December 30, 2019

PharmacologyOnLine

Archives • 2019 • vol.3 • 224-235

THE ANTITUMOR ACTIVITY IN LYMPHOMA L5178Y IN MICE, ACUTE TOXICITY AND PHYTOCHEMICAL OF *Decatropis bicolor* Zucc. (Radlk)

Almaguer, V.G.[1 2*], Resendiz, E.E.[3], Galnares, N.M.L.[4], Montejano, A.B.V.[1], Becerril, F.M.A.[5], Imbert, P.H.[5], Molina, T.E.M.[5], Balderas, D.C.[6], Bautista, A.M.[1], Montejano, R.J.R.[1]

[1]Pharmacy Area. Autonomous University of the State of Hidalgo. Hidalgo, Mexico.

[2]Higher School of Medicine National Polytechnic Institute

[3]Pharmaceutical Services. Children´s Hospital Teleton of Oncology. Queretaro, Mexico.

[4]Pharmaceutical Services. Angels Chihuahua Hospital. Chihuahua, Mexico.

[5]Medicine Area. Autonomous University of the State of Hidalgo. Hidalgo, Mexico.

[6]Odontology Area. Autonomous University of the State of Hidalgo. Hidalgo, Mexico.

*georginaalmaguervargas@yahoo.com.mx

Abstract

Cancer represents a challenge for all nations in the world because of the high human and economic cost it represents. The study of plants with active secondary metabolites for cancer would allow these patients other therapeutic options that can contribute to the treatment. Within the traditional Hidalguense medicine *D. bicolor* is a plant with anticancer activity that has been used for generations. It is objective of this work to evaluate the antitumor effect of the ethanolic and aqueous extract of *D. bicolor*, as well as performing the qualitative phytochemistry and acute toxicity of both extracts. To achieve this objective, the L5178Y model was used, the positive control was vincristine, the tumour growth and the days of life were valued, colorimetric tests were used for the phytochemistry, and toxicity was carried out using Lorke's oral acute toxicity method. Within the results of the present work, *D. bicolor* provided antitumor activity with both extracts, presenting a better effect with the aqueous extract than with the ethanol extract, with the first one tumour growth was inhibited up to 84% in relation to the control, so this extract increased the days of life by 50% . When the qualitative phytochemistry was made, the metabolites found in both extracts were alkaloids, coumarins, flavonoids and triterpenes. When the toxicity tests were performed, the ethanolic extract was toxic, while no toxicity was found for the aqueous extract. In the present work, the aqueous and ethanolic extracts of *D. bicolor* showed antitumor activity in lymphoma L5178Y. The presence of flavonoids, coumarins, triterpenes and alkaloids were observed, the aqueous extract is not considered toxic.

All components of the manuscript must appear within a single electronic file: references, figure legends and tables must appear in the body of the manuscript.

Keywords: *Decatropis bicolor, cancer, toxicity, phytochemical*

Introduction

Cancer is a group of diseases whose importance lies in its high morbidity and mortality and continues to increase in such a way that in the next 20 years it could increase up to 70%, with highest mortality in poor or middle-income countries[1] and this is related to the lack of resources and infrastructure for prevention, diagnosis and treatment. Many times patients abandon treatments or do not receive them due to economic circumstances[2].

Although it is necessary to bet to prevent this group of diseases, it is also convenient to seek more affordable and safe treatments. In this sense, the WHO proposes to promote the safe and effective use of traditional medicine in order to improve the state of health worldwide[3].

A viable option is the study of plants with active secondary metabolites for cancer, which would allow offering adjuvant alternatives to treatment for patients and at the same time favoring the safe and effective rational use of natural resources traditionally used in developing countries such as Mexico. Among these species is *Decatropis bicolor* Zucc. (Radlk) of the RUTACEAS family, whose common names are aranthó, arandhó[4], gold leaf, among others. The importance of this plant is that its leaflets are sold in markets and tianguis in the state of Hidalgo, Mexico for different types of cancer, skin infections and liver problems, other registered uses are as a pesticide, for rituals[5] and kidney pain[6].

