December 30, 2020

PharmacologyOnLine

Archives • 2020 • vol.3 • 7-18

ANTIPROLIFERATIVE ACTIVITY OF TOTAL EXTRACTS FROM *ANNONA SQUAMOSA*, *PETIVERIA ALLIACEA* AND *PUNICA GRANATUM* ON CANCER CELL LINES

Gil Carrillo Mónica[1], Mendez Callejas Gina Marcela[2*], Celis Zambrano Crispin Astolfo[1], Vera Bravo Ricardo[1*]

Pontificia Universidad Javeriana, Facultad de Ciencias, Departamento de Química, Carrera 7 #43-82, Bogotá, Colombia

Universidad de Ciencias Aplicadas y Ambientales, Facultad de Medicina, Programa de Medicina, Calle 222 # 55-37, Bogotá, Colombia

gmendez@udca.edu.co, verar@javeriana.edu.co

Abstract

The highest cancer mortality rates in Colombia are associated with prostate, breast, cervix, lung, and colon cancer. The treatments used such as chemotherapy, radiotherapy, and surgery, in some cases are not efficient and cause collateral effects that deteriorate the life quality of the affected people. Currently, in the world, there has been an increase in research directed to the treatment of cancer using secondary metabolites obtained from plants, either as a direct treatment or as a complement to traditional treatments. In the present research work, we determined the cytotoxic activity of total extracts of *Annona squamosa, Petiveria alliacea, and Punica granatum* on breast, lung, cervix, prostate, and colon cancer cell lines. A preliminary phytochemical analysis was carried out on the total extracts obtained from the plants under study to determine the type of molecule associated with the activity. The cytotoxic activity was determined by the Tetrazolium Bromide method (MTT) on the cell lines MDA-MB231 (breast cancer), PC3 (prostate cancer), SiHa (cervical cancer), A549 (lung cancer), and HT29 (colon cancer). As result of the study, it was found that the ethanolic extract of *Annona squamosa seeds* presented the highest cytotoxic activity against the different cancer cell lines. The mean inhibitory concentration of the cell population (IC50) corresponded to 17.54 µg / mL for MDA-MB231; 13.07 µg / mL for PC3; 39,68 µg / mL for A549; 16.09 µg / mL for SiHa, and 19.74 µg / mL for HT29. Additionally, morphological changes were evidenced on the cytoskeleton and mitochondria of the different cancer cells treated with the ethanolic extract of *Annona squamosa* seeds using immunofluorescence microscopy. The results obtained are promising for the development of an adjunctive treatment to traditional treatments for different types of cancer and suggest that these extracts could also have an effect on other types of cancer.

Keywords: Prostate, breast, cervix, lung, and colon cancer, *Annona squamosa, Petiveria alliacea, Punica granatum*

Introduction

Cancer is one of the leading cause of death worldwide according to the World Health Organization (WHO). The most frequent types of cancer that cause the highest death rates are lung, liver, gastric, colorectal, and breast [1]. In Colombia, the International Agency for Research on Cancer (IARC) reported that in 2018, there were 101,893 cases of cancer in the country; this amount includes both sexes and all ages [2]. Breast cancer was the most diagnosed with 13,380 patients (13.1%), followed by prostate cancer with 12,712 cases, corresponding to 12.5% of all cancers presented. Colorectal cancer was the most recurrent in men and women with 9.2% and 8.8% respectively [2].

Treatments for different types of cancer are based on the removal of cancer cells or affected tissue through chemotherapies, surgeries, radiotherapies, hormonal therapies, among others [3]. However, these treatments are not easily accessible to the Colombian population due to the high cost of treatment, in addition to generating side effects in those who suffer from it, deteriorating their quality of life. At present, the use of new natural alternatives using plant extracts as an adjunct to conventional treatment methods for cancer have been implemented. Studies with the *Annona squamosa, Petiveria alliacea,* and *Punica granatum* plants have shown the potential of their extracts against various diseases due to their anti-inflammatory, antioxidant, antiproliferative, antimicrobial properties, among others biological activities [4-8].

