



## **In Vitro Studies on Essential Oils Against Cancer Cell Lines**

### **Myrrh**

*Phytother Res.* 2016 Mar;30(3):418-25. doi: 10.1002/ptr.5543. Epub 2015 Dec 15.

**$\beta$ -Bisabolene, a Sesquiterpene from the Essential Oil Extract of Opoponax (*Commiphora guidottii*), Exhibits Cytotoxicity in Breast Cancer Cell Lines.**

#### **Abstract**

The essential oils from *Commiphora* species have for centuries been recognized to possess medicinal properties. Here, we performed gas chromatography-mass spectrometry on the essential oil from opoponax (*Commiphora guidotti*) and identified bisabolene isomers as the main constituents of this essential oil. Opoponax essential oil, a chemical component;  $\beta$ -bisabolene and an alcoholic analogue,  $\alpha$ -bisabolol, were tested for their ability to selectively kill breast cancer cells. Only  $\beta$ -bisabolene, a sesquiterpene constituting 5% of the essential oil, exhibited selective cytotoxic activity for mouse cells (IC<sub>50</sub> in normal Eph4: >200  $\mu$ g/ml, MG1361: 65.49  $\mu$ g/ml, 4T1: 48.99  $\mu$ g/ml) and human breast cancer cells (IC<sub>50</sub> in normal MCF-10A: 114.3  $\mu$ g/ml, MCF-7: 66.91  $\mu$ g/ml, MDA-MB-231: 98.39  $\mu$ g/ml, SKBR3: 70.62  $\mu$ g/ml and BT474: 74.3  $\mu$ g/ml). This loss of viability was because of the induction of apoptosis as shown by Annexin V-propidium iodide and caspase-3/7 activity assay.  $\beta$ -bisabolene was also effective in reducing the growth of transplanted 4T1 mammary tumours in vivo (37.5% reduction in volume by endpoint). **In summary, we have identified an anti-cancer agent from the essential oil of opoponax that exhibits specific cytotoxicity to both human and murine mammary tumour cells in vitro and in vivo, and this warrants further investigation into the use of  $\beta$ -bisabolene in the treatment of breast cancers.**

### **Frankincense**

*Asian Pac J Cancer Prev.* 2015;16(16):7179-88.

## **Phytochemical Analysis and Anti-cancer Investigation of *Boswellia serrata* Bioactive Constituents In Vitro.**

### **Abstract**

Cancer is a major health obstacle around the world, with hepatocellular carcinoma (HCC) and colorectal cancer (CRC) as major causes of morbidity and mortality. Nowadays, there is growing interest in the therapeutic use of natural products for HCC and CRC, owing to the anticancer activity of their bioactive constituents. *Boswellia serrata* oleo gum resin has long been used in Ayurvedic and traditional Chinese medicine to alleviate a variety of health problems such as inflammatory and arthritic diseases. The current study aimed to identify and explore the in vitro anticancer effect of *B. Serrata* bioactive constituents on HepG2 and HCT 116 cell lines. Phytochemical analysis of volatile oils of *B. Serrata* oleo gum resin was carried out using gas chromatography-mass spectrometry (GC/MS). Oleo-gum-resin of *B. Serrata* was then successively extracted with petroleum ether (extract 1) and methanol (extract 2). Gas-liquid chromatography (GLC) analysis of the lipoidal matter was also performed. In addition, a methanol extract of *B. Serrata* oleo gum resin was phytochemically studied using column chromatography (CC) and thin layer chromatography (TLC) to obtain four fractions (I, II, III and IV). Sephadex columns were used to isolate  $\beta$ -boswellic acid and identification of the pure compound was done using UV, mass spectra,  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR analysis. Total extracts, fractions and volatile oils of *B. Serrata* oleo-gum resin were subsequently applied to HCC cells (HepG2 cell line) and CRC cells (HCT 116 cell line) to assess their cytotoxic effects. GLC analysis of the lipoidal matter resulted in identification of tricosane (75.32%) as a major compound with the presence of cholesterol, stigmasterol and  $\beta$ -sitosterol. Twenty two fatty acids were identified of which saturated fatty acids represented 25.6% and unsaturated fatty acids 74.4% of the total saponifiable fraction. GC/MS analysis of three chromatographic fractions (I, II and III) of *B. Serrata* oleo gum resin revealed the presence of pent-2-ene-1,4-dione, 2-methyl-levulinic acid methyl ester, 3,5-dimethyl-1-hexane, methyl-1-methylpentadecanoate, 1,1-dimethoxy cyclohexane, 1-methoxy-4-(1-propenyl)benzene and 17 $\alpha$ -hydroxy-17 $\alpha$ -cyano, preg-4-en-3-one. GC/MS analysis of volatile oils of *B. Serrata* oleo gum resin revealed the presence of sabinene (19.11%), terpinen-4-ol (14.64%) and terpinyl acetate (13.01%) as major constituents. The anti-cancer effect of two extracts (1 and 2) and four fractions (I, II, III and IV) as well as volatile oils of *B. Serrata* oleo gum resin on HepG2 and HCT 116 cell lines was investigated using SRB assay. Regarding HepG2 cell line, extracts 1 and 2 elicited the most pronounced cytotoxic activity with IC<sub>50</sub> values equal 1.58 and 5.82  $\mu\text{g}/\text{mL}$  at 48 h, respectively which were comparable to doxorubicin with an IC<sub>50</sub> equal 4.68  $\mu\text{g}/\text{mL}$  at 48 h. With respect to HCT 116 cells, extracts 1 and 2 exhibited the most obvious cytotoxic effect; with IC<sub>50</sub> values equal 0.12 and 6.59  $\mu\text{g}/\text{mL}$  at 48 h,

