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Evaluation of the sex enhancement and fertility properties of *Waltheria indica* ethanol root extract in *Drosophilia melanogaster* (Fruit flies)

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Abstract
Folklore medical practitioners have indicated the roots of *Waltheria indica* in the management of erectile dysfunction but there seems to be no sufficient scientific evidence to support the claim. This study investigated the aphrodisiac and fertility enhancing potentials of the ethanol root extract of *Waltheria indica* using the fruit fly (*Drosophilia melanogaster*) model. A total of 24 sexually inexperience male flies were randomly allotted to four groups. The negative control (group A) was fed on 0.2 ml of 10% ethanol only and groups B, C, and D received 0.025% w/w, 0.05% w/w and 0.1% w/w of extract respectively. Mating studies showed a significant difference in mating latency of treated flies at ($p< 0.01$, $p< 0.001$ and $p<0.01$) for groups B, C and D respectively, when compared with control. Copulation duration results did not show any significant difference between treated groups and control although there were observable dose related differences. The extract had no effect on fecundity. In conclusion, the extract enhances sexual performance without a corresponding enhancement in fertility in *D. melanogaster*. This has implication on its current use in ethnomedicine.

Keywords: aphrodisiac, *Waltheria indica*, *Drosophilia melanogaster*, fertility, fecundity

Introduction
Aphrodisiacs refer to foods, drinks or drugs that increase sexual desire or arouse sexual response (Malviya *et al*., 2011) [13]. These substances may be obtained from plants, animals or minerals. Aphrodisiacs may be expected to act in either or all of these three ways; increase libido via the endocrine system, improve erection (which may be through its effects on neurotransmitters and some enzymes involved in sexual function), and enhance of sexual pleasure (may be via psychologically-mediated pathway of sexual function) (Kotta *et al*., 2013) [10].
Some aphrodisiacs have been indicated in the management of erectile dysfunction (Malviya *et al*., 2011) [13] The discovery of this class of agents can be traced to the early 1980s when injectable vasoactive drugs (papavarine, phentolamine) capable of producing firm and lasting erections were introduced (Brindley, 1983) [5]. Currently available drugs for the treatments of ED have limited efficacy, unpleasant adverse effects and contraindications. Sildenafil citrate is a one of such successful drugs that modifies penile hemodynamics (Segraves, 2003) but headache, flushing, dyspepsia and nasal congestion have been reported with its use (Liu *et al*., 2009) [11]. There have been quests for herbal formulations for management of ED but with very minimal adverse effects. Aside from possessing aphrodisiac property, such herbal formulations may also possess fertility enhancing property since there is a strong relationship between both (Watcho *et al*., 2007).
Waltheria indica L. (syn. *Waltheria americana*) belongs to the family *Sterculiaceae*. It is also known as velvet leaf, marsh-mallow, monkey bush, boater bush, leather coat, and buff coat.
It is a plant with robust medicinal properties that has been indicated locally in the treatment of diseases such as syphilis, wounds, coughs and as general tonic. All parts of the plant are said to be employed for medicinal purposes in Africa; as anti-diarrhea (Ragasa *et al.*, 1997) [17], as analgesic (Mohammed *et al*., 2007) [14], roots and aerial parts found to exhibit a moderate anti-plasmodial activity (Jansen *et al*., 2010) [8]. In Nigeria, the plant is used traditionally against hemorrhoids, syphilis, anemia, cough (Sabo *et al*., 2017) [20]. This study was designed to investigate the aphrodisiac activity of the ethanol root extract of the plant in fruit flies.

Materials and Methods
Plant material
The roots of *W. indica* were obtained in April 2018 from a forest near the stadium in the campus of University of Ibadan, Oyo State, Nigeria. The plant was identified and authenticated by Mr. Donatus O. Esimekhuai of the Department of Botany, University of Ibadan. A voucher specimen with number UIH-22850 has been deposited in the herbarium of the Department of Botany, University of Ibadan.

Preparation of plant extract
The roots of *W. indica* were washed clean of sand and chopped into smaller sizes. The pieces of roots were air dried for two weeks and then oven dried at 70℃ for 72 h. The dried samples were ground to powder using a mechanical grinder. The powdered root material (1.5 kg) was soaked in 5 L of 96% ethanol for 72 h with intermittent vigorous stirring. The material was filtered using Whatman #1 filter paper. The filtrate were then concentrated *in vacuo* using a rotary evaporator at 37 ℃ to about one tenth of the original volume. The concentrate was further

concentrated to dryness in a water bath at 40 ℃ (yield = 2.5%w/w). The reddish coarse powder substance and was stored in the refrigerator for future use (Atangwho *et al*., 2010) [11].

