Original article

Anti-quorum sensing and anti-biofilm activity of Amomum tsaoko (*Amommum tsao-ko* Crevost et Lemarie) on foodborne pathogens

Md Ramim Tanver Rahman, Zaixiang Lou, Fuhao Yu, Peng Wang, Hongxin Wang

PII: S1319-562X(15)00229-6
DOI: http://dx.doi.org/10.1016/j.sjbs.2015.09.034
Reference: SJBS 570

To appear in: *Saudi Journal of Biological Sciences*

Received Date: 2 June 2015
Revised Date: 27 September 2015
Accepted Date: 27 September 2015

Please cite this article as: M.R.T. Rahman, Z. Lou, F. Yu, P. Wang, H. Wang, Anti-quorum sensing and anti-biofilm activity of Amomum tsaoko (*Amommum tsao-ko* Crevost et Lemarie) on foodborne pathogens, *Saudi Journal of Biological Sciences* (2015), doi: http://dx.doi.org/10.1016/j.sjbs.2015.09.034

Anti-quorum sensing and anti-biofilm activity of Amomum tsaoko (*Amommum tsao-ko* Crevost et Lemarie) on foodborne pathogens

Md Ramim Tanver Rahman[a,b], **Zaixiang Lou**[*a, b, c], **Fuhao Yu**[a, b], **Peng Wang**[a, b], **Hongxin Wang**[a, b]

[a]*State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, Wuxi 214122, P.R.China*

[b]*Research Center for Food Nutrition & Functional Factors, School of Food Science and Technology, Jiangnan University, Wuxi 214122, P.R.China*

[c]*Department of Food Science and Technology, University of California, Davis, USA*

[*]Corresponding author:

louzaixiang@126.com ; + 8651085917795; fax: + 8651085876799;

Anti-quorum sensing and anti-biofilm activity of Amomum tsaoko (*Amommum tsao-ko* Crevost et Lemarie) on foodborne pathogens

Abstract : Cell-to-cell communication or quorum sensing （QS) leads to biofilm formation and causing other virulence factors which are extreme problem for food safety, biofilm related infectious diseases etc. This study evaluated the anti-QS activity of the *Amomum tsaoko* extract (0.5 - 4 mg/ml) by using *C. violaceum* a biosensor strain and biofilm formation by crystal violate assay. Experimental results demonstrated that the overall yield of *Amomum tsao-ko* extract was 11.33±0.3 % (w/w). MIC for *Staphylococcus aureus* (Gram positive), *Salmonella* Typhimurium *and Pseudomonas aeruginosa* (Gram negative) were 1, 2 and 2 mg/ml respectively. A concentration of 4 mg/ml extract showed highest biofilm inhibition 51.96 % on *Salmonella* Typhimurium when 47.06%, 45.28% were showed by *Staphylococcus aureus, Pseudomonas aeruginosa* respectively. The damage of biofilm architecture were observed by Confocal laser scanning microscopy(CLSM). A level of 44.59% inhibition of violacein production was demonstrated when the dose was 4 mg/ml. Swarming motility inhibition were observed on dose dependent manner. Taken together, the treatment of *Amomum tsaoko* extract can deliver value to food product and medicine by controlling pathogenesis.

Keywords: Amomum tsaoko, biofilm, drug, food spoilage, quorum sensing.

Introduction

Amomum tsao-ko (Zingiberaceae) is commonly known as “Black Cardamom” or “Tsaoko Amomum” which is grown in China (Guangxi, Guizhou, Yunnan), North Vietnam and some other parts of Asia (Delin W. and Larsen K., 2000). It is widely used as Chinese Traditional

Medicine in the treatment of hemorrhoids, windy, vomiting, diarrhea, malaria, throat infections, stomach disorders, dyspepsia, nausea, and abdominal pain and also in many dishes e.g. Chinese medicinal soup dish, beef herbal dish, hotpot etc. (Feng et al., 2010 ; Wu et al. 2012; Lim, 2013). As it has commercial value, some other fruits of this genus are being mixed with *Amomum tsao-ko* (*A. tsaoko*) as adulterants. *A. tsaoko* is being over-harvested and has been declared as "Near Threatened" species by the International Union for Conservation of Nature (IUCN) (Leong-Skornickova et al., 2012). In past, a lots of studies have been done on its anti-cancer, anti-inflammatory, antioxidant, anti-microbial activities and anti- Hepatitis B effect(Lim 2013) but the anti-QS and biofilm inhibition activities are not yet been investigated.

Bacterial spoilage of food and other infections are a carefully orchestrated process controlled by QS (cell to cell communication mechanism through gene expression) - which is regulated by the activity (production / detection) of small molecules called autoinducers (AIs). When the environmental concentration of AIs (correlated to bacterial population density) reaches a certain threshold, the QS is activated. The expression of a variety of genes is modulated by the AI receptors which finally control the behavior of virulence factors expression - biofilm formation, bioluminescence, sporulation, conjugation and swarming motility (Annous et al., 2009 ; Skandamis and Nychas , 2012 ; Solano et al., 2014 ; Wu et al., 2014).

Biofilms are considered as sources of infections and provide safety challenges in a wide range of food factories including dairy processing, sea food processing, poultry and meat processing; hence a critical hindrance to the food preservation system. The biofilm allows bacteria to grow in a rhythmic way into a extracellular polymeric network without any environmental depression

even protecting them from antimicrobials and attack from the immune system (Srey et al., 2013 ; Solano et at., 2014).

Considering the impact of QS and biofilm, several strategies (cleaning agents , disinfectants, CIP, ultrasonication, phages, irradiation, mixed vibriophages, $scCO_2$, essential oil etc.) have been developed in the food factory and in drug discovery (pharmaceuticals) (Srey et al., 2013). But to minimize the food spoilage even developing of new edible packaging materials, removal of biofilm from membrane filter of water treatment/pipe lines and to prevent the bacterial infection in human, we have to find the natural, healthy and "generally regarded as safe"(GRAS) anti-QS and anti-biofim agent. Traditional medicinal plants are popular for their new bioactive compounds and therapeutic values. So we have made an attempt to find the activity of *A. tsao-ko* which could potentially be used as an *de novo* natural anti QS and anti biofilm inhibitor or natural chemotherapeutic agent.

