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In-vitro anticoccidial potential of *Saccharum officinarum* extract against *Eimeria* oocysts

[Potencial in vitro anti coccidial de un extracto de *Saccharum officinarum* contra oocistos de Eimeria]

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Abstract: Present study was conducted to evaluate the effect of aqueous methanolic extract from *Saccharum officinarum* on the sporulation and morphology of oocysts of four Eimeria species (*Eimeria tenella*, *E. necatrix*, *E. mitis*, *E. brunetti*) of poultry. Sporulation inhibition bioassay was used to evaluate the activity of *Saccharum officinarum* extract (SOE) on the sporulation of coccidian oocysts. In this assay, unsporulated oocysts were exposed to six concentrations of *S. officinarum* in 10% dimethyl sulfoxide solution (w/v; 10, 5, 2.5, 1.25, 0.625 and 0.31%) while DMSO and potassium dichromate solution ($K_2Cr_2O_7$) served as control groups. The Petri dishes were partially covered to allow the passage of oxygen and incubated at 25-29° C for 48 h, providing 60-80% humidity. The sporulation of the oocyst was confirmed by examining sporocysts under inverted microscope at 40x. Results showed anticoccidial activity of SOE against all *Eimeria* species as proved by its ability to inhibit the sporulation of the oocysts under laboratory conditions. Inhibition of sporulation was observed in dose dependent manner. *S. officinarum* extract at higher dose also damaged the normal morphology and shape of oocysts of *Eimeria* species.

Keywords: *Saccharum officinarum*, *in vitro*, sporulation, *Eimeria*, oocysts

Resumen: El presente estudio se llevó a cabo para evaluar el efecto del extracto metanólico acuoso a partir de *Saccharum officinarum* en la esporulación de los ooquistes y la morfología de cuatro especies de Eimeria tenella (*Eimeria*, *E. necatrix*, *E. mitis*, *E. brunetti*) de aves de corral. Bioensayos de la inhibición de la esporulación se utilizaron para evaluar la actividad de extracto de *Saccharum officinarum* (SOE) en la esporulación de ooquistes de coccidios. En este ensayo, los ooquistes no esporulados se expusieron a seis concentraciones de *S. officinarum* en solución de dimetil sulfóxido 10% (w / v; 10, 5, 2,5, 1,25, 0,625 y 0,31%), mientras DMSO y una solución de dicromato de potasio ($K_2Cr_2O_7$) sirvió como grupos de control. Las placas de Petri se cubren parcialmente para permitir el paso de oxígeno y se incubaron a 25-29° C durante 48 h, proporcionando el 60-80% de humedad. La esporulación de los ooquistes fue confirmado mediante el examen de esporoquistes bajo microscopio invertido a 40x. Los resultados mostraron actividad anticoccidial de SOE contra todas las especies de *Eimeria* como se ha demostrado por su capacidad para inhibir la esporulación de los ooquistes en condiciones de laboratorio. Se observó una inhibición de la esporulación de manera dependiente de la dosis. Extracto de *S. officinarum* en dosis más alta también dañó la morfología normal y la forma de ooquistes de las especies de *Eimeria*.

Palabras clave: *Saccharum officinarum*, *in vitro*, esporulación, *Eimeria*, ooquistes.

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INTRODUCTION

Avian coccidiosis, is probably the most expensive parasitic disease of poultry. Its causative agent is single-celled protozoon belonging to genus *Eimeria* having different species (Blake & Tomley, 2014; Chapman, 2014; Shivaramaiah *et al*., 2014). Seven species of *Eimeria* have been recognized to infect poultry and each species has its own characteristics according to site of infection, immunogenicity and pathogenicity. Coccidiosis causes heavy economic losses to commercial poultry farming and is thought to be the one of the most expensive infectious diseases of poultry (Masood *et al*., 2013). According to an estimate (Chapman, 2009), coccidiosis causes about $127 million losses to US poultry industry annually and likewise similar losses may occur worldwide. The disease is clinically characterized by bloody diarrhea, poor feed conversion ratio, low growth rate or poor weight gain. This has also been considered a contributory factor in the pathogenesis of other diseases (Bachaya *et al*., 2012). Infection to bird occurs when it ingests a sporulated oocyst from litter.

