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ANTIMICROBIAL NATURAL PRODUCTS AGAINST  
ORAL PATHOGENS

A PROJECT REPORT PRESENTED BY

W. D. SAMANTHI GUNATHILAKA  
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MASTER OF SCIENCE OF ANALYTICAL CHEMISTRY

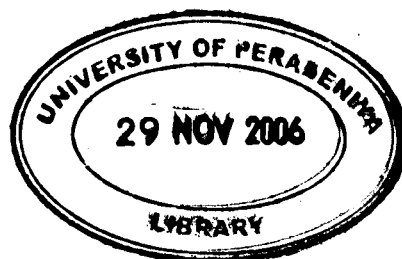
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## ANTIMICROBIAL NATURAL PRODUCTS AGAINST ORAL PATHOGENS

W.D.S. Gunathilaka.

Jinananda Mawatha, Karandugoda, Ahangama. Sri Lanka.

Higher plants have been shown to serve as a source of anti-microbial agents, however, fewer reports are available on their activity against oral pathogens. The oral care product industry is keen in finding natural antibacterial agents for mouth rinses, toothpastes and other oral care products that are effective in fighting against oral pathogens. In Sri Lanka, extracts of *Terminalia arjuna*, *Terminalia chebula*, *Terminalia bellirica*, *Adhatoda vasica*, *Mimusops elengi*, *Tephrosia purpurea*, *Phyllanthus emblica* and essential oils of *Zingiber officinale*, *Kaempferia galanga*, *Piper nigrum*, *Cinnamomum zeylanicum*, *Syzygium aromaticum* are used in oral care products. However, scientific evidences of those plants are limited to prove the effectiveness of these plants against oral care pathogens are limited. The objectives of the present study are to identify anti-microbial properties of extracts of above plants against oral pathogens and to investigate the active extracts to isolate and identify the active principles.

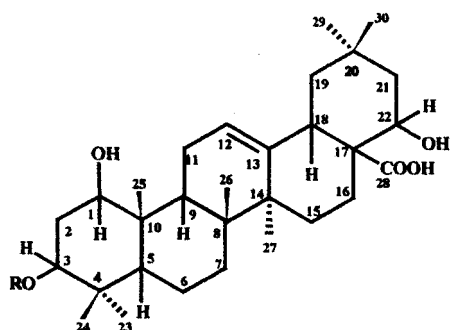
Anti-microbial activity of twelve plant extracts was tested against *Staphylococcus aureus* and *Candida albicans*. Out of those, *T. arjuna* was extensively studied for its anti-microbial constituents. Dry Column Flash Chromatographic technique was used in fractionation to isolate the active compounds. Structure elucidation of compounds was achieved mainly by NMR spectroscopic techniques including 2D NMR techniques such as HMQC, HMBC and COSY. For the anti-microbial bioassay, the disc diffusion method was followed, and Minimum Inhibitory Concentration (MIC) of fractions and compounds was determined by the agar dilution method.

Qualitative bioassays of total ethanol extracts of *T. arjuna*, *T. bellirica*, *T. chebula*, *Z. officinale*, *K. galanga*, *P. emblica*, and *S. aromaticum* showed antibacterial

activity against *S. aureus* but significant inhibition was noted only in *T. arjuna*, *T. bellirica*, *T. chebula* and *S. aromaticum*.

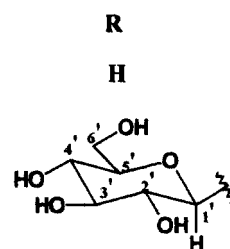
*T. arjuna*, *T. bellirica*. *T. chebula* showed large growth inhibition zones of 4.60, 3.01 and 4.35 mm at 2000 ppm, respectively and MICs was 0.1 mg/ mL against *S.aureus*. Out of twelve plants only the ethanol extract of *S. aromaticum* showed growth inhibitory activity against *C. albicans* observing inhibition zone diameter of 1.31 mm at 2000 ppm.

The bioassay guided fractionation of the ethyl acetate extract of the stem bark of *T. arjuna* resulted in the isolation of two antibacterial compounds 1 and 2. Compounds 1 and 2 showed MIC of 0.041 and 0.2 mg/mL, respectively, against *S. aureus*. The structure of compound 1 was tentatively established as olean-12-ene type triterpene, which is olean-1 $\alpha$ , 3 $\beta$ , 22 $\beta$ -triol-12-en-28-oic acid. The structure of 2 was established as olean-1 $\alpha$ , 3 $\beta$ , 22 $\beta$ -triol-12-en-28-oic acid-3 $\beta$ -D-glucopyranoside which was a previously reported compound from *T. arjuna*.



Compound 1

Compound 2



### Compounds from *Terminalia arjuna*