2 Materials and method

2.1 Plant material

Dried *A. tsaoko* (Black Cardamom) fruits were purchased from nearest supermarket (Auchan, wuxi, China). Fruits were authenticated by Dr. Jian Tang (Professor, Jiangnan University, Wuxi, China), a voucher with specimen no. JNU201409S05 has been deposited at herbarium of Food Nutritional and Functional Factors Research Center of Jiangnan University, Wuxi, China. Then, fruits were well cleaned and washed with water, dried in the shade at room temperature. After this period, *A. tsaoko* has been grinded (FW100, Tai Si Te Instrument Com.

Ltd., Tianjin, China) and transformed to powder. The powders were preserved in cleans, sealed plastic containers and kept away from light, heat and moisture until use.

2.2 Preparation of extract and % of yield

For extraction, 100g of powdered *A. tsaoko* were blended (JJ-1, JianTan city Antoulin Feng Equipment Co. Ltd, China) with 1:20 solvents (ethanol: water = 80 : 20) for 3 h with agitation at 45°C. After that, the extracts were taken out and vacuum filtered using a Buchner funnel through Whatman (No. 1) filter paper. Again same process was followed for second and third time extractions. Then, the extracts were concentrated using a vacuum rotary evaporator (BC-W203, Shanghai Biochemical Equipment Co. Ltd, China). Following vacuum drying (DZF-650 Shanghai Yi Heng Sci. Equipment Co. Ltd, China) at 40°C, the extracts were scraped out, weighed to obtain percentage of yield and stored in desiccator.

2.3 Strains and Culture Conditions

Staphylococcus aureus (ATCC 6538), *Salmonella* Typhimurium (ATCC 50013), *Pseudomonas aeruginosa* (ATCC 9027) were purchased from China General Microbiological Culture Collection Center (Beijing, China). *Choromobacterium violaceum* ATCC 12472 was purchased from Spanish Type Culture Collection (CECT-494), Paterna (Valencia) . They were cultured as per recommended procedure from the purchase center.

2.4 Bioassay for Anti-Quorum Sensing

2.4.1 Preliminary screening (Disk diffusion assay)

To evaluate the anti QS capacity of the *A. tsaoko* extract, the qualitative disk diffusion method was used as previously published procedure (Khan et al., 2009). An aliquot of 100 µl fresh *C. violaceum* culture dilution was plated on Luria-Bertani agar (CM159, Beijing Land Bridge Technology Co. Ltd, China) Petri dishes (LB broth supplemented with 1.5% bacteriological agar). Sterile paper disks (Whatman No. 40; 6.0 mm in diameter) with *A. tsaoko* extract (10 µl extract/ disk) were plotted above the agar. The Petri dishes were incubated at 30 ^{o}C for 30 h. Results were determined by observing the growth inhibition colorless ring diameter, opaque circle around the extract loaded paper disk and around positive control (vanillin, 250 µg/ml from Sino Pharma Chemical Reagent Co. , China) loaded disc. Negative control were LB broth loaded paper disk.

2.4.2 Flask incubation assay for quantification of violacein production

With slight modifications, Truchado et al.'s (2012) previously described procedure was used to quantify the anti-QS activity of *A. tsaoko* extract. *C. violaceum* ATCC 12472 (1×10^{8} CFU/ml) was inoculated in Erlenmeyer flasks containing LB supplemented with *A. tsaoko* extract to obtain different concentrations (0.5 , 1 , 2 , 4 mg/ml). The flasks were incubated at 30°C in a shaking incubator (SPH-1102, Xi'an Heb Biotechnology Co. Ltd, Shaanxi, China) for 30 hours. 1 ml culture of each test tube was centrifuged at 18630 x g (Scilogex D3024R, USA) for 15 min to precipitate the insoluble pigment. The pellet was resuspended in 1 ml of dimethyl sulfoxide (DMSO; Aladdin Industrial Corporation, Shanghai, China) and homogenized by

vortexing. Violacein absorbance at 585 nm was determined using a UV–visible spectrophotometer (Epoch, BioTek Instruments Inc, USA). The control sample consisted of incubating the *C. violaceum* in LB broth (CM158, Beijing Land Bridge Technology Co. Ltd) without adding extract. Inhibition was calculated respect to control and triplicate measurements were performed.

2.5 *Minimum Inhibitory Concentration (MIC) & Anti-Biofilm Assay*

The 30% ethanol elution fraction of *A. tsaoko* (100 µl) which had serial dilutions (0.5, 1.0, 2.0,3.0, 4.0 mg/ml) were added to the wells of a 24-well culture plate (Sarstedt, Newton, NC) containing 800µl of fresh LB broth (CM158, Beijing Land Bridge Technology Co. Ltd) and 100µl bacteria suspension (10^8 cfu/ml). After incubation for 24 h, growth medium in the every well was observed very carefully. The well which is clear than other well to look at confirmed the inhibition of bacterial growth. The clear well containing concentration of extract declared the MIC value for tested bacterial strain. Anti biofilm assay was performed as previously described by Gao et al. (2015) with modification. After following MIC, suspension cultures were removed. The wells were rinsed 3 times with PBS (Sigma, China), fixed by drying for 3h at 37 ^{o}C in incubator. Once the wells were fully dried, 1000 µl of 0.1% crystal violet(Sinopharm chemical reagent co. ltd) stain was added to each well to stain for 15 min. The excess stain was rinsed off with tap water and 1000 µl of 95% (v/v) ethanol was added to each well for 1 h to release the stain. 100 µl from each well was then transferred to a new plate for spectrophotometric (Epoch, BioTek Instruments Inc, USA) analysis (OD570nm). For all the assays, controls without A.

tsaoko fractions and without inoculation were prepared. The procedure was performed in triplicate and the means±SD were calculated.

2.6 CLSM(Confocal Laser Scanning Microscopy) observation

To observe the biofilm structure, samples were prepared according to Zhang et al. (2014a) and CLSM (Leica TCS SP2; Leica Microsystems, Heidelberg, Germany) were used. Cover slips were treated with and without *A. tsaoko* extract. The L13152 LIVE/DEAD® BacLight Bacterial Viability Kit (Molecular Probes, Inc., USA) were used to stain the cover slip according to the manufacturer's instruction. Finally 3D view was performed to visualize the architecture of biofilm, surface coverage of cover slips and the density of bacteria.

