RESEARCH PAPER

Boswellia Carterii Gum Resin Induced Apoptosis on Human Colon Cancer Cell line HCT-116

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Abstract

Colorectal cancer (CRC) represents one of the highest malignancies causing death globally. Although the cytotoxic and apoptotic-inducing effect of the chemotherapy-based drug,5-Fluorouracil (5-FU), its side effects are severe. Indeed, *Boswellia carterii* (B.C.) gum resin, with its active components possess anti-inflammatory and anti-cancer activities. This study aims to develop new natural-based anticancer drug via evaluating the cytotoxic, anti-proliferative and apoptotic effects of B.C. gum resin on the HCT-116 colon cancer cell line.B.C. gum resin active components were characterized using Gas Chromatography coupled Mass Spectrometry (GC/MS) and heavy metals analyses. The cytotoxic effect of B.C was evaluated via cell viability assay and morphological changes. Apoptotic-inducing effect of B.C. was assessed via caspase-3 activity and flow cytometry assay. Results revealed that the four terpenes; 24-Norursa-3,12-dien-11-one (56.53%), 24-Norursa-3,12-diene (20.66%), 24-Norursa-3,9(11),12-triene (11.278%) and 24-Noroleana-3,12-diene (7.678%) represents >96% of the total active B.C. components. Heavy metals results showed78% calcium, 18% magnesium with an excellent Selenium concentration. B.C. gum resin exhibited IC_{50} level of 1.74 ± 0.1 µg/ml accompanied with cell shrinking, organelles degradation and vacuoles formation in a concentration dependent manner. caspase-3 activity was significantly increased (p 0.03) by 3- and 4-fold in cells treated with 1.7 and 5 µg/ml from B.C. gum resin respectively, compared to the untreated cells. Flow cytometry analysis showed that cells treated with 1.7 and 5µg/ml of B.C. gum resin exhibited 78% and 84% of apoptotic cells respectively. In conclusion, B.C. gum resin is a promising natural therapeutic agent against CRC through its potent cytotoxic and apoptotic-inducing effects.

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Introduction

CRC, one of the most common tumors throughout the world, was the prominent cause of death from cancer among adults (Aman *et al.,* 2016). The classical chemotherapy agent, 5-Fluorouracil (5-FU), was previously the first choice for CRC treatment (Meyerhardt and Mayer, 2005). Its apoptotic effect leads to cancer cells growth inhibition. However, the drawbacks of 5-FU uses are its limited response rate in the advanced CRC stages (Tebbutt *et al.,* 2002) in addition to its severe impact on many organs, including skin and gastrointestinal tract (Barbara *et al.,* 2011). Thus, novel effective and safe therapeutic agents, multitargeted to several mechanisms of carcinogenesis, are severely needed. Traditional medicine from natural compounds have been used for thousands of years and still until now considered the most promising source of therapeutic agents (Gusella *et al.,* 2009).

Boswellia (family Burseraceae) are short-lived trees having enormous and diverse species. A unique type of resin with specific chemical composition was produced by each species (Al-Harrasi and Al-Saidi, 2008). Olibanum (frankincense), are exuded from the trunks of Boswellia genus trees in a milk-like form, which subsequently hardens into orange-brown gum upon exposing to air (Fig.1). Aromatic gum resin has been widely used as traditional medicine for various diseases treatment. Actually, more than three quarters of its composition was alcohol-soluble resin, while the essential oil and the water-soluble gums represent the remaining amount. *Boswellia* resins have been used in ancient remedies since the Ancient Egyptian time (Nagwa *et al.,* 2013). Several studies have recorded various biological activities of compounds and extracts isolated from these resins. Trees of the *Boswellia* genus are mainly distributed over the northeast Africa countries, the Arabian Peninsula as Yemen, Oman, in addition to its prevalence in India.

The most economically important species are *Boswellia sacra* (Arabian frankincense; Yemen, Oman) (Sunnichan *et al.,* 2005), *Boswellia*

carterii (Somalian Frankincense; Somalia), *Boswellia papyrifera* (Ethiopia, Eritrea, Sudan) and *Boswellia serrata* (Indian Frankincense) (Ogbazghi *et al.,* 2006).

The present study focused on the *boswellia Carterii* (B.C.) gum resin as a safe and effective natural compound-based therapeutic agent for the human colon cancer cell line. B.C. gum resin and its bioactive constituents including boswellic acids, several triterpenoids and essential heavy metals have powerful anti-oxidant, anti-inflammatory as well as anti-cancer activities (Nishimura *et al.,* 2006). Triterpenoids, isolated from B.C. gum resin have shown to possess anti-proliferative, cytotoxic and cytostatic effects (Poeckel and Werz, 2006). Many previously *in vitro* findings have reported the potent anticancer activity of boswellic acid via its apoptotic and anti-proliferative impacts in a number of human cancer cell lines, in particular, the human colon cancer one(Nishimura *et al.,* 2006).

The crucial proteases activity of Caspase-3 and Caspase-7 during apoptosis have been previously demonstrated. Caspase-3 activation lead to many morphological changes including; cell disassembly, organelles degradation, cell shrinking and DNA fragmentation (Aman *et al.,* 2016). Hence, several studies demonstrated that successful anticancer agents should exert their effects by inducing apoptosis and inhibit cell proliferation with a good IC_{50} level (Stein *et al.,* 2011). However, the mechanism induced by B.C. gum resin against colon cancer is not yet elucidated. Therefore, the present study aims to evaluate the cytotoxic, the anti-proliferative as well as the apoptotic effects of B.C. gum resin with referring to the standard 5-FU drug on the human colon cancer cell line (HCT-116).

