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Assessment of Kenaf Hibscus Cannabinus and Moringa Oleifera Against Haemonchus Contortus

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ABSTRACT

Helminthiasis and anthelmintic resistance in goat farming in malaysia. The objective of this study were to Assess, antioxidant and in vitro larvicidal activity of Kenaf (Hibiscus cannabinus) and Moringaoleifera leaves against Haemonchuscontortus. The Level of antioxidant in the leaves of Kenaf and Moringa leaves was determined by using HPLC and compared with quercetin as standard. The level of antioxidant for Kenaf, Moringa and quercetin (the standard) gave 353 ppm, 455 ppm and 375 ppm respectively. There was no significant difference among the means for the three. Larvicidal activity at different concentrations (10%, 20%, 30%, 40%, and 50%) of ethanolic extract of Kenaf and Moringa leaves were evaluated against the first and second stage larva of Haemonchuscontortus. The ethanol leaf extract of Kenaf and Moringa leaves inhibited 33.2% and 44.0% of the larva at 30% concentration respectively at ($P < 0.05$). In conclusion, the ethanol extracts of Kenaf and Moringa leaves possessed larvicidal activities against first (L1) and second (L2) stage larva ofH. contortus. Further in vivo studies are necessary to confirm the anthelminthic property of the leaf of these plants.

Keywords: KenafHibscusCannibus, HaemonchusContortus, MoringaOleifera

I. INTRODUCTION

Haemonchuscontortus is also one of the main causes of substantial economic losses in global small ruminants' production (Cantacessi et al., 2012). In Malaysia, helminthiasis is the second most important disease of small ruminants. Sani and Chandrawathani (1996) noted that the disease is responsible for high morbidity and mortality. Small ruminant production in Malaysia has been hindered by this parasite, causing deficiencies and imbalances of nutrients (Kaplan and Vidyashankar, 2012).

The acuteness of helminthiasis is often detected in both sheep and goats however; goats are more susceptible compared to sheep (Sani et al., 2004). In goats, mild infestation by helminth can result in malabsorption of nutrients, anorexia, poor digestion and diarrhea which resulted in poor growth. Local goat's farmers depend heavily on anthelmintics to control internal parasites in their small ruminants (Basripuzi et al., 2012). The frequent and indiscriminate use of anthelminthics by goat farmers has made the helminths resistant to anthelmitics. Thus resulted in remarkable increased in researches towards finding alternative control methods for helminthiasis (Kaplan and Vidyashankar, 2012).

In Malaysia, a wide range of anthelmintics, encompassing all the varieties of chemical groups, have been tested on nematode parasites of goats. These chemical groups of drugs include: macrocyclic lactones (e.g. ivermectin), benzimidazoles (e.g. albendazole, fenbendazoleetc.) imidothiazoles (e.g. ievamisole) and salicylanilides (e.g. closantel) (Chandrawathani et al., 1994). In a survey carried out on 9 goats and 39 sheep farms, it was found that majority of the flocks were infected with worms that were resistant to all categories of drugs

(Chandrawathani et al., 1999). Locally available herbal plants have the potential to be used as an alternative and non-chemical method for controlling gastrointestinal parasites in ruminant animals, thus enhancing their productivity. These include Kenaf (Hibiscus cannabinus) a newly introduced plant as a substitute for tobacco. Kenaf is an annual tropical plant belonging to the hibiscus family and closely related to cotton (Gossypiumhirsutum), okra (Abelmochusesculentus) and "bungaraya" (Hibiscus). Kenaf is tolerance to drought and adaptable to various local agro-climatic conditions. It is a fast growing plant, rising to heights of 3.0-3.2 meter in about 44.5 months. The dry matter yield of Kenaf can go up to 30/tons/ha/year, depending on variety, age, soil condition and rate of fertilizer application. Thus it has a great potential to be utilized as a fodder source for ruminant livestock (Wan Zahari et. al., 1999).

