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Induction, immobilization, modification and natural inhibitors of α-glucosidase from *Penicillum chrysogenum*

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Abstract

α -glucosida se (EC: 3.2.1.20) was isolated from *Penicillum chrysogenum*. The enzyme was enhanced by plant growth regulators such as gibberellic acid (GA_3), benzylaminopurine (BAP) and kinetin. Dansyl chloride inhibited the enzyme at 1, 2, 3, 4 and 5 mM with $T_{0.5}$ 67, 52.2, 34.4 and 23.3 min, respectively. The substrate offered partial protection for the enzyme against dansyl chloride inhibition. The enzyme was activated by Ca^{2+} and Mg^{2+}. However, Pb^{2+}, Cd^{2+}, Zn^{2+}, Ni^{2+} and Hg^{2+} inhibited α -glucosidase activity. The enzyme was immobilized on Ca alginate and the optimal concentration for 3% w/v. The optimal concentration of $CaCl_2$ was recorded at 3 mM. The optimal $CaCl_2$ concentration and the optimum time for immobilization was 3mM and 4hr. The enzyme was inhibited by aqueous extracts of *Datura stramonium, Trigonella foenum-graecum, Hyoscymus muticus and Cynodon dactylon*. The IC_{50} values for the four extracts were 59.1, 73.6, 68.5 and 77.1 μg ml^{-1}, respectively.

Keywords: P chrysogenum; glucosidase; Induction; Modification; Immobilization; Inhibition

Introduction

It is difficult to recover the soluble enzyme from the reaction mixture. Thus, it cannot be applied for catalyzing more reactions, however, the procedure of immobilization can make it feasible. The loss of some activity for the immobilized biological catalyst in repeated use is a common phenomenon [1].

The aim of immobilization of any enzyme is to increase its stability against different factors of incubation. This due to the fact that the mobility of immobilized enzyme is restricted. Thus, the immobilized enzyme could act under various environmental conditions with little loss of activity compared to the free enzyme [2].

Immobilized α -glucosidase may be applied repeatedly since it retains its activity. Thus, there is intensive work to use the immobilized enzymes to be retained in a biochemical reactor and allow operation in continuous way and consequently lower cost of its use [3].

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Thus, the present work aimed to induce, immobilize and modif y the purified enzyme from *Penicillum chrysogenum* and testing some plant extracts regarding their inhibitory effects on α -glucosidase.

Material and methods

Materials

Glycerol and other chemicals mentioned in the investigation were purchased from Sigma Chemicals (Sigma Aldrich, Steinheim, Germany). All media cultures including Plate Count Agar (PCA) and others were obtained from Merck Company (Merck, Darmstadt, Germany).

Experimental

Penicillium chrysogenum Thom ATCC 10106 was provided by Salwa A. Khalaf, Prof. of Microbiology, Botany Department, Faculty of Science, Zagazig University.

Growth medium

Modified Czapak´s dox agar (CDA)

This medium used for growth of *P. chrysogenum* at a final pH 7.3 was described by Eaton et al. [4] includes the following in g/L: 2g sodium nitrate, 30g glucose, 1g potassium dihydrogen phosphate, 0.5g potassium, 0.5g chloride, 0.5g magnesium sulphate, 20g agar, 0.01g ferrous sulfate and 1L distilled water. Boil to dissolve the medium completely. The medium was sterilized by autoclaving at 15 Ibs pressure (121ºC) for a period of 15 min. The medium was mixed well and poured into sterile petri plates. Cultural characteristics observed after an incubation at 25ºC - 30ºC for 48-72 h and were kept in the refrigerator at 4ºC for storage.

Potato-dextrose agar medium (PDA)

This medium used for the inoculum preparation at final pH 5.6 was that Vanderzant and Splittstoesser [5]. It included of the following in g/L: 20g dextrose, 4g potato extract, 15g agar and 1L distilled water. Boil to dissolve the medium completely and sterilize by autoclaving at 15 Ibs pressure (121ºC) for 15 min. Mix well before dispending. The volume of required acid for 100 ml of sterilized medium was approximately 1ml. The medium should not be heated after the addition of acid. Cultural characteristics recorded after incubation at 25ºC to 30ºC days. Rate of recovery is considered as 100 % for the growth of *P. chrysogenum* on Sabouraud Dextrose Agar.

