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(54) Titre : COMPOSITIONS DE SOINS CAPILLAIRES CONTENANT UNE PREPARATION DE METABOLITE
EXTRACELLULAIRE ISSU DE BACILLUS COAGULANS
(54) Title: HAIR CARE COMPOSITIONS CONTAINING EXTRACELLULAR METABOLITE PREPARATION FROM
BACILLUS COAGULANS

(57) **Abrégé/Abstract:**

Disclosed are hair care compositions containing partially purified extracellular metabolite preparation from strains of *Bacillus coagulans*. Specifically, the uses of compositions containing extracellular metabolite preparation from a strain of *Bacillus coagulans* for increasing hair growth, inhibition of 5 α -reductase and proliferation of follicle dermal papilla cells and in the management of androgenic alopecia, are disclosed.

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(54) Title: HAIR CARE COMPOSITIONS CONTAINING EXTRACELLULAR METABOLITE PREPARATION FROM BACILLUS COAGULANS

(57) Abstract: Disclosed are hair care compositions containing partially purified extracellular metabolite preparation from strains of *Bacillus coagulans*. Specifically, the uses of compositions containing extracellular metabolite preparation from a strain of *Bacillus coagulans* for increasing hair growth, inhibition of 5 α -reductase and proliferation of follicle dermal papilla cells and in the management of androgenic alopecia, are disclosed.

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HAIR CARE COMPOSITIONS CONTAINING EXTRACELLULAR METABOLITE PREPARATION FROM BACILLUS COAGULANS

CROSS-REFERENCE TO RELATED APPLICATIONS

This is a PCT application claiming priority from US provisional application no. 62516077 filed on 6th June 2017

BACKGROUND OF THE INVENTION

Field of the invention

[PARA 001] The invention in general relates to hair care compositions. More specifically the invention relates to hair care composition containing partially purified extracellular metabolite preparation from *Bacillus coagulans*.

Description of prior art

[PARA 002] Hair is one of the fastest growing tissues of the human body. Hair follicles undergo repetitive regenerative cycles consisting of three stages: anagen (rapid growth, active stage), catagen (apoptosis-driven regression, physiological involution stage), and telogen (resting stage). Reduction in anagen stage can result in miniaturization of hair follicles and hair loss. Thus extending the anagen phase is an effective treatment for the prevention of hair loss.

[PARA 003] Growth and proliferation of follicle papilla cells are important for increasing the number of hair follicles and thereby to promote hair growth. Dermal papilla cells accumulate below undifferentiated epidermis which is then stimulated to form the hair “peg”. Interaction between the hair peg and the dermal papilla cells promotes differentiation into a mature hair follicle. During the anagen phase the dermal papilla cells secrete several growth factors, like insulin like growth factor (IGF-1), fibroblast growth factor (FGF-7) which stimulates the proliferation and elongation of hair shaft. Hence, the dermal papilla has to be present in good numbers to ensure sufficient hair growth. Certain clinical conditions like androgenic alopecia, seborrhoeic dermatitis (dandruff) etc reduce the number of dermal papilla and increase hair fall. TGF- β 2 is specifically expressed in human dermal papilla cells. TGF- β has been shown to exert unique multidirectional effect by inducing premature hair follicle regression in adult hair growth

cycle and is required for hair folliculogenesis. The role of TGF- β is well documented in the following references:

1. Foitzik K, Paus R, Doetschman T, et al. The TGF- β 2 isoform is both a required and sufficient inducer of murine hair follicle morphogenesis. *Dev Biol.* 1999; 212:278–89
2. Hibino T, Nishiyama T. Role of TGF- β 2 in the human hair cycle. *J Dermatol Sci.* 2004; 35: 9-18.
3. Jamora C, Lee P, Kocieniewski P, et al. A signaling pathway involving TGF- β 2 and snail in hair follicle morphogenesis. *PLoS Biol.* 2004; 3: e11.
4. Keita Inoue, Noriyuki Aoi, Yuji Yamauchi, Takahiro Sato, Hirotaka Suga, Hitomi Eto, Harunosuke Kato, Yasuhiko Tabata, Kotaro Yoshimura. TGF- β 2 is specifically expressed in human dermal papilla cells and modulates hair folliculogenesis *J. Cell. Mol. Med.* Vol 13, No 11-12, 2009 pp. 4643-4656

[PARA 004] Vascular endothelial growth factor (VEGF) is essential for angiogenesis and vascular permeability and is responsible for maintaining proper vasculature around the hair follicle during the anagen growth phase. VEGF mRNA is strongly expressed in dermal papilla cells (DPC) in the anagen phase, but during the catagen and telogen phases. VEGF mRNA is less strongly expressed (Kozłowska et al., Expression of vascular endothelial growth factor (VEGF) in various compartments of the human hair follicle *Arch Dermatol Res* (1998) 290 :661–668; Yano et al., Control of hair growth and follicle size by VEGF-mediated angiogenesis *J Clin Invest.* 2001;107(4):409-417). Both VEGF and TGF- β 2 promote the proliferation of dermal papilla cells and thereby increase hair growth.

[PARA 005] The dermal papilla also produces an enzyme called 5 α -reductase (3-oxo-5 α -steroid- Δ 4-dehydrogenase;), a NADPH-dependent enzyme, which converts Testosterone to dihydrotestosterone (DHT). Overproduction of 5 α -reductase increases the conversion of testosterone to DHT in hair follicles, thereby disrupting the natural hair cycle leading to hair fall and development of androgenic alopecia.

[PARA 006] Natural 5 α -reductase inhibitors like mushrooms, green tea etc. slow down the production of DHT and prevent hair loss. The use of probiotic in hair care is now garnering

attention owing to the fact that probiotics can alter the microbiome of the skin responsible for hair loss. US7374750, discloses a composition containing a probiotic organism for hair care. The beneficial effects of probiotics for hair growth and increasing follicle dermal papilla is also well documented by Levkovich et al. (2013); Probiotic Bacteria Induce a 'Glow of Health', PLoS One. 2013; 8(1): e53867. Probiotics and their extracellular products are also reported to exhibit excellent anti-fungal activity, making them a valuable addition individually or as an adjuvant in anti-dandruff compositions.

[PARA 007] It is well known in the scientific art that biological effects of probiotics or products thereof are strain specific and cannot be generalised among genera, species and strains (Probiotics: In Depth/NCCIH, U.S. Department of Health and Human Services, National Institutes of Health). Hence, there exists a need to find a superior probiotic strain and its extracellular product which promotes the growth of dermal papilla cells and prevents hair loss by inhibiting the 5 α -reductase. Also, the beneficial effects of extracellular product obtained from probiotics on hair growth remain to be validated. The present invention solves the above problem by disclosing the beneficial effect of partially purified extracellular metabolite preparation of *Bacillus coagulans* on dermal papilla proliferation.

[PARA 008] It is the principle objective of the invention to disclose a composition containing the partially purified extracellular metabolite preparation of *Bacillus coagulans* to increase hair growth.

[PARA 009] It is another objective of the invention to disclose a composition containing the partially purified extracellular metabolite preparation of *Bacillus coagulans* to inhibit the activity of 5 α -reductase.

[PARA 0010] It is yet another objective of the invention to disclose a composition containing the partially purified extracellular metabolite preparation of *Bacillus coagulans* for use in the therapeutic management of androgenic alopecia.