respectively which were comparable to 5-fluorouracil with an IC50 equal 3.43 µg/ mL at 48 h. In conclusion, total extracts, fractions and volatile oils of *B. Serrata* oleo gum resin proved their usefulness as cytotoxic mediators against HepG2 and HCT 116 cell lines with different potentiality (extracts > fractions > volatile oil). **In the two studied cell lines the cytotoxic activity of each of extract 1 and 2 was comparable to doxorubicin and 5-fluorouracil, respectively. Extensive in vivo research is warranted to explore the precise molecular mechanisms of these bioactive natural products in cytotoxicity against HCC and CRC cells.**

BMC Complement Altern Med. 2012 Dec 13;12:253. doi: 10.1186/1472-6882-12-253.

**Frankincense essential oil prepared from hydrodistillation of *Boswellia sacra* gum resins induces human pancreatic cancer cell death in cultures and in a xenograft murine model.**

Ni X<sup>1</sup>, Suhail MM, Yang Q, Cao A, Fung KM, Postier RG, Woolley C, Young G, Zhang J, Lin HK.

#### **Author information**

#### **Abstract**

#### ***BACKGROUND:***

Regardless of the availability of therapeutic options, the overall 5-year survival for patients diagnosed with pancreatic cancer remains less than 5%. Gum resins from *Boswellia* species, also known as frankincense, have been used as a major ingredient in Ayurvedic and Chinese medicine to treat a variety of health-related conditions. Both frankincense chemical extracts and essential oil prepared from *Boswellia* species gum resins exhibit anti-neoplastic activity, and have been investigated as potential anti-cancer agents. The goals of this study are to identify optimal condition for preparing frankincense essential oil that possesses potent anti-tumor activity, and to evaluate the activity in both cultured human pancreatic cancer cells and a xenograft mouse cancer model.

#### ***METHODS:***

*Boswellia sacra* gum resins were hydrodistilled at 78°C; and essential oil distillate fractions were collected at different durations (Fraction I at 0-2 h, Fraction II at 8-10 h, and Fraction III at 11-12 h). Hydrodistillation of the second half of gum resins was performed at 100°C; and distillate was collected at 11-12 h (Fraction IV). Chemical compositions were identified by gas chromatography-mass spectrometry (GC-MS); and total boswellic acids contents were quantified by high-performance liquid chromatography (HPLC). Frankincense essential oil-modulated pancreatic tumor cell viability and cytotoxicity were determined by colorimetric assays. Levels of apoptotic markers, signaling molecules, and cell cycle regulators expression were characterized by Western blot analysis. A heterotopic (subcutaneous) human pancreatic cancer

xenograft nude mouse model was used to evaluate anti-tumor capability of Fraction IV frankincense essential oil in vivo. Frankincense essential oil-induced tumor cytostatic and cytotoxic activities in animals were assessed by immunohistochemistry.

**RESULTS:**

Longer duration and higher temperature hydrodistillation produced more abundant high molecular weight compounds, including boswellic acids, in frankincense essential oil fractions. Human pancreatic cancer cells were sensitive to Fractions III and IV (containing higher molecular weight compounds) treatment with suppressed cell viability and increased cell death. Essential oil activated the caspase-dependent apoptotic pathway, induced a rapid and transient activation of Akt and Erk1/2, and suppressed levels of cyclin D1 cdk4 expression in cultured pancreatic cancer cells. In addition, *Boswellia sacra* essential oil Fraction IV exhibited anti-proliferative and pro-apoptotic activities against pancreatic tumors in the heterotopic xenograft mouse model.

