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Abstract

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Characteristics and Stability of Nanostructured Lipid Carrier (NLC) Aleurites Moluccana Seed Oil (AMS oil) Using Various Combinations of Beeswax and Oleum Cacao

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ABSTRACT

This study aims to determine the effect of the combination of beeswax and oleum cacao on the characteristics and stability of nanostructured lipid carrier-aleurites moluccana seed oil (NLC-AMs oil). The combination ratio of beeswax-oleum cacao and AMs oil is 3:1 which total lipid 20%, while the ratio of beeswax-oleum cacao used were F1(100:0); F2 (50:50); F3 (25:75); and F4 (0:100). These preparations are made by the high shear homogenization method because the processing technique is relatively easier, faster and it is possible to get nanoparticle size. Then characterization and physical stability test (real time, thermal cycling test, and centrifugation) were carried out. The results of this study can be concluded that: 1) NLC-AMs oil with combinations of beeswax and oleum cacao has smaller particle size than those using only single solid lipids (oleum cacao or beeswax). 2) Increased concentration of oleum cacao in the NLC-AMs oil system increases its viscosity. 3) The ratio of beeswax and oleum cacao affects the recrystallization index of the NLC-AMs oil. The lowest recrystallization index is in the NLC-AMs oil with a combination of beeswax-oleum cacao in F2 (50:50). 4) The results of the real time stability test for 8 weeks of storage revealed the NLC-AMs oil system with a combination of solid fat beeswax and oleum cacao on F3 (25:75) had the best stability. 5) All formulas are not stable against extreme temperature changes (thermal cycling test) and shocks (centrifugation) indicated by system separation.

Keyword: Aleurites moluccana seed oil (AMs oil), Beeswax, Nanostructured lipid carrier (NLC), Oleum cacao.

INTRODUCTION

Aleurites Moluccana seed oil (AMs oil) is known can increase male rabbit hair growth as long as 11.26 mm in 18 days observation¹. But AMs oil have some weaknesses that is easily evaporated when applied because it contains essential oils and is easily oxidized in storage because it contains a lot of unsaturated fatty acids indicated by a high iodine number of 136-167². To overcome the shortage of AMs oil, which prevents evaporation and oxidation, a system that is able to maintain the stability is needed, namely using a nanostructured lipid carrier (NLC) system consisting of a mixture of solid lipid, liquid lipid and stabilized with surfactants or several surfactant mixtures. In this study 5% of AMs oil was formulated in the NLC-AMs oil system with a solid lipid combination of beeswax-oleum cacao and AMs oil in a ratio of 3:1 and a total lipid of 20%. Whereas the ratios of beeswax and oleum cacao are 100: 0; 50:50; 75:25 and 0: 100.

RESEARCH METHODS

Research Material

The materials used in this study if not stated other have the purity degree of Pharmaceutical Grade. The materials used were beeswax (PT Kurniajaya Multisentosa), oleum cacao

(Research Center for Coffee and Cocoa, Jember-Indonesia), Aleurites moluccana seed oil (Natures), Span 80 (Sigma Aldrich), Tween 80 (Sigma Aldrich), Propylenglycol (PT Kurniajaya Multisentosa), Sodium benzoate (PT Brataco), Sodium acetate and Glacial Acetic acid pro analysis (E.Merck),
NLC-AMs oil Preparation

Table 1: Concentration of material in formula NLC-AMs oil.

Material	Concentration of material in formula (%)			
	F1	F2	F3	F4
AMs oil	5	5	5	5
Oleum cacao	–	7.5	11.25	15
Beeswax	15	7.5	3.75	–
Span 80	3.98	5.73	6.60	7.48
Tween 80	6.02	4.27	3.40	2.52
Propilenglicol	15	15	15	15
Na Benzoate	0.1	0.1	0.1	0.1
Acetic buffer pH	ad	ad	ad 100	ad
5 ± 0.2	100	100		100

Table 2: Characteristic of NLC-AMs oil.