2.7 Swarming motility assay

Swarming motility assay were carried out as previously described method by Kuchma et al. (2015). 25 µl of molten soft top agar (3% agar, 1% tryptone, 5% yeast extract powder, 5% sodium chloride, deionized water) was prepared containing 0.5 mg/ml, 1 mg/ml, 2 mg/ml and 4 mg/ml *A. tsaoko* extract. Then, poured it immediately over the surface of a solidified LB agar plate as an overlay. The center of plate was inoculated with an overnight culture of different tested bacterium. Then plates were incubated at 37 ^{o}C. After 30 h, swimming and swarming migration zones of the bacterial cells were observed. The control plates were prepared without *A. tsaoko* extract. Photographs were taken using iPhone 4s from the same distance of each disc and picture contrasts were adjusted.

2.8 Statistical analysis

The results were analyzed statistically by ANOVA using IBM SPSS Statistics 20.0. , OriginPro 9.0 ; $P < 0.05$ was considered to indicate statistical significance.

3. Results

3.1 % yield of Amomum tsao-ko

We selected methanol as solvent for extraction. The extract yields from the dried fruit of *A. tsaoko* were 11.33±0.3 % .

3.2.1 Preliminary screening (qualitative agar diffusion assay)

Colorless, opaque circle were observed (**Figure 1**) around the extract (concentration 4 mg/ml) loaded disk. Negative control (LB broth loaded disk) did not show any inhibition when the positive control showed very clear, visible inhibition.

3.2.2 Flask incubation assay for quantification of violacein production:

Loss of purple pigmentation in *C. violaceum* 12472 on the presence of tested extract is an indication of QS inhibition. The inhibitory effect of the *A. tsaoko* extract on violacein pigment production was measured and quantified (**Supplementary 1**). A concentration dependent but not proportional inhibition were found (**Figure 2**). A concentration of 4 mg/ml extract inhibited

about 44.59% violacein production which is about 4 times higher inhibition than 2 mg/ml concentration's inhibition (10.81% inhibition).

3.3 MIC and Anti-Biofilm Assay

The minimum concentration that cause a decline in growth of bacteria is considered to be the MIC value and in this experiment it was evaluated through careful visual inspection. Biofilm is the product of quorum sensing mechanism. In the present study, the anti-biofilm effect was determined through crystal violet assay. The results are summarized in **Table 1.** *Salmonella* Typhimurium and *Pseudomonas aeruginosa* presented higher MIC values than *Staphylococcus aureus*. The obtained results for biofilm showed a concentration-dependent inhibition. The highest (51.96 %) anti-biofilm activity showed by 4 mg/ml *A. tsaoko* extract on *Salmonella* Typhimurium.

3.4 CLSM(Confocal Laser Scanning Microscopy) observation of biofilm

A. tsaoko extract treatment were applied on *Staphylococcus aureus*, *Salmonella* Typhimurium, *Pseudomonas aeruginosa* biofilm. From control and treated sample observation(**Figure 3.**), it is clear that *A. tsaoko* extract has effect on biofilm inhibition(break down the biofilm architecture, less microcolonies).

3.5 Swarming motility inhibition

Swarming motility has been characterized as remarkable feature to formation of biofilm which is flagella-dependent movement of bacterium. **Figure 4** indicated that *A. tsaoko* has significant influence on the swarming motility by dose dependent manners. Best anti-swarming activities were showed by 4 mg/ml extract on *Salmonella* Typhimurium and *Staphylococcus aureus.* But in the same extract's concentration (4 mg/ml) *Pseudomonas aeruginosa* showed less swarming inhibition activity.

4. Discussion

The present study focused on the anti-QS and biofilm inhibitory effect of *A. tsaoko* extract. The % yield of extraction is very important on commercial prospect. Usually, plant extract contains biologically active compounds at low concentration. Minimal changes to the functional properties of the extract depend on extraction techniques. The percentage of extraction yields will increase or decrease with the ratio of solvent, temperature of extraction and extraction methods (green extractions, microwave assisted solvent extraction , ultrasound etc.). Crude extracts of *Alpinia conchigera* a species from the Malaysian Ginger (Zingiberaceae) family showed 10.76% extractions yield when the ratio of powdered rhizomes and ethanol was 1:10 (w/v) (Ujang et al., 2013). This is consistent with our result. Our study showed that *A. tsaoko* extract, similar to *Eleutherine americana Merr.* (*Iridaceae*), *Rhodomyrtus tomentosa (Aiton) Hassk. (Myrtaceae)* extract (Limsuwan and Voravuthikunchai, 2008) , inhibited the production of violacein both in agar diffusion assay and flask incubation assay. but *Boesenbergia pandurata (Roxb.) Schltr* another family member of Zingiberaceae (chloroform extracted) did not show any inhibition activity. Kumar et al., (2014) observed that phenolic derivatives of ginger (

member of Zingiberaceae family) can inhibit QS in pathogenic bacteria and zingerone showed 35% inhibition, [6]-gingerol showed 52.5% inhibition at concentration 500ppm. As an effect of *A. tsaoko* extract, *Salmonella* Typhimurium and *Pseudomonas aeruginosa* showed higher MIC values than *Staphylococcus aureus* (**Table 1**). Yang et al.(2008) found the essential oil of *A. tsaoko* has strongest bactericidal activity against *Staphylococcus aureus*, with MIC and MBC of 0.20 g/l. This was due to that we have used the crude extract of *A. tsaoko* but Yang et al.(2008) used the essential oil.

