(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
28 June 2018 (28.06.2018)

WIPO | PCT

(10) International Publication Number
WO 2018/116146 A1

(51) International Patent Classification:
A61K 36/36 (2006.01) *A61K 31/353* (2006.01)
A61P 35/00 (2006.01) *A61K 31/12* (2006.01)

(21) International Application Number:
PCT/IB2017/058097

(22) International Filing Date:
19 December 2017 (19.12.2017)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
2016/08789 21 December 2016 (21.12.2016) ZA

(71) Applicant: **BIOPHARM NZ LIMITED** [NZ/NZ]; 11 Hardley Street, Whitiora, Hamilton 3200 (NZ).

(71) Applicant *(for UG only)*: **GERNTHOLTZ, Otto Carl** [ZA/ZA]; Post Box 8, Cape Town (ZA).

(72) Inventor: **BARRY, Michael-John Joseph**; 11 Hardley Street, Whitiora, Hamilton 3200 (NZ).

(74) Agent: **GERNTHOLTZ, Otto Carl**; Post Box 8, 8000 Cape Town (ZA).

(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, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, 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))*
— *in black and white; the international application as filed contained color or greyscale and is available for download from PATENTSCOPE*

(54) Title: COMPOSITIONS DERIVED FROM *GALENIA AFRICANA* AND METHODS OF USE FOR CANCER TREATMENT

(57) Abstract: The present invention discloses a cancer treatment method which includes the step of treating a patient having cancer with an extract from *Galenia Africana L.* plant. The extract may include pinocembrin and/or 2',4' dihydroxychalcone and/or 7- hydroxyflavanone. The cancer may be breast cancer or melanoma. The composition may be solubilized with MPG and/or Suganate.

WO 2018/116146 A1

COMPOSITIONS DERIVED FROM *GALENIA AFRICANA* AND METHODS OF USE FOR CANCER TREATMENT

FIELD OF INVENTION

The present invention relates to a cancer treatment method and composition.

More particularly, the present invention relates to a cancer treatment method and composition for treating breast cancer and melanoma.

BACKGROUND TO INVENTION

Kraalbos (KB) extracts from *Galenia Africana L.* plant are known to be rich in pinocembrin, 2',4' dihydroxychalcone, 7-hydroxyflavanone and 2',4' dihydroxydihydrochalcone compounds. These molecules have previously been shown to exhibit varying degrees of cytotoxity on cancer cells.

It is an object of the invention to suggest a novel cancer treatment method and composition which includes an extract of *Galenia Africana*.

SUMMARY OF INVENTION

According to the invention, a cancer treatment method includes the steps of treating a patient having cancer with an extract from *Galenia Africana L.* plant.

Also according to the invention, a cancer treatment composition includes an extract from *Galenia Africana L.* plant

The extract may include pinocembrin and/or 2',4' dihydroxychalcone and/or 7-hydroxyflavanone.

The cancer may be breast cancer.

The cancer may be melanoma.

The composition may be solubilized.

The composition may be solubilized with MPG and/or Suganate.

DETAILED DESCRIPTION OF INVENTION

The invention will now be described by way of example.

According to the invention, a cancer treatment method includes the steps of treating a patient having cancer with an extract from *Galenia Africana L.* plant.

Also according to the invention, a cancer treatment composition includes an extract from *Galenia Africana L.* plant

The extract may include pinocembrin and/or 2',4' dihydroxychalcone and/or 7-hydroxyflavanone.

The cancer may be breast cancer.

The cancer may be melanoma.