Materials and methods

Plant materials

Dried B.C. gum-resin, were purchased from one of the Egyptian spices herbal store, Cairo, Egypt. The dried gum resins were crushed into fine powder using electric blender. The powder was freshly used after

reconstitution.

GC-MS analysis of Boswellia Carterii gum resin
The phytochemical examination of the B.C. gum resin was accomplished on a GC-MS equipment (Agilent technologies model; GC-MS-5977 A/7890 B). Analytes were loaded on a 30 m × 0.25 mm nonpolar capillary column with 0.25 µm thickness and interfaced with a quadrupole mass spectrometer. Injector and interface temperature were adjusted at 300 °C and 275 °C respectively. The carrier gas, Helium was used with 10.066 psi and the total flow rate was 46.419 ml/min.

Heavy metals analysis of Boswellia Carterii gum resin
The total contents of heavy metals in the B.C. gum resin sample was evaluated through samples digestion according to the method of (Yeihseu *et al.,* 2002) with HNO_3-H_2O_2 mixture in a microwave ovens of Inductively Coupled Argon plasma, iCAP 6500 Duo, Thermo Scientific, England.

HCT-116 cell lines and cultures
The human colon cancer cell lines (HCT-116) were obtained from VACSERA Tissue Culture Unit. Dulbecco's Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, HEPES buffer and 50µg/ml gentamycin was used for cells propagation. Cells were maintained at 37ºC in a humidified atmosphere with 5% CO_2 and were sub-cultured twice a week.

Cell viability assay and morphological changes examination
In order to determine the cytotoxic effect of B. C. gum resin on the HCT-116 cell line, viable cells percentage was evaluated using the tetrazolium salt according to (Mosmann, 1983) method.

In order to examine the morphological changes resulted from the effect of various B.C. gum resin concentrations on the HCT-116 cells. Cell viability staining assay was conducted according to the method of (Gomha *et al.,* 2015).

Apoptotic-inducing effect of Boswellia Carterii gum resin
In order to evaluate the apoptotic inducing effect of the B.C. gum resin on the HCT-116 cell line, Caspase-3 activity level was determined according to the instructor's manual of the quantitative ELISA human active Caspase-3 immunoassay kit R&D systems; Cat. No. KM300 (Minneapolis, MN, USA).

Apoptosis assay using flow cytometry
HCT-116 cells were cultured for 2 days, medium was replaced with fresh one and two different concentrations of B.C. gum resin; 1.7 µg/ml and 5 µg/ml were added. 48 hours post-treatment, both floating and adherent cells were collected for apoptosis assay using Annexin V-FITC Apoptosis Detection Kit (Beckman Coulter, PN IM3546), according to the manufacturer's instructions.

Statistical analysis
Statistical analysis of data was carried out using the Statistical Package for the Social Science (SPSS) for Windows (version 22.0, Chicago, IL, USA). Data were presented as mean values ± SD. Results were analyzed by t-student test. $P<0.05$ was considered statistically significant.

Results
B.C. gum resin characterization
The GC/MS results of the B. C. gum resin revealed the identification of 14 different compounds (table 1). Results showed that four triterpernes represent the highest percentage (>96%)of the total B.C. active constituents.

These four main triterpenes are 24-Norursa-3,12-dien-11-one (56.53%), 24-Norursa-3, 12-diene (20.66%), 24-Norursa-3,9(11),12-triene (11.278%) and 24-Noroleana-3, 12-diene (7.678%). They represent 93% of the total contents of the B.C. gum resin.

Their deconvoluted mass spectra enabled us to identify these compounds as dehydrated, deacetoxylated and decarboxylated forms of boswellic

acids (Fig. 2).

Heavy metals contents of Boswellia carterii gum resin

Results shown in Table 2 and Fig.3 revealed the presence of 15 metals with different concentrations. It is also interested to denote that calcium (3.4 g/kg) represents the highest percentage (78%) of the total

metals found in B.C. gum resin sample. In addition, magnesium (793.5 mg/kg), iron (78.88 mg/kg), and aluminum (87.825 mg/kg) represent 18%, 2% and 2% respectively of the total heavy metals identified. Moreover, selenium which is a trace essential element was detected with a good level of 430.4 µg/kg in B.C. gum resin sample.

Table 1. Compounds identified in the *Boswellia carterii* by GC-MS analysis.

