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In Vitro Anti-Acetylcholinesterase Activity of Crude Fruits Sap Extract of *Solanum incanum* in Green Peach Aphids

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Abstract

Acetylcholinesterase is a key enzyme that terminates nerve impulses by catalyzing the hydrolysis of neurotransmitter, acetylcholine, in the nervous system in various organisms. Irreversible inhibitors have been developed as insecticides such as organophosphates and carbamates. Aqueous crude fruit sap extract of *S. incanum* was evaluated for acetylcholinesterase activity in green peach aphids using Ellman's method and is found active in inhibiting the AChe of green peach aphids. All tested concentrations of crude fruits sap extract of *S. incanum* possessed the ability to inhibit acetylcholinesterase of green peach aphids at dose dependent manner and IC_{50} of 49.9 was calculated. This study suggest that aqueous crude fruit sap extract of *S. incanum* inhibit the acetylcholinesterase of the green peach aphids and it is possible the extract has compounds that increase both the level and duration of the neurotransmitter action.

Keywords: Acetylcholinesterase; *Solanum incanum*; Green peach aphids; Organophosphates

Introduction

Acetylcholinesterase (AChE) is an enzyme that terminates nerve impulses by catalyzing the hydrolysis of neurotransmitter, acetylcholine, in the nervous system in various organisms [1]. Acetylcholinesterase is also the target site of organophosphate and carbamates insecticides [2].

Solanum incanum is a perennial, wild shrub like herb that belongs to family Solanaceae, which grows in many regions of Africa, Middle East and Far East Asia. It is an erect or spreading perennial shrub with leaves and stem occasionally having small prickles. The fruits are small berries of 2-3 cm in diameter and yellowish orange or brown in color when ripe [3]. It is common as a weed around houses, in overgrazed grass land and on road sides. It also found at forest edges and in bush land and grasslands, from sea-level up to 2500 m altitude [4].

The plant is used in many parts of Africa in the treatment of various illnesses such as sore throat, angina, stomach pain, colic, headache, painful menstruation and liver pain. For this purposes leafs, roots and fruit decoctions are drank, roots are chewed and sap swallowed, leaf sap is used for washing painful areas, and ash of burnt plants is mixed with fat and applied externally [4]. Leaves are added to soup to improve the flavor. The fruit and the seed are used in Africa and Asia to curdle milk and to make cheese. Further, the plant is employed in East and Southern Africa for the treatment of skin diseases, general infections, abdominal pains, fever, stomachache and indigestion.

Many plants have been reported to possess insecticidal activity against different insect pests, *E. tirucalli* and *J. curcas* and sap from *P. dodecandra* [5]; *Mentha piperita* [6]; *Peganum harmala* [7], but the mode of insecticidal action of these botanicals is unknown. Therefore this study is therefore designed to evaluate the anti – acetylcholinesterase activity of aqueous crude fruit sap extract of *S. incanum* in green peach aphids. This is the first report of *in vitro* antiacetylcholinesterase activity of aqueous crude fruit sap extract of *S. incanum* inhibited the acetylcholinesterase of green peach aphids (*Myzus persicae*).

Materials and Methods

In vitro acetylcholinesterase assay

Assay reagents: The assay reagents used was acteylcholinesterase assay kit (colourmetric) ab138871 which contains acetylcholinesterase standard, assay buffer, 5, 5′-dithio-bis (2-nitrobenzoic acid) (DTNB) and acetylthiocholine were purchased from Abcam (UK), bovine serum albumin (BSA), Commassie brilliant blue and Phosphoric acid (Sigma aldrich, USA), Triton X-100 (Lobal Chemie, India) and EDTA (Duchefa Haarlem, Netherlands).

Isolation of acetylcholinesterase enzyme: Acetylcholinesterase (AChE) was isolated by collecting about 0.5 g of adult aphids and homogenizing them in 6 ml phosphate buffer (pH 8.0, 0.01M, containing 1 mM EDTA, 1% Triton X-100, 1M NaCl). After filtration through a Whatman filter paper No. 1, the homogenate was centrifuged at 15,000 g for 10 min at 4°C. The supernatant were used as crude enzyme extract.

Protein quantification: The protein content of the isolated acetylcholinesterase was determined according to the protein-dye- binding method of Bradford [8] using bovine serum albumin as standard. The dye solution was made by dissolving 100 mg of Coomassie Brilliant Blue G-250 in 50 ml 95% ethanol and 100 ml 85% (w/v) phosphoric acid. The absorbance was measured at 595 nm in a 72 N spectrophotometer after mixing samples in 3 ml cuvettes against the blank. The protein concentration was estimated from a standard curve of concentration of BSA.

