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Thin Layer Chromatographic Profiling and Antioxidant Activity of Extracts from a Muscadine Grape Skin Commercial Preparation

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INTRODUCTION

The muscadine grape *Vitis rotundifolia*:

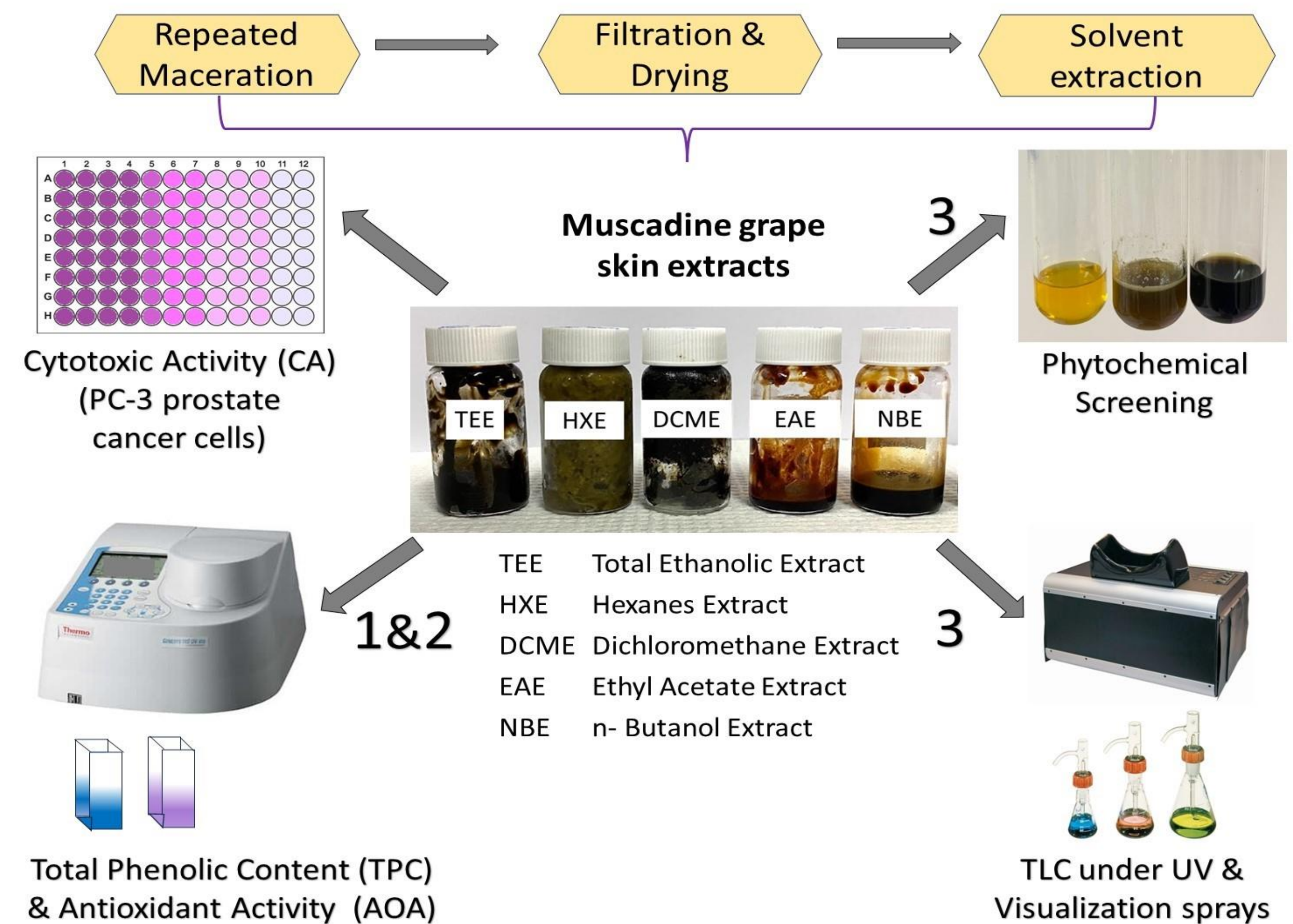
- is a rich source of bioactive secondary metabolites - potential for development into anticancer therapeutic agents;
- has increased levels of polyphenolic compounds relative to other grape species - thus, able to suppress the growth of cancer cells (Mendonca *et al.*, 2019);
- contains high amount of natural antioxidants (phenolic compounds & other phytochemical compounds) - ability to reduce/prevent free radical-mediated oxidative damage of cells; protective effect against certain cancers (Dawish *et al.*, 2023, Sandhu and Gu, 2010).

