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IN VITRO ANTHELMINTIC STUDY OF BAUHINIA ACUMINATA LINN. LEAF EXTRACTS AGAINST THE HOUSEFLY WORMS

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Abstract:

Bauhinia acuminataLinn. (Caesalpiniaceae) or its local name "Dwarf White Orchid", "TapakKuda" or "Safed Kachnar" is a recent medicinal plant discovered which has been employed by the folks in treating of different types of ailment. Therefore, the present study was made an attempt to investigate its anthelmintic quality against the housefly worms. The housefly worms were used as the experimental model due to its resemblance to parasitic pinworms. The advantages of this method are easy, economic, eco-friendly, prominent and reproducible method compared to conventional method. Aqueous and ethanolic extracts with different concentrations (12.5, 25, 50, 100 and 200 mg/ml) were tested on the Musca domesticaworms in vitro assay. Determination of paralysis and death time wasrecorded. Albendazole was used as standard and distilled water as a control. From the preliminary phytochemical evaluation, tannins and saponins were detected in both extracts. In vitro anthelmintic assay revealed both extracts have shown significant effect in paralysis and death of the housefly worms in a dose dependent manner. In comparison of test solutions, ethanolic extract was more effective than aqueous extract. As a conclusion, the leaves of Bauhinia acuminata could be used as an anthelmintic remedy, however, further studies are suggested to carry out in vivo using laboratory animals to confirm the safety, efficacy, and toxicity profile of this plant. Further screening investigation is recommended to isolate the bioactive and total phytochemical compounds contribute to the anthelmintic property of this plant.

Keywords: *Bauhinia acuminate Linn, Dwarf White Bauhinia, Albendazole and anthelmintic activity*

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INTRODUCTION:

Worm parasitic infection or medically termed as helminthiasis is a common infection in human beings as well as animals in which they might get infected through air, food, and water [1]. Several types of helminths known to be infested the human intestinal are generally from cestodes such as tapeworms (*Taeniasolium*), nematodes such as roundworm (*Ascarislumbricoids*), and hookworms (*Ancylostomaduodenale*), and trematodes such as flukes (*Schistomamansoni, Schistomahematobolium*) [2]. Current medications act as anthelmintic are included Albendazole, Ivermectin and combination therapy of Diethylcarbamazepine and albendazole which is categorized mainly as microfilaricidal. However, the treatment regimens possessed a little effect in killing the adult worms and it leads to another serious problem which is the development of resistance [3][4].

Dwarf White Bauhinia is one of the recent medicinal plants often called as "TapakKuda" or "Bunga Perak" by Malays, "Safed Kachnar" by Indians and scientifically named as *Bauhinia acuminata* Linn. It belongs to the more than 200 species in the genus Bauhinia. This plant comes from Caesalpiniaceae family which is discovered throughout India and nowadays widely planted in the tropics and warm regions [5] including Malaysia. It grows as a tree which has root, bark, leaves, flowers and fruits. It occurs widely in deciduous forests and scrubs. The leaves are green, bilobed, shaped like an ox or cow hoof; long and broad, with the apical cleft. The flowers are white in color with five petals, ten yellow tipped stamens, a green stigma and it has a fragrant smell. The fruit is a pod with 7.5 to 15 cm long and 1.5 to 1.8 cm broad, hard, flat, dehiscent and 10 to 15 seeded [6][7][8]. Recently, this plant is undergoing extensive cultivation due to its various uses. Over the years, the natives were using different parts of this plant as a traditional medicine. It is used traditionally as treatment of headache and high blood pressure by its flower and to relieve coughs by its root as well as various skin diseases, worms, tumors and diabetes [9][10].

However, there is no anthelmintic study has scientifically been tested on *Bauhinia acuminata* species. Therefore, this study evaluates the *in vitro* anthelmintic activity of the leaf extracts of the plant with the newly developed method using house fly worms.

MATERIALS AND METHODS:

Research design

The present study design involves collection and processing of *Bauhinia acuminata* leaf, screening of its phytochemical constituents and anthelmintic investigation of the standard reference and the leaf extracts against the housefly worms. Extraction, phytochemical screening, preparation of nutrient agar and anthelmintic activity were carried out.

Plant Material Collection and Identification:

Fresh leaves of *Bauhinia acuminata* is collected from Borneo part of Malaysia, specifically in Keningau area, Sabah. The plant sample was brought to Asia Metropolitan University and identified and confirmed by a botanist from the Biodiversity Department of University Putra Malaysia.

