EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 11
Issue: 5
143-151
Year: 2024

STUDY OF ANTI-PROLIFERATIVE ACTIVITY OF TRI-ACETOXY CUCURBITACIN ISOLATED FROM DRY SEED OF WILD CUCUMBER (JANGLI KHIRA)

Ajoy K. Bauri*[1], Gerardo Anaya Eugenio[2] and Esperanza J. Carcache De Blanco*[2]

[1]Bio-Organic Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400085, India.
[2]College of Pharmacy, The Ohio State University, Ohio, Columbus, OH 43210, USA.

***Corresponding Author: Ajoy K. Bauri**
Bio-Organic Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400085, India.

Article Received on 03/03/2024 **Article Revised on 23/03/2024** **Article Accepted on 13/04/2024**

ABSTRACT
Chemical profiling of methanol extract of seed of wild cucumber revealed the presence of a triterpenoid as major constituent. Structural determination of this triterpenoid has been carried out by chemical, spectral, and spectrometric methods and was identified as tri-acetoxy cucurbitacin. The anti-proliferative activity of this compound has been carried out on four different human cancer cell lines. It exhibited potent inhibitory activity against different human cancer cell lines named as prostate (DU-145), breast (MCF-7), cervical (HeLa) and colon (HT-29) cancer cell lines using SRB assay in comparison with therapeutically used standard drug, paclitaxel as control. Bioassay result exhibited the most significant activity against aforesaid cell lines with inhibitory concentration, IC50 values 27.1±1.5, 24.9 ±0.8, 34.8 ±3.4, 26.3 ± 2.4 µM level respectively.

KEYWORD: Wild Cucurbita seed; isolation; structural characterization; triterpenoid; tri-acetoxy cucurbitacin; cell cytotoxicity; SRB assay.

INTRODUCTION
Cucurbitaceae, a plant family consisting of about 965 species in around 95 genera. Most of them are perennial herbs and some of them are edible, use as vegetable, raw eatery item or in the form of salad. The seed of wild cucumber is used in Ayurveda as folklore medicine to cure cancer reported in literature.[1,2] Wild variety of cucumber is a well-known medicinal plant belonging to *Cucurbitaceae* family widely distributed to the central forestry part of India. The fruits of the plant are very much useful for health benefits claimed by local communities as well as reported in literature. The seed are used as folklore medicine by the people of Gaulle and Dhungar communities in the central part of India and prescribed as folklore remedial agent for the treatment of cancer and preventive measure for different kinds of ailments.

Now a days, the major health issue in our society is cancer and it is increasing in rapid rate as compare to other diseases.[3] It is a very severe life-threatening disease to human beings in our modern society. Of course, in the modern pharmacy, some synthetic drugs and other therapies are being used to provide relief temporarily, but it is difficult or not possible to total eradicate this disease. These drugs are very much expensive beyond affordability of common people, prolong uses of these synthetic drugs, adaptation of chemo-prevention, radio-prevention for therapeutic uses reflected lot of side effects. In this regard, we are looking for suitable botanical medicines with low cost, not over burden to the common people, easy availability from natural resources, consider as the replacement of expensive and harmful synthetic drugs for therapeutic uses for treatment of this severe disease. In this connection, a combating process has been adopted world-wise to look for suitable botanical medicine from natural resources to prevent this disease. Medicinal plants and herbs extracts are now generally considered as effective and reliable medicines in modern pharmacy as remedies to cure many acute and chronic diseases due to their least side effects and easy availability in least prices comparatively. Majority of world population relies therapeutically on medicinal plants and herbs for their primary healthcare.[4] Although botanical medicines have been used as remedial agent to cure various ailments since from antiquity, but due to lack of full scientific proof and unclearness of the functionaries of plants crude products in human physiology, it remains less reliable in modern society. Since there is no proper medication so

far in modern pharmacy as curative agent against many acute and chronic diseases, therefore, herbal products and botanical supplements may be considered as the last hope to survive in struggleful life performing diet and supplementary therapy. Many phytochemical investigations are going on all over the world in various aspects in order to combat suitable curative agent based on the ancient information available in ethnopharmacology.[5-7] In this regard, we have chosen the seed of wild cucumber belonging to *Cucurbitaceae* family for its proliferation prevention activity reported in literature and evidence-based knowledge of local tribal communities in central forestry part of India.[8] Details phytochemical investigation combated on methanol extract of the seed of wild cucumber to looking for the bio-active ingredients which could be responsible to prevent cancer, their structural characterization of active constituent and study of anti-proliferative activity against four different human cancer lines using SRB assay.