This is a little studied bush between 1.2 to 3 meters high, its leaves are composed with 5 to 10 leaflets, which in the dorsal part are green and in the underside are brown-yellowish, with blooms between February and April[4]. In Hidalgo, it is located in the municipalities of Ixmiquilpan, San Salvador and Tepetitlan[5]. Due to the popular use of this species as an antitumor and the little information about it, in the present work the antineoplastic effect was observed in a murine lymphoma model, as well as its secondary metabolites and acute toxicity in aqueous and ethanolic extracts, since a wider knowledge about this resource could lead to a safer and more effective use by the population.

Methods

Biological material

The plant was collected in Tlacotlapilco municipality of Chilcuautla Hidalgo coordinates 20 ° 22¨26.8 ”N 99 ° 12´37.3” W altitude 1798 msnm, at the beginning of its flowering. An example was deposited in the herbarium of the center of biological research at the Institute of Basic Sciences and engineering of the UAEH with Manuel González Ledesma with voucher number GAV7.

The leaflets were selected by discarding those with other coloration and were dried in the shade. Those were fragmented for the elaboration of the ethanolic and aqueous extracts, first they were left to macerate in 70% ethanol[7] for 7 days at room temperature and the extract was obtained by filtration. This was concentrated to dryness at 70 ° C, the aqueous one was done every day before the administration in the form of decoction following an ethnopharmacological criterion according to the population of the Mezquital Valley, which uses decoction of 1 and 3 leaves in a litter of water[8, 9], which was boiled for 20 minutes[10], subsequently concentrated to dryness. The yield found was 1.1%.

2.2 Antitumor

Were used BALB/c male mice, with an average weight of 22 g. They underwent a 7-day adaptation period, subsequently they were weighed and inoculated intraperitoneally (IP) with approximately 6 x10^6 viable tumour cells of murine lymphoma L5178Y and three days later to assess the effect of *D. bicolor* 6 random batches were formed with at least 5 animals each. The groups were: 1) negative control (vehicle 1 ml/oral gavage OG); 2) positive control (vincristine sulphate 2 mg/m^2 IP)[11]; 3) Ethanolic extract 0.2 mg/kg/every 24hrs OG; 4) Ethanolic extract 0.5 mg mg/every 24hrs/kg; 5) aqueous extract 1 mg/kg/every 24hrs OG; 6) Aqueous extract 2 mg/kg/every 24hrs OG.

For the evaluation of the antitumor effect in this model, the weight (g) gained by the animals 13 days after the inoculation of the tumour cells was considered, taking this as an indicator of tumour growth. Was also evaluated post-inoculation lifetime. Statistical analysis of the data was carried out by the Variance Analysis test (ANOVA) followed by the Tukey multiple comparison test. A value of $p < 0.05$ was considered statistically significant.

Another indicator was the probability of survival by comparing survival curves through the Log-rank test.

Qualitative phytochemical analysis of ethanolic and aqueous extracts

Was performed the preliminary phytochemical screening of *D.bicolor* leaflets from ethanolic and aqueous extracts through qualitative chemical reactions. Were identified secondary metabolites after evaporating the extracts. The tests used for the determination were alkaloids (Dragendorff, Mayer, Wagner), triterpenes and steroids (Liebermann-Burchard, Solkowski, Rosemheim), coumarins (Baljet, Legal, Erlich), Flavonoids (concentrated sulfuric acid, Shinoda), Resins, oils essentials (white paper, sweat IV), reducing sugars (Fehling, Benedic), phenols and tannins (ferric chloride), free amino acids and amines in general (ninhydrin). The results were interpreted as negative (-) or positive (+ little; ++ moderate; +++ abundant)[12, 13].

Toxicity

Because one of the objectives of this work is to promote the safe use of this plant, was performed the evaluation of acute toxicity with the modified Lorke methodology[14].

This study was carried out in 12 male BALB/c mice for each extract, the animals were divided into batches of three individuals and fasted 12 hrs before intragastric vehicle administration, 10, 100, 1000 mg/Kg of live weight (LW) of the ethanolic and aqueous extract of the plant. For this last extract was also administered an additional dosage of 2000 mg/kg. They were kept under observation for 24 hours after the administration of the extracts.

The animals that did not die after 15 days were euthanized with sodium pentobarbital (150 mg/kg IP) for subsequent necropsy.

As for the interpretation of the results this was carried out using the formula $LD50= \sqrt{[(D0*D100)]}$

Where:

D0 = higher dose that does not give mortality

D100= lower dose that produced mortality[15].