DEPOSIT OF BIOLOGICAL MATERIAL

[PARA 0011] The deposit of biological material *Bacillus coagulans* bearing accession number MTCC 5856, mentioned in the instant application has been made on 19th September 2013 at Microbial Type

Culture Collection & Gene Bank (MTCC), CSIR-Institute of Microbial Technology, Sector 39-A, Chandigarh – 160036, India.

SUMMARY OF THE INVENTION

[PARA 0012] The present invention discloses a hair care composition containing the partially purified extracellular metabolite preparation isolated from a strain of *Bacillus coagulans*. More specifically, the invention discloses the use of a composition containing the extracellular metabolite isolated from a strain of *Bacillus coagulans* for the enhancing hair growth by increasing the proliferation follicle dermal papilla cells and inhibition of 5 α -reductase .

BRIEF DESCRIPTION OF THE DRAWINGS

[PARA 0013] Fig.1a is the graphical representation of the percentage increase in dermal papilla cell proliferation compared to control by partially purified extracellular metabolite preparation from *Bacillus coagulans* MTCC 5856.

[PARA 0014] Fig.1b and 1c depict the dose dependent increase in the proliferation of dermal papilla cells in the presence of partially purified extracellular metabolite preparation from *Bacillus coagulans* MTCC 5856.

[PARA 0015] Fig.2 is the graphical representation of the percentage increase in the expression of TGF- β 2 in the dermal papilla cells in the presence of partially purified extracellular metabolite preparation from *Bacillus coagulans* MTCC 5856.

[PARA 0016] Fig.3 is the graphical representation of the percentage increase in the expression of VEGF in the dermal papilla cells in the presence of partially purified extracellular metabolite preparation from *Bacillus coagulans* MTCC 5856.

[PARA 0017] Fig.4a depicts the percentage inhibition of 5 α -reductase by partially purified extracellular metabolite preparation from *Bacillus coagulans* MTCC 5856.

[PARA 0018] Fig.4b is the graphical representation showing decrease in concentration of dihydrotestosterone due to the inhibition of 5 α -reductase activity by partially purified extracellular metabolite preparation from *Bacillus coagulans* MTCC 5856.

DESCRIPTION OF THE MOST PREFERRED EMBODIMENTS

[PARA 0019] In a most preferred embodiment, the invention discloses a hair care composition containing the partially purified extracellular metabolite preparation from a strain of *Bacillus coagulans* formulated with pharmaceutically/cosmeceutically acceptable excipients, adjuvants, bases, diluents, carriers, conditioning agents and preservatives. In a related embodiment, the composition containing the partially purified extracellular metabolite preparation from a strain of *Bacillus coagulans* can be combined with and/or incorporated into formulations containing hair care ingredients. In another related embodiment, the hair care composition is administered topically in the form of creams, gels, lotions, shampoo, serum, oil, suspensions, emulsions, and compacts. In another embodiment, the strain of *Bacillus coagulans* is selected from the group consisting of *Bacillus coagulans* MTCC 5856, *Bacillus coagulans* ATCC 31284 and *Bacillus coagulans* ATCC 7050

[PARA 0020] In another related embodiment, the invention relates to a method of increasing hair growth in mammals, said method comprising steps of administering effective concentration of partially purified extracellular metabolite preparation from a strain of *Bacillus coagulans* to said mammals, to bring about increase in hair growth. In a related embodiment, the increase in hair growth is brought about by a) increasing the expression of secretory factors and b) increasing the proliferation of follicle dermal papilla cells. In another related embodiment, the secretory factors are selected from the group consisting of vascular endothelial growth factor (VEGF) and transforming growth factors – β (TGF- β). In another related embodiment, the effective concentration of the partially purified extracellular metabolite preparation is 0.01% v/v to 2.0% v/v of the total composition. In another embodiment, the strain of *Bacillus coagulans* is selected from the group consisting of *Bacillus coagulans* MTCC 5856, *Bacillus coagulans* ATCC 31284 and *Bacillus coagulans* ATCC 7050

[PARA 0021] In another preferred embodiment, the invention relates to a method of inhibiting 5 α -reductase activity, said method comprising step of bringing into contact said follicle dermal papilla cells with effective concentration of partially purified extracellular metabolite preparation from a strain of *Bacillus coagulans* to bring about 5 α -reductase inhibition in follicle dermal papilla cells. In a related embodiment, the effective concentration of the partially purified extracellular metabolite preparation in the composition is 0.01% v/v to 2.0% v/v of the total

composition. In another embodiment, the strain of *Bacillus coagulans* is selected from the group consisting of *Bacillus coagulans* MTCC 5856, *Bacillus coagulans* ATCC 31284 and *Bacillus coagulans* ATCC 7050.

[PARA 0022] In another preferred embodiment, the invention discloses a method for therapeutic management of androgenic alopecia in mammals, said method comprising steps administering an effective concentration of a composition containing partially purified extracellular metabolite preparation from a strain of *Bacillus coagulans* to mammals in need of such therapy. In a related embodiment, the composition is formulated with pharmaceutically/cosmeceutically acceptable excipients, adjuvants, bases, diluents, carriers, conditioning agents and preservatives and/or incorporated into formulations containing hair care ingredients and administered topically in the form of creams, gels, lotions, shampoo, serum, oil, suspensions, emulsions, and compacts. In a related embodiment, the effective concentration of the partially purified extracellular metabolite preparation in the composition is 0.01% v/v to 2.0% v/v of the total composition. In another embodiment, the strain of *Bacillus coagulans* is selected from the group consisting of *Bacillus coagulans* MTCC 5856, *Bacillus coagulans* ATCC 31284 and *Bacillus coagulans* ATCC 7050. In another related embodiment, the mammal is preferably human.

[PARA 0023] In another preferred embodiment, the invention discloses a method for therapeutic management of seborrhoeic dermatitis in mammals, said method comprising steps administering an effective concentration of a composition containing partially purified extracellular metabolite preparation from a strain of *Bacillus coagulans*, along with standard anti-dandruff ingredients to mammals in need of such therapy. In a related embodiment, the composition is formulated with pharmaceutically/cosmeceutically acceptable excipients, adjuvants, bases, diluents, carriers, conditioning agents and preservatives and administered topically in the form of creams, gels, lotions, shampoo, serum, oil, suspensions, emulsions, and compacts. In another embodiment, the anti-dandruff ingredients are selected from the group consisting of, but not limited to, coleus oil, tea tree oil, clove oil, basil oil and extract, rosemary oil, neem extract, cedarwood oil, selenium sulphide, Zinc Pyrithione, Salicylic acid, Ketoconazole, Climbazole and Ciclopirox Olamine. In a related embodiment, the effective concentration of the partially purified extracellular metabolite preparation in the composition is 0.01% v/v to 2.0% v/v of the total composition. In another embodiment, the strain of *Bacillus coagulans* is selected from the group consisting of

Bacillus coagulans MTCC 5856, *Bacillus coagulans* ATCC 31284 and *Bacillus coagulans* ATCC 7050. In another related embodiment, the mammal is preferably human.

[PARA 0024] Specific illustrative examples enunciating the most preferred embodiments are included herein below

[PARA 0025] EXAMPLE 1: Proliferation of dermal papilla

[PARA 0026] Isolation of extracellular metabolite

[PARA 0027] The extracellular metabolite from *Bacillus coagulans* MTCC 5856 was isolated as per the steps outlined in US9596861. The product is commercially available under the tradename LACTOSPORIN[®] (INCI: Bacillus ferment filtrate extract) from Sabinsa Corporation, USA.

