



SDI Review Form 1.6

Journal Name:	<u>Journal of Advances in Medical and Pharmaceutical Sciences</u>
Manuscript Number:	Ms_JAMPS_20557
Title of the Manuscript:	Molecular detection of azurin: a powerful anticancer protein from native Pseudomonas isolates
Type of the Article	Original Research Article

General guideline for Peer Review process:

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.

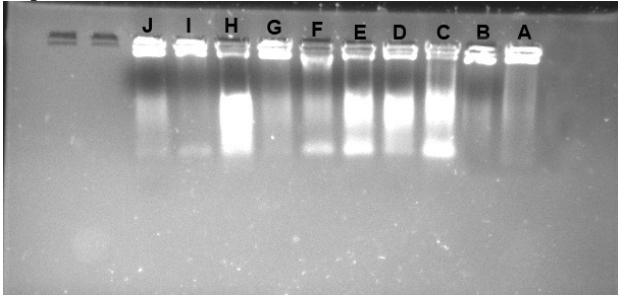
To know the complete guideline for Peer Review process, reviewers are requested to visit this link:

(<http://www.sciencedomain.org/page.php?id=sdi-general-editorial-policy#Peer-Review-Guideline>)



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PART 1: Review Comments

	Reviewer's comment	Author's comment (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)
Compulsory REVISION comments	<u>Material and methods</u> Where were the controls? I suggest put azurin (i think it commercially available inspite of its highly price) and mixture without DNA as positive and negative control. Controls of this kind test were very important. <u>Result</u> After 3.1 subsection NO 3.2 subsection , direct to 3.3 . genomic DNA extraction... missed any data or miss typed? Please check. Figure 1. I do not think isolates "C" reveal correct DNA extraction; relevant band was not looked as clear as others. Please check and enlarge the figure.	No control was kept for this PCR. Presence of azurin gene determined with DNA ladder according to the size of the amplicons(545bp).Some works on PCR of azurin gene reported by Yehiya <i>et al</i> have been done without the use of control[29]. Subsection numbering corrected from 3.2 onwards Whole DNA extraction procedure and agarose gel electrophoresis was repeated several times. From the many photos taken I am attaching here one photo in which DNA of isolate C is seen as clear band, but isolates J and I reveal bands stuck in the gel. From this it is clear that the absence of azurin in isolate C (fig. 2) was not due to problem in whole DNA extraction. 



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	Figure 1 and 2: Please re arrange the title of the figure in common manner (noun form) and followed by relevant explanation regarding the subject. <u>Discussion</u> Several issues might be interest to rise: -Two isolates (B and C) of eight were not revealed for azurin. Is it because <i>Pseudomonas aeruginosa</i> B and C did not produce azurin? or something not working well in extraction and PCR steps? You can give some explanations. - About azurin detection methods, are there any methods other than PCR and followed by SDS PAGE protein profile, you can compare with? I suggest you to rise this issue regarding assuring the azurin protein originated from <i>Pseudomonas aeruginosa</i>. -What different you already performed (may be the alternative condition of the PCR amplification as stated in 162 line) might be interest to discussed. -NO CONTROL in PCR REACTION: such a lacked step. If any reason you might consider please put in the discussion section.	Title of figures are rearranged and corrected. Some <i>Pseudomas aeruginosa</i> strains with mutations or deletions in the azurin gene that will not produce azurin. Necessary citations are made in discussion session[28,29]. PCR is the accurate and rapid test to identify azurin gene presence in large number of isolates. Other methods are SDS PAGE, Western blotting and N terminal sequencing but they require high degree of purity or anti azurin antibody . Necessary citations are made in discussion session [30,31]. Different temperature and time combinations were practiced using gradient PCR and 60°C for 45 seconds was found to be more specific. Some works on PCR of azurin gene reported by Yehiya et al have been done without the use of control[29].
<u>Minor</u> REVISION comments		
<u>Optional/General</u> comments		