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Anti-Infective Potential of Selected Philippine Medicinal Plant Extracts against Multidrug-Resistant Bacteria

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Abstract : From the various medicinal plants available in the Philippines, crude ethanol extracts of twelve (12) Philippine medicinal plants, namely: Senna alata L. Roxb. (akapulko), Psidium guajava L. (bayabas), Piper betle L. (ikmo), Vitex negundo L. (lagundi), Mitrephora lanotan (Blanco) Merr. (Lanotan), Zingiber officinale Roscoe (luya), Curcuma longa L. (Luyang dilaw), Tinospora rumphii Boerl (Makabuhay), Moringga oleifera Lam. (malunggay), Phyllanthus niruri L. (sampa-sampalukan), Centella asiatica (L.) Urban (takip kuhol), and Carmona retusa (Vahl) Masam (tsaang gubat) were studied. In vitro methods of evaluation against selected Gram-positive and Gram-negative multidrug-resistant (MDR), bacteria were performed on the plant extracts. Although five of the plants showed varying antagonistic activities against the test organisms, only Piper betle L. exhibited significant activities against both Gram-negative and Gram-positive multidrug-resistant bacteria, exhibiting wide zones of growth inhibition in the disk diffusion assay, and with the lowest concentrations of the extract required to inhibit the growth of the bacteria, as supported by the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays. Further antibacterial studies of the Piper betle L. leaf, obtained by three extraction methods (ethanol, methanol, supercritical CO2), revealed similar inhibitory activities against a multitude of Gram-positive and Gram-negative MDR bacteria. Thin layer chromatography (TLC) assay of the leaf extract revealed a maximum of eight compounds with Rf values of 0.92, 0.86, 0.76, 0.53, 0.40, 0.25, 0.13, and 0.013, best visualized when inspected under UV-366 nm. TLC- agar overlay bioautography of the isolated compounds showed the compounds with Rf values of 0.86 and 0.13 having inhibitory activities against Gram-positive MDR bacteria (MRSA and VRE). The compound with an Rf value of 0.86 also possesses inhibitory activity against Gram-negative MDR bacteria (CRE Klebsiella pneumoniae and MBL Acinetobacter baumannii). Gas Chromatography-Mass Spectrometry (GC-MS) was able to identify six volatile compounds, four of which are new compounds that have not been mentioned in the medical literature. The chemical compounds isolated include 4-(2-propenyl)phenol and eugenol; and the new four compounds were ethyl diazoacetate, tris(trifluoromethyl)phosphine, heptafluorobutyrate, and 3-fluoro-2-propynenitrite. Phytochemical screening and investigation of its antioxidant, cytotoxic, possible hemolytic activities, and mechanisms of antibacterial activity were also done. The results showed that the local variant of Piper betle leaf extract possesses significant antioxidant, anti-cancer and antimicrobial properties, attributed to the presence of bioactive compounds, particularly of flavonoids (condensed tannin, leucoanthocyanin, gamma benzopyrone), anthraquinones, steroids/triterpenes and 2-deoxysugars. Piper betle L. is also traditionally known to enhance wound healing, which could be primarily due to its antioxidant, anti-inflammatory and antimicrobial activities. In vivo studies on mice using 2.5% and 5% of the ethanol leaf extract cream formulations in the excised wound models significantly increased the process of wound healing in the mice subjects, the results and values of which are at par with the current antibacterial cream (Mupirocin). From the results of the series of studies, we have definitely proven the value of Piper betle L. as a source of bioactive compounds that could be developed into therapeutic agents against MDR bacteria.

Keywords : Philippine herbal medicine, multidrug-resistant bacteria, Piper betle, TLC-bioautography
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