Preparation of Aqueous Extract:

The collected fresh leaves of *Bauhinia acuminata* are cleaned with water and shade dry for 15 days. The leaves extracts are prepared using maceration technique by adding 25g of the coarsely ground leaves in 1L beaker with the ratio 1:20 *w/v* of distilled. The mixture is soaked for 5 days in a refrigerator and filtered using a filter funnel with Whatmann filter paper No. 1. After filtration, the filtrates are placed on a water bath at 60^{o}C for concentration.

Preparation of Ethanolic Extract:

During maceration, 25g of *Bauhinia acuminata* coarsely ground leaves are dissolved in 1litre beaker with 500ml of 95% ethanol. The beaker is closed with aluminium foil and kept at room temperature for 5 days. After 5 days, the mixture is filtered using a filter funnel with Whatmann filter paper No. 1. The filtrates then are placed on water bath at 60^{o}C for evaporation.

Collection of Housefly Eggs:

*Musca domestica*sp. housefly eggs are placed in petri dishes and obtained from Institute of Medical Research, Kuala Lumpur with the permission of Dr.Lee Han Lim, Head of Entomology Department.

Preparation of Housefly Worms Medium:

The medium preparation is done by suspending 14g of nutrient agar powder in a 1L beaker with 500 ml of distilled water. The mixture is placed on the laboratory hotplate and stirred to allow all components to be fully dissolved. Once dissolved, the mixture is sterilized in laboratory autoclave at 121^{o}C for 15min. Amoxicillin 100µg/ml and Itraconazole 75µg/ml are added into the medium to prevent other microbial growth. After sterilization, 20 ml media are pouring into 20 petri dishes and allow for solidification. After solidified, a well at the centre is made with help of a borer which having 1cm diameter and keep in fridge until use.

Culturing of Housefly Worms:
Musca domestica housefly eggs are placed and culture in a well of each petri dish of nutrient agar media with equal age (3 days), size (3mm length) and weight (0.01 mg). The range of worms in each plate is 25 to 48 worms, but only 5 worms are used for control and each concentration of reference drug and extracts.

Qualitative Phytochemical Screening of *Bauhinia acuminate* Extracts:
About 1g of both aqueous and ethanolic extracts are dissolved in 50ml of distilled water in different test tubes and subjected to qualitative phytochemical screening applying the standard screening tests [11].

Carbohydrates Test (Fehling's Test):
To 5ml of distilled water, aqueous and ethanolic extracts solution is dissolved individually and filtered. The filtrates are then hydrolyzed with dil. Hydrochloric acid, followed by neutralizing with alkali and finally heat with Fehling's A and B solutions. The presence of reducing sugars is indicated by the formation of a red precipitate [11].

Cardiac Glycosides Test (Keller-Killani Test):
The aqueous and ethanolic extracts solution is treated with 2ml of glacial acetic acid containing one drop of Ferric Chloride solution. The mixture is added with 1 ml of conc. Sulphuric acid. Upon the appearance of a brown ring or the interface indicates the deoxysugar characteristic of cardenolides. Cardiac glycosides component is confirmed upon the appearance of a violet ring below the brown ring and a formation of greenish ring in the acetic acid layer [11].

Phytosterols and Terpenoids test (Salkowski's Test):
To 2 ml of the aqueous and ethanolic extracts solution are added with few drops of Chloroform and filtered. The filtrates then are treated with few drops of conc. Sulphuric acid, shake and allowed to stand. The appearance of a golden yellow colour confirmed the presence of a component of phytosterol, triterpenes [11].

Saponin Test (Foam Test):
Both aqueous and ethanolic extracts solution are shaking vigorously to produce foam. Saponin test is positive if the foam produced persists last for ten minutes [11].

Tannins Test:
About 5ml of both the extracts solution are boiled on a hotplate for few minutes and filtered. Few drops of 0.1% Ferric Chloride are added into the filtrates. The presence of tannin is confirmed with the development of a brownish green which indicates of condensed tannins or blue-black which indicates of hydrolyzed tannins [11].

Flavonoids and Phenolic Compound Test (Ferric Chloride Test):
Few drops of Ferric Chloride solution were added into the 5ml aqueous and ethanolic extracts solution. The appearance of a bluish black color confirmed the presence of flavonoids as well as phenolic compound [11].

