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Research Article

ISOLATION AND CHARACTERIZATION TRITERPENOID COMPOUND FROM LEAVES MANGROVE PLANT (*Sonneratia Alba*) AND ANTIBACTERIAL ACTIVITY TEST

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ABSTRACT

Mangrove plant *(Sonneratia alba)* is easily found in Indonesia and has the potential of being a herb medicine. General phytochemical screening revealed the presence of flavonoid, steroid, triterpenoid, and tannin compounds. Mangrove plant variously used in ethnomedicine to treat wounds, diarrhea, and fever disease. Lup-20(29)-en-3β-ol (lupeol) compound is pentacyclic triterpenoid group. Lupeol was isolated from the methanol extract of the leaves of mangrove (*S. alba*). Extraction was done by maceration method using methanol 96% as solvent. Its isolation was carried out by a combination of column chromatography and combination of n-hexana, ethyl acetate, and methanol solvent. The structure was determined by analysis of IR, ^{1}H-NMR, ^{13}C-NMR, 2D NMR and MS spectroscopies data, as well as comparison with various reference. The result of antibacterial activity test showed that isolated compound effectively inhibited the growth of these bacterial pathogens with inhibition zone 18 mm for *Staphyloccocus aureus,* 14 mm for *Pseudomonas aeruginosa*, and 13 mm for *Escherichia coli.* This is the first report of isolation lupeol compound from the leaves of *Sonneratia alba* of this species and antibacterial activity test against *Staphyloccocus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* pathogen bacterial.

Keywords: *Sonneratia alba,* antibacterial, triterpenoid, and mangrove.

INTRODUCTION

Mangroves are a group of plants high or shrubs that grow in coastal areas tropical and subtropical. This plant has a distinctive morphological features and can survive in environments with high salinity[1,2]. Mangroves grow in coastal areas and have a unique adaptation to cope with environmental stresses such as high salinity, high temperature and strong sunlight radiation, as well as the abundance of microorganisms and insects[3]. Some mangroves have been used as herbs and extracts have biological activity in humans, animals and harmful bacteria but a study of the womb secondary metabolites responsible the biological activity is still limited[4-6].

Sonneratia alba is one of mangrove plants in the family of *lythraceae*. *Sonneratia alba* widely known in Indonesia with the name coastal Pidara white and widely distributed in the coastal regions of Southeast Asia and the Indian Ocean[7]. This plant has been used traditionally in coastal communities of Indonesia to the treatment of wounds, diarrhea, and fever[8]. In previous study phytochemical investigation *Sonnetaria* has been reported contained triterpenoid, steroid, and flavonoid compounds.

Infectious disease and parasites are one of the major disease in the world. According to World Health Organization (WHO) data in 2011, infectious diseases and parasites are the third leading cause of death in the world[9]. In Indonesia, infection deseases is one of the major caused of death especially in north Indonesia and still a health problem in all levels of society from low to high socioeconomic levels. Infectious disease of the skin tissue that commonly affects the public caused by various microbes. Bacteria cause the most common skin disease and infection is *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Escherichia coli*[10,11]. Based on reports of various studies have not revealed the active compounds antibacterial, diarrhea and in skin disease derived from the leaves of plants *Sonneratia alba.* Therefore in this study, isolation of antibacterial compounds from plant leaf parts and antibacterial activity test against bacterial pathogen causing skin diseases are needed.

MATERIAL AND METHODS

Material

The research specimen is *S. alba* collected from Dulupi village, Boalemo district, Gorontalo province, Indonesia in july 2016. The chemicals used in this research were ethyl acetate, *n*-hexane, methanol, distilled water, silica gel G60 (70-320 mesh), thin layer chromatography (TLC), silica plate, octadecylsilane (ODS) RP-18, 10% H_2SO_4 in ethanol, alcohol 70%, ciprofloxacin 100 ppm, amoxylin 100 ppm, bacto agar, and Mueller-Hinton agar.

Instrumentation

Spectrum measurements were performed using a variety of spectroscopy tools. Infrared (IR) spectra were measured with Shimadzu FTIR, ^{1}H and ^{13}C-NMR spectra were measured using JEOL JNM A-500 which works at 500 MHz (for ^{1}H-NMR spectrum) and at 125 MHz (for ^{13}C-NMR spectrum) with TMS as an internal standard, ES-MS spectrometry (UPLC MS/MS TQD type Waters) and laminar air flow.

