[PHAR06]

Anti *Helicobacter pylori* from extracts of ten species of *Phyllanthus* with special emphasis on chloroform extracts of *Phyllanthus pulcher*

Wan Iryani Wan Ismail, Shaida Fariza Sulaiman, Uyub Abdul Manaf

School of Biological Sciences, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia.
E-mail: waniryani@yahoo.com

Some species of *Phyllanthus* have been traditionally used as a remedies for gastric ailments which may be associated with *H. pylori*. In the present study 10 species of *Phyllanthus* such as *P. acidus, P. columnaris, P. debilis, P. emblica, P. myrtifolius, P. niruri, P. oxyphyllus, P. pulcher, P. reticulatus* and *P. urinaria* were evaluated for their anti-*H. pylori* activity. Dried whole plants (*P. debilis, P. myrtifolius, P. niruri* and *P. urinaria*) or leaves (*P. acidus, P. columnaris, P. emblica, P. oxyphyllus, P. pulcher* and P. *reticulatus*) were extracted using soxhlet extraction with hexane followed by chloroform and methanol. By using disc diffusion assay, the extracts were tested against *H. pylori* (S179) and nine other common bacterial pathogens such as *Enterobacter sp*. (E114), *Escherichia coli, Escherichia coli* (ATCC 25920), *Pseudomonas slutzeri, Salmonella sp., Shigella bodyii, Shigella dysenteriaceae, Staphylococcus aureus* and *Vibrio cholerae*. Chloroform extract of *P. pulcher* exhibited the biggest inhibition zone diameter against *H. pylori* (S179) but was non-inhibitory towards the other nine bacteria tested suggesting that the extract may act specifically against *H. pylori*. Other extracts possess relatively lower anti-*H. pylori* activity and were therefore ignored. Chloroform extract of *P. pulcher* also showed the high toxicity value on *Artemia salina* (LC_{50} acute = 2.124 ± 0.752 mg/ml and LC_{50} chronic = 1.252 ± 0.378 mg/ml). By column chromatography, the chloroform extract was fractionated into 30 fractions. Further evaluation by disc diffusion assay revealed that all the fractions specifically inhibited *H. pylori* (S179) with one of the fractions (F18) exhibited the biggest inhibition zone diameter. This fraction (fraction no. 18) also gave the high toxicity value on *A. salina* (LC_{50} acute =1.350 ± 0 mg/ml and LC_{50} chronic = 1.000 ± 0.245 mg/ml). Minimum inhibition concentration (MIC) test from fraction no. 18 onto 20 strains *H. pylori* showed MIC_{50} = 4 μg/ml and MIC_{90} = 256 μg/ml. The MIC value for *H. pylori* (S179) was 32 μg/ml. From the time kill assays test, the best concentration fraction to kill *H. pylori* was 16 μg/ml. The difference values between MIC and time kill assays test were caused by different kind of media used in both experiments. For further observation, time factor was the main factor in the time kill assays test compared to media factor. Killing mechanism by fraction 18 on *H. pylori* was determined by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Following exposure of *H. pylori* to the fraction, the morphology was turned from spiral form to coccoid form. Lastly, phytochemistry test revealed the presence of terpenoids in the fraction.