Acetylcholinesterase inhibition assay: The AChE inhibitory activity was preformed according to the colorimetric method of Ellman's method using acetylcholine iodide as a substrate [9]. The principle of this method is the measurement of the rate of production of thiocholine as acetylthiocholine is hydrolyzed. Thiocholine then reacts with the reagent DTNB and a yellow anion of 5-thio-2-nitrobenzoic acid is produced which has maximum absorbance at 412 nm.

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Acetylthiocholine iodide reaction mixture was prepared by addition of 150 µl of sodium phosphate buffer, 10 µl of acetylthiocholine iodide and 10 µl of DTNB. Briefly, 10 µl of acetylthiocholine iodide reaction mixture was added to 10 µl of crude aphid acetylcholinesterase and 20 µl of the different concentrations of the extract. This was incubated for 30 mins at room temperature and the absorbance was read three times with 5 mins interval at 412 nm with spectrophotometer. Dimethoate was used as a standard insecticide. All assays were corrected for non-enzymatic activity using the same mixtures except replacing the enzyme with 10 µl of sodium phosphate buffer. Enzyme activity was calculated from the absorbance value using Beer lamberts law.

Qualitative phytochemical screening

The crude fruit sap extract of *S. incanum* was subjected to qualitative phytochemical screening to identify presence or absence of selected chemical constituents using methods of analysis as described by [10,11]. Standard screening tests for detecting the presence of different chemical constituents were employed. Secondary metabolites tested included alkaloids, saponins, steroids, cardiac glycosides, terpenoids, flavonoids, phenolics, and tannins.

Data management and statistical analysis

Analysis of the data was done using Microsoft Excel. The enzyme activity was calculated using Beer Lamberts law. Dose response curve was plotted using Graph pad Prism software.

Results

In vitro anti-acetylcholinesterase activity of crude fruits sap extract of S. incanum in green peach aphids

Crude fruits sap extract of *S. incanum* was tested for inhibitory activity against green peach aphids' acetylcholinesterase at increasing concentrations of 10%, 25%, 50% and 75% by using Ellman's *in vitro* method. All tested concentrations of crude fruits sap extract of *S. incanum* possessed the ability to inhibit acetylcholinesterase of green peach aphids at dose dependent manner (Figure 1). The enzyme activity decreases with the increase in the concentration of the extract. The enzyme activity is expressed as µM/min/mL.

Meanwhile, from the graph shown in Figure 2, an IC_{50} value of 49.9 was calculated from plotting a regression line through the linear portion of the graph and using the plot equation to calculate the concentration (x) at which 50% inhibition was observed. The protein content of the isolated acetylcholinesterase was 13.5 mg/ml as determined using Bradford method.

Qualitative phytochemical screening

A number of compounds were found in the crude fruits sap extract of *S. incanum*. As Table 1 show, the extract contained alkaloids, saponins, cardiac glycosides, terpenoids, flavonoids and tannins while steroids and phenolics were absent.

Discussion

Acetylcholinesterase affects the cholinergic system because it regulates the level of acetylcholine and terminates nerve impulses by catalyzing the hydrolysis of acetylcholine. Its inhibition causes death. Irreversible inhibitors have been developed as insecticides such as organophosphates and carbamates [12].

In this study, crude fruits sap extract of *S. incanum* inhibited the acetylcholinesterase of green peach aphids. The acetylcholinesterase activity was dose dependent and decreased with the increase in the extract concentration. The inhibition of AChE in the present study is in line with previous studies by other researchers. The inhibition of AChE was observed in the cockroach, *Periplaneta americana* L [13] and the snail, *Limnaea acuminate* Lamarck, at 40% and 80% concentrations of neem oil [14]. It was also observed that 25 g of distilled water extracts of the botanicals *Punica granatum* L., *Thymus vulgaris* L., and *Artemisia absinthium* L., significantly inhibited the AChE activity of nematodes at 100% concentrations [15].

Aqueous crude fruit sap extract of *S. incanum* shows it potency in inhibiting the acetylcholinesterase of green peach aphids with IC_{50} of

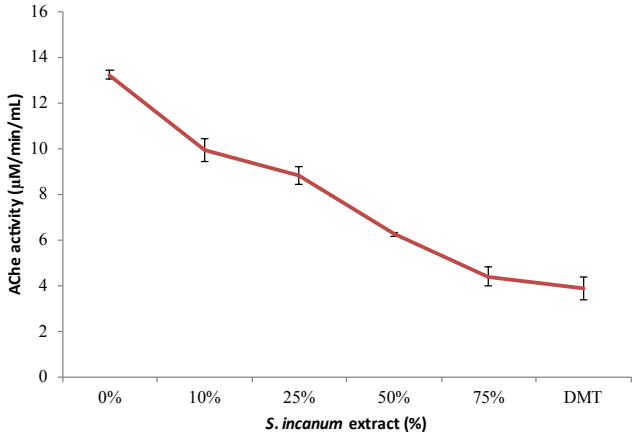

Figure 1: *In vitro* anti-acetylcholinesterase activity of crude fruits sap extract of *S. incanum* in green peach aphids. DMT=Dimethoate.

