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In Vitro Anti-Acetylcholinesterase Activity of Dichloromethane Leaf Extracts of *Carphalea glaucescens* in *Chilo partellus* Larvae

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Abstract

Acetlycholinesterase (AChE) hydrolyses the neurotransmitter acetylcholine resulting in the termination of nerve impulse at the synapse. Anti-acetylcholinesterase activities stop the passage of the nerve impulse at the synapse resulting in continuous stimulation which can lead to death. The manufacturers of many pesticides target the AChE because it interferes with the passage of the nerve impulse. *In vitro* study revealed that DCM leaf extract of *Carphalea glaucescens* has anti-acetylcholinesterase activity against crude acetlycholinesterase (AChE) enzyme extracted from *Chilo partellus* and an IC_{50} of 12.02 mg/ml was calculated. After qualitative phytochemical screening was carried out the phytochemicals which were present were tannins, phenols, flavonoids, steroids, terpenoids and alkaloids.

Keywords: *Chilo partellu; Carphalea glaucescens;* Anti-acetylcholinesterase

Abbreviations: AChE: Acetlycholinesterase ; ANOVA: Analysis of Variance; DCM: Dicholomethane; WHO: World Health Organization; ACTI : Acetylthiocholine Iodide; DTNB: 5,5'-Dithiobis-(2-Nitrobenzoic Acid)

Introduction

Acetylcholinesterase is the target for insecticides belonging to organophosphorus and carbamate group. Organophosphates act upon the nervous system of the pests interfering with the passage of impulse. Due to the negative effects of synthetic insecticides, there is need to develop cheaper and safer insecticides.

The *Chilo partellus* (spotted stem borer) is one of the major constraints in maize and sorghum production worldwide. The yield losses reported due to stem borers vary greatly. Melaku [1] reported 49% grain yield losses due to stem borers in northern Ethiopia but on average yield losses can be estimated between 20% and 50%. *C. partellus* is very invasive and once it invades an area it displaces native species and is widely distributed. In coastal Kenya, there is evidence that *C. partellus* has partially displaced the indigenous stem borer, *Chilo orichalcociliellus* [2-4].

The distribution of *C. partellus* now includes Ethiopia, Kenya, Tanzania, Mozambique, Swaziland, Lesotho, and Bostwana [5,6]. Different conventional insecticides have been used to control stem borer resulting in high productivity but there are shortcomings in their application, including the high residue levels of pesticides in agricultural produce, pest resistance as well as environmental pollution [7]. The management of *C. partellus* has been typically carried out by synthetic insecticides, which are non-biodegradable and not environmentally safe [8,9].

In this perspective, plants are considered the alternative sources of insect-control because they contain a range of bioactive compounds and many of which are selective. In Africa and elsewhere, plants extracts are still widely used in the treatment of many ailments and up to 80% of the African population use traditional medicines for health care [10]. The present study aimed to investigate the mode of action of *C. glaucescens* in the inhibition of acetylcholinesterase.

Materials and Methods

Collection and preparation of the plant materials

Fresh leaves of *C. glaucescens* were harvested from Siakago division, Mbeere North Sub County, in Embu County guided by ethnobotanical information from local farmers and herbalists. An acknowledged taxonomist authenticated the identity of the plant under study. They leaves were dried at room temperature (25°C) and they were ground to powder using an electric mill. The ground sample was labeled and stored at room temperature ready for extraction.

Extraction procedure

A sample of 500 g of powdered plant leaves was soaked in 1 litre of dichloromethane for 24 hours. The mixture was then filtered under pressure using a vacuum pump. The filtrate was concentrated using a rotary evaporator at 40°C to obtain dry extract which was stored at 4°C.

Crude acetlycholinesterase enzyme extraction procedure

Crude acetlycholinesterase (AChE) enzyme was extracted from *C. partellus* obtained from Kenya Agricultural and Livestock Research Organization (KARLO), Katumani Station, Kenya. Third and fourth instar larvae were used for this study. After washing the larvae three times with distilled water, they were homogenized for 3 minutes (0.5 g) in 6 ml sodium phosphate buffer (pH 8, 0.1 M, containing 1.0 M Na_2HPO_4 and 1.2 M NaH_2PO_4). After filtration through Whatman filter paper No. 1, the homogenate was centrifuged at 2,000 g for 20 min at 4°C. The supernatant was used as a crude enzyme extract.

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The concentration of the isolated AChE was determined by method described by Bradford [11].

