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# Studi biologik efek getah jatropha curcas (Euphorbiaceae) terhadap jaringan gigi dan periapeks

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Abstrak

Tujuan umum: Mengetahui profil keamanan dan efek getah J. curcas terhadap jaringan gigi dan periapeks dalam persiapan untuk memanfaatkan pemakaian bahan alami getah J. curcas pada radang pulpa.
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Tujuan khusus (1) Mengetahui kandungan golongan senyawa getah J. curcas. (2) Mengetahui sitotoksisitas getah J. curcas. (3) Mengetahui toksisitas akut pemberian secara oral dosis tunggal getah J. curcas pada hewan percobaan. (4) Mengetahui aktivitas hemolisis getah J. curcas pada darah manusia secara in vitro. (5) Mengetahui sifat mutagenisitas getah J. curcas. (6) Mengetahui efek getah J. curcas terhadap pembebasan interleukin-1β oleh sel makrofag. (7) Mengetahui efek getah J. curcas terhadap pembebasan kolagenase pada set fibroblast. (8) Mengetahui efek histopatologik getah J. curcas terhadap pulpa dan jaringan periapeks gigi pada hewan percobaan. (9) Mengetahui efek getah J. curcas terhadap kekerasan macro jaringan keras gigi manusia secara in vitro. (10) Mengetahui efek getah J. curcas terhadap jaringan keras gigi manusia dalam hal kelarutan unsur kalsium dan fosfat secara in vitro.
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Metode penelitian: Disain penelitian eksperimental dan eksplorasi. Penelitian dibagi atas (1) skrining fitokimia, (2) tahap 1 dan (3) tahap 2 evaluasi biologik getah J. curcas. Untuk standardisasi getah J. curcas diambil dari satu petak tanaman dalam satu musim, kemudian diukur pH, volume basah, diliofilisasi, diukur berat kering, dan disimpan pada -20°C sebagai sampel.
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(1). Skrining fitokimia getah J. curcas. Analisis kualitatif golongan senyawa diidentifikasi dari ekstrak eter, etil asetat, dan air.
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(2). Uji toksisitas
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1. Uji sitotoksisitas. (1) Metoga agar overlay. Getah J. curcas dan kontrol diserap oleh cakram selulosa, kemudian diletakkan di atas permukaan agar yang menutupi selapis sel Fib L929 yang telah diwarna neutral red. Evaluasi berdasar luas zona dekolorisasi dan zona lisis yang terbentuk setelah 24 jam. (2) Assay MTT. Getah J. curcas dalam medium diberikan pada kultur set Fib L929 cell line dan sel primer fibroblast gingiva manusia yang tumbuh dalam mikroplat 96-sumur. Setelah 1-4 hari, dilakukan assay MTT. Evaluasi berdasar perbandingan nilai OD kontrol dan perlakuan.
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2. Uji toksisitas akut. Mencit diberi getah J. curcas secara intragastrik sebanyak 1 kali. Dihitung LD5O berdasar jumlah mencit yang mati. Dibandingkan antara kelompok perlakuan dan kontrol dalam hal tanda toksisitas, berat badan selama 2 minggu, pemeriksaan makroskopik dan mikroskopik organ tubuh.
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3. Uji hemolisis. Darah dicampur dengan berbagai konsentrasi getah J. curcas. Evaluasi berdasar pembebasan hemoglobin, dibandingkan OD kelompok perlakuan dengan kontrol positif air, dan kontrol negatif salin.
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4. Uji mutagenisitas. Getah J. curcas dikultur dengan bakteri S. typhi dan E. coil mutan. Evaluasi berdasar penghitungan koloni reversi bakteri, dibandingkan kelompok perlakuan, kontrol positif dan kontrol negatif.