Our preliminary research revealed that the dichloromethane extract from a commercially available muscadine grape skin has high cytotoxic potency against PC-3 prostate cancer cells [the concentration that inhibits cell growth by 50% (IC₅₀) = 0.714µg/mL].

OBJECTIVES

1. To determine the phenolic content and the *in vitro* antioxidant activity of various extracts from the grape skin of a muscadine cultivar.
2. To calculate their subsequent correlation with the cytotoxic activities.
3. To assess the phytochemical constituents of the extracts with the highest content/activity.

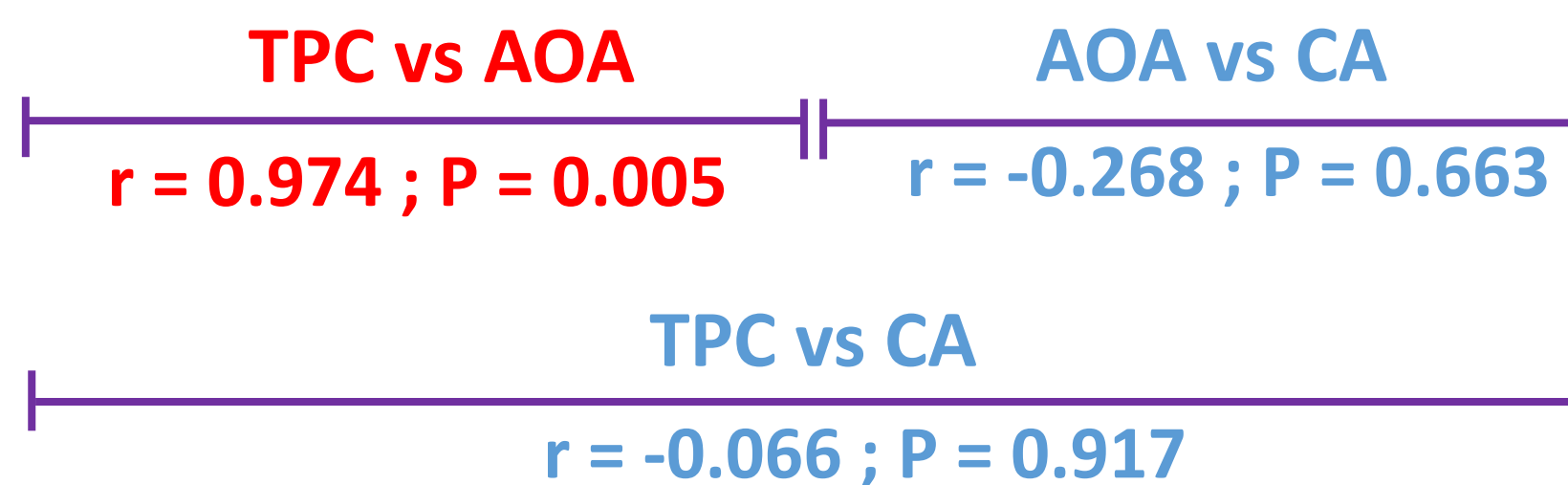
METHODOLOGY



RESULTS & SUMMARY

Table 1. Muscadine grape skin extracts TPC (mg gallic acid equivalents (GAE)/g), AOA and CA (tested previously)

Extract	TPC (mgGAE/g)	AOA (IC ₅₀ mg/mL)	CA (IC ₅₀ µg/mL)
TEE	57.059 ± 0.02	1.548	4.549
HXE	7.255 ± 0.01	685.004	6.538
DCME	120.850 ± 0.02	0.902	0.714
EAE	325.817 ± 0.07	0.116	38.960
NBE	73.399 ± 0.004	1.507	≥ 400



- Phenolic compounds contribute to the antioxidant activity of *V. rotundifolia* extracts
- TPC vs AOA : **strong positive & significant correlation**
- The observed *in vitro* cytotoxic activity of all extracts might not be only due to their TPC and AOA
- AOA vs CA and TPC vs CA: **weak negative & non-significant correlation**
- The extract with the highest cytotoxic activity - DCME - displays the second highest TPC and AOA

