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Molecular and Cellular Life Sciences: Infectious Diseases, Biochemistry and Structural Biology 2015 Conference

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Molecular and Cellular Life Sciences: Infectious Diseases, Biochemistry and Structural Biology 2015 Conference

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Bauke W. Dijkstra, Niels van Oosterwijk, Ali Rohman
Pages 3-11

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Abstract

Abstract

3-Ketosteroid dehydrogenases (KSTDs) are FAD-dependent enzymes that introduce a double bond in the A ring of 3-ketosteroid substrates to initiate degradation of the steroid nucleus. Δ^1-KSTD desaturates the C1-C2 bond of the steroid, while Δ^4-KSTD targets the C4-C5 bond. Crystal structures with bound products showed that Δ^1- and Δ^4-KSTD use different amino acid residues to catalyze an otherwise mechanistically very similar reaction (Δ^1-KSTD: Tyr318, Tyr119, and Tyr487; Δ^4-KSTD: Ser468, Tyr319, and Tyr466). However, the substrates are rotated by ~40° about an axis perpendicular to their plane to bring the target bond (C1-C2 or C4-C5) in the right position.

Research article *Open access*

Extracellular Enzymes Produced by *Vibrio alginolyticus* Isolated from Environments and Diseased Aquatic Animals

Supansa Bunpa, Natthawan Sermwittayawong, Varaporn Vuddhakul
Pages 12-17

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Abstract

Abstract

A total of 17 *Vibrio alginolyticus* isolates were obtained from environments, diseased fish and shrimp using CHROMagar Vibrio and confirmed by biochemical tests and PCR targeted to the *gyrB* gene. They were investigated for production of exoenzymes. All of the isolates from diseased fish and shrimp (n = 8) showed gelatinase, lecithinase, and caseinase activities, while 75% (6/8) of them possessed amylase and lipase activities. In environmental isolates (n = 9), the gelatinase and lecithinase activities were detected in all isolates. Four out of nine isolates (44%) possessed lipase, while 67% (6/9) of environmental isolates were positive for both caseinase and amylase activities. Interestingly, α-hemolysin activity was detected in all *V. alginolyticus* isolates from diseased fish and shrimp but it was detected in only 44% (4/9) of the environmental

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Recombinant LipL32 Protein for Leptospirosis Detection in Indonesia

Sumarningsih, Simson Tarigan, Susanti, Kusmiyati

Pages 18-25

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Abstract

Abstract

Leptospirosis is an endemic zoonotic disease in tropical countries. However, detection using the microagglutination test (MAT) as a gold standard is difficult to perform in Indonesia. Therefore, recombinant LipL32 protein (rLipL32) has been studied as an antigen for an ELISA test to detect Leptospirosis. We produced rLipL32 using the pRSET-C vector and expressed it in *E. coli* BL21 (DE3) competent cells. Under native conditions, we purified 2 mg rLipL32 protein from 500 ml culture. Analysis using western blotting and ELISA showed that serum from the bovine positive MAT serovar Hardjo could recognize pure rLipL32 protein. This result confirms an earlier study that indicates that rLipL32 protein is a good antigen for Leptospirosis detection. The diagnostic assay using rLipL32 is safe because it does not use infectious bacteria as an antigen and because

Research article *Open access*

Enhancing Stability and Purity of Crude Chitinase of *Achatina fulica* by Crystallization

Afaf Baktir, Nira Ambar Arum, Suyanto, Bambang Suprijanto

Pages 26-30

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Abstract

Abstract

A crystallization method was developed to enhance the purity and stability of hydrolase mixtures from the digestive gland of the snail *Achatina fulica*, as demonstrated by chitinase activity. Crude chitinase was concentrated by freeze drying and then crystallized at 10 °C. Crystal formation was observed under the microscope. The best concentration for crystallization was obtained with 1.5-fold concentrated crude chitinase. Crystallization enhanced the chitinase specific activity from 0.87 U mg^{-1} to 0.95 U mg^{-1}. The loss of chitinase activity from liquid and crystals of crude chitinase on four days storage at 10 °C was 83.0% and 17.7%, respectively. It was concluded that the crude chitinase crystals showed a significant increase in stability and purity.

Research article *Open access*

Application of Cassava Peel and Waste as Raw Materials for Xylooligosaccharide Production Using Endoxylanase from *Bacillus subtilis* of Soil Termite Abdomen

Anak Agung Istri Ratnadewi, Agung Budi Santoso, Erma Sulistyaningsih, Wuryanti Handayani

Pages 31-38

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Abstract

Abstract

Xylooligosaccharides (XOS) are the sugars produced from xylan hydrolysis. XOS have a prebiotic characteristic by promoting the growth of probiotic microorganisms. Xylan containing agriculture wastes *e.g.* rice straw, sugarcane bagasse, corncobs, cassava peel and waste can be used to produce XOS by a consecutive process of alkali-pretreatment and enzymatic hydrolysis. In this study, we focused on enzymatic production of XOS from cassava peel and waste, which is a low cost material with a relatively high xylan content. The dried cassava peel and waste were ground and sieved to be <100 mesh size, and were then subjected to pretreatment with 0.5% (w/v) sodium hypochlorite solution for 5 h to remove the lignin in the sample. In the next stage, the xylan was extracted by soaking in 10% sodium hydroxide (NaOH) for 24 h, followed by

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Mutation Analysis of the pK_a Modulator Residue in β-D-xylosidase from *Geobacillus Thermoleovorans* IT-08: Activity Adaptation to Alkaline and High-Temperature Conditions

Lanny Hartanti, Ali Rohman, Ami Suwandi, Bauke W. Dijkstra, ... Ni Nyoman Tri Puspaningsih

Pages 39-48

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Abstract

Abstract

β-D-xylosidases are hemicellulases that catalyze the release of xylose units from short xylooligosaccharides. Their activity is the rate-limiting step in xylan hydrolysis. A major application of these enzymes is as eco-friendly biobleaching agents in the pulp and paper

industry, where thermostable, alkaliphilic xylosidases are much preferred. Hence, the isolation and characterization of new thermo-alkaliphilic xylosidases from nature, or re-engineering existing ones is of importance for industrial applications. The thermophilic bacterium *Geobacillus thermoleovorans* IT-08 produces a meso-thermophilic β-D-xylosidase (EC 3.2.1.37), which has a unique primary structure compared to other xylosidases with only 32-35% amino

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Autolytic Isolation of Chitin from White Shrimp (*Penaues Vannamei*) Waste

Achmad Sjaifullah, Agung Budi Santoso

Pages 49-52

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Abstract

Abstract

White shrimp waste comprised of shrimp head, tail and shell contains valuable materials such as chitin, protein, enzymes, minerals, natural pigments, etc. Isolation of chitin from white shrimp waste through an autolysis enzymatic deproteination is described in this paper. The deproteination rate was estimated by measuring nitrogen content during the autolysis and the structure was analyzed by FTIR. The results show that autolysis of white shrimp waste was effectively performed at pH 2 with the highest rate observed in the first two days of autolysis, showing it contains 13.0% protein and 14.7% chitin. The chitin produced after a 9-day autolysis indicated a less clean result than chitin which is chemically digested.

Research article *Open access*

Effects of Fermentation and Storage on Bioactive Activities in Milks and Yoghurts

Irma Sarita Rahmawati, Worapot Suntornsuk

Pages 53-62

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Abstract

Abstract

Fermentation of milk enhances its nutritional value through improved bioavailability of nutrients and production of bioactive substances which have biological functions. The goals of this research were to study the effect of fermentation and storage on antioxidant and antimicrobial activities in buffalo, goat and cow milks and yoghurts. Samples of buffalo, goat, cow milks and their yoghurts during their fermentation and storage were determined for proximate analysis and bioactive activities including antioxidant activities of DPPH, ABTS and reducing power assays, and antimicrobial activities against *Staphylococcus aureus*, *Bacillus cereus*,

Research article *Open access*

Scanning Electron Microscope Analysis of Rice Straw Degradation by a Treatment with α-L-arabinofuranosidase

Anita Kurniati, Handoko Darmokoesoemo, Ni Nyoman Tri Puspaningsih

Pages 63-68

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Abstract

Abstract

The purpose of this research was to analyze surface structure modification of rice straw after degradation by α-L-arabinofuranosidase. Analysis of surface structure modification was performed by scanning electron microscope. Alpha-L-arabinofuranosidase was collected from intracellular enzyme and extracellular enzyme; the ratio of enzyme mixture and incubation time were then optimized. The optimum ratio of enzyme mixture was 1:1. The optimum incubation time of rice straw was 8 hours. Rice straw, after degradation by α-L-arabinofuranosidase, was analyzed by scanning electron microscope. The rice straw, before degradation by α-L-arabinofuranosidase, was used as the control of the analysis. The result of this research indicated that enzymatic hydrolysis damaged surface structure of rice straw

