



(86) **Date de dépôt PCT/PCT Filing Date:** 2014/09/05
(87) **Date publication PCT/PCT Publication Date:** 2015/03/12
(85) **Entrée phase nationale/National Entry:** 2016/02/08
(86) **N° demande PCT/PCT Application No.:** EP 2014/068992
(87) **N° publication PCT/PCT Publication No.:** 2015/032922
(30) **Priorité/Priority:** 2013/09/06 (EP13306227.3)

(51) **Cl.Int./Int.Cl. A61K 31/35** (2006.01),
A61K 31/025 (2006.01), **A61K 31/045** (2006.01),
A61K 31/05 (2006.01), **A61K 31/085** (2006.01),
A61K 31/56 (2006.01), **A61K 36/23** (2006.01),
A61K 36/53 (2006.01), **A61K 36/54** (2006.01),
A61K 36/61 (2006.01), **A61P 33/02** (2006.01)

(71) **Demandeur/Applicant:**
MARS, INCORPORATED, US

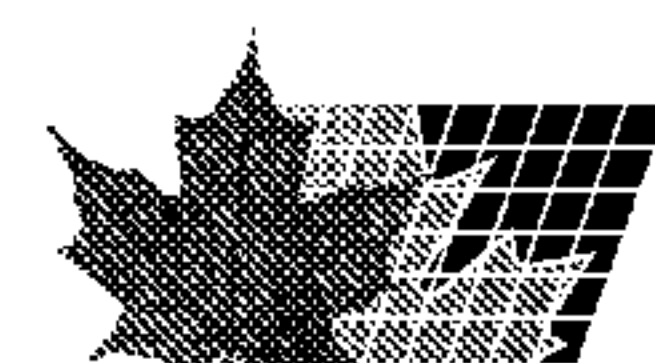
(72) **Inventeurs/Inventors:**
FEUGIER, ALEXANDRE, FR;
LEROUXEL, NICOLAS, FR

(74) **Agent:** CASSAN MACLEAN

(54) **Titre : COMPOSITION ANTIPARASITAIRE ORALE**
(54) **Title: ORAL ANTI-PARASITIC COMPOSITION**

(57) **Abrégé/Abstract:**

The present invention relates to a composition for use as an oral anti-parasitic, the composition comprises one or more of an essential oil which contains gamma-terpinene, and/or carvacrol, and/or thymol, and/or terpinenol, and/or eucalyptol and/or eugenol, an essential oil of genus Cinnamomum, Eugenia, Eucalyptus or a source of saponin. It also relates to a method of preventing or treating parasitic infection in an animal, the method comprising orally administering to said animal a composition comprising one or more of an essential oil which contains gamma-terpinene, and/or carvacrol, and/or thymol, and/or terpinenol, and/or eucalyptol and/or eugenol, an essential oil of genus Cinnamomum, Eugenia, Eucalyptus or a source of saponin.



(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)
(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
12 March 2015 (12.03.2015)

(10) International Publication Number
WO 2015/032922 A1

(51) International Patent Classification:

A61P 33/02 (2006.01) *A61K 36/53* (2006.01)
A23L 1/30 (2006.01) *A61K 36/54* (2006.01)
A61K 31/7034 (2006.01) *A61K 36/61* (2006.01)
A61K 36/23 (2006.01)

(21) International Application Number:

PCT/EP2014/068992

(22) International Filing Date:

5 September 2014 (05.09.2014)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

13306227.3 6 September 2013 (06.09.2013) EP

(71) Applicant: **MARS, INCORPORATED** [US/US]; 6885 Elm Street, McLean, Virginia 22101 (US).

(71) Applicant (*for TT only*): **MARS PETCARE UK** [GB/GB]; 3D Dundee Road, Slough, Berkshire SL1 4LG (GB).

(72) Inventors: **FEUGIER, Alexandre**; Royal Canin S.A., F-30470 Aimargues (FR). **LEROUXEL, Nicolas**; Royal Canin S.A., F-30470 Aimargues (FR).

(74) Agents: **CORNISH, Kristina Victoria Joy** et al.; Kilburn & Strode LLP, 20 Red Lion Street, London, Greater WC1R 4PJ (GB).

(81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

(54) Title: ORAL ANTI-PARASITIC COMPOSITION

(57) Abstract: The present invention relates to a composition for use as an oral anti-parasitic, the composition comprises one or more of an essential oil which contains gamma-terpinene, and/or carvacrol, and/or thymol, and/or terpinenol, and/or eucalyptol and/or eugenol, an essential oil of genus Cinnamomum, Eugenia, Eucalyptus or a source of saponin. It also relates to a method of preventing or treating parasitic infection in an animal, the method comprising orally administering to said animal a composition comprising one or more of an essential oil which contains gamma-terpinene, and/or carvacrol, and/or thymol, and/or terpinenol, and/or eucalyptol and/or eugenol, an essential oil of genus Cinnamomum, Eugenia, Eucalyptus or a source of saponin.



WO 2015/032922 A1

Oral anti-parasitic composition

The present invention relates to a composition for use as an oral anti-parasitic, the composition comprises one or more of an essential oil which contains gamma-terpinene, and/or carvacrol, and/or thymol, and/or terpinenol, and/or eucalyptol and/or eugenol, an
5 essential oil of genus *Cinnamomum*, *Eugenia*, *Eucalyptus* or a source of saponin. It also relates to a method of preventing or treating parasitic infection in an animal, the method comprising orally administering to said animal a composition comprising one or more of an essential oil which contains gamma-terpinene, and/or carvacrol, and/or thymol, and/or
10 terpinenol, and/or eucalyptol and/or eugenol, an essential oil of genus *Cinnamomum*, *Eugenia*, *Eucalyptus* or a source of saponin.

Parasitic infection can affect all animals. In particular, dogs and cats are major hosts for parasites, at least 80% of dogs and cats are infected by a type of endoparasite. In
15 breeding environments, between 20-30% of dogs and cats are particularly subject to some internal parasites, such as coccidians. Parasite infection can cause symptoms such as worm infestation, abdominal pain, bowel obstructions, diarrhea and vomiting eventually leading to dehydration, sleeping problems, anemia, aching muscles or joints, allergies and/or fatigue. In particular, cats and dogs are hosts to many parasites and thus close
20 contact can lead to increasing risks of parasite infection to humans (zoonosis).