The butanol fractions of both leaf and stem/root of *Quercus cerris L.* (Fagaceae) exhibited the biofilm inhibition of *Staphylococcus aureus* about 63± 10% and 74±4% respectively at a test dose of 200 mg/ml (Hobby et al., 2012) . This concentration is higher than our study. An interesting mechanism which interferes with biofilm formation *in S. aureus* involves the heptapeptide RIP (receptor-interacting protein) kinases. This peptide inhibits biofilm formation of *S. aureus* in vivo, possibly by blocking the *agr* (accessory gene regulation)-dependent QS system (Landini et al.,2010). Segev-Zarko et al. (2015) suggested that decreased biofilm growth are due to the peptide's ability to coat either the biomaterial surface or the bacterium itself. *Rosa rugosa* tea polyphenol damaged the biofilm architecture of *Escherichia coli* K-12 and *Pseudomonas aeruginosa* PAO1 about 67.02% and 72.90% respectively (Zhang et al., 2014a). Our CLSM observation (**Figure 3**) is also similar with them. Flagellar-mediated swimming motility is associated with biofilm formation by instigating the cell-to-surface attachment, and plays an important role in the virulence of pathogens (Wu et al.,2014). *A. tsaoko* extract reduced the swarming motility zone of *Salmonella* Typhimurium, *Staphylococcus aureus* and *Pseudomonas aeruginosa* (**Figure 4**). It might be due to the effect of phytochemicals of *A.*

tsaoko on flagella-related processes, namely, flagella biosynthesis, rotation, rafting and chemotaxis etc. Interestingly Tremblay and Deziel (2008) reported that swarming motility assays of *P. aeruginosa* is influenced by incubation temperature , %agar , p^H , and drying time under laminar flow. In this study, we used molten soft top agar for all experiments about swarming motility.

Through a phytochemical investigation on *A. tsaoko* extract by Zhang et al. (2014b), eight main chemical components (sitosterol, daucosterol, *meso*-hannokinol, quercetin, epicatechin, quercetin-7-O-β-glucoside, quercetin-3-O- β -glucoside, and catechol) were isolated. GC/MS analysis showed seventy-three compounds mainly containing 1,8-cineole (45.24%), *ρ*-propylbenzaldehyde (6.04%), geraniol (5.11%), geranial (4.52%), α-terpineol (3.59%) and α-phellandrene (3.07%) from the essential oil of *A. tsaoko* fruit. Now it is well established that most of these compounds have antimicrobial (more or less) and even antifungal activity (Martin et al., 2006; Yang et al., 2008; Yang et al., 2010; Zhang et al., 2014b). Interestingly, studies associated with some natural products have shown that plant extracts with poor antimicrobial properties could also be effective against bacterial biofilms (Upadhyay A., 2014). Plant extract(major or minor components) inhibit prokaryotic and eukaryotic microorganisms with different action mechanism (Evren et al., 2015). Without further molecular analysis, it is hard to explain which components are responsible for such inhibition mechanism.

In clinical and food borne pathogenesis biofilm associated infection are known as trigger to chronic diseases, food spoilage and leads to billions of dollars in healthcare, food industry cost. Even dairy spoilage, refrigerated food spoilage also created by the bacterial biofilm related enzyme (Teh et al., 2014; Mizan et al., 2015). Developed studies are demonstrating that there is a

biological rational between QS and biofilm which work on a coordinate manner leads to spoilage (Bai and Vittal, 2014). On the other hand, biofilm formation, swarming motility, cellulose synthesis etc are also regulated by newly found second messengers c-di-GMP in Gram positive and Gram negative bacteria. Some constituent/constituents of *A. tsaoko* extract may accelerate the activity of proteins with EAL (Simm et al., 2004) or HD-GYP domains (Ryan et al., 2006) resulting the degradation of c-di-GMP ; some enzymes can also react with this plant extract and reduce the activity of c-di-GPM. If there is a similarity between the QS signals(AHL) and chemical structure of QSI, it will show anti-QS activity. Because they have the ability to degrade the signal receptor (LuxR/LuxR). So tested *A. tsaoko* extract (major & minor components, tsaokoaryline – a cytotoxic diarylheptanoid [7 - (4 – hydroxyl - 3 -methoxyphenyl) -1 - (4 hydroxyphenyl) – hepta -4*E*,6*E*-dien-3-one]) (Moon et al., 2005) may affect the gene expression pathway and inhibit biofilm formation, swarming motility, violacein production etc.

5. Conclusion

Future study is necessary to unveil the detailed mechanism of *A. tsaoko* extract (isolating single compound from crude extract) on c-di-GPM level and mode of actions with proteolytic activity, bioluminescence inhibition etc to confirm the realistic action on vivo research considering symbiotic, synergistic, antagonistic growth inhibition. In this study, we demonstrated the anti QS and anti biofilm activity of *A. tsaoko* extract on Gram positive and Gram negative bacterium by well established methods. These anti-QS and biofilm inhibition evidence can be an alternative to combat with food spoilage, on antibiotic therapy and many other fields where natural compounds are required for human health safety. The results of this study are a

benchmark for the application of *A. tsaoko* extract as a *de novo* anti-QS and anti-biofilm agent. It can be expected more with its marvelous functions.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Acknowledgements

The authors gratefully acknowledge the financial support provided by Project 31201433 of the National Natural Science Foundation of PR China, the Project BK2012555 of Jiangsu Provincial Natural Science Foundation. And we are grateful to Prof. Dr Syed A. Morshed, Mount Sinai School of Medicine, NY 10468,USA for his critical comments and suggestions to improve the manuscript.

References

Annous, B.A., Fratamico P.M., Smith J.L., 2009. Quorum sensing in biofilms: why bacteria behave the way they do. J Food Sci. 74(1): R24-R37.

Bai, A.J., Vittal, R.R., 2014. Quorum sensing inhibitory and anti-biofilm activity of essential oils and theirin vivo efficacy in food systems. Food Biotechnol. 28:269-292.

Delin, W., Larsen,K., 2000. Zingiberaceae. Flora of China. 24:322–377.

Evren, E., Yurt Erkan Y.E., 2015. In vitro effects on biofilm viability and antibacterial and antiadherent activities of silymarin. Folia Microbiol (praha). 60(4):351-6

Feng, X., Jiang, Z.T., Wang, Y., Li R., 2010. Composition comparison of essential oils extracted by hydrodistillation and microwave-assisted hydrodistillation from *Amomum tsao-ko* in china. J Essent Oil Bear Pl .13:286-291.

