Cytotoxic effect of hydroalcoholic extract of Annona Muricata against a human cell line of gastric adenocarcinoma

Efecto citotóxico del extracto hidroalcoholico de la Annona Muricata frente a la línea celular humana de adenocarcinoma gástrico

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ABSTRACT

Background: Therapeutic advances against cancer have not been as successful as expected and have adverse effects that patients rarely tolerate. A study in Peru identified favorable anticancer effects of Annona muricata (AM), a medicinal plant known as soursop, in C-678 mouse gastric adenocarcinoma. However, to date, no results have been reported in human cells. Objective: The objective of this study was to determine the cytotoxic effect of AM extract against a human gastric adenocarcinoma cell line (AGS). Methodology: Experimental in vitro analytical study using a hydroalcoholic extract of AM (AMOH) leaves collected in the Amazonas. Chemical functional groups were identified by phytochemical screening. To obtain the cytotoxic effect, different dilutions of extract were added to the plates containing the cell lines and the data were extrapolated to GraphPad employing an observation card. Finally, the cytotoxic effect was expressed as the half-maximal inhibitory concentration (IC50) and nonlinear regression analysis was performed to determine the growth inhibition of cancer cells. Results: Phytochemical screening showed the presence of reducing carbohydrates, alkaloids, phenols, tannins, triterpenes, steroids, saponins, flavonoids, proteins, cardiac glycosides, and anthocyanins. A calibration curve with gallic acid was used to determine the total phenol content and, quercetin was used to identify the flavonoid content. The AGS cell line showed cytotoxic activity with AMOH with an IC50 at 24 hours of 45.81 µg/mL and 19.05 µg/mL at 48 hours. Conclusion: Several chemical functional groups of AM were identified. In addition, the AMOH showed a cytotoxic effect against the AGS cell line.

Keywords: Cancer; stomach; Annona muricata; cytotoxicity; hydroalcoholic extract
RESUMEN

Antecedente: Los avances terapéuticos frente al cáncer no han tenido el éxito esperado y presentan efectos adversos pocas veces tolerados por el paciente. Un estudio en Perú identificó el efecto anticanerigene de la *Annona muricata* (AM), planta medicinal conocida como guanábana, en adenocarcinoma gástrico de ratón C-678 con resultados favorables, sin embargo, no se ha encontrado evidencia previa en células humanas. **Objetivo:** El objetivo de este estudio fue determinar el efecto citotóxico del extracto de AM frente a la línea celular de adenocarcinoma gástrico humano (AGS). **Materiales y métodos:** Estudio experimental in vitro tipo analítico con extracto hidroalcohólico de hojas de AM (AMOH) recolectadas en Amazonas. Mediante screening fitoquímico se identificaron los grupos funcionales químicos. Para obtener el efecto citotóxico, se añadieron diferentes diluciones de extracto a las placas que contienen las líneas celulares y mediante una ficha de observación los datos fueron extrapolados a GraphPad. Finalmente se expresó como la concentración inhibidora media máxima (IC50) y se hizo un análisis de regresión no lineal con la finalidad de encontrar la cantidad de inhibición de crecimiento de células oncológicas. **Resultados:** En el screening fitoquímico se pudo identificar la presencia de carbohidratos reductores, alcaloides, fenoles, taninos, triterpenos y esteroïdes, saponinas, flavonoides, proteínas, glicósidos cardiotónicos y antocianinas. Para identificar el contenido total de fenoles se utilizó la curva de calibración con ácido gálico el cual nos comprobó la presencia de una buena cantidad de estos metabolitos. Adicionalmente se utilizó quercetina para identificar el contenido de flavonoides, obteniendo resultados favorables ya que se hizo evidente su presencia. La línea celular AGS mostró una actividad citotóxica frente al AMOH con un IC50 a las 24 horas de 45.81ug/mL y 19.05ug/mL a las 48 horas. **Conclusión:** Se identifica a los grupos funcionales de la AM. Además, AMOH demostró un efecto citotóxico contra la línea celular AGS. **Palabras claves:** Cáncer; estómago; *Annona muricata*; citotoxicidad; extracto hidroalcoholico

INTRODUCTION

According to the National Cancer Institute, cancer is one of the leading causes of deaths worldwide (1). The incidence of gastric cancer in Peru is 15.2 per 100,000 inhabitants, being the highest value in all Latin America: Haiti (13.5), Chile (13.0), Colombia (12.8), and Costa Rica (12.8). However, this value is surpassed by Asian countries such as Mongolia (32.5), Japan (31.6), and South Korea (27.9) (2). The 5-year survival of gastric cancer is less than 27%, and it has been estimated that in 2030 it will cause the death of 1.4 million individuals (3).

