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**Submission date:** 05-Dec-2021 07:58PM (UTC+0700)
**Submission ID:** 1720951270
**File name:** 2017a.pdf (383.28K)
**Word count:** 1944
**Character count:** 11179

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# Effects of ethanolic extract of Miana (*Coleus scutellariodes* [L] Benth) leaf on IgM profile in Balb/c mice with systemic of vulvovaginal candidiasis

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**ABSTRACT**

*Coleus scutellariodes [L] Benth (Miana or Mayana) is commonly used in Indonesian folk medicine to treat vaginal discharge. This study was aimed to evaluating effect of ethanolic extract of Miana leaf on IgM profiles in Balb/C mice with systemic vulvovaginal candidiasis. In experiment, 25 mice were divided into five groups, five mice per each group, for IgM serum analysis. Animals received a dose of 500 and 750 mg/kg of Miana extracts with orally and then intravaginal inoculated 1 x $10^{-2}$ C. albicans. Control vulvovaginal candidiasis group and positive group received 0.2 mL saline and ketoconazole (200 mg/kg), respectively, and inoculation. Normal group received 500 mg/kg of Miana extract and no infection with C. albicans. IgM level was measured at 0, 2, 3, 5 and 14 days by ELISA method with using albicans antibody kit according to the manufacturer's protocol. The results indicated that the Miana-treated mice (dose of 750 mg/kg) produced lower IgM level than vulvovaginal candidiasis control groups at 0-14 days ($p < 0.001$) with values of 0.22-0.80 pg/mL and 0.25-1.74 pg/mL, respectively. This infected group showed similarly potency for IgM level in mice of ketoconazole positive control group (values, 0.22 to 0.78 pg/mL) at $p < 0.001$. Our results provide important information about the potential application of Miana ethanolic extract in the treatment of Balb/C mice with systemic vulvovaginal candidiasis.*

***Keywords**: Coleusscutellariodes[L], Benth, Miana, Vulvovaginal candidiasis, IgM*

## INTRODUCTION

Vulvovaginal candidiasis is a common disorder in women (estimation of 75%) and caused by fungi of *Candida albicans*[1]. *C. albicans* is a polymorphic opportunistic fungal pathogen in human[2] and causes a clinical significant problem in immunocompromised patients[3]. Thus, the combination therapy with antibody and antifungal could be beneficial to immunosuppressed patients who suffer from *Candida* infection. Generally, the azoles are the treatment of choice for vulvovaginal candidiasis, but there is a certain limit to use azoles because the antifungal drug causes resistance[4]. Since the number patients the use antibiotics increase every day, therefore, it is important to find a safe and also a proper treatment. The medicinal plants are the choice to treatment in vulvovaginal candidiasis because plant-derived drugs have been reported to be safe and without side-effect[5]. In traditionally (Toraja community, South Sulawesi, Indonesia), leaves of *Coleus scutellariodes* [L] Benth, namely Miana or Mayana in Indonesia, has been used for vaginal discharge. Some investigators have been reported the potential pharmaceuticals properties of Miana such as immunostimulatory[6], antibacterial[7,8,9], and antihyperglycemic[10]. The aim of this study was to evaluation the immunostimulatory effect (IgM profiles) of oral administration of Miana ethanolic extracts against systemic candidiasis in a mouse model.

## MATERIALS AND METHODS

The leaves of Miana were obtained from Indonesian Spices and Medicinal Crops Research Institute, Bogor, Indonesia. The plant was identified in the Herbarium Bogoriense of the Indonesia Institute of Sciences where a specimen was deposited. After drying, leaves of Miana were ground to give a powder. The leaf powder of Miana was macerated separately in ethanol (1:5 w/v) for 24 h. After evaporation, extract was collected and tested for in vivo analysis of IgM profile in Balb/C mice.