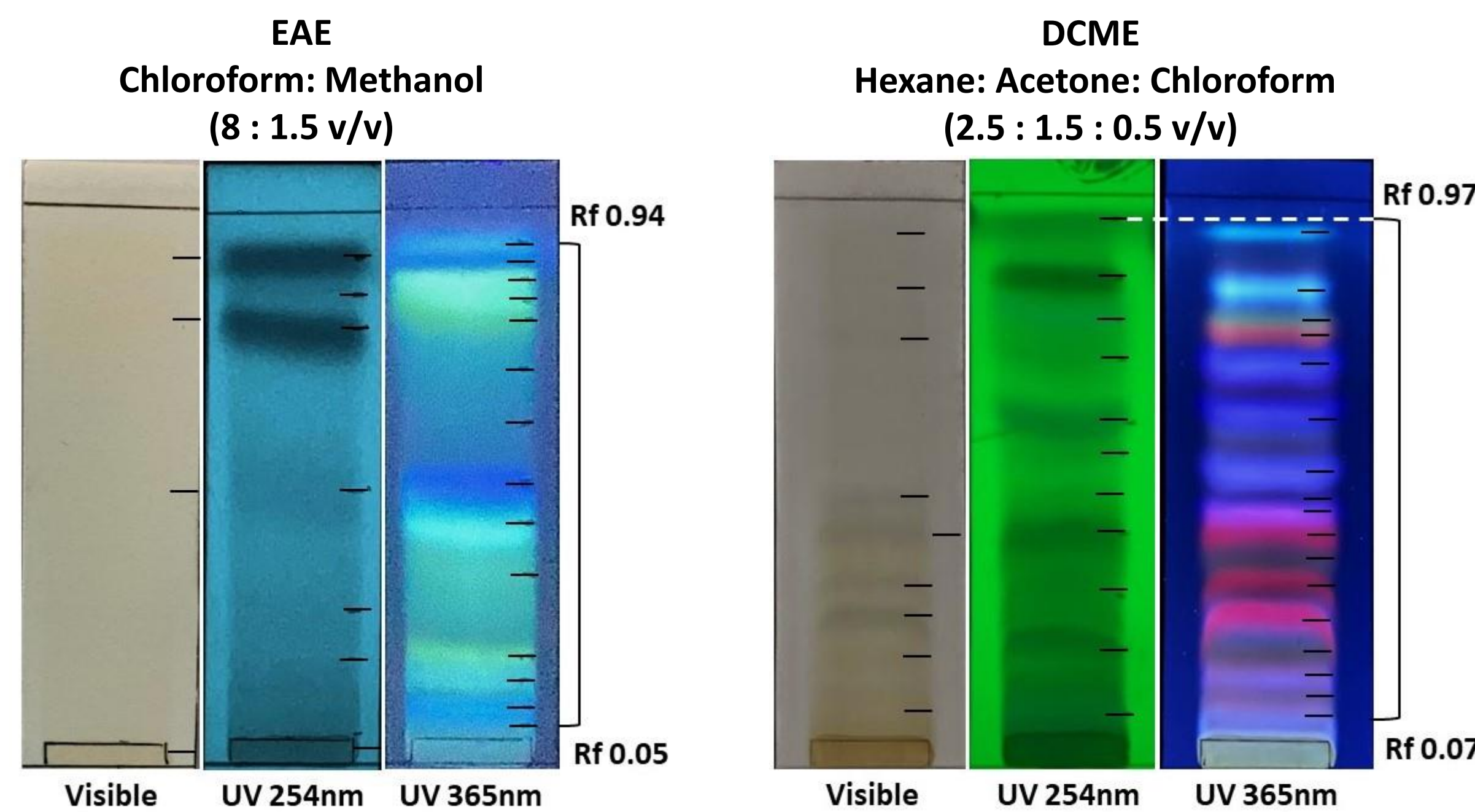


Figure 1. Reverse phase TLC of EAE (the highest AOA) and normal phase TLC of DCME (the highest CA).

