

The safety and tolerability of *Annona muricata* leaf product in people living with cancer: study protocol

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Abstract

Background: *Annona muricata*, also known as graviola, soursop and guanabana is a herbal product used by people living with cancer. Anecdotal clinical evidence suggests this herb has potential anti-cancer activity. There are numerous pre-clinical studies detailing *Annona muricata*'s main bioactive constituents. To date, clinical studies evaluating the efficacy, safety and tolerability are limited. This study aims to investigate the safety and tolerability of *Annona muricata* leaf in people living with advanced malignancy.

Methods: This open label pilot study will involve people living with stage III and IV cancers of any type, who are not undergoing chemotherapeutic treatment. The primary outcome is safety and tolerability of an *Annona muricata* leaf product in this population assessed by participants' self-reporting of adverse effects experienced and the proportion of participants completing the study. Secondary outcomes include biomarkers of inflammation and immune function, disease status and self-reported quality of life scores. Participants (n = 24) will be allocated to one of two groups for 12 weeks. Participants will commence at different timepoints and be assigned to either 530 mg or 1060 mg of *Annona muricata* daily. The study has been approved by the Human Research Ethics Committee Bellberry (2022-04-420) and will be conducted in compliance with the SPIRIT statement.

Discussion: The results of this study will provide safety and tolerability data for clinicians involved in the care of people living with cancer who choose to use *Annona muricata* products as an integrative approach to their healthcare. The results of this study will inform further research about the potential role of *Annona muricata* in cancer.

Trial registration: This study has been registered with the Australian New Zealand Clinical Trials Register (ACTRN 12622001288718)

Background

There has been an increasing global trend of traditional and complementary medicines (T&CM) use by people living with cancer. A systematic review and meta-analysis reporting the prevalence of T&CM use across 18 countries found an average of 40% (n = 65, 000) of people living with cancer had or were currently using T&CM as part of their healthcare [1]. T&CM use in people living with cancer had increased over the last 30 years, particularly since the year 2000 [1]. The reasons for T&CM use by people living with cancer include a perceived beneficial response (38.4%), wanting control (17.3%), a strong belief in T&CM (17.3%), as a last resort (9.6%) and finding hope (9.6%) (n = 20, 656) [2]. In Australia, 65% of people living with cancer (n = 248) reported that they used at least one form of T&CM. Among the T&CM users, 72% had undergone surgery, 75% had been treated with chemotherapy, 57% had received radiotherapy and 31% had received hormone therapy [3]. Given the prevalence of T&CM use by this population as either a complementary, or as an alternative to standard care, this raises important questions about the appropriate and safe use of specific T&CM.

The development of the clinical trial reported in this paper was prompted by a case report [4]. The case involved an Australian woman living with ovarian cancer who integrated a herbal product containing *Annona muricata* into her standard care. Due to the clinically measurable benefits observed, the authors concluded clinical research is required to explore mechanisms of action, effective and tolerable dose, and any adverse effects including potential drug interactions with *Annona muricata* to inform use in the management of ovarian and other cancers, either alone or as part of an integrated treatment approach.

Annona muricata, also known as graviola, soursop and guanabana [5], is prevalently used by people living with cancer in the Caribbean and South American region [6–8]. In Trinidad, of the 150 people surveyed who were living with prostate, breast, and colorectal cancers (n = 150), 80.9% reported they had used *Annona muricata*, and 87% of these people believed that herbal medicines were effective in ‘destroying cancer cells’ [6]. In Jamaica, *Annona muricata* was the most prevalently used (58.8%) herb by people living with cancer who were attending an oncology clinic (n = 100) [7]. Participants in this study identified the reason of using herbal products was due to traditional practices (40%), high availability (33%) and high level of “faith” in its efficacy (25%) [7]. In Peru, *Annona muricata* is also a popular herb used by people living with liver cancer. Of the 88 people surveyed with liver cancer, 14% of participants reported taking *Annona muricata* specifically for the treatment of cancer related symptoms [8]. Although *in vitro* and *in vivo* anti-cancer properties of *Annona muricata* have been comprehensively reviewed [5], human data remains limited.