Gao, T., Foulston, L., Chai, Y., Wang, Q., Losick, R., 2015. Alternative modes of biofilm formation by plant-associated Bacillus cereus. MicrobiologyOpen. 4(3): 452–464

Hobby, G.H., Quave, C.L., Nelson, K., Compadre, C.M., Beenken, K.E., Smeltzer, M.S., 2012. *Quercus cerris* extracts limit *Staphylococcus aureus biofilm formation,* J Ethnopharmacol. 144(3): 812–815.

Khan, M.S., Zahin, M., Hasan, V.S., Husain, F.M., Ahmad, I., 2009. Inhibition of quorum sensing regulated bacterial functions by plant essential oils with special reference to clove oil. Lett Appl Microbiol, 49(3): 354-60.

Kumar, N.V., Murthy, P.S., Manjunatha, J.R., Bettadaiah, B.K., 2014. Synthesis and quorum sensing inhibitory activity of key phenolic compounds of ginger and their derivatives. Food Chem. 15(159)451-457.

Kuchma, N.J.D., Filkins, L.M., Snavely, E.A., Armitage, J.P., O'Toolea, G.A., 2015. Cyclic Di-GMP-mediated repression of swarming motility by Pseudomonas aeruginosa PA14 requires the motab stator. Journal of Bacteriol. **197**(3): p. 420-430.

Landini, P., Antoniani, D., Burgess, J.G., Nijland, R., 2010. Molecular mechanisms of compounds affecting bacterial biofilm formation and dispersal, Appl Microbiol Biotechnol. 86(3):813-23.

Leong-Skornickova, J., Tran, H.D., Newman, M., Lamxay, V., Bouamanivong, S., 2012. Amomum tsao-ko. The IUCN Red List of Threatened Species.

Lim, T.K., 2013. Amomum tsao-ko. Handbook of Herbs and Spices (Second edition) 813-817.

Limsuwan, S., Voravuthikunchai S.P., 2008. Boesenbergia pandurata (Roxb.) Schltr., Eleutherine americana Merr. and Rhodomyrtus tomentosa (Aiton) Hassk. as antibiofilm producing and antiquorum sensing in Streptococcus pyogenes. FEMS Immunol Med Mic. 53:429-436.

Martin, T.S., Kikuzaki, H., Hisamoto, M., Nakatani, N., 2006. Constituents of *Amomum tsao-ko* and their radical scavenging and antioxidant activities. J Am Oil Chem Soc.77:667-673.

Mizan, M.F.R., Jahid, I.K., Ha, S-D.. 2015. Microbial biofilms in seafood: A food-hygiene challenge. Food Micro.49:41-55.

Moon, S.S., Cho,. S.C., Lee, J.Y., 2005. Tsaokoarylone, a cyto-toxic diarylheptanoid from Amomum tsao-ko fruits. Bull. Korean Chem Soc.26(3):447–450

Ryan, R.P., Fouhy, Y., Lucey, J.F., Crossman, L.C., Spiro, S., He, Y.W., Zhang, L.H., Heeb, S., Cámara, M., Williams, P., Dow, J.M., 2006. Cell-cell signaling in Xanthomonas campestris involves an HD-GYP domain protein that functions in cyclic di-GMP turnover. Proc Natl Acad Sci U S A 103: 6712-6717.

Segev-Zarko, L., Saar-Dover, R., Brumfeld, V., Mangoni, M.L., Shai, Y., 2015. Mechanisms of biofilm inhibition and degradation by antimicrobial peptides. Biochem J. 468(2):259-70.

Simm, R., Morr, M., Kader, A., Nimtz, M., Römling, U., 2004. GGDEF and EAL domains inversely regulate cyclic di-GMP levels and transition from sessility to motility. Mol Microbiol. 53: 1123-1134.

Skandamis, P.N., Nychas, G.J., 2012. Quorum sensing in the context of food microbiology. Appl Environ Microb. 78:5473-5482.

Solano, C., Echeverz, M., Lasa, I., 2014. Biofilm dispersion and quorum sensing. Curr Opin Microbiol. 18:96-104.

Srey, S., Jahid, I.K., Ha, S-D., 2013. Biofilm formation in food industries: a food safety concern. Food Control.31:572-585.

Teh, K.H., Flint, S., Palmer, J., Andrewes, P., Bremer, P., Lindsay, D., 2014. Biofilm – An unrecognised source of spoilage enzymes in dairy products? Int Dairy J. 34:32-40.

Tremblay, J., Deziel, E., 2008. Improving the reproducibility of Pseudomonas aeruginosa swarming motility assays. J Basic Microbiol. 48:509-515.

Truchado, P., Gimenez-Bastida, J.A., Larrosa, M., Castro-Ibanez, I., Espin, J.C., Tomas-Barberan F.A., Garcia-Conesa, M.T., Allende, A.. 2012. Inhibition of quorum sensing (QS) in Yersinia enterocolitica by an orange extract rich in glycosylated flavanones. J Agri Food Chem. 12(60) 8885-8894.

Ujang, Z.B., Subramaniam, T., Diah, M.M., Wahid, H.B., Abdullah, B.B., Rashid, A,A, Appleton, D., 2013. Bioguided fractionation and purification of natural bioactives obtained from *Alpinia conchigera* water extract with melanin inhibition activity . J Biomater Nanobiotechnol. 4(3):265-272.

Upadhyay, A., 2014. Investigating the potential of plant-derived antimicrobials and probiotic bacteria for controlling *Listeria monocytogenes*, *Doctoral Dissertations.* Paper 326.

Wu, H., Moser, C., Wang, H.Z., Hoiby, N., Song, Z.J., 2014. Strategies for combating bacterial biofilm infections. Int J Oral Sci. 7(1):1-7.

Wu, M., Guo, P., Tsui, S.W., Chen, H., Zhao, Z., 2012. An ethnobotanical survey of medicinal spices used in Chinese hotpot. Food Res Int.48:226-232.

Yang, Y., Yan, R.W., Cai, X.Q., Zheng Z.L., Zou, G.L., 2008. Chemical composition and antimicrobial activity of the essential oil of *Amomum tsao-ko*. J Sci Food Agr. 88:2111-2116.