Plant extracts have been used in combination with chemical drugs, in particular for applying on the skin of the animals as spot on anti-parasitic medicines, for example EP0116401. The problem with these spot-on methods is that the skin of the animal
25 becomes irritated and can cause skin damage.

There is thus a need to develop further alternative medicines and/or foodstuffs for animals to reduce parasite infection, in particular endoparasites in dogs and/or cats to reduce further increasing risks of zoonosis.

30

The present invention provides a composition that addresses this need.

The present invention provides a composition comprising one or more of an essential oil which contains gamma-terpinene, and/or carvacrol, and/or thymol, and/or terpinenol, and/or eucalyptol and/or eugenol, an essential oil of genus *Cinnamomum*, *Eugenia*,
35

Eucalyptus or a source of saponin for use orally as anti-parasitic in animals. The composition of the present invention has shown to be effective in reducing parasite infection in animals.

- 5 Further, the composition of the invention has shown a beneficial effect when comprising two or more of an essential oil which contains gamma-terpinene, and/or carvacrol, and/or thymol, and/or terpinenol, and/or eucalyptol and/or eugenol, an essential oil of genus *Cinnamomum*, *Eugenia*, *Eucalyptus* or a source of saponin. The effect may be synergistic.
- 10 The present invention relates, for all aspects, to any animal, including a human. In particular, the present invention relates to a companion animal such as a dog or a cat.

The first aspect of this invention relates to a composition for use as an oral anti-parasitic, the composition comprising one or more of an essential oil which contains gamma-
 15 terpinene, and/or carvacrol, and/or thymol, and/or terpinenol, and/or eucalyptol and/or eugenol, an essential oil from genus *Cinnamomum*, *Eugenia*, *Eucalyptus* or a source of saponin.

Use as an anti-parasitic includes treating and/or preventing the symptoms of parasitic
 20 infection. Treating includes ameliorating the symptoms of parasitic infection.

The composition of the present invention can be used against parasites, in particular belonging to the phylum Apicomplexa. Apicomplexa is a large group of unicellular protists. This group of protists include organisms classified as coccidian, gregarines, piroplasms,
 25 haemogregarines and plasmodia. Preferably, the parasite is from the class conoidasida.

The composition of the invention can be used against parasites from the class conoidasida. Conoidasida includes two subclasses coccidia or gregarinia. Preferably, the parasite is from the subclass coccidia.

30

The composition of the present invention can be for use to treat coccidiosis. Preferably, the composition treats parasite infection against parasites from the genus *Isospora*, *Neospora* or *Toxoplasma*. Most preferably, the composition of the present invention can treat coccidiosis against the parasite species *Neospora caninum* or *Toxoplasma gondii*.

35

The composition of the present invention comprises one or more of an essential oil which contains gamma-terpinene.

5 Terpinenes are a group of isomeric hydrocarbons classified as terpenes that are naturally found in plants and flowers. Terpinenes have the molecular formula $C_{10}H_{16}$ and differ from one another in the position of the carbon double bonds. Terpinenes include (α) alpha-terpinene, (β) beta-terpinene, (γ) gamma-terpinene or (δ) delta-terpinene, in particular gamma-terpinene.

10 Essential oils are volatile mixture of esters, aldehydes, alcohols, ketones, terpenes, monoterpenes, sesquiterpenes, phenols and oxides, which can be prepared from any of the parts of the plant. Methods for extracting essential oils from plants, for example distillation or solvent extraction, are readily known in the art.

15 Essential oils can be extracted from any parts of the plants, such as from the leaves, flowers, stems, bark, seeds or roots. Preferably, the essential oil is extracted from the leaves.

20 Essential oils containing gamma-terpinene, and/or carvacrol, and/or thymol, and/or terpinenol, and/or eucalyptol and/or eugenol include the genus *Satureja*, *Thymus*, *Trachyspermum*, *Origanum* or *Melaleuca*. Compounds can also be synthetic, or isolated from natural sources. Mix of compounds from natural or synthetic sources can be used.

25 In particular, winter savory oil can be obtained from the leaves of *Satureja Montana*, thyme oil can be obtained from the leaves of *Thymus vulgaris* or *Thymus zygis*, oregano oil can be obtained from the leaves of *Origanum vulgare*.

The essential oil containing gamma-terpinene can be of any of the genus *Satureja*, *Thymus*, *Trachyspermum*, *Origanum* or *Melaleuca*.

30

The composition of the present invention comprises one or more of an essential oil of genus *Cinnamomum*, *Eugenia*, *Eucalyptus*.

The essential oil of genus *Cinnamomum* can be cinnamon. The essential oil of genus *Eugenia* can be clove. The essential oil of genus *Eucalyptus* can be eucalyptus.. Preferably, the essential oil is cinnamon.

- 5 Cinnamon is a spice that is obtained from the inner bark of *Cinnamomum* trees. Cinnamon includes *cinnamomum verum*, *cinnamomum burmanii*, *cinnamomum loureiros* or *cinnamomum cassia*. Preferably, the cinnamon oil is extracted from *Cinnamomum cassia* (also called Chinese cassia). Most preferably, the cinnamon oil is extracted from the leaves of *Cinnamomum cassi*.

10

Clove is a spice that is obtained from the flower buds of a tree in the family of *Myrtaceae*. In particular, the clove oil is extracted from the flower buds of *Syzygium aromaticum*. Preferably, the clove is *Eugenia caryophyllus*.

- 15 Eucalyptus is a genus of flowering trees and shrubs in the family of *Myrtaceae* with over 700 species. In particular, the eucalyptus oil is extracted from *Eucalyptus globulus*. Preferably, the eucalyptus oil is extracted from the leaves of *Eucalyptus globulus*.

- 20 Saponins are chemical compounds naturally found in various plants and may also be found in marine organisms. Saponins can be found in the botanical families of Quillajaceae, Caryophyllaceae, Sapindaceae, Aceraceae, Hippocastanceae, Cucurbitanaceae, Araliaceae or Asparagaceae.