Research article *Open access*

Secretion of *Geobacillus Thermoleovorans* IT-08 α-L-Arabinofuranosidase (AbfA) in *Saccharomyces Cerevisiae* by Fusion with HM-1 Signal Peptide

I Nengah Wirajana, Tetsuya Kimura, Kazuo Sakka, Eddy Bagus Wasito, ... Ni Nyoman Tri Puspaningsih

Pages 69-74

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Abstract

Two different expression vectors were constructed to investigate two signal peptides on secretion of *Geobacillus thermoleovorans* IT-08 α-L-arabinofuranosidase (AbfA) *in Saccharomyces cerevisiae*. They were designed to direct the secretion of AbfA by the aid of one of the following signal peptides, the αF signal peptide in the plasmid YEpFLAG1-Af and HM-1 signal peptide in the plasmid pYHM1-Af. Although some successful results have been reported in proteins secretion with αF leader sequence, in this research no α-L-arabinofuranosidase activity could be observed in recombinants *S. cerevisiae* YEpFLAG1-Af. The HM-1 leader sequence, originated from *Hansenula mrakii* IFO 0895 killer toxin, showed the capability to AbfA secretion.

Research article *Open access*

Hydrolysis of Corncob Xylan using β-xylosidase GbtXyl43B from *Geobacillus Thermoleovorans* IT-08 Containing Carbohydrate Binding Module (CBM)

Ni Nyoman Purwani, Handoko Darmokoesoemo, Ni Nyoman Tri Puspaningsih

Pages 75-81

Abstract

Abstract

Corncob is mainly compose of lignocellulose rich in xylan. Corncob collected from Tanjung Anom village, East Java, was extracted by alkaline method. Corncob was used as substrate for β-xylosidase GbtXyl43B and oat spelt xylan was used as standard for xylan substrate. GbtXyl43B has molecular mass of 72 kDa and is composed of Catalytic Module (CM) and Carbohydrate Binding Module (CBM). GbtXyl43B was purified from culture supernatant using Ni-NTA affinity chromatography. The enzyme was purified 26.785 fold over the crude extract of soluble proteins with a specific activity of 14.9641 Unit mg^{-1}. Enzymatic hydrolysis product of corncob and oat spelt xylan was analyzed by HPLC, producing xylose, arabinose and xylooligosaccharide.

Research article *Open access*

Biochemical Potential of α-L-Arabinofuranosidase as Anti-Tuberculosis Candidate

One Asmarani, Much Zaenal Fanani, Ni Nyoman Tri Puspaningsih
Pages 82-89

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Abstract

Abstract

Mycobacterium tuberculosis (MTB), the main causative organism of tuberculosis (TB), is a successful pathogen that overcomes the numerous challenges presented by the immune system of the host. The situation regarding control of tuberculosis has significantly worsened over the last decade with the spread of strains resistant to multiple antimycobacterial agents. Mycobacterial cell wall has potential as a target for developing protein therapeutic enzybiotic to overcome resistancy case in TB. One of the compiler of mycobacterial cell wall is arabinogalactan which is compiled by arabinose with unusual stereochemical. α-L-arabinofuranosidase (Abfa) is an enzyme that can breakdown arabinofuranosidic bond. However, its ability to breakdown the arabinofuranosidic from D-arabinose as monomer is still unknown

Research article *Open access*

Shortening of Amino Acids from C-terminal of PZase as Basis of Pyrazinamide Resistance in P14 Isolate of *Mycobacterium Tuberculosis* Strain

Purkan, Redianti Galuh Novarizka, Rizka Aziz Ayuningsih, Presty Nurdiana, Wiwin Retnowati
Pages 90-95

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Abstract

Abstract

Pyrazinamide (PZA) is one of the mainstays WHO-recommended drugs for therapy of tuberculosis (TB). The emergence of PZA resistance in clinical isolates of *M. tuberculosis* is often associated with *pncA* gene mutations encoding PZase. A local clinical isolate of *Mycobacterium tuberculosis* strain showed phenotipe resistant to PZA at concentration of 10 µg/mL. The ORF of *pncA* gene of the isolate showed deletion of guanine base at position 81, then followed by shortening of 70 amino acids from C-terminal of PZAse which has 186 amino acid residues. The mutant of PZase took frame shift of amino acids after the residue at position 27. The *pncA* gene mutation at the level of genotype, that produced a physical-chemical alteration of the active site

Research article *Open access*

Elimination of SCMV (*Sugarcane Mozaik Virus*) and Rapid Propagation of Virus-free Sugarcane (*Saccharum officinarum* L.) Using Somatic Embryogenesis

Parawita Dewanti, Laily Ilman Widuri, Choirul Ainiyati, Purnama Okviandari, ... Bambang Sugiharto

Pages 96-102

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Abstract

Abstract

The use of apical buds and in-vitro shoot for elimination of SCMV and shoot proliferation in sugarcane was assessed. The purpose of this research was to determine the level of virus elimination and to obtain virus-free sugarcane. Research were using explants of apical buds and in vitro shoots of sugarcane PS-881 cultured on MS medium supplemented with antiviral acyclovir and ribavirin consisted of 0, 20 and 40 mg l^{-1} with incubation duration 4, 5 and 6 weeks. Elimination of virus-free plantlets was detected by DAS-ELISA and RT-PCR. The results showed that the detection of RT-PCR using apical explants treated with acyclovir 40 mg l^{-1} for 6 weeks was not effective to eliminate SCMV, while the use of in vitro shoot explants treated with 40 mg l^{-1} of acyclovir or 40 mg l^{-1} ribavirin eliminated the SCMV for about 100% resulting virus-

Research article *Open access*

Antimicrobial Activities and In silico Analysis of Methoxy Amino Chalcone Derivatives

Hery Suwito, Ni'matuzahroh, Alfinda Novi Kristanti, Salwa Hayati, ... Ni Nyoman Tri Puspaningsih

Pages 103-111

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Abstract

Abstract

A series of methoxy-4'-amino chalcone derivatives were tested for their antimicrobial activities against *Escherichia coli ATCC 25923*, *Staphylococcus aureus ATCC 25922* and *Candida albicans ATCC 10231*. Furthermore, their molecular interactions with dihydropteroate synthase (DHPS) of *E. coli* and *S. aureus* were studied with a docking experiment. Compound 4 ((*E*)-1-(4-aminophenyl)-3-(2,3-dimethoxyphenyl)prop-2-en-1-one) exhibited the strongest activity, in which its activity was equal to sulfamerazine and sulfadiazine used as positive controls. In addition, it showed a good potential to be used as a wide spectrum antimicrobial agent. The in

silico experiment showed that the prepared compounds had higher affinity to DHPS of *S. aureus*

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Potential Application of Oleylamine-encapsulated $AgInS_2$-ZnS Quantum Dots for Cancer Cell Labeling

Mochamad Zakki Fahmi, Jia-Yaw Chang

Pages 112-121

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Abstract

Abstract

This study improves a simple strategy for phase transfer process via ultrasonication approach. Water-soluble $AgInS_2$-ZnS quantum dots (QDs) encapsulated with oleylamine have been successfully prepared without the presence of surfactant or polymer. During the propagation process, ultrasonication creates harmonic pressure (relative to the equilibrium hydrostatic pressure of the liquid), which was helpful for the dispersion of nanomaterials in two immiscible phases. The highly photoluminescent ZnS coated $AgInS_2$ QDs (up to 55.3% QY) was synthesized with a one-pot two-step process with narrow particle distribution and successfully transferred to water phase without significant effect on optical properties. Beside QDs characterization, some factors such as pH stability, ionic strength, and bonding properties were investigated to reach a

Research article *Open access*

Macrophage Activity and Capacity Following Oral Administration of Cocoa Extract to Mice

Ariza Budi Tunjung Sari, Teguh Wahyudi, Misnawi, Diana Chusna Mufida, I Wayan Suardita

Pages 122-126

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Abstract

Abstract

The activity of an ethanolic extract from cocoa bean (*Theobroma cacao* L.,) towards the non-specific immune response in mice being challenged with *Staphylococcus epidermidis* was studied. Mice (Swiss-Webster, 12 weeks old, 35 ± 1.9 g) received oral administration of cocoa extract (CE),

positive control or negative control, every day for seven consecutive days. Cocoa extract (CE) was in three different doses, i.e. CE1 7.14 mg/30 g body weight (BW), CE2 14.28 mg/30 g BW, and CE3 28.57 mg/30 g BW. The positive control was *Phyllanthus niruri* Linn. (PN) extract (Stimuno®) 17.55 mg/30 g BW, while the negative control was sterile water (SW). On day 8, mice were given intraperitoneal injections of *S. epidermidis* suspension (0.5 ml, 105 CFU). After being settled for

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The Role and Efficiency of Ammonium Sulphate Precipitation in Purification Process of Papain Crude Extract

Maria Goretti M. Purwanto

Pages 127-131

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Abstract

Abstract

It has been common to do fractionation (for example using ammonium sulphate as a precipitating agent) before doing a more sophisticated method for purification of a protein. The logic behind this is easy to understand, but in fact, the precipitation step often causes severe loss in yield and activity of the protein, making the whole purification effort too costly. In this work we evaluated the specific activity (thus, purification factor) and total activity (yield) during the purification process of papain from a crude extract using ion exchange chromatography (IEC), with and without prior fractionation using ammonium sulphate. Detail assays in each step were recorded and SDS-PAGE was also done to reveal the protein profile of the purification products.