The statistical analysis of the data was carried out by the t test. A value of $p < 0.05$ was considered statistically significant.

The zootechnical management and the slaughter of the animals used in this research was carried out in accordance with Mexican NOM-062-ZOO-199916 and according to the guide for the care and use of laboratory animals, of the committee of the national investigation council of EEUU[17].

Results

D. bicolor has antitumor activity

The antitumor effect in murine lymphoma L5178Y produced by the ethanol and aqueous extracts of the leaflets of *D. bicolor* was quantified as the post-transplant weight gain, which is interpreted as ascites tumour growth, so the survival obtained was also quantified.

The results regarding tumour growth showed that while the vehicle reached an average of 18.6 g, the treatment with the positive vincristine control obtained a tumour growth of 4.06 g, which represented 79% less than the tumour size of the vehicle ($p = 0.001$) .

On the other hand, between both extracts: ethanolic and aqueous, the one with the best effect was the latter. Regarding the ethanolic extract, with the dose of 0.5 mg/kg/every 24 hours, the tumour growth was inhibited 57% in relation to the control ($p = 0.01$). Effect surpassed by the aqueous extract since in an interesting way caused a lower development of the tumour in relation to the vehicle, presenting better effect the dose of 1 mg/kg with an inhibition of 83% ($p = 0.0001$). See fig. 1

The figure shows the effect on tumour growth (g) of BALB/c mice obtained after intraperitoneal administration of $6x10^6$ L5178Y lymphoma cells in the vehicle groups, vincristine (2 mg/m^2), of ethanol and aqueous extracts of *D. bicolor* in mouse BALB/c. The vincristine positive control showed lower tumour development than the control group ($p = 0.01$), the ethanol extract had an antitumor effect at a dose of 0.5 mg/kg/every 24hrs. While the aqueous extract was the one that exhibited the best result considering the dose of 1.0 mg /kg/every 24 hrs as the one that obtained greater significance in relation to the control ($p = 0.0001$). Each bar

represents the mean ± ES with n ≥5. The data were analysed by the statistical test of variance analysis (ANOVA) followed by the Tukey multiple comparison test. p values <0.05 were considered statistically significant.

Importantly in this work the commercial chemotherapeutic vincristine was statistically similar to the different treatments with *D. bicolor* shown here.

Regarding the average number of days after the transplant, the treatments administered had an increase in survival compared to the vehicle (p = 0.01), which showed 13 days on average. While with vincristine 25 days were reached, the groups of *D. bicolor* also increased the lifetime reaching and interestingly the dose of 1 mg/kg the aqueous extract was statistically similar to the antineoplastic vincristine. See fig. 2

The figure shows the effect of the vehicle, vincristine (2 mg/m^2) and the ethanol and aqueous extracts of *D. bicolor* in BALB/c mice with L5178Y lymphoma in the lifetime. While the control reached 13 days of survival, with vincristine 25 were obtained, all the groups with *D. bicolor* shown here presented a greater number of days lived than the control and interestingly with the dose of 1 mg/kg of the aqueous survival is statistically similar to that obtained with vincristine. Each bar represents the mean ± ES with n ≥ 5. The data were analysed by the statistical test of variance analysis (ANOVA) followed by the Tukey multiple comparison test. P values <0.05 were considered statistically significant.

After the calculation of days lived, the comparison of logrank survival curves was performed in the treatments with the best-observed effect. See fig 3.

The comparison of survival curves of mice inoculated with 6x10^6 lymphoma cells L5178Y via subcutaneous route in BALB/c mice with vehicle and with extracts of *D. bicolor* is shown. When comparing the curves of the negative control against the ethanol extract (etho) (0.2 mg/kg), it is observed that the vehicle has a median of 16 days, while with etho it is 21 (Radius is 0.76; 95% CI: 0.29 to 1.94), which can be interpreted as that the ethanolic extract is related to a significant increase in survival of 23.80% compared to the vehicle. (P = 0.0001 log rank). Meanwhile the comparison of vehicle curves against the 1 mg/kg aqueous extract shows a significant increase in survival (P = 0.0001 log rank) since this extract has a median of 22 days (/R of 0.72; 95% CI radius : 0.26 to 2), which in turn is associated with a 28% increase in survival compared to the negative control (P = 0.0001). Kaplan-Meier curve. (longrank). n ≥ 5.

