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Methanol extract from *Psidium sartorianum* (arrayán) berries induces *in vitro* damage on *Taenia crassiceps* WFU cysticerci

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Abstract
Taenia crassiceps is an asexually proliferating cestode, may give rise to a high number of cysts in the experimental conditions and become a suitable model organism for drug screening in the absence *T. solium* cysts, responsible for human and swine cysticercosis. Although there is no conclusive evidence on resistant cestodes, alternative treatments are relevant for the human and animal health. A methanol extract from *Psidium sartorianum* (O. Berg) Nied. Berries (AME) was used to evaluate anti-parasitic effect on *T. crassiceps* cysticerci. Metacestodes were exposed to 25, 50 or 100 mg/ml of AME for up to 72 h. The tissue impairment at the tegumentary and subtegumentary levels was seen after 2.5 h of treatment with 100 mg/ml or 24 h after when treated with 50 mg/ml of AME. Here we report denuded microtriches and blebbing on the surface of cysts. The results indicate that high concentrations of AME must be employed in order to achieve relevant tissue damage in the short time. Therefore active components must be characterized for their anti-parasitic effect.

Keywords: Plant extracts, *Psidium sartorianum*, parasitic helminth, *Taenia crassiceps* WFU, ultrastructural damage

1. Introduction
Human and swine parasitic cestode *Taenia solium* is one of the six helminths, which cause the neglected tropical diseases (NTDs). State-of-the art given by WHO/TDR in 2012 for NTDs points out also that the social and economic conditions, which favor the maintenance of the life-cycle of *T. solium*, are not likely to be changed in the short term [1]. Thereby, control of this helminthiasis requires tools such are pig vaccination and concomitant chemotherapy based on known or developing drugs. ABZ is widely used in mass drug administration (MDA) by the WHO program to control the soil-transmitted helminths and lymphatic filariasis, as well as large-scale initiative for cestodes. Human *Taenia* species are spread mainly in Latin America, Asia and Africa, affecting around 40 million people [2]. Infection caused by *T. solium* is important due to neurocisticercosis. In addition, recent cases of human infected by *T. asiatica* [3], *T. saginata* [4], *T. crassiceps* [5-7] and *T. multiceps* [8-10] should not be underestimated in poverty conditions or globalized world. Both, humans and animals infected with larval stages of taeniids and treated with either ABZ or praziquantel have shown variable responses due to differences in drug absorption after oral administration [11-13]. Although there are no reports related to ABZ resistance in cestodes, positive outcome of treated cysticercosis also depends on the number, type and location of the cysts [14, 15].
Plant extracts and its purified active components may become relevant natural substitutes of chemically designed drugs and optimize existing methods to combat infectious diseases [16]. *Psidium* species are commonly known as “guava” and have been used in worldwide traditional medicine. Oil or water extract from leafs, bark, roots or fruits from the different species of *Psidium* have been recommended to treat a number of human and animal diseases. Extract or purified active components from *Psidium* sp. are known to be effective against the pathogenic fungi, bacteria, protozoa, and also have an anti-oxidant, anti-inflammatory, cardio-active, hepato-protective and anti-diabetic properties [17].
Taenia crassiceps WFU is an asexually proliferating cestode that grows in the peritoneal cavity of experimentally infected mice, and may give rise to many hundreds of cysts after serial passages [18]. This model has been frequently used for *in vitro* drug screening [19, 20], in substitution of *T. solium* cysticerci, which must be found and then resected from the infected

porcine host. The present study reports damage in the tegument of *T. crassiceps* WFU cysterci, for the first time, the blebbing together with denuded subsequent layers, under *in vitro* effect of methanol extract from *P. sartorianum*.