[PARA 0028] Materials and Method

[PARA 0029] Cells: Dermal Papilla Cells (human HFDPC) were purchased from Promocell (Germany), and maintained as a monolayer culture in Fibroblast growth media (Promocell, Germany) at 37 °C in a humidified 5% CO₂ incubator. Dermal Papilla Cells were cultured in DMEM supplemented with 10% FBS. The confluent cultures were harvested by trypsinization and expanded during two more passages before they were used for the experiments. Medium and other culture components were renewed after 48–72 h. All cell cultures were maintained at 37°C in 95% air and 5% CO₂ in a CO₂ incubator.

[PARA 0030] The Dermal Papilla Cells were used for the assay at a seeding density of 2000 cells per well of 24 -well clear bottom microplate in DMEM and incubated at 37°C in 95% air and 5% CO₂ in a CO₂ incubator for 24 hours. The cell monolayers were fixed with 50% (w/v) trichloroacetic acid for 1 hour at 4°C and stained with 0.4% SRB for 30 min. The excess dye was removed by washing repeatedly with 1% (v/v) acetic acid. The protein-bound dye is dissolved in 10 mM Tris base solution and the optical density (OD) was read at 492 nm using a microplate reader. Cell proliferation in the presence of the samples was calculated based on the increased optical density due to the viable cells, as percentage of proliferation compared to the controls in the absence of test samples.

[PARA 0031] Results

[PARA 0032] The results indicated that the extracellular metabolite preparation increased the proliferation of Dermal papilla cells (**Fig. 1a, 1b and 1c**). It also stimulated the anagen phase of follicular cell growth indicating its potential as a hair care cosmetic.

[PARA 0033] Expression of secretory factors**[PARA 0034] Materials and Method**

[PARA 0035] Cells : Dermal Papilla Cells (human HFDPC) were purchased from Promocell (Germany), and maintained as a monolayer culture in Fibroblast growth media (Promocell, Germany) at 37 °C in a humidified 5% CO₂ incubator. Dermal Papilla Cells were cultured in DMEM supplemented with 10% FBS. The confluent cultures were harvested by trypsinization and expanded during two more passages before they were used for the experiments. Medium and other culture components were renewed after 48–72 h. All cell cultures were maintained at 37°C in 95% air and 5% CO₂ in a CO₂ incubator.

[PARA 0036] Dermal Papilla Cells were used for the assay at a seeding density of 5000 cells per well of 96 -well clear bottom microplate in DMEM and incubated at 37°C in 95% air and 5% CO₂ in a CO₂ incubator for 24 hours. Culture supernatants were collected and stored at -80°C until use. TGF- β and VEGF -1 were detected using ELISA kits (Quantikine® ELISA kit for human TGF- β 2 and human VEGF kit ,Krishgen biosystems) as per manufacturer's instructions

[PARA 0037] Result

[PARA 0038] The extracellular metabolite increased the expression of TGF- β 2 (**Fig. 2**) by 70.3% at a concentration of 0.5% v/v while a 30% increase in VEGF expression (**Fig. 3**) was observed, indicating that the extracellular metabolite from *Bacillus coagulans* MTCC 5856 increases the proliferation of dermal papilla and thereby promoting hair growth.

[PARA 0039] EXAMPLE 2: 5 α -reductase Inhibition**[PARA 0040] Isolation of extracellular metabolite**

[PARA 0041] The extracellular metabolite from *Bacillus coagulans* MTCC 5856 was isolated as per the steps outlined in US9596861. The product is commercially available under the tradename LACTOSPORIN[®] (INCI: Bacillus ferment filtrate extract) from Sabinsa Corporation, USA.

[PARA 0042] Materials and Method

[PARA 0043] Cells: Dermal Papilla Cells (human HFDPC) were purchased from Promocell (Germany), and maintained as a monolayer culture in Fibroblast growth media (Promocell, Germany) at 37 °C in a humidified 5% CO₂ incubator. Dermal papilla cells (DPC) were cultured in DMEM medium with 10% FBS and seeded in 96 well tissue culture plate. 24 hrs post seeding, DPC were treated with 50nM testosterone with & without the test samples and incubated for 48 hrs in a 5% CO₂ incubator at 37°C. After 48hrs the supernatant was collected from each well. The amount of 5 α -DHT produced by the various treated DPCs was quantified using ELISA and absorbance was measured at 450 nm using a microplate reader

[PARA 0044] Conclusion

[PARA 0045] The results indicated that the extracellular metabolite preparation brought about 16.2% inhibition in 5 α -reductase activity (**Fig. 4a**), and dihydrotestosterone levels (**Fig. 4b**) in vitro at 0.5% indicating its potential as a potent 5 α -reductase inhibitor for treating clinical conditions like androgenic alopecia.

[PARA 0046] EXAMPLE 3: Hair care formulations containing extracellular metabolite preparation from *Bacillus coagulans*

[PARA 0047] Tables 1- provide illustrative examples of hair care formulations containing partially purified extracellular metabolite preparation from *Bacillus coagulans* MTCC 5856 (Bacillus ferment filtrate extract)

[PARA 0048] Table 1: Hair serum

Active Ingredients
Bacillus ferment filtrate extract 0.01% - 2%
Excipients

Cationic polymers (Galsilk 700), Disodium EDTA, glycerin, Preservatives, non-ionic surfactant (Tween 20), non-ionic solubilizers and emulsifying agents (Cremophor RH 40), Bioavailability enhancers (Piperine extract or Tetrahydropiperine (Cosmoperine®)), Fragrance, Thickeners (Cellulose derivatives or Acrylates Cross Polymer)

[PARA 0049] Table 2: Hair serum

Active Ingredients

Bacillus ferment filtrate extract 0.01% - 2%

Amla extract, *Cocus nucifera* extract, Cosmetic peptides, Selenium sulphide, Vitamin A, C

Excipients

Cationic polymers (Galsilk 700), Disodium EDTA, glycerin, Preservatives, non-ionic surfactant (Tween 20), non-ionic solubilizers and emulsifying agents (Cremophor RH 40), Bioavailability enhancers (Piperine extract or Tetrahydropiperine (Cosmoperine®)), Fragrance, Thickeners (Cellulose derivatives or Acrylates Cross Polymer)

[PARA 0050] Table 3: Hair oil

Active Ingredients

Bacillus ferment filtrate extract 0.01% - 2%

Tea tree oil/coleus oil, Cedarwood oil

Excipients

Flavouring agent, Preservatives, Carrier oils, Bioavailability enhancers (Piperine extract), Selenium containing amino acids, Selenium sulfide, Antioxidants (Vitamins, rosmarinic acid)

[PARA 0051] Table 4: Hair oil

Active Ingredients

Bacillus ferment filtrate extract 0.01% - 2%

Almond oil, Amino acids (Methionine, Cysteine), Selenium Sulfide, Vitamins

E, A
Excipients Flavouring agent, Preservatives, Carrier oils, Bioavailability enhancers (Piperine extract, Tetrahydropiperine (Cosmoperine®)), Antioxidants (rosmarinic acid)

[PARA 0052] Table 5: Anti-dandruff shampoo

Active Ingredients Bacillus ferment filtrate extract 0.01% - 2% Antifungal agents (ketoconazole), Tea tree oil/Coleus oil, Amino acids (Cysteine, Methionine)
Excipients Selenium sulfide, Cooling agents (Menthol), non-ionic Surfactants (Tween 20), Humectants, Conditioning agents, Preservatives, Antioxidants, Thickeners, Chelating agents or sequestering agents, pH Neutralizer, Detergents

[PARA 0053] The above formulations are merely illustrative examples; any formulation containing the above active ingredient intended for the said purpose will be considered equivalent.