PK	RT	Area Pct (% value)	Name of compound
1	16.2845	0.3072	(S,E)-8,12,15,15-Tetramethyl-4-methylenebicyclo[9.3.1]pentadeca-7,11-diene
2	17.169	1.8058	Isopropyl-1,5,9-trimethyl-15-oxabicyclo[10.2.1]pentadeca-5,9-dien-2-ol
3	17.2714	1.4064	3-Nonen-2-one, 3-ethyl-
4	17.5694	0.2466	Thunbergol
5	17.9139	1.7649	1-Isopropyl-5,9,13-trimethyl-4,16-dioxatricyclo[11.2.1.03,5]hexadec-8-en-12-ol
6	17.9465	0.3	3-Cyclopentylpropionic acid, undec-2-enyl ester
7	18.2211	0.8371	3-Cyclopentylpropionic acid, undec-2-enyl ester
8	18.4725	0.2246	Thunbergol
9	18.5051	0.2684	Pyridine, 3-(5-furan-2-yl-[1,2,4]oxadiazol-3-yl)-2-methoxy-6-(4-methoxyphenyl)-
10	18.6122	0.8107	trans-Geranylgeraniol
11	19.1382	0.2666	(E)-3-Methyl-5-((1R,4aR,8aR)-5,5,8a-trimethyl-2-methylenedecahydronaphthalen-1-yl)pent-2-en-1-ol
12	19.9622	0.331	24-Norursa-3,12-dien-11-one
13	19.9808	1.0456	24-Norursa-3,12-dien-11-one
14	20.5721	23.2164	24-Norursa-3,12-dien-11-one
15	21.2983	1.204	5H-Dibenz[b,f]azepine-5-carbonitrile
16	21.5822	1.9607	24-Norursa-3,9(11),12-triene
17	21.964	9.3173	24-Norursa-3,9(11),12-triene
18	22.0524	7.6784	24-Noroleana-3,12-diene
19	22.3923	12.9817	24-Norursa-3,12-diene
20	23.6213	0.6709	6-Chlorothiocarbonyl-3-nitro-1-methyl-2-pyridone
21	23.8121	1.2253	A:D-Neooleana-12,14-diene, (3.xi.,5.alpha.)-
22	24.1892	1.7595	24-Norursa-3,12-dien-11-one
23	24.4034	0.1869	MDMA methylene homolog
24	24.706	30.1841	24-Norursa-3,12-dien-11-one

Evaluation of the anticancer effect of Boswellia carterii against HCT- 116

In order to elucidate the inhibitory effects of B.C. gum resin on the colorectal cancer in vitro, different concentrations of B.C. were used on the HCT-116 cell lines for 48 hrs.

Table 2. Heavy metals content in the *Boswellia carterii* gum resin.

Parameter	Results
Aluminum, mg/kg	87.825
Calcium, mg/kg	3369.5
Cadmium, mg/kg	0,0006
Cobalt, mg/kg	0.1325
Chromium, mg/kg	3.8925
Copper, mg/kg	7.07
Iron, mg/kg	78.875
Magnesium, mg/kg	793.5
Manganese, mg/kg	2.3925
Molybdenum, mg/kg	0.1975
Nickel, mg/kg	3.015
Lead, mg/kg	1.105
Vanadium, mg/kg	$\angle$0.01
Zinc, mg/kg	6.635
Selenium, µ/kg	430. 4

Table 3. Caspase-3 activity level in HCT-116 cells treated with different concentrations of *B.C* and 5-FU.

Groups / Parameter	untreated Cells Control group	Cells treated with B.C.			Cells treated with 7.5 µg/ml 5-fluorouracil
		0.5 µg/ml	1.7 µg/ml	5 µg/ml	
Caspase-3 conc. (ng/mg protein)	0.31± 0.17	0.49± 0.25	0.95± 0.34*	1.26± 0.42*	0.87± 0.21*

Data are expressed as mean ± SD, *t-test*, *p<0.05 was s*tatistically significant.

The results showed that the low concentration of B.C. gum resin (up to 0.5 µg/ml) exerted moderate inhibition of cell viability (0-36%), whereas, 90% of HCT-116 cells were inhibited upon treatment with 60 µg/ml of B.C.

The inhibitory effect of B.C. gum resin was dose dependent. Moreover, the treated HCT-116 cells showed a good IC$_{50}$ level of 1.74 µg/ml after 48 hours treatment with the gum resin of B.C. and indicated its promising cytotoxic effect on the colorectal cancer cells as shown in Fig.4a.As shown in Fig.4b, the anti-proliferative impact of the B.C. gum resin on the HCT-116 cell line was morphologically observed. The effect of 1µg/ml of B.C. treated cells showed more dead cells and low confluence compared to the control group. Additionally, some cells contained small vacuoles. By increasing the concentration of the B.C. gum resin, treatment (3.9, 7.8 µg/ml), the number of dead cells increase accompanied with a decrease in the cell confluence. Whereas, the 15.6µg/ml of the B.C. gum resin showed high number of dead cells with cell shrinking and appearance of many vacuoles.

The most effective concentration of the B.C. gum resin; 31.25µg/ml showed death of most cells. These results showed the inhibitory impact of the B.C. gum resin on the HCT-116 cells via apoptotic induction.

Apoptotic-inducing effect of B.C. via caspase-3 activation
HCT-116 colon cancer cells treated with 0.5 µg/ ml, 1.7µg/ml and 5µg/ml from the gum resin of B.C.,

showed a significant increase in the caspase-3 activity (p 0.03) by 1.5-fold, 3-fold and 4-fold respectively compared to the untreated control cells. Whereas, 7.5 µg/ml of 5-FU induces a significant increase in the caspase-3 activity level by 3-fold on the HCT-116 cells, compared to the control group (Table3 and Fig.5a).

