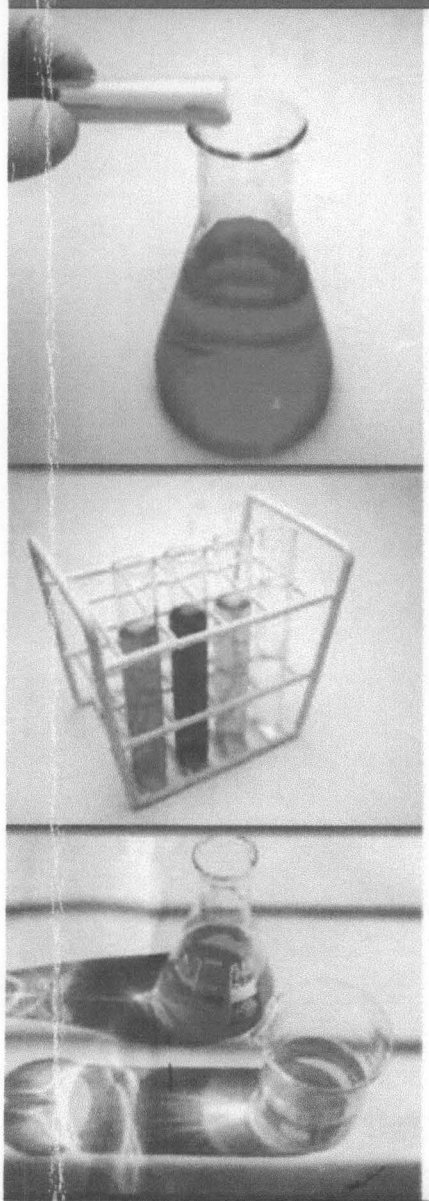
CHEMISTRY PROGRESS

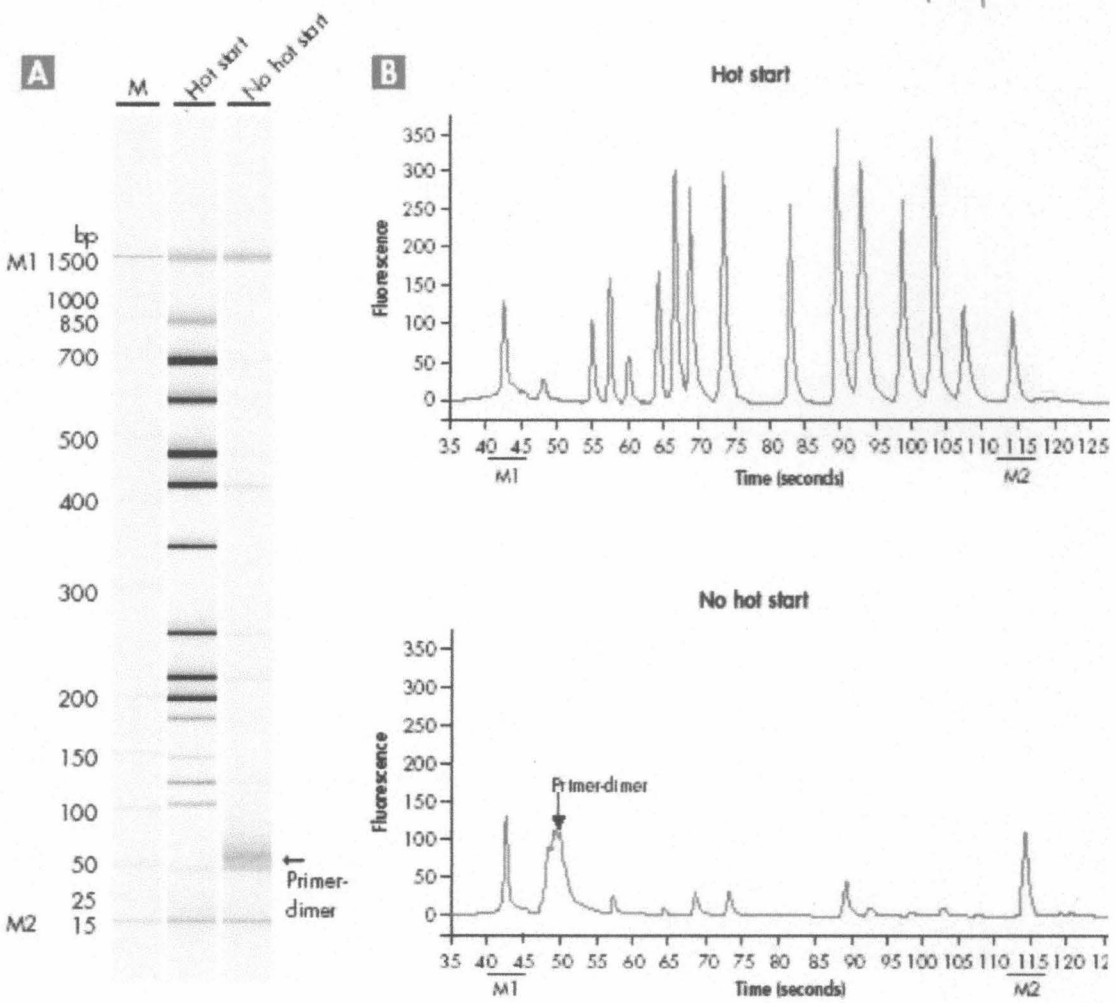
Majalah Publikasi Ilmu Kimia

Volume 4, Nomor 2
November 2011

ISSN : 1979-5920



44



Chem.Prog	Vol. 4	No. 2	Hal. 55-96	Manado, November 2011	ISSN : 1979-5920

Diterbitkan Oleh :

JURUSAN KIMIA
FAKULTAS MATEMATIKA DAN ILMU PENGETAHUAN ALAM
UNIVERSITAS SAM RATULANGI
HIMPUNAN KIMIA INDONESIA CABANG SULAWESI UTARA

 ISSN: 1979-5920

CHEMISTRY PROGRESS

Majalah Publikasi Ilmu Kimia

Majalah Ilmiah Chemistry Progress merupakan media untuk menyebarkan informasi ilmiah dan sarana komunikasi bagi para ilmuan dan cendekiawan melalui tulisan-tulisan ilmiah. Majalah Ilmiah Chemistry Progress terbit pertama kali tahun 2008 dengan frekuensi dua nomor dalam satu tahun (Mei dan November) berisi kajian penelitian dalam lingkup ilmu kimia (organik, anorganik, analitik, biokimia, fisika, bahan alam, lingkungan, pangan, kelautan, pertambangan, farmasi dan komputasi). Jumlah halaman pervolume adalah 80-100 halaman.

