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# THE ANTITUBERCULOSIS DRUG RIFAMPICIN IS ACTIVATED BY 2', 5'-DIMETHYL BENZOPELARGONOLACTONE FROM THE LEAF OF *Coleus atropurpureus* L. BENTH

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## ABSTRACT

Anti-mycobacterial bioactivity of non polar compounds from *Coleus atropurpureus* L. Benth was carried out. An cyclic ester terpenoid, 2', 5'-dimethyl benzopelargonolactone has been isolated from the chloroform fraction from the leaves of *Coleus atropurpureus* L. Benth, and its structure was determined by spectroscopic evidences including FTIR and 2D NMR and compared to previous data. The *in vitro* anti-mycobacterial activities of its whole extracts, chloroform extract, and pure compound were evaluated by testing their MIC (minimal inhibitory concentration). The isolate alone was active against *M. tuberculosis* with MIC at concentration 200 µg/mL, and could also increase the sensitivity of this pathogen to rifampicin drug at concentration 0.5 µg/mL. These studies suggest that anti-mycobacterial 2', 5'-dimethyl benzopelargonolactone in combination with rifampicin may play an important role for alternative treatment combination against *M. tuberculosis* strain H37Rv. Further studies aimed to structural modification and semi-synthesis of active compound which exhibited increasing anti-mycobacterial activities, need to be carried out in the future.

**KEYWORDS: Antimycobacterial activity, *Coleus atropurpureus* L. Benth,, 2', 5'-dimethyl benzopelargonolactone.**

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# INTRODUCTION

Tuberculosis is a disease that causes a high mortality every year to the world's population. According to WHO, 1.1 million people died from TB in 2010 among HIV negative cases, and an additional 0.35 million deaths among people who were HIV-positive were registered. In the recently year there were estimated 8.8 million incident cases of TB globally. Although TB can be treated and even cured with chemotherapy, treatment is exceedingly lengthy and takes 6-9 months[1]. In addition to significant toxicity, lengthy therapy also causes poor patient compliance, which is a frequent cause for selection of drug resistant and often deadly multidrug resistant TB (MDR-TB) bacteria[2]. Additionally, the number of cases of multi-drug resistant tuberculosis (MDRTB) was 440,000 in 2008 and by 2010, 58 countries and territories had reported at least one case of extensively drug-resistant tuberculosis (XDR-TB)[3]. Tuberculosis with a different drug resistance (DDRTB) involves *M. tuberculosis* strains displaying mono- or poly-resistance which is not including associated resistance against isoniazid and rifampicin[4]. Very recently, patterns of resistance to commonly used anti-tuberculosis drugs among *M. tuberculosis* complex isolates from patients attending government urban TB diagnostic in Makassar, the capital of the South Sulawesi Province in Indonesia, was found to be highly potential risk factors for MDR-TB[5]. According to these data, strong efforts should be directed to find new antimycobacterial drugs, taking into account that MDR-TB and XDR-TB represent serious threats to the world's population health. To eliminate this situation and problem from every corner of the world, a safe, non-toxic and cost effective drug with novel mode of action is immediately required. The need of new leads for such drugs, sustain to consider natural products from plants as sources of antimycobacterial therapeutically useful substances[6]. Plants are good source of a wide variety of compounds, such as phenolic compounds, terpenoids, steroids, alkaloids, nitrogen containing compounds, vitamins, and primary or secondary metabolites which have antioxidant, antimicrobial, anti-inflammatory, antitumor, antimutagenic, anti-carcinogenic, and diuretic activities. Natural product from plants are important for pharmacological research and drug development, not only when the plant constituents are used directly as therapeutic agents, but also as starting materials for the synthesis of drugs or as model for the lead compounds[7].

Among the plants known for medicinal value, the plants of the genus *Coleus* belonging to the family Lamiaceae or Labiatae are well known for their therapeutic potentials. The plants of Lamiaceae are usually aromatic and known for kitchen herbs like R*osemary* and *Ocimum sanctum*. Many of the plants of this family are used in traditional medicine because of their antimicrobial, antioxidant, antiseptic and other pharmacological activities. *Coleus* is a large and wide spread genus containing 300 species and is found in different parts of tropical Africa, Australia and Asia, especially in Indonesia[8,9]. *Coleus* species are found as herbs, subshrubs or shrubs. They are often succulent with opposite leaves. In flourescence is terminal or in the upper leaf axils is in compact cymose clusters. *Coleus atropurpureus L.* Benth, one of the plants of genus *Coleus* which is native to Indonesia, especialy in South Sulawesi Province. The leaves decoction is indicated for the traditional treatment of bronchitis, hemorrhoids, antioxidants, and tuberculosis[10,11] Nevertheless, only few studies have focused on the biological activity of the extracts and components of the leaves of Coleus. The methanol extract of the bark showed *in vitro* bactericidal activity against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Enterococcus faecalis* [12].

