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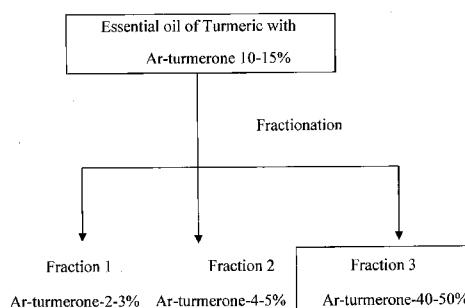
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Fig: 5: Method of preparation of Essential oil of turmeric with varying concentration of Ar-turmerone.



(57) Abstract: Invention 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. The invention 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.

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FORMULATION OF CURCUMIN WITH ENHANCED BIOAVAILABILITY OF CURCUMIN AND METHOD OF PREPARATION AND TREATMENT THEREOF

OBJECTIVE OF THE INVENTION

5 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

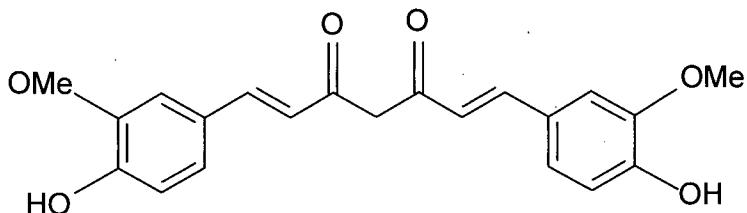
10 volunteers. The present invention also relates to an application of curcuminoid with essential oil of turmeric to enhance the bioavailability of curcumin for oral supplementation against a variety of diseases and method of doing the same. In particular the present invention relates to oral supplementation of curcuminoid with essential oil of turmeric to enhance the bioavailability of curcumin for the prophylaxis, treatment,

15 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

20 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]



is the 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 chemo preventive, antineoplastic and anti-inflammatory properties¹. 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^{+/−}) mouse⁴, 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 kinases¹⁰, c-Jun/AP-1 activation¹¹, prostaglandin biosynthesis¹² 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 complex¹⁵.

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 functions¹⁶ 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.

5 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 10 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 15 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 mice²⁰. Curcumin (10 μ 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, PGF2 α and 20 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 C γ I, and PGE2 levels²¹. In this study, dietary curcumin also decreased enzyme activity in the colonic mucosa and tumors for the formation of PGE2, PGF2 α , PGD2, 6-keto- PGF2 α 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 this impressive array of beneficial bioactivities, the bioavailability of curcumin in animals and man remains low. In rodents, curcumin demonstrates poor systemic 5 bioavailability after p.o. dosing²² 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 patients²³. Indirect evidence suggests that curcumin is metabolized in the intestinal tract. Curcumin undergoes metabolic O-conjugation to curcumin glucuronide and curcumin 10 sulfate and bioreduction to tetrahydrocurcumin, hexahydrocurcumin and hexahydrocurcuminol in rats and mice *in vivo*^{24,25} in suspensions of human and rat hepatocytes²⁶ and in human and rat intestine²⁷. Metabolic conjugation and reduction of curcumin was more in human than in rat intestinal tissue. It has been suggested that the 15 intestinal tract plays an important role in the metabolic disposition of curcumin. This is based predominantly on experiments in which [³H] labeled curcumin was incubated with inverted rat gut sacs²⁸. This was later confirmed in intestinal fractions from humans and rats. Intestinal mucosa, as well as liver and kidney tissue from the rat, can glucuronidate and sulfate curcumin, as judged by the analysis of differential amounts of curcumin present before and after treatment of tissue extracts with conjugate-hydrolyzing 20 enzymes²⁹. 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 tissues³⁰.

Although p.o. administered curcumin has poor bioavailability and only low or non-measurable blood levels were observed³¹, 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 tumors³⁴. Similarly, in a study 5 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 gut³⁵.

Earlier Shobha et al³⁶ had observed that administering piperine along with curcumin enhances the bioavailability of curcumin. However, the level of enhancement was only 10 modest and no curcumin could be detected after 3 hours even when supplemented with piperine.

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. 15 The quantity of curcumin that reaches tissues outside the gut is probably pharmacologically insignificant. Several studies have failed to demonstrate the positive *invitro* results with curcumin in *invivo* 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 20 concentration in the blood and remain in the system for a sufficient period at an effective concentration level.

SUMMARY

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.

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

Some embodiments provide a method of reducing visual analogue scale for pain by administering a composition having curcuminoid mixture and added essential oil of 10 turmeric.

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 15 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 20 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

5 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

10 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

15 rate scale by 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 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

20 curcuminoid mixture and added essential oil of turmeric.

BRIEF DESCRIPTION OF THE DRAWINGS

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:

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

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, 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. The x-axis shows time in hours and y-axis shows the concentration of curcumin (ng/g) in blood

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

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 5 concentration of curcumin (ng/g) in blood

Fig. 5 provides Method of preparation of Essential oil of turmeric with varying concentration of Ar-turmerone.

DETAILED DESCRIPTION

The disclosure relates to a product to enhance the bioavailability of curcumin by mixing a 10 suitable portion of the volatile oil obtained from turmeric with the curcuminoids isolated from turmeric.

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 15 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.

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 20 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.

As stated herein, the term "a" or "an" refers to one or more.

As stated herein, the terms "isolated" and "purified" are referred to interchangeably.

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

5 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 10 crystals of curcuminoid mixture, showed that, in some embodiments, 95% of the product was composed of curcumin, demethoxycurcumin and bisdemethoxycurcumin.

The disclosure provides a composition having curcuminoid and an essential oil of turmeric.

15 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.

20 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 25 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 5 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 10 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 of the curcuminoid to the essential oil of turmeric is about 1:2. In some embodiments, the weight ratio of the curcuminoid to the 15 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.

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 20 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%.

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%.

5 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%.

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%.

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.

In some embodiments, a composition of a curcuminoid and added essential oil of turmeric 5 is orally administered to a human.

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, 10 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.

Curcumin is isolated from the de-oiled turmeric by solvent extraction. Suitable solvents for this purpose include acetone, hexane, ethyl acetate, dichloroethane, chloroform, etc. 15 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 20 essential oil plus other constituents such as carbohydrates, etc, which were not characterized.

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;

5 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.

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

10 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

15 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.

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

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.

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

- 5 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
- 10 heat and vacuum can be performed using a vaccumized desolventiser with a stirrer.

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

- 15 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.

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

- 20 a composition having a homogeneous mixture of the curcuminoid and water.

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.

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 5 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 10 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 15 ranges from about 5 mg to about 200 mg.

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.

The disclosure provides a method of preparing a gelatin capsule having a curcuminoid and 20 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.

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 filling machine manufactured by Pam Pharmaceuticals, Mumbai, India.

5 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
10 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
15 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
20 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
25 45% Ar-turmerone in 10:1 ratio or curcuminoids 95% were given to patients with active

rheumatoid arthritis for 2 months duration in a dose of 500mg capsules twice daily. Formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio were able to significantly decrease disease activity score, total number of swollen and painful joints and erythrocyte sedimentation rate. The patients administered formulation of

5 Curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio, 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

10 essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio. 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 ratio alone after 2 months continued to be asymptomatic during the follow up phase of 4 more months.