- 25 Preferably, a source of saponin is derived from the family of Asparagaceae. In particular, the source of saponin is derived from the genus *Yucca*. Most preferably, the source of saponin is derived from the species *Yucca schidigera*.

- 30 The source of saponin can be found in various parts of the plant such as: leaves, stems, roots, bulbs, blossom and fruit. Methods of extracting the source of saponin from plants are known.

Saponins are amphipathic glycosides composed of one or more hydrophilic glycoside moieties combined with a triterpene derivative. The number of saccharide chains attached can vary in length from 1 to 11, preferably from 2 to 5. The saccharide chains can be linear and/or branched.

5

The source of saponin can be steroidal.

Preferably, the saponin is a steroid sapogenin like diosgenine, sarsapogenine, smilagenine, tigogenine, hecogenine, 9-dehydrohecogenine, gitogenine, chlorogenine,
10 yuccagenine, samogenine, manogenine, 9-dehydromanogenine or kammogenine.

A preferred composition includes one or more of an essential oil containing gamma-terpinene and one or more of an essential oil of cinnamon, clove or eucalyptus. Preferably, the composition contains oregano as the essential oil containing gamma-
15 terpinene. Most preferably, the composition includes essential oils of oregano and cinnamon or oregano and clove.

The composition of the invention is any composition which an animal may consume or may consume as part of its diet.

20

The composition can be a liquid, a tablet or a foodstuff. Preferably, the composition can be a foodstuff. It can be any foodstuff, such as dry product, semi moist product, wet food product or a liquid and includes food supplement, a snack or a treat. Thus, the invention covers standard food products including liquids, as well as pet food snacks (for example,
25 snack bars, pet chew, crunchy treat, cereal bars, snacks, biscuits and sweet products) and supplements.

The composition may be incorporated in a gelatinised starch matrix, or in any dry or wet foodstuffs or supplements. Methods of incorporation are known in the art.

30

The foodstuff is preferably a commercial pet product. Such a product is preferably sold as a product for feeding/administering to a pet animal, in particular a pet cat or a pet dog.

A typical dry pet foodstuff contains about 10-40% crude protein and about 5-40% fat, the

remainder being carbohydrate, including dietary fibre and ash. A typical wet or moist product contains (on a dry matter basis) about 40% fat, 50% protein and the remainder being fibre and ash. The foodstuff of the invention may be a dry product (with approximately 5 to approximately 15% moisture), a semi-moist product (with
 5 approximately 15 to approximately 70% moisture) or a wet product (with approximately 70 to approximately 90% moisture). The foodstuff of the invention is preferably produced as a dry product containing from approximately 5% to approximately 15% moisture. The preferred dry food is more preferably presented as a small biscuit – like kibbles.

10 The remaining components of the foodstuff are not essential to the invention and typical standard products can be included. The combined ingredients of the foodstuff according to the invention can provide all of the recommended vitamins and minerals for the particular animal in question (a complete and balanced food).

15 The foodstuff can be provided as a food supplement. The food supplement can be a powder, sauce, topping, biscuit, kibble, pocket or tablet that can be administered with or without an additional foodstuff. Where the food supplement is administered with an additional foodstuff, the food supplement can be administered sequentially simultaneously or separately. The food supplement may be mixed with the foodstuff, sprinkled over the
 20 foodstuff or served separately. Alternatively, the food supplement can be added to a liquid provided for drinking such as water or milk.

The foodstuff is preferably a cooked product. It may incorporate meat or animal derived material (such as beef, chicken, turkey, lamb, fish, blood plasma, marrow bone etc. or one
 25 or more thereof). The product alternatively may be meat free (preferably including a meat substitute such as soya, maize gluten or a soya product) in order to provide a protein source. The foodstuff may contain additional protein sources such as soya protein concentrate, milk proteins, gluten etc. The foodstuff may also contain a starch source such as one or more grains (e.g. wheat, corn, rice, oats, barley etc.), or may be starch
 30 free.

The foodstuff of the invention may be or may be used in combination with a complete and balanced food which provides all the recommended vitamins and minerals for the dog in questions, for example, as described in National Research Council, 1985, Nutritional
 35 Requirements for Dogs, National Academy Press, Washington DC (ISBN:0-309-03496-5); or Association of American Feed Control Officials, Official Publication 1996.

These values apply to a composition for feeding to an animal, in particular a companion animal, such as a dog or a cat.

5

The total amount of essential oil /saponin is from 0,01 to 1000 mg/kg (ppm) of foodstuff (solid or liquid).

The amount of essential oil /saponin can have a range of 0.01 to 500 mg/kg (ppm) 0.01 to 100 mg/kg (ppm), 0.01 to 50 mg/kg (ppm), 0.01 to 1 mg/kg (ppm), 100 to 1000 mg/kg (ppm) 100 to 500 mg/kg (ppm) 50 to 100 mg/kg (ppm), or to 50mg/kg (ppm) or any combination thereof of food stuff (solid liquid).

The second aspect of the invention relates to a method of preventing or treating parasitic infection in an animal.

The composition of the invention has demonstrated to provide, *inter alia*, a decrease in parasite infection and proliferation *in vitro*. The composition of the invention prevents and/or treats parasite infection in animals, including the prevention of zoonosis and prevention and/or treatment in a breeding kennel environment.

In particular, it is a desire in the area of pet foodstuff and companion animal health to provide foodstuff including supplements suitable to support the health of the companion animals. In particular, it is a desire to provide diets suitable to promote or maintain the health of already healthy companion animals as preventative diets.

In particular, the second aspect of the invention provides a method for preventing and/or treating parasitic infection in an animal, including ameliorating the symptoms of parasite infection. The method comprises administering to said animal a composition which comprises one or more of an essential oil which contains gamma-terpinene, gamma-terpinene, and/or carvacrol, and/or thymol, and/or terpinenol, and/or eucalyptol and/or eugenol, an essential oil of genus *Cinnamomum*, *Eugenia*, *Eucalyptus* or a source of saponin The animal may be in need thereof.

