Anthelmintic Activity Ethanol Extract of *Ocimum sanctum* Linn. Leaves Against *Ascaridia galli In Vitro*

Aktivitas Antelmintik Ekstrak Etanol Daun Kemangi *Ocimum sanctum* Linn. Terhadap *Ascaridia galli* Secara *In Vitro*

[1)]Vanna Lidya Kharisma, [2)]Setiawan Koesdarto, [3)]Koesnoto Supriandono, [2)]Lucia Tri Suwanti, [4)]Sri Agus Sudjarwo, [2)]Kusnoto

[1)]Student, [2)] Department of Parasitology, [3)]Departement of Agriculture Veterinary Medicine,
[4)] Department of Basic Medical Veteriner. Faculty of Veterinary Medicine, Universitas Airlangga

Abstract

The aims of this research are to determine concentration, exposure time, interaction between concentration and exposure time of ethanol extract of *Ocimum sanctum* Linn. Leaves to cause death toward *Ascaridia galli* in vitro, and the value of LC_{50} and LC_{90} ethanol extract of *Ocimum sanctum* Linn. Leaves. Research design that has been used in the research was completely randomized design. This research used 200 samples of *Ascaridia galli* with length 7-11 cm without differentiating their sex. The concentration ethanol extract of *Ocimum sanctum* Linn. leaves were 1.25%, 2.5%, 5%, 10%. The control was using CMC-Na 0.5%. Each treatment then being replicated four times. The observation and recording of dead worm were done at 0, 3, 6, 12 and 24 hours. *Ascaridia galli* were declared dead if there was no movement while disturbed by anatomy tweezers and dipped in slightly warm water (50ºC). The obtained data was analyzed using Anova Factorial and continued with Duncan Multiple Range Test by *SPSS for Windows 22*. The result were 10% concentration and exposure time for 24 hours caused the most mortality toward *Ascaridia galli.* Interaction between concentration and exposure time resulted 10% concentration ethanol extract of *Ocimum sanctum* Linn. leaves in 24 hours caused the most mortality towards *Ascaridia galli.* Probit analysis was used to calculate the LC_{50} and LC_{90} of *Ocimum sanctum* Linn. leaves. The results were LC_{50} ethanol extract of *Ocimum sanctum* Linn. leaves at 6 hours was 14.8%, at 12 hours was 4.8% and at 24 hours was 3.0% and the LC_{90} at 24 hours was 9.1%.

Key words: *Ocimum sanctum* Linn. leaves, *Ascaridia galli,* ethanol extract, in vitro.

Introduction

Nematodosis prevalence especially ascaridiasis caused by infection of *A. galli*, this worm attacks small intestine of domestic chicken especially in the traditional farm method and cause decreasing meat productions, which make farmers have financial loss (Parmin *et.al.,* 2002 and Subekti, 2011).

The disease is highly prevalent particular in world countries due to poor management practices. FAO (2001) showed that prevalence number of round-worms in Indonesia is ranged about 92.3% and 48% of them is *Ascaridia galli.* One of helminthiasis caused by Nematodes.

Most common drugs for anthelmintic treatment to treat ascaridiasis are Piperazine. Although these medicine are the most common drugs which used in field, various problems have been found in the parasite control using synthetic anthelmintic drugs, such as chemical residues, toxicity issues, long withdrawal period, possibility lead to resistance, not economical and unavailability of these drugs in remote areas (Hussain, 2008 and Veerakumari, 2015). Small holder farmers in many developing countries often do not have access to expensive anthelmintic drugs and low income farms are not able to prophy-

lactically treat animals with synthetic drugs. Therefore, there is an urgent need to investigate alternative or complementary options for the control of this parasite (Andrew et al., 2014).

Many researchers have been working to find the solutions. One of the works is by researching the anthelmintic activity derived from various herbal, included using *Ocimum sanctum* as one kind of herbal. Researchers have found many efficacies of *Ocimum sanctum,* including as anthelmintic. Recent work of its consumption has shown that holy basil had no genotoxic or organ toxic effects (Chandrasekaran *et al.,* 2013). Its anthelmintic activity has been tested against *Pheretema posthuma, Cotylophoron cotylophorum, Syphacia muris, Setaria digitata, Caenorhabditis elegans* (Goswami *et al*., 2016; Buchineni *et al.,* 2015; Pandey *et al.,* 2016; Karumari *et al.,* 2014; Joshi *et al.,* 2013; Verma *et al.,* 2013). However, it has not been done against Ascaridia galli. The anthelmintic activity presents because there are some phytochemical constituents which responsible for this efficacy. Those are tannins, alkaloids, flavonoids and saponin (Athanasiadou *et al*, 2001).