Annona squamosa has antiproliferative, antirheumatic, insecticidal properties, among others; It is attributed to its content of terpenes, alkaloids, and glycosides. Its anticancer potential is mainly related to its polyphenol content. Total ethanolic and ethyl acetate extracts of *A. squamosa* showed cytotoxic activity on cell lines of lung cancer (A549), breast cancer (MCF-7), liver cancer (HepG2), and cervical cancer (Hela), at low concentrations [9]. *Punica granatum* is characterized by its antioxidant, antihypertensive, and chemopreventive properties in different tissues, due to its content of phenolic compounds, flavonoids, tannins, glycosides, and fatty acids [10-13]. The inhibitory and antioxidant effect of the ethanolic extract of *P. granatum* seeds has been studied on different cell lines such as renal adenocarcinoma (ACHN), amelanotic melanoma (C32), malignant melanoma (A375), lung carcinoma (COR-I23), hormone-dependent prostate carcinoma (LNCap), breast cancer (MCF-7) and cervical adenocarcinoma (HeLa), obtaining IC50 values of 8.6 µg / mL for LNCap, and 9.6 µg / mL for MCF-7 [13]. *Petiveria alliacea* is a Jamaican plant used in alternative medicine for the treatment of diseases due to its diuretic, antispasmodic, anti-inflammatory, antitumor, and analgesic properties [14]. Likewise, its cytotoxic effect has been highlighted on various cell lines such as K562 for human myeloid erythroleukemia, A375 for human melanoma, 4T1 for murine sinus adenocarcinoma; all at very low concentrations of its ethanolic extract from its leaves and stem [15-17].

In the present work, we evaluated the cytotoxic effect of the polar and apolar extracts of *Annona squamosa, Petiveria alliacea,* and *Punica granatum* on the cell lines MDA-MB231 for breast cancer, PC3 for prostate cancer, HT29 for colon cancer, SiHa of cervical cancer and A549 of lung cancer, using the Tetrazolium Bromide (MTT) method, as potential adjuvant extracts in cancer treatment.

Methods

Samples and extraction: The *Petiveria alliacea* plant and the *Punica granatum* and *Annona squamosal* fruits were acquired at a Square farmer's market in Bogotá. The leaves and stems of *P. alliacea*, peel and seeds of *P. granatum*, and seeds of *A. squamosa* were separated and left to dry for 2 days at room temperature. The dry parts of the plants and the seeds of the fruits were macerated with liquid nitrogen and immediately ground in a ball mill (Biobase, Zhangqiu, China). For *Punica granatum* (peel and seeds), the dry and ground material (50 gr) was macerated with 100 mL of three different solvents: ethanol, chloroform, and hexane (Merck KGaA, Darmstadt, Germany). After 24 hours, the solvent was filtered to remove the solid and the solvent was evaporated in a vacuum chamber

(ProLab, Jalisco Mexico) in order to concentrate the extract [18]. 90 grams of ground material of *A squamosa* seeds was macerated with 200 ml of petroleum ether (Merck KGaA, Darmstadt, Germany) followed by ethanol both for 24 hours. Then the solid material was filtered and concentrated in a rotary evaporator under reduced pressure (Biobase, Zhangqiu, China). 300 grams of powdered stems and leaves of *Petiveria alliacea* were macerated in 1.5 liters of methanol for a period of 3 days. The ethanol extract was evaporated on a rotary evaporator under reduced pressure [18].

Phytochemical analysis: The concentrated extracts were dissolved, and subsequently, the Liebermand-Burchard, Salkowsky, Baljet, Ferric Hydroxamate, Shinoda, Ferric Chloride, Antrona, Draggendorf, and foam tests were performed with the respective reagents to establish the presence of steroids, sterols, terpenes, flavonoids, phenols, glycosides, flavonoids, alkaloids, and saponins [18]. Plate chromatography was also carried out using silica gel as the stationary phase. The samples of the total extracts were run with solvents of different polarities and subsequently developed with vanillinic acid 5% w / v H_2SO_4.

