Section A-Research paper

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Morinda lucida extract composition for treatment of melanoma cancer and anti-malarial activity its preparation ¹Mrs. Archana, ¹Bhavana Dubey*, ²Mohit Chadha, ³Saurabh Jawahar Sanghavi, ⁴Dr. Mohd Mazha, ⁵Mr. Mohammad Muztaba, ⁶Mr. Brijesh Kumar Saroj, ⁷Dr. Kiran Sharma

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ABSTRACT

The present invention generally relates to a process for *Morinda lucida* extract for treatment of melanoma cancer comprises collecting powder extract of Morinda lucida plant; mixing 50-150 grams powdered material with petroleum ether followed by methanol in a Soxhlet; treating marc with lukewarm water for 24 h and then filtering it with filter paper to prepare the aqueous extract; recovering the solvent from respective extracts at low temperature (<40°C) under reduced pressure; grinding fresh plant material in a mixture cum grinder and staining the paste with thick cotton cloth; and keeping the extract overnight for sedimentation and decanting thereby drying at room temperature to get the swaras.

FIELD OF THE INVENTION

The present disclosure relates to an *Morinda lucida* extract composition and a preparation process for treatment of melanoma cancer. In more detail, the process is used for exploring in vivo antitumor potential of *I. Morinda lucida extract* on melanoma cancer[1-2].

BACKGROUND OF THE INVENTION

Herbs and their extracts are inexpensive and rich resources of active compounds that can be utilized as novel cytotoxic agents as well as can be used for the treatment of dermatological disorders associated with melanin hyperpigmentation. In vivo antitumor activity is an emerging research area in cancer biology. Process in the development of antitumor drugs,

Section A-Research paper

worldwide, has been focused on screening therapeutically effective and safe, synthetic and herbal molecules. There are numerous natural or synthetic drugs which were studied for their antitumor potentials, but none of them have uniqueness to meet all the desired requisites, that is, freedom from cumulative or irreversible toxicity, effective long-term protection, prolong stability and ease of administration[3-4].



Figure.1 Morinda lucida plant

Morinda lucida as shown in figure 1, an important plant in traditional medicine in West Africa, has been shown to possess a variety of biological activity, such as anti-inflammatory, antipyretic, analgesia, anti-malarial, anti-diabetic, anticancer, and insecticidal activity, as well as promoting gastric emptying and intestinal motility. The plant is astringent, acrid, refrigerant, mucilaginous, somatic, laxative, diuretic and tonic and used in the treatment of skin diseases, boils, swelling, wounds, ulcer, carbuncle, dropsy, menorrhagia, haemorrhoids, colic, flatulence, dyspepsia, cramp, and burning sensation. Many activities have also been reported for *I. Morinda lucida extract* revealing its antioxidant, analgesic and anti-inflammatory, antispasmodic, antinociceptive activities, antihistaminic, immunostimulant, insulinogenic, hypoglycaemic, antimicrobial, antifungal and antibacterial characteristics. The researches have reported in the inhibition of platelet aggregation, diarrhea, vomiting, and piles[5-6].

Although enough literature is available on medicinal aspects of *I. Morinda lucida extract*, but there is still few research evidence supporting its antitumor activity. In the view of the forgoing discussion, it is clearly portrayed that there is a need to have an *Morinda lucida* extract composition and a preparation process for treatment of melanoma cancer.