[PARA 0054] Other modifications and variations to the invention will be apparent to those skilled in the art from the foregoing disclosure and teachings. Thus, while only certain embodiments of the invention have been specifically described herein, it will be apparent that numerous modifications may be made thereto without departing from the spirit and scope of the invention. The scope of the invention is to be interpreted only in conjunction with the appended claims.



सुक्ष्मजीव प्ररूप संवर्धन संग्रह एवं जीन बैंक
MICROBIAL TYPE CULTURE COLLECTION & GENE BANK

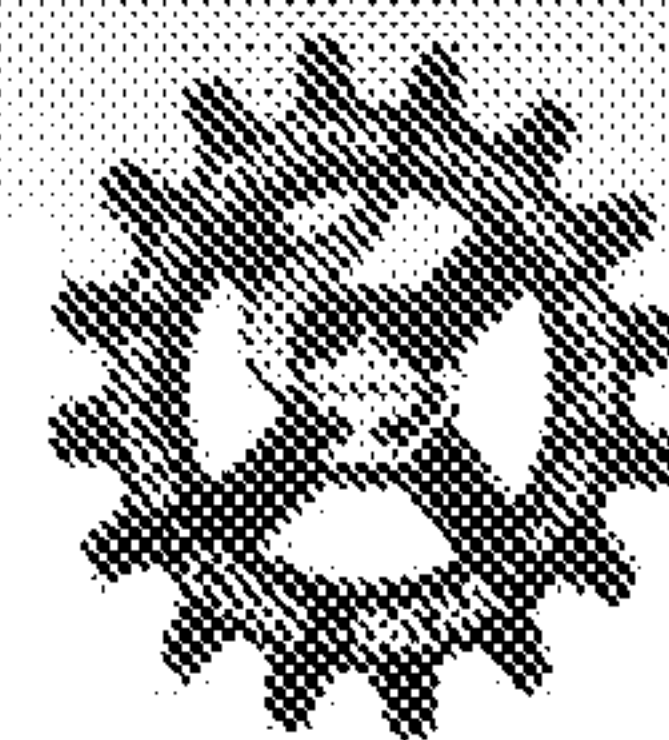
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सीएसआईआर - इन्स्टीट्यूट
CSIR - IMTECH

Dr. G.S. Prasad
Senior Principal Scientist

By Courier
17.12.2013

To

Dr. Muhammed Majed
Sami Labs. Limited
19/1 & 19/2, I Mai, II Phase
Peenya Industrial Area
Bangalore - 560 058
Karnataka, India

Dear Dr. Muhammed Majed,

Your microbial culture has been accepted for deposit in MTCC-IDA under Budapest Treaty. It was assigned MTCC number and preserved in the patent collection of MTCC. The details are as follows.

<u>Taxonomic designation</u>	<u>Identification Reference</u>	<u>MTCC Number Assigned</u>	<u>Date of Accession</u>
<i>Bacillus coagulans</i>	SBC-37-01	MTCC 5856	19.09.2013

Enclosed here with are the relevant documents of the above strain.

Form BP14 - Receipt and acceptance of the culture in MTCC

Form BP19 - Viability statement of the culture

Please acknowledge the receipt of the forms.

Sincerely yours,

(G. S. PRASAD)

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**BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE**



INTERNATIONAL FORM

To Dr. Muhammed Majeed Sami Labs. Limited 19/1 & 19/2, I Mai, II Phase Peenya Industrial Area Bangalore - 560 058 Karnataka, India NAME AND ADDRESS OF THE DEPOSITOR	RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT issued pursuant to Rule 7.1 by the INTERNATIONAL DEPOSITARY AUTHORITY identified at the bottom of this page				
I. IDENTIFICATION OF THE MICROORGANISM <table style="width: 100%;"> <tr> <td style="width: 50%;">Identification reference given by the DEPOSITOR:</td> <td style="width: 50%;">Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:</td> </tr> <tr> <td style="text-align: center;"><i>Bacillus coagulans</i> SBC-37-01</td> <td style="text-align: center;">MTCC 5858</td> </tr> </table>		Identification reference given by the DEPOSITOR:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:	<i>Bacillus coagulans</i> SBC-37-01	MTCC 5858
Identification reference given by the DEPOSITOR:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:				
<i>Bacillus coagulans</i> SBC-37-01	MTCC 5858				
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION The microorganism identified under I above was accompanied by: ☒ a scientific description ☐ a proposed taxonomic designation (Mark with a cross when applicable)					
III. RECEIPT AND ACCEPTANCE This International Depositary Authority accepts the microorganism identified under I above, which was received by it on 19.09.2013 (date of the original deposit)					
IV. RECEIPT OF REQUEST FOR CONVERSION The microorganism identified under I above was received by this International Depositary Authority on NOT APPLICABLE (date of the original deposit) and a request to convert the original deposit under the Budapest Treaty was received by it on NOT APPLICABLE (date of receipt of request for conversion)					
V. INTERNATIONAL DEPOSITARY AUTHORITY <table style="width: 100%;"> <tr> <td style="width: 50%; vertical-align: top;"> Name: Dr. G.S. PRASAD Address: Microbial Type Culture Collection & Gene Bank Institute of Microbial Technology Sector 39-A, Chandigarh - 160 036 India </td> <td style="width: 50%; vertical-align: top;"> Signature(s) of person(s) holding the power to represent the International Depositary Authority or of authorized official(s) Date: 19.09.2013 Stamp of the International Depositary Authority (MTCC) </td> </tr> </table>		Name: Dr. G.S. PRASAD Address: Microbial Type Culture Collection & Gene Bank Institute of Microbial Technology Sector 39-A, Chandigarh - 160 036 India	Signature(s) of person(s) holding the power to represent the International Depositary Authority or of authorized official(s) Date: 19.09.2013 Stamp of the International Depositary Authority (MTCC)		
Name: Dr. G.S. PRASAD Address: Microbial Type Culture Collection & Gene Bank Institute of Microbial Technology Sector 39-A, Chandigarh - 160 036 India	Signature(s) of person(s) holding the power to represent the International Depositary Authority or of authorized official(s) Date: 19.09.2013 Stamp of the International Depositary Authority (MTCC)				

Where Rule 9.4(i) applies, each date is the date on which the status of International Depositary Authority was acquired



**BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE**

INTERNATIONAL FORM

To Dr. Muhammed Majeed Sami Labs. Limited 19/1 & 19/2, I Mai, II Phase Peenya Industrial Area Bangalore - 560 058 Karnataka, India NAME AND ADDRESS OF THE PARTY TO WHOM THE VIABILITY STATEMENT IS ISSUED	VIABILITY STATEMENT issued pursuant to Rule 10.1 by the INTERNATIONAL DEPOSITARY AUTHORITY identified on the following page
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I. DEPOSITOR Name: Dr. Muhammed Majeed Address: Sami Labs. Limited 19/1 & 19/2, I Mai, II Phase Peenya Industrial Area Bangalore - 560 058 Karnataka, India	II. IDENTIFICATION OF THE MICROORGANISM Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY MTCC 5856 Date of the deposit or of the transfer: 19-09-2013
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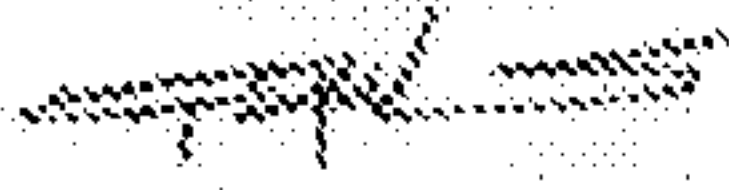
III. VIABILITY STATEMENT	
The viability of the microorganism identified under II above was tested on 17-09-2013 ² on that date, the said microorganism was ☒ ¹ viable ☐ ¹ no longer viable	

¹ Indicates the date of the original deposit or, where the new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

² In the cases referred to in Rule 10.2(a)(ii) and (b), refer to the most recent viability test.