The effect of phytohormones on α-glucosidase activity

Three phytohormones including kinetin, gibberellic acid (GA_3) and benzyl amino purine were tested at different concentrations (20, 40, 60, 80 and 100 μmole) followed by measuring α-glucosidase activity spectrophotometrically.

Modification of α-glucosidase.

Stock solution of DnsCl in acetonitrile were freshly prepared 0.5 ml of the enzyme was incubated for 1h at 4 0C with different concentrations of DnsCl (1, 2, 3, 4 and 5 mM) in 100 mM Tris (pH 7). The enzyme was incubated in 100 mM Tris (pH 7) containing various concentrations of DnsCl for 15 min. Terminate the reaction by adding 20 mM β-mercaptoethanol and 30 mM lysine.

Effect of various cations on α-glucosidase activity

Different metal cations were tested regarding their effects on α-glucosidase activity. These cations were Zn^{2+}, Cd^{2+} ,Mn^{2+} ,Co^{2+}, Na^{2+} and Ca^{2+} and tested at 5 mM in the reaction medium followed by measuring enzyme activity.

Immobilization of α-glucosidase

α-glucosidase was immobilized by alginate) Sodium alginate (5 % w/v) in 150 mM sodium phosphate buffer (pH 8.0) was prepared by heating at 50 ºC then cooled down to room temperature. The solution containing enzyme was mixed with sodium alginate solution in total volume 10 ml. The mixture was put into a syringe, and beads were formed by dropping the solution into 100 mM $CaCl_2$ solution after gentle stirring for 2 h at 4ºC. The beads were then filtrated and washed thoroughly with distilled water, dried using filter paper (whatman no.1). The beads were exposure to the open air for a period of 1 h before being used. The filtered solution of $CaCl_2$ was used for determination of α-glucosidase activity.

Determination of α-glucosidase immobilization yield.

The yield of immobilization was identified as the yield for enzyme which was immobilized by entrapment and expressed by the following equation:

Immobilization yield (%) = (Activity of immobilized enzyme / Activity added) $\times$ 100.

Reusability of immobilized α-glucosidase.

Reusability was done by measuring the enzyme activity throughout 10 cycles.

Effect of extracts from various plants on α-glucosidase

Experimental

Datura stramonium L. is belonging to family: Solonaceae and was collected from Mansoura University area. *Trigoella foenum-graecum* L. belongs to family: Fabaceae and was brought from the local market of Mansoura. *Hyoscymus muticus* L. belongs to family : Solanaceae and was collected from Wadi Hagul region of the northern part of the Eastern Desert of Egypt, (The Isthmic Desert). *Cynodon dactylon* L. belongs to family: Poaceae and was collected from cultivated area of Mansoura Governotare.

Preparation of plant extracts

Plant leaves were air-dried and extracted aqueously by distilled water then various concentrations (20, 40, 60, 80, 100 μg ml^{-1}) were prepared and tested for their effects on α-glucosidase activity.

Results

Effect of plant growth regulators on α-glucosidase activity from P. chrysogenum

The results in Fig. 1 reveal that the three tested phytohormones GA_3, BAP and kinetin induced α-glucosidase activity and GA_3

was the best inducer followed by BAP and kinetin. Also, it was remarkable that there was continuous increment in α -glucosidase activity with increasing the time of incubation. GA_3 as growth regulator induced the activity of α-glucosidase. GA_3 induced the activities of other enzymes such as phosphoenol pyruvate carboxy lase [6], myrosinase [7], NADH-glutamate synthetase [8], sucrose synthase [9] and acid phosphatase [10]. The enhancement of α-glucosidase by GA3 might be attributed to enhancing of α-glucosidase synthesis through controlling the transcription and translation.

Benzylaminopurine (BAP) induced α-glucosidase of *P. chrysogenum*. BAP enhanced the activities of other enzymes including glutamate oxaloacetic acid transaminase [11], and RNA polymerase [12]. α-glucosidase activity was induced in presence of zeatin in the growth medium. Also, L-asparaginase activity of *Aspergillus niger* was found to be induced by zeatin in growth medium [13]. The induction of α-glucosidase by zeatin could be explained on the bases that zeatin may act at the transcriptional or posttranscriptional level including posttranslational regulation, however, this require more investigation for clarification of the actual mechanism of induction.