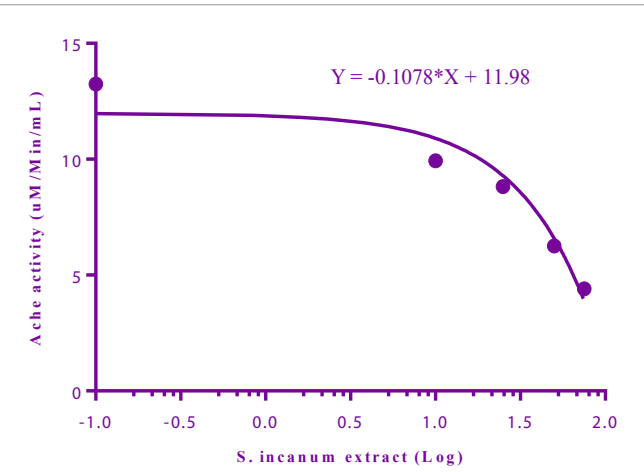

Figure 2: Linear regression from which IC_{50} was calculated.

Phytochemical compound	Result
Alkaloids	+
Saponins	+
Steroids	–
Cardiac glycosides	+
Terpenoids	+
Flavonoids	+
Phenolics	–
Tannins	+

Table 1: Qualitative phytochemical screening of crude fruit sap extract of *S. incanum*. Key: +: Present; –: Absent.

49.9. In addition, the classes of phytochemicals in crude fruit sap extract of *S. incanum* have previously been associated with the inhibiting of acetylcholinesterase.

Conclusion

The results from the experiment suggest that aqueous crude fruit sap extract of *S. incanum* inhibit the acetylcholinesterase of the green peach aphids at a dose dependent manner. It is therefore, possible the extract has compounds that increase both the level and duration of the neurotransmitter action.

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References

1. Wang JJ, Cheng WX, Ding W, Zhao ZM (2004) The effect of the insecticide dichlorvos on esterase activity extracted from the psocids, Liposcelis bostrychophila and L. entomophila. J Insect Sci 4: 23.
2. Li F, Han Z (2002) Purification and characterization of acetylcholinesterase from cotton aphid (Aphis gossypii Glover). Arch Insect Biochem Physiol 51: 37-45.
3. Mwonjoria JK, Ngeranwa JJ, Kariuki HN, Githinji CG, Sagini MN, et al. (2014) Ethno medicinal, phytochemical and pharmacological aspects of Solanum incanum (lin.). I J Pharma Tox 2: 17-20.
4. Habtamu A, Tamene G, Adane L (2014) Phytochemical investigation on the roots of Solanum incanum, Hadiya Zone, Ethiopia. J Med Pla Stu 2: 83-93.
5. Mwine J, Ssekyewa C, Kalanzi K, Van Damme P (2013) Evaluation of selected pesticidal plant extracts against major cabbage insect pests in the field. J Med Pla Res 7: 1580-1586.
6. Mersha W, Ayele N, Fentahun G (2014) Repellent and insecticidal activity of Mentha piperita (L.) plant extracts against cabbage aphid [Brevicoryne brassicae Linn. (Homoptera: Aphididae)]. Amer-Eur J Sci Res 9: 150-156.
7. Elham S, Kamal A, Reza ZD, Amin P, and Haji MT (2012) Toxic and repellent effect of Harmal (Peganum harmala L.) acetonic extract on several aphids and Tribolium castaneum (Herbst). C J Agric Res 72: 147-151.
8. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248-254.
9. Ellman G, Courtney KD, Andres V Jr, Feather-Stone RM (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7: 88-95.
10. Harbone JB. Phytochemical Methods (1998) A Guide to Modern Techniques of Plant Analysis. Chapman and Hal Publishers, London, UK 3: 60-66.
11. Kokate CK (2000) Practical Pharmacognosy. 4th edn. Vallabh Prakashan, New Delhi, India, 107-111.
12. Praveena A, Sanjayan KP (2011) Inhibition of acetylcholinesterase in three insects of economic importance by linalool, a monoterpene phytochemical. Ins Pes Managt 56: 340-345.
13. Shafeek A, Jaya Prasanthi RP, Reddy GH, Chetty CS, Reddy GR (2004) Alterations in acetylcholinesterase and electrical activity in the nervous system of cockroach exposed to the neem derivative, azadirachtin. Ecotoxicol Environ Saf 59: 205-208.
14. Singh K, Singh DK (2000) Toxicity to the snail Limnaea acuminata of plant-derived molluscicides in combination with synergists. J Pes Managt Sci 56: 889-898.
15. Korayem AM, Hasabo SA, Ameen HH (1993) Effects and mode of action of some plant extracts on certain plant parasitic nematodes. Anzeiger Fur Schadlingskunde. J Pes Sci 66: 32-36.