Antiprolifertaive assay: extracts were dissolved in Dimethyl sulfoxide (DMSO) (Sigma Aldrich) for the test of antiproliferative activity. Cultured cells were maintained as follow PC-3 (prostate adenocarcinoma) (ATCC® CRL1435 ™) a in EMEM (Lonza) medium MDA-MB231 (breast cancer), A549 (lung cancer) and SiHa (cervical cancer) in RPMI-1640 (Lonza); and HT29 (colon cancer) in DMEM-High glucose (Lonza), all of them suplemmented with 10% (v/v) Fetal Bovine Serum (Biowest), 2 mM L-glutamine, 5,000 IU/mL penicillin and 5 mg/mL streptomycin (Lonza). Incubation was per-formed at 37 °C and 5% CO_2 [19].

The 3-(4,5-methyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) cell viability assay: Cancer cells were cultured to 80% confluence, then, approx-imately seven thousand cells were seeded per well in a 96-well plate, and were incubated at 37 °C, 5% CO_2 for 24 hours allowing cell adhesion. Extracts treatment concentrations ranged be-tween 10-250 µg/mL, and 0,0625-1,562 µg/mL for the positive control Vincristine, followed for incubation for 48 h. Then, 100 µl of 0.5 mg/mL of the MTT solution (Sigma-Aldrich), dissolved in a medium without phenol red was added per well , later the plate was incubated for 4 h. Formazan crystals were dissolved with 100 µl of DMSO. The results were determined by the optical density (OD) determined by the absorbance at 570 nm in a Microplate Reader (BioRad) [19]. Estimation of the half-maximal inhibitory concentration (IC50), was done using non-linear regression from plotting cell sur-vival (%) versus treatment concentration [µg/mL]. The IC50 values were submitted to a one-way analysis of variance (ANOVA) with post-hoc Tukey and Scheffé. Tests were considered statistically significant at $p < 0.05$. All the experiments were performed in triplicate and in at least two independent replicates, results are presented as mean ± SEM [20]

Detection of morphological changes by immunofluorescence: Approximately seven thousand cells per well were seeded in a 96-well plate and subsequently incubated at 37 °C, 5% CO_2 for 24 hours before treatment. Cells were treated at half of the IC50 determined in the previous MTT analysis and then incubated for another 24 hours. The cells were fixed in methanol (-20 °C), and then permeabilized with a 1:1 PBS/acetone mixture (-20 °C) for 20 seconds. To evaluate the integrity of the microtubules, the monoclonal anti-α-tubulin (Sigma-Aldrich), and the goat anti-mouse Alexa Fluor 488 antibodies were used. (Molecular Probes), both diluted in 5% BSA/TTBS (w/v) (bovine serum albumin/Tris HCl pH 7.5 NaCl, and Tween 20) blocking solution. DNA staining was done with 1.0 mg/mL of DAPI (Invitrogen) [19-20]. Fluorescence was monitored using an epifluorescence microscope (Motic AE31), and the images were captured with MoticCamPro 282A and analyzed using Motic Image plus 2.0 software.

Results and Discussion

After the extraction processes, 9 total extracts were obtained in different polarities that are summarized in table 1. A higher yield was obtained in the ethanol extracts from the peel of *Punica*

granatum with 18.78% and of petroleum ether from the seeds *of Annona squamosa* with 10.80%.

Phytochemical assays: The main groups of molecules identified in the extracts, according to the phytochemical progress, are summarized in table 2. The low presence of sterols was evidenced, except for *P. alliacea*. The presence of terpenes and sesquiterpenes lactones was common for all the extracts and in all the plants, and the presence of flavonoids in the methanol extracts of the plants under study. The presence of alkaloids is not evidenced in the extracts made to the plants.

