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(54) **Title**: FULVIC ACID COMPOSITIONS AND THEIR USE

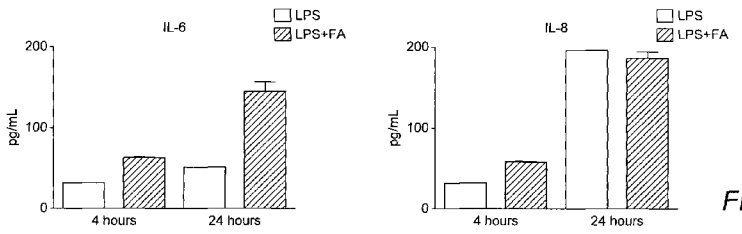

FIG. 3

(57) **Abstract**: Carbohydrate-derived fulvic acid (CHD-FA), a salt, ester or derivative thereof for use in the prevention, treatment or inhibition of infection by oral pathogens in a mammal, compositions, particularly compositions suitable for oral administration, comprising the same.

FULVIC ACID COMPOSITIONS AND THEIR USE

FIELD OF INVENTION

[0001] This invention relates to a use of fulvic acid in the prevention and treatment of infection by oral pathogens.

BACKGROUND OF THE INVENTION

[0002] Fulvic acid (FA) is a humic substance along with humic acid and humin that is formed during the decay of organic matter (MacCarthy et al., 1985). These substances are characterised on the basis of their solubility in water as a function of pH and FA is the fraction that is soluble in water under all pH conditions. In general, FA is also lower in molecular size and weight and lower in colour intensity than the humic acids.

[0003] Although soil and water naturally contain low levels of FA, this is difficult to isolate. Wet oxidation of bituminous coal, as described in US Patent No. 4,912,256 yields oxifulvic acids. Use of these oxifulvic acids for treatment of inflammation, acne, eczema, bacterial, fungal and viral infections has been described in US Patent Nos. 4,999,202 and 5,204,368 and International Patent Publication No. WO00/19999. However, oxifulvic acids contain high concentrations of heavy metals, including mercury, aluminium, chromium, lead and cadmium and are therefore not appropriate for use in medical, pharmaceutical and cosmetic preparations.

[0004] Carbohydrate sources such as saccharides including glucose, sucrose and fructose, starches and cellulose can also be treated by wet oxidation and produce a carbohydrate derived fulvic acid composition. This carbohydrate derived fulvic acid is suitable for use in medical, pharmaceutical and cosmetic preparations as it contains only a low content of the harmful elements. International Patent Publication No. WO2007/125492 describes the

carbohydrate derived fulvic acid composition and a method for producing the composition (hereinafter referred to as CHD-FA).

DISCLOSURE OF THE INVENTION

[0005] It has been found that fulvic acid as described and claimed in International Patent Publication No. WO 2007/125492 (CHD-FA), a salt, ester or derivative thereof is effective in preventing, treating or inhibiting infection by oral pathogens in mammals.

[0006] Thus, the invention provides, according to one aspect, CHD-FA, a salt, ester, or derivative thereof for use in the prevention, treatment or inhibition of infection by oral pathogens in a mammal, which may be human or animal.

[0007] The CHD-FA, salt, ester or derivative thereof may be administered in an amount from about 8% to 60% v/v of CHD-FA in an oral formulation. Preferably, the amount is about 35% v/vof CHD-FA in an oral formulation.

[0008] The CHD-FA, salt, ester or derivative thereof can have any pH, from acid to basic. For example, the pH of the CHD-FA can be raised by converting the acid into a salt, such as the sodium or potassium salt. This may be achieved by adding a suitable hydroxide to the CHD-FA.

[0009] According to a further embodiment of the invention, there is provided a composition comprising CHD-FA, salt, ester or derivative thereof and a suitable carrier for oral administration.

[0010] The composition may be in the form of a mouthwash, toothpaste, denture cleanser, impregnated dental floss or dental tape, endodontic irrigant or the like. The CHD-FA, salt, ester or derivative thereof may be an active ingredient or an adjuvant. The composition may further comprise flavourants and the like.

[0011] According to a further aspect of the invention, there is provided a method of preventing, treating or inhibiting infection by oral pathogens in a mammal including the step of administering an effective amount of the CHD-FA, salt, ester or derivative thereof to the mammal.

