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Antioxidant and Anti-Inflammatory Activities of Combined Menthol and Capsaicin as the Active Ingredient in Moringa Balm

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Abstract
In this study, menthol and capsaicin were selected to enhance the antioxidant and anti-inflammatory activity of moringa balm that used for pain and inflammation relief. However, the combination of both compounds might increase or decrease some of the antioxidant and anti-inflammatory properties. To identify the combined effect of capsaicin and menthol on the antioxidant and anti-inflammatory activity of moringa balm, the balm was made using moringa leaf extract together with 3% and 5% menthol as well as 0.3% and 0.2% capsaicin. Then, the antioxidant activity of the balm was assessed via DPPH radical scavenging assay and Ferric Reducing Antioxidant Power (FRAP) assay while the anti-inflammatory activity was evaluated via anti-lipoxygenase assay. MB1 that had 3% menthol and 0.3% capsaicin, exhibited the highest radical scavenging activity and the highest inhibition of lipoxygenase although is MB4 that had 5% menthol and 0.2% capsaicin, shown the highest reducing capability.
Keywords: menthol, capsaicin, antioxidant activity, anti-inflammatory activity

Introduction

Moringa Oleifeira or commonly known as Drumstick tree, is a plant native to sub-Himalayan tracts of India, Pakistan, Bangladesh, and Afghanistan (Fahey, 2005). *M. oleifera* had been known as 'the miracle tree' among commoners due to its medicinal uses in various ailments and diseases (Gupta et al., 2018). This plant also exhibits anti-inflammatory and antioxidant properties (Xu et al., 2019; Yan et al., 2019) which made it extract especially leaf extract suitable to be used in making of pain and inflammation relief balm.

Anti-inflammation properties and antioxidant properties are important properties for pain and inflammation relief balm. Hence, active ingredients such as menthol and capsaicin were selected to enhance these 2 properties of moringa balm. Menthol is a naturally occurring monoterpene that could be found in mint plant or *Mentha* genus plant (Farco & Grundmann, 2013). Menthol had shown to possess antioxidant and anti-inflammatory properties. Menthol can increase the level of antioxidants such as glutathione, glutathione peroxidase and glutathione reductase as well as anti-inflammatory cytokine interleukin-10 while reduce the activity of oxidation enzyme, myeloperoxidase and levels of the pro- inflammatory cytokines: tumour necrosis factor-a and interleukin-6 (Rozza et al., 2014). Capsaicin is a capsaicinoid which is a form of alkaloid that could be found in the *Capsicum* family (Hayman & Kam, 2008). Capsaicin displays its anti-inflammatory properties by inhibit NF-kB and MAPK signalling pathways that lead to reduce production of inflammatory cytokines, interleukins, nitric oxide and TNF-α in human macrophages (Li et al., 2021). Capsaicin also exhibit antioxidant properties as capsaicin could induce antioxidants such as activities of superoxide dismutase, catalase, and glutathione-S-transferase (Hassan et al., 2012).

Despite the benefits of menthol and capsaicin hold, the combination of 2 natural compounds might lead to synergistic interaction or antagonistic interaction (Caesar & Cech, 2019). Thus, the combined effect of menthol and capsaicin sometimes could possibly increase or decrease some of the antioxidant and anti-inflammatory properties. However, there is no studies that studied the antioxidant and anti-inflammatory effect of combined menthol and capsaicin in moringa balm. Therefore, this study aims to determine the antioxidant and anti-inflammatory activity of Moringa balm with the addition of menthol and capsaicin. This study will help to produce a better formulation of moringa balm with a better combination of active ingredients, so the moringa balm might have an enhanced pain and inflammation relief effect.

Materials and methods

The moringa leaves that were essential for moringa balm were harvested from the residential area at Ulu, Tiram, Johor Bahru, Malaysia. Then the leaves were oven dried at 50℃ until a constant mass and blended into powder. Methanolic extraction of *M. oleifera* leaf powder was carried out according to the method from Ali et al. (2013) with modification. 3g of *M. oleifera* powder was extracted with 150mL of aqueous methanol (80%) for 6 hours at 25°C in a mechanical mixer. The extract was filtered, re-extracted with 75mL 80% methanol and underwent agitation for 6 hours at 25°C. Then, the extracts from the residue were mixed with the previous extract. The combined extract was concentrated under reduced pressure at 45°C via a rotatory evaporator. After the methanolic extract become dry, the dried extract was resuspended in 50% DMSO to get a concentration of 100mg/mL.

