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ANTHELMINTIC ACTIVITY OF *MELASTOMA MALABATHRICUM* LINN LEAVES AND *NIGELLA SATIVA* LINN SEEDS EXTRACT

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ABSTRACT

In this study, the in vitro anthelmintic activity of the Melastoma malabathricum leaves and Nigella sativa seeds extract were tested. Extracts from both plants were prepared using methanol extraction method. Five concentrations of each set of individual Melastoma malabathricum extract, individual Nigella sativa extract and a 1:1 mixture of both plants extracts were prepared using adult motility assay. Eudrilus eugeniae was used in the experiment while albendazole 40 mg/ml and distilled water were used as positive and negative controls, respectively. The time required for paralysis and motility of the worms were recorded. The results were recorded in mean and were analyzed using the one-way ANOVA. The extracts of the Melastoma malabathricum leaves and Nigella sativa seeds were shown to possess significant anthelmintic effect. However, the effects were more prominent at higher concentrations of the extracts. Meanwhile, the combination of both plant extracts had demonstrated a better anthelmintic effect than their individual plant extract. This could be due to the presence of the synergistic effect from the combination of both extracts. Thus, combination of Melastoma malabathricum and Nigella sativa extracts has the potential to be used as natural remedy to treat parasitic infestation.

Keywords: *Anthelmintic, Melastoma malabathricum, Nigella sativa, Eudrilus eugeniae*

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AKTIVITI ANTIHELMINTIK DARIPADA EKSTRAK DAUN *MELASTOMA MALABATHRICUM* DAN BIJI *NIGELLA SATIVA*

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ABSTRAK

Dalam kajian ini, aktiviti antihelmintik daripada ekstrak daun Melastoma malabathricum dan biji Nigella sativa telah diuji. Kedua-dua tumbuhan diestrak menggunakan kaedah metanol. Lima kepekatan daripada setiap set ekstrak individu Melastoma malabathricum, ekstrak Nigella Sativa dan campuran dalam nisbah 1:1 kedua-dua ekstrak disediakan untuk proses biocerakinan . Eudrilus eugeniae telah digunakan dalam eksperimen ini manakala Albendazole 40 mg/ml dan air suling, masing-masing digunakan sebagai kawalan positif dan juga kawalan negatif. Masa yang diperlukan untuk pengesahan lumpuh dan motiliti setiap cacing telah direkodkan. Keputusan telah direkodkan dalam min dan dianalisa menggunakan One-way ANOVA .Ekstrak daun Melastoma malabathricum dan biji Nigella sativa telah dikenalpasti untuk mempunyai kesan antihelmintik yang ketara. Walau bagaimanapun, kesan lebih menonjol pada kepekatan ekstrak yang lebih tinggi. Sementara itu, gabungan kedua-dua ekstrak tumbuhan itu telah menunjukkan kesan yang lebih baik daripada kesan ekstrak tumbuhan secara individu .Ini mungkin disebabkan oleh kehadiran kesan sinergi daripada gabungan kedua-dua ekstrak.Oleh itu, gabungan ekstrak Melastoma malabathricum dan Nigella sativa mempunyai potensi sebagai ubat semula jadi untuk merawat serangan parasit.

Kata kunci: *Anthelmintic, Melastoma malabathricum, Nigella sativa, Eudrilus eugeniae*

1.0 INTRODUCTION

Parasites which include the intestinal protozoa and helminthes are the organisms that infect the humans and animals. These pathogens penetrate the living things; causing damage to the host which favors the infections .The parasitic diseases which had infected almost majority of the world populations during the past century had been associated with many factors such as improper hygiene, bad eating behavior and poor socioeconomics (Ngui et al., 2011). Nevertheless, 4.5 billion peoples are estimated to be at risk of developing this infections and more than one billion peoples are claimed to be infected with parasites, majority of them are adolescent (Vercruysse et al., 2011). Most of peoples that were infected with the parasites came from those who have low income or poor (Lim et al., 2009).

Anthelmintic is the circumstances of eliminating the parasitic infections by using either synthetic drugs or natural remedies. However, the limited availability and expensive synthetic medicines make it difficult to treat the disease. Thus, the necessity of using traditional and cheap method could contribute to a better source of treatment. A part from that, due to the anthelmintic resistance that had developed, finding of effective natural remedies for curing the parasitic infections has also become necessary (Harhay et al., 2010).

