean 75 77 2.6%
EOT with 10-15% Ar-t Mean 77 78 1.3%
C + E with 45% Ar-t Mean 79 77 2.5%
in 1:10 ratio
C + E with 45% Ar-t Mean 78 76 2.6%
in 1:1 ratio
C 24% + E with 45% Mean 76 74 2.6%
Ar-t in 10:1 ratio
C + E with 10-15% Mean 75 71 5.3%
Ar-t in 10:1 ratio
C + E with 45% Ar-t Mean 79 27.5 65%
in 10:1 ratio
Curcuminoids 95% Mean 77 70 9%
C + E with 45% Ar-t Mean 78 24 69%
in 12:1 ratio

TABLE 10
Treatment efficacy results - CRP
End of Baseline Treatment %
Group (mg/L) (mg/L) Change
Raw turmeric Mean 13 13 0
EOT with Mean 11 11.5 4%
45% Ar-t
EOT with 10-15% Mean 12.5 13.5 7%
15% Ar-t
C + E with 45% Mean 12 12.5 4%
Ar-t in 1:10
ratio
C + E with 45% Mean 13 13 0
Ar-t in 1:1
ratio
C 24% + E Mean 12 12 0
with 45% Ar-t
in 10:1 ratio
C + E with 10-15% Mean 11.5 11.5 0
Ar-t in
10:1 ratio
C + E with 45% Mean 12 5.7 53%
Ar-t in 10:1
ratio

Curcuminoids Mean 11.5 11.4 1%
9.5%
C + E with 45% Mean 12 5.3 56%
Ar-t in 12:1
ratio

TABLE 11
Treatment efficacy results - Rheumatoid Arthritis Factor
End of Baseline Treatment %
Group (IU/L) (IU/L) Change
Raw turmeric Mean 23 25 8%
EOT with 45% Mean 26 28 7%
Ar-t
EOT with 10-15% Mean 25 26 3.8%
Ar-t
C + E with 45% Mean 23 24 4%
Ar-t in 1:10 ratio
C + E with 45% Mean 22 22 0
Ar-t in 1:1 ratio
C 24% + E with Mean 21 21 0
45% Ar-t in
10:1 ratio
C + E with 10-15% Mean 24 24 0
Ar-t in 10:1
ratio
C + E with 45% Mean 24 15 38%
Ar-t in 10:1 ratio
Curcuminoids Mean 22 24 9%
95%
C + E with 45% Mean 23 13 39%
Ar-t in 12:1 ratio

Example 21

Human Clinical Study of Different Turmeric Extracts in Patients with Osteo Arthritis

[0116] In a human clinical trial to determine the effectiveness of formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio in relieving symptoms and clinical conditions of osteoarthritic patients compared with raw turmeric powder, essential oil of turmeric with 45% Ar-turmerone, essential oil of turmeric with 10-15% Ar-turmerone, curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 1:10 ratio, curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 1:1 ratio, curcuminoid 24% with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio, curcuminoid with essential oil of turmeric with 10-15% Ar-turmerone in 10:1 ratio, formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 12:1 ratio and curcuminoids 95% capsules, patients of either sex diagnosed to have osteoarthritis according to ACR criteria were selected for the study. The patients were divided into nine groups of 5 patients each.

Gr 1: Oral administration of raw turmeric powder 500 mg capsules, prepared as described in Example 18, in twice daily dosage

Gr 2: Oral administration of essential oil of turmeric with 45% Ar-turmerone (EOT with 45% Ar-t) 500 mg capsules prepared as described in Example 14, in twice daily dosage.
Gr3: Oral administration of essential oil of turmeric with 10-15% Ar-turmerone (EOT with 10-15% Ar-t) 500 mg capsules prepared as described in Example 15, in twice daily dosage.
Gr4: Oral administration of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 1:10 ratio (C+E with 45% Ar-t in 1:10 ratio), 500 mg capsules prepared as described in Example 11, in twice daily dosage.
Gr5: Oral administration of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 1:1 ratio (C+E with 45% Ar-t in 1:1 ratio), 500 mg capsules prepared as described in Example 12, in twice daily dosage.
Gr6: Subjects receiving curcuminoid 24% with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio (C 24%+E with 45% Ar-t in 10:1 ratio), 500 mg capsules prepared as described in Example 17 twice daily
Gr7: Oral administration of curcuminoid with essential oil of turmeric with 10-15% Ar-turmerone in 10:1 ratio (C+E with 10-15% Ar-t in 10:1 ratio), 500 mg capsules prepared as described in Example 13, in twice daily dosage.
Gr8: Oral administration of formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio (C+E with 45% Ar-t in 10:1 ratio), 500 mg capsule prepared as described in Example 10, in twice daily dosage
Gr 9: Oral administration of curcuminoids 95% 500 mg capsule prepared as described in Example 16, in twice daily dosage.
Gr10: Oral administration of formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 12:1 ratio (C+E with 45% Ar-t in 12:1 ratio), 500 mg capsule prepared as described in Example 19, in twice daily dosage

[0117] Each patient was given treatment for 12 weeks. The efficacy of the use of the study drugs over the treatment period was evaluated by symptom scoring and clinical examination. Symptom refers to the complaints expressed by the patient and scored depending on severity. Symptom scoring includes joint pain measurements and walking distance measurements. Joint pain in osteoarthritis is a deep pain localized to the joint and is measured by querying the patient and scoring it as No/mild/moderate/severe during each visit. Results of this analysis for the eight treatment groups are presented in Table 12 (FIG. 7). Walking distance refers to the maximum distance a person is able to walk at a stretch without limiting pain. Walking distance measurements were recorded and are given Table 13 (FIG. 8). Joint line tenderness was elicited by palpating along the joint line and was measured by querying the patient and recording the response as No/mild/moderate/severe and was recorded and results are presented in Table 14 (FIG. 9).

[0118] Crepitus (crackling or grating feeling or sound in joints) is elicited by palpating the joint on movement and scoring it as No/Mild/Moderate/Severe. Range of movement of the knee is measured for flexion/extension movement and the normal range is from 0 to 135 degrees (0 being neutral position and increasing flexion of the joint is normally up to 135 degrees). It is measured using a Goniometer and is measured by asking the patient to flex the joint to the maximum extent possible and the maximum value was recorded.

[0119] The results showed that the % response of patients taking formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 and 12:1 ratios were significantly better than patients taking raw turmeric powder, essential oil of turmeric with 45% Ar-turmerone, essential oil of turmeric with 10-15% Ar-turmerone, curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 1:10 ratio, curcuminoid with essential oil

of turmeric with 45% Ar-turmerone in 1:1 ratio, curcuminoid 24% with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio, curcuminoid with essential oil of turmeric with 10-15% Ar-turmerone in 10:1 ratio and curcuminoids 95% capsules. At the beginning of study all patients had joint pain and after treatment with formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 12:1 ratio, 19% patients did not have any joint pain. The percentage of patients with moderate joint pain decreased from 80% at baseline to 29% at the end of treatment and majority of the patients (52%) had only mild pain at the end of 3 months of treatment in patients given formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 12:1 ratio. Before the treatment 7% of patients had severe joint pain and after treatment none of the patients given formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 12:1 ratio had severe joint pain.

[0120] At the beginning of study all patients had joint pain and after treatment with formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio, 17% patients did not have any joint pain. The percentage of patients with moderate joint pain decreased from 78% at baseline to 30% at the end of treatment and majority of the patients (53%) had only mild pain at the end of 3 months of treatment in patients given formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio. Before the treatment 7% of patients had severe joint pain and after treatment none of the patients given formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio had severe joint pain.

[0121] Before the treatment 87% of patients given formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 12:1 ratio had joint line tenderness and after treatment 52% of patients no longer had pain and the remaining 48% patients showed improvement and none of the patient's condition worsened or remained same without change.

[0122] Before the treatment 86% of patients given formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio had joint line tenderness and after treatment 50% of patients no longer had pain and the remaining 50% patients showed improvement and none of the patient's condition worsened or remained same without change.

[0123] Before the treatment with formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 12:1 ratio 8% of patients could not walk even up to 100 meters. And after the treatment 75% of patients could walk over 1000 meters and 22% could walk 500-1000 meters.

[0124] Before the treatment with formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio 7% of patients could not walk even up to 100 meters. And after the treatment 72% of patients could walk over 1000 meters and the remaining 28% could walk 500-1000 meters.