This research was aimed to know the concentration, exposure time, interaction between concentration and exposure time of ethanol extract of *Ocimum sanctum* linn. leaves which cause the most mortality toward *Ascaridia galli*, and to know its value of LC_{50} and LC_{90}.

Materials and Methods

Research Location and Date

This research was held at Laboratory of Parasitological Department Faculty of Veterinary Medicine Universitas Airlangga, Badan Penelitian dan Konsultasi Industri Surabaya, UPT Materia Medica Batu and Wonokromo Slaughter House in Surabaya. It was started in January 2017 and has been done in March 2017.

Research Materials and Equipments

Materials used in this research were leaves of *Ocimum sanctum* Linn. which was obtained from UPT Materia Medica Batu, *Ascaridia galli* from Wonokromo Slaughter House in Surabaya, PBS solution, 96% ethanol and CMC Na.

Equipments used in this research were petri dish with 15 cm diameter, anatomy tweezers, thermometer, baker glass, glass rod, oven, stove, grinder, rotavapor, plastic bag, electronic scale, incubator with 120°C capacity and camera for documentation.

Preparation of *Ocimum sanctum* Linn Leaves Ethanol Extract

Fresh *Ocimum sanctum* Linn leaves were collected from UPT Materia Medica Batu in January 2017. The *Ocimum sanctum* Linn leaves were washed thoroughly with fresh water and dried with oven in 50-60 °C. The dried *Ocimum sanctum* Linn leaves were mashed up with a grinder into powder and the powder was used for the extraction. The extraction was done in Badan Penelitian dan Konsultasi Industri, Surabaya.

Ocimum sanctum Linn leaves powder were macerated in 96 % ethanol for five days. Filtration was done to separate the dregs from the solution. Then the dregs were macerated again in 96 % ethanol (remacerated), maceration was performed three times and the pooled macerated then evaporated using a rotavapor at 50 °C for 4-5 hours to obtain a viscous extract. The ethanol extract was stored in 4°C until being used (Bachaya *et al.,* 2009).

Preparation of *Ascaridia galli*

Ascaridia galli were collected from the small intestine of domestic chicken in domestic chicken slaughtered house in Wonokromo traditional market, Surabaya then brought to Parasitology Laboratory, Faculty of Veterinary Medicine, Universitas Airlangga. *Ascaridia galli* were washed with PBS until they are separated from blood, tissue, debris or any attached particles (Amin *et al.,* 2009 and Bachaya *et al.,* 2009). Then, the alive and cleaned were used as the sample and were placed in the petri dish then ready to be treated.

Determination Ethanol Extract of *Ocimum sanctum* Linn Leaves Suspension Concentration

This research used four different concentration, those were 1.25%, 2.5%, 5% and 10%. These concentration was based on previous research which was done by Goswami *et al*., (2016).

Experimental Design

All 50 *Ascaridia galli* were divided into five groups by simple random sampling and done for four replication.

Control (C) : Ten *Ascaridia galli* were put in 40 ml of 0.5% CMC-Na solvent. Treatment 1 (T1) : Ten *Ascaridia galli* were put in 40 ml ethanol extract of *Ocimum sanctum* Linn leaves suspension with 1.25% concentration. Treatment 2 (T2) : Ten *Ascaridia galli* were put in 40 ml ethanol extract of *Ocimum sanctum* Linn leaves suspension with 2.5% concentration. Treatment 3 (T3) : Ten *Ascaridia galli* were put in 40 ml ethanol extract of *Ocimum sanctum* Linn leaves suspension with 5% concentration.Treatment 4 (T4) : Ten *Ascaridia galli* were put in 40 ml ethanol extract of *Ocimum sanctum* Linn leaves suspension with 10% concentration.