That is 0.2 mg/kg of ethanolic extract and 1 mg/kg of aqueous extract against the vehicle, observing a significant increase with both treatments against control (p = 0.0001). This analysis of the curves showed the probability of life over time, for the ethanolic extract a radius (R) of 0.76 (95% CI: 0.29 to 1.94) and for the aqueous R of 0.72 (95% CI radius: 0.26 to 2). Which can be associated with an increase in survival prognosis of 23.8% and 28% respectively compared to the control[18, 19].

Qualitative phytochemistry

To know the main secondary metabolites present in *D. bicolor*, qualitative phytochemical was performed with both extracts and it was observed that both showed the presence of alkaloids, triterpenes, abundant coumarins, and flavonoids. However in the aqueous one, a lower presence of these were found, only positive result was observed for alkaloids with Mayer, for triterpenes in Liebermann-Buchard, in coumarins for Baljet and Erlich and for flavonoids with Shinoda. See Table 1.

The tests were negative for resins, essential oils, phenols and tannins, free amino acids and amines in general.

Toxicity

When carrying out the toxicity of the ethanolic extract, it was observed that from 100 mg/kg the mice presented anxiety, open mouth, ataxia, dyspnea, abdominal breathing, cyanosis, piloerection, abdominal contractions and shrieks during the first 60 minutes. The animals did not die and were kept 15 days under observation, then autopsy and macroscopic observation of the liver and kidney was performed, the kidneys were congested, splenomegaly and weight loss were observed.

When administering the dose of 1000 mg/kg, the mice showed incoordination, tachycardia, apnoea and two mice died at four hours, the third died 24 hours later, and the latter one presented dark stools, dehydration, cyanosis, weight loss. At necropsy were observed renal congestion, hemorrhagic gastric mucosa, dark intestine and concentrated urine.

The LD_{50} for the ethanol extract was 316 mg/kg PV that according to the Globally Harmonized System of Classification and Labeling of Chemicals of the United Nations presents toxicity [20].

With different doses of the aqueous extract a habitual behavior was observed, without weight loss during the following 15 days, at the end of these, the necropsy was performed and there were no apparent changes in heart, liver, spleen and kidneys.

Discussion

Cancer is a global public health problem according to GLOBOCAN. The number of deaths in 2018 was 9, 555, 02721. Developing countries have the greatest impact on mortality, which is why it is important to find more affordable and cheaper alternative therapies, in relation to this it is convenient to mention that the search for antitumor activity in plants is not random, since 60% of the active principles for chemotherapy comes from plants[22]. *D. Bicolor* is used by the Hidalguense population for different types of cancer, in order to collaborate with the knowledge of this species and to favor its effective and safe use by the population. In this work, antitumor activity, qualitative phytochemical and toxicity of two extracts ethanol and aqueous obtained from this plant were studied.

The results indicated that *D. bicolor* had an antitumor effect on lymphoma L5178Y, decreasing the size of the tumour and increasing the lifetime. As far as we know this is the first report of the antitumor effect *in vivo*.

Phytochemistry showed the presence of coumarins, alkaloids, flavonoids and triterpenes. The ethanol extract is considered toxic, however the aqueous extract showed no toxicity up to 2000 mg/kg.

There is a toxicity study of the ethanolic extract in saline artemia obtaining a Lethal $Concentration_{50}$ (LC_{50}) of 565 mg/ml, this same team mentions not having found cytotoxic activity with this same ethanol extract in Hela cells[8]. On the other hand, was not observed this activity with essential oil (EO) in the MCF-10 breast cancer line. However, in MDA-MB-231 cells Estanislao and team report an Inhibitory concentration IC_{50} of 128 g/ml for ethanolic extract, observing the best effect with essential oil with an IC_{50} of 53.81 g/ml, they themselves mention not having found cytotoxicity with the aqueous extract[23]. Finding effect or not in extracts similar to those studied in the present work with different models may be a consequence of the variation in the presence or quantity of secondary metabolites in the plant due to pharmacoergastic factors[24] or by the cell line used[25].