There are 4 different types of moringa balm been synthesised which are MB1 that have 3% menthol and 0.3% of capsaicin, MB that consist of 3% menthol and 0.2% of capsaicin, MB 3 that have 5% menthol and 0.3% of capsaicin, MB4 that consist of 5% menthol and 0.2% of capsaicin as well as control that have neither capsaicin nor menthol. The synthesis of 10mL of moringa balm required ingredients such as 4mL of 187.5mg/mL HPMC, 0.5mL of 60% (v/v) Oleic acid, 0.5mL of 100% (v/v) Tween 80,1.5mL of 1 to 1 ethanol: dichloromethane, 0.5mL of 60% (w/v) PEG, 0.375mL or 0.625mL of 80% (w/v) menthol, 0.3mL or 0.2mL of 10% (w/v) capsaicin, 2mL of moringa leaves methanolic extract and ultra- pure water. The HPMC was first added and heated to 4°C then the ingredients were added one by one with stirring via a mechanical mixer. After this, the mixture was stirred for 20 minutes.

The antioxidant activity of the moringa balm were evaluated with 2 tests: DPPH radical scavenging assay and Ferric Reducing Antioxidant Power (FRAP) assay. DPPH radical scavenging activity was performed as described by Sánchez-Moreno et al. (1999) and the ferric reducing activity of the sample was determined using the FRAP method by Pulido et al. (2000) with modification. The anti- inflammatory activity of the moringa balm were evaluated with anti-lipoxygenase assay and the assay was conducted according to the method of Sarveswaran et al. (2017) with some modifications.

Results and discussion

Figure 1 had demonstrated the radical scavenging activities (RSA) value for each moringa balm that been tested in DPPH radical scavenging assay. All the moringa balm has higher radical scavenging activities (RSA) than moringa balm. Among the 4 different types of moringa balm, the order of moringa balm that show increasing RSA: MB2 <MB4 <MB3 <MB1. The results had shown that the amount of capsaicin present in the balm will affect the radical scavenging abilities of the balm as MB1 which had the 0.3% of capsaicin exhibit highest radical scavenging activities while MB2 that had 0.2% of capsaicin exhibit lowest radical scavenging activities while both having the same amount of menthol. One study showed that capsaicin has similar DPPH radical scavenging ability as α-tocopherol and even performed better than α-tocopherol in scavenging radicals at or near the membrane surface and in the interior of the membrane (Kogure et al., 2002). MB3 and MB4 that had the highest amount of menthol, have worse radical scavenging abilities compare to MB1 but better radical scavenging abilities than MB2 which had the least menthol and capsaicin concentration. The results suggested that the concentration of menthol used can affect the radical scavenging abilities, but the concentration

of menthol used and the interaction between capsaicin and menthol might impact the radical scavenging capability of the balm negatively. Menthol had shown to be one of the essential oil components that have the least active antioxidant activity and radical scavenging activities compared to other phenolic compounds, carvacrol, thymol, and eugenol (Sharopov et al., 2015).

