

Research Article

Evaluation of aqueous and ethanol extracts of *Cyperus rotundus* L. on sexual behaviours and reproductive fitness in *Drosophila melanogaster*

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ABSTRACT

Cyperus rotundus L. has been used in Ayurvedic and Egyptian folk medicine as an aphrodisiac, no experimental prove has been confirmed for this affirmation. In this study, we aimed to evaluate aqueous and ethanol extract of *C. rotundus* L. on sexual behaviors and reproductive fitness in *Drosophila melanogaster*. *C. rotundus* was extracts in water (WCE) and ethanolic (HECE). Their extracts were supplemented in diets in *D. melanogaster*. The results revealed that the 5 and 10 mg/mL HECE treatment significantly decreased the duration time of mating latency, significantly prolonged the copulation duration and produced the maximum number of eggs laying of *Drosophila*, particularly at 10 mg/mL HECE supplemented diet. Moreover, as to fertility, supplementation with 5 and 10 mg/mL HECE in *Drosophila* significantly increased the number of progenies produced from the mated females. In contrast, 5 and 10 mg/mL WCE treatment didn't have effect on the duration time of mating latency and the number of progenies in *Drosophila* compared to the untreated controls. In conclusion, an ethanolic extract of *C. rotundus* exhibits potential of sexual behaviors and reproductive fitness in *Drosophila*. Our finding might contribute to the potential pharmacological effect of *C. rotundus* ethanol extract as aphrodisiac agent.

Keywords:

Cyperus rotundus L., Aphrodisiac, *Drosophila melanogaster*, Reproductive fitness

1. INTRODUCTION

Sexual dysfunction or sexual disorder is one of the significant health problems that affects both males and females. Sexual dysfunction can be a cause of infertility in both genders¹, which might associate with a decreasing birth rate around the world. The number of infertile couples is increasing worldwide. In 2010, 1.9% of women aged 20-44 years who wanted to have children were unable to have their first live birth (primary infertility), and 10.5% of women with a previous live birth were unable to have an additional live birth (secondary

infertility)². For males, erectile dysfunction (ED) has been marked as the most common sexual disorder. A study on global male sexual disorder revealed that the global prevalence of ED was 3-76.5%³. It has been reported that the intervention by aphrodisiac agents is one of the most complaints in male patients who suffer with sexual disorders⁴. Sildenafil citrate is a standard drug that is widely used as an aphrodisiac to improve the sexual ability of males. However, several side effects such as loss of accommodation due to blurred vision, urinary tract infection, stomach disorder, and nasal congestion have been reported⁵.

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Nowadays, the researchers are searching for aphrodisiac agents from natural compounds because of their efficacy, accessibility, affordability, and minimal side effect⁶. Several medicinal plants used as aphrodisiacs have been reviewed^{7,8}.

Cyperus rotundus L. or purple nutsedge is a plant of the Cyperaceae family. This plant is widely distributed in tropical and subtropical regions worldwide⁹. The tuber of this plant has been used in traditional medicine in many regions worldwide¹⁰. The results from *in vitro* and *in vivo* studies suggest that *C. rotundus*'s extracts are the potential bioactive substances for the prevention and treatment of many diseases¹¹. The pharmacological properties of *C. rotundus* included antioxidant¹², wound healing¹³, anti-microbial¹⁴, anti-convulsant¹⁵, anti-allergy¹⁶, anti-hyperglycemic¹⁷, cardio-protective and anti-hyperlipidemic¹¹, anti-viral activity¹⁸, anti-inflammation¹⁹, anti-obesity²⁰, anti-diarrhea²¹, and anti-pyretic²². According to aphrodisiac property, the hot water extract of its tuber has been used in Ayurvedic and Egyptian folk medicine^{10,23}. In addition, the tuber of *C. rotundus* has been described to use as spermatogenic property²⁴. Although the tuber of *C. rotundus* is described as an aphrodisiac, there is no experimental prove confirming this affirmation.

For aphrodisiac agent testing, *Drosophila melanogaster* or fruit fly is one of the most effective models for the evaluation of natural compounds. This model organism shares a large amount of conserved biological pathways and disease-causing genes with humans^{25,26}. *D. melanogaster* is a respectable model for studying sexual behaviors because of its behavioral plasticity²⁷. Also, the fruit fly has evidenced to be a fast and cost-effective model system. Because of it has short lifespan, the low number of chromosomes, high fecundity, and easy to handle²⁵. Hence, *D. melanogaster* was used as a model to study the aphrodisiac property of *C. rotundus* in the different solvent extracts.