Extraction and Purification

Dried leaves of *S. alba* (240 g) was extracted successively with methanol 96% (3 × 24 hours), followed by filtration. The filtrates

were combined and evaporated by rotary evaporator at a temperature of 45°C using a buchi rotary evaporator to give a residu. Concentrate of methanol extract obtained as much 13 g of a gummy concentrate of the crude extract.

The methanol extract (10 g) was subjected to liquid chromatography over silica gel using a gradient elution mixture of n-hexane-EtOAc (10:0-0:10) as an eluting solvent, yielding 7 fractions (A–G). Fraction C (0.15 g) was subjected to column chromatography over silica gel using a mixture of n-hexane :EtOAc (9:1) as an eluting solvent, affording 30 fractions (E01–E30) and give pure isolated. The purification results of these compounds were determined by TLC on silica gel and ODS with several solvent systems and showed a single spot.

Chromatographic Separation

The column was packed with fine TLC grade siliga gel G60 was used as the packing material. A column having 50 cm leght and 5 cm in diameter was packed with the silica gel G60 under reduced pressure. The column was washed with methanol and then with n-hexane to facilitate compact packing. The methanol extract was subjected to column chromatography. The column was then eluted using n-hexane (150 mL) followed by mixture of *n*-hexane-ethyla cetate (10:0-0:10). A total of 10 fractions (A-J) were collected each in 250 mL beakers.

The fraction C (0.15 g) was subjected to column chromatography over silica gel (Kieselgel G60, mesh 70-230) using a mixture of n-hexane :Ethyl acetate (9:1) as an eluting solvent, affording 30 fractions (C01–C30). Fraction C19 was found to yield crystal on the wall of the beakers. The crystals were washed with n-hexane carefully. As a result mother solution was obtained leaving back the needle shape crystals which were isolate as compound. The purification results of compound were determined by TLC on silica gel and ODS with several solvent systems and showed a single spot (>95% pure).

Test for Triterpenoid with Liebermann-Burchard Reaction

A few crystals of compound 1 and 2 were dissolve in chloroform and a few drops of concentrated sulfuric acid were added to it followed by the addition of 2-3 drops of anhydride acetid. In this case isolated compound turned to violet blue and finally formed green color which indicates the presence of triterpenoid[10].

Characterization compound

Different spectroscopic methods were used to elucidate the structure of isolated compound. Among the spectroscopic techniques IR, ^{1}H and ^{13}C-NMR, HMQC, HMBC and H-H COSY were carried out. The infrared spectrum was recorded on Shimadzu affinity-1, ^{1}H and ^{13}C-NMR spectra were recorded using $CDCl_3$ as solvent on JEOUL NMR 500 MHz spectrometer.

Isolated compound : white needles. IR (KBr) U_{max} /cm^{-1}: 3590, 2935, 1687, 1462, 1385, 1236, and 897. ^{1}HNMR (500 MHz, $CDCl_3$) δ: 2.22 (2H, m, H1), 1.65 (2H, m, H2), 3.15 (1H, dd, J 15.0, 8.4 Hz, H3), 0.70 (1H, d, H5), 1.42 (2H, m, H6), 1.44 (2H, m, H7), 1.07 (1H, H9), 140 (2H, m, H11), 1.41 (2H, m, H12), 0.75 (1H, s, H13), 1.20 (2H, m, H15), 1.39 (2H, m, H16), 0.96 (1H, d, H18), 2.23 (1H, d, H19), 2.25 (2H, m, H21), 2.22 (2H, m, H22), 0.94 (3H, s, H23), 0.96 (3H, s, H24), 0.85 (3H, s, H25), 0.75 (3H, s, H26), 1.00 (3H, s, H27), 1.59 (3H, s, H28), 4.58 & 4.60 (2H, s, H29), 1.69 (3H, s, H30). ^{13}CNMR (125 MHz, $CDCl_3$) δ: 39.7 (CH_2, C1), 28.1 (CH_2, C2), 79.7 (CH, C3), 40.1 (C_q, C4), (CH, C5), 19.6 (CH_2, C6), 35.7 (CH_2, C7), 43.3 (C_q, C8), 56.9 (CH, C9), 38.4 (Cq, C-10), 26.9 (CH_2, C11), 28.8 (CH_2, C12), 40.2 (CH, C13), 48.6 (C_q, C14), 30.9 (CH_2, C15), 38.3 (CH_2, C16), 49.2 (C_q, C17), 52.1 (CH, C18), 50.5 (CH, C19), 152.2 (C_q, C20), 35.5 (CH_2, C21), 42.2 (CH_2, C22), 31.8 (CH_3, C23), 16.2 (CH_3, C24), 16.9 (CH_3, C25), 16.7 (CH_3, C26), 15.2 (CH_3, C27), 19.5 (CH_3, C28), 110.2 (CH_2, C29), 22.2 (CH_3, C30).