Percetakan

Sam Ratulangi Press, Manado

Isi diluar tanggungjawab percetakan

Alamat Redaksi :

Jl. Kampus UNSRAT Kleak Manado, Sulawesi Utara. Kode Pos : 95115
Telp. (0431) 827964, 085256311981, Fax. (0431) 827964.,
Email: *chemistryprogress@yahoo.com*
Website: *http://ejournal.unsrat.ac.id/index.php/chemprog*

PENERBIT

Jurusan Kimia, Fakultas Matematika Dan Ilmu Pengetahuan Alam,
Universitas Sam Ratulangi
Himpunan Kimia Indonesia Cabang Sulawesi Utara

Harga : Rp. 50.000,- /eksemplar ditambah 20% ongkos kirim
Berlangganan Rp. 90.000,-/tahun untuk 2 nomor ditambah 20% ongkos kirim
Rekening Bank BNI 46 kantor cabang Manado, No. 0066410783 a.n. Chemistry Progress.

ISSN : 1979-5920

CHEMISTRY PROGRESS

Majalah Publikasi Ilmu Kimia

Volume 4. Nomor 2, November 2011

Daftar Isi :

ISSN: 1979-5920

EDITORIAL

Petunjuk Bagi Penulis : Pastikan naskah yang dikirim ke redaksi selalu sesuai dengan dengan petunjuk penulisan.

