Review of: "Developing a Novel Solvent System to Separate Polar and Nonpolar Leaf Pigments of Copperleaf (Acalypha wilkesiana) Using Thin Layer Chromatography"

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Potential competing interests: No potential competing interests to declare.

The article “Developing a novel solvent system to isolate plant pigments of different polarities using thin layer chromatography” by Vishwajit Adiga, Samanyu Chandra P, Varnika Venkatesh Kikkeri et al. deals with interesting problem of separation polar and nonpolar pigments of plants by TLC-method. The article can be published, but some questions and comments were during consideration of the work.

1. Part “Introduction”, P. 2: Declaration about toxicity of methanol in comparison with solvents of mobile phase proposed in work is questionable, because methanol is in the same hazard group as ethyl acetate and isopropyl alcohol.
2. Part “Experimental", “'Materials”, P. 3: It will be better to write qualification of solvents.
3. Part “Experimental", “'Chromatographic separation”, P. 4: TLC-Plates' cipher (TLC Silica gel 60 F_{254}, Merck, Germany) has been done already on P.3.
4. How correct was to use individual water as mobile phase with polar stationary phase (cullulose)?
5. Part “Results”, “'Thin-layer chromatography (TLC)”, P. 6, Fig. 2, second photo: How did you inject samples on plate “in point” or “in line“? It seems that you had deformation of chromatographic zones due to edge effects. It is not good to inject samples near edge of plates, nearer than 1 cm from edge.
6. Part “Results”, “'Thin-layer chromatography (TLC)”, P. 6, Table 1, “Probable photopigment”: It will be better to identify chromatographic zones comparing they with standards. However, if you compare zones with literature data, you need to write this data with a link to them. The same comments are to Fig. 3 and text on P. 8 (It is well established that the green, yellow/orange, and red colors of leaves are due to the presence of chlorophylls (a and b), xanthophylls, ß-carotene, and anthocyanin, respectively.), P. 9 (We recorded the UV-Vis absorption spectra using each separated fraction and compared them with the literature.).
7. Part “Results”, “'Thin-layer chromatography (TLC)”, P. 7, Fig. 3, D: It seems that discussion about absorbance peak for ß-carotene absolutely does not have sense.
8. Part “Discussion”, P. 8: References [4, 5, 13, 14] are very old literature (1963, 1981, 1961, 1965). However, authors discuss the advantages of their new method with data in references [4, 5, 13, 14]. It will be better to consider more novel TLC-techniques in addition.
9. Part “Discussion”, P. 8, P.9: What effects are responsible for tailing of pigments' zone in paper chromatography? It is not enough to explain this effect only as influence of stacionar phase texture. It will be better to discuss this problem by consideration physico-chemical causes more careful.

10. Part “Discussion”, P.9: Authors compared retention factor of pigments with literature data. It is well known that retention factor can be difference for the same compounds from plate to plate even from the same batch. Consequently, it seems useful to write Rf with confidence range and, of course, to write Rf from literature, with which authors explained their data (for example, to write Rf for plant pigments from literature in Table 1).

These remarks do not diminish the significance of the study in the article and do not prevent it publication in Qeios.

Sincerely yours,

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