Melastoma malabathricum that is known as *senduduk* among the locals belong to the family of Melastomataceae. The *Melastoma malabathricum* can be easily found in Malaysia and also in the tropic and moist region worldwide. Various part of the plant is used traditionally for the treatment of diarrhea (Suteki & Dwatmadji, 2011), anti-ulcers, to heal scars from smallpox and to treat piles (hemorrhoids) (Joffry et al., 2012). Some phytochemical compounds isolated from *Melastoma malabathricum* have also been reported to have antiulcerogenic (Zabidi et al., 2012), antiviral (Joffry et al., 2012), anthelminthic (Suteki & Dwatmadji, 2011) and anticoagulant effects (Joffry et al., 2012). The phytochemical compounds such as tannins, saponins and flavonoids are the major compounds that contributed in the anthelmintic activity of the plant (Tiwari et al., 2011).

Black cumin or scientifically known as *Nigella sativa* is an aromatic plant of the Ranunculaceae family (Khan, 1999). The *Nigella sativa* is widely grown in several part of the world including Europe, Africa and some parts in Asia. The parts of *Nigella sativa* such as, seed, oils and its extraction has been used widely in ancient Islamic system of herbal medicine as valuable remedies for different kinds of ailments (Javed et al., 2012).

Previous studies have shown that the seeds contain activities such as anti-inflammatory (Alemi et al., 2013), antitumor activities (Paraakh, 2010), antifungal (Mashahadian & Rakhshandeh, 2005) , antibacterial (Zuridah et al. , 2008), anthelmintic activities (Agrawal, Kharya, & Shrisstava ,1979; Al-Shaibani et al., 2008), antioxidant and hepatoprotective activities (Kanter, Coskun & Budancamanak, 2005). The phytochemical compound found in the seeds of the *Nigella sativa* are the essential oil which includes thymoquinone, tannins,

Flavonoids, saponins, glycosides and alkaloids that may have the activity of anthelmintic activity against parasites (Khan, 1999; Tiwari et al., 2011).

Previous researches have stated that *Melastoma malabathricum* has anthelmintic property this was followed by *Nigella sativa* that has also been demonstrated to have anthelmintic activity (Suteki & Dwatmadji , 2011; Al-Shaibani et al., 2008; Agarwal et al .,1979). Therefore, this study is designed to investigate the possibility of using both types of plant extracts as alternative anthelmintic agent.

2.0 OBJECTIVES:

1) To investigate the presence of anthelmintic activities of *Melastoma malabathricum* leaves and *Nigella sativa* seeds extract using *in vitro m*ethod.
2) To detect the presence of anthelmintic activity in *Melastoma malabathricum* leaves and *Nigella sativa* seeds extracts.
3) To investigate the effectiveness of the anthelmintic activity demonstrated by the combination of *Melastoma malabathricum* leaves and *Nigella sativa* seeds.

3.0 METHODOLOGY

3.1 Plants materials

Approximately 2 kg of the leaves of *Melastoma malabathricum* were collected at Phase 2 of Puncak Alam and within University of Technology Mara (UiTM) Puncak Alam campus, Selangor Darul Ehsan. This plant has already been identified and authenticated, and the voucher has been deposited at the Forest Research Institute Malaysia (FRIM), Kepong, Selangor. While the seeds of *Nigella sativa* was purchased at local store in Kuantan, Pahang.

3.2 Selection of earthworm

Earthworms selected for this study was the adult earthworms of *Eudrilus eugeniae* as it is easy availability and widely used in the *in vitro* anthelmintic study (Ogu, 2012; Fred-Jaiyesimi, Adepoju & Egbebunmi, 2011; Lakshmanan et al., 2011; Pagariya , Chatur & Nawab, 2013). The earthworms were purchased from local store in Puncak Alam area. The supplier supplied the earthworms of *Eudrilus eugeniae* species. The average measurement in length was about 5 to 10 cm that was used in the study. The bioassay of the anthelmintic activity was done *in vitro* with the used of the adult earthworms as resembled the anatomical and physiological of the intestinal worms (Danquah et al., 2012; Lakshmanan et al., 2011; Wadekar et al., 2010; Ali et al., 2011; Sprirdonov, 1992).

3.3 Preparation of the *Melastoma malabathricum* and *Nigella sativa* extraction

The clean *Melastoma malabathricum* leaves were shade dried for three days and followed by drying in an incubator at 55oC for 2 hours to complete the drying process (Lopez et al., 2003). The dried materials were weighed using an analytical balance and were ground in an electrical blender to powder form. Approximately 400 grams (400 g) of the powder material were dissolved in 500 ml of methanol in a container. The extracts was shaken using a horizontal shaker at 120 rpm and the extracts was allowed to sediment at room temperature with agitation for 72 hours (Zakaria et al., 2011). While, the *Nigella sativa* seeds were ground using electrical blender. Preparation of the *Nigella sativa* seeds extract was performed using the method of Mashhadian & Rakhshandeh (2005) and Zuridah, Fairuz, Zakri & Rahim (2008) with minor modification. 1500 ml of methanol (HmbG Chemicals, Germany) and 600 grams of *Nigella sativa* seeds powder were mixed and incubated for 1 week at 25oC with at least 5 times vibration per day.