The present invention relates, for all aspects, to any animal, including a human. In particular the present invention relates to a companion animal such as a dog, a cat or any other such animal that suffers or is prone to suffer from parasite infection. Since a significant number of dogs and/or cats are hosts to parasites, thus are readily prone to
5 parasitic infection.

In particular embodiments, the method comprises administering to said animal a composition comprising one or more of an essential oil which contains gamma-terpinene, and/or carvacrol, and/or thymol, and/or terpinenol, and/or eucalyptol and/or eugenol, an
10 essential oil of genus *Cinnamomum*, *Eugenia*, *Eucalyptus* or a source of saponin. The composition can include one or more of an essential oil containing gamma-terpinene, and/or carvacrol, and/or thymol, and/or terpinenol, and/or eucalyptol and/or eugenol include the genus *Satureja*, *Thymus*, *Trachyspermum*, *Origanum* or *Melaleuca* and one or more of an essential oil of cinnamon, clove or eucalyptus. Preferably, the composition
15 contains oregano as the essential oil containing gamma-terpinene and carvacrol. Most preferably, this composition includes essential oils of oregano and cinnamon or oregano and clove.

Further, the method is preferably administered to an animal, in particular a companion
20 animal such as a dog and/or a cat, that suffers from parasite infection and is in need of ameliorating the symptoms of parasite infection or in need of preventing further symptoms of parasite infection. This may be to, for example a young pet animal, such as a puppy, or an older companion animal. Where the composition is a foodstuff, the foodstuff may be administered in a dietary regime in accordance with the usual dietary regime of the
25 companion animal. The foodstuff may comprise 100% of the diet of the companion animal or a lesser proportion, depending on the level of prevention or treatment required. The foodstuff allows the composition to be administered with ease thus avoiding a need to supplement the companion animal's food. In addition, the foodstuff can be administered by the animal's owner thus avoiding constant veterinary supervision. The foodstuff may be
30 available at any outlet selling pet food products or may be available from a veterinarian, or a breeder.

As used herein, the term "administration" also includes feeding or any other method of oral administration. Other means of administration may include tablets, capsules,
35 injection, suppositories or any other suitable means.

Preferred features of the first aspect of the invention apply as for the second aspect of the invention *mutatis mutandis*.

5 In a third aspect of the invention there is provided a composition comprising two or more of the following essential oils from the genus *Cinnamomum*, *Eugenia*, *Satureja*, *Thymus*, *Trachyspermum*, *orignum*, *Eucalyptus* or *Melaleuca*.

Preferably, the composition comprises an essential oil selected from winter savory, thyme, oregano or tea tree oil and an essential oil selected from cinnamon, clove or eucalyptus.
10 Preferably, the composition contains oregano. Most preferably, the composition includes essential oils of oregano and cinnamon or oregano and clove.

Preferred features of the first and second aspect of the invention apply as for the third aspect of the invention *mutatis mutandis*.
15

In a fourth aspect of the invention there is provided a composition comprising a source of saponin and one or more of an essential oil which contains gamma-terpinene and/or carvacrol, and/or thymol, and/or terpinenol, and/or eucalyptol and/or eugenol, or an essential oil of genus *Cinnamomum*, *Eugenia* or *Eucalyptus* .
20

Preferably, the composition can include one or more of an essential oil containing gamma-terpinene and one or more of an essential oil of cinnamon, clove or eucalyptus. Preferably, the composition contains oregano as the essential oil containing gamma-terpinene. Most preferably, this composition includes essential oils of oregano and
25 cinnamon or oregano and clove.

Preferred features of the first, second and third aspect of the invention, apply as for the fourth aspect of the invention *mutatis mutandis*.

30 The present description includes a method for preparing the composition of the invention.

The composition can include suitable carriers which are known in the art, for example silica dioxide, maltodextrine, Arabic gum or aluminosilicate minerals. The composition can be incorporated in a gelatinised starch matrix.

35

The composition can be sprayed onto foodstuff, mixed in with foodstuff or incorporated into foodstuff in a gelatinised starch matrix. Methods of inclusion of the composition are known in the art.

- 5 The present description includes a method for preparing the foodstuff of the invention.

The foodstuff can be made according to any method known in the art such as in Waltham Book of Dog and Cat Nutrition, Ed. ATB Edney, Chapter by A. Rainbird, entitled "A Balanced Diet" in pages 57 to 74 Pergamon Press Oxford.

10

For example, a process for the manufacture of a foodstuff as defined herein comprises mixing together ingredients with the composition which comprises one or more of an essential oil which contains gamma-terpinene, an essential oil of cinnamon, clove, eucalyptus or a source of saponin and forming a foodstuff, in particular a pet foodstuff.

- 15 Heating/cooking may be applied to any one or more of the ingredients prior to, during or following the mixing.

- The importance of the present invention is the beneficial properties of one or more of the essential oils and/or a source of saponin. In particular, an effect which is more than the
20 cumulative effect may be seen.

- The combination of the essential oils and/or source of saponin in the composition of the present invention can provide a synergistic effect in terms of one or more of reducing parasite infection and/or proliferation, preventing parasite infection and/or proliferation,
25 decreasing risks of zoonosis and treating parasite infection, in particular coccidiosis.

- A further benefit is seen when an essential oil contain gamma-terpinene and an essential oil of cinnamon, clove or eucalyptus are combined in the composition. In particular, when the essential oil containing gamma-terpinene is winter savory, thyme, oregano or tea tree
30 oil and the second essential oil is cinnamon, clove or eucalyptus. Preferably, the composition contains oregano. Most preferably, the composition includes the essential oils oregano and cinnamon or oregano and clove.

- The invention will now be further described by way of reference to the following Examples
35 and Figures, which are provided for the purpose of illustration only and are not to be construed as being limiting on the invention.

Figure 1: shows the 7 solutions of essential oils and source of saponin used. Each solution has been labeled A through to H.

- 5 Figure 2A: shows a schematic diagram of the proliferation protocol followed. Figure 2B shows fluorescence microscopy of HFF cells infected by different parasitical inoculums of *Toxoplasma gondii*.