The multipurpose utilization and development of Kenaf as a potential crop has been the subject of numerous researchers in many countries including Malaysia. The main use of Kenaf is for making paper, paperboard, newsprint, carpet backing, automobile dashboards, high absorbing materials, insulators, packing materials and fabrics. The use of Kenaf as a feed source has been extensively reported (Chow et al., 2000).

Moringaoleifera is another plant which has potential to be utilized as an anthelminthic agent. Nearly all parts of this plant, the leaves, bark root, gum and fruit (pods), flowers, seed and seed oil have been utilised for varieties of ailments. Moringa plant has been used to treat infectious diseases and inflammation together with cardiovascular, haematological, gastrointestinal, and hepatorenal disorders (Amabye, 2015, and Onyekwere et.al, 2014. Additionally, seeds of Moringaoleifera are considered to be antipyretic, acrid, and bitter and were reported to show antimicrobial activity (Vidya et al., 2008).

Determination of active compounds /antioxidant

The leaves of the plants were collected around Bachok district, Kelantan. The Kenaf and Moringa leaves were collected from different plants respectively and sub sampled again for further analysis. The leave samples were oven-dried at 70°C for 16 h until a constant weight and ground to uniform powder using pestle and mortar. The aqueous extract of the sample was prepared by soaking 20g of dried powdered sample in 150 ml of distilled water for 24 h, on the bioshaker with 150 rpm/min. The extract was thereafter filtered using Whatman filter paper No 42 (125 mm). A total of 20g of the leave sample was extracted with 100 ml of 80% aqueous ethanol at room temperature. The solution was then filtered through Whatman filter paper No 42 (125 mm). The filtrate was later transferred into a rotary evaporator and evaporated into dryness over a water bath and weighed to a constant weight.

Phenolic compounds in the leaves extract of Kenaf and Moringa were analyzed using the HPLC-UV system (Agilent 1100 series, U.S.A.), equipped with a binary pump, array detector (diode array detector [DAD]) (200 to 600 nm range; 5 nm bandwidth) and auto sampler. The chromatographic separation was done through the prepacked LUNA C18 (4 × 250 mm, 5 μm); Phenomenex (Calif., U.S.A). HPLC conditions were as follows: Solvent A was distilled water; Solvent B was 50:50 (v/v) methanol: distilled water. The profile of the gradient program: 0 to 10 min, 50% solvent B. The injection volume was set to 20 μL at a flow rate of 1.0 mL/min. The UV detection wavelength was optimized to 254 nm.

Collection of Haemonchuscontortus Larvae.

The larvae of Haemonchuscontortus were collected from abomasum of infected goat in PengkalanChepa an Abattoir,then taken to the veterinary laboratory Universiti Malaysia Kelantan for recovery, quantification and identification of H.contortus using the procedure described by Hansen and Perry 1994.

Worms were washed and crushed to liberate eggs. The eggs were then cultured in a glass jar filled with autoclaved goat faeces for eight days at room temperature. At the end of 8th day, infective larvae were harvested by rinsing the side of the culture jar with a drop of water. About 3000 larvae were inoculated. The H.contortus the larvae were viewed under the microscope at x40 magnification.

Determination of the Larvicidal Activity

Aqueous and hydro-alcoholic extracts of the plant materials were used as the active treatment. Albendazole (99.8% pure standard reference) obtained from Drug Administration and Control Authority (DACA) was used as positive control. For the effect of plant extracts on L1 and L2 larvae of the nematode, using the procedures described above was followed 1ml of solution containing 20-30 larvae was used. Death or immobilization of the larval was assessed under a microscope (at 4× magnification) after 24hours. Larvae that showed no movement after 5-10 seconds of continuous observation were recorded as dead. The mortality rate (M%) was assessed using the formula, adopted from Hubert and kerboeuf. All these were replicate three times for treatment at different concentrations. 10,20,30,40,50.respectivitly.

Statistical Analysis.