Annonaceous acetogenins are thought to be the bioactive constituents of *Annona muricata* for inducing cytotoxicity. Annonacin is the most abundant Annonaceous acetogenins in *Annona muricata* leaves [5]. These compounds act by inhibition of mitochondrial complex I, which can inhibit oxidative phosphorylation and adenosine triphosphate (ATP) synthesis [9]. Due to the high ATP demand in cancerous cells, this conveys selectivity against cancerous cells.

The aim of this study is to investigate the safety and tolerability of *Annona muricata* within an Australian healthcare setting in people living with advanced cancer who are not receiving chemotherapy. This study will also explore the impact of treatment with *Annona muricata* on quality of life, disease status and markers of inflammation. This study will provide evidence to guide the design and implementation of a larger randomised controlled trial (RCT) to evaluate the safety and efficacy of *Annona muricata* in people with cancer.

Methods

Study design

This is an open-label pilot study investigating the safety and tolerability of *Annona muricata* leaf product in people with stage III and IV cancers who are either ineligible for, or who have chosen not to undertake chemotherapeutic treatments. Ethics approval has been granted by Human Research Ethics Committee Bellberry (2022-04-420). The ethics approved protocol has been registered with the Australian New

Zealand Clinical Trials Register (ACTRN 12622001288718) and will be conducted in compliance with the SPIRIT statement [10].

Setting and research team

The setting for this study is the Concord Cancer Centre based at Concord Repatriation General Hospital, New South Wales, Australia. The research team includes a hospital oncologist and a clinical pharmacist, who both have extensive experience in herbal medicine cancer clinical trials. The extended research team contribute expertise in the areas of complementary medicines, pharmacology, pharmacy and phytochemistry. Participants will be closely monitored by the clinical trial oncologist.

Investigational product

The study medication selected for this study is a commercially available product listed with The United States Food and Drug Administration (FDA) as Graviola (brand name Swanson Inc.). This product is manufactured in Layton, UST, Inc., a Good Manufacturing Practice (GMP) certified company. The research team have also conducted an independent analysis for quality and safety of the product. This included testing by an Australian National Association of Testing Authorities (NATA) approved laboratory (the Australian Laboratory Services, Smithfield, NSW, Australia) for microbial contaminants prior to use. The trial herbal medicine product was also analysed by the research team for contamination with heavy metals. In addition, quantification of annonacin, one of the most abundant and active compounds was conducted. Cell-line studies were also conducted prior to the study commencement for analysing its cytotoxicity in various cancer cell-lines.

Study Objectives

Primary outcome

The primary outcome of this study will be the safety and tolerability of the *Annona muricata* product. The primary outcome will be measured by participants self-reporting of adverse effects experienced and the proportion of participants completing the study. In addition, participants' safety measurements including carcinoembryonic antigen (CEA), full blood count, liver and renal function will be evaluated. Participants' cognitive function will also be assessed by conducting the standardised mini-mental state examination (SMMSE) and standard neurological assessment to ensure no neurotoxic effect is experienced.

Secondary outcomes

The secondary outcomes are markers of inflammation, immune function, quality of life and disease status. Inflammatory markers including serum neutrophil-lymphocyte ratio, C-reactive protein and albumin will be measured. These inflammatory markers are associated with cancer prognosis [11–13]. Immune function changes will be measured using flow cytometry to assess changes in CD3, CD4, CD8, CD16/56, CD19 and lymphocyte helper:suppressor ratio. These white blood cells are indirect markers of tumour activity [14]. The ratio of T-cells subsets (CD4 and CD8) and natural killer cells (CD16/56) indicates a pro- or anti-tumour effect [14].

Quality of life of participants will be assessed by conducting the Functional Assessment of Cancer Therapy – General (FACT-G) version 4 and Functional Assessment of Chronic Illness Therapy – Fatigue (FACIT-Fatigue) version 4 questionnaires. Both FACT-G and FACIT-Fatigue have demonstrated reliability and validity and enables the assessment of participants' quality of life in the previous seven days [15, 16]. The 28-question FACT-G questionnaire comprehensively evaluates the quality of life of participants evaluating physical, social/family, emotional and functional well-being aspects. Each FACT-G questionnaire is accompanied by a set of additional questions specific to a cancer type. Depending on participants' cancer types, they will be provided with the relevant additional questions specific to their cancer [17]. The 16-question FACIT-Fatigue questionnaire has also been utilised by previous herbal-cancer study as part of quality of life assessment [18].