[0125] The safety of the test drug was evaluated by measuring vital signs (systolic and diastolic blood pressure, pulse rate, respiratory rate), haemogram measurement (Hb, TC, DC, ESR), liver function tests (SGOT, SGPT, SAP, bilirubin), renal function tests (blood urea, serum creatinine). None of these parameters were adversely modified by the study drugs. There were also no adverse events reported in the study.

[0126] In conclusion, formulation of curcuminoid with essential oil of turmeric with 45% Ar-

turmerone in 10:1 and 12:1 ratios were significantly effective compared to raw turmeric powder, essential oil of turmeric with 45% Ar-turmerone, essential oil of turmeric with 10-15% Ar-turmerone, curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 1:10 ratio, curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 1:1 ratio, curcuminoid 24% with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio, curcuminoid with essential oil of turmeric with 10-15% Ar-turmerone in 10:1 ratio and curcuminoids 95% capsules in relieving symptoms and clinical conditions of osteoarthritic patients when given over a period of 3 months. There was significant improvement in pain scores, walking distance, joint line tenderness, crepitus, range of movement of the knee and joint swelling measurements in osteoarthritic patients receiving formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 and 12:1 ratios for 3 months compared to patients receiving raw turmeric powder, essential oil of turmeric with 45% Ar-turmerone, essential oil of turmeric with 10-15% Ar-turmerone, curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 1:10 ratio, curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 1:1 ratio, curcuminoid 24% with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio, curcuminoid with essential oil of turmeric with 10-15% Ar-turmerone in 10:1 ratio and curcuminoids 95% capsules in the similar dosage. The study drugs were well tolerated and no dose-related toxicity was found.

Example 22

Human Clinical Study in Patients with Alzheimers Disease

[0127] A double-blind, placebo-controlled, pilot clinical trial formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 and 12:1 ratio capsules compared with raw turmeric powder, essential oil of turmeric with 45% Ar-turmerone, essential oil of turmeric with 10-15% Ar-turmerone, curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 1:10 ratio, curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 1:1 ratio, curcuminoid 24% with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio, curcuminoid with essential oil of turmeric with 10-15% Ar-turmerone in 10:1 ratio and curcuminoids 95% capsules was done in patients with progressive decline in memory or cognitive function and diagnosed with probable or possible Alzheimer's disease (AD). Patients were randomized to 10 groups to receive 3.0 grams of each study drug capsules twice daily for 12 months.

[0128] Parameters measured at baseline and end of study include plasma isoprostanes, Vit E, Aβ and clinical assessment with Mini-Mental State Examination Scores (MMSE). Isoprostanes are the products of non-enzymatic oxidation of arachidonic acid and so this, along with the antioxidant Vit E levels is indicative of the level of oxidative stress. Aβ are a 39-43 amino acid peptide fragment derived from the f-amyloid precursor protein (APP) and are the predominant component of the neuritic plaques, an invariant pathological hallmark of AD. Aggregated forms of Aβ are believed to be the real culprits of the disease. Mini-Mental State Examination Scores (MMSE) is a measure of cognitive function. The pharmacokinetics of curcumin from the ingested drugs and adverse events, if any, associated with the drug were also recorded.

[0129] Serum Aβ levels was significantly higher in formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 and 12:1 ratio capsules compared to results following administration of raw turmeric powder, essential oil of turmeric with 45% Ar-turmerone, essential oil of turmeric with 10-15% Ar-turmerone, curcuminoid with essential

oil of turmeric with 45% Ar-turmerone in 1:10 ratio, curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 1:1 ratio, curcuminoid 24% with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio, curcuminoid with essential oil of turmeric with 10-15% Ar-turmerone in 10:1 ratio and curcuminoids 95% capsules, reflecting the increased ability of formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 and 12:1 ratio capsules to disaggregate Aβ deposits in the brain. The MMSE scores of patients given formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio capsules increased significantly from baseline value at 16/30 to 23/30 at the end of the study. The MMSE scores of patients given formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 12:1 ratio capsules increased significantly from baseline value at 17/30 to 25/30 at the end of the study and in patients given raw turmeric powder, essential oil of turmeric with 45% Ar-turmerone, essential oil of turmeric with 10-15% Ar-turmerone, curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 1:10 ratio, curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 1:1 ratio, curcuminoid 24% with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio, curcuminoid with essential oil of turmeric with 10-15% Ar-turmerone in 10:1 ratio and curcuminoids 95% capsules there was a marginal deterioration in the MMSE score (Table 15). Isoprostanes arc products of non-enzymatic oxidation of arachidonic acid and are indicative of oxidative stress. Plasma isoprostane levels were significantly lowered between baseline and at 12 months in patients taking formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 and 12:1 ratios. Vitamin E levels increased in the formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 and 12:1 ratio groups from baseline to end of treatment, (Table 16). The curcumin level in patients taking formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio (baseline at 12 to 653 at the end of treatment period) was 15 times higher than patients taking curcuminoids 95% capsules (baseline at 13 to 42 at the end of treatment) (Table 17). The curcumin level in patients taking formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 12:1 ratio (baseline at 14 to 875 at the end of treatment period) was 20 times higher than patients taking curcuminoids 95% capsules (baseline at 13 to 42 at the end of treatment) (Table 17). In patients taking raw turmeric powder, essential oil of turmeric with 45% Ar-turmerone, essential oil of turmeric with 10-15% Ar-turmerone, curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 1:10 ratio, curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 1:1 ratio, curcuminoid 24% with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio, curcuminoid with essential oil of turmeric with 10-15% Ar-turmerone in 10:1 ratio and curcuminoids 95% capsules there was no decrease noticed in the plasma isoprostane levels and Vitamin E levels remained more or less the same in all the groups except in patients taking formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 and 12:1 ratios.

[0130] This study thus reveals that the formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 and 12:1 ratio capsules confer greater clinical benefits as observed by significant increase in the MMSE score, increase in Vit E levels, high levels of serum Aβ levels, and lowered plasma isoprostane levels in patients consuming formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 and 12:1 ratio capsules compared with patients consuming raw turmeric powder, essential oil of turmeric with 45% Ar-turmerone, essential oil of turmeric with 10-15% Ar-turmerone, curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 1:10 ratio, curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 1:1 ratio, curcuminoid 24% with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio, curcuminoid with essential oil

of turmeric with 10-15% Ar-turmerone in 10:1 ratio and curcuminoid 95% capsules for 12 months.

TABLE 15

MMSE levels of patients in each group over 12 months

Groups	Baseline 0 month	Study End 12 months
Raw turmeric	MMSE 16/30	14/30
EOT with 45% Ar-t	MMSE 15/30	14/30
EOT with 10-15% Ar-t	MMSE 18/30	16/30
C + E with 45% Ar-t in 1:10 ratio	MMSE 16/30	15/30
C + E with 45% Ar-t in 1:1 ratio	MMSE 18/30	17/30
C 24% + E with 45% Ar-t in 10:1 ratio	MMSE 16/30	16/30
C + E with 10-15% Ar-t in 10:1 ratio	MMSE 17/30	17/30
C + E with 45% Ar-t in 10:1 ratio	MMSE 16/30	23/30
Curcuminoids 95%	MMSE 17/30	16/30
C + E with 45% Ar-t in 12:1 ratio	MMSE 17/30	25/30

TABLE 16

Vitamin E levels of patients in each group over 12 months

Groups	Baseline 0 month	Study End 12 months
Raw turmeric	Vit E in mg %	0.4 0.4
EOT with 45% Ar-t	Vit E in mg %	0.3 0.3
EOT with 10-15% Ar-t	Vit E in mg %	0.3 0.3
C + E with 45% Ar-t in 1:10 ratio	Vit E in mg %	0.4 0.4
C + E with 45% Ar-t in 1:1 ratio	Vit E in mg %	0.3 0.3
C 24% + E with 45% Ar-t in 10:1 ratio	Vit E in mg %	0.4 0.4
C + E with 10-15% Ar-t in 10:1 ratio	Vit E in mg %	0.3 0.4
C + E with 45% Ar-t in 10:1 ratio	Vit E in mg %	0.30 2.1
Curcuminoids 95%	Vit E in mg %	0.4 0.4

C + E with 45% Vit E in 0.3 2.8
Ar-t in 12:1 ratio mg %

TABLE 17
Plasma level of curcumin in patients in each group over 12 months
Baseline Study End
Groups 0 month 12 months
Raw turmeric Curcumin in 11 20
nMol/L
EOT with Curcumin in 9 11
45% Ar-t nMol/L
EOT with 10-15% Curcumin in 12 11
Ar-t nMol/L
C + E with 45% Curcumin in 10 13
Ar-t in 1:10 ratio nMol/L
C + E with 45% Curcumin in 21 14
Ar-t in 1:1 ratio nMol/L
C 24% + E with Curcumin in 14 28
45% Ar-t in 10:1 nMol/L
ratio
C + E with 10-15% Curcumin in 15 82
Ar-t in 10:1 nMol/L
ratio
C + E with 45% Curcumin in 12 653
Ar-t in 10:1 ratio nMol/L
Curcuminoids Curcumin in 13 42
95% nMol/L
C + E with 45% Curcumin in 14 875
Ar-t in 12:1 ratio nMol/L