Next, both of the products were filtered using Whatmann No 1 filter paper. The extracts were then concentrated under reduced pressure in a rotary evaporator where the temperature setting was at 84oC. The *Nigella sativa* crude extract was then dried further in a 50oC oven for 24 hours. Both of the *Melastoma malabathricum* and *Nigella sativa* crude extract obtained were prepared using five different concentrations of 100 mg/ml, 80 mg/ml, and 60 mg/ml, 40 mg/ml and 20 mg/ml.

3.4 Anthelmintic assay

The adult motility assay was carried out on individual extracts and on a 1:1 mixture of both extracts. The assay method from Ajaiyeoba et al, 2001 with simple modifications was done. Before starting the experiment worms were washes with normal saline to remove any interfering substances. The commercial Albendazole (Zentel) which was the standard drug solution was freshly prepared. The adult worms were released in a petri dish and the plant extracts for each concentration was poured into the dish and the time taken for the adult worm to show paralysis and death was recorded. Five petri dishes with six earthworms each were used with 15 ml of different concentrations to the extracts. For each concentration of plant extracts, the time of reaction of the adult worms were compared. Another two petri dishes used were for positive control that used a concentration of 40 mg/ml of albendazole and the negative control which uses the distilled water.

3.5 Statistical Analysis

The data obtained were analyzed using the one-way ANOVA of the SPSS (Statistical Package for Social Sciences) version 18.

4.0 RESULTS

The results of adult motility testing for all plants extract are shown on the table 1, 2 and 3. Based on the Table 1 it shows the recoded time in mean, concentration for *Melastoma malabathricum* methanol leaves extract at 20 mg/ml took about 2 hours and 38 minutes for paralysis and 3 hours and 50 minutes for death. As for 40 mg/ml concentration of methanol extract, the paralysis time was 2 hours and 4 minutes and the death time was 3 hours and 28 minutes. The 60 mg/ ml concentration showed that the paralysis time was 1 hour and 36 minutes followed by the death time of 2 hours and 45 minutes. The paralysis time taken for 80 mg/ml was 1 hour and 6 minutes and the death time was 1 hour and 43 minutes. As for the highest *Melastoma malabathricum* extract concentration which was 100 mg/ml, the paralysis time was decreased with 52 minutes and death time was 1 hour and 29 minutes.

Table 1: Anthelmintic activity of *Melastoma malabathricum* leaves extract

Treatment	**Concentrations**	**Mean(SD)time taken for paralysis (minutes)**	**Time taken for death (minutes)**
***Melastoma malabathricum* leaves methanolic extract**	20 mg/ml	158.39(4.17)	230.10(17.89)
	40 mg/ml	124.45(1.25)	208.39(5.50)
	60 mg/ml	96.45(7.38)	145.56(4.24)
	80 mg/ml	66.00(2.82)	103.31(6.88)
	100 mg/ml	52.38(9.69)	89.03(4.89)
Albendazole (control)	40 mg/ml	15.39(0.01)	36.47(2.82)

For each values it represents mean (Standard deviation); n=2 for each group.The one-way ANOVA reveals that the different concentrations of *Melastoma malabathricum* extract was significant as ($p< 0.05$).

While for *Nigella sativa* the first concentration at 20 mg/ml consumed the immobility time of the worms with 2 hours and 5 minutes and the death time was 3 hours and 30 minutes. As for 40 mg/ml concentration, the paralysis time was 1 hour and 26 minutes and the death time is 2 hours and 31 minutes. The 60 mg/ ml

concentration shows that the paralysis time was 1hour and 17 minutes followed by the death time of 1 hour and 44 minutes. The paralysis time taken for 80mg/ml was 53 minutes and the death time was 1 hour and 35 minutes. The highest concentration that was 100 mg/ml demonstrated at paralysis time was 38 minutes and the death of the worms was at 1 hour and 15 minutes.