*Annona Muricata* (AM) is a medicinal plant belonging to the class *Equisetopsida* *C. Agardhy* and subclass *Magnoliidae Novák ex Takht* (4) and is commonly known as soursop (5). It has been studied for its therapeutic potential and the medicinal properties produced by its secondary metabolites synthesized and located in different parts of the plant (6). Among its various bioactive components, annonaceous acetogenins have anti-proliferative effects and, therefore, potential anti-cancer properties (7,8). These components predominate in the leaves and shell; their extract is being used to search for anticancer properties (9).

The objective of cancer treatment is to inhibit the growth of cancer cells. However, some treatments can damage healthy cells leading to adverse effects that can compromise the patient’s life or do not produce the expected results (10,11). Moreover, these adverse effects can cause greater susceptibility to infections, anemia, neuropathies such as numbness and tingling, decreased mental function, and heart damage (12). To date, many articles have been published on medicinal plants to combat this disease and control its adverse effects (13). According to the World Health Organization (WHO), traditional medicine can reduce costs and be accessible for low-income populations (14).

A preclinical study in mice demonstrated the protective effects of the hydroalcoholic extract of AM on the gastric mucosa reducing the ulceration process by activating prostaglandins and reducing aggressive factors (15). In addition, the cytotoxic effect of the ethanolic extract of AM has been studied in the mouse gastric adenocarcinoma cell line C-678, obtaining favorable results (16). However, no previous study has demonstrated this activity in human gastric adenocarcinoma cells.

Despite the wealth of 5,000 different species of plants that can be used in multiple ways to treat various ailments and consumed as therapy even before approaching a health institution (17,18), there are very few studies demonstrating the real utility of these plants.

Taking all the above into account, the main objective of this study was to determine the cytotoxic effect of AM hydroalcoholic extract (AMOH) and determine the chemical functional groups and the half-maximal inhibition concentration (IC_{50}) against a human gastric adenocarcinoma cell line (AGS).
MATERIALS AND METHODS

We performed an analytical, prospective in-vitro experimental study, as part of a pre-clinical trial design including the AGS cell line.

Preparation of the hydroalcoholic extract from AM

The AM leaves were collected in Alto Amazonas, Bagua and identified at the Centro Nacional de Salud Intercultural of Instituto Nacional de Salud (CENSI/INS). Two hundred grams of leaves were weighed in powder, and 1,500 mL of alcohol 70° were added to the sample and allowed to macerate for 14 days. Then the mix was filtered and reduced to 40° with 20 rpm and 70 mbar in a rotary evaporator RV10C (IKA®) to obtain a resin. The data was collected on an observation sheet (Table 1).

Solubility assay

Methanol, ethanol, chloroform, N-hexane, ethyl acetate, and distilled water were added to 50 mg of the dry drug in a proportion of 1 mL to each test tube to determine the solubility of the active components of the sample (19) (Table 2, Figure 1).

Phytochemical screening

Phytochemical screening of the resin of the AMOH was carried out to identify the chemical functional groups (Table 3, Figure 2).

Determination of the total phenolic content in hydroalcoholic extract

Total phenols were determined by the Folin-Ciocalteu method using gallic acid as a reference standard (20). 21.4 mg of the resin was weighed, and a mix of dimethyl sulfoxide (DMSO) and ultrapure water (1:1) was used for resuspension. 100 µL of the sample was taken and diluted in 900 µL of ultrapure water, and the reaction system was performed. The sample was treated three times; 1mL of Folin-Ciocalteau and 0.1 mL of AMOH were added to each sample and set for 5 minutes. Then 1 mL of 7.5% sodium carbonate was added and the sample was placed in a water bath at 45° for 15 minutes. Finally, it was read at 725 nm in a UV/VIS Pharo 300 spectrophotometer (Spectroquant®) with 570 and 600 nm (Figure 4).