Example 23

Human Clinical Study of Patients with Depression

[0131] In a randomized, double blind, active control, parallel group study, formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 and 12:1 ratios were studied against raw turmeric powder, essential oil of turmeric with 45% Ar-turmerone, essential oil of turmeric with 10-15% Ar-turmerone, curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 1:10 ratio, curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 1:1 ratio, curcuminoid 24% with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio, curcuminoid with essential oil of turmeric with 10-15% Ar-turmerone in 10:1 ratio and curcuminoid 95% capsules in patients with depression to compare the efficacy and tolerability of the eight formulations. Patients with a Score greater than 7 but less than 24 on the 17-item Hamilton Depression (HAM-D) Scale and assessed by Structured Clinical Interview or DSM-IV Axis I Disorders without any concurrent treatment were selected for the study. 50 patients selected were randomized into 10 groups and were given treatment for 8 weeks.

Gr 1: raw turmeric powder 500 mg capsules prepared as described in Example 18 twice daily
Gr 2: essential oil of turmeric with 45% Ar-turmerone (EOT with 45% Ar-t) 500 mg capsules

prepared as described in Example 14 twice daily.
Gr3: essential oil of turmeric with 10-15% Ar-turmerone (EOT with 10-15% Ar-t) 500 mg capsules prepared as described in Example 15 twice daily
Gr4: curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 1:10 ratio (C+E with 45% Ar-t in 1:10 ratio), 500 mg capsules prepared as described in Example 11 twice daily
Gr5: curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 1:1 ratio (C+E with 45% Ar-t in 1:1 ratio), 500 mg capsules prepared as described in Example 12 twice daily.
Gr6: Subjects receiving curcuminoid 24% with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio (C 24%+E with 45% Ar-t in 10:1 ratio), 500 mg capsules prepared as described in Example 17 twice daily.
Gr7: curcuminoid with essential oil of turmeric with 10-15% Ar-turmerone in 10:1 ratio (C+E with 10-15% Ar-t in 10:1 ratio), 500 mg capsules prepared as described in Example 13 twice daily.
Gr 8: curcuminoids 95% (500 mg) capsules prepared as described in example 16 twice daily.
Gr 9: Formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio (C+E with 45% Ar-t in 10:1 ratio) (500 mg) capsules prepared as described in Example 10 twice daily.
Gr 10: Formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 12:1 ratio (C+E with 45% Ar-t in 12:1 ratio) (500 mg) capsules prepared as described in Example 19 twice daily.

[0132] Efficacy was evaluated by using 17 point—Hamilton depression scale and clinical global impression by Global Severity (CGI-S) and Global change (CGI-I) scales. Tolerability of the drugs was assessed clinically and by biochemical parameters like SGOT, SGPT, Urea and Creatinine (measured at the start and at the end of study).

[0133] Results: The proportion of responders as measured by the HAM-D17 scale was significantly (97%) higher in the formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 12:1 ratio group than other groups (Table: 18). The proportion of responders as measured by the HAM-D17 scale was significantly (93%) higher in the formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio group than other groups (Table: 18). The change in HAM-D17 scores at the end of 8 weeks from baseline at 20 to 7 at the end of treatment was higher for formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 12:1 ratio group (65%) than other groups (Table: 19). The change in HAM-D17 scores at the end of 8 weeks from baseline at 21 to 10 at the end of treatment was also higher for formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio group (52%) than other groups (Table: 19). In Clinical Global Impression assessment scale, the formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 12:1 ratio group showed a decrease in CGI-S score from baseline at 4 to 1 at the end of treatment. That is 75% improvement in CGI-S (Table: 20). In Clinical Global Impression assessment scale, the formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio group showed a decrease in CGI-S score from baseline at 5 to 2 at the end of treatment. That is 60% improvement in CGI-S (Table: 20). Formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 12:1 ratio group showed a decrease in CGI-I score from baseline 5 to 2 at the end of treatment. That is 60% improvement in CGI-I scale (Table: 21). Formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio group showed a decrease in CGI-I score from baseline 4 to 2 at the end of

treatment. That is 50% improvement in CGI-I scale (Table: 21). Whereas the other groups showed no change at all at the end of 8 weeks of treatment. Overall the study medications were well tolerated and there was no significant difference in vital signs, physical examination, laboratory tests and electrocardiogram from baseline and had 'excellent' tolerability.

TABLE 18

Proportion of responders in each group over 2 months

Raw turmeric HAM-D17 scale	% response rate on 6%
EOT with 45% Ar-t HAM-D17 scale	% response rate on 4%
EOT with 10-15% Ar-t HAM-D17 scale	% response rate on 4%
c + E with 45% Ar-t in 1:10 ratio HAM-D17 scale	% response rate on 7%
C + E with 45% Ar-t in 1:1 ratio HAM-D17 scale	% response rate on 9%
C 24% + E with 45% Ar-t in 10:1 ratio HAM-D17 scale	% response rate on 8%
C + E with 10-15% Ar-t in 10:1 ratio HAM-D17 scale	% response rate on 12%
C + E with 45% Ar-t in 10:1 ratio HAM-D17 scale	% response rate on 93%
Curcuminoids 95% HAM-D17 scale	% response rate on 10%
C + E with 45% Ar-t in 12:1 ratio HAM-D17 scale	% response rate on 97%

TABLE 19

Hamilton Depression Scoring Scale - 17 point scale in patients in each group over 2 months

Groups	Baseline 0 month	Study End 2 months
Raw turmeric HAM-D17 scale	20	19
EOT with 45% Ar-t HAM-D17 scale	19	19
EOT with 10-15% Ar-t HAM-D17 scale	22	22
C + E with 45% Ar-t in 1:10 ratio HAM-D17 scale	18	18
C + E with 45% Ar-t in 1:1 ratio HAM-D17 scale	20	19
C 24% + E with 45% Ar-t in 10:1 ratio HAM-D17 scale	19	19
C + E with 10-15% Ar-t in 10:1 ratio HAM-D17 scale	19	16
C + E with 45% Ar-t in 12:1 ratio HAM-D17 scale	21	10

t in 10:1 ratio scale
Curcuminoids 95% HAM-D17 19 17
scale
C + E with 45% Ar- HAM-D17 20 7
t in 12:1 ratio scale

TABLE 20
Clinical Global Impression - Severity Scale in patients in each group over 2 months
Baseline Study End
Groups 0 month 12 months
Raw turmeric CGI-S score 5 5
EOT with 45% Ar-t CGI-S score 4 4
EOT with 10-15% Ar-t CGI-S score 4 4
C + E with 45% Ar-t in CGI-S score 5 5
1:10 ratio
C + E with 45% Ar-t in CGI-S score 4 4
1:1 ratio
C 24% + E with 45% CGI-S score 4 4
Ar-t in 10:1 ratio
C + E with 10-15% Ar- CGI-S score 5 5
t in 10:1 ratio
C + E with 45% Ar-t in CGI-S score 5 2
10:1 ratio
Curcuminoids 95% CGI-S score 5 5
C + E with 45% Ar-t in CGI-S score 4 1
12:1 ratio

TABLE 21

Clinical Global Impression - Improvement/Change Scale in patients in each group over 2 months
Baseline Study End
Groups 0 month 12 months
Raw turmeric CGI-I score 4 4
EOT with 45% Ar-t CGI-I score 4 4
EOT with 10-15% Ar-t CGI-I score 4 4
C + E with 45% Ar-t in CGI-I score 5 5\
1:10 ratio
C + E with 45% Ar-t in CGI-I score 4 4
1:1 ratio
C 24% + E with 45% CGI-I score 4 4
Ar-t in 10:1 ratio
C + E with 10-15% Ar-t CGI-I score 5 5
in 10:1 ratio
C + E with 45% Ar-t in CGI-I score 4 2
10:1 ratio
Curcuminoids 95% CGI-I score 4
C + E with 45% Ar-t in CGI-I score 5 2
12:1 ratio

[0134] Other modifications and variations to the invention will be apparent to those skilled in the art from the foregoing disclosure and teachings. Thus, while only certain embodiments of the invention have been specifically described herein, it will be apparent that numerous modifications may be made thereto without departing from the spirit and scope of the invention.