Yang, Y., Yue, Y., Runwei, Y., Guolin, Z., 2010. Cytotoxic, apoptotic and antioxidant activity of the essential oil of Amomum tsao-ko. Bioresource Technol. 101:4205-4211.

Zhang, J., Rui, X, Wanga, L., Guana, Y., Sunc, X., Dong, M., 2014a. Polyphenolic extract from Rosa rugosa tea inhibits bacterial quorum sensing and biofilm formation. Food Control, 42: 125-131.

Zhang, T.T., Lu, C.L, Jiang, J.G., 2014b. Bioactivity evaluation of ingredients identified from the fruits of Amomum tsaoko Crevost et Lemaire, a Chinese spice. Food & Func. 5:1747-1754.

Figure captions

Figure 1 : Anti-quorum sensing (anti-QS) activity by *Amomum tsaoko* extract against bioreporter strain CV12472, using agar disc diffusion method. *Amomum tsaoko* extract (4 mg/ml) and positive control (vanillian, 250 µg/ml) loaded disc are showing colorless, opaque circle.

While, negative control (LB broth loaded disc) is showing no inhibition of violacein production around the disc.

Figure 2 : Effect of *A. tsaoko* extract on violacein production. Data are represented as percentage of violacein inhibition. Control (untreated) was set as 100% production. Mean values of triplicate independent experiments and SD are shown. Not significantly different at $P \leq 0.05$ of treatment.

Figure 3 : Observation of biofilm on CLSM. (a-c) Untreated biofilm of *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella* Typhimurium (ta-tc) treated biofilm of *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella* Typhimurium respectively.

Figure 4 : The migration distance of *Staphylococcus aureus*, *Salmonella* Typhimurium, *Pseudomonas aeruginosa* were observed while they were incubated for 30 h at different concentrations of *A. tsaoko* extract. Swarming motility of (a) *Pseudomonas aeruginosa* (b) *Salmonella* Typhimurium (c) *Staphylococcus aureus* in presence of *Amomum tsaoko* extracts (left-right ; 0.5, 1, 2 , 4 mg/ml , control : without extract).

Table caption

Table 1. Minimum Inhibitory Concentration (MIC) and % biofilm inhibition by the *Amomum tsao-ko* extract for *Staphylococcus aureus, Salmonella* Typhimurium*, Pseudomonas aeruginosa* [a]

Supplementary

Supplementary 1 : Flask incubation assay for quantification of violacein production. Left – right (containing 4, 2, 1, 0.5, 0(control) mg/ml *A. tsaoko* extract)

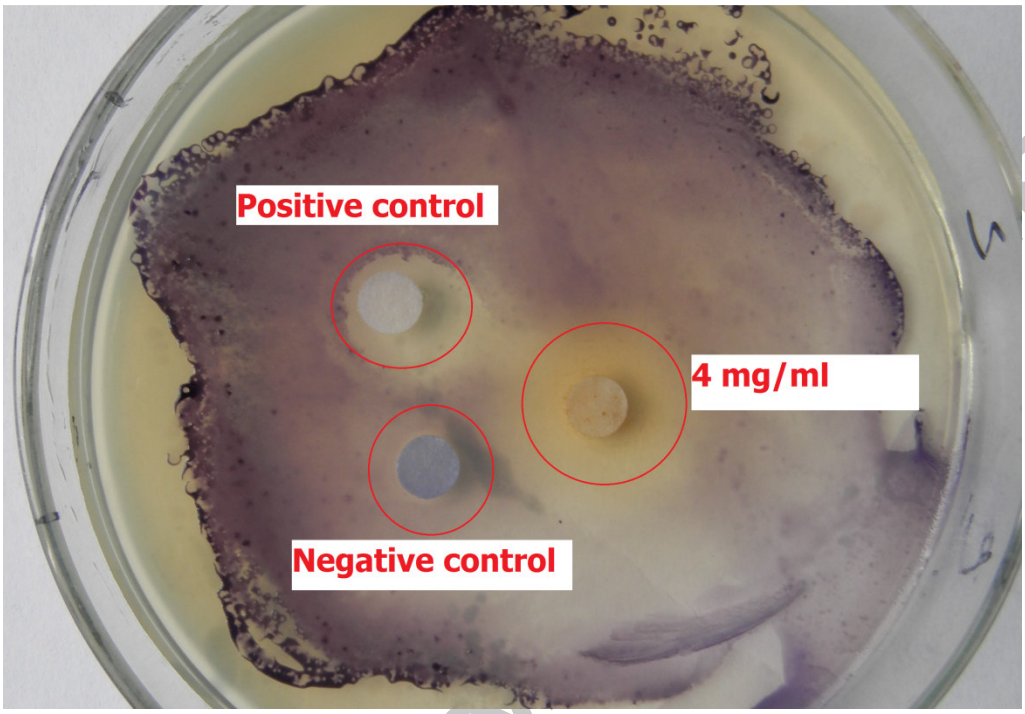

Figure 1 : Anti-quorum sensing (anti-QS) activity by *Amomum tsaoko* extract against bioreporter strain CV12472, using disc diffusion method. *Amomum tsaoko* extract (4 mg/ml) and positive control (vanillian, 250 µg/ml) disc are showing colorless, opaque circle. While, negative control (LB broth loaded disc) is showing no inhibition of violacein production around the disc.