Cytotoxic effect

The AMOH dilutions were calculated from a laboratory stock concentration of 32 mg/mL dimethyl sulfoxide (DMSO) stored at -80°C. The human gastric adenocarcinoma cells were counted in the Neubauer chamber (5x103 cells) and placed in a 96-well plate. All the cells were incubated for 12 hours and then 20, 40, 80, and 160 µg/mL of AMOH were added. Morphological changes were microscopically observed and photographed (Nikon Eclipse TI®) after further incubation for 24 and 48 hours. A control group to which 0.5% DMSO was added without the extract was also used. 20 µL of resazurin was added to the wells, and the sample was incubated again for 3 hours to determine cell viability at a concentration of 0.15 mg/mL. Finally, the plate was read by a Synergy LX spectrophotometer (Biotek®) (Figure 5).

Statistical analysis

Data obtained from cellular viability absorbance were extrapolated to Microsoft Excel and expressed in percentages considering the control group. Regarding the cytotoxic effect, the response to the different doses and the IC50 were determined by non-linear regression analysis. Significant differences among values were compared with the one-way ANOVA test with post hoc of Tukey test (p<0.05), using GraphPad Prism 5 Project software (Figure 5).
RESULTS

Table 1. Observation sheet of hydroalcoholic extract of Annona Muricata

| Hydroalcoholic extract of Annona Muricata |         |
|------------------------------------------|---------|
| Initial amount                           | 1500 mL of 70° alcohol and 200 g of AM |
| Final amount                             | 32 gr of resin                           |
| Yield percentage                         | 16%                                             |

Table 2. Results of solubility assay of the resin of Annona Muricata

| Solvent          | Results |
|------------------|---------|
| Methanol         | ++++    |
| Chloroform       | +++     |
| Ethyl acetate    | -       |
| N hexane         | -       |
| Ethanol 70%      | ++      |
| Distilled water  | ++      |

Legend: ++++: very soluble, +++: soluble in medium proportion, +: soluble in low proportion, -: absent

Table 3. Phytochemical screening of fresh leaves of Annona Muricata.

| Metabolite                           | Reactive | Indicator     | Results |
|--------------------------------------|----------|---------------|---------|
| Free amino acids                     | Ninhydrin| Coloration    | (- -)   |
| Carbohydrates (reducing sugar)       | Benedict | Coloration    | (+++    |
|                                      | Fehling  | Coloration    | (++      |
| Alkaloids                            | Dragendorf| Coloration  | (++      |
|                                      | Wagner   | Precipitation | (++      |
| Phenols and Tannins                  | Ferric trichloride | Coloration | (++      |
| Triterpenes and steroids             | Liberman-Buchard | Coloration | (++      |
| Saponins                             | Foam test| Foam         | (++      |
| Quinones                             | Borntrager| Coloration  | (- -)   |
| Flavonoids                           | Shinoda  | Coloration    | (++      |
| Catechins                            | Sodium carbonate | Coloration | (- -)   |
| Proteins                             | Bluret   | Coloration    | (++      |
| Tannins                              | Gelatin  | Precipitation | (++      |
| Cardiotoxic Glycosides               | Kedde    | Coloration    | (++      |
| Anthocyanin                          | Anthocyanin | Coloration | (++      |
| Resin                                | H₂O      | Precipitation | (- -)   |

(++) Positive, (- -) negative

Figure 1. Solubility assay

The results of the phytochemical screening are shown in Table 3 and Figure 2 according to the group of metabolites identified.
The phenolic compound content in AMOH is shown in Table 4, expressed as mg of gallic acid.

Table 4. Total phenolic and flavonoid content

| Flavonoid content (mg/g of flavonoids sample equivalent to quercetin) | Phenolic content (mg/g of polyphenols sample equivalent to gallic acid) |
|--------------------------------------------------------|---------------------------------------------------------------|
| 0.249 mg/g                                              | 1.013 mg/g                                                   |

**Cell morphology**

Changes were observed in both cell morphologies. At 24 hours, at a 20 µg/mL concentration, some cells were suspended while others adhered at 24 hours, becoming more notable with the cells being amorphous and fragmented at higher concentrations of 40, 80, and 160 µg/mL. At 48 hours, the effect was much greater, with fragmented cells being observed at the minimum concentration (20 µg/mL) (Figure 3).