Kebijakan Editor : Jurusan Kimia Fakultas MIPA UNSRAT dan para Editor tidak bertanggung jawab atas segala pernyataan dan pandangan yang dinyatakan oleh para pengarang.

Pengiriman naskah : Kirimkan naskah ke Dewan Redaksi, Prof. Dr. Edi Suryanto, M.Si, Jurusan Kimia Fakultas MIPA-UNSRAT Manado, Jl. Kampus Bahu Unsrat Manado, Sulawesi Utara. Kode Pos : 95115 Telp. 0431-827964, 0852-56311981, Fax. 0431-827964 atau E-mail: chemistryprogress@yahoo.com. Naskah yang perlu dikoreksi, akan dikembalikan kepada penulis untuk diperbaiki. Penulis diwajibkan mengembalikan naskah yang telah dikoreksi ke Dewan Editor dalam jangka waktu 3 hari sejak dikembalikan.

Biaya : Membayar biaya administrasi sebesar Rp. 150.000,- ke rekening Bank BNI 46 kantor cabang Manado, No. 0066410783 a.n. Chemistry Progress atau pembayaran langsung.

Perubahan alamat : Perubahan alamat pelanggan hendaknya diberitahukan secepatnya ke redaksi dalam kurun 3 bulan setelah penerbitan.

ANTIBACTERIAL ACTIVITY OF PROPOLIS *Trigona* spp. FROM BUKITTINGGI WEST SUMATERA AGAINST *Salmonella* sp.

A. E. Zainal Hasan[1], I Made Artika[1], Amin Fatoni[2], Kuswandi[3], Budi Haryanto[3]

[1]*Department of Biochemistry, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Darmaga Campus, Bogor16680, Indonesia*
[2]*Departement of Chemistry, Faculty of Science and Technique, Jenderal Soedirman University, Purwokerto 53123, Indonesia*
[3]*Animal Husbandry Research Institute, Jalan Pajajaran, Bogor 16151, Indonesia*

ABSTRACT

Zaenal Hasan, A. E. *et al.* 2011. Antibacterial activity of propolis *Trigona* spp. from Bukittinggi West Sumatera against *Salmonella* sp.

Propolis is a resinous hive product consists of exudates from plants mixed with beeswax and used by bees as glue. The objective of this study was to investigate the antibacterial activity of ethanol extract of *Trigona* spp. propolis collected from Bukittinggi West Sumatera on *Salmonella* sp. using agar well diffusion method. The compound group of the propolis was also investigated on the existence of alkaloids, flavonoids, saphonins, tannins, steroids, and terpenoids. Ethanol extract of *Trigona* spp. propolis showed antibacterial activity on *Salmonella sp.* with minimum inhibitory concentration of 0,87% (w/w). The compound groups detected in ethanol extract *Trigona* spp. propolis were flavonoids and tannins, which suggests that the antibacterial activity of *Trigona* spp. propolis may be due to these compounds.

Keywords: propolis, *Trigona* spp., *Salmonella* sp., antibacterial

ABSTRAK

Zaenal Hasan, A. E. *et al.* 2011. Aktivitas antibakteri propolis *Trigona* spp. dari Bukittinggi Sumatera Barat terhadap *Salmonella* sp.

Propolis adalah produk resin sarang lebah yang mengandung cairan tumbuhan yang bercampur dengan lilin lebah dan bagi lebah digunakan sebagai perekat. Tujuan penelitiian ini untuk mengetahui aktivitas antibakteri ekstrak etanol dari propolis *Trigona* spp. yang peroleh dari Bukittinggi Sumatera Barat terhadap *Salmonella* sp. Kelompok senyawa dalam propolis diuji terhadap keberadaan alkaloid, flavonoid, saphonin, tanin, steroid dan terpenoid. Ekstrak etanol Propolis *Trigona* spp. menunjukkan aktivitas antibakteri terhadap *Salmonella sp.* dengan konsentrasi penghambatan minimum 0,87% (b/b). Golongan senyawa yang terdapat dalam ekstrak etanol propolis *Trigona* spp. adalah flavonoid and tanin. Aktivitas antibakteri propolis *Trigona* spp. propolis mungkin disebabkan oleh senyawa-senyawa tersebut.