In this study, we intended to test whether water and ethanolic extracts of *C. rotundus* exhibit sexual behaviors and reproductive fitness in *D. melanogaster*, a respectable model for studying sexual behaviors. The effects of *C. rotundus* extracts on the fecundity and fertility of *D. melanogaster* was also investigated. The study might have valuable for alternative medical plant as aphrodisiac agent.

2. MATERIALS AND METHODS

2.1. Preparation of *C. rotundus* extract

C. rotundus tuber was collected in Singburi Province, Thailand during April 2019. Plant materials were dried in a hot air oven at 50°C for 48 hours and ground into a fine powder. A hundred grams of fine powder was extracted with 500 mL distilled water by

decoction using round bottom flask at 95±0.7°C for 30 minutes. The extracts were filtered with a No.1 Whatman filter paper, and concentrated by using a lyophilizer at -110°C. The dried materials were stored at -20°C until use.

Another 100 grams of powder was extracted with 500 mL 70% ethanol by using the maceration method for 7 days at ambient temperature (25-30)°C. The extracts were obtained by filtration (Whatman filter paper 40) and concentrated on a rotary evaporator (Buchi R-210, Flawil, Switzerland). The derived extracts were stored at -20°C until use.

2.2. *C. rotundus* extract analysis

The analysis was performed on high-performance liquid chromatography (Water 486 model with UV/Vis detector). The HPLC separation was performed on a Hypersil C18 column (150×4.5 mm, 5 µm) and quantified on a UV detector at 272 and 336 nm. The mobile phase was an isocratic of 0.1% phosphoric acid: ACN (45:55). The flow rate was 1 mL/min. The peaks were identified according to the retention time of standards

2.3. Culturing of *Drosophila*

The wild type *D. melanogaster* strain was obtained from the Department of Biology, Khon Kaen University. The flies were cultured in a standard wheat cream agar media seeded with yeast granules and maintained under the laboratory condition at a temperature of 25±1.2°C of relative humidity 70-80% on a 12:12 light/dark cycle with survivors transferred to fresh food vials every 2 days. The animal study protocol was approved by The Animal Ethic Committee of Ubon Ratchathani Rajabhat University, Thailand (Ethical Clearance No. AN63008).

2.4. Sexual behavior assay

To evaluate the aphrodisiac effect of water (WCE) and ethanolic (HECE) extracts of *C. rotundus*, the analysis of sexual behavior patterns was used in the study. The method was modified from the previously described with some slightly modification^{28,29}. In order to obtain virgin females and bachelor males, the pupa stage of flies was isolated into a separated vial and maintained them separately in standard media for 5 days (the flies enclosed within 5 hours after isolation). After that, 20 flies of both genders were starved for 2 hours and fed for 40 hours with WCE or HECE extracts at a concentration of 5 and 10 mg/mL (mixed to the diet). For observation of the aphrodisiac effect, the mating latency (time between the introduction of males and females into the mating chamber and initiation of copulation of each pair) and copulation duration (time between initiation and termination of copulation of each pair) were investigated. The latency to initiate courting

and the occurrence of specific behaviors (tapping, wing vibration, licking, and attempting copulation) were recorded. The courtship index (CI) was calculated by dividing the time spent in courtship divided by the total time until copulation. The experiments were done in the morning hours between 6.00-8.00 AM. The data were expressed as mean \pm SD.

2.5. The reproductive fitness assays

The fecundity was analyzed by counting the number of eggs laid by mated paired *Drosophila* for ten days after mating. The females of mated *Drosophila* were individually transferred into a vial containing a standard diet and allowed to lay eggs for 24 hours. After 24 hours, the number of eggs laid by *Drosophila* from each experimental group was counted using a stereomicroscope. The mated *Drosophila* were transferred to the new food vial diet every day. The data of ten days were pooled to calculate the number of eggs per *Drosophila*. The data were expressed as mean \pm SD.

The fertility was analyzed by counting the total number of *Drosophila* that emerged from each vial. All vials collected from ten consecutive changes were kept and the new emerged *Drosophila* were counted until no

fly emerging. The data of total number of new flies from the same experimental group were pooled and the number of flies per female were calculated. The data were expressed as mean \pm SD. The experiment was performed in triplicate.

