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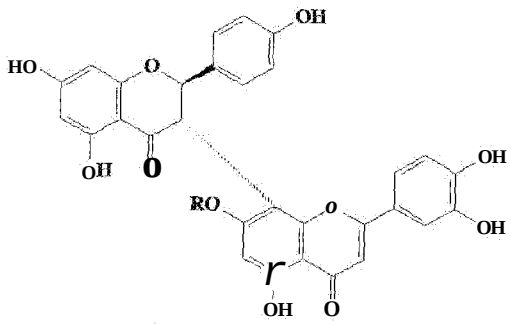
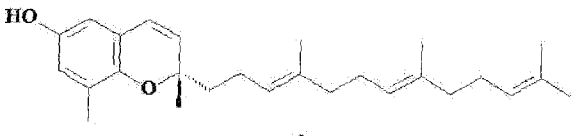
[Continued on nextpage]

(54) Title: DERMATOLOGICAL COMPOSITIONS

(57) Abstract: The invention provides a skin-lightening composition which includes at least one compound selected from morelloflavone, morelloflavone-7"-sulphate and sargaol.



morelloflavone R = H
morelloflavone-7"-sulphate 7 R = SO_3H



sargaol

FIG 1

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DERMATOLOGICAL COMPOSITIONS

THIS INVENTION relates to invention relates to dermatological compositions. It relates, in particular, to skin-lightening compositions.

There is a perception in some societies that fair skin is more attractive than darker skin and for this reason skin-lightening creams are often used for cosmetic reasons and not to treat underlying skin disorders. The management of hyperpigmentation disorders constitutes a serious challenge for dermatologists. Amongst the common disorders of hyperpigmentation are conditions such as melasma, lichen planus, solar lentigo, freckles, and post inflammatory hyperpigmentation. The disorders of hyperpigmentation are challenging to treat[1]. The gold standard for the treatment of hyperpigmentation is hydroquinone. However, hydroquinone is associated with long term side effects such as ochronosis, as well as irritant contact dermatitis post inflammatory hyperpigmentation, and confetti hypopigmentation. [1,2,3]

Exogenous ochronosis is cosmetically induced pigmentation of the skin due to the deposition of polymerized products of homogentisic acid in the connective tissue, as a result of the prolonged application of skin lightening creams containing hydroquinone (HQ), and was initially described by Findlay [2,3] in 1975 in thirty five black South African females. The largest number of cases of exogenous ochronosis was found in South Africa where epidemic proportions were reached due to the lack of regulation of the use of HQ. The largest study was undertaken by Phillips *et al.* in 1986 where, over a period of a year, 395 patients in Johannesburg were diagnosed with ochronosis.[4] In some cases bleaching creams in concentrations of 3.5% -7% had been used for periods of up to eight years[3].

HQ is still widely used as a depigmentation agent in dermatology. In the United States, the FDA designated 2% hydroquinone as a safe concentration in OTC creams.

The designation was based on Findlay's article which showed that 3.5% to 7.5% concentrations of HQ caused exogenous ochronosis[3]. In South Africa, the concentration of HQ in over the counter creams may not legally exceed 2%[5]. Hoshaw *et al.*[6] and Conner and Braunstein[7] questioned the safety of 2% HQ preparations because patients had acquired ochronosis despite using over the counter HQ preparations. This suggests that a significant number of manufacturers did not comply with labelling requirements. Even if the concentration of HQ in a preparation does not exceed 2%, the amount, frequency, and overenthusiastic application of the creams as well as exposure to sunlight[2] also play a role. In South Africa skin damage resulting from the abuse of skin lightening creams which are illegally imported and improperly labelled is still a major problem. This is due to poor regulation and lack of consumer education (conference proceedings N.C. Dlova Annual Congress of the South African Dermatology Society, Johannesburg 2007 and Continental Congress of Dermatology , ICC Durban, 24-27 Oct 201 2). In addition in Singapore, Tan *et al.* have reported two cases of hydroquinone induced exogenous ochronosis in Chinese patients using the lower concentration of 2%. This report casts some doubts on the safety of 2% hydroquinone[5]. For this reason the cosmetic and pharmaceutical industries are constantly searching for safer products for treating hyper pigmentation.