Yellow arrow: suspended cells; Green arrow: adhered cells; and White arrow: dead cells.
Cytotoxic effect

The AGS cell line showed cytotoxic activity to AMOH with an IC₅₀ at 24 hours of 45.81 µg/mL and 19.05 µg/mL at 48 hours (Figure 4). No significant difference was obtained between the cellular control and the response to 20 µg/mL of the extract, with the same being observed for 80 µg/mL versus 160 µg/mL. However, significant differences were observed with the remaining concentrations at 24 hours. At 48 hours, a significant value was obtained between the cellular control and the different concentrations: 20, 40, 80, and 160 µg/mL, with no significant difference among the remaining concentrations (Figure 5).

The Shinoda reaction was used to identify flavonoids showing a positive faint yellow coloration. Flavonoids have antioxidant activity thanks to a combination of their properties that sequester free iron and chelators, protecting against inflammation. In addition, the flavonoid compounds protect against the formation of malignant tumors, reduce the risk of developing heart disease, and have an antiviral and antibacterial effect (22–24). Alkaloids were identified using the Dragendorff reaction showing a brownish-orange precipitate. The pharmacological spectrum of alkaloids was wide, and the pharmacological properties of these compounds include modification of the nervous system and analgesic, anticancer and antimicrobial activities (25-27). The presence of tannins and phenols was confirmed using the reaction of ferric trichloride, presenting a grayish-green color, identifying the presence of adjacent 2-OH. The defining characteristic of tannins is their astringent capacity, and they are commonly used to treat flu symptoms and bronchitis. They also have pharmacological applications as antimicrobials, antifungals, and antidiarrheals and are used as an antidote for heavy metals. On the other hand, phenols have a long list of properties, the main properties being: antioxidant, anti-allergic, anti-tumoral, and antiasthma, among others (28).

**DISCUSSION**

One of the most important and interesting aspects of our study was determining the chemical and functional groups of AM in the cytoplasm of the plant cell.

The absence of free amino acids and catechins, which have several medicinal properties, was of note, despite having been reported in previous studies (41,42). Amino acids have regulatory
functions in health, such as maintaining immune, gastric, neuromuscular, and cognitive protective functions (43). In the case of catechins, these are excellent antioxidants, help in weight control, prevent cardiovascular diseases, and have anticancer activity (44).

On the other hand, reducing carbohydrates’ presence is unclear since some studies have identified them but not in others. This can be explained by the extract studied; for example, methanol extracts show greater solubility of chemical compounds than ethanolic extracts. In our study, these carbohydrates are related to their high presence in the plant (45).

In the present study, AMOH showed potent cytotoxic activity against the AGS cell line confirmed by our negative control (DMSO 10%). The IC₅₀ at 24 hours was 45.81 µg/mL and 19.05 µg/mL at 48 hours, showing greater cytotoxic activity in vitro at 48 hours. The anti-tumor activity of AM has been studied in various cell lines, showing its cytotoxic activity against breast, prostate, liver, endometrial, skin, colorectal cancer, and hematological neoplasms (46). However, the cytotoxic potential against AGS was evidenced for the first time in this study. This finding is important considering that currently, the regions with the highest mortality per 100,000 inhabitants in Peru are Huánuco (21.7); Huancavelica (17.7), and Junín (16.8), which have a high poverty index and less access to health services, and thus, easy access to this plant could be beneficial (47).

Cytotoxic effects of different treatments have been widely studied in different types of cancer and are due to different mechanisms, such as: stimulation of apoptosis through mitochondrial damage, proteins that stop the cell cycle in the G0, G1, and G2 phases, and an increase in the cleavage of caspase-3, with anti-proliferation effects (46). Further studies on the mechanisms inducing cytotoxic effects in gastric cancer are needed.

Additionally, the anti-tumor activity of AMOH can be explained by the total phenol content identified in the present study. In vitro studies have shown that polyphenols have antioxidant effects and can delay or inhibit the oxidation of other molecules making the initiation or propagation of free radical chain reactions impossible and demonstrating their relationship with the anti-proliferative activity of cancer cells (48). Similarly, flavonoids were identified as part of total phenol content using quercetin as a reference, and together these polyphenols enhance the anticancer effects of AMOH.

CONCLUSIONS
AM has chemical functional groups such as flavonoids, reducing carbohydrates, alkaloids, phenols, tannins, triterpenes, steroids, saponins, proteins, cardiotonic glycosides, and anthocyanins with medicinal properties. The cytotoxic effect of AMOH was demonstrated against the AGS cell line with an IC₅₀ of 45.81 µg/mL at 24 hours, achieving the highest effect of 19.05 µg/mL at 48 hours, demonstrating that the effect was maintained. This effect can be explained by metabolites with anticancer properties, mainly phenols, and flavonoids, identified in the present study. Further studies are needed to determine the mechanisms inducing this effect.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS
All the authors discussed the results, contributed to the final manuscript, and assume responsibility for the content of the manuscript.

