To get your target compound quickly with the TLC-based GUESS working chart

Choosing a suitable solvent system for a counter-current chromatography separation presents a challenge for many researchers. In this study we introduce a quick method of separating a target compound from the bark of Zanthoxylum myriacanthum var. pubescens by counter-current chromatography. This method relies on the TLC-based the generally useful estimation of solvent systems (GUESS) method. This paper presented how to quickly choose a suitable solvent system with a TLC-based the generally useful estimation of solvent systems (GUESS) working chart as following.

> *Proceeding TLC-based the generally useful estimation of solvent systems (GUESS) method working chart.*

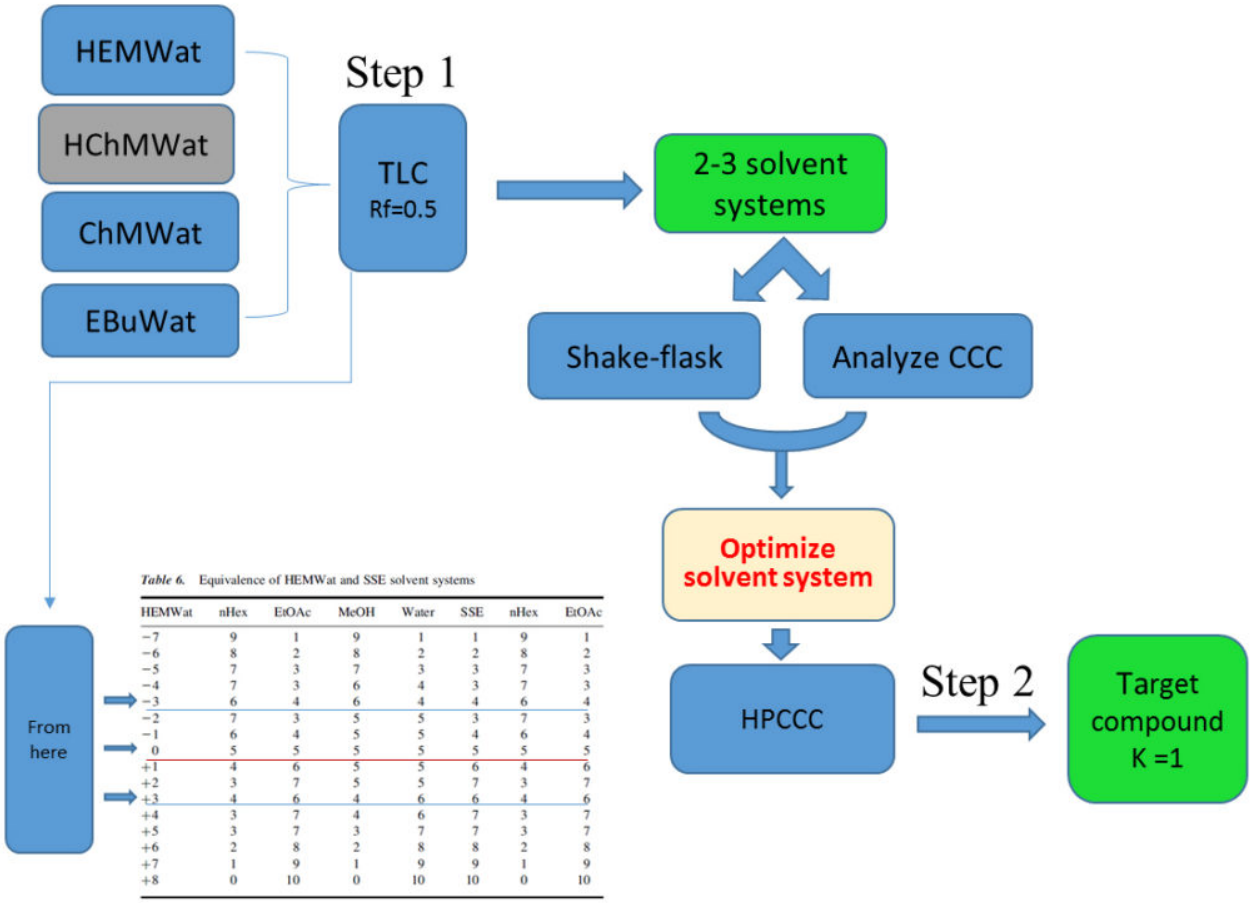

Table 6. Equivalence of HEMWat and SSE solvent systems

HEMWat	nHex	EtOAc	MeOH	Water	SSE	nHex	EtOAc
−7	9	1	9	1	1	9	1
−6	8	2	8	2	2	8	2
−5	7	3	7	3	3	7	3
−4	7	3	6	4	3	7	3
−3	6	4	6	4	4	6	4
−2	7	3	5	5	3	7	3
−1	6	4	5	5	4	6	4
0	5	5	5	5	5	5	5
+1	4	6	5	5	6	4	6
+2	3	7	5	5	7	3	7
+3	4	6	4	6	6	4	6
+4	3	7	4	6	7	3	7
+5	3	7	3	7	7	3	7
+6	2	8	2	8	8	2	8
+7	1	9	1	9	9	1	9
+8	0	10	0	10	10	0	10

Fig. 1. The TLC-based GUESS method working chart.

1.1 Choosing suitable solvent system family for target compound
Generally, there are three useful solvent system families for laboratory technician. The laboratory technician can choose solvent system family according the polarity of the target compound. The three CCC solvent system families: hexane/ethyl acetate/methanol/water, chloroform/methanol/water and ethyl acetate/ butyl alcohol /water are recommended as the HEMWat, ChMWat and EBuWat methods