

ENRICHED WATER FORMULATIONS AND PROCESSES FOR PREPARATION THEREOF

RELATED PATENT APPLICATION(S)

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This application claims the priority to and benefit of Indian Patent Application No. 201841040967 filed on January 30, 2019; the disclosures of which are incorporated herein by reference.

10 FIELD OF THE INVENTION

The present invention pertains to the field of nutritionally enriched beverages. More particularly, the invention relates to enriched water formulations comprising herbal extracts, vitamin D and essential oils.

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BACKGROUND OF THE INVENTION

High paced lifestyles of people have led to a major shift in beverage consumption habits. People are increasingly dependent upon packaged beverages, which are not only nutritionally poor but
20 contains free sugars and unhealthy preservatives.

Regular consumption of such sweetened beverages is associated with unhealthy dietary nutrient profiles which leads to several diet-related diseases such as obesity, type 2 diabetes, heart disease and other chronic diseases.

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In view of the disadvantages of sweetened beverages, water remains the best beverage choice for replenishing the body fluids.

However, modern packaged drinking water is subjected to several treatments such as reverse
30 osmosis and distillation to remove contaminants and pathogens. Though processes like reverse osmosis and distillation remove contaminants and pathogens, these processes also remove all dissolved solids from water, including critical micronutrients and minerals necessary for important bodily functions.

The inventors have identified the above challenges and have addressed the same by developing a nutritionally enriched water formulation composition comprising herbal extracts, vitamin D3 and essential oils, having an enhanced shelf life. The invention would cater to the beverage preference for packaged drinking water while supplying daily requirements of essential
5 micronutrients and nutritional supplements for maintaining a balanced nutritional profile.

Packaged sweetened beverages, as well as packaged drinking water, cannot supply recommended requirements of essential micronutrients and nutritional supplements. So, there is a need for packaged drinking water having an enhanced nutritional profile and enhanced
10 shelf life.

The problem has been solved by a multi-dimensional approach, which involves developing enriched water formulation for supplying recommended requirements of essential micronutrients and nutritional supplements.
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OBJECTIVE OF THE INVENTION

The main objective of the present invention is to provide enriched water formulation comprising herbal extracts, vitamin D3 and essential oils.
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Another objective of the present invention is to provide method for preparation of water formulation comprising herbal extracts, vitamin D3 and essential oils.

Yet another objective of the invention is to provide enriched water formulation having longer
25 shelf life and safe for human consumption.

SUMMARY OF THE INVENTION

The present invention provides for enriched water formulations comprising herbal extracts such
30 as *Ocimum sanctum* (Linn) extract, *Withania somnifera* (L) extract, *Tinospora cordifolia* (Thunb) Miers extract, *Garcinia cambogia* (L) extract and *Bacopa monnieri* (L) extract, vitamin D3 nanogels and essential oils such as rosemary oil, spearmint oil, menthol mint oil and cardamom oil. The enriched water formulation supplies recommended requirements of

essential micronutrients and nutritional supplements for maintaining a balanced nutritional profile.

5 In one aspect of the invention, there is provided a water formulation comprising a herbal composition, Vitamin D3 and an essential oil composition.

10 In some embodiment, the herbal composition in the water formulation according to invention comprises *Ocimum sanctum* extract, *Withania somnifera* extract, *Tinospora cordifolia* extract, *Garcinia cambogia* extract, *Bacopa monnieri* extract or mixture thereof.

In some other embodiment, the essential oil in the water formulation according to invention comprises rosemary oil, spearmint oil, menthol mint oil, cardamom oil or mixture thereof.

15 In yet another embodiment, the *Ocimum sanctum* extract, *Withania somnifera* extract, *Tinospora cordifolia* extract, *Garcinia cambogia* extract and *Bacopa monnieri* extract are each present in the water formulation according to invention at a concentration in the range from 0.25 g/L to 2.28 g/L.

20 In some embodiment, the vitamin D3 in the water formulation according to invention is present at a concentration in the range from 1012 IU/L to 6072 IU/L.

25 In some embodiments, the rosemary oil, spearmint oil, menthol mint oil and cardamom oil are each present in the essential oil composition at a concentration in the range from 1.26 μ L/L to 11.38 μ L/L.

In some embodiment, each of *Ocimum sanctum* extract, *Withania somnifera* extract, *Tinospora cordifolia* extract, *Garcinia cambogia* extract and *Bacopa monnieri* extract is present in the herbal composition at a concentration range of 7% w/w to 60% w/w.

30 In some other embodiment, each of rosemary oil, spearmint oil, menthol mint oil and cardamom oil are present in the essential oil composition at a concentration range of 8% v/v to 25% v/v.

In another aspect, there is provided a method for preparation of water formulation comprising a herbal composition, Vitamin D3 and an essential oil composition, said method comprising

- a. preparing an herbal composition, wherein the herbal composition comprises *Ocimum sanctum* extract, *Withania somnifera* extract, *Tinospora cordifolia* extract, *Garcinia cambogia* extract and *Bacopa monnieri* extract;
- b. preparing a vitamin D3 nanogel;
- c. preparing an essential oil composition, wherein the essential oil composition comprises rosemary oil, spearmint oil, menthol mint oil and cardamom oil;
- d. homogenizing the mixture of herbal composition obtained in step a), vitamin D3 nanogel obtained in step b) and essential oil composition obtained in step c) to obtain composite composition; and
- e. dissolving the composite composition obtained in step (d) in water to obtain a water formulation.

In some embodiment, the *Ocimum sanctum* extract, *Withania somnifera* extract, *Tinospora cordifolia* extract, *Garcinia cambogia* extract or *Bacopa monnieri* extract as used in the above described process for the preparation of water formulation are prepared by a method comprising:

- a. treating raw *Ocimum sanctum*, *Withania somnifera*, *Tinospora cordifolia*, *Garcinia cambogia* or *Bacopa monnieri* with methanol-water solvent, wherein methanol and water are present at a ratio of 7:3 to obtain a supernatant containing the extract; and
- b. evaporating the solvent from the supernatant to obtain *Ocimum sanctum* extract, *Withania somnifera* extract, *Tinospora cordifolia* extract, *Garcinia cambogia* extract or *Bacopa monnieri* extract.