2. Material and Methods

2.1 Cysticerci of *Taenia crassiceps* WFU

Taenia crassiceps WFU cysticerci were maintained in female BALB/c mice by intra-peritoneal passages [21]. Parasites were obtained from the peritoneum of infected host, after cervical dislocation. For drug assay six cysts with 2 mm long invaginated scolex and transparent contractile bladder were selected. Cysts were rinsed in PBS containing 0.2% antibiotic-antimycotic (Invitrogen, Carlsbad, CA, USA) and deposited per each well in a 24-well plate (Corning, Sigma-Aldrich, St. Louis, MO, USA) for the subsequent steps.

2.2 *Psidium sartorianum* (arrayán) methanol extracts (AME)

Psidium sartorianum "arrayán" is a native to México and Central America myrtaceae plant, and is used in the traditional medicine to treat diarrhea, dysentery, scabies and intestinal parasites. The arrayán tree can reach a height of 20 m in the warm and temperate climates, 0-1600 m.a.s.l. The ripening season occurs mainly from September to March. Mature berries are yellow, 1.0-2.5 cm in diameter and taste similar to the *P. guajava* (guava). The 5-8 mm long seeds are angular (1-5 per fruit; 1-2 per lobe). Voucher specimens (accession number VAR2636) of *P. sartorianum* were deposited at the Herbarium, Facultad de Agronomía, Universidad Autónoma de Sinaloa, Culiacán, and México. Patent application "Procedimiento para la obtención de extractos del fruto de arrayán y sus usos como anti-parasitarios" by Díaz Camacho S.P., Delgado Vargas F. and Willms Manning K. Was submitted as MX/a/2009/004174.

Arrayán berries were collected in the valley of Culiacan, Mexico, grounded, sieved through a 0.425 mm mesh (Gilson, OH, USA) and powder stored at -20 ºC. Extract was prepared as described with modifications in [22]. Briefly, 20% (w/v) of arrayán powder in methanol was mixed using an orbital shaker (Thermo Fisher Scientific, Waltham, MA, USA) at 150 rpm during 72 h. Each 24 h, the supernatant was substituted with a fresh methanol, and three collected methanol fractions were concentrated in a vacuum evaporator (Büchi, Labortechnik AG, Switzerland) at 40 ºC. Residual methanol was removed in a vacuum oven (Precision Scientific Thelco, Cortland St. Chicago, IL, USA) at 40 °C and the obtained arrayán extract was stored at -20 °C until use. AME yielded in 55% (w/w) of *P. sartorianum* dry weight; was oily, viscous and dark colored.

2.3 Treatment of *T. crassiceps* cysts with AME, ABZ or nitazoxanide (NTZ)

Six cysts were pre-incubated in 2 ml of Hank´s Balanced Salt Solution (HBSS; Sigma-Aldrich, MO, USA) complemented with 0.5% of peptone (Bioxon, Mexico) and 0.2% antibiotic-antimycotic for 24 h at 37 ºC in 5% CO_2. AME was diluted to 0.5 g/ml of HBSS, adjusted to pH 7.4 and insoluble particles eliminated by centrifugation for 2 min at 2500 rpm/min. Cysticerci of *T. crassiceps* were treated with the AME at 25, 50 or 100 mg/ml.

Anti-helminthics ABZ (>97%) and NTZ were kindly donated by Dr. Rafael Castillo, Facultad de Química, UNAM, and used as a damage-control drugs. ABZ and NTZ were dissolved in 0.05% DMSO (Sigma-Aldrich, St. Louis, MO, USA) in HBSS at concentration of 0.08 µg/ml or 0.13µg/ml, respectively. Viability of the cysts based on the motility and morphometric parameters, was monitored by light microscopy between 2-72 h post-administration. Experiments were carried out in triplicate. Procedures were performed in accordance with the guidelines established by the Comité de Ética e Investigación de la Facultad de Medicina, UNAM, and animals were manipulated according to the Mexican Official Regulation NOM-062-ZOO-1999.