SUMMARY OF THE INVENTION

The present disclosure seeks to provide an *Morinda lucida* extract composition and a preparation process for treatment of melanoma cancer. The process for Exploring in vivo antitumor potential of *I. Morinda lucida extract* on melanoma cancer. Petroleum ether ($60^{\circ}C-80^{\circ}C$), methanolic and aqueous extracts, and swaras prepared from the whole herb of

Section A-Research paper

I. Morinda lucida extract are assessed for their antitumor activity. The extracts and swaras at doses of 25 and 50 mg/kg b. wt. are administered intraperitoneal along with chemo and radiotherapy for 40 days for exploring antitumor activity against melanoma cancer (B16F10) in male C57BL mice. The results obtained from tumour volume, and histopathological studies are compared with the control and dacarbazine used as a standard. Antitumor effect of *I. Morinda lucida extract* extracts and swaras on melanoma cancer is found to be significant (P < 0.01) compared to normal control. The tumour volume inhibition against tumour-bearing mice, although differed from each other, is concentration dependent. Administration of plant extracts and swaras from the day 1 since tumour inducted. The induction of tumour is found delayed by 10–15 days and the tumour volume on the day 40 is similar to the Dacarbazine treatment used as a standard[7-8].

In an experimental work, an *Morinda lucida* extract composition for treatment of melanoma cancer is disclosed. The composition includes a powder extract of *Morinda lucida*, from 50-150 grams; an aqueous extract of petroleum ether, from 10-100 milliliters; and an aqueous extract of methanol, from 1-10 milliliters.

In another experimental work, a process for *Morinda lucida* extract for treatment of melanoma cancer is disclosed. The process includes collecting powder extract of *Morinda lucida* plant. The process further includes mixing 50-150 grams powdered material with petroleum ether followed by methanol in a Soxhlet. The process further includes treating marc with lukewarm water for 24 h and then filtering it with filter paper to prepare the aqueous extract. The process further includes recovering the solvent from respective extracts at low temperature (<40°C) under reduced pressure. The process further includes grinding fresh plant material in a mixture cum grinder and staining the paste with thick cotton cloth. The process further includes keeping the extract overnight for sedimentation and decanting thereby drying at room temperature to get the swaras[9-10].

An object of the present disclosure is to reveal the antitumor potential of *I. Morinda lucida extract* on melanoma cancer.

Another object of the present disclosure is to explore in vivo antitumor activity of *I*. *Morinda lucida extract* against mice melanoma (B16F10) cancer cells.

Yet another object of the present invention is to deliver an expeditious and costeffective *Morinda lucida* extract composition.

To further clarify advantages and features of the present disclosure, a more particular description of the invention will be rendered by reference to specific experimental works thereof, which is illustrated in the appended drawings. It is appreciated that these drawings depict only typical experimental works of the invention and are therefore not to be considered

Section A-Research paper

limiting of its scope. The invention will be described and explained with additional specificity and detail with the accompanying drawings.

Material and Methods

Analytical grade dacarbazine (daczin, 200 mg) MEM Media, Tris-HCl, Hydroxylamine hydrochloride, nitro blue tetrazolium (NBT), sodium azide, ethylene diamine tetra acetic acid (EDTA), Hydrogen peroxide, reduced glutathione, 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB), Sodium citrate, Sodium dodecyl sulfate, thiobarbituric acid (TBA) and trichloroacetic acid (TCA) are used for this process.

These and other features, aspects, and advantages of the present disclosure will become better understood when the following detailed description is read with reference to the accompanying drawings in which like characters represent like parts throughout the drawings, wherein[11-12]:

Figure 2 illustrates a process for *Morinda lucida* extract for treatment of melanoma cancer in accordance with an experimental work of the present disclosure.

Further, skilled artisans will appreciate that elements in the drawings are illustrated for simplicity and may not have necessarily been drawn to scale. For example, the flow charts illustrate the method in terms of the most prominent steps involved to help to improve understanding of aspects of the present disclosure. Furthermore, in terms of the construction of the device, one or more components of the device may have been represented in the drawings by conventional symbols, and the drawings may show only those specific details that are pertinent to understanding the experimental works of the present disclosure so as not to obscure the drawings with details that will be readily apparent to those of ordinary skill in the art having benefit of the description herein[13-14].

DETAILED DESCRIPTION:

For the purpose of promoting an understanding of the principles of the invention, reference will now be made to the experimental work illustrated in the drawings and specific language will be used to describe the same. It will nevertheless be understood that no limitation of the scope of the invention is thereby intended, such alterations and further modifications in the illustrated system, and such further applications of the principles of the invention as illustrated therein being contemplated as would normally occur to one skilled in the art to which the invention relates.