Alkaloids Test (Wagner's Test):
About 0.5g of both aqueous and ethanolic extracts are dissolved individually in 5 ml dil. hydrochloric acid on water bath then filtered. Then, few drops of Wagner's reagent (Iodine in Potassium Mercuric Iodide) are adding into the particular mixture. The presence of alkaloids are confirmed with the formation of a brown/reddish colored precipitate [11].

Preparations of Drug and Extracts Solutions:
The standard drug, albendazole is used as the reference in the evaluation of anthelmintic study. Therefore, albendazole, aqueous and ethanolic extracts are prepared in five different concentrations from 12.5mg/ml, 25mg/ml, 50mg/ml, 100mg/ml and 200mg/ml using water as solvent respectively.

***In vitro* Anthelmintic Assay with Housefly Worms:**
Distilled water is used as a control solution; albendazole solution is used as the reference standard drug. Aqueous and ethanolic extracts of *Bauhinia acuminata*are used as the test solutions. About 0.2ml of each solution is added into the respective containers and time is noted for standard and test solutions. In each of the petridish consisting of 20 ml of the prepared, solidified and sterilized housefly medium, the housefly eggs are placed, covered and cultured for 72 hours. After 72 hours, an average of 32 white colour worms with an equal age, size and weight is hatched from the eggs. From these, five worms are taken into each small cubic shaped container named as control (C), albendazole, aqueous and ethanolic. The concentrations of the drug and extracts are noted on the respective container. Activity such as worm motility and paralysis activity are observed in each container. The motility is done after tapping the edges of the containers allowing the worms to move freely in the solutions. The arrest in the movement can be seen after paralysis while applying little pressure by tapping the edges. Meanwhile in the control container, the motility of

the worms is viable for at least 5 days. Parameters such as number of worms, age of worms, paralysis and death time are noted.

Data Analysis:
Statistical data is analyse using IBM SPSS version 22 and worm counts in each solution are generated, summarized in table and expressed as mean ± SEM. Meanwhile, the significance of different between standard reference, ethanolic and aqueous extracts are determined by independent t-test and it is considered as significant if $P < 0.05$.

RESULT AND DISCUSSION:

Phytochemical constituents of *Bauhinia acuminate*:
The results of phytochemical screening of both aqueous and ethanolic extracts of *B. acuminata* are shown in Table 1. The major phytoconstituents present in *B. acuminata* in both extracts are saponins and tannins.

Table 1: Results of preliminary phytochemical screening of different extracts of Bauhinia acuminata.

Constituent(s)	Test	Inference		Observation
		Aqueous	Ethanolic	
Carbohydrates	Fehling's Test	+	-	Formation of red precipitation
Cardiac Glycosides	Keller-Killani Test	-	-	-
Phytosterols	Salkowski's Test	+	-	Golden yellow coloration
Terpenoids				
Saponins	Foam Test	+	+	Persistent foams
Tannins		+	+	Greenish brown coloration
Flavonoids	Ferric Chloride Test	-	+	Bluish black coloration
Phenolic Compound				
Alkaloids	Wagner's Test	-	+	Formation of brown/reddish precipitate

(+) = Test component present; (-) = Test component absent

Anthelmintic effect of different extracts on *M. domestica* worms:
On the experimental observations, all housefly worms were cultured with same age (72 hours), and length (0.30 mm). The housefly worms can be seen motile and known to be appeared similar to parasitic pinworms in the intestine of human [12]. After 0.2ml of each prepared solution added into the respective containers, time of paralysis and death were noted. The results of the anthelmintic effect of reference drug and test solutions in different concentrations were generated by using IBM SPSS version 22 and shown in Table 2.
From the table 2, both ethanolic and aqueous extracts were showed their anthelmintic efficacy in a dose-dependent manner. Besides that, both were shown significantly different from standard reference drug albendazole. Ethanolic extract of *Bauhinia acuminata* in the concentration of 200 mg/ml has shown its anthelmintic efficacy at 47.53 for paralysis time and 55.10 for death time which is almost as effective as albendazole in the concentration of 12.5 mg/ml, which is illustrated in figure 1 & 2. As a comparison to albendazole, both extracts have low anthelmintic efficacy, but it possesses a great potential of anthelmintic properties. Whereas, in comparison between the tests solution, ethanolic extracts have great anthelmintic effect than aqueous extract.