Coccidiosis is generally controlled by using anticoccidial drugs which are administered in feed of chickens (Blake & Tomley, 2014; Shivaramaiah *et al*., 2014). Success has been achieved by using these drugs but, main problem associated with their poor response is development of resistance in *Eimeria* species to the commonly available anticoccidial drugs (Abbas *et al*., 2012). In external environment, farmers usually fight against this disease applying disinfecttant compounds. Commonly used disinfect-tants include some phenolic products such as ammonia, methyl bromide and carbon disulfide. Toxic effects of these products represent a danger to the staff and health of birds and therefore their use has been restricted (Hilbrich, 1975; Williams, 1997). Because of widespread drug resistance constraint (Akhter *et al*., 2014), residual effects of drugs in meat of birds and toxic effects of disinfectants, scientist all over the world are shifting towards alternative approaches for the control of parasitic problems (Hamad *et al*., 2014).

In this context, many plants and herbal products have been found to have chemotherapeutic effect against coccidiosis in poultry and are being commercialized after a series of experimental trials for their validation and economically cheaper approach for the control of coccidiosis (Abbas *et al*., 2012; Zaman *et al*., 2012).

Saccharum officinarum is commonly known as the sugar cane as it produces abundant sweet juice. Immunological and therapeutic activities of sugar cane derived constituents and extracts have been exhibited in different animal studies. These include anti-thrombotic (Molina *et al*., 2000), anti-inflammatory (Ledon *et al*., 2007), anti-oxidant (Takara *et al*., 2002), anti-stress and immunomodulatory activities (El-Abasy *et al*., 2003), protective effects against avian coccidiosis (Akhtar *et al*., 2008), radiation induced injury (Amer *et al*., 2005) and reconstituting effects on the B-cells in cyclophosphamide induced immunosuppression in chickens (El-Abasy *et al*., 2003).

Recently, a number of *in vitro* experiments have proved remarkable anticoccidial effects of different herbal extracts and essential oils on inhibition of sporulation of coccidian oocysts. These results suggest formulate a herbal remedy for control of coccidiosis in birds (Remmal *et al*., 2011; Remmal *et al*., 2013). Keeping in view the diverse biological activities of sugar cane extracts, present study was conducted to evaluate *in vitro* potential of *S. officinarum* extract against sporulation and morphology of oocysts of *Eimeria* species.

MATERIALS & METHODS

Plant material

Stalks of fresh sugar cane (*Saccharum officinarum*) plants were purchased from the local market of Faisalabad (Pakistan), authenticated by a botanist of University of Agriculture, Faisalabad, Pakistan. Plant material was dried under shade and extracted with methanol in a Soxhlet's apparatus at 80º C. The crude methanolic extract was evaporated in a rotary evaporator under reduced pressure at 35º C. The extract was further dried by using freeze dryer and then stored at 4º C until used.

Collection of coccidial oocysts

Chicken guts naturally infected with coccidia were collected from outbreak cases of poultry farms and different poultry shops of Faisalabad. Contents collected from intestines were examined microscopically. The contents were placed in separate desiccators containing 25% laboratory grade sodium hypochlorite @ 4:1 for 25 minutes to discard debris. To remove the chemical, about four times more water was added to the desiccators and sediment was obtained. Coccidial oocysts were

extracted following the method described by Ryley *et al*. (1976).

Experimental design and sporulation inhibition assay

The experimental design used in the present study was approved by Department of Parasitology, University of Agriculture, Faisalabad review board, in accordance with approved published research ethics guidelines. An *in vitro* sporulation inhibition assay was used to examine the effect of *S. officinarum* on oocysts sporulation of different *Eimeria* species. In this assay, unsporulated oocysts were preserved in 2.5% potassium dichromate solution in Petri dishes obtaining a thickness of 6 mm and exposed to six concentrations (two fold serial dilutions) of *S. officinarum* in 10% DMSO solution (w/v; 10, 5, 2.5, 1.25, 0.625 and 0.31%) while, DMSO and potassium dichromate solution($K_2Cr_2O_7$) served as control groups. The Petri dishes were partially covered to allow the passage of oxygen and incubated at 25-29º C for 48 h, providing 60-80% humidity and maintained by placing water in two Petri dishes in the incubator. The contents of the Petri dishes were stirred off and on to ensure the oxygenation. The sporulation of the oocysts was confirmed by examining sporocysts under inverted microscope at 40x.

