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PART A:

Journal Name:	International Journal of Biochemistry Research & Review
Manuscript Number:	MS: 2012/IJBcRR/1562
Title of the Manuscript:	<i>Phytochemical screening and biological activities of Hypotrigona squamuligera raw honey.</i>
Manuscript received on (Date)	
Review comment submitted (Date)	



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PART B: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part and write here 'Corrected'/ if not agreed, give suitable justifications)
General comment:	The topic of the manuscript is novel and interesting since phytochemical composition, and functional (antioxidant and antibacterial) activities of honey produced by <i>Hypotrigena squamuligera</i> has not been investigated before. However, there are several major issues related to the methodology and results that have to be clarified and improved.	
Specific comments:		
Title and abstract	"The phytochemical screening" should be omitted from the title since only simplified and preliminary qualitative testes were conducted in this study that did not convincingly proved the presence of certain compounds.	Standard chemical test used in this study affords a cheap reliable method of showing the types of phytochemical compounds present in crude extracts without necessary fractionating it. Initial separation may result in loss of information as some characteristic of compounds is due to synergism. This test gives reliable results and the phrase phytochemical screening should remain. There is no need to carry out the test with standards as this is a validated method in favour with many authors.
Introduction	Introduction is concise and provides a good rationale for undertaking of the study.	
Review of literature (Heading may differ in the case of review paper)	Only a minor suggestion: A review of honey as antibacterial agent and wound healing aid should include Peter Molan's papers. He is a pioneer in research on antibacterial activity of honey.	



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Materials & methods (Heading may differ in the case of review paper)	1. There is no sufficient information provided relating to the methodology used. A. Extraction of honey with methanol. What was the final concentration of extract after filtration? For example, some researchers would air-dry 1 ml of filtrate and weight it to express the extract concentration in mg/ml. B. Phytochemical screening: The authors provided a list of methods used to screen honey for phytochemicals but the methods lack descriptions of reactions and the expected outcomes of these qualitative tests. The references to the original method should be provided. C. Determination of antioxidant activity: lack of reference to the original method. What was the initial extract concentration from which series of dilutions (0.0125, 0.025, 0.05 and 0.1 mg/ml) were prepared? What was the concentration of quercetin and ascorbic acid standards? What was the blank sample? D. Antibacterial activity. Line 71: Nutrient agar cannot be melted at 30°C. The sentence should be corrected to read: Nutrient agar was melted and then kept in a stem bath set at	Corrected. Please note that the honey used is crude including pollen and spores. This honey is amazing and its not coming from a bee type. The insect cannot be classified as a bee. Even its appearance is very different from that of Apis mellifera honey. We are not sure of all the types of plants visited by the insect. The insect habitats the wild consisting of many plants. The reactions of phytochemicals and the procedures are available in previous studies such that describing them would be repeating issues. Ascorbic acid and quercetin are in solid form which was used to prepare the successive concentrations as shown in the result table.
Results & discussion (Heading may differ in the case of review paper)	1. Phytochemical screening Results of preliminary qualitative tests for honey	Corrected.



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	phytochemicals indicated the presence of tannins, saponins and cardiac glycosides. To the best of my knowledge, these compounds have never been found or described in any honey from any geographical origin before. If the authors have some references which would support their findings, these references should be included into the text. The reliability of the qualitative tests is under question since the authors have not described how the tests were conducted and whether the standard positive controls were used (saponins, tannins or cardiac glycosides) to assess the intensity of the reaction (weak, medium, strong). Could these simplified qualitative methods generate false positive results? For example, could gallic acid which is a component of tannins, and flavonoid give similar results in the reaction with FeCl_3? Both flavonoids and gallic acids are common compounds in honeys while tannins are not. Cardiac glycosides could only contaminate honeys via bee pollen and only if plants that produce it (for example, foxglove, oleander, lily of the valley) grow close to the beehives. On the other hand, several honey compounds are linked to glycosides (phenolic acids, flavonoids, proteins). 2. Antioxidant activity What was the concentration of quercetin and ascorbic acid in the DPPH assay? The Table 1 shows that there is almost no change in antiradical activity of these standards. Was the calibration curve generated to show the relationship between concentrations of quercetin or ascorbic acid and their radical scavenging activity? If yes, what was the regression line equation and R value?	
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	There is no standard deviation included in the Table 1. Does it mean that the experiment was conducted only one time? Recently, honey antioxidant activity has been standardized (Berreta et al, 2005). The authors should consult these methodologies and compare results. 3. Antibacterial activity The quality of the Figure 2 is poor and either should be replaced by a better picture or omitted. It is not clear whether Table 3 represents the mean diameter of inhibition zones obtained from n = 6 repetition? If yes, what was the standard deviation? It would be beneficial to express antibacterial activity of this honey in the Minimum Inhibitory Concentrations (MIC) values based on the zone of inhibition. What is the MIC value of amoxicillin against E. coli and S. aureus used in the study? What are ATCC numbers of these microorganisms?	
Conclusion	Due to uncertainty of results from phytochemical screening and the antiradical activity of this honey, the conclusions and the abstract might have to be modified.	corrected
References		