15 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

20 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 and curcuminoids 95% in a dose of 500mg twice daily for 3 months. Almost all patients in formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 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.

10 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. 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 or curcuminoids 95% in a dose of 3gm/day. The patients on curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio formulation 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 ratio reflecting the ability of formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio to disaggregate Amyloid beta deposits in the brain

5 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 ratio, the values being significantly higher for the formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio group. Another human study which supplemented formulation of curcuminoid with

10 essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio 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 ratio therapy while majority of the patients in curcuminoids 95% progressed to dementia and 50 % to

15 Alzheimers disease within 2 years.

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 in patients with depression and an improved method of delivering curcumin in the human blood and tissues and ensuring bioavailability in humans for

20 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,

25 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 and curcuminoids 95% in a dose of 500mg twice daily for 8 weeks. Almost all patients in formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio group had significant reduction in the severity of depression as 5 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.

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; 10 Jayaprakasha, G.K. et al, *Z.Naturforsch.*, 2002, 57:828-35) and thus are expected to synergistically enhance the bioactivity of curcumin.

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 15 examples as described are not intended to be construed as limiting the scope of the present invention.

EXAMPLES

Example 1

Nine healthy human volunteers aged between 25 and 45 years of age were selected for the 20 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 25 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 x 4.5 mm) using methanol as solvent and UV detection at 420 nm. The 5 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 10 procedure. The efficiency of recovery of curcumin by the above extraction procedure was estimated to range between 80.12% and 86.49%.

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A typical result is given in Table 1.

Table 1

Time (h)	Curcumin content in blood (ng/g)	
	Curcumin composition	Enhanced curcumin bioavailability composition
0.0	0.0	0.0
0.5	3.17	0.5
1.0	7.57	1.0
1.5	4.42	1.5
2.0	13.81	2.0
2.5	9.61	2.5
3.0	5.67	3.0
4.0	8.2	4.0
6.0	1.62	6.0
8.0	1.11	8.0

5 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.

5 Example 2

Human subjects were administered capsule (4X500mg) 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 10 groups were administered an enhanced curcumin bioavailability composition (4X500mg). 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 15 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

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

5 3. The unit of curcumin content in blood is provided as area under the curve (AUC).

Table3. Ratio of curcuminoids to added essential oil in compositions for enhanced curcumin bioavailability

Ratio of Curcuminoids to added essential oil of turmeric	Ratio of curcuminoids to added essential oil of turmeric
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	35:1

Example 3

Bioavailability of curcumin from essential oil of turmeric alone, raw turmeric powder, curcuminoid alone and curcuminoid with essential oil of turmeric with 45% Ar-turmerone
5 etc.

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 50mg 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 10 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 50mg 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 15 per gram of blood for the subjects, which was averaged for each time point.

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 50mg of essential oil 20 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 50mg 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 50mg of raw turmeric powder/kg body weight of the human subject.

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 (25x4.5mm) using methanol as solvent and UV detection at 420nm. The eluent flow rate was 1ml/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.

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. 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 was 6.7 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

Time in hours	Curcumin content in blood (ng/g)				
	Raw turmeric powder	Essential oil of turmeric (45 % Ar-turmerone)	Essential oil of turmeric (10 – 15 % Ar - turmerone)	Curcuminoid mixture without added Essential oil of turmeric	Curcuminoid mixture with added essential oil of turmeric (45% Ar-turmerone) 10:1
0	0	0	0	0	0
0.5	Nd	Nd	Nd	3.17	7.85
1	1.05	Nd	Nd	7.57	6.23
1.5	Nd	Nd	Nd	4.42	4.84
2	2.1	Nd	Nd	13.81	11.95
2.5	Nd	Nd	Nd	9.61	19.22
3	Nd	Nd	Nd	5.67	92.59
4	Nd	Nd	Nd	8.2	24.33
6	Nd	Nd	Nd	1.62	8.43
8	Nd	Nd	Nd	1.11	5.09

Example 4

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

10 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 5 calculated from the figure. In Table 5 and Fig: 3, AUC is provided as nanograms of curcumin per gram of blood.

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 10 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 12.8:1; 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 15 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.

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 20 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.

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.

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

Group	Ratio of C:E	Dosage 4 caps each	C without added E		C with added E		
			C (mg) per capsule	C (ng) per gm of blood (AUC)	C (mg) per capsule	E (mg) per capsule	C (ng) per gm of blood (AUC)
A	99:1	500mg	475	771	495	5	3855
B	95:5 or 19:1	500mg	475	786	475	25	5515
C	90:4 or 22.5:1	500mg	475	725	478.72	21.28	5147.5
D	90:5 or 18:1	500mg	475	820	473.68	26.32	5904
E	90:6 or 15:1	500mg	475	750	468.75	31.25	5475
F	90:7 or 12.8:1	500mg	475	900	463.77	36.23	6300
G	90:8 or 11.3:1	500mg	475	752	459.35	40.65	5367.6
H	90:9 or 10:1	500mg	475	782	454.55	45.45	5552.2
I	90:10 or 9:1	500mg	475	760	450	50	5320
J	90:20 or 4.5:1	500mg	475	765	409.1	90.9	5469.75
K	89:9 or 9.8:1	500mg	475	696	453.7	46.3	5080.8
L	80:9 or 8.8:1	500mg	475	726	448.98	51.02	5227.2
M	80:20 or 4:1	500mg	475	754	400	100	5315.7
N	70:20 or 3.5:1	500mg	475	810	388.89	111.11	5147.5
O	70 :1	500mg	475	769	493	7	5124
P	60:1	500mg	475	725	491.8	8.2	5200
Q	50:1	500mg	475	749	490.2	9.8	5284
R	40:1	500mg	475	737	487.8	12.2	5310
S	75:25 or 3:1	500mg	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	500mg	475	726	125	375	1276

Example 5

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

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.

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).

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.

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.

5 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.

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
10 45.45 mg E (45% Ar-t) in Table 6.

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 454.55mg curcuminoids and 45.45mg 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
15 45% Ar-t) in Table 6.

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 (25x4.5mm) using methanol as solvent and UV detection at 420nm. The eluent flow rate was 1ml/min. Curcumin content in the blood was determined for each group at each time
20 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.

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 5 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.

10 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 15 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 curcuminoid mixture to essential oil of turmeric

Time h	Nanograms of curcumin per gram of blood								
	20 mg C alone (10-15% Ar-t)	200 mg E C: 200 mg E = 1:10 (E had 10-15% Ar-t)	20 mg C: 200 mg E = 1:10 (E had 45% Ar-t)	20 mg C: 2 mg E = 10:1, (E had 45% Ar-t)	454.55 mg C without added E	45.4 mg E (45% Ar-t)	454.55 mg C: 45.45 mg E = 10:1 (E had 45% Ar-t)		
0	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	

5 Example 6

Method of preparation of curcuminoid mixture with 95% Curcuminoids

The rhizomes of turmeric (300Kg) 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 78⁰C 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 4⁰ C to obtain crystals of curcuminoid 5 (12Kg) 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

10 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 hrs at a temperature of about 83⁰ 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 15 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.