It will be understood by those skilled in the art that the foregoing general description and the following detailed description are exemplary and explanatory of the invention and are not intended to be restrictive thereof.

Section A-Research paper

Reference throughout this specification to "an aspect", "another aspect" or similar language means that a particular feature, structure, or characteristic described in connection with the experimental work is included in at least one experimental work of the present disclosure. Thus, appearances of the phrase "in an experimental work", "in another experimental work" and similar language throughout this specification may, but do not necessarily, all refer to the same experimental work[15-16].

The terms "comprises", "comprising", or any other variations thereof, are intended to cover a non-exclusive inclusion, such that a process or method that comprises a list of steps does not include only those steps but may include other steps not expressly listed or inherent to such process or method. Similarly, one or more devices or sub-systems or elements or structures or components proceeded by "comprises...a" does not, without more constraints, preclude the existence of other devices or other sub-systems or other elements or other structures or other components or additional devices or additional sub-systems or additional elements or additional structures or additional structures or additional components.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. The system, methods, and examples provided herein are illustrative only and not intended to be limiting.

Experimental works of the present disclosure will be described below in detail with reference to the accompanying drawings[17-18].

In an experimental work, an *Morinda lucida* extract composition for treatment of melanoma cancer is disclosed. The composition includes a powder extract of *Morinda lucida*, from 50-150 grams; an aqueous extract of petroleum ether, from 10-100 milliliters; and an aqueous extract of methanol, from 1-10 milliliters.

In another experimental work, weight percentage of *Morinda lucida* is preferably 100 grams.

Referring to **Figure 2**, a process for *Morinda lucida* extract for treatment of melanoma cancer is illustrated in accordance with an experimental work of the present disclosure. At step 102, the process 100 includes collecting powder extract of *Morinda lucida* plant.

The process 100 includes mixing 50-150 grams powdered material with petroleum ether followed by methanol in a Soxhlet. Followed by process 100 includes treating marc with lukewarm water for 24 h and then filtering it with filter paper to prepare the aqueous extract.

Section A-Research paper

Followed by the process which includes recovering the solvent from respective extracts at low temperature ($<40^{\circ}$ C) under reduced pressure.

Includes grinding fresh plant material in a mixture cum grinder and staining the paste with thick cotton cloth. keeping the extract overnight for sedimentation and decanting thereby drying at room temperature to get the swaras[19].

In another experimental work, collecting powder extract of *Morinda lucida* plant comprises collecting plant material and shade-drying. Then, grinding the shade-dried plant material. Then, sieving the powder extract of *Morinda lucida* plant through 20 mesh size.

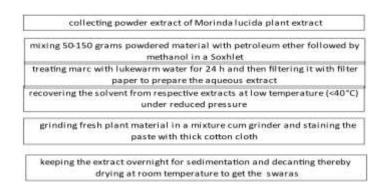
In another experimental work, 100 g powdered material is Soxhlet with petroleum ether at $60^{\circ}C-80^{\circ}C$ followed by methanol.

In another experimental work, the marc is obtained after methanolic extraction.

In another experimental work, grinding plant material with double distilled water to convert into a pasty consistency.

Plant material

Morinda lucida with its leaves, stems, and flowers is collected on December 2008 and authenticated by Botanical Survey with reference number: BSI/WC/ Tech/09/447 and voucher specimen [20].





Preparation of extracts

Freshly collected plant material is shade-dried, powdered, and sieved through 20 mesh size. 100 g powdered material is Soxhlet with petroleum ether (60°C-80°C) followed

Section A-Research paper

by methanol. The aqueous extract is prepared by treating marc (obtained after methanolic extraction) with lukewarm water for 24 h and then filtering it with filter paper as shown in Figure.1. The solvent from respective extracts was then recovered at low temperature (<40°C) under reduced pressure. Swaras is prepared by taking fresh plant material in a mixture cum grinder and grinding it well with double distilled water to convert it to a pasty consistency and then straining the paste with thick cotton cloth. The extract so obtained is kept overnight for sedimentation after which it is decanted and dried at room temperature to get the swaras[21].