In some embodiment, the treatment with methanol-water solvent in the above described extraction process is carried at a temperature in a range from 30°C to 50°C. In some other embodiment, the treatment with methanol-water solvent is carried for a period in a range from 0.5 hours to 3.5 hours.

In some other embodiment, in the method for preparation of water formulation according to invention each of *Ocimum sanctum* extract, *Withania somnifera* extract, *Tinospora cordifolia* extract, *Garcinia cambogia* extract and *Bacopa monnieri* extract is present in the herbal composition at a concentration range of 7% w/w to 60% w/w.

In some embodiment, in the method for preparation of water formulation according to invention each of rosemary oil, spearmint oil, menthol mint oil and cardamom oil are present in the essential oil composition at a concentration range of 8% v/v to 25% v/v.

- 5 In some other embodiment, in the method for preparation of water formulation according to invention vitamin D3 is present in the water formulation at a concentration in the range from 1012 IU/L to 6072 IU/L.

BRIEF DESCRIPTION OF THE DRAWINGS

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Figure 1: Flow diagram of various formulations and complexes of vitamin D and Plant extracts enriched water

DETAILED DESCRIPTION OF THE INVENTION

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Embodiments described herein can be understood more readily by reference to the following detailed description, examples, and drawings. Elements, apparatus and methods described herein are merely illustrative of the principles of the present invention and are not limited to the specific embodiments presented in the detailed description, examples, and drawings.

- 20 Numerous modifications and adaptations will be readily apparent to those of skill in the art without departing from the spirit and scope of the invention.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the methods belong.

- 25 Although any water formulations, compositions or methods similar or equivalent to those described herein can also be used in the practice or testing of the embodiments of the present invention, representative illustrative methods and compositions are now described.

- 30 Where a range of values is provided, it is understood that each intervening value between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within by the methods and compositions. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges and are also encompassed within by the methods and compositions, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges

excluding either or both of those included limits are also included in the methods and compositions.

5 It is appreciated that certain features of the methods, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the methods and compositions, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination. It is noted that, as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates
10 otherwise. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely," "only" and the like in connection with the recitation of claim elements or use of a "negative" limitation.

15 As will be apparent to those of skill in the art upon reading this disclosure, each of the individual embodiments described and illustrated herein has discrete components and features which may be readily separated from or combined with the features of any of the other embodiments without departing from the scope or spirit of the present methods. Any recited method can be carried out in the order of events recited or in any other order that is logically
20 possible.

DEFINITIONS

The term "nanogel" or "Vitamin D3 nanogel" means a vitamin D3 particle prepared with one
25 or more polymeric component having any shape with an equivalent diameter of approximately a few to about 100 nm as described in Indian Patent Application No. 201841027257 entitled "A novel aqueous composition with water-insoluble vitamins". Vitamin D3 nanogels are typically soluble in the solvent in which they are made and nanogels may be further made to be soluble in various liquids as necessary depending on the monomers or polymers used in
30 their manufacture.

The term "water formulation" as used herein means a formulation comprising herbal composition, vitamin D3 nanogels and essential oil composition as described herein.

The term “herbal composition” as used herein is referred to a composition comprising bioactive fractions or extracts of one or more plant or plant parts of *Ocimum sanctum* (Linn), *Withania somnifera* (L), *Tinospora cordifolia* (Thunb) Miers, *Garcinia cambogia* (L) and *Bacopa monnieri* (L), as described herein. The “herbal composition” as used herein refers to both
5 lyophilized or liquid plant extract.

The term “essential oil” or “oil” or “essential oil composition” encompasses all botanical oils, lipids and active ingredients present in such oils, polyphenols, aldehydes, terpenes and lipids, as described herein. The term may also mean the individual active ingredient of the botanical
10 oils and lipids which can be defined chemically and synthesized using commercially available reagents, without the use of natural products or extracts. As used herein, essential oils may be extracted from one or more species such as *Rosmarinus officinalis* (L), *Mentha spicata* (L), *Mentha arvensis* (L) and *Elettaria cardamomum* (Maton).

15 The term “composite composition” as used herein refers to a composition comprising herbal composition, vitamin D3 nanogels and essential oil composition, as described herein. The composite composition is dissolved in water to obtain water formulations of the present invention.

20 The present invention discloses water formulations and methods for the effective preparation of water formulations. In particular, the invention discloses water formulations comprising herbal extracts, vitamin D nanogels and essential oils.

The invention contemplates a multidimensional approach in the development of highly
25 efficacious and cost-effective water formulations which can be used for supplying essential micronutrients. The water formulations of the present invention overcome the problems of the prior art and can be used for supplying recommended requirements of essential micronutrients and nutritional supplements for maintaining a balanced nutritional profile.

30 The water formulations of the present invention are characterized by the following advantages:
(a) Supply of essential micronutrients and nutritional supplements: The water formulations can be used for supplying recommended requirements of essential micronutrients and nutritional supplements, which are absent in commercially available beverages such as sweetened drinks and packaged drinking water.

(b) Improved shelf-life/stability: The water formulations comprising essential micronutrient and nutritional supplement retains essential properties and have a considerable shelf life.

5 (c) No alteration in organoleptic properties: The water formulations, even while containing essential herbal extracts, vitamin D nanogels and essential oil, have been formulated in such a manner that there is no distinctive taste. A person consuming the water formulation cannot differentiate it from packaged drinking water.

10 Before the water formulations and methods of the present disclosure are described in greater detail, it is to be understood that the invention is not limited to particular embodiments and may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

15 In one embodiment, the invention provides a water formulation comprising an herbal composition, Vitamin D3 and an essential oil composition.

In another embodiment, the herbal composition comprises extracts obtained from one or more plants.