Formula (Beeswax : oleum cacao)	pH value	Viscosity (cps)	Particle Size (nm)	% RI
F1 (100 : 0)	4.88 ± 0.06	81.40 ± 6.12	694.77 ± 5.23	0.34
F2 (50 : 50)	5.34 ± 0.04	2702.33 ± 42.00	219.60 ± 16.81	0.27
F3 (25 : 75)	5.63 ± 0.02	14275.67 ± 982.37	280.13 ± 5.06	36.23
F4 (0 : 100)	5.92 ± 0.02	19996.67 ± 886.36	337.37 ± 15.53	-

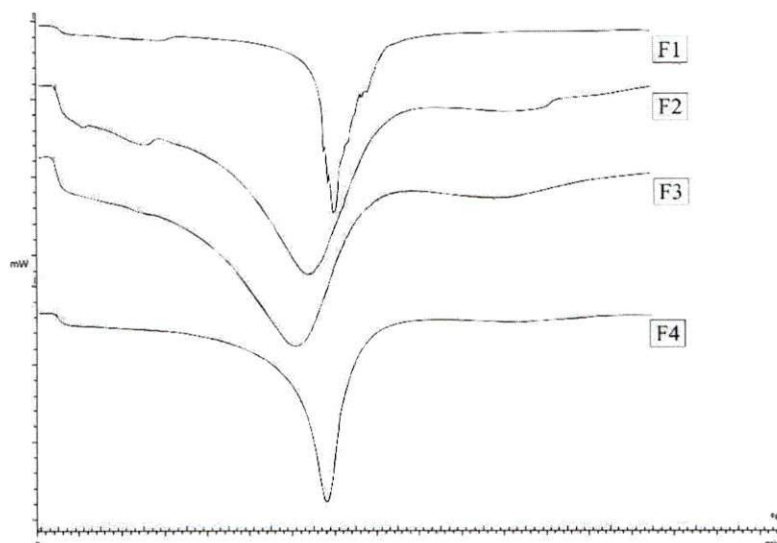


Figure 1: Thermogram Differential Scanning Calorimetric (DSC) of NLC-AMs oil system.

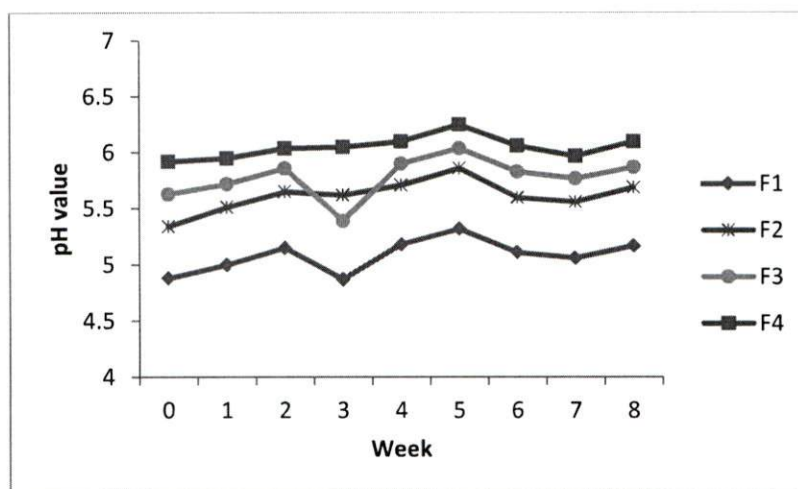


Figure 2: The pH value of NLC-AMs oil at week 0, 1, 2, 3, 4, 5, 6, 7, and 8.

The NLC-AMs oil formula consists of various comparisons of beeswax and oleum cacao, namely: F1 (100: 0), F2 (50:50), F3 (25:75) and F4 (0: 100) in table 1, made by the High Shear Homogenization method.³

NLC-AMs oil physical stability test includes

- 1) real time stability test at room temperature ($25 \pm 2^\circ\text{C}$) for 8 weeks,
- 2) thermal cycling test, sample was stored at 40°C for 48 hours then at $2-8^\circ\text{C}$ for 48 hours, for 3 cycles,
- and 3) centrifugation test at 3500 rpm for 5 hours

Characterization of NLC-AMs oil includes

- 1) pH value was evaluated by pH meter Schott glass mainz type CG842,
- 2) viscosity was evaluated by viscosimeter Brookfield Cone and Plate HADV-1 + CP,
- 3) particle size

was evaluated by Particle Analyzer DeltaTM Nano Submicron Particle Size, and 4) recrystallization index value (%RI).

