

Abstract

Antimicrobial resistance (AMR) observed in microorganisms against synthetic antibiotics, along with the adverse effects associated with these pharmaceutical drugs, pose significant challenges in their application for treating various medical conditions. Many microorganisms have developed resistance to specific antibiotics due to factors like inappropriate drug use and suboptimal treatment approaches. Consequently, the management of several diseases has become more intricate and problematic due to the declining effectiveness of synthetic antibiotics. AMR emergence jeopardizes individuals' well-being, and the cost of treating AMR-related infections has risen due to reduced drug efficacy. Addressing the AMR dilemma may involve the development of novel medicinal compounds that bacteria cannot easily develop resistance against. An assortment of diseases is on the rise due to oxidative stress within biological systems, with Diabetes Mellitus being a notable example. Oxidative stress disrupts the physiological processes within biological systems, resulting in metabolic disorders. While various synthetic antioxidants are available in the market for scavenging free radicals, including reactive oxygen species, it is vital to recognize that using these synthetic antioxidants carries potential health risks and may lead to various health-related complications. The World Health Organization (WHO) has officially reported that 80% of the population in developing nations relies on various plant species to address a range of medical conditions. Consequently, researchers have been actively engaged in studying plants that contain pharmaceutical agents, particularly those with antimicrobial and antioxidant properties. Prior research has uncovered highly potent antioxidant and antimicrobial compounds among the secondary metabolites found in plants. However, it's important to note that the comprehensive analysis of these metabolites is an ongoing endeavor. As a result, natural products derived from plants are emerging as a promising alternative to synthetic pharmaceuticals for addressing diseases related to antimicrobial and antioxidant challenges. The primary objective of this study is to quantify the inherent antioxidant and antimicrobial potential present in the leaves of Mantha Royleana. The research can be divided into two distinct components: the first involves the metabolic screening and profiling of essential oils extracted from Mantha Royleana leaves, while the second focuses on the metabolic screening and profiling of the Mantha Royleana extract. Additionally, the study includes the assessment of the antioxidant and antimicrobial potential of this extract. The essential oil extraction process from the leaves employed a hydro distillation apparatus of the Clevenger type. In this procedure, 200 grams of leaves were finely chopped and placed in a round-bottom flask, along with distilled

water. The extraction of essential oils was carried out at a temperature of 70°C. Following this, the resultant extract underwent a solvent extraction process using dichloromethane (DCM) and was further treated with sodium sulfate (Na₂SO₄) to eliminate any remaining water content. This resulting sample then underwent Fourier-transform infrared spectroscopy (FTIR) and gas chromatography-mass spectrometry (GC-MS) analyses. In contrast, for the leaf extract preparation, freshly harvested leaves were rapidly frozen using liquid nitrogen after thorough cleaning. Subsequently, a solvent mixture was created by blending ethanol and water in various proportions, including aqueous compositions of 20%, 40%, 60%, 80%, and 100% ethanol, as well as methanol. The frozen leaf powder was subjected to an extraction process using a series of solvent compositions through ultrasonication for 30 minutes. Following this, rotary evaporation and lyophilization were employed to completely remove any remaining water content. After calculating the extract yield percentage, the antioxidant potential of these extracts was evaluated using the ABTS Assay and DPPH assay. Additionally, the antimicrobial activities of the extracts were determined using the well-diffusion method, targeting two bacterial strains: *Klebsiella pneumonia* and *Bacillus coagulans*. The analysis results indicated that peaks corresponding to various functional groups, such as N-H, C=O, C-H, C-N, C-O, O-H, C=C, and others, were detected in the essential oils (EOs) derived from *Mantha Royleana* leaves, as confirmed by Fourier-transform infrared (FTIR) analysis. Furthermore, gas chromatography-mass spectrometry (GC-MS) analysis identified a total of 24 compounds in the EOs of *Mantha Royleana* leaves. The extract yields obtained from *Mantha Royleana* leaves were as follows: 40.7%, 18.41%, 18.32%, 18.11%, 18.20%, and 18.27% for aqueous, 20% ethanol, 40% ethanol, 60% ethanol, 80% ethanol, and pure ethanol, respectively. In contrast, the yields obtained from *Mantha Royleana* leaves extracts were 33.81%, 33.27%, 32.89%, 32.53%, and 32.64% for 20% methanol, 40% methanol, 60% methanol, 80% methanol, and pure methanol, respectively. Notably, the extraction yield from methanol was higher than that from ethanol. The DPPH radical scavenging percentages were as follows: 66.9%, 57.2%, 55.7%, 54.3%, 57.8%, and 18.5% for aqueous, 20% methanol, 40% methanol, 60% methanol, 80% methanol, and pure methanol, respectively. The Trolox equivalent antioxidant capacity (TEAC) values, as determined by the ABTS assay, were 7.41, 7.48, 7.56, 7.61, 7.74, and 8.5 mM for aqueous, 20% ethanol, 40% ethanol, 60% ethanol, 80% ethanol, and pure ethanol, respectively. Notably, only the aqueous sample exhibited antibacterial activity against *K. pneumonia*, with an inhibition zone diameter measuring 1.3 cm, while none of the samples showed activity against *B. Coagulans*. The highest yielding sample (aqueous) underwent methylation using methanol and was subsequently subjected to GC-MS analysis, which identified a total of 29 compounds in the derivatized extract.