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(3) Efek getah J. curcas terhadap makrofag dan fibroblast
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1. Efek getah J. curcas terhadap pembebasan IL-1β. Lima dosis getah J. curcas dimasukkan ke dalam kultur makrofag peritoneum mencit BALB/c, secara bersamaan, sebelum, atau sesudah pemberian LPS. Setelah 1 dan 2 hari, IL-1β dalam supernatan diukur secara ELISA dengan Quantikine IL-1β for mouse kit.
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2. Efek getah J. curcas terhadap pembebasan kolagenase oleh fibroblast. Empat dosis getah J. curcas dan IL-1β dimasukkan dalam kultur sel primer fibroblast gingiva manusia. Setelah 1-4 hari kolagenase dalam supematan diukur dengan assay kolagenase. Hasil degradasi kolagen dipisahkan dengan SDS-PAGE. Pita 3/4 αA diukur dengan program komputer Adobe Photo.
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(4) Efek histopatologik getah J. curcas pada jaringan pulpa dan periapeks. Getah J. curcas dimasukkan ke dalam kavitas gigi monyet. Setelah 3 hari, gigi diproses untuk pembuatan sediaan histologik. Evaluasi berdasar perbandingan pemeriksaan keadaan mikroskopik jaringan pulpa dan peripeks dalam hal inflamasi dan nekrosis, antara kelompok kontrol dan perlakuan.
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(5) Efek getah J. curcas terhadap jaringan keras gigi.
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1. Efek getah J. curcas terhadap kekerasan mikro dentin dan email. Mahkota gigi premolar dibelah 4 longitudinal, lalu ditanam di dalam akrilik dengan 1 permukan tidak tertutup akrilik. Setelah direndam dalam 3 konsentrasi getah J. curcas, permukaan dentin dan email diberi indentasi oleh intan Knoop. Evaluasi berdasar perbandingan KHN kelompok kontrol dan perlakuan.
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2. Efek getah J. curcas terhadap kelarutan kalsium dan fosfat. Mahkota gigi premolar utuh dibelah 4 secara longitudinal, lalu direndam dalam 3 konsentrasi getah J. curcas. Setelah 1-3 hari, kalsium dan fosfat yang larut dalam rendaman diukur berturut-turut dengan alat atomic absorption spectrophotometer (AAS) dan spektrofotometer (metoda asam askorbat).
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Hasil penelitian pH getah J. curcas rata-rata 3,49 ± 0,09 dan perbandingan berat kering/volume basah 15,12 ± 0,31%.
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(1) Skrining fitokimia: getah J. curcas mengandung golongan senyawa sterol, aglikon flavon, tanin, senyawa pereduksi, glikosida steroid, poliose, dan saponin.

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(2) Uji toksisitas
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1.(1) Sitotoksisitas getah J. curcas pada metoda agar overlay ditemukan zona dekolorisasi indeks 2 dari 5 indeks zona. Tak ada lisis sel, bentuk sel masih jelas.
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(2) Assay MTT: pads getah J. curcas kadar 0,25% terhadap Fib L929 dan kadar 0,12% terhadap fibroblast gingiva, sel nekrosis.
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2.(1) LD50 > 5 g/kg BB, sehingga getah J. curcas dapat diklasifikasi dalam toksik ringan. (2) Tidak ada perbedaan berat badan. (3) Tidak ada perbedaan makroskopik dan mikroskopik organ tubuh yang diperiksa. (4) Terjadi inaktivitas pada hari 1 pada kelompok perlakuan, selanjutnya tidak ada perbedaan.
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3. Aktivitas hemolisis getah J. curcas 15% adalah 6,5% dibanding air. Tidak ada hemolisis pada konsentrasi getah J. curcas yang lebih rendah.
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4. Tidak ada aktivitas mutagenisitas getah J. curcas.
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(3) Efek getah J. curcas terhadap makrofag dan fibroblast
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1. (1) LPS meningkatkan pembebasan 1L-1β oleh makrofag. (2) Pemberian getah J. curcas menghambat pembebasan 1L-1β oleh makrofag.
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2. (1) Makin lama waktu kultur, produksi kolagenase makin banyak. (2) Getah J. curcas menurunkan pembebasan kolagenase oleh fibroblast.
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(4) Efek histopatologik getah J. curcas terhadap jaringan pulpa dan periapeks
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(1) Inflamasi dan nekrosis terj adi pads daerah yang terbatas dekat dengan daerah yang kontak dengan getah J. curcas. Di bawahnya terdapat jaringan pulpa normal. (2) Tingkat inflamasi pulpa kelompok perlakuan tidak lebih parah dari kelompok kontrol. (3) Tidak ada radang periapeks pads kelompok kontrol dan perlakuan.
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(5) Efek getah J. curcas terhadap jaringan keras gigi.
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1. Efek getah J. curcas terhadap kekerasan mikro dentin dan email. (1) Kekerasan mikro dentin tidak berbeda bermakna pada 1 dan 2 hari perendaman getah J. curcas antara kelompok kontrol dan perlakuan. Namur lebih kecil setelah 3 hari pada konsentrasi getah 15%. (2) Kekerasan mikro email tidak berbeda antara kelompok kontrol dan perlakuan pada 1 dan 3 hari, Namun lebih kecil setelah 2 hari pada konsentrasi getah J. curcas 15%.
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2. Kadar kalsium dan fosfat yang larut meningkat sesuai dengan kenaikan konsentrasi getah J. curcas.