20 In another embodiment, the one or more plants used for preparing the herbal composition are selected from, but not limited to, *Ocimum sanctum*(Linn) (Basil), *Withania somnifera* (L) (Ashwagandha/ Winter cherry), *Tinospora cordifolia* (Thunb) Miers (Guduchi), *Garcinia cambogia*(L) (Malabar tamarind) and *Bacopa monnieri* (L) (Brahmi). In some embodiment, the herbal composition in the water formulation according to invention comprises *Ocimum*
25 *sanctum* extract, *Withania somnifera* extract, *Tinospora cordifolia* extract, *Garcinia cambogia* extract, *Bacopa monnieri* extract or mixture thereof.

30 In some embodiment, the plant extract is lyophilised to make dry powder form. In some other embodiment, the herbal composition is lyophilized plant extract. The percentage of extracts yield is calculated on dry weight basis.

Ashwagandha (*Withania somnifera*) or commonly known as winter cherry. Its roots and flowers have been major source of health benefits. It has been historically known to treat problems like arthritis, diabetes, gastro-intestinal problems, fevers, nervous breakdown and

snake bites etc. Modern studies have revealed that it is also useful in treating the problems like anxiety, depression, heart diseases and impeding the growth of cancerous cell.

Garcinia cambogia (Malabar tamarind) is a fruit that enhances the culinary experience of food.

5 It is also used as fat burner.

Tinospora cordifolia (Guduchi) is used to enhance vitality. It is useful for a variety of health effects, including its effect on diabetes, glucose metabolism, inflammation, immune system support, and neurology.

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Bacopa monnieri (Bramhi) is an herb improves cognition, by means of reducing anxiety. It is also reliable for improving memory formation. *B. monnieri* appears to affect young people as well, making it a useful nootropic.

15 *Asparagus racemosus* (sathavari) aids in digestion, since the plant has anti-ulcer effects. It also aids the immune system when antibodies are fighting off a threat i.e. antigens. *A. racemosus* also has aphrodisiac, antidepressant and anxiety-reducing effects. *A. racemosus* has been used as a galactagogue to increase breast milk production.

20 *Ocimum sanctum* (Holy Basil) is used to treat a variety of general ailments. It recently has been shown to hold scientific worth in the areas of liver protection and general anti-oxidant activity, as well as being classified as an adaptogen.

In another embodiment, the one or more plant extracts are present in the water formulation at
25 a concentration in the range from about 0.25 g/L to 0.5 g/L, about 0.5 g/L to 0.75 g/L, about 0.75 g/L to 1.0 g/L, about 1.0 g/L to 1.25 g/L, about 1.25 g/L to 1.5 g/L, about 1.5 g/L to 1.75 g/L, about 1.75 g/L to 2.0 g/L, about 2.0 g/L to 2.25 g/L, about 2.25 g/L to 2.5 g/L.

In yet another embodiment, the *Ocimum sanctum* extract, *Withania somnifera* extract,
30 *Tinospora cordifolia* extract, *Garcinia cambogia* extract and *Bacopa monnieri* extract are each present in the water formulation according to invention at a concentration in the range from 0.25 g/L to 2.28 g/L.

In some embodiment, each of *Ocimum sanctum* extract, *Withania somnifera* extract, *Tinospora cordifolia* extract, *Garcinia cambogia* extract and *Bacopa monnieri* extract is present in the herbal composition at a concentration range of 7% w/w to 60% w/w.

- 5 In another embodiment, the one or more plant extracts are present in the herbal composition at a weight percentage from about 5% to 10%, about 10% to 15%, about 15% to 20%, about 20% to 25%, 25% to 30%, about 30% to 35%, about 35% to 40%, about 40% to 45%, about 45% to 50%, about 50% to 55%, about 55% to 60%, about 60% to 65%.

- 10 In another embodiment, plant extracts of the present invention can be prepared by suitable solvent extraction methods known in the art.

In another embodiment, the plant extracts are prepared by subjecting plants or plant parts to solvent extraction using methanol-water as a solvent.

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In another embodiment, the methanol-water solvent comprises methanol and water in the ratio from 1:2 to 3:1, preferably 7:3.

- 20 In another embodiment, the plant or plant part and methanol-water solvent are present at a weight ratio in the range from 1:10 to 2:5, preferably 1:5, 1:4 or 1:3 during treatment.

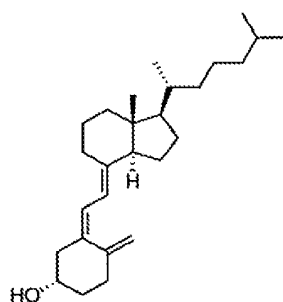
In another embodiment, the temperature of the reaction mixture during the solvent extraction treatment is maintained at a range from about 30°C to 35°C, 35°C to 40°C, 40°C to 45°C, 45°C to 50°C.

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In another embodiment, the solvent extraction treatment is done for a period in a range from about 0.5 hours to 1 hour, about 1 hour to 1.5 hours, about 1.5 hours to 2 hours, about 2 hours to 2.5 hours, about 2.5 hours to 3 hours, about 3 hours to 3.5 hours.

- 30 In another embodiment, the plant extract is subjected to a treatment in a rotary vacuum evaporator to evaporate the methanol-water solvent and obtain a plant extract.

In another embodiment, the water formulation comprises vitamin D3 nanogel.

**Vitamin D3**

Vitamin D is a fat soluble and its major functions are the increase of intestinal absorption of calcium and promotion of normal bone formation and mineralization. Vitamin D has evidenced that it has great importance and is linked with a wide range of diseases, such as cardiovascular diseases, diabetes, rickets, osteoporosis, osteomalacia and secondary hyperparathyroidism and cancer. Vitamin D cannot be directly dispersed into an aqueous solution. Instead, it needs to be combined into an appropriate colloidal system prior to dispersion. In order to explore for targeting purpose and also to improve Vitamin D bioavailability it is necessary to review the carrier systems to perform a sustainable release.

Encapsulation method is used as a preferred process to capture active agents, i.e. vitamins, into a carrier material, as a way to improve and protect bioactive molecules for further delivery. The advantages of encapsulating drugs in nanocarriers comprise an increase in the dissolution rate of hydrophobic or drugs with poor solubility. In some embodiment, vitamin D3 in the water formulation according to invention is present either as micelles or nano-gels or both along with various other components such as emulsifiers and salts.