- 10 Figure 3: shows results of a test of inhibition of the proliferation of *Neospora caninum* parasite in the presence of different essential oils at different concentrations. Summary of results and IC50 are set out in Table 1.

- 15 Figure 4: shows comparative results of IC50 of 7 essential oils (oregano (E) tested at 50 ppm and 100 ppm) and the cell viability.

Figure 5: shows results from a test of inhibition of proliferation of *Toxoplasma gondii* with a cocktail of essential oils EB (Oregano and Clove).

Examples

20

The study aimed to determine an anti-parasitic activity from seven essential oils on the intracellular microorganism *Neospora caninum*, but also combinations of these drugs on *Toxoplasma gondii*. The work focused on the capacity of proliferation of the parasite in the presence of these solutions containing the essential oils and invasion after pre-treatment.

25

Seven solutions of essential oils and one solution with a source of saponin were tested (see Figure 1: A to H).

- 30 In order to solubilize the essential oils, the oils were diluted in dimethyl sulfoxide (DMSO) and then spread in the cell culture medium (DMEM) that was supplemented with 10% fetal calf serum (D10). DMSO was also used at a final concentration not exceeding 0.1%.

- 35 Separate cultures of both parasites, *Toxoplasma gondii* and *Neospora caninum*, were prepared *in vitro* in human cell type HFF (Human Foreskin Fibroblasts), grown at 37°C under 5% CO₂ in a D10 medium. Both cultures were similar, the inoculum used for the various tests were the same, 105 parasites per well. It was observed that growing *Neospora caninum* is slower than that of *Toxoplasma gondii*.

Example 1: Proliferation assay of *Neospora caninum* parasite in the presence of essential oils

Proliferation of the parasite in the cells is measured by counting the specific radioactivity of the incorporated tritiated uracil and compared to the control without essential oil (100% proliferation). The test is performed in 24-well plates and the whole test lasts about 24 hours. The positive control is achieved using pyrimethamine (IC50: 0.1µg/mL). Figure 3 shows IC50 of each essential oil solution tested at different concentrations in the presence of *Neospora caninum*.

Table 1 (below) summarises the results from example 1 in Figure 3. Values represent inhibition of proliferation. The dotted results show the IC50 of each essential oil tested.

Essential Oil	12,5ppm	25ppm	50ppm	100ppm	200ppm	400ppm	800ppm
A (Cinnamon)	43,7%	84,3%	95,3%	94,5%			
B (Clove)			21,0%	58,7%	78,0%		
C (Winter savory)		4,6%	-3,1%	42,2%	85,2%		
D (Thyme)		6,0%	43,8%	41,1%			
E (Oregano)		26,7%	53,9%	75,9%			
F (Eucalyptus)			-1,6%	23,9%	15,0%	20,7%	69,5%
G (Tea tree)			7,9%	18,7%	11,6%	24,5%	

IC 50

Example 2

The inhibition of proliferation was measured by incorporating tritiated uracil in both *Toxoplasma gondii* () and *Neospora caninum* () and the viability of HFF cells was assessed using MTT assay (). The results are shown in Figure 4.

Figure 4 shows for each essential oil, having a concentration of proliferation inhibition properties for each of the parasites while maintaining cell viability HFF near 80%.

It can be observed that oils having a pesticidal activity, the IC50 concentrations obtained from *Toxoplasma gondii* are the same as for *Neospora caninum*. However, F and G oils

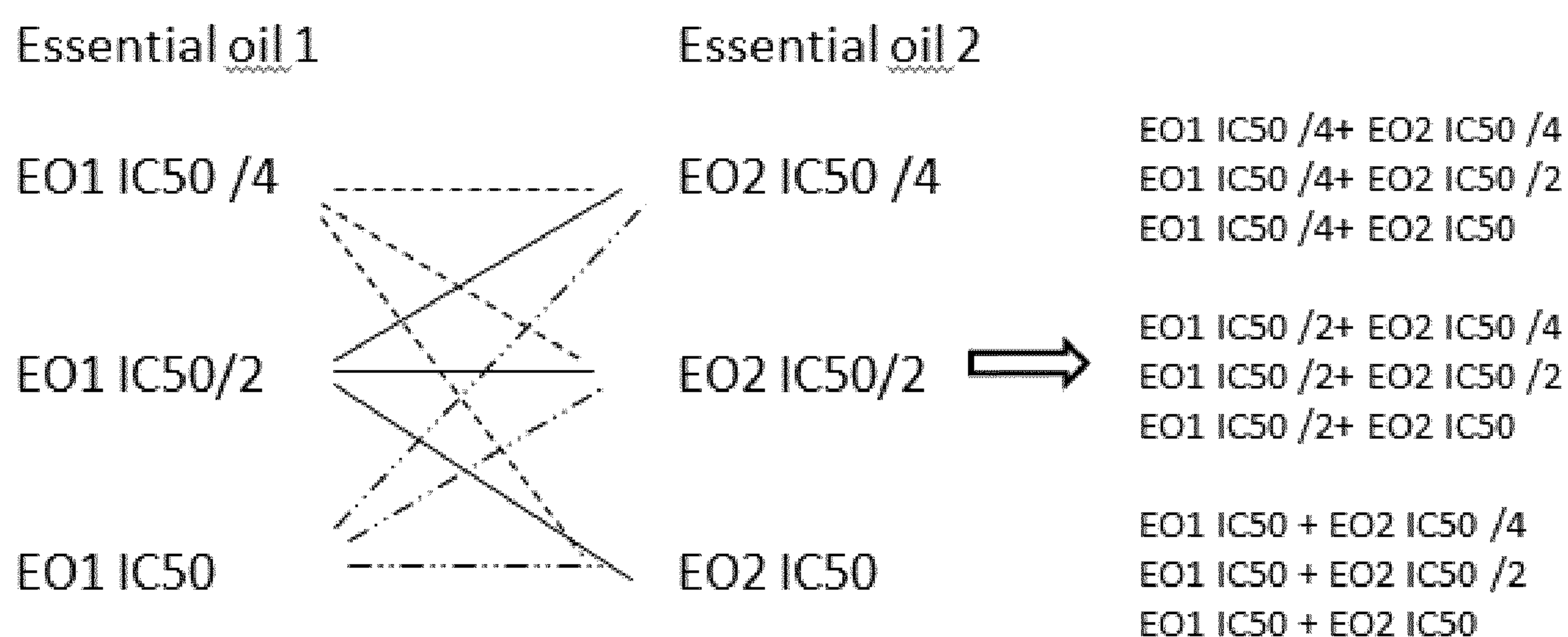
have little or no action on the inhibition of the proliferation of *Neospora caninum*.