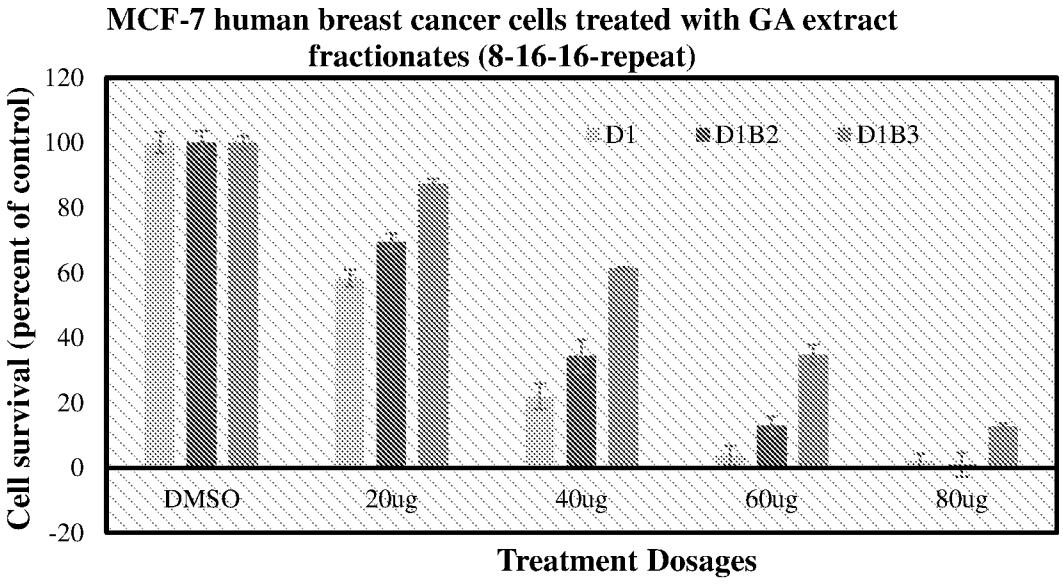
The composition may be solubilized.

The composition may be solubilized with MPG and/or Suganate.

Experiment 1

The objective of the experiment was to test the role of Galenia Africana (GA) extracts on the proliferation of MCF-7 human breast cancer cells. Cells were treated with B1, B2, B3, D1, D1B2 or D1B3. D1 exhibited the most cytotoxicity (IC50 of 26.53 ug/ml), followed by B2 (IC50 of 32.28 ug/ml). Based on the chemical composition of these extracts, it is clear that those that contain high levels of 2',4' dihydroxychalcone have the most effect. Indeed, a combination of D1 and B3 at 50:50 ratio had minimal effect as compared to a combination of D1 and B2 at 70:30 ratio.

The results are shown below:



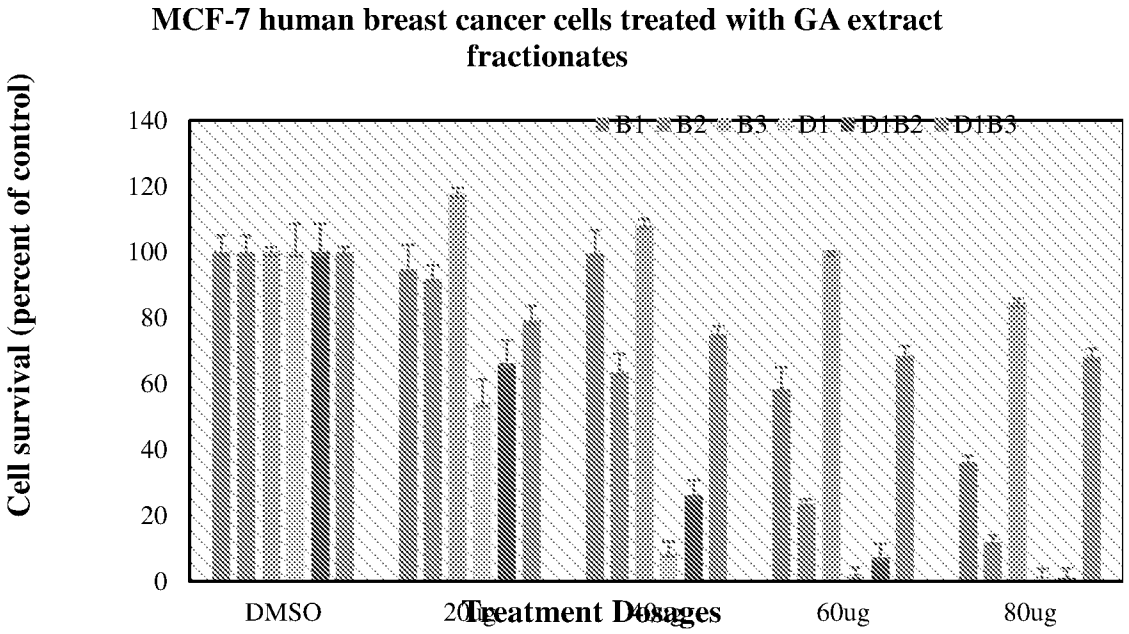


Table 1: IC50s of GA extracts on MCF-7 human breast cancer cells

B1	73.84ug/ml
B2	46.68ug/ml
D1	26.53ug/ml
D1B2	32.28ug/ml

Table 2: Chemical compositions of GA extracts with emphasis on pinocembrin and 2',4' dihydroxychalcone

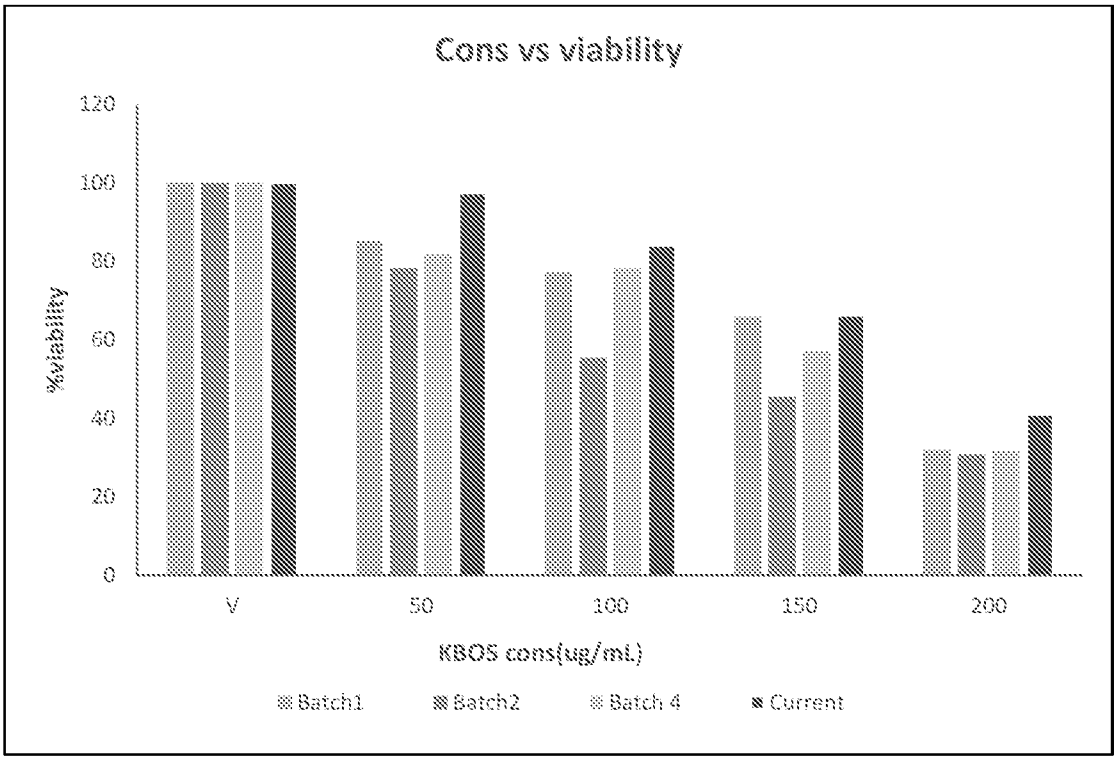
Items, %	pinocembrin	2',4'-dihydroxychalcone
B1	20.46	14.95
B2	40.86	42.97
B3	90.09	1.38
D1		75

