Antibacterial activity of asiaticoside towards enterrococcus faecalis in periapical infections

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ANTIBACTERIAL ACTIVITY OF *ASIATICOSIDE* TOWARDS *ENTERROCOCCUS FAECALIS* IN PERIAPICAL INFECTIONS

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ABSTRACT : This experiment aim is to examine the antibacterial activity of *Asiaticoside* towards *E. faecalis* which is associated with persistent periradicular lesions after root canal treatment. Two methods, PASS Prediction and Disc Diffusion, were used to assess antibacterial asiaticoside activity against E.faecalis. PASS Prediction assessed by Pa value and Disc diffusion system assessed by inhibition zones presence or absence. The Pa value results of *asiaticoside* is 0,57 and the presence of inhibition zones ranged from 9-14mm. This preliminary study indicate that *asiaticoside* has antibacterial effects towards *E .faecalis*.

Key words : *Asiaticoside*, *E. faecalis*, antibacteria, medicine, disc diffusion method.

INTRODUCTION

Nowadays, the use of medicinal plants and their derivatives as a source for antibacterial drugs is becoming more relevant due to increasing insidences of drug-resistant pathogens. Various plants have been used as therapeutic agents for various human diseases, including *Centella asiatica* L. (*C. asiatica*) and its derivatives (Bylka *et al*, 2013; Hashim *et al*, 2011; Rahman *et al*, 2012). *C. asiatica* is herb that belongs to India , Sri Lanka, Malaysia, South Africa, Madagascar, China, Australia , Japan, etc. This plant is very popular in Asia due to its therapeutic potential (Patel *et al*, 2016; Singh *et al*, 2010). These plants reported to have a wide range of antioxidant, anti-ulcer, anti-stress, anti-microbial and wound healing effects. The beneficial impact *C. asiatica* is caused by various pure phytoconstituents such as madecassic acid, asian acid, madecassoside, asiaticoside and many more that are triterpenes (Hashim *et al*, 2011; Patel *et al*, 2016). Among them, asiaticoside and asian acid are the most important bioactive compounds responsible for the medicinal values, but less knowledge about the potential for asiaticoside as antibacterial (Norzaharaini *et al*, 2011). Many studies of this plant's antibacterial agent rely more on plant extracts. Therefore, this research is to investigate asiaticoside's possible antibacterial activity against *E. fecalis* that is related to chronic periradicular lesions following root canal treatment.

MATERIALS AND METHODS

WAY2DRUG PASS prediction

Biological activity of *asiaticoside* was predicted with Biological Activity Spectrum (BAS). Prediction of activity spectra for substances (PASS) is hosted by the V. N. Orechovich Institute of Biomedical Chemistry under the aegis of the Russian Foundation of Basic Research (Parasuraman, 2011). The web-based application predicted the biological activity spectrum of *asiaticoside* based on its structure. PASS tool interpreted the biological active spectrum using 2D structure of *asiaticoside* molecule. Activity of the *asiaticoside* molecule was predicted by "comparing" the structure of *asiaticoside* with structure of well-known biological active substance existing in the Dr.Duke PhytoChemical database (Lagunin *et al*, 2000). The PASS prediction tool predicted the Pa:Pi (active, inactive ratio) at prediction threshold of Pa < 0,3; 0,5 < Pa < 0,7 and Pa > 0,70 (Filimonov *et al*, 2014). Average accuracy of prediction is about 95% according to leave-one-out cross validation (LOO CV) estimation (Parasuraman, 2011).

In vitro

In this analysis *E. faecalis* obtained from Universitas Airlangga Research Center was used. *E. faecalis* was inoculated in Brain Heart Infussion Broth and incubated for 48-72 hours in microaerobic surroundings at 37°C. The inoculum had been calibrated to a turbidity level of

0.5 Mc Farland. The Disc Diffusion Assay suggested by the National Clinical Laboratory Norm Commitee (NCCLS) was used for antibacterial test. Six mm sterilized filter paperdisk was impregnated with 10ìL of asiaticoside diluted to 1000ìg/ml concentration. As a result, 500ìg/ml, 250ìg/ml and 125ìg/ml each produce 50 percent asiaticoside, 25 percent as well as 12.5 percent. Trypticase Soy Broth steril was used as the control negative. The experiment was conducted in triplicates and the mean diameters of the inhibition zones were determined based on the nearest millimeter of the clear zone around the disks (Norzaharani *et al*, 2011; Lagunin *et al*, 2000).