RESULTS AND DISCUSSION

The ethnobotanical survey shows that dry powder of the seed of wild cucumber is used as folk botanical remedy to cure cancer claimed by local communities in the central forestry region of India.[8] From previous evidence-based experience of local communities in this region, the seed powder of wild cucumber would be considered as botanical medicine to save life for replacement of harmful synthetic drugs prescribed to prevent and treat the cancer disease. The dry powder of seed of wild cucumber possesses preventive performance against proliferation.[8] In Hindi, it is known as ′Jangle khira` and in English known as wild cucumber (*Cucurbita andreana*). The fruit and seed of the wild cucumber are owing to prevent cell proliferation.[9-19,21] The major therapeutic credentialism of different parts of the fruit of wild cucumber are antistress, anti-oxidant, antiinflammatory, antidiabetics, antiproliferative, immunomodulatory, antitumour, antimelanoma, hepatoprotective, anticovid, antiviral, etc. But detailed chemical profiling of the seed of wild cucumber indicated the presence of a tetra-cyclic triterpene known as cucurbitacin in the form of triacetates as major chemical constituents (about 4% of crude extract of its dry seed) in methanol extract of its seed isolated by column chromatography over silica gel with gradient solvent elution. The structural determination of the major chemical constituent, tri-acetoxy cucurbitacin has been carried out by chemical (using different spray reagents followed by heating at 120 ºC for a span of 5 min and appeared as pink coloured spot in micro thin layer chromatography plates), spectral and spectrometric methods on comparison with published values of ^{1}H NMR and ^{13}C NMR, MS spectral data reported in literature.[39,46-50] The chemical structure of the major active ingredient is depicted below in figure 1.

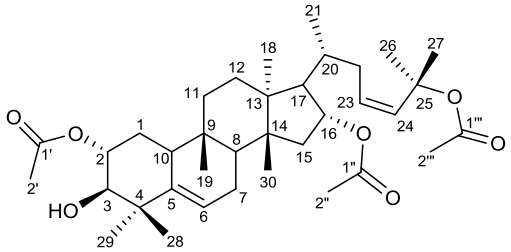


Figure 1: Chemical structure of tri-acetoxy cucurbitacin (compound 1).

This manuscript deals with the isolation of active ingredient, tri-acetoxy cucurbitacin from methanol extract of the dried seed of wild cucumber by column chromatography, its structure elucidation by chemical, spectral, spectrometric methods and study of cell cytotoxicity on four different human cancer cell lines using sulforhodamine B (SRB) assay in comparison with paclitaxel as control.[51,52]

MATRIALS AND METHODS

General experimental procedures

Melting points were determined using a Buchi melting point apparatus (Model number M560). Specific rotations were obtained using a JASCO DIP 1000 digital polarimeter. UV spectra were measured on Shimadzu UV-2100 UV-Vis spectrophotometer. NMR spectra were recorded in $CDCl_3$ (manufactured by Board of Radiation and Isotope Technology, INDIA) on a Bruker Advance 200 and Varian 600 NMR spectrometer using residual $CHCl_3$/HDO as an internal standard. Chemical shifts are given in ppm (δ_H & δ_C), relative to residual $CHCl_3$/HDO at 7.26 and 77.00 ppm. UHPLC/MS analysis of the samples was performed using a Vanquish UHPLC system coupled to a Q-Exactive quadrupole orbitrap mass spectrometer (Thermo Fisher Scientific, USA) at the Ohio State University, College of Pharmacy, Instrumentation Facility. Silica gel 60 (Merck, Germany) was used for analytical TLC. Silica gel 60 (70-230 mesh, Merck, Germany) was used for column chromatography. This compound was visualized by TLC using vanillin-perchloric acid-EtOH followed by heating at 110 °C for 5 min, expose in iodine vapour, spraying with alkaline $KMnO_4$ followed by heating at 110°C, anisaldehyde in EtOH and neutral $FeCl_3$ in MeOH.

Plant material

The dried seeds (10 gm) of wild *Cucurbita* used in this study has been gifted to us on March, 2019 by the eminent naturopath Mr. Vivek Rao Godbole of Bhopal Pattanum locality in Beejapur district of Chhattisgarh State, India for phytochemical analysis to combat the active ingredient owing anti-proliferation property. The seeds of wild cucurbita possessed anti-cancer property (knowledge-based evidence) also claimed by local Vaidya (naturopath) of Baster region of Chhattisgarh State. The material was authenticated by Dr. Hussain Barbhuiya, Landscape and Cosmetic Maintenance Section, A & SED Division, Bhabha Atomic Research Centre, Trombay, Mumbai. A voucher specimen was

deposited in the Herbarium of the Landscape & Cosmetic Maintenance Division, BARC, Mumbai-400085, India.

Extraction and Isolation

The dried seeds (10 gm) of wild cucumber are finely powdered and extracted three times at room temperature with AR grade methanol (3 x 20 ml x 24 h). Removal of solvent afforded a colourless viscous residue (250 mg, extractable amount 2.5 % of dry seed) from methanol extract. Initial screening of the crude extract, it has revealed that crude extract of methanol possessed excellent anti-proliferative activity on different human cancer cell lines. In order to find out the active constituents, the crude extract was fractionated over silica gel (5 gm, 230-400 mesh, Aldrich) and eluted with a step gradient of chloroform followed by a binary mixture of methanol in chloroform to furnish five fractions, volume of each aliquot 5 mL. In some cases, the volume of aliquot collected is about 10-15 mL. Fractions were monitored by TLC to examine the chemical constituents. The fractions having similar chemical profiles were combined and, in some case, further purified by using repetitive column chromatograph on open column over silica gel, gel permeation chromatography (GPC) with solvent gradual elution followed by preparative thin layer chromatography (PTLC).

