Reviewer's report

**Title:** Cytotoxic effects of Euterpe oleracea Mart. in malignant cell lines

**Version:** 1

**Date:** 20 February 2014

**Reviewer:** Steve Talcott

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**Abstract:**
Common name for “açai” is also “juçara”. I will leave this one to the locals, but it was my understanding that juçara was the common name for Euterpe edulis, not Euterpe oleracea. Also a small point, but are açai fruits actually “berries” as stated on page 9? They more resemble a drupe than a true berry.

**Methods**
Methods for extraction of (just the) seeds was no clear. Seeds boiled in water (water kept or tossed) and then extracted in MeOH (ground, particle sized, etc are not explained). How were seeds obtained. Why give these details for seeds and not for the bark and “total fruit”. Not sure what “total fruit” means. Was this a whole açai fruit, treated in a like-manner? A bit confusing, overall.

Was a cytotoxicity test run on the cells first, to determine the dosing ranges to be used for additional assays? (As I read on, I see Figures 1 and 2; however, I am not seeing a dosing response from 0 control to a high concentration). Was any attempt to normalize among the plant extracts made, since seeds would logically contain low phenolic concentrations in açai compared to fruit pulp….meaning it may take 100 grams of seeds to get the same total phenolic content of 1 gram of “total fruit” extract. Many additional co-extractives could be present in such a situation.

Quantification of polyphenolics in the seed, “total fruit”, and bark are not clear. How was a concentration of 28, 25, and 15% obtained? 28% of what? By weight? By final volume? Previous reports with açai seeds showed them to contain relatively low polyphenolics concentrations. Some basic indication of content or extract description could be given here (ie. colorless, brown, yellow, red) colored extracts, which will give some indication of what was present.

Figure 1 is a bit confusing, and I get the impression that a true dynamic dosage range was not obtained here. There is a narrow window from which efficacy is being tested, and there is illogical trends based on concentration (and between 24 and 48 hrs). Results on Page 7 are being “cherry picked” as to their importance/significance. I am also confused at the comparisons among the test samples. Without knowledge of the chemistry/composition of these extracts, the amount of biomass it took to attain these concentration, etc it makes the comparisons difficult.
Morphology. What caused the physical changes? Were there tannins present in any extracts?

Discussion:
Page 10 indicates that there are phytoestrogen in acai….however, the reference cited has no data in reference to such estrogens in acai. However, acai has been reported to contain lignans, but then it would be up to the authors to show that lignans were first present in their extracts and second that these compounds were absorbed or acted on surface receptors in the breast cells. Absorption issues are also problematic for breast vs colon cells, since there should be some indication of phytochemical absorption/penetration by the compounds as opposed to the colon cells. Something to think about.

It is unclear why caspase 3 or 7 was not activated. Most classes of polyphenolics will readily kill cancer cells by apoptosis, and this a widely used assay for confirming cell death mechanisms by phenolics. Why this research did not show such a response is puzzling.

Overall, this paper seems a little pre-mature for publication in its current form. The authors generated more questions than answers, which will obviously lead to many future elucidations of the mechanism of action. The chemical characterizations need to be completed, since much of the discussion is based on supposition based on composition. It would be nice to see a standard polyphenolics run in the assays, ie. something with a known dose-response in cytotoxicity and induction of capases. This 'positive control' helps to further elucidate mechanisms. Please double-check your references, since the one paper I did look up did not have the correct content/data cited in this work.

Level of interest: An article of limited interest

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
This paper is too basic and lacking in details for anyone or any company to have a competing interest.
'I declare that I have no competing interests'