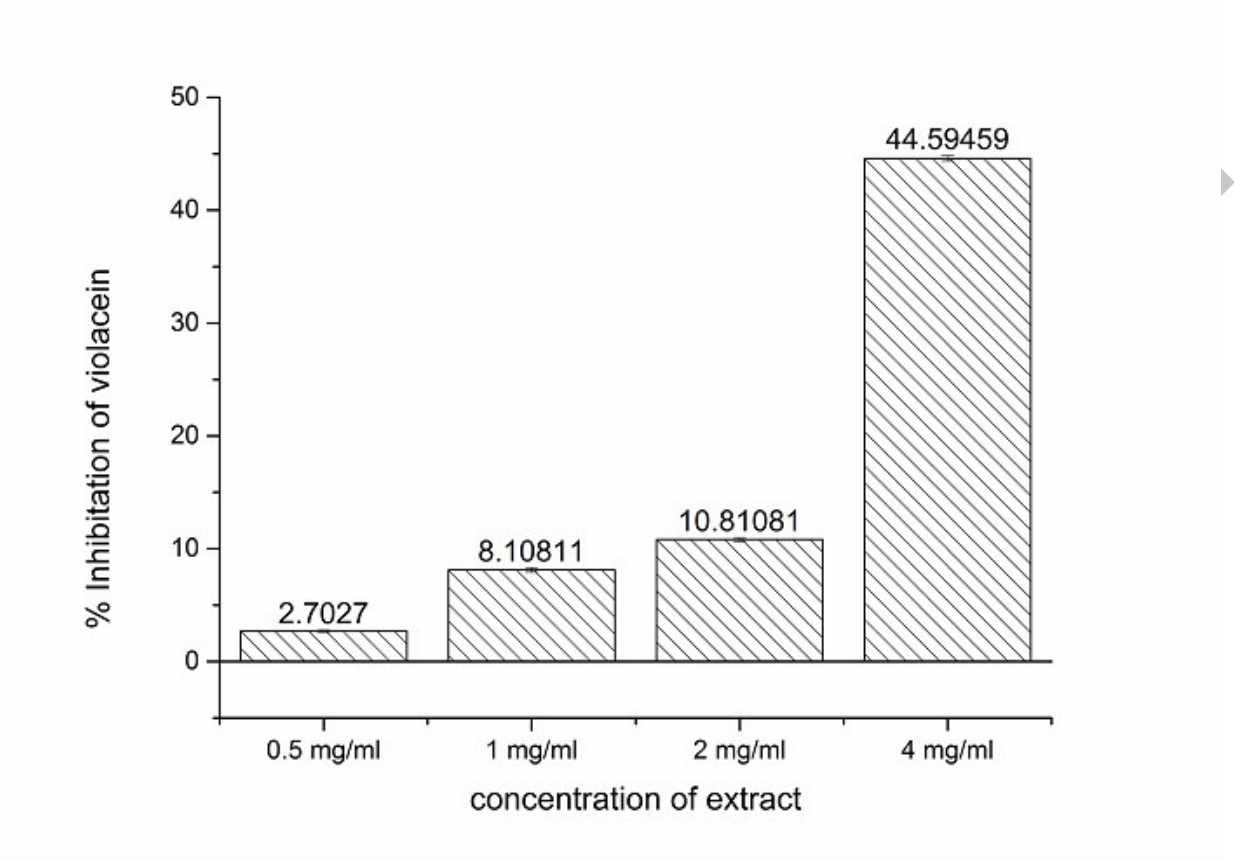

Figure 2 : Effect of *A. tsaoko* extract on violacein production. Data are represented as percentage of violacein inhibition. Control (untreated) was set as 100% production. Mean values of triplicate independent experiments and SD are shown. Not significantly different at $P \leq 0.05$ of treatment.

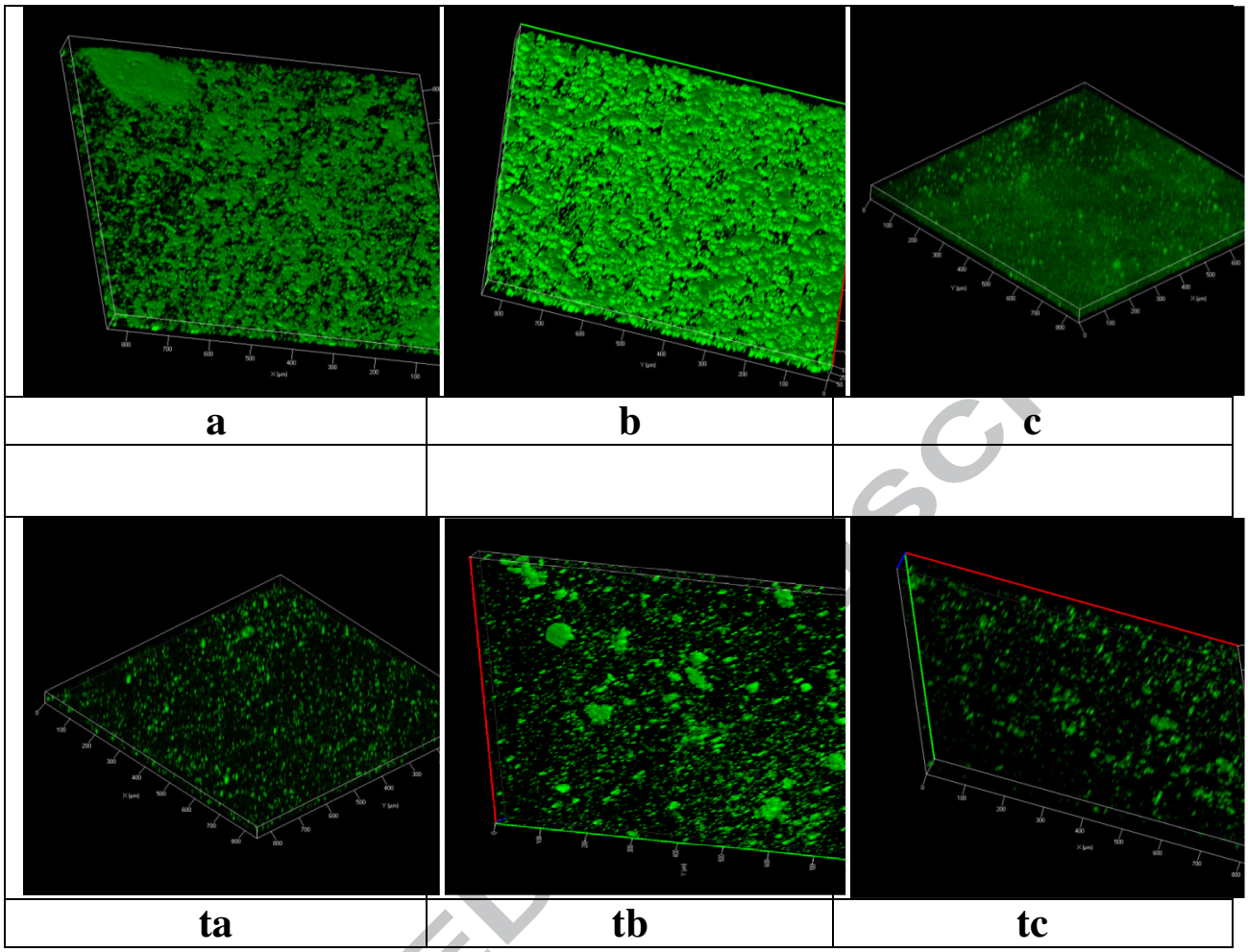

Figure 3 : Observation of biofilm on CLSM. (a-c) Untreated biofilm of *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella* Typhimurium (ta-tc) treated biofilm of *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella* Typhimurium respectively.