Time observations was in 0, 3, 6, 12 and 24 hours. During the observation, all the petri dishes were put in incubator with temperature of 37°C (Ali *et al.*, 2012; Chang *et al.,* 2015; Nassef *et al.,* 2014).

Observation of the Changes

In this research, death of the worms were observed by seeing its movement. If *Ascaridia galli* did not show any movement while disturbed by anatomy tweezers and while dipped in slightly warm water (50°C), the worm was confirmed dead (Ali., *et al,* 2012).

In this research, the obtained data was analyzed using Anova Factorial and continued with Duncan Multiple Range Test. While the LC_{50} and LC_{90} were calculated using probit analysis. SPSS 22 for windows software was used as statistical analysis program.

Result and Discussion

Effect of *Ocimum sanctum* Linn. Leaves Ethanol Extract Concentration toward Dead *Ascaridia galli*

The statistical analysis result showed that the death of *Ascaridia galli* influenced by the variation of ethanol extract of *Ocimum sanctum* linn. leaves concentrations, that are 1.25% (T1), 2.5% (T2), 5% (T3) and 10% concentration (T4). Based on Table 4.1 and Figure 4.1, the highest number of dead *Ascaridia galli* is in 10% concentration (T4).

According to transformation data in Table 4.1, control is significantly different with 1.25% (T1). T1 has significant difference with 2.5% (T2). T2 has significant difference with 5% (T3), and significant difference also occur between T3 and 10% (T4). It can be concluded that significant difference happens between every treatment groups.

Table 1. The Effect of Ethanol Extract of *Ocimum sanctum* Linn. Leaves to *Ascaridia galli* Which Cause Death Based on Concentration Treatment Groups.

Treatment Group	Mean ($\bar{x}$)	
	Data in Percent	Transformation Data
Control	00.00	0.71^{a}
1.25% (T1)	05.50	1.70^{b}
2.5% (T2)	16.00	3.04^{c}
5% (T3)	22.00	3.70^{d}
10% (T4)	36.50	4.77^{e}

^{a,b,c,d,e} Different superscript in the same column showed significant difference ($p < 0.05$).

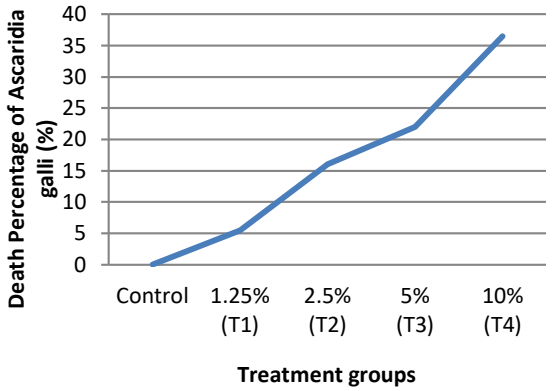

Figure 1. Graphic of Death Percentage of *Ascaridia galli* Based on Treatment groups.

Figure 2. Graphic of Death Percentage of *Ascaridia galli* Based on Observation Time.

Effect of Exposure Time toward Dead *Ascaridia galli*

Ascaridia galli death in this research is influenced by the exposure time of *Ascaridia galli* to the extract of *Ocimum sanctum* linn. leaves. The observation and recording of dead *Ascaridia galli* were done at 0, 3, 6, 12 and 24 hours.

Based on Table 1.1 and Figure 1.1, data in percent (%) and transformation data, the highest number of dead *A. galli* is at 24 hours. The column of transformation data showed that there is no significant difference between observation at 0 to 3 hours. The significant difference occur between observation at 3 to 6 hours, 6 to 12 hours, also between 12 to 24 hours.

Table 2. The Effect of Ethanol Extract of *Ocimum sanctum* Linn. Leaves to *Ascaridia galli* Which Cause Death Based on Observation Time

Observation Time (hours)	Mean ($\bar{x}$)	
	Data in Percent (%)	Transformation Data
0	00.00	0.71[a]
3	00.00	0.71[a]
6	06.50	2.03[b]
12	29.50	4.58[c]
24	44.00	5.90[d]

[a,b,c,d] Different superscript in the same column showed significant difference ($p < 0$.