The numbers of sporulated and non-sporulated oocysts were counted and the percent sporulation was estimated by counting the number of sporulated oocysts in a total of 40 oocysts for each *Eimeria* species (*E. tenella, E. nectrix, E. mitis* and *E. brunetti*). In addition, the number of sporocysts within each sporulated oocyst and the number of abnormal sporocysts (in terms of shape and size) were counted. Three replications were made for each concentration and the whole experiment was repeated to confirm the results. The oocysts with 4 sporocysts was considered sporulated regardless the shape and size of the sporocysts. The oocysts were slightly flattened under the pressure of a cover slip to better illustrate morphology.

Statistical Analysis

Data were analyzed by mean ± SEM or one way analysis of variance (ANOVA) followed by Duncan's multiple range test used for detection of significance among groups. $P < 0.05$ was considered as statistically significant.

RESULTS

Different concentrations of SOE showed dose dependent inhibition for the sporulation of coccidial oocysts of different *Eimeria* species as compared to control groups, Control-I (DMSO) and Control-II ($K_2Cr_2O_7$), as shown in Figure 1. The statistical analysis showed that all dilutions of *S. officinarum* significantly inhibited the sporulation in all *Eimeria* species as compared to both control groups.

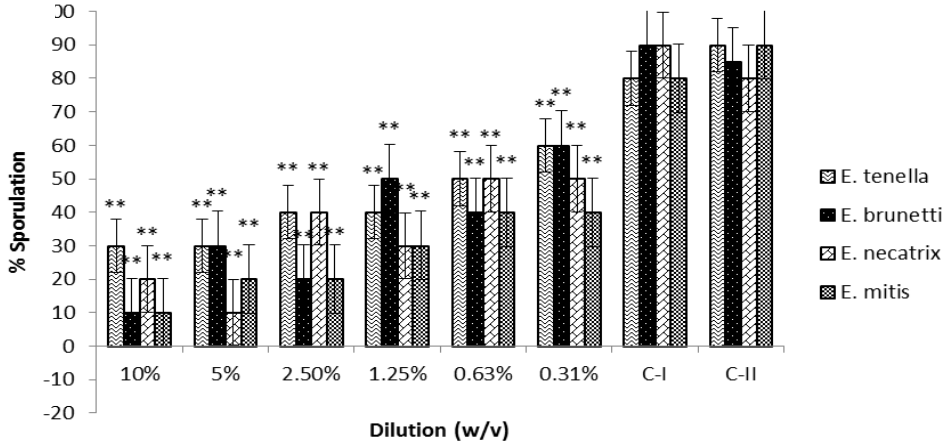

Figure 1

Effect of *S. officinarum* on percentage sporulation of oocysts of *E. tenella, E. brunetti, E. necatrix* and *E. mitis*. C-1 and C-2 served as percent groups containing DMSO (C-1) and $K_2Cr_2O_7$ (C-2). Results are the mean and standard error of means. **P < 0.0001, level of significance of the inhibitory effect was before compared with the untreated control groups.

Figure 1 shows that different dilutions of *S. officinarum* (10, 5, 2.5, 1.25, 0.62 and 0.31%) caused the inhibition of percent sporulation in dose dependent manner. Higher dose 10% of *S. officinarum* restricted sporulation percentage by 80% as shown in figure 1. As dose of *S. officinarum* decreased sporulation inhibition percentage also decreased respectively.

Figure 2 shows that there were no damaged oocysts in both control groups. However, like that of sporulation inhibition anticoccidial effect of SOE, dose dependent response of different dilutions of *S. officinarum* (10, 5, 2.5, 1.25, 0.62 and 0.31%) was also observed in terms of oocysts damage (abnormal size and shape) of *E. tenella*, *E. necatrix*, *E. mitis*and *E. brunetti*.