REFERENCES
National Cancer Institute, Cancer statistics. https://www.cancer.gov/about-cancer/understanding/statistics 2015 (accessed 28 October 2021).

GLOBOCAN, Cancer today. https://gco.iarc.fr/today/about 2020 (accessed 28 October 2021).

National Cancer Institute, SEER Cancer Statistics Review (CSR) 1975-2018. https://seer.cancer.gov/csr/1975_2018/ 2021 (accessed 28 October 2021).

Tropicos, Annona Muricata. https://www.tropicos.org/name/1600001 (accessed 28 October 2021).

Ortiz-Septién G, Campos-Ortiz S. Healing properties of soursop (annonamuricata) leaves and their potential pharma-industrial impact [Grade work]. [Puebla, Mexico]: Universidad Autonoma de Puebla: 2018: 12p.

Gavamukulya Y, Wamunyokoli F, El-Shemy HA. Annona muricata: Is the natural therapy for most disease conditions including cancer growing in our backyard? A systematic review of its research history and future prospects. Asian Pacific Journal of Tropical
Smith RA, Andrews K, Brooks D, et al. Synergistic interactions among flavonoids and acetogenins in Graviola (Annona muricata) leaves confer protection against prostate cancer. Carcinogenesis. 2015;36(6):656–65. DOI: https://doi.org/10.1093/carcin/cbg406

Bermúdez, Alexis, Oliveira-Miranda, María A., Velázquez, Dilia. La investigación etnobotánica sobre plantas medicinales: una revisión de sus objetivos y enfoques actuales. Intercepción [Internet]. 2005 [cited 2021 Oct 28];30(8):453-459. Available from: https://www.redalyc.org/articulo.oa?id=33917030

Rodríguez Lado DC, Sorí Díaz Y, Barceló Tellería JA. Necesidad de un protocolo integrado de Medicina Natural y Tradicional dirigido al paciente oncológico. Medicentro Electrónica [Internet]. 2019 Mar [cited 2021 Oct 28];23(1):75–8. Available from: http://scielo.sld.cu/scielo.php?script=sci_arttext&amp;pid=S1029-2800;41(4):18–27. Available from: https://www.redalyc.org/articulo.oa?id=57916060003

Armaz Velasco F, Calderay Domínguez M, Córdoba Largo S, Crespo Massieu C, Fuentes Castro P, González Martín A, et al. Cáncer de mama. Temas actuales, Ergon, Madrid; 2008. 240p

Lorraine SA, Alberto M-EJ. Medicinal plants as potential agents against cancer, relevance for Mexico. Revista Mexicana de Ciencias Farmaceuticas [Internet]. 2010 [cited 2021 Oct 28];41(4):18-27. Available from: https://www.redalyc.org/articulo.oa?id=57916060003

Guance SH, Marino L, Isern DM, Coria ID, Irurzun I. Flavonoides: aplicaciones medicinales e industriales. Invenio: Revista de Investigación académica [Internet]. 2019 [cited 2021 Oct 28];(40):11–27. Available from: http://sedici.unlp.edu.ar/bitstream/handle/10915/113738/Documento_completo.pdf-PDFA.pdf?sequence=1&isAllowed=y

Calle EE, Rodríguez C, Jacobs EJ, Almon ML, Chao A, McCullough ML, et al. The American Cancer Society Cancer Prevention Study II Nutrition Cohort: rationale, study design, and baseline characteristics. Cancer. 2002 Jan 15;94(2):500–511. DOI: https://doi.org/10.1002/cncr.10197

Rivero N, Gomez M, Medina J. Search for Bioactive Alkaloids in Hymenocallis Species: Pharmaceutical Biology. 2004;42:4-5. DOI: https://doi.org/10.1080/13880200490511774

Gonzalez Chavarro, C. F., Cabezas Gutiérrez, M., Pulido Blanco, V. C., & Celis Ruiz, X. M. Amaryllidaceae: Potential Source of Alkaloids. Biological and Pharmacological Activities. Ciencia y Agricultura [Internet] 2020 [cited 2021 Oct 28];17(3):78–94. Available from: https://doi.org/10.19053/01228420.v17n3.2020.11379