Interaction between Exposure Time and Ethanol Extract of *Ocimum sanctum* linn. Leaves Concentration

The death of *Ascaridia galli* is affected by the interaction of ethanol extract of *Ocimum sanctum* Linn. leaves concentration and exposure time. From Table 4.3 and Figure 4.3, can be known that there is no significant difference of dead *Ascaridia galli* between every treatment group at 0 and 3 hours. At 6 hours, the result showed that there is no significant difference of dead *A. galli* between control to 1.25% concentration (T1) and T1 to 2.5% concentration (T2). Significant difference occur in T2 to 5% concentration (T3), and T3 also showed significant difference to 10% concentration (T4). Observation at 12 hours showed that control and 1.25% concentration (T1) have no significant difference in between. Comparing to control and T1, T1 to 2.5% concentration (T2) showed significant difference while T2 is not significantly difference to 5% concentration (T3). T3 showed significant difference to 10% concentration (T4). The last observation hours, at 24 hours showed that significant difference happen between almost every treatment groups, control to 1.25% concentration (T1), T1 to 2.5% concentration (T2), and between T3 to 10% concentration (T4). But not with T2 to 5% concentration

Table 3. The Interaction Between Observation Time and Concentration Level of Ethanol Extract of *Ocimum sanctum* Linn. Leaves to *Ascaridia galli* Death

Observation Time (hours)	Treatment	Mean ($\bar{x}$)	
		Data in Percent	Transformation Data
0	Control	00.00	0.71^{a}
	1.25% (T1)	00.00	0.71^{a}
	2.5% (T2)	00.00	0.71^{a}
	5% (T3)	00.00	0.71^{a}
	10% (T4)	00.00	0.71^{a}
3	Control	00.00	0.71^{a}
	1.25% (T1)	00.00	0.71^{a}
	2.5% (T2)	00.00	0.71^{a}
	5% (T3)	00.00	0.71^{a}
	10% (T4)	00.00	0.71^{a}
6	Control	00.00	0.71^{a}
	1.25% (T1)	00.00	0.71^{a}
	2.5% (T2)	02.50	1.34^{a}
	5% (T3)	10.00	2.93^{b}
	10% (T4)	20.00	4.45^{c}
12	Control	00.00	0.71^{a}
	1.25% (T1)	05.00	1.66^{a}
	2.5% (T2)	32.50	5.73^{d}
	5% (T3)	42.50	6.54^{de}
	10% (T4)	67.50	8.23^{g}
24	Control	00.00	0.71^{a}
	1.25% (T1)	22.50	4.70^{c}
	2.5% (T2)	45.00	6.74^{ef}
	5% (T3)	57.50	7.61^{fg}
	10% (T4)	95.00	9.77^{h}

^{a,b,c,d,e,f,g,h} Different superscript in the same column showed significant difference ($p < 0.05$).

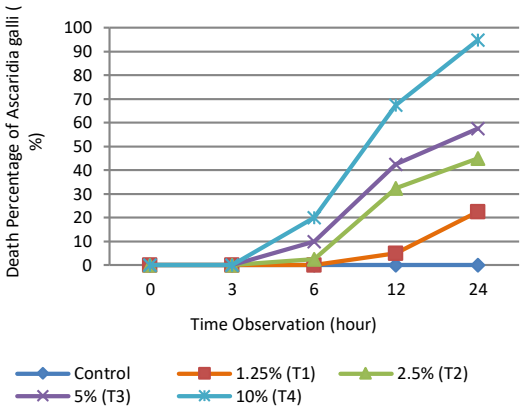

Figure 3. Showing The Interaction Between Observation Time and Concentration of Ethanol Extract of *Ocimum sanctum* Linn. Leaves to *Ascaridia galli* Death.

LC_{50} and LC_{90}

Ethanol extract of *Ocimum sanctum* linn. leaves LC_{50} and LC_{90} calculation is using probit analysis. From the calculation, the LC_{50} and LC_{90} can be known in every observation time that can be seen in Table 4.4. Based on Table 4.4, can be concluded that LC_{50} of ethanol extract of *Ocimum sanctum* Linn. leaves at 6 hours is 14.8%, at 12 hours is 4.8%, and at 24 hours is 3.0% concentration. The 10% concentration is enough to kill 50% of *Ascaridia galli* population at 6, 12 and 24 hours. The LC_{90} of ethanol extract of *Ocimum sanctum* Linn. leaves at 6 hours is 45.5%, at 12 hours is 14.7%, and at 24 hours is 9.1% concentration. The 10% concentration is enough to kill 90% of *Ascaridia galli* total population in the longest observation time, which is 24 hours.