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US8859020
Treatment of alzheimer's with a curcuminoid mixture and essential oil of turmeric having 45% Ar-turmerone

Disclosure provides a formulation of curcuminoid with essential oil of turmeric to enhance the bioavailability of curcumin and to augment the biological activity of curcumin, wherein curcumin is the main constituent of curcuminoid and wherein Ar-turmerone is the main constituent of the essential oil of turmeric. An application of curcuminoid with essential oil of turmeric to enhance the bioavailability of curcumin for oral supplementation against a variety of diseases and method of doing the same is provided.

US2008312333
Agent for Preventing/Ameliorating Life Style-Related Diseases Containing Turmeric Essential Oil Component

The present invention has its object to provide an agent for preventing and/or ameliorating life style-related diseases which contains, as an active ingredient, a substance derived from a safe food material having a long history of being eaten as a food and which is capable of being utilized as functional foods such as health foods or functional health foods (specific health foods, functional nutritive foods). The agent for preventing and/or ameliorating life style-related diseases according to the invention contains, as an active ingredient, at least one compound selected from the group consisting of ar-turmerone, alpha-turmerone, beta-turmerone, curlone, bisacumol and beta-sesquiphellandrene, or at least one compound selected from among the bisabolane type sesquiterpenoids derived from Curcuma longa L., and therefore is useful for preventing and/or ameliorating diabetes, visceral fat obesity, metabolic syndrome and obesity, among others.

TECHNICAL FIELD

[0001] The present invention relates to an agent for preventing and/or ameliorating life style-related diseases which contains, as an active ingredient, an essential oil component derived from a plant material of the genus Curcuma origin, and a functional food containing the same.

BACKGROUND ART

[0002] Life style-related diseases resulting from changes for the worse in life style, such as excessive nutrition and lack of exercise, are now a great social problem. Among such life style-related diseases, there may be mentioned obesity, diabetes, hyperlipidemia and hypertension, among others. A plurality of such morbid states may develop in combination and such combination is also termed metabolic syndrome, obesity, syndrome X, deadly quartet, insulin resistance syndrome or visceral fat syndrome, among others. The onset of metabolic syndrome is said to be based on insulin resistance and, further, it is said that there is the accumulation of visceral fat as a further upstream cause. Therefore, it is considered that such life style-related diseases as mentioned above may be prevented and/or alleviated by preventing and/or ameliorating the accumulation of visceral fat or the insulin resistance.

[0003] The peroxisome proliferator-activated receptor (PPAR) is a transcriptional regulatory factor serving to control the expression of a group of genes for maintaining the metabolism of sugars and lipids; it is a ligand-dependent transcriptional regulatory factor belonging to the nuclear receptor family. The PPAR includes three subtypes, namely PPAR[alpha], PPAR[gamma] and PPAR[delta]. Among them, PPAR[gamma] is expressed in adipose tissues and is a master regulator controlling the differentiation and maturation of adipocytes. Such thiazolidine derivatives as troglitazone, pioglitazone and rosiglitazone developed as antidiabetics and agents for alleviating insulin resistance (insulin sensitizers) are PPAR[gamma] ligands activating PPAR[gamma] and showing a hypoglycemic activity and an insulin resistance-alleviating activity. It has been confirmed that these agents clinically reduce the visceral fat level; they are known to be effective not only against diabetes but also against life style-related diseases, typically metabolic syndrome.

[0004] Turmeric (Curcuma longa L.) is a perennial herb of the family Zingiberaceae, genus Zingiber and is generally known as turmeric, one of curry spices; it is used not only for food but also a coloring agent for food, clothing, etc. It is also used medicinally in Chinese medicine and in such traditional medicine as Ayurveda in India or Jamu in Indonesia owing to its hemostatic, stomachic, antibacterial and anti-inflammatory activities.

[0005] It is known that the main components of turmeric are yellow coloring matters curcuminoids, namely curcumin and its derivatives demethoxycurcumin and bisdemethoxycurcumin. While various physiological effects are known as the effects of turmeric or turmeric extracts, as mentioned above, most of the effects coincide with the physiological effects of the curcuminoids, in particular curcumin and, therefore, it is believed that the curcuminoids are principal active ingredients.

[0006] It is also known that turmeric contains essential oil components as well and most of them are bisabolane type sesquiterpenoids, for example ar-turmerone, [alpha]-turmerone and [beta]-turmerone. Known physiological activities of turmeric essential oil component include mosquitocidal activity (cf. Non-Patent Document 1), apoptosis-inducing activity (cf. Non-Patent Document 2), prostaglandin and nitrogen oxide production-inhibiting activity (cf. Non-Patent Document 3 and 4) and liver function-improving activity.

[0007] On the other hand, among the plants of the family Zingiberaceae, genus Zingiber (Curcuma sp.), there are not only turmeric (autumn turmeric: Curcuma long L.) but also such varieties as wild turmeric (spring turmeric: Curcuma aromatica Salisb.), zedoary (purple turmeric: Curcuma zedoaria Rosc.) and xanthorrza (Curcuma xanthorrhiza Roxb.). These are herbs belonging to the same genus but differ in components contained therein. Thus, turmeric and xanthorrza are rich in the curcuminoids, typically curcumin, and all the species contain essential oil components but the compounds contained therein differ from species to species and are characteristic.

[0008] It has been disclosed that curcumenone (cf. Patent Document 1) and (4S,5S)-(+)-germacrone-4,5-epoxide (cf. Patent Document 2) contained in wild turmeric (spring turmeric: Curcuma aromatica Salisb.) have glucose tolerance improving activity and are useful as antidiabetics. Further, it has been disclosed that [alpha]-curcumene, a bisabolane type sesquiterpenoid contained in xanthorrza (Curcuma xanthorrhiza Roxb.), has serum triglyceride lowering activity and is useful as a lipid metabolism improving agent (cf. Patent Document 3). However, it is not known as yet that turmeric (Curcuma longa L.)-derived essential oil components, in particular the bisabolane type sesquiterpenoids ar-turmerone,

[alpha]-turmerone and [beta]-turmerone, have blood sugar lowering activity or blood sugar increase inhibiting activity, and visceral fat reducing activity.

[0009] Non-Patent Document 1: Roth, G. N., et al., J. Nat. Prod., 61, 542-545, 1998

[0010] Non-Patent Document 2: Aratanechemuge, Y., et al., Int. J. Mol. Med., 9, 481-484, 2002

[0011] Non-Patent Document 3: Hong, C. H., et al., Planta Med., 68, 545-547, 2002

[0012] Non-Patent Document 4: Lee, S. K. et al., J. Environ. Pathol. Toxicol. Oncol., 21, 141-148, 2002

[0013] Patent Document 1: Japanese Kokai Publication Hei-01-233217

[0014] Patent Document 2: Japanese Kokai Publication Hei-06-192086

[0015] Patent Document 3: Japanese Kokai Publication Hei-07-149628

SUMMARY OF THE INVENTION

[0016] It is an object of the present invention to provide an agent for preventing and/or ameliorating life style-related diseases which contains, as an active ingredient, a substance derived from a safe food material having a long history of being eaten as a food and which is capable of being utilized as functional foods such as health foods or functional health foods (specific health foods, functional nutritive foods).

[0017] The present inventors made intensive investigations to accomplish the above object and, as a result, found that a compound selected from among ar-turmerone, [alpha]-turmerone, [beta]-turmerone, curlone, bisacumol and [beta]-sesquiphellandrene, and the bisabolane type sesquiterpenoids derived from Curcuma longa L. has a blood sugar increase inhibiting activity and visceral fat reducing activity in an obesity-accompanied type II diabetes model and has PPAR[gamma] ligand activity.

[0018] The present invention has now been completed based on the above finding.

[0019] Thus, in a first aspect, the invention relates to

[0020] an agent for preventing and/or ameliorating life style-related diseases

[0021] which contains, as an active ingredient, at least one compound selected from the group consisting of ar-turmerone, [alpha]-turmerone, [beta]-turmerone, curlone, bisacumol, and [beta]-sesquiphellandrene.

[0022] The above-mentioned compound is preferably obtained from an essential oil component derived from a plant material of the genus Curcuma origin. In a second aspect, the invention relates to

[0023] an agent for preventing and/or ameliorating life style-related diseases

[0024] which contains, as an active ingredient, at least one compound selected from among the bisabolane type sesquiterpenoids derived from Curcuma longa L.