2.4 Transmission (TEM) and scanning electron microscopy (SEM)

Samples were prepared for TEM and SEM [23]. Briefly, parasites were rinsed in HBSS, fixed for 24 h in Karnovsky solution [24], washed in 0.15 M cacodylate buffer for further 24 h and post-fixed in 1% osmium tetroxide during 2 h at 4 °C. Fixed parasites were included in poly form (Polysciences, Warrington, PA, USA) 1 µm thick sections were stained with 0.5% toluidine and examined by light microscopy [25]. Samples were submitted to TEM at JEOL-1200EX. SEM-sample preparation consisted of steps including 1% osmium tetroxide post-fixation, dehydration, drying at the critical point and gold sputtering [26]. Images were taken using JEOL-JSM35CF.

3. Results

3.1 Effect of AME on cysts viability

Taenia crassiceps WFU cysticerci showed 90% evagination after one-day pre-incubation in supplemented HBSS. Both untreated or treated only with 0.05% DMSO parasites, showed characteristic bladder contractions and evagination-invagination scolex movements during 72 h of experimental period. Under light microscopy, cysticerci became completely paralyzed after 2.5 h of treatment with 100 mg/ml of AME. In the presence of 50 mg/ml of AME, movements of 95% of cysts were reduced after 2.5 h and were absent in 100% of metacestodes after 24 h of incubation. Treatments with 25 mg/ml of extract, as well as 0.08 µg/ml of ABZ or 0.13 µg/ml of NTZ, had slowed the movement of all parasites during 72 h of assay.

3.2 SEM and TEM findings

Cysticerci incubated in HBSS, as well as in the solution supplemented with DMSO only, had an intact tegument and microtriches seen by SEM (Fig. 1A and B). Microvilli and tegument with dense granules and mitochondria (Fig. 1C) and parenchymal tissue with organized myofibrils (Fig. 1D) were also seen by TEM. After 2.5 h of incubation with 100 mg/ml of AME, randomly selected and submitted to SEM cysts showed blebs and collapsed tegument microtriches (Fig. 2A and B). Cysts under TEM had a brush border partially substituted by blebs of different size, protruding from the membrane surface (Fig. 2C and D). Blebs were also seen in the close proximity of an apparently intact microtriches (Fig. 2C). The lack of tegumentary organization, a swollen and thickened basal matrix enclosing the parenchymal tissue, as well as dispersed mitochondria without typical crests, were seen in the Fig. 2D. Bundles of myofibers were normally situated below the basal matrix, while compacted glycogen particles were organized in sacs in the proximity of tegumenta myocytons in untreated cysticerci (Fig. 3A). Distended and swollen sacs with less compacted material were found in the parenchyma of cysts treated with 100 mg/ml of AME (Fig. 3B). Cysts incubated for 24 h in 50 mg/ml of AME presented damaged tegument sections as well, characterized by a partial

loss of microtriches and blebbing of the membranous material on the surface (not shown). Cysts treated with 0.13 µg/ml NTZ or 0.08 µg/ml of ABZ and examined by light microscopy had an apparently intact tegument with normal microvilli and parenchymal organization (not shown).