In the process, the in vivo antitumor activity of *I. Morinda lucida extract* against mice melanoma (B16F10) cancer cells has been explored. Petroleum ether extract and swaras of *I. Morinda lucida extract* also showed antitumor activity but compared to methanolic and aqueous the effect is found less. The activity of *I. Morinda lucida extract* is found in following order methanolic extract > aqueous extract > swaras > petroleum ether extract. Further, the antitumor activity of *I. Morinda lucida extract* in is attributed to the presence of polar phytoconstituents such as alkaloids, flavonoids, tannins, terpenoids, and glycosides present in the crude extract (maximum in methanolic and aqueous) of *I. Morinda lucida extract*. Further processes are needed to elucidate the molecular mechanism of action and potential usefulness of *I. Morinda lucida extract* as an agent for cancer therapy.

The drawings and the forgoing description give examples of experimental works. Those skilled in the art will appreciate that one or more of the described elements may well be combined into a single functional element. Alternatively, certain elements may be split into multiple functional elements. Elements from one experimental work may be added to another experimental work. For example, orders of processes described herein may be changed and are not limited to the manner described herein. Moreover, the actions of any flow diagram need not be implemented in the order shown; nor do all of the acts necessarily need to be performed. Also, those acts that are not dependent on other acts may be performed in parallel with the other acts. The scope of experimental works is by no means limited by these specific examples. Numerous variations, whether explicitly given in the specification or not, such as differences in structure, dimension, and use of material, are possible. The scope of experimental works is at least as broad as given.

Utilization of Morinda lucida for the treatment of ailments such as malaria, diarrhea, infertility in women, and dysentery in many countries including Nigeria is on increase due to its efficiency, availability, and affordability. However, its cytogenotoxicity has not been elucidated.

Benefits, other advantages, and solutions to problems have been described above with regard to specific experimental works. However, the benefits, advantages, solutions to problems, and any component(s) that may cause any benefit, advantage, or solution to occur

Section A-Research paper

or become more pronounced are not to be construed as a critical, required, or essential feature or component of any or all the claims.

Results and Discussion

An Morinda lucida extract composition for treatment of melanoma cancer, the composition comprises: a powder extract of *Morinda lucida*, from 50-150 grams; an aqueous extract of petroleum ether, from 10-100 milliliters; and an aqueous extract of methanol, from 1-10 milliliters. The composition wherein weight percentage of *Morinda lucida* is preferably 100 grams. A process for *Morinda lucida* extract for treatment of melanoma cancer, the process comprises: collecting powder extract of Morinda lucida plant; mixing 50-150 grams powdered material with petroleum ether followed by methanol in a Soxhlet; treating marc with lukewarm water for 24 h and then filtering it with filter paper to prepare the aqueous extract; recovering the solvent from respective extracts at low temperature (<40°C) under reduced pressure; grinding fresh plant material in a mixture cum grinder and staining the paste with thick cotton cloth; and keeping the extract overnight for sedimentation and decanting thereby drying at room temperature to get the swaras. The process as, wherein collecting powder extract of Morinda lucida plant comprises: collecting plant material and shade-drying; grinding the shade-dried plant material; and sieving the powder extract of Morinda lucida plant through 20 mesh size. The process as claimed in, wherein 100 g powdered material is Soxhlet with petroleum ether at 60°C–80°C followed by methanol. The process wherein the marc is obtained after methanolic extraction. The process wherein grinding plant material with double distilled water to convert into a pasty consistency.