[0025] The life style-related disease so referred to with respect to the first aspect and second aspect of the invention includes diabetes, visceral fat obesity, metabolic syndrome and obesity, among others.

[0026] In a third aspect, the invention relates to

[0027] a PPAR ligand agent

[0028] which contains, as an active ingredient, at least one compound selected from the group consisting of ar-turmerone, [alpha]-turmerone, [beta]-turmerone, curlone, bisacumol, and [beta]-sesquiphellandrene.

[0029] The above-mentioned compound is preferably obtained from an essential oil component derived from a plant material of the genus Curcuma origin. In a fourth aspect, the invention relates to

[0030] a peroxisome proliferator-activated receptor ligand agent

[0031] which contains, as an active ingredient, at least one compound selected from among the bisabolane type sesquiterpenoids derived from Curcuma longa L.

[0032] The PPAR so referred to herein is PPAR[gamma], for example.

DETAILED DESCRIPTION OF THE INVENTION

[0033] In the following, the embodiments of the present invention will be described in detail.

[0034] The agent for preventing and/or ameliorating life style-related diseases according to the first aspect of the invention contains, as an active ingredient, at least one compound selected from the group consisting of ar-turmerone, [alpha]-turmerone, [beta]-turmerone, curlone, bisacumol, and [beta]-sesquiphellandrene. The agent for preventing and/or ameliorating life style-related diseases according to the second aspect of the invention contains, as an active ingredient, at least one compound selected from among the bisabolane type sesquiterpenoids derived from Curcuma longa L. The life style-related disease so referred herein includes diabetes, visceral fat obesity, metabolic syndrome and obesity, among others. The compound selected from among ar-turmerone, [alpha]-turmerone, [beta]-turmerone, curlone, bisacumol and [beta]-sesquiphellandrene, and the bisabolane type sesquiterpenoids derived from Curcuma longa L. has a blood sugar lowering activity, blood sugar increase inhibiting activity, and visceral fat reducing activity, and therefore is useful for preventing and/or ameliorating diabetes, and/or for preventing and/or ameliorating visceral fat obesity. Accordingly, the compound mentioned above is also useful for preventing and/or ameliorating such a life style-related disease as metabolic syndrome comprising two or more of diabetes (in particular type II diabetes), obesity (in particular visceral fat obesity), hyperlipidemia and hypertension, among others, or obesity.

[0035] The PPAR ligand agent, in particular the PPAR[gamma] ligand agent, according to the third aspect of the invention contains, as an active ingredient, at least one compound selected

from the group consisting of ar-turmerone, [alpha]-turmerone, [beta]-turmerone, curlone, bisacumol, and [beta]-sesquiphellandrene. The PPAR ligand agent, in particular the PPAR[gamma] ligand agent, according to the fourth aspect of the invention contains, as an active ingredient, at least one compound selected from among the bisabolane type sesquiterpenoids derived from Curcuma longa L. The compound selected from among ar-turmerone, [alpha]-turmerone, [beta]-turmerone, curlone, bisacumol and [beta]-sesquiphellandrene, and the bisabolane type sesquiterpenoids derived from Curcuma longa L. activates PPAR[gamma] by binding to PPAR ligand-binding region, in particular PPAR[gamma] ligand-binding region, and therefore is useful for alleviating insulin resistance, and for preventing and/or ameliorating such a life style-related disease as metabolic syndrome comprising two or more of diabetes (in particular type II diabetes), obesity (in particular visceral fat obesity), hyperlipidemia and hypertension, among others, or obesity.

[0036] Turmeric is a safe food material having a long history of being eaten as a food. Twenty or more compounds are known as essential oil components derived from a plant material of the genus Curcuma origin. The ar-turmerone, [alpha]-turmerone, [beta]-turmerone, curlone, bisacumol, and [beta]-sesquiphellandrene, which are to be used in the present invention, are known as species of the bisabolane type sesquiterpenoids, which are essential oil components derived from turmeric (autumn turmeric: Curcuma long L.). The ar-turmerone, [alpha]-turmerone, [beta]-turmerone, curlone, bisacumol, and [beta]-sesquiphellandrene, which are to be used in the present invention, may be obtained from essential oil components derived from a plant material of the genus Curcuma origin, or chemically synthesized as long as they conform to food or food additive manufacturing standards, among others. Those obtained from an essential oil component derived from a plant material of the genus Curcuma origin are preferred. These 6 compounds may be used each as a single compound in the practice of the invention, or a mixture of two or more of them may also be used in the practice of the invention. As the compound mentioned above, ar-turmerone, [alpha]-turmerone and [beta]-turmerone are preferred, and ar-turmerone is more preferred.

[0037] As the compound selected from among the bisabolane type sesquiterpenoids derived from Curcuma longa L., there may be mentioned, for example, ar-turmerone, [alpha]-turmerone and [beta]-turmerone mentioned above, in addition curlone, bisacumol, and [beta]-sesquiphellandrene. As the compound selected from among the bisabolane type sesquiterpenoids derived from Curcuma longa L., ar-turmerone, [alpha]-turmerone and [beta]-turmerone are preferred, and ar-turmerone is more preferred.

[0038] The method for preparing the above-mentioned compounds to be used in the practice of the invention is not particularly restricted but any of those methods known in the art can be used. For example, an essential oil component can be obtained directly from a plant material of the genus Curcuma origin by such a method as solvent extraction using a hydrophobic solvent such as hexane, supercritical carbon dioxide extraction, or steam distillation. An essential oil component can also be obtained as a sesquiterpenoid fraction by subjecting a curcuma extract (extract, oleoresin) obtained by extraction with a solvent such as ethanol to column chromatography using silica gel or a resin for purification. The thus-obtained essential oil component derived from a plant material of the genus Curcuma origin generally contains about 50 to 60% by weight of a sum total of the bisabolane type sesquiterpenoids ar-turmerone, [alpha]-turmerone, [beta]-turmerone, curlone, bisacumol, [beta]-sesquiphellandrene. In addition, the method for obtaining such bisabolane type

sesquiterpenoid compounds as ar-turmerone, [alpha]-turmerone and [beta]-turmerone from the essential oil component derived from a plant material of the genus Curcuma origin is not particularly restricted but may comprise column chromatography using silica gel or a resin for purification, by which the compounds can be separated as a mixture or can be purified as respective single compounds.

[0039] The content of the compound mentioned above in the agent for preventing and/or ameliorating life style-related diseases according to the invention may be properly selected depending on the intended application but is preferably about 1 to 100% by weight and more preferably about 10 to 90% by weight. The agent for preventing and/or ameliorating life style-related diseases according to the invention may contain other ingredients for the purpose of improving the nutrition, taste, odor, flavor, property, etc. thereof.

[0040] When the an agent for preventing and/or ameliorating life style-related diseases according to the invention is taken for the above-mentioned compound(s) as an active ingredient(s) to produce the effects thereof, the total amount of the compound(s) per day per adult is desirably such that preferably about 0.1 to 1,000 mg/kg body weight, more preferably about 1 to 100 mg/kg body weight, be taken continuously.

[0041] The agent for preventing and/or ameliorating life style-related diseases of the invention can be utilized as or in functional foods such as health foods or functional health foods (specific health foods, functional nutritive foods). Such foods are not restricted in shape or form but the above agent can be utilized in supplement forms such as capsules and tablets; drink forms such as refreshing drinks and health drinks; or food forms such as processed foods and nutrient-adjusted foods. Such functional foods containing the agent for preventing and/or ameliorating life style-related diseases mentioned above also constitute an aspect of the present invention.

EFFECT OF THE INVENTION

[0042] According to the present invention, an agent for preventing and/or ameliorating life style-related diseases, which can be utilized as or in functional foods such as health foods or functional health foods (specific health foods, functional nutritive foods), may be provided. The agent for preventing and/or ameliorating life style-related diseases according to the invention contains, as an active ingredient, at least one compound selected from the group consisting of ar-turmerone, [alpha]-turmerone, [beta]-turmerone, curlone, bisacumol, and [beta]-sesquiphellandrene, or at least one compound selected from among the bisabolane type sesquiterpenoids derived from Curcuma longa L. The compound has a blood sugar increase inhibiting activity and visceral fat reducing activity, and therefore is useful for preventing and/or ameliorating diabetes, visceral fat obesity, metabolic syndrome and obesity, among others.

BEST MODE FOR CARRYING OUT THE INVENTION

[0043] In the following, the present invention is described further in details by means of examples. However, these examples are no limitative of the present invention.