Phytochemical screening

Preliminary phytochemical screening of the root extract of *W. indica* was carried out using standard methods (Trease and Evans, 1996; Sofowora, 1993) [25, 23]. The extract was screened for the presence of flavonoids, alkaloids, tannins, terpernoids, saponins and glycosides.

Feed preparation and dose concentrations

About 700 mL of water was heated to 100℃ after which 7.8 g of agar-agar was measured out and added into the pot containing boiling water and stirred continuously for 10 min to prevent particle aggregation and lump formation. Upon saturation, 52 g of corn meal was measured out and dissolved in a beaker containing 200 mL of clean water at room temperature and added into the boiling water containing agar-agar with continuous stirring for an additional 10 min. The yeast solution 3.5 g in 25 mL ethanol was then added at this point to the solution of agar-agar and corn meal. The mixture of ingredients was allowed to boil for 5 min with continuous stirring. The feed was removed from the heat source (hot plate), and nipangin solution (0.5 g nipangin dissolved in 5 mL of ethanol) was then incorporated into the mixture with thorough stirring. The feed was covered and allowed to cool naturally before being weighed into the treatment vials.

Preparation of offsprings used for the study

The eggs of *D. melanogaster* were obtained from *Drosophila* stock center of the Department of Biochemistry, University of Ibadan, Nigeria. It was transferred to Madonna University, Elele, Rivers State, in modified icepack and kept at the Pharmacognosy laboratory extension of Madonna University, Elele. Flies were bred on corn agar media. After seven days of breeding, the eggs developed into adult fruit flies. Breeding of flies continued for about 10 days during which the stock generation was withdrawn and the daughter generation (off-springs) was allowed to acclimatize at 25 ± 2℃ until the required number of flies for treatment was obtained. Four groups of 25 flies (both sexes) were used for the study. Three replicates of each group were made and the three groups were fed with three different concentrations of ethanol root extract of *W. indica*; 0.025% w/w, 0.05% w/w and 0.1% w/w The number of dead flies were counted and recorded daily throughout the study and the percentage surviving flies determined until the mortality of all flies occurred.

Survival test and LC_{50} determination

For the test, four different groups A, B, C, D, (n=25 flies) were exposed to 0% w/w, 0.05% w/w, 0.1% w/w, and 0.2% w/w of the extract incorporated in the feed respectively, and observed for 28 days. Each of the days, the groups were checked for dead flies. The median lethal concentration (LC_{50}) was calculated as described by (Bagu *et al*., 2020) [2].

Mating experiment

A total of 25 male flies were randomly isolated less than 8 h after eclosure into 4 separate vials (n=6) labelled A (untreated), B, C, and D. Groups B-D received 0.025% w/w, 0.05% w/w and 0.1% w/w of ethanol root extract of *W. indica* incorporated in the feed respectively. Each group consisted of single vial containing one virgin female paired with a treated virgin male. Mating behavior was assessed for 1 h immediately after the flies were paired. The duration of copulation was scored for each of the 24 pairs.

Fertility studies

Each pair was then transferred to a fresh agar-corn meal diet and the female flies were allowed to lay eggs for a period of 2 days after which the pair was transferred into another treatment vial with fresh feed for an additional 2 days. Thereafter, parent flies were killed off by dumping in a bottle containing methanol. The progeny from each replicate vial labeled A1-A6, B1-B6, C1-C6 and D1-D6 were scored after 8 days for a period of 7 days until there was no additional emergence.

Statistical analysis

The results were analyzed using one-way ANOVA followed by Dunnett's post hoc test (GraphPad Prism version 5.01 software). Values are mean ± S.E.M (standard error of mean) and $p<0.05$ indicates statistical difference between compared groups.

Results

Phytochemical constituents of *W. indica*

Table 1: Shows the results of phytochemical tests on the ethanol root extract of *W. indica.* While alkaloids, flavonoids, sterols, terpenes, tannins, saponins, anthraquinones and carbohydrates were present, only cardiac glycosides were absent.