Experiment 2

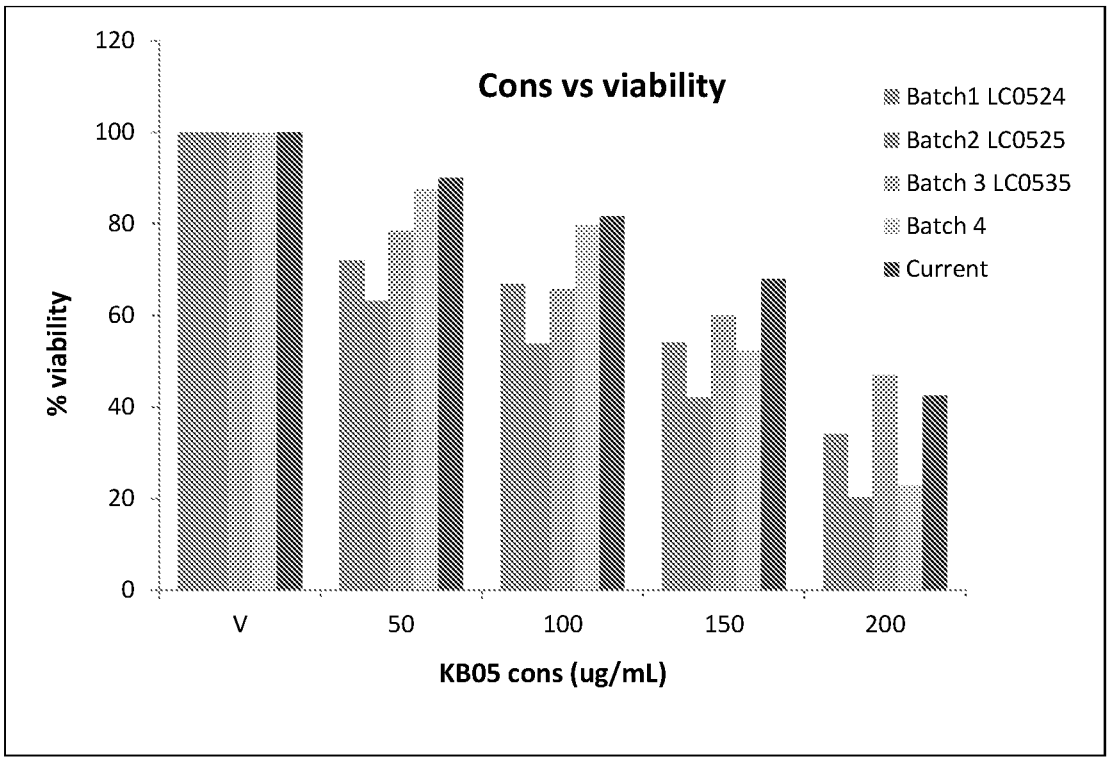
The objective of the experiment was to test the role of Galenia Africana extracts on the proliferation of MCF-7 human breast cancer cells.

The results are shown below:

MCF-7 human breast cancer cells treated with GA extracts (MTT Assay 1)