In one embodiment, the method for preparation of Vitamin D3 nanogel is provided in Indian Patent Application No. 201841027257 entitled "A novel aqueous composition with water-insoluble vitamins".

In another embodiment, the Vitamin D3 nanogels in the formulation comprises vitamin D3 crosslinked with agar-agar and another suitable monomer or polymer such as poly(acrylamidoglycolic) acid, pectin and the like using a suitable cross-linker such as methylene bisacrylamide and glutaraldehyde.

In another embodiment, Vitamin D3 nanogels are present in the water formulation at a concentration in the range from about 1000 IU/L to 1500 IU/L, about 1500 IU/L to 2000 IU/L, 2000 IU/L to 2500 IU/L, about 2500 IU/L to 3000 IU/L, about 3000 IU/L to 3500 IU/L, about 3500 IU/L to 4000 IU/L, about 4000 IU/L to 4500 IU/L, about 4500 IU/L to 5000 IU/L, about
5 5000 IU/L to 5500 IU/L, about 5500 IU/L to 6000 IU/L.

In some embodiment, the vitamin D3 in the water formulation according to invention is present at a concentration in the range from 1012 IU/L to 6072 IU/L.

10 In another embodiment, the water formulation comprises one or more essential oil or active ingredient extracted from *Rosmarinus officinalis* (L), *Mentha spicata* (L), *Mentha arvensis* (L) and *Elettaria cardamomum* (L), and the like.

In one embodiment, the essential oil is Rosemary oil from *Rosmarinus officinalis* (L).
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In another embodiment, the essential oil is spearmint oil from *Mentha spicata* (L).

In another embodiment, the essential oil is menthol mint oil from *Mentha spicata* (L).

20 In another embodiment, the essential oil is cardamom oil from *Elettaria cardamomum* (L)

In some embodiment, the water formulation according to invention comprises essential oil composition comprising rosemary oil, spearmint oil, menthol mint oil, cardamom oil or mixture thereof.
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In another embodiment, each essential oil is present in the essential oil composition at a concentration of area % range of about 6-8% v/v, about 8-10% v/v, about 10-12% v/v, about 12-14% v/v, about 14-16% v/v, about 16-18% v/v, 18 -20% v/v, about 20-22% v/v, about 22-24% v/v, about 24-26% v/v. In some other embodiment, each of rosemary oil, spearmint oil,
30 menthol mint oil and cardamom oil are present in the essential oil composition at a concentration range of 8% v/v to 25% v/v.

In some embodiments, the rosemary oil, spearmint oil, menthol mint oil and cardamom oil are each present in the essential oil composition at a concentration in the range from 1.26 $\mu\text{L/L}$ to 11.38 $\mu\text{L/L}$.

- 5 Extraction method is also an essential element to improve the total yield and quality of essential oil. Commonly used extraction methods are hydro distillation, hydro and steam distillation, and steam distillation. Plant parts which were having aromatic oils were used to extract the essential oils. The essential oils were dried over anhydrous sodium sulphate and stored at 4°C until used for the formulation.

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In other embodiments, the essential oil compositions may further comprise acceptable carriers or excipient or may be microencapsulated, as may be deemed suitable.

In another aspect, there is provided a method for preparation of water formulation comprising
15 a herbal composition, Vitamin D3 and an essential oil composition, said method comprising

- a. preparing an herbal composition, wherein the herbal composition comprises *Ocimum sanctum* extract, *Withania somnifera* extract, *Tinospora cordifolia* extract, *Garcinia cambogia* extract and *Bacopa monnieri* extract;
- b. preparing a vitamin D3 nanogel;
- 20 c. preparing an essential oil composition, wherein the essential oil composition comprises rosemary oil, spearmint oil, menthol mint oil and cardamom oil;
- d. homogenizing the mixture of herbal composition obtained in step a), vitamin D3 nanogel obtained in step b) and essential oil composition obtained in step c) to obtain composite composition; and
- 25 e. dissolving the composite composition obtained in step (d) in water to obtain a water formulation.

In some embodiment, the *Ocimum sanctum* extract, *Withania somnifera* extract, *Tinospora cordifolia* extract, *Garcinia cambogia* extract or *Bacopa monnieri* extract as used in the above
30 described process for the preparation of water formulation are prepared by a method comprising:

- a. treating raw *Ocimum sanctum*, *Withania somnifera*, *Tinospora cordifolia*, *Garcinia cambogia* or *Bacopa monnieri* with methanol-water solvent, wherein methanol and water are present at a ratio of 7:3 to obtain a supernatant containing the extract; and

- b. evaporating the solvent from the supernatant to obtain *Ocimum sanctum* extract, *Withania somnifera* extract, *Tinospora cordifolia* extract, *Garcinia cambogia* extract or *Bacopa monnieri* extract.

5 In some embodiment, the treatment with methanol-water solvent in the above described extraction process is carried at a temperature in a range from 30°C to 50°C. In some other embodiment, the treatment with methanol-water solvent is carried for a period in a range from 0.5 hours to 3.5 hours.

10 In some other embodiment, in the method for preparation of water formulation according to invention each of *Ocimum sanctum* extract, *Withania somnifera* extract, *Tinospora cordifolia* extract, *Garcinia cambogia* extract and *Bacopa monnieri* extract is present in the herbal composition at a concentration range of 7% w/w to 60% w/w.

15 In some embodiment, in the method for preparation of water formulation according to invention each of rosemary oil, spearmint oil, menthol mint oil and cardamom oil are present in the essential oil composition at a concentration range of 8% v/v to 25% v/v.

In some other embodiment, in the method for preparation of water formulation according to
20 invention vitamin D3 is present in the water formulation at a concentration in the range from 1012 IU/L to 6072 IU/L.

In one embodiment, the method for extraction of essential oils comprises subjecting the raw *Rosmarinus officinalis* (L), *Mentha spicata* (L), *Mentha arvensis* (L) and *Elettaria*
25 *cardamomum* (L) to hydro-distillation, followed by drying.

