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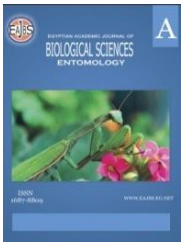

Antioxidant and Anticoagulant Activities of Red Palm Weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) Extracts

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ABSTRACT

The study focused on analyzing the antioxidant properties of extracts from red palm weevil larvae *Rhynchophorus ferrugineus*, obtained using hexane, n-butanol, dichloromethane, and diethyl ether. Additionally, the anticoagulant effects of these extracts were evaluated through activated partial thromboplastin time (APTT) and prothrombin time (PT) assays. Results showed that the hexane, n-butanol, dichloromethane, and diethyl ether extracts from *Rh. ferrugineus* larvae exhibited antioxidant activity comparable to ascorbic acid, with IC_{50} values of 142.67, 122.24, 231.77 and 74.95 µg/ml, respectively, compared to 3.16 µg/ml for ascorbic acid. Also, the anticoagulant activity of *Rh. ferrugineus* (larvae) tested extracts using APTT assay showed 33.8, 44.3 and 50.9 sec. At 25, 50 and 75 µg/ml of hexane extract, respectively. Meanwhile, *Rh. ferrugineus* n-butanol extract recorded 46.9, 57.2 and 65.5 sec. At the same concentrations. Also, dichloromethane and diethyl ether recorded anticoagulant activity reached 31.2, 40.6, 48.7 and 44.3, 52.9, 62.1 sec. at 25, 50 and 75 µg/ml, respectively, vs. 59.8, 86.2 and 109.7 sec. For the Heparin sodium salt, use the same concentrations. Generally, all *Rh. ferrugineus* larval extracts tested proved to be antioxidant agents. The anticoagulant potential of *Rh. ferrugineus* tested extracts were weaker than those of heparin and needed more concentration to reach the commercial anticoagulant (Heparin sodium salt) in both assays (APTT and PT).

INTRODUCTION

Oxidative reaction has a crucial function in human health. An antioxidant is a chemical that can inhibit the oxidation of other molecules, hence preventing harmful changes. Free radicals are commonly found in the body and are produced by certain physiological processes to combat pathogenic germs. Various disorders such as neurological diseases, cardiovascular diseases, autoimmune skin diseases, chronic renal failure, and cancer result from oxidative stress activation and the lack of intracellular antioxidant defenses (Zhu *et al.*, 2013; Roy *et al.*, 2015; Hasaballah *et al.*, 2019). Antioxidants can diminish unstable and reactive free radicals, protecting cells from their harmful effects (Souri *et al.*, 2008).

Conversely, any vascular injury will result in quick bleeding. The bleeding is

quickly halted by the blood coagulation mechanism, which is then followed by the repair of the hemostatic system. Accurate regulation of blood coagulation is crucial for the survival of all animals. Failure to stop bleeding promptly can provide a life-threatening risk. Unwanted clot development, such as in cases like stroke or DIC, can also lead to death. To address various bleeding disorders, a procoagulant or anticoagulant molecule is required. Anticoagulants decrease blood coagulation to avoid deep vein thrombosis, pulmonary embolism, myocardial infarction, and stroke (Hossain *et al.*, 2018).

Arthropods, especially insects, are valuable and untapped sources of bioactive compounds for modern medicine. Insects make up over 50.0% of total biodiversity and more than 80.0% of all animal diversity, inhabiting various ecological systems (Chernysh *et al.*, 2002; Roy *et al.*, 2015; Hassan *et al.*, 2018; Hasaballah *et al.*, 2019; Mekhlif 2021).

The current study focused on examining the antioxidant and anticoagulant properties of different extracts obtained from red palm weevil larvae *(Rhynchophorus ferrugineus)* to contribute to the understanding of potential insect-derived materials.

MATERIALS AND METHODS

***Rhynchophorus ferrugineus* Tested:**

The last instar larvae of *Rhynchophorus ferrugineus,* a type of palm weevil, were initially obtained from the Central Laboratory for Date Palm Research and Development in Giza. They were subsequently bred for multiple generations in a controlled environment within the insectary-rearing laboratory at the Zoology Department, Faculty of Science, Al-Azhar University (Cairo). The larvae were maintained under specific conditions of temperature (25-27°C), relative humidity (60-70%), and photoperiods (12-12 hours light-dark rhythm) in a wooden cage measuring 200×120×300 cm (Shahina *et al.* 2009).