[0012] The administration will be oral. For human administration, the CHD-FA, salt, ester or derivative thereof may be formulated into a form such as a mouthwash, toothpaste, denture cleanser, impregnated dental floss or dental tape, endodontic irrigant or the like. The CHD-FA, salt, ester or derivative thereof may be the active ingredient or an adjuvant. The formulated form may further comprise flavourants and the like.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the effectiveness of CHD-FA in killing oral biofilms in comparison to other treatments i.e. Tea Tree Oil and the commercially available products, Oral B and Corsodyl.

Figure 2 shows the relative lack of toxicity of CHD-FA when applied to the OKF6 cell line.

Figure 3 shows the immuno-modulatory effect of CHD-FA on IL-6 and IL-8.

Figure 4 shows the relative lack of toxicity of CHD-FA compared to Chlorhexadine, an ingredient commonly used to treat oral pathogens.

DESCRIPTION OF PREFERRED EMBODIMENTS

[0013] CHD-FA, a salt, ester or derivative thereof for use in a method of preventing, treating or inhibiting infection by oral pathogens in mammals is described herein.

[0014] The optimal dose of the CHD-FA, a salt, ester or derivative thereof is an amount of about 35% of CHD-FA in an oral formulation maintained in the mouth for about 60 seconds. However a range of from about 8% to about 60%

v/v CHD-FA in an oral formulation maintained in the mouth from about 30 seconds to about 60 seconds could also be used.

[0015] The CHD-FA, salt, ester or derivative thereof can have any pH, from acid to basic. For example, the pH of the CHD-FA can be raised by converting the acid into a salt, such as the sodium or potassium salt. This may be achieved by adding a suitable hydroxide to the CHD-FA.

[0016] The administration would generally be oral. Typically, the CHD-FA, salt, ester or derivative thereof would be formulated into a form such as a mouthwash, toothpaste, denture cleanser, impregnated dental floss or dental tape, or endodontic irrigant or the like, with the CHD-FA, salt, ester or derivative thereof as the active ingredient, or an adjuvant. Additional ingredients, including flavourants may also be included in the oral formulation.

[0017] An *in vitro* study was conducted to evaluate the antimicrobial activity of CDH-FA against a range of oral pathogens associated with caries, periodontal disease, endodontic infection and soft tissue infections. In addition, the toxicological properties of the compound were evaluated.

[0018] The following example is for the purpose of illustration only and is not to be construed as limiting on the invention in any way.

EXAMPLES

Methods

[0019] A panel of oral pathogens, including Gram-positive, Gram-negative and yeasts were grown planktonically and tested with CHD-FA using standardised CLSI methodology. In addition, biofilm populations were grown using a 96-well peg plate method, challenged with CHD-FA, and the sessile MIC's evaluated. Static and cidal activity was assessed. A TR146 oral epithelial cell line was used to assess the toxicity of CHD-FA using both metabolic (XTT) and LDH assays.

Results

[0020] CHD-FA (0.5% active matter) was effective against the entire panel of planktonic Gram positive, Gram negative bacteria and yeasts, which included *Streptococcus mutans*, *Porphymonas gingivalis* and *Candida albicans*, and it was shown to exhibit cidal activity.

[0021] Against biofilms the CHD-FA was less effective, with a range of activity of 2- ≥ 4% active matter. However, CHD-FA was shown to be more effective in killing oral biofilms in comparison to other treatments i.e. Tea Tree Oil and the commercially available products, Oral B and Corsodyl as illustrated in Figure 1.

[0022] In addition, CHD-FA was shown to be relatively non-toxic when applied to the OKF6 cell line (see Figure 2). When the cell line was exposed a concentration of 0.5% active matter CHD-FA, the planktonic MIC, it did not exhibit any cellular toxicity by either assay. Furthermore, CHD-FA is relatively non-toxic compared to Chlorhexadine, an ingredient commonly used to treat oral pathogens (see Figure 4).

[0023] As indicated in Figure 3, CHD-FA was shown to have an immune-modulatory effect on the cytokine IL-6 and the chemokine IL-8 which are often associated with inflammation.