2.6. Statistical analysis

Statistical analysis of the data was done with the SPSS 23.0 (SPSS Inc., Chicago, USA). The comparison between means was analyzed using One-Way ANOVA. Differences were considered significant when $P < 0.05$. For all assays, P values for levels of significance are symbolized as * <0.05 , and ** <0.01 .

3. RESULTS

3.1. Phytochemical analysis

The HPLC chromatogram of *C. rotundus* extract was shown in Figure 1. It was found that HECE used in this study contained quercetin (0.252 ug/mg), gallic acid (0.499 ug/mg) and tannic acid (7.939 ug/mg). However, these phytochemical compounds were not detected in WCE.

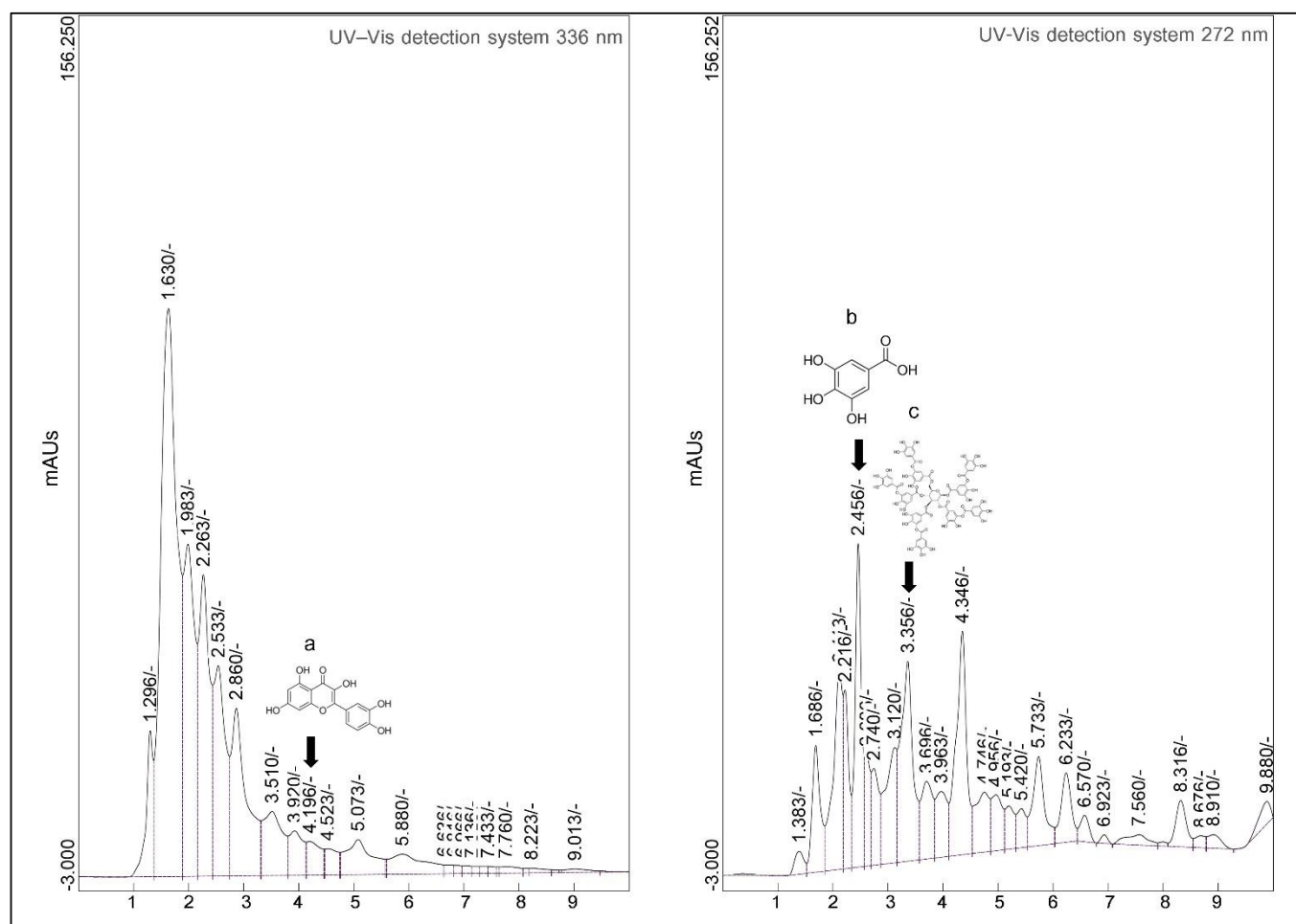


Figure 1. Chromatogram of *C. rotundus* extract: (a) quercetin, (b) gallic acid and (c) tannic acid.