References

- Adewole KE, Attah AF, Adebayo JO. Morinda lucida Benth (Rubiaceae): A review of its ethnomedicine, phytochemistry and pharmacology. J Ethnopharmacol. 2021 Aug 10;276:114055. doi: 10.1016/j.jep.2021.114055. Epub 2021 Mar 19. PMID: 33753141.
- Oladeji OS, Oluyori AP, Dada AO. Antiplasmodial activity of Morinda lucida Benth. Leaf and bark extracts against Plasmodium berghei infected mice. Saudi J Biol Sci. 2022 Apr;29(4):2475-2482. doi: 10.1016/j.sjbs.2021.12.017. Epub 2021 Dec 13. PMID: 35531230; PMCID: PMC9073002.
- **3.** Jolayemi AK, Adeyemi DO, Awoniran PO. Lead nitrate toxicity: its effects on hepatic extracellular matrix fibers, filamentous cytoskeleton and the mitigative potentials of *Morinda lucida* extract. Vet Anim Sci. 2022 Jun 26;17:100260. doi: 10.1016/j.vas.2022.100260. PMID: 35800154; PMCID: PMC9253832.

Section A-Research paper

- 4. Ayertey F, Ofori-Attah E, Antwi S, Amoa-Bosompem M, Djameh G, Lartey NL, Ohashi M, Kusi KA, Appiah AA, Appiah-Opong R, Okine LK. Anti-inflammatory activity and mechanism of action of ethanolic leaf extract of *Morinda lucida* Benth. J Tradit Complement Med. 2020 Aug 4;11(3):249-258. doi: 10.1016/j.jtcme.2020.07.001. PMID: 34012871; PMCID: PMC8116761.
- Ayertey F, Ofori-Attah E, Antwi S, Amoa-Bosompem M, Djameh G, Lartey NL, Ohashi M, Kusi KA, Appiah AA, Appiah-Opong R, Okine LK. Anti-inflammatory activity and mechanism of action of ethanolic leaf extract of *Morinda lucida* Benth. J Tradit Complement Med. 2020 Aug 4;11(3):249-258. doi: 10.1016/j.jtcme.2020.07.001. PMID: 34012871; PMCID: PMC8116761.
- Mancuso RI, Foglio MA, Olalla Saad ST. Artemisinin-type drugs for the treatment of hematological malignancies. Cancer Chemother Pharmacol. 2021 Jan;87(1):1-22. doi: 10.1007/s00280-020-04170-5. Epub 2020 Nov 3. PMID: 33141328.
- Omeiza FO, Ademowo GO, Ayeni FA. Evaluation of in vivo anti-malarial potential of omidun obtained from fermented maize in Ibadan, Nigeria. Malar J. 2020 Nov 19;19(1):414. doi: 10.1186/s12936-020-03486-0. PMID: 33213477; PMCID: PMC7678239.
- Salimi-Sabour E, H Shirazi F, Mahboubi A, Mojab F, Irani M. Biological Activities and the Essential Oil Analysis of *Cousinia harazensis* and *C. calocephala*. Iran J Pharm Res. 2021 Summer;20(3):140-150. doi: 10.22037/ijpr.2020.114155.14697. PMID: 34903977; PMCID: PMC8653680.
- 9. Xiao D, Liu Z, Zhang S, Zhou M, He F, Zou M, Peng J, Xie X, Liu Y, Peng D. Berberine Derivatives with Different Pharmacological Activities via Structural Modifications. Mini Rev Med Chem. 2018;18(17):1424-1441. doi: 10.2174/1389557517666170321103139. PMID: 28325147.
- **10.** Abdullah -, Zuberi MH, Haroon U, Tajammul A, Tabassum A, Sultana A. Development and evaluation of herbal formulation AKIGTU01 and AKIGCL03 against acne producing microbes. Pak J Pharm Sci. 2022 May;35(3):819-825. PMID: 35791482.
- 11. Abdel Fattah NS, Darwish YW. In vitro antibiotic susceptibility patterns of Propionibacterium acnes isolated from acne patients: an Egyptian university hospitalbased study. J Eur Acad Dermatol Venereol. 2013 Dec;27(12):1546-51. doi: 10.1111/jdv.12057. Epub 2012 Dec 20. PMID: 23279041.