³ Mark with a cross the applicable box.

⁴ Form MTCC 15/93 (first page)

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED ¹	
- NOT APPLICABLE -	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: Dr. G.S. PRASAD Microbial Type Culture Collection & Gene Bank Address: Institute of Microbial Technology Sector 39-A, Chandigarh - 160 036 India	 Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Date: 19.09.2019 Dr. G.S. Prasad, Director Institute of Microbial Technology Sector 39-A, Chandigarh - 160 036, India

¹ Fill in if the information has been requested and if the results of the test were negative.

We Claim;

1. A hair care composition containing extracellular metabolite preparation from a strain of *Bacillus coagulans* formulated with pharmaceutically/cosmeceutically acceptable excipients, adjuvants, bases, diluents, carriers, conditioning agents and preservatives.
2. The composition as in claim 1, wherein the composition can be combined with and/or incorporated into formulations containing hair care ingredients and administered topically in the form of creams, gels, lotions, shampoo, serum, oil, suspensions, emulsions, and compacts.
3. The composition as in claim 1, wherein the strain of *Bacillus coagulans* is selected from the group consisting of *Bacillus coagulans* MTCC 5856, *Bacillus coagulans* ATCC 31284 and *Bacillus coagulans* ATCC 7050.
4. A method of increasing hair growth in mammals, said method comprising steps of administering effective concentration of extracellular metabolite preparation from a strain of *Bacillus coagulans* to said mammals, to bring about increase in hair growth.
5. The method as in claim 4, wherein the increase in hair growth is brought about by a) increasing the expression of TGF- β 2 and VEGF in dermal papilla and b) increasing the proliferation of follicle dermal papilla cells.
6. The method as in claim 4, wherein the effective concentration of the extracellular metabolite preparation is 0.01% v/v to 2.0% v/v of the total composition.
7. The method as in claim 4, wherein the strain of *Bacillus coagulans* is selected from the group consisting of *Bacillus coagulans* MTCC 5856, *Bacillus coagulans* ATCC 31284 and *Bacillus coagulans* ATCC 7050.
8. A method of inhibiting 5 α -reductase activity, said method comprising step of bringing into contact said follicle dermal papilla cells with effective concentration of extracellular metabolite preparation from a strain of *Bacillus coagulans* to bring about 5 α -reductase inhibition in follicle dermal papilla cells.

9. The method as in claim 8, wherein the effective concentration of the extracellular metabolite preparation is 0.01% v/v to 2.0% v/v of the total composition.
10. The method as in claim 8, wherein the strain of *Bacillus coagulans* is selected from the group consisting of *Bacillus coagulans* MTCC 5856, *Bacillus coagulans* ATCC 31284 and *Bacillus coagulans* ATCC 7050.
11. A method for therapeutic management of androgenic alopecia in mammals, said method comprising steps administering an effective concentration of a composition containing extracellular metabolite preparation from a strain of *Bacillus coagulans* to mammals in need of such therapy.
12. The method as in claim 11, wherein the composition is formulated with pharmaceutically/cosmeceutically acceptable excipients, adjuvants, bases, diluents, carriers, conditioning agents and preservatives and/or incorporated into formulations containing hair care ingredients and administered topically in the form of creams, gels, lotions, shampoo, serum, oil, suspensions, emulsions, and compacts.
13. The method as in claim 11, wherein the effective concentration of the extracellular metabolite preparation in the composition is 0.01% v/v to 2.0% v/v of the total composition.
14. The method as in claim 11, wherein the strain of *Bacillus coagulans* is selected from the group consisting of *Bacillus coagulans* MTCC 5856, *Bacillus coagulans* ATCC 31284 and *Bacillus coagulans* ATCC 7050.
15. The method as in claim 11, wherein the mammal is preferably human.
16. A method for therapeutic management of seborrhoeic dermatitis in mammals, said method comprising steps administering an effective concentration of a composition containing extracellular metabolite preparation from a strain of *Bacillus coagulans*, along with standard anti-dandruff ingredients to mammals in need of such therapy.
17. The method as in claim 16, wherein the composition is formulated with pharmaceutically/cosmeceutically acceptable excipients, adjuvants, bases, diluents,

- carriers, conditioning agents and preservatives and administered topically in the form of creams, gels, lotions, shampoo, serum, oil, suspensions, emulsions, and compacts.
18. The method as in claim 16, wherein the anti-dandruff ingredients are selected from the group consisting of, but not limited to, coleus oil, tea tree oil, clove oil, basil oil and extract, rosemary oil, neem extract, cedarwood oil, selenium sulphide, Zinc Pyrithione, Salicylic acid, Ketoconazole, Climbazole and Ciclopirox Olamine.
 19. The method as in claim 16, wherein the effective concentration of the extracellular metabolite preparation in the composition is 0.01% v/v to 2.0% v/v of the total composition.
 20. The method as in claim 16, wherein the strain of *Bacillus coagulans* is selected from the group consisting of *Bacillus coagulans* MTCC 5856, *Bacillus coagulans* ATCC 31284 and *Bacillus coagulans* ATCC 7050.
 21. The method as in claim 16, wherein the mammal is preferably human.