Analysis of major chemical constituent isolated from methanol extract of the dry seed of wild cucumber

The major chemical constituent has been isolated from methanol extract of the seed of wild cucumber is a tetracyclic triterpene known as tri-acetoxy cucurbitacin (compound **1**). It is a colourless sticky substance present as major chemical constituent. The mass of the compound has been determined as 599.3920 au $[M-H]^-$ (obs. m/z in negative mode) $[M-H]^-$ by LRESI mass spectrometry; calc. m/z in negative mode $[M-H]^-$ 599.829 au. Therefore, the molecular formula of compound **1** (tri-acetoxy cucurbitacin) has been established as $C_{34}H_{54}O_6$; UV (MeOH), λ_{max} (log ε): 210 nm; IR (neat), ν_{max} (cm^{-1}): 2924 (symmetric stretching -CH_2-), 2854 (asymmetric stretching -CH_2-), 1739 (very strong peak for ester carbonyl), 1644 (>CH-O- stretching), 1452 (>C=C< stretching), 1374, 1163, 1097, 906, 724. ^{1}H NMR ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz) data see in Table 1: LRESI- MS (negative mode) mass interpretation and spectrum given below depicted in figure 2 & 3.

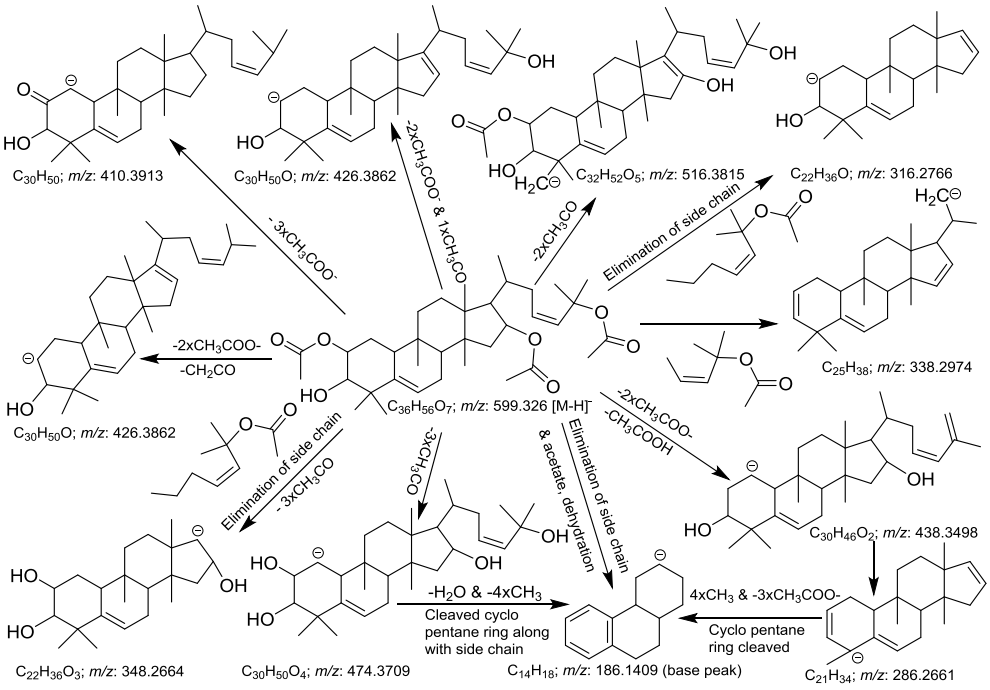


Figure 2: Interpretation of LRESI-MS of tri-acetoxy cucurbitacin.

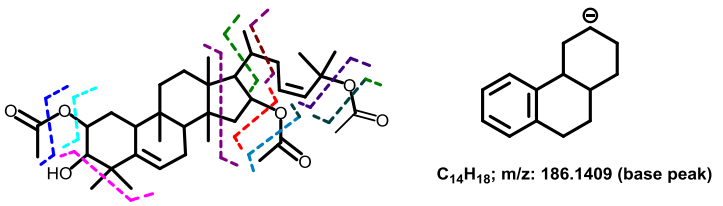


Figure 3: Probable cleavage of bonds of tri-acetoxy cucurbitacin while mass fragmentation.