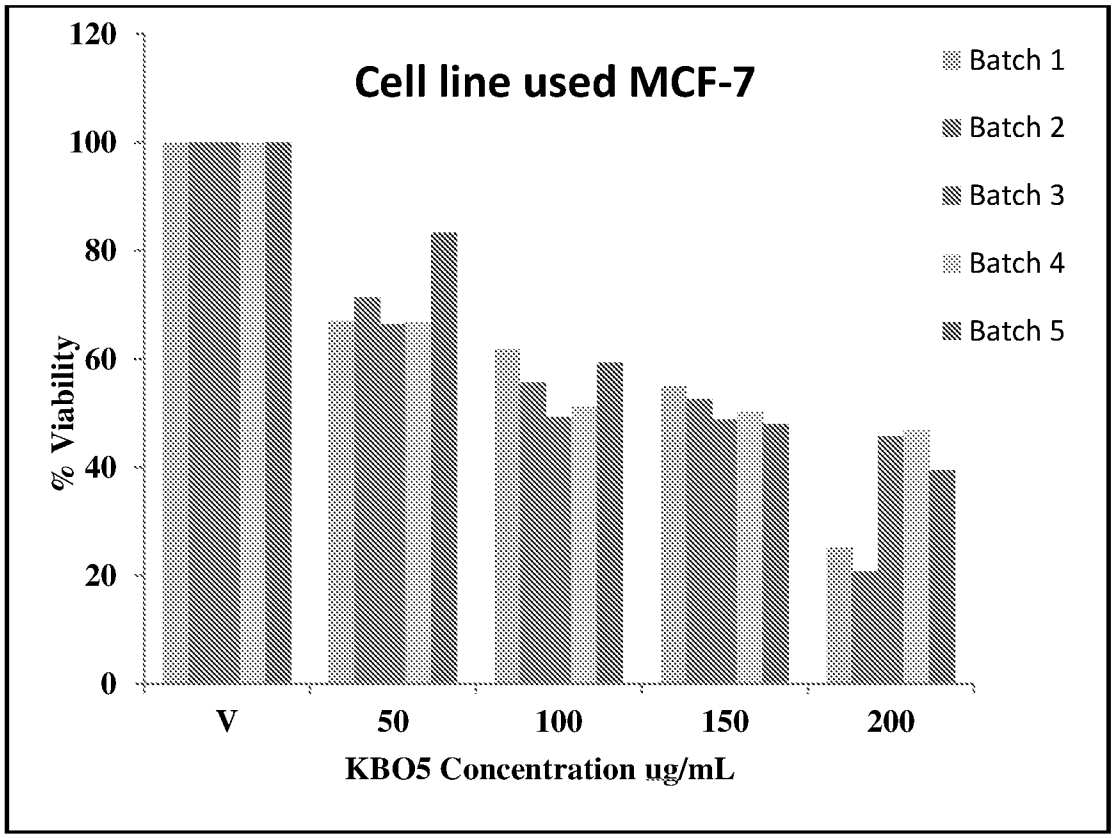
MCF-7 human breast cancer cells treated with GA extracts (MTT Assay 2)



Chemical compositions of GA extracts				
Batches	7-hydroxyflavanone	pinocembrin	2',4'-dihydroxychalcone	2',4'-dihydroxydihydrochalcone
LC0524(1)	3.3%	9.6%	7.6%	3.1%
LC0525(2)	**3.0%**	**8.4%**	**6.8%**	**2.8%**
LC0535(3)	2.4%	4.3%	3.4%	0.7%
Batch 4	2.4%	12.4%	8.9%	3.2%

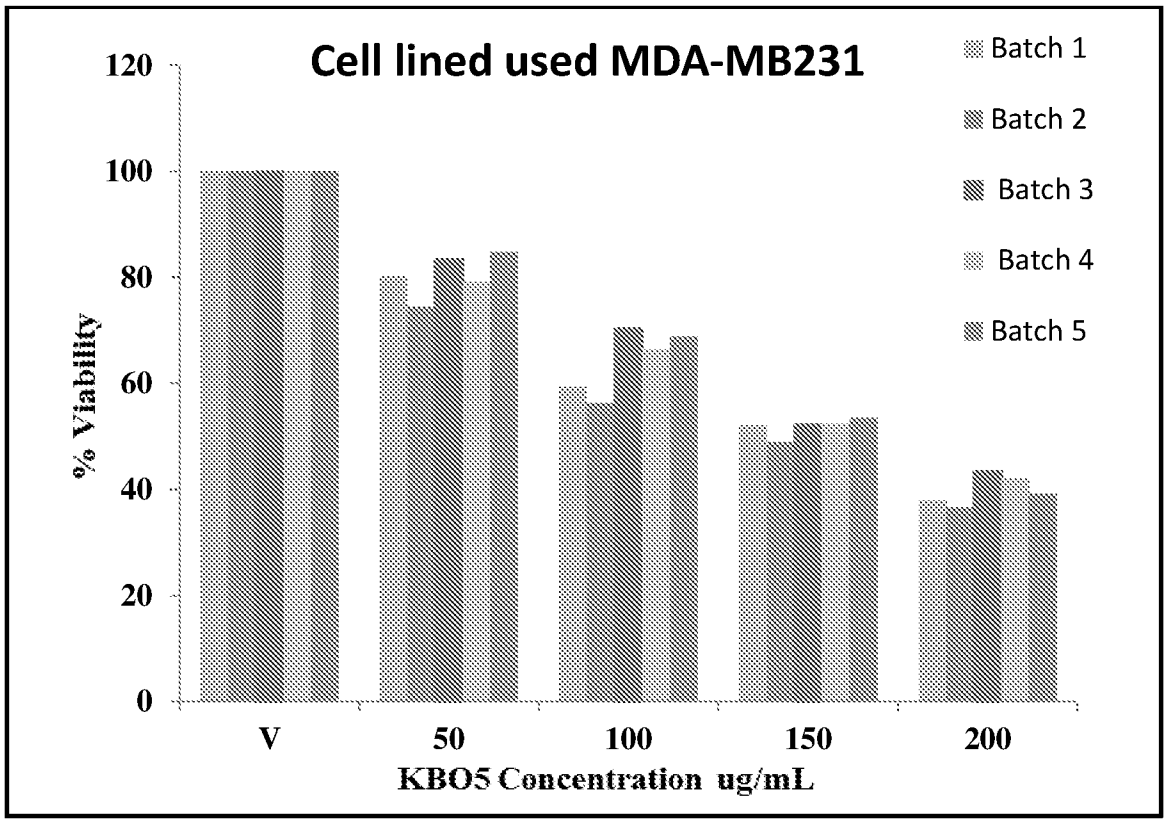
Batches	IC50 value (ug/mL)
LC0524(1)	146.0
LC0525(2)	93.39
LC0535(3)	195.6
Batch 4	148.1
Current	187.1