Kata kunci : propolis, *Trigona* spp., *Salmonella* sp. antibakteri

INTRODUCTION

Propolis is a sticky dark-colored material that honeybees collect from living plants, mix with wax and use in construction and adaptation of their nests (Bankova et al., 2000). Bees use propolis not only as a building material but also as a means of maintaining low levels of bacterial and fungal concentrations in the hive. The action against microorganisms is an essential characteristic of propolis and it has been used by human beings since ancient times for its pharmaceutical properties. Propolis possesses antibacterial, antifungal and antiviral properties and many other beneficial biological activities: anti-inflammatory, antiulcer, local anesthetic, hepatic-protective, antitumor, and immunostimulant. For this reason propolis is widely used as a popular remedy in folk medicine, in apitherapy, as a constituent of "biocosmetics", "health food" and for numerous further purposes (Bankova et al., 2000).

In common propolis was obtained from *Apis* spp. honeybee. One of the others bee that collect less honey and more propolis is *Trigona* spp., member of stingless bee. Hasan (2006) found that propolis from *Trigona* spp. was effective against B. *subtilis,* S. *aureus,* and E. *coli.* Sabir (2005) showed that flavonoids Trigona sp propolis inhibits *S. mutans.* We have also previously shown that propolis produced by *Trigona* spp. is active against *Campylobacter* spp. (Fatoni et al., 2008).

Salmonella serovars are responsible for human diseases that range from mild gastroenteritis to host-

Korespondensi dialamatkan kepada yang bersangkutan :
[1]Departemen Biokimia. FMIPA Institut Pertanian Bogor.
Phone : -, E-mail : -

disseminated enteric fever (Ohl & Miller 2001). Although most non-typhoidal strains associated with food-borne infection are self-limiting and do not require antibiotics, complicated or systemic infection and infection with specific serovars (such as typhi) are indications for antibiotic treatment and often hospitalization. The long-term usage of antibiotics in both the poultry and beef industries may have created a strain of salmonella which is potentially resistant to antibiotics (Brisabois et al., 1997). Bacterial drug resistance is an important world problem (Asna & Haq 2000; WHO 1995). Poppe et al. (2001) verified that Salmonella serovars, isolated from food or infected animals, were resistant to several antibiotics. Lewin (1991) and Stoner et al. (2000) also reported an increased resistance of Salmonella serovars to several antimicrobial drugs. The World Health Organisation has recently pointed out an alarming increase in the incidence of antibiotic resistant strains of *Salmonella*, which are due to the use of antibiotics in intensive breeding. The Laboratoire de Recherche Moléculaire sur les d'Antibiotiques (LRMA) has collaborated with CNEVA-Paris to compare the phenotypes of antibiotic resistance and the distribution of genes encoding for beta-lactamase of *S. typhimurium* strains isolated either from humans or from animals, mainly cattle (Lee *et al.* 1994). Different types of beta-lactamase have already been identified in salmonella: TEM-1, TEM-2, OXA-1 types are the commonest, SHV-1 type seems to predominate in Africa, and PSE-1 and PSE-2 types are usually detected in *Pseudomonas aeruginosa* (Brisabois et al., 1997).

According to many reasons above, it's to be important to found new antibiotic that can effectively inhibit *Salmonella* growth. We suggest that propolis from *Trigona* spp. may be one of new natural resource antibiotic can be used to achieve this intention. Main objective of this research is to identify antibacterial effect or *Trigona* spp. propolis against *Salmonella* sp.

METHODOLOGY

Materials and Tools

Chemicals used in this experiment are p.a. grade of silica gel (Merck), aluminium hydroxide (Merck), and sodium hydroxide (Merck). The primary equipment is infra red spectrophotometer Shimadzhu FTIR-8201PC.

