Reviewer's report

Title: Anti-ageing potential of Vayasthapana Rasayana correlates with decreasing oxidative stress by free-radical quenching action

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Reviewer: Nishant P Visavadiya

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Major Compulsory Revisions:

Paper regarding “Anti-ageing potential of Vayasthapana Rasayana… is based on in-vitro free radical scavenging activity, It is interesting that “rasayana” demonstrates antioxidant potential; however, after careful reviewed this paper, there are several concerns raised, particular in result and discussion section, which are as below.

(1) Add ref. at page # 3, “Recent investigations have shown that the antioxidant… different human diseases and aging process”.

(2) Page # 3: “In Indian traditional Ayurved system, there remains therapies exist for both timely and untimely ageing”, sentence should be revised accordingly, it confusing!

(3) Results section is confusing including M & M section of same! Such as Method did not explain how ABTS assay is carried out for VRF formula, except for ABTS solution preparation along with author did not describe the dosage (concentration of VRF used for assay), it should be in the form of µg/ml (or mg/ml), in other word, it should be weight/volume, not in volume/volume (in case of FRPA assay described by author in M & M section). Because only in this study, weight/volume gives good and standard comparison. Also, Result for ABTS is basis of % inhibition for VRF formula (first paragraph of result section), while standard is on the basis of µM/L of ascorbic acid and trolox. That should be in uniform, as it is known that plant extracts does not suitable for molar basis, therefore, author should have to describe uniformly such as mg/ml or µg/ml for the standard. Further, ABTS result should be given in the form of “Figure”

(4) Author described only % scavenging = [(A0-A1)/A0] X100 as a statistic or % inhibition activity of respect assay, how IC50 was calculated?

(5) DPPH graph is not “representative”, such as VRF does not explain the lower to higher concentration effects on DPPH assay, it look like “plateau” is already at lowest conc., and that given the 90 % inhibition. In this context, author described in result section, first paragraph - for DPPH assay, “IC50 value of the formulation was 5.51 µg/ml,” how this calculation made???

(6) first paragraph Also, “X” line of graph should be in µg or mg/ml not as
describe by author “conc. in 0.01 mg to 0.1 mg/ml”

(7) Author described in result section, first paragraph “VRF was also found to be a potential DPPH-free radical scavenger, 0.1 mg/ml, the activity was 94%...IC50 value of the formulation was 5.51 μg/ml. The percentage scavenging activity of standard (ascorbic acid) was only 84% at 0.1 mg/ml and IC50 value was 39 μg/ml (Fig. 1”). In this case, 94 % vs. 84 % is not a good comparison; because, most of components at higher concentration become “plateau” (stable). Further at “Interrelation between DPPH radical and ABTS radical scavenging” author compared the % value, it is also not a good comparison, author should have to compare by using IC50 value, other word, at the level of 50 % inhibition of std. vs. VRS.

(8) Similarly, as mention above (# 3 comments), author noted, page # 8 last line “The scavenging activity of ABTS+ radical by the formulation was found 69.55 %).” What is the concentration of formula?

(9) Page # 9 second paragraph: Author describe “Several authors have also reported that some compounds may have ABTS+ activity or may not have DPPH radical scavenging activity at all. Our results did not support ... potent DPPH-radical and ABTS+ radical scavenger.” This entire sentence should be potentially revised or removed; it does not make any sense!!!. This comment is suitable IF references - authors (16, 17) have used same VRS formula.

(10) Page # 9 “It is known that proton radical scavenging .... antioxidants” and “which plays an important role in (a) adsorbing .....decomposing peroxides”, add appropriate reference/s.

(11) Page # 9, “This implies that the plant extract may be useful for treating radical-related pathological damage, especially at higher concentration [13]”. What does mean “higher concentration”?

(12) Sub title “Relation between phenol content and total anti-antioxidant capacity” should be removed, as author did not use any polyphenol (ex. Gallic acid) as a positive control in FRAP assay.

(13) What is the principle of reducing assay? How it is differs from FRAP assay, explain it...

(14) M & M section should have name of solvent used for preparation of formula as well standard for assays.

(15) Reference section should be re-checked according to Journal style, such as some of ref. are not co-related, ex., Food Chemistry and Toxicology as well as Food chem., also be sure for short form of journal name, ex., J Agricult Food Chem

**Level of interest:** An article whose findings are important to those with closely related research interests
Quality of written English: Needs some language corrections before being published

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

Declaration of competing interests:

I declare that I have no competing interests'