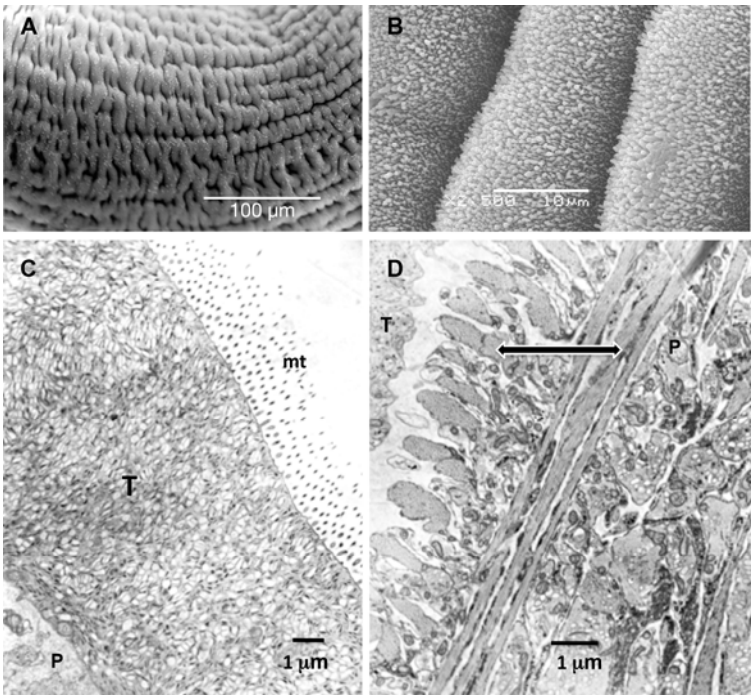

Fig 1: Intact tegument (A) and microtriches (B) of untreated *T. crassiceps* WFU cysts seen by SEM, and dense granules together with microtriches in distal cytoplasm (C) and organized longitudinal myofibrils (D; double head arrow) documented by TEM. T, tegument; P, parenchyma; mt, microtriches.

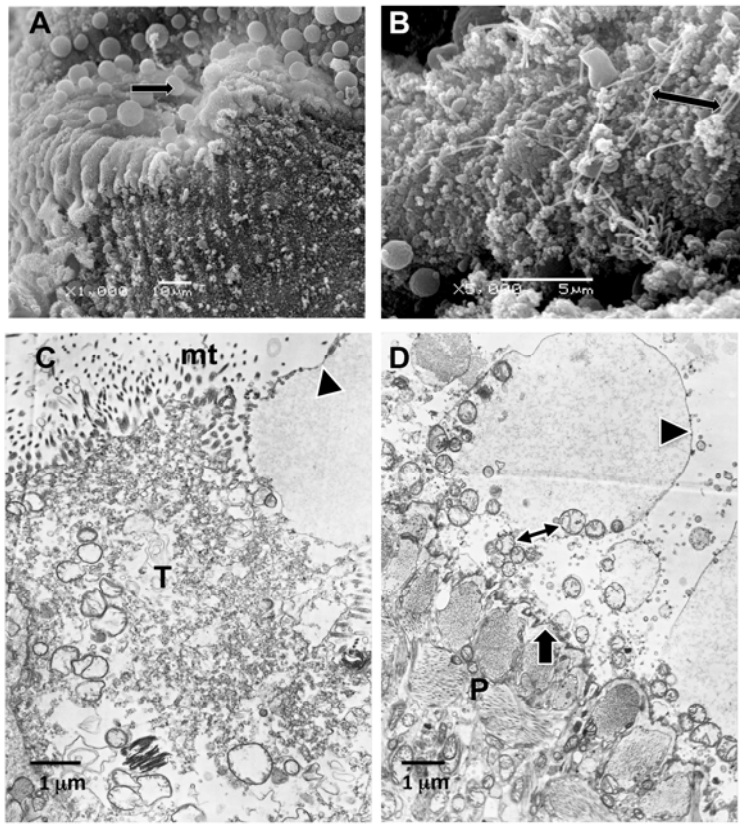

Fig 2: Scanned micrographs of cysts surface after treatment with 100 mg/ml AME with partially obliterated and balloon shaped tegumentary microtriches (A; arrow) and bundles of protruded myofibrils in denuded tegument (B; double head arrow). Electron micrographs treated with AME cysts showing apparently normal microtriches intercalated with disarranged distal cytoplasm, swollen mitochondria and blebbing surface (C; arrowhead). Tissue fragment (D) with absent tegument and dispersed above basal membrane (filled arrow) mitochondria (double head arrow) and blebs (arrowhead). T, tegument; P, parenchyma; mt, microtriches.