Evaluation of B.C. apoptotic-inducing effect by flow cytometry

The apoptotic inducing impact of B.C.gum resin was confirmed by flow cytometry after staining with Annexin V/PI. The present results evaluate the apoptotic activity of 1.7 µg/ml and 5 µg/ml B.C.gum resin on the HCT-116 cells 48 hours after treatment compared to the untreated cells. Fig. 5bshows that the apoptotic cells, including those both in early (lower right quadrant, c4) and late (upper right quadrant, c2) apoptosis, induced by 1.7µg/ml and 5 µg/ml B.C. gum resin were 78% and 84% respectively. While, the cytogram indicates that only 21.9% of apoptotic cells were detected in the untreated cells.
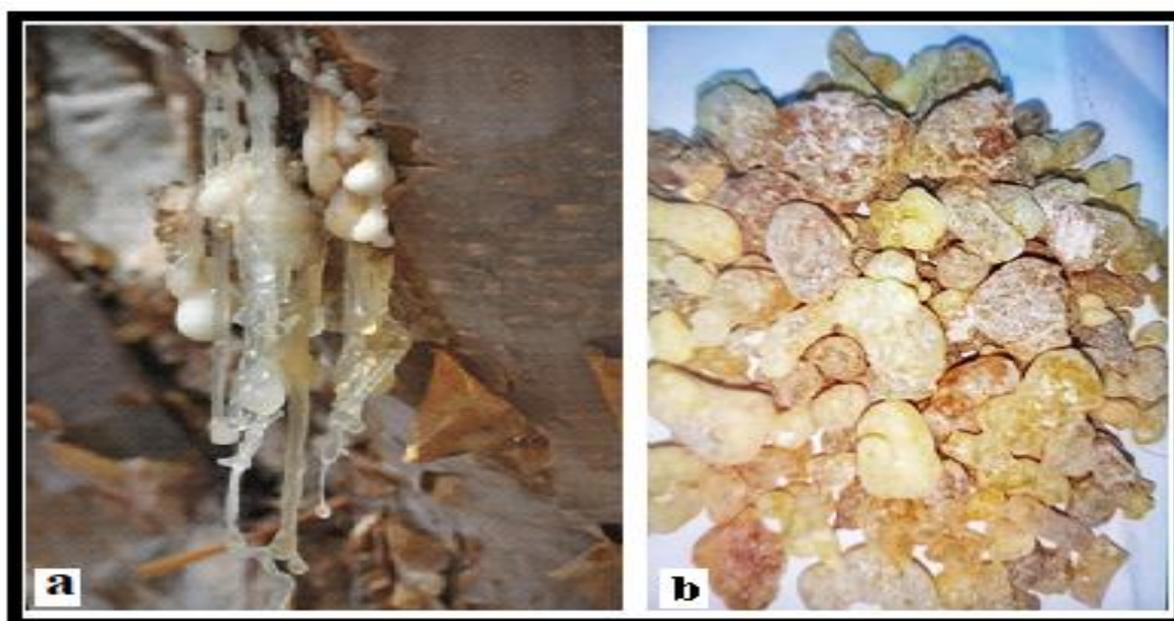
Fig. 1. *Boswellia carterii* gum resins. Frankincense tree incision and tears (a), Somalia *B.C.* gum resin (b).

These results prove and confirm the potent inducing apoptotic effect of B.C. gum resin on the HCT-116 cell line.

Discussion

CRC is one of the uppermost occurrences with highest mortality frequency of any cancer. Surgery only, or synergized with 5-FU-based chemotherapy is the current and mainstay treatment option for the different CRC stages (Stein *et al.,* 2011). 5-FU induces apoptosis via the activation of caspases level in the target cells (Flanagan *et al.,* 2016). However, 5-FU has many limitations. Besides, it's low response rate, it exhibits multiple undesirable side effects in the CRC treatment such as; severe hepatotoxicity as well as general organ toxicity; including myelotoxicities,

neurotoxicities and cardiotoxicities (Alvarez *et al.,* 2007).At the same time, it was found to cause highly elevated levels in the liver enzymes, bilirubin, associated with hyperammonemia (Zorzi *et al.,* 2007). Additionally, Serious bone marrow suppression, gastrointestinal toxicity, including stomatitis was also demonstrated especially when it's administrated via intravenous bolus injection. Moreover, multiple histopathological abnormalities in the liver were revealed apoptotic cell death, necrotic cells and appearance of numerous areas of inflammatory cells after intraperitoneal administration (Ouyang *et al.,* 2012).