Parameters	Inference
Alkaloids	+
Flavonoids	+
Sterols	+
Terpenes	+
Tannins	+
Cardiac glycosides	-
Saponins	+
Anthraquinones	+
Carbohydrates	+

+ Presence
- Absence

Effect of *W. indica* ethanol root extract on the survival of *D. melanogaster*

Survival studies revealed mortality was lowest among the control (untreated) group in comparison with others (Figure 1). All 25 flies in the vials of 0.5 % w/w, 0.1 % w/w and 0.2 % w/w of the extract in the feed died before the 28th day of treatment. The 8^{th}-day LC_{50} was found to be ≤ 0.1 % w/w.

Effect of *W. indica* ethanol root extract on the survival of *D. melanogaster*

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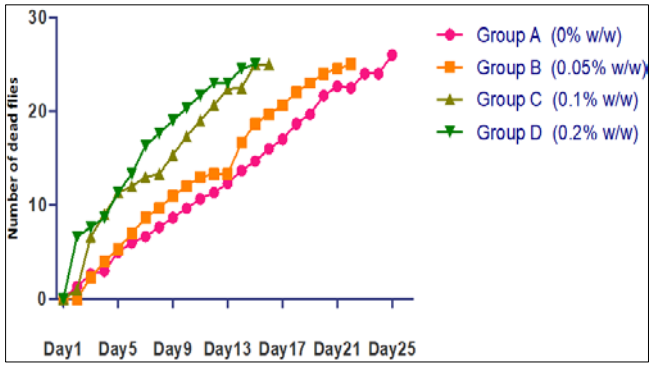

Fig 1: Survival of *D. melanogaster* after 28-day exposure to feed containing different concentrations of the ethanol root extract of *W. indica.* n=25

Effect of *W. indica* ethanol root extract on mating latency and duration of copulation of *D. melanogaster*

Figure 2 shows that the mating latencies of the groups fed with extract-containing feeds were significantly ($p<0.01$) different from untreated (control) group but duration of copulation was not significantly different when compared with control group.

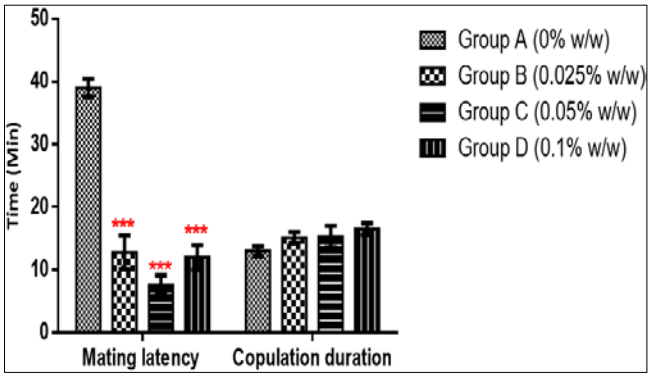

Fig 2: Effect of *W. indica* ethanol root extract on mating latency and duration of copulation duration in *D. melanogaster*. $^{***}p < 0.01$ versus control (mating frequency). n=6; mean ± S.E.M.

Effect of *W. indica* ethanol root extract on the number of eggs laid by *D. melanogaster*

The numbers of eggs laid by the treated groups of *D. melanogaster* were not significantly different from that laid by the control group (Figure 3).

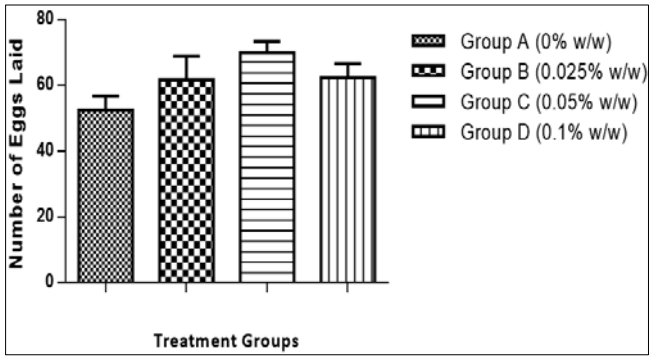

Fig 3: Effect of *W. indica* ethanol root extract on number of eggs laid by *D. melanogaster*. Values are not significantly different across the groups. (n = 6). The values are mean ± S.E.M.

Effect of *W. indica* ethanol root extract on fecundity of *D. melanogaster*

Figure 4 shows that although the extract-fed groups produced more flies than the control, these values are not significantly different.