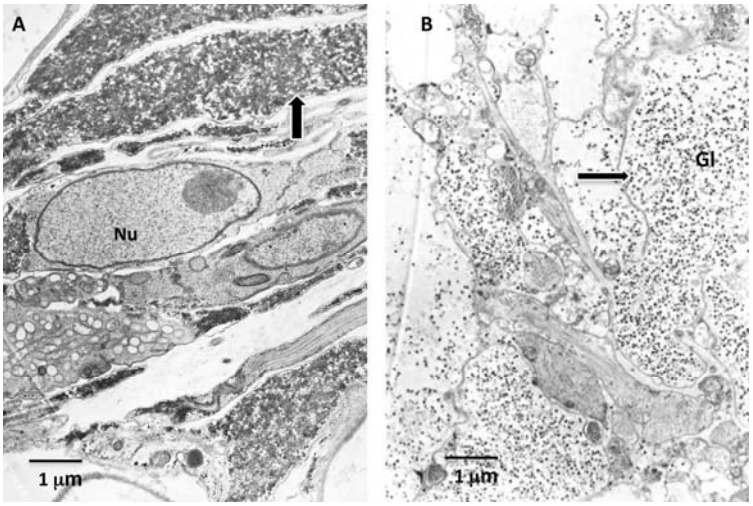

Fig 3: Transmission electron micrographs of parenchymal tissue of *T. crassiceps* WFU cysticerci. Subtegumentary myocyton surrounded by compact glycogen sacs (arrow) in untreated cysticercus (A). Reduced in thickness parenchymal tissue with distended, swollen or dispersed in cytoplasm glycogen particles seen in cysticercus treated with 100 mg/ml AME (B; arrows). GI, glycogen particles; Nu, nucleus of a tegumentary cyton.

4. Discussion

Anti-helminthic compounds are known to have a partial efficacy due to the: a) applications of single-dose treatment, b) grade of endemicity of certain parasites, c) a low bioavailability of the used drug and, c) eventual development of resistance toward the treatment. Recent politics in health care have been emphasizing on the development of alternative drugs, especially in and for the countries whose population also relies on the traditional medicine. Mexican plant locally called "arrayán" is a member of the Myrtaceae family and belongs to the compendium of the worldwide medicinal plants. Based on anti-parasitic effect on the *Hymenolepis nana* and pathogenic fungi [27], AME obtained from *P. sartorianum* is now in process of patenting by UNAM. Similar to other traditional remedies, AME may contain more than one compound with therapeutic values; known for *P. sartorianum* berries are tannins, phytic acid and a high content of vitamin C [28]. Complete chemical definition and lack of rigorous toxicity testing may complicate any patenting process; however efforts to evaluate and develop botanical preparations with a medicinal value should not be diminished [29].

The absence of helminth-cell lines, difficulties for *in vitro* helminthes culturing, as well as lack of complete egg-to-egg cycle, are considered a major problem for drug screening [30]. The continuous maintenance of *T. crassiceps in vivo* may represent a good whole-organism-based screening helminthiasis model. Besides developing metacestodes in mice, a scolex-presenting WFU strain may give rise to adult tapeworms in experimental hamsters (*Mesocricetus auratus*) and therefore, become a suitable for drug screening model well. Cysticerci proliferate in the peritoneum of the experimentally infected mice, yielding in a hundreds of phenotypically similar individuals. After extraction, cysts with apparently similar in scolex size were selected for *in vitro* AME treatment. A transparent bladder and the body contractions were considered as viability criterions, including scolex intussusception movements. Dying or partially calcified individuals slow down their movements or are completely rigid also at physiological temperature. At microscopic examination, exposed to AME *T. crassiceps* cysticerci resulted in slowed contractions, blurred aspect of vesicular fluid and the tegument damage with a parenchymal disorganization. After 2.5 h of treatment with 100 mg/ml of AME, parasites were dead. Anti-helminthics such are ABZ or ABZ-sulfoxide, NTZ or tizoxanide are known to cause death linked to the relevant tissue damage of these metacestodes [31]. While those authors have used a multiple doses for *in vitro* cyst´s treatment, only a single dose of ABZ or NTZ was used during 72 h of our experimental design, which apparently did not affect the tegument integrity, when compared to the single dose of AME. Although drug-related damage has been documented for *T. crassiceps*, this parasite has high proliferation properties and, any regenerative loci situated on the tegument, may give rise to the new buds [32, 19]. A deeply denuded parasite surface, with a damage seen at the subtegumentary level and relevant membranous blebbing, caused by the highest concentration of AME, suggest good anti-helminthic effect on this experimental model. In the absence of 100% efficacy of existing anti-helminthic drugs, AME must show also potent cysticidal effect on *T. solium* cysts, which lack of asexual proliferation. *Taenia solium* metacestodes are expected to present similar to *T. crassiceps* damage, if cultured in the presence of AME, whose effective concentration must be previously determined. Moreover, active components of AME must be characterized in the future in order to reduce high concentrations of extract.