Example 8

Method of analysis of total curcuminoids by HPLC method

20 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 μ m membrane filter before injection. Standard was prepared by weighing accurately 25mg 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 μ m membrane filter before injection.

The total Curcuminoids was analyzed by high performance liquid chromatography
5 (HPLC) on a C18 column ((250X4.6mm Shimadzu Co., Japan.) using methanol as the mobile phase and UV detection at 420 nm. The eluent flow rate was 1ml/min.

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

$$\% \text{ of total Curcuminoid} = \frac{\text{Area of sample} \times \text{amount of std} \times \text{Purity of std}}{10 \times \text{Area of Std} \times \text{weight of the sample}}$$

Example 9

Method of preparation of Essential oil of turmeric with varying concentration of Ar-turmerone

The rhizomes of turmeric (500Kg) were dried. The dried turmeric rhizomes were
15 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 78⁰C 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 4⁰ C to obtain crystals of curcuminoid
20 (20Kg) and a liquid. The crystals of curcuminoid were isolated from the liquid by filtration.

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 (9.3Kg) was obtained as fraction 1.(Fig: 5)

5 **Example 10**

Method of preparation of combination of curcuminoids and essential oil of turmeric with 45 % Ar turmerone in 10:1 ratio.

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 10 as per Example 9 (0.27Kg) 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).

A 500 mg capsule containing 454.55mg of curcuminoid and 45.45mg of Essential oil with 45% Ar-turmerone in a weight ratio of about 90:9 (10:1) (curcuminoids 95%, curcumin 15 70%, demethoxy curcumin 23% and bisdemethoxycurcumin 7%)was prepared by encapsulating the above blended extract powder in hard gelatin capsules done in an air-conditioned at 21degreeC 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 20 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.

The powdered curcuminoid mixture prepared as per Example 6 (0.27Kg) was suspended

in water (1 L) to form a suspension. Fraction of essential oil of turmeric containing 45 %

5 Ar-turmerone prepared as per Example 9(2.7Kg) 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).

Capsule containing curcuminoid and Essential oil of turmeric with 45% Ar-turmerone in a

weight ratio of about 1: 10 (curcuminoids 95%, curcumin 70%, demethoxy curcumin

10 23% and bisdemethoxy curcumin 7%)was prepared by encapsulating the above blended

extract powder in soft gelatin capsules done in an air-conditioned at 21degreeC and de-

humidified room. 3 kg of extract powder 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 weights of capsules were

15 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.

20 The powdered curcuminoid mixture prepared as per Example 6 (1.5Kg) 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 (1.5Kg) 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. (3Kg).

A 500 mg capsule containing 250mg of curcuminoid and 250mg of Essential oil of turmeric with 45% Ar-turmerone in a weight ratio of about 1:1 (curcuminoids 95%, curcumin 70%, demethoxy curcumin 23% and bisdemethoxy curcumin 7%) was prepared by encapsulating the above blended extract powder in hard gelatin capsules done in an air-conditioned at 21degreeC 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.

The powdered curcuminoid mixture prepared as per Example 6 (2.7Kg) 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 (0.27Kg) 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).

A 500 mg capsule containing 454.55mg of curcuminoid and 45.45mg of Essential oil of turmeric with 10-15% Ar-turmerone in a weight ratio of about 90:9 (10:1) (curcuminoids 95%, curcumin 70% ,demethoxy curcumin 23% and bisdemethoxy curcumin 7%)was

prepared by encapsulating the above blended extract powder in hard gelatin capsules done in an air-conditioned at 21degreeC 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-
10 turmerone.

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 21degreeC 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.

20 **Example 15**

Method of preparation of capsules containing Essential oil of turmeric with 10-15 % Ar-
turmerone.

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 (2.5kg) in soft gelatin capsules done in an air-conditioned at 21degreeC and de-humidified room. 2.5 kg essential oil of turmeric with 10-15 % Ar-turmerone was charged 5 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.

10 **Example 16**

Method of preparation of capsules containing curcuminoids 95%.

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 21degree C and de- humidified room. 3 kg of powder was charged into the 15 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.

20 **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.

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 (0.27Kg) 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 5 slurry under heat and vacuum to form a uniform blend (3 Kg).

A 500 mg capsule containing 454.55mg of curcuminoid and 45.45mg of Essential oil with 45% Ar-turmerone in a weight ratio of about 90:9 (10:1) (curcuminoids 24%, curcumin 72%, demethoxy curcumin 22% and bisdemethoxy curcumin 6%)was prepared by encapsulating the above blended extract powder in hard gelatin capsules done in an air- 10 conditioned at 21degreeC 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 15 of 500 mg each.

Example 18

Method of preparation of raw turmeric powder.

The raw turmeric rhizomes (10Kg) were collected and cleaned. The rhizomes were dried and pulverized to get turmeric powder (2.5Kg). The turmeric powder was sieved through 20 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 21degreeC 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.

5 **Example 19**

Human clinical study of different turmeric extracts in patients with Rheumatoid arthritis

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 and curcuminoids 95% in patients with rheumatoid arthritis, forty five patients diagnosed with rheumatoid arthritis were randomized into 9 groups viz.,

- Group1: Subjects receiving raw turmeric powder 500mg 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) 500mg capsules prepared as described in Example 14 twice daily
- 20 Group3: Subjects receiving essential oil of turmeric with 10-15% Ar-turmerone (EOT with 10-15% Ar-t) 500mg 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), 500mg capsules prepared as described in Example 11 twice daily.

5 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), 500mg 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), 500mg capsules prepared as described in Example 17 twice daily.

10 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), 500mg capsules prepared as described in Example 13 twice daily

Group8: Subjects receiving curcuminoids 95% 500mg capsules prepared as described in Example 16, twice daily.

15 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) 500mg capsules prepared as described in Example 10, twice daily dose after food with water for a period of 8 weeks.

Subjects aged 18-65 years of either sex diagnosed to have rheumatoid arthritis (RA) 20 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.

Efficacy and safety evaluations were performed at biweekly intervals. Patients were 5 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 10 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 15 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, 20 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.

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 79mm at baseline to 27.5mm at the end of treatment.