**CONCLUSION:**

All fractions of frankincense essential oil from *Boswellia sacra* are capable of suppressing viability and inducing apoptosis of a panel of human pancreatic cancer cell lines. Potency of essential oil-suppressed tumor cell viability may be associated with the greater abundance of high molecular weight compounds in Fractions III and IV. Although chemical component(s) responsible for tumor cell cytotoxicity remains undefined, crude essential oil prepared from hydrodistillation of *Boswellia sacra* gum resins might be a useful alternative therapeutic agent for treating patients with pancreatic adenocarcinoma, an aggressive cancer with poor prognosis.

BMC Complement Altern Med. 2011 Dec 15;11:129. doi: 10.1186/1472-6882-11-129.

**Boswellia sacra essential oil induces tumor cell-specific apoptosis and suppresses tumor aggressiveness in cultured human breast cancer cells.**

Suhail MM<sup>1</sup>, Wu W, Cao A, Mondalek FG, Fung KM, Shih PT, Fang YT, Woolley C, Young G, Lin HK.

**Author information**

**Abstract**

**BACKGROUND:**

Gum resins obtained from trees of the Burseraceae family (*Boswellia* sp.) are important ingredients in incense and perfumes. Extracts prepared from *Boswellia* sp. gum resins have been shown to possess anti-inflammatory and anti-neoplastic effects. Essential oil prepared by distillation of the gum resin traditionally used for aromatic therapy has also been shown to have tumor cell-specific anti-proliferative and pro-apoptotic activities. The objective of this study was to optimize conditions for preparing *Boswellia sacra* essential oil with the highest biological activity in inducing tumor cell-specific cytotoxicity and suppressing aggressive tumor phenotypes in human breast cancer cells.

**METHODS:**

Boswellia sacra essential oil was prepared from Omani Hougari grade resins through hydrodistillation at 78 or 100 °C for 12 hours. Chemical compositions were identified by gas chromatography-mass spectrometry; and total boswellic acids contents were quantified by high-performance liquid chromatography. Boswellia sacra essential oil-mediated cell viability and death were studied in established human breast cancer cell lines (T47D, MCF7, MDA-MB-231) and an immortalized normal human breast cell line (MCF10-2A). Apoptosis was assayed by genomic DNA fragmentation. Anti-invasive and anti-multicellular tumor properties were evaluated by cellular network and spheroid formation models, respectively. Western blot analysis was performed to study Boswellia sacra essential oil-regulated proteins involved in apoptosis, signaling pathways, and cell cycle regulation.

**RESULTS:**

More abundant high molecular weight compounds, including boswellic acids, were present in Boswellia sacra essential oil prepared at 100 °C hydrodistillation. All three human breast cancer cell lines were sensitive to essential oil treatment with reduced cell viability and elevated cell death, whereas the immortalized normal human breast cell line was more resistant to essential oil treatment. Boswellia sacra essential oil hydrodistilled at 100 °C was more potent than the essential oil prepared at 78 °C in inducing cancer cell death, preventing the cellular network formation (MDA-MB-231) cells on Matrigel, causing the breakdown of multicellular tumor spheroids (T47D cells), and regulating molecules involved in apoptosis, signal transduction, and cell cycle progression.

**CONCLUSIONS:**

Similar to our previous observations in human bladder cancer cells, Boswellia sacra essential oil induces breast cancer cell-specific cytotoxicity. Suppression of cellular network formation and disruption of spheroid development of breast cancer cells by Boswellia sacra essential oil suggest that the essential oil may be effective for advanced breast cancer. Consistently, the essential oil represses signaling pathways and cell cycle regulators that have been proposed as therapeutic targets for breast cancer. Future pre-clinical and clinical studies are urgently needed to evaluate the safety and efficacy of Boswellia sacra essential oil as a therapeutic agent for treating breast cancer.

BMC Complement Altern Med. 2009 Mar 18;9:6. doi: 10.1186/1472-6882-9-6.

## **Frankincense oil derived from *Boswellia carteri* induces tumor cell specific cytotoxicity.**

### **Abstract**

#### ***BACKGROUND:***

Originating from Africa, India, and the Middle East, frankincense oil has been important both socially and economically as an ingredient in incense and perfumes for thousands of years. Frankincense oil is prepared from aromatic hardened gum resins obtained by tapping *Boswellia* trees. One of the main components of frankincense oil is boswellic acid, a component known to have anti-neoplastic properties. The goal of this study was to evaluate frankincense oil for its anti-tumor activity and signaling pathways in bladder cancer cells.

#### ***METHODS:***

Frankincense oil-induced cell viability was investigated in human bladder cancer J82 cells and immortalized normal bladder urothelial UROtsa cells. Temporal regulation of frankincense oil-activated gene expression in bladder cancer cells was identified by microarray and bioinformatics analysis.

