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ANTHELMINTIC ACTIVITY OF *ARTOCARPUS ALTILIS* EXTRACTS

Nissanti Goindasami*¹, Anandarajagopal Kalusalingam¹, Abdullah Khan¹, Krupavaram Bethala¹

¹School of Pharmacy, KPJ Healthcare University College, Persiaran Seriemas, Kota Seriemas, Nilai 71800, Negeri Sembilan, Malaysia.

ABSTRACT

The use of medicinal plants as traditional medicine to fight against diseases were in practice since ancient time. Medicinal plants are better choice for the treatment of various diseases including helminthic infections due to the increasing cost, side effects and multidrug resistance of modern anthelmintic drugs. *Artocarpus altilis* (family, Moraceae) has been empirically used by the people of Indonesia as traditional medicine for the treatment of various diseases. The present study was aimed to scientifically evaluate the anthelmintic potency of *A. altilis* leaf and fruit extracts. Ethanol and water extracts of *A. altilis* leaves and fruits were prepared by cold maceration method. The anthelmintic activity of *A. altilis* was assessed against the earth worms, *Pheretima posthuma*. The time taken to paralyze and kill the earthworms were recorded. All the results were compared with the standard drug, piperazine citrate. Ethanol extract of leaves and fruits of *A. altilis* exhibited higher *in vitro* anthelmintic activity in dose-dependent manner. The findings of this study scientifically justify the anthelmintic activity of *A. altilis* to eradicate nematodes from intestines.

KEYWORDS

Artocarpus altilis, *Pheretima posthuma*, Ethanol extract and Anthelmintic.

Author for Correspondence:

Nissanti Goindasami,
School of Pharmacy,
KPJ Healthcare University College,
Persiaran Seriemas, Kota Seriemas,
Nilai 71800, Negeri Sembilan, Malaysia.

Email: anandkarg@gmail.com

INTRODUCTON

Medicinal plants were used since ancient time for various therapeutic effects. Although there are many synthetic drugs available in the market, natural products from medicinal plants are still on demand¹. There has been a tremendous increase in finding novel drugs from natural sources². It is essential to discover new drugs from medicinal plants to treat helminthic infections because they are cheaper, have lower side effects and also due to an increase in parasitic resistance to conventional anthelmintic drugs³.

Artocarpus is one of the genus consumed as food and also as a traditional medicine. Due to their benefits to human health, there is tremendous scientific interest on phytochemicals present in this genus⁴. Artocarpus species such as *A. heterophyllus* (jackfruit), *A. altilis* (bread fruit), *A. hirsutus* (wild jack), *A. lakoocha* and *A. camansi* (bread nut) provides edible fruit. *A. altilis* belongs to *Moraceae* family and it is native of New Guinea, Moluccas (Indonesia) and Philippines. It is cultivated in most Pacific islands except in New Zealand and Easter Island⁵. The leaves, fruit, root bark and sap of *A. altilis* has been used as medicine. The people of Indonesia empirically used this plant as traditional medicine to treat various human ailments⁶. *A. altilis* has been reported for their therapeutic effects such as antifungal and antibacterial⁶, antiausteric⁷, anti-inflammatory⁸, anti-atherogenic⁹, antihyperglycemic¹⁰ and anti-hypertensive effects¹¹. It also can be used as an antioxidant¹² and mosquito-repellent against *Aedes aegypti*¹³.

The anthelmintic activity of the Artocarpus species in *Moraceae* family such as seeds of *A. heterophyllus*¹⁴, decoctions of *A. heterophyllus*¹⁵ and crude extract of *A. lakoocha*¹⁶ has been reported. *A. altilis* is used traditionally in Indonesia for killing worms, but there is no scientific investigation for the anthelmintic activity of *A. altilis* against the earth worms, *Pheretima posthuma*. Hence, the present study was undertaken to investigate the efficacy of leaves and fruits of *A. altilis* against helminthic infections.

MATERIAL AND METHODS

Plant Material

The leaves and fruits of *Artocarpus altilis* were collected from the local area of Nilai, Negeri Sembilan, Malaysia in the month of October 2017. The plant was authenticated by Miss. Rosni Ludin, Faculty of Forestry, University Putra Malaysia, Selangor, Malaysia (Ref No.: UPMFH/HERBARIUM / 08/ 17).

