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# Isoledene from *Mesua ferrea* oleo-gum resin induces apoptosis in HCT 116 cells through ROS-mediated modulation of multiple proteins in the apoptotic pathways: A mechanistic study

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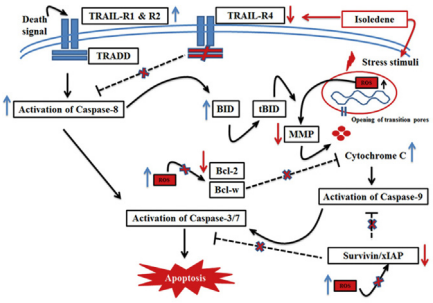
## HIGHLIGHTS

- Isoledene induces selective cytotoxic effects towards human colorectal carcinoma cell lines.
- Isoledene induces typical apoptotic changes in the morphology of colon cancer cells.
- Isoledene elevates the levels of caspases-3/7, −8 and −9 and ROS in colon cancer cell line.
- Isoledene modulates the expression of Bid, Bim, Bcl-2, Bcl-w, survivin, xIAP and HSPs in HCT 116 cell line.

## GRAPHICAL ABSTRACT




The blue arrows represent up-regulation of apoptosis mediators as a result of exposure to IR- SF, while red arrows illustrate down-modulation of negative regulators of apoptosis. Dotted lines with blunt end represent the negative role of proteins and red-cross represent a blockage of mechanisms as a result of exposure to IR-SF.

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## ABSTRACT

Colorectal cancer (CRC) is one of the most common human malignant tumors worldwide. Arising from the transformation of epithelial cells in the colon and/or rectum into malignant cells, the foundation of CRC pathogenesis lies in the progressive accumulation of mutations in oncogenes and tumor-suppressor genes, such as *KRAS* and *APC*. Resistance to apoptosis is one of the key mechanisms in the development of CRC as it is for any other kind of cancer. Natural products have been shown to induce the expression of apoptosis regulators that are blocked in cancer cells. In the present study, a series of *in vitro* assays were employed to study the apoptosis-inducing attributes of Isoledene rich sub-fraction (IR-SF) collected from

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Bim
Survivin
xIAP
ROS
Isoledene
*Mesua ferrea*

the oleo-gum resin of *M. ferrea*. Data obtained, showed that IR-SF inhibited cell proliferation and induced typical apoptotic changes in the overall morphology of all the CRC cell lines tested. Fluorescent staining assays revealed characteristic nuclear condensation, and marked decrease in mitochondrial outer membrane potential in the treated cells. In addition, an increment in the levels of ROS, caspase-8, −9 and −3 was observed. Proteomic analysis revealed that IR-SF up-regulated the expression of pro-apoptotic proteins, i.e., Bid, Bim and cytochrome c. Cytochrome c in turn activated caspases cascade resulting in the induction of apoptosis. Moreover, IR-SF significantly down-regulated Bcl-2, Bcl-w, survivin, xIAP and HSPs pro-survival proteins and induced DNA fragmentation and G0/G1-phase arrest in HCT 116 cells. Chemical characterization of IR-SF by GC–MS and HPLC methods identified Isoledene as one of the major compounds. Altogether, results of the present study demonstrate that IR-SF may induce apoptosis in human colorectal carcinoma cells through activation of ROS-mediated apoptotic pathways.



## 1. Introduction

Colorectal carcinoma (CRC) which involves the formation of a malignant mass in the colon and/or rectum parts of the large intestine is the third most common cancer worldwide. In Malaysia, according to the National Cancer Registry (NCR) report 2007, CRC was recognized as the second most common cancer in men and women, accounting for 12.3% of all the cancer cases reported. Worldwide high prevalence of CRC highlights the urgent need to find new treatments to combat this multifactorial syndrome (Ariffin and Saleha, 2011; Torre et al., 2015).

One of the hallmarks of cancers is the abnormalities in cell death mechanisms including apoptosis. Apoptosis is orchestrated by a series of well-balanced and interlinked energy dependent events which play a vital role in the removal of injured and abnormal cells from the body, thus maintaining body homeostasis. Inadequate apoptosis is one of the major reasons for the development of autoimmune and neoplastic disorders, including CRC (Ashkenazi, 2008; Prasad and Prabhakar, 2003). The role of faulty apoptotic machinery in the progression and spread of cancer has been highlighted by numerous studies. Inadequate apoptosis not only extends the life span of neoplastic cells, but also helps in the further

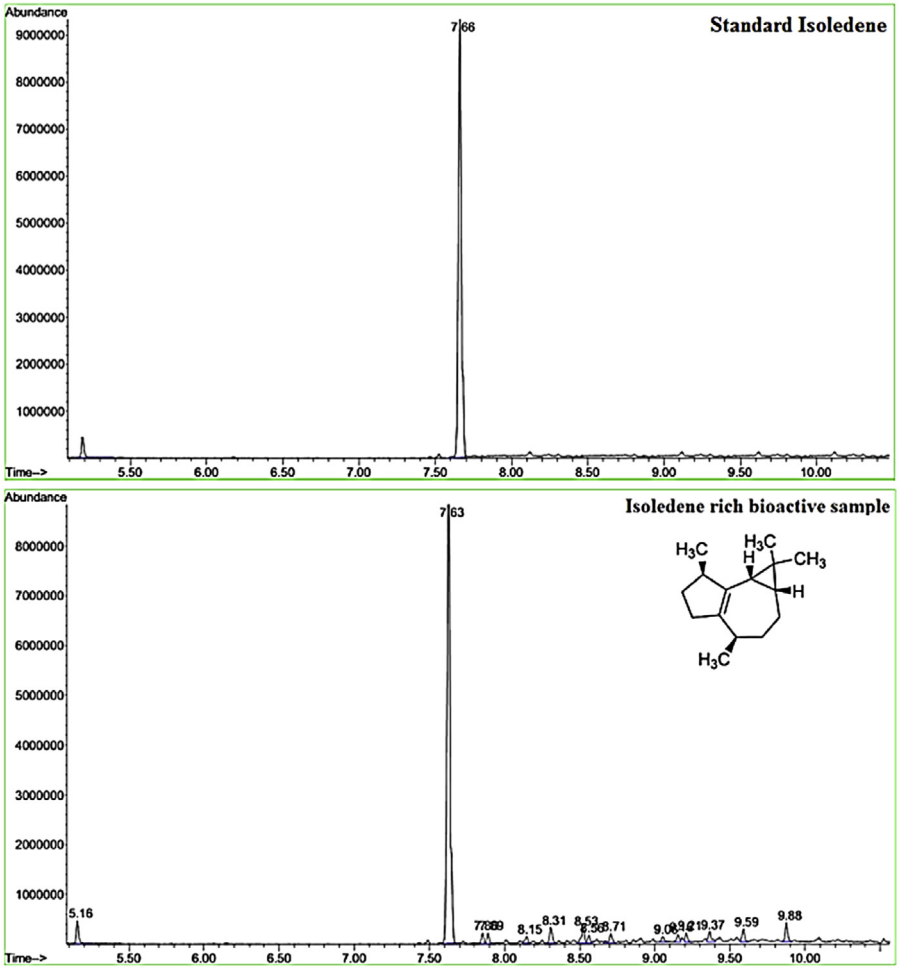


**Fig. 1.** GC–MS spectra of standard Isoledene and Isoledene rich sub-fraction. Both the samples were analysed at the same time using the same GC-MS method. The retention time of standard Isoledene was 7.66 minutes while the retention time of the Isoledene peak in IR-SF was 7.63 minutes. Slight changes in the retention time might be due to the presence of other compounds in minor amounts.