#### ***RESULTS:***

Within a range of concentration, frankincense oil suppressed cell viability in bladder transitional carcinoma J82 cells but not in UROtsa cells. Comprehensive gene expression analysis confirmed that frankincense oil activates genes that are responsible for cell cycle arrest, cell growth suppression, and apoptosis in J82 cells. However, frankincense oil-induced cell death in J82 cells did not result in DNA fragmentation, a hallmark of apoptosis.

#### ***CONCLUSION:***

Frankincense oil appears to distinguish cancerous from normal bladder cells and suppress cancer cell viability. Microarray and bioinformatics analysis proposed multiple pathways that can be activated by frankincense oil to induce bladder cancer cell death. Frankincense oil might represent an alternative intravesical agent for bladder cancer treatment.

Chin Med. 2014 Jul 2;9:18. doi: 10.1186/1749-8546-9-18. eCollection 2014.

### **Differential effects of selective frankincense (Ru Xiang) essential oil versus non-selective sandalwood (Tan Xiang) essential oil on cultured bladder cancer cells: a microarray and bioinformatics study.**

Dozmorov MG<sup>1</sup>, Yang Q<sup>2</sup>, Wu W<sup>3</sup>, Wren J<sup>1</sup>, Suhail MM<sup>4</sup>, Woolley CL<sup>5</sup>, Young DG<sup>5</sup>, Fung KM<sup>6</sup>, Lin HK<sup>7</sup>.

## **Author information**

### **Abstract**

#### ***BACKGROUND:***

Frankincense (*Boswellia carterii*, known as Ru Xiang in Chinese) and sandalwood (*Santalum album*, known as Tan Xiang in Chinese) are cancer preventive and therapeutic agents in Chinese medicine. Their biologically active ingredients are usually extracted from frankincense by hydrodistillation and sandalwood by distillation. This study aims to investigate the anti-proliferative and pro-apoptotic activities of frankincense and sandalwood essential oils in cultured human bladder cancer cells.

#### ***METHODS:***

The effects of frankincense (1,400-600 dilutions) (v/v) and sandalwood (16,000-7,000 dilutions) (v/v) essential oils on cell viability were studied in established human bladder cancer J82 cells and immortalized normal human bladder urothelial UROtsa cells using a colorimetric XTT cell viability assay. Genes that responded to essential oil treatments in human bladder cancer J82 cells were identified using the Illumina Expression BeadChip platform and analyzed for enriched functions and pathways. The chemical compositions of the essential oils were determined by gas chromatography-mass spectrometry.

#### ***RESULTS:***

Human bladder cancer J82 cells were more sensitive to the pro-apoptotic effects of frankincense essential oil than the immortalized normal bladder UROtsa cells. In contrast, sandalwood essential oil exhibited a similar potency in suppressing the viability of both J82 and UROtsa cells. Although frankincense and sandalwood essential oils activated common pathways such as inflammatory interleukins (IL-6 signaling), each essential oil had a unique molecular action on the bladder cancer cells. Heat shock proteins and histone core proteins were activated by frankincense essential oil, whereas negative regulation of protein kinase activity and G protein-coupled receptors were activated by sandalwood essential oil treatment.

#### ***CONCLUSION:***

The effects of frankincense and sandalwood essential oils on J82 cells and UROtsa cells involved different mechanisms leading to cancer cell death. While frankincense essential oil elicited selective cancer cell death via NRF-2-mediated oxidative stress, sandalwood essential oil induced non-selective cell death via DNA damage and cell cycle arrest.

### **Carvacrol**

Anticancer Drugs. 2015 Sep;26(8):813-23. doi: 10.1097/CAD.0000000000000263.

## **Carvacrol inhibits proliferation and induces apoptosis in human colon cancer cells.**

Fan K<sup>1</sup>, Li X, Cao Y, Qi H, Li L, Zhang Q, Sun H.

### **Author information**

#### **Abstract**

Colon cancer is one of the most common malignancies worldwide and has a high mortality rate. Carvacrol is a major component of oregano and thyme essential oils and shows antitumor properties. Here, we investigated the effects of carvacrol on the proliferation and apoptosis of two human colon cancer cell lines, HCT116 and LoVo, and studied the molecular mechanisms of its antitumor properties. We found that carvacrol inhibited the proliferation and migration of the two colon cancer cell lines in a concentration-dependent manner. Cell invasion was suppressed after carvacrol treatment by decreasing the expression of matrix metalloprotease-2 (MMP-2) and MMP-9. Carvacrol treatment also caused cell cycle arrest in the G2/M phase and decreased cyclin B1 expression. Finally, carvacrol induced cell apoptosis in a dose-dependent manner. At the molecular level, carvacrol downregulated the expression of Bcl-2 and induced the phosphorylation of the extracellular-regulated protein kinase and protein kinase B (p-Akt). In parallel, carvacrol upregulated the expression of Bax and c-Jun N-terminal kinase. These results indicate that carvacrol might induce apoptosis in colon cancer cells through the mitochondrial apoptotic pathway and the MAPK and PI3K/Akt signaling pathways. Together, our results suggest that carvacrol may have therapeutic potential for the prevention and treatment of colon cancer.