It is an object of the invention to provide skin-lightening compositions which do not have long-term side effects. The Applicant has found that the compounds morelloflavone, morelloflavone-7''-sulphate and sargaol can be used to produce such compositions.

According to a first aspect of the invention there is provided a skin-lightening composition, the composition including at least one compound selected from morelloflavone, morelloflavone-7''-sulphate and sargaol.

According to a second aspect of the invention there is provided a method of lightening the skin, the method including the step of applying to the skin a skin-lightening composition which includes at least one compound selected from morelloflavone, morelloflavone-7''-sulphate and sargaol.

According to a third aspect of the invention there is provided the use of at least one compound selected from morelloflavone, morelloflavone-7''-sulphate and sargaol in the preparation of a composition for lightening the skin.

According to a fourth aspect of the invention there is provided a method of preparing a skin lightening composition, the method including combining at least one compound selected from morelloflavone, morelloflavone-7''-sulphate and sargaol with one or more pharmaceutically acceptable excipients.

The composition may include one, two or all three of the compounds.

The composition may be in the form selected from creams, ointments, lotions or gels.

The concentration of the compound or compounds in the composition may be between about 2% and 7% and is preferably between about 2% and 4% on a mass to mass basis.

The invention is now described, with reference to the accompanying examples and Figures in which

Figure 1 shows the structures of the compounds morelloflavone, morelloflavone-7''-sulphate and sargaol;

Figure 2 is a graph of the log of the concentration of hydroquinone (HQ) as a function of cell death and melanin concentration;

Figure 3 shows bar graphs indicating cell death for HQ, morelloflavone, morelloflavone-7''-sulphate and sargaol; and

Figure 4 shows graphs of the logs of concentrations of morelloflavone, morelloflavone-7''-sulphate and sargaol as functions of cell death and melanin concentration.

Example

Toxicoloqical studies of morelloflavone, morelloflavone-7''-sulphate and sargaol.

MeWo cells, a human malignant melanoma cell line, were selected to investigate the action of the compounds in vitro, both in terms of impact on melanin levels and overall cytotoxicity. A human granular fibroblast cell line derived from malignant melanoma was used in all in vitro assays (MeWo; ECACC No: 93082609) and were purchased from ECACC (Porton Down, UK). Cells were grown in EMEM supplemented with 2mM glutamine, 1% non-essential amino acids, 10% foetal bovine serum and 100U/ml penicillin, 100μg/ml streptomycin. All cell culture medium and supplements were purchased from Invitrogen (Paisley, UK). MeWo cells were seeded into 96-well plates (Nunc International, Leicestershire, UK) at a concentration of 10,000 cells/well and incubated at 37 °C for 48 hours in a humidified container for attachment. The compounds or vehicle (0.1 % DMSO) were then added to the wells at the indicated concentrations and the cells were incubated for a further forty-eight hours. Following exposure, medium was collected and cytotoxicity determined by LDH-leakage using the cytotoxicity detection kit (Roche, Lewes, Sussex, UK), as per the manufacturer's instructions. For evaluation of melanin content, cells were washed with PBS, lysed with 1N NaOH and centrigued to remove cellular debris. The melanin content of the supernatant was then determined by optical density at 405 nm (Takiwaki et al., 2004).

Morelloflavone 1, morelloflavone-7''-sulphate 2, and sargaol 3 were screened for both cytotoxicity and activity on mammalian melanocytes *in vitro.* The melanin content of the melanocytes was determined after dosing these cells with known concentrations of the compounds, while the cytotoxicity assay was based on the measurement of lactate dehydrogenase (LDH) released from the cytosol of the damaged cells into the supernatant culture medium.