Namun lama perendaman tidak berpengaruh secara bermakna terhadap kelarutan kalsium.
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Kesimpulan (1) Getah J. curcas mengandung sterol, aglikon flavon, tanin, senyawa pereduksi, glikosida steroid, poliose, dan saponin. (2) Tahap 1 evaluasi biologik: getah J. curcas relatif aman pada hewan percobaan berdasar LD50>5 g/kg BB sehingga termasuk dalam klasifkasi toksik ringan; hemolisis 6,5% dibanding air; tidak mutagen; dan sitotoksik dengan nekrosis koagulasi. (3) Uji tahap 2: getah J. curcas cukup efektif dalam menanggulangi pulpalgia, berdasar nekrosis pulpa terbatas, tidak ada kelainan periapeks; kekerasan mikro email dan dentin tidak turun pada 1 hari; menghambat pembebasan IL-1β dan kolagenase. Namun getah melarutkan kalsium dan fosfat.
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Kesimpulan penelitian: penelitian dapat dilanjutkan ke tahap uji klinik atau tahap 3.
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Biological Study on the Effects of Jatropha Curcas (Euphorbiaceae) Latex on Dental and Periapical TissuesObjective: The objective of this study was to evaluate the safety level and the effects of J. curcas latex on dental and periapical tissues. The aims in details were (1) to identify the main classes of chemical constituent in J. curcas latex; (2) to evaluate the cytotoxicity of J. curcas latex; (3) to determine the acute toxicity of J. curcas latex after single oral administration on mice; (4) to assess hemolytic activity of J. curcas latex; (5) to evaluate mutagenic activity of J. curcas latex; (6) to evaluate the effect on J. curcas latex of IL-1 il release from macrophages; (7) to evaluate the effect of J. curcas latex on collagenase release from fibroblasts; (8) to assess the histopathological effects of J. curcas latex on monkey dental pulp and periapical tissues; (9) to determine the effects of J. curcas latex to dentin and enamel micro-hardness; (10) to assess the effects of J. curcas latex on dissolving calcium and phosphate.
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Methods: Research design was experimental and explorative. To standardize the sample, J. curcas latex was collected from Balittro, Bogor in 1997, then the pH and wet volume were measured, the latex was lyophilized, dry weight was measured, and latex was stored at-20°C as sample. Biological evaluation was grouped into (1) phytochemical sreening, (2) toxicity test, (3) effects of J.curcas latex on cell, (4) effects of J.curcas latex on dental pulp and periapical tissues, and (5) effects of J.curcas latex on dental hard tissues,
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(1). Phytochemical screening: the main classes of chemical constituents of J. curcas latex were analyzed qualitatively from ether, ethyl acetate, and water extracts.
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(2). Toxicity test
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1. Cytotoxicity test. (1) Agar overlay technique. J. curcas latex was imbibed in cellulose discs and put on the surface of agar overlaying a neutral red stained Fib L929 cell monolayer. Evaluation was judged on zone index and lysis index after 24 hours incubation. (2) MT assay. J. curcas latex was added to human gingival fibroblasts and Fib L929 cell culture in 96-well micro-plates. After 1-4 days of incubation, MTT assay was performed. Evaluation was based on comparing the OD values of control and test groups.
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2. Acute toxicity. A single dose of J. curcas latex was given to male and female mice, intragastrically. LD50 was determined based on mortality rate. Assessment was also performed on 2 weeks observations of body