In another embodiment, the hydro distillation is performed using a Clevenger apparatus for a period in the range from about 3 to 5 hours.

30 In another embodiment, the essential oils collected in Clevenger apparatus were dried over anhydrous sodium sulphate.

In another embodiment, a composite composition comprising herbal extracts, vitamin D3 and essential oils is prepared.

In another embodiment, the method for preparation of the composite composition comprises homogenizing the herbal extracts, vitamin D3 and essential oils.

- 5 In another embodiment, the composite composition is dissolved in water to prepare the water formulation.

In some embodiment, the essential oil composition is first mixed with the lyophilised powder or liquid herbal extract or vitamin D3 nanogel.

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In another embodiment, the composite composition can also be used for fortifying any beverage or food, other than drinking water. The usage of the composite composition does not result in the development of undesirable colour, solubility or flavour. The composite composition is completely dispersed in water and does not result in any changes in organoleptic properties.

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The water formulation of the present invention can be prepared from a variety of water source. Most preferred sources are deionized water, softened water, water treated by commercially available reverse osmosis processes or distilled water.

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In one embodiment, the water formulation of the present invention may be optionally carbonated to suit the requirements of carbonated, also known as sparkling water.

- 25 In another embodiment, the water formulation of the present invention may be formulated as an alkaline water formulation comprising an herbal composition, Vitamin D3 and an essential oil composition.

- 30 In one embodiment, small amounts of colouring agents can be optionally used in the water formulation. Such coloring agents are added to the water for aesthetic reasons only. The exact amount of colouring agent used will vary, depending on the agents used and the intensity desired in the finished product. The amount can be readily determined by one skilled in the art.

In another embodiment, the water formulation can optionally comprise a flavouring agent or sweetener consisting of any natural or synthetically prepared fruit or botanical flavours or with

mixtures of botanical flavours and fruit juice blends. Such flavouring agents or sweeteners are added to the water for aesthetic reasons only. The amount can be readily determined by one skilled in the art.

- 5 Various combinations of stabilizers were also added to enhance shelf-life of the mineralised water. These stabilizers include rosemary, and flavouring agents vetiver, patchouli, geranium, palmarosa, menthol mint, spear mint, cardomomum.

10 The water formulation according to invention provides a stable and healthy mineralized water comprising plant extracts, essential oils and vitamin D3 nanogel.

Formulation also includes emulsifiers, salts, sweetening agents i.e., natural sweetening agents and artificial sweetening agents, antioxidants, colours etc. A flow diagram of various formulations is depicted in Figure 1.

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In another embodiment, the invention provides a method for preventing or treating micronutrient deficiency by the administration of a therapeutically effective amount of water formulation as disclosed herein.

- 20 In reference to the treatment or prevention of micronutrient deficiency, an effective amount refers to that amount which has the effect of reducing or inhibiting (that is, slowing to some extent, preferably stopping) one or more signs or symptoms characterizing micronutrient deficiency.

- 25 In certain embodiments, water formulations may be administered in one or more dosage forms.

Those skilled in the art will be able to determine, according to known methods, the appropriate amount, dose or dosage of the formulation for administration to a subject taking into account factors such as age, weight, general health, the compositions administered, the route of
30 administration, the nature and advancement of the micronutrient deficiency requiring treatment, and the presence of other medications.

The water formulations may be administered by oral route. In certain embodiments, the water formulations are administered at a pre-determined daily dosage. This dosage regimen may be

adjusted to provide the optimal therapeutic response. For example, the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation.

5 The practice of the method of this invention may be accomplished through various administration or dosing regimens. The water formulations of the present invention can be administered intermittently, concurrently or sequentially with other prescribed pharmaceutical compositions.

10 Repetition of the administration or dosing regimens may be conducted as necessary to achieve the sufficiency of micronutrient levels in the body.

EXAMPLES

Example 1: Preparation of extract from *Ocimum sanctum* (Linn)

15 For preparing an extract of *Ocimum sanctum* (Linn) (basil), 100 grams healthy leaves were treated with a methanol-water solvent.

20 To prepare the solvent, methanol and water were mixed at a ratio of 7:3. The solvent was added to the leaves at leaves to solvent ratio of 1:5 and the treatment was done in a maceration and soxhlet apparatus for a period of 2.5 hours at a temperature of 40°C.

The supernatant was collected and stored separately. The residue was treated at 40°C for a period of 1.5 hours with the methanol-water solvent at a residue to solvent ratio of 1:4.

25 The supernatant was collected again and stored separately. The residue was treated at 40°C for a period of one hour with the methanol-water solvent at a residue to solvent ratio of 1:3 and the supernatant was collected.

30 The supernatant was subjected to a rotary vacuum evaporator to evaporate the solvent and obtain a semi-solid soft extract of *Ocimum sanctum* weighing about 20 grams.

Example 2: Preparation of extract from *Withania somnifera* (L)

For preparing an extract of *Withania somnifera* (L) (Ashwagandha/ winter cherry), 100 grams raw *Withania somnifera* was treated with a methanol-water solvent.

To prepare the solvent, methanol and water were mixed at a ratio of 7:3. The solvent was added
5 to the leaves at leaves to solvent ratio of 1:5 and the treatment was done in a maceration and soxhlet apparatus for a period of 2.5 hours at a temperature of 40°C.

The supernatant was collected and stored separately. The residue was treated at 40°C for a period of 1.5 hours with the methanol-water solvent at a residue to solvent ratio of 1:4.
10 The supernatant was collected again and stored separately. The residue was treated at 40°C for a period of one hour with the methanol-water solvent at a residue to solvent ratio of 1:3 and the supernatant was collected.

The supernatant was subjected to a rotary vacuum evaporator to evaporate the solvent and
15 obtain a semi-solid soft extract of *Withania somnifera* weighing about 20 grams.

Example 3: Preparation of extract from *Tinospora cordifolia* (Thunb) Miers

For preparing an extract of *Tinospora cordifolia* (Thunb) Miers (Guduchi), 100 grams raw
20 *Tinospora cordifolia* was treated with a methanol-water solvent.