Prior to examining the compounds the response of MeWo cells to the classical pro-oxidant hydroquinone was examined. As can be seen from Figure 2, hydroquinone was able to elicit a dose-dependent decrease in melanin content of the cells, with an EC50 = 87 μM. This effect on melanin content was accompanied by dose-dependent cytotoxicity, with an IC50 = 45 μM.

Following characterization of the response of MeWo cells to hydroquinone, the effect of the 25 μM concentrations of the compounds on both melanin content and cytotoxicity was determined. The results are set out in Figure 3. Exposure of the cells to morrelloflavone, morelloflavone -7''-sulphate at concentrations of 25 μM caused between 10 - 20% cell death (Figure 3A). In addition, as can be seen from Figure 3B, all of the compounds were able to decrease the melanin content of the cells, although to differing extents.

Morelloflavone, morelloflavone-7''-sulphate and sargaol all caused significant decreases in melanin content (approximately 60% of control; Figure 3B), while causing less than 20% cell death. Figure 4 shows dose response curves for each compound (1 μM to 100 μM), with both cytotoxicity (LDH) and melanin content measured. Morelloflavone, morelloflavone-7''-sulphate and sargaol can all be seen to elicit a dose-dependent effect on cytotoxicity and melanin content, with morelloflavone-7''-sulphate having the most promising profile. Figure 4B shows a clear separation of the curves for cytotoxicy (IC50 = 50.5 μM) and melanin content (EC50 = 5.7 μM), suggesting that this compound has the most favourable balance of maximal pharmacological effect with minimal toxicological liability. It should be noted, however, that of the three compounds tested, morelloflavone-7''-sulphate also elicited the smallest absolute change in melanin content, being 74% of control, compared to 13% for sargaol. The dosage range was insufficient to determine a robust maximal effect for morelloflavone, but it is expected to be in excess of 60%.

From these results it can be seen that morelloflavone, morelloflavone-7''-sulphate and sargaol are all more effective in reducing melanin content of MeWo human melanoma cells than hydroquinone. In addition, these compounds are less cytotoxic than HQ with

morelloflavone-7''-sulphate showing the best separation between the reduction in melanin and induction of cell death.

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Claims

1. A skin-lightening composition, the composition including at least one compound selected from morelloflavone, morelloflavone-7''-sulphate and sargaol.

2. A method of lightening the skin, the method including the step of applying to the skin a skin-lightening composition which includes at least one compound selected from morelloflavone, morelloflavone-7''-sulphate and sargaol.

3. The use of at least one compound selected from morelloflavone, morelloflavone-7''-sulphate and sargaol in the preparation of a composition for lightening the skin.

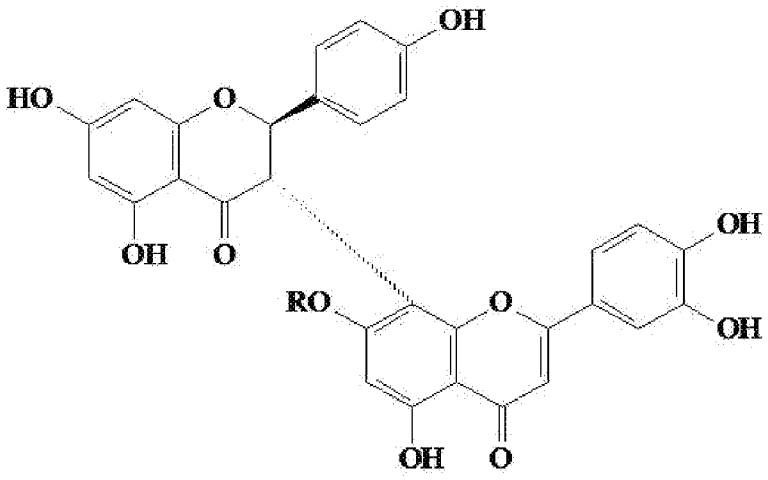
4. A method of preparing a skin lightening composition, the method including combining at least one compound selected from morelloflavone, morelloflavone-7''-sulphate and sargaol with one or more pharmaceutically acceptable excipients.