RESULTS

The PASS Prediction Tool predicted Pa value of *Asiaticoside* as antibacterial. Pa value of *asiaticoside* is presented in Fig. 1.

PASS prediction results show that *C. asiatica* has potential as an antibacterial with a Pa value of 0.47. This can be interpreted that *C. asiatica* has antibacterial potential, but further laboratory tests are needed to ensure the anti-bacterial potential that exists in *C. asiatica*. Followed by an antibacterial prediction of all active ingredients *C. asiatica*. The PASS prediction results found that *asiaticoside* has anti-bacterial potential with Pa value of 0.54. This can be interpreted that *asiaticode* has potential as an anti-bacterial (Fig. 2).

To ensure the anti microbial activity of *asiaticoside*, an *in vitro* test was carried out using the diffusion method. The antibacterial activity of *asiaticoside* towards *E. faecalis* is presented in Fig. 3.

Asiaticoside inhibited the growth of *E. faecalis* with inhibition effects means 9,53 mm at 12, 5%, 12,23 mm at 25% and 14,38 mm at 50% which analyzed by Wilcoxon test and presented at Table 1.

Table 1 : Inhibition zone (mm) of *E.faecalis* by *asiaticoside*.

Concentration	Inhibition zone means (mm)	p-value ($p < 0.05$)
12.5%	9.5333	0.001926
25%	12.2333	0.07652
50%	14.3833	0.07652

DISCUSSION

E. faecalis is a persistent organism that plays an important role in the etiology of periapical lesions after root canal treatment. According to previous study found that 63% of failed root canal treatments were due to root canals being infected by *E. faecalis*. This is due to *E. faecalis* virulence factor which can survive at low temperatures, in an environment lacking nutrition, resistant to antibacterial and can form biofilms (Peciuliene *et al*, 2008; Gajan *et al*, 2009). *Asiaticoside* is one of the triterpenoid in *C. asiatica*. Several studies have suggested that *asiaticoside* has many pharmacological effects. One pharmacological effect that can be used in root canal treatment is the anti-bacterial effect of *asiaticoside* (Norzaharaini *et al*, 2011; Singh *et al*, 2010).

The anti-bacterial effect of a bioactive can be tested *in vitro*, *in vivo* and insilico. *In vitro*, anti-bacterial activity test can be performed by disk diffusion and dilution method. In the disk diffusion method, the assessment of anti-bacterial activity is seen from the measurement of

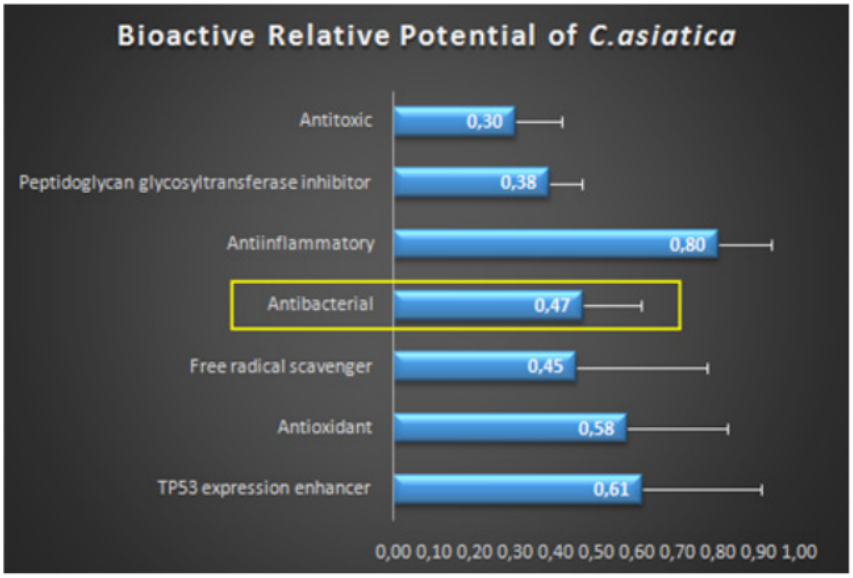

Fig. 1 : Bioactive Relative Potential of *C.asiatica*.