Example 1

[0044] A turmeric powder (Kaneka Sun Spice Co., Ltd.; 700 g) was immersed in 3.5 liters of

n-hexane, allowed to stand in the dark at room temperature for 3 days and then filtered to give a primary extract. The residue after filtration was immersed in 3.5 liters of n-hexane, allowed to stand in the dark at room temperature for 1 day and then filtered to give a secondary extract. The primary extract and secondary extract were combined and concentrated under reduced pressure to give 50.4 g of a hexane extract of turmeric.

[0045] As a result of silica gel thin-layer chromatography (TLC), it was confirmed that the hexane extract of turmeric contained essential oil components but did not contain any curcuminoids. The TLC was carried out using Silica Gel 60F254 (Merck Ltd.) plates, with a 9:1 (v/v) chloroform-methanol mixture as a developing solvent.

Example 2

[0046] The blood sugar increase inhibiting effect of the hexane extract of turmeric as prepared in Example 1 was evaluated using KK-A<[gamma]> mice known as type II diabetes model animals.

[0047] KK-A A<[gamma]> mice (females, 6 weeks of age) were divided into two groups (5 animals per group), which were used as a control group and a hexane extract-dosed group. The control group was given a purified powder feed (Oriental Yeast Co.), and the hexane extract-dosed group was given the purified powder feed supplemented with 0.5% by weight of the hexane extract of turmeric as prepared in Example 1. The purified powder feed had the following composition: 20% by weight of casein, 49.948% by weight of corn starch, 10% by weight of sucrose, 10% by weight of soybean oil, 5% by weight of cellulose powder, 3.5% by weight of AIN-93 mineral mix, 1% by weight of AIN-93 vitamin mix, 0.25% by weight of choline bitartrate, 0.002% by weight of tert-butylhydroquinone and 0.3% by weight of L-cystine.

[0048] Small blood samples were collected from the mice via the caudal vein at the start of feeding and at 2 weeks and 4 weeks later. Each blood sample was measured for blood sugar using a Glutest Ace portable blood sugar meter (SANWA KAGAKU KENKYUSHO CO., LTD.). The results are shown in Table 1.

TABLE 1
Blood sugar level (mg/dl, mean ± standard error, n = 5)

	Control group	Hexane extract group
Initial	177 ± 15	193 ± 13
After 2 weeks	360 ± 36	262 ± 17 ($P < 0.05$)
After 4 weeks	393 ± 18	299 ± 46 ($P < 0.1$)

[0049] In the control group, the blood sugar levels after 2 weeks and 4 weeks were higher as compared with the time of start of feeding, whereby it was confirmed that the animals became hyperglycemic. On the other hand, the sugar levels in the hexane extract-dosed group after 2 weeks and 4 weeks were clearly lower than those in the control group; thus, a blood sugar increase inhibiting effect was observed.

Example 3

[0050] A turmeric oleoresin (Maruzen Pharmaceuticals Co., Ltd.; 30 g) was subjected to silica gel column chromatography, followed by elution with 10% (by volume) ethyl acetate/n-

hexane. The eluate was concentrated to dryness to give 13.5 g of a sesquiterpenoid fraction of turmeric.

[0051] As a result of high-performance liquid chromatography (HPLC), it was confirmed that the sesquiterpenoid fraction of turmeric contained such bisabolane type sesquiterpenoids as ar-turmerone, [alpha]-turmerone and [beta]-turmerone but did not contain any curcuminoids. The HPLC was carried out at 30[deg.] C. using a TSKgel ODS-80Ts column (4.6*75 mm) (Tosoh Corporation) and an acetonitrile (A)-distilled water (B) system as the mobile phase under gradient conditions such that the concentration of A was increased from 45% to 70% at a constant rate from minute 0 to minute 15 and then maintained at 70% from minute 15 to minute 30. The flow rate was 0.7 ml/minute, the injection size was 20 [mu]l, and the detection wavelength was 254 nm.

Example 4

[0052] The blood sugar increase inhibiting effect and visceral fat reducing effect of the sesquiterpenoid fraction of turmeric as prepared in Example 3 was evaluated using KK-A<[gamma]> mice known as type II diabetes model animals.

[0053] KK-A<[gamma]> mice (females, 6 weeks of age) were divided into two groups (6 animals per group), which were used as a control group and a sesquiterpenoid fraction-dosed group. The control group was given a high-fat powder feed (Oriental Yeast Co.), and the sesquiterpenoid fraction-dosed group was given the high-fat powder feed supplemented with 0.24% by weight of the sesquiterpenoid fraction as prepared in Example 3. The high-fat powder feed had the following composition: 25% by weight of casein, 14.869% by weight of cornstarch, 20% by weight of sucrose, 2% by weight of soybean oil, 14% by weight of lard, 14% by weight of tallow, 5% by weight of cellulose powder, 3.5% by weight of AIN-93 mineral mix, 1% by weight of AIN-93 vitamin mix, 0.25% by weight of choline bitartrate, 0.006% by weight of tert-butylhydroquinone and 0.375% by weight of L-cystine.

[0054] Small blood samples were collected from the mice via the caudal vein at the start of feeding and at 2 weeks and 4 weeks later. Each blood sample was measured for blood sugar using a Glutest Ace portable blood sugar meter (SANWAKAGAKUKENKYUSHO CO., LTD.). The results are shown in Table 2. After 5 weeks of feeding, the perirenal fat and the mesenteric fat within the abdominal cavity were excised from each mouse by anatomy and weighed. The results are shown in Table 3.

TABLE 2

Blood sugar level (mg/dl, mean ± standard error, n = 6)

	Control group	Sesquiterpenoid fraction group
Initial	155 ± 7	156 ± 6
After 2 weeks	389 ± 27	309 ± 16 ($P < 0.05$)
After 4 weeks	465 ± 22	334 ± 32 ($P < 0.01$)

TABLE 3

Weight of fat (g, mean ± standard error, n = 6)

	Control group	Sesquiterpenoid fraction group
Perirenal fat	2.43 ± 0.07	1.94 ± 0.16 ($P < 0.05$)
Mesenteric fat	1.90 ± 0.05	1.63 ± 0.02 ($P < 0.01$)

[0055] In the control group, the blood sugar levels after 2 weeks and 4 weeks were higher as compared with the time of start of feeding, whereby it was confirmed that the animals became hyperglycemic. On the other hand, the sugar levels in the sesquiterpenoid fraction-dosed group after 2 weeks and 4 weeks were clearly lower than those in the control group; thus, a blood sugar increase inhibiting effect was observed.

[0056] The weights of the perirenal fat and the mesenteric fat in the sesquiterpenoid fraction-dosed group were clearly lower than in the control group; thus, a visceral fat reducing effect was observed.

Example 5

[0057] A 3-g portion of the sesquiterpenoid fraction of turmeric prepared in Example 3 was subjected to ODS column chromatography, followed by elution with 65% (by volume) acetonitrile, whereby 0.7 g of ar-turmerone was isolated and purified. That the isolated and purified compound was ar-turmerone was confirmed by structural analysis by <1> H-NMR and <13> C-NMR.

Example 6

[0058] The hexane extract of turmeric as prepared in Example 1, the sesquiterpenoid fraction of turmeric as prepared in Example 3 and the ar-turmerone prepared in Example 5 were measured for PPAR[gamma] ligand activity levels.

[0059] CV-1 cells (male African green monkey kidney-derived cultured cells) were seeded onto a 96-well culture plate (6*10<3 > cells/well) and cultured under conditions of 37[deg.] C. and 5% CO2 for 24 hours. The medium used was DMEM (Dulbecco's modified Eagle medium; GIBCO) supplemented with 10% FBS (fetal bovine serum), 10 ml/L of a solution of penicillin and streptomycin (5,000 IU/ml and 5,000 [mu]g/ml, respectively; GIBCO) and 37 mg/L of ascorbic acid (Wako Pure Chemical Industries, Ltd.). Cells were washed with OPTI-MEM (GIBCO), a serum-free medium for transfection, and then transfected with two plasmids, namely pM-PPAR[gamma] and 4xUASg-luc, using Lipofectamine Plus (Invitrogen Corporation), a gene transfer reagent. pM-PPAR[gamma] is a chimera protein expression plasmid resulting from joining of the yeast-derived transcription factor GAL4 gene (amino acid sequence 1-147) and the PPAR[gamma] ligand binding site gene (amino acid sequence 204-505), and 4xUASg-luc is a reporter plasmid with 4 repetitions of a GAL4 responsive element (UASg) as inserted upstream of the luciferase gene. At about 24 hours after transfection, the medium was replaced with a medium containing the sample (hexane extract of Example 1, sesquiterpenoid fraction of Example 3 or ar-turmerone of Example 5) (4 wells), followed by 24 hours of cultivation. Each sample was dissolved in dimethyl sulfoxide (DMSO) and the solution, or DMSO used in a no treatment control group, was added to the medium to each concentration given in Table 4. Cells were washed with phosphate-buffered saline (PBS+) containing Ca and Mg, then luclite (PerkinElmer), a luciferase chemiluminescence reagent, was added, and the luciferase-due chemiluminescence intensity was measured using a TopCount microplate scintillation/luminescence counter (PerkinElmer).