[0024] As shown in Table 1, when the efficacy of CHD-FA is compared to the natural product Tea Tree Oil, or existing over-the counter products, Oral B and Corsodyl, we see that CHD-FA is superior to all the other treatments for a range of oral pathogens.

[0025] The values in percent in the Figures refers to % active matter.

Table 1: Efficacy of CHD-FA against a range of oral pathogens

	NATURALS						OTC's					
DRUG	Fulvic Acid %			Tea Tree Oil %			Oral B %			Corsodyl® %		
Bacterium	PMIC	PMBC	SMIC	PMIC	PMBC	SMIC	PMIC	PMBC	SMIC	PMIC	PMBC	SMIC
S. *sanguinis*	0.0625	0.0625	0.25	>10	>10	10	0.3125	5	10	0.3125	0.625	5
S. *salivarius*	0.0625	0.125	0.25	>10	>10	>10	>10	>10	10	0.3125	0.3125	5
S. *mutans*	0.125	0.25	0.25	>10	>10	>10	2.5	>10	10	0.3125	0.625	5
E. *faecalis*	0.125	0.25	0.25	>10	>10	>10	1.25	2.5	10	0.3125	>10	5
Aggregatibacter actinomycetemcomitans	0.0625	0.125	0.25	>10	>10	>10	0.625	5	>10	0.625	0.625	10
F. *nucleatum*	0.0625	0.0625	1.25	>10	>10	5	2.5	2.5	0.625	2.5	2.5	0.3125
P. *gingivalis*	0.03125	0.25	0.25	>10	>10	10	10	>10	1.25	1.25	2.5	1.25
C. *albicans*	0.125	0.125	0.25	0.625	2.5	>10	0.156	0.156	5	0.625	1.25	5
C. *glabrata*	0.125	0.125	0.25	0.625	>10	0.25	0.156	0.3125	2.5	0.625	1.25	5
C. *tropicalia*	0.03125	0.125	0.25	0.3125	>10	5	0.078	0.078	2.5	0.3125	0.3125	2.5

Conclusions

[0026] This study has shown that the natural antimicrobial CHD-FA is non-toxic and has cidal activity against a range of microbes associated with a variety of oral diseases. Therefore, as a mouthwash, this product has potential as an adjunct to mechanical disruption to minimise the microbial burden in the oral cavity, subject to further studies.

Claims:

1 Carbohydrate-derived fulvic acid (CHD-FA), a salt, ester or derivative thereof for use in the prevention, treatment or inhibition of infection by oral pathogens in a mammal.

2 CHD-FA according to claim 1, wherein the mammal is human.

3 A composition comprising CHD-FA, a salt, ester or derivative thereof for use in the prevention, treatment or inhibition of infection by oral pathogens.

4 A composition according to claim 3, in which the CHD-FA, a salt, ester or derivative thereof is present in an amount from about 8% to 60% v/v.

5 A composition according to claim 4, in which the amount is about 35% v/v.

6 A composition comprising CHD-FA, a salt, ester or derivative thereof and a suitable carrier for oral administration.

7 A composition according to claim 6, in the form of a mouthwash.

8 A composition according to claim 6, in the form of a toothpaste.

9 A composition according to claim 6, in the form of impregnated dental floss or dental tape.

10 A composition according to claim 6, in th form of an endodontic irrigant.

11 A composition according to any one of claims 6 to 10, in which the CHD-FA, salt, ester or derivative thereof is an active ingredient.

12 A composition according to any one of claims 6 to 11, in which the CHD-FA, salt, ester or derivative thereof is an adjuvant.

13 A composition according to any one of claims 6 to 12, further comprising flavourants.

14 A method of preventing, treating or inhibiting infection by oral pathogens in a mammal including the step of administering an effective amount of CHD-FA, a salt, ester or derivative thereof to the mammal.

