

Nitric Oxide Inhibitory and Cytotoxic Activities of Spice Essential Oils

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ABSTRACT

This study aims to investigate the effects of anise oil, lemongrass oil and cassia oil on nitric oxide production from nitric oxide donor and stimulated macrophage cells. Furthermore, this study also evaluates the cytotoxic effect of essential oils on cell viability of macrophage and human colorectal cancer cells. The results showed that anise oil and lemongrass oil presented higher nitric oxide scavenging capacity by reduction of nitrite formation from NO donor with IC_{50} of 406.90 and 413.50 $\mu\text{g/ml}$. In vitro study, cassia oil presented lower NO scavenging capacity. For cellular study, lemongrass oil and cassia oil at concentration of 6.25-25 $\mu\text{g/ml}$ and all concentration of anise oil (6.25-100 $\mu\text{g/ml}$) presented NO inhibitory activity with no cytotoxic effect of the macrophages. For the cancer cell study, lemongrass oil and cassia oil reduced cell viability of human colorectal cancer cells after 48 h of treatment with IC_{50} of 77.91 and 32.72 $\mu\text{g/ml}$, and IC_{50} was better in 72 h of treatment with 67.96 and 21.94 $\mu\text{g/ml}$. Nevertheless, anise oil displayed insignificant effect on HT-29 cell viability. Anethole, citral and cinnamaldehyde were identified as main composition of anise oil, lemongrass oil and cassia oil using gas chromatography-mass spectrometry (GC-MS). The results from this study suggested the different effects of essential oils on nitric oxide inhibition of in vitro and cellular study as well as the cytotoxic effect to macrophage and colorectal cancer cell. These results are beneficial for further study of anise oil, cassia oil and lemongrass oil in pharmaceuticals and natural therapies.

Keywords: Anise oil, Lemongrass oil, Cassia oil, Nitric oxide, Macrophage, Human colorectal cancer

INTRODUCTION

Nitric oxide (NO) is one of oxidative molecule which involved in inflammatory processes and cancer development. Nitric oxide acts as a signaling molecule in the normal physiological control. However, under hypoxic conditions, NO is produced by the mitochondrial respiratory chain which can generate other reactive nitrogen species (RNS). The excess production of NO causes of oxidative stress and chronic inflammation which further associated to increase the risk of several human cancer development (Reuter et al., 2010).

Nitric oxide (NO) is accumulated in chronic inflammatory processes by phagocytic cells, including macrophages. Excessive NO interacts with O_2^- to contribute cytotoxic oxidant peroxynitrite (ONOO⁻), which is a powerful oxidant to initiate lipid peroxidation and cleavage DNA, resulting in the risk of cancer (Choudhari et al., 2013). NO presents different roles in the development stage of cancers, including functions as a signaling molecule in the regulation of cancer formation, progression and metastasis as well as application in cancer therapy. However, high level of NO modulates matrix metalloproteinase (MMP) expression in malignant cells to support tumor cell invasion in the cancer invasive stage. This event is supported by the previous study that found the angiogenesis in tumor cell was suppressed afterwards the inhibition of nitric oxide production (Cheng et al., 2014).

Three of spice essential oils (anise oil, lemongrass oil and cassia oil) have been demonstrated the pharmacological activities. Anise oil is separated from star anise (*Illicium verum* Hook. fil.) and commonly used in cooking and utilization for antifungal agents in medical and food (Matan et al., 2012). Anise oil and anise seed extract also presented anti-proliferative effect on gastric cancer cells (Rahamooz-Haghighi & Asadi, 2016).

Lemongrass oil is the volatile oil from *Cymbopogon citratus* D.C. (*C. citratus*) which has been used as a remedy for the treatment of various health conditions. In Asia and Africa, lemongrass oil has been used for pain and inflammation treatment, e.g. backache, sprains and anti-rheumatic as well as antiseptic and antitussive (Boukhatem et al., 2014). The pharmacological properties of *C. citratus* have been exhibited antimicrobial and antifungal (Adukwu et al., 2016; Liakos et al., 2016) and anti-proliferative effect on human colon carcinoma (HCT-116), breast carcinoma (MCF-7 and MDA-MB 231), ovarian carcinoma (SKOV-3 and COAV), and a normal liver cell line (WRL 68) (Halabi & Sheikh, 2014) and HeLa and ME-180 cervical cell line (Ghosh, 2013).

Cassia oil is the volatile oil from the leaves, branches or barks of *Cinnamomum cassia* Blume (Lauraceae) and has been traditionally used as a spice and aromatic (Geng et al., 2011). The pharmacological activity of cassia