Evaluation of Antioxidant Activity by DPPH Radical Scavenging Method:

The free radical scavenging activity of various leaf plant extracts was assessed using 1,1-diphenyl-2-picryl hydrazyl (DPPH). A 0.1 mM DPPH solution in ethanol was produced. A 1 ml solution was combined with 3 ml of various ethanol extracts at concentrations ranging from 3.9 to 1000 µg/ml. Only the extracts that are soluble in ethanol were employed, and different quantities were generated using a dilution procedure. The mixture was briskly agitated and then left at room temperature for 30 minutes. The absorbance was measured at 517 nm using a UV-VIS Milton Roy spectrophotometer. The experiment was conducted using ascorbic acid as the reference standard chemical and was repeated three times (Shekhar and Anju, 2014; González-Palma *et al.*, 2016).

The IC_{50} value of the sample, representing the concentration needed to inhibit 50% of the DPPH free radical, was determined using a Log dosage inhibition curve. Decreased absorbance of the reaction mixture suggested increased free radical activity. The DPPH scavenging effect percentage was determined using the formula: Percent inhibition = [(A0 − A1) / A0] × 100, Where A0 represents the absorbance of the control, and A1 represents the absorbance in the presence of the test or standard sample.

Evaluation of the Anticoagulant Activity of Tested Extracts:

The anticoagulant effects of the studied extracts were evaluated utilizing both activated partial thromboplastin time (APTT) and prothrombin time (PT).

The APTT assay was conducted according to the procedure outlined by Seedevi *et al.* (2017). The APTT was measured using the commercial kit Plasmatrol H-II, Liquicellin-E, and BioMed. The citrated blood plasma was combined with 10 µl of tested extracts at concentrations of 25, 50 and 75 µg/ml and incubated at 37°C for 10 minutes in a glass vial in this assay. 100 µl of APTT reagent was added to the mixture, which was then incubated for 3 minutes at 37°C. Following this, 100 µl of pre-heated 0.02 mol/l $CaCl_2$ solution was

added to the mixture, and the clotting time in seconds was recorded in comparison to Heparin sodium salt.

The PT potential of the studied extracts was determined using the method outlined by Seedevi *et al.* (2015). The prothrombin time was evaluated using the commercial kit plasmatrol H-II from BioMed. Preheat the reagent to 37°C for 5-10 minutes before using it in the test procedure. 90 µl of citrated normal human plasma was combined with 10 µl of extracts at concentrations of 25, 50, and 75 µg/ml. The combination was then incubated for 10 minutes at 37°C. Following incubation, 200 µl of PT reagent was combined with the mixture, and the clotting time in seconds was measured and compared to Heparin sodium salt used as a standard.

Statistical Analysis:

The data was encoded and inputted with the statistical software SPSS V.22. Data underwent testing to ensure they met the assumptions of parametric tests. Continuous variables were assessed for normality using the Shapiro-Wilk and Kolmogorov-Smirnov tests. The probability and percentile data were normalized for normal distribution using the Arcsine Square Root transformation. The data were given as the mean and standard deviation. Data were displayed graphically, when feasible, with R Studio version 2022.02.4.

RESULTS

Antioxidant Activity Of Tested Extracts by DPPH Radical Scavenging Method:

The results showed that hexane, n-butanol, dichloromethane, and diethyl ether extracted from *Rhynchophorus ferrugineus* larvae exhibited antioxidant activity comparable to ascorbic acid, with IC_{50} values of 142.67, 122.24, 231.77, and 74.95 µg/ml, respectively, compared to 3.16 µg/ml for ascorbic acid (Tables 1-3 and Figs. 1-3).

Table 1: The DPPH% of *Rhynchophorus ferrugineus* hexane and n-butanol larval extract.

