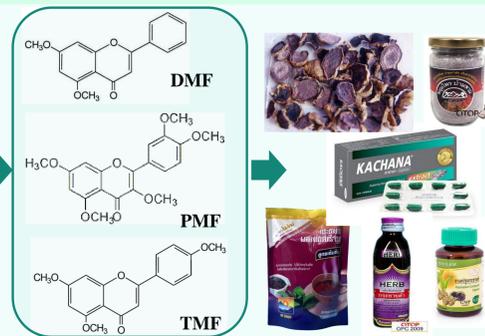


Abstract Black ginger (*Kaempferia parviflora*) was promoting as herbal Champion product. But there was a lack of reference standards for the quality control of raw materials, extracts and health products from Black ginger. For further step of herbal Department of Medical Sciences reference standards production, the isolation of three major compounds; 5,7-Dimethoxyflavone (DMF), 5,7,4'-Trimethoxyflavone (TMF) and 3,3',4',5,7-Pentamethoxyflavone (PMF) from Black ginger extract was studied by Column chromatography technique. Thin layer chromatography (TLC) and Ultra-performance liquid chromatography-photodiode detector mass spectrometry (UPLC-PDA-MS) were used for identification. According to the results, the considered fraction (F4) containing three major compounds was obtained as 22.21% w/w of crude extract. After the separation of F4 fraction by flash column chromatography, TMF was isolated as 7.11% w/w of F4 and a combination fraction of DMF and PMF (F4-2) was obtained as 76.52% w/w of F4. Then the final step of column chromatograph was also operated for the separation of DMF from PMF as well. Base on the study, this efficient method of isolation will be led to next steps in the production of herbal reference standards.

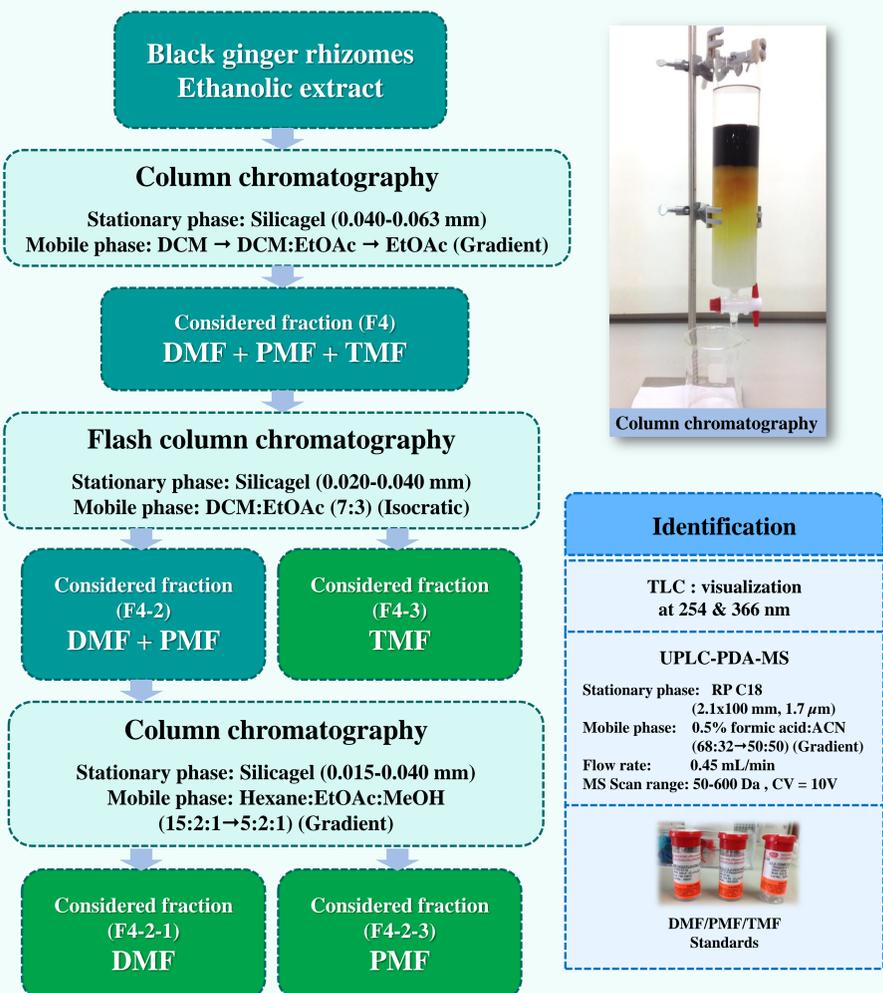
INTRODUCTION & PURPOSE

“Black ginger” or “Krachai Dam” (*Kaempferia parviflora*) is a famous herb in Thai traditional medicine. Due to their health promoting potentiality, safety and pharmacological reports, it was promoted as one of five champion herbal products by Thailand government[1]. The rhizomes of Black ginger contains at least 11 bioactive methoxyflavones which 3,5,7,3',4'-pentamethoxy flavone (PMF), 5,7-dimethoxyflavone (DMF) and 5,7,4'-trimethoxy flavone (TMF) are considered as the major compounds in an ethanolic extract[2-5]. These three compounds can be used as markers of Black ginger as well. However, there was a lack of reference standards for the quality control of raw materials/extracts and health products from Black ginger. The purpose of this study is to isolate the three purified compounds by column chromatography methods in the production of herbal Department of Medical Science reference standards that is applied for the quality control of Black ginger in the future.