Disease status will be measured by changes in appropriate biomarkers during standard care such as carcinoembryonic antigen (CEA) and imaging.

Study procedures

Participants and recruitment

Participant recruitment will be conducted at the Concord Cancer Centre at Concord Repatriation General Hospital by a medical oncologist and clinical trials team. Potential participants meeting the eligibility criteria will be approached by a member of the research team to participate in this clinical trial. The target sample size is 24 participants across two dosing levels.

Eligibility

Inclusion criteria include the diagnosis of metastatic or recurrent cancer at stage III or IV; willing and able to undertake the trial treatment and clinical follow-up; aged ≥ 18 years of age; able to understand the English language to give consent and complete questionnaires. Patients who are clinically stable after central nervous system and/or cranial radiation will be included. This is supported by a lack of evidence of progression by CT scan or MRI or on clinical grounds.

Exclusion criteria include participants who are actively undergoing (or intend to receive) chemotherapeutic and/or radiotherapeutic treatments; pregnant or breastfeeding; experience dysphagia; Eastern Cooperative Oncology Group (ECOG) performance status ≥ 3 ; life expectancy status ≤ 12 weeks; full blood counts with white blood cells $< 3.0 \times 10^9/L$, absolute neutrophil count $< 1.5 \times 10^9/L$, platelets $< 100 \times 10^9/dL$; inadequate hepatic (either aspartate transaminase: ALT $> 2.5 \times$ upper limit of normal (ULN), or $> 5 \times$ ULN for liver metastatic patient or bilirubin $> 1.5 \times$ ULN) and/or renal function (< 50 mL/min/1.73 m²); mucositis (disrupted gastrointestinal integrity); grade 2 or higher peripheral neuropathy toxicity; diagnosis of any major psychiatric or neurodegenerative disorders such as Parkinson's disease or dementia; other major serious medical illness such as poorly controlled diabetes or cardiovascular disease. Participants will also be excluded if they are concurrently taking other T&CM and unable to cease these medicines two weeks before the trial, already enrolled in other cancer treatment trials, plan to undergo major surgery during study, documented allergy to study treatment.

Intervention

Participants (n = 24) will be allocated to one of two treatment groups, with each group allocated to receive a different dose and commence at different time points as illustrated in Table 1 and Fig. 1. Following the initial 3-week period, each group will be evaluated for safety and tolerability of the allocated dose before continuing for a further three weeks. The trial will run for a total period of 12 weeks for each group.

The rolling recruitment and three weekly assessments will allow for each dose to be evaluated for safety and tolerability prior to commencing the subsequent groups onto a higher dose.

Table 1
Dosage regimen of *Annona muricata*

Group	1	2
n	12	12
Dosage regimen	1 capsule daily	1 capsule twice daily
Total daily dose	530 mg	1060 mg

Dose regimen justification

The proposed dose regimen in this study is based on the available information from clinical and animal studies. A previous clinical trial conducted in people with type II diabetes (n = 30) utilised the ethanolic leaf extract of *Annona muricata* in a daily dose range of 180 to 540 mg. Mild adverse effects were experienced by participants including mild epigastric burn (n = 2) and nausea (n = 4) [19]. Another study conducted in people with colorectal cancer (n = 30) employed a daily dose of 300 mg ethanolic leaf extract of *Annona muricata*. Adverse effects were reported to be experienced by one participant in this study included anal pain [20].

In toxicological studies, the minimum oral dosage of the ethanolic *Annona muricata* leaf extract to achieve toxicity in mice was 1000 mg/kg [21]. The calculated oral LD₅₀ of the ethanolic leaf extract in mice is 1670 mg/kg [22]. Our previous systematic review also suggests a favourable safety and tolerability profile in clinical use [21].