In vivo evaluation of cysticidal properties of arrayán extract represents the most important challenge in porcine cysticercosis. Mexican backyard pigs are heavily exposed to the *T. solium* eggs, and 3-25% of rural porcine population is reported as positive for cysticercosis [33]. Treatment of infected *T. solium* pigs is based on oxfendazole, but also development of TSOL18 [34] and S3Pvac [35] vaccines to control this zoonotic disease in concomitant way. Other *Psidium* species, such as *Psidium guajava* commonly known as "guava", can be a part of the natural diet in omnivorous animals from *Suidae* family [36]. The hog industry in some pork-meat demanding countries, use a "guava" to treat diarrheic diseases [37, 38]. If AME cysticidal effect on *T. solium* cysts is maintained also *in vivo*, then pigs fed with processed arrayán, may have a reduced risk of cysticercosis. This strategy together with a better sanitation and health education may give, as states WHO, a promising result on eradication taeniasis-cysticercosis.

5. Conclusions

A myrtaceae plant *Psidium sartorianum* has been used to evaluate cysticidal effect on *Taenia crassiceps*. Metacestodes of this Taeniidae parasite have been used as a model organism for anthelmintic drug screening. Methanol extract obtained from *P. sartorianum* at concentration of 100 mg/ml resulted in the death of *T. crassiceps* after 2.5 h of *in vitro* incubation. Scanning and transmission electron microscopy revealed an extensive tegumentary and sub-tegumentary damage in tissue organization of parasite. The cysticidal components of AME and *in vitro/in vivo* assays on cestodes, may provide an alternative anti-helminthic treatment.

5.1 Conflict of interest

The authors declare no conflict of interest.