Extract	Concentrations (µg/ml)	Optical Density (Mean)	DPPH scavenging (%)	SD	SE	IC_{50} (µg/ml)
Hexane	1000	0.359	72.0	0.003	0.001	142.67
	500	0.453	64.7	0.005	0.001	
	250	0.558	56.5	0.003	0.001	
	125	0.660	48.6	0.006	0.002	
	62.5	0.763	40.6	0.004	0.001	
	31.25	0.859	33.1	0.005	0.001	
	15.625	0.975	24.0	0.005	0.001	
	7.8125	1.078	16.0	0.003	0.001	
	3.9	1.158	9.7	0.003	0.001	
	1.95	1.282	0.1	0.005	0.001	
	0	1.283	0.0	0.002	0.001	
n-butanol	1000	0.334	74.0	0.003	0.001	122.24
	500	0.415	67.7	0.004	0.001	
	250	0.532	58.5	0.003	0.001	
	125	0.648	49.5	0.002	0.000	
	62.5	0.761	40.7	0.004	0.001	
	31.25	0.846	34.1	0.002	0.000	
	15.625	0.949	26.0	0.002	0.000	
	7.8125	1.048	18.3	0.006	0.002	
	3.9	1.128	12.1	0.004	0.001	
	1.95	1.241	3.3	0.003	0.001	
	0	1.283	0.0	0.002	0.001	

SD: standard deviation; SE: standard error.

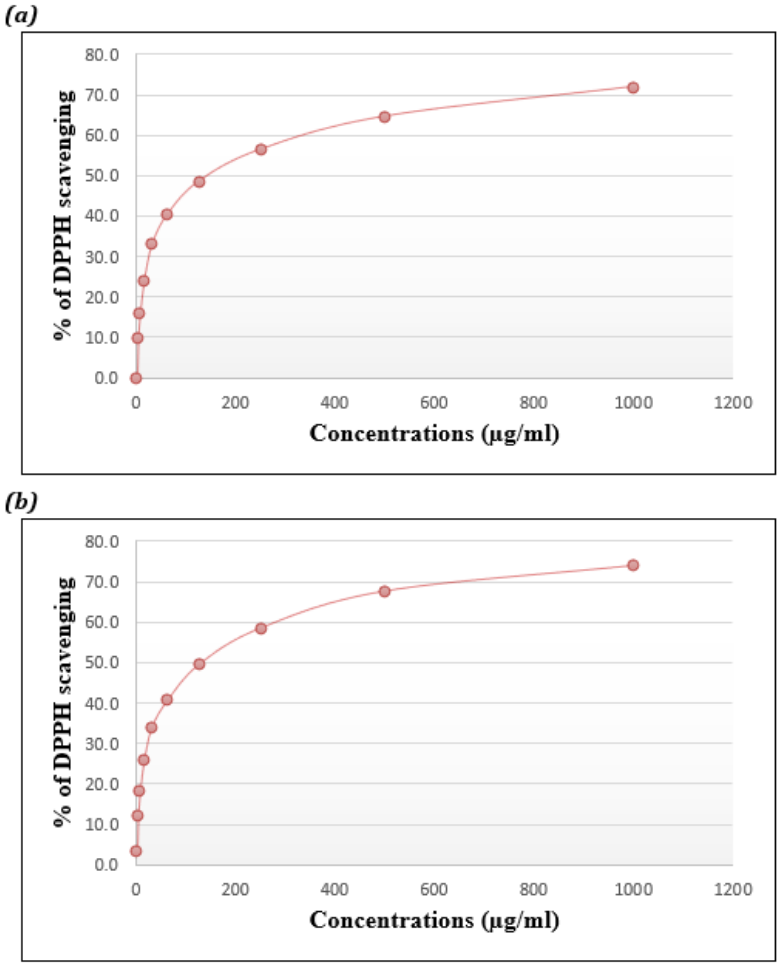

Fig. 1: Antioxidant activity of *Rhynchophorus ferrugineus* larval extracts ***(a)*** hexane extract and ***(b)*** n-butanol extract.

Table 2: The DPPH% of *Rhynchophorus ferrugineus* dichloromethane and diethyl ether larval extract.