The current study utilises an *Annona muricata* daily dose range from 530 to 1060 mg. The minimum dose (530 mg) is based on the evidence obtained from a previous clinical trial [19]. The incremental increases in doses for each group is to establish safety and tolerability at the lower dose. The highest dose (1060 mg) is based on a case study [4]. The initial dose utilized in the current study is below the human equivalent dose of rat (12.2 mg/kg) [23, 24]. Participants will be closely monitored at 3-week intervals to assess the safety profile of the herb at specified doses.

Demographic and related medical data collection

Demographic and medical information including age, gender, ethnicity, educational level, medical history, cancer diagnosis, cancer stage and previous cancer treatment will be collected at baseline. Concurrent medication, blood glucose, blood pressure, CEA, full blood count, serum creatinine, liver function including gamma glutamyl transferase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin levels will be recorded at baseline, weeks 3, 6, 9 and week 12. Adherence of *Annona muricata* capsules will be assessed by participant dosing diary and by counting returned medication as compared to the prescribed dose at the week 6 and week 12. Additional 5 mL of plasma samples will be collected through routine blood sampling and stored frozen for later analyses. These include evaluation of annonacin metabolite and validation of annonacin detection in the blood sample.

Data collection

Samples and data will be collected during visits at baseline, weeks 3, 6, 9 and 12 according to the schedule presented in Table 2. Participants will be requested to keep a participant dosing diary to record any symptoms experienced. One standard tube of blood collected from participants will be frozen for later analysis, including evaluation of annonacin metabolite and validation of annonacin detection in blood sample.

Table 2 Timeline of dosage regimen and data collection

Time point	Screening	Baseline	Week 3	Week 6	Week 9	Week 12
Self-report symptom diary	Daily- Throughout the study					
Questionnaires (FACT-G, FACIT-fatigue and SMMSE)		X	X	X	X	X
Blood tests (serum neutrophil-lymphocyte ratio, C-reactive protein, albumin, CD3, CD4, CD8, CD16/56 lymphocyte helper: suppressor ratio, serum creatinine, liver function, full blood count, CEA)		X	X	X	X	X
Blood tests (serum creatinine, liver function, pregnancy, full blood count, fasting blood glucose)	X					
Adherence				X		X
Blood glucose and blood pressure	X	X	X	X	X	X
Demographic and other medical information		X				
Standard neurological assessment	X	X	X	X	X	X

*Tests included in standard care and results data will be analysed

Data Analysis and data presentation

Potential adverse reactions reported by participants will be assessed by employing the Naranjo algorithm to assess causality (definite, probable, possible, doubtful) [25]. Adverse drug reactions will be graded using the National Cancer Institute Common Terminology Criteria for Adverse Events V5 [26].

Data integrity will be maintained by regularly reviewing the data for missing data and errors and any inconsistencies will be resolved. Descriptive statistics will be reported for baseline data including participants' characteristics. For continuous outcome data including serum neutrophil-lymphocyte ration, C-reactive protein, albumin, CD3, CD4, CD16/56, CD19, lymphocyte helper: suppressor ratio, renal and liver function, linear mixed models will be used to examine changes in outcome measures over time. Dose effects will be examined by fitting dosage group as a factor. If there is evidence of an effect of time, post-hoc independent sample t-test will be conducted to compare the mean at follow-up time-points (week 3, 6, 9 and 12) to baseline. If the continuous data is not normally distributed, non-parametric equivalents tests of the above data will be used. For categorical data, proportion and frequencies will be presented in tables. For binary outcomes including whether renal, liver function, full blood count and CEA falls within or out of the reference range will be assessed by mixed-effect logistic regression.

Statistical data including *p*-value, 95% confidence interval will be reported. Statistically significant results are considered to have a *p*-value < 0.05. Data analysis will be conducted using SPSS version 27.0.

Modification of protocol

Any modification of the protocol such as changes in study design will require a formal protocol amendment. These amendments will be discussed among investigators for consensus. Ethical approval will be sought before implementation. Members of the study team, clinicians and participants will be informed of any changes.

