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Journal Name:	International Journal of Biochemistry Research & Review
Manuscript Number:	2014_IJBcRR_13617
Title of the Manuscript:	Isolation of Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase from Young Leaves of <i>Phytolacca dioica</i> L.
Type of the Article	Original Research Article

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This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound.

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PART 1: Review Comments

	Reviewer's comment	Author's comment <i>(if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)</i>
Compulsory REVISION comments	Abstract In the abstract, line 3 replace "Therefore, the present study aimed" by " The present study was aimed" In the Study design, replace "considering high value" by "based on high value " In the Methodology replace "RubisCO from P. dioica" by"RubisCO from P. dioica leaves" In the conclusion, replace "could be used as the starting point for biological studies aimed to characterize this enzyme". by "could be used as the starting point for biological characterization of this enzyme". 1. INTRODUCTION Page 2 (lines 36-37 replace "The structure of this holoenzyme is typically 550,000 in molecular weight and presumably consists of eight of each of the two types of subunits (LS) 8" [6]" by "The molecular weight of this holoenzyme is typically 550,000. The structure presumably consists of eight of each of the two types of subunits (LS)8" [6]"	As request the sentences have been revised. In addition, the English has been improved. The authors are grateful to the referee!.



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	Page 2 (line38) replace “of 70-90 %” by “approximatly of 70-90 %” Page 2 (line 39) replace “are only for the 30-35 % identical in amino acid sequenc” by “are approximatly 30-35 % identical in amino acid sequenc” page2 (line 42-43) replace “RuBisCO is usually only active during the day as ribulose 1,5-bisphosphate. It is not regenerated in the dark” by “RuBisCO is usually only active during the day as ribulose 1,5-bisphosphate is not regenerated in the dark.” Page 2 (line 47) replace “proficiency” by “efficiency” 2. MATERIAL AND METHODS Page 2(line 61) replace “eaves “ by “leaves “ page 2 (line 63) delete “were” from “were from” page 3 (line 66) add “grade” after “analytical” and place between two braket (analytical grade) page 3 (line 72), replace “NaCl 0,3 M by “NaCl 0.3 M” Page 3(line 80) replace “protein were” by “The homogenate were” (since proteins are not PPT by ammonium sulfate) page 3 (line 83) it is stated that” Superdex 75 (GE Healthcare, Milan, Italy; separation range 100-3 kDa)”, please check the separation range of the gel page 3 (line 84) replace 'AKTA Purifier' by “ AKTA prime” page 3 (line 95-96) replace “condition and after electrophoresis,” by “condition. After electrophoresis,”	
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	page 3(line 114). Briefly describe the method of measuring RubiscCO activity 3. RESULTS AND DISCUSSION Page 4 (123-130 and 139-144) replace “Although the presence of RubisCO in <i>P. dioica</i> has not been investigated so far, crude extracts from young leaves of <i>P. dioica</i> were assayed (data not shown). The preliminary data showed a high RubisCO activity from young leaves of <i>P. dioica</i>, thus heading towards the purification of RubisCO. Therefore, a purification procedure was undertaken in order to isolate potential novel RubisCO. The protein fraction extracted from young leaves of <i>P. dioica</i> with Tris●Cl saline buffer was submitted to gel-filtration chromatography, at pH 8.2. Each 128 fraction showing RubisCO activity was analysed by SDS-PAGE (data not shown) and the 129 homogeneous samples (fraction numbers 39-43) were pooled (Peak 1, Fig. 1). Peak 1, showing RubisCO activity, with an eution volume with a molecular mass higher than 100 kDa was concentrated and subjected to partial enzymatic and structural characterisation (hereafter named RubisCO-Pd). Isolated RubisCO-Pd, analysed by SDS-PAGE, showed two bands of 56 and 15 kDa in the presence of β-mercaptoethanol (Fig. 2 A, lanes 1-2). On the other hand, when characterized by gel-filtration, a higher value than 100 kDa was obtained.” By “The presence of RubisCO in <i>P. dioica</i> has not been investigated so far. Based on that, extracts from young leaves of <i>P. dioica</i> were assayed (data not shown). The preliminary data showed a high RubisCO activity from young leaves of <i>P. dioica</i>. Therefore, a purification procedure using young leaves was undertaken in order to isolate potential novel RubisCO. Fractionation of the concentrated portion of the homogenate was carried out by gel-filtration chromatography. Detection of the biological activity was carried out as	
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	described above. fractions showing RubisCO activity were analysed by SDS-PAGE (data not shown) and the homogeneous samples (fraction numbers 39-43) were pooled (Peak 1, Fig. 1). Peak 1, showing RubisCO activity (hereafter was named RubisCO-Pd) was concentrated and subjected to further analysis. Subjection of Isolated RubisCO-Pd to SDS-PAGE (in the presence of β-mercaptoethanol), revealed two bands of 56 and 15 kDa (Fig. 2 A, lanes 1-2). On the other hand, when characterized by gel-filtration, a higher value of more than 100 kDa was obtained.” page 6 (line 169) delete “in a novel set of experiment” Page 6 (line 171-172) replace “This analysis provided two different sequences (up to residue seventeen), 1-AGVKDYKLTY YTPQYKP-17 and 1-QVWPDLLGKK FFETLSY-17 for band 1 and band 2, respectively (Fig. 3).” By “ The analysis of band 1 and band 2, revealed two different sequences; 1-AGVKDYKLTY YTPQYKP-17 and 1-QVWPDLLGKK FFETLSY-17 respectively (Fig. 3)” page 6 (line 177) replace “ is reported” by 'is presented” page 6 (line 181)replace “results” by “ shows” Page 6 (line 192-197) replace, “The RubisCO-Pd showed two predominant bands by SDS-PAGE; the Large subunit, whose molecular weight was about 56 kDa and the other was the Small subunit, whose molecular was about 16 kDa, whereas a high molecular weight was obtained by gel-filtration. N-terminal sequences of both large and small subunits of RubisCO-Pd show share a high percentage of identity with those of other plants RubisCO enzymes. “ By “Analysis of RubisCO-Pd by SDS-PAGE showed two predominant bands. The molecular weight of large and small bands are; 56 kDa and 16 kDa respectively. A molecular weight of more than 100 was obtained by gel-filtration. N-terminal sequences of both large and small subunits of RubisCO-Pd showed	
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	a high percentage of identity with those of other plants RubisCO enzymes.” The English in general needs special attention throughout the manuscript. There are many editing needs to be done, please give serious attention to this. In general, I agree that this paper worth publication.	
<u>Minor</u> REVISION comments		
<u>Optional/General</u> comments		