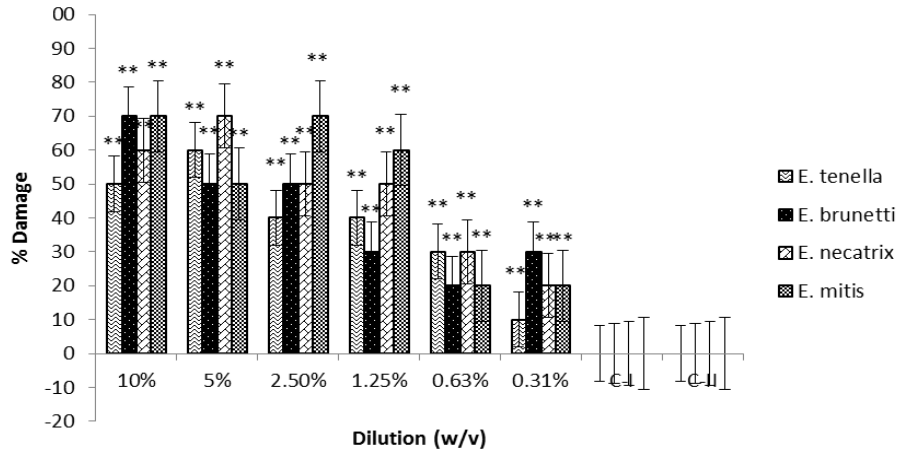

Figure 2
Effect of *S. officinarum* on % damage of oocysts of *E. tenella, E. brunetti, E. necatrix* and *E. mitis* oocysts. C-1 and C-2 served as control groups containing DMSO (C-1) and $K_2Cr_2O_7$ (C-2). Results are the mean and standard error of means. **P < 0.0001, level of significance of the damage oocysts was before compared with the untreated control groups.

DISCUSSION

In the recent years, botanicals have got great attention for the control and treatment of infectious diseases of animals (Pieri *et al*., 2014; Xiao *et al*., 2014). Many botanicals have anticoccidial potential like, *S. officinarum* (Fornazier *et al*., 2000), *Pinus radiata* (Wang *et al*., 2008; Molan *et al*., 2009) and *Aloe vera* (Molan *et al*., 2004, Narsih *et al*., 2012). *Saccharum officinarum* is rich in phenolic compounds like flavones (luteolin, apigenin and tricin derivatives), caffeic, hydroxycinnamic and sinapic acids (Fornazier *et al*., 2000). These components are known to have antioxidant, antiviral, antitumor, anti-proliferative, anti-inflammatory, anti-parasitic and antibacterial potential (Fujiki, 2005; Awais *et al*., 2011). Moreover, prophylactic activities of sugar cane (extracts/components) against infectious diseases may be exploited to minimize the use of antibiotics and/or anthelmintics in poultry birds. Earlier, sugar cane extracts have been reported for various biological activities including immunostimulation (Awais *et al*., 2011; Akhtar *et al*., 2012), anti-inflammatory (Ledon *et al*., 2007), vaccine adjuvant (El-Abasy *et al*., 2003), anti-oxidant (Takara *et al*., 2002), anti-thrombosis (Molina *et al*., 2000), modulation of acetylcholine release and anti-stress activities (Barocci *et al*., 1999).

Molan and Thomas (2007) reported similar *in vitro* effects of aqueous extracts from green tea on the sporulation of *E. tenella*, *E. acervulina* and *E. maxima* and found that addition of 10% and 25% (v/v) of tea extracts to the incubations containing unsporulated oocysts resulted in a significant reduction in sporulation rate. In addition, up to 30% of the oocysts recovered from incubations containing

25% of *S. officinarum* extract were with abnormal sporocysts (Molan & Thomas, 2007).

In an experiment *in vitro* effect of aqueous extract of *Thonningia sanguinea* on *E. tenella* and *E. necatrix* sporozoites cells invasions was evaluated and results showed that concentrations above 2.5 mg/mL inhibited invasions of sporozoites of *E. tenella* and *E. necatrix* on bovine kidney cells (Séverin *et al*., 2012). Similar *in vitro* results were reported by Molan *et al*. (2009), who evaluated effect of aqueous Pine bark extract on sporulation inhibition of *Eimeria* oocysts and results showed that Pine bark extract have potential to inhibit sporulation of *Eimeria* oocysts. Sugar cane (*Saccharum officinarum*) extract, a well known natural immunostimulant, is reported to have protective effects against *E. tenella* infection in chickens (El-Abasy *et al*., 2003; Hikosaka *et al*., 2007).

Present study also demonstrated the inhibitory potential of *S. officinarum* on sporulation of coccidian oocysts. Such findings demand for further investigations on sugar cane to identify the component(s) responsible for such activities. Results of present study suggests promoting further *in vivo* research to provide to a cheaper and less time consuming remedy for control of coccidiosis.