Extraction of propolis

Trigona spp. bee hive as raw propolis was collected from Bukittinggi West Sumatera. The sample was cut into small pieces, grounded and extracted with 70% ethanol (1:5 w/v) in a shaker (EYELA, Japan) at speed 130 rpm, room temperature for 14 days. The ethanol extract solution was then filtered through a filter paper, and then the solvent evaporated using freeze-drier to get ethanol extract of propolis (EEP) free-solvent (Hasan 2006).

Antibacterial activity

Antimicrobial activity of propolis ethanol extract was investigated by the agar well diffusion method (Andrews, 2001). The bacterial isolates were first grown in PYG broth (pepton-yeast extract-glucose) for 18 h. 0.2 ml of the log phase culture was aseptically used to seed a molten PYG agar which had been cooled to about 45 °C, mixed gently and poured into sterile petri dishes and allowed to set. The extracts were tested at a series dilutions concentration (16.7; 8.3; 4.1; 2.0; 1.0; 0.2; 0.1; and 0.06 % w/w). This was delivered into wells (5 mm in diameter) bored unto the surface of the already seeded nutrient agar plates. Standard antibiotics concentration of ampicillin 100 ppm was assayed using the agar-well diffusion technique. Plates were incubated at 37° C for 24 h. Inhibitory zone diameters were measured with caliper. All experiments were performed in triplicates. The data were submitted to analysis of variance using general linear model procedure of statistical software. The chosen level of significance for all statistical tests was $P< 0.05$.

Compound groups test.

Ethanol extract propolis was investigated any contain of flavonoids, alkaloids, terpenoids, steroids, saphonins, and tannins compound groups (Harborne, 1996).

RESULTS AND DISCUSSIONS

Ethanol extract propolis was obtained very sticky, with dark brown colored. The average of extraction yield was 24,66% (w/w). Ethanol extract propolis was obtained still content ethanol in a small amount (0,05%). The diameters of bacterial growth inhibited by different concentration of EEF and others controls are shown in Table 1 and Figure 1. The result showed that at a concentration of 16,67%, EEP was more effective than the commercial propolis, and less active then ampicillin (100 ppm) on *Salmonella* sp. growth. Among the series concentration of EEP tested, the less concentration that still showed inhibitory effect is 1,04% (two replicate), and 0,52% (one sample), thus the average of MICs was 0,87% (w/w). Orsi et al. (2006) verify that *Salmonella* Typhi is susceptible to *Apis* spp. propolis from Brazil (MIC = 9,9% v/v) and Bulgaria (MIC = 10,0% v/v).

The propolis activity and chemical compositions depend on plant origin, season of propolis harvesting and geographical location of bee hive collected (Bankova et al., 2000; Banskota et al., 2000), thus researchers use this reasons to investigated new propolis active compounds. The pharmacological activities of propolis are more numerous in tropical regions than in temperate climates, reproducing the richer vegetal diversity observed in the former (Bankova 2005). There is less reference about propolis activity from Indonesia, especially from *Trigona* spp. bee. Sabir (2005) investigated the activity of flavonoids from *Trigona* spp. propolis collected from South Sulawesi on *Streptococcus mutans*.