Antibacterial Activity Test

The antibacterial activity test was conducted using the Kirby-Bauer method, where in the bacterial growth inhibition zone was used as a parameter to determine the antibacterial activity. Bacteria that have grown on solid media were given a test compound solution on a paper disk with concentration: 100 µg/ml. Ciprofloxacin was used as a positive control at a concentration of 100 µg/ml for *P. aeruginosa* and amoxylin 100 µg/ml for *E. coli* and *S. aureus* in the solvent water, and methanol/water are used as negative controls (3:1). After the incubation for 24 hours at a temperature of 35-37 °C in aerobic and anaerobic, clear zone around the paper disk which has been given a test solution (test compound, positive control and negative control), was observed and measured using calipers). This clear zone indicates the bacterial growth inhibition zone produced by the test compound[11,12].

RESULT AND DISCUSSION

The leaves of *S. alba* was dried and successively extracted with methanol 96%. Therefore, the subsequent phytochemical analysis was focused on the methanol extract, which was chromatographed over a coloumn packed with silica gel G60 with gradient elution. The fractions were repeatedly subjected to normal-phase and reverse-phase column chromatography, yielding one triterpenoid pentacyclic (Figure 1). The compound (20 mg), appeared as white needles.

Spectral data

The IR spectrum (KBr) of isolated showed characteristic absorption frequencies at 3590 and 1236cm^{-1} typical of the O-H stretching and C-O bond vibrations respectively; The C-C vibrations was at 1687 cm^{-1}. The absorption observed at 897cm^{-1} was due to an unsaturated out of plane C-H vibration; stretching and bending vibrations due to methyl groups were represented by the bands at 2935cm^{-1} and 1462cm^{-1} and the signal at 1385cm^{-1} was due to methylenic vibration (cycloalkane) [13,14].

The ^{1}H-NMR spectrum of compound showed the presence of seven singlet methyl protons at δ 0.75, 0.85, 0.94, 0.96, 1.00, 1.59 and 1.69 ppm. Isolated compound also showed protons at δ 2.23 ppm ascribable to 19β -H is indicated of lupeol. The H-3 proton showed a multiplet at δ 3.15 ppm while a pair of broad singlets at δ 4.58 and δ 4.60 (1H, each) was indicative of olefinic protons at (H-29). The methylene proton Sp^3 showed at δ_H 1.20, 1.39, 1.40, 1.41, 1.42, 1.44, 1.65, 2.22, and 2.25 ppm. These assignments are in good agreement belonging the structure of lupeol[14-16].

The ^{13}C-NMR spectrum showed seven methyl groups at δ: 31.8 (C-23), 19.5 (C-28), 16.8 (C-25), 16.7 (C-26), 16.2 (C-24), 15.2 (C-27) and 22.2 (C-30); the signals due to an exomethylene group at δ: 110.2 (C-29) and 152.0 (C- 20). The DEPT 135º indicated and belonging to ten methylene, five methine and five quaternary carbons were assigned with the aid of DEPT 135º spectrum[15,16]. The deshielded signal at δ 79.0 was due to C-3 with a hydroxyl group attached to it. The confirmation of the structure of isolated was accomplished through the 2D-NMR experiments (COSY and HMBC).