To prepare the solvent, methanol and water were mixed at a ratio of 7:3. The solvent was added
to the leaves at leaves to solvent ratio of 1:5 and the treatment was done in a maceration and soxhlet apparatus for a period of 2.5 hours at a temperature of 40°C.
25

The supernatant was collected and stored separately. The residue was treated at 40°C for a period of 1.5 hours with the methanol-water solvent at a residue to solvent ratio of 1:4.

The supernatant was collected again and stored separately. The residue was treated at 40°C for
30 a period of one hour with the methanol-water solvent at a residue to solvent ratio of 1:3 and the supernatant was collected.

The supernatant was subjected to a rotary vacuum evaporator to evaporate the solvent and obtain a semi-solid soft extract of *Tinospora cordifolia* weighing about 20 grams.

Example 4: Preparation of extract from *Garcinia cambogia* (L)

For preparing an extract of *Garcinia cambogia* (L), 100 grams raw *Garcinia* rind was treated with a methanol-water solvent.

5

To prepare the solvent, methanol and water were mixed at a ratio of 7:3. The solvent was added to the leaves at leaves to solvent ratio of 1:5 and the treatment was done in a maceration and soxhlet apparatus for a period of 2.5 hours at a temperature of 40°C.

The supernatant was collected and stored separately. The residue was treated at 40°C for a period of 1.5 hours with the methanol-water solvent at a residue to solvent ratio of 1:4.

10

The supernatant was collected again and stored separately. The residue was treated at 40°C for a period of one hour with the methanol-water solvent at a residue to solvent ratio of 1:3 and the supernatant was collected.

15

The supernatant was subjected to a rotary vacuum evaporator to evaporate the solvent. The aqueous extract was precipitated with calcium hydroxide. Thereafter, the pH was adjusted by dissolving the extract in aqueous potassium hydroxide.

Thereafter, the extract was subjected to activated charcoal at a temperature of 80°C and stirred to filter the concentrate. The filtrate was spray-dried and subjected to calcium potassium double salt (HCA salt) to obtain a powder extract of *Garcinia cambogia* weighing about 20 grams.

20

Example 5: Preparation of extract from *Bacopa monnieri* (L)

25

For preparing an extract of *Bacopa monnieri* (L) (Brahmi), 100 grams raw *Bacopa monnieri* was treated with a methanol-water solvent.

To prepare the solvent, methanol and water were mixed at a ratio of 7:3. The solvent was added to the leaves at leaves to solvent ratio of 1:5 and the treatment was done in a maceration and soxhlet apparatus for a period of 2.5 hours at a temperature of 40°C.

30

The supernatant was collected and stored separately. The residue was treated at 40°C for a period of 1.5 hours with the methanol-water solvent at a residue to solvent ratio of 1:4.

The supernatant was collected again and stored separately. The residue was treated at 40°C for a period of one hour with the methanol-water solvent at a residue to solvent ratio of 1:3 and the supernatant was collected.

- 5 The supernatant was subjected to a rotary vacuum evaporator to evaporate the solvent and obtain a semi-solid soft extract of *Bacopa monnieri* weighing about 20 grams.

Example 6: Preparation of Vitamin D3 nanogels

- 10 Vitamin D nanogels were prepared according to a procedure described in Indian Patent Application No. 201841027257 entitled “A novel aqueous composition with water-insoluble vitamins”.

Briefly, a reaction mixture comprising agar-agar, poly(acrylamidoglycolic) acid, methylene
15 bisacrylamide and vitamin D was prepared. For preparing the reaction mixture, 1 gm of agar-agar was dissolved in 100 mL water by heating to obtain a homogenous mixture. 1.5 gm poly(acrylamidoglycolic) acid was added to the reaction mixture. In another setup, 1.5 gm pectin was used instead of poly(acrylamidoglycolic) acid. Thereafter, 200 mg methylene bisacrylamide was added as a crosslinker to the reaction mixture. 200 mg of cholecalciferol
20 (vitamin D3) dissolved in 1 mL acetone-water mixture (80:20) was added. 20 mg potassium persulphate was added as an initiator for the polymerization reaction.

The reaction mixture was transferred to a three-necked round bottom flask equipped with a reflux condenser and a nitrogen inlet containing 10 mL sodium dodecyl solution at a
25 concentration of 0.5 wt%. The mixture was bubbled with nitrogen gas for 30 min, heated to 80°C, and stirred well at a rotation speed of 1000 rpm for 5 hrs. 2 mL glutaraldehyde at a concentration of 1 wt% was added to the reaction mixture as an amine-reactive homobifunctional crosslinker and stirred for a period of 4 hrs.

- 30 The nanogels were collected by centrifuging the reaction mixture at 15,000 rpm speed for 10 min. The nanogels were re-dispersed in double distilled water, and the centrifugation cycles were repeated three times to remove any unreacted monomers, polymers and crosslinkers. The purified Vitamin D nanogels were dried at 40°C and stored in a desiccator for further use.

Example 7: Preparation of essential oil extracts

Rosemary oil [from *Rosmarinus officinalis* (L)], spearmint oil [*Mentha spicata* (L)], menthol mint oil [from *Mentha arvensis* (L)], and cardamom oil [from *Elettaria cardamomum* (Maton)]
5 were extracted.

In order to extract the essential oils, raw *Rosmarinus officinalis* (L), *Mentha spicata* (L), *Mentha arvensis* (L) and *Elettaria cardamomum* (Maton) were obtained and filled into a round bottom flask. Water was added until the raw materials were submerged.

10 The raw materials were subjected to hydro-distillation for 4 hours in a Clevenger apparatus and the essential oil extracts were collected and dried over anhydrous sodium sulphate.

Example 8: Preparation of water formulation

15 For preparation of 500 mL enriched water formulation, a herbal extract composition, vitamin D nanogels and essential oil mixture was used.

To prepare the herbal extract composition, 20 gm of extract of each *Ocimum sanctum* (Linn), *Withania somnifera* (L), *Tinospora cordifolia* (Thunb) Miers, *Garcinia cambogia* (L) (in
20 powder form) and *Bacopa monnieri* (L) as obtained in Examples 1-5 were mixed to form a 100 grams herbal extract composition.