For this experiment, 25 Balb/C mice females were purchased from Faculty of Medicine, Hasanuddin University, Indonesia and kept 7 days at animal house with 25 ± 2°C temperature, 12/12 hours dark-light cycle and had access to tap water *ad libitum*. Animal housing and treatments were performed according to the approved ethical protocol of Faculty of Medicine, Hasanuddin University (UH16010010 in March 7, 2016). In experimental model, mice were divided into five groups as follow:

- VCC, Vulvovaginal candidiasis control group (n = 5): mice were orally given normal saline (0.2 mL) via cannula and inoculated intravaginal with *C. albicans* ($1x10^{-2}$ CFU /mouse, ATCC = 10231)[11];
- VCK, ketoconazole-treated in vulvovaginal candidiasis group (n = 5): mice were orally given ketoconazole (200 mg/kg body weight) and inoculated intravaginal with *C. albicans* ($1x10^{-2}$ CFU/mouse, ATCC = 10231);
- VCM500, Miana leaf ethanolic extract-treated in vulvovaginal candidiasis group: mice were orally given Miana leaf ethanolic extract (500 mg/kg body weight) and inoculated intravaginal with *C. albicans* ($1x10^{-2}$ CFU/mouse, ATCC = 10231);
- VCM750, Miana leaf ethanolic extract-treated in vulvovaginal candidiasis group: mice were orally given Miana leaf ethanolic extract (750 mg/kg body weight) and inoculated intravaginal with *C. albicans* ($1x10^{-2}$ CFU/mouse, ATCC = 10231);
- M500, Miana leaf ethanolic extract-treated group: mice were orally given Miana leaf ethanolic extract (500 mg/kg body weight) and received 0.1 mL saline intravaginal;

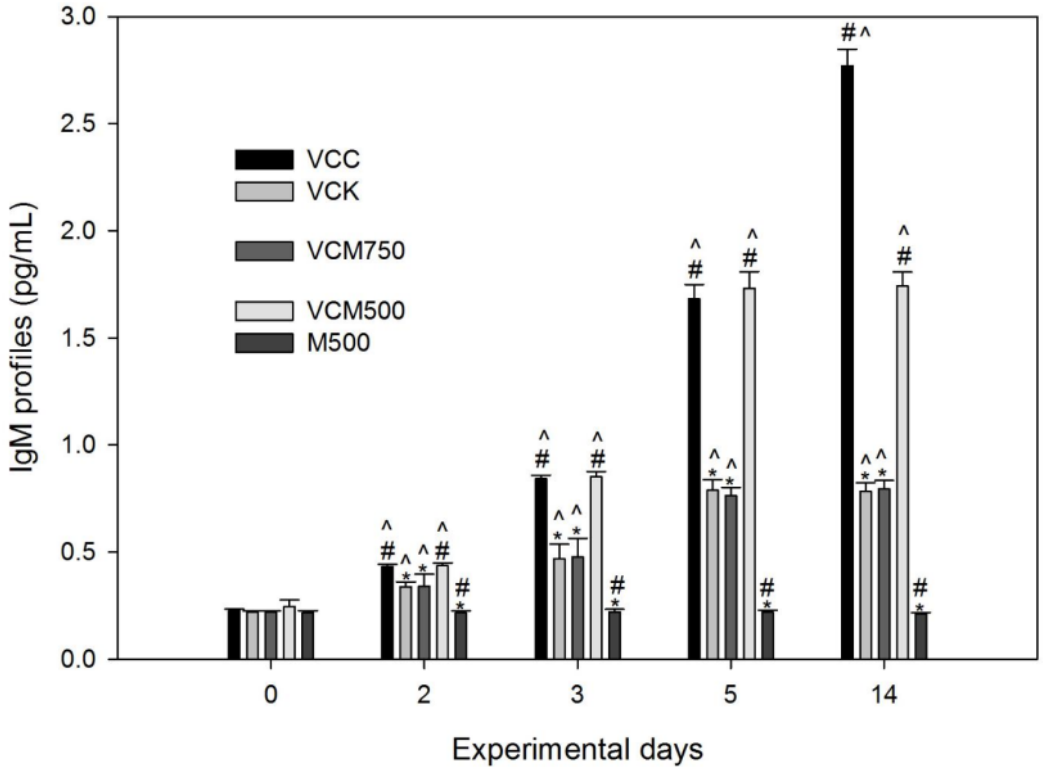
These groups were monitored for 14 days. Serum samples were collected from the submaxillary vein of all the mice in each of the five models (5 mice per group) at different days (0, 2, 3, 5 and 14). IgM concentration in serum (n = 5 mice from each group) were analyzed by anti-candida albicans antibody kit (ab53891, Abcam, USA) according to the manufacturer's protocol. Samples were analyzed using a microplate reader at 450 nm. IgM concentrations were determined by a standard curve and expressed in pg/mL.