of solvent system selection. The HEMWat method was designed to provide a systematic process of choosing a CCC solvent system for separating a wide range of organic compounds of low and medium polarity. The ChMWat method was designed for the separation a wide range of organic compounds of medium and high polarity. The EBuWat method was designed for the separation a wide range of organic compounds of high polarity. As the sample for HPCCC work was prepared from ethyl acetate extract, The HEMWat method was chosen for the work.

1.2 Making target compound near Rf=0.5
Confirm target compound on silica gel TLC plates. According the ‘GUESS’ method, modify the target compound’s Rf value near 0.5.

1.3 Optimizing solvent systems
The solvent system of *n*-hexane–ethyl acetate–methanol–water (6:4:6:4) for CCC was chosen. Firstly, one mixture of TLC solvents with *n*-hexane/EtOAc (5:5) was tested, the Rf value of target compound was above 0.5. Another mixture TLC solvents with *n*-hexane/EtOAc (6:4) to make Rf value of target compound near 0.5. Secondly, different ratios of two solvent systems including *n* -hexane–ethyl acetate–methanol–water (6:4:5:5) and *n*-hexane–ethyl acetate–methanol–water (6:4:6:4) were tested. The upper phase of both of solvent systems could make the Rf value of the target compound near 0.5. Generally, there are two ways to select the optimized solvent system. One way is using the CCC to compare the performance of the two solvent systems; another way is to test the settling times of the two solvent systems. Finally, *n*-hexane–ethyl acetate–methanol–water (6:4:6:4) was chosen for CCC by comparing settling times of two solvent systems.