weight, macroscopic and microscopic examinations of several organs.
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3. Hemolysis test. Blood was mixed with several concentrations of J. curcas latex. The result was the extent of hemolysis expressed based on the absorbance of the test samples, negative and positive controls.
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4. Mutagenicity test. L curcas latex was added to the S. ryphi and E. coil mutans culture. Assessment was based on bacterial revertant colonies, compare to positive and negative controls.
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(3) Effects of J.curcas latex on macrophages and fibroblasts
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1. Effects of .T. curcas latex on the release of IL-1 β from macrophages. Five doses of J. curcas latex from 75-1200 μg/ml were added into the culture of BALB/c mice peritoneal macrophages, along with, after, or before addition of LPS. Following 1-3 days of incubation, IL-1P presence in supernatant was measured by ELISA using Quantikine ]L-1P for mouse kit.
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2. Effects of J. curcas latex on the release of collagenase. Four doses of J. curcas latex from 37.5-300 μg/ml were added to human gingival fibroblasts cell culture. After 1-4 days of incubation, collagenase in the supernatant was assayed with collagen. The degradation products were then separated by SDS-PAGE and the density of 3/4 αA bands was measured semi quantitatively by Adobe Photo computer program.
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(4) Effects of J.curcas latex on dental pulp and periapical tissues. The latex of J. curcas was brought in contact with dental pulp and sealed. Assessment was based on the presence of inflammation and necrosis in dental pulp and periapical tissues, histopathologically.
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(5) Effects of J.curcas latex on dental hard tissues
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1. Effects of J. curcas latex on dentin and enamel micro-hardness. Intact premolar crowns were cut longitudinally into 4 fragments, followed by embedding of each fragment in acrylats leaving 1 free surface. The fragments were then soaked in 3 concentrations of J. curcas latex from 3.7-15% for 1-3 days. The dentin and enamel micro-hardness were assessed by Knoop hardness measurement.
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2. Effects of J. curcas latex on dissolved calcium and phosphate. Intact premolar crowns were cut longitudinally into 4 fragments, followed by soaking the fragments in 3 concentration of J. curcas latex from 3.7-15% for 1-3 days. The dissolved calcium and phosphate were measured according to atomic absorption spectrophotometer and spectrophotometer (ascorbic acid method), respectively.
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Results: The mean ± SD of J. curcas latex pH was 3.49 ± 0.09. The dry weight/wet volume was 15.12 ± 0.31%.
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(1). Phytochemical screening: sterols, flavone aglycones, tannins, reducing compounds, sterol glycosides, poliose, and saponins were identified in J. curcas latex.
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(2) Toxicity test
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1. (1) Agar overlay technique. 2-5 mm decoloration zones were observed, indicating that J. curcas latex was cytotoxic. No lysis of cells was observed within the decolorized zone. (2) MTT assay. At 2.5 mg/ml J. curcas latex no living Fib L929 cells were observed, while the same result was shown at 1.2 mg/ml J. curcas latex on human gingival fibroblasts.
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2. LD50 was more than 5 g/kg BW, hence dry J. curcas latex may be classified into mildly toxic substance. No significant body weight difference was observed. Macroscopic and microscopic examination on several organs showed no differences between test and control groups.
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3. 6,5% hemolytic activity of 15% J. curcas latex compared to water was observed, while no hemolisis was observed with lower concentrations of latex.
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4. No mutagenic ativity was observed with J. curcas latex.
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(3) Effects of J.curcas latex on macrophages and fibroblasts
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1. (1) LPS increased the release of IL-1β. (2) J. curcas latex inhibited the release of IL-lβ from macrophages.
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2. (1) The longer the duration of incubation, the more collagenase was released. (2)
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J. curcas latex decreased collagenase release by human gingival fibroblast.
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(4) Effects of I. curcas latex on dental pulp and periapical tissues. Inflammation and necrosis were observed in a limited area, which was in direct contat with J. curcas latex, underneath was normal pulp. Inflammation in the pulp of test group was not greater than in the control group. No inflammation or necrosis in periapical tissues was observed in all groups.
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(5) Effects of J. curcas latex on dental hard tissues
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1. (1) The micro-hardness of dentin was not lowered after 1 and 2 days treatment, but lower after 3 days at 15% J. curcas latex. (2) The enamel microhardness was not decreased after 1 and 3 days immersion in J. curcas latex, but decreased after 2 days at 15% J. curcas latex.
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2. The calcium and phosphate release were increased in accordance to the concentration of J. curcas latex. The duration of treatment did not influence the release of calcium, while it influenced the release of phosphate.
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Conclusions (1) J. curcas latex contains sterols, flavone aglycones, tannins, reducing compounds, sterol glycosides, poliose, and saponins. (2) Level 1 biological evaluation: J. curcas latex is relatively safe in

animals based on LD50>5 g/kg BW, 6,5% hemolysis compared to water, not mutagenic, but cytotoxic with coagulative necrosis. (3) Level 2 biological evaluation: J. curcas latex seems to be effective in relieving pulpal pain. It caused coagulative necrosis in pulp, which was in direct contact with J. curcas latex while the tissue underneath was normal. It did not cause inflammation of periapical tissues, and did not lower the dentin and enamel micro-hardness after 1 day of exposure, but it lowered the microhardness after 3 days. It inhibited IL-1β and collagenase release. It dissolved dental calcium and phosphate.