Data were evaluated for statistical significance of differences by one-way ANOVA followed by Duncan test. All data were expressed as mean ± SD. Level of statistical significance of differences: $p < 0.001$.

## RESULTS AND DISCUSSION

Figure 1 shows effect of ethanolic extract of Miana (*C. scutellariodes*) leaf on IgM profiles in Balb/c mice with systemic of vulvovaginal candidiasis. In Group-M500 (normal control), IgM levels of animals not changed throughout the 14 days' experiment from 0.22 to 0.21 pg/mL. In comparison with the normal control (Group-M500), the level of IgM in all experiments (Groups: VCC, VCK, VCM500 and VCM750) was increased significantly ($p < 0.001$). Infected *C. albicans* of the mice can induce level of IgM, which may be attributed to an improvement in immune functions[12]. There was no significant difference ($p < 0.001$) in IgM levels between group VCC (vulvovaginal candidiasis control group) and VCM500 at 14 days experiments from 0.23-2.77 pg/mL and 0.25-1.74 pg/mL, respectively. On the other hand, serum levels of IgM in group VCM750 and VCK showed decreased at 14 days (0.22 to 0.80 pg/mL and 0.22 to 0.78 pg/mL) as compared to group VCC ($p < 0.001$). However, there was no significant difference ($p < 0.001$) between group VCM750 and VCK (as a positive control using ketoconazole). These results indicate that dose of 750 mg/kg body weight of leaf ethanolic extract of Miana has the same potency with ketoconazole (200 mg/kg body weight). These data indicate that ingestion of leaf ethanolic extract of Miana (750 mg/kg body weight) during *C. albicans* infection is associated with significantly lower fungal titer in serum at 0, 2, 3, 5, and 14 days after infection. Our results confirm the earliest study by Pakadang et al.[6] that oral administration of Miana extract in rats can enhanced immune response by resistance against tuberculosis infection.

**Figure-1: Effect of ethanolic extract of Miana (Coleus scutellariodes [L] Benth) leaf on IgM profile in Balb/c mice with systemic of vulvovaginal candidiasis. Results are presented as the mean ± SD (n=5). One-way ANOVA followed by Duncan test was used for statistical significance. The animal groups are as follows: VCC, Vulvovaginal candidiasis control group; VCK, 200 mg/kg b.w. ketoconazole-treated in vulvovaginal candidiasis group; VCM500, 500 mg/kg b.w. Miana leaf ethanolic extract-treated in vulvovaginal candidiasis group; VCM750, 750 mg/kg b.w. Miana leaf ethanolic extract-treated in vulvovaginal candidiasis group; M500, 500 mg/kg b.w. Miana leaf ethanolic extract-treated in normal group. Notes: *p<0.001 significant difference from the vulvovaginal candidiasis control group (VCC); #p<0.001 significant difference from the ketoconazole-treated in vulvovaginal candidiasis group (VCK); ^p<0.001 significant difference from the Miana leaf ethanolic extract-treated in normal group.**




## CONCLUSION

It can be concluded that leaf ethanolic extract of Miana (750 mg/kg body weight) has a potent to decrease of IgM profiles in mice infected with *C. albicans* and its potent to use as antifungal for *C. albicans* infection.

## ACKNOWLEDGMENTS

This work was sponsored by the Ministry of Research, Technology, and Higher Education of the Republic of Indonesia via the Doctoral scholarship program (Beasiswa Pendidikan Pascasarjana Dalam Negeri/ BPPDN)

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