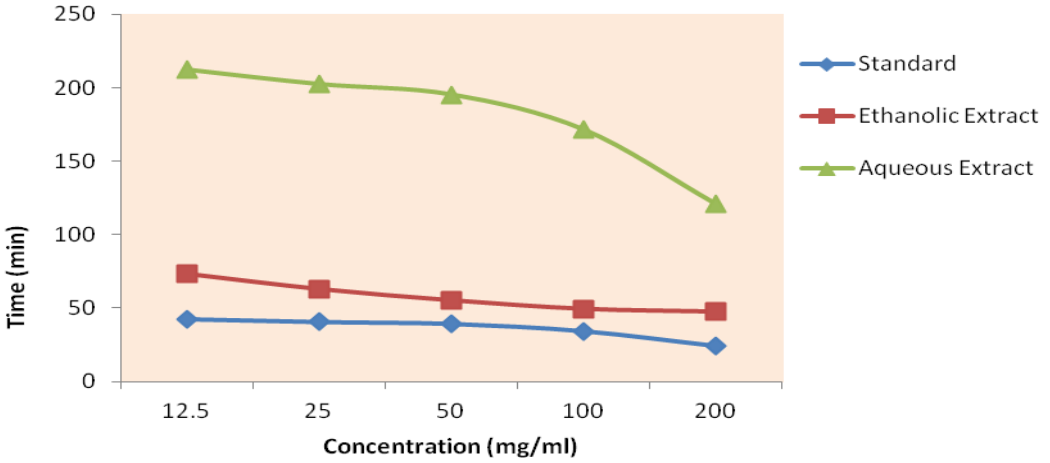

Figure 1: Comparison of Paralysis Time

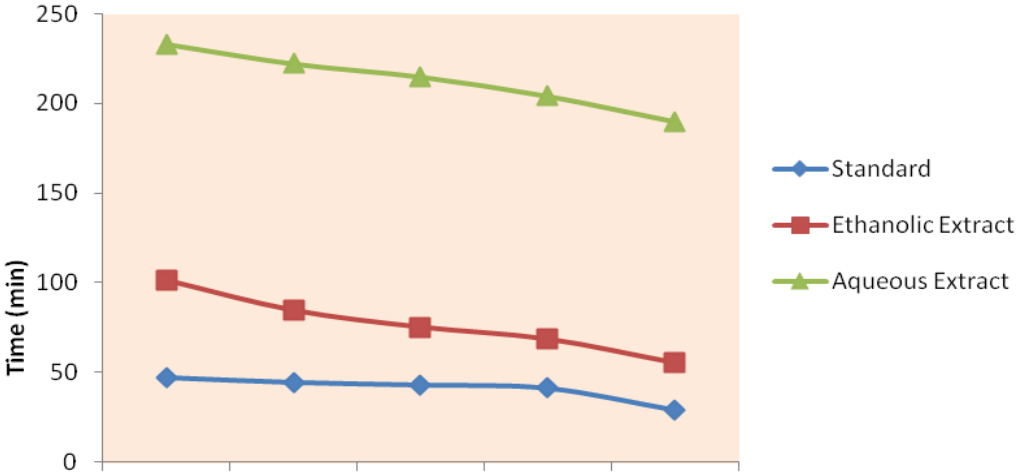

Figure 2: Comparison of Death Time

Study of phytoconstituents basically refers to chemical components that exist naturally in plants that shaped the colour and organoleptic characteristic. The essential nutrients in the plant are not established, but they possess the biological significance [13].The ethanolic extract possessflavonoids, phenolic compound and alkaloids. Carbohydrates, phytosterols, and terpenoids are present in aqueous extract. The phytochemical evaluation reveals that tannins and saponins are present in both extracts. The phytoconstituents like phenols, tannins and alkaloids are important in the anthelmintic properties of plants [14].