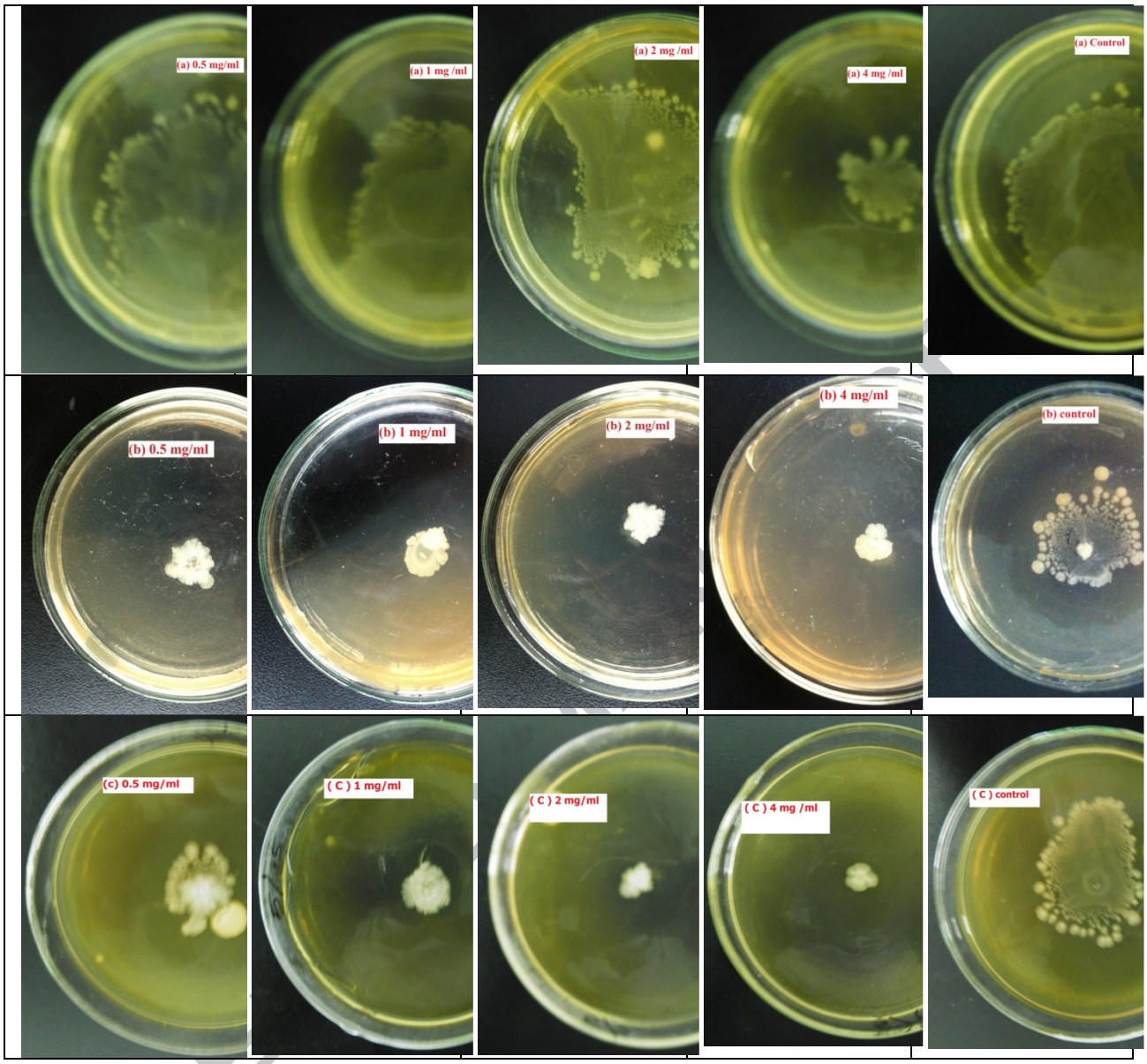

Figure 4 : The migration distance of *Staphylococcus aureus*, *Salmonella* Typhimurium, *Pseudomonas aeruginosa* were observed while they were incubated for 30 h at different concentrations of *A. tsaoko* extract. Swarming motility of (a) *Pseudomonas aeruginosa* (b) *Salmonella* Typhimurium (c) *Staphylococcus aureus* in presence of *Amomum tsaoko* extracts (left-right ; 0.5, 1, 2 , 4 mg/ml , control : without extract).

Table 1. Minimum Inhibitory Concentration (MIC) and % biofilm inhibition by the *Amomum tsao-ko* extract for *Staphylococcus aureus, Salmonella* Typhimurium, *Pseudomonas aeruginosa* [a]

	Staphylococcus aureus		*Salmonella* Typhimurium		*Pseudomonas aeruginosa*	
MIC (mg/ml)	1		2		2	
Concentration of extract	Total biofilm	% inhibition	Total biofilm	% inhibition	Total biofilm	% inhibition
Control	0.68±0.06	0	1.02±0.08	0	0.53±0.02	0
0.5 mg/ml	0.57±0.02	16.18	0.97±0.02	4.9	0.51±0.01	3.77
1 mg/ml	0.42±0.02	38.24	0.89±0.01	12.47	0.43±0.01	18.87
2 mg/ml	0.39±0.03	42.64	0.65±0.07	38.2	0.31±0.02	41.51
4 mg/ml	0.36±0.02	47.06	0.49±0.02	51.96	0.29±0.003	45.28

[a] Data are the mean ± SD of three independent experiments.