5 The results are summarized in Table 8. All components of ACR response criteria viz., Total Painful Joints (19 at baseline to 3 at end of analysis), Total Swollen Joints (12 at baseline to 0.5 end of analysis), Patient's GA (84 at baseline to 31 at the end of analysis), Physician's GA (80 at baseline to 28 at end of the study), Disability Index and HAQ (4.5 at baseline to 1.0 at the end of the study) showed a significant reduction from baseline to 10 end of study in the formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio. The results are summarized in Table 9. Treatment with 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 15 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 ratio can provide significant improvement in treatment efficacy in active RA. All the patients who 20 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 % 25 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 95%	Mean	6.5	6	8%

Table 8. Treatment efficacy results – VAS

Group		Baseline (mm)	End of Treatment (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 in 1:10 ratio	Mean	79	77	2.5%
C+ E with 45% Ar-t in 1:1 ratio	Mean	78	76	2.6%
C 24% + E with 45% Ar-t in 10:1 ratio	Mean	76	74	2.6%
C+ E with 10-15% Ar-t in 10:1 ratio	Mean	75	71	5.3%
C+ E with 45% Ar-t in 10:1 ratio	Mean	79	27.5	65%
Curcuminoids 95%	Mean	77	70	9%

Table 9. ACR response

Parameter	Raw turmeric	EDT with 45%Ar-t	EDT with 10-15% Ar-t	C+ E with 45% Ar-t in 1:10 ratio	C+ E with 45% Ar-t in 1:1 ratio	C 24%+ E with 45% Ar-t in 10:1 ratio	C+ E with 10-15% Ar-t in 10:1 ratio	C+ E with 45% Ar-t in 10:1 ratio	Curcuminoids 95%
Total Painful Joints	Base line	E O T	Base line	EO T	Base line	EO T	Base line	EO T	Base line
	18	18	17	16	18	20	21	17	19
Total Swollen Joints	10	11	12	12	11	10	11	13	12
Patient's GA	86	82	79	75	82	80	84	79	76
Physician's GA	84	79	74	72	79	76	81	75	79
Disability Index, HAQ [#]	4	4	3.5	3.5	3.5	4	4	4.5	3.5

Table 10. Treatment efficacy results – CRP

Group		Baseline (mg/L)	End of Treatment (mg/L)	% Change
Raw turmeric	Mean	13	13	0 5
EOT with 45%Ar-t	Mean	11	11.5	4%
EOT with 10- 15% Ar-t	Mean	12.5	13.5	7%
C+ E with 45% Ar-t in 1:10 ratio	Mean	12	12.5	4%
C+ E with 45% Ar-t in 1:1 ratio	Mean	13	13	0 10
C 24% + E with 45% Ar-t in 10:1 ratio	Mean	12	12	0
C+ E with 10- 15% Ar- t in 10:1 ratio	Mean	11.5	11.5	0
C+ E with 45% Ar-t in 10:1 ratio	Mean	12	5.7	53%
Curcuminoids 95%	Mean	11.5	11.4	15 1%

Table 11. Treatment efficacy results- Rheumatoid Arthritis Factor

Group		Baseline (IU/L)	End of Treatment (IU/L)	% Change
Raw turmeric	Mean	23	25	8%
EOT with 45% Ar-t	Mean	26	28	7% 5
EOT with 10-15% Ar-t	Mean	25	26	3.8%
C+ E with 45% Ar-t in 1:10 ratio	Mean	23	24	4%
C+ E with 45% Ar-t in 1:1 ratio	Mean	22	22	0
C 24% + E with 45% Ar- t in 10:1 ratio	Mean	21	21	0 10
C+ E with 10- 15% Ar-t in 10:1 ratio	Mean	24	24	0
C+ E with 45% Ar-t in 10:1 ratio	Mean	24	15	38%
Curcuminoids 95%	Mean	22	24	9%

15 Example 20

Human clinical study of different turmeric extracts in patients with Osteo arthritis.

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, 5 curcuminoid with essential oil of turmeric with 10-15 % Ar-turmerone in 10:1 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.

10 Gr 1: Oral administration of raw turmeric powder 500mg 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) 500mg capsules prepared as described in Example 14, in twice daily dosage.

15 Gr3: Oral administration of essential oil of turmeric with 10-15% Ar-turmerone (EOT with 10-15% Ar-t) 500mg 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), 500mg capsules prepared as described in Example 11, in twice daily dosage.

20 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), 500mg 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), 500mg 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), 500mg 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), 500mg capsule prepared as described in Example 10, in twice daily dosage

10 Gr 9: Oral administration of curcuminoids 95% 500mg capsule prepared as described in Example 16, in twice daily dosage.

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.

15 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. Walking distance refers to the maximum distance a person is able to walk at a stretch 20 without limiting pain. Walking distance measurements were recorded and are given table 13. 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.

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.

The results showed that the % response of patients taking formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio was significantly better than patients taking raw turmeric powder, essential oil of turmeric with 45% Ar-turmerone, 10 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 15 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 20 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.

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 5 change.

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.

10 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.

15 In conclusion, formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio at 500 mg capsules twice a day was 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 20 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 ratio 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.

Table 12: Joint pain measurements and % response of patients in each group over 3months

Time Points	Condition of illness	Raw turmeric	EOT with 45%Ar-t	EOT with 10-15%Ar-t	C+ E with 45% Ar-t in 1:1 ratio	C+ E with 45% Ar-t in 1:10 ratio	C 24%+ E with 45% Ar-t in 1:1 ratio	C+ E with 45% Ar-t in 10:1 ratio	C+ E with 45% Ar-t in 10:1 ratio	Curcuminoids 95%
T0 Base line	No Mild Moderate Severe	0% 20% 74% 6%	0% 22% 69% 9%	0% 15% 80% 5%	0% 18% 76% 6%	0% 13% 80% 7%	0% 20% 75% 5%	0% 20% 75% 5%	0% 14% 78% 7%	0% 13% 80% 6%
T12 End of Study	No Mild Moderate Severe	1% 18% 75% 6%	0% 23% 68% 9%	0% 14% 78% 8%	0% 16% 77% 7%	0% 14% 79% 7%	0% 21% 74% 5%	0% 22% 73% 5%	0% 17% 30% 0%	2% 16% 76% 6%

Table13: Joint line tenderness and % response of patients in each group over 3 months

Time Points	Condition of illness	Raw turmeric	EOT with 45%Ar-t	EOT with 10-15%Ar-t	C+E with 45%Ar-t in 1:10 ratio	C+E with 45%Ar-t in 1:1 ratio	C 24% + E with 45%Ar-t in 10:1 ratio	C+E with 15%Ar-t in 10:1 ratio	C+E with 45%Ar-t in 10:1 ratio	Curcuminoids 95%
T0 Base line	No Joint line tenderness	12%	15%	14%	11%	15%	11%	13%	14%	12%
	Joint line tenderness present									
	No Improv ed	13%	15%	14%	11%	15%	12%	15%	50%	14%
	Same	2%	1%	2%	1%	3%	1%	6%	50%	7%
T12 End of Study	Worsen ed	85% 0%	84% 0%	84% 0%	88% 0%	82% 0%	87% 0%	79% 0%	0% 0%	79% 0%

Table 14: – Walking distance scores and % response of patients in each group over 3 months

Time Points	Condition of illness	Raw turmeric	EOT with 45%Ar-t	EOT with 10-15%Ar-t	C+ E with 45% Ar-t in 1:10 ratio	C+ E with 45% Ar-t in 1:1 ratio	C 24% + E with 45% Ar-t in 10:1 ratio	C+ E with 15% Ar-t in 10:1 ratio	C+ E with 45% Ar-t in 10:1 ratio	Curcuminoi ds 95%
T0 Base line	<100 m	7%	9%	10%	8%	7%	9%	10%	7%	8%
	100-500 m	29%	31%	29%	35%	34%	33%	27%	37%	23%
	500-1000 m	33%	40%	38%	42%	41%	44%	45%	35%	46%
	>1000 m	31%	20%	23%	15%	18%	14%	18%	21%	23%
T12 End of Study	<100 m	7%	9%	10%	8%	6%	9%	9%	0%	7%
	100-500 m	28%	30%	29%	33%	33%	32%	25%	0%	21%
	500-1000 m	33%	41%	38%	40%	42%	44%	47%	28%	47%
	>1000 m	32%	20%	23%	15%	19%	15%	19%	72%	25%

Example 21

Human Clinical Study in patients with Alzheimers disease.