6. Acknowledgements

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7. References

1. WHO/TDR. Research priorities for helminth infections. Technical Report of the TDR disease reference group on helminth infections. WHO Technical Report Series. 2012, 972.
2. WHO. Working to overcome the global impact of neglected tropical diseases. First WHO report on neglected tropical diseases, 2010.
3. Galan-Puchades MT, Fuentes MV. *Taenia asiatica*: left out by globalization. Trends in Parasitology. 2014; 30:54-55.
4. Cho J, Jung BK, Lim H, Kim MJ, Yooyen T, Lee D *et al*. Four cases of *Taenia saginata* infection with an analysis of COX1 gene. Korean Journal of Parasitology. 2014; 252:79-83.
5. Tappe D. Subcutaneous *Taenia crassiceps* infection in a patient with non-Hodgkin's lymphoma. American Journal of Tropical Medicine and Hygiene. 2006; 75:108-111.
6. Goesseringer N, Lindenblatt N, Mihic-Probst D, Grimm F, Giovanoli P. *Taenia crassiceps* upper limb fasciitis in a patient with untreated acquired immunodeficiency syndrome and chronic hepatitis C infection the role of surgical debridement. Journal of Plastic and Reconstructive Aesthetic Surgery. 2011; 64:174-176.
7. Ntoukas V, Tappe D, Pfütze D, Simon M, Holzmann T. Cerebellar cysticercosis caused by larval *Taenia crassiceps* tapeworm in immunocompetent woman. Germany Emerging Infectious Diseases. 2013; 19:2008-2011.
8. El-On J, Shelef I, Cagnano E, Benifla M. *Taenia multiceps*: a rare human cestode infection in Israel. Veterinaria Italiana. 2008; 44:621-631.
9. Collomb J, Machouart M, Biava MF, Brizion M, Montagne K, Plénat F *et al*. Contribution of NADH dehydrogenase subunit I and cytochrome C oxidase subunit I sequences toward identifying a case of human coenuriasis in France. Journal of Parasitology. 2007; 93:934-937.
10. Ing MB, Schantz PM, Turner JA. Human coenurosis in North America: case reports and review. Clinical Infectious Diseases. 1998; 27:519-523.
11. Lange H, Eggers R, Bircher J. Increased systemic availability of albendazole when taken with a fatty meal. European Journal of Clinical Pharmacology. 1988; 34:315-317.
12. Sotelo J, Diaz-Olavarrieta C. Neurocysticercosis changes after 25 years of medical therapy. Archives of Medical Research. 2010; 41:62-63.
13. Zanetti Lopes WD, Cruz BC, Soares VE, Nunes JLN, Pires Teixeira WF, Maciel WG *et al*. Pereira de Oliveira G, da Costa AJ. Historic of therapeutic efficacy of albendazol sulphoxide administered in different routes, dosages and treatment schemes, against *Taenia saginata* cysticercus in cattle experimentally infected. Experimental Parasitology. 2014; 137:14-20.
14. Van den Enden E. Pharmacotherapy of helminth infection. Expert Opinion on Pharmacotherapy. 2009; 10:435-451.
15. Garcia HH. Antiparasitic drugs in neurocysticercosis: albendazole or praziquantel? Expert Review of Anti-Infective Therapy. 2008; 6:295-298.
16. WHO. World Health Organization Traditional Medicine Strategy, 2014-2023, 2013.
17. Alipour G, Dashti S, Hosseinzadeh H. Review of pharmacological effects of *Myrtus communis* L. and its active constituents. Phytotherapy Research. 2014; 28:1125-1136.
18. Everhart ME, Kuhn RE, Zelmer DA. Infrapopulation dynamics of a wild strain of *Taenia crassiceps* (WFU) (Cestoda: Taeniidae) in BALB/cJ mice. Journal of Parasitology. 2004; 90:79-84.
19. Zurabian R, Aguilar-Vega L, Terrones Vargas E, Cervera Hernández ME, Willms K, Ruíz-Velasco S. In vivo albendazole treatment of *Taenia crassiceps* cysticerci strain WFU: proliferation, damage, and recovery. Parasitology Research. 2013; 112:3961-3968.
20. Palomares-Alonso F, Palencia Hernández G, Rojas-Tomé IS, Jung-Cook H, Pinzón-Estrada E. Murine cysticercosis model: influence of the infection time and the time of treatment on the cysticidal efficacy of albendazole and praziquantel. Experimental Parasitology. 2015; 149:1-6.
21. Zurabian R, Aguilar L, Jiménez JA, Robert L, Willms K. Evagination and infectivity of *Taenia crassiceps* metacestodes in experimental animals. Journal of Parasitology. 2008; 94:1-6.
22. Wall ME, Wani MC, Brown DM, Fullas F, Olwald JB, Josephson FF *et al*. Effect of tannins on screening of plant extracts for enzyme inhibitory activity and techniques for their removal. Phytomedicine. 1996; 3:281-285.
23. Díaz Camacho SP, Willms K, Ramos MZ, del Carmen de la Cruz Otero M, Nawa Y, Akahane H. Morphology of *Gnathostoma* spp. Isolated from natural hosts in Sinaloa, Mexico. Parasitology Research. 2002; 88:639-645.
24. Karnovsky MJA. Formaldehyde-glutaraldehyde of high osmolality for use in electron microscopy. Journal of Cell Biology. 1965; 27:137-138.
25. Willms K, Robert L. Ultrastructure of a spermatid transport system in proglottids of experimental *Taenia crassiceps* (WFU strain). Parasitology Research. 2007; 101:967-973.
26. Watson LP, McKee AE, Merrell BR. Preparation of microbiological specimens for scanning microscopy. Ed. Murphy JA, Roomans GM. Preparation of biological specimens for scanning microscopy Scanning Electron Microscopy, Inc., AMF O´Hare, 1980, 45-57.
27. Camacho-Hernández L, Cisneros-Rodríguez C, Uribe-