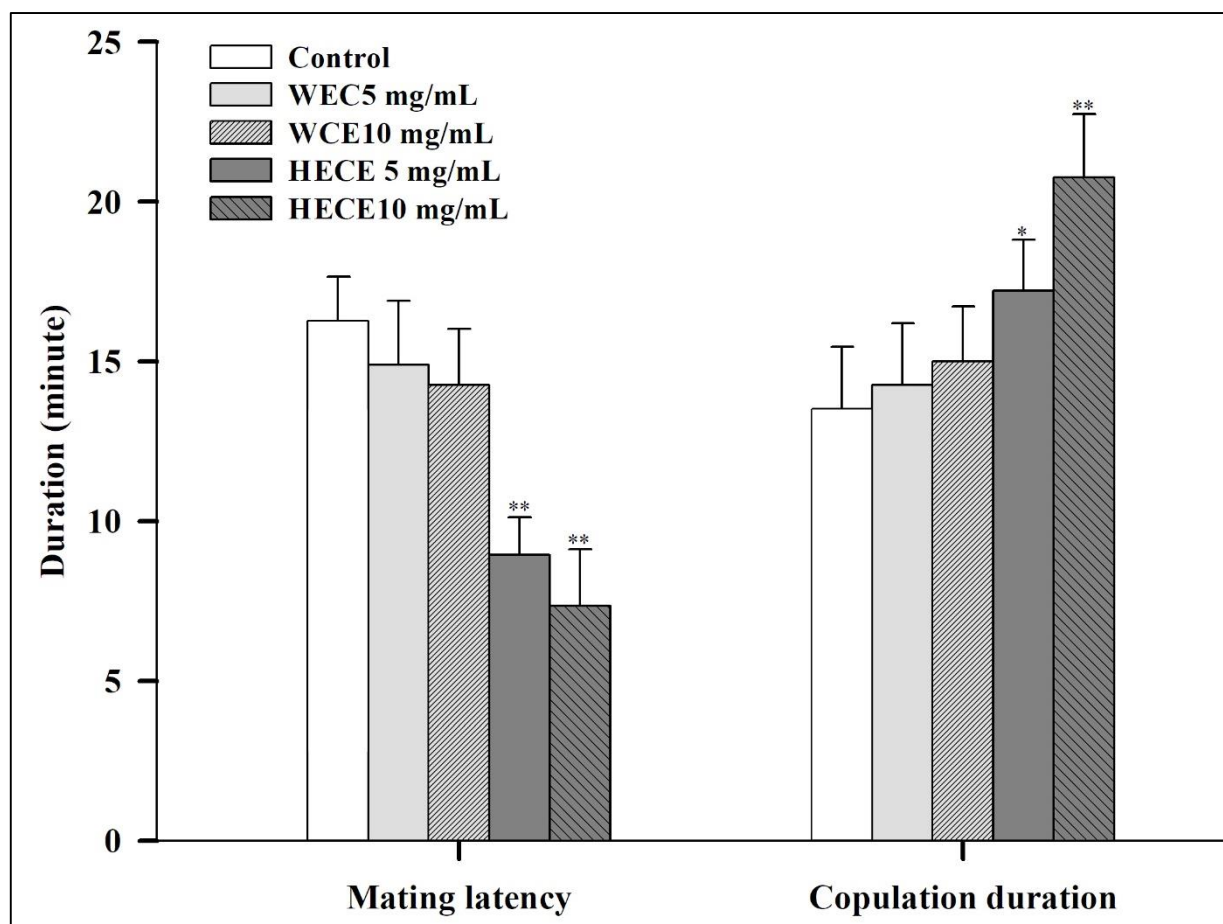


Figure 2. Effect of *C. rotundus* extracts on mating latency and copulation duration in *D. melanogaster* (*; $p<0.05$, and **; $p<0.01$).