Research article *Open access*

Isolation and Antibacterial Activity Test of Lauric Acid from Crude Coconut Oil (*Cocos nucifera* L.)

Febri Odel Nitbani, Jumina, Dwi Siswanta, Eti Nurwening Solikhah

Pages 132-140

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Abstract

Abstract

Isolation of lauric acid from crude coconut oil (CCO) has been done. Neutralization of CCO using 30% Na_2CO_3 solution could decrease its acid value from 1.69 to 0.48. Transesterification reactions of neutral coconut oil with methanol and K_2CO_3 at 55 °C in 3 hours produced methyl laurate in 52% purity. Methyl laurate with 87% purity could be isolated by fractionatal distillation at 130-140 °C. Hydrolysis of methyl laurate with NaOH produced solid lauric acidin 84% yield. Lauric acid at 5% concentration could inhibit the growth of all bacteria tested but it is still lower than Ciprofloxacin.

Research article *Open access*

Effect of Butyric Acid on p53 Expression and Apoptosis in Colon Epithelial Cells in Mice after Treated with 9,10-dimethyl-1,2-benz(a)anthracene

Cherry Siregar, Eddy Bagus Wasito, I Ketut Sudiana

Pages 141-146

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Abstract

Abstract

As the most common cancer, colorectal cancer is the fourth leading cause of death among this malignancy disease. Surgery procedure with chemotherapy and radiotherapy for colorectal cancer treatment may cause unpleasant side effects. Therefore, prevention and early detection of the disease is important. Butyrate, a short chain fatty acid, has a protective effect against colon cancer by inhibiting cell proliferation and inducing apoptosis. We conduct a research to investigate the effect of butyrate as a possible agent to decreased mutant p53 gene expression.

Research article *Open access*

Deep Eutectic Solvent (DES) as a Pretreatment for Oil Palm Empty Fruit Bunch (OPEFB) in Sugar Production

Nur Atikah Md Nor, Wan Aida Wan Mustapha, Osman Hassan

Pages 147-154

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Abstract

Abstract

Oil Palm Empty Fruit Bunch (OPEFB) was pretreated using Deep Eutectic Solvent (DES) at different parameters to enable a highest yield of sugar. DES is a combination of two or more cheap and safe components to form an eutectic mixture through hydrogen bond interaction, which has a melting point lower than that of each component. DES can be used to replace Ionic Liquids (ILs), which are more expensive and toxic. In this study, OPEFB was pretreated with DES mixture of choline chloride: urea in 1:2 molar ratio. The pretreatment was performed at temperature 110 °C and 80 °C for 4 hours and 1 hour. Pretreatment A (110 °C, 4 hours), B (110 °C, 1 hour), C (80 °C, 4 hours) and D (80 °C, 1 hour). Enzymatic hydrolysis was done by using the combination of two enzymes, namely, Cellic Ctec2 and Cellic Htec2. Morphology surface of

Research article *Open access*

Pretreatment of Oil Palm Empty Fruit Fiber (OPEFB) with Aquaeous Ammonia for High Production of Sugar

Nursyafiqah Zulkiple, Mohamad Yusuf Maskat, Osman Hassan

Pages 155-161

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Abstract

Abstract

Corncob Oil Palm Empty Fruit Bunch (OPEFB) is an agricultural residue that has the potential to become a good source for renewable feedstock for production of sugar. This work evaluated the effectiveness of aqueous ammonia as pretreatment at low (soaking, SAA) and elevated temperature (Pressurized Chamber) to deconstruct the lignocellulosic feedstock, prior to enzymatic hydrolysis. The ammonia pretreatments were compared against the standard NaOH method. The best tested Pressurized Chamber method conditions were at 100 °C with 3 hour retention time, 12.5% Ammonium hydroxide and 1:30 solid loading. The digestibility of the feedstock is determined with enzymatic hydrolysis using Cellic Ctech2 and Cellic Htech2. The sugars produced by Pressurized Chamber method within 24 hour of enzyme hydrolysis are

Research article *Open access*

Antibacterial Activity of Pyrogallol, a Polyphenol Compound against *Vibrio parahaemolyticus* Isolated from The Central Region of Thailand

Tran Huu Tinh, Taiyeebah Nuidate, Varaporn Vuddhakul, Channarong Rodkhum

Pages 162-168

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Abstract

Abstract

Vibrio parahaemolyticus is a notorious marine bacterium that causes disease to human and marine animal. Polyphenols are plant-derived products that are commonly found in fruits and plants. These products are well-known for their antioxidant properties due to the ability to scavenge free radicals. In addition, many polyphenols have been proved to possess bactericidal effects against both Gram-negative, and Gram-positive bacteria. In this work, a total of 30 isolates of *V. parahaemolyticus* including pathogenic (n = 9) and non-pathogenic (n = 21) strains were isolated from Pacific White shrimps obtained from the central area of Thailand. Susceptibility of *V. parahaemolyticus* to pyrogallol, a polyphenol compound commonly found in mango and many citrus plants, was compared to antibiotics. Antibacterial activity of V

Research article *Open access*

AntiHepatitis C Virus Activity of *Alectryon serratus* Leaves Extract

Lidya Tumewu, Evhy Apryani, Mei Ria Santi, Tutik Sri Wahyuni, ... Hak Hotta

Pages 169-173

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Abstract

Abstract

Hepatitis C Virus (HCV) has infected approximately 2-3% (130-170 million) of the world's population. No vaccine is available to prevent HCV infection. Investigation of anti-HCV agent is thus deemed necessary. Various plants have been explored for their anti-HCV activity. *A. serratus* is a member of Sapindaceae family, which fruit and seed were traditionally used as insecticide. Anti-HCV activity tested on *A.serratus* leaves extract has been done. The result showed that leaves extract exhibited anti-HCV with IC_{50} value of 14.9 μg/ml and 9.8 μg/ml against HCV J6/JFH1 and JFH1a, respectively. The cytotoxicity assay results showed that *A.serratus* leaves extract was not toxic and has CC_{50} >100 μg/ml. Mode of action experiment results suggested that *A.serratus* extract inhibited HCV at the post-entry step. Further fractionation of leaves extract by open

Research article *Open access*

Toxicity Test n-Hexane: Ethyl Acetate (3:7) Fraction of Sudamala (*Artemisia vulgaris L.*)

Ira Arundina, S. Theresia Indah Budhy, Intan Nirwana, Retno Indrawati, Muhammad Luthfi

Pages 174-178

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Abstract

Abstract

Sudamala (*Artemisia vulgaris* L.) is commonly used in the community as anti-tumor in digestive organ, including in oral cavity. However, there have been no scientific studies about oral anti-carcinogenic active substances of Sudamala. The purpose of this study was to analyse the effect of per oral administration of n-hexane: ethyl acetate (3:7) fraction of *Artemisia vulgaris* L. on healthy mice. This experimental study used male Swiss Webster (Balb C) strain mice (*Mus musculus*) 2.5 months old, 20-30 grams by weight. Group 1, the mice received 0.5% CMC- Na fraction solvent, as a control. Group 2, the mice received 200 mg/kgbw n-hexane: ethyl acetate (3:7) fraction of Sudamala (*Artemisia vulgaris* L). The fraction was given once daily for 8 weeks per oral according to the mice's weight. At the end of 8th week, the mice were killed and oral

Research article *Open access*

Activities of *Ficus fistulosa* Leave Extract and Fractions against Hepatitis C Virus

Achmad Fuad Hafid, Adita Ayu Permanasari, Lidya Tumewu, Myrna Adianti, ... Hak Hotta