MCF-7 human breast cancer cells treated with GA extracts (Cell Titer Glo Assay)



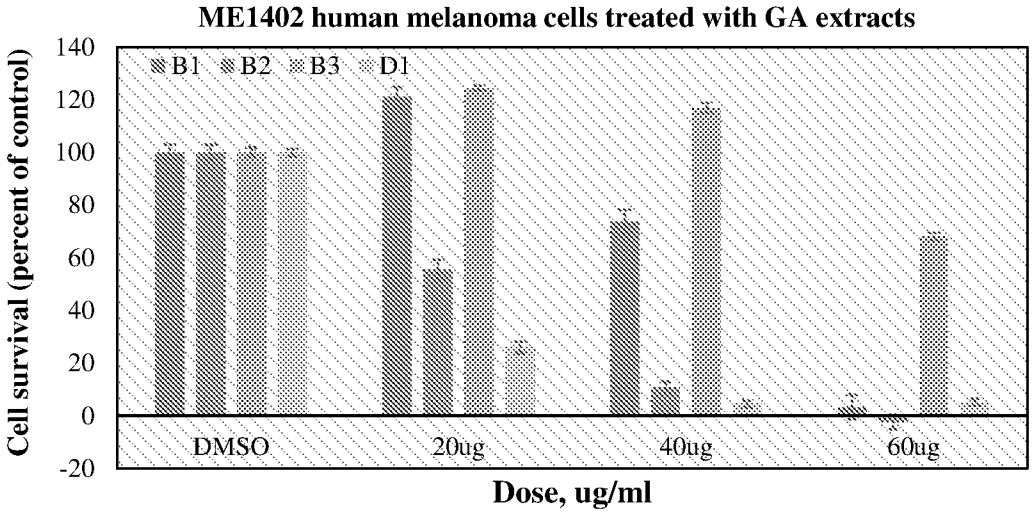
Batches	IC 50 value (ug/mL)
LC0524(1)	124.2
LC0525(2)	114
LC0535(3)	132
Batch 4	144.1
Batch 5	141.6

MCF-7 human breast cancer cells treated with GA extracts (Cell Titer Glo Assay)