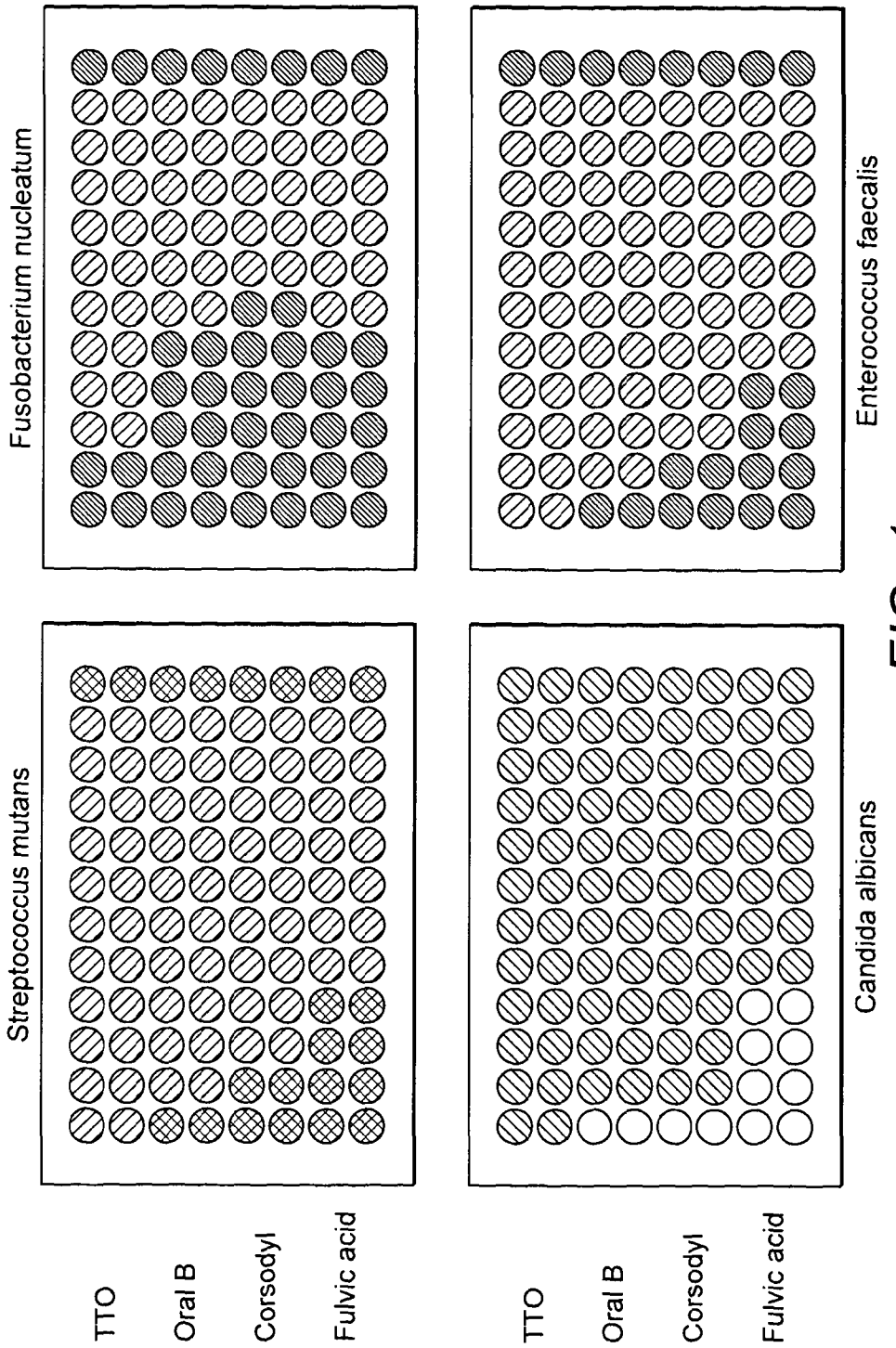
Fusobacterium nucleatum
Enterococcus faecalis
Streptococcus mutans
Candida albicans
TTO
Oral B
Corsodyl
Fulvic acid
TTO
Oral B
Corsodyl
Fulvic acid