5. The composition, method or use of any one of claims 1 to 4 inclusive, in which the composition includes one, two or all three of the compounds.

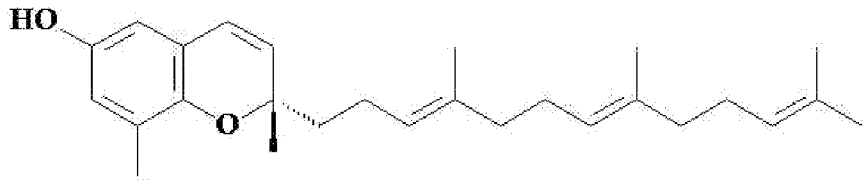
6. The method or the use of any one of claims 1 to 5 inclusive wherein the composition is in the form selected from creams, ointments, lotions or gels.

7. The method or the use of any one of claims 1 to 6 inclusive wherein the concentration of the compound or compounds in the composition is between 2 and 7% mass per mass.

8. The method or the use of claim 7, wherein the concentration of the compound or compounds in the composition is between 2 and 4% mass per mass.



morelloflavone R = H
morelloflavone-7''-sulphate 7 R = SO_3H



sargaol

FIG 1

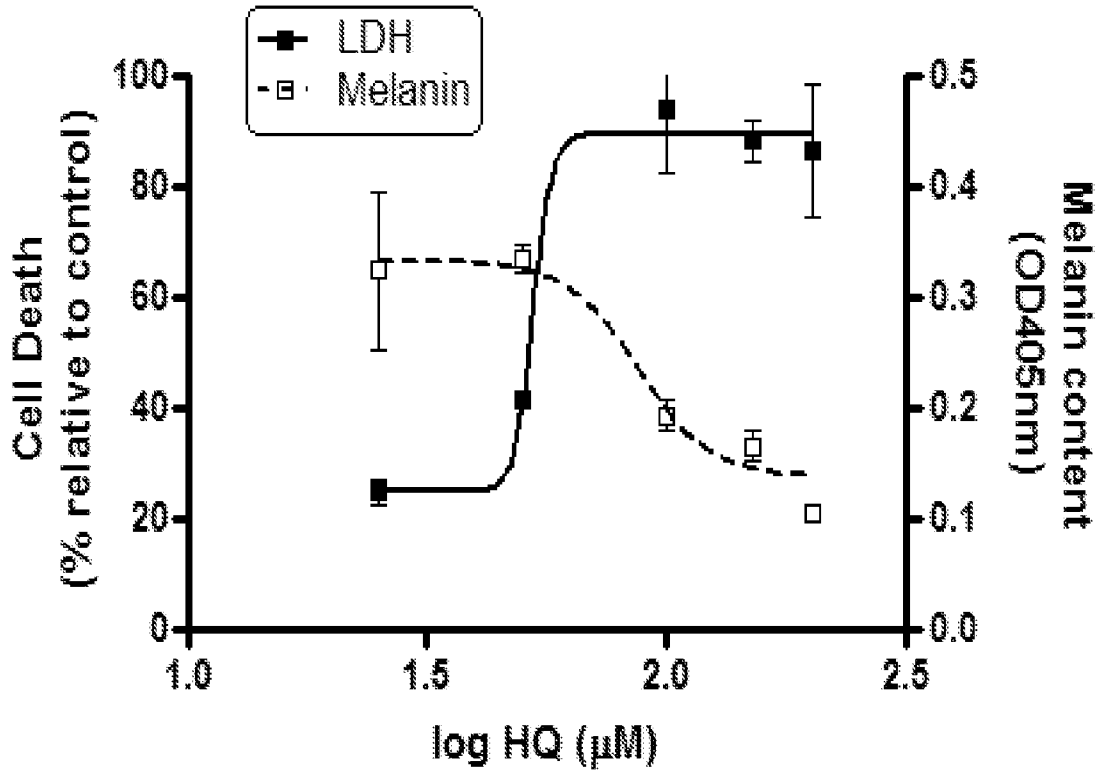

LDH
Melanin
Cell Death
(% relative to control)
Melanin content
(OD405nm)
log HQ (µM)
100
80
60
40
20
0
0.5
0.4
0.3
0.2
0.1
0.0
1.0
1.5
2.0
2.5