Example 3: Proliferation assay of *Toxoplasma gondii* in the presence of a set of combinations of essential oils

5

The set of essential oils combinations are: AB, AE, AD and BE.

For each combination a 3 time 3 factorial design was drawn. The theoretical IC50 is adjusted to the measured inhibition proliferation rate and then is used as the basis to
10 make dilutions and to make the subsequent 9 different ratios of the 2 essential oils combined in a cocktail.



15 For each association, 9 tests of the inhibition of proliferation of *Toxoplasma gondii* were performed (all data not shown). Again, the inhibition of proliferation was measured by incorporation of tritiated uracil.

Cocktail BE (see Figure 5): Shows the synergistic effect of cocktail BE on *Toxoplasma*
20 *Gondii* proliferation.

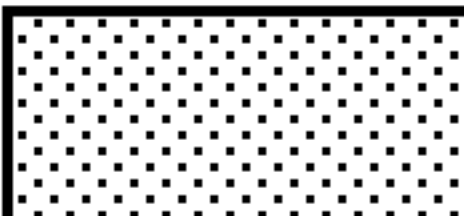

Essential oil B at 100ppm alone corresponds to an IC58 (58% of proliferation inhibition). Essential oil E at 75ppm alone corresponds to an IC88 (88% of proliferation inhibition). Assuming an independent relationship between the two oils on proliferation inhibition, a
25 cocktail of essential oil B at $IC58/2 = IC29$ with the essential oil E at $IC88/4 = IC22$ leads to an expected additional effect of $IC22 + IC29 = IC51$ (51% of proliferation inhibition). The measured of proliferation inhibition of such a cocktail (B at IC22 combined to E at IC29) revealed a surprising measurable proliferation inhibition of 87% (IC87). This highly

significant difference clearly highlights a synergistic effect of the cocktail BE on *Toxoplasma Gondii* proliferation.

Bilan Cocktail

Table 2 (below) includes all the results on the inhibition of the proliferation of *Toxoplasma gondii* in the presence of the 36 batches of essential oils.

	IC 50/4 IC 50/4	IC 50/4 IC 50/2	IC 50/4 IC 50	IC 50/2 IC 50/4	IC 50/2 IC 50/2	IC 50/2 IC 50	IC 50 IC 50/4	IC 50 IC 50/2	IC 50 IC 50
AB	16,4%	34,3%	63,5%	16,7%	39,3%	63,2%	47,6%	75,2%	82,4%
AE	45,5%	53,3%	85,7%	23,1%	53,5%	86,0%	57,0%	67,0%	91,9%
BE	27,7%	52,1%	79,6%	87,2%	93,4%	81,5%	73,8%	85,2%	96,4%
AD	19,4%	0,3%	31,9%	16,0%	20,2%	53,5%	54,1%	60,0%	69,6%

	50-90% proliferation inhibition
	>90% proliferation inhibition

Values represent inhibition of proliferation. Dotted area shows the combinations of inhibiting between 50-90% of the proliferation of the parasite. The striped area shows those for inhibiting the proliferation of more than 90%.

Conclusion

Various tests on the two parasites have yielded highly similar results. The combination of essential oils has in some cases increased the capacity of inhibiting the proliferation of the compounds. This suggests that essential oils can act synergistically. This is particularly true for the combinations AE (data not shown) and BE, where the measured effect of the cocktail is significantly higher than the expected additional effect of both essential oil taken alone. It is also important to note that all items tested had no cytotoxic effects on HFF cells (monolayer integrity).

CLAIMS

1. A composition for use as an oral anti-parasitic, the composition comprises one or more of
 - 5 i) an essential oil which contains gamma-terpinene, and/or carvacrol, and/or thymol, and/or terpinenol, and/or eucalyptol and/or eugenol
 - ii) an essential oil from genus *Cinnamomum*, *Eugenia*, *Eucalyptus*
 - iii) a source of saponin.
- 10 2. The composition for use as claimed in claim 1, which comprises an essential oil containing gamma-terpinene and/or carvacrol, and/or thymol, and/or terpinenol, and/or eucalyptol and/or eugenol and an essential oil from genus *Cinnamomum*, *Eugenia*, *Eucalyptus*.
- 15 3. The composition for use as claimed in any one of claims 1 or 2, wherein the essential oil that contains gamma-terpinene , and/or carvacrol, and/or thymol, and/or terpinenol, and/or eucalyptol and/or eugenol include the genus *Satureja*, *Thymus*, *Trachyspermum*, *Origanum* or *Melaleuca*.
- 20 4. The composition for use as claimed in any one of claims 1 to 3, wherein the composition comprises one or more of the essential oils from genus *Cinnamomum*, *Eugenia*, *Eucalyptus*.
- 25 5. The composition for use as claimed in any one of claims 1 to 4, wherein the source of saponin contains a mixture of steroid saponigenin.
6. The composition for use as claimed in any one of claims 1 to 5, for use in a cat or a dog.
- 30 7. The composition for use as claimed in any one of claims 1 to 6, wherein the composition is liquid, a tablet or a foodstuff.
8. The composition for use as claimed in any one of claims 1 to 7, wherein the composition is incorporated in a gelatinised starch matrix, or a wet product.