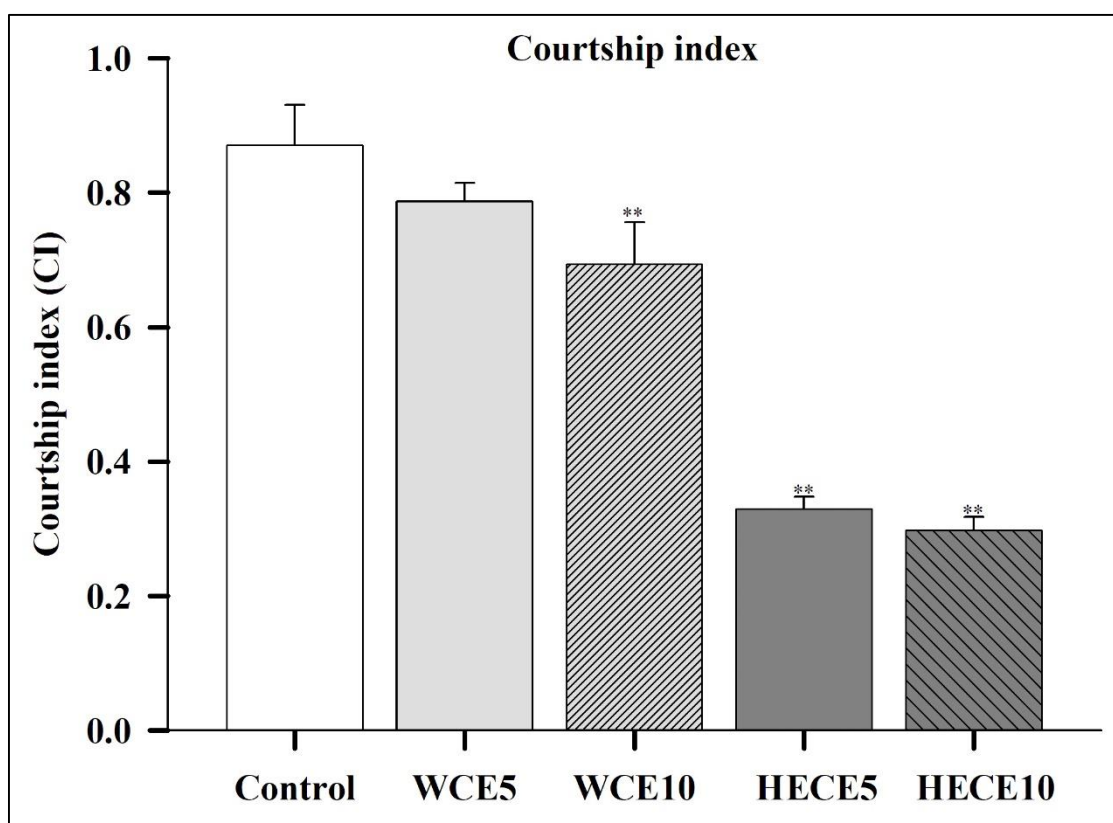


Figure 3. Effect of *C. rotundus* extracts on courtship index in *D. melanogaster* (**; $p<0.01$).