## **Hedychium**

Nat Prod Res. 2016;30(10):1224-7. doi: 10.1080/14786419.2015.1049176. Epub 2015 Jul 21.

### **Composition and in vitro cytotoxic activities of essential oil of Hedychium spicatum from different geographical regions of western Himalaya by principal components analysis.**

#### **Abstract**

The rhizome of Hedychium spicatum has been widely used in traditional medicines. The present study deals with the evaluation of the cytotoxic potential of rhizome essential oils from four different regions of the Western Himalaya (India) along with comparative correlation analysis to characterise the bioactive cytotoxic component. The essential oils were coded as MHS-1, MHS-2, MHS-3 and MHS-4, and characterised using GC-FID and GC-MS. The main volatile compounds identified were 1,8-cineol, eudesmol, cubenol, spathulenol and  $\alpha$ -cadinol. In vitro cytotoxic activities were assessed against

human cancer cell lines such as, the lung (A549), colon (DLD-1, SW 620), breast (MCF-7, MDA-MB-231), head and neck (FaDu), and cervix (HeLa). **MHS-4 is significantly active in comparison to other samples against all cancer cell lines.** Sample MHS-4 has major proportion of monoterpene alcohol mainly 1,8-cineol. Principal components analysis was performed for the experimental results and all four samples were clustered according to their percentage inhibition at different doses.

## **Bergamot**

Fitoterapia. 2013 Sep;89:48-57. doi: 10.1016/j.fitote.2013.05.014. Epub 2013 May 23.

### **Implication of limonene and linalyl acetate in cytotoxicity induced by bergamot essential oil in human neuroblastoma cells.**

#### **Abstract**

Bergamot (*Citrus bergamia*, Risso et Poiteau) essential oil (BEO) is a widely used plant extract showing anxiolytic, analgesic and neuroprotective effects in rodents; also, BEO activates multiple death pathways in cancer cells. Despite detailed knowledge of its chemical composition, the constituent/s responsible for these pharmacological activities remain largely unknown. Aim of the present study was to identify the components of BEO implicated in cell death. To this end, limonene, linalyl acetate, linalool,  $\gamma$ -terpinene,  $\beta$ -pinene and bergapten were individually tested in human SH-SY5Y neuroblastoma cultures at concentrations comparable with those found in cytotoxic dilutions of BEO. None of the tested compounds elicited cell death. However, significant cytotoxicity was observed when cells were cotreated with limonene and linalyl acetate whereas no other associations were effective. Only cotreatment, but not the single exposure to limonene and linalyl acetate, replicated distinctive morphological and biochemical changes induced by BEO, including caspase-3 activation, PARP cleavage, DNA fragmentation, cell shrinkage, cytoskeletal alterations, together with necrotic and apoptotic cell death. **Collectively, our findings suggest a major role for a combined action of these monoterpenes in cancer cell death induced by BEO.**

## **Agarwood**

BMC Complement Altern Med. 2016 Jul 22;16:236. doi: 10.1186/s12906-016-1210-1.

### **In vivo toxicity and antitumor activity of essential oils extract from agarwood (*Aquilaria crassna*).**

#### **Abstract**

#### **BACKGROUND:**

*Aquilaria crassna* has been used in traditional Asian medicine to treat vomiting, rheumatism, asthma, and cough. Furthermore, earlier studies from our laboratory have

revealed that the essential oil extract from agarwood inhibited colorectal carcinoma cells. Despite of the wide range of ethno-pharmacological uses of agarwood, its toxicity has not been previously evaluated through systematic toxicological studies. Therefore, the potential safety of essential oil extract and its in vivo anti-tumor activity had been investigated.

#### **METHODS:**

In the acute toxicity study, Swiss female mice were given a single dose of the essential oil extract at 2000 mg/kg/day orally and screened for two weeks after administration. Meanwhile, in the sub-chronic study, two different doses of the extract were administered for 28 days. Mortality, clinical signs, body weight changes, hematological and biochemical parameters, gross findings, organ weights, and histological parameters were monitored during the study. Other than that, in vivo anti-tumor study was assessed by using subcutaneous tumors model established in nude mice.

#### **RESULTS:**

The acute toxicity study showed that the LD50 of the extract was greater than 2000 mg/kg. In the repeated dose for 28-day oral toxicity study, the administration of 100 mg/kg and 500 mg/kg of essential oil per body weight revealed insignificant difference in food and water intakes, bodyweight change, hematological and biochemical parameters, relative organ weights, gross findings or histopathology compared to the control group. Nevertheless, the essential oil extract, when supplemented to nude mice, caused significant growth inhibition of the subcutaneous tumor of HCT 116 colorectal carcinoma cells.