Thus, development of novel safe therapeutic strategies is critically needed.
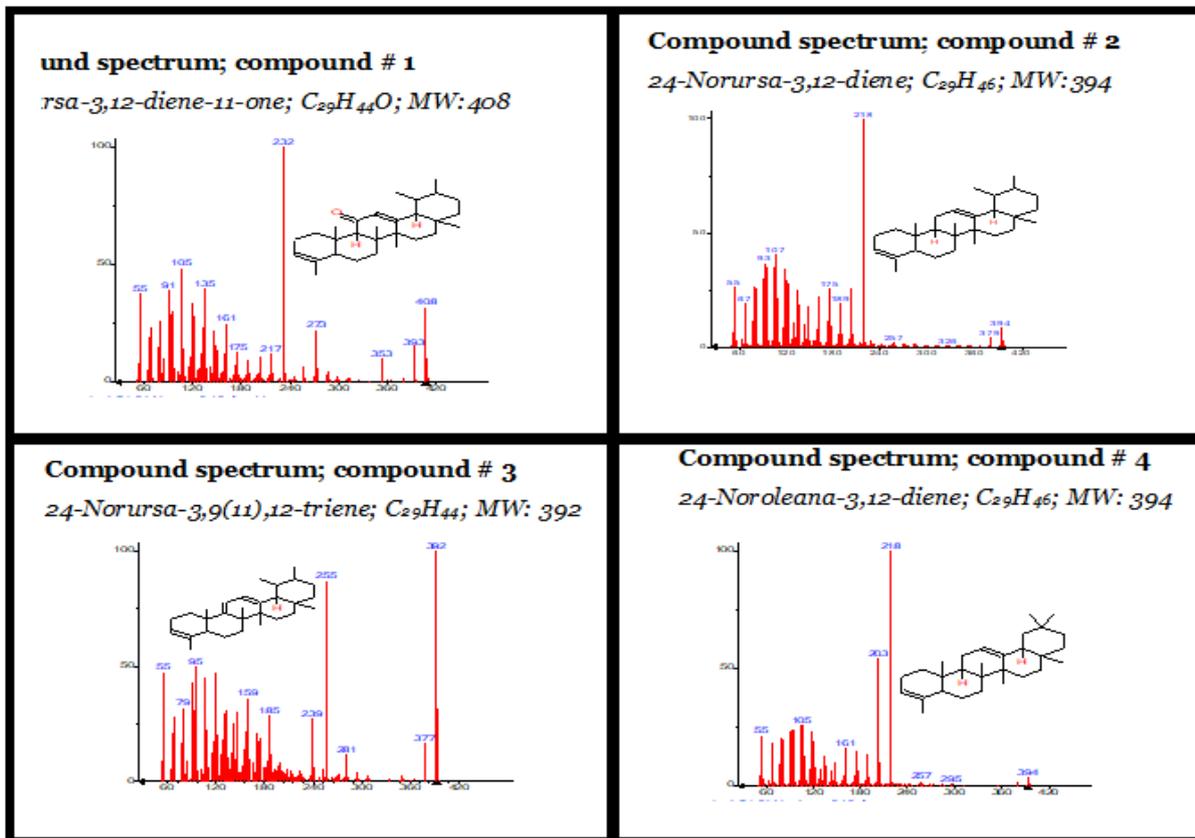
Fig. 2. Mass spectral deconvolution of the four main terpenes identified in the *B.C.* using GC/MS analysis.

The increasingly promising source of therapeutic agents is the traditional treatment with natural agents (Yadav *et al.,* 2012). B.C. gum resin has been historically used as a traditional medicine for many years. It has been shown to alleviate various inflammatory conditions and were used as antibacterial, antifungal, immunomodulatory and anti-hyperlipidemic agents (Takahashi *et al.,* 2012).
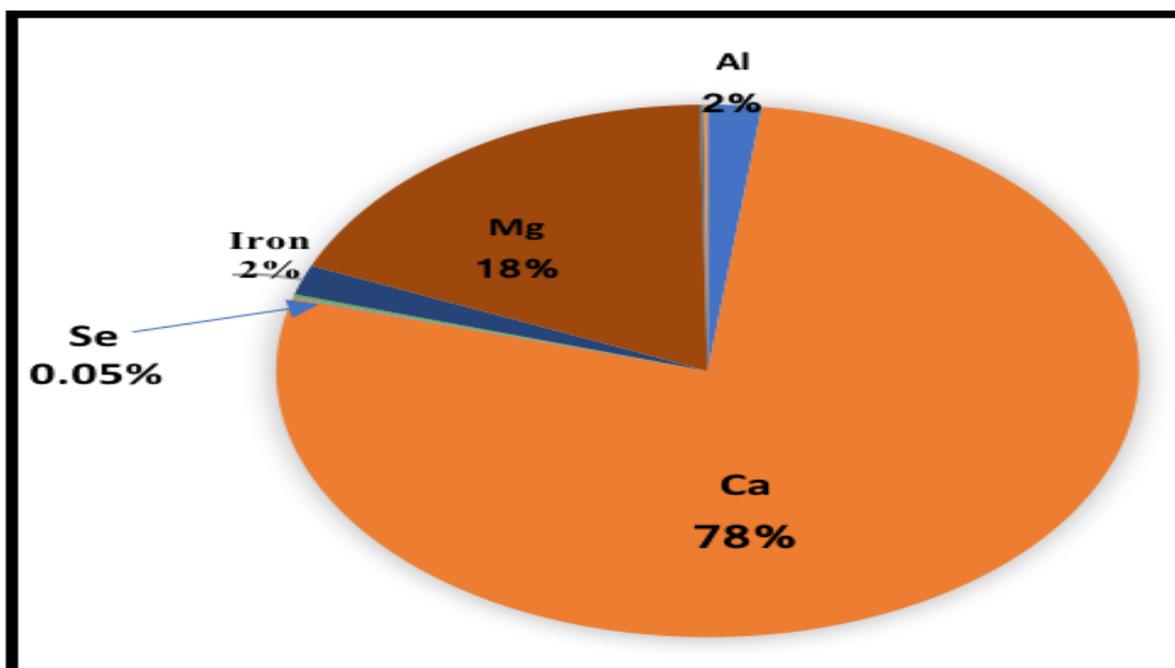
Fig. 3. The percentage value of the heavy metals identified in the *Boswellia Carterii* gum resin.