Comparison of the Mean percentage of larval mortality rate after exposure to Moringa and Kenaf ethanol extracts were calculated also. Analysis of variance (ANOVA) was used to compare significant differences of the mean of larvae mortality rate that were exposed to the extracts of the two leaves. Statistical analysis was performed by using the software SPSS version 17.0. The post hoc statistical significance test employed was Duncan, differences between the means were considered significant at $P < 0.05$.

II. RESULTS

Level of antioxidant of Kenaf and Moringa Leaves

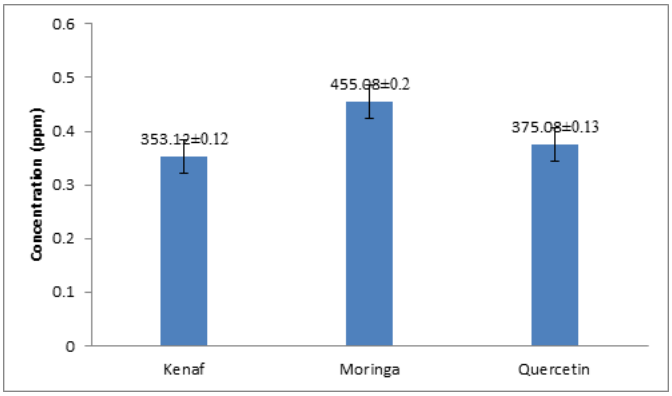

Figure 1. Concentration of antioxidant compounds in Kenaf and Moringa Leave extracts using Quercetin as a standard

*values are expressed as mean±SD of triplicates.

Natural antioxidants, including the carotenoids and vitamins A, E and C are important to sheep and goat health. They function as natural antioxidants to remove harmful free radicals that affect structural integrity of immune cells. However they regulate cellular events. Carotenoids and vitamins are of great importance to sheep and goat health by enhancing immunity. A compromised immune system will result in increased morbidity and mortality. The level of anti-oxidant in Moringa (Moringaoleifera) and Kenaf (Hibiscus cannabinus) leaves was higher than the standard quercetin with values of 455 ppm, 353ppm and 375 ppm respectively.

Larvicidal activity of ethanol extract of Moringa and Kenaf leaf

The result of the larvicidal activity of the ethanol extract of Moringa and Kenaf leaves is presented in Table 4.4. The result showed that, ethanol extract of Kenaf leaf at 20mg/ml concentration was more effective than that of Moringa with mortality rate of 31.6% and 22.3% respectively. While, the ethanol extract of Moringa leaf at 30mg/ml concentration was more effective than Kenaf with a mortality rate of 33.3% and 27.3% respectively. There was significant

difference (P<0.05) between Kenaf and Moringa leave extracts at 20mg/ml and 30mg/ml respectively.

Table 1. Larvicidal activities of ethanol extract of Kenaf, Moringa leaf and Albendazole at different concentrations.

Concentration (Mg/ml)	Mortality rate of Haemonchuscontortus larva (%)			
	Moringa	Kenaf	P value	Albendazole positive control
10	23.3±4.0*	29.3±4.0*	P<0.05	37.6±5.0
20	22.3±3.0*	31.6±5.0*	P<0.05	30.3±3.0
30	33.3±6.2	27.3±6.0	P>0.05	44.0±8.1
40	19.1±2.0*	27.0±5.0*	P<0.05	42.6±7.0
50	26.0±2.0	25.0±7.1	P>0.05	36.3±6.0

Note : Values are expressed as means ± SD of three replicates of mortality rate at different concentrations.
*significant at P<0.05

At concentration of 10, 20 and 40mg/ml, there was a significantly higher larvicidal activity of Kenaf ethanol extract on Haemonchuscontortus larvae in comparison to Moringa ethanol extract. Kenaf extract larvicidal activity was highest at 20mg/ml while performing better than both the control drug (albendazole) and Moringa extract at this concentration. There was no significant difference in the larvicidal activity of both Kenaf and Moringa ethanol extracts at the concentration of 30 and 50 mg/ml.The control drug (albendazole) had a better lavicidal activity than the extracts at these concentration.