Pages 179-184

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Abstract

Abstract

Hepatitis C Virus (HCV) is a major global disease which often leads to chronicity and is potential to liver failure. There is no anti-HCV vaccine and the high diversity of viral genotypes will probably make it very difficult to develop a vaccine. Therefore, the development of new drugs for HCV treatment is highly required. It is commonly known that numerous important modern drugs have been developed from molecules originally isolated from natural sources. In this study, we tested the leave extract and fractions of *Ficus fistulosa* for their anti-HCV activities by cell culture method using Huh7it cells and HCV JFH1a. The result showed that ethanol extract of *Ficus fistulosa* (FFL) inhibited HCV JFH1a with IC_{50} value of 20.43±4.51 µg/ml. Toxicity

Research article *Open access*

Characterization of Tryptophanase from *Vibrio cholerae* O1

Taiyeebah Nuidate, Natta Tansila, Kanda Panthong, Varaporn Vuddhakul

Pages 185-189

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Abstract

Abstract

Tryptophanase (Trpase) encoded by the *tnaA* gene catalyzes the conversion of tryptophan to indole, which is an extracellular signaling molecule detected in various bacteria including *Vibrio cholerae*. Indole has been demonstrated to regulate biofilm formation, drug resistance, plasmid maintenance and spore formation of bacteria. In the present study, the *tnaA* gene from *V. cholerae* O1 (VcTrpase) was cloned and expressed in *E. coli* BL21(DE3) *tn5:tnaA* (a Trpase-deficient competent). VcTrpase was purified by Ni^{2+}-NTA chromatography. The obtained VcTrpase had a molecular mass of approximately 49 kDa, a specific activity of 3 U/mg protein, and absorption peaks at 330 and 435 nm. Using a site-directed mutagenesis technique, replacement of Arg419 by Val resulted in a VcTrpase completely devoid of activity. Thus, this site can be a target for

Research article *Open access*

Curcuminoid Prevents Protein Oxidation but not Lipid Peroxidation in Exercise Induced Muscle Damage Mouse

Bambang Purwanto, Harjanto, I. Ketut Sudiana

Pages 190-193

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Abstract

Abstract

Oxidative stress is believed as underlined mechanism of exercise induced muscle damage. This study was aimed to investigate curcuminoid effect on protein oxidation and lipid peroxidation muscle of exercised induced model. Adult male healthy mice were used as experiment models, grouped in to curcuminoid treated, placebo (corn oil only treated) and untreated group. Protein carbonyl level was significantly lower in curcuminoid treated group compared with untreated

group (p =0.003). In the contrary, the malondialdehyde level was not significantly different between those groups (p = 0.092).

Research article *Open access*

The Correlation between Pulmonary Function Tests and the Salivary MMP-9 Activity among Chronic Obstructive Pulmonary Disease (COPD) Patients

Mulyadi, Sunnati, Mulkan Azhary

Pages 194-198

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Abstract

Abstract

The spirometry test is routinely performed to assess FEV_1, FVC, and FEV_1/FVC ratio among chronic obstructive pulmonary disease (COPD) patients with the increased activity of MMP-9. Saliva is less invasive to assess the MMP-9 activity. This study aimed to compare Pulmonary Function Tests to estimate the MMP-9 activity. The respondents were 30 COPD outpatients from Pulmonary Policlinic. Results showed mean ratio of FEV_1, FVC, FEV_1/FVC (SD) and that of the salivary MMP-9 activity were 1.67 (0.12) L, 2.97 (0.43) L, 56.15 (8.43) % and 1.85 (1.54) μM respectively. The correlation between FEV_1, FVC, and FEV_1/FVC ratio and the salivary MMP-9 activity was insignificant (p>0.05). The pulmonary function tests were not able to estimate the salivary MMP-9 activity. The findings suggest further activities of MMP-9 from other samples

Research article *Open access*

Multiple Intracranial Tuberculomas: Diagnosis Difficulties in a Clinical Case

Evita Mayasari, Sufida

Pages 199-204

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Abstract

Abstract

Intracranial tuberculoma is the second most common manifestation of central nervous system (CNS) involvement in immunocompetent tuberculosis (TB) patients and the major cause of death and disability in developing countries due to diagnostic difficulties. Tuberculosis is the

most common infectious cause of CNS space-occupying lesions (SOLs) in Indonesia. However, we should consider other infectious causes for the differential diagnosis of CNS SOLs. Clinical presentations, histopathological and microbiological findings of brain biopsy specimens may confirm intracranial tuberculoma diagnosis. We discuss a case of multiple intracranial tuberculomas in a 23-year-old female with diagnosis difficulties at the early stage of illness.

Research article *Open access*

Study of Tree-sparrow (*Passer montanus*) as Natural Spreader of H5N1 Virus

Emmanuel Djoko Poetranto, Anna Lystia Poetranto, Aldise Mareta Nastri, Adhitya Yoppy Ro Candra, ... Kazufumi Shimizu

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Abstract

Abstract

To understand sparrow‘s ability in spreading H5N1 avian influenza virus, especially compared to backyard chicken, we performed an experimental infection study. Nine sparrows and backyard chickens were inoculated with H5N1 viruses isolated from sparrow and chicken. We observed the incubation period, clinical signs, and time of death after the exposure. We also carried out pathological anatomy and examined any histopathologyical changes. The results indicate that sparrows are more resistant to the H5N1 virus infection than chicken. The striking organ changes of sparrows died because of the virus infection were lungs and brains haemorrhage. Lung damage seemed to be the main cause of the chicken's death. Neuronal necrosis and gliosis seemed to cause the torticollis of sparrow. The H5N1 virus isolated from sparrow that was more

Research article *Open access*

The Effect of Spirulina as Feed Additive to Myocardial Necrosis and Leukocyte of Chicken with Avian Influenza (H5N1) Virus Infection

Widya Paramita Lokapirnasari, Andreas Berny Yulianto, Djoko Legowo, Agustono

Pages 213-217

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Abstract

Abstract

The aim of this research was to examine the effect of *Spirulina sp.* as feed additive to myocardial necrosis and leukocytes which were infected by Avian Influenza H5N1 virus. This research comprised three level treatment of Spirulina 0%, 10%, 20% of the fresh water algae as a liquid supplement, each of which consisted of seven replicates given to 7 day to 32 day old broiler chicken. Artificial infection of Avian Influenza virus H5N1 by entering the respiratory tract (nose drops) using a dose of 0.1 ml inoculum. Blood samples were collected from brachialis vein 0.5–1 ml to calculate leukocyte cell. Heart tissue of chicken were taken to histopathologic and immunohistochemistry examination. The results showed that there was no significant difference ($p>0.05$) in myocardial necrosis and significant difference ($p<0.05$) in leukocyte in the

Research article *Open access*

Potency of Attenuated *Eimeria tenella* in Protective Immunity Induction on Homologous and Heterologous Challenges

Muchammad Yunus, Endang Suprihati

Pages 218-224

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Abstract

Abstract

The aim of this study was to know the protective immunity of broilers immunized with attenuated *Eimeria tenella* (*E. tenella*) on homologous and heterologous challenges. The protective immunity was represented by oocyst production and histopathological changes of cecum. The previous experiment already produced attenuated *E. tenella* through serial passages of precocious lines in naive chicken. Four groups of chickens were separately divided into two non-immunized control groups and two immunized with attenuated *E. tenella* groups, and each group was challenged with homologous and heterologous strains. The results showed significant difference in oocysts production ($p<0.01$) and histopathological changes between control and immunized groups

Research article *Open access*

Sequence Analysis of the Gene Region Encoding ESAT-6, Ag85B, and Ag85 C Proteins from Clinical Isolates of *Mycobacterium tuberculosis*

Ni Made Mertaniasih, Didik Handijatno, Agnes Dwi Sis Perwitasari, Desak Nyoman Surya Suameitria Dewi, ... Ika Qurrotul Afifah

Pages 225-230

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Abstract

Abstract

Mycobacterium tuberculosis secreted proteins in culture filtrate and early phase of infection, such as early secretory antigen target 6 (ESAT-6), culture filtrate protein 10 (CFP-10), and antigen 85 complex i.e. Ag85A, Ag85B, and Ag85C which played roles in adherence, invasion, cytolysis, and evading cytosol of macrophage, were virulence factors that determined the immune responses important on pathogenesis of Tuberculosis (TB), including granuloma formation or tissue that determine the degree of disease. The purpose of this research was to analyze the gene region sequence encoding ESAT-6, Ag85B, and Ag85C of *Mycobacterium tuberculosis*. *Mycobacterium tuberculosis* strain analyzed was taken from sputum of pulmonary TB patients in East Java, Indonesia. Sequenced DNA analyzed using GENETYX Ver.10. There were no SNPs both inside

Research article *Open access*

Detection of *Mycobacterium leprae* in Formalin-Fixed Paraffin-Embedded Sample by Fite-Faraco Staining and Polymerase Chain Reaction

Willy Sandhika, Dinar Adriaty, Indropo Agusni

Pages 231-236

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Abstract

Abstract

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae*. The identification of mycobacteria in tissue sections can be made through a microscopic examination with fite-faraco staining or PCR method. Paraffin blocks from four patients with leprosy were retrieved from The Pathologic Department of Dr.Soetomo Hospital, Surabaya. Two cases were from paucibacillary leprosy patients with no mycobacteria stained by fite-faraco. PCR assay showed a negative result. The other two cases were multibacillary leprosy with many bacteria stained by fite-faraco. PCR assay showed a positive result.