#### **CONCLUSION:**

Collectively, the data obtained indicated that essential oil extract from agarwood might be a safe material, and this essential oil is suggested as a potential anti-colon cancer candidate.

### **Catnip**

Asian Pac J Cancer Prev. 2016;17 Spec No.:125-30.

#### **Growth Inhibition and Apoptosis Induction of Essential Oils and Extracts of *Nepeta cataria* L. on Human Prostatic and Breast Cancer Cell Lines.**

##### **Abstract**

*Nepeta cataria* L. has been used in traditional medicine of some countries. Here the cytotoxic and apoptogenic activity of methanol extracts, n-hexane, dichloromethane, ethyl acetate, n-butanol, and aqueous extracts and the essential oil obtained from the aerial parts of the plant were evaluated with PC3, DU-145 and MCF-7 cell lines. Cell viability, histograms of PI stained fragmented DNA in apoptotic cells and Western blot analysis of proteins involved in the cascade of apoptosis were compared in all samples.

Thirty components were identified as volatile, representing 99.7% of essential oil composition after GC-MS analysis of the oil obtained from aerial parts of the *N. cataria* by hydro-distillation. The major oil components of the essential oil were nepetalactone stereoisomers. Comparing IC50 values showed estrogen receptor positive PC3 cells were more sensitive to the cytotoxic effects of *N. cataria* in comparison with low hormone-receptor presenting DU-145 cells. Among multiple extracts and essential oils of the plant, only the ethyl acetate extract could significantly decrease cell viability in PC3 cells, in a concentration dependent manner. Ethyl acetate extract of *N. cataria* treated cells showed a sub-G1 peak in PC3 cells in a concentration dependent manner that indicates the involvement of an apoptotic process in ethyl acetate extract-induced cell death. Western blotting analysis showed that in PC3 cells treated with ethyl acetate (48 h) caspase 3 and PARP were cleaved to active forms. **Overall, the results suggest that further analytical elucidation of *N. cataria* in respect to finding new cytotoxic chemicals with anti-tumor activity is warranted.**

## **Geraniol**

Int J Oncol. 2016 May;48(5):1772-82. doi: 10.3892/ijo.2016.3427. Epub 2016 Mar 9.

### **The antitumor effects of geraniol: Modulation of cancer hallmark pathways (Review).**

#### **Abstract**

Geraniol is a dietary monoterpene alcohol that is found in the essential oils of aromatic plants. To date, experimental evidence supports the therapeutic or preventive effects of geraniol on different types of cancer, such as breast, lung, colon, prostate, pancreatic, and hepatic cancer, and has revealed the mechanistic basis for its pharmacological actions. In addition, geraniol sensitizes tumor cells to commonly used chemotherapy agents. Geraniol controls a variety of signaling molecules and pathways that represent tumor hallmarks; these actions of geraniol constrain the ability of tumor cells to acquire adaptive resistance against anticancer drugs. **In the present review, we emphasize that geraniol is a promising compound or chemical moiety for the development of a safe and effective multi-targeted anticancer agent.** We summarize the current knowledge of the effects of geraniol on target molecules and pathways in cancer cells. Our review provides novel insight into the challenges and perspectives with regard to geraniol research and to its application in future clinical investigation.

Int J Oncol. 2012 May;40(5):1683-90. doi: 10.3892/ijo.2011.1318. Epub 2011 Dec 23.

### **Geraniol induces cooperative interaction of apoptosis and autophagy to elicit cell death in PC-3 prostate cancer cells.**

#### **Abstract**

Geraniol, an acyclic dietary monoterpene, suppresses prostate cancer growth and enhances docetaxel chemosensitivity in cultured cell or xenograft tumor models. However, the mechanisms of the geraniol action against prostate cancer are largely unknown. In this study, we investigated the cellular and molecular mechanisms of geraniol-induced cell death in PC-3 prostate cancer cells. Among the examined structurally and functionally similar monoterpenes, geraniol potently induced apoptosis and autophagy. Although independent processes, apoptosis and autophagy acted as cooperative partners to elicit geraniol-induced cell death in PC-3 cells. At a molecular level, geraniol inhibited AKT signaling and activated AMPK signaling, resulting in mTOR inhibition. **Combined treatment of AKT inhibitor and AMPK activator markedly suppressed cell growth compared to either treatment alone.** Our findings provide insight into future investigations that are aimed at elucidating the role of apoptosis and autophagy in prostate cancer therapy and at developing anticancer strategies co-targeting AKT and AMPK.