Little is also known about the chemical constituents of the plant. The essential oil composition was studied[13, 14] and saponins were isolated from the seeds[15]. From an aqueous extract, catechins were identified as the main components[16]. The objectives of the present study were to evaluate the *in vitro* antimycobacterial activity of the chloroform extract obtained from the leaves of *Coleus atropurpureus* L. Benthand characterization of components by Spectroscopy FTIR and 2D NMR, in order to improve the knowledge about

the chemical structural of compound from the leaves of *Coleus atropurpureus* L. Benth and to explore its potential as a source of new drugs against *M. tuberculosis*. The election of the Spectroscopy FTIR and NMR technique was supported by the fact that the extract was obtained from the leaves of *Coleus atropurpureus* L. Benth, which possesses a high content of terpenoid compounds. In this study, we report for the first time the isolation, purification, and structural determination of cyclic ester terpenoid, 2', 5'-dimethyl benzopelargonolactone from the leaves of *Coleus atropurpureus* L. Benth. We also tested the activity of 2', 5'-dimethyl benzopelargonolactone alone and in combination with the anti-tuberculosis drug rifampicin against *M. tuberculosis* strain H37Rv (ATCC 27294) to increase sensitivity to anti-tuberculosis drugs.

## MATERIALS AND METHODS

### *Materials*

The leaves of *Coleus atropurpureus* L. Benth was collected in the region of Malino and Tana Toraja, South Sulawesi Province, Indonesia. Samples plant identification was conducted in Plant Taxonomy Laboratory Faculty of Natural Sciences Hasanuddin University, Indonesia. Chloroform, rifampicin, and methanol were purchased from Sigma Chemical Co. (USA).

### *Extraction and Isolation of active compound*

Dry powder of 3.0 kg of the leaves of *Coleus atropurpureus* L. Benth, was macerated with MeOH for 72 h, filtered, and solvent evaporated to obtain a dark brown extract amounting 59.28 g. The extracts were partitioned with chloroform resulting in 9.96 g product. The chloroform extract was fractionated by VLC and produced ten major fractions (I-X). Colourless red precipitate found in the second fraction was filtered by filtration resulting in 58.3 mg product. Purity test was performed by TLC analysis using three solvent systems.

### *Instrumentation*

FTIR spectra determination was done with a Shimadzu spectrometer (Japan). NMR spectra of $^{1}H$, $^{13}C$ and HMBC were obtained using a Bruker, Germany DPX-500 spectrometer at 300 MHz ($^{1}H$) and 125 MHz ($^{13}C$) in $CDCl_3$ using TMS as internal reference. Separations and identification of compound were conducted with VLC by Merck Si gel 60 (230-400 mesh), and TLC on aluminum or glass plates coated with Merck Si gel 60 F254 and thickness of 0.25 mm.

### *Anti-mycobacterial activity assays*

For the initial bioactivity screening, standard colony assays on Löwenstein-Jensen (LJ) medium were performed to assess the anti-mycobacterial activity of methanol whole extract, chloroform fraction, and compound I with the final concentrations of 200 μg/mL, respectively, using the strain *M. tuberculosis* H37Rv (ATCC 27294) and solvent DMSO as negative control plus rifampicin as positive control. For testing the anti-mycobacterial activity and MIC value of compound I and in combination with rifampicin, the strain *M. tuberculosis* H37Rv (ATCC 27294) were grown on Middlebrook 7H19-OADC (oleic acid, albumin, dextrose, catalase) (Difco Laboratories, USA) at 37 °C for 3 weeks until midlog phase. The turbidity of culture were adjusted with 7H9 broth to a 0.5 McFarland standard using a nephelometer, and cell cultures was inoculated on 4 mL MGIT medium[17,18], using solvent (DMSO) as negative control, rifampicin (0, 0.5, 1.0, and 2.0 μg/mL), and combination with compound I were indicated. Tubes were incubated at 37 °C and inhibition growth documented after 3 days; MGIT tubes were removed from the incubator and placed on a 365 nm UV transilluminator (micro MGIT reader). It is important to read the tube everyday beginning on day 3, until results can be interpreted. The positive tubes results if there was high level fluorescens from the bottom of the tube when the tube put in the micro MGIT reader and negative tubes showed low level or no flourescens. . Once the MGIT growth control is positive, the drug-containing tubes will be read for interpreting results. We could start read the MGIT tube with containing drug (first line drugs or second line drugs) on the same day when MGIT GC is positive and interpret the result for up the third days, not to exceed fourteen days. . If the MGIT GC tube is not positive by the twelfth day of the test, the test is invalid. Interpret the MGIT result as