Discussion

To date a range of studies have been conducted with *Annona muricata* which has been traditionally used for the management of various disorders such as diabetes, gastric disorders and malaria [5]. The leaf, bark, seeds and fruit of the plant are commonly used [5]. Annonaceous acetogenins are thought to be the bioactive constituents of *Annona muricata*. These compounds are known to inhibit mitochondrial complex I, resulting in inhibition of oxidative phosphorylation and ATP synthesis [27]. Cancer cells require rapid ATP production, therefore interruption of ATP synthesis enables the Annonaceous acetogenins selectivity for cancer cells [27]. Promising anticancer properties of Annonaceous acetogenins and *Annona muricata* have also been demonstrated in *in vitro* and *in vivo* studies [9]. In recent years, *Annona muricata* leaf product has become available in retail outlets and online. These products are purported for their 'free radical scavenging' activity, 'supporting healthy cell growth and function' and 'encouraging a healthy immune system'. With emerging evidence such as case reports and preclinical studies demonstrating promising outcomes in cancer management [9, 28], a larger study evaluating the safety and tolerability of *Annona muricata* leaf extract is warranted.

A RCT utilising *Annona muricata* leaf extract was previously conducted in colorectal cancer patients (n = 30) [29]. This trial utilised the Zirzak Orac brand at a daily dose of 300 mg for eight weeks. No cases of mortality or significant adverse effects were reported in this study [29]. An important distinction between previous studies and the current protocol is the baseline dose and incremental increase in dosage design that aims to achieve the highest tolerable dose. The use of a commercially available product, and the inclusion of people living with a range of cancers provides a broader evaluation that may result in more generalisable clinical implications. This study also excludes participants who are currently on chemotherapeutic agents or taking other T&CM, to avoid confounding factors.

Another important distinction between previous studies and this study is the pretesting of the product quality and safety. The product is manufactured according to the GMP and the safety is monitored by the FDA at post-marketing stage [30]. However, to further ensure the quality and safety of this product, an independent quality and safety analysis is built into the study design. Pretesting includes quantification of annonacin by employing thin layer chromatography, high performance liquid chromatography, liquid chromatography-mass spectrometry, nuclear magnetic resonance and the determination of the presence of heavy metal contaminants using inductively coupled plasma mass spectrometry. The services of a NATA approved laboratory were engaged to conduct screening for bacterial contamination.

In regard to known adverse effects, previous studies have reported *Annona muricata* leaf extract induced nausea, epigastric burn and anal pain [19, 20]. In addition, one review identified *Annona muricata* leaf extract to be associated with potential neurotoxic effects caused by Annonaceous acetogenins [21]. It is estimated that the daily consumption of one fruit or can of nectar for one year is comparable to intravenously infusing a rat with annonacin for 28 days at 3.8 mg/kg/day to cause widespread neurodegeneration (106 mg/kg) [31]. Annonacin was quantified in this product to contain an estimate of 2.42 ± 0.10 mg/g. The maximum total daily annonacin intake will be 2.66 ± 0.12 mg (Data not published). Considering the low bioavailability ($F = 3.2 \pm 0.3\%$) [32] and low blood brain barrier penetration [33] in preclinical studies, the dosage, formulation and duration of the current study is unlikely to cause any neurotoxic effects in participants. To further ensure safety within this present study, participants with neurodegenerative disorders will be excluded. The same review also identified the potential hypotensive and hypoglycemic effects of *Annona muricata* leaf extract in animal studies [21]. Therefore, participants' blood pressure and blood glucose level will be monitored.

Conclusion

The study is a phase 1 open label trial to assess the safety and tolerability of *Annona muricata* leaf extract in cancer patients. The results of this study will inform clinicians and people living with cancer about the safety and tolerability of *Annona muricata* leaf extract. Future research may include performing larger randomised trials based on the results of this study.