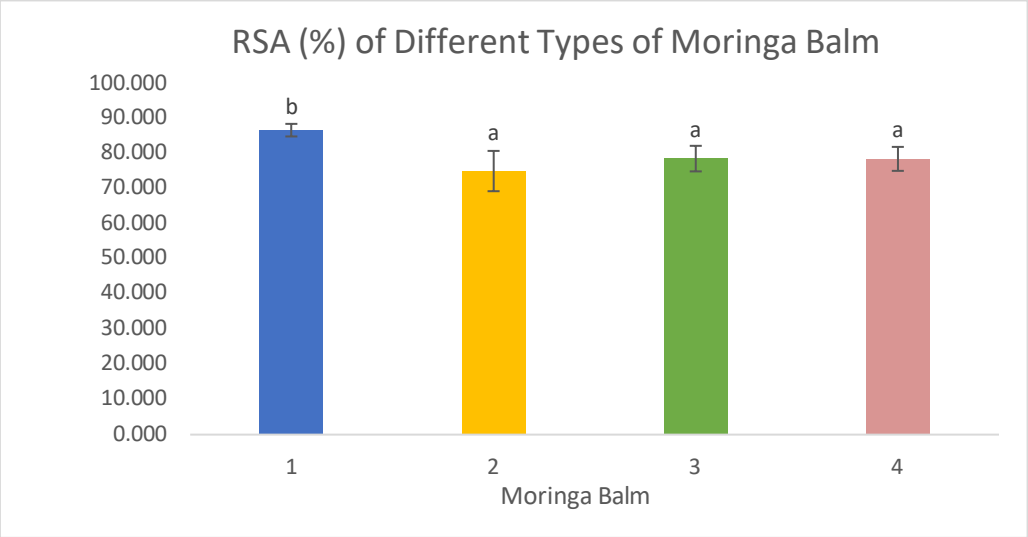

Figure 1 Comparison of the RSA values of different types of moringa balms. Each bar represents mean± standard errors (n = 3) except control. For each moringa balm, bars denoted by the same letters are not significantly different (p>0.05), according to SPSS's one way ANOVA test and Duncan post hoc test.

Figure 2 show the FRAP values of different moringa balm obtained through the standard curve of FeSO4.H2O. The moringa balm control had achieved a FRAP value of 10.091. Among all 4 moringa balms, the only moringa balm that has a higher FRAP value than moringa balm control is MB 4 while the rest are lower than the moringa balm control. Capsaicin had shown to improve plasma's ferric reducing potential in the capsaicin supplemented male Wistar rats (Chaudhary et al., 2019) and the ferric reducing potential of the capsaicin are work in a concentration-dependent manner (Kumar et al., 2015). Even though menthol was shown to possess the ferric reducing capability, menthol has the lowest reducing ability compared to other essential oil components or monoterpenes (Lado et al., 2004; Sharopov et al., 2015). Thus, the result obtained is unexpected and the probable reason behind this result might be due to menthol and capsaicin might act antagonistically in ferric reducing activity.

Figure 3 show the capabilities of different types of Moringa Balm (MB) in inhibiting lipoxygenase activity. All 4 types of moringa balm have a higher value than the control. The capability of inhibition of lipoxygenase is increase in the order: MB4 < MB2 < MB3 < MB1. The result showed that the presence of capsaicin affects the anti-lipoxygenase activity greatly since MB has the highest (MB1) and second highest (MB3) anti lipoxygenase activity has 0.3% capsaicin. 5-lipoxygenase was regulated by the transcription factor nuclear factor-kappa B (NF-κB) (Manjunatha & Srinivasan, 2006) and capsaicin had been proven to be able to inhibit activation of NF-κB (Mori et al., 2006). While menthol also able to enhance the anti-lipoxygenase capability of the balm since MB 1 to 4 that consist of menthol has a higher percentage of inhibition of lipoxygenase than control. Menthol exhibited remarkable capability in inhibition of 5-lipoxygenase (79.9%) in a study conducted by Demirci et al. (2021). Based on figure 3, the addition of menthol with capsaicin affects the anti-lipoxygenase capability negatively especially when the balm has a higher concentration of menthol (5%). This outcome is probably due to capsaicin and menthol acting antagonistically, menthol might reduce the anti-lipoxygenase activity of capsaicin when in higher concentration.