[0060] For each sample, the mean of luminescence intensities (4 wells) was determined, the ratio thereof to the value for the no treatment control was calculated and the relative activity was reported as the PPAR[gamma] ligand activity of the sample. The results obtained by

carrying out the experiment in triplicate are shown in Table 4.

TABLE 4
Addition PPAR[gamma] ligand activity
level (mean ± standard error, n = 3)
No treatment control (DMSO) (0.1%) 1.00
Positive control 0.5 [mu]M 2.10 ± 0.31
troglitazone 1 [mu]M 3.33 ± 0.73
Hexane extract 5 [mu]g/ml 1.81 ± 0.13
10 [mu]g/ml 2.14 ± 0.52
Sesquiterpenoid fraction 2.5 [mu]g/ml 1.79 ± 0.54
5 [mu]g/ml 2.30 ± 0.84
10 [mu]g/ml 2.49 ± 0.55
ar-turmerone 2 [mu]g/ml 1.51 ± 0.14
5 [mu]g/ml 2.33 ± 0.59

[0061] When troglitazone, a PPAR[gamma] ligand, was used as a positive control, a concentration-dependent PPAR[gamma] ligand activity was confirmed. Similarly, the hexane extract of turmeric, the sesquiterpenoid fraction and the ar-turmerone were found to have PPAR[gamma] ligand activity.

Example 7

[0062] Using the sesquiterpenoid fraction of turmeric similarly prepared as in Example 3, a soft capsule was prepared by the common method according to the following composition.

Sesquiterpenoid fraction 40 parts by weight
Olive oil 60 parts by weight
Vitamin E 1 part by weight

INDUSTRIAL APPLICABILITY

[0063] According to the present invention, an agent for preventing and/or ameliorating life style-related diseases, which can be utilized as or in functional foods such as health foods or functional health foods (specific health foods, functional nutritive foods), may be provided. The agent for preventing and/or ameliorating life style-related diseases according to the invention contains, as an active ingredient, at least one compound selected from the group consisting of ar-turmerone, [alpha]-turmerone, [beta]-turmerone, curlone, bisacumol, and [beta]-sesquiphellandrene, or at least one compound selected from among the bisabolane type sesquiterpenoids derived from Curcuma longa L. The compound has a blood sugar increase inhibiting activity and visceral fat reducing activity, and therefore is useful for preventing and/or ameliorating diabetes, visceral fat obesity, metabolic syndrome and obesity, among others.

KR20020081003
COMPOSITION CONTAINING TURMERONE AND USE THEREOF

PURPOSE: A composition containing an effective amount of ar-turmerone inhibiting COX-

2(cyclooxygenase-2) and iNOS(inducible nitric oxide synthase) activity is provided. Therefore, the ar-turmerone separated from Zedoariae Rhizoma can reduce or alleviate inflammation and suppress cancer. CONSTITUTION: A Zedoariae Rhizoma extract containing ar-turmerone of the formula 1 is obtained by extracting in water or alcohol and then subjected to silica gel column chromatography with a mixture of chloroform and methanol. For example, 600g Zedoariae Rhizoma is extracted in 100% methanol three times every 3hr, concentrated under reduced pressure to produce 28.55g extract, which is dispersed in distilled water, extracted in methylene chloride, ethylacetate and n-butanol.

US2007148263
A COMPOSITION TO ENHANCE THE BIOAVAILABILITY OF CURCUMIN

TECHNICAL FIELD

This invention relates to a composition of curcumin with the essential oil of turmeric, with Ar-turmerol as the main constituent, to enhance the bioavailability of curcumin and to augment the biological activity of curcumin. Such enhanced bioavailability has been demonstrated in human volunteers.

Curcumin [1 ,7-bis (4-hydroxy-3-methoxyphenyl)-1 ,6-heptadiene-3,5-dione is a major yellow pigment of turmeric, a commonly used spice, derived from the rhizome of the herb Curcuma longa Linn. In the Indian subcontinent and Southeast Asia, turmeric has traditionally been used as a treatment for inflammation, skin wounds, and tumors. Clinical activity of curcumin is yet to be confirmed; however, in preclinical animal models, curcumin has shown cancer chemopreventive, antineoplastic and antiinflammatory properties (for a review, see, Kelloff, G.I., et al, J. Cell Biochem., 1996, 265:54-71). Especially interesting is its ability to prevent the formation of carcinogen-induced intestinal premalignant lesions and malignancies in rats (Rao, CV. et al, Cancer Res., 1995, 55:259-66; Kawamori, T. et al, Cancer Res., 1999, 59:597-601), and in the multiple neoplasia (Min/+) mouse (Mahmood, N.N. et al, Carcinogenesis, 2000, 31 :921-27), a genetic model of the human disease familial adenomatous polyposis. Curcumin acts as a scavenger of oxygen species such as hydroxyl radical, superoxide anion and singlet oxygen (Subramanian, M. et al, Mutat Res., 1994, 311 :249-55; Tonnesen, H. H. et al, int. J. Pharm., 1992, 87:79-87; Reddy, A.C.P. et al, MoI. Cell Biochem., 1994, 137:1-8) and interferes with lipid peroxidation (Donatus, I.A., Biochem. Pharmacol., 1990, 39:1869-75; Sharma, S. C. et al, Biochem. Pharmacol., 1972, 21 :1210-14). Curcumin suppresses a number of key elements in cellular signal induction pathways pertinent to growth, differentiation and malignant transformations. Among signalling events inhibited by curcumin are protein kinases (Liu, J.V. et al, Carcinogenesis, 1993, 14:857-61), c- Jun/AP-1 activation (Huang, T.S. et al, Proc. Natl. Acad. Sci., 1991 , 88:5292-96), prostaglandin biosynthesis (Huang, M-T. et al, In L.W. Battenberg (ed.) Cancer Chemoprevention, CRC Press, Boca Raton, 1992, pp375-91) and activity and expression of the enzyme cyclooxygenase-2 (Huang, M. T., et al, Cancer Res., 1991 , 51 :813-19; Zhang, F. et al, Carcinogenesis, 1999, 20:445-51). This latter property is probably mediated by the ability of curcumin to block activation of the transcription factor NF-KB at the level of the NF-[kappa]B inducing kinase/IKK[alpha]/[beta] signalling complex (Plummer, S. et al, Oncogene, 1999, 18:6013-20).

Curcumin directly inhibit the cyclooxygenase-2 and also inhibits the transcription of the gene responsible for its production. Cyclooxygenases (COX) catalyze the synthesis of prostaglandins (PGs) from arachidonic acid. There are two isoforms of COX, designated COX-1 and COX-2. COX-1 is expressed constitutively in most tissues and appears to be responsible for housekeeping functions (Funk, CD. et al, FASEB J., 1991 , 5:2304-12) while COX-2 is not detectable in most normal tissues but is induced by oncogenes, growth factors, carcinogens and tumor promoters (Subbaramiah, K. et al, 1996, Cancer Res., 1996, 56:4424-29; DuBois, R.N. et al, J. Clin, invest, 1994, 93:493- 98; Kelley, D.J. et al, Carcinogenesis, 1997, 18:795-99). Several different mechanisms account for the link between COX-2 activity and carcinogenesis.

BACKGROUND ART

Curcumin is not simply an alternative to non-steroidal anti-inflammatory drugs (NSAIDS), which also have anti-inflammatory and cancer chemopreventive properties. This is so because COX is a bifunctional enzyme with cyclooxygenase and peroxidase activities. Aside from being important for PG synthesis, the peroxidase function contributes to the activation of procarcinogens. Therefore, the failure of NSAIDS to inhibit the peroxidase function of COX potentially limits their effectiveness as anticancer agents. Curcumin, in contrast, down-regulates levels of COX-2 and thereby decreases both the cyclooxygenase and peroxidase activities of the enzyme.