A double-blind, placebo-controlled, pilot clinical trial formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio capsules compared with raw 5 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 10 progressive decline in memory or cognitive function and diagnosed with probable or possible Alzheimer's disease (AD). Patients were randomized to 9 groups to receive 3.0 grams of each study drug capsules twice daily for 12 months.

Parameters measured at baseline and end of study include plasma isoprostanes, Vit E, A β 15 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. A β are a 39-43 amino acid peptide fragment derived from the β -amyloid precursor protein (APP) 20 and are the predominant component of the neuritic plaques, an invariant pathological hallmark of AD. Aggregated forms of A β 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.

Serum A β levels was significantly higher in formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 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 ratio capsules to disaggregate A β 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 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 are 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 ratio. Vitamin E levels increased in the formulation of curcuminoid with essential oil of turmeric with 45% Ar-turmerone in 10:1 ratio group, the values being significantly higher for the formulation of curcuminoid with essential oil of turmeric with

45% Ar-turmerone in 10:1 ratio group from a baseline at 0.30 to 2.1 at the end of treatment. (Table16). 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). 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 ratio.

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

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

Table 17: Plasma level of curcumin in patients in each group over 12 months

5

Groups		Baseline 0 month	Study End 12 months
Raw turmeric	Curcumin in nMol/L	11	20
EOT with 45%Ar-t	Curcumin in nMol/L	9	11
EOT with 10- 15%Ar-t	Curcumin in nMol/L	12	11
C+ E with 45% Ar-t in 1:10 ratio	Curcumin in nMol/L	10	13
C+ E with 45% Ar-t in 1:1 ratio	Curcumin in nMol/L	21	145
C 24% + E with 45% Ar-t in 10:1 ratio	Curcumin in nMol/L	14	28
C+ E with 10- 15% Ar-t in 10:1 ratio	Curcumin in nMol/L	15	82
C+ E with 45% Ar-t in 10:1 ratio	Curcumin in nMol/L	12	653
Curcuminoids 95%	Curcumin in nMol/L	13	42

Example 22

Human Clinical Study of patients with Depression

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 ratio was 10 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 5 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. 45 patients selected were randomized into nine groups and were given treatment for 8 weeks.

10 10 Gr 1: raw turmeric powder 500mg capsules prepared as described in Example 18 twice daily

Gr 2: essential oil of turmeric with 45% Ar-turmerone (EOT with 45% Ar-t) 500mg capsules prepared as described in Example 14 twice daily.

15 15 Gr3: essential oil of turmeric with 10-15% Ar-turmerone (EOT with 10-15% Ar-t) 500mg 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), 500mg capsules prepared as described in Example 11 twice daily

20 20 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), 500mg 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), 500mg 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), 500mg capsules prepared as described in Example 5 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 10 in Example 10 twice daily.

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).

15 Results: 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 21 to 10 at the end of treatment was higher for formulation of curcuminoid with essential oil of turmeric with 45% Ar- 20 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 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 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	% response rate on HAM-D17 scale	6%
EOT with 45%Ar-t	% response rate on HAM-D17 scale	4%
EOT with 10-15%Ar-t	% response rate on HAM-D17 scale	4%
C+ E with 45% Ar-t in 1:10 ratio	% response rate on HAM-D17 scale	7%
C+ E with 45% Ar-t in 1:1 ratio	% response rate on HAM-D17 scale	9%
C 24% + E with 45% Ar-t in 10:1 ratio	% response rate on HAM-D17 scale	8%
C+ E with 10-15% Ar-t in 10:1 ratio	% response rate on HAM-D17 scale	12%
C+ E with 45% Ar-t in 10:1 ratio	% response rate on HAM-D17 scale	93%
Curcuminoids 95%	% response rate on HAM-D17 scale	10%

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 10:1 ratio	HAM-D17 scale	21	10
Curcuminoids 95%	HAM-D17 scale	19	17

Table 20: Clinical Global Impression –Severity Scale in patients in each group over 2 months

Groups		Baseline 0 month	Study End 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 1:10 ratio	CGI-S score	5	5
C+ E with 45%Ar-t in 1:1 ratio	CGI-S score	4	4
C 24% + E with 45% Ar-t in 10:1 ratio	CGI-S score	4	4
C+ E with 10-15% Ar-t in 10:1 ratio	CGI-S score	5	5
C+ E with 45% Ar-t in 10:1 ratio	CGI-S score	5	2
Curcuminoids 95%	CGI-S score	5	5

Table 21: Clinical Global Impression – Improvement/Change Scale in patients in each group over 2 months

Groups		Baseline 0 month	Study End 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 1:10 ratio	CGI-I score	5	5
C+ E with 45% Ar-t in 1:1 ratio	CGI-I score	4	4
C 24% + E with 45% Ar-t in 10:1 ratio	CGI-I score	4	4
C+ E with 10-15% Ar-t in 10:1 ratio	CGI-I score	5	5
C+ E with 45% Ar-t in 10:1 ratio	CGI-I score	4	2
Curcuminoids 95%	CGI-I score	4	4

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.

References:

1. Kelloff, G.I., et al, *J. Cell Biochem.*, 1996, 265:54-71.
2. Rao, C.V. et al, *Cancer Res.*, 1995, 55:259-66.
3. Kawamori, T. et al, *Cancer Res.*, 1999, 59:597-601.
- 5 4. Mahmood, N.N. et al, *Carcinogenesis*, 2000, 31:921-27.
5. Subramanian, M. et al, *Mutat. Res.*, 1994, 311:249-55.
6. Tonnesen, H.H. et al, *Int. J. Pharm.*, 1992, 87:79-87.
7. Reddy, A.C.P. et al, *Mol. Cell Biochem.*, 1994, 137:1-8.
8. Donatus, I.A., *Biochem. Pharmacol.*, 1990, 39:1869-75.
- 10 9. Sharma, S.C. et al, *Biochem. Pharmacol.*, 1972, 21:1210-14.
10. Liu, J.V. et al, *Carcinogenesis*, 1993, 14:857-61.
11. Huang, T.S. et al, *Proc. Natl. Acad. Sci.*, 1991, 88:5292-96.
12. Huang, M-T. et al, In L.W. Battenberg (ed.) *Cancer Chemo prevention*, CRC Press, Boca Raton, 1992, pp375-91.
- 15 13. Huang, M. T., et al, *Cancer Res.*, 1991, 51:813-19.
14. Zhang, F. et al, *Carcinogenesis*, 1999, 20:445-51.
15. Plummer, S. et al, *Oncogene*, 1999, 18:6013-20.
16. Funk, C.D. et al, *FASEB J.*, 1991, 5:2304-12.
17. Subbaramiah, K. et al, 1996, *Cancer Res.*, 1996, 56:4424-29.
- 20 18. DuBois, R.N. et al, *J. Clin. Invest.*, 1994, 93:493-98.
19. Kelley, D.J. et al, *Carcinogenesis*, 1997, 18:795-99.
20. Huang, M.T., et al *Cancer Res.*, 1988, 48:5941-46; 1991, 51:813-19.