In figure 1-A, the lilac, violet, and yellow tones, for the extracts of *Annona squamosa* seeds (lines 1 and 2) indicate the presence of triterpenes, sesquiterpenes lactones, and flavonoid glycosides respectively; this corroborates the information obtained by the preliminary phytochemical analysis [22]. Other studies have confirmed the presence of terpenes, phenols, acetogenins, lactones in the seeds as the main constituents of *A. squamosa* extracts, highlighting their antioxidant, and anticancer activity [8, 23]. A greater presence of triterpenes is evidenced (figure 1A) for the extracts of hexane and chloroform from seed (lines 3, and 4) and peel of *P. granatum* (lines 6, and 7), than for the ethanolic extracts of seed and peel of this plant (lines 5 and 8). The separation of compounds is not evidenced for the ethanolic extract of *Petiveria alliacea* (line 9). In the results for *P granatum* peel, figure 1B (lines 6, 7 and 8), violet, pink and brown tones refer to the presence of triterpenes, lactone ring, phenolic compounds, and flavonoids [22]. It is reported that P. *granatum* seeds are rich in steroids, sterols, terpenes, and fatty acids [22], related to antioxidant activity and cancer management due to their efficacy in eliminating free radical oxygen species [7, 24]. Other studies reported that fruit parts of the *P. granatum* have a high content of phenols, glycosides, and terpenes [3, 7]. In line 9, there is the presence of sesquiterpenes lactones in the extract of stems and leaves of *P. alliacea*, recognized by their violet color. The presence of flavonoids, steroids, coumarins, flavonoid glycosides have also been reported and involved in the alteration of metabolic functions of 4T1 breast cancer cells line [17, 25].

Cytotoxic activity: The effect of nine extracts of different polarities on cell viability of five cancer cell lines (MDA-MB231, HT29, A549, SiHa, and PC3) and the non-tumorigenic cell line HEK-293, was evaluated and expressed as the capacity to inhibit 50% of the cell population. The IC50 values were calculated from non-linear regression (Table 3).

The ethanolic seed extract of A. squamosa (SAE) was the most cytotoxic extract, with IC50 values less than 50 µg/mL on the five cancer cell lines, being the lowest value obtained against PC-3 prostate cancer cell line: 13.08 µg/mL. It is noted that the effect on HEK-293 cells was lower, which could indicate that the active compounds present in the SAE extract are more selective for cancer cells possibly terpenes or acetogenins. Another important result obtained with SAE corresponds to the effect on the cell Line SiHa, 16 µg/mL, which is low considering that this cervical cancer cell line is highly resistant to most of the extracts. It is consistent with previous results obtained with extracts containing acetogenins that shown an antiproliferative effect even on cell lines with multi-drug resistance, which is why it has stood out as a possible adjuvant therapy [26]

Previously, the anticancer effect of organic seed extracts from *A. squamosa* was evaluated on the AK-5 mouse cancer cell line, and on MCF-7 breast cancer cell lines, COLO-205 colon cancer, and K-562 of myelogenous erythroleukemia, where the cytotoxic effect was attributed to the acetogenins, considered as one of the most active compounds in the *Annonaceae* family, with which besides, nuclear condensation and DNA fragmentation were evident [27].

The results obtained for *P. granatum* seed extracts showed low cytotoxic activity on evaluated cancer cell lines (Table 3), however, the most active extract was SGE on MDA-MB231 and HT29 with IC50 values of 130,914 µg/mL and 133.77 µg/mL respectively. Previously the cytotoxic effect of *P. granatum's* ethnic seed extract was evaluated on MCF-7 cancer cell line obtaining an IC50 of 320 µg/mL after 24 hours and 63 µg/mL after 72 hours [28].

In relation with the ethanolic-aqueous extract of *Petiveria alliacea* (AE) that shown an IC50 of 38.8

µg/mL on HT-29 colon cancer cells, the activity seems to be relevant, however, on HEK-293 cells the activity is higher, which indicates that active compounds present in AE could cause damage even to non-tumorigenic cells.

Morphological changes: The effect of *Annona squamosa* seed ethanolic extract (SAE) that showed the highest cytotoxicity was analyzed on the cellular integrity of A549, MDA-MB23, PC3, and SiHa cell lines.

The fluorescence microscopy permits to evidence the loss of the integrity of cellular structures due to the activity of compounds present in SAE. The analysis of tubulin (Figure 2A) allowed seeing how this structure involved in cell dynamics and mitosis, loses its integrity after treatment with SAE as with vincristine, which confirms the cytotoxicity established for this extract. The most obvious damage to microtubules is seen in cervical cancer cells SiHa, whereas the vincristine altered mainly A549 lung cancer cells. Nuclear condensation was observed in SiHa and MDA-MB-231, which is related to the induction of an apoptotic process [29].