susceptible if the drug-containing tube does not fluoresce within two days of onset of fluorescence in the GC tube. Interpret the MGIT result as resistant if the drug-containing tube fluoresces on or within two days of the day of onset of fluorescence in the GC tube. When interpreting resistance, finalize the result as soon as the MGIT GC and the drug-containing tubes fluoresce. All inoculated MGIT tubes after 56 days or negative results should be autoclaved prior to disposal. For positive MGIT media results should add disinfectant (5 % Vesphene) before put in the autoclave bag. MGIT tubes are kept in the autoclave bag place in the autoclave. After finish autoclaving, send them for the incineration. The growth control tube was compared to the positive and negative controls; positivity was indicated by bright orange fluorescence on the bottom of the tube and an orange reflection at the meniscus; negative tubes on the other hand showed low level or no fluorescence. The assays was conducted in duplicates and repeated three times to produce representative experimental data.

## RESULTS AND DISCUSSION

The amount 59.28 g of methanol extract of the dried and powdered form of the leaves of *Coleus atropurpureus* L., was partitioned with chloroform solvent. The chloroform fraction was further separated by silica gel chromatography and purified to obtain compound I. The compound obtained, compound I is an colourless red paste, giving an indication of terpenoid on Liebermann-Burchard test. Its FTIR spectrum showed the absorption bands for aliphatic groups (2956, 2927 and 2856), C=C aromatic (1598 and 1579), =C-H aromatic (3068), $CH_2$ (1463), $CH_3$ (1379), C=O from ester groups in the molecule (1728, 1266) and while strong absorption band C-O-C (1072) and disubstitution aromatic (742) were characterized as an cyclic ester terpenoid compound. The $^{13}C$ NMR and DEPT spectrum exhibited 15 carbon signals ($2\times CH_3$, $4\times CH_2$, $6\times CH$ including =C-H aromatic, $3\times C$ including C=O), and the molecular formula of compound I was deduced to be $C_{15}H_{20}O_2$. The $^1H$ NMR spectrum (Table 1) observed the presence of two tertiary methyl groups on saturated carbons at 0.89 (3H, *m*) and 0.91 (3H, *m*), also two unit isoprenoid of aliphatic signals at δ 1.43 (H-1', 2H, *d*, *J* = 5,8 Hz), 1.68 (H2', 1H, *m*), 1.31 (H3', 2H, *m*), 1.35 (H-4', 2H, *m*), 1.68 (H-5', 2H, *m*), 4.21 (H-6', 2H, *m*), 1.30 (H-7', 2H, *m*), 0.89 (H-8', 3H, *m*), 1.41 (H9', 2H, *m*), 0.91 (H-10', 3H, *m*). Two signals methyl carbons at d 14.2 and 11.1 in the $^{13}C$ NMR (Table 1) spectrum indicated that compound I belongs to the cyclic ester terpenoid, and four methylen signals carbons at δ 68.3 ppm (C-6'), 30.5 ppm (C-4'), 23.1 ppm (C-3'), 27.1 ppm (C-1'), and six methyn signals at δ 38.9 ppm (C-5'), 38.9 ppm (C-2'), 128.9 ppm (C-3 and C-6), 131.0 ppm (C-4 and C-5). The correlation of $^{13}C$ carbon with $^1H$ proton were observed in the HMBC and COSY spectrum (Table 1). Based on the data description above and by comparison of their physico-chemical and spectroscopic data with those of previously known related compounds[19], the molecular structure of compound I is in Figure 1 with name of 2', 5'-dimethyl benzopelargonolactone.

**Table 1**
***$^1H$ (300 MHz) and $^{13}C$ (125 MHz) NMR data of compound I in $CDCl_3$***

| Position of C | Type of C | $^1H$-NMR δ ppm (H, multiplicity, J) | $^{13}C$-NMR δ ppm | HMBC (H→C) | COSY (H↔H) |
|---|---|---|---|---|---|
| 1 | C | - | 132.6 | - | - |
| 2 | C | - | 132.6 | - | - |
| 3 & 6 | CH | 7.70 (1H &2H, *dd*, *J* = 9,15 & 2.45 Hz) | 128.9 | C-4, C-5, C-1'' | H-4, H-5 |
| 4 & 5 | CH | 7.25 (1H, *ddd*, *J* = 9.15; 7.5 & 2.45 Hz) | 131.0 | C-3, C-6 | H-3, H-6 |
| 1' | $CH_2$ | 1.43 (2H, *d*, *J* = 5.8 Hz) | 27.1 | - | H-2' |
| 2' | CH | 1.68 (1H, *m*) | 38.9 | - | H-1' |
| 3' | $CH_2$ | 1.31 (2H, *m*) | 23.1 | C-7' | - |
| 4' | $CH_2$ | 1.35 (2H, *m*) | 30.5 | C-8' | H-5' |
| 5' | CH | 1.68 (1H, *m*) | 38.9 | C-6' | H-4', H-6' |
| 6' | $CH_2$ | 4.21 (2H, *m*) | 68.3 | C-1'', C-5', C-4' | H-5' |
| 7' | $CH_3$ | 0.89 (3H, *m*) | 14.2 | C-7' | H-7' |
| 8' | $CH_3$ | 0.91 (3H, *m*) | 11.1 | C-5' | H-9' |
| 1'' | C | - | 167.9 | - | - |