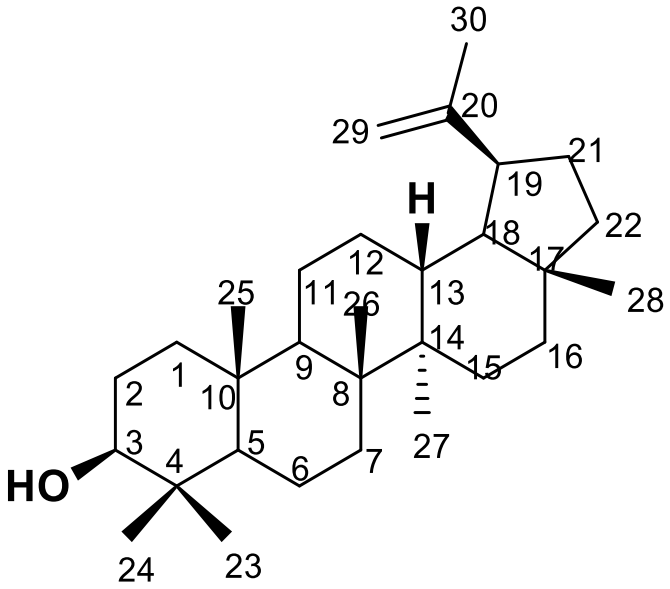

Figure 1. Structure of isolated compound: Lup-20(29)-en-3*β*-ol)

Table 1. NMR data (500 MHz for ^{1}H and 125 MHz for ^{13}C, in CDCl3) for isolated compound and Compared with references

Position C	^{13}C-NMR δ_C (ppm)	DEPT 135°	^{1}H -NMR δ_H (Int., mult)	^{13}C-NMR δ_C (ppm) ref.	^{1}H -NMR δ_H (Int., mult) ref.
1	39.7	CH_2	2.22 (2H, *m*)	38.0	2.37 (2H, *m*)
2	28.1	CH_2	1.65 (2H, *m*)	25.3	1.65 (2H, *m*)
3	79.7	CH	3.15 (1H, *dd*)	78.4	3.20 (1H, *dd)*
4	40.1	Cq	-	38.6	-
5	57.7	CH	0.70 (1H, *d*)	55.1	0.69 (1H, *d*)
6	19.6	CH_2	1.42 (2H, *m*)	18.1	1.42 (2H, *m*)
7	35.7	CH_2	1.44 (2H, *m*)	34.1	1.43 (2H, *m*)
8	43.7	Cq	-	41.2	-
9	56.9	CH	1.07 (1H, *d*)	49.7	1.06 (1H, *d*)
10	38.4	Cq	-	37.3	-
11	26.9	CH_2	1.40 (2H, *m*)	21.1	1.40 (2H, *m*)
12	28.7	CH_2	1.41 (2H, *m*)	27.5	1.41 (2H, *m*)
13	40.2	CH	0.75 (1H, *s*)	39.2	0.76 (1H, *s*)
14	48.6	Cq	-	42.6	-
15	30.9	CH_2	1.20 (2H, *m*)	27.6	1.22 (2H, *m*)
16	38.3	CH_2	1.39 (2H, *m*)	35.6	1.38 (2H, *m*)
17	49.2	Cq	-	43.2	-
18	52.1	CH	0.96 (1H, *d*)	48.2	0.97 (1H, *d*)
19	50.5	CH	2.23 (1H, *d*)	47.8	2.38 (1H, *d*)
20	152.2	Cq	-	151.6	-
21	35.5	CH_2	2.25 (2H, *m*)	30.2	2.40 (2H, *m*)
22	42.2	CH_2	2.22 (2H, *m*)	40.2	2.39 (2H, *m*)
23	31.8	CH_3	0.94 (3H, *s*)	28.2	0.91 (3H, *s*)
24	16.2	CH_3	0.96 (3H, *s*)	16.0	0.94 (3H, *s*)
25	16.8	CH_3	0.85 (3H, *s*)	16.8	0.74 (3H, *s*)
26	16.7	CH_3	0.75 (3H, *s*)	16.4	0.78 (3H, *s*)
27	15.2	CH_3	1.00 (3H, *s*)	15.1	1.06 (3H, *s*)
28	19.5	CH_3	1.59 (3H, *s*)	18.0	1.59 (3H, *s*)
29	110.2	CH_2	4.58 & 4.60 (2H, *s*)	108.6	4.56 & 4.70 (2H, *s*)
30	22.2	CH_3	1.69 (3H, *s*)	19.5	1.72 (3H, *s*)

Table 2. Antibacterial activity test result

Bakteri	lupeol compound (mm)	Positive control (mm)	Negative control (mm)
S. aureus	18	22	0
P. aeruginosa	14	25	0
E. coli	13	22	0

The ^{1}H-^{1}H COSY spectrum is used to identify protons that are correlated with three bond spacing. COSY spectrum of isolated compound indicates peaks such as between δ 2.23, H-19 and one Sp^3 methylene proton signal (δ 2.25, H-21) and another Sp^3 methine proton signal (δ 0.96, H-18); and oxygenated methine proton signal belonging to (δ 1.69, H- 30 and Sp^3 methylene signal (δ 1.65, H-2)[16-19].