FIG. 1

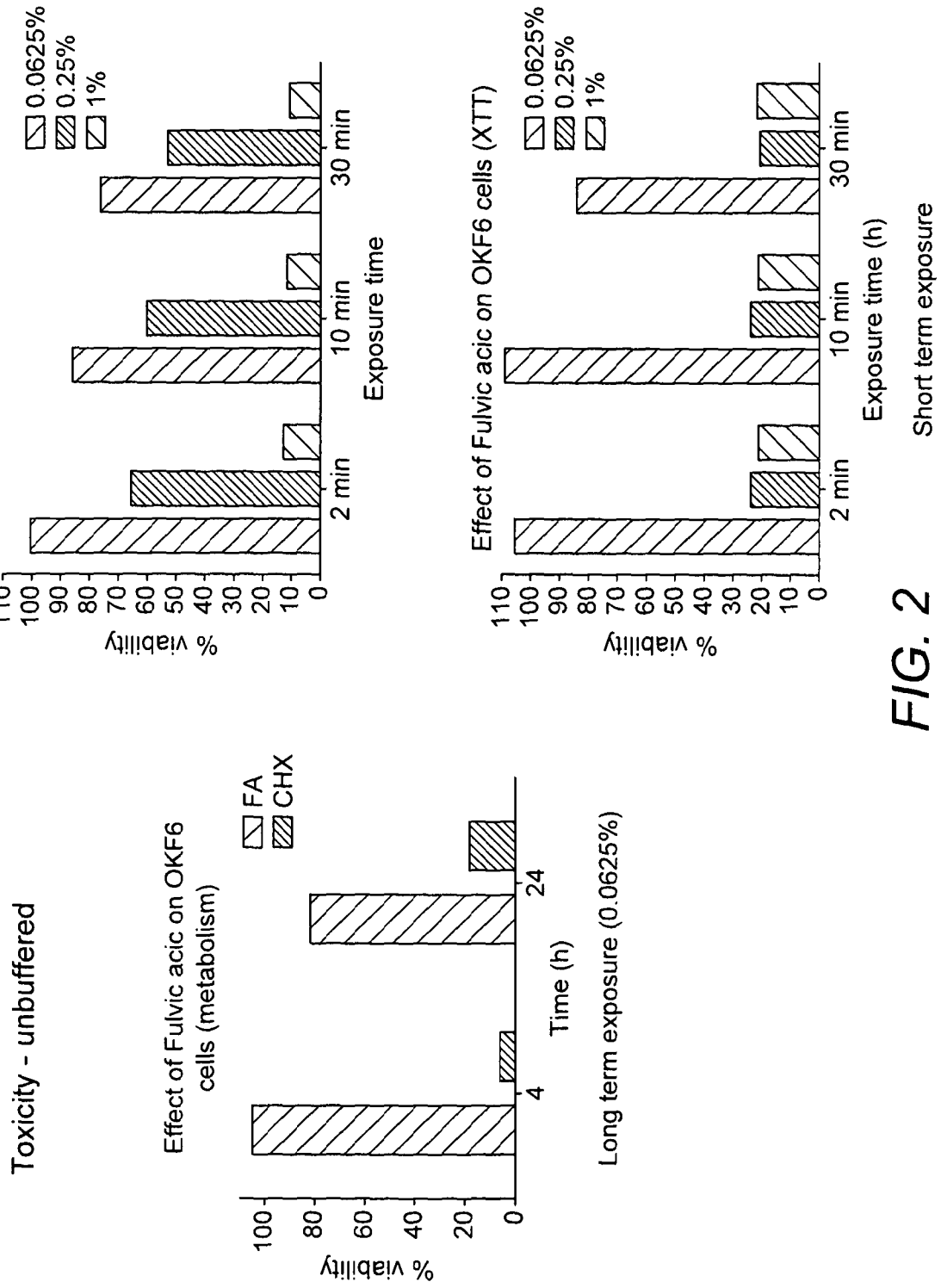
Toxicity - unbuffered
Effect of Fulvic acic on OKF6 cells (metabolism)
FA
CHX
% viability
Time (h)
Long term exposure (0.0625%)
Effect of Fulvic acic on OKF6 cells (LDH)
0.0625%
0.25%
1%
% viability
Exposure time
2 min
10 min
30 min
Effect of Fulvic acic on OKF6 cells (XTT)
Exposure time (h)
Short term exposure