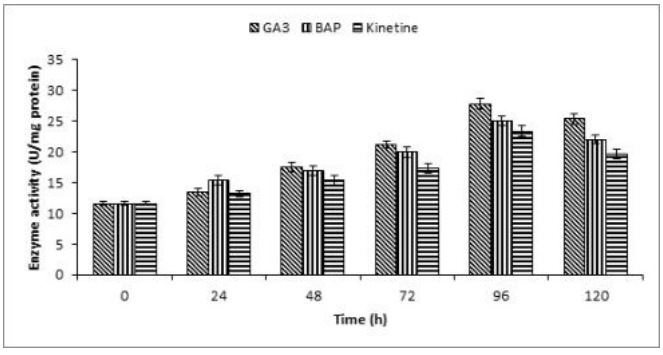

Figure 1 Effect of phytohormones on α -glucosidase from *P. chrysogenum*.

*Effect of dansyl chloride on thermostability of α-glucosidase from P. chrsogenu*m

Dansyl chloride is a reagent used for the detection of lysyl residue in the protein [14]. The obtained results in Fig. 2 show that da nsyl chloride inhibited α -glucosidase activity at all tested concentrations. The inhibition was dependent on both the concentration and the time of incubation. The T0.5 values were 67, 52.2, 34.4 and 23.3 min. Plotting log conc. against log $t_{0.5}$ (Fig. 3) resulted in straight line with slope 0.7. The effect of substrate of α -glucosidase on the enzyme protein against dansyl chloride was carried out using 5 and 10 mM substrate in presence of dansyl chloride (Fig. 4). The resulting reveal that the substrate of α -glucosidase offered appreciable protection against dansyl chloride and the protection was dependent on the substrate concentration. Dansyl chloride is known as a reagent for lysyl group in proteins [15]. In this investigation d ansyl chloride inhibited α -glucosidase activity revealing that lysyl residue is present in the active site and taking essential part in the enzyme catalysis.

Generally, modification of enzyme chemically plays a role in probing the mechanism of enzyme activity. This technique is applied for identification of the amino acid in the enzyme molecules which are responsible for the catalytic properties of the entire protein. During the chemical modification, the enzyme activity may be altered [16]. Generally, it was observed that the chemical modification of the enzyme could selectively target the residues specifically at the active site, as demonstrated by substrate protection. The obtained results in this paper are valuable for determination of the active groups which are taking part for enzyme catalysis for possible regulation of the enzyme activity.

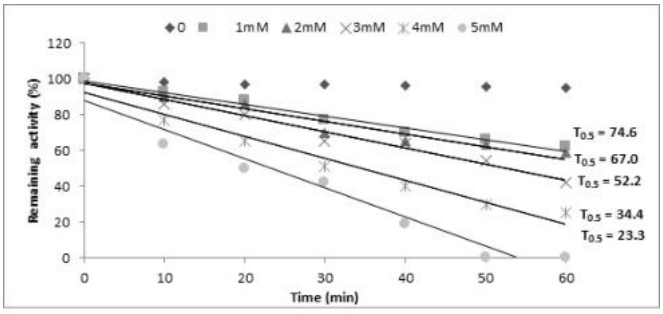

Figure 2 Effect of dansyl chloride on α -glucosidase activity from *P. chrysogenum*.

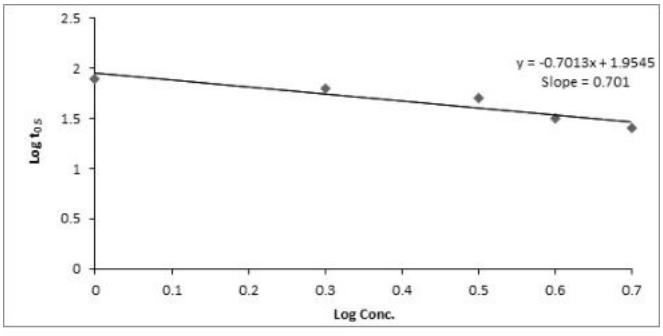

Figure 3 Plot of log $t_{0.5}$ against log dansyl chloride concentrations.