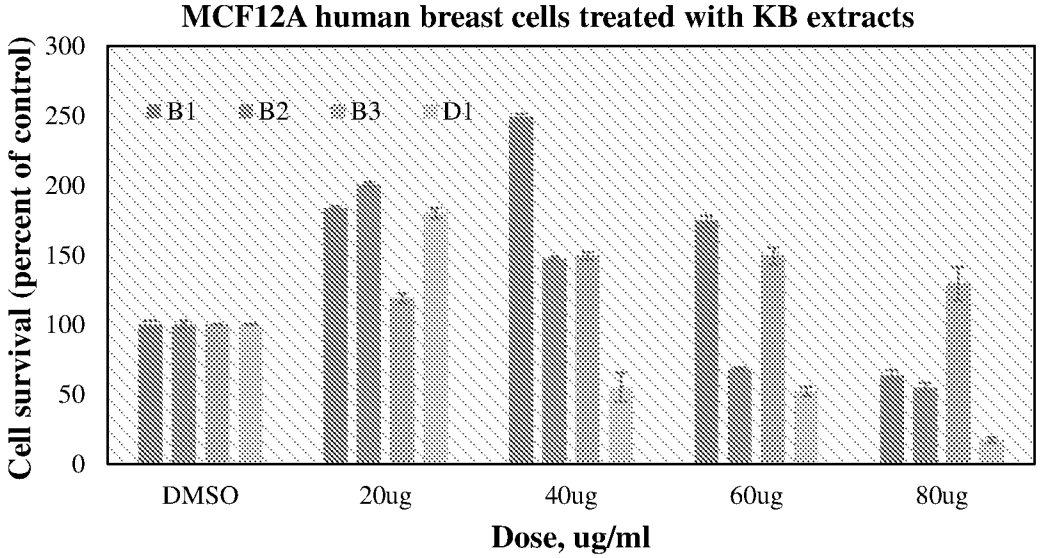


Batches	IC 50 value (ug/mL)
LC0524(1)	144.2
LC0525(2)	130.5
LC0535(3)	166.3
Batch 4	160.8
Batch 5	158.4

The following figure depicts ME1402 human melanoma cells treated with GA extracts after 48 hrs of incubation. Mean cell survival calculated as percentage of the mean vehicle control.



The following figure depicts MCF12A human breast cells treated with KB extracts after 48 hrs of incubation. Mean cell survival calculated as percentage of the mean vehicle control.



PATENT CLAIMS

1. A cancer treatment method which includes the step of treating a patient having cancer with an extract from *Galenia Africana L.* plant.
2. A method as claimed in claim 1, in which the extract includes pinocembrin and/or 2',4' dihydroxychalcone and/or 7-hydroxyflavanone.
3. A method as claimed in claim 1 or claim 2, in which the cancer is breast cancer.
4. A method as claimed in claim 1 or claim 2, in which the cancer is melanoma.
5. A cancer treatment composition which includes an extract from *Galenia Africana L.* plant.
6. A composition as claimed in claim 5, in which the extract includes pinocembrin and/or 2',4' dihydroxychalcone and/or 7-hydroxyflavanone.
7. A composition as claimed in claim 5 or claim 6, in which the cancer is breast cancer.
8. A composition as claimed in claim 5 or claim 6, in which the cancer is melanoma.
9. A composition as claimed in any one of claims 5 to 8, which is solubilized.
10. A composition as claimed in claim 9, in which the composition is solubilized with MPG and/or Suganate.
11. A cancer treatment method substantially as hereinbefore described.
12. A cancer treatment composition substantially as hereinbefore described.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2017/058097

A. CLASSIFICATION OF SUBJECT MATTER

A61K 36/36 (2006.01) A61P 35/00 (2006.01) A61K 31/353 (2006.01) A61K 31/12 (2006.01)

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)

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 practicable, search terms used)

IP Australia Internal Databases, Espacenet, AusPat, Patentscope, Google, PubMed for; Inventor/Applicant name search. Mintel, Traditional Knowledge Digital Library (TKDL), PATENW, NPL, XPTK, NAPRALERT, CAplus, BIOSIS, REGISTRY, EMBASE, FSTA, Medline for; Galenia Africana, Kraalbos, Geelbos, Perdebos, cancer, tumour, melanoma, metastasis, pinocembrin, 7-hydroxyflavanone, 2',4'-dihydroxychalcone, Suganate, monopropylene glycol, A61K31/353, A61K36/36, C07D311, A61P35/00 and similar terms.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

[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

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

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

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 February 2018	21 February 2018
Name and mailing address of the ISA/AU	**Authorised officer**
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustralia.gov.au	Jodi Matic AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. +61262832671