Research article Open access

Immunogenicity and Specificity of Anti recombinant Protein Fim-C-*Salmonella typhimurium* Antibody as a Model to Develop Typhoid Vaccine

Muktiningsih Nurjayadi, Dea Apriyani, Umar Hasan, Imam Santoso, ... Wibowo Mangunwardoyo

Pages 237-245

Abstract

Abstract

Salmonella typhimurium, that cause typhoid fever in mice was used as a model to study typhoid fever in humans. This study is aimed to determine the immunogenicity of recombinant protein of Fim-C-*S. typhimurium* and specificity of antibody anti-recombinant protein of Fim-C-*S. typhimurium* in ddY mice. The immunogenicity studies by ELISA indicated that recombinant protein of Fim-C-*S. typhimurium* evokes strong immune response in ddY mice, particularly in experiment group. The specificity evaluated by Western Immunobloting technique indicated that the antibody anti-recombinant protein of Fim-C-*S. typhimurium* can detect the 31 kDa of recombinant protein of Fim-C-*S. typhimurium* as an antigen. This data reported that recombinant protein of Fim-C-*S. typhimurium* is immunogenic and the antibody anti-

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Antimicrobial Activities and In silico Analysis of Methoxy Amino Chalcone Derivatives

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Abstract

A series of methoxy-4'-amino chalcone derivatives were tested for their antimicrobial activities against *Escherichia coli ATCC 25923*, *Staphylococcus aureus ATCC 25922* and *Candida albicans ATCC 10231*. Furthermore, their molecular interactions with dihydropteroate synthase (DHPS) of *E. coli* and *S. aureus* were studied with a docking experiment. Compound 4 ((*E*)-1-(4-aminophenyl)-3-(2,3-dimethoxyphenyl)prop-2-en-1-one) exhibited the strongest activity, in which its activity was equal to sulfamerazine and sulfadiazine used as positive controls. In addition, it showed a good potential to be used as a wide spectrum antimicrobial agent. The in silico experiment showed that the prepared compounds had higher affinity to DHPS of *S. aureus* than to DHPS of *E. coli*. The tested compounds showed high similarity interaction with hydroxymethylpterin pyrophosphate (natural substrate of DHPS) in building intermolecular interactions.

Peer-review under responsibility of the organizing committee of the Molecular and Cellular Life Sciences: Infectious Diseases, Biochemistry and Structural Biology 2015 (MCLS 2015)

Keywords: Methoxy amino chalcones; antimicrobial; dihydropteroate synthase

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1. Introduction

Besides their simple preparation procedures, the increasing interest in chalcones is due to their various pharmacological potentials, such as antitumor[1], anticancer[2,3], antimalarial[4], antihepatotoxic[5], topoisomerase I inhibitor[6], antinflammation[7], antioxidant[8] and antimicrobial activities[9].

Overuse and misuse of antimicrobial agents increases cases of antimicrobial drug resistance[10]. Sulfanilamide derivatives as the first generation of antibiotics and penicillin as one of the next generation (Patrick, 2001) have been replaced gradually by new generations of antibiotics[11]. However, the needs for more potential antibiotics are still to be explored due to the global health problems worldwide caused by antimicrobial resistance, especially in the developing countries. Suwito *et al.*[14] have designed and synthesized a series of methoxy-4'-amino chalcone derivatives as antimicrobial agents mimicking the structure of PABA (*p*-amino benzoic acid) in the 4-amino benzoyl moiety[12]. PABA is a substrate of dihydropteroate synthase (DHPS) in the biosynthesis of 7,8-dihydropteroate, which is very important in folic acid biosynthesis needed for cell proliferation[13]. The rationale of the design was that the synthesized compounds could act as a competitive inhibitor of PABA.

In this work we report the study of intermolecular interactions between the prepared compounds with DHPS to develop better understanding of the molecular interactions, so that in the future we can design compounds possessing better anti-microbial activities.

2. Methods

2.1. Synthesis of chalcone derivatives

The target molecules (compound 1-7) were synthesized using the Claisen-Schmidt reaction as reported by Suwito *et al.*[12] Structure characterization of the prepared compounds was based on spectroscopic evidence.

2.2. Antimicrobial activity assay

Seven prepared compounds were tested for their antimicrobial activities against *Escherichia coli* ATCC 25923, *Staphylococcus aureus* ATCC 25922 and *Candida albicans* ATCC 10231. The antimicrobial test was performed using the disc diffusion method and the diameter of the inhibition zone was observed. The data obtained were then analyzed statistically using the Kolmogorov-Smirnov test and the Kruskal-Wallis test. Sulfadiazine and sulfamerazine were used as positive controls.

2.3 Software and program

The ligand structures were drawn in ChemBioDraw Ultra 11.0. DS visualizer 2.5 (Accelerys, Inc., USA) and PyMOL (DeLano Scientific LLC, USA) were used to modify the ligand and to visualize the receptor structure and docking results. The preparation of the DHPS *pdbqt* file and determination of the grid box size and position were carried out using AutoDock Tools version 1.5.6. AutoDock 4 was the sole docking program employed in this work[15].

2.4 Preparation of the receptors and ligand structure

The three-dimensional structure of DHPS of *E. coli* complexed with sulfanilamide was retrieved from the Protein Data Bank [PDB:1AJ0], while the three-dimensional structure of DHPS of *S. aureus* complexed with 6-hydroxymethylpterin-diphosphate was downloaded from Protein Data Bank [PDB:1AD4]. The DHPS structure of *E. coli* was prepared for molecular docking by removing the sulphate ions and saved as a *pdb* file for the docking experiment. The DHPS three-dimensional structure of *S. aureus* was prepared for the docking experiment by removing K^+ and Mn^{2+} ions and then saved as *pdb* file for molecular docking. The ligand structures were drawn using ChemBioDraw Ultra 11.0, subsequently modified using DS visualizer 2.5 and saved as a *pdb* file.

2.5 Docking experiment

Molecular docking was carried out using AutoDock 4[15]. AutoDock Tools 1.5.6 was utilized to prepare the input *pdbqt* files of DHPS of *E. coli* and *S. aureus* and to set the size and center of the grid box. The *E. coli* DHPS binding site was set at 41.903 x 8.143 x 2.045 Å, while the *S. aureus* binding site was set at 33.106 x 8.125 x 41.335 Å in the dimensions of x, y, z using 1.000 Å spacing for *E. coli* and 3.75 Å spacing for *S. aureus*. The *pdbqt* input file required for AutoDock4 was prepared by AutoDock Tools 1.5.6. The predicted binding affinity (kcal/mol), which describes the binding strength between ligand and receptor, is calculated based on the scoring function employed in AutoDock4. A more negative binding affinity indicates stronger binding. The docking experiment was performed according to the procedures provided by the AutoDock4 protocol.

3. Results and discussion

3.1. Antimicrobial activity

The compounds used in this work were methoxy-4'-amino chalcone derivatives, the molecular structures of which are displayed in Table 1. Their synthesis and structure characterization have been reported by Suwito *et al.*[12]

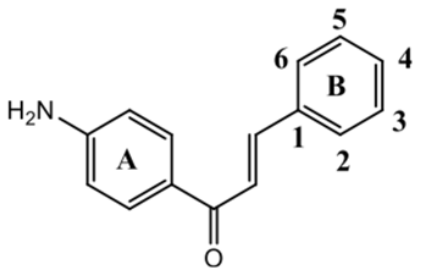

Table 1. Structure of the tested chalcones

Comp	Ring B					
	1	2	3	4	5	6
1	-	OMe	-	-	-	-
2	-	-	OMe	-	-	-
3	-	-	-	OMe	-	-
4	-	OMe	OMe	-	-	-
5	-	OMe	-	OMe	-	-
6	-	OMe	-	-	OMe	-
7	-	-	-	-	-	-

The data of antimicrobial tests of the entire compounds against *E. coli* ATCC 25923, *S. aureus* ATCC 25922, and *C. albicans* ATCC 10231 have been reported.[14] Dose dependence of the antimicrobial activity of the tested compound was observed. The higher the concentration of the tested compound, the larger the inhibition zone diameter.[14] The effect of the substituent of the prepared chalcone derivatives without considering the variation of concentration toward its activity was studied and is reported in this article. Based on the Kolmogorov-Smirnov test, it was shown that the diameters of the inhibition zones were not normally distributed ($\alpha = 0.05$). The test was then followed with the Kruskal-Wallis test. The results of the tests are presented in Fig. 1.