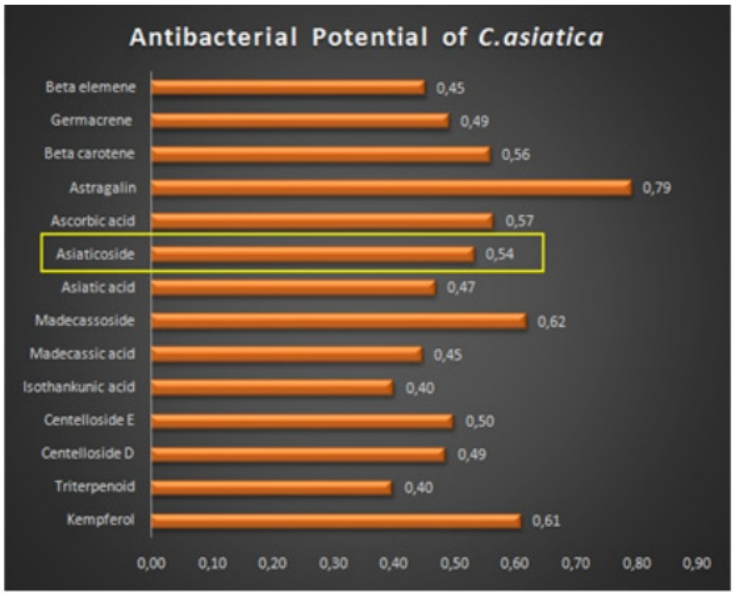

Fig. 2 : Bioactive potential *Asiaticosid*e as antibacterial.

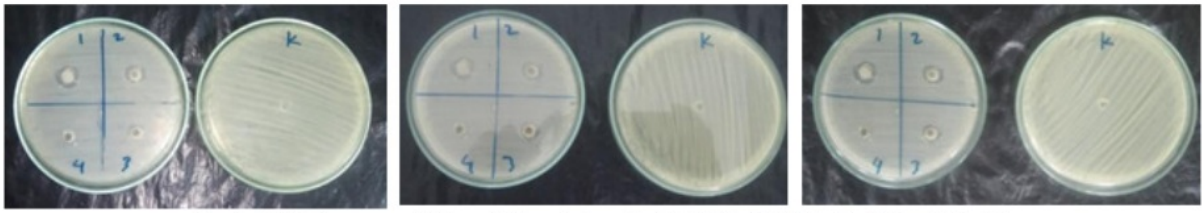

Fig. 3 : Inhibition zone of *E.faecalis* by *asiaticoside* (1) 12,5%, (2) 25%, (3) 50% and (K) 0%.

inhibition zones affected by the solubility and diffusion of the material being tested. While the dilution method is carried out to observe the anti-bacterial activity by means of the material being tested in direct contact with microorganisms. In this research, disk diffusion method is used to determine the anti-bacterial activity by measuring the diameter of the bacterial inhibitory zone formed (Balouiri *et al*, 2016; Norzaharani *et al*, 2011; Idris and Nadzir, 2017). *Asiaticoside* has antibacterial activity towards various pathogens and fungi. Though less research focus in antibacterial activity of *asiaticoside*. Most are still focused on the anti-bacterial activity of *C.asiatica* plant extracts (Norzaharaini *et al*, 2011).

Another study to determine asiaticoside's antibacterial activity was conducted to analyze asiaticoside and asiatic acid's antibacterial activity against Gram-negative and Gram-positive bacteria (*H.pylori*, *E. coli*, *S. aureus*, *S. pneumonia*, *P. aeroginosa*). The study states that *asiaticoside* did not present bacterial towards these 5 bacteria (Norzaharaini *et al*, 2011). However, our study using *E. faecalis* as a target bacterium showed that *asiaticoside* was able to form bacterial inhibition zones and after analyzing the results of the study showed that there were significant differences from each treatment group so that it could be stated that *asiaticoside* had anti-bacterial activity in vitro against *E.faecalis*. This may occur because of differences in the types of target bacteria used.

Based on the interpretation of the Pa threshold, the Pa value of 0.54 means that *asiaticoside* is potential of anti-bacterial activity and this anti-bacterial activity supported by in vitro. Results of this research show that *asiaticoside* has a potential anti-bacterial activity. However, the anti-bacterial mechanism of *asiaticoside* towards *E.faecalis* need further analyze. Anti-bacterial mechanism of *asiaticoside* towards *E.faecalis* can be further analyze by molecular docking (Ekins *et al*, 2007). In molecular docking, simulated how the *asiaticoside* membrane protein can bind to the *E. faecalis* membrane protein right on its target to lysis *E. faecalis*.

CONCLUSION

The results of this preliminary study indicate that *asiaticoside* has a potential anti-bacterial effects towards *E. faecalis*.

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