FIG. 1a

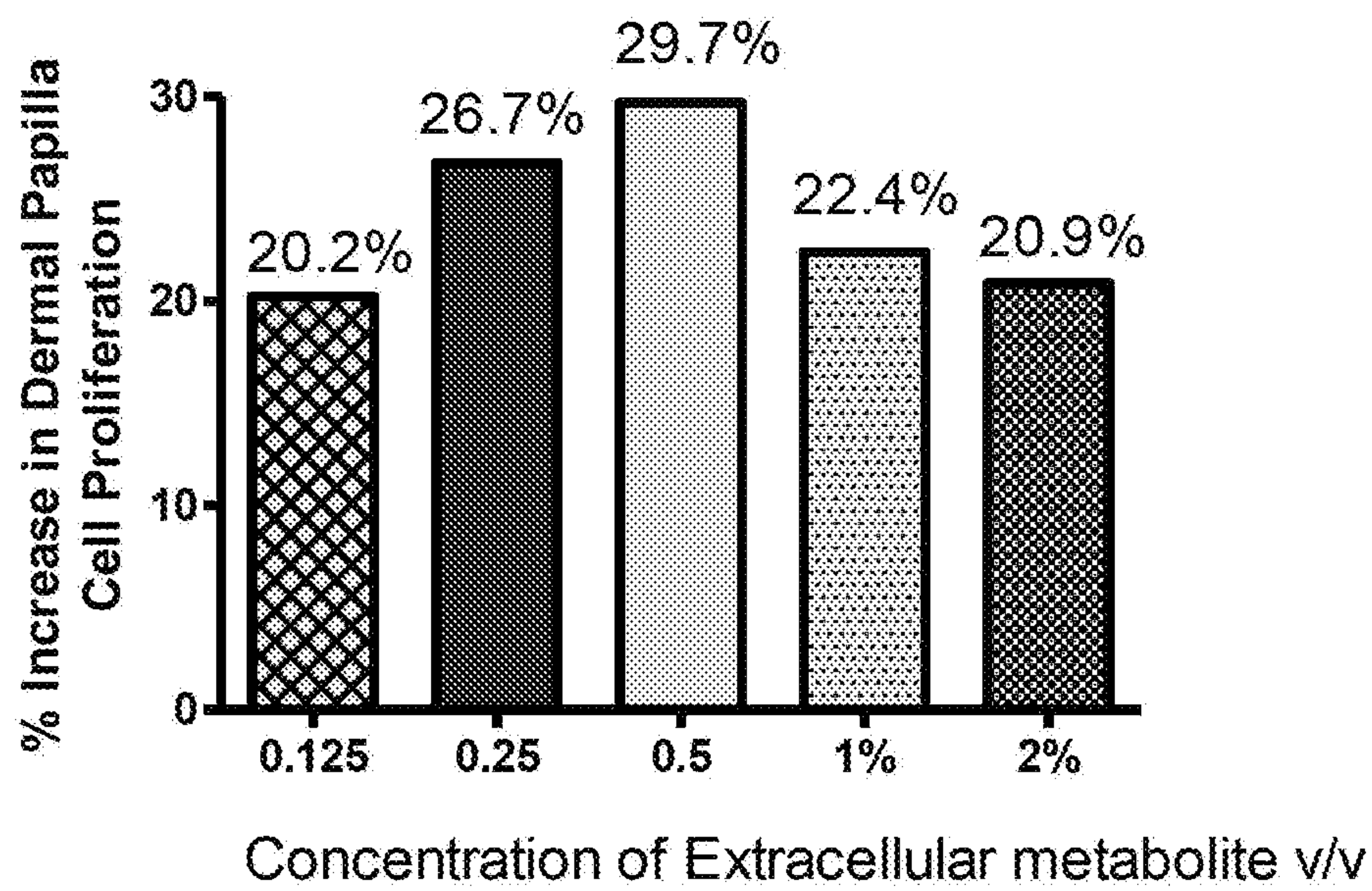


FIG. 1b

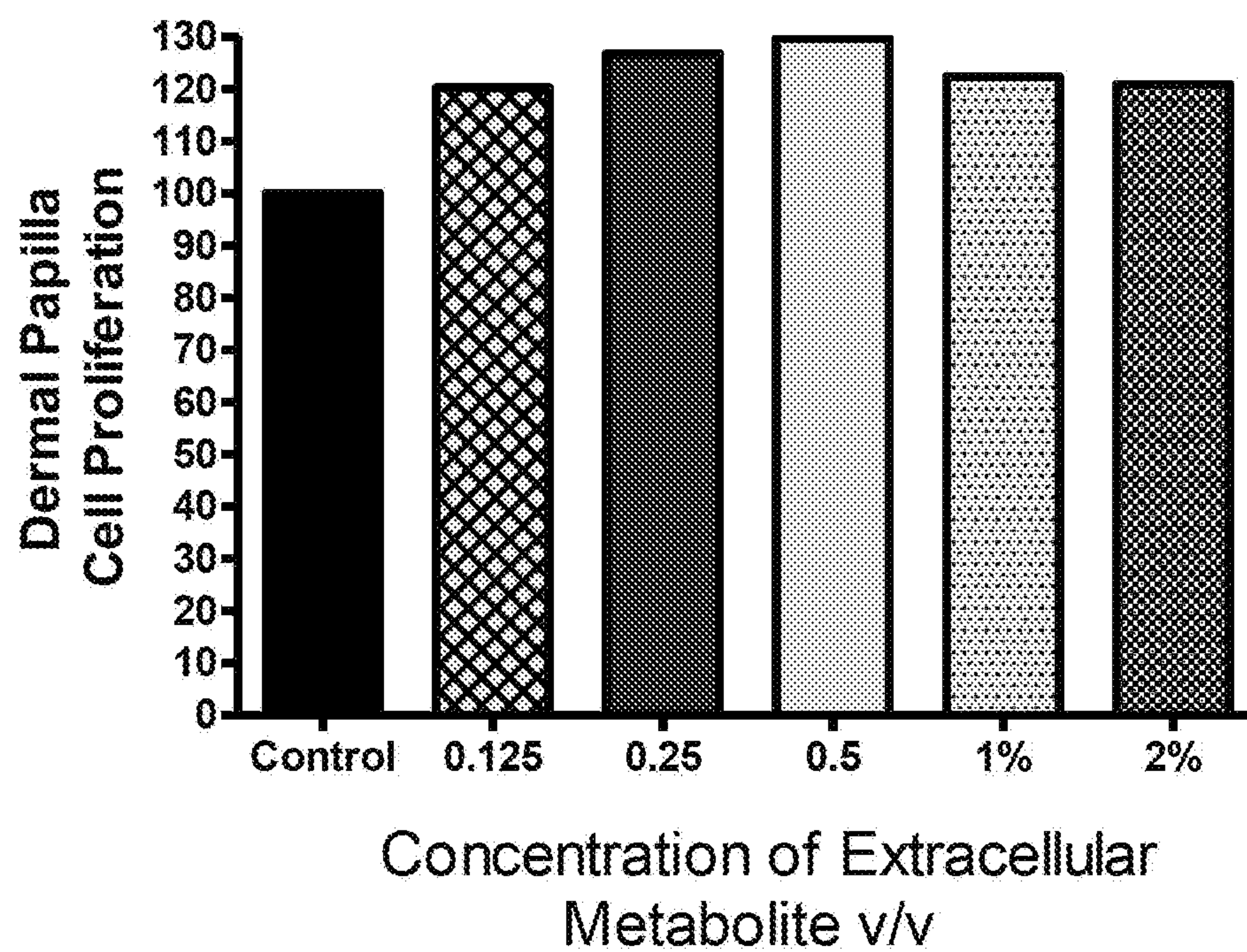