Based on the analysis using the Kruskal-Wallis test toward *E. coli*, sulfadiazine showed the strongest inhibition activity, followed by sulfamerazine, compound 7 and then compound 4. However, the inhibition activity of compound 7 did not differ significantly from sulfamerazine and compound 4. Statistical analysis of the antimicrobial test toward *S. aureus* showed that compound 4 exhibited the strongest activity. However its activity did not differ significantly from sulfamerazine. The inhibition activity of compounds 7, 1, 5, 3 and 2 also did not differ significantly from sulfadiazine. The inhibition activity analysis toward *C. albicans* showed that sulfadiazine possessed the strongest activity, although its activity did not differ significantly from compounds 5, 3 and 4. Structure-activity relationship analysis employing Kruskal-Wallis test gave us information that the amino group played an important role in the antimicrobial activity, while the methoxy group on ring B played a less significant role. Among the tested compounds, compound 4, (*E*)-1-(4-aminophenyl)-3-(2,3-dimethoxyphenyl)prop-2-en-1-one, exhibited a promising wide spectrum of antimicrobial activity as strong as the positive controls.

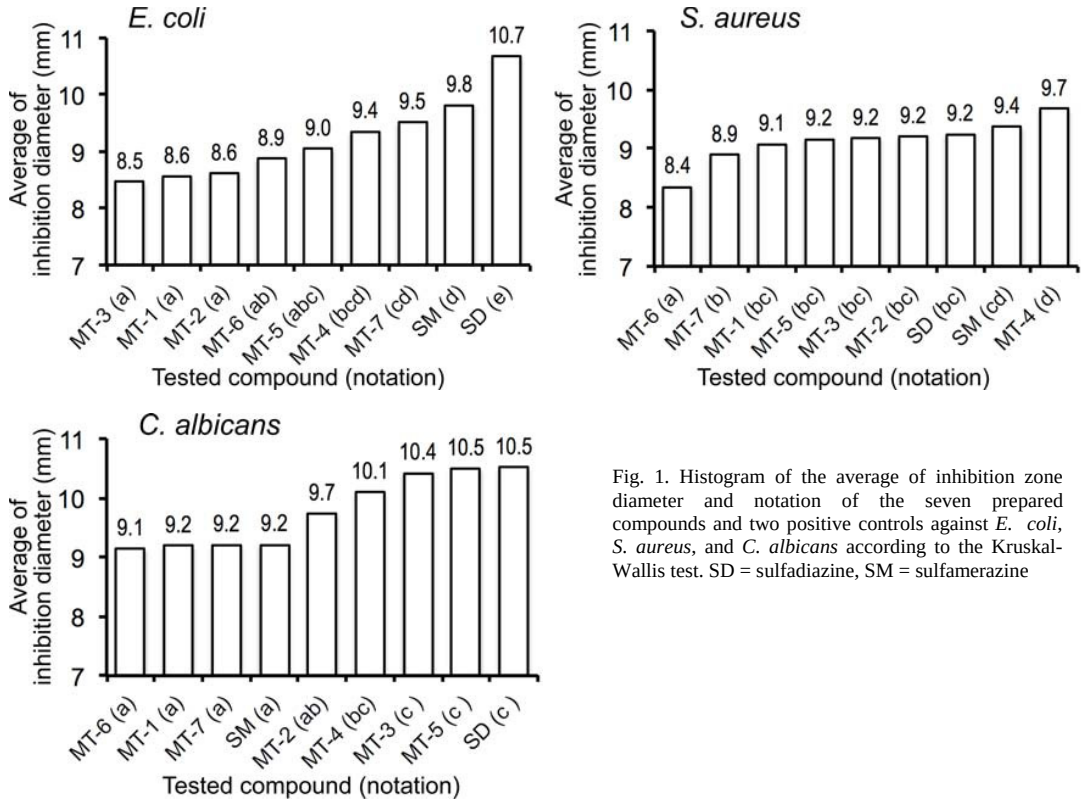

Fig. 1. Histogram of the average of inhibition zone diameter and notation of the seven prepared compounds and two positive controls against *E. coli*, *S. aureus*, and *C. albicans* according to the Kruskal-Wallis test. SD = sulfadiazine, SM = sulfamerazine

3.2. In silico analysis

This article discusses the molecular interaction between DHPS of *E. coli* and *S. aureus* with the title compounds, as determined by in silico analysis, while the interaction with DHPS of *C. albicans* could not be discussed because the three dimensional structure of DHPS of *C. albicans* was not available in the PDB.

3.3. Validation of the docking

In the molecular docking experiment, to ensure that ligand conformations bound correctly with the binding pocket of the target protein, the size and position of the grid box had to be validated. In this work, docking validation was carried out by redocking the co-crystallized DHPS-sulfanilamide complex (PDB:1AJ0)[16] for *E. coli*, and the co-crystallized DHPS-6-hydroxymethylpterin diphosphate complex for *S. aureus* (PDB:1AD4).[17] We found that the binding conformations of both redocked complexes reproduced the binding modes of the co-crystallized complexes with binding affinities –2.91 kcal/mol (RMSD = 1.58 Å) for *E. coli*, and –7.33 kcal/mol (RMSD = 0.83 Å) for *S. aureus*.

According to Achary *et al.*[16], the 7,8-dihydropterin pyrophosphate substrate bound in a deep cleft in the barrel, while sulphanilamide bound closer to the surface. Precisely, the sulphanilamide in the *E. coli* DHPS binding site was sandwiched between the main chain of Arg220 and the side chain of Lys221 on one side and the side chain of Arg63 on the other. The sulphonamide NH_2 formed a hydrogen bond to the carbonyl of Ser219, while sulphonamide oxygen accepted a hydrogen bond from the guanidinium of Arg63. Our redocking experiment provided the following observation: the sulphanilamide was flanked between the side chain of Lys221 and the main chain of Thr62. The hydrogen of aminophenyl moiety of sulphanilamide NH_2 donated a hydrogen bond with the carbonyl of Thr62, while the hydrogen of sulphonamide donated a hydrogen bond with the carbonyl of Ser219. Hydrophobic interactions were observed between sulphanilamide and Thr62, Phe190, Arg220, and Lys221.

According to Hampele *et al.*[17], the interactions of hydroxymethylpterin pyrophosphate with amino acid residues of the *S. aureus* binding site occurred through polar and hydrophobic interactions. Amino acid residues involved in the polar interactions with the pterin moiety of the ligand were Asp167, Asn103, Lys203 and Asp84. The primary amine on C-2 and the protonated nitrogen at N-3 donated hydrogen bonds to the carboxylate oxygen atom of Asp167. Again the 2-NH_2 group donated a hydrogen bond with the side-chain oxygen of Asn103, while the NH group of Asn103 built a hydrogen bond with N-1 of the pterin. Lys203 donated its hydrogen atom to form a hydrogen bond with the oxygen atom of the carbonyl group of the pterin and with the N-5. Asp84 donated a hydrogen bond to the N-8 of the pterin moiety. Our redocking experiment conferred the following observation: the interactions observed between *S. aureus* DHPS binding site with 6-hydroxymethylpterin diphosphate are explained as follows: 6-hydroxymethylpterin diphosphate built hydrophobic interactions with Ile9, Asn11, Arg52, Asp84, Asn103, Met128, Asp167, Phe172, Lys203, Arg239 and His241, while hydrogen bonds existed between the pyrophosphate moiety and four amino acid residues, which were Arg52, Lys203, Arg239, and His241; Arg52 with 4-OH; Asp167 with NH_2, Asn103 with 1-N-pterin; Arg52 with 4-N-pterin; and Asp84 with H of the N-8-pterin moiety.