Abbreviations

T&CM: Traditional and complementary medicines; ATP: Adenosine triphosphate; RCT: randomised controlled trial; FDA: Food and Drug Administration; GMP: Good Manufacturing Practice; NATA: National Association of Testing Authorities; CEA: carcinoembryonic antigen; SMMSE: Standardised mini-mental state examination; FACT-G: Functional Assessment of Cancer Therapy – General; FACIT-Fatigue: Functional Assessment of Chronic Illness Therapy – Fatigue; ECOG: Eastern Cooperative Oncology Group; ULT: upper limit of normal; GGT: Gamma glutamyl transferase; ALT: Alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase

Declarations

Ethics approval and consent to participate: Ethics approval has been granted by Human Research Ethics Committee Bellberry (2022-04-420). All methods will be performed in compliance with Good Clinical Practice (GCP) and in accordance with the guidelines of the Australian National Health and Medical Research Council and Declaration of Helsinki (as revised in 2004). Australian New Zealand Clinical Trials Register (ACTRN 12622001288718). Informed consent will be obtained from all subjects and/or their legal guardian(s).

Consent for publication: Not applicable

Availability of data and materials: Not applicable

Competing interests: The authors declare that they have no competing interests

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References

1. Horneber M, Bueschel G, Dennert G, Less D, Ritter E, Zwahlen M. How many cancer patients use complementary and alternative medicine: a systematic review and metaanalysis. *Integr Cancer Ther* 2012;11:187-203. <https://doi.org/10.1177/1534735411423920>
2. Verhoef MJ, Balneaves LG, Boon HS, Vroegindewey A. Reasons for and characteristics associated with complementary and alternative medicine use among adult cancer patients: a systematic review. *Integr Cancer Ther* 2005; 4:274-86. <https://doi.org/10.1177/1534735405282361>.
3. Oh B, Butow P, Mullan B, Beale P, Pavlakis N, Rosenthal D, Clarke S. The use and perceived benefits resulting from the use of complementary and alternative medicine by cancer patients in Australia. *Asia Pac J Clin Oncol* 2010;6:342-9. <https://doi.org/10.1111/j.1743-7563.2010.01329.x>.

4. Pham TTD, Sayed S, Baele, P. Graviola in metastatic ovarian cancer: A case report of sustained disease stability. *Sci Rep* 2019;2:3-3. <http://dx.doi.org/10.31487/j.COR.2019.06.03>.
5. Coria-Tellez AV, Montalvo-González E, Yahia EM, Obledo-Vázquez EN. *Annona muricata*: A comprehensive review on its traditional medicinal uses, phytochemicals, pharmacological activities, mechanisms of action and toxicity. *Arab J Chem* 2018;11: 662-691. <http://dx.doi.org/10.1016/j.arabjc.2016.01.004>.
6. Clement YN, Mahase V, Jagroop A, Kissoon K, Maharaj A, Mathura P, Quan CM, Ramadhin D, Mohammed C. Herbal remedies and functional foods used by cancer patients attending specialty oncology clinics in Trinidad. *BMC Complement Altern Med* 2016;16:399. <https://doi.org/10.1186/s12906-016-1380-x>.
7. Foster K, Younger N, Aiken W, Brady-West D, Delgoda R. Reliance on medicinal plant therapy among cancer patients in Jamaica. *Cancer Causes Control* 2017;28:1349-1356. <https://doi.org/10.1007/s10552-017-0924-9>.
8. Rojas Rojas T, Bourdy G, Ruiz E, Cerapio J-P, Pineau P, Gardon J, Doimi F, Deparis X, Deharo E, Bertani S. Herbal medicine practices of patients with liver cancer in Peru: a comprehensive study toward integrative cancer management. *Integr Cancer Therapies* 2018;17:52-64. <https://doi.org/10.1177/1534735416681642>.
9. Rady I, Bloch MB, Chamcheu RCN, Banang Mbeumi S, Anwar MR, Mohamed H, Babatunde AS, Kuiate JR, Noubissi FK, El Sayed KA, Whitfield GK, Chamcheu JC. Anticancer Properties of Graviola (*Annona muricata*): A Comprehensive Mechanistic Review *Oxid Med Cell Longev* 2018; (1826170). <https://doi.org/10.1155/2018/1826170>
10. Chan AW, Tetzlaff JM, Gotzsche PC, Altman DG, Mann H, Berlin JA, Dickersin K, Hrobjartsson A, Schulz KF, Parulekar WR, Krleza-Jeric K, Laupacis A, Moher D. SPIRIT 2013 explanation and elaboration: guidance for protocols of clinical trials. *Bmj* 2013;346:e7586. <https://doi.org/10.1136/bmj.e7586>.
11. Templeton AJ, McNamara MG, Šeruga B, Vera-Badillo FE, Aneja P, Ocaña A, Leibowitz-Amit R, Sonpavde G, Knox JJ, Tran B. Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic review and meta-analysis. *J Natl Cancer Inst* 2014;106. <https://doi.org/10.1093/jnci/dju124>.
12. Allin KH, Nordestgaard BG. Elevated C-reactive protein in the diagnosis, prognosis, and cause of cancer. *Crit Rev Clin Lab Sci* 2011;48:155-70. <https://doi.org/10.3109/10408363.2011.599831>.
13. Espinosa E, Feliu J, Zamora P, Gonzalez Baron M, Sanchez JJ, Ordon ez A, Espinosa J. Serum albumin and other prognostic factors related to response and survival in patients with advanced non-small cell lung cancer. *Lung Cancer* 1995;12:67-76. [https://doi.org/10.1016/0169-5002\(95\)00407-r](https://doi.org/10.1016/0169-5002(95)00407-r).
14. Ostrand-Rosenberg S. Immune surveillance: a balance between protumor and antitumor immunity. *Curr Opin Genet Dev* 2008;18:11-8. <https://doi.org/10.1016/j.gde.2007.12.007>.
15. Cella DF, Tulsky DS, Gray G, Sarafian B, Linn E, Bonomi A, Silberman M, Yellen SB, Winicour P, Brannon J. The Functional Assessment of Cancer Therapy scale: development and validation of the general measure. *J Clin Oncol* 1993;11:570-579. <https://doi.org/10.1200/JCO.1993.11.3.570>.