FIG. 1c

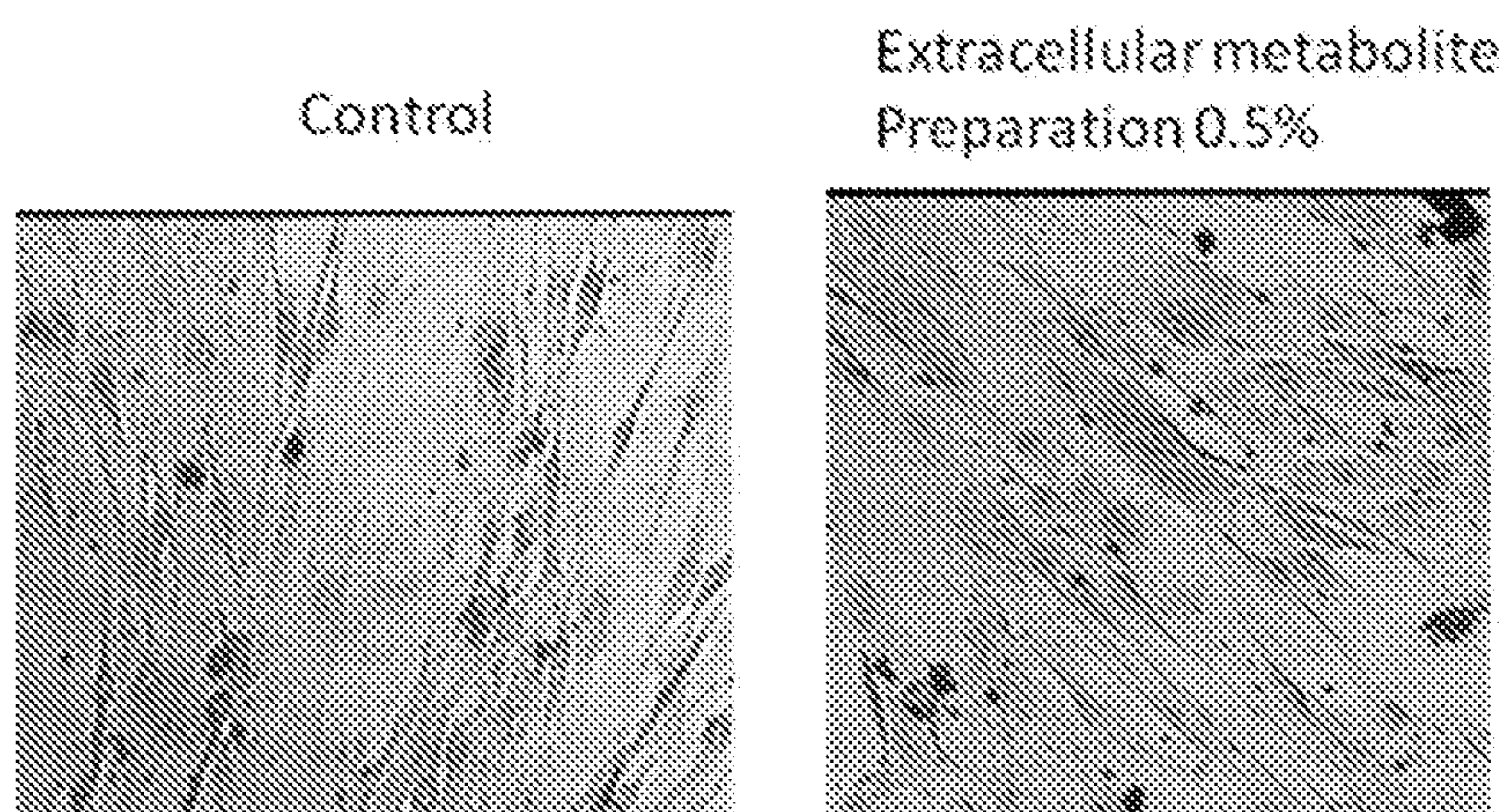


FIG. 2

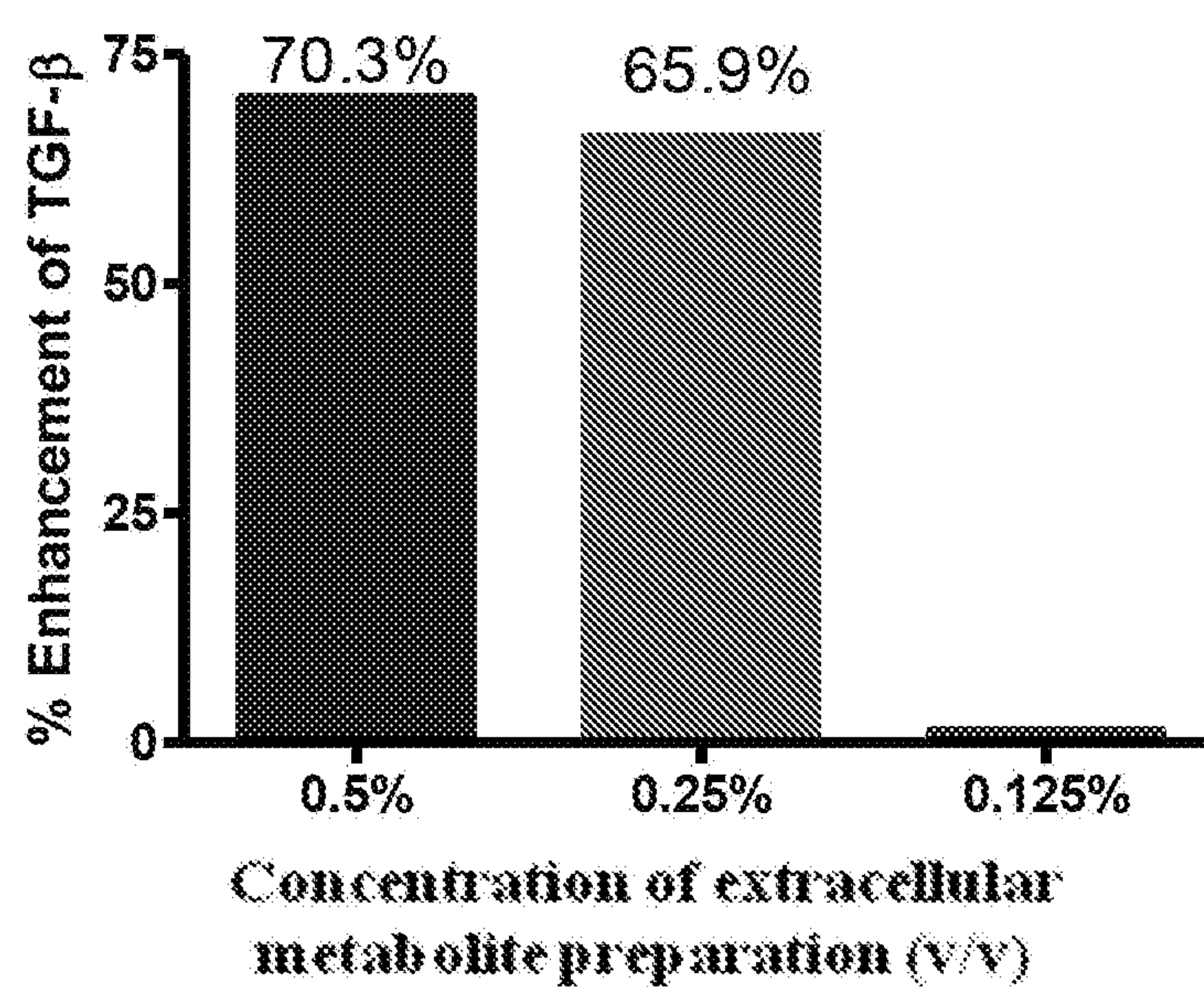


FIG. 3

