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Exploration of phytochemicals, GC-MS analysis, and assessment of *in vitro* cytotoxic potential of *Justicia adhatoda* (L.) against Dalton's Lymphoma Ascites (DLA) murine tumor model

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Researchers worldwide are presently delving into natural substances as prospective solutions for cancer treatment to develop drugs that minimize side effects. In this context, the present study investigated the *in vitro* cytotoxic potential of *Justicia adhatoda* (L.), a medicinal plant traditionally employed to address a range of health concerns. The phytochemical analysis using standard tests showed the presence of important metabolites such as carbohydrates, alkaloids, proteins, polyphenols, tannins, saponins, terpenoids, flavonoids, phenols, and triterpenoids. Additionally, GC-MS analysis exhibited the presence of about 20 phytocompounds. Antioxidant potency determined through phosphomolybdate reduction and DPPH (2,2-diphenyl 1-picrylhydrazyl) free radical scavenging assays showed a dose-dependent antioxidant activity compared to the standard ascorbic acid. Moreover, the *in vitro* cytotoxicity assays, such as the trypan blue exclusion method, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium), and LDH (Lactate dehydrogenase) demonstrated an effective cytotoxic response of the extract proportional to dose, indicated with a low IC₅₀ value. Furthermore, morphological observation of the extract-treated cells through dual fluorescence staining of ethidium bromide/acridine orange (EtBr/AO) showed apoptotic features. These findings highlight the antitumor efficacy of *J. adhatoda* (L.) extract specifically against the DL cell line. Further *in vivo* studies and analysis of its active constituents are recommended.

Keywords: Anticancer, antioxidant, apoptosis, natural product, secondary metabolites