RESULT AND DISCUSSION

The result of NLC-AMs oil characterization

the characteristic of NLC-AMs oil included pH value, viscosity, particle size and %RI was shown in table 2 and figure 1

From table 2 it was known that the pH values of all formulas of the NLC-AMs oil are in the range of skin pH at 4.9-6.3. The results of statistical analysis of viscosity value with one-way ANOVA with 95% confidence degree,

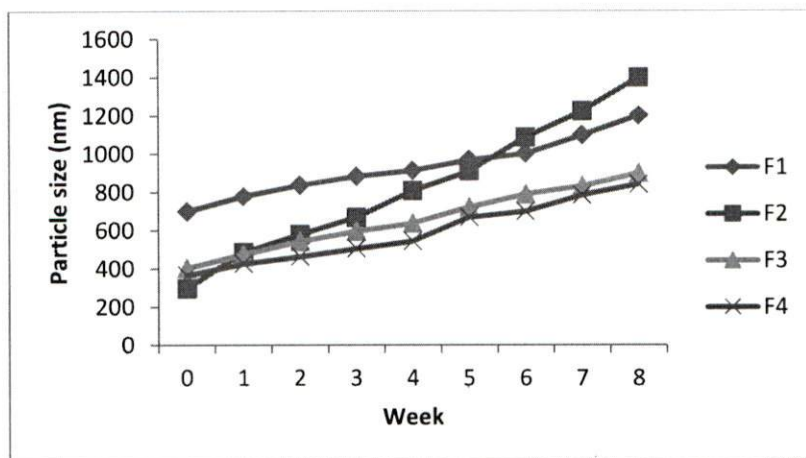


Figure 3: The particle size of NLC-AMs oil at week 0, 1, 2, 3, 4, 5, 6, 7, and 8.

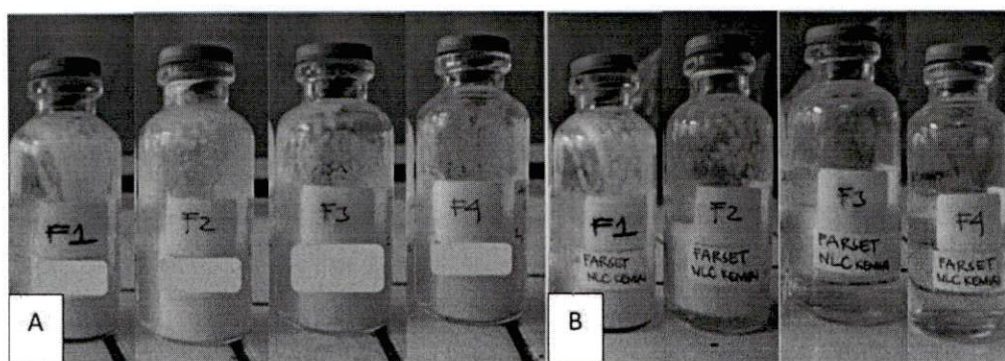


Figure 4: Photo of NLC-AMs oil before (A) and after 3 cycle of thermal cycling test (B).

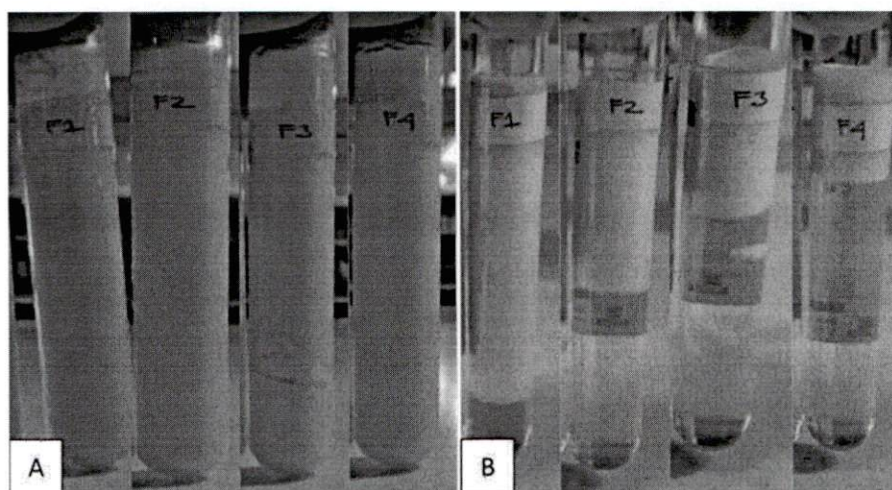


Figure 5: Photo of NLC-AMs oil before (A) and after centrifugation test 3500 rpm, 5 h (B).