The HMBC spectrum used to determine the correlation between proton and carbon from two to three bonds (2J and 3J). From the spectrum it can be observed that H-13 (δH = 0.75 ppm) correlates with C-12 (δC = 28.7 ppm), H-26 (δH = 0.75 ppm) has a correlation with C-10 (δC = 38.4 ppm), H-23 (δH = 0.95 ppm) has a correlation with C-24 (δC = 16.2 ppm), and H-28 (δH = 1.0 ppm) has correlation with C-15 (δC = 30.9 ppm). The pair of broad singlets of olefinic proton at δ_H 4.58 and 4.60 showed cross peaks with a methylene carbon signal [δ 50.5 (C-19) and δ 22.2 (C-30)] by J3 correlation. The forgoing spectral analysis and comparison with reported data (table 1), led us to propose the structure of isolated compund as lupeol, a pentacylic triterpenoid, (figure 1) below.

Antibacterial Test Result

The results of antibacterial activity testing of isolated compound based on the inhibition zone of isolated compounds on bacterial growth of *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* is shown in Table 2.

Different responses from three classes of bacteria to isolated compounds is caused by differences in sensitivity in Gram positive bacteria (*S. aureus* and *E. coli*) and Gram negative bacteria (*P. aeruginosa)* against isolated compound. Gram-positive bacteria tend to be more sensitive to antibacterial components. This is caused by the Gram positive cell wall structure is simple making it easier for the antibacterial compounds to enter the cells and to find goals for work.

Lupeol compound were successfully isolated is a compound of the triterpenoid group. Triterpenoids are compounds that the carbon framework is derived from six isoprene units and synthesized derived from C hydrocarbons 30 acyclic, which is skualena. Based on literature review, triterpenoid group compounds and steroids has antibacterial activity with the mechanism of action inhibiting synthesis protein[20-21].

CONCLUSION

In this research we successfully isolated pentacyclic triterpenoid compound Lup-20(29)-en-3*β*-ol) from methanol extract of leaves *Sonneratia alba.* The result of antibacterial activity test showed that isolated compound effectively inhibited the growth of these bacterial pathogens with inhibition zone 18 mm for *Staphyloccocus aureus,* 14 mm for *Pseudomonas aeruginosa*, and 13 mm for *Escherichia coli.* This is the first report of isolation lupeol compound from the leaves of *Sonneratia alba* and test antibacterial activity against pathogens bacteria of this species.

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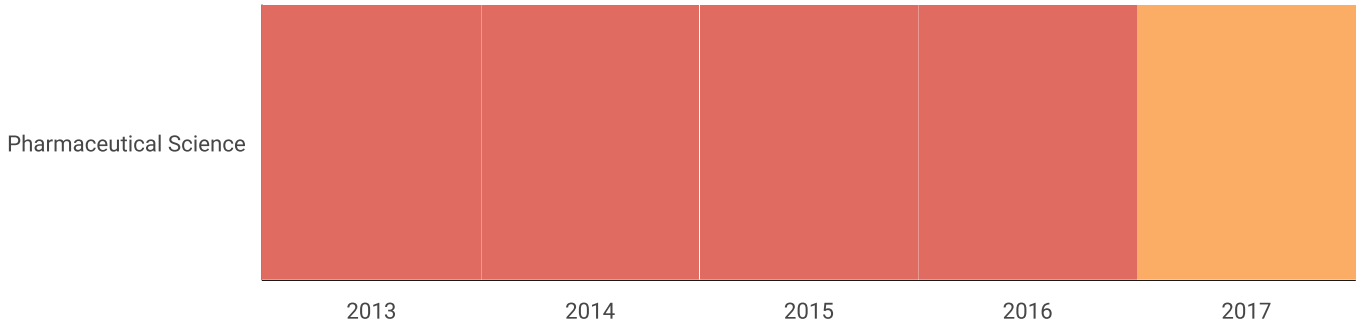