Table 4. Various Concentration of Ethanol Extract of *Ocimum sanctum* Linn. Leaves Suspension That Cause Death of 50% and 90% *Ascaridia galli* Population in Every Observation Time

Observation Time (hours)	LC_{50}	LC_{90}
0	-	-
2	-	-
6	14.8%	45.5%
8	4.8%	14.7%
10	3.0%	9.1%

Discussion

Based on statistical analysis of the research result, there is no significant difference of dead *Ascaridia galli* between every treatment groups at 0 and 3 hours. At 6 hour, the result showed that the ethanol extract of *O. sanctum* Linn. leaves at 2.5% concentration (T2), 5% concentration (T3) and 10% concentration (T4) already has potential anthelmintic activity to kill *Ascaridia galli*, which means the onset of action of the ethanol extract of *O. sanctum* Linn. leaves has started at 6 hours. Those concluded based on the statistical analysis data which showed that control has no significant difference to T1 and T2 while T2 has significant difference to T3, also the T3 which significantly difference to T4.

Statistical analysis result at 12 hours showed there is no significant difference between control to 1.25% concentration (T1). T1 showed significant difference to 2.5% concentration (T2). But T2 is not significantly difference to 5% concentration (T3) which can be concluded that control to T1 has similar activity with T2 to T3. T3 showed there is significant difference to10% concentration (T4).

The result of observation at 24 hours revealed that the number of dead *Ascaridia galli* are keep increasing based on statistical analysis result. The interaction between observation of exposure time and concentration in control showed significant difference to all of treatments, so are in between 1.25% concentration (T1) to 2.5% concentration (T2). But not between 2.5% concentration (T2) to T3. This can be concluded that T2 has similar anthelmintic activity with T3. While 10% concentration (T4) is significantly difference to other treatments, control, T1, T2 and T3. From the data above, can be concluded that the highest average of dead *Ascaridia galli* were in 10% concentration (T4) at 24 hours of time observation..

Dead *Ascaridia galli* are not found in control group, which was CMC-Na dissolved in PBS. This might be happening because of CMC-Na is a suspensator that does not effect *Ascaridia galli* ability to live outside its host and also because of the duration of *A.galli* ability to live outside the host which known by the preliminary test result that was done before the main experiment started. The preliminary test resulted that *Ascaridia galli* could live outside its host up to ±40 hours while the main experiment was done in 24 hours because the anthelmintic effect of *O. sanctum* Linn. leaves towards *Ascaridia galli* only take 24 hours to kill almost all of the *Ascaridia galli* population in the highest concentration which was 10% concentration.

Ethanol extract of *O. sanctum* Linn. leaves has been proved to have phytochemical constituent that were beneficial to be anthelmintic. According to Karumari *et al.* (2014), *O. sanctum* Linn. leaves has anthelmintic activity. Its phytochemical constituents contain *tannin, phenol, flavonoid* and *saponin. Tannins* and *phenol* are known to interfere with the energy generation in helminth parasites by uncoupling oxidative phosporilation. Thus blocking ATP synthesis in helminth parasite then causing paralysis and death, also works bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of parasite and leading to death (Athanasiadou *et al.,* 2001). *Flavonoid* have pharmacological effect to vascular and causing capillary vasoconstriction and decreasing vascular permeability, because of these pharmacological effects, it causing vascular disturbance so the worm's nutrition and oxygen that required for the parasite sustainability will be disturbed and quickly quicken the mortality of the parasite. *Saponin* as an anthelmintic works by increasing the permeability and pore formation of the worm body wall, it causing vacuolization and disintegration cuticle (Sea, 2016).

Based on the statistical analysis of the result, the higher concentration of the extract and the longer the time of observation hours causing higher number of dead *Ascaridia galli.*

According to statistical analysis of the research result to determine the effective concentration can be concluded that 10% concentration for 24 hours is the most effective concentration to kill *Ascaridia galli* population.