The treatment with SAE also induced the loss of mitochondria stability evaluated by COX-IV protein expression (Figure 2B). The acetogenins present in SAE may have antiproliferative capacity based on inhibition of the ATP production by the mitochondria, and therefore, the intracellular biological functions such as biological oxidations as well as inhibition of the electron transport system in the mitochondria, which leads to the loss of cell viability, as has been previously described for several plants of the *Annonaceae* family [30].

Conclusions

The most active extract against cancer cell lines evaluated corresponds to the ethanolic extract of Annona squamosa that exhibited IC50 values between 16-40 µg / mL, in which the formation of apoptotic bodies, loss of microtubular dinamics, nuclei condensation, and mitochondrial viability were evidenced.

Acknowledgments

Thanks to MinCiencias-Colombia contract project 407-2020.

References

1. World Health Organization. 2020; https://www.who.int/newsroom/fact-heets/detail/the-top-10-causes-of-death

2. International Agency for Research on Cancer (IARC). (2018), https://gco.iarc.fr/today/data/factsheets/populations/170-colombia-fact-sheets.pdf

3. World Health organization. (2015); https://www.who.int/cancer/treatment/en/

4. Chaturvedi, A. K., Luqman, S., Dubey, V., Thakur, J. P., Saikia, D., Chanotiya, C. S., . . . Negi, A. S. (2013). Inhibition of cathepsin D protease activity by punica granatum fruit peel extracts, isolates, and semisynthetic analogs. Medicinal Chemistry Research, 22(8), 3953-3958.

5. Kim, N. D., Mehta, R., Yu, W., Neeman, I., Livney, T., Amichay, A., Jiang, W. (2002). Chemopreventive and adjuvant therapeutic potential of pomegranate (punica granatum) for human breast cancer. Breast Cancer Research and Treatment, 71(3), 203-217.

6. Vivek, R., Thangam, R., Muthuchelian, K., Gunasekaran, P., Kaveri, K., & Kannan, S. (2012). Green biosynthesis of silver nanoparticles from annona squamosa leaf extract and its in vitro cytotoxic effect on MCF-7 cells. Process Biochemistry, 47(12), 2405-2410.

7. Manoharan, S., Nagar, A., Panjamurthy, K., & Senthil, N. (2007). Modifying effects of annona squamosa on glycoconjugates levels in 7, 12-dimethylbenz (a) anthracene induced hamster buccal pouch carcinogenesis. J.Med.Sci, 7(1), 100-105.

8. Lansky, E. P., & Newman, R. A. (2007). Punica granatum (pomegranate) and its potential for prevention and treatment of inflammation and cancer. Journal of Ethnopharmacology, 109(2), 177-206.

9. Chen, Y., Xu, S., Chen, J., Wang, Y., Xu, H., Fan, N., & Li, X. (2012). Anti-tumor activity of Annona

squamosa seeds extract containing annonaceous acetogenin compounds. Journal of Ethnopharmacology, 142(2), 462-466.

10. Agargun, M.Y., Cartwright, R., REM sleep, dream variables and suicidality in depressed patients. (2003). Psychiatry Res 2003;119:33-39

11. Jadon, G., Nainwani, R., Singh, D., Soni, P. K., & Diwaker, A. (2012). Antioxidant activity of various parts of punica granatum: A review. Journal of Drug Delivery and Therapeutics, 2(6)

12. Adams, L. S., Zhang, Y., Seeram, N. P., Heber, D., & Chen, S. (2010). Pomegranate ellagitannin-derived compounds exhibit antiproliferative and antiaromatase activity in breast cancer cells in vitro. Cancer Prevention Research (Philadelphia, Pa.), 3(1), 108-113.

13. Lucci, P., Pacetti, D., Loizzo, M. R., & Frega, N. G. (2015). Punica granatum cv. dente di cavallo seed ethanolic extract: Antioxidant and antiproliferative activities. *Food Chemistry, 167*, 475-483.