In the present work, the positive vincristine control had the expected effect achieving a tumour inhibition of 79% in relation to the vehicle. On the other hand, when using the highest dose of ethanol extract, the tumour produced was 57% smaller than the vehicle group, the group that presented the best effect was 1.0 mg/kg of the aqueous extract with a tumour inhibition of 83% followed by 65% for double this dose. This last extract was obtained by decoction. This cytotoxic effect found in an aqueous extract obtained with heat has also been found in other species such as *Trichilia hirta*[26] and *Plumbago pulchella* Bois, studied by this investigation team, a plant also used by the Hidalguense population which significantly reduced the tumour size, without increasing life time[11]. Contrary to *P. pulchella* the different extracts of *D. bicolor* in the present work showed an increase in the life time different from the control and in the case of the low dose of the aqueous extract this presents 20.8 days (50%) against 13.8 of the vehicle, resulting Statistically similar to vincristine. Which was 25.75 days.

When analysing the probability of survival of this plant by means of the logrank test, a prognosis higher than the vehicle of 24% was observed for the ethanolic with 95.5 Confidence Interval (CI 0.28-1.94) and 29% (95% CI 0.26-2.0) for the aqueous.

The mechanism of action attributed by the Estanislao team to the essential oil of this plant in MD-MB-231 cells was through apoptosis with the

expression of proteins such as Bax and caspases 3 and 9[23].

The inhibition or stimulation of molecular pathways involved in cancer has been observed with coumarins, flavonoids, triterpenes and alkaloids, secondary metabolites found when performing the phytochemical of both extracts in this work, which coincides with Cortez[8]. The coumarins that have been reported from the aerial part of *D. bicolor* are heracline, seseline, psoralene, imperatorine, and the skimmianine and dictamnine alkaloids[27]. Of these coumarins the soraleno has been involved in breast cancer as a cyclin inhibitor with p21 and regulating p53 and intervening in the PI3 pathway/Akt[28] and the dictaminine alkaloid has been linked to cytotoxicity through mTOR, and MAPK, regulating HIF-1[29].

Within the RUTACEAS family is *Pilocarpus alvaradoii* from which triterpene lupeol[30] has been isolated. Which has been obtained from a hydroalcoholic solution[31], and showed cytotoxic activity in PCNA-1 pancreatic cell carcinoma, causing cell cycle arrest by regulating p21 and p27 and inhibited tumour growth *in vivo* by decreasing phosphorylated Akt and phosphorylated ERK [32].

As in the present work, Cortez reports abundant flavonoids[8] and due to the polarity of the extracts these could be within the group of glycosylates such as flavanones among which is the hesperidine present in the citrus genus of the RUTACEAS family[33]. To which cytotoxicity has been attributed in breast and gastric cancer cell lines involving proteins such as Fas, caspases 3 and 9, reducing BAX and decreasing mitochondrial membrane potential[34]. It is interesting that coumarin, flavonoid, triterpene and alkaloid are involved in cancer molecular pathways found in *D. bicolor*, especially those that have been previously reported as is the case of the coumarin solareno and the alkaloid dictamnine, which when intervening directly on the IP3/Akt/mTOR pathway. They become very important because AKT/PKB is a serine/threonine kinase protein that can be hyperactivated in different types of cancer. This can modulate different targets promoting proliferation, growth and survival through regulating the cell cycle directly with p21 and p27 or modulating levels of cyclin D1 and p53, as well as inhibiting proapoptotic signals such as BAD or FOXO and favoring resistance to treatment[35]. Which turn this route into a striking anti-tumor pharmacological mechanism that has taken great relevance in the creation of such therapies. Such is the case of derivatives of wortamine and others that are still in clinical studies, as well as some that are already in use: everolimus and temsirolimus. However, due to the presence of adverse reactions observed[36, 37] the search continues and turns *D. bicolor* into a striking alternative.

In the ethnopharmacological investigations carried out by Martinez Pérez and team, they mention that the plant is of delicate use[38] and on the other hand, Estanislao mentions that in normal epithelial cells the viability in normal epithelial cells was reduced by 25% by increasing the effective dose 2.84 times by 72 hrs[23].