Extract	Concentrations (µg/ml)	Optical Density (Mean)	DPPH scavenging (%)	SD	SE	IC_{50} (µg/ml)
dichloromethane	1000	0.422	67.1	0.003	0.001	231.77
	500	0.531	58.6	0.004	0.001	
	250	0.618	51.8	0.002	0.000	
	125	0.728	43.3	0.002	0.001	
	62.5	0.833	35.1	0.002	0.001	
	31.25	0.942	26.6	0.003	0.001	
	15.625	1.054	17.8	0.003	0.001	
	7.8125	1.143	10.9	0.004	0.001	
	3.9	1.245	2.9	0.003	0.001	
	1.95	1.280	0.2	0.006	0.002	
	0	1.283	0.0	0.002	0.001	
diethyl ether	1000	0.251	80.5	0.003	0.001	74.95
	500	0.345	73.1	0.004	0.001	
	250	0.452	64.8	0.003	0.001	
	125	0.560	56.4	0.003	0.001	
	62.5	0.672	47.6	0.004	0.001	
	31.25	0.778	39.3	0.003	0.001	
	15.625	0.890	30.7	0.003	0.001	
	7.8125	1.005	21.7	0.005	0.002	
	3.9	1.091	15.0	0.006	0.002	
	1.95	1.176	8.3	0.005	0.001	
	0	1.283	0.0	0.002	0.001	

See footnote of table (1).

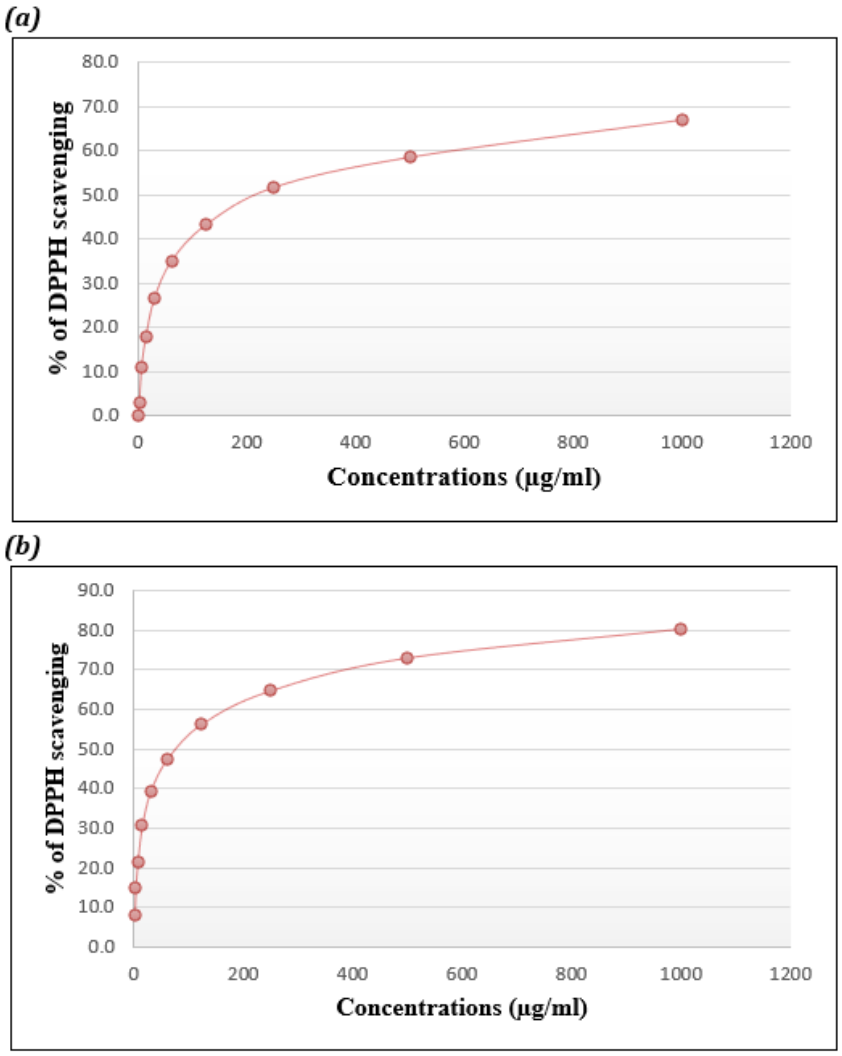

Fig. 2: Antioxidant activity of *Rhynchophorus ferrugineus* larval extracts ***(a)*** dichloromethane extract and ***(b)*** diethyl ether extract.