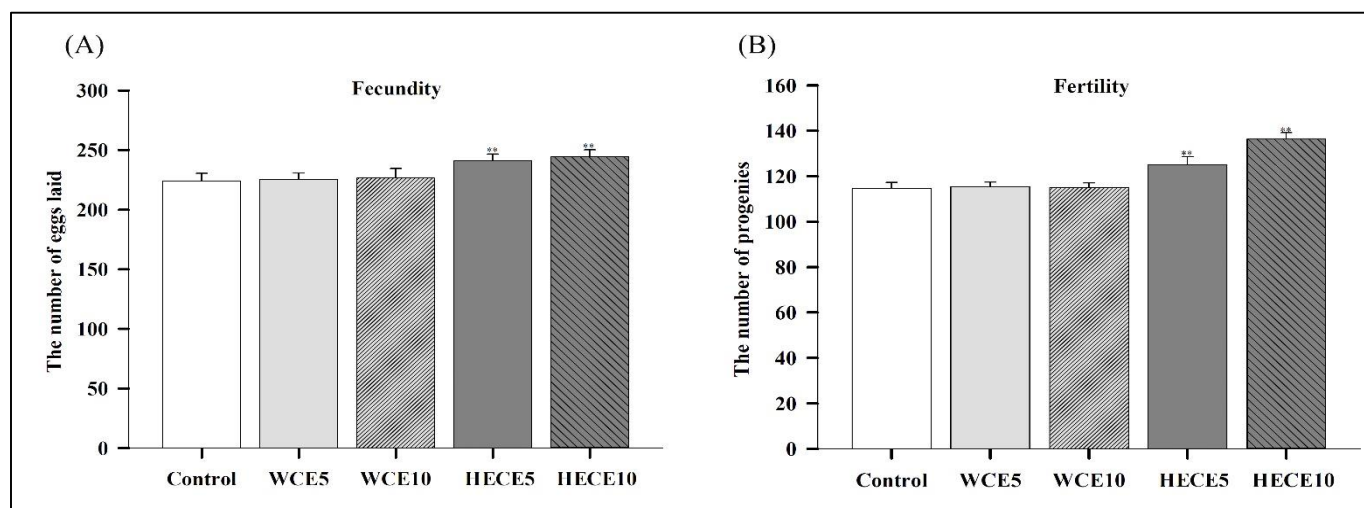


Figure 4. The effect of *C. rotundus* extracts on the productive fitness of *D. melanogaster*. (A) Number of eggs laid. (B) Number of progenies (**; $p < 0.01$).

3.2. Effect of *C. rotundus* extracts on the sexual behavior of *Drosophila*

The effect of *C. rotundus* extracts on the sexual behavior of *Drosophila* is shown in Figure 2. The minimum duration time for mating was observed in *Drosophila* treated with a diet containing ethanolic extract of HECE at 10 mg/mL. Results from the One-Way ANOVA test showed that the 5 and 10 mg/mL HECE treatment significantly decreased the duration time of mating latency of *Drosophila* (8.95 ± 1.16 and 7.35 ± 1.77 min, respectively) compared with the control group (16.25 ± 1.39 min). No significant difference was found in 5 and 10 mg/mL WCE treatment *Drosophila* when compared with the control group.

The copulation duration of *Drosophila* with or without treatment by *C. rotundus* was also investigated as shown in Figure 2. Treatment with 5 and 10 mg/mL HECE significantly prolonged the copulation duration of *Drosophila* (17.20 ± 1.60 and 20.75 ± 1.98 min, respectively) compared with the control group (13.50 ± 1.95 min). Treatment by 5 and 10 mg/mL WCE in *Drosophila* had a trend towards prolonging the copulation duration without statistically different compared to untreated controls.

As shown in Figure 3, the mean of the courtship index was significantly lower in *Drosophila* fed with the diet containing 5 and 10 mg/mL HECE (0.33 ± 0.02 and 0.23 ± 0.02 , respectively) than in untreated control (0.87 ± 0.06). WCE treatment was dose dependent effect on the courtship index in *Drosophila* at a concentration of 10 mg/mL, but was not 5 mg/mL WCE compared to untreated controls.

3.3. Effect of *C. rotundus* extracts on the reproductive fitness of *Drosophila*

The effect of *C. rotundus* extracts on the produc-

tive fitness of *Drosophila* is shown in Figure 4. The maximum number of eggs laid was observed in the *Drosophila* fed with a diet containing 10 mg/mL HECE. Treatment of 5 mg/mL HECE also significantly increased in the number of eggs laid in *Drosophila* (240 ± 5.74 and 244.35 ± 5.85 , respectively) compared to untreated control (224.25 ± 6.11). In contrast, WCE treatment at the concentration of 5 and 10 mg/mL was not statistically different from untreated controls. As to fertility, supplementation with 5 and 10 mg/mL HECE in *Drosophila* significantly increased the number of progenies produced from the mated females with dose-dependent manner (125.05 ± 3.59 and 136.40 ± 2.82 , respectively) compared to untreated control (114.55 ± 2.70). In contrast, no statistical difference in the number of progenies in *Drosophila* was observed after WCE at the concentration of 5 and 10 mg/mL supplemented diet compared to the untreated controls.

4. DISCUSSION

Numerous medicinal plants have historically been known as aphrodisiacs, including tuber of *C. rotundus*, however, the experiment proves this is limited. Here, we demonstrated the different extraction solvents by water and ethanol of *C. rotundus* on sexual behaviors and reproductive fitness in *Drosophila*. This animal is an attractive model to study behavior because of its complex behavioral repertoires and sensory system^{30,31}. The expression of sexual behavior of the *Drosophila* is comparable to that of mammals because *Drosophila* receptors have been shown to mediate similar behavioral responses to the same effector pathway as mammalian³². Therefore, this animal model can be used to analyze aspects of human disease-related behaviors³³.

