

## Formulation and Characterization of Cinnamon Bark Essential Oil (*Cinnamomum burmanii*) Nanoemulsion as Poultry Feed Additive Candidate

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### ABSTRACT

This study was conducted to formulate and to characterize nanoemulsion of cinnamon (*Cinnamomum burmanii*) bark essential oil (CBO). The compositions of cinnamon essential oil were qualitatively analyzed using Gas Chromatography-Mass Spectrophotometry (GC-MS). The formulations which consisted of CBO, virgin coconut oil (VCO), surfactant (Tween 80), and co-surfactant (PEG 400) were investigated to prepare emulsion in nano-meter size. The nanoemulsion was developed using low energy emulsification method. The transmittance value was carried out to choose the best nanoemulsion formulation. Droplet size, polydispersity index (PI), zeta potential, and morphology were studied to characterize the nanoemulsion formulation. Based on the transmittance value after dispersion into aqueous phase, formulation consisting of CBO, VCO, Tween 80, PEG 400 (0.75:0.25:4:1 v/v) was chose. The formulation of nanoemulsion having mean droplet diameters of 20.2 nm with polydispersity index (PI) 0.463 and potential zeta -0.8 mV. The nanoemulsion morphology using transmission electron microscopy (TEM) imaging was demonstrated the oil droplets in nano-scale. This study showed that nanoemulsion of cinnamon bark essential oil could be applied as poultry feed additive.

**Keywords:** Cinnamon, Essential Oil, Nanoemulsion.

### INTRODUCTION

The introduction briefly justifies the research and specifies the hypotheses to be tested. Antimicrobial agents are critically important in the prevention and treatment of diseases in poultry production (Landoni and Albarellos, 2015). Various plant extracts, especially essential oils (EOs), have been studied for their antimicrobial abilities (Griggs and Jacob, 2005; Jayasena and Jo, 2013). One of edible and medicinal plants with high antimicrobial effects is cinnamon. The use of cinnamon would be with regards to its antimicrobial properties, especially antibacterial activity (Nabavi *et al.*, 2015). The antimicrobial and other biological properties of essential oils are correlated to the presence of their bioactive volatile components (Mahmoud and Croteau, 2002). Those volatile components also become limiting factor in essential oils application due to its volatile properties and poor bioavailability.

Nanoemulsions carriers are increasingly being utilized in the food and pharmaceutical industries by encapsulating, protecting, and delivering lipophilic bioactive components (McClements, 2012). Nanoemulsions has also been shown to increase the bioavailability of certain types of lipophilic substances encapsulated within them (Acosta, 2009), which may be useful for increasing the bioactivity. This study was conducted to formulate and to

characterize nanoemulsion of cinnamon (*Cinnamomum burmanii*) bark essential oil (CBO) as poultry feed additive candidate.

## MATERIALS AND METHODS

**Materials.** Cinnamon bark essential oil was obtained from Lansida, Indonesia; virgin coconut oil (VCO) from Mutia VCO, Indonesia; Tween 80, PEG 400 and distilled water from Brataco, Indonesia.

**GC-MS Analysis of Cinnamon Oil.** CBO constituents were qualitatively analyzed using gas chromatography-mass spectrometry (GC-MS QP 2010 S, Shimadzu, Kyoto, Japan). Helium was used as carrier gas at specific injection temperature of 300 °C, pressure of 13 kPa and total flow of 80 ml/min. The samples were injected with certain split ratio of 14.6. Mass spectra were recorded with start time 3 min and end time 60 min. Identification of components of cinnamon bark essential oil was done by comparing the results with MS data bases WILEY229.LIB and NIST12.LIB (Adams, 1995).

**Nanoemulsion Preparation.** The major components that used to formulate nanoemulsions were oil phase, aqueous phase and stabilizers (surfactants/co-surfactants) according to McClements and Rao (2011). Nanoemulsion was initially prepared by adding Tween 80 and PEG 400 into oil phase (VCO). The formulation ratio 1:4:1 1:5:1; 1:6:1 and 1:7:1 (v/v) of oil phase (VCO) were chosen and prepared by Tween 80 and PEG 400 respectively using magnetic stirrer (Cimarec Digital Stirring Hot Plate SP131320-33Q, Thermolyne Thermo Scientific, Waltham, USA) at 100 rpm. The emulsions were then diluted using magnetic stirrer at 400 rpm 3 times with distilled water to produced nanoemulsions. Their transmittance values were evaluated at 650 nm (Patel *et al.*, 2011) by a UV/VIS spectrophotometer (Optima SP-3000 nano, Optima, Tokyo, Japan) using distilled water as a blank. Last step, the concentration of CBO in the formulation was optimized by adding into oil phase (VCO) using magnetic stirrer at 100 rpm in chosen formulation and the transmittance values were evaluated at 650 nm by a UV/VIS spectrophotometer.

