Available online at http://www.ifgdg.org

Int. J. Biol. Chem. Sci. 12(5): 2101-2108, October 2018

ISSN 1997-342X (Online), ISSN 1991-8631 (Print)

International Journal of Biological and Chemical Sciences

Original Paper http://ajol.info/index.php/ijbcs http://indexmedicus.afro.who.int

Anticoccidial activity of *Carica papaya* and *Vernonia amygdalina* extract

Hervé Brice DAKPOGAN[1*], Serge MENSAH[2], Sabbas ATTINDEHOU[1], Christophe CHYSOSTOME[3], André ABOH[2], Muriel NACIRI[4], Sahidou SALIFOU[1] and Guy Apollinaire MENSAH[2]

[1]*National Veterinary Parasitology Laboratory, Polytechnic School of Abomey-Calavi P.O.BOX 2009, University of Abomey-calavi, Benin.*
[2]*National Agricultural Research Institute of Benin (INRAB), 01 P.O. BOX 884, Cotonou, Benin.*
[3]*Faculty of Agronomic Sciences 01 P.O.BOX 526, University of Abomey-calavi, Benin.*
[4]*INRA – UR 86 Bio-Agresseurs Santé et Environnement, 37380 Nouzilly – France.*
[*]*Corresponding author; E-mail: dakpogan2002@yahoo.fr; Tel: (00229) 94 62 58 36.*

ACKNOWLEDGMENTS

The Benin Ministry of high Education and Scientific Research is to be thanked for the technical and financial support.

ABSTRACT

The anticoccidial effect of *C. papaya* and *V. amygdalina* crude juice was tested on 100 *Eimeria tenella* experimentally infected day-old Isa-brown male chicks in a completely randomized design, as an alternative coccidiosis control measure. Each chick received 3 x 10^4 *E. tenella* oocysts doses. The first and the second groups were orally treated with papaya and vernonia juice, for consecutive 5 days. The third and the fourth groups were medicated (sulfadimidine) and unmedicated controls. The papaya treatment improved the survivability by 20% compared with the unmedicated control group. Neither death nor bloody feces were found in the medicated control chick group. Similar body weight gains were observed in all groups at the end of the second week post inoculation. However, the papaya and vernonia effect represents only 59.31 and 40.78% of the medicated control efficacy, respectively, in terms of oocysts excretion reduction. *Carica papaya* did demonstrate in this first herein preliminary study an anticoccidial effect, however, the active substance need to be extracted and its dose and toxicity threshold to be further investigated.

Keywords: Coccidiosis, chick; anticoccidian, medicinal plant, oocysts.

INTRODUCTION

Coccidiosis is one of the most expensive and common disease of poultry production systems (Dakpogan and Salifou, 2013; Lucas and Zainab, 2016), in spite of advances in chemotherapy, management, nutrition and genetics (McDougald, 2003). The disease is caused by a protozoan parasite belonging to the Apicomplexa phylum, the family of the Eimeridae and genus of *Eimeria*. There are 7 species of coccidia of pathological importance in