14. Lowe, H. I., Facey, C. O., Toyang, N. J., & Bryant, J. L. (2014). Specific RSK kinase inhibition by dibenzyl trisulfide and implication for therapeutic treatment of cancer. Anticancer Research, 34(4), 1637-1641.

15. Hernández, J. F., Urueña, C. P., Cifuentes, M. C., Sandoval, T. A., Pombo, L. M., Castañeda, D., Fiorentino, S. (2014). A petiveria alliacea standardized fraction induces breast adenocarcinoma cell death by modulating glycolytic metabolism. Journal of Ethnopharmacology, 153(3), 641-649.

16. Chaturvedi, A. K., Luqman, S., Dubey, V., Thakur, J. P., Saikia, D., Chanotiya, C. S., . . . Negi, A. S. (2013). Inhibition of cathepsin D protease activity by punica granatum fruit peel extracts, isolates, and semisynthetic analogs. Medicinal Chemistry Research, 22(8), 3953-3958.

17. Uruena, C., Cifuentes, C., Castaneda, D., Arango, A., Kaur, P., Asea, A., & Fiorentino, S. (2008). Petiveria alliacea extracts uses multiple mechanisms to inhibit growth of human and mouse tumoral cells. BMC Complementary and Alternative Medicine, 8, 60-6882-8-60. doi:10.1186/1472-6882-8-60.

18. Thaís, P., Fosquiera, E., Esmerino, L., Brasil, E., Farago, P., Santos, F., Groppo., F. (2011). Phytochemical screening, antioxidant, and antimicrobial activities of the crude leaves' extract from Ipomoea batatas (L.) Lam. Pharmacogn Mag. 7(26), 165–170.

19. Méndez-Callejas G, Rodríguez-Mayusa J, Riveros-Quiroga M, Mahete-Pinilla K, Torrenegra-Guerrero R. (2017). Antiproliferative activity of chloroformic fractions from leaves and inflorescences of Ageratina gracilis. Emir. J. Food Agric. 29 (1), 59–68.

20. Delgado-Carreño C, Méndez-Callejas G. (2019). Topological properties and in vitro identification of essential nodes of the Paclitaxel and Vincristine interactomes in PC-3 cells. Biomed J. 42(5), 307–16.

21. Wang C, Huang SB, Yang MC, Lin YT, Chu IH, Shen YN. (2015). Combining Paclitaxel with ABT-263 has a synergistic effect on Paclitaxel resistant prostate cancer cells. PloS one, ;10:e0120913. doi:10.1371/journal.pone.0120913.

22. Sabine P. Plant drug analysis: a thin layer chromatography atlas, Springer Science & Business Media. USA. 1996.

23. Yunianto, P., Rosmalawati, S., Rachmawati, I., Suwarso, W. P., & Sumaryono, W. (2012). Isolation and identification of endophytic fungi from srikaya plants (*annona squamosa*) having potential secondary metabolites as anti-breast cancer activity. Microbiology Indonesia, 6(1), 23-29.

24. Jadon, G., Nainwani, R., Singh, D., Soni, P. K., & Diwaker, A. (2012). Antioxidant activity of various parts of punica granatum: A review. Journal of Drug Delivery and Therapeutics, 2(6), 138-141.

25. Hernández, J. F., Urueña, C. P., Cifuentes, M. C., Sandoval, T. A., Pombo, L. M., Castañeda, D., Fiorentino, S. (2014). A petiveria alliacea standardized fraction induces breast adenocarcinoma cell death by modulating glycolytic metabolism. Journal of Ethnopharmacology, 153(3), 641-649.

26. Schlie-Guzmán, M. A., González-Esquinca, A. R., & Luna-Cazáres, L. M. (2009). Las acetogeninas de annonaceae: Efecto antiproliferativo en líneas celulares neoplásicas. Bol Latinoam Caribe Plant Med Aromat, 8, 245-257.

27. Pandey, N., & Barve, D. (2011). Phytochemical and pharmacological review on annona squamosa linn. International Journal of Research in Pharmaceutical and Biomedical Sciences, 2(4), 1404-1412.