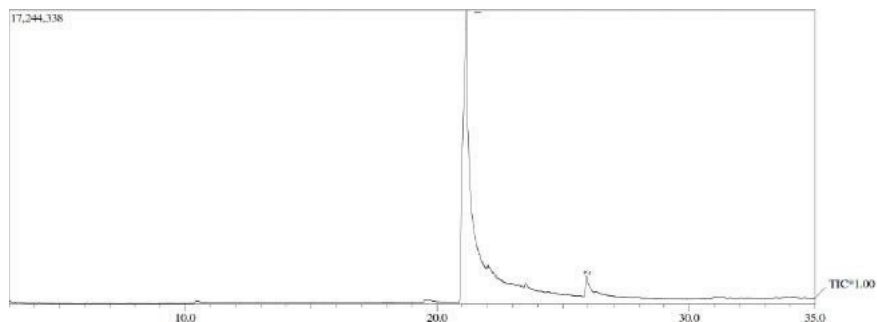
**Droplet Size and Zeta Potential Measurement.** The droplet size of the selected formulations was determined by dynamic light scattering (DLS). The nanoemulsion, 100 µL was diluted to 250 mL in a beaker and gently mixed and then subjected to particle size analyzer (Horiba Scientific SZ-100, Horiba, Kyoto, Japan). For zeta potential determination, The nanoemulsion was diluted with a ratio of 1:2500 (v/v) with distilled water and mixed for 1 min and measured using a particle size analyzer (Jyothi and Sreelakshmi, 2011).

**Morphology of Droplets.** Transmission Electron Microscopy (JEOL JEM 1400 Plus, JEOL, Peabody, USA) was carried out to visualize the shape and morphology of the nanoemulsion. After nanoemulsion dilution with double distilled water (1:50), a sample drop was placed on a carbon coated copper grid and air-dried for 10 min at room temperature. The excess was instantly drawn off with a filter paper. Samples were then negatively stained with 1% (w/v) phosphotungstic solution for 60 s and excess of solution was also removed, before loading in the microscope. The shape and surface characteristics were evaluated subsequently at appropriate magnifications, according to Kassem *et al.* (2016).

## RESULTS AND DISCUSSION

Production for therapeutic purposes, disease prevention and growth promotion, and this may select for drug resistant microorganisms known to spread to humans through consumption of contaminated food (Hawkey, 2008). Therefore, non-antibiotic alternatives

which can control disease and promote growth of poultry are of great interest (Pan and Yu, 2014). Cinnamon has shown potential anti-microbial action against a wide variety of bacteria (Ranasinghe *et al.*, 2013). Volatile compound in as obtained CBO was identified by GC-MS analysis. Figure 1 showed the gas chromatograph of CBO. Cinnamaldehyde (Peak 1) and cinnamyl acetate Peak 2) were found to be the components of the oil with similarity index 97 and 96 respectively by comparing with MS data system. In CBO, cinnamaldehyde was the most represented substance, with a content ranging from 62 to 90% (Nabavi *et al.*, 2015).



**Figure 1.** GC-MS chromatograph of cinnamon bark oil

**Nanoemulsion Preparation.** Limitation of essential oils application as feed additives that majority of essential oils were unstable, volatile, and insoluble in water also limits the possible routes of administration. The neglectable solubility of EOs in biological fluids impedes their absorption leading to a very low bioavailability (Pedro *et al.*, 2013; Natrajan *et al.*, 2015). Novel technologies such as nanoemulsions could offer possible solutions to solve challenges facing the applications of EOs due to the enhanced distribution and solubility of the encapsulated EO (Calo *et al.*, 2015). Nanoemulsion is an isotropic mixture of oil, surfactants and co-surfactants that form fine oil-in-water nanoemulsion, upon mild agitation, followed by administration into aqueous media, such as GI fluid (Wang *et al.*, 2009). In this research, CBO and VCO as oil phase, Tween 80 as surfactant and PEG 400 as co-surfactants were used to produced nanoemulsion. As seen in Table 1, four formulations were prepared and evaluated the transmittance values to produced good nanoemulsion.