35

9. The composition for use as claimed in any one of claims 1 to 8, wherein the parasite is from the phylum Apicomplexa, optionally wherein the parasite is from the class conoidasida.
- 5 10. The composition for use as claimed in claim 9, wherein the parasite is *Neospora caninum* or *Toxoplasma gondii*.
- 10 11. A method of preventing or treating parasitic infection in an animal, the method comprising orally administering to said animal a composition comprising one or more of
- iv) an essential oil which contains gamma-terpinene, and/or carvacrol, and/or thymol, and/or terpinenol, and/or eucalyptol
 - v) an essential oil from genus *Cinnamomum*, *Eugenia*, *Eucalyptus*
 - vi) a source of saponin.
- 15 12. A composition comprising two or more of the following essential oils from the genus *Cinnamomum*, *Eugenia*, *Satureja*, *Thymus*, *Trachyspermum*, *orignum*, *Eucalyptus* or *Melaleuca*.
- 20 13. A composition comprising a source of saponin and one or more of
- i) an essential oil which contains gamma-terpinene and/or carvacrol, and/or thymol, and/or terpinenol, and/or eucalyptol and/or eugenol or
 - ii) an essential oil from genus *Cinnamomum*, *Eugenia*, *Eucalyptus*.
- 25 14. A process for the preparation of a composition as claimed in any one of claims 12 or 13 comprising mixing together the ingredients and optionally presenting the mixture in a form suitable for composition and administration.
- 30 15. The process as claimed in claim 14, wherein the composition is added to the mixture in the form of a spray, coating or incorporated in a gelatinised starch matrix.
- 35 16. The composition as claimed in any one of claims 12 or 13 for use as an anti-parasitic.

Figure 1

A		B		C		D	
EO Cinnamon China (leaves) <i>Cinnamomum cassia</i>		EO Clove Madagascar (leaves) <i>Eugenia caryophyllus</i>		EO Winter savory Balkans (leaves) <i>Satureja montana</i>		EO Red Thyme Spain (leaves) <i>Thymus zygis</i>	
	%		%		%		%
Cinnamaldehyde	70-90	Eugenol	75-85	Carvacrol	40-60	Thymol	40-60
o-Methoxycinnamaldehyde	<10	beta-Caryophyllene	<15	p-Cymene	<20	p-Cymene	<20
Cinnamyl acetate	<5	Eugenyl acetate	<5	gamma-Terpinene	<10	gamma-Terpinene	<15
Coumarin	<5	Methyl eugenol	<5	Thymol	<10	Linalool	<5
Phenylethyl alcohol	<5			Myrcene	<5	Carvacrol	<5
Benzaldehyde	<5			beta-Caryophyllene	<5	beta-Caryophyllene	<5
Cinnamic acid	<5			alpha-Pinene	<5	Myrcene	<5
Salicylaldehyde	<5			alpha-Terpinene	<5	Terpinene 1 ol 4	<5
Cinnamyl alcohol	<5			Terpinene 1 ol 4	<5	alpha-Terpinene	<5
Styrene	<5			alpha-Thuyene	<5	alpha-Pinene	<5
Acetophenone	<5			beta-Pinene	<5	alpha-Thuyene	<5
Eugenol	<5					Methyl carvacrol ether	<5
						Limonene	<5
						trans-Hydrate Sabinene	<5

E		F		G		H	
EO Origanum (leaves) <i>Origanum vulgare</i>		EO Eucalyptus China (leaves) <i>Eucalyptus globulus</i>		EO Australian Tea Tree (leaves) <i>Melaleuca alternifolia</i>		Saponin sources <i>Yucca schottigera</i>	
	%		%		%		
Carvacrol	65-85	Eucalyptol	70-90	4-Terpinenol	30-50	Diosgénine	
gamma-Terpinene	<5	Limonene	<10	gamma-Terpinene	70-30	sarsapogénine	
p-Cymene	<5	gamma-Terpinene	<10	alpha-Terpinene	<15	simlagénine	
beta-Caryophyllene	<5	p-Cymene	<5	alpha-Terpineol	<5	tigonenine	
Linalool	<5	alpha-Pinene	<5	Terpinolene	<5	hecogenine	
Thymol	<5			alpha-Pinene	<5	9-dehydhecogenine	
Beta phellandréne+limoné	<5			Eucalyptol	<5	glogénine	
beta-Pinene	<5			p-Cymene	<5	chlorogénine	
Borneol	<5			Limonene	<5	yuccagenine	
Campher	<5			delta-Cadinene	<5	samogénine	
Terpinene 1 ol 4	<5			Viridiflorol (Viridiflorol gamma)	<5	manogénine	
alpha-Terpineol	<5			Sabinene	<5	9-dehydromanogénine	
alpha-Humulene	<5			beta-Pinene	<5	kammogénine	
alpha-Terpinene	<5						