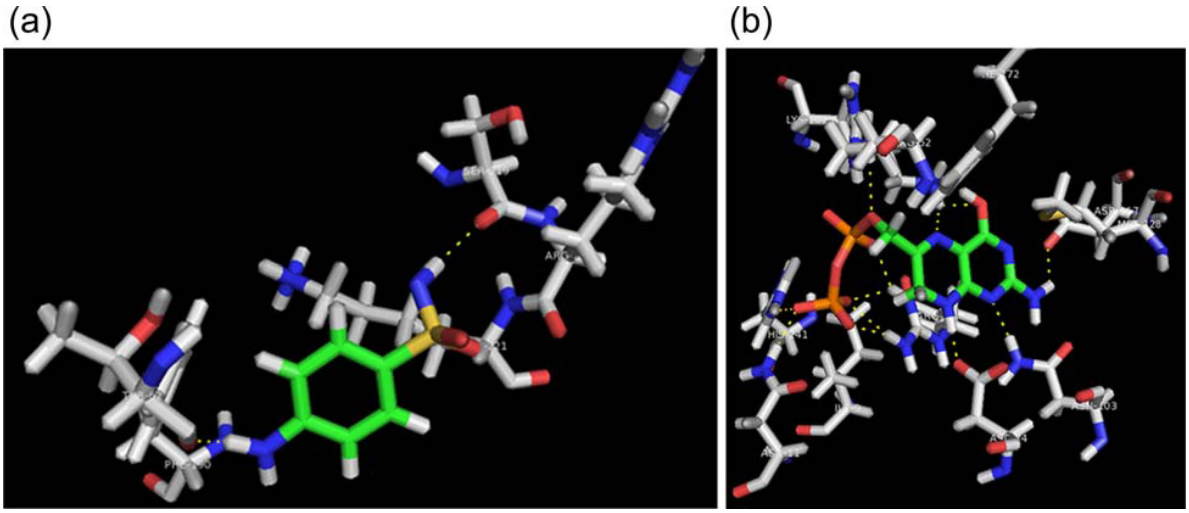

Fig. 2. Redocking of sulphanilamide into *E. coli* DHPS (a) and 6-hydroxymethylpterin diphosphate into *S. aureus* DHPS (b). The docking poses of the ligand are shown in green carbons. Residues with hydrophobic contacts with sulphanilamide and 6-hydroxymethylpterin diphosphate are labelled in grey while hydrogen bonds are shown in yellow with dashed lines.

3.4. Docking of the title compounds

In this work we performed molecular docking on 9 compounds, which were 7 derivatives of methoxy-4'-amino chalcone, sulfadiazine, and sulfamerazine. The results presenting the docking poses are displayed in Fig. 2, while the docking results and molecular interactions between tested compounds with amino acid residues of DHPS binding site are tabulated in the Table 2.

General observation of the docking results provided that the tested chalcone derivatives exhibited preponderant affinity with DHPS of *S. aureus* than with *E. coli* (based on the data of binding affinity). Three dimensional structural complementarities between the protein binding site and the ligands is also one of the important factors determining the binding affinity. Based on the sequence alignment of DHPS of *E. coli* and *S. aureus*, only 36% of homology was identified. This results provided that DHPS of both microbes were similar but not identical (Fig. 3), and the three dimensional structures of their binding sites differed consequently. In addition, all the tested compounds were bound in the deep cleft of the binding site, which was in accordance with the binding location of hydroxymethylpterin pyrophosphate. This information gave us an understanding that the prepared compounds, which were designed as competitive inhibitors of PABA, were more suitable to dock into a hydroxymethylpterin pyrophosphate binding location than into a sulphanilamide binding location.

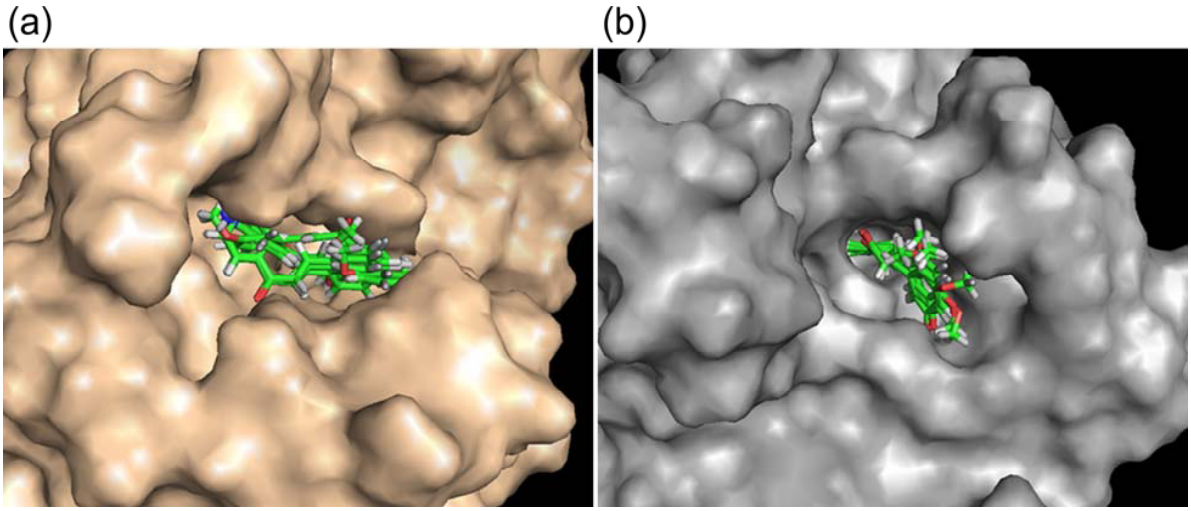

Fig. 3. Docking poses of the tested compounds in the DHPS binding site, (a) *E. coli*, (b) *S. aureus*.

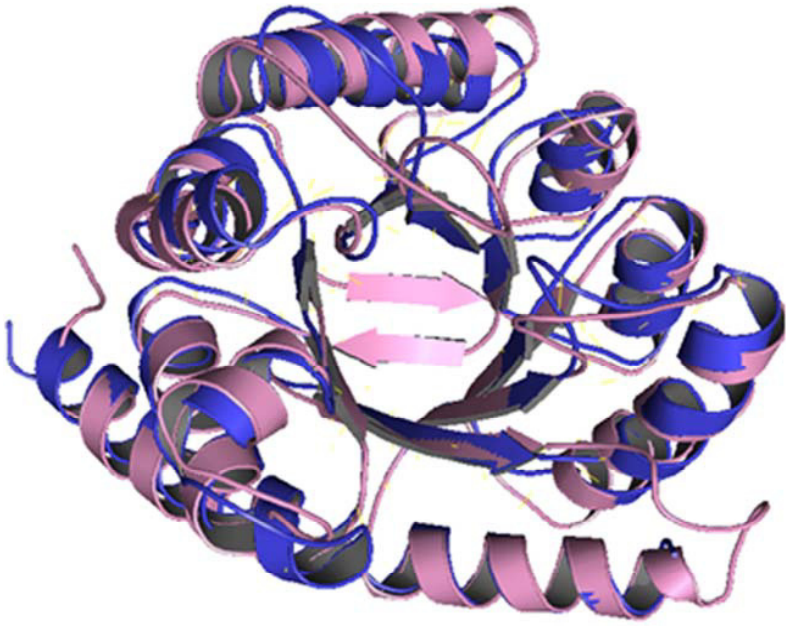

Fig. 4. Superimposed 3D-structure between DHPS of *E. coli* (pink) and *S. aureus* (blue).

In this article we discuss the docking results of compounds 4, 2 and 7. The reasons for the choice of these compounds were shown by the antimicrobial test, in which compound 4 (a dimethoxy-4'-amino chalcone derivative) displayed good potential to be used as a wide spectrum antimicrobial agent, compound 2 representing a monomethoxy-4'-amino chalcone derivative, and compound 7 representing 4'-amino chalcone derivative.

The factors considered for the scoring function in AutoDock4 were van der Waals interactions, electrostatic interactions, hydrogen bonds, desolvation, and rotations[16]. Besides hydrogen bonds, from the docking experiment we also obtained data about van der Waals and electrostatic interaction, which are not displayed in the Table 2.

Table 2. Docking results and molecular interactions between tested compounds with amino acid residues of DHPS.