B.C. gum resin characterization

The GC/MS analysis of the B.C. gum resin result in the identification of triterpenes, sesquiterpenes, alcohols as well as esters. The four main identified triterpenes; 24-Norursa-3, 12-dien-11-one, 24-Norursa-3,12-diene, 24-Norursa-3,9(11),12-triene and 24-Noroleana-3,12-diene were exhibited to account for the highest percentage of the components present in the tested sample (93%).
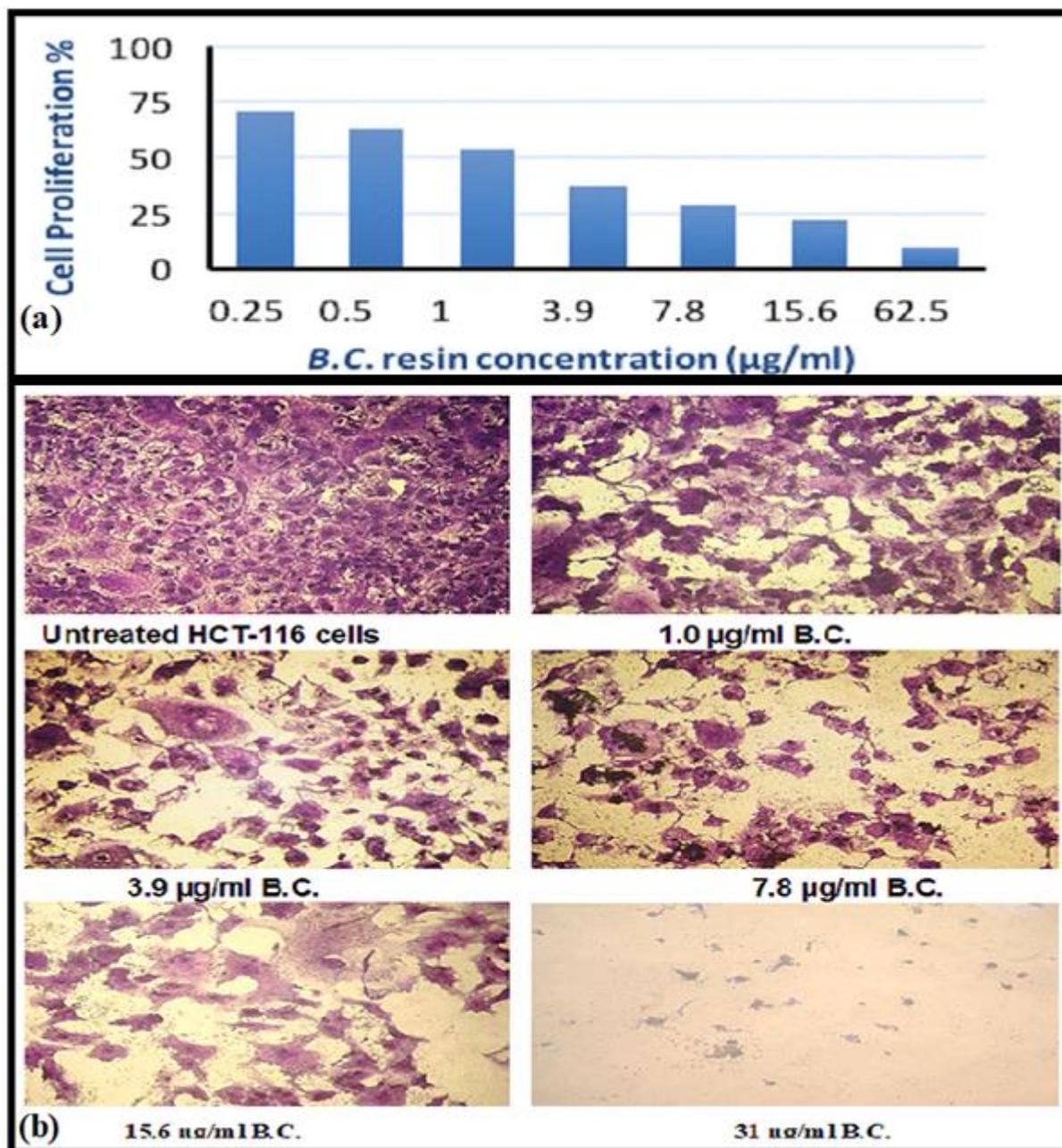
Fig. 4(a &b). Morphological and anti-proliferative effect of different concentrations of *B.C.* on the HCT-116 cell line.

The inhibitory effect of *B.C.* gum resin on the growth of the HCT-116 CRC cells. The cells were exposed to different concentrations of *B.C.* gum resin treatment for 48 hrs. And the cell proliferation % was determined. The data showed a highly anti-proliferative effect of *B.C.* gum resin on the HCT- 116 cells viability with a good IC_{50} level of1.74±0.1 µg/ml (a). Morphological changes on the HCT-116 cells following treatment with 1 µg/ml *B.C*, 3.9 µg/ml *B.C*, 7.8 µg/ml *B.C*, 15.6 µg/ml *B.C* and 31 µg/ml *B.C.* gum resins were photographed with a microscope after staining. The results showed that the inhibitory effect of *B.C.* gum resin was concentration dependent (b).

These main triterpenes reflect the high content of boswellic acids and alpha-amyrin found in the fresh resin. *Boswellia sp.* produced many crucial phytochemicals as amyrins and their acetates and oxidized forms (Chudzik *et al.,* 2015).By contrast, 24-nortriterpenoids such as 24-Norursa-3,12-dien-11-one, 24-Norursa-3,12-diene, 24-Norursa-3,9(11),12-triene are much more specific to the *Boswellia* resin and are produced throughchemical modification of