Enrichment of target compound by high performance CCC

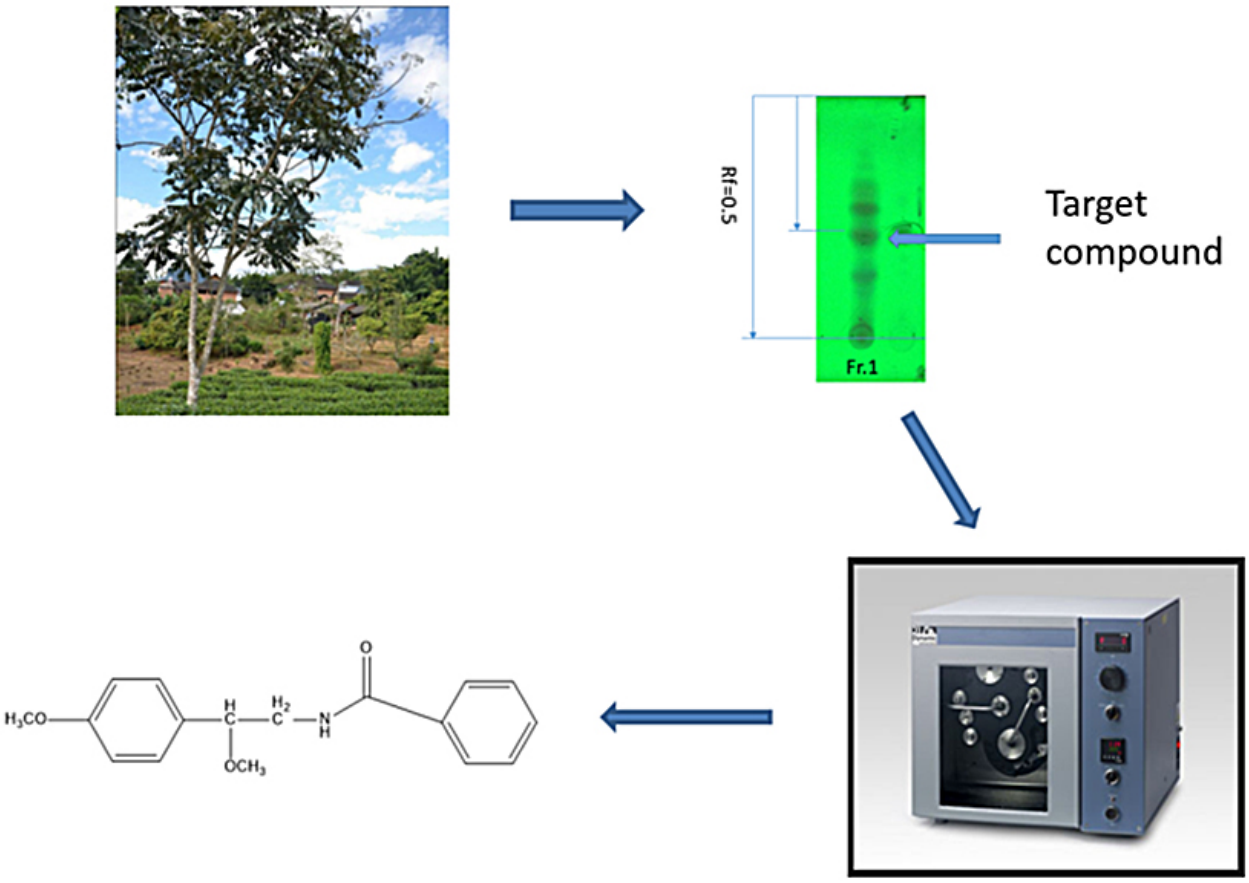

Fig. 2. How to go with “TLC 0.5 GUESS” method.

According the TLC-based ‘GUESS’ method and previous studies, compounds with Rf values between 0.29 and 0.71 (optimal 0.5) will have K values between 0.4 and 2.5 (optimal = 1) and be eluted in the sweet spot of an HSCCC run. With the chosen ratios of solvents, the target compound would come out after one column volume elution, as its K-value may be close to one. The target compound were detected at fraction 25 fration from CCC where the elution volume equals to one column volume.

Purification of the target compound

O-Methyltembamide (1) was purified by HPLC with purities 98.2% and identified by ESI-HRMS and NMR.

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Publication

Quick method for separating target compounds from the bark of Maqian (Zanthoxylum myriacanthum var. pubescens) by high-performance countercurrent chromatography.
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