21. Rao, C.V. et al., *Cancer Res.*, 1995, 55:259-66.
22. Ireson, C.R. et al, *Cancer Res.*, 2001, 41:1058-64.
23. Sharma, R.A. et al, *Clin. Cancer Res.*, 2001, 7:1834-1900.
24. Pan, M.H. et al, *Drug Metabol. Dispos.*, 1999, 27:486-94.
- 5 25. Asai, A., et al, *Life Sci.*, 2000, 67:2785-93.
26. Ireson et al, loc. cit.
27. Ireson, C.R. et al, *Cancer Epidemiol. Biomark. Prev.*, 2002, 11:105-11.
- 28.3 Ravindranath, V. and Chandrasekhara, N., *Toxicology*, 1981, 20:251-57.
29. Asai et al, loc cit.
- 10 30. Ireson, C.R., et al, *Cancer Epidemiol. Biomark. Prev.*, 2002, 11:105-11.
31. Perkins, S. et al, *Cancer Epidemiol. Biomark. Prev.*, 2002, 11:535-40.
32. Limtrakul, P., et al, *Cancer Lett.*, 1997, 116:197-203.
33. Chiang, S.E. et al, *Carcinogenesis*, 2000, 21:331-35.
34. Inano, H. et al, *Carcinogenesis*, 2000, 21:1835-41; *Int. J. Radiat.Oncol. Biol. Phys.*,
15 2002, 52:212- 23; *ibid*, 2002, 53:735-43.
35. Garcea, G. et al, *Cancer Epidemiol. Biomark. Prev.*, 2005, 14:120-25.
36. Shobha et al, *Planta Med.*, 1998, 64:353-56

I claim:

1. A method of treating rheumatoid arthritis comprising administering a composition of a curcuminoid mixture and added essential oil of turmeric, wherein the weight ratio of the curcuminoid mixture to the added essential oil of turmeric ranges from about 1:3 to about 99:1, wherein the curcuminoid mixture comprises curcumin, demethoxycurcumin and bisdemethoxycurcumin, and wherein the essential oil of turmeric comprises ar-turmerone.
2. A method of reducing visual analogue scale for pain comprising administering a composition of a curcuminoid mixture and added essential oil of turmeric, wherein the weight ratio of the curcuminoid mixture to the added essential oil of turmeric ranges from about 1:3 to about 99:1, wherein the curcuminoid mixture comprises curcumin, demethoxycurcumin and bisdemethoxycurcumin, and wherein the essential oil of turmeric comprises ar-turmerone.
3. A method of decreasing disease activity score comprising administering a composition of a curcuminoid mixture and added essential oil of turmeric, wherein the weight ratio of the curcuminoid mixture to the added essential oil of turmeric ranges from about 1:3 to about 99:1, wherein the curcuminoid mixture comprises curcumin, demethoxycurcumin and bisdemethoxycurcumin, and wherein the essential oil of turmeric comprises ar-turmerone.
- 20 4. A method of improving patient response to ACR criteria comprising administering a composition of a curcuminoid mixture and added essential oil of turmeric, wherein the weight ratio of the curcuminoid mixture to the added essential oil of turmeric ranges from about 1:3 to about 99:1, wherein the curcuminoid mixture comprises curcumin,

demethoxycurcumin and bisdemethoxycurcumin, and wherein the essential oil of turmeric comprises ar-turmerone.

5. A method of reducing C-reactive protein levels comprising administering a composition of a curcuminoid mixture and added essential oil of turmeric, wherein the 5 weight ratio of the curcuminoid mixture to the added essential oil of turmeric ranges from about 1:3 to about 99:1, wherein the curcuminoid mixture comprises curcumin,

demethoxycurcumin and bisdemethoxycurcumin, and wherein the essential oil of turmeric comprises ar-turmerone.

6. A method of reducing rheumatoid Arthritis Factor comprising administering a 10 composition of a curcuminoid mixture and added essential oil of turmeric, wherein the weight ratio of the curcuminoid mixture to the added essential oil of turmeric ranges from about 1:3 to about 99:1, wherein the curcuminoid mixture comprises curcumin, demethoxycurcumin and bisdemethoxycurcumin, and wherein the essential oil of turmeric comprises ar-turmerone.

15 7. A method of decreasing joint pain comprising administering a composition of a curcuminoid mixture and added essential oil of turmeric, wherein the weight ratio of the curcuminoid mixture to the added essential oil of turmeric ranges from about 1:3 to about 99:1, wherein the curcuminoid mixture comprises curcumin, demethoxycurcumin and bisdemethoxycurcumin, and wherein the essential oil of turmeric comprises ar-turmerone.

20 9. A method of improving walking distance scores comprising administering a composition of a curcuminoid mixture and added essential oil of turmeric, wherein the weight ratio of the curcuminoid mixture to the added essential oil of turmeric ranges from about 1:3 to about 99:1, wherein the curcuminoid mixture comprises curcumin,

demethoxycurcumin and bisdemethoxycurcumin, and wherein the essential oil of turmeric comprises ar-turmerone.

10. A method of treating osteoarthritis comprising administering a composition of a curcuminoid mixture and added essential oil of turmeric, wherein the weight ratio of the curcuminoid mixture to the added essential oil of turmeric ranges from about 1:3 to about 99:1, wherein the curcuminoid mixture comprises curcumin, demethoxycurcumin and bisdemethoxycurcumin, and wherein the essential oil of turmeric comprises ar-turmerone.
11. A method of treating Alzheimer's disease comprising administering a composition of a curcuminoid mixture and added essential oil of turmeric, wherein the weight ratio of the curcuminoid mixture to the added essential oil of turmeric ranges from about 1:3 to about 99:1, wherein the curcuminoid mixture comprises curcumin, demethoxycurcumin and bisdemethoxycurcumin, and wherein the essential oil of turmeric comprises ar-turmerone.
12. A method of improving mini mental state exam scores comprising administering a composition of a curcuminoid mixture and added essential oil of turmeric, wherein the weight ratio of the curcuminoid mixture to the added essential oil of turmeric ranges from about 1:3 to about 99:1, wherein the curcuminoid mixture comprises curcumin, demethoxycurcumin and bisdemethoxycurcumin, and wherein the essential oil of turmeric comprises ar-turmerone.
- 20 13. A method of increasing Vitamin E levels comprising administering a composition of a curcuminoid mixture and added essential oil of turmeric, wherein the weight ratio of the curcuminoid mixture to the added essential oil of turmeric ranges from about 1:3 to about 99:1, wherein the curcuminoid mixture comprises curcumin, demethoxycurcumin

and bisdemethoxycurcumin, and wherein the essential oil of turmeric comprises ar-turmerone.

