



Research Article

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Preliminary phytochemical and HPLC screening of triterpenoids fraction from bark extract of *Callicarpa arborea* Roxb

M. Amin*¹, Yunazar Manjang², Sanusi Ibrahim², Mai Efdi² and Adlis Santoni²

¹Faculty of Health, University of Muhammadiyah Bengkulu, Bengkulu, Indonesia

²Department of Chemistry, Faculty of Mathematics and Natural Science, University of Andalas, Padang, Indonesia

ABSTRACT

Triterpenoids extracted and preliminary identified from raw bark extract of *Callicarpa arborea* Roxb by applying phytochemical screening test and high performance liquid chromatography (HPLC) identification have been done. Triterpenoids found that contained in the extract have a semi-polar polarity direction. One dominant triterpenoid compounds were observed in the observation by HPLC. Application for the use of preparative HPLC can be suggested for the next stage of structure elucidation.

Key words: Triterpenoid, *Callicarpa arborea* Roxb, phytochemical, HPLC

INTRODUCTION

Chemistry of natural resources play a supportive field of bioindustry. Therefore, it is necessary to explore chemical compounds from plants [1]. This activity can provide effect a multiple beneficial on the development of plants for the production, preservation and argotourism [2]. Phytochemical screening performed as the initial act of exploration intended.

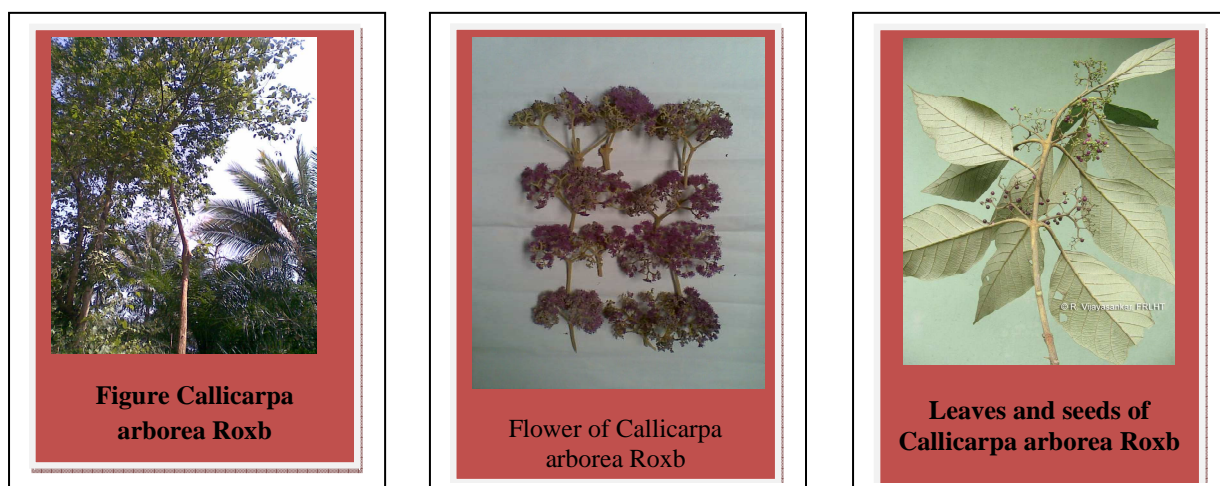


Figure 1. *Callicarpa arborea* Roxb

Callicarpa arborea Roxb including the family Verbenaceae is a small evergreen plant. The spread of this plant: the Himalayas, India, Burma, South China, Indo-China and Malaya. Pictures of these plants can be seen below [3]. This plant in Bengkulu used as traditional medicine for jaundice, while in India and Nepal used for fever, head, stomach, skin and scorpion bites disease [4],[5].

In the present study, an identification method has been developed by optimizing the extraction, separation, qualitative and HPLC analytical conditions for simultaneous estimation the terpenoids. As we know HPLC technique gives a finger print analysis of terpenoids for its components. This information can be usefull for isolation of the single compound with HPLC preparative activity.