FIG 2

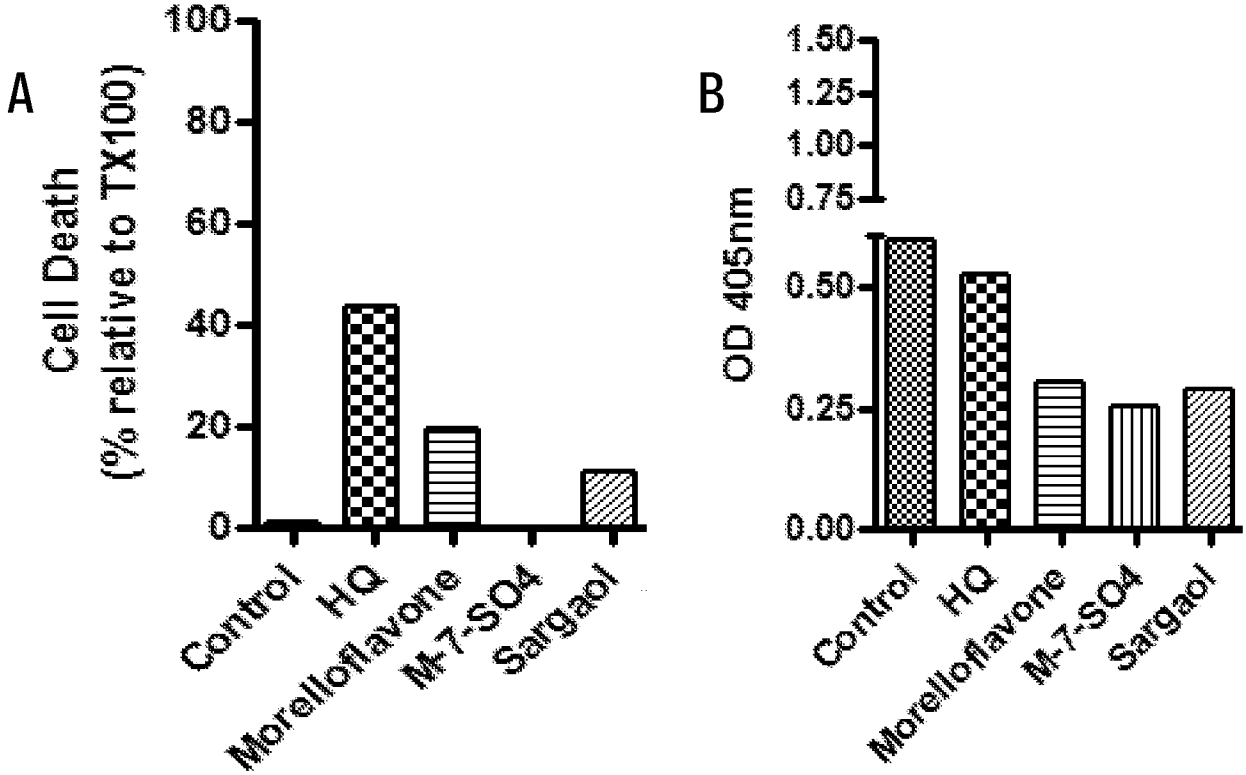

A
Cell Death
(% relative to TX100)
100
80
60
40
20
0
Control
HQ
Morelloflavone
M-7-SO4
Sargaol
B
OD 405nm
1.50
1.25
1.00
0.75
0.50
0.25
0.00
Control
HQ
Morelloflavone
M-7-SO4
Sargaol