DOI: https://dx.doi.org/10.4314/ijbcs.v12i5.12

chicken: *E. acervulina, E. brunetti, E. maxima, E. necatrix, E. tenella, E. praecox* and *E. mitis*. The macroscopic lesions in the digestive tract are some predisposing factors to many gastrointestinal bacterial poultry diseases such as clostridiosis, salmonellosis and colibacillosis (Bostvironnois and Zadjian, 2011). Coccidiosis is also exacerbated by certain immunosuppressive viral diseases such as Infectious bursal disease, Marek disease and chick anemia infectious viral disease (Lanckriet et al., 2010). It remains a big concern for commercial chicken production, because of the high costs of its control. Sørensen et al. (2006) estimated by extrapolation the total cost of loss due to coccidiosis, its clinical and subclinical forms and its control, at 2.3 Billion € world-widely, with 70% of the loss attributable to the unapparent sub-clinical form of the disease that depress considerably weight gain and feed conversion ratio. Various anticoccidial feed additives; predominantly ionophorous antibiotics have been developed and used (Chapman et al., 2005). The routinely use and misuse of these drugs, in one hand, has led to coccidial parasite drug resistant strains (Shirley et al., 2007) and on the other hand, is prejudicial to consumer health because of the presence of anticoccidial drug residues in poultry products (Cannavan et al., 2000; Youn and Noh, 2001; Mortier et al., 2005; Danaher et al., 2008). The recent introduction of live drug-tolerant anticoccidial vaccine used in rotational basis with anticoccidial drugs (Chapman et al., 2005; Lee et al., 2009; Tewari et al., 2010; Berezin et al., 2010) is of great advantage for poultry industries in terms of effectiveness, but the vaccine in its ability to replicate in bird intestinal tract constituted a predisposing factor to other opportunistic disease agents (Bostvironnois and Zadjian, 2011). The pharmaceutical industry has shown little interest in developing new products for anti-parasitic use, and it is unlikely that we will have replacements for the products already available (McDougald, 2003). Medicinal plants are considered as alternative new tools to control coccidiosis. The incorporation of dried leaf of *Andrographis paniculata* at 10, 20, 30 and 40% in feed proved to be efficient on mortality reduction (Sujikara, 2000). Among the 15 medicinal plants tested by Youn and Noh (2001), only *Sophora flavescens* was more efficient in reducing bloody diarrhea, lesion scores and oocysts excretion. Arczewska and Swiatkiewicz (2010) tested the extracts of *Allium sativum, Salvia officinalis, Echinacea purpurea, Thymus vulgaris* and *Origanum vulgare* on *Eimeria tenella* coccidiosis and obtained similar result compared with that of the conventional anticoccidiostatic in terms of weight gain and oocyst production. The anthelmintic effect of *Carica papaya* (Ekanem et al., 2004; Okeniyi et al., 2007; Sacramento et al., 2010) and *Venonia amygdalina* (Huffman, 2003; Hamil et al., 2003; Ojiako and Nwanjo, 2006; Erasto et al., 2007; Arhoghro et al., 2009) was reported. These herbs are classified in the group of medicinal plants used by rural communities to cure many human and animal diseases in Benin (Attindehou et al., 2011). The aim of the current study was to test the anticoccidial effect of *Carica papaya* and *Venonia amygdalina* fresh leaves extract on chick *Eimeria tenella* coccidiosis.

MATERIALS AND METHODS

Day-old chick

One hundred (100) day-old Isa-brown male chicks were used. The birds were housed in a deep litter-floured starting pen, under 22 hours lighting and held at initially 27 °C up to 20 day-old before being transferred in experimental cages. The chicks had free access to feed and drinking water. Vaccination against Newcastle disease, Infectious bronchitis and Infectious bursal disease was the basic applied biosecurity measures.

***Eimeria tenella* and inoculation**

Eimeria tenella oocysts preserved in 2% potassium dichromate solution were generously provided by the infectiology laboratory of INRA, Tour, France and kept in a refrigerator (2-5 °C) until use. All the feces produced by each cage of birds, during the 24 h preceding the experimental infection, were examined to confirm the absence of any oocysts. Each coccidia-free chick was challenged orally with a dose of 30,000 oocysts.

Herb extracts and anticoccidial drug

Herb extracts were obtained by hand squeezing and pressing of the fresh green leaves of *Carica papaya* and *Venonia amygdalina* (1 g of leave for 2 ml of drinking water) to obtain the crude juice after sieving. Sulfadimidine was the conventional anticoccidial molecule used. Each chick in the treatment group received orally 1 ml of the leaves juice three times per day with 4 hours time period between each treatment and this, during 5 days after challenge. Sulfadimidine was given following the drug administration posology.

Experimental groups and collected data

Twenty five experimentally infected male Isa-brown day-old chicks were used (5 per cage with 5 replications) per group. There were papaya, venonia, medicated and unmedicated control groups. The effectiveness of herb extracts was accessed on the basis of bloody diarrhea, survival rate, oocysts excretion and body weight gain. The proportion of blood in feces from the third to seventh day post inoculation was evaluated. The survival rate was estimated from the number of surviving chicks divided by the number of initial chicks. Oocysts excretion (Soulsby, 1986) was recorded from 6 to 14 day post inoculation. Chick body weights in each group were recorded at the starting of the experiment and at the end of the first and the second week after challenge.

Statistical analysis

The descriptive and inferential analyses applied to oocyst excretion and body weight gain, were made using the General Linear Model (GLM) procedure of SAS (vo. 9.2). Frequency procedure with fisher test was used for survivability estimation and comparison.

RESULTS

The survivability of the infected medicated control (100%) and *C. papaya* (80%) treated chicks were significantly higher ($p < 0.05$) than that observed in *V. amygdalina* treated chicks (65%) and the infected unmedicated control groups (60%). Bloody feces of all experimental and control groups were observed from the fourth to sixth day post infection with *Eimeria tenella* oocysts. There were no bloody feces the third and the seventh day post infection. No bloody feces were ever found in the infected medicated control group. In the groups treated with the extract of *C. papaya* and *V. Amygdalina*, the extent of bloody diarrhea was similar but milder than that observed in the infected unmedicated control group.

