Summary

The authors tested the hydroethanolic extracts of the leaves and twigs of *Lippia adoensis* against various parasitic protozoa namely *Plasmodium falciparum* 3D7 and Dd2, *Leishmania donovani* and *Trypanosoma brucei* brucei . They also tested the cytotoxic effects of the extracts using Vero cells. They then did *in vitro* phytochemical analysis of the extracts as well as identifying molecular clusters using GNPS.

General comments

The paper falls short of publication in many ways however these can be rectified through labwork as well as additional information. The idea of testing the extracts of the plant against the protozoan parasites is good but the execution is not done well. It is important to find the active principle in an extract or at least attempt to especially when you have the assays at your disposal. Simply doing phytochemical analysis and using LCMS based software to try and identify compounds in the extracts does not help in identification of the active principle and simply leads to a speculative discussion. In the introduction the authors also show inconsistencies in the chemical composition of the essential oil of the plant that was tested and this leaves one wondering whether the plant could be used in drug discovery as the difference in chemical composition may lead to inconsistence bioactivities. The merit of the work is the bioassays but unfortunately without attempting to identify the active principle, it simply isn't enough to do an invitro phytochemical analysis as well as identifying molecular clusters.

Specific comments

Line 9: The authors used 70 % ethanol for extraction, from my experience 80 % methanol gives the best extraction. Did they try other solvents for extraction and what was the basis of using 70 % ethanol?

Line 15: the statement 'As a result' needs to be changed as it gives the impression the GNPS phytochemical screening gave the growth inhibition results.

Line 25: Bioassay guided fractionation would be the best approach to identify the active compound(s). Metabolomic studies alone cannot give conclusive results as to what the active principle(s) is(are).

Liebe 33, 34: 'These diseases claim the lives of a billion people worldwide annually'. This statement is incorrect.

Line 36: What do the authors mean by 'Environmental changes'?

Line 36, 37: How does eliminating one NTD in each of 50 countries indicate that we are at the midway point of eradicating NTDs in 100 countries by 2030?

Line 38: 'This notorious disease', which disease are the authors referring to? You have to write Malaria and not 'This notorious disease' as you have not mentioned malaria in your previous sentence.

Line 42, 44: Please cite the source of this data before beginning the next sentence.

Line 50: I would not begin a sentence with 'Caused'.

Line 62: The authors wrote human American trypanosomiasis, this is Chagas disease but in the previous sentences they are referring to human African trypanosomiasis, 'Sleeping sickness'.

Line 74: Change revolutionise to revolutionised.

Line 78, 79: change the word derivative to derivatives for both diamidine and nitrofuran.

Line 82, 83: The statement 'there is interest of natural product-based drug screening over high throughput screening of combinatorial libraries' is debatable as natural products also bring challenges like adherence to Lipinskis rules, intellectual property issues, sustainability, changes in chemical composition due to seasonal changes, just to mention a few.

Line 98: This is rather unexpected. Can the authors provide a possible explanation as to why linalool was not found in the wild type plant.

Line 100- 110:The variability in the chemical composition of the essential oil raises questions as to whether this plant can be a reliable source of active compounds.

Line 128-131: Investigating the antiparasitic effects was a good idea, but the authors should have followed up by assaying the fraction by partitioning with n-hexane/ heptane, chloroform, ethylacetate and n-butanol. Partitioning and testing the fractions would have given an indication as to which compounds may be responsible for the activity.

Line 165: Where was the human blood obtained?

Line 172: *P. falciparum* in italics.

Line 181: Why were the tests done in duplicate not triplicate, with duplicate it is not easy to identify an outlier if you have only two readings.

Line 223: why did the authors use varying concentrations of the positive control. Normally you use a concentration where you expect 100 % mortality of your parasite. Why use a range of concentrations?

Line 246: *Trypanosoma brucei* in italics.

Line 260: The authors should have done triplicates.

Line 322: the in vitro phyto chemical screening does not really add much value to the paper as it confirms the presence of compounds that occur in plants in general.

Line 325: On what basis do the authors think carbohydrates could be responsible for the antiparasitic activity?

Line 353: Experiments should be performed in triplicates

Line 360: Table 1, may the authors be consistent on the number of decimal points. Also give a numerical figure for the CC_{50} of vero cells rather than saying it is greater than 100 as this figure is used in calculating the SI.

Line 366, 369: In line 366 the authors refer to values of 10.008 and 97.467 as relevant. In line 369 the authors refer to 29.48 and 26.96 as moderate, yet these lie in between 10 and 96. Should they also not be relevant?

Line 385: Shouldn't the molecular networking have confirmed the presence of the compounds known to be abundant in the essential oil of *Lippia adoensis*?

Line418: 10.008 micrograms per ml is referred to as significant. In line 420 and 421, 97.467, 29.48 and 26.96 micrograms per ml are referred to as moderate. What criteria did the authors use to determine what moderate and significant activity is?

Line 422-443: This part of the discussion is very speculative and the activity of the compounds mentioned cannot be linked to the activity of the extracts as the compounds were not identified in the extract.

Line 443-445: The authors should have attempted to isolate the compound linalool through column chromatography in order to test this claim.

Line 452-460: The LC-MS feature based detection and molecular networking flow on GNPS did not give any valuable information pertaining to the activity of the extract and what the authors state here is speculative.

Line 462: The non toxicity on vero cells is not an indication that the extracts are safe to use as this is just one cell line. Further tests would be needed to make this justification that the plant is safe to use. The authors did however mention the need for further testing.

Line 484: The authors cannot conclude that the compounds identified using GNPS were responsible for the antiparasitic effects.

Line 485: The authors state that the IC50 values are 'significantly low', how do they come up with the criterion to determine whether activity is high, low, significant, insignificant, moderate etc?

There is no supplementary data attached. The raw data used to calculate the IC_{50} and CC_{50} values has to be presented in the supplementary data.