Lozano R. N, Solís Q. L, Bonilla R. P. Sustancias químicas bioactivas de la Peperomia flavamenta. Ciencia e investigación [Internet]. 1998 June [cited 2021 Oct 28];1(1):32-4. Available from: https://revistasinvestigacion.unmsm.edu.pe/index.php/farma/article/view/4749

Marina D, Avela G, Alberto C, García O, Cisneros A. Medición de Fensoles y Actividad Antioxidante en Malezas Usadas para Alimentación Animal. Centro Nacional de Metrología [Internet]. 2008 [cited 2021 Oct 28]; Available from: https://www.semanticscholar.org/paper/Medici%C3%B3n-de-Fensoles-y-Actividad-Antioxidante-en-Marina-Avela/ f41204da2a19ab71374ccf4516bfdaa11267f043c

Colina Ramos AC. Análisis fitoquímico, determinación cualitativa y cuantitativa de flavonoides y lenbeckia hastulata (J. E. Sm) I. M. Johnst "de la zona de Yucay (Cusco). [Grade work]. [Cusco, Perú]: Universidad Nacional Mayor de San Marcos; 2016. 96p.

Jiménez CIE, Martínez EYC, Fonseca JG. Flavonoides y sus acciones antioxidantes. Rev Fac Med UNAM [Internet]. 2008 [cited 2021 Oct 28];32(3):81-91. Available from: https://www.redalyc.org/articulo.oa?id=71709266724

González, Paola Natalia Alvarado Mayor, Alexandra Bautista-Flores, Amanda Asunción Lovera Arellano. 2021. La investigación etnobotánica sobre plantas medicinales: una revisión de sus objetivos y enfoques actuales. Intercepción [Internet]. 2021 [cited 2021 Oct 28];30(8):453-459. Available from: https://www.redalyc.org/articulo.oa?id=33917030

Armaz Velasco F, Calderay Domínguez M, Córdoba Largo S, Crespo Massieu C, Fuentes Castro P, González Martín A, et al. Cáncer de mama. Temas actuales, Ergon, Madrid; 2008. 240p

Lorraine SA, Alberto M-EJ. Medicinal plants as potential agents against cancer, relevance for Mexico. Revista Mexicana de Ciencias Farmaceuticas [Internet]. 2010 [cited 2021 Oct 28];41(4):18-27. Available from: https://www.redalyc.org/articulo.oa?id=57916060003

Smith RA, Andrews K, Brooks D, et al. Cancer screening in the United States, 2016: A review of current American Cancer Society guidelines and current issues in cancer screening. CA Cancer J Clin. 2016;66(2):96-114. DOI: https://doi.org/10.3322/caac.21336

Llopard Carles N. Las plantas medicinales como fuente de compuestos antineoplásicos. [Grade work]. [Madrid, España]: Universidad Complutense: 2016. 25p.

World Health Organization, Estrategia de la OMS sobre medicina tradicional 2014-2023. https://apps.who.int/iris/bitstream/handle/10665/95008/9789243506098_spa.pdf?sequence=1&isAllowed=y

Bento EB, Júnior FE de B, de Oliveira DR, Fernandes CN, de Araújo Delmondes G, Cesário FRAS, et al. Antiulcerogenic activity of the hydroalcoholic extract of leaves of Annona muricata Linnaeus in mice. Saudi Journal of Biological Sciences. 2018;25(4):609–2. DOI: https://doi.org/10.1016/j.sjbs.2016.01.024

Quispe A, Zavala D, Posso M, Rojas J, Vaisberg A. Efecto citotóxico de Annona muricata (guanabana) en cultivo de líneas celulares de adenocarcinoma gástrico y pulmonar. CIMEL. Ciencia e Investigación Médica Estudiantil Latinoamericana [Internet] 2007 [cited 2021 Oct 28];12(1):19–22. Available from: https://www.redalyc.org/pdf/717/71712105.pdf

Egg AB. Diccionario enciclopédico de plantas útiles del Perú. Programa de las Naciones Unidas para el Desarrollo, Texas; 1999. 588p.