In this sense, it should be considered that medicinal plants are not exempt from adverse reactions, so it is necessary to seek the safe use of these. One of the objectives of this work was to evaluate the acute toxicity of *D. bicolor* in mice. The results in the toxicity with the ethanolic extract showed it is considered toxic according to the Harmonized Global system20. No toxic signs were observed in the aqueous extract. On the other hand, *D. bicolor* is a plant that does not exist in abundance, so it is recommended to seek its sustainability before its widespread use.

Acknowledgments

Thanks to the students of the degree in Pharmacy of the Autonomous University of the State of Hidalgo for his support for the performance of this work.

The Autonomous University of the State of Hidalgo funded this work.

References

1. Informe mundial sobre el cáncer 2018 [Internet]. OMS. 2018 [cited September 2019]. Available from: https://www.who.int/es/news-room/fact-sheets/detail/cancer
2. Allemani C., Weir H.K., Carreira H., et al ; World Surveillance of Cancer Survival

1995-2009: analysis of individual data from 25,676,887 patients from 279 population registries in 67 countries (CONCORD-2) Working Group. The Lancet 2015; 385(9972): 977-1010,
3. Estrategia de la OMS sobre medicina tradicional 2002-2005 Ginebra. [Internet]. OMS. 2002 [cited September 2019]. Available from: https://apps.who.int/medicinedocs/pdf/s2299s/s2299s.pdf
4. Villavicencio N.M.A., Pérez E.B.E., Ramirez A.A. Listado de las Plantas útiles del estado de Hidalgo. 1 ed. Pachuca: Universidad Autónoma del Estado de Hidalgo; 2003.
5. Sánchez-González A., Granados-Sánchez D., Simón-Nabor R.· Uso medicinal de las plantas por los otomíes del municipio de Nicolás Flores, Hidalgo, México. Revista Chapingo serie horticultura 2008; 14 (3) 271-279.
6. Villavicencio N.M.A., Pérez E.B.E. Plantas Útiles del Estado de Hidalgo. 1 ed. Pachuca: Editor Universidad Autónoma del Estado de Hidalgo. 1995.
7. Arroyo J, Almora Y, Quino M, et al; Efecto protector en cirrosis hepática inducida en ratas del extracto etanólico de las hojas de *Piper aduncum* comparado con silimarina. Anales de la Facultad de Medicina 2012; 73 (2): 85-91.
8. Cortez J. Actividad biológica de extractos de plantas usadas para el tratamiento de cáncer e infecciones en Tepatepec, Hidalgo [licenciatura]. Área académica de Biología, Universidad Autónoma del Estado de Hidalgo; 2005.
9. Villavicencio NMA, Pérez EBE. Plantas medicinales del estado de Hidalgo. 1 ed. Pachuca. Universidad Autónoma del Estado de Hidalgo; 2013.
10. Farmacopea Argentina, [Internet] Argentina 7° ed. Capitulo IV, pag 239 decocción. Disponible en: http://www.anmat.gov.ar/webanmat/fna/flip_pages/Farmacopea_Vol_IV/files/assets/basic-html/page239.html
11. Almaguer G, Villavicencio MA, Pérez BE, Cjheue RA., Gonzalez MG., Figueroa L., Altamirano BDA., Montejano JR. Antitumor effect of the infusion of pañete (*Plumbago pulchella* Boiss.) and its plumbagin metabolite in lymphoma L5178y in misse CD-1. Int J Pharm 2015; 5(1):1-8.
12. Ochoa PA, Escalona AJC, Fechine TJ, et al; Evaluación fitoquímica de hojas de *Excoecaria lucida* Sw. (Aité) (Euphorbiaceae) y Aislamiento e Identificación de un hemiterpenoide. Revista Cubana de Plantas Medicinales 2015; 20(1): 117-130.
13. Martínez N, Almaguer G., Vázquez-Alvarado P. et al; Análisis fitoquímico de *Jatropha dioica* y determinación de su efecto antioxidante y quimioprotector sobre el potencial genotóxico de ciclofosfamida, daunorrubicina y metilmetanosulfonato evaluado mediante el ensayo cometa. Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas 2014; 13 (5): 437-457.
14. Lorke D. A new approach to practical acute toxicity testing. Arch Toxicol 1983. 54:275–87.
15. Chinedu E, Arome DD, Ameh FS. A New Method for determining acute toxicity in animal models. Toxicol Int. 2013; 20(3): 224–226.
16. NOM-062-ZOO-1999 Mexicana. Especificaciones técnicas para la producción uso y cuidado de los animales de laboratorio. [Internet]. Diario oficial de la Federación. 2001 [cited September 2019] Available from: http://publico.senasica.gob.mx/?doc=743
17. Guide for the Care and Use of Laboratory Animals. [Internet]. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. 2011 [cited September 2019] Available from: https://www.ncbi.nlm.nih.gov/books/NBK54050/ doi: 10.17226/12910