III. DISCUSSION

The present study indicated that the level of antioxidant in Moringa leaf was higher (455 ppm) than the standard quercetin (353 ppm) and Kenaf leaf (375 ppm). Natural antioxidants including carotenoids, vitamins A, E and C are important to goat health by enhancing immunity. They function as natural antioxidants to remove harmful free radicals produced through normal cellular activity and from environmental stressors, thereby maintaining the structural integrity of immune cells. In addition, they may regulate cellular events. A compromised immune system will result in increased morbidity and mortality rates and thereby decrease animal production efficiency (Chow, 2000).

The balance between cellular oxidants and antioxidants is critical, with an imbalance leading to oxidative stress (OS) and damage to cellular lipids, proteins and DNA (Halliwell 2007). The antioxidant action of phenolic compounds is essentially due to their contributions in redox reactions by which they can absorb and neutralize free radicals. Animals with compromised antioxidant dynamics and with greater metabolic activity are more likely to be affected, since a sufficient supply of dietary antioxidants underpins favourable immune responses (Finch and Turner 1996), efficient energy utilisation and optimum mitochondrial function (Bottje and Carstens 2009).

Haemonchuscontortus (H. contortus) resistance against almost all chemical anthelmintics available for its control had been reported. H. contortus has a remarkable ability to develop resistance and threatens the viability of the goat industry in many regions of the world. Consequently, there is an urgent need to understand the genetic mechanisms underlying anthelmintic resistance and to discover new alternative methods of chemical and non-chemical control. With chemical anthemintics failing; this has led to the evaluation of plants as a natural source of anthemintic. Researchers are evaluating the

effectiveness of phytochemical compounds, called tannins. Condensed tannins (CT) and hydrolyzable tannins (HT) exist, but HT is metabolized to toxic by-products.

The larvicidal activities of ethanol extracts of Kenaf and Moringa leaves on Haemonchuscontortus larvae were assessed at 10, 20, 30, 40 and 50 mg/ml concentrations. Previous studies have indicated that Kenaf ethanol extract had significally higher (15.25%) larvicidal activity at 20mg/ml (Ravalli,et al., 2015). In this study, the larvicidial activity of Kenaf ethanol extract was highest at 20mg/ml in concordance with Ravailli et al., (2015). The extract however had a 16.42% which is 31.67% higher larvicidal activity than that reported by Ravailli et al., (2015). The variation in findings in this study is due to the lesser number of larvae per culture (22 – 30) used in contrast to 30 – 40 used by previous authors. This indicates that at 20mg/ml the 30 – 40 larvae in a culture would require more volume of the extract for it to render its larvicidal activity. Hence in the present study, the larval number has been reduced thereby resulting in a higher activity at the same volume and concentration as Ravalli et al., (2015). While larvicidial activity of Moringa leaf extract was highest at 30mg/ml in concordance with Mbogning, et al., 2014). Uniform activity of infused ethanolic extract of M. oleifera on larvae of H. contortus observed in this study was 33.33% at 30mg/ml. Hence in the present study, the larval number has been reduced thereby resulting in a higher activity at the same volume and concentration. These may be attributed by the presence of similar or related chemicals that possess the property in proportions that are almost equivalent. However, a different result was obtained by Wabo et al., (2013) who evaluated in vitro activities of acetonic extracts of leaves of three forage legumes on H. contortus because they obtained LC50s values below 0.9 and above 1 mg/ml for L1 and L2 larvae respectively. Macerated aqueous extract indicated a good activity on almost all the four stages of parasites. The minor differences that occur on ethanolic extracts may be as a result of the variance in solubility of the active compounds in the solvent or quite lesser in number of larvae cultured or number of larvae exposed to ethanol extract.Therefor, a highest concentration of the extract is require achieving the larvicidal goal.

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