Table 3: The DPPH% of ascorbic acid (standard).

Concentrations (µg/ml)	Optical Density (Mean)	DPPH scavenging (%)	SD	SE	IC_{50} (µg/ml)
1000	0.025	98.0	0.003	0.001	
500	0.043	96.7	0.002	0.001	
250	0.096	92.5	0.001	0.000	
125	0.152	88.2	0.004	0.001	
62.5	0.220	82.8	0.006	0.002	
31.25	0.320	75.1	0.007	0.002	3.16
15.625	0.438	65.8	0.007	0.002	
7.8125	0.535	58.3	0.005	0.002	
3.9	0.633	50.7	0.005	0.002	
1.95	0.755	41.2	0.003	0.001	
0	1.283	0.0	0.002	0.001	

See footnote of table (1).

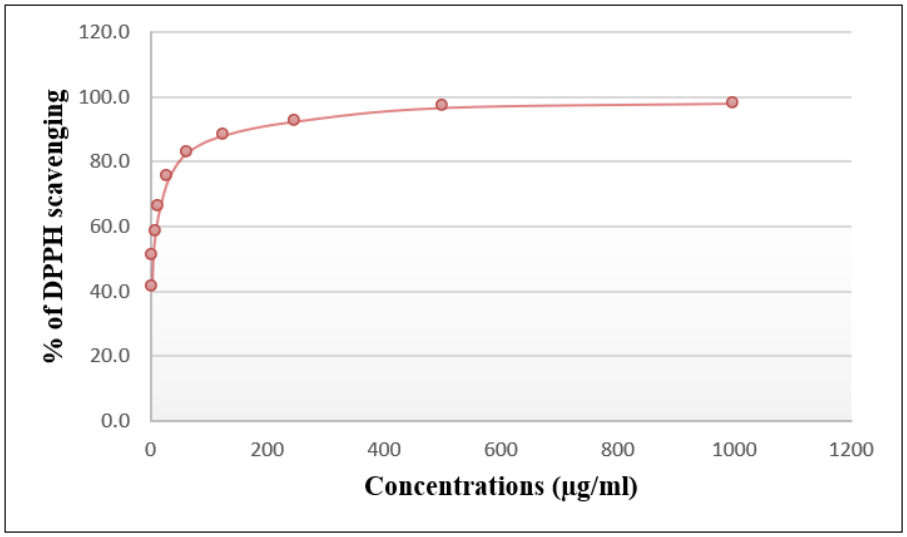

Fig. 3: Antioxidant activity of ascorbic acid (standard).

Anticoagulant Activity of Tested Extracts:

The anticoagulant properties of *Rh. ferrugineus* larvae extracts were evaluated using the usual coagulation tests, Activated Partial Thromboplastin Time (APTT) and Prothrombin Time (PT), with heparin sodium salt as a control. The typical range for the PT commercial kit is 10 to 12 seconds, while for the APTT kit is 28 to 35 sec.

The results given in Table (4) and Figure (4) evoked the anticoagulant activity of *Rh. ferrugineus* (larvae) tested extracts using APTT assay which showed 33.8, 44.3 and 50.9 sec. At 25, 50 and 75 µg/ml of hexane extract, respectively. Meanwhile, *Rh. ferrugineus* n-butanol extract recorded 46.9, 57.2 and 65.5 sec. at the same concentrations. Also, dichloromethane and diethyl ether recorded anticoagulant activity reached 31.2, 40.6, 48.7 and 44.3, 52.9, 62.1 sec. at 25, 50 and 75 µg/ml, respectively, vs 59.8, 86.2 and 109.7 sec. for the Heparin sodium salt at the same concentrations. In addition, the results showed that the anticoagulant activity of *Rh. ferrugineus* (larvae) tested extracts using PT assay recorded 13.5, 15.1 and 18.7 sec. by hexane extract; 15.8, 17.3 and 22.5 sec. by n-butanol extract; 11.7, 13.1 and 18.2 sec. by dichloromethane extract; 14.1, 16.5 and 20.9 sec. by diethyl ether extract at 25, 50 and 75 µg/ml, respectively compared with 27.1, 38.5 and 52.2 sec. recorded by Heparin sodium salt at the same concentrations (Table 4 and Fig. 4).