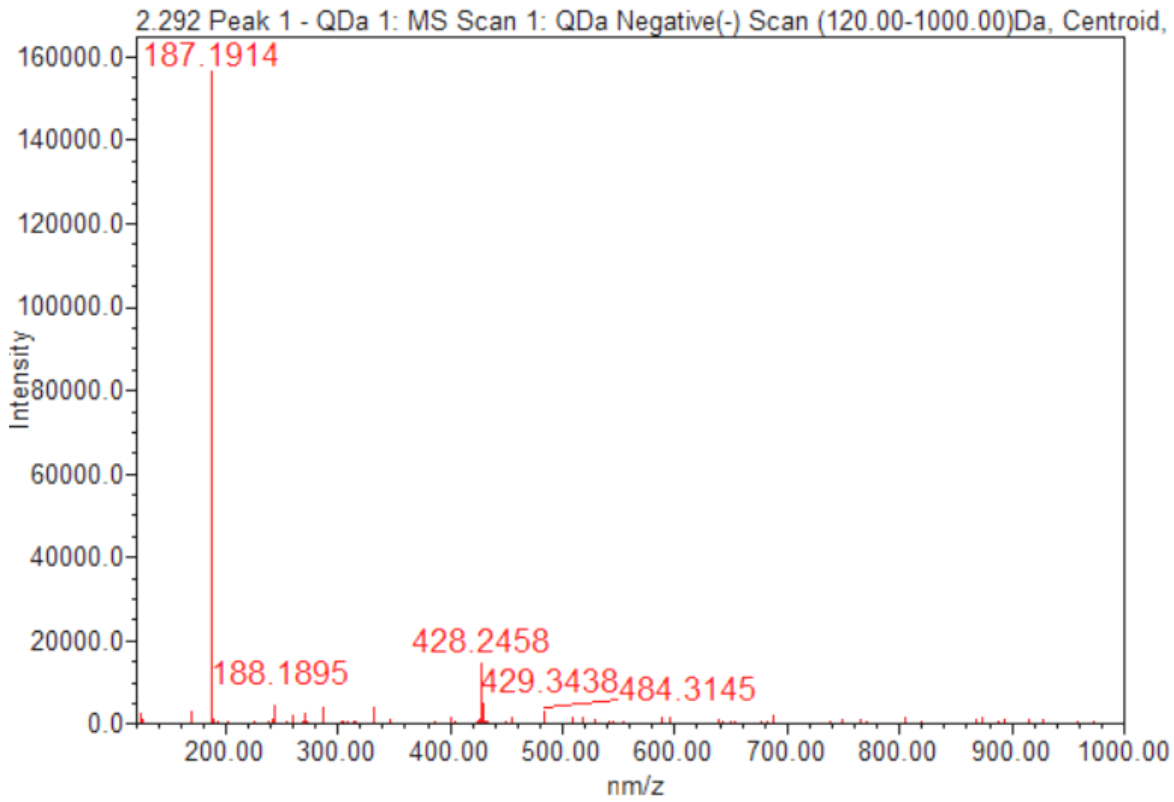


Figure 4: LRESI-MS of tri-acetoxy cucurbitacin isolated from wild cucumber.

Table 1: Chemical shift values for ^{1}H & ^{13}C nuclei of tri-acetoxy cucurbitacin isolated from dry seed of wild Cucumber (*Cucurbita andreana*).

Chemical shift values (in ppm) for ^{1}H ($CDCl_3$, 600 MHz) spectrum and ^{13}C NMR spectrum ($CDCl_3$, 150 MHz) respectively											
Position	**No. of H**	**Orientation**	**δ_H, (J_{H-H} values)**	**Position**	**No. of H**	**Orientation**	**δ_H (J_{H-H} values)**	**Position**	**δ_C**	**Position**	**δ_C**
1a	1	H-1α	1.60, m	18	3	H-18	0.89, s, CH_3	C-1	14.25	C-18	24.98
1b	1	H-1β	2.32, m	-	-	-	-	-	-	-	-
2	1	H-2	4.22, m	19	3	H-19	1.25, s, CH_3	C-2	62.23	C-19	14.15
3	1	H-3	3.98, m	20	1	H-20	0.88, dd (9.2, 13.5)	C-3	69.04	C-20	29.30
4	0	H-4	-	21	3	H-21	0.98, d (6.6), CH_3	C-4	34.17	C-21	22.64
5	0	H-5	-	22	2	H-22	2.04, m	C-5	130.14	C-22	29.24
6	0	H-6	5.38, m	23	1	H-23	7.50, dd (10, 2.4)	C-6	128.20	C-23	128.02
7a	1	H-7a	2.33, m	24	1	H-24	7.76, d (10.0 Hz)	C-7	24.98	C-24	130.36
7b	1	H-7b	2.02, m	-	-	-	-	-		C-25	62.23
8	1	H-8	2.00, m	25	0	H-25	-	C-8	32.04	C-26	29.45
9	0	H-9	-	26	3	H-26	1.26, s, CH_3	C-9	29.89	C-27	14.20
10	1	H-10	1.04, m	27	3	H-27	1.27, s, CH_3	C-10	29.82	C-28	22.81
11a	1	H-11α	1.05, m	28	3	H-28	1.30, s, CH_3	C-11	29.61	C-29	25.75
11b	1	H-11β	1.60, m	-		-	-	-	-	-	-
12a	1	H-12α	2.01, m	29	3	H-29	1.28, s, CH_3	C-12	29.40	C-30	22.70
12b	1	H-12β	2.00, m	-	-	-	-	-	-	-	-
13	0	H-13	-	30	3	H-30	0.88, s, 3H	C-13	29.21	C-1′	173.01
14	0	H-14	-	2′	3	C2-OAc	2.22, s, 3H	C-14	29.49	C-1″	173.46
15a	1	H-15α	1.37, dd (9.2, 13.5)	2″	3	C16-OAc	2.22, s, 3H	C-15	27.32	C-1‴	173.46
15b	1	H-15β	2.03, dd (9.2, 13.5)	-	-	-	-	-	-	C-2′	22.81
16	1	H-16	4.30, m	2‴	3	C25-OAc	2.22, s, 3H	C-16	62.23	C-2″	22.81
17	1	H-17	2.77, dd (9.2, 13.5)	C3-OH	1	-OH	3.25, brs, 1H	C-17	29.80	C-2‴	22.81