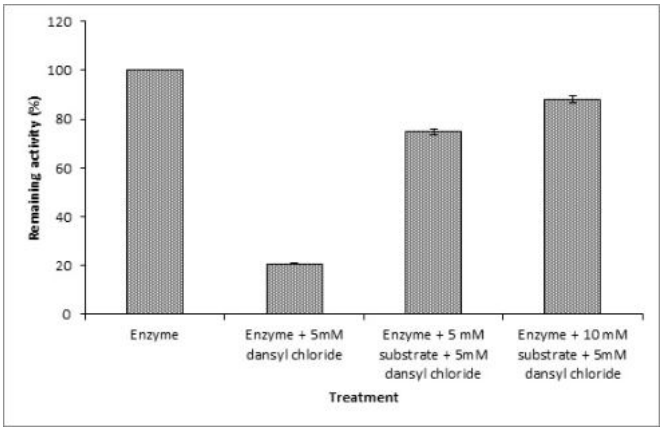

Figure 4 Protection of α -glucosidase by its substrate against inactivation by dansyl chloride.

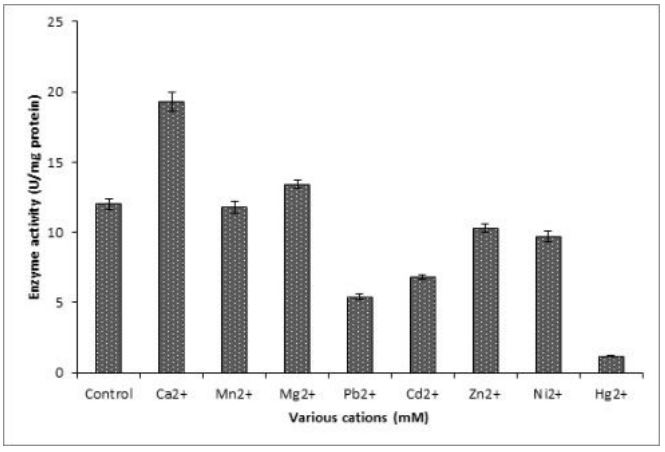

Figure 5 Effect of different cations on α -glucosidase activity from *P. chrysogenum.*

Effect of different cations on α-glucosidase activity from P. chrysogenum

The effect of different metal cations on α-glucosidase activity was studied. The cations tested were Ca^{2+}, Mn^{2+}, Mg^{2+}, Pd^{2+}, Cd^{2+}, Zn^{2+}, Ni^{2+} and Hg^{2+}. These cations were examined at 5 mM in the reaction mixture. The enzyme activity was expressed as unitsmg^{-1} protein (Fig. 5). The relative activity was also calculated. The results revealed that Ca^{2+} was the best activator for α -glucosidase whereas Hg^{2+} was the most suppressive cations re duced the activity to 1.2 units mg^{-1} protein. The other remaining cations expressed variable inhibitory effect on the enzyme activity. α -glucosidase was activated by Ca^{2+} and Mg^{2+}, however Pb^{2+}, Cd^{2+}, Zn^{2+}, Ni^{2+} and Hg^{2+} were inhibitors. The activation of α-glucosidase by Ca^{2+} could be due to the in forcing of the interactions inside α-glucosidase molecules and by the binding of Ca^{2+} to the autolysis site. Ca^{2+} activated other enzymes such as phytase [17] and glucose isomerase [18].

The inhibition of α -glucosidase by Cd^{2+} and Hg^{2+} might be due to oxidation of sulfhydryl groups of α -glucosidase. Heavy metals could inhibit α -glucosidase activity by binding to sulfhydryl groups in proteins or might disrupt enzyme protein structure or displace of an essential element [19].

Immobilization α-glucosidase activity from P. chrysogenum on calcium alginate

The pure α -glucosidase was immobilized on calcium alginate and the immobilized yield was calculated. The results in Fig. 6 indicate that immobilization yield increased up to 3 % w/v of calcium alginate then declined at 4 and 5 % w/v where the immobilization yield were 31 % w/v and 28 % w/v, respectively. This critical point α -glucosidase activity is at its maximum and this temperature is called optimal temperature. α-Glucosidase was immobilized using Ca alginate and the optimal concentration was 3% w/v. After which the immobilization decreased at the higher concentrations. The best time for immobilization of α-glucosidase was 4h. The present results revealed that α -glucosidase immobilized on Ca alginate was reusable for 10 cycle where the enzyme retained 50% of its activity. The immobilized α -glucosidase in the present investigation could be preferred to avoid costly purification processes and to increase catalytic stability as mentioned by [3].