EXPERIMENTAL SECTION

Plant Material Preparation

Plants are used as samples taken from Forest Park Rajolelo city of Bengkulu. The identification of plants is done in the laboratory of Biology / Natural Sciences Herbarium UNAND. Fresh bark is taken in moderation, then wind dried without sunlight for 3 weeks. Samples were dried crushed into powder by using a blender and stored in preparation for the extraction plant.

Preparation and Extraction

50 g powder of sample was macerated with 350 ml of methanol in erlemeyer as much as 6 repetitions and each repetition for 24 hours while stirring a few times periodically. Collected filtrate filtered through filter paper and then concentrated by using a rotary evaporator at temperatures of 50 ° C. Successive extraction done by using n-hexane, dichloromethane, ethyl acetate and n-butanol, respectively of 6 repetitions. Extracts from the variation of solvent respectively separately collected and concentrated by using a rotary evaporator the temperature adjusted. Concentrated extracts subsequently treated for phytochemical screening and HPLC identification of triterpenoid compounds.

Phytochemical Screening of Triterpene Compounds

Phytochemical screening is an early detection of chemical compounds that may be present in a plant .. phytochemical screening performed by the methods commonly used and to variations extract [6], [7], [8]. Test of Terpenoids by Liebermann-Burchard methode; some drops of the extract is placed into the plate drops, add a few drops of acetic acid anhydride and add 1-2 drops of concentrated sulfuric acid. The occurrence of reddish color / pink indicates terpenoids and the color purple to blue or green indicate the presence of steroids.

HPLC analysis

Chromatographic analysis was performed at room temperature using Shimadzu UFLC system. Chromatograms were recorded by Lab solution was achieved on C₁₈-column (150×4.6 mm, 5.0 mm) using acetonitrile: water (40:60, v/v) delivered at a flow rate of 1.0 mL/min. After a 10 min of equilibration period, the samples were injected into the HPLC system. Peaks were recorded at 210nm.

The analysis running with step gradient polarity elution (Table 1) :

Table 1. HPLC step gradient polarity programme

Minutes	A	B
0	40	60
20	90	10
30	40	50
40	40	60
End time = 40 minutes		

RESULTS AND DISCUSSION

From the results of phytochemical screening showed that there are triterpenoid compounds in dichloromethane and ethyl acetate extracts. The results can be seen in the table 2.

Tabel 2. Results of Phytochemical screening of triterpene compounds of Solvent Variation from Bark *Callicarpa arborea* Roxb

Compound Group	Hexane	DCM	EtOAc	Butanol	Metanol-Water
Triterpenoid	-	+	+	-	-

There are two things can be expressed in the extraction of triterpenoids. The first, there is no specific solvents to extract a class of compounds in this case are triterpenoids. The second is that there is little difference in polarity between fellow members terpenoid compounds. The result of the extraction of triterpenoids found in more than one type of solvent in this case is Ethyl acetate and Dichloromethane.

On the table 2. Shown that triterpenoid can be extracted by semi polar solvents, this shows that the possibility of triterpenoid compounds that exist in the extract are triterpenoid compounds which have the arrangement of medium chain length atoms C. Based on this information, it can be predicted that the next step must be done, such as the selection HPLC method.

The use of step gradient polarity will be good for crude extracts that contain many compounds, because it will be able to perform perfectly separation. On HPLC data (figure 2) shown the compounds that containing in the dichloromethane extract. From three fractionations, it can be concluded that there in one major compound that can be collected by using HPLC preparative. The compound on retention time at 28 minutes give a big presence. It will be very efficient if separated by preparative HPLC