At the end of the first week following *E. tenella* oocyst inoculation, the average body weight gain in the medicated control group, *C. papaya* and the unmedicated control chick groups were significantly higher than that of the *V. amygdalina* treated chick group ($p < 0.05$). At the end of the second week post infection, the body weight gain of all groups were statistically similar.

Lower excreted oocysts were observed with the medicated control (18,814) and higher with the infected unmedicated control chick group (349,935). The oocysts per gram in the groups treated with *C. papaya* and *V. amygdalina* were milder and statistically similar ($p > 0.05$).

Table 1: Survivability, Oocysts Per Gram and Body Weight Gain (mean ± SE).

Groups	Survivability (%)	Oocysts Per Gram	Body Weight Gain (g)	
			Week 1	Week 2
Medicated control	100[a]	18814[a] ± 3716	54[a] ± 7.4	73[a] ± 8.1
C. papaya	80[b]	153547[b] ± 53312	50[a] ± 9.2	65[a] ± 8.5
V. amygdalina	65[c]	214,904[b] ± 88109	22[b] ± 7.5	67[a] ± 10.4
Unmedicated control	60[c]	349935[c] ± 104560	48[a] ± 10.1	55[a] ± 8.4

SE: Standard Error, (values in the same column that not share the same superscript letters are significantly different, $p < 0.05$)

Table 2: Proportion of bloody feces.

Groups	Proportion (%) of blood in feces (day after infection)				
	3	4	5	6	7
Medicated control	0	0	0	0	0
C. papaya	0	3.1	21	27.5	0
V. amygdalina	0	3.5	23.8	36.1	0
Unmedicated control	0	4.6	20.2	43.4	0

DISCUSSION

Carica papaya treatment reduced significantly the oocysts per gram down to 56.13% compared with the unmedicated control oocysts per gram. This result is in line with previous experiments showing similar activity of papaya latex, seeds and leaves against intestinal worm (Arvind et al., 2013). In an experimental infection, Ekanem et al. (2004) observed a significant reduction effect of petroleum-ether extract of seeds of *C. papaya* against a protozoan fish parasite *Ichthyophthirius multifiliis*, with a reduction rate of 90% *in vivo* and 100% *in vitro*, compared with the untreated control. Likely, *C. papaya* seeds were found efficacious in treating human intestinal parasites, without significant side effects, with a parasite clearance rate of 76.7% (Okeniyi et al., 2007). The anthelmintic activity of papaya seed might be ascribed to carpaine, carpasemine (Kermanchai et al., 2001) and proteolytic enzymes such as cysteine proteinases (Stepek et al., 2005) and papaine (Arvind et al., 2013) from the fruit. Furthermore, *C. papaya* is known to have an anti-inflammatory properties, certainly due to its riches in Vitamin A, used against tumors, ulcers and can accelerate wound healing (Beuth et al., 2001). The oocysts per gram reduction induced by *C. papaya* crude juice treatment might be ascribed in one hand to the direct *E. tenella* protozoits digestion by a synergistic action of pancreas chymotrypsin and papaine. The anti-inflammatory property of the *C. papaya* concentrated vitamin A on the other hand might act in caecal epithelium cell protection, detrimental to the coccidia reproductive activities. This can justify the improvement of survival rate recorded in *C. papaya* treated chick group compared with that of the infected untreated control

and *V. amigdalina* treated chick groups. The *C. papaya* anticoccidial effect was significant in oocysts excretion and mortality reduction. However, the observed oocysts per gram reduction of *C. papaya* constituted only 59.31% of the infected medicated control oocysts reduction.