Anti-proliferative Bioassay

Anti-proliferative activity of cucurbitacin against different cancer cell lines has been evaluated by using sulforhodamine B (SRB) assay and was performed at The College of Pharmacy, The Ohio State University, Ohio, Columbus, USA.

Sulforhodamine B (SRB) cell cytotoxicity assay is one of the most widely used method to detect cell viability. This assay is independent of cell metabolic activity. The incorporated dye released from stained cells after washing is directly proportional to the cell biomass and can be measured at 515 nm. Four different types of cancer cell lines such as prostate (DU-145), breast (MCF-7), cervical (HeLa) and colon (HT-29) using SRB assay in comparison with paclitaxel as control drug were used to carry out bioassay for cell cytotoxicity study (Table 1).

Sample preparation

Test samples and controls (paclitaxel) were dissolved in 100% DMSO to prepare stock solutions of 10 mg/ml. Dilutions were first prepared using 10% DMSO in water and then 100% water.

Cell culture

Cancer cells such as prostate (DU-145), breast (MCF-7), HeLa (cervical) and colon (HT-29) were obtained from American Type Culture Collection, Manassas, VA, USA. Monolayer cells were cultured using T75 tissue culture flasks in Roswell Park Memorial Institute medium (RPMI) or Dulbecco's Modified Eagle Medium (DMEM), containing 10% fetal bovine serum and 1% antibiotic-anti-mycotic from Gibco. Also, HPAC used DMEM: F12 with insulin, transferrin, hydrocortisone, epidermal growth factor and MDAT-32 used RPMI-1640 with 1% NEAA (Nonessential amino acid). Cells were kept at 37 °C and in an atmosphere with 5% of CO_2.

Table 2: Anti-proliferative activity of cucurbitacin against different cancer cell lines.

Bioassay results of tri-acetoxy cucurbitacin on cancer cell lines using SRB assay (µM)		
Cell lines used	**IC_{50} value of the isolate**	**IC_{50} value of Paclitaxel**
Prostate (DU-145)	9.47±2.0	13.17±0.5
Breast (MCF-7)	8.32±1.3	0.92±3.3
Cervical (HeLa)	34.8 ±1.3	0.011±0.0005
Colon (HT-29)	6.10 ± 0.6	0.0028±0.00007

[a] Samples tested in triplicate and in two separate experiments at **20µg/mL**
[b] % Inhibition <50 deemed inactive
[c] IC_{50} ≥20 deemed inactive

CONCLUSION AND SUMMARY OF THE WORK

Details phytochemical analysis revealed that methanol extract of the dry seed of wild cucumber contained a tetracyclic triterpenoid as major chemical constituent and identified as tri-acetoxy cucurbitacin by means of chemical, spectral, and spectrometric study. For evaluation of anti-proliferation activity, this compound has been bio-assayed against four different human cancer cell lines named as prostate (DU-145), breast (MCF-7), cervical (HeLa) and colon (HT-29) cancer cell lines to check its potentiality using SRB assay in comparison with therapeutically used standard drug, paclitaxel as control. Bioassay results exhibited highly potent activity against aforesaid human cancer cell lines with IC_{50} values 27.1±1.5, 24.9±0.8, 34.8±3.4, 26.3±2.4 µM level.

ORCID
Ajoy Bauri: 0000-0002-0941-5638

ORCID
Esperanza J. Carcache de Blanco: 0000-0002-9519-3292

ORCID
Gerardo Anaya Eugenio: 0000-0002-5872-201X

Associated Content

Supporting Information (Spectroscopic data consisting IR, UV, ^{1}H NMR, ^{13}C NMR, DEFT, ESIMS) of compound cucurbitacin were enclosed in attached file (**Annexure 1**).

ACKNOWLEDGEMENT

We express our sincere gratitude to Dr. David G. I. Kingston, Department of Chemistry, M/C 0212, Virginia Tech, Blacksburg, Virginia 24061, USA to bring this opportunity for collaboration with The College of Pharmacy, The Ohio State University, Ohio, Columbus, USA. We are also thankful to Dr. Dmitriy Uchenik and Dr. Deepa Krishnan, OSU College of Pharmacy instrumentation facility for allowing acquisition of MS data and Mamata V. Joshi for her excellent effort to acquire NMR data from National Instrumental Facility, Tata Institute of Fundamental Research (TIFR), Mumbai. Authors very much thankful to Head of Division, Bio-Organic Division and Group Director, Bioscience Group, BARC for their continuous encouragement and support.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