boswellic acids and their corresponding acetates(Van Bergen *et al.,* 1997). Triterpenes have caught great research attention. Several studies have demonstrated the apoptotic, cytotoxic, cytostatic, and anti-proliferative activity of triterpenoids isolated from B.C. gum resin against a multiple number of cancer cell lines, especially the colon cancer cell line(Nishimura *et al.,* 2006).
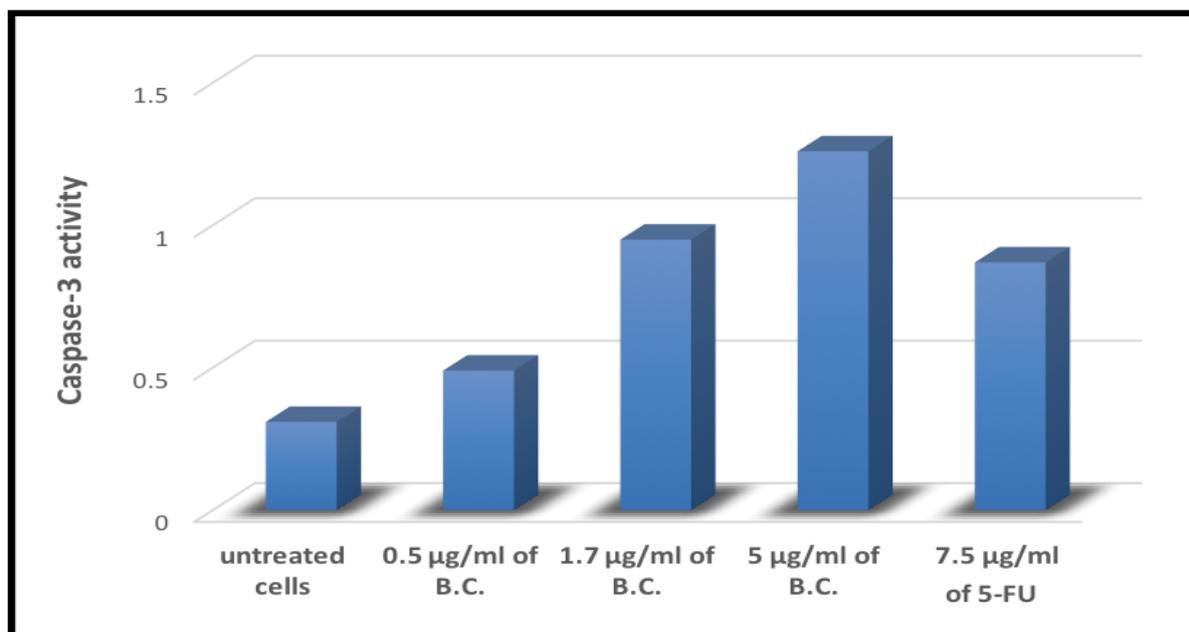
Fig. 5(a). *Boswellia carterii* gum resin induces caspase-3 activity.

Caspase-3 activity was determined in the HCT-116 cell supernatants after 48 hrs. post treatment with 3 different concentrations of *B.C.* (0.5, 1.7 and 5 μg/ml) as well as 7.5 μg/ml of 5-FU using the human caspase-3assay ELISA kit. Data represented as mean ±SD. Significant results were detected; **P<0.05* compared to the untreated control cells.

The potent effect of boswellic acids, in preventing or blocking the CRC metastasis has been previously reported (Takahashi *et al.,* 2012). Boswellic acids exerts its anticancer property by inhibitingthe 5-lipoxygenase (5-LO) enzyme. It is an essential enzyme in the leukotrienes biosynthesis from arachidonic acid.

These findings are in agreement with Badria *et al.,* 2003, who previously demonstrated that the total extract of B.C. exhibited better cytotoxic, anti-proliferative as well as antiviral activities than that of any of the isolated pure compounds. This may be due

to the synergistic effect among the different components of the total extract.

Heavy metals contents of Boswellia carterii gum resin

In addition to terpenes, the dietary intake of certain nutrients has been associated with a reduction in the risk of CRC, especially, Ca, Mg and Se. The heavy metals identified in our sample, revealed the presence of calcium 78%, magnesium 18 % with a ratio of 4:1. Moreover, B.C. gum resin was shown to contain a good level of selenium (430.4 μg/kg). Calcium was the most abundant metal identified in our B.C. gum resin natural component. Its relationship with

colorectal cancer has constituted an intense area of research. It's great ability to decrease the risk for colorectal cancer was closely correlated to the inflammation (Garland *et al.,* 1985). Calcium binds to the free fatty acids and bile acids, lead to their precipitating in the colon, which is turn reduce oxidative stress and inflammation in the colon, and hence block their tumor promoting effect by sequestering them and so they can no longer activate the protein kinase C (PKC), which thought to play a