Curcumin is among the few agents to block both the COX and LOX (lipoxygenase) pathways of inflammation and carcinogenesis by directly modulating arachidonic acid metabolism. In a study to evaluate the effect of curcumin on the metabolism and action of arachidonic acid in mouse epidermis, it was found that topical application of curcumin inhibited arachidonic acid-induced ear inflammation in mice.(Huang, M.T., et al Cancer Res., 1988, 48:5941-46; 1991 , 51 :813-19). Curcumin (10 [mu]M) inhibited the conversion of arachidonic acid to 5- and 8-hydroxyeicosatetraenoic acid by 60% and 51 %, respectively (LOX pathway) and the metabolism to PGE2, PGF20 and PGD2 by 70%, 64% and 73%, respectively (COX pathway). In another study, dietary administration of 0.2% curcumin to rats inhibited azoxymethane-induced colon carcinogenesis and decreased colonic and tumor phospholipase A2, phospholipase CyI, and PGE2 levels (Rao, CV. etal, Cancer Res., 1995, 55:259-66). In this study, dietary curcumin also decreased enzyme activity in the colonic mucosa and tumors for the formation of PGE2, PGF2[alpha], PGD2, 6-keto- PGF2n and thromboxane B2 via the COX system and production of 5(S)-, 8(S)-, 12(S)-, and 15(S)-hydroxy-eicosatetraenoic acid via the LOX pathway was also inhibited.

Despite its impressive array of beneficial bioactivities, the bioavailability of curcumin in animals and man remains low. In rodents, curcumin demonstrates poor systemic bioavailability after p.o. dosing (Ireson, CR. et al, Cancer Res., 2001 , 41 :1058- 64) which may be related to its inadequate absorption and fast metabolism. Curcumin bioavailability may also be poor in humans as seen from the results of a recent pilot study of a standardized turmeric extract in colorectal cancer patients (Sharma, R.A. et al, CHn. Cancer Res., 2001 , 7:1834-1900). Indirect evidence suggests that curcumin is metabolized in the intestinal tract. Curcumin undergoes metabolic O-conjugation to curcumin glucuronide and curcumin sulfate and bioreduction to tetrahydrocurcumin, hexahydrocurcumin and hexahydrocurcuminol in rats and mice in vivo (Pan, M. H. et al, Drug Metabol. Dispos., 1999, 27:486-94; Asai, A., et al, Life ScL, 2000, 67:2785-93), in suspensions of human and rat hepatocytes (Ireson et al, loc. cit) and in human and rat intestine (Ireson , CR. et al, Cancer Epidemiol. Biomark. Prev.,

2002, 11 :105-11). Metabolic conjugation and reduction of curcumin was more in human than in rat intestinal tissue. It has been suggested that the intestinal tract plays an important role in the metabolic disposition of curcumin. This is based predominantly on experiments in which [<3>H] labeled cucrcumin was incubated with inverted rat gut sacs (Ravindranath, V. and Chandrasekhara, N., Toxicology, 1981 , 20::251-57). This was later confirmed in intestinal fractions from humans and rats. Intestinal mucosa, as well as liver and kidney tissue from the rat, can glucurodinate and sulfate curcumin, as judged by the analysis of differential amounts of curcumin present before and after treatment of tissue extracts with conjugate-hydrolyzing enzymes (Asai et al, loc cit). Thus, gut metabolism contributes substantially to the overall metabolic yield generated from curcumin in vivo. In human intestinal fractions, conjugation with activated sulfuric or glucuronic acids was much more abundant, whereas conjugation in human hepatic tissues was less extensive, than in the rat tissues (Ireson, C. R., et al, Cancer Epidemiol. Biomark. Prev., 2002, 11 :105-11).

Although p.o. administered curcumin has poor bioavailability and only low or non-measurable blood levels were observed (Perkins, S. et al, Cancer Epidemiol. Biomark. Prev., 2002, 11 :535-40), this route of administration inhibits chemically induced skin and liver carcinogenesis (Limtrakul, P., et al, Cancer Lett, 1997, 116:197-203; Chiang, S.E. et al, Carcinogenesis, 2000, 21 :331-35). Oral administration of curcumin also inhibits the initiation of radiation-induced mammary and pituitary tumors (Inano, H. et al, Carcinogenesis, 2000, 21 :1835-41 ; Int. J. Radiat.Oncol. Biol. Phys., 2002, 52:212-23; ibid, 2002, 53:735-43). Similarly, in a study to assess the curcumin levels in the colorectum, a daily dose of 3.6 g curcumin achieves pharmacologically effective levels in the colorectum with negligible distribution of curcumin outside the gut (Garcea, G. et al, Cancer Epidemiol. Biomark. Prev., 2005, 14:120-25). Earlier Shobha et al (Planta Med., 1998, 64:353-56) had observed that administering piperine along with curcumin enhances the bioavailability of curcumin. However, the level of enhancement was only modest and no curcumin could be detected after 3 hours even when supplemented with piperine.

DISCLOSURE OF THE INVENTION

Thus, in order to derive full benefits from the administration of curcumin in human subjects, ways and means to enhance its bioavailability needs to be explored. The present invention is an effort in this direction. It was found that when the essential oil of turmeric was added to curcumin, the bioavailability of curcumin is significantly enhanced. Accordingly, a composition of curcumin admixed with a suitable proportion of turmerone (the main component of the turmeric essential oil) is provided. This composition was administered to 9 human volunteers and blood samples were collected at zero hour and then at hourly or half-hourly intervals upto 8 hours. Maximum absorption was observed at 3 hours after ingestion and consumption of the said composition resulted in curcumin levels that were 5-16 fold higher compared to curcumin alone. Earlier Shobha et al (Planta Med., 1998, 64:353-56) had observed that administering piperine along with curcumin enhances the bioavailability of curcumin. However, the level of enhancement was only modest and no curcumin could be detected after 3 hours even when supplemented with piperine. With turmerone as the adjuvant, as in the present invention, peak absorption occurred at 3 hours and persisted at low levels at least until 8 hours, beyond which no measurements were made. The invention relates to a product to enhance the bioavailability of curcumin by mixing a suitable portion of the volatile oil obtained from turmeric with the curcuminoids isolated from turmeric. For this purpose, the volatile oil of turmeric was isolated by conventional methods of steam distillation to isolate essential oils and is well known in the art.. Curcumin is isolated from the

de-oiled turmeric by solvent extraction. Suitable solvents for this purpose include acetone, hexane, ethyl acetate, dicholoroethane, chloroform, etc. The extraction is conveniently carried out at moderate temperatures (40-55<0>C) and the solvent is partially removed to yield a concentrate containing 30-60% solids. This solution is cooled to obtain crystals of curcumin which are isolated by any suitable method such as filtration or centrifugation. This product was analyzed to contain 95% curcumin. Curcumin and the volatile oils of curcumin are mixed and blended to get a uniform product. The ratio of curcumin to oil can be varied between 3:1 to 99:1 , preferably in the ratio 85:15. A more preferred ratio is 95:5. Gelatin capsules containing 500 mg of the blend were prepared. Curcumin capsules without the essential oil were similarly prepared.

Nine healthy human volunteers aged between 25 and 45 years of age were selected for the study. They were given curcumin and the inventive composition in capsules at the dosage of 50 mg/kg body weight. They were advised to take curcumin first. Blood samples were collected at zero hour and periodically at one-hour or half-hour intervals for 8 hours. After a washout period of one week, the same protocol was followed with the inventive composition. The whole blood was extracted exhaustively with ethyl acetate. Recoveries ranged from 80.12 to 86.49. The ethyl acetate extract was analyzed by HPLC on a RP-C18 column (25 x 4.5 mm) using methanol as solvent and UV detection at 420 nm. The eluant flow rate was 1 ml/min. A typical result is given in the following Table.

Time (h) Curcumin content in blood (ng/g)

Curcumin Inventive compositioN

0.0 0.0 0.0 0.5 3.17 7.85 1.0 7.57 6.23 1.5 4.42 4.84 2.0 13.81 11.95 2.5 9.61 19.22 3.0 5.67 92.59 4.0 8.42 24.33 6.0 1.62 8.43 8.0 1.11 5.09

The results are also graphically represented in Fig. 1. The peak absorption of curcumin occurred at 3 h and in the case of the Inventive composition, curcumin persisted in small amounts in the blood till 8 h beyond which measurements were not made. This is significant. At peak absorption the enhancement of bioavailability ranged among the 9 persons between 5 and 16-fold with a mean value of 10.62.

The inventive composition has the additional benefit that the essential oil components are themselves bioactive (for example, see Yue, A et al, Int. J. MoI. Med., 2002, 9:481-84; Jayaprakasha, G.K. et al, Z.Naturforsch., 2002, 57:828-35) and thus is expected to synergistically enhance the bioactivity of curcumin.