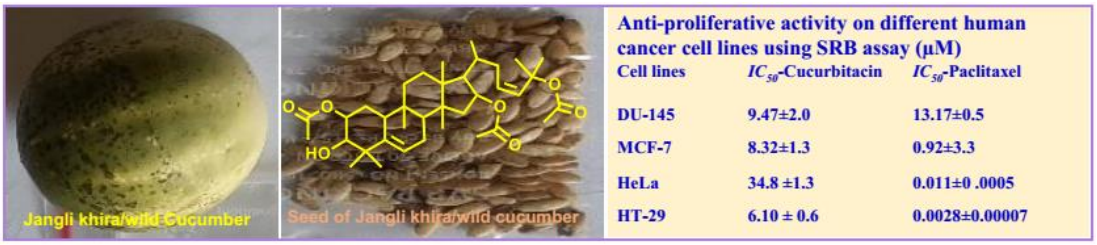
Data Availability Statement

The data that support the findings of this study are available from corresponding author upon reasonable request.

Author Contribution Statement

AKB performed isolation, structural characterization, chemical transformation/reaction and drafted manuscript. Gerardo Anaya Eugenio contributed to the samples/reagents/materials /analysis tools and analysed anti-proliferative assay the data. EJCB supervised biological experimental, analysed result of anti-proliferative assay, managed to acquire HRESI-MS data with the help from Gerardo Anaya Eugenio.

GRAPHICAL ABSTRACT



REFERENCES

1. Jian CC, Ming HC, Rui LN, Cordel GA, Qiuz SX. Cucurbitacins and cucurbitane glycosides: Structures and biological activities. Nat. Prod. Rep., 2005; 22: 386-399.
2. Jing S, Zou H, Wu Z, Ren L, Zhang T, Zhang J, Wei Z. Cucurbitacins: Bio-activities and synergistic effect with small-molecule drugs. J. Funct. Foods, 2020; 72: 104042.
3. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J. Clin., 2021; 71: 209-249.
4. Rolnik A, Olas B. Vegetable from the Cucurbitaceae family and their products: Positive effect on human health. Nutrition, 2020; 78: 110788.
5. Kaushik U, Aeri V, Mir SR. Cucurbitacins-an insight into medicinal leads from nature. Pharmacogn. Rev., 2015; 9: 12-8.
6. Kirtonia A, Gala K, Fernandes SG, Pandya G, Pandey AK, Sethi G, Khattar E, Garg M. Repurposing of drugs: An attractive pharmacological strategy for cancer therapeutics. Semin. Cancer Biol., 2021; 68: 258-278.
7. Tariq A, Sadia S, Pan K, Ullah I, Mussarat S, Sun F, Abiodun OO, Batbaatar A, Li Z, Song D, Xiong Q, Ullah R, Khan S, Basnet BB, Kumar B, Islam R, Adman M. A systematic review on ethnomedicines of anti-cancer plants. Phytother. Res., 2017; 31: 202-264.
8. Yang SF, Weng CJ, Sethi G, Hu DN. Natural bio-actives and phytochemicals serve in cancer treatment and prevention. Evidence-based Complementary and Alternative Medicine, 2013; 6981-90.
9. Bishayee A, Sethi G. Bioactive natural products in cancer prevention and therapy: Progress and promise. Semin. Cancer Biol., 2016; 40-41: 1-3.
10. Sak K. Anti-cancer action of plant products: Changing stereotyped attitudes. Explor. Target. Anti-Tumor Ther., 2022; 3: 423-427.
11. Chen T, Ma B, Lu S, Zeng L, Wang H, Shi W, Zhou L, Xia Y, Zhang X, Zhang J, Chen J. Cucumber-Derived Nanovesicles Containing Cucurbitacin B for Non-Small Cell Lung Cancer Therapy. Int. J. Nanomed, 2022; 17: 3583-3599.
12. Vidal-Gutiérrez M, Torres-Moreno H, Arenas-Luna V, Loredo-Mendoza ML, Tejeda-Dominguez F, Velazquez C, Vilegas W, Hernández-Gutiérrez S, Robles-Zepeda RE. Standardized phyto-preparations and cucurbitacin IIb from Ibervillea sonorae (S. Watson) greene induce apoptosis in cervical cancer cells by Nrf2 inhibition. J. Ethnopharmacol, 2022; 298: 115606.
13. Üremiş N, Üremiş MM, Çiğremiş Y, Tosun E, Baysar A, Türköz Y. Cucurbitacin-I exhibits anti-cancer efficacy through induction of apoptosis and modulation of JAK/STAT3, MAPK/ERK, and AKT/mTOR signalling pathways in HepG2 cell line. J. Food Biochem, 2022; 46: e14333.
14. Raghunath A, Sundarraj K, Arfuso F, Sethi G, Perumal E. Dysregulation of Nrf2 in Hepatocellular Carcinoma: Role in Cancer Progression and Chemoresistance. Cancers, 2018; 10: 481.
15. Ma Z, Xiang X, Li S, Xie P, Gong Q, Goh BC, Wang L. Targeting hypoxia-inducible factor-1, for cancer treatment: Recent advances in developing small-molecule inhibitors from natural compounds. Semin. Cancer Biol., 2022; 80: 379-390.
16. Liang J, Chen D. Advances in research on the anti-cancer mechanism of the natural compound cucurbitacin from Cucurbitaceae plants: A review. Tradit. Chin. Med., 2019; 4: 68.
17. Ren Y, Kinghorn AD. Natural Product Triterpenoids and Their Semi-Synthetic Derivatives with Potential Anti-cancer Activity. Planta Med., 2019; 85: 802-814.
18. Torres-Moreno H, Marcotullio MC, Velázquez C, Ianni F, Garibay-Escobar A, Robles-Zepeda RE. Cucurbitacin IIb, a steroidal triterpene from Ibervillea sonorae induces anti-proliferative and apoptotic effects on cervical and lung cancer cells. Steroids, 2020; 157: 108597.
19. Liu M, Yan Q, Peng B, Cai Y, Zeng S, Xu Z, Yan Y, Gong Z. Use of cucurbitacin for lung cancer