V. amygdalina treatment reduced significantly the infected unmedicated control group oocysts per gram down to 38.6%. This reduction was slightly lesser than that observed in *C. papaya* treatment. Works done by Ademola and Eloff (2011) *in vitro*, revealed that the extract of *V. amygdalina* inhibited egg hatching and inhibited larval development and killed larvae of *Haemonchus contortus* in a concentration-dependent manner. Best-fit 50% lethal concentration (LC(50)) values were 957.0, 76.0, 524.0, 309.0 and 224.0 µg/ml for the acetone extract, and the butanol, hexane, chloroform and 35% water in methanol fractions, respectively, when tested against nematode eggs. Huffman (2003) noted that multiple parasitic affected chimpanzees chewed *V. amygdalina* pith. Further analyses have revealed chemical compounds such as sesquiterne lactones (Cimanga et al., 2004), vernoniosides and flavenoides, namely: luteolin, luteonin $7-O-\beta-$ glucuronoside and $7-O-\beta-$ glucoside, which are known for their anti-tumor activities (Prabhakar et al., 2006). The bitterness and the chemical compounds especially the alkaloids of *V. amygdalina* might enhance the gastrointestinal enzymes (chymotrypsin) production and the digestion of the sporozoits. In addition the vernonioside antiparasitic activity against *Eimeria* is also possible, for, many antiparasitic effect of *V. amigdalina* have been reported. Anti-protozoan and anthelmintic properties (Abosi and Raseroka, 2003; Adiukwu et al., 2011; Ogni et al., 2014), anti-tumour properties (Izevbigie et al., 2004; Sweeney et al., 2005; Song et al., 2005; Opata and Izevbigie, 2006), hepato-protective activity (Ojiako and Nwanjo, 2006; Arhoghro et al., 2009) and anti-bacterial effect (Hamil et al., 2003; Bolou et al., 2011) have been demonstrated. It is also reported hat *V. amygdalina* provide anti-oxidant benefits (Erasto et al., 2007) and enhance the immune system through cytokines and chemokines regulation (Sweeney et al., 2005). The herein observed *V. Amydalina* anticoccidial effect was only significant in oocysts excretion. However, this constituted 40% of the infected medicated control efficacy in terms of oocysts excretion reduction.

The treatment effect was significant on body weight gain in the first week following experimental infection and parallels previous reports (Youn and Noh, 2001). Bloody diarrhea was only affected by the conventional anticoccidial drug.

Conclusion

This preliminary anticoccidial activity study of *Carica papaya* and *Vernonia amygdalina* fresh leaves aqueous extract did reveal a *Eimeria tenella* oocyst reduction potential. But some parameters pertaining to the effective dose, the effective extraction procedure, the most effective part of the plant in terms of antiparasitic activity and the toxicity threshold need to be further investigated.

REFERENCES

Abosi AO, Raseroka BH. 2003. *In-vivo* antimalarial activity of *Vernonia amygdalina*. *British Journal of Biomedical Science*, **60**(2): 89-91.

Adang LK, ISAH Z. 2016. Prevalence of Eimeria species in local breed chickens in Gombe metropolis, Gombe State, Nigeria. *Int. J. Biol. Chem. Sci.*, **10**(6): 2667-2676.

Ademola IO, Eloff JN. 2011. Anthelmintic activity of acetone extract and fractions of *Vernonia amygdalina* against *Haemonchus contortus* eggs and larvae. *Tropical Animal Health and Production*, **43**(2) 521-527.

Arczewska-Wlosek A, Swiatkiewicz S. 2010. Response of Chickens Infected With Coccidiosis to Herbal Extracts

Mix Fed Singly or In Combination with Additives. XIIIth European Poultry Conference pp: 45- 50.

Adiukwu PC, Amon A, Nambatya G. 2011. Pharmacognostic, antiplasmodial and antipyretic evaluation of the aqueous extract of *Vernonia amygdalina* leaf. *Int. J. Biol. Chem. Sci.,* **5**(2): 709-716.

Arczewska-Wlosek A, Swiatkiewicz S. 2010. Response of chickens infected with coccidiosis to herbal extracts mix fed singly or in combination with additives. XIIIth European Poultry Conference pp: 45-50.

Arhoghro E, Ekpo KEM, Anosike EO Ibeh GO. 2009. Effect of Aqueous Extract of Bitter Leaf (*Vernonia amygdalina*) on Carbon Tetrachloride (CCl4) Induced Liver Damage in Albino Wistar Rats. *European Journal of Scientific Research*, **26**(1): 122-130.

Arvind G, Bhowmik D, Duraivel S, Harish G. 2010. Traditional and medicinal uses of *Carica papaya*. *J. Med. Car. Pap,* **1**(1): 2320-3862.

Attindéhou S, Houngnimassoun MA, Salifou S, Biaou CF. 2012. Inventory of herbal remedies used to control small ruminant parasites in Southern Benin. *International Multidisciplinary Research Journal*, **2**(8): 14-16.

Berezin VE, Bogoyavlenskyi AP, Khudiakova SS, Alexuk PG, Omirtaeva ES, Zaitceva IA, Tustikbaeva GB, Barfield RC, Fetterer RH. 2010. Immunostimulatory complexes containing *Eimeria tenella* antigens and Low toxicity plant saponins induce antibody response and provide protection from challenge in broiler chickens. *Veterinary Parasitology*, **167**(1): 28–35.