28. Kim, N. D., Mehta, R., Yu, W., Neeman, I., Livney, T., Amichay, A., Jiang, W. (2002). Chemopreventive and adjuvant therapeutic potential of pomegranate (punica granatum) for human breast cancer. Breast Cancer Research and Treatment, 71(3), 203-217.

29. Shen, Y., Liu, Q., Sun, H., Li, X., Wang, N., & Guo, H. (2013). Protective effect of augmenter of liver regeneration on vincristine-induced cell death in jurkat T leukemia cells. International Immunopharmacology, 17(2), 162-167.

30. Pandey, N., & Barve, D. (2011). Phytochemical and pharmacological review on annona squamosa linn. International Journal of Research in Pharmaceutical and Biomedical Sciences, 2(4), 1404-1412.

Annona squamosa	**Solven**	**Weight (gr)**
Seeds Extracts	*Petroleum Ether*	9.175
	Ethanolic	1.615
Punica granatum		
Peel Extracts	*Hexane*	*0.133*
	Choloroform	*0.056*
	Ethanolic	*9.390*
Seeds Extracts	*Hexane*	*0.086*
	Choloroform	*0.428*
	Ethanolic	*3.550*
Petiveria alliacea		
Stems and Leaves Extracts	*Ethanolic*	*1.880*

Table 1. Weight of total extracts for the three plants under study

ISSN: 1827-8620

Assays	Metabolites Analyzed	*Annona squamosa* Seeds		*Punica granatum* Peel			*Punica granatum* Seeds			*Petiveria alliacea* Stems and Leaves Extracts
		Petroleum Ether	EtOH	Hexan	*Choloroform*	*EtOH*	Hexan	*Choloroform*	*EtOH*	EtOH
Lieberman-Burchard	Steroids and Sterols	(-)	(-)	(+)	(-)	(-)	(-)	(-)	(+)	(+)
Salwosky	Terpenes	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Baljet	Terpenes and Sterols	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Ferric hydroxamate	Sesquiterpene lactones	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Shinoda	Flavonoids and Phenols	NA	(-)	(+)	(+)	(+)	NA	NA	(-)	(-)
Ferric chloride	Flavonoids and Phenols	NA	(+)	NA	NA	(+)	NA	NA	(+)	(+)
Antrona	flavonoid glycosides and terpenes	NA	(+)	NA	NA	(-)	NA	NA	(+)	(+)
Draggendorf	Alkaloids	NA	(-)	NA	NA	(-)	NA	NA	(-)	(-)
Foam formation	Saponins	NA	(-)	NA	NA	(-)	NA	NA	(-)	(-)

Table 2. Preliminary phytochemical analysis for the plants *Annona squamosal, Punica granatum and Petiveria alliacea*, and their respective extracts.

Extract	IC_{50} (µg/mL) cancer cells					IC_{50} normal cells
	MDA-MB231	PC3	A549	SiHa	HT29	HEK293
SAP	148,413	209,67	224,31	>250	123,57	186,307
SAE	17,540	13,0755	39,683	16,09	19,740	48,3
SGH	176,4039	246,96	>250	>250	146,05	94,342
SGC	209,377	241,93	>250	>250	>250	190,939
SGE	130,914	>250	>250	>250	133,77	162,143
CGH	155,039	113,71	212,84	165,268	134,39	68,241
CGC	183,305	239,448	209,116	157,452	81,056	36,2504
CGE	>250	>250	170,069	>250	172,84	227,882
AE	>250	>250	>250	160,5742	38,791	99,4726

Table 3. Cytotoxic activity expressed as the IC50 values of: SAP extracts from *A. squamosa* seed oil ether extract; SAE from A. squamosa seed ethanol extract; SGH from P granatum seed hexane extract; SGC from P granatum seed chloroform extract; SGE from P granatum seed ethanol extract; CGH from *P. granatum* shell hexane extract. CGC from P. granatum shell chloroform extract; CGE from P. granatum shell ethanol extract; AE from ethanolic-aqueous extract of Petiveria alliacea. ($p = 0.000$; $\alpha=0.05$)