research and therapy. Cancer Chemother. Pharmacol, 2021; 88: 1-14.
20. Chen X, Bao J, Guo J, Ding Q, Lu J, Huang M, Wang Y. Biological activities and potential molecular targets of cucurbitacin: a focus on cancer. Anti-cancer Drugs, 2012; 23: 777-87.
21. Jayaprakasam B, Seeram NP, Nair MG. Anti-cancer and anti-inflammation activities of cucurbitacins from Cucurbita andreana. Cancer Letters, 2003; 189: 11-6.
22. Li R, Xiao J, Tang S, Lin X, Xu H, Han B, Yang M, Liu F. Cucurbitacin-I induces apoptosis in ovarian cancer cells through oxidative stress and the p190B-Rac1 signalling axis. Mol. Med. Rep., 2020; 22: 2545-2550.
23. Xie H, Tuo X, Zhang F, Bowen L, Zhao W, Xu Y. Dietary cucurbitacin E reduces high-strength altitude training induced oxidative stress, inflammation and immunosuppression. An. Acad. Bras. *Cienc*., 2020; 92: 1-14.
24. Tannin-Spitz T, Bergman M, Grossman S. Cucurbitacin glucosides: Anti-oxidant and free-radical scavenging activities. Bioch. Biophys. Res. Commun, 2007; 364: 181-6.
25. Recio MC, Prieto M, Bonucelli M, Orsi C, Manez S, Giner RM, Cerda-Nicolas M, Rios JL. Anti-inflammation activity of two cucurbitacins isolated from Cayaponia tayuya roots. Planta Med., 2004; 70: 414-20.
26. Siqueira JM, Peters Jr RR, Gazola AC, Krepsky PB, Farias MR, Rae GA, Brum-Fernandes AJD, Ribeiro-do-Valle RM. Anti-inflammation effects of a triterpenoid isolated from Wilbrandia ebracteata Cogn. Life Sci., 2007; 80: 1382-1387.
27. Huerta-Reyes M, Tavera-Hernández R, Alvarado-Sansininea JJ, Jiménez-Estrada M. Selected species of the Cucurbitaceae family used in Mexico for the treatment of diabetes Mellitus. Molecules, 2022; 27: 3440.
28. Attard E, Brincat MP, Cuschieri A. Immunomodulatory activity of cucurbitacin E isolated from Ecballium elaterium. Fitoterapia, 2005; 76: 439-41.
29. Sadzuka Y, Fujiki S, Itai S. Enhancement of doxorubicin-induced anti-tumour activity and reduction of adverse reactions by cucurbitacin I. Food Res. Int., 2012; 47: 64-69.
30. Hsu H. S, Huang PI, Chang YL, Tzao C, Chen YW, Shih HC, Hung SC, Hen YC, Tseng LM, Chiou SH. Cucurbitacin-I inhibits tumorigenic ability and enhances radio-chemosensitivity in nonsmall cell lung cancer-derived CD133-positive cells. Cancer, 2011; 117: 2970-2985.
31. Aiswarya SUD, Gowda V, Haritha NH, Liju VB, Shabna A, Swetha M, Rayginia TP, Keerthana CK, Nath LR, Reshma MV, Sundaram S, Anto NP, Lankalapalli RS, Anto RJ, Bava SV. Cucurbitacin B, purified and characterized from the rhizome of Corallocarpus epigaeus Exhibits Anti-Melanoma Potential. Front. Oncol, 2022; 12: 903832.
32. Arjaibi HM, Ahmed MS, Halaweish FT. Mechanistic investigation of hepato-protective potential for cucurbitacins. Med. Chem. Res., 2017; 26: 1567-1573.
33. Kapoor N, Ghorai SM, Kushwaha PK, Shukla R, Aggarwal C, Bandichhor R. Plausible mechanisms explaining the role of cucurbitacins as potential therapeutic drugs against coronavirus 2019. Inform. Med. Unlocked, 2020; 21: 100484.
34. Sathya TN, Mehta VA, Senthil D, Navaneethakrishnan K, Murugan S, Kumaravel T. Cytotoxicity evaluation of CELNORM, a nutritional health supplement, on MCF7 breast cancer cells. Indian J. Sci. Technol, 2020; 13: 3070-3075.
35. Ren G, Sha T, Guo J, Li W, Lu J, Chen X. Cucurbitacin B induces DNA damage and autophagy mediated by reactive oxygen species (ROS) in MCF-7 breast cancer cells. J. Nat. Med., 2015; 69: 522-530.
36. Kaushik U, Aeri V, Mir SR. Cucurbitacins-An insight into medicinal leads from nature. Pharmacogn. Rev., 2015; 9: 12-18.
37. Ramezani M, Hasani M, Ramezani F, Abdolmaleki MK. Cucurbitacins: A focus on Cucurbitacin E as a natural product and their biological activities. Pharm. Sci., 2020; 27: 1-13.
38. Omokhua-Uyi AG, Van Staden J. Phytomedicinal relevance of South African Cucurbitaceae species and their safety assessment: A review. J. Ethnopharmacol, 2020; 259: 112967.
39. Pan L, Yong Y, Deng Y, Lantvit DD, Ninh TN, Chai H, Carcache de Blanco EJ, Soejarto DD, Swanson SM, Kinghorn AD. Isolation, structure elucidation, and biological evaluation of 16, 23-epoxycucurbitacin constituents from Eleaocarpus chinensis. J. Nat. Prod., 2012; 75: 444-52.
40. Nobre CP, Raffin FN, Moura TF. Standardization of extract from Monordica charantia L (Cicurbitaceae) by total flavonoids content determination. Acta farm Bonaerense, 2005; 24: 562-566.
41. Siciliano T, Tomaamsi ND, Morelli I, Braca A. Study of flavonoids of Sechium edule (Jacq) Swartz (Cucurbitaceae) different edible organs by liquid chromatography photodiode array mass spectrometry. J. Agri. Food. Chem., 2004; 52: 6510-6515.
42. Mukherjee PK, Singha S, Kar A, Chanda J, Banerji S, Dasgupta B, Haldar PK, Sharma N. Therapeutic importance of Cucurbitaceae: A medicinally important family. J. Ethnopharm, 2002; 282: 114599.
43. Dinan L, Harmatha J, Lafont R. Chromatographic procedures for the isolation of plant steroids. J. Chromato. A., 2001; 935: 105-123.
44. Yakubu J, Sodipo AA, Abubakar SU. Phytochemical profiling, toxicity study and abortifacient activity of seed and whole plant of Momordica charantia Linn. (Cucurbitaceae). Chem Rev. Lett., 2022; 5: 200-2006.