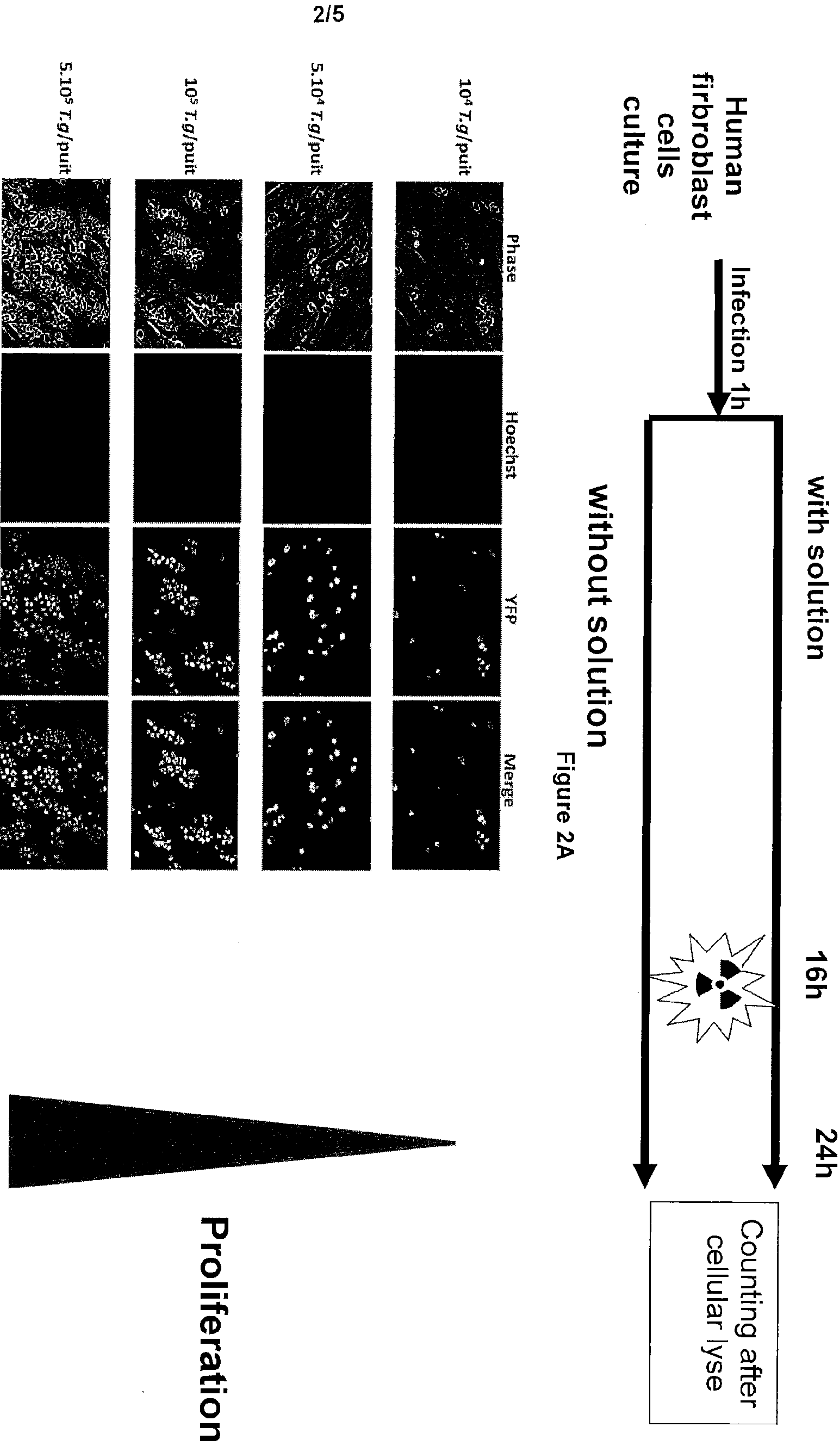


Figure 4

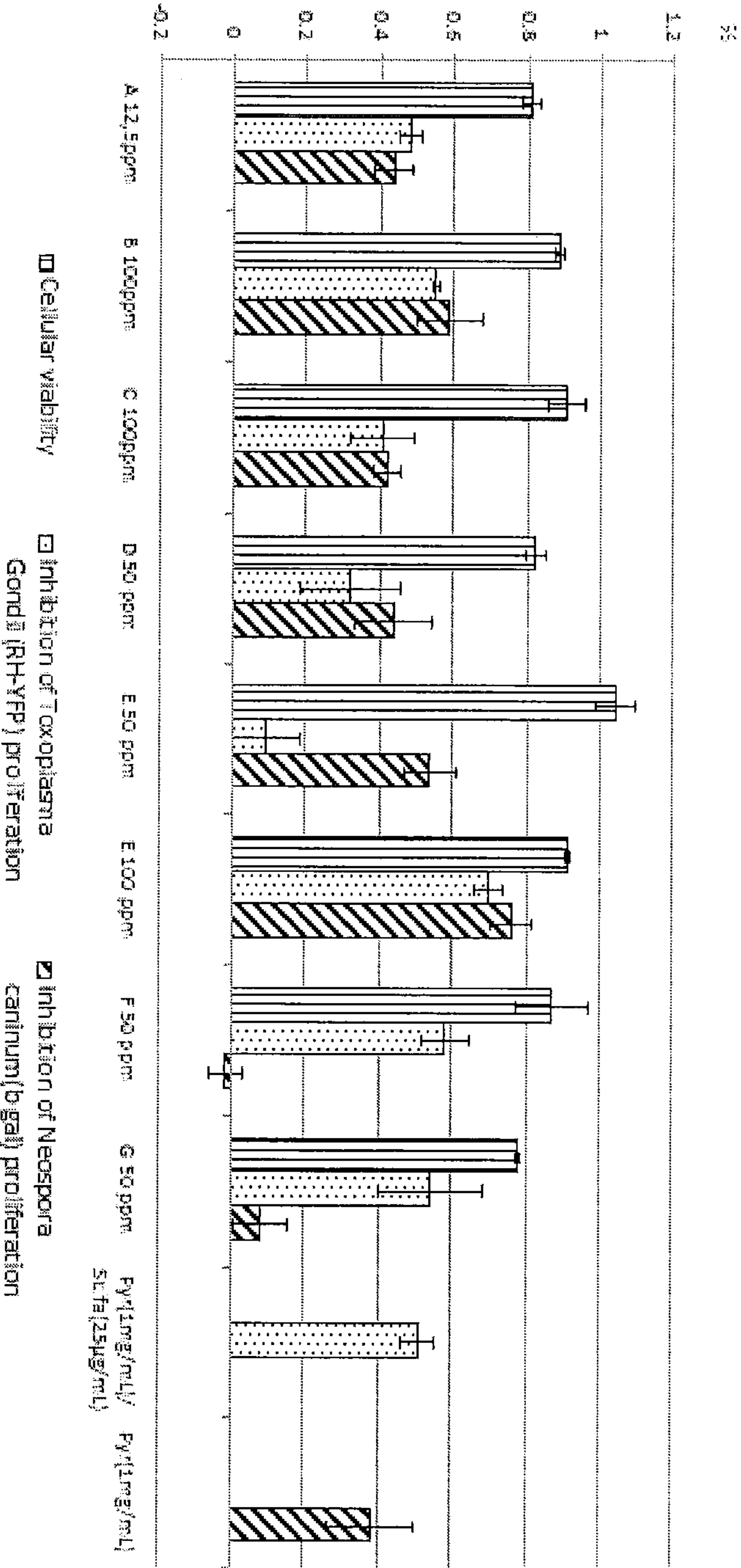


Figure 5