1.5 mg of Vitamin D nanogels (60,000 IU) as obtained in Example 6 was used.

25 100 μ L of each essential oil (rosemary oil, spearmint oil, menthol mint oil and cardamom oil) were mixed to obtain a 400 μ L essential oil mixture.

The Herbal extracts (100 g), essential oils (400 μ L) and Vitamin D3 (1.5 mg (60,000 IU)) were added to form a composite composition (weighing 105.4 g), which was then homogenized.

30 2 grams of the homogenized extract was then dissolved in 480 mL water to obtain an enriched water formulation of 500 mL.

Table 1 provides the concentration of each component and a concentration range in which the components may be present in the enriched water formulation.

Table 1: Composition of enriched water formulation			
Component	Amount per 500 mL	Concentration	Concentration Range
<i>Ocimum sanctum</i> extract	0.38 grams	0.76 g/L	0.25 g/L to 2.28 g/L
<i>Withania somnifera</i> extract	0.38 grams	0.76 g/L	0.25 g/L to 2.28 g/L
<i>Tinospora cordifolia</i> extract	0.38 grams	0.76 g/L	0.25 g/L to 2.28 g/L
<i>Garcinia cambogia</i> extract	0.38 grams	0.76 g/L	0.25 g/L to 2.28 g/L
<i>Bacopa monnieri</i> extract	0.38 grams	0.76 g/L	0.25 g/L to 2.28 g/L
vitamin D3 nanogels	1138 IU	2277 IU/L	1012 IU/L to 6072 IU/L
<i>Rosmarinus officinalis</i> oil	1.9 μ L	3.8 μ L/L	1.26 μ L/L to 11.38 μ L/L
<i>Mentha spicata</i> oil	1.9 μ L	3.8 μ L/L	1.26 μ L/L to 11.38 μ L/L
<i>Mentha arvensis</i> oil	1.9 μ L	3.8 μ L/L	1.26 μ L/L to 11.38 μ L/L
<i>Elettaria cardamomum</i> oil	1.9 μ L	3.8 μ L/L	1.26 μ L/L to 11.38 μ L/L

5

Example 9: Organoleptic Characterization of enriched water formulation

An organoleptic characterization of the enriched water formulation was carried out over a period of 30 days at room temperature ($27 \pm 2^\circ\text{C}$) to assess any changes in the organoleptic properties such as colour, taste, aroma as provided in Table 2. The pH profile was also monitored over a period of 30 days.

10

Table 2: Organoleptic characterization of enriched water formulation at room temperature				
Organoleptic properties	Day 0	Day 10	Day 20	Day 30
pH	5.79	5.73	5.54	5.74
Colour	Light brown Colour	Light brown Colour	Light brown Colour	Light brown Colour
Tasteless	No distinctive taste	No distinctive taste	No distinctive taste	No distinctive taste
Aroma	Herbal	Herbal	Herbal	Herbal

Organoleptic characterization of the enriched water formulation was also carried out over a period of 30 days at accelerated temperature ($42 \pm 2^\circ\text{C}$), which is equivalent to 90 days of storage at room temperature to assess any changes in the organoleptic properties such as colour, taste, aroma as provided in Table 3.

15

Table 3: Organoleptic characterization of enriched water formulation at accelerated temperature				
Organoleptic properties	Day 0	Day 10	Day 20	Day 30
pH	5.92	5.95	5.77	6.02
Colour	Light brown Colour	Light brown Colour	Light brown Colour	Light brown Colour
Tasteless	No distinctive taste	No distinctive taste	No distinctive taste	No distinctive taste
Aroma	Herbal	Herbal	Herbal	Herbal

The results indicate that the enriched water formulation of the present invention retain all major organoleptic properties and there is no major alteration in palatability, colour, taste, aroma etc. over a period of 90 days at room temperature.

5

Example 10: Chemical characterization and Stability Studies of enriched water formulation

Chemical characterization of the enriched water formulation was carried out over a period of 30 days at room temperature ($27 \pm 2^\circ\text{C}$) to assess any changes in the concentration levels of different components present in the formulation as provided in Table 4.

10

Table 4: Chemical characterization of enriched water formulation at room temperature				
Components	Day 0	Day 10	Day 20	Day 30
Rosmarinic Acid (mg/L)	0.51	0.49	0.45	0.44
Total Withanolides mg/L(HPLC)	1	<1.0	<1.0	<1.0
Withanolides (Gravimetric) (mg/L)	0.08	<0.1(0.079)	<0.1(0.076)	<0.1(0.070)
Epi-tinosporide (mg/kg)	0.43	0.42	0.4	0.35
Hydro citric acid (Garcinia) %	0.05	0.04	0.04	0.035
Saponins %	<0.1	<0.1	<0.1	<0.1
Vitamin D3 IU/ G	1.76	1.71	1.68	1.62
1,8 Cineole %	20.85	28.91	23.81	13.14
Camphor %	Absent	Absent	Absent	Absent
Dihydro carvone, %	1.61	1.68	1.58	1.26
Carvone %	Absent	Absent	Absent	Absent
Menthol, %	4.14	6.2	5.37	2.68
Menthon %	3.42	4.39	3.47	2.2
Menthol Acetate %	0.89	1.71	1.61	0.68
Alpha-terpinile acetate %	4.5	7.35	5.32	1.6

Chemical characterization of the enriched water formulation was carried out over a period of 30 days at accelerated temperature ($42 \pm 2^\circ\text{C}$), which is equivalent to 90 days of storage at room temperature to assess any changes in the concentration levels of different components present in the formulation as provided in Table 5.