and the Tukey's HSD test obtained a significant figure value of $0.000 < 0.05$. So it can be concluded that the viscosity of $F1 < F2 < F3 < F4$, the higher concentration of oleum cacao produce higher viscosity of the NLC-AMs oil. The particle size of $F2 < F3 < F4 < F1$, a decrease of particle size in F2 and F3 which is containing combination of solid lipids can be affected by the viscosity differences of solid lipid while melting in the NLC manufacturing process. The formula with ratio of beeswax-oleum cacao 50:50 has the lowest recrystallization index value (%RI)

compared to formula consisting only a single lipid beeswax. The decrease in the recrystallization index indicates less ordered matrix which causes the entrapment ability of active ingredients increased⁴. There was an increase of recrystallization index at the formula with ratio of beeswax-oleum cacao 25:75 indicates an increasingly regular crystal lattice change. This crystal lattice change is thought to be due to the formation of new crystals or the presence of chemical interactions between the lipid structures formed in the system.

Based on the DSC profile in figure 1, pure beeswax has an enthalpy value greater than the pure oleum cacao. This indicates that the beeswax crystal lattice is more ordered compared to the oleum cacao. The melting enthalpy value is the energy needed in the endothermic melting process, a melting point examination is carried out to ensure that the NLC system is formed in the presence of a constituent endothermic lipid. The melting point of beeswax in each formula undergoes a shift indicating the system has been formed. The thermogram (figure 1) shows the presence of new endothermic peaks on each formula which indicates changes in polymorphism. The surfactant modulates the polymorph transition during the compaction process, to maintain the dispersion stability of the preparation so that the formed crystals are more stable. However, with the high number of surfactants causing surfactants adsorbed on the surface of the particles so that the possibility of new endothermic phases appears as a result of the process⁶. In this study a mixture of tween 80 and span 80 was used as the highest amount of surfactant, which was 15%⁵. So that the emergence of new endothermic peaks was suspected due to the high amount of surfactant. One factor that causes changes in polymorphism is the high number of surfactant use⁶.

The result of NLC-AMs oil organoleptic observation (visually) at week 0 to 8

known in formula 1, formula 3 and formula 4 there was no separation, whereas formula 2 in four weeks storage separated into two phase. The smell of all formulas does not change during storage. The consistency of formula 2 decreased after separation at week four, while the other formulas the consistency did not change.

The result of NLC-AMs oil pH value measurement at week 0 to 8

in the figure 2 there was decreased in the pH of formula 1 (F1) and formula 3 (F3) at the third week but still within the pH range of the skin.

The result of NLC-AMs oil particle size measurement at week 0 to 8

there was an increase in NLC-AMs oil particle size for 8 weeks of storage, as seen in figure 3. Based on the results of the one-way ANOVA statistical test, the p value (sig.) $0,000 < 0,05$ means that there are significant differences in particle size of the NLC system during storage. Then followed by Post Hoc Tukey HSD to find out which groups had significant differences. The result known particle size of formula 2 (50:50) increased significantly at week 0 to 1, week 5 to 6, and week 7 to 8, while in formulas 1, 3, and 4 did not increase significantly. The largest particle size after 8 weeks of storage is formula 2. Even though the 50:50 ratio of beeswax and oleum cacao solid lipid combination has the smallest particle size at week 0 compared to the other three formulas. It is suspected that in this combination an eutectic point is formed. The difference in particle size at the week 0 and 8 is large and the significant increase from week to week in formula 2 shows that the preparation is not stable. The instability of particle size in these formula can cause agglomeration and result in phase separation. This is supported by

organoleptic observations at the fourth week of storage formula 2 was separated.

The result of thermal cycling test and centrifugation test

The thermal cycling test and centrifugation stability test in figure 4 and 5 results for all formulas were unstable due to separation, so formula improvement was necessary.

CONCLUSION

1) NLC-AMs oil with combinations of beeswax and oleum cacao has smaller particle size than those using only single solid lipids (oleum cacao or beeswax). 2) Increased concentration of oleum cacao in the NLC-AMs oil system increases its viscosity. 3) The ratio of beeswax and oleum cacao affects the recrystallization index of the NLC-AMs oil. The lowest recrystallization index is in the NLC-AMs oil with a combination of beeswax-oleum cacao in F2 (50:50). 4) The results of the real time stability test for 8 weeks of storage revealed the NLC-AMs oil system with a combination of solid fat beeswax and oleum cacao on F3 (25:75) had the best stability. 5) All formulas are not stable against extreme temperature changes (thermal cycling test) and shocks (centrifugation) indicated by system separation.