Determination of AChE concentration

After crude AChE was extracted from the larvae of *C. Partellus,* the concentration was determined by the method described by Bradford [11] and bovine serum albumen (BSA) was the used as the standard for protein. The dye was made by dissolving 100mg coomassie Brilliant Blue G-250 in 50 ml 95% ethanol followed by 100ml 85% (w/v) phosphoric acid. Absorbance was measured at 595nm and the absorbance values were used to plot the standard curve. The standard curve was used to determine the concentration of the AChE.

Anti-acetylcholinesterase activity assay

Acetylcholinesterase activity was determined as described by Ellman method [12], with some modification that allowed the use of 1 ml cuvette glass. In 2 ml eppendorf tube, 150 µl of 0.1 M sodium phosphate buffer (pH 8) was put in which 10 µl of the plant extract was added, followed by 20 µl larvae homogenate (crude enzyme). Addition of 10 µl of 14 mM of acetythiocholine iodide was used as substrate to initiate the reaction. The eppendorf tube with the mixture was incubated for 30 minutes at 25ºC. The principle was to measure the production of thiocholine from the hydrolysis of acetylthiocholine iodide. Thereafter 10 µl of 10 mM of DTNB was added which was used for the measurement of AChE activity. The reaction mixture was then incubated for 5 minutes at room temperature (25ºC). The absorbance is read at 412 nm with spectrophotometer against a blank and reference pesticide used was cyclone. The optical density (OD) was read after one minute and the fourth minutes. Then the change optical density with time (OD/min) was calculated to estimate substrate hydrolysis overtime. One unit of AChE activity is defined as 1 µl of substrate hydrolyzed per minute. The activity of the AChE was calculated using Beer lamberts law.

Qualitative phytochemical screening

Qualitative phytochemical screening was done to determine the presence of selected secondary metabolites. This was done according to the standard methods as described by Harborne [13] and Kotake [14].

Treatment (mg/ml)	Mean change in absorbance	Enzyme activity (µmoles/min/mg protein)	Mean % inhibition
Normal control	0.032±0.058	0.000	0.00±0.00
Positive control	0.001±0.055	3.744	96.97±1.84^{a}
2.5	0.009±0.003	4.444	73.64±8.67^{ab}
5	0.013±0.028	1.836	64.80±28.00^{ab}
7.5	0.018±0.002	0.902	45.45±6.19^{ab}
25	0.021±0,004	0.021	35.50±13.4^{b}
50	0.028±0.036	0.039	35.09±4.66^{b}
75	0.037±0.033	0.032	34.54±6.05^{b}

Values are expressed as mean ± SEM for triplicate readings. Values with different superscripts are significantly different calculated by one way ANOVA followed by Tukey's post hoc test (p>0.05).

Table 1: Effects of DCM leaf extracts of *C. glaucescens on* anti- acetylcholinesterase of *C. partellus* larvae.

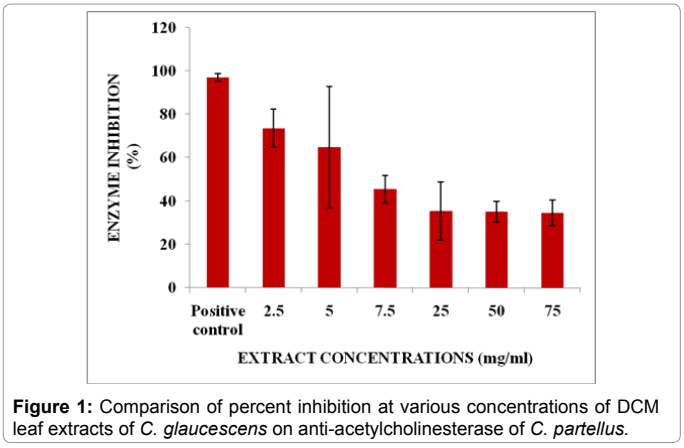

Figure 1: Comparison of percent inhibition at various concentrations of DCM leaf extracts of *C. glaucescens* on anti-acetylcholinesterase of *C. partellus.*

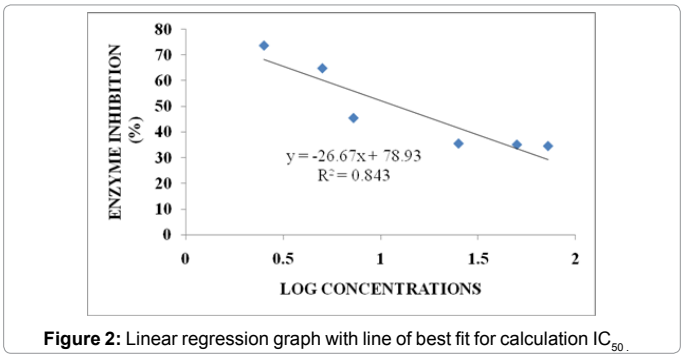

Figure 2: Linear regression graph with line of best fit for calculation IC_{50}.