Comp	DHPS *E. coli* ΔG (kcal/mol)	Hydrogen bond interactions	DHPS *S. aureus* ΔG (kcal/mol)	Hydrogen bond interactions
NS	-2.91	Thr62, Ser219	-7.33	Asn11, Asp84, Asn103, Lys203, Arg239, His241
1	-3.13	Pro145	-6.23	Asn103, Asp167, Lys203
2	-2.19	Thr62, Pro145	-6.48	Asn103, Asp167, Lys203
3	-1.05	Thr62, Pro145	-6.19	Asn103, Asp167, Lys203
4	-2.05	Thr62, Pro145	-6.10	Asn103, Asp167, Lys203
5	-1.09	Thr62, Pro145	-6.11	Asn103, Asp167, Lys203, Arg239
6	-0.66	---	-6.47	Asn103, Asp167, Lys203
7	-3.57	Pro145	-6.23	Asn103, Asp167, Lys203
SD	-3.25	Thr62, Arg63	-6.31	Val49, Asp84, Lys203
SM	-3.12	Thr62, Arg63	-6.08	Asn103, Asp167, Arg239

SD = sulfadiazine, SM = sulfamerazine, NS = natural substrate (as in PDB).

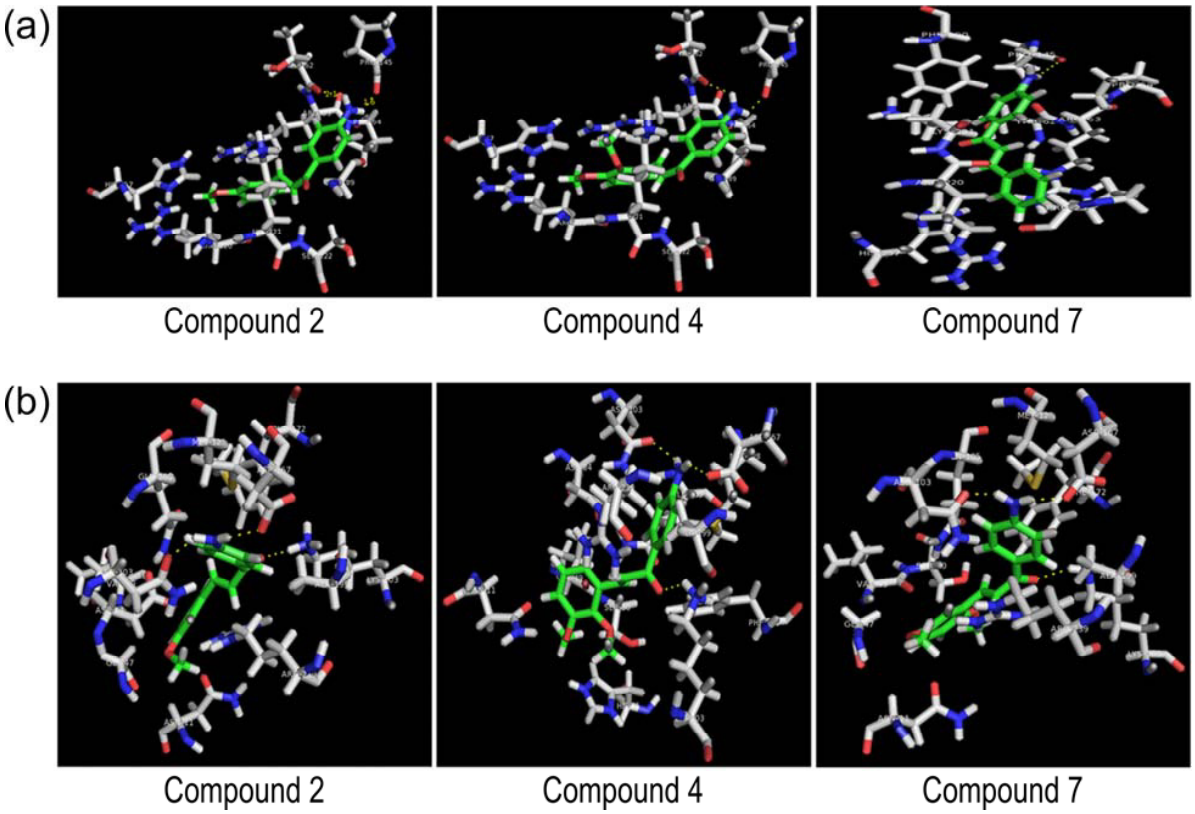

Fig. 5. Docking poses and intermolecular interactions of compound 2, 4 and 7 in DHPS binding sites. (a) *E. coli*, (b) *S. aureus*. The docking poses of the ligand are shown in green carbons. Residues with hydrophobic contacts with tested compounds are labelled in grey while hydrogen bonds are shown in grey with dashed lines.

3.4.1. Docking into E. coli DHPS (Figure 4a)

Compound 2: The location of ring A of compound 2 was flanked by the side chain of Gly189 and Arg63, whilst the ring B was located near the side chain of Lys221. The primary amino group of compound 2 donated hydrogen bonds to the carbonyl groups of Thr62 and Pro145. The position of the methoxy group was pointing to the

guanidinium moiety of Arg220. The amino acid residue Thr62, Arg63, Pro64, Pro145, Gly189, Arg220, Lys221, Ser222, and His257 contributed to hydrophobic interactions.

Compound 4: The position of ring A of compound 4 was flanked by the side chain of Arg63 and Pro64, whilst ring B was near the side chain of Lys221. The primary amino group of compound 4 made a bifurcated hydrogen bond with the carbonyl groups of Thr62 and Pro145. One methoxy group pointed to the guanidinium moiety of Arg63 and another pointed to the guanidinium moiety of Arg220. Amino acid residues Thr62, Arg63, Pro64, Pro145, Gly189, Arg220, Lys221, Ser222 and His257 contributed to hydrophobic interactions.

Compound 7: The side chain of Phe190 and Arg63 flanked the ring A of compound 7, while ring B was flanked by the side chains of Pro232 and Arg220. The primary amino group of compound 7 donated a hydrogen bond to the carbonyl group of Pro145. The amino acid residues involved in the formation of hydrophobic interactions were Thr62, Arg63, Pro64, Pro145, Phe190, Arg220, Lys221, Pro232 and His257.

3.4.2. Docking into S. aureus DHPS (Figure 4b)

Compound 2: The ring A of compound 2 was in a twisted conformation relative to ring B and was flanked by the side chains of three amino acid residues, Gln105, Asp167, and Ala199, whilst ring B was flanked by Asn103 and Arg239. The methoxy group pointed to the side chain of Asn11. The primary amino group of compound 2 made a bifurcated hydrogen bond with the carbonyl group of side chains of Asp167 and Asn103, while the carbonyl group of compound 2 acted as a hydrogen acceptor in the hydrogen bond with the amino group of the side chain of Lys203. Non-polar interactions were contributed by Asn11, Gly47, Val49, Asp84, Asn103, Gln105, Met128, Asp167, Phe172, Ala199, Lys203 and Arg239.

Compound 4: The ring A of compound 4 was flanked by the side chains of the amino acid residues Gln105, Arg239, and Ala199, whilst the ring B was flanked by Val49 and Ser40. The position of the two methoxy groups was pointing to Asn11 and His55. The amino group of compound 4 donated its two hydrogen atoms to the formation of hydrogen bonds with the side chain carbonyl groups of Asn103 and Asp167, while the carbonyl group of compound 4 acted as a hydrogen acceptor of the side chain amino group of Lys203 in hydrogen bond formation. The following amino acid residues Asn11, Val49, Ser50, His55, Asp84, Asn103, Gln105, Met128, Asp167, Phe172, Ala199, Lys203 and Arg239 contributed to hydrophobic interactions.

Compound 7: The ring A of compound 7 was located in the area flanked by Phe172, Asn103, and Ala199, while ring B was flanked by Val19 and Arg239. The amino group of compound **7** donated hydrogen bonds with the side chain carbonyl group of Asn103 and Asp167. The carbonyl group of compound **7** acted as a hydrogen acceptor of the side chain amino group of Lys203 in hydrogen bond formation. The hydrophobic interactions were contributed by the following amino acid residues: Asn11, Val49, Asp84, Asn103, Gln105, Met128, Asp167, Phe172, Ala199, Lys203 and Arg239.

The docking results showed that the amino acid residues involved actively in the molecular interaction from the docking experiment were in accordance with the results of protein-ligand complexes that were co-crystallized.

4. Conclusions

The antimicrobial activities of the methoxy amino chalcone derivatives have been assayed and their molecular interaction with the *E. coli* and *S. aureus* DHPS were studied with a docking experiment using the AutoDock4 program. Compound 4, (*E*)-1-(4-aminophenyl)-3-(2,3-dimethoxyphenyl)prop-2-en-1-one), exhibited a promising wide spectrum antimicrobial activity. The docking experiment gave us information that the prepared compounds were docked in the binding location of hydroxymethylpterin pyrophosphate in the DHPS binding site.

Acknowledgment

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