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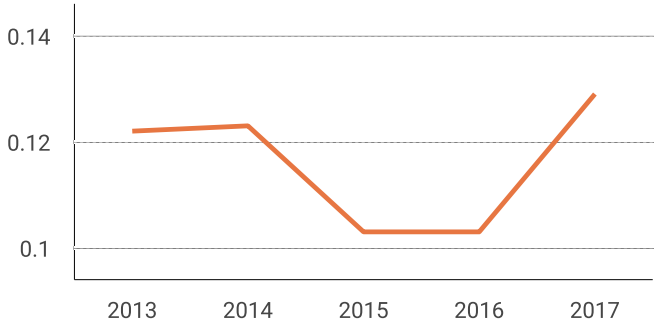

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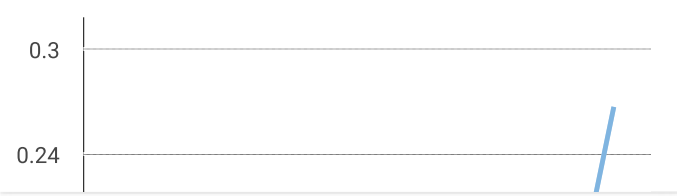

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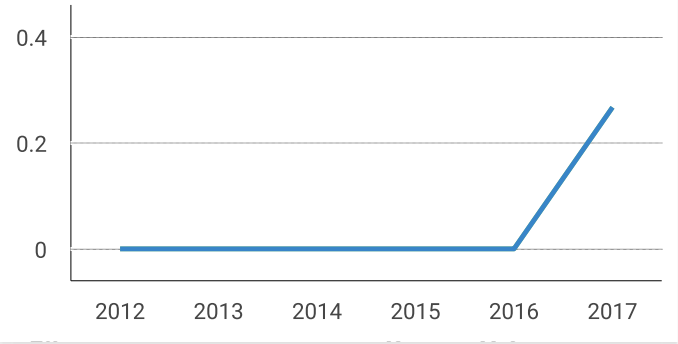

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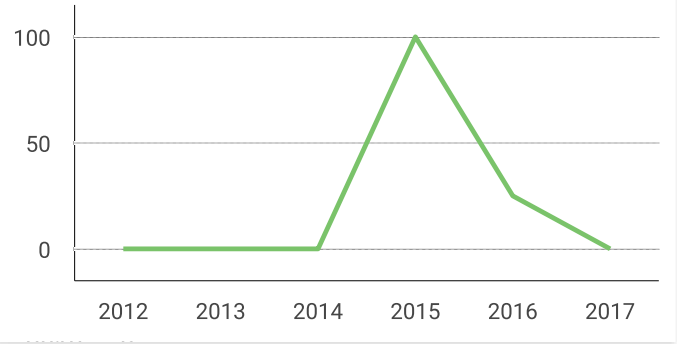

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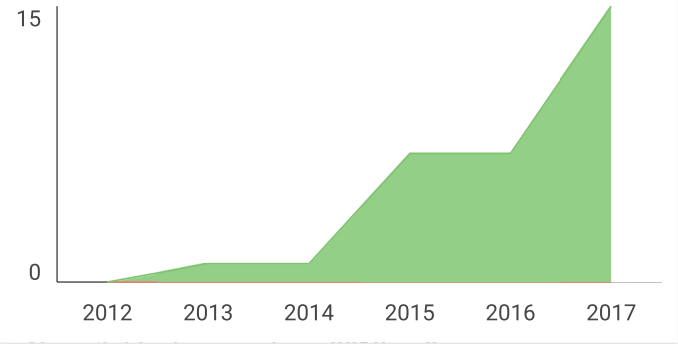

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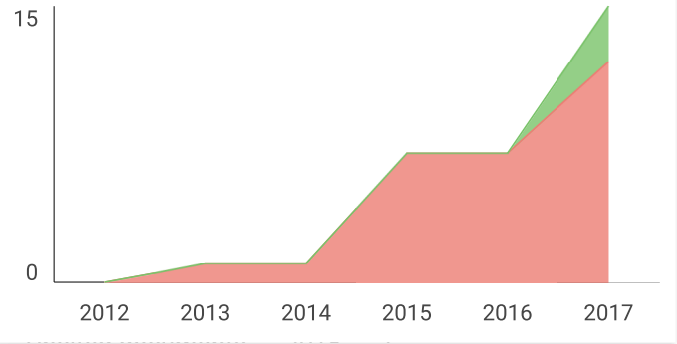

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