Data collection, management and statistical analysis

Data collected included the efficacy of the extract against the AChE, change in absorbance, and qualitative phytochemical screening was also done. Data on phytochemical was presented on a table showing presence or absence of the phytochemicals. Data on different extract concentrations, and controls were analyzed using one way ANOVA, which was followed by Tukey's post hoc for pairwise separation and comparison of means. Minitab version 17 software was used for statistical analysis.

Results and Discussion

The DCM leaf extract of *C. glaucescens* showed efficacy against AChE activity in *C. partellus* as indicated by percent inhibition of the enzyme activity by various extract concentrations (Table 1 and Figure 1) using method described by [12]. Acetylcholinesterase plays a critical role in terminating synaptic transmission so that the next nerve impulse can be transmitted across the synapse. Therefore, the leaf extract has the potential to prevent the AChE of *C. partellus* in termination of nerve impulse. Among the six different extract concentrations, the 2.5 mg/ml had the highest percent inhibitory effects on activity of acetylcholinesterase with a value of 73.64% and 75mg/ml had the lowest percentage enzyme activity inhibition of 34.54% (Table 1 and Figure 2). The normal control group had no effect against the enzyme activity. This is similar to a study conducted by Orhan [15], which demonstrated that all *Fumaris* species studied showed the most potent inhibitory activity against AChE. The high SEM for concentration 5mg/ml (Figure 1) can be interpreted to mean that it was not a representative of the population however despite the high SEM the results were consistent with results of other concentrations.

From this study, any change in absorbance was also associated

with inhibition of enzyme activity. The IC_{50} for DCM leaf extract of *C. glaucescens* was computed by regression line and found to be 12.02 mg/ml (Figure 2). At lower extract concentrations the extract was more sensitive to enzyme activity than at higher concentrations (Figure 3) which mean that to be effective only low concentrations of the extract would be required.

Results revealed that anti acetylcholinesterase activity of DCM leaf extracts of *C. glaucescens* in *C. partellus* is dose dependent because the extract concentration increased the percent enzyme inhibition decreased. This was comparable to a study by [16] which observed that Foliar application of semi-solid crude extract of *T. orientalis* on maize was found to be effective against *C. partellus*. In this study, the leaf extract showed enzyme inhibitory effects. The inactivated enzyme is no longer capable of hydrolyzing acetylcholine, resulting in the build up of ACh in the nerve synapse, leading to death [17,18].

Qualitative phytochemical screening

In DCM extract of *C. glaucescens* phytochemicals which were present after screening were tannins, phenols, flavonoids, steroids, terpenoids and alkaloids. However saponins and cardiac glycosides were absent (Table 2). The bioactivity of DCM leaf extracts of *C. glaucescens* can be attributed to constituent phytochemicals such as phenols and flavonoids in the extracts which are associated with phenols and flavonoids [19]. Similarly, a study conducted by [15] found out that since most of the acetylcholinesterase inhibitors are known to contain nitrogen, the higher activity of these extracts may be due to their rich alkaloidal content.

Conclusion

In conclusion, this study has revealed that the DCM leaf extracts of *C. glaucescens* has the potential of *in vitro* anti acetylcholinesterase activity in *C. partellus* and it is possible for the studied plants to possess bioactive compounds. Therefore *C. glaucescens* can be used as biopesticide in the control of *C. partellus*. However the bioactivity could have been higher if pure extract were used and would recommend purification of AChE through processes such as chromatography.

Acknowledgement

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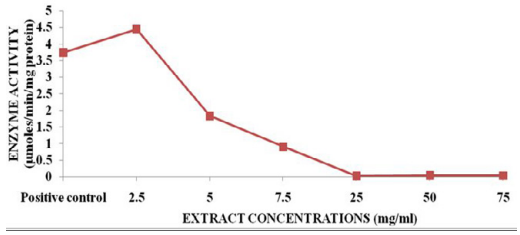

Figure 3: The dose dependent curve for *in vitro* AChE inhibition.

Phytochemical	*C. glaucescens*
Phenols	+
Flavonoids	+
Terpenoids	+
Saponins	-
Alkaloids	+
Cardiac glycosides Steroids	-
Tannins	+
	+

(KEY: + = Present, - = Absent)

Table 2: Results of qualitative phytochemical screening of *C. glaucescens*.

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