Our results revealed that ethanol extract of *C. rotundus* exhibited more potential than water extract on sexual behaviors and reproductive fitness in *Drosophila*.

These finding was in agreement with many previous reported in the *Drosophila* model that aphrodisiac agents exhibit a decrease in mating latency while increasing copulation duration^{34,35}.

The main parameters for studying sexual behavior in *Drosophila* are mating latency and copulation duration. These parameters associate with the sexual desire of males. It has been clearly demonstrated that a male with strong sexual desire reacts quickly in the presence of a female^{36,37}. Copulation duration indicates the sexual performance and associates with the reproductive fitness of *Drosophila*. The duration of copulation positively relates to the number of sperm transferring from male to female³⁵. The extension of copulation duration improves the fitness of both males and females. As shown in Figure 1, reduced the duration time for mating latency and courtship index indicates that supplementation of *C. rotundus* extract could stimulate sexual desire in the *Drosophila*. In addition, an improvement in sexual performance was detected in *Drosophila* fed with *C. rotundus* extract through prolonged the copulation duration, which was agreement with the previous reports^{35,36}.

Fecundity and fertility are a significant parameter for evaluating fitness in *Drosophila*. These two parameters influence the reproductive fitness of females and can be influenced by environmental factors³⁸. In this study, our results showed that a significant increase of fecundity and fertility of *Drosophila* fed with *C. rotundus* extract (Figure 3) were supported to the effect of supplementation extract for improving the reproductive fitness.

The molecular mechanism by which *C. rotundus* extract exhibits an aphrodisiac effect and improves reproductive fitness is still unclear. The possible explanation for these properties involves its phytochemical contents. Based on our results showed that the more significant ethanolic extract gave more than water extract, suggesting that compounds that soluble in ethanol may be the primary active ingredients. The active compounds of *C. rotundus* are well studied³⁹. We primarily considered that the polyphenols or flavonoids existing in our extract might demonstrate its effects. Our results showed that HECE used in this study contained gallic acid, quercetin, and tannic acid. Zhou and Fu (2012) have reported that *C. rotundus* rhizomes contain quercetin, kaempferol, luteolin, ginkgetin, and isoginkgetin⁴⁰. Moreover, polyphenolic substances, including leucocyanidin, leucocyanidinglucoside, catechine and chlorogenic acid were also identified in tubers of *C. rotundus*⁴¹. Although the direct proof does not exist in the *Drosophila* model, a previous study revealed that supplementation with an extract of *C. esculent* tubers that contains quercetin could stimulate sexual motivation and improve sexual performance in male rats⁴². In rats, quercetin treatment also showed to improve sexual

behavior, sperm quantity, and quality in streptozotocin-induced diabetic erectile dysfunction⁴³. In addition, *Tamarindus indica* extract containing gallic acid as a component has been proven to increase the aphrodisiac potential in Wistar rats⁴⁴. Based on the results from previous studies and this study, quercetin and gallic acid contained in the *C. rotundus* extract may be one of the key agents for improving reproductive fitness.

Taken together the results from this study and the previous studies contribute to the potential pharmacological effect of *C. rotundus* extract as an aphrodisiac agent. Nevertheless, this study's limitation is the use of the crude extract, which should have to be the purification of *C. rotundus* extract use and test its activity in *Drosophila*. In addition, further studies in the mammal model are now required to verify the role of *C. rotundus* extract in aphrodisiac property. Ultimately, the supplementation with *C. rotundus* extract may help to improve sexual function and reproductive fitness.

5. CONCLUSIONS

An ethanolic extract of *C. rotundus* exhibits potential of sexual behaviors and reproductive fitness in *Drosophila*. Our finding might contribute to the potential pharmacological effect of *C. rotundus* ethanol extract as aphrodisiac agent.

6. ACKNOWLEDGEMENT

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Conflict of interest

The authors declare no conflict of interest.

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Ethics approval

The animal study protocol was approved by The Animal Ethic Committee of Ubon Ratchathani Rajabhat University, Thailand (Ethical Clearance No. AN63008).

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