16. Salsman JM, Beaumont JL, Wortman K, Yan Y, Friend J, Cella D. Brief versions of the FACIT-fatigue and FAACT subscales for patients with non-small cell lung cancer cachexia. *Supportive Care Cancer* 2015;23:1355-1364. <https://doi.org/10.1007/s00520-014-2484-9>.
17. FACIT. FACIT, <https://www.facit.org/FACITOrg/Questionnaires>, 2010 [accessed 10th October 2022].
18. Sekhon BK, Beale P, Vardy JL, Charles KA, Brown RD, Liang HL, Clarke SJ, McLachlan AJ. AL study: traditional Chinese medicine combination *Astragalus membranaceus* and *Ligustrum lucidum* in people with advanced malignancy: research protocol for an open labelled pilot study. *Int J Clin Trials* 2017;4:111-115. <https://dx.doi.org/10.18203/2349-3259.ijct20173614>.
19. Arroyo J, Martínez J, Ronceros G, Palomino R, Villarreal A, Bonilla P, Palomino C, Quino M. Efecto hipoglicemiante coadyuvante del extracto etanólico de hojas de *Annona muricata* L (guanábana), en pacientes con diabetes tipo 2 bajo tratamiento de glibenclamida. *An Fac med* 2009;70.
20. Indrawati L, Purwastyastuti P, Abdullah M, Surono IS, Basir I. Safety of *Annona muricata* extract supplementation for colorectal cancer patients. *Indones J Gastroenterol Hepatol Dig Endosc* 2017;17:170-175.
21. Chan W-JJ, McLachlan AJ, Hanrahan J, Harnett JE. The safety and tolerability of *Annona muricata* leaf extract: A systematic review. *J Pharm Pharmacol* 2019;72:1-16. <https://doi.org/10.1111/jphp.13182>.
22. de Sousa OV, Vieira GD, de Jesus RGdPJ, Yamamoto CH, Alves MS. Antinociceptive and anti-inflammatory activities of the ethanol extract of *Annona muricata* L. leaves in animal models. *Int J Mol Sci* 2010;11:2067-78. <https://doi.org/10.3390/ijms11052067>.
23. Florence NT, Benoit MZ, Jonas K, Alexandra T, Desire DD, Pierre K, Theophile D. Antidiabetic and antioxidant effects of *Annona muricata* (Annonaceae), aqueous extract on streptozotocin-induced diabetic rats. *J Ethnopharmacol* 2014;151:784-90. <https://doi.org/10.1016/j.jep.2013.09.021>.
24. Nair AB, Jacob S. A simple practice guide for dose conversion between animals and human. *J Basic Clin Pharm* 2016;7:27. <https://doi.org/10.4103/0976-0105.177703>.
25. Naranjo CA, Busto U, Sellers EM, Sandor P, Ruiz I, Roberts EA, Janecek E, Domecq C, Greenblatt DJ. A method for estimating the probability of adverse drug reactions. *Clin Pharmacol Ther* 1981;30:239-45. <https://doi.org/10.1038/clpt.1981.154>.
26. National Cancer Institute. Common Terminology Criteria for Adverse Events (Version 5), https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_50; 2017 [accessed 10 October 2022].
27. McLaughlin JL. Paw paw and cancer: annonaceous acetogenins from discovery to commercial products. *J Nat Prod* 2008;71:1311-1321. <https://doi.org/10.1021/np800191t>.
28. Hansra DM, Silva O, Mehta A, Ahn E. Patient with metastatic breast cancer achieves stable disease for 5 years on graviola and xeloda after progressing on multiple lines of therapy. *Adv Breast Cancer Res* 2014;3:84. <https://doi.org/10.4236/abcr.2014.33012>.
29. Surono IS, Lienggonegoro LA, Indrawati L, Wibowo H. Inflammatory response of *Annona Muricata* Linn leaves extract in colorectal cancer patients. *J Glob Pharma Technol* 2017;9:150-157.