role in tumor carcinogenesis (Fitzer *et al.,* 1987). The potent anti-inflammatory effects of calcium supplementation have been proven to refer to its reducing effect on the tumor-promoting pro-inflammatory markers. Moreover, calcium has a great role in the cell cycle and differentiation events and by activating the calcium sensing receptor, and promoting the cell-cell and cell-matrix adhesion (Lamprecht and Lipkin, 2003).
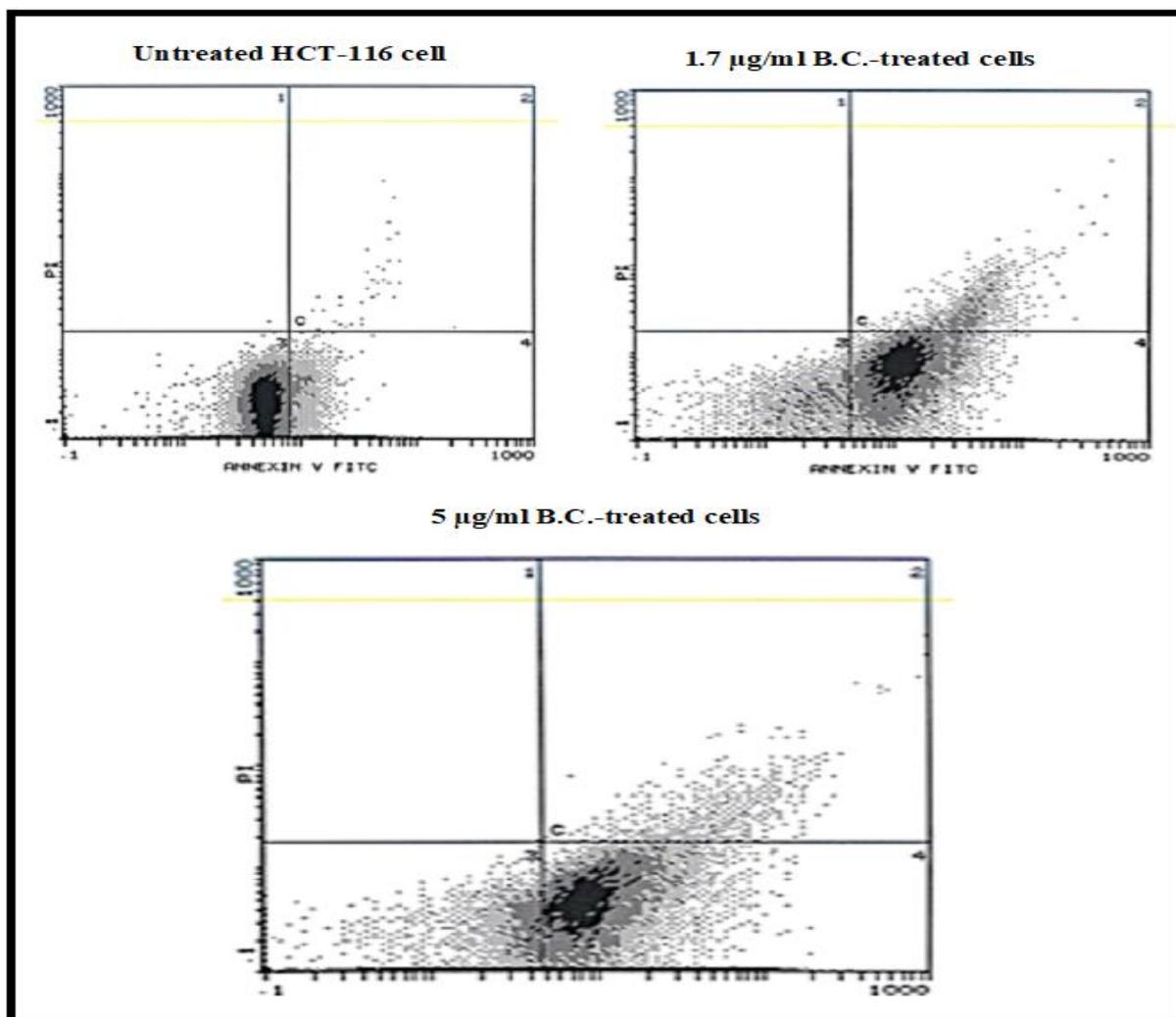
Fig. 5b. The effect of *Boswellia carterii* gum resinon the HCT-116 cell line.

Mg plays an essential role in reducing colorectal carcinogenesis by means of various inflammatory responses (Kuno *et al.,* 2013). Moreover, it controls crucial cell functions, such as proliferation, differentiation, migration and apoptosis. Several studies have linked high intake of calcium(Dai *et al.,* 2007), to magnesium ratio to a reduced risk of

colorectal cancer. However, the observed beneficial effect of magnesium intake against CRC appeared to be independent of calcium intake and restricted to colon cancer only (Ma *et al.,* 2010).

The dietary intake of selenium (Se) has been associated with a reduction in the risk of CRC (Zeng,

2009). As a single agent, Se has been proven to inhibit tumor growth and induce apoptosis in many cancer cells [61].It arrests cells in the G1-phase of the cell cycle by inhibiting cyclins and cyclin-dependent kinases (CDKs), and many other transcriptional factors. Se, decrease the free radical' slevels generated by cancer cells due to its antioxidant activity. The anticancer effectively of Se compounds, proven in many types of cancer, especially CRC, is correlated with their chemical conjugations and doses (Valdiglesias *et al.,* 2010).

Evaluation of the anticancer effect of Boswellia carterii against HCT- 116

Cancer cells have known to grow rapidly and multiply uncontrollably as well as to evade the apoptotic pathways through different mechanisms. Therefore, the basic features essential to be present in the powerful anticancer drug, is its apoptotic inducing effect andanti-proliferative impacton the cancer cells (Abraha and Ketema, 2016). In the present study, the investigation of the cytotoxic activity of B.C. gum resin on the HCT-116 cell line revealed its potent inhibitory effect on the cancer cell proliferation. Cell growth was shown to be significantly inhibited with a satisfactory IC_{50} level of 1.74 ± 0.1 µg/ml. This excellent IC_{50} level obtained in this study indicates that B.C. gum resin lies within the range of biological availability. Additionally, the anti-proliferative effect of the natural B.C. resin on the HCT-116 cells was shown to be a concentration dependent. Hence it can be inferred that B.C. gum resin treatment led to the lysis of HCT