Preparation of Extract

The fresh leaves and fruits of *A. altilis* were separately cleaned, washed, and shade dried. The dried plant materials were ground into coarse powder. The coarsely powdered leaves and fruits were equally divided into two portions and

macerated separately with ethanol and distilled water by cold maceration method for 7 days. The extract was filtered using Whatmann grade 1 filter paper and concentrated using Rotary vacuum evaporator under reduced pressure¹⁷. The efficiency of the extraction was found out on dry weight basis of the concentrated extracts. The colour, consistency and the percentage yield of leaf and fruit extracts are tabulated in Table No.1. Both leaf and fruit extracts were stored at 4°C in refrigerator until further use¹⁸.

Collection of earthworms

The earthworms, *Pheretima posthuma* were collected from moist soil of Bagan Lalang, Sepang. The earthworms were washed and removed of the dirt and fecal matter using normal saline solution. According to the experimental protocol of this method, the earthworms of 2-4 cm in length and 0.2-0.3 cm in width was used. The earthworms, *P. posthuma* were used because it's anatomy and physiology resembles the human intestinal parasitic roundworms¹⁴.

Anthelmintic Activity

Two different concentrations (50mg/mL and 100mg/mL) of each extract of leaf and fruit of *A. altilis* and reference standard, piperazine citrate¹⁹ were used to assess the anthelmintic activity *in vitro* against *Pheretima posthuma* earthworms. The worms were divided into 11 groups of 6 worms each. The selected earthworms were placed in the respective petri dishes containing 10mL of distilled water, two different concentrations of the extracts and piperazinecitrate. The worms of group I were released into a plate containing distilled water which was served as control. The worms of group II-III were treated using piperazine citrate (50 mg/mL and 100 mg/mL) and served as standard. The worms of group IV-VII and VIII-XI were released into the petridish containing the respective extracts of *A. altilis* leaf and fruit (50 mg/mL and 100mg/mL). The time taken to paralysis and death of individual earthworms were observed and recorded. Time taken to paralyze the earthworms was noted when there was no movement of the worms even after transferred to distilled water. Death was determined when the worms lost their motility completely by either failed to respond even after touched with the needle or placed in warm

water (50°C). This was followed by fading of their body color. The experiment was conducted for 120 minutes²⁰. All the results as mean \pm SEM are tabulated in Table No.3.

Statistical Analysis

Statistical analysis was carried out using Dunnett's One-Way ANOVA test using SPSS software version 20. The respective extracts were compared with the corresponding concentration of reference standard by calculating the significant difference between the groups (P values) at the statistical significant levels of 0.01 and 0.001²¹.

RESULTS

The colour, consistency, and percentage yield of *Artocarpus altilis* leaf and fruit extracts are tabulated in Table No.1. Water extract of leaf was non-sticky semisolid, whereas ethanol extract of leaf and both water and ethanol extracts of fruit were sticky and semisolid in nature. Among the four extracts, water extract of leaf had highest percentage yield (16.42%) whereas the ethanol extract of fruit had lowest percentage yield (5.68 %).

In the present study, *A. altilis* leaf and fruit extracts were assessed to demonstrate their anthelmintic efficacy in paralyzing and killing the earthworms and the results are recorded in Table No.2. An effective anthelmintic activity was observed against worms treated with ethanol extract of leaf and fruit by paralyzing and killing the earthworms. Ethanol extract of leaf and fruit at a concentration of 50 and 100mg/mL showed non-significant difference ($p>0.05$) in time to paralyze and to kill the earthworms when compared with the standard drug, piperazine citrate. A dose-dependent anthelmintic activity was also noted in the group treated with ethanol extract of *A. altilis*. No considerable anthelmintic activity was observed against worms treated with water extract of leaf and fruit (100mg/mL) as a high significant difference ($p<0.001$) was noted when compared to the standard drug. Also no anthelmintic activity was observed against worms treated with water extract of leaf and fruit (50mg/mL) because there was no paralysis or death of worms was noted even when the experiment was carried out for 120 min.