Section A-Research paper

- Kamboj A, Sihag B, Brar DS, Kaur A, Salunke DB. Structure activity relationship in β-carboline derived anti-malarial agents. Eur J Med Chem. 2021 Oct 5;221:113536. doi: 10.1016/j.ejmech.2021.113536. Epub 2021 May 13. PMID: 34058709.
- Nguyen PTV, Van Dat T, Mizukami S, Nguyen DLH, Mosaddeque F, Kim SN, Nguyen DHB, Đinh OT, Vo TL, Nguyen GLT, Quoc Duong C, Mizuta S, Tam DNH, Truong MP, Huy NT, Hirayama K. 2D-quantitative structure-activity relationships model using PLS method for anti-malarial activities of anti-haemozoin compounds. Malar J. 2021 Jun 11;20(1):264. doi: 10.1186/s12936-021-03775-2. PMID: 34116665; PMCID: PMC8196453.
- Pabón A, Escobar G, Vargas E, Cruz V, Notario R, Blair S, Echeverri F. Diosgenone synthesis, anti-malarial activity and QSAR of analogues of this natural product. Molecules. 2013 Mar 14;18(3):3356-78. doi: 10.3390/molecules18033356. PMID: 23493102; PMCID: PMC6270258.
- 15. Kim TH, Kim HK, Hwang ES. Novel anti-adipogenic activity of anti-malarial amodiaquine through suppression of PPARγ activity. Arch Pharm Res. 2017 Nov;40(11):1336-1343. doi: 10.1007/s12272-017-0965-3. Epub 2017 Oct 25. PMID: 29071567.
- 16. Ma W, Zhang Y, Yu M, Wang B, Xu S, Zhang J, Li X, Ye X. In-vitro and in-vivo anti-breast cancer activity of synergistic effect of berberine and exercise through promoting the apoptosis and immunomodulatory effects. Int Immunopharmacol. 2020 Oct;87:106787. doi: 10.1016/j.intimp.2020.106787. Epub 2020 Jul 21. Erratum in: Int Immunopharmacol. 2020 Aug 19;88:106899. PMID: 32707493.
- Hazafa A, Rehman KU, Jahan N, Jabeen Z. The Role of Polyphenol (Flavonoids) Compounds in the Treatment of Cancer Cells. Nutr Cancer. 2020;72(3):386-397. doi: 10.1080/01635581.2019.1637006. Epub 2019 Jul 9. PMID: 31287738.
- Elias SO, Ladipo CO, Oduwole BP, Emeka PM, Ojobor PD, Sofola OA. Morinda lucida reduces contractility of isolated uterine smooth muscle of pregnant and nonpregnant mice. Niger J Physiol Sci. 2007 Jun-Dec;22(1-2):129-34. doi: 10.4314/njps.v22i1-2.54891. PMID: 18379632.
- Adewole KE, Ishola AA. Phytosterols and triterpenes from *Morinda lucida* Benth (*Rubiaceae*) as potential inhibitors of anti-apoptotic BCL-XL, BCL-2, and MCL-1: an in-silico study. J Recept Signal Transduct Res. 2019 Feb;39(1):87-97. doi: 10.1080/10799893.2019.1625062. Epub 2019 Jun 19. PMID: 31215288.

Section A-Research paper

Anifowoshe AT, Abdulkareem AO, Opeyemi OA, Aina OM, Makanjuola DE, Abel JO, Majolagbe JO, Babamale OA. Evaluation of cytogenotoxic potential of Morinda lucida leaf extract on Swiss albino male mice using two bioassays. J Basic Clin Physiol Pharmacol. 2019 Dec 19;31(1). doi: 10.1515/jbcpp-2019-0079. PMID: 31855566.

21.