5

Table 5: Chemical characterization of enriched water formulation at accelerated temperature				
Components	Day 0	Day 10	Day 20	Day 30
Rosmarinic Acid (mg/L)	0.48	0.48	0.44	0.43
Total Withanolides mg/L(HPLC)	1.1	0.96	<0.1	<0.1
Withanolides (Gravimetric) (mg/L)	<0.1(0.08)	<0.1(0.076)	<0.1(0.07)	<0.1(0.066)
Epi-tinosporide (mg/kg)	0.37	0.37	0.35	0.35
Hydro citric acid (Garcinia) %	0.05	0.04	0.035	<0.035
Saponins %	<0.1	<0.1	<0.1	<0.1
Vitamin D3 IU/ G	1.72	1.604	1.6	1.57
1,8 Cineole %	29.36	23.79	25.41	29.06
Camphor %	Absent	Absent	Absent	Absent
Dihydro carvone, %	2.88	1.51	2.07	1.43
Carvone %	Absent	Absent	Absent	Absent
Menthol, %	6.73	5.08	6.32	5.09
Menthon %	4.65	3.53	3.71	4.39
Menthol Acetate %	1.05	0.74	1.29	0.97
Alpha-terpinile acetate %	7.29	0.68	6.66	1.69

The results indicate that the enriched water formulation of the present invention is extremely stable to retain all major components and there is no major alteration in the desired concentration of components over a period of 90 days at room temperature.

10

Example 11: Pathogenic Load Determination of enriched water formulation

A microbial load determination study was conducted over 30 days to check the presence of major pathogenic microorganisms at room temperature ($27 \pm 2^\circ\text{C}$) and accelerated temperature ($42 \pm 2^\circ\text{C}$) in packaged enriched water formulation as shown in Table 6.

15

Table 6: Microbial Load Determination				
Organism	Room temperature		Accelerated temperature	
	Day 0	Day 30	Day 0	Day 30
<i>Salmonella</i> sp	Absent	Absent	Absent	Absent
<i>Escherichia coli</i> (Castellani and Chalmers)	Absent	Absent	Absent	Absent
<i>Staphylococcus aureus</i> (Rosenbach)	Absent	Absent	Absent	Absent
<i>Pseudomonas aeruginosa</i> (Migula)	Absent	Absent	Absent	Absent

No pathogenic microbe was observed in the packaged enriched water formulation indicating that the water formulation composition is safe for consumption.

We claim:

1. A water formulation comprising a herbal composition, Vitamin D3 and an essential oil composition.
2. The water formulation as claimed in claim 1, wherein the herbal composition comprises *Ocimum sanctum* extract, *Withania somnifera* extract, *Tinospora cordifolia* extract, *Garcinia cambogia* extract, *Bacopa monnieri* extract or mixture thereof.
3. The water formulation as claimed in claim 1, wherein the essential oil composition comprises rosemary oil, spearmint oil, menthol mint oil, cardamom oil or mixture thereof.
4. The water formulation as claimed in claim 2, wherein *Ocimum sanctum* extract, *Withania somnifera* extract, *Tinospora cordifolia* extract, *Garcinia cambogia* extract and *Bacopa monnieri* extract are each present in the water formulation at a concentration in the range from 0.25 g/L to 2.28 g/L.
5. The water formulation as claimed in claim 1, wherein vitamin D3 is present at a concentration in the range from 1012 IU/L to 6072 IU/L.
6. The water formulation as claimed in claim 3, wherein rosemary oil, spearmint oil, menthol mint oil and cardamom oil are each present in the essential oil composition at a concentration in the range from 1.26 μ L/L to 11.38 μ L/L.
7. The water formulation as claimed in claim 2, wherein each of *Ocimum sanctum* extract, *Withania somnifera* extract, *Tinospora cordifolia* extract, *Garcinia cambogia* extract and *Bacopa monnieri* extract is present in the herbal composition at a concentration range of 7% w/w to 60% w/w.
8. The water formulation as claimed in claim 3, wherein each of rosemary oil, spearmint oil, menthol mint oil and cardamom oil are present in the essential oil composition at a concentration range of 8% v/v to 25% v/v.
9. A method for preparation of water formulation as claimed in claim 1, comprising:

- a. preparing an herbal composition, wherein the herbal composition comprises *Ocimum sanctum* extract, *Withania somnifera* extract, *Tinospora cordifolia* extract, *Garcinia cambogia* extract and *Bacopa monnieri* extract;
 - b. preparing vitamin D3 nanogel;
 - c. preparing an essential oil composition, wherein the essential oil composition comprises rosemary oil, spearmint oil, menthol mint oil and cardamom oil;
 - d. homogenizing the mixture of herbal composition obtained in step a), vitamin D3 nanogel obtained in step b) and essential oil composition obtained in step c) to obtain composite composition; and
 - e. dissolving the composite composition obtained in step (d) in water to obtain a water formulation.
10. The method as claimed in claim 9, wherein *Ocimum sanctum* extract, *Withania somnifera* extract, *Tinospora cordifolia* extract, *Garcinia cambogia* extract or *Bacopa monnieri* extract are prepared by a method comprising:
- a. treating raw *Ocimum sanctum*, *Withania somnifera*, *Tinospora cordifolia*, *Garcinia cambogia* or *Bacopa monnieri* with methanol-water solvent, wherein methanol and water are present at a ratio of 7:3 to obtain a supernatant containing the extract; and
 - b. evaporating the solvent from the supernatant to obtain *Ocimum sanctum* extract, *Withania somnifera* extract, *Tinospora cordifolia* extract, *Garcinia cambogia* extract or *Bacopa monnieri* extract.
11. The method as claimed in claim 10, wherein the treatment with methanol-water solvent is carried at a temperature in a range from 30°C to 50°C.
12. The method as claimed in claim 10, wherein the treatment with methanol-water solvent is carried for a period in a range from 0.5 hours to 3.5 hours.
13. The method as claimed in claim 9, wherein each of *Ocimum sanctum* extract, *Withania somnifera* extract, *Tinospora cordifolia* extract, *Garcinia cambogia* extract and *Bacopa monnieri* extract is present in the herbal composition at a concentration range of 7% w/w to 60% w/w.

14. The method as claimed in claim 9, wherein each of rosemary oil, spearmint oil, menthol mint oil and cardamom oil are present in the essential oil composition at a concentration range of 8% v/v to 25% v/v.
15. The method as claimed in claim 9, wherein vitamin D3 is present in the water formulation at a concentration in the range from 1012 IU/L to 6072 IU/L.