Beuth J, Ost B, Pakdaman A, Rethfeldt E, Bock PR, Hanish J, Schneider B. 2001. Impact of complementary oral enzyme application on the postoperative treatment results of breast cancer patients. Results of an epidemiological multicentre retrospective cohort study. *Cancer Chemotherapy and Pharmacology,* **47**: 45-54.

Bolou GEK, Bagré I, Ouattara K, Djaman AJ. 2011. Evaluation of the Antibacterial Activity of 14 Medicinal Plants in Côte d'Ivoire. *Tropical Journal of Pharmaceutical Research,* **10**(3): 335-340.

Bostvironnois C, Zadjian C. 2011. Coccidiose sub-cliniques en production de poulet de chair: Bilan et prospectives. Neuvième Journée de Recherche Avicole, Tours, 29 et 30 Mars 2011. pp: 585-588.

Cannavan A, Ball G, Kennedy DG. 2000. Nicarbazin contamination in feeds as a cause of residue in eggs. *Food Additive Contamination*, **25**: 829-836.

Chapman HD, Matsler PL, Muthavarapu VK, Chapman ME. 2005. Acquisition of immunity to *Eimeria maxima* in newly hatched chickens given 100 oocysts. *Avian Disease*, **49**(3): 426-429.

Cimamga RK, Tona L, Mesia K, Musuamba CT, De Bruyne T, Apers S, Hernan N, Miert VS, Pieters L, Totte J, Vlietink AJ. 2004. *In vitro* antiplasmodial activity of extracts and fractions of seven medicinal plants used in the Democratic Republic of Congo. *J. Ethnopharmacol*., **94**: 27-32.

Dakpogan HB, Salifou S. 2013. Coccidiosis prevalence and intensity in litter based high stocking density layer rearing system of Benin. *J. Anim. Plant. Sci.,* **17**(2): 2522-2526.

Danaher M, Campbell K, O'Keeffe M, Capurro E, Kennedy G, Elliott CT. 2008. Survey of the anticoccidial feed additive nicarbazin (as dinitrocarbanilide residues) in poultry and eggs. *Food Additive Contamination*, **25**: 32-40.

Ekanem AP, Obiekezie A, Kloas W, Knopf K. 2004. Effects of crude extracts of *Mucuna pruriens*

(Fabaceae) and *Carica papaya* (Caricaceae) against the protozoan fish parasite *Ichthyophthirius multifiliis*. *Parasitology Research*, **92**(5): 361-6.

Erasto P, Grierson DS, Afolayan AJ. 2007. Evaluation of Antioxidant activity and The fatty acid profile of the leaves of *Vernonia amygdalina* growing in South Africa. *Food Chemistry*, **104**: 636-642.

Hamill FA, Apio S, Mubiru NK, Bukenya-Ziraba R, Mosango M, Maganyi OW, Soejarto DD. 2003. Traditional herbal drugs of southern Uganda, 2: Literature analysis and antimicrobial assays. *Journal of Ethnopharmacology*, **84**: 57–78.

Huffman MA. 2003. Animal self-medication and ethno-medicine: Exploration and exploitation of medicinal properties of plants. *Proceedings of the Nutrition Society*, **62**: 371-381.

Izevbigie EB, Bryant TL, Walker A. 2004. A novel natural inhibitor of extracellular signal- regulated kinases and human breast cancer cell growth. *Experimental Biology and Medicine*, **229**(2): 163-169.

Kermanshai R, McCarry BE, Rosenfeld J, Summers PS, Weretilnyk EA, Sorger GJ. 2001. Benzyl isothiocyanate is the chief of sole anthelminthic in papaya seed extracts. *Phytochemistry*, **57**(3): 427-235.

Lanckriet A, Timbermont L, De Gussem M, Marien M, Vancraeynest D, Haesebrouk F, Ducatelle R, Van Immerseel F. 2010. The effect of commonly used anticoccidials and antibiotics in a subclinical necrotic enteritis model. *Avian Pathology*, **39**: 63 – 68.

Lee SH, Lillehoj HS, Park DW, Jang SI, Morales A, García D, Lucio E, Larios R, Victoria G, Marrufo D, Lillehoj EP. 2009. Induction of passive immunity in broiler chickens against *Eimeria acervulina* by hyper immune egg yolk immunoglobulin Y. *Poultry Sciences*, **88**: 562–566.