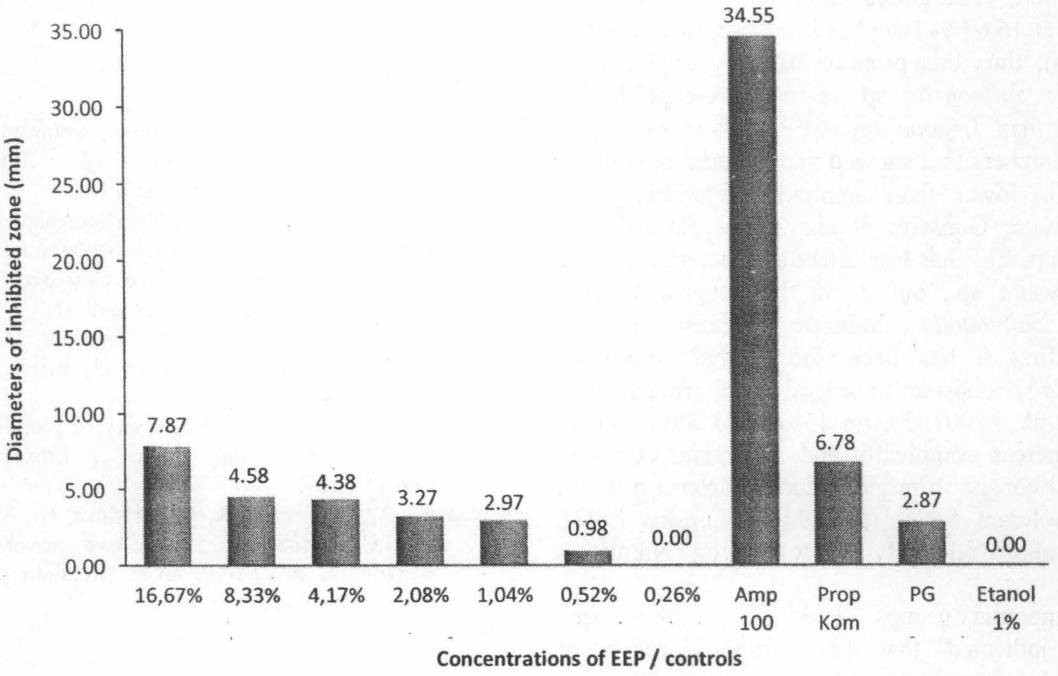


Figure 1. The means of diameters (mm) of bacterial growth inhibited by different concentrations of *Trigona* spp. propolis and other controls

The sample was also analyzed for compound groups of alkaloids, flavonoids, tannins, saphonins, steroids, and terpenoids. The compounds groups identified in *Trigona* spp. propolis are flavonoids and tannins (Table 1).

Table 1. Compound groups test results for *Trigona* spp. propolis

Compound groups	Test results	Color produced by test
Flavonoids	+++	Yellow
Alkaloids	-	
Saphonins	-	
Tannins	+++	Greenish violet
Steroids	-	
Terpenoids	-	

+ : identified by visual observation (+++ : strong, ++ : fair, + slightly);
- : unidentified by visual observation.

Hasan (2006) showed that *Trigona* spp. propolis from Pandeglang-Banten against *Stapylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*. The yield obtained in this research (24,66%) is larger than *Trigona* spp. propolis obtained from Pandeglang (8,2 %) (Hasan 2006). This difference may because of diversity of plant around the *Trigona* spp. hive. *Trigona* spp. ethanol extract obtained in this research contains ethanol in small amount (0,05) %. The extraction solvent cannot be remove clearly by freeze drying on the chance of sticky resinous of the propolis

properties, thus some solvent entrapped in this substance.

Antibacterial activity of *Trigona* spp. EEP at 16,67 % (w/w) is larger than commercial propolis may be due to two reasons, first this propolis has higher antibacterial activity than commercial propolis, and second this propolis is more concentrated than commercial propolis (unknown active compound concentration). The antibacterial activity of *Trigona* spp. EEP at 16,67 % (w/w) is smaller than ampicillin (100 ppm), thus this propolis has low antibacterial activity or *Salmonella* sp. is more susceptible for ampicillin than *Trigona* spp. EEP. This results agree many researchers that showed antibacterial activity of propolis is lower than ampicillin (Katircioglu & Mercan 2006; Gonsales et al., 2006). Nevertheless *Trigona* spp. EEP has low effect antibacterial activity on *Salmonella* sp., but it can be suggest as new effective *Salmonella* antibiotic, because of two reasons: first It has been shown that strains of *Salmonella sp.* resistant to several antimicrobial drugs (Poppe et al., 2001; Lewin 1991; and Stoner *et al.* 2000), whereas complexity and synergism effect of compound consist in propolis cause bacteria difficult to built tolerant for it (Mizrahi & Lensky 1997); second propolis relatively non toxic drug (Nikulin et al., 1979).