Galvez JAM, R EC, R JLM, S SAF. Conocimiento, aceptación y uso de medicina tradicional peruana y de medicina alternativa/complementaria en usuarios de consulta externa en Lima Metropolitana. Revista Peruana de Medicina Integrativa. 2017;2(1):47-57. DOI: http://dx.doi.org/10.26722/rpmi.2017.21.44

Lozano R. N, Solís Q. L, Bonilla R. P. Sustancias químicas bioactivas de la Peperomia flavamenta. Ciencia e investigación [Internet]. 1998 June [cited 2021 Oct 28];1(1):32-4. Available from: https://revistasinvestigacion.unmsm.edu.pe/index.php/farma/article/view/4749

Marina D, Avela G, Alberto C, García O, Cisneros A. Medición de Fensoles y Actividad Antioxidante en Malezas Usadas para Alimentación Animal. Centro Nacional de Metrología [Internet]. 2008 [cited 2021 Oct 28]; Available from: https://www.semanticscholar.org/paper/Medici%C3%B3n-de-Fensoles-y-Actividad-Antioxidante-en-Marina-Avela/ f41204da2a19ab71374ccf4516bfdaa11267f043c

Colina Ramos AC. Análisis fitoquímico, determinación cualitativa y cuantitativa de flavonoides y lenbeckia hastulata (J. E. Sm) I. M. Johnst “de la zona de Yucay (Cusco). [Grade work]. [Cusco, Peru]: Universidad Nacional Mayor de San Marcos; 2016. 96p.

Jiménez CIE, Martínez EYC, Fonseca JG. Flavonoides y sus acciones antioxidantes. Rev Fac Med UNAM [Internet]. 2009 [cited 2021 Oct 28];52(2):73–5. Available from: https://www.medigraphic.com/pdfs/facmed/un-2009/un092g.pdf

Guance SH, Marino L, Isern DM, Coria ID, Irurzun I. Flavonoides: aplicaciones medicinales e industriales. Invenio: Revista de investigación académica [Internet]. 2019 [cited 2021 Oct 28];(40):11–27. Available from: http://sedici.unlp.edu.ar/bitstream/handle/10915/113738/Documento_completo.pdf-PDFA.pdf?sequence=1&isAllowed=y
Benítez R, Ibarz Ribas A, Pagan i Gilabert J. Hidrolizados de proteína: procesos y aplicaciones. Acta bioquímica clínica latinoamericana [Internet]. 2008 [cited 2021 Oct 28];42(2):227-236. Available from: https://repositori.udl.cat/handle/10459.1/49268

SCRIBD, Identificación de Saponinas Marcha. https://es.scribd.com/document/373329382/Identificacion-de-Saponinas-Marcha 2018 (accessed 28 October 2021).

Cardozo Pinzón, J. S., & Gómez Barrera, M. Contribución al estudio fitoquímico del extracto etanólico de las hojas de Kalanchoe daigremontiana Raym. Revista de la Asociacion Colombiana de Ciencias Biologicas [Internet]. 2019 Jan [cited 2021 Oct 28];130: 74–83. Available from: https://www.revistaaccb.org/r/index.php/accb/article/view/159/152

Reyes NM. Análisis de características diferenciales entre antocianinas y betacianinas en extractos de plantas mediante pruebas de color. Ambiocientificas- Revista de divulgación científica [Internet]. 2018 Dec [cited 2021 Oct 28];25(16):38-48. Available from: http://revpub.unileon.es/index.php/ambio/article/view/5754/4433

Poma EM, Requis ER, Gordillo GC, Fuertes CM. Estudio fitoquímico y actividad antiinflamatoria de la Annona muricata L. (guanábana) de Cuzco. Ciencia e investigación divulgación científica [Internet]. 2018 Dec [cited 2021 Oct 28];39(5):350–353. Available from: https://www.redalyc.org/pdf/339/33930879008.pdf

Vit P, Santiago B, Pérez-Pérez EM. Composición química y actividad antioxidante de pulpa, hoja y semilla de guanábana Annona muricata L. Interenciencia [Internet]. 2014 [cited 2021 Oct 28];14(2):29-33. Available from: https://revistasinvestigacion.unmsm.edu.pe/index.php/farma/article/view/3168

Cunha HR, Cantuária P de C, Costa EVM da, Farias ALF, Miranda Vit P, Santiago B, Pérez-Pérez EM. Composición química de Hojas de Kalanchoe daigremontiana Raym. Revista de la Asociacion Colombiana de Ciencias Biologicas [Internet]. 2018 Jan [cited 2021 Oct 28];130: 74–83. Available from: https://www.revistaaccb.org/r/index.php/accb/article/view/159/152