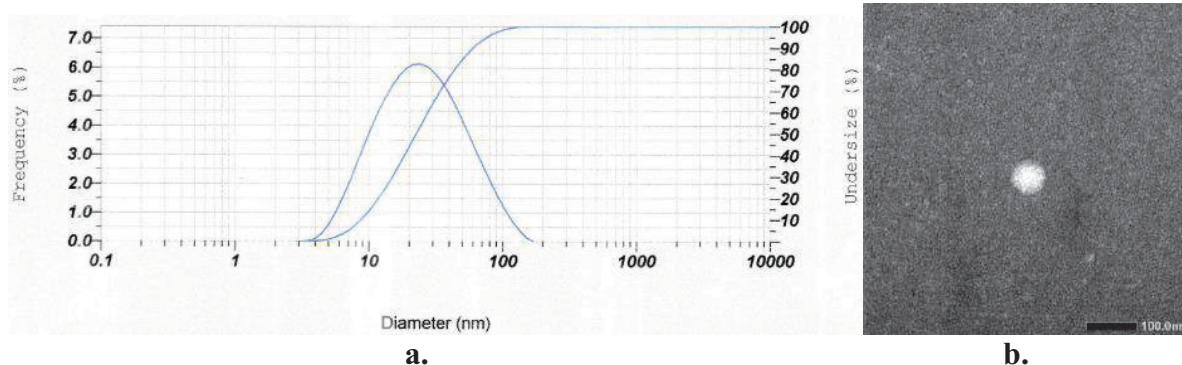
**Table 1.** The optimization of nanoemulsion and the transmittance values after diluted 3 times in distilled water

Formulation code	Oil:surfactant:co-surfactant ratio (v/v)	Transmittance (%) ± SD	Formulation code	CBO:VCO:surfactant:co-surfactant ratio (v/v)	Transmittance (%) ± SD
F1	1:4:1	97.43 ± 0.40	F1.1	0.25:0.75:4:1	96.90 ± 0.80
F2	1:5:1	98.97 ± 0.05	F1.2	0.5:0.5:4:1	98.60 ± 0.10
F3	1:6:1	99.80 ± 0.22	F1.3	0.75:0.25:4:1	98.75 ± 0.25
F4	1:7:1	100.03 ± 0.31			

Nanoemulsion with percentage transmittance closer to 100% gives an indication of globule size in nanometer range (Yadav *et al.*, 2014). The F1 was chosen due to had lowest concentration of surfactant. The concentration of CBO was optimized by adding into chosen formulation to produced good CBO nanoemulsion (Table 1). The final formulation of CBO nanoemulsion was 0.75:0.25:4:1 (v/v) consisting of CBO, VCO, Tween 80, PEG 400 respectively.

**Droplet Size and Zeta Potential Measurement.** The particle size and polydispersity index value of the chosen nanoemulsion formulation were 20.2 nm and 0.463 respectively

(Figure 2.a.). Most common size was stated vary from 20 up to 500 nm (Solans and Solé, 2012). Smaller particle size of the nanoemulsion droplets could enhanced absorption rate and improved bioavailability (Balakumar *et al.*, 2013). Polydispersity index was less than 0.5, indicating uniform globule size distribution (Kassem *et al.*, 2016). The measurement showed that zeta potential value was  $-0.8$  mV. The zeta potential is often a better representation of the electrical characteristics of a droplet than the surface potential because it inherently accounts for the adsorption of any charged counter-ions (McClements and Rao, 2011).



**Figure 2. a.** The particle size and polydispersity index value of the CBO nanoemulsion. **b.** The morphology of the CBO nanoemulsion

**Morphology of Droplets.** The morphology of the CBO nanoemulsion formulation was observed using TEM (**Figure 2.b.**). It reveals spherical oil globules, with no signs of coalescence, less than 100 nm in size, which was in accordance to the findings using particle size analyzer. The droplets morphology appeared distinct and non-aggregated with no signs of precipitation inferring the physical stability of formed nanoemulsions. TEM images reveals that globule was surrounded by a layer indicating the formation of monolayer around the droplets, reducing the interfacial energy, and forming a barrier to coalescence (Villar *et al.*, 2012).

## CONCLUSIONS

It can be concluded that cinnamon bark essential oil could be used as poultry feed additive, especially for the potential of anti-microbial action. Unfortunately, essential oils were unstable, volatile, and insoluble in water also limits the possible routes of administration. Nanoemulsion was a promising strategy to facilitate the applicability of cinnamon bark essential oil as poultry feed additive. The result of current research showed that the formulation had mean droplet diameters of 20.2 nm with polydispersity index 0.463 and spherical oil globules inferred the physical stability. Further research was needed before the cinnamon bark essential oil nanoemulsion could be applied as poultry feed additive.

## REFERENCES

- Acosta, E. 2009. Bioavailability of nanoparticles in nutrient and nutraceutical delivery. *Curr. Opin. Colloid Interface Sci.* 14: 3–15.
- Adams, R. P. 1995. Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy. Allured Publishing Corporation. Carol Stream, Illinois.