Biochem Biophys Res Commun. 2011 Apr 1;407(1):129-34. doi: 10.1016/j.bbrc.2011.02.124. Epub 2011 Mar 1.

### **Geraniol inhibits prostate cancer growth by targeting cell cycle and apoptosis pathways.**

#### **Abstract**

The progression of prostate cancer is associated with escape from cell cycle arrest and apoptosis under androgen-depleted conditions. Here, we found that geraniol, a naturally occurring monoterpene, induces cell cycle arrest and apoptosis in cultured cells and tumor grafted mice using PC-3 prostate cancer cells. Geraniol modulated the expression of various cell cycle regulators and Bcl-2 family proteins in PC-3 cells in vitro and in vivo. **Furthermore, we showed that the combination of sub-optimal doses of geraniol and docetaxel noticeably suppresses prostate cancer growth in cultured cells and tumor xenograft mice.** Therefore, our findings provide insight into unraveling the mechanisms underlying escape from cell cycle arrest and apoptosis and developing therapeutic strategies against prostate cancer.

### **Nigella**

Ultrason Sonochem. 2016 Jul;31:449-55. doi: 10.1016/j.ultsonch.2016.01.035. Epub 2016 Jan 29.

### **Anticancer activity of an ultrasonic nanoemulsion formulation of Nigella sativa L. essential oil on human breast cancer cells.**

#### **Abstract**

*Nigella sativa* L. (NS) is a plant renowned in traditional holistic medicine systems for almost 1400 years because of its remarkable antioxidant, antimicrobial, anti-inflammatory and anti-cancer properties. The essential oil of *N. sativa*, in particular, possesses these significant biological properties. However, *N. sativa* essential oil has many insoluble constituents with properties that have not been fully explored. Nanoemulsion-based insoluble formulations are a widely used carrier system for lipophilic materials. In the present study, we used ultrasonic emulsification, polysorbate 80 and water to formulate a highly stable *N. sativa* essential oil nanoemulsion (NSEO-NE). To optimize the NSEO-NE preparation, we changed the surfactant concentration, the oil-surfactant mixing ratio and the emulsification time. The droplet size distribution and morphology of the prepared NE was analyzed using dynamic light scattering and scanning electron microscopy, respectively. The droplet size of the NSEO-NE was approximately 20-50 nm in diameter. The anticancer properties of the NE preparation were studied using a modified methyl-thiazolyl-diphenyl tetrazolium bromide (MTT) assay as well as cellular uptake and nuclear morphological analyses. The NSEO-NE significantly reduced the viability of Michigan Cancer Foundation-7 (MCF-7) breast cancer cells. The nucleocytoplasmic morphological features of NSEO-NE-treated cells included cell membrane blebbing, cytoplasmic vacuolation, marginalization of chromatin, and fragmentation of the nucleus. **The results clearly indicate that NSEO-NE induced apoptosis in MCF-7 cells. These findings support the potential application of NSEO-NE in breast cancer therapy, and also merit future translational research.**

## **Calamus**

Sci Rep. 2016 Feb 19;6:21148. doi: 10.1038/srep21148.

### **Volatile Oil of *Acori Graminei* Rhizoma-Induced Apoptosis and Autophagy are dependent on p53 Status in Human Glioma Cells.**

#### **Abstract**

*Acori Graminei* Rhizoma is well known for the beneficial effects on CNS disorders in traditional medicine. Though it is frequently prescribed in formulations for brain tumors, the anti-glioma effect has not been examined. We used volatile oil of *Acori Graminei* Rhizoma (VOA) and human glioblastoma multiforme (GBM) cells in this study. We found that VOA exhibited greater growth suppression in p53 wild-type cells than p53 mutant cells and very low effect on fibroblasts and human glial HEB cells. Apoptosis was triggered by VOA with a caspase-dependent way in p53 wild-type A172 cells, while a caspase-independent way in p53 mutant U251 cells. Meanwhile, both A172 and U251 cells treated by VOA displayed autophagic features. Furthermore, p53 decrease was observed along with VOA-induced apoptosis and autophagy in A172 cells.

VOA-induced autophagy was mediated through a p53/AMPK/mTOR signaling pathway in A172 cells, while an mTOR-independent signaling pathway in U251 cells. Finally, blockage of autophagy potentiated the proapoptotic effect in both A172 and U251 cells, indicating a protective role of autophagy in VOA-induced cell death. **Together, VOA exhibited anti-tumor activity in human GBM cells and induced apoptotic cell death and protective autophagy, which is cell type specific and dependent on p53 status.**

## **Cinnamon**

J BUON. 2015 Nov-Dec;20(6):1518-25.