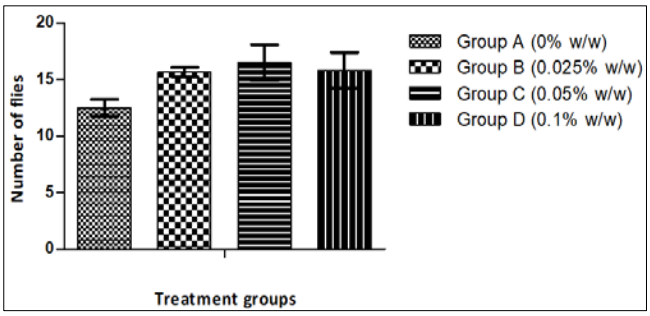

Fig 4: Effect of *W. indica* ethanol root extract on number of emerged flies by *D. melanogaster*. Values are not significantly different. (n= 6). The values are mean ± S.E.M.

Discussion

This study has shown that the ethanol root extract of *W. indica* contains alkaloids, flavonoids, saponins, anthraquinones, and terpenoids while cardiac glycosides are absent. This is in tandem with a previous report (Rafiu *et al*., 2019) [16]. Of these constituents saponins have been associated with aphrodisiac property (Sing and Gupta, 2011) [22]. Therefore, the presence of saponins in the ethanol root extract is a likely basis of its aphrodisiac action. Also, the flavonoids in *Moringa oleifera* have been reported to increase androgen levels leading to increased libido and sexual performance (Padashetty and Mishra, 2007) [15]. Interestingly, there are related compounds in this plant. More so, Increased androgenic hormones are associated with increase sexual libido (Rahmawati and Bachri, 2012) [18]. It is logical to infer that some of the phytochemical constituents present in *W. indica* root extract may be responsible for the observed sexual behaviour. The reduced mating latency in the treated flies, indicate an increase in libido.

The flies that were exposed to the extract-constituted feeds (0.05%w/w to 0.2%w/w) experienced high death. It has previously been reported that there is an inverse relationship between compounds that can positively influence reproduction and lifespan (Lopez *et al*., 2014) [12]. This inverse relationship is likely due to the fact that reproduction is a high energy and resource dependent expenditure, in which fruit flies trade late life survival for increased reproduction. Female fruit flies exhaust their energy resources in the production of eggs. Male fruit flies, however, require an exertion of energy during mating, which involves a complex ritual of courtship behaviors (Branco *et al*., 2017) [4]. Disorders of sexual desire (libido) may involve either a deficient or compulsive desire for sexual activity. The significant decrease in mating latency in the extract-treated flies compared to the control group may indicate that libido increased, which underscores an aphrodisiac activity (Malviya *et al*., 2011) [13]. The results of fertility and fecundity, in this study, did not show any significant difference between control and treatment groups even though there were observable dose dependent increases in the results. It is therefore possible that the extract may enhance sexual desire without a corresponding enhancement of fertility and fecundity.

Conclusion
Ethanol root extract of *W. indica* has a sex enhancing property that does not translate to increased fertility and fecundity. The mechanisms that underscore the aphrodisiac property are dependent on the phyto-constituents.

Author Contributions
Ben Enoluomen Ehigiator designed and worked on data analysis for the study, Raymond Ozolua wrote the protocol and the manuscript's first draft. Rose Egbogu managed laboratory experiments and data analysis of the study. All authors read and approved the final manuscript.

Conflict of interest
The authors declare no conflict of interest.

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Employment or Leadership
None declared.