14. A method of increasing serum amyloid beta levels comprising administering a composition of a curcuminoid mixture and added essential oil of turmeric, wherein the weight ratio of the curcuminoid mixture to the added essential oil of turmeric ranges from about 1:3 to about 99:1, wherein the curcuminoid mixture comprises curcumin, demethoxycurcumin and bisdemethoxycurcumin, and wherein the essential oil of turmeric comprises ar-turmerone.

15. A method of disaggregating amyloid beta comprising administering a composition of a curcuminoid mixture and added essential oil of turmeric, wherein the weight ratio of the curcuminoid mixture to the added essential oil of turmeric ranges from about 1:3 to about 99:1, wherein the curcuminoid mixture comprises curcumin, demethoxycurcumin and bisdemethoxycurcumin, and wherein the essential oil of turmeric comprises ar-turmerone.

15 16. A method of lowering plasma isoprostane levels comprising administering a composition of a curcuminoid mixture and added essential oil of turmeric, wherein the weight ratio of the curcuminoid mixture to the added essential oil of turmeric ranges from about 1:3 to about 99:1, wherein the curcuminoid mixture comprises curcumin, demethoxycurcumin and bisdemethoxycurcumin, and wherein the essential oil of turmeric comprises ar-turmerone.

17. A method of treating depression comprising administering a composition of a curcuminoid mixture and added essential oil of turmeric, wherein the weight ratio of the curcuminoid mixture to the added essential oil of turmeric ranges from about 1:3 to about

99:1, wherein the curcuminoid mixture comprises curcumin, demethoxycurcumin and bisdemethoxycurcumin, and wherein the essential oil of turmeric comprises ar-turmerone.

18. A method of improving response rate on Hamilton Depression rate scale comprising administering a composition of a curcuminoid mixture and added essential oil of turmeric, wherein the weight ratio of the curcuminoid mixture to the added essential oil of turmeric ranges from about 1:3 to about 99:1, wherein the curcuminoid mixture comprises curcumin, demethoxycurcumin and bisdemethoxycurcumin, and wherein the essential oil of turmeric comprises ar-turmerone.

19. A method of improving clinical global impression by Global Severity comprising administering a composition of a curcuminoid mixture and added essential oil of turmeric, wherein the weight ratio of the curcuminoid mixture to the added essential oil of turmeric ranges from about 1:3 to about 99:1, wherein the curcuminoid mixture comprises curcumin, demethoxycurcumin and bisdemethoxycurcumin, and wherein the essential oil of turmeric comprises ar-turmerone.

15 20. A method of improving clinical global impression by Global Change scale comprising administering a composition of a curcuminoid mixture and added essential oil of turmeric, wherein the weight ratio of the curcuminoid mixture to the added essential oil of turmeric ranges from about 1:3 to about 99:1, wherein the curcuminoid mixture comprises curcumin, demethoxycurcumin and bisdemethoxycurcumin, and wherein the 20 essential oil of turmeric comprises ar-turmerone.

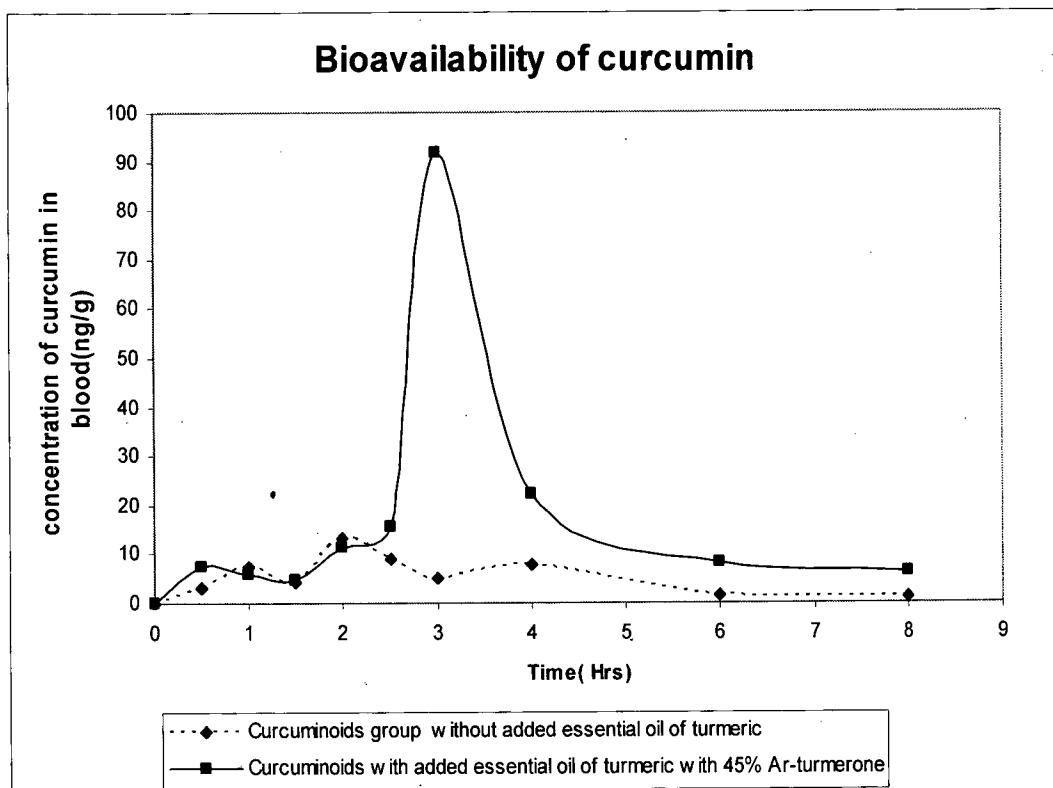
Fig 1.