Compound groups tested for *Trigona* spp. propolis indicated that this propolis reach of polyphenol compounds that are flavonoids and tannins. This result agree with numerous researchers that have been reported caffeic acids, flavonoids and phenolic esters are the main biologically active compounds in propolis (Kartal et al., 2003; Kujumgiev et al., 1993; Marcucci et al., 2001; Park et al., 1998). But their biological effects cannot be attributed solely to these components, because, the chemical composition of propolis is complicated. Some authors attribute the complex composition of propolis as a reason for its antimicrobial activity, and some mechanisms of action have been proposed (Mirzoeva et al., 1997; Park et al., 1998; Simuth et al., 1986; Strehl et al., 1994, Takaishi-Kikuni & Schilcher 1994). The antimicrobial properties of propolis are related to the synergistic effect of its compounds (Santos et al., 2002). The bee glue affects the cytoplasmic membrane and inhibits bacterial motility and enzyme activity (Mirzoeva et al., 1997). Propolis exhibits bacteriostatic activity against different bacterial genera and can be bactericidal in high concentrations (Drago et al., 2000, Mirzoeva et al., 1997).

CONCLUSIONS

The results indicate that there is antibacterial activity of ethanol extract of *Trigona* spp. propolis on *Salmonella sp.* growth, and the MICs of this propolis is 0,87 % (w/w). The compound groups identified in this *Trigona* spp. propolis are flavonoids and tannins, which suggest that this compounds responsible for antibacterial activity of this *Trigona* spp. propolis.

REFERENCES

Andrews, J. M. 2001. Determination of minimum inhibitory concentrations. *Journal of Antimicrobial Chemotherapy 48* Suppl. S1:5–16.

Asna, S. M. Z. H., & Haq, J. A. 2000. Decrease of antibiotic resistance in *Salmonella* Typhi isolated from patients attending hospitals of Dhaka City over a 3 years period. *Int J Antim Agents, 16*:249-51.

Bankova, V. S., Castro, S. L., & Marcucci, M. C. 2000. Propolis: recent advances in chemistry and plant origin. *Apidologie, 31*:3–15.

Bankova, V. 2005. Chemical diversity of propolis and the problem of standardization. *J Ethnopharmacol, 100*:114-117.

Banskota, A. H., Tezuca, K, Midorikawa, K., Matsushige, K., & Kadota, S. 2000. Two novel cytotoxic benzofuran derivatives from Brazilian propolis. *J Nat Prod, 63*:1277-1279.

Brisabois, A., Cazin, I., Breuil, J., & Collatz, E. 1997. Surveillance of antibiotic resistance in *Salmonella*. *2*(3).

Drago, L., Mombelli, B., Vecchi, E. D., Fassina, M. C., Tocalli, L., & Gismondo, M. R. 2000. In vitro antimicrobial activity of propolis dry extract. *J Chemother, 12*: 390–395.

Fatoni, A., Artika, I. M., Hasan, A. E. Z., & Kuswandi. 2008. Antibacterial activity of propolis produced by Trigona spp. against *Campylobacter* spp. *Hayati J Biosci, 15*: 161-164

Gonsales, G. Z., Orsi, R. O., Fernandes, Jr. A., Rodrigues, P., & Funari, S. R. C. 2006. Antibacterial activity of propolis collected in different regions of Brazil. *J Venom Anim Toxins incl Trop Dis, 12*(2):276-284.

Hasan, A. E. Z. 2006. *Potensi Propolis Lebah Madu Trigona spp sebagai Bahan Antibakteri.* Seminar Nasional HKI, Bogor.