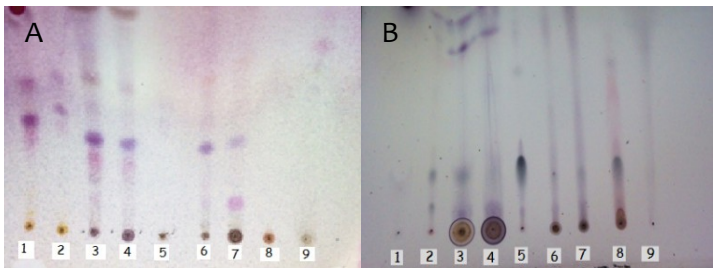

Figure 1. Plate chomatography of seeds of *A. squamosa* (1) Peroleum ether, (2) EtOH, *P. granatum* seeds (3) hexane, (4) Chloroform, (5) EtOH; *P. granatum* Peel (6) Hexane, (7) Chloroform, (8) EtOH; *P. alliacea* Stems and Leaves (9) EtOH. A) Ethil acetate (7:3), B) Chloroform:Ethanol (9:1). Plates were developed with 5% vanillinic acid in H_2SO_4.

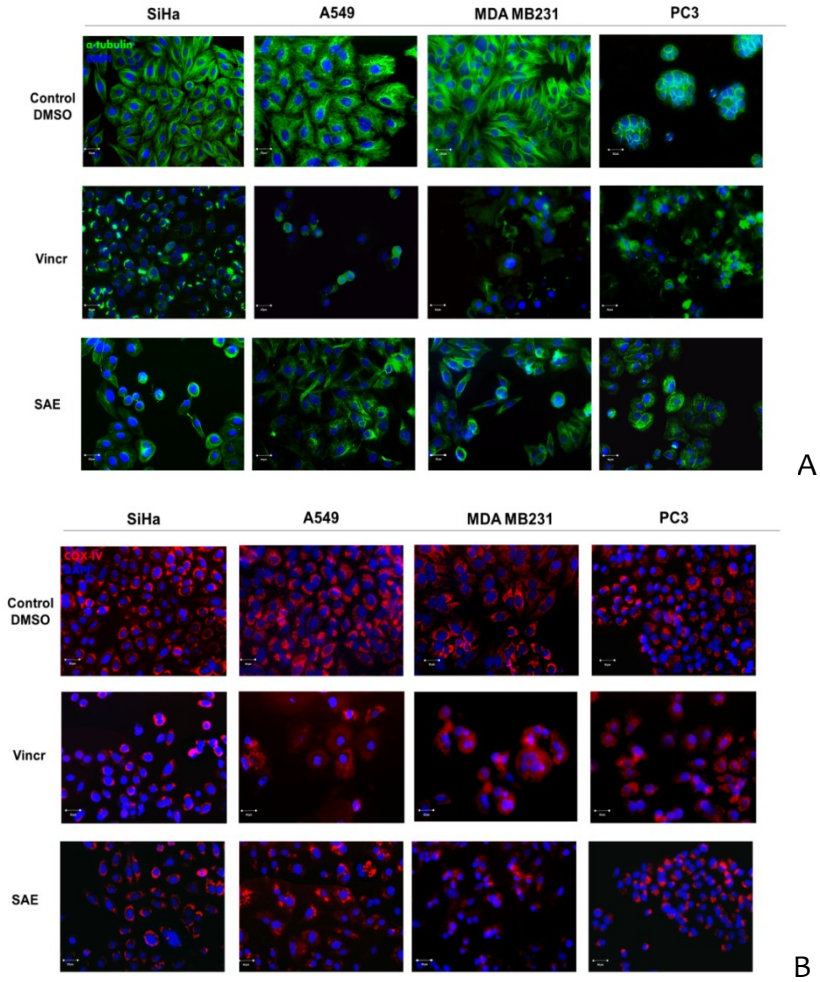

Figure 2. Morphological analysis of nuclei (DAPI), cytoskeleton (anti-alfa tubulin – alexa488) and mitochondria (anti-COX IV – TexasRed) of SiHa, A549, MDA-MB-231 and PC3 cancer cell lines exposed to SAE (ethanolic extract of Annona squamosa seed), DMSO 0.1% as negative control and Vincristine as positive control.