Based on Table 4.4, can be concluded that LC_{50} of ethanol extract of *Ocimum sanctum* Linn. leaves at 6 hours is 14.8%, at 12 hours is 4.8%, and at 24 hours is 3.0% concentration. The 10% concentration is enough to kill 50% of *A. galli* population at 6, 12 and 24 hours. The LC_{90} of ethanol extract of *Ocimum sanctum* Linn. leaves at 6 hours is 45.5%, at 12 hours is 14.7%, and at 24 hours is 9.1% concentration. The 10% concentration is enough to kill 90% of *A. galli* total population in the longest observation time, which is 24 hours.

Conclusion

Based on research result, it can be concluded that ethanol extract of *Ocimum sanctum* linn. leaves with 10% concentration and exposure time for 24 hours caused the most mortality toward *Ascaridia galli.* Means that the interaction between concentration and exposure time stated that 10% concentration for 24 hours is the effective concentration and time to cause the most mortality towards *Ascaridia galli*. Meanwhile, the LC_{50} of ethanol extract of *Ocimum sanctum* linn. leaves is 14.8% at 6 hours, 4.8% at 12 hours and 3.0% at 24 hours. While the LC_{90} of ethanol extract of *Ocimum sanctum* linn. leaves is 9.1% at 24 hours.

References

Agarwal, C., N. L. Sharma and S.S. Gaurav. 2012. An Analysis of Basil (*Ocimum sp.*) to Study The Morphological Variability. Indian Journal of Fundamental and Applied Life Science, 3 (3), 521-525.

Ali, S.A., G.E. Mohammed and A.A. Gamel. 2012. In vitro Adulticidal Efficacy of Albendazole, *Capparis decidua* Stems and *Moringa oleifera* Leaves against *Fasciola gigantica.* Sudan University of Science and Technology Journal of Science and Technology, 13 (2), 59-67.

Andrew, R.W., C. Fryganas, A. Ramsay, Irene Mueller-Harvey, and M.T.Stig. 2014. Direct Anthelmintic Effects of Condensed Tannins from Diverse Plant Soures against *Ascaris suum.* PLoS One, 9 (5).

Athanasiadou, S., I. Kyriazakis, F. Jackson and R. L. Coop. 2001. Direct Anthelmintic Effects of Condensed Tannins towards Different Gastrointestinal Nematodes of Sheep: In vitro and In vivo Studies. Veterinary Parasitology, 99 (3), 205–219.

Buchineni, M., R.M. Pathapati, and J. Kandati. 2015. Anthelmintic Activity of Tulsi Leaves (*Ocimum sanctum* Linn.) An In vitro Comparative Study. Saudi J. of Med and Pharm. Sci, 2, 47–49.

Chandrasekaran, C.V., H.S. Srikanth, M.S. Anand, J.J. Allan, M.M. Viji and A. Amit. 2013. Evaluation of the Mutagenic Potential and Acute Oral Toxicity of Standardized Extract of *Ocimum sanctum* (OciBest™). Human and Exp. Toxicology, 32: 992-1004.

Chang, A.C.G. and M.J.C. Flores. 2015. Morphology and Viability of Adult *Fasciola gigantica* (Giant Liver Flukes) from Philippine Carabaos (*Bubalus bubalis*) upon in vitro Exposure to Lead. Asian Pacific J. of Trop Biomed, 5 (6), 493-496.

Chitwood, D. J. 2002. Phytochemical Based Strategies For Nematode Control[1]. Annual Review of Phytopathology, 40 (1), 221–249.

Ekeanyanwu, R. C and O. F. Etienajirhevwe. 2012. In vitro Anthelmintic Potentials of *Xylopia aethiopica* and *Monodora myristica* from Nigeria. African Journal of Biochemistry Research, 6 (9), 115–120.

Goswami, S., K. N. Mishra, A. Mishra, A.P. Singh, P. Singh, P. Singh. 2016. Comparative Assessment of In vitro Anthelmintic Studies of Some Plants From Indian Origin. Journal of Pharmacy Research, 10 (7), 514-518.

Hendrawati, A.R.E. 2009. Uji Toksisitas Akut Ekstak Etanol Daun Kemangi (*Ocimum sanctum* Linn.) Terhadap Larva *Artemia salina* Leach dengan Metode Brine Shrimp Lethality Test (BST), 1–35.