Table 2: Anthelmintic activity of *Nigella sativa* seeds extract

Treatment	**Concentrations**	**Mean (SD)time taken for paralysis (minutes)**	**Mean(SD) time taken for death (minutes)**
***Nigella sativa* seeds methanolic extract**	20 mg/ml	125.71(13.24)	210.21(29.63)
	40 mg/ml	86.30(5.33)	151.91(4.89)
	60 mg/ml	77.30(7.11)	104.35(12.94)
	80 mg/ml	53.08(0.01)	95.12(1.53)
	100 mg/ml	38.75(9.32)	75.15(15.50)
Albendazole (control)	40 mg/ml	15.39(0.01)	36.47(2.82)

For each values it represents mean (Standard deviation); n=2 for each group. The one-way ANOVA reveals that the different concentrations of *Nigella sativa* extract was significant as ($p < 0.05$).

The table 3 shows at the 20 mg/ml concentration took about 1 hour and 17 minutes for paralysis and 2 hours for death. As for 40 mg/ml concentration of methanol extract, the paralysis time was 52 minutes and the death time was 2 hours and 26 minutes. The 60 mg/ ml concentration showed that the paralysis time was 42 minutes followed by the death time of 1 hour and 5 minutes. The paralysis time taken for 80mg/ml was 35 minutes and the death time is 49 minutes. The 100 mg/ml concentration given the paralysis time of 26 minutes and the death time was 37 minutes. On the other hand, paralysis time for the control was 15 minutes followed by the death time with 36 minutes. On top of that, the negative control that used the distilled water shows no effect on earthworms even after 5 hours of experiment.

Table 3: Anthelmintic activity of combination of *Melastoma malabathricum* leaves and *Nigella sativa* seeds extract.

Treatment	**Concentrations**	**Mean(SD) time taken for paralysis (minutes)**	**Mean(SD) time taken for death (minutes)**
***Melastoma malabathricum* leaves and *Nigella sativa* seeds methanolic extract**	20 mg/ml	77.66(5.04)	120.14(8.37)
	40 mg/ml	51.97(2.23)	86.96(19.28)
	60 mg/ml	42.43(4.05)	65.07(3.49)
	80 mg/ml	34.64(4.84)	49.50(6.96)
	100 mg/ml	26.49(1.44)	37.27(2.45)
Albendazole (control)	40 mg/ml	15.39(0.01)	36.47(2.82)

For each values it represents mean (Standard deviation); n=2 for each group. The one-way ANOVA reveals that the combination of both plants extract were significant as ($p < 0.05$).

5.0 DISCUSSION:

Anthelmintic is a condition of which elimination or expulsion of parasitic infections take place when exposed to certain medications or substance. In the recent days, the use of drugs in curing the parasitic infections had been used widely and effectively. According to Mali and Mehta (2007), the best anthelmintic drug should appealingly be of effective broad spectrum with reasonable price and causes less toxic to the living things. In addition, the best medicine to treat parasitic infections from the above statement is close to the natural remedies of herbal medicine. Hence, in this study, the potential of the *Melastoma malabathricum* and *Nigella sativa* extracts as candidate anthelmintic drug was evaluated.

An *in vitro* study which was done by Suteky and Datmadji (2011), had indicated that *Melastoma malabathricum* aqueous, hydro-ethanolic and chloroform leaves extracts had anthelmintic activity. The hydro-ethanolic extract was found to give the most potent effect in inhibiting egg hatch and larvae development of *Haemonchus contortus* (Suteky & Dwatmadji, 2011). However, an *in vivo* study as well as evaluation on the methanol extract of this plant has not been tested. As for the *Nigella sativa* seeds, a study by Shaibani et al. (2008), on anthelmintic activity was conducted using *in vivo* and also *in vitro* techniques with the aqueous and methanolic extraction method, had shown that the seeds have potential anthelmintic activity.

The biochemical components of the plants are the one that contributed to the process that inhibit the anthelmintic activity against the helminth. Previous study has demonstrated that the *Nigella sativa* seeds extract contained the phytochemical compounds such as tannins, alkaloids, polyphenols, saponine, glycoside, lipids, terpenoids, sesquiterphenoids, steroids, thymoquinone , dithymoquinone-cymeno and α pine that was contributed to its anthelmintic activity (Javed et al., 2012; Tiwari et al., 2011;Michel et al., 2010 ; Mali & Mehta, 2007; Gali-Muhtasib et al., 2006 ; Chitwood, 2002).

The anthelmintic assay was performed *in vitro* on adult African nightwalker (*Eudrilus eugeniae*) earthworms as it resembled the anatomical and physiological of the intestinal worms (Danquah et al., 2012; Lakshmanan et al., 2011; Wadekar et al.,2010 ; Ali et al., 2011; Sprirdonov, 1992). The earthworms are capable of moving using cilliary movements and its outer layer is mucilaginous layer that comprised of complex polysaccharides. The slimy layers enable the earthworm to easily moves. The damage to the mucopolysaccharides membranes will cause the outer membrane to be exposed this will lead to restriction of its movement and cause paralysis and later, death of the worms (Mulla, Thorat, Patil & Burade, 2010).