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CONFLICT OF INTEREST

No conflict of interest is associated with this work

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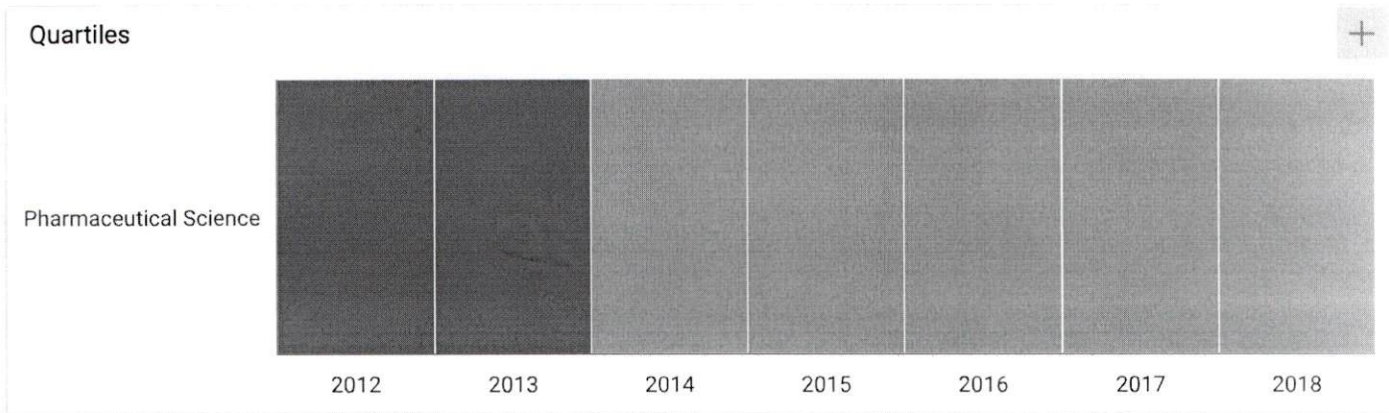