Beltrán MJ, Ríos-Morgan A, Delgado-Vargas F. Antifungal activity of fruit pulp extract from *Psidium sartorianum*. Fitoterapia. 2004; 75:401-404.
28. Delgado-Vargas F, Díaz-Camacho SP, Salazar-Zamora G, Uribe-Beltran MJ, Vega-Aviña R. *Psidium sartorianum* (O.Berg) Nied, an indigenous plant to Mexico, from Biology to biological activity, Ed. Govil JN, Singh VK, Arunachalam C. Search for Natural Drugs. Recent Progress in Medicinal Plants. Studium Press LLC Houston, 2005, 81-114.
29. Geary TG, Chibale K, Abegaz B, Andrae-Marobela K, Ubalijoro E. A new approach for anthelmintic discovery for humans. Trends in Parasitology. 2012; 28:176-181.
30. Geary TG, Sakanari JA, Caffrey CR. Anthelmintic drug discovery: into the future. Journal of Parasitology. 2015; 101:125-133.
31. Palomares-Alonso F, Piliado JC, Palencia G, Ortiz- Plata A, Jung-Cook H. Efficacy of nitazoxanide, tizoxanide and tizoxanide/albendazole sulphoxide combination against *Taenia crassiceps* cysts. Journal of Antimicrobial Chemotherapy. 2007; 59:212-218.
32. Toledo A, Cruz C, Fragoso G, Laclette JP, Merchant MT, Hernández M, Sciutto E. *In vitro* culture of *Taenia crassiceps* larval cells and cyst regeneration after injection into mice. Journal of Parasitology. 1997; 83:189-193.
33. Flisser A, Rodríguez-Canul R, Willingham ALIII. Control of the taeniosis/cysticercosis complex: future developments. Veterinary Parasitology. 2006; 139:283-192.
34. Lightowlers MW. Eradication of *Taenia solium* cysticercosis: A role for vaccination of pigs. International Journal for Parasitology. 2010; 40:1183-119.
35. Sciutto E, Fragoso G, Hernández M, Rosas G, Martínez JJ, Fleury A, *et al*. Development of the S3Pvac vaccine against porcine *Taenia solium* cysticercosis: a historical review. Journal of Parasitology. 2013; 99:686-692.
36. Pinna W, Nieddu G, Moniello G, Cappai MG. Vegetable and animal food sorts found in the gastric content of Sardinian Wild Boar (*Sus scrofa meridionalis*). Journal of Animal Physiology and Animal Nutrition. 2007; 91:252-255.
37. Kanbutra P, Borisutpeth P, Porntrakulpipat S, Sarachoo K, Jivaganon J, Aromdee C *et al*. Anti-bacterial activity of alcoholic extract of Thai medicinal plants on *Escherichia coli* F18+ Proceeding in STT 29th KKU Thailand, 2003.
38. Azevêdo JAG, Valadares Filho SC, Pina DS, Detmann E, Valadares RFD, Pereira LGR. Costa e Silva LF. Intake, total digestibility, microbial protein production and the nitrogen balance in diets with fruit by-products for ruminants. Revista Brasileira Zootecnia. 2011; 40:1052-1060.