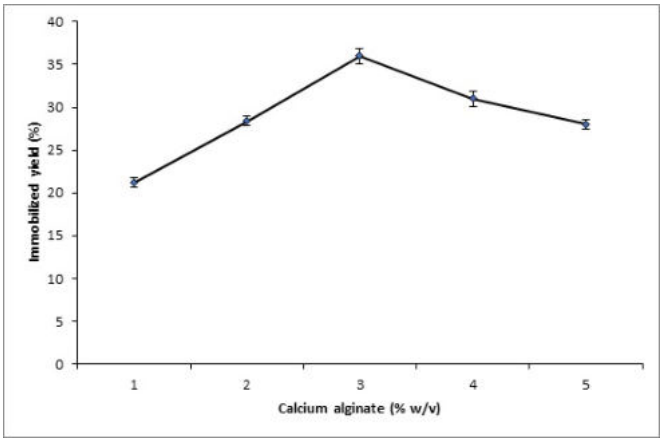

Figure 6 Immobilization α -glucosidase activity from *P. chrysogenum* on calcium alginate.

Effect of CaCl 2 immobilization of α-glucosidase activity from P. chrysogenum

The recorded results in Fig. 7 show continuous increase in the immobilization yield with the increase in $CaCl_2$ concentration

up to 3 mM followed by reduction at 4 and 5 mM where the immobilization yield values were 62 and 54 %, respectively.

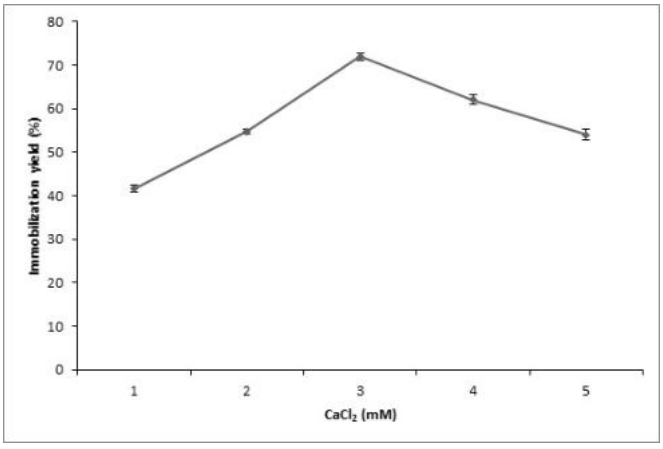

Figure 7 Effect of $CaCl_2$ on immobilization yield of α -glucosidase activity from *P. chrysogenum*

Effect of immobilized time of immobilization yield on α-glucosidase activity from P. chrysogenum.

The results in Fig. 8 indicate continuous increase in the immobilization yield up to 4 h after which the immobilization yield decreased at 6 and 5 h where the immobilization yield was 52.8% and 40.4%, respectively.

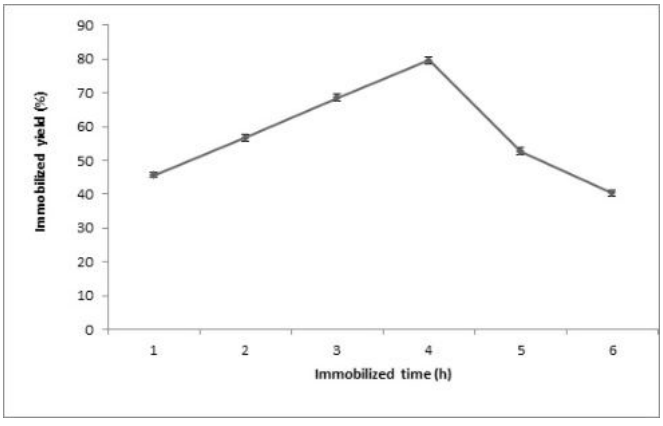

Figure 8 Effect of immobilized time on immobilization yield of α -glucosidase activity from *P. chrysogenum.*

Reusability of immobil ized α-glucosidase enzyme from P. chrysogenum.

This experiment was done to study the reusability of alginate-immobilized α-glucosidase throughout 10 cycles (Fig. 9). The results of the reusability experiment reveal that α-glucosidase activity decreased gradually throughout the 10 cycles, however 50% of its initial activity after the 10th cycle.