McDougald LR. 2003. In: Diseases of Poultry 11th edition, Y. M. Saif (eds) Iowa State press, Ames, IA USA.

Mortier L, Huet AC, Charlier C, Daeseleire E, Delahaut P, Van Peteghem C. 2005. Incidence of residues of nine anticoccidians in eggs. *Food Additive Contamination*, **22**: 1120-1125.

Ogni CA, Kpodekon MT, Dassou HG, Boko CK, Koutinhouin BG, Dougnon JT, Youssao AKI, Yedomonhan H, Akoegninou A. 2014. Inventaire ethno-pharmacologique des plantes utilisées dans le traitement des pathologies parasitaires dans les élevages extensifs et semi-intensifs du Bénin. *Int. J. Biol. Chem. Sci.*, **8**(3): 1089-1102.

Ojiako OA, Nwanjo HU. 2006. Is *Vernonia amygdalina* hepatotoxic or hepatoprotective? Response from biochemical and toxicity studies in rats. *African Journal of Biotechnology*, **5**(18): 1648-1651.

Okeniyi JA, Ogunlesi TA, Oyelami OA, Adeyemi LA. 2007. Effectiveness of dried *Carica papaya* seeds against human intestinal parasitosis a pilot study. *J. Med. Food*, **10**(1): 194-196.

Opata MM, Izevbigie EB. 2006. Aqueous *Venonia. amygdalina* Extracts Alter MCF-7 Cell Membrane Permeability and Efflux. *International Journal of Environmental Research and Public Health*, **3**(2): 174-179.

Prabhakar KR, Veeresh VP, Vipan K, Sudheer M, Priyadarsini KI, Satish RBSS, Unnikrishnan MK. 2006. Bioactivity guided fractionation of *Coronopus didymus*: A free radical scavenging perspective. *Phytomedicine*, **13**: 591-595.

Sacramento TI, Mensah GA, Adote HS. 2010. Effet antiparasitaire des graines de papaye (*Carica papaya*) chez l'aulacode (*Thryonomys swinderianus* Temminck, 1827) d'élevage : cas des

aulacodicultures du Sud-Bénin. *Int. J. Biol. Chem. Sci.*, **6**(4) : 2280-2293.

Shirley MW, Smith AL, Blake DP. 2007. Challenges in the successful control of the avian coccidian. *Vaccine*, **25:** 5540-5547.

Song YJ, Lee DY, Kim SN, Lee KR, Lee HW, Han JW, Kang DW, Lee HY, Kim YK. 2005. Apoptotic potential of sesquiterpene lactone ergolide through the inhibition of NF-κB signaling pathway. *Journal of Pharmacy and Pharmacology*, **57**(12): 1591-1597.

Soulsby EJL. 1986. *Helminths, Arthropods and Protozoa of domestic Animals* (7th Edn). Beiliere Tindal II: London; 231.

Stepek G, Buttle DJ, Duce IR, Lowe A, Behnke JM. 2005. Assessment of the anthelmintic effect of natural plant cysteine proteinases against the gastrointestinal nematode, *Heligmosomoides polygyrus, in vitro*. *Parasitology*, **130**(2): 203-211.

Sujikara l. 2000. *Andrographis paniculata* A paper presented at an International Conference on Tropical Agriculture for better health and environment at Kasetsart, University, Kampaengsaen, Nakornpathom, 29 Nov.-1 Dec. 2000. Thailand. pp. 7.

Sweeney CJ, Mehrotra S, Sadaria MR, Kumar S, Shortle NH, Roman Y, Sheridan C, Campbell RA, Murray DJ, Badve S, Nakshatri H. 2005. The sesquiterpene lactone parthenolide in combination with docetaxel reduces metastasis and improves survival in a xenograft model of breast cancer. *Molecular Cancer Therapeutics*, **4**(6): 1004- 2005.

Sørensen JT, Edwards S, Noordhuizen J, Gunnarson S. 2006. Animal production system in the industrialized world. Scientific and technical review. *OIE*, **25**(2): 493 – 503.

Tewari AK, Singh H, Sudan V, Rao JR. 2010. Recombinant surface antigen 2 (SAG 2) based serodetection of toxoplasmosis in cattle. In: Proceedings of XX national congress of veterinary parasitology, pp 42.

Youn HJ, and Noh JW. 2001. Screening of the anticoccidial effect of herb extracts against *Eimeria tenella*. *Veterinary Parasitology*, **96**: 257-263.