The results indicated that *Rh. ferrugineus* has anticoagulant properties. Larval extracts were less powerful than heparin and required higher concentrations to achieve the same level of anticoagulation in both APTT and PT testing.

Table 4: Anticoagulant activity of *Rhynchophorus ferrugineus* larval crude extracts and heparin based on APTT and PT Values (seconds).

Essay	Concentrations (µg/ml)	Anticoagulant Values in sec. caused by tested extracts				Heparin sodium salt (standard)
		hexane	n-butanol	dichloromethane	diethyl ether	
APTT	25	33.8	46.9	31.2	44.3	59.8
	50	44.3	57.2	40.6	52.9	86.2
	75	50.9	65.5	48.7	62.1	109.7
PT	25	13.5	15.8	11.7	14.1	27.1
	50	15.1	17.3	13.1	16.5	38.5
	75	18.7	22.5	18.2	20.9	52.2

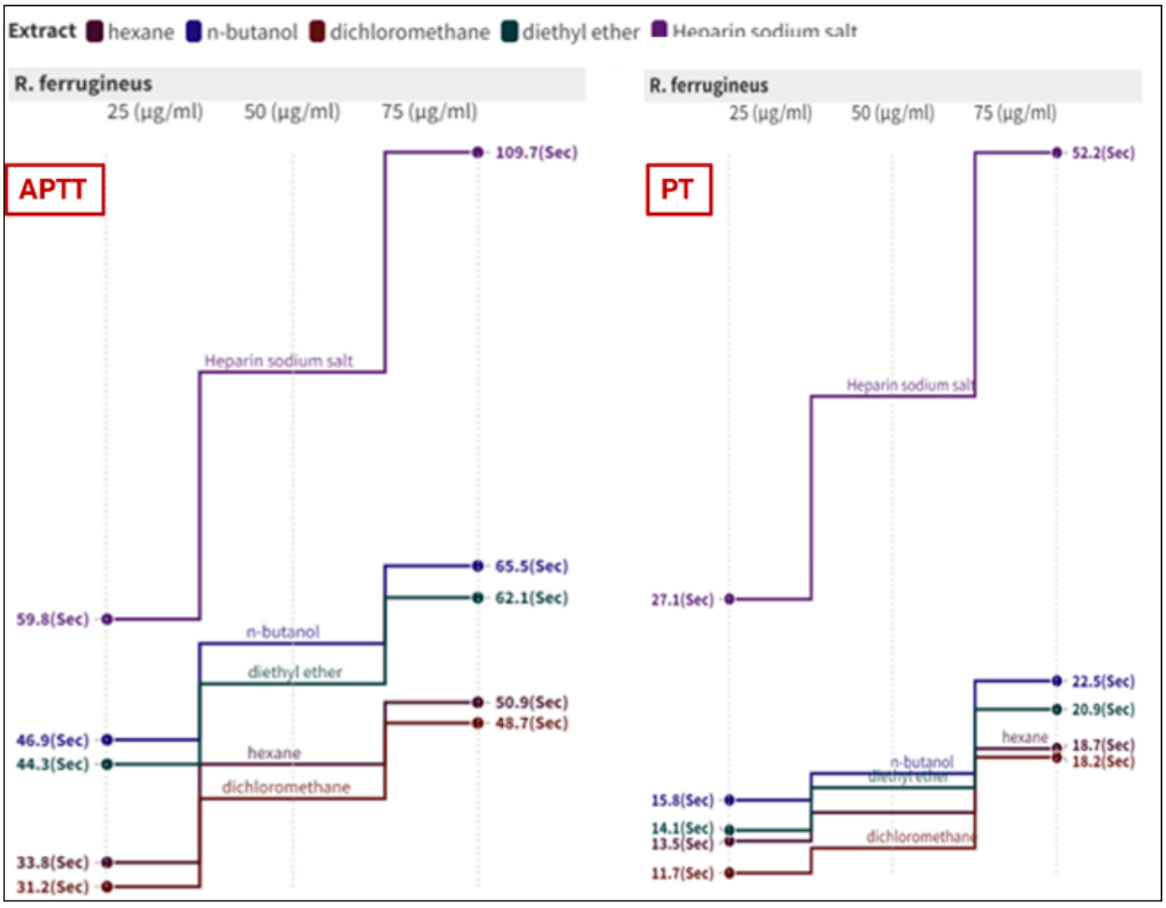

Fig. 4: Step slope chart represents the anticoagulant activity of *Rhynchophorus ferrugineus* extracts using APTT and PT assays at different concentrations.

DISCUSSION

The present study proved the capability of *Rhynchophorus ferrugineus* larval extracts as antioxidant agents. The hexane, n-butanol, dichloromethane, and diethyl ether were extracted from *Rh. ferrugineus* larvae recorded antioxidant activity with values of 142.67, 122.24, 231.77 and 74.95 µg/ml as compared with 3.16 µg/ml for the standard ascorbic acid, respectively. Free radicals such as hydroxyl groups, peroxyl radicals, and single oxygen are detrimental because they can damage cell components, leading to various illnesses (Bozin *et al.*, 2008). Antioxidants can reduce chronic diseases such as cancer and cardiovascular diseases by neutralizing free radicals. Synthetic antioxidants have been utilized to decrease biological toxicity by causing various harmful effects (Hasaballah *et al.*, 2019). Therefore, there is a high desire to substitute synthetic antioxidants with natural oxidizing agents. Original substances like ground-up bodies have been commonly utilized in medical sectors and are generally deemed safe because they include numerous chemicals with antioxidant and anticancer properties (Suh *et al.*, 2010). The findings support the results of Orhan *et al.* (2007), who utilized various extracts of *Lycopodium clavatum* and its alkaloid fraction, showing insignificant antiradical effects on DPPH. Hasaballah *et al.* (2019) also utilized methanol crude extract from maggots of *Musca domestica, Lucilia sericata,* and Chrysomya albiceps.

