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BIOASSAY-GUIDED ISOLATION AND CHARACTERIZATION OF ANTIBACTERIAL COMPOUNDS FROM *Calotropis procera* (Ait.)

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ABSTRACT

Infectious diseases have been the leading cause of human mortality in the past and yet are challenging public health concerns worldwide. Thus, there is a huge demand for exploring new and effective antimicrobial leads from all sources to combat the infectious diseases. The medicinal plant *Calotropis procera* has been traditionally used in the treatment of various ailments, including infectious diseases. Although a wide array of biological activities and rich phytochemistry of *C. procera* are reported, a systematic investigation to isolate antibacterial compounds of the plant has not been reported so far. Considering the pressing need for new antibacterial leads, present bioassay-guided study was designed to explore antibacterial compounds of *C. procera* twigs, leaves, and flowers. The plant materials collected from the Dungun district of Terengganu, Malaysia, were extracted with *n*-hexane, dichloromethane, and methanol, applying a successive extraction method to prepare nine crude extracts. The obtained extracts were tested for their *in-vitro* antibacterial activity against a panel of ten Gram-positive and ten Gram-negative bacteria, applying the agar well diffusion (AWD) method. The methanolic extracts of leaves (LM) and flowers (FM) showing broader antibacterial effects were selected for further bioassay-guided studies applying different chromatographic techniques. The LM and FM yielded a total of eighteen compounds (**1**–**18**), which were elucidated by using different spectroscopic and spectrometric approaches, e.g., UV, IR, LCMS, 1D, and 2D NMR techniques along with a literature survey. The isolated compounds include seven flavonoids [kaempferol 3-*O*-robinoside (**1**), kaempferol 3-*O*-rutinoside (**2**), 8-bromoisorhamnetin 3-*O*-rutinoside (**8**), 8-chloroisorhamnetin 3-*O*-rutinoside (**10**), isorhamnetin 3-*O*-rutinoside (**11**), kaempferol 3-*O*-*β*-D-glucopyranoside (**12**), and quercetin (**18**)], seven cardenolides [calotoxin (**3**), frugoside (**4**), 15*β*-hydroxycalotropagenin (**5**), 12*β*-hydroxycalotropagenin (**6**), 19-carboxyl-2*α*,15*β*-dihydroxyuzarigenin (**7**), 12*β*-hydroxycoroglaucigenin (**9**), and calotropagenin (**16**)], two lignans [(+)-pinoresinol 4-*O*-(6″-*O*-*para*-hydroxybenzoyl)-*β*-D-glucopyranoside (**13**) and (+)-pinoresinol 4-*O*-(6″-*O*-vanillyl)-*β*-D-glucopyranoside (**14**)], and two benzene derivatives [benzyl *β*-primeveroside (**15**) and protocatechuic acid (**17**)]. All of the compounds were tested against *Bacillus cereus*, *Staphylococcus epidermidis*, and *Proteus vulgaris* at dose of 30 µg/well using the AWD method. The compounds (except **12)** showed varied antibacterial potential (ZOI: 7–17.5 mm) against the tested bacteria. Conclusively, the present study justified the ethnopharmacological value of *C. procera* as a traditional remedy for infectious diseases. The study also pointed out that the antibacterial metabolites were largely accumulated in both the leaves and flowers compared to stems of the plant. Amongst the eighteen isolated phytochemicals, five new compounds (**6**–**8**, **10**, and **13**) showed strong antibacterial effects (ZOI of 11.5–17.5 mm) and were isolated for the first time from nature. Meanwhile, two known compounds (**15** and **17**) showing ZOI of 11–13 mm were reported for the first time from the genus *Calotropis*, whereas **5** producing ZOI of 12–17 mm against the tested G+ve bacteria and **12** (inactive in this study) were new for *C. procera*. Overall, the three isolated cardenolides (**5**, **7**, and **9**), one flavonoid (**10**), and two lignans (**13** and **14**) were among the potent compounds (ZOI: up to 17.5 mm), while *S. epidermidis* was the most sensitive strain. The three cardenolide aglycones (**5**, **7**, and **9**), two halogenated flavonoids (**8** and **10**), and the two lignans (**13** and **14**) could be emphasized for further antibacterial drug discovery research.

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CHAPTER 1
INTRODUCTION

1.1 Background of the Study

Almighty Allah grows plants on the earth, which are providing all kinds of life with the prominent necessities and life requirements on Earth. Ancient humans were using plants and their products not only as food but also as shelter, hunting tools, fuel for baking food, and remedies to alleviate their illnesses. That is to say, the use of natural products and plants to combat health problems has been practiced since time immemorial (El-Seedi et al., 2019; Walesch et al., 2023).

All those plants which are used for medicinal purposes, e.g. prevention and treatment of health problems, are called medicinal plants (MPs) (WHO, 2007). Based on WHO estimations, around 80% of the world population, especially in developing countries, are still relying on traditional medicinal approaches, particularly medicinal plants (Fatima et al., 2018; Mohamad et al., 2019).

Interestingly, even in current modern age, MPs and herbal products still retain their dignity and prestige (WHO, 2007), as in many developed countries, herbalism and herbal medicines are reviving, and the acceptance of herbal as well as traditional medicines is gradually increasing worldwide (Jamshidi-Kia et al., 2018; Jatau et al., 2018). Several reasons, such as: ease of accessibility, efficiency of traditional medicines for health promotion and self-health care, dissatisfaction with conventional therapy, belief in traditional medicines' compatibility with the values, beliefs, philosophical perceptions about health and wellness, could be counted for the on growing use of traditional medicines even in developed countries (Jatau et al., 2018).

The medicinal values and pharmacological activities of MPs are attributed to their bioactive chemical ingredients, also called "phytochemicals" or "secondary metabolites". However, there are different factors, such as developmental, geo-climatic, or environmental, as well as genetic factors that have crucial quantitative and qualitative influences on MPs' secondary metabolites (Barbieri et al., 2017; Farhadi et al., 2018; Jamshidi-Kia et al., 2018; Li et al., 2020).

The MPs, besides being used as spices and local remedies in the treatment of various maladies, are greatly valued as promising sources of bioactive natural products