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REFERENCES

Abbas RZ, Colwell DD, Gilleard J. 2012. Botanicals: An alternative approach for the control of avian coccidiosis. **World Poult Sci J** 68: 203 - 215.

Akhtar M, Hafeez MA, Muhammad F, Haq AU, Anwar MI. 2008. Immunomodulatory and protective effects of sugar cane juice in chickens against *Eimeria* infection. **Turk J Vet Anim Sci** 32: 463 - 467.

Akhtar M, Hai A, Awais MM, Iqbal Z, Muhammad F, Haq AU, Anwar MI. 2012. Immunostimulatory and protective effects of *Aloe vera* against coccidiosis in industrial broiler chickens.**Vet Parasitol** 186: 170 - 177.

Akhter N, Arijo AG, Phulan MS, Iqbal Z. 2014. In vivo and in vitro studies on the efficacy of anthelmintics against *Haemonchus contortus* in goats. **Pak Vet J** 34: 329 - 332.

Amer S, Na KJ, Motubu M, El-Abasy M, Nakamura K, Koge K, Hirota Y. 2005. Radioprotective effect of sugar cane extract in chickens. **Phytother Res** 19: 496 - 500.

Awais MM, Akhtar M, Muhammad F, Haq AU, Anwar MI. 2011. Immunotherapeutic effects of some sugar cane (*Saccharum officinarum L.*) extracts against coccidiosis in industrial broiler chickens. **Exp Parasitol** 128: 104 - 110.

Bachaya HA, Raza MA, Khan MN, Iqbal Z, Abbas RZ, Murtaza S, Badar N. 2012. Predominance and detection of different *Eimeria* species causing coccidiosis in layer chickens. **J Anim Plant Sci** 22: 597 - 600.

Barocci S, Re L, Capotani C, Vivani C, Ricci M, Rinaldi L, Paolucci G, Scarpantonio A. 1999. Effects if some extracts on the acetylcholine release at the mouse neuromuscular joint. **Pharmacol Res** 39: 239 - 245.

Blake DP, Tomley FM. 2014. Securing poultry production from the ever-present *Eimeria* challenge. **Trends Parasitol** 30: 12 - 19.

Chapman HD. 2009. A landmark contribution to poultry science prophylactic control of coccidiosis in poultry. **Poult Sci** 88: 813 - 815.

Chapman, HD. 2014. Milestones in avian coccidiosis research: A review. **Poult Sci** 93: 501 - 511.

El-Abasy M, Motobu T, Sameshima K, Koge T, Onodera, Hirota Y. 2003. Adjuvant effects of sugar cane extracts (SCE) in chickens. **J Vet Med Sci** 65: 117 - 119.

Fornazier RF, Ferreira RR, Vitoria AP, Molina SMG, Lea PJ, Azevedo RA. 2000. Effects of cadmium on antioxidant enzyme activities in sugar cane. **Biologia Plantarum** 45: 91 -97.

Fujiki H. 2005. Green tea: health benefits as cancer preventive for humans. **Chem Res** 5: 119 - 132.

Hamad KK, Iqbal Z, Sindhu ZUD, Abbas RZ, Khan A, Muhammad G, Epperson B. 2014. Combination of *Nicotiana tabacum* and *Azadirachta indica*: A novel substitute to control levamisole and ivermectin resistant *Haemonchus contortus* in ovine. **Pak Vet J** 34: 24 - 29.

Hikosaka IK, EL-Abasy M, Koyama Y, Motobu M, Koge K, Isobe T, Kang C.B, Hayashidani H, Onodera T, Wang PC, Mastumura M, Hirota. 2007. Immunostimulating effects of the polyphenol-rich fraction of sugar cane (*Saccharum officinarum* L.) extract in chickens. **Phytother Res** 21: 120.

Hilbrich P. 1975. 'Disinfection Tests on *Eimeria Tenella* Oocysts. **Berliner Und Munchener Tierarztliche Wochenschrift** 88: 144 - 148.

Ledon N, Casaco A, Remirez D, Gonzalez A, Cruz J, Gonzalez R, Capote A, Tolon Z, Rojas E, Rodriguez VJ, Merino N, Rodriguez S, Ancheta O, Cano MC. 2007. Effects of a mixture of fatty acids from sugar cane (*Saccharum officinarum* L.) wax oil in two models of inflammation: Zymosan-induced arthritis and mice tail test of psoriasis. **Phytomed** 14: 690 - 695.