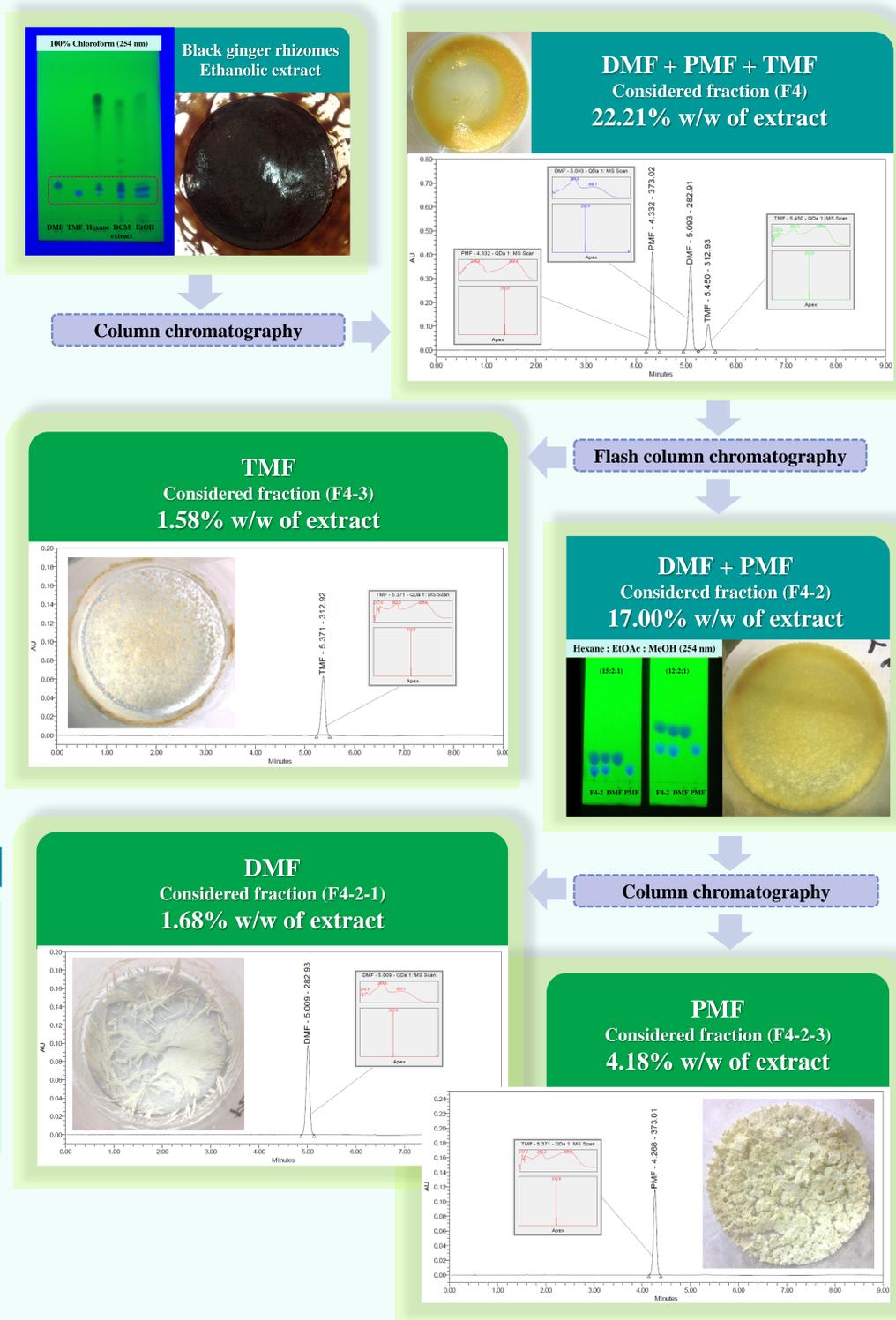


MATERIALS & METHODS

The isolation of DMF, PMF and TMF from Black ginger rhizomes ethanolic extract by column chromatography technique was modified from previous study[6]. The eluting conditions were prior investigated by TLC. And the identification of considered fractions also proceeded by TLC and UPLC-PDA-MS in comparison to reference standards with modified liquid chromatography systems[5,7].



RESULTS



DISCUSSION & CONCLUSION

DMF, PMF and TMF can be isolated from Black ginger ethanolic extract by normal phase column chromatography using the suitable mobile phase (MP) type and ratio. The two solvents mixture, dichloromethane (DCM) and ethyl acetate (EtOAc), was fail to separate DMF from PMF but proficient in an isolation of TMF from them. Whereas a three-type solvent mixture: hexane, EtOAc and methanol (MeOH), is better for the separation of DMF from PMF in F4-2. In addition, column chromatography is more applicable than preparative TLC used in previous method[6], when the large amount of substance required for reference standard production. Base on the study, this efficient method of isolation will be led to next steps in the production of herbal reference standards. A recrystallization process is still required for purification step of the remaining compounds in F4-3, F4-2-1 and F4-2-3. And the developed UPLC analytical method for these substances should be considered in a further validation.

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