A part from that, *Melastoma malabathricum* leaves extract has also been reported to possess chemical compounds such as tannins, polyphenols, saponins, glycosides, terpenoids and steroids that favor the anthelmintic effect (Balamurugan, Nishantini & Mohsn, 2013; Joffry et al. , 2012, Tiwari et al.,2011, Zakaria, Rafiee, Mohamed, Teh & Salleh,2011; Susanti et al., 2008) Tannins is the polyphenolic compound possess anthelmintic effect that is capable of binding to the free proteins or glycoprotein inside the host gastrointestinal tract that can result to death of the parasites (Vidyadhar, et al., 2010).

In this present study, six earthworms were used for each petri dish of the different extract concentrations including the positive and negative controls. The experiment was done in replicate which includes two recorded time for paralysis and death for each assay. Approximately after 5 hours the experiment was terminated. Paralysis or immobility means that, when observed there were no wriggling movements except when vigorously shaken and the death were concluded when the worms lost their motility when placed into distilled water.

Based on the results of the anthelmintic activity of the *Melastoma malabathricum* methanol leaves extract can be observed. However, the activity was not comparable to the positive control. Also noted was that, as the concentration increased, it resulted in reduction of the paralysis and death time. Similar anthelmintic effect was seen with *Nigella sativa* seeds extract, whereby once the concentrations were increased, the effect was intensified. However, the anthelmintic activity was better as compared to *Melastoma malabathricum* leaves extract.

Interestingly, when the two plants extracts were combined, a better anthelmintic activity compared to those of the individual extract was observed. Figure 5.1 and figure 5.2 shows the paralysis and death of the worms that were achieved much faster for the combination of both extract respectively. As for the highest combination extracts concentration, 100 mg/ml, the paralysis time was 26 minutes and death time was 37 minutes. During this concentration, the death time was similar with the death time taken when using the albendazole 40 mg/ml as control. This proven that the anthelmintic activity from the combination extracts could effectively reach the time taken for the control but only at higher concentration.

The variety of the different result obtained can be due to different compound that responsible with the tested anthelmintic activity. Components proportion obtained could be differing in each extract. The several bioactive components in different plants are important and play a role in giving the variations of anthelmintic activity (Hrckova & Velebny, 2013). A recent study conducted by Wink (2012) had discussed on the synergistic effect from mixture of metabolites that contribute to the synergistic interactions of medicinal plants. This is supported with a study on green tea compound (epigallocatechin gallate) and membrane permealibising digitonin (*Digitalis purpurea*) on survival and death of *Plasmodium berghei* sporozoite. The typical green tea's polyphenol that combined with saponins compound from the digitonin decreased the death and survival of the *Plasmodium berghei* due to the synergistic effect (Hellmann, Münter, Wink & Frischnecht, 2010).

Similar observation of effective anthelmintic effect due to synergistic activity, that has been reported by other groups which involved the combination of coconut and onion using the polyethylene glycol/propylene carbonate (PEG/PC) extraction method which proved that they able to terminate all of the cestode inside the host. (Abdel-Ghafar et al., 2011). Another study by Caner et al. (2008) that used the methanol extracts of *Artemisia absinthium* and *Artemisia vulgaris* had demonstrated the reduction in the muscles of the *Trichinella spiralis* larval in rats which shown that it could be due to the synergistic effect of other plant components. Hence, in this study combination of the *Melastoma malabathricum* and *Nigella sativa* extracts may have exhibited a mild synergistic effect as at higher concentrations the effectiveness of the anthelmintic effect shown to be more effective compare to individual extracts.

6.0 CONCLUSION:

From this study, it can be concluded that the Melastoma malabathricum leaves, Nigella sativa seeds and combination of Melastoma malabathricum and Nigella sativa extracts possess potential anthelmintic activity. The bioassay performed showed that the combination of the two plant extracts exerted more anthelmintic effect than the individual plant extracts. However, the effects were more prominent

at higher concentrations of the extracts. The one-way ANOVA statistical analysis for the different concentration displayed a significant result as p-value is lowers than 0.05. This proved that all of the plants extracts exerted the potential anthelmintic activity. The combination of Melastoma malabathricum and Nigella sativa are possible to have anthelmintic effect. However, the experiment also needs to be supplemented with the in vivo investigation to ensure effectiveness of the extracts.

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