In the first, to screening antimycobacterial activity of all fractions, including compound I at concentrations 200 µg/mL was measured by incubating with the mycobacterial cells on LJ medium. MeOH fraction at concentration 200 µg/mL was able to decrease mycobacterial cells growth, where as chloroform fraction and compound I in the same concentration, like rifampicin as positive control with no colony growth of *M. tuberculosis* on LJ medium (data not shown). To identify the MIC value of compound I, anti-mycobacterial activity of this compound against

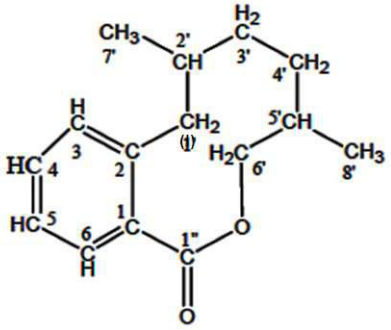

**Figure 1**
***Structure of 2',5'-dimethyl benzopelargonolactone (I) from the leaves of Coleus atropurpureus L.***

**Table 2**
***Effect of compound I in combination with rifampicin in MGIT medium on growth inhibition of M. tuberculosis H37Rv (ATCC 27294) strain.***

| | | Rifampicin (µg/mL) | | | |
|---|---|---|---|---|---|
| | | 0 | 0.5 | 1.0 | 2.0 |
| Compound I (µg/mL) | 0 | P1 (+++) | P2 (+) | P3 (-) | P4 (-) |
| | 100 | P5 (+) | P6 (-) | P7 (-) | P8 (-) |
| | 200 | P9 (-) | P10 (-) | P11 (-) | P12 (-) |
| | 400 | P13 (-) | P14 (-) | P15 (-) | P16 (-) |

*M. tuberculosis* test was performed with MGIT medium. The combinations of compound I and rifampicin were tested in the anti-mycobacterial assay described above by a checkerboard method in order to identify those combinations that could enhance bioactivity effect. The antimycobacterial activity of compound I at different concentrations 0, 100, 200, and 400 µg/mL in combination with rifampicin at variation concentrations 0, 0.5, 1.0, and 2.0 µg/mL was measured by incubating the mycobacterial cells on MGIT medium. Compound I alone at concentration 100 µg/mL was not able to inhibited mycobacterial cells growth. Furthermore, at concentration 200 and 400 µg/mL showed significant inhibition of growth of *M. tuberculosis* (Table 2). The combinations with the rifampicin at concentration 0.5 µg/mL was able to exhibit enhanced mycobacterial effect at the lowest concentration (100 µg/mL) of compound I (Table 2). The results of this study demonstrated that compound I was activated against *M. tuberculosis* when used in combination with other antimycobacterial agents (rifampicin), and increased the sensitivity of the anti-TB drug. This result was in accordance with previously reported research by Woldemichael *et al* (2003), where terpenoid compounds from *Calceolaria pinnifolia* (Scrophulariaceae) showed significant inhibition of growth of *M.*

*tuberculosis* with MIC values 128 μg/mL, although mechanism of anti-mycobacterial action by direct and/or indirect of terpenoid compounds is not fully clear, but is speculated to involve membrane disruption of mycobacterial cells by lipophilic compounds [18,20, 21].

## CONCLUSION

Compound I, an cyclic ester terpenoid, 2', 5'-dimethyl benzopelargonolactone has been isolated from the chloroform fraction from leaves of *Coleus atropurpureus* L. Benth. The compound was not only active against *M. tuberculosis* with MIC values 200 μg/mL, but also could activated the sensitivity of rifampicin drug for treated patient with TB. In conclusion, our results demonstrated that the activity rifampicin drug could be activated and enhanced through administration with 2', 5'-dimethyl benzopelargonolactone. Further studies aimed to structural modification and semi-synthesis of compound I which exhibited increasing anti-mycobacterial activities, and also using *in vivo* methods are required to explore the compound responsible for the activity which might prove important for improved therapies for the treatment of tuberculosis disease.

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