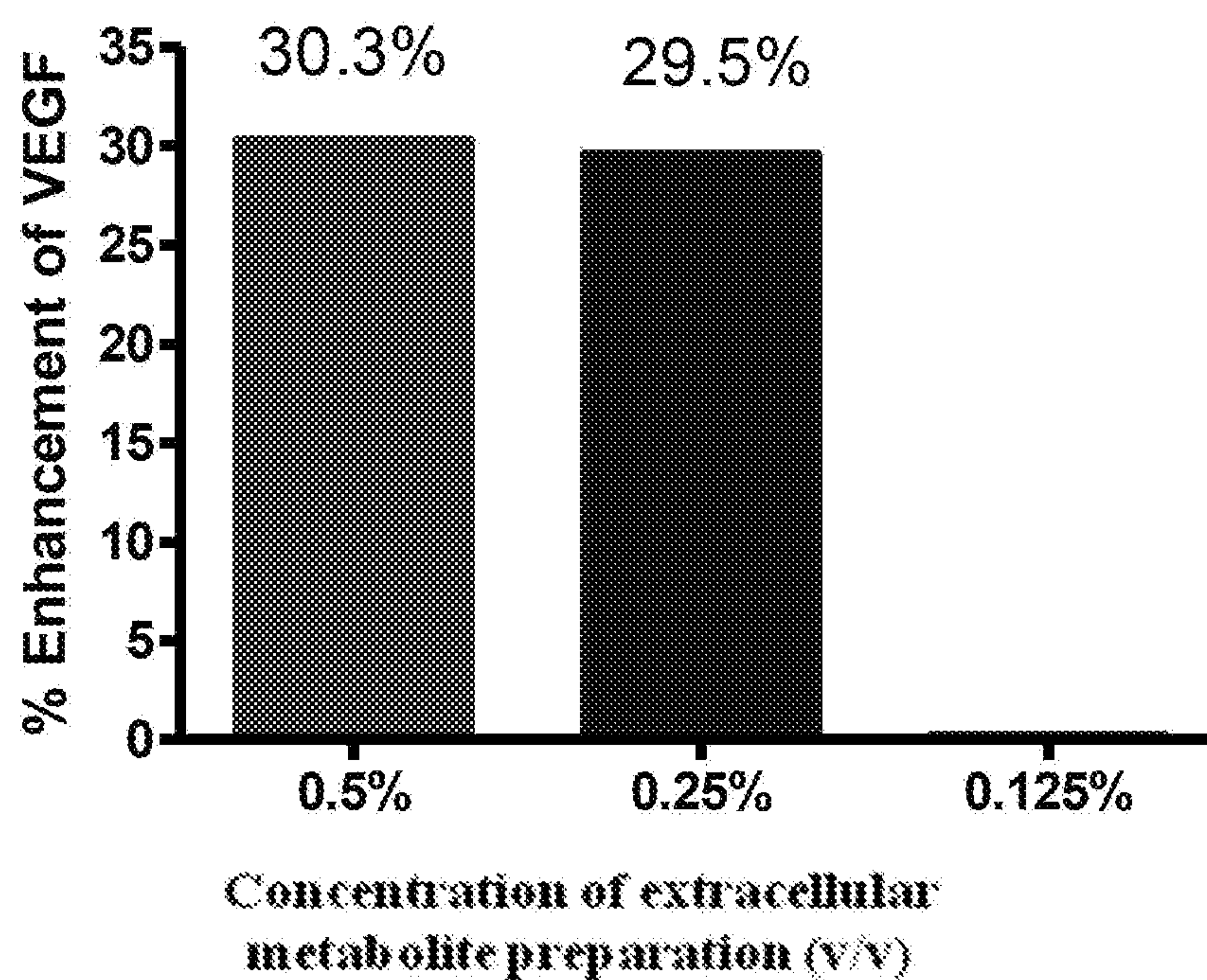


FIG. 4a

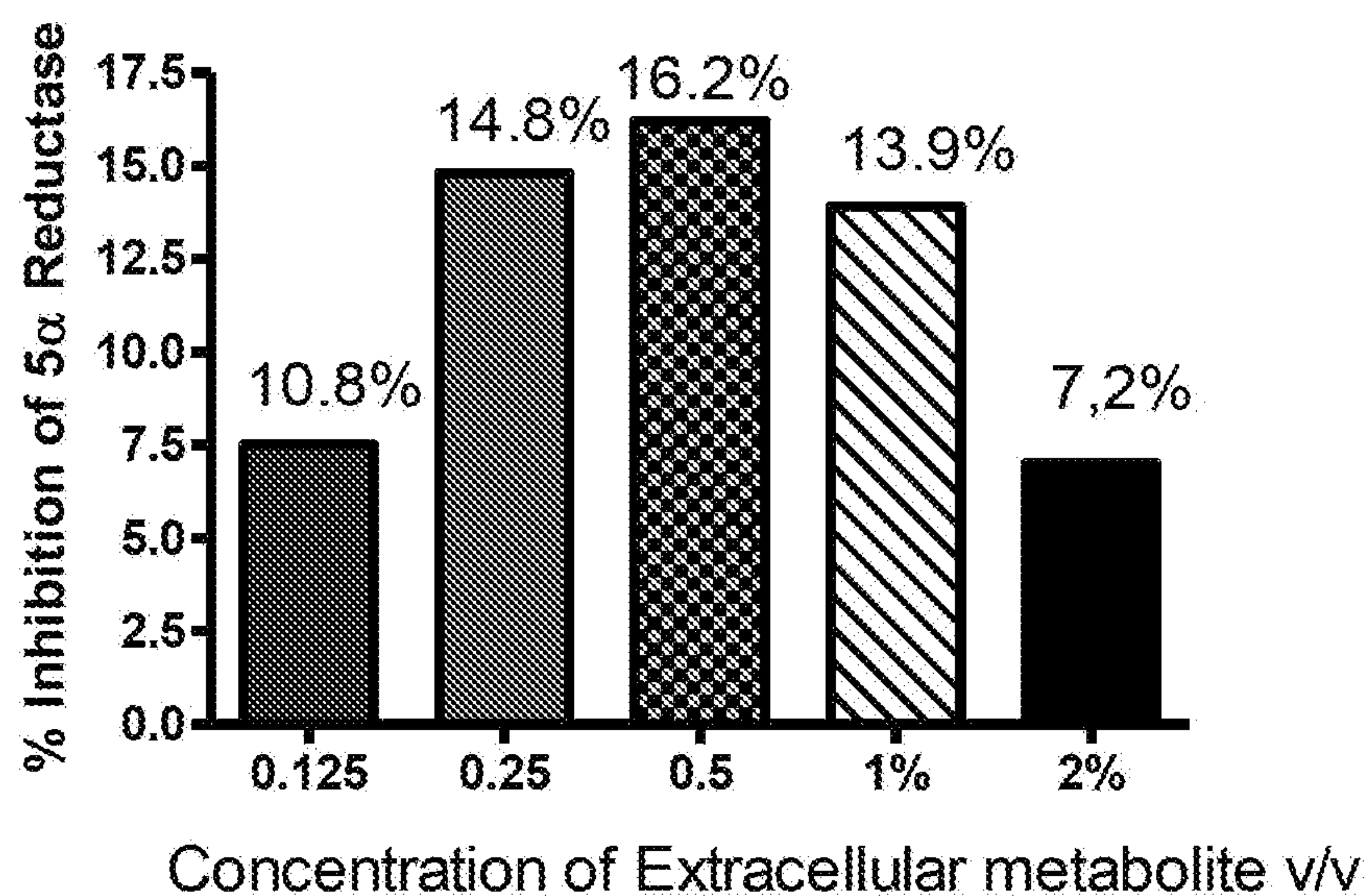


FIG. 4b