Hostettmann, K., and A. Marston. 2005. Saponins. Cambridge University Press, Medical, 564.

Hussain, A., D. O. F. Philosophy, F. Science. 2008. Evaluation of Anthelmintic Activity of Some Ethnobotanicals. Department of Parasitology, Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan.

Iglesias, L., V. Adela., and F. Javier. 1997. Some Factors which Influence The In vitro Maintenance of *Anisakis simplex* (Nematoda). Departemento de Parasitologia, Facultad de Farmacia, Universidad de Granada, Granada, Spain. Folia Parasitologica 44, 297 – 301.

Joint FAO/WHO Expert Committee on Food Additives (JECFA). 2001. Sodium Carboxymethyl Cellulose. 15 November 2017.

Joseph, B., and V. Nair. (2013). Ethanopharmacological and phytochemical aspects of *Ocimum sanctum* Linn. The Elixir of Life. British Journal of Pharmaceutical Research, 3 (2), 273–292.

Joshi, K. C., D. Nanda, P. Nainwal, and P. Saini. 2013. In vitro Anthelmintic Activity of *Ocimum sanctum*, International Journal of Pharma Sciences, 3 (4), 287–288.

Kamel, S., N. Ali., K. Jahangir, S. M. Shah and A. A. El-Gendy. 2008. Pharmaceutical Significance of Cellulose: A Review. Express Polymer Letters, 2 (11), 758-778.

Karumari, R. J., K. Vijayalakshmi and S. E. Balasubramanian. 2014. Preliminary Phytochemical Analysis and Anthelmintic Activity of Aqueous Extract of *Ocimum sanctum* (Linn.eaus, 1767) Leaves (Green and Black) Against *Cotylophoron cotylophorum* (Fischoeder, 1901). International Journal of Pharma and Bio Sciences, 5 (2), 580-587.

Katoch R., A. Yadav, R. Godara, J. K. Khajuria, S. Borkataki and S. S. Sodhi. 2012. Prevalence and Impact of Gastrointestinal Helminthes on Body Weight Gain in Backyard Chickens in Subtropical and Humid Zone of Jammu, India.

Khaled, B. and B. Abdelbaki. 2012. Rheological and Electrokinetic Properties of Carboxymethylcellulose-water Dispersion in the Presence of Salts. International Journal of Physical Science, 7 (11), 1790-1798.

Koche, D., S. Imran, R. Shirsat, and D. Bhadange. 2011. Comparative Phytochemical and Nutritional Studies of Leaves and Stem of Three Lamiaceae Members. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2 (3), 1-4.

Kombe, L., P. A. Abdu, J. O. Ajanusi. S. J. Oniye, and A.U. Ezealor. 2012. Effects of *Ascaridia galli* Infection on Body Weight and Blood Parameters of Experimentally Infected Domestic Pigeons (*Columba livia domestica*) in Zaria, Nigeria. Revista Cientifica UDO 960 Agricola, 12 (4), 960-964.

Kumar, N., A. Yadav, N. Aggarwal, and R. Gupta. (2016). Protective Effect of *Ocimum sanctum* Plant Extract Against DNA Damage Induced by Malathion in Cultured Human Peripheral Blood Lymphocytes. International Journal of Current Microbiology and Applied Sciences, 5 (5), 840–847.

Kusriningrum, R.S. 2008. Perancangan Percobaan. Airlangga University Press, Surabaya.

Luka S. A., I. S. Ndams. 2007. Gastrointestinal Parasites of Domestic Chicken *Gallus-gallus domesticus* Linnaeus 1758 in Samaru, Zaria Nigeria.Science World Journal, 2 (1), 27-29.

Matsuda. M., N. Suzuki, and S. Taniguchi. 2006. Small Molecule Inhibitors of α-Synuclein Filament Assembly. Biochemistry, 45 (19), 6085-6094.

Meyer, B.N., N.R. Ferrigni, J.E. Putnam, L.B. Jacobsen, D. E. Nicholas, and J.L. Mc Laughlin. 1982. Brine Shrimp: A Convenient General Bioassay for Active Plant Constituents. Planta Med May, 45 (5), 31-34.