**Essential oil of Cinnamon exerts anti-cancer activity against head and neck squamous cell carcinoma via attenuating epidermal growth factor receptor - tyrosine kinase.**

### **Abstract**

#### **PURPOSE:**

Impressed by the exceptional anticancer activity of cinnamon, the present study was conducted to elucidate the anticancer potential of essential oil of Cinnamon (EOC).

#### **METHODS:**

EOC was tested against various cell lines (FaDu, Detroit-562 and SCC-25) of head and neck squamous cell carcinoma (HNSCC) using MTT assay. The Hep-2 cell xenograft model was used to assess the positive bio-activity of EOC. EGFR-TK inhibitory assay was also carried out to explain the possible mechanism of action of EOC. Moreover, to rationalise the key contacts responsible for attenuating EGFR, the major component of EOC, i.e., trans-cinnamaldehyde, as identified by GC-MS analysis, was subjected to molecular docking experiments with the catalytic domain of EGFR protein model.

#### **RESULTS:**

EOC exhibited significant anticancer activity with percent inhibition 66.12, 87.32, and 99.34%, against FaDu, Detroit-562 and SCC-25, respectively. Moreover, EOC reduced the tumor burden to 43.5% in Hep-2 cell xenograft model along with 89% inhibition of EGFR-TK activity in the EGFR-TK inhibitory assay. Docking experiments showed that trans-cinnamaldehyde was proficiently fitted into the inner groove of the active site of EGFR by making close inter-atomic contacts with the key catalytic residues Val702, Ala719, Lys721, Leu764, Thr766 and Leu820 and with inhibition constant  $K_i = 775.93 \mu\text{M}$ .

#### **CONCLUSION:**

**EOC exhibits significant anticancer activity against HNSCC cells in vitro.** The mechanism underlying its anticancer action was attributed to the suppression of EGFR-TK. It also significantly suppressed the tumor growth in Hep-2 cell xenograft model.

(Antibacterial Essential Oils Reduce Tumor Smell and Inflammation in Cancer Patients  
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Tumor necrosis with associated malodor in cancer patients is a serious problem in oncology and palliative care throughout the world.<sup>1</sup> Necrotic neoplastic ulcers are usually superinfected with anaerobic bacteria such as *Bacteroides*, *Enterobacter*, or *Escherichia coli* species,<sup>2</sup> especially when the ulcers communicate with the oral or nasal cavity. Patients suffering from tumor malodor suffer the additional stress of both perceived and real social isolation in the hospital wards and in the community as a result of their smell.

In an attempt to address this problem, we have been conducting a pilot study in the use of topically applied essential oils. Essential oils such as tea tree and eucalyptus oils have recently gained acceptance as safe and effective antiseptics.<sup>3</sup> Their potent bactericidal activity has already been proven in in vitro and clinical trials.<sup>4-8</sup>

We have previously reported that the elimination of tumor-related malodor is possible with an essential oil mixture, the application of which led to an improved quality of life for our cancer patients.<sup>9</sup> We are now able to report that we have observed an additional beneficial effect of the topical application of essential oils in patients with head and neck tumor-related ulcers: they promote ulcer healing and re-epithelization.

A 67-year-old man presented with an extremely large and inoperable squamous cell cancer of the left buccal mucosa that had eroded through to the extraoral skin, resulting in a fistula. Having previously declined surgery, radiotherapy, and chemotherapy, his reason for presenting was the foul smell emanating from the ulcer. This was a result of a suppurative superinfection. Our approach to management was two-fold: In addition to a 5-day course of oral clindamycin (600 mg twice daily), the fistula was rinsed twice a day with 5 mL of a *Eucalyptus*-based oil mixture (KielMix-PT 70; Klonemax, Central Tilba, Australia). After 3 days, the patient's malodour had completely resolved. The patient was discharged home after 14 days. Oil therapy was continued postdischarge, and administered daily by his wife, a former nurse. After 2 and a half weeks, clinical signs of superinfection in terms of inflammation and purulence were markedly reduced. The fistula appeared clean, and there was evidence of healing by secondary intention: a layer of fibrin had formed, deep in the fistula. After 6 weeks of the essential oil regimen and no further antibiotics, the fistula had completely closed. This is not the usual course of events for ulcers associated with cancers of the head and neck. The oil therapy did

not retard the growth of the tumor, and by the eighth week it had grown along the path of the former fistula to the skin surface. Despite this, the offensive smell that had prompted his presentation to the emergency department did not recur. His quality of life had been improved by the use of the essential oils, and he was able to live at home with his family for the next 10 weeks until he died.

We have now treated more than 30 patients with a similar regimen of topical essential oils. Adverse effects are uncommon and are usually limited to minor irritation at the time of application. The beneficial effects, however, have been quite pronounced. We are in the process of planning a randomized controlled trial to confirm our observations.