Table 2: Anthelmintic activity of standard reference drug and test solutions

Solution	Strength (mg/ml)	*Musca domestica* worms	
		Paralysis Time(min) Mean ± SEM	**Death Time (min) Mean ± SEM**
Control (Distilled Water)	–	–	–
Albendazole (Standard)	12.5	42.46 ± 0.30	47.18 ± 0.39
	25	40.59 ± 0.38	44.51 ± 0.93
	50	39.35 ± 0.98	43.05 ± 1.14
	100	34.45 ± 1.72	41.24 ± 1.40
	200	24.52 ± 1.89	29.06 ± 2.07
Ethanolic (Test)	12.5	73.46 ± 1.26**	101.14 ± 5.99**
	25	63.04 ± 4.51**	84.43 ± 3.18**
	50	55.31 ± 3.16**	75.03 ± 5.79**
	100	49.47 ± 1.74**	68.37 ± 4.04**
	200	47.53 ± 2.26**	55.10 ± 3.05**
Aqueous (Test)	12.5	212.04 ± 0.88**	233.25 ± 1.86**
	25	202.30 ± 1.29**	222.30 ± 4.05**
	50	195.26 ± 4.89**	215.02 ± 5.72**
	100	171.59 ± 8.23**	204.24 ± 7.02**
	200	121.27 ± 9.24**	190.02 ± 9.88**

Each value expresses as Mean ± SEM (N=5);
** = Values are significantly different from the standard reference solution

According to a review on the mechanism of action of some phytochemicals in a plant, the anthelmintic effect is might be due to the particular chemical compound detected during evaluation takes place. Tannins produce the anthelmintic effect by forming the protein complexes in rumen, thus to increase the digestible protein supply, cause the interference of energy generation by uncoupling oxidative phosphorylation and leads to reduction in the metabolism of gastrointestinal tract. In another study, anthelmintic activity produced by tannins is through binding of the free protein in the gastrointestinal tracts of host animal [15] or through glycoprotein on the parasite cuticle leads to death [16].

Meanwhile, alkaloids act as one of the important anthelmintic components by possessing anti-oxidating effect, responsible in the reduction of nitrate generation which is important in protein synthesis, suppression of sucrose transfer from the stomach to the small intestine, as well as the elimination of glucose support to the helminths. Apart from the two components, another important phytochemical is saponins in which it produces its anthelmintic effect by causing vacuolization and disintegration of teguments[14].

In the study of an anthelmintic activity of *B. acuminata,* housefly worm *M. domestica* was selected as in-vitro model and Albendazole was used as the standard reference drug. Albendazole produces the worms to starve and death by binding to the parasite protein tubulin and interfering with polymerization of microtubules [17][18]. The housefly worm model is the newly developed method and it was preferred over the conventional method due to its adaptability to laboratory conditions in which earthworm takes lesser time to paralyze and dead compare to housefly worm even though housefly worm is smaller and lighter than the

earthworm. Apart from that, this new method is easily obtained, prominent and reproducible in a sense of equality in size, age and weight of the cultured worms [12].

Based on the analysis result, both ethanolic and aqueous extracts were statistically significant ($P<0.05$). This shows that the plant possess pharmacological activity against housefly worms. In comparison of the test solution, ethanolic extract was more effective than aqueous extract where the lesser time is taken in paralysis and death of the housefly worms.

CONCLUSION:

The present study demonstratesthat both ethanolic and aqueous leaf extracts of *Bauhinia acuminata* have significant anthelmintic effect against the housefly worm *Musca domestica.*This study also revealsthe presence of carbohydrates and phytosterols in aqueous extract.Flavonoids, phenolic compounds, and alkaloids in ethanolic extract and presence of saponins and tannins in both extracts. Thus, based on the overall result of the present study, null hypothesis which stated earlier that leaf extracts of *Bauhinia acuminata* has no anthelmintic activity against the housefly worms is rejected. In-vivo studies using laboratory animals should be carried out so as to evaluate the efficacy of *Bauhinia acuminata.* Toxicity study of this plant should be studied in order to determine at which concentration of the plant extracts that could cause a toxic effect to the laboratory animals, thus the safety profile of this plant could be established. Further analysis should be conducted to isolate the bioactive compound which has the worm killing effect to be used as a safe anthelmintic drug.

The present study provides a brief idea which could be benefited to the researchers in further developing the study of this plant. Further information on this plant could be used as a platform for the pharmaceutical companies to develop a new alternative remedy in the treatment of worm infection.

Besides that, this study could help the society by assuring the safety of the plant to be used as part of traditional treatment or for quick medicament. This is because rural society usually takes some time to reach to the nearest hospitals or clinics. Therefore, with the information available on this plant and its efficacy, they could apply as temporary relief before they could get a proper treatment from the hospital or clinic.

The study would be useful to the traditional practitioners who seeking for a new remedy that is important in their practice and to update their knowledge. This is important in preventing interactions between the conventional and alternative treatment. The traditional practitioners could brief, suggest or discourage the use of this plant in combination of conventional therapy to the patients.

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