45. Salehi B, Sharifi-Rad J, Capanoglu E, Adrar N, Catalkaya G, Shaheen S, Jaffer M, Giri L, Suyal R, Jugran AK, Calina D, Docea AO, Kamiloglu S, Kregiel D, Antolok H, Pawlikowska E, Sen S, Acharya K, Bashiry M, Selamoglu Z, Martorell M, Sharopov F, Martins N, Namiesnik J, Cho WC. Cucurbita plants: from farm to industry. Appl. Sci-Basel., 2019; 9: 21.
46. Jacobs H, Singh T. Isolation and ^{13}C NMR assignments of cucurbitacin's from Cayaponia angustiloba, Cayaponia racemosa and Gurania subumbellata. J. Nat. Prod., 1990; 53: 1600-1605.
47. Velde VV, Lavie D. ^{13}C NMR Spectroscopy of Cucurbitacin. Tetrahedron, 1983; 39: 317-321.
48. Attar UA, Ghane SG. Optimized extraction of anti-cancer compound-Cucurbitacin I and LC-MS identification of major metabolites from wild bottle gourd (*Lagenaria siceraria* Molina Standl.). S. Afr. J. Botany, 2018; 119: 181-187.
49. Chaves DC, Assuncao JCC, Braz-Filho R, Lemos TLG, Monte FJQ. Cucubitacins from Cyaponia racemose: Isolation and total assignment of ^{1}H and ^{13}C NMR spectra. Magn. Reson. Chem., 2007; 45: 389-392.
50. Ul Haq F, Ali A, Khan MN, Shah SMZ, Kandel RC, Aziz N, Adhikari A, Choudhary MI, Rahman AU, El-Seedi HR, Musharraf SG. Metabolite profiling and quantitation of cucurbitacins in Cucurbitaceae plants by liquid chromatography coupled to tandem mass spectrometry. Sci. Rep., 2019; 9: 1-11.
51. Vichai V, Kirtikara K. Sulfo-rhodamin B colorimetric assay for cytotoxicity screening. Nat Protoc, 2006; 1: 1112-1116.
52. Anaya-Eugenio GD, Addo EM, Ezzone N, Henkin M, Ninh TN, Ren Y, Soejarto DD, Kinghorn AD. Car cache de Blanco EJ. Caspase-dependent apoptosis in prostate cancer cells and zebrafish by corchorusoside C from Streptocaulon juventas. J. Nat. Prod., 2019; 82: 1645-1655.