On the other hand, the obtained results exhibited the anticoagulant activity of *Rh. ferrugineus* (larvae) tested extracts using APTT assay showed 33.8, 44.3 and 50.9 sec. at 25, 50 and 75 µg/ml of hexane extract, respectively, while *Rh. ferrugineus* n-butanol extract recorded 46.9, 57.2 and 65.5 sec. at the same concentrations. The results align with

those reported by Xu *et al.* (2016) for the crude extract of *Holotrichia diomphalia* larvae and by Arasukumar *et al.* (2019), who studied the anticoagulant activity of chitosan extracted from *Thenus unimaculatus* using APTT and PT assays with heparin sodium as the standard. The results showed that the anticoagulant activity of extracted chitosan was recorded at 49.7 and 20.9 s and at 50 µg/ml by APTT and PT assays, compared with 61.2 and 31.4 s. recorded by heparin, respectively and Hamdi *et al.* (2020), who reported that anticoagulant activity of the different types of blue crab chitosan was recorded at 31.2 s., 14.8 and 14.4 s. using APTT, Quick time (QT) and thrombin time (TT) *in vitro* assays.

Conclusion

The study examined the antioxidant and anticoagulant properties of several extracts from the larvae of the red palm weevil, *Rhynchophorus ferrugineus,* using hexane, n-butanol, dichloromethane, and diethyl ether extracts. The current investigation demonstrated that all *Rh. ferrugineus* larval extracts tested have antioxidant properties. The anticoagulant properties of *Rh. ferrugineus* tested extracts were less powerful than heparin and required higher concentrations to achieve the same level of anticoagulation in both APTT and PT experiments. Further research is required to clarify the bioactivity of *Rh. ferrugineus* larval extracts.

Declarations:

Ethical Approval: Ethical Approval is not applicable.

Competing interests: The authors declare no conflict of interest.

Funding: No funding was received.

Availability of Data and Materials: All datasets analysed and described during the present study are available from the corresponding author upon reasonable request.

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