DISCUSSION

The yield of various extracts of *A. altilis* leaf and fruit were ranged from 5.68% to 16.42% (Table No.1). The water extract of *A. altilis* leaf and fruit marked the highest percentage yield than ethanol extract of *A. altilis* leaf and fruit. This indicated that the secondary metabolites may be greatly extracted and highly soluble in polar solvent²². As medicinal plant under study is claimed to have numerous therapeutic activities such as anthelmintic efficacy, various extracts of *A. altilis* were investigated for their anthelmintic potency. *Pheretima posthuma* adult earthworms were used in this study because it has anatomy and physiology that resemblance to intestinal parasitic nematodes¹⁴.

The ethanol extract of leaf and fruit of *A. altilis* (50mg/mL and 100mg/mL) showed comparable time to paralyze and to kill the worms with the standard drug, piperazine citrate which is evident by non-significant difference ($p>0.05$) in anthelmintic activity (Table No.2). Ethanol extract exhibited effective anthelmintic activity by paralyzing and killing the earthworms than water extract, and also showed dose-dependent anthelmintic activity. This may be due to the higher concentration of phytochemicals present to produce the effective anthelmintic activity. The results revealed that ethanol extract of leaf and fruit of *A. altilis* have more pronounced anthelmintic activity than water extract by inhibiting motility and extermination of the earthworms.

Table No.1: Colour, consistency and % yield of *Artocarpus altilis* extracts

S.No	Extracts	Colour	Consistency	% yield
1	Ethanol extract of Leaf	Reddish brown	Sticky semisolid	5.82
2	Water extract of Leaf	Reddish dark brown	Non-sticky semisolid	16.42
3	Ethanol extract of Fruit	Pale green	Sticky semisolid	5.68
4	Water extract of Fruit	Brownish yellow	Sticky semisolid	9.57

Table No.2: Anthelmintic activity of *Artocarpus altilis* extract

S.No	Groups	Treatment	Concentration (mg/ mL)	Time taken for paralysis (min)	Time taken for death (min)
1	I	Control (normal saline)	-	-	-
2	II	Piperazine citrate	50	5.52 ± 0.2	6.56 ± 0.3
	III		100	3.91 ± 0.1	5.08 ± 0.3
3	IV	Ethanol extract of Leaf	50	6.19 ± 0.7 ^{NS}	6.97 ± 0.2 ^{NS}
	V		100	3.23 ± 0.3 ^{NS}	4.20 ± 0.3 ^{NS}
4	VI	Water extract of Leaf	50	-	-
	VII		100	15.72 ± 1.4 ^{**}	28.21 ± 3.4 ^{**}
5	VIII	Ethanol extract of Fruit	50	4.78 ± 0.2 ^{NS}	6.74 ± 0.5 ^{NS}
	IX		100	3.14 ± 0.1 ^{NS}	4.04 ± 0.2 ^{NS}
6	X	Water extract of Fruit	50	-	-
	XI		100	18.62 ± 0.4 ^{**}	23.14 ± 0.9 ^{**}

Values were expressed as mean ± SEM; (n=6).

The mean time (min) taken by each extract to paralyze or kill worms was compared to that of the same concentration of standard drug piperazine citrate by using Dunnett’s test of One-Way ANOVA analysis.

*p< 0.01 - significant difference, **p<0.001 - high significant difference, and ^{NS}p>0.05 - non significant difference

CONCLUSION

The present study suggest that crude ethanol extract of *A. altilis* of leaf and fruit possess significant *in-vitro* anthelmintic activity against *Pheretima posthuma* earthworms. *A. altilis* with proclaimed anthelmintic activity is likely to have possible efficacy in treating helminthic infections. Therefore, *A. altilis* has the potential for the use as an alternative anthelmintic agent after carrying out *in vivo* research to evaluate their safety and effectiveness. The present study recommends that *A. altilis* may have beneficial anthelmintic activity to expel intestinal parasitic roundworms. The findings of this study is a key reference for further study on isolation of phytoconstituents and their mechanism at cellular level in order to develop potent natural anthelmintic agent.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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