30. Food and Drug administration. Dietary Supplement Current Good Manufacturing Practices (CGMPs) and Interim Final Rule (IFR) Facts, <http://wayback.archive-it.org/7993/20170111070358/http://www.fda.gov/Food/GuidanceRegulation/CGMP/ucm110858.htm>; 2017 [accessed 10th October].
31. Champy P, Melot A, Guérineau Eng V, Gleye C, Fall D, Höglinger GU, Ruberg M, Lannuzel A, Laprévotte O, Laurens A, Hocquemiller R. Quantification of acetogenins in *Annona muricata* linked to atypical parkinsonism in guadeloupe. *Mov Disord* 2005;20:1629-33. <https://doi.org/10.1002/mds.20632>.
32. Bonneau N, Schmitz-Afonso I, Brunelle A, Touboul D, Champy P. Method development for quantification of the environmental neurotoxin annonacin in Rat plasma by UPLC-MS/MS and application to a pharmacokinetic study. *J Chromatogr B Analyt Technol Biomed Life Sci* 2015;1004:46-52. <https://doi.org/10.1016/j.jchromb.2015.09.039>.
33. Bonneau N, Schmitz-Afonso I, Brunelle A, Touboul D, Champy P. Quantification of the environmental neurotoxin annonacin in Rat brain by UPLC-MS/MS. *Toxicon* 2016;118:129-33. <https://doi.org/10.1016/j.toxicon.2016.03.015>.

Figures

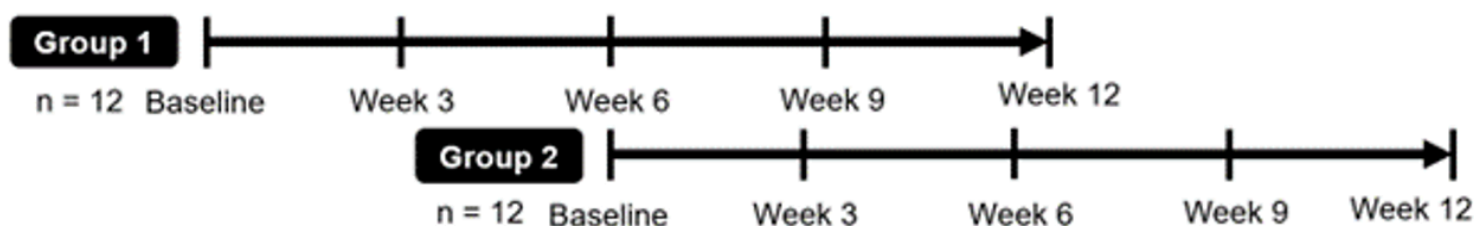


Figure 1

Dosage regimen