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(54) Benævnelse: **Fremgangsmåde til forbedring af biotilgængeligheden af curcumin**

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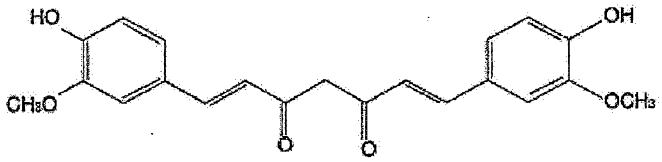
**DATABASE WPI Week 200520 Thomson Scientific, London, GB; AN 2005-183710 XP002558520 & CN 1 548 121 A (GUANGZHOU BAIYUNSHAN MEDICINE SCI TECH D) 24 November 2004 (2004-11-24)**

# DESCRIPTION

## TECHNICAL FIELD

**[0001]** 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.




**[0002]** 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, C.V. 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, Mol. 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-KB inducing kinase/IKKα/β signalling complex (Plummer, S. et al, Oncogene, 1999, 18:6013-20).

**[0003]** 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, C.D. 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**

**[0004]** 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.

**[0005]** 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 $PGE_2$, $PGF_{2\alpha}$ and $PGD_2$ 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 $A_2$, phospholipase Cyl, and $PGE_2$ levels (Rao, C.V. 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 $PGE_2$, $PGF_{2\alpha}$, $PGD_2$, 6-keto- $PGF_{2\alpha}$ and thromboxane $B_2$ 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.

**[0006]** 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, C.R. 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, Clin. 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 Sci., 2000, 67:2785-93), in suspensions of human and rat hepatocytes (Ireson et al, *loc. cit)* and in human and rat intestine (Ireson , C.R. 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).

**[0007]** 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.

**[0008]** Methods and compositions known in the art include those disclosed in US 2002/0136786, US 6235287, US 5861415 and WO 03/051380. US 2002/0136786 discloses a method for effecting smoke detoxification in a human by using a composition that is made of effective amounts of supercritical extract and hydroalcoholic extract of turmeric. US 6235287 discloses certain diterpenes for use as medicaments and to extracts or concentrates of the plant Curcuma amada containing them. US 5861415 disclose a composition containing three curcuminoids, i.e., curcumin, demethoxy curcumin and bis demethoxy curcumin, extracted from roots of turmeric. WO 03/051380 discloses a method of producing lipid soluble extract called Curcuma oil in high yield.

**DISCLOSURE OF THE INVENTION**

**[0009]** 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 method of making a composition consisting essentially of curcumin admixed with a suitable portion of turmerone (the main component of the turmeric essential oil) according to claim 1 and a composition prepared by the method of claim 1 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.

**[0010]** 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 essential oil obtained from turmeric comprising tumerone with the curcuminoids isolated from de-oiled turmeric by solvent extraction, wherein the weight ratio of the curcuminoid to the essential oil of turmeric ranges from 3:1 to 99:1. For this purpose, the essential 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°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.

**[0011]** 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-$C_{18}$ column (25 x 4.5 mm) using methanol as solvent and UV detection at 420 nm. The eluant flow rate was 1 ml/min.

**[0012]** 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 |

**[0013]** 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 1.0.62.

**[0014]** The inventive composition has 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 is expected to synergistically enhance the bioactivity of curcumin.

# REFERENCES CITED IN THE DESCRIPTION

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**PATENTKRAV**

1. Fremgangsmåde til fremstilling af en sammensætning, som omfatter trinene:
   (a) isolering af en flygtig olie fra gurkemeje, der omfatter turmeron, ved hjælp af dampdestillation;
   (b) isolering af et ekstrakt, der omfatter curcumin, fra den afolierede gurkemeje ved hjælp af opløsningsmiddelekstraktion;
   (c) delvis fjernelse af opløsningsmidlet fra ekstraktet, der omfatter curcumin, til opnåelse af et koncentrat, der indeholder 30 til 60 % faststoffer;
   (d) nedkøling af koncentratet til opnåelse af krystaller af curcumin;
   (e) isolering af krystallerne af curcumin til opnåelse af et produkt, der omfatter 95 % curcumin; og
   (f) blanding og blending af den flygtige olie fra gurkemeje opnået i trin (a) og produktet i trin (e) til opnåelse af et ensartet produkt, hvor vægtforholdet mellem curcumin og den flygtige olie fra gurkemeje kan varieres mellem fra 3:1 til 99:1.

2. Fremgangsmåde ifølge krav 1, hvor opløsningsmidlet, der anvendes i opløsningsmiddelekstraktionen i trin (b), er valgt fra gruppen, der består af acetone, hexan, ethylacetat, dichlorethan eller chloroform.

3. Fremgangsmåde ifølge krav 1 eller krav 2, hvor opløsningsmiddelekstraktionen i trin (b) udføres ved en temperatur på 40 til 55 °C.

4. Fremgangsmåde ifølge krav 1, hvor isolering af krystallerne af curcumin i trin (e) udføres ved hjælp af filtrering eller centrifugering.

5. Fremgangsmåde ifølge krav 1, hvor vægtforholdet i trin (f) mellem curcumin og den æteriske olie fra gurkemeje er 95:5.

6. Fremgangsmåde ifølge krav 1, hvor vægtforholdet mellem curcumin og den æteriske olie fra gurkemeje er 85:15.

7. Sammensætning fremstillet ved hjælp af fremgangsmåden ifølge krav 1, der i det væsentlige består af en blanding af curcumin og en passende del af turmeron.

8. Sammensætning ifølge krav 7 til anvendelse i medicin til forbedring af biotilgængeligheden af curcumin.

9. Gelatinekapsel, der omfatter 500 mg af sammensætningen ifølge krav 7.

# DRAWINGS

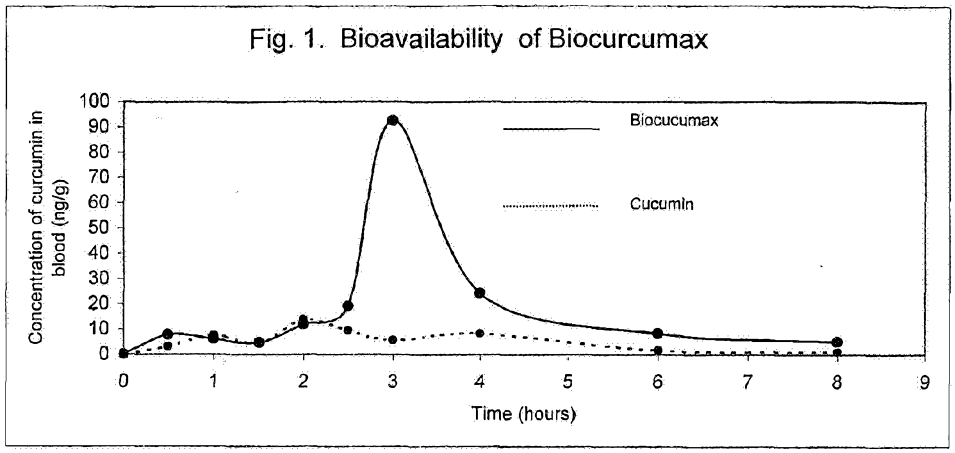
Fig. 1. Bioavailability of Biocurcumax
Concentration of curcumin in blood (ng/g)
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
7
8
9
Time (hours)
Biocucumax
Cucumin