18. Arribalzaga BE. Interpretación de las curvas de supervivencia. Revista chilena de cirugía 2007; 59(1): 75-83.
19. Gramatges OA. Aplicación y técnicas del análisis de supervivencia en las investigaciones clínicas. Revista Cubana de Hematología, Inmunología y Hemoterapia 2002; 18(2).
20. Sistema globalmente armonizado de clasificación y etiquetado de productos químicos [Internet]. Naciones Unidas. Nueva York y Ginebra. 2011 [cited September 2019] Available from: https://www.unece.org/fileadmin/DAM/trans/danger/publi/ghs/ghs_rev04/Spanish/ST-SG-AC10-30-Rev4sp.pdf
21. World Health Organization International agency for research on cancer [Internet]. Globocan. 2018 [cited September 2019]. Available from: https://gco.iarc.fr/today/online-analysis-pie?v=2018&mode=cancer&mode_population=continents&population=900&populations=900&key=total&sex=0&cancer=39&type=1&statistic=5&prevalence=0&population_group=0&ages_group%5B%5D=0&ages_group%5B%5D=17&nb_items=7&group_cancer=1&include_nmsc=1&include_nmsc_other=1&half_pie=0&donut=0&population_group_globocan_id=
22. Sultana SP, Asif HM, Nazar HM, *et al*. Medicinal plants combating against cancer--a green anticancer approach. Asian Pacific J Cancer Prevention 2014;15 11: 4385-94.
23. Estanislao G.C.C., Aquino C.A., Pérez I.D.G., *et* al; *Decatropis bicolor* (Zucc.) Radlk essential oil induces apoptosis of the MDA-MB-231 breast cancer cell line. BMC complementary and alternative medicine 2016; 16: 266.
24. Sánchez G.E., León F.M., Chávez F.D. *et al*; Caracterización farmacognóstica de *Melissa officinalis* L. (toronjil). Revista Cubana de Plantas Medicinales 2010; 15(4): 198-208.
25. Ravindranath M.H., Saravanan T.S., Monteclaro C.C., et al; Epicatechins Purified from Green Tea (*Camellia sinensis*) Differentially suppress growth of gender-dependent human cancer cell lines. Evid Based Complement Alternat Med 2006; 3(2): 237-47.
26. Hernández S.E., Mora G.N., Morris Q.H..J, Delgado C.L., Martínez M.C.E. Actividad citotóxica de extractos acuosos de hojas de *Trichilia hirta* sobre células tumorales humanas. Rev Cubana Invest Bioméd 2013; 32(1): 93-101.
27. García-Argáez A.N., Ramírez A.T..O. Parra DH., Velázquez G., Martínez-Vázquez M. Anti-Inflammatory Activity of Coumarins from *Decatropis bicolor* on TPA Ear Mice Model Planta Med 2000; 66(3): 279-281
28. Panno M.L., Giordano F. Effects of psoralens as anti-tumoral agents in breast cancer cells. World journal of clinical oncology 2014; 5(3): 348–358.
29. Wang J.Y., Wang Z., Li M.Y., et al., Dictamnine promotes apoptosis and inhibits epithelial-mesenchymal transition, migration, invasion and proliferation by downregulating the HIF-1α and Slug signaling pathways. Chem Biol Interact 2018; 296:134-144.
30. Cuca L., Ávila M. Nueva benzoil lactona y otros constituyentes de *Pilocarpus alvaradoii* (Rutaceae). Revista Colombiana de Química 2007; 36 (3):285-290.
31. Bracho P.J.C., Rodríguez B.C., Llanes F. Triterpenos pentacíclicos en propóleo. Revista de la Sociedad Química del Perú 2009; 75(4): 439-452.
32. Liu Y., Bi T., Wang G. Lupeol inhibits proliferation and induces apoptosis of human pancreatic cancer PCNA-1 cells through AKT/ERK pathways Naunyn-Schmiedeberg's Arch Pharmacol 2015; 388: 295.
33. de la Rosa-Hernández M., Wong-Paz J., Muñiz-Márquez D., Carrillo-Inungaray M., Sánchez-González J. Compuestos fenólicos bioactivos de la toronja (*Citrus paradisi*) y su importancia en la industria farmacéutica y alimentaria. Revista Mexicana de Ciencias Farmacéuticas (2016); 47 (2): 22-35.