Hoyos Roldán AF, Mora Páez GA, Gómez Barrera M. Análisis fitoquímico preliminar y evaluación de la actividad biológica del extracto etanólico de las semillas de la guanábana (Annona muricata L.). Revista de investigaciones Carmenta [Internet] 2018 [cited 2021 Oct 28]. Available from: https://repositorio.sena.edu.co/handle/11040/6759

Bravo A, Rivera C, Ale D, Huamán M, Muñoz H, Delmás R, et al. OBTENCION DE PLAGUICIDAS NATURALES A PARTIR DE SEMILLAS DE CHIRIMÓRIA (Annona cherimolia Mill.) Y GUANÁBANA (Annona muricata L.). Revista Peruana de Química e Ingeniería Química [Internet]. 2010 Dec 31 [cited 2021 Oct 28];13(2):96–103. Available from: https://revistasinvestigacion.unmsm.edu.pe/index.php/quim/article/view/4714/3796

Dueñas Cely DP. Estudio fitoquímico y evaluación de la actividad citotóxica de un extracto de hojas de Annona muricata (Guanábanab) frente a las líneas celulares MCF-7, 4T1, B16 y 3T3. [Grade work]. [Bogotá, Colombia]: Pontificia Universidad Javeriana: 2019. 56p.

Alegre A, Iannacone J, Carhuapoma M, Alegre A, Iannacone J, Carhuapoma M. TOXICIDAD DEL EXTRACTO ACUOSO, ETÁNOLICO Y HEXÁNICO DE Annona muricata, Minthostachys mollis, Lupinus mutabilis, Y Chenopodium quinoa SOBRE Tetranychus urticae Y Chrysoperla externa. Chilean journal of agricultural & animal sciences [Internet]. 2017 [cited 2021 Oct 28];33(3):273-284. Available from: http://dx.doi.org/10.4067/S0719-389020170050000705

Matos Alejo MM. Evaluación del efecto pediculicida del extracto etánolico de las semillas de Annona muricata L. “guanábana” sobre Pediculus humanus capitis (piojo humano). [Grade work]. [Lima,Perú]: Universidad Nacional Mayor de San Marcos: 2013.

Acosta Vásquez RC, Díaz Peña BJ. Evaluación composicional, capacidad antioxidantes de pulpa y cáscara de la Annona muricata l. (Guanábanab). [Grade work]. [Lambayeque, Perú]: Universidad Nacional de la Amazonía Peruana: 2016. 150p.

Cardona Serrate F. Proteínas y aminoácidos. Propiedades físico-químicas y funcionales. Universitat Politécnica de València [Internet]. 2020 Jun 30 [cited 2021 Oct 28]. Available from: http://hdl.handle.net/10251/147138

Salinero C, Barreiro R, Regueira N, Vela P. TÉ, CATEQUINAS Y SALUD. Sociedad de Ciencia de Galicia [Internet]. 2018 [cited 2021 Oct 28];99:47-56. Available from: http://mol-en.scg.org.es/wp-content/uploads/2019/05/Mol-18.pdf#page=47

Mio Roque VM, Cunyarahe Pangalima D. Formulación y Caracterización de un Filtrante a Partir de las Hojas de Guanábanab (Annona Muricata L.). [Grade work]. [Lima,Perú]: Universidad Nacional Pedro Ruiz Gallo: 2018. 108p.

Alvarado Mayor PN, Bautista Flores A. Propiedades medicinales de la annona muricata: una revisión de la literatura. [Grade work]. [Lima, Perú]: Universidad Científica del Sur: 2021. 21p.

Alvarado Tolentino C, Venegas Ojeda D. Sobrevivencia de pacientes con cáncer gástrico en el Perú, 2009–2010. Rev Panam Salud Publica [Internet]. 2015 [cited 2021 Oct 28];37(3):133–139. Available from: https://www.revistaaccb.org/r/index.php/accb/article/view/5754/4438

Rivas-Morales C, Orandyad-Cádenas MA, Verde-Star MJ. Investigación en plantas de importancia médica [Internet]. OmniaScience Monographs; 2016 [cited 2021 Oct 28]. 452p. Available from: https://www.omniascience.com/books/index.php/monographs/catalog/view/97/409/812-1 DOI: https://doi.org/10.3926/oms.313