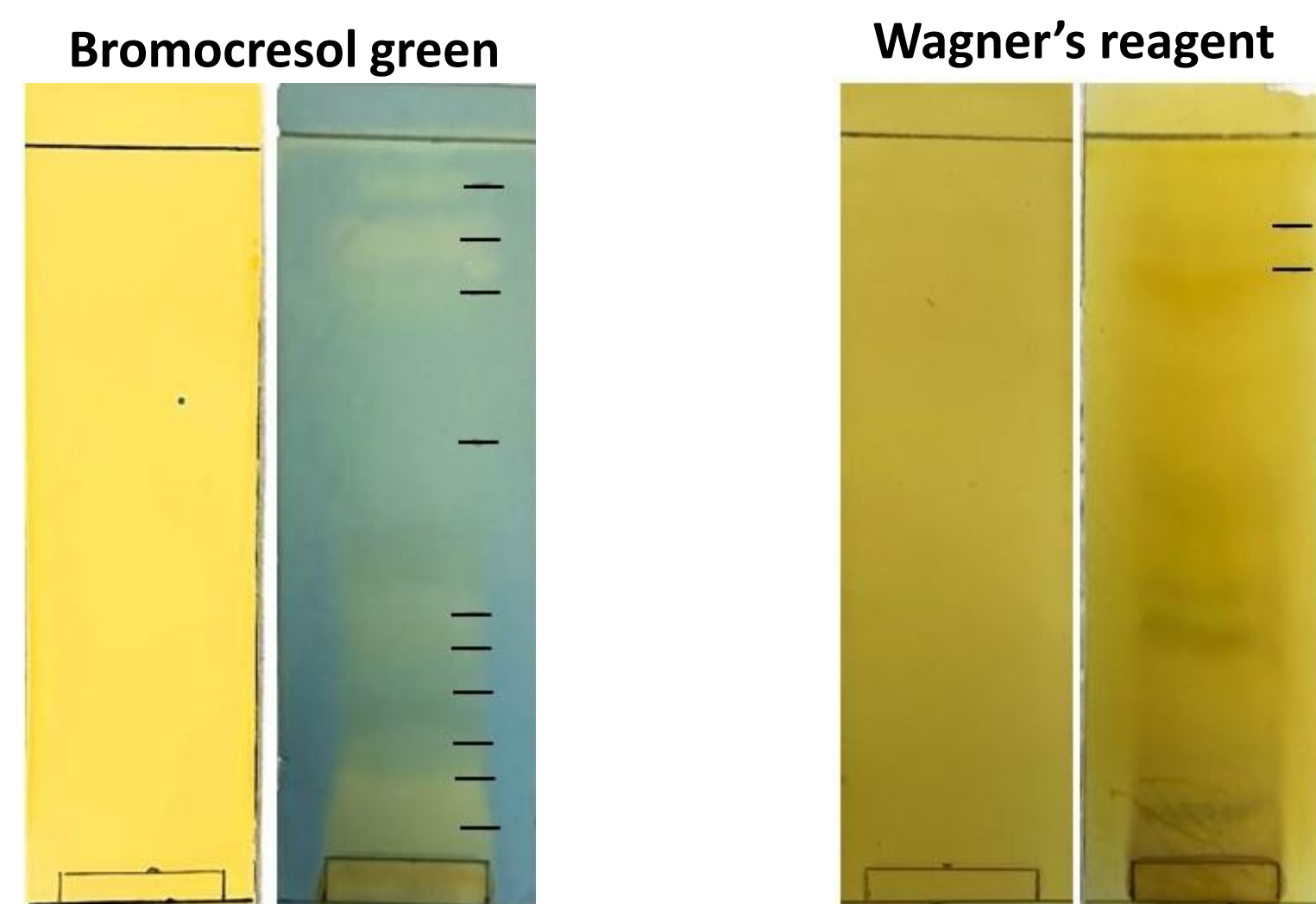


Figure 2. Visualization of TLC plates (EAE vs DCME) for carboxylic acids (Bromocresol green) and alkaloids (Wagner's reagent).

- Carboxylic acids and alkaloids are present in DCME (a number of bands for both visualization sprays) but absent from EAE.

Table 2. TLC Visualization of chemical groups & determination of band quantity (#)

Active Constituents	Visualization Sprays	EAE # of bands	DCME # of bands
Alkenes, alkynes, alcohols, aldehydes	Potassium permanganate	9	9
Aldehydes, ketones, alcohols, amines	Vanillin-EtOH-H ₂ SO ₄	9	11
Alkaloids	Wagner's reagent	---	2
Anthraquinones, Coumarins	Potassium Hydroxide	3	6
Antioxidants	DPPH	7	12
Carboxylic Acids	Bromocresol green	---	10
Flavonoids	Aluminium Chloride	3	3
Phenols	Iron (III) chloride	3	8
Organic compounds - aromatics	Iodine Vapour	5	9
Steroids and Triterpenoids	Liebermann Burchard	8	13