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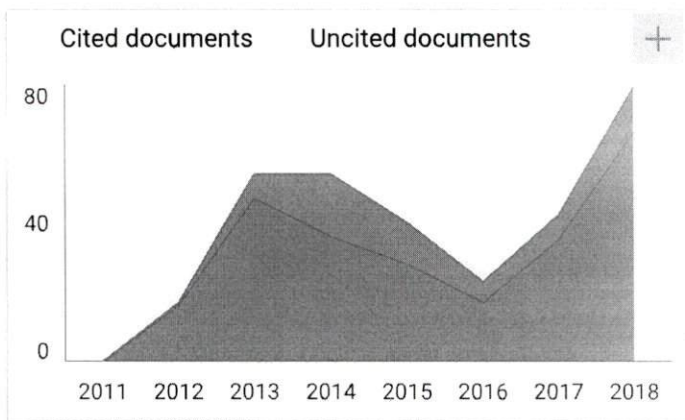
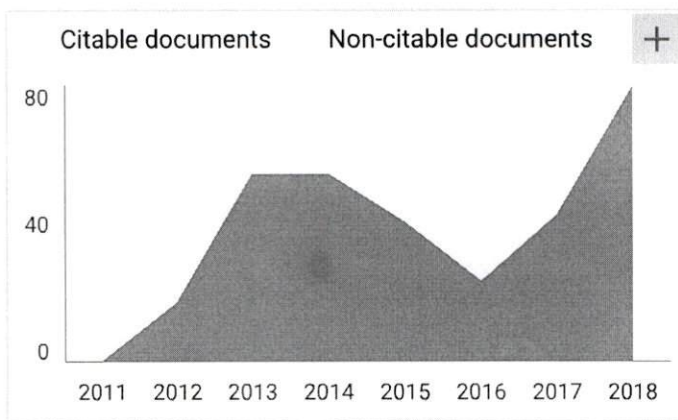
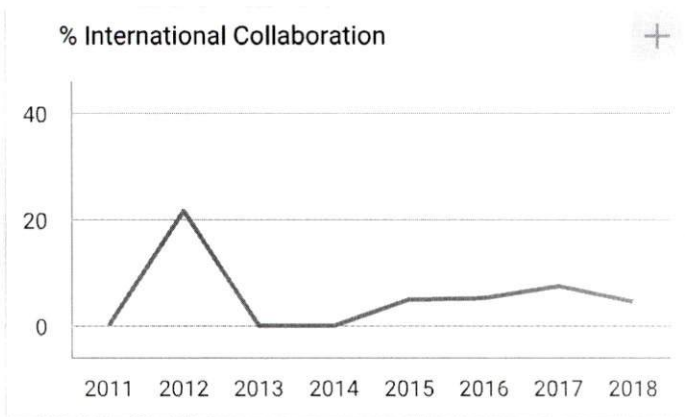
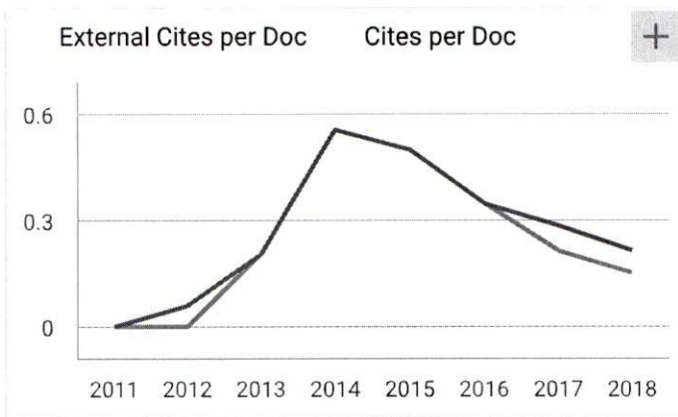
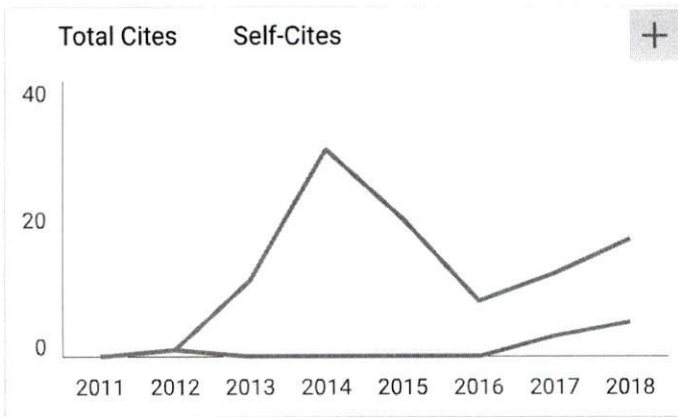
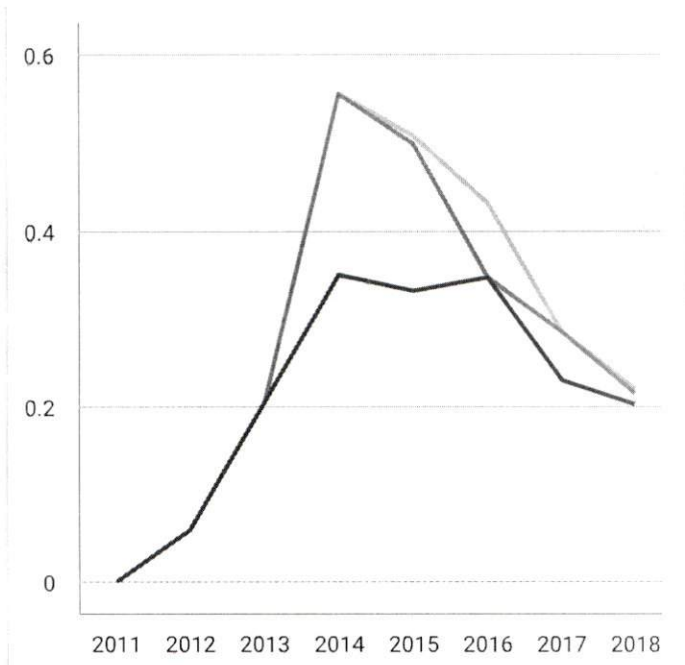
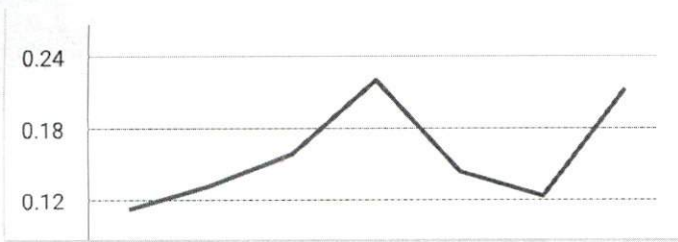
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