FIG 3

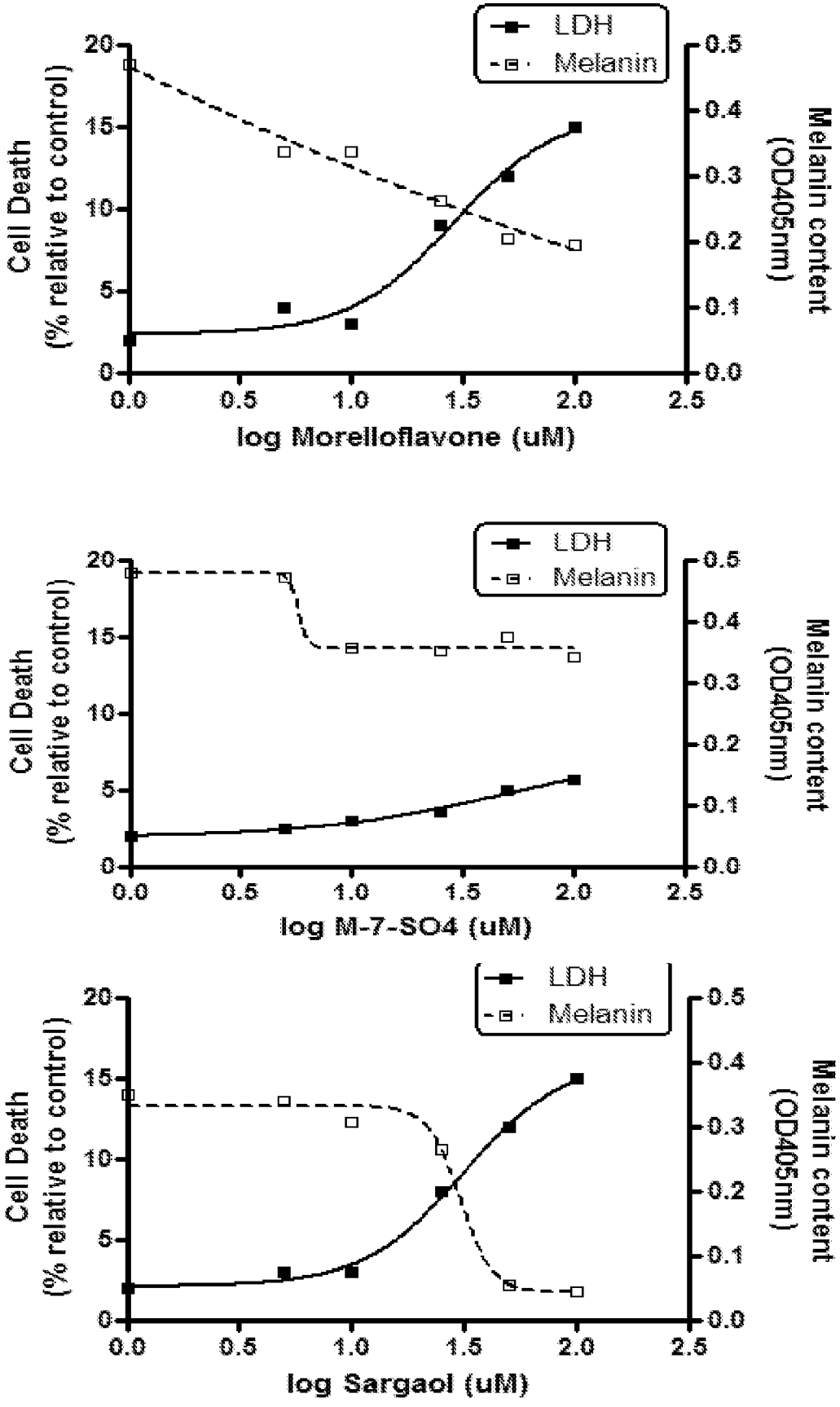

LDH
Melanin
Cell Death
(% relative to control)
Melanin content
(OD405nm)
log Morelloflavone (uM)
log M-7-SO4 (uM)
log Sargaol (uM)

FIG 4