- Balakumar, K., C. V. Raghavan, N. T. Selvan, R. H. Prasad, and S. Abdu. 2013. Self-nanoemulsifying drug delivery system (SNEDDS) of Rosuvastatin calcium: Design, formulation, bioavailability and pharmacokinetic evaluation. *Colloids Surf., B.* 112: 337–343.
- Calo, J. R., P. G. Crandall, C. A. O'Bryan, and S. C. Ricke. 2015. Essential oils as antimicrobials in food systems - A review. *Food Control.* 54: 111-119.
- Griggs, J. P. and J. P. Jacob. 2005. Alternatives to antibiotics for organic poultry production. *J. Appl. Poult. Res.* 14: 750-775.
- Hawkey, P. M. 2008. The growing burden of antimicrobial resistance. *J. Antimicrob. Chemother.* 62: i1–i9.
- Jayasena, D. D. and C. Jo. 2013. Essential oils as potential antimicrobial agents in meat and meat products: a review. *Trends Food Sci. Technol.* 34: 96-108.
- Jyothi, J. B. and K. Sreelakshmi. 2011. Design and evaluation of self-nanoemulsifying drug delivery system of flutamide. *J. Young Pharm.* 3: 4-8.
- Kassem, A. A., A. M. Mohsen, R. S. Ahmed, and T. M. Essam. 2016. Self-nanoemulsifying drug delivery system (SNEDDS) with enhanced solubilization of nystatin for treatment of oral candidiasis: design, optimization, in vitro and in vivo evaluation. *J. Mol. Liq.* 218: 219–232.
- Landoni, M. F. and G. Albarellos. 2015. The use of antimicrobial agents in broiler chickens. *Vet. J.* 205: 21–27.
- Mahmoud, S. S. and R. B. Croteau. 2002. Strategies for transgenic manipulation of monoterpene biosynthesis in plants. *Trends Plant. Sci.* 7: 366-373.
- McClements, D. J. 2012. Nanoemulsions versus microemulsions: terminology, differences, and similarities. *Soft Matter.* 8: 1719–1729.
- McClements, D. J. and J. Rao. 2011. Food-grade nanoemulsions: formulation, fabrication, properties, performance, biological fate, and potential toxicity. *Crit. Rev. Food Sci. Nutr.* 51: 285 – 330.
- Nabavi, S. F., A. Di Lorenzo, M. Izadi, E. Sobarzo-Sánchez, M. Daglia, and S. M. Nabavi. 2015. Antibacterial effects of cinnamon: from farm to food, cosmetic and pharmaceutical industries. *Nutrients.* 7: 7729-7748.
- Natrajan, D., S. Srinivasan, K. Sundar, and A. Ravindran. 2015. Formulation of essential oil-loaded chitosan-alginate nanocapsules. *J. Food. Drug. Anal.* 23: 560-568.
- Pan, D. and Z. Yu. 2014. Intestinal microbiome of poultry and its interaction with host and diet. *Gut Microbes.* 5: 108–119.
- Patel, J., A. Patel, M. Raval, and N. Sheth. 2011. Formulation and development of a self-nanoemulsifying drug delivery system of irbesartan. *J. Adv. Pharm. Technol. Res.* 2: 9-16.
- Pedro, A. S, I. E. Santo, C. V. Silva, C. Detoni and E. Albuquerque. 2013. The use of nanotechnology as an approach for essential oil-based formulations with antimicrobial activity. *Formatex.* 1364-1374.

- Ranasinghe, P., S. Pigera, G. A. S. Premakumara, P. Galappaththy, G. R. Constantine, and P. Katulanda. 2013. Medicinal properties of 'true' cinnamon (*Cinnamomum zeylanicum*): a systematic review. *BMC Complement Altern. Med.* 13: 275-285.
- Solans, C. and I. Solé. 2012. Nano-emulsions: formation by low-energy methods. *Curr. Opin. Colloid Interface Sci.* 17: 246–254.
- Villar, A. M. S., B. C. Naveros, A. C. C. Campmany, M. A. Trenchs, C. B. Rocabert, and L. H. Bellowa. 2012. Design and optimization of self-nanoemulsifying drug delivery systems (SNEDDS) for enhanced dissolution of gemfibrozil. *Int. J. Pharm.* 431: 161–175.
- Wang, L., J. Dong, J. Chen, J. Eastoe, and X. Li. 2009. Design and optimization of a new self-nanoemulsifying drug delivery system. *J. Colloid Interface Sci.* 330: 443–448.
- Yadav, P. S., E. Yadav, A. Verma, and S. Amin 2014. Development, characterization, and pharmacodynamic evaluation of hydrochlorothiazide loaded self-nanoemulsifying drug delivery systems. *Scientific World J.* 2014: 1-10.