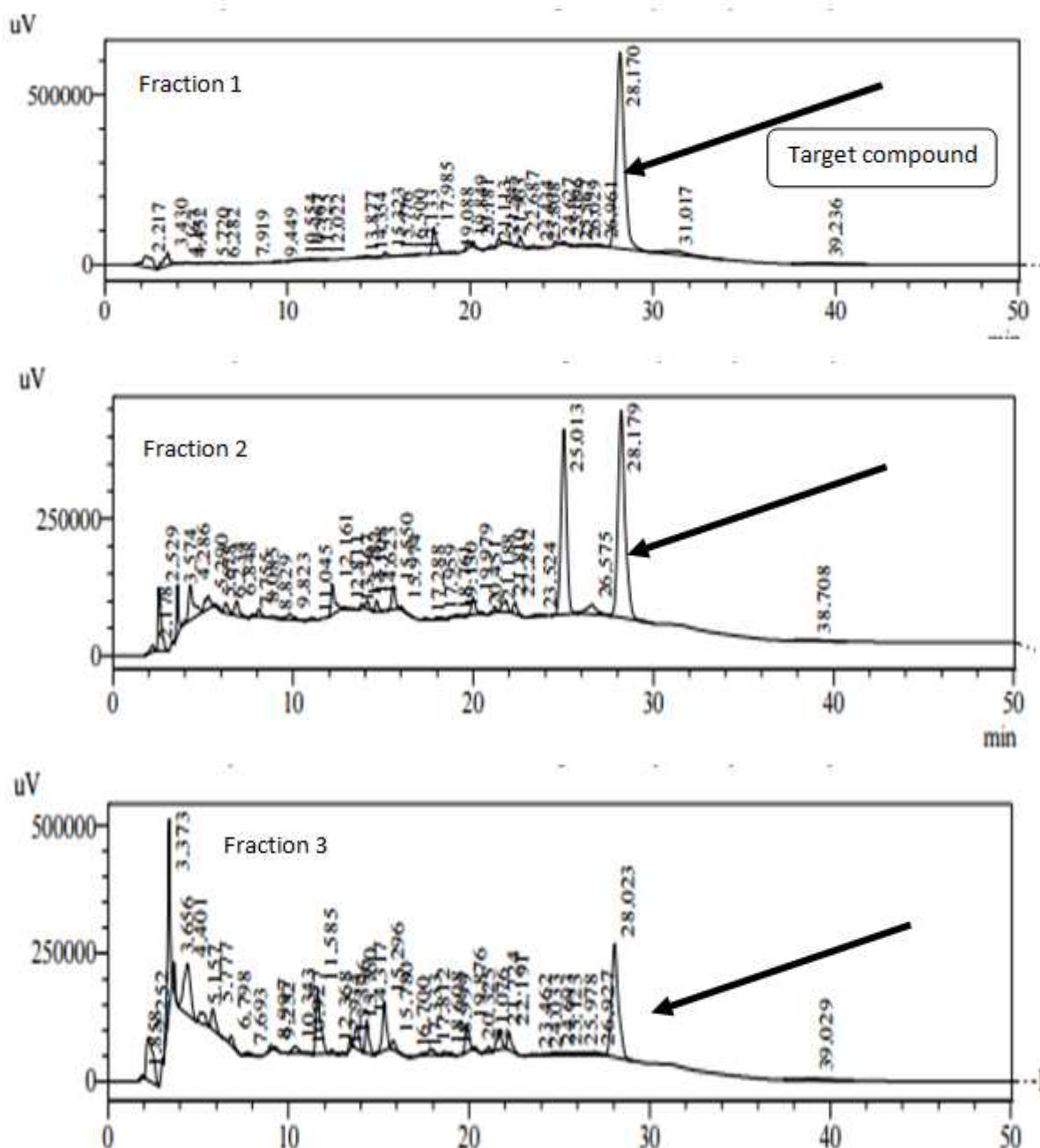


Figure 2. Chromatogram data of Dichloromethane fraction of *Callicarpa arborea* Roxb bark extract

CONCLUSION

Phytochemical screening test of *Callicarpa arborea* Roxb bark extract shown that triterpenoids found. The compounds have a semi-polar polarity direction. One dominant triterpenoid compounds were observed in the

observation by HPLC. Application for the use of preparative HPLC can be suggested for the next stage of structure elucidation determination

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