34. Abotaleb M., Samuel S.M., Varghese E., et al;. Flavonoids in Cancer and Apoptosis. Cancers 2018; 11(1): 28.
35. Pinzón C.E., Serrano M.L., Sanabria M.C. Papel de la vía fosfatidilinositol 3 kinasa (PI3K/Akt) en humanos. Rev. Cienc. Salud. Bogotá 2009; 7(2):47-66.
36. Khan K.H., Yap T.A., Yan L., Cunningham D. Targeting the PI3K-AKT-mTOR signaling network in cancer. Chinese journal of cancer 2013; 32(5): 253–265.
37. Cravedi P., Ruggenenti P., Remuzzi G. Sirolimus for calcineurin inhibitors in organ transplantation: contra. Kidney International 2010; 78: 1068–1074
38. Martinez P.E.R., Perez E.B.E., Villavicencio P.M.A. Plantas medicinales de Plomosas, Actopan Hidalgo. CDMEX: Universidad Autónoma del Estado de Hidalgo. 2010.

Table 1. Phytochemical of ethanolic and aqueous extract of *D. bicolor*

Secondary metabolites	Test	Etho	Aqueous
Alkaloids	Dragendorff	++	-
	Mayer	++	++
	Wagner	++	-
Triterpenes	Solkowsky	++	-
	Lieberman-Buchard	+	+
	Rosemheim	+	-
Coumarins	Baljet	+++	+++
	Legal	++	-
	Erlich	+	+
Flavonoids	Ac. Sulfúrico	+++	-
	Shinoda	+++	+++
	Rosemheim	+++	-

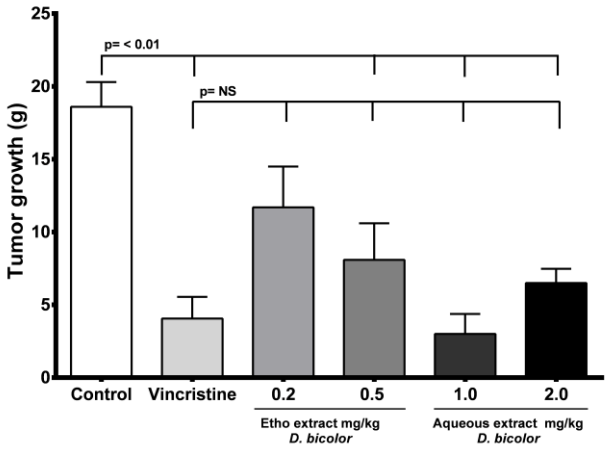

Figure 1. *D. bicolor* inhibits tumour growth

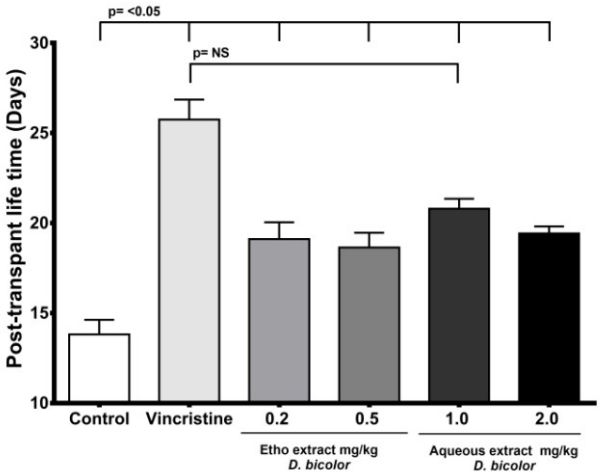

Figure 2. *D. bicolor* increased lifetime

Figure 3. *D. bicolor* increases the probability of survival in lymphoma L5178Y