FIG. 2

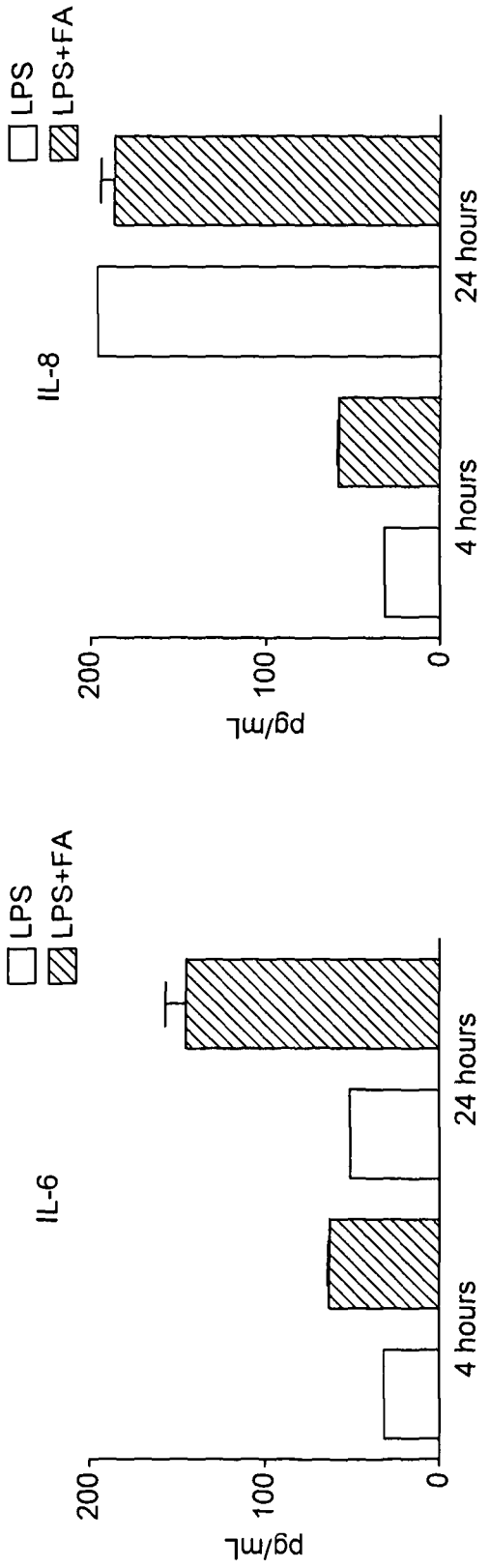

FIG. 3

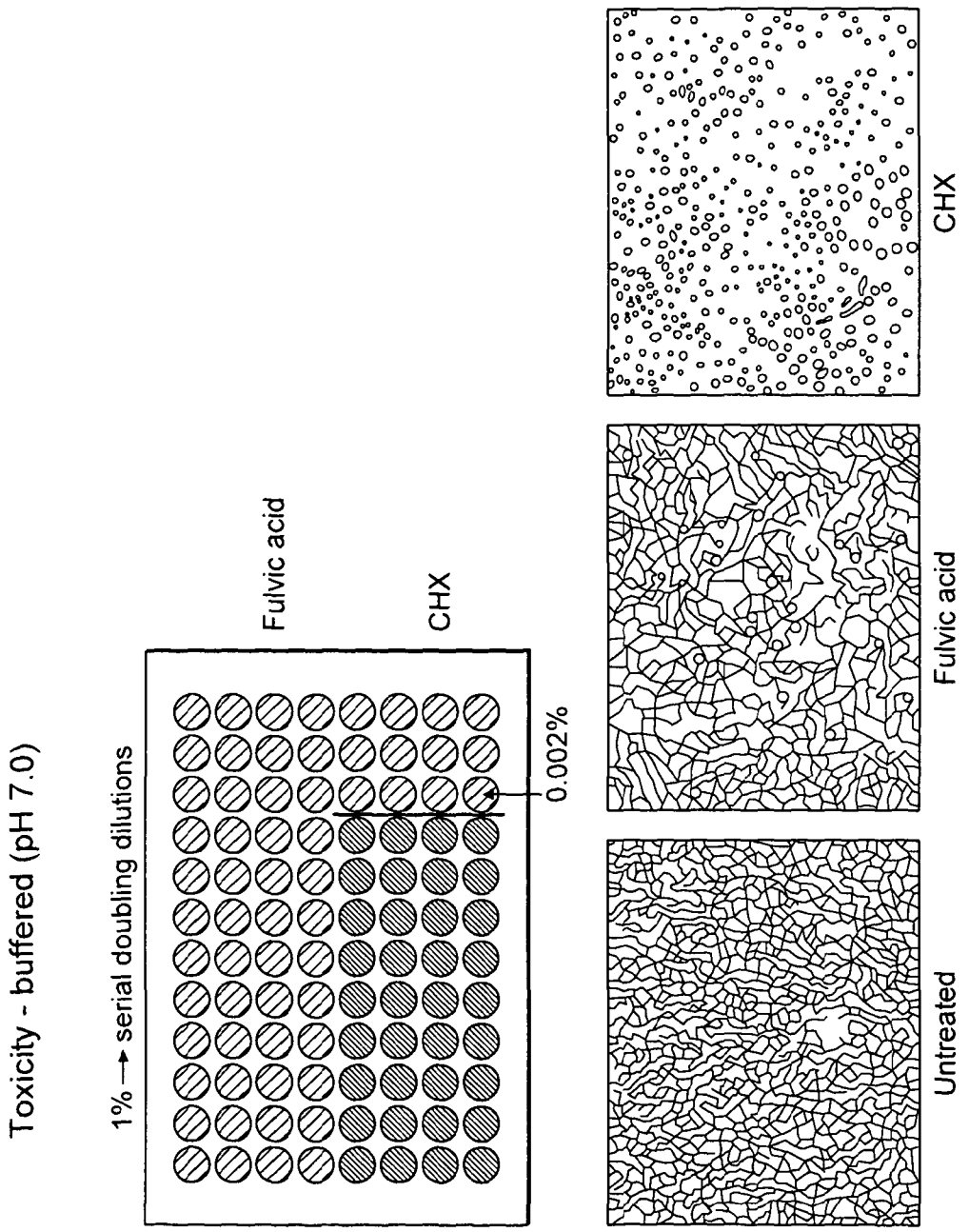
Toxicity - buffered (pH 7.0)
1% → serial doubling dilutions
Fulvic acid
CHX
0.002%
CHX
Fulvic acid
Untreated

FIG. 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2010/001641

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/352 A61P31/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, EMBASE, CHEM ABS Data, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2007/125492 A2 (PFEINSMITH S A PTY LTD [ZA]; LOXTON EARLE JOHN [ZA]; MALAN RUDOLPH JOH) 8 November 2007 (2007-11-08) page 3, paragraph 5 page 12, lines 21-24	1-3,6, 11,12,14
X	WO 2007/102813 A1 (ADVANTAGE MARKETING INC [US]; DAY KENNETH S [US]; HANSEN GEORGE A [US]) 13 September 2007 (2007-09-13) claims 15-17,23,24; examples 8,16	6,8, 10-13
X	WO 00/19999 A1 (ENERKOM PTY LTD [ZA]; DEKKER JOHANNES [ZA]; MEDLEN CONSTANCE ELIZABETH) 13 April 2000 (2000-04-13) claims 8,9	1-3,6, 11-14

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

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Date of the actual completion of the international search	Date of mailing of the international search report
30 November 2010	07/12/2010

Name and mailing address of the ISA/	Authorized officer
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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2010/001641

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO 2009/147635 A1 (PFEINSMITH S A PTY LTD [ZA]; FERNANDES ANTONIO CELESTINO [ZA]; MEDLEN) 10 December 2009 (2009-12-10) page 2, line 17; claims 1,16,22	1-3,6, 11,12,14

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2010/001641

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2007125492 A2	08-11-2007	CN 101437509 A	20-05-2009
		EP 2023916 A2	18-02-2009
		RU 2008147213 A	10-06-2010
WO 2007102813 A1	13-09-2007	NONE	
WO 0019999 A1	13-04-2000	AT 410158 T	15-10-2008
		AT 410159 T	15-10-2008
		AT 410160 T	15-10-2008
		AT 336997 T	15-09-2006
		AU 766198 B2	09-10-2003
		AU 5992399 A	26-04-2000
		DE 69932939 T2	07-12-2006
		EP 1126837 A1	29-08-2001
		JP 2002526407 T	20-08-2002
		US 6569900 B1	27-05-2003
WO 2009147635 A1	10-12-2009	NONE	