Honorarium
None declared

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References
1. Atangwho IJ, Ebong PE, Eyong EU, Eteng MU. Combined administration of extracts of *W. indica* Mimic insulin in time course body weight and glucose regulation in rats. Nigerian Journal of Biochemistry & Molecular biology,2010:25(1): 44-49.
2. Bagu GD, Omale S, Iorjiim WM, Uguru MO, Gyang SS. Determination of LD50, Fecundity and locomotor effects of methanol root extract of *Ximenia americana* Linn, in *Drosophila melanogaster*. Asian Journal of Biochemistry, Genetics and Molecular Biology,2020:5(2):1-9.
3. Baskaran K, Ranjani R. Aphrodisiac activity of aqueous root extract of *Waltheria indica* in male Wistar albino rats. International Journal of Pharmacology Research,2016:6(2):141-143.
4. Branco AT, Schilling L, Silkaitis K, Dowling2 DK LemosB. Reproductive activity triggers accelerated male mortality and decreases lifespan: genetic and gene expression determinants in Drosophila. *Heredity*, 2017:118:221-228.
5. Brindley GS. Carvernous alpha blockade: A new technique for investigating and treating erectile dysfunction. British Journal of Psychiatry,1983:143(41): 332.
6. Buchon N, Silverman N, Cherry S. "Immunity in *Drosophila melaganoster* from microbial recognition to whole-organism physiology". Nature reviews immunology,2014:14(12):796-810.
7. Jaiswel M, Sandoval H, Zhang K, Bayat V, Bellen HJ. "Probing mechanisms that underlie human neurodegenerative diseases in Drosophila". Annual Review of genetics,2002:46:371-96.
8. Jansen O, Angenot L, Tits M, Nicolas JP, De Mol P, Nikiema JB *et al*. In-vitro antiplasmodial activity of ethnobotanically selected plants from Burkina Faso. Journal of Ethnopharmacology,2010:130:143-150.
9. Kaun KR, Devinerin AV, Heberlein U. *Drosophila melaganoster* as a model to study drug addiction". Human Genetics,2014:131(6):959-75.
10. Kotta S, Ansari SH, Ali J. Exploring scientifically proven herbal aphrodisiacs. Pharmacognosy Reviews,2013:7(13):1-10.
11. Liu F, Huang XF, Qi Q, Dai Q, Yang L, Nie F *et al*. Asparanin A induces G2/M cell cycle arrest and apoptosis in human hepatocellular carcinoma HepG2 cells, Biochemical and Biophysical Research Communications,2009:381(4) 700-705.
12. Lopez T, Schrine SE, Okoro M, Lu D, Chiang BT, Huey J *et al*. Green tea polyphenols extend the lifespan of male *Drosophila melanogaster* while impairing reproductive fitness. Journal of Medicinal Food,2014:17(12):1314-1321.
13. Malviya N, Jain S, Gupta V B, Vyas S. Recent studies on aphrodisiac herbs for the management of male sexual dysfunction: A review. Acta Poloniae Pharmaceutica,2011:68(1):3-8.
14. Mohammed Z, Shok M, Ilyas N, Musa KY, Yaro AH. Analgesic activity of Waltheria indica. European Journal of Scientific Research,2007:16(1):1-6.
15. Padashetty SA, Mishra SH. Aphrodisiac studies of *Tricholepis glaberrima* with supportive action from antioxidant enzyme. Journal of Pharmaceutical Biology,2007:45(7):580-586.
16. Rafiu BO, Lawal IO, Olaniyi MB. Chemical composition and antioxidant activity of *Waltheria indica* Linn whole plant. Nigerian Journal of Natural Products and Medicine,2019:23:1-9.
17. Ragasa CY, Cruz CA, Chiong ID, Tada M, Rideout JA. Antifungal flavonoids from Waltheria Americana. Philippine Journal of Science,1997:126:243-25.
18. Rahmawati N, Bachri NM. The aphrodisiac effect and toxicity of combination *Piper retrofractum* L, *Centella asiatica*, and *Curcuma domestica* infusion. Aphrodisiac Effect of Infusion,2012:3:19-22.
19. Reiter LT, Potocki L, Chiens S, Gribskov M, Bier E. "A systematic analysis of human disease associated gene sequences in *Drosophila melaganoster*. Genome Research,2001:11(6):1114-25.
20. Sabo SY, Otimenyin SO, Uguru MO, Bukar BB. Ethnobotanical survey of medicinal plants used as aphrodisiacs in Bauchi Local Government Area. Journal of Complementary and Alternative Medical Research,2017:4(4):1-13.
21. Segraves RT. Pharmacologic management of sexual dysfunction, benefits and limitations. CNS Spectrum,2014:8:225-229.8.
22. Singh S, Gupta YK. Aphrodisiac activity of *Tribulus terrestris* Linn in experimental models in rats. Journal of Men's Health,2011:8(1):575-577.

23. Sofowora A. Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd., Ibadan,1993:191-289.
24. Suchitra G, Shakunthala V. Effect of *Glycyrrhiza glabra* root extract on behaviour and fitness of *Drosophila melanogaster* and vestigial wing mutant. International Journal Current Microbiology and Applied Sciences,2014:3(7):1047-1054.
25. Trease EG, Evans CW. Pharmacognosy (14th edition). Saunders Publishers WB: London,1996:41:423.
26. Watcho P, Zelefack F, Nguelefack TB, Ngouela S, Telefo PB, Kamtchouing P *et al*. Effects of the aqueous and hexane extracts of *Mondia whitei* on the sexual behaviour and some fertility parameters of sexually inexperienced male rats. African Journal of Traditional, Complementary, and Alternative Medicine,2006:4(1):37-46.