INTERNATIONAL SEARCH REPORT C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		International application No. **PCT/IB2017/058097**
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	Ticha LA et al. Phytochemical and Antimicrobial Screening of Flavanones and Chalcones from Galenia africana and Dicerothamnus rhinocerotis. Nat Prod Commun. 2015; 10(7): 1185-90. Whole document, Abstract, Fig.1, Intro [1], Determination of MIC99 Whole document, Abstract, Fig.1, Intro [1], Determination of MIC99	5-9 1-10
X Y	Mativandlela SP et al. Antimycobacterial flavonoids from the leaf extract of Galenia africana. J Nat Prod. 2009; 72(12): 2169-71. Introduction [3-4], Fig. 1, Extraction and Isolation. Introduction [3-4], Fig. 1, Extraction and Isolation.	5-9. 1-10
X	Ghani NA et al. Chemical Constituents and Cytotoxic Activity of Polyalthia cauliflora var. cauliflora. Research Journal of Medicinal Plant. 2012; 6 (1): 74-82. Whole document, Abstract, Pg. 76-77, Pg. 78 [2]), Table 3, Discussion [last].	1-10
Y	Suresh Kumar MA et al. Pinocembrin Triggers Bax-dependent Mitochondrial Apoptosis in Colon Cancer Cells. Molecular Carcinogenesis. 2007; 46: 231-241. Abstract, results [3], Figures 2A, Methods.	1-10
Y	Lou C et al. 2',4'-Dihydroxychalcone-induced apoptosis of human gastric cancer MGC-803 cells via down-regulation of survivin mRNA. Toxicology in Vitro. 2010; 24: 1333–1337. Introduction [2], Methods and Results	1-10
Y	Yang S et al. Antimetastatic potentials of flavones on oral cancer cell via an inhibition of matrix-degrading proteases. Archives of Oral Biology. 2008; 53: 287-294. Methods and Results, Pg. 292 [3].	1-10
A	Zaki MA et al. Bioactive Formylated Flavonoids from Eugenia rigida: Isolation, Synthesis, and X-ray Crystallography. Journal of Natural Products. 2016, Sept; 79: 2341-2349. Whole document	1-10
A	Tan KW et al. Identification of novel dietary phytochemicals inhibiting the efflux transporter breast cancer resistance protein (BCRP/ABCG2). Food Chemistry. 2013; 138: 2267–2274. Whole document	1-10
A	Chen Z et al. Antiproliferative and apoptotic effects of pinocembrin in human prostate cancer cells. Bangladesh J Pharmacology. 2013; 8: 255-262. Whole document	1-10
A	Rasul A et al. Pinocembrin: A Novel Natural Compound with Versatile Pharmacological and Biological Activities. BioMed Research International. 2013; Volume 2013, Article ID 379850, 9 pages. Whole document	1-10
A	Chen K et al. Pinocembrin suppresses TGF-β1-induced epithelial mesenchymal transition and metastasis of human Y-79 retinoblastoma cells through inactivating αvβ3 integrin/FAK/p38α signaling pathway. Cell & Bioscience. 2014; 4: 41. Whole document	1-10
A	Awasthi, M. et al. Molecular docking and 3D-QSAR-based virtual screening of flavonoids as potential aromatase inhibitors against estrogen-dependent breast cancer. Journal of Biomolecular Structure and Dynamics. 2015; 33(4): 804-819. Whole document	1-10

INTERNATIONAL SEARCH REPORT C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		International application No. **PCT/IB2017/058097**
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Benguedouar, L. et al. Ethanolic Extract of Algerian Propolis and Galangin Decreased Murine Melanoma Tumour Progression. Anti-Cancer Agents in Medicinal Chemistry. 2016; 16(9): 1172-1183. Whole document.	1-10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2017/058097

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:

because they relate to subject matter not required to be searched by this Authority, namely:

the subject matter listed in Rule 39 on which, under Article 17(2)(a)(i), an international search is not required to be carried out, including

2. ☒ Claims Nos.: **11 and 12**

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

See Supplemental Box

3. ☐ Claims Nos:

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT	International application No. PCT/IB2017/058097

Supplemental Box

Continuation of **Box II**

1. The claims do not comply with Rule 6.2(a) because they rely on references to the description and/or drawings.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/IB2017/058097**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**

End of Annex

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.