Harborne, J. B. 1996. *Metode Fitokimia*. Edisi ke-2, cetakan ke-2. Padmawinata K and Soediro I, translator. Bandung: Penerbit ITB. Translate from: *Phytochemical Methods.*

Katircioglu, H. & Mercan, N. 2006. Antimicrobial activity and chemical compositions of Turkish propolis from different regions. *African J Biotechno. 5*(11):1151-1153.

Kujumgiev, A., Bankova, V., Ignatova, A., & Popov, S. 1993. Antibacterial activity of propolis. some of its components and their analogs. *Pharmazie, 48*:785-786.

Lee L. A., Puhr, N. D., Malonet, E. K., Bean, N. H, & Tauxe, R. V. 1994. Increase in antimicrobial-resistant *Salmonella* infections in the United States, 1989-1990. *J Infect Dis, 170*: 128-34.

Lewin, C. E. 1991. Treatment of multiresistant *Salmonella* infection. *The Lancet, 47*:337.

Mirzoeva, O. K., Grishanin, R. N., & Colder, P. C. 1997. Antimicrobial action of propolis and some of its components: the effect on growth, membrane potential and motility of bacteria. *Microbiol Res, 152*:239–246.

Mizrahi, A., & Lensky, Y. 1997. *Bee Products : Properties, Appication and Apitherapy*. London, UK: Plenum.

Nikulin, I. M., Lisitsyna, L. Y., Tiknonov, A. I., Shcherbina, V. D., & Stebliuk, P. N. 1979. Propolis in the treatment of Inflammatory diseases of the airways. *Zh Ushn Nos Gorl Bolezn* Nov-Dec:9-12.

Ohl, M. E., & Miller, S. I. 2001. *Salmonella*: a model for bacterial pathogenisis. *Ann. Rev. Med., 52*, 259-274.

Park, Y. K., Koo, M. H., Jose,´ A. S. A., Masaharu, I., Jaime, A. C., & Pedro, L. R. 1998. Antimicrobial activity of propolis on oral microorganisms. *Curr Microbiol, 36*:24–28.

Poppe, C *et al.* 2001. Trends in antimicrobial resistance of *Salmonella* isolated from animals, foods of animal origin, and the environment of animal production in Canada, 1994-1997. *Microbial Drug Resistance-Mechanisms Epidemiol Dis* 7:197-212.

Sabir, A. 2005. Aktivitas antibakteri flavonoid propolis *Trigona* sp. terhadap bakteri *Streptococcus mutans* (*in vitro*). *Majalah Kedokteran Gigi (Dent J) 38*(3):135-141.

Santos, F. A *et al.* 2002. Antibacterial activity of Brazilian propolis and fractions against oral anaerobic bacteria. *J Ethnopharmacol,* 80:1–7.

Simuth, J., Trnovsky, J., & Jelokova, J. 1986. Inhibition of bacterial DNA-dependent RNA polymerases and restriction endonuclease by UV- absorbing components from propolis. *Pharmazie, 41*:131-132.

Strehl, E., Volpert, R., & Elstner, E. F. 1994. Biochemical activities of propolis-extracts. III. Inhibition of dihydrofolate reductase. *Z Naturforsch Sect C Biosci, 49*:39-43.

Stoner, M. C., Forsythe, R., Mills, A. S., Ivatury, R. R., & Broderick, T. J. 2000. Intestinal perforation secondary to *Salmonella* Typhi: case reported and review of the literature. *Amer Surg, 66*:219-222.

Takaisi-Kikuni, N. B. & Schilcher, H. 1994. Electron microscopic and microcalorimetric investigations of the possible mechanism of the antibacterial action of a defined propolis provenance. *Planta Med, 60*:222-227.

World Health Organization (WHO). Surveillance Programme for Control of Foodborne Infections and Intoxications in Europe. Sixth report 1990-1992, 32-38. 1995.

$10/28 \times 100\% = 36\% < 10$ th

$64\% > 10$ th

ISSN 1979-5920

9 771979 592094

Percetakan :

Sam Ratulangi University Press

Jl. Kampus Bahu UNSRAT Manado

Telp. (0431) 827386