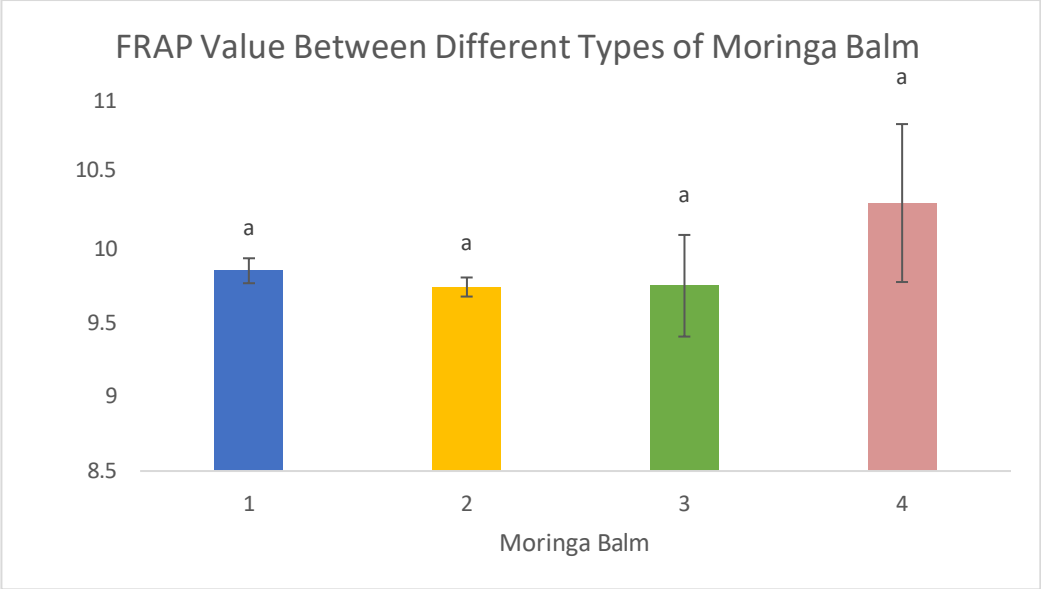

Figure 2 Comparison of FRAP value among different types of MB. Each bar represents mean ± standard errors (n = 3). For each moringa balm, bars denoted by the same letters are not significantly different (p>0.05), according to SPSS's one way ANOVA test and Duncan post hoc test.

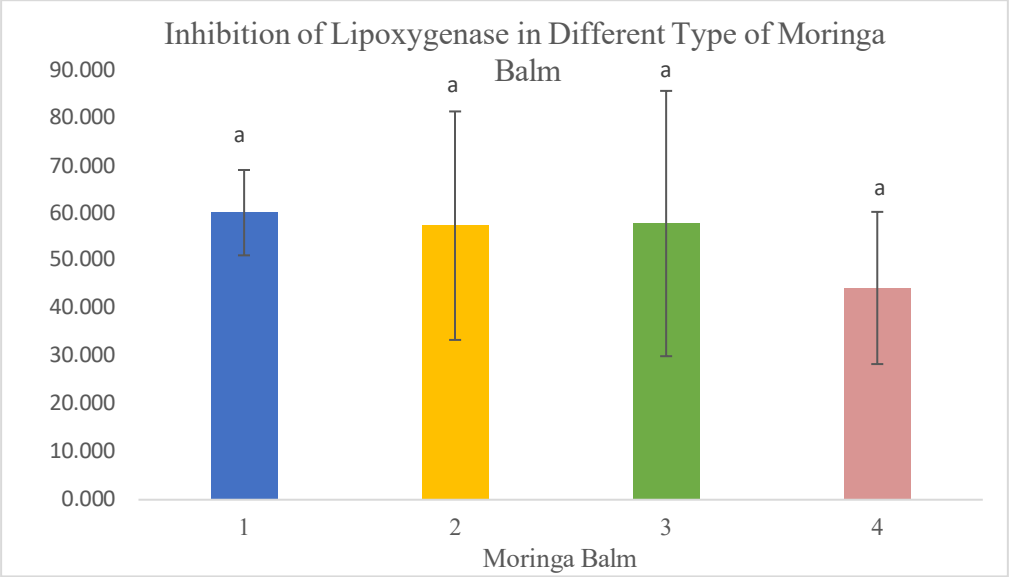

Figure 3 Capabilities of different types of Moringa Balm (MB) in inhibiting lipoxygenase activity. Each bar represents mean ± standard errors (n = 3). For each moringa balm, bars denoted by the same letters are not significantly different (p>0.05), according to SPSS's one way ANOVA test and Duncan post hoc test.

MB 1 had scored the highest radical scavenging activities and inhibition of lipoxygenase in the DPPH assay and anti-lipoxygenase assay. This result suggested that the radical scavenging activity and anti-lipoxygenase activity are indirectly correlated to each other. In an inflammation site, inflammatory cells will release ROS at the site inflammation-causing exaggerated oxidative stress and oxidative damage to cells as well as tissues. While on another side, ROS can initiate an intracellular signalling cascade that promotes the expression of proinflammatory genes (Biswas, 2016). The interdependency of inflammation and oxidative stress had proposed an interdependent relationship between antioxidant and anti-inflammatory activity, but further exploration of how menthol and capsaicin work in this interdependency relationship needs to be determined.

Conclusion

The addition of capsaicin and menthol into the moringa balm able to improve the antioxidant and anti- inflammatory capability of the balm. The moringa balm that show the best DPPH radical scavenging and anti-lipoxygenase activity is MB 1 which contain 3% menthol and 0.3% capsaicin. While the moringa balm that show to have the best antioxidant activity in FRAP assay is MB4 that consist of 5% menthol and 0.2% capsaicin. Among all 4 types of moringa balm, the most suitable combination of menthol and capsaicin that should be added into moringa balm is 3% menthol and 0.3% capsaicin.

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