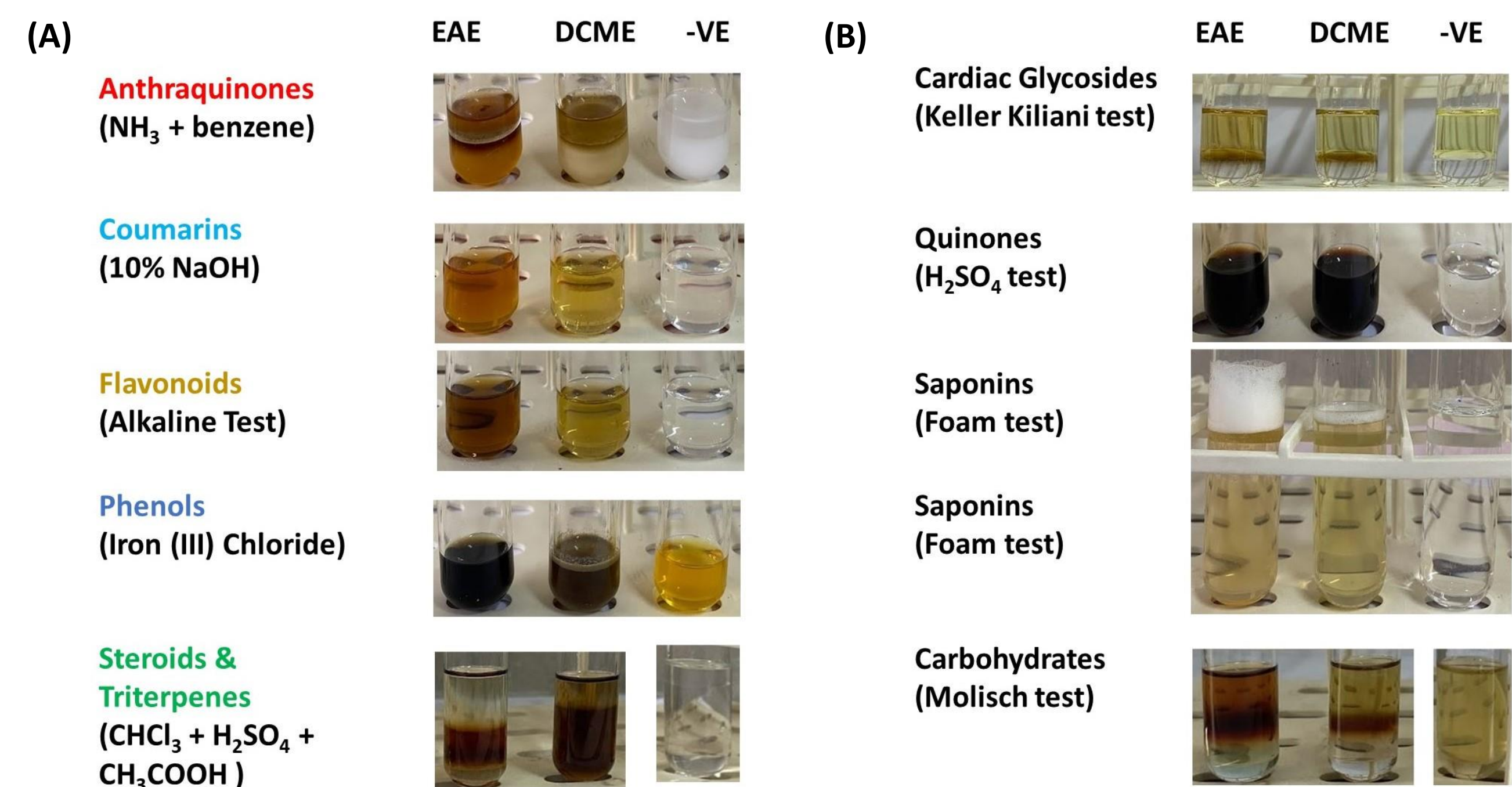


Figure 3. Qualitative phytochemical tests of EAE vs DCME for (A) bioactives revealed by TLC visualization sprays, and (B) additional chemical groups. -VE: negative control

CONCLUSIONS

The present findings demonstrate that the phenolic compounds contribute directly to the AOA (weak to moderate activity) of the extracts, but both TPC and AOA are not correlated to the *in vitro* cytotoxic activity against PC-3 cells. Therefore, the anticancer effect of the DCME may be due to the other medicinally relevant bioactive compounds which have been confirmed in our study by both TLC and phytochemical testing.

The muscadine grape skin extracts show potential for drug development. Future work is carded to fractionate DCME, and to purify and identify the active components with anticancer activity. Our work is contributing to the UN SDG #3, which focuses on good health and well-being.

ACKNOWLEDGEMENTS

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