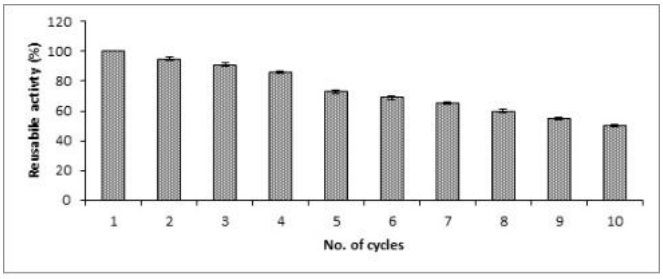

Figure 9 Reusability of immobilized α- glucosidase enzyme.

Effect of plant extracts on α-glucosidase activity from P. chrysogenum.

In trial to find a natural inhibitors for α -glucosidase activity it was decided to study the influence of extracts from various plant species on α -glucosidase activity. The tested plants *Datura stramonium L., Trigonella foenum-graecum L., Hyoscymus muticus L. and Cynodon dactylon L.* Each plant extract was examined at different concentrations 20, 40, 60, 80 and 100 μg ml^{-1}.

The illustrated results in Fig. 10 showed the influence of these plant extracts extract on α-glucosidase activity. These results showed continuous inhibition of the enzyme activity by the plant 1 extract in a concentration dependent manner. The activity was expressed as units mg^{-1} protein and the relative activity was calculated. The IC_{50} of three plant extracts were 59.1, 73.6, 68.5 and 77.1 μg ml^{-1}, respectively. The aqueous extracts from tested plants (*Datura stramonium, Trigonella foenum-graecum, Hyoscymus muticus and Cynodon dactylo*n) inhibited α-glucosidase activity. The inhibition of α -glucosidase activity by the four tested plants might be due to the presence of several phytochemicals, saponin and tannin in these extracts. Previous studies on inhibitors of α -glucosidase inhibitors isolated from various medicinal plants suggested that flavonoids and potential inhibitors for α -glucosidase [20].

Conclusion

T he present results revealed successful method for induction of α -glucosidase and its immobilization on alginate. In addition, the results introduce four plant extracts as a natural candidates for inhibiting of α -glucosidase. However, it needs to know the

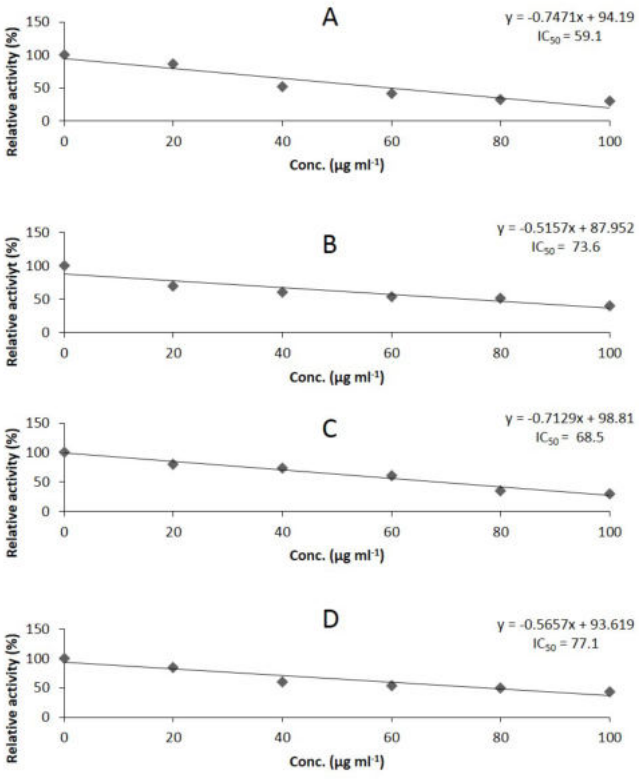

Figure 10 The relative activity of α- glucosidase in presence of various concentrations of plant extracts. A: *Datura stramonium*, B: *Trigonella foenum-graecum*, C: *Hyoscymus muticus* and D: *Cynodon dactylon.*

main compounds of which exhibit the bioactivity of these extracts.

Author's contributions

Hamed M. El-S hora, Mohsen E. Ibrahim and Mohammad W. Alfakharany performed the experiments together and participated in manuscript for publications.

C onflicts of interest

None.

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