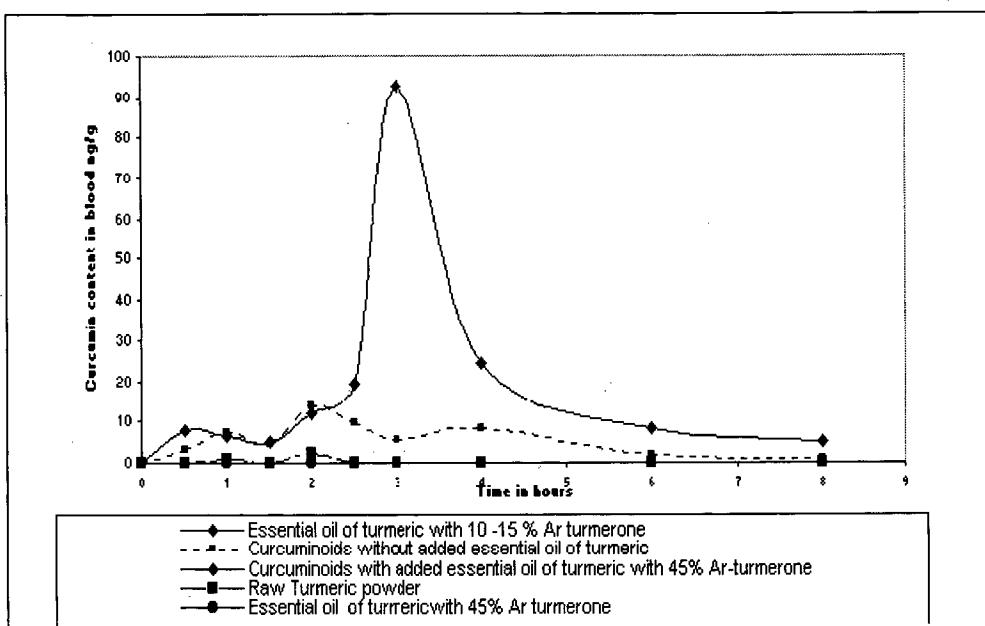
Fig:2

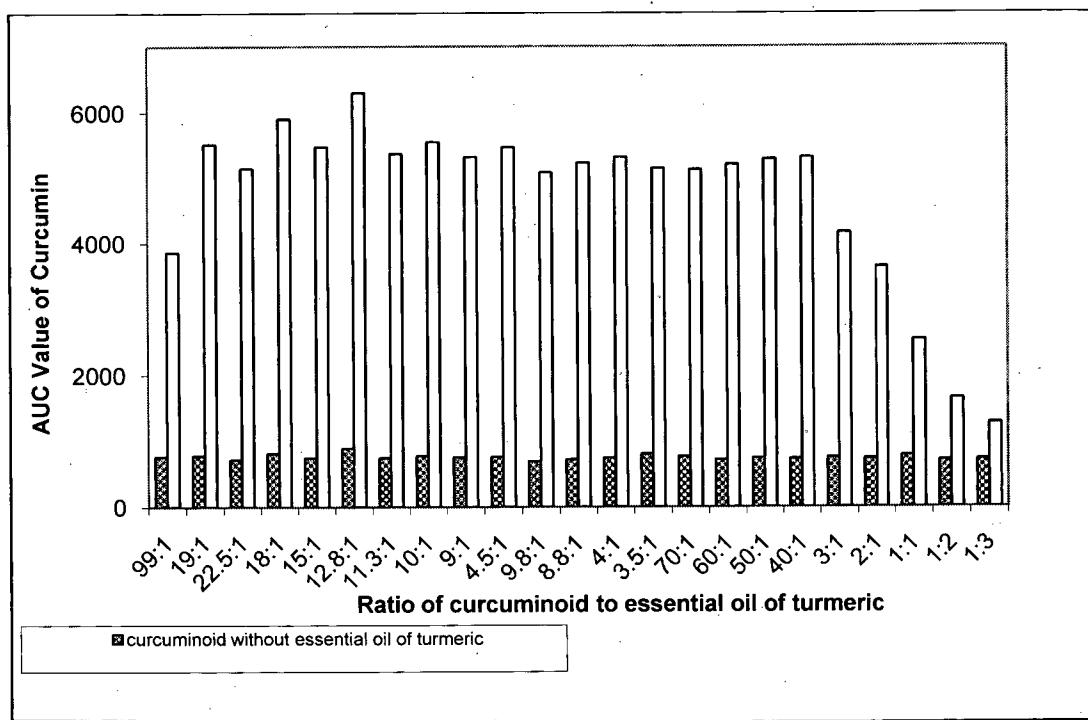
Fig: 3

Fig: 4

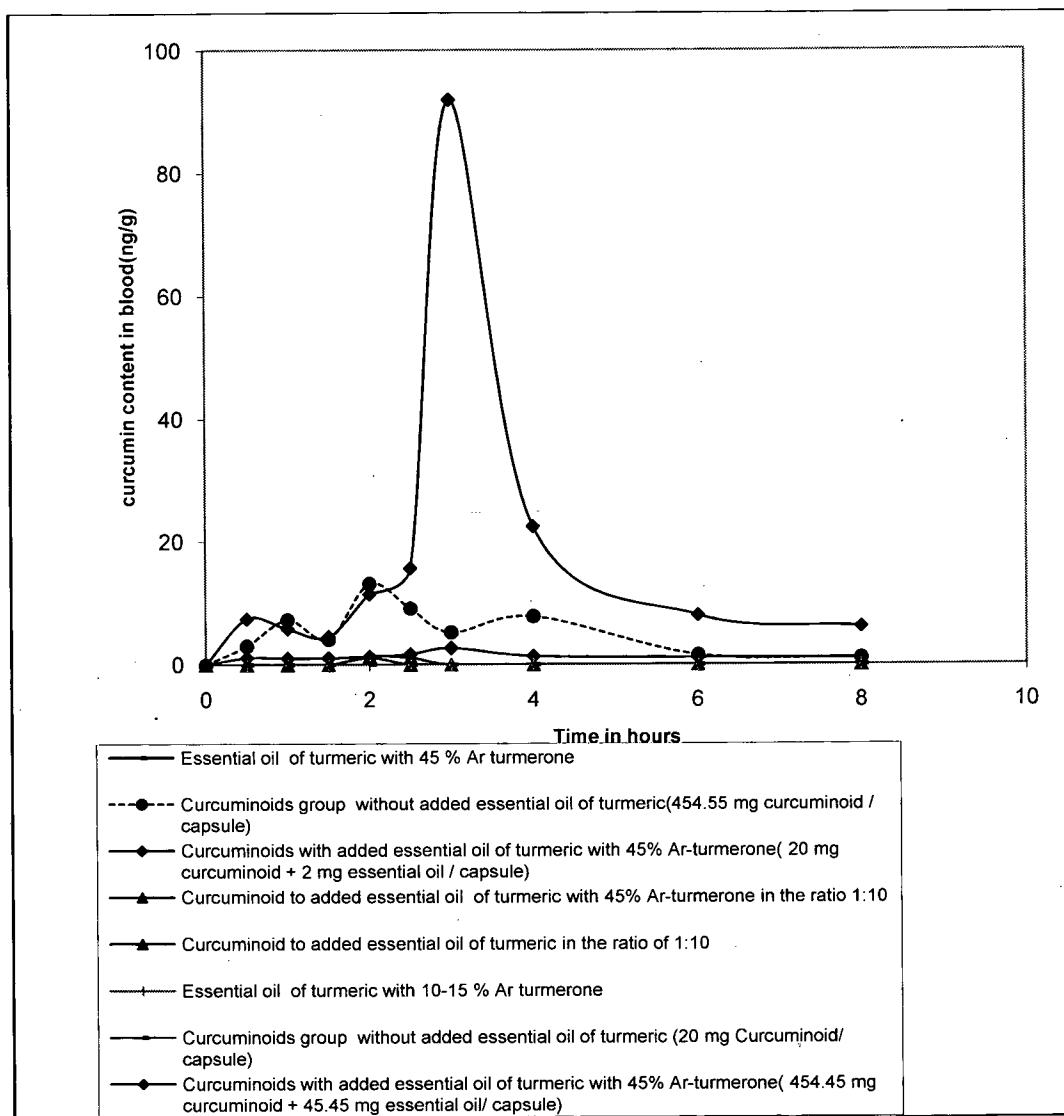


Fig: 5: Method of preparation of Essential oil of turmeric with varying concentration of Ar-turmerone.