Masood S, Abbas RZ, Iqbal Z, Mansoor MK, Sindhu ZD, Zia MA, khan JA. 2013. Role of natural antioxidants for the control of coccidiosis in poultry. **Pak Vet J** 33: 401 - 407.

Molan AL, Sivakumaran S, Spencer PA, Meagher LP. 2004. Green tea flavan-3-ols and oligomeric proanthocyanidins inhibit the motility of infective larvae of *Teladorsagia circumcincta* and *Trichostrongylus colubriformisin vitro*. **Res Vet Sci** 77: 239 - 243.

Molan AL, Thomas DV. 2007. Green tea water extracts inhibit the sporulation of *Eimeria* oocysts *in vitro*. Advancing Poultry Production – **Proceedings of the Massey Technical Update Conference** 9: 89 - 97.

Molan AL, Zhuojian L, De S. 2009. Effect of pine bark (*Pinus radiata*) extracts on sporulation of coccidian oocysts. **Folia Parasitol** 56: 1 - 5.

Molina V, Arruzazabala ML, Carbajal D, Mas RS, Valdes S. 2000. Antiplatelet and antithrombotic effect of D-003. **Pharmacol Res** 42: 137 - 143.

Narsih, Kumalaningsih S, Wignyanto, Wijana S. 2012. Identification of aloin and saponin and chemical composition of volatile constituents from *Aloe vera (L.)* Peel. **J Agric Food Tech** 2: 79 - 84.

Pieri FA, Silva VO, Vargas FS, Veiga Junior VF, Moreira MAS. 2014. Antimicrobial activity of *Copaifera langsdorffii* oil and evaluation of its most bioactive fraction against bacteria of dog's dental plaque. **Pak Vet J** 34: 165 - 169.

Remmal A, Sanaa A, Latifa B, Najat C, Fouzia C. 2011. In vitro destruction of *Eimeria* oocysts by essential oils. **Vet Parasitol** 182: 121 - 126.

Remmal A, Sanaa A, Latifa B, Fouzia C, Najat C. 2013. Oocysticidal Effect of essential oil components against chicken *Eimeria* oocysts. **Int J Vet Med** 2: 133 - 139.

Ryley JF, Meade R, Burst JH, Robinson TE. 1976. Methods in coccidiosis research: Separation of oocysts from faeces. **J Parasitol** 73: 311 - 326.

Séverin KK, Alassane T, Karamoko O, Joseph DA, David NJ. 2012. *In vitro* anticoccidial activity of *Thonningia sanguinea* extract on *Eimeria tenella* and *Eimeria necatrix* sporozoites cells. **Afr J Microb Res** 6: 6247 - 6251.

Shivaramaiah C, Barta JR, Hernandez-Velasco X, Téllez G, Hargis BM. 2014. Coccidiosis: recent advancements in the immunobiology of *Eimeria* species, preventive measures, and the importance of vaccination as a control tool against these Apicomplexan parasites. **Vet Med Res Reports** 5: 23 - 34.

Takara K, Matsui D, Wada K, Ichiba T, Nakasone Y. 2002. New antioxidative phenolic glycosides isolate from Kokuto, non centrifuged cane sugar. **Bio Sci Biotech Biochem** 66: 29 - 35.

Wang ML, Suo X, Gu JH, Zhang WW, Fang Q, Wang X. 2008. Influence of grape seed proanthocyanidin extract in broiler chickens: effect on chicken coccidiosis and antioxidant status. **Poult Sci** 87: 2273 - 2280.

Williams RB. 1997. Laboratory Tests of Phenolic Disinfectants as Oocysticides Against the Chicken Coccidium *Eimeria Tenella*. **Vet Record** 141: 447 - 448.

Xiao CW, Ji QA, Rajput ZI, Wei Q, Liu Y, Bao GL. 2014. Antifungal efficacy of *Phellodendron amurense* ethanol extract against Trichophyton mentagrophytes in rabbits. **Pak Vet J** 34: 219 - 223.

Zaman MA, Iqbal Z, Abbas RZ, Khan MN. 2012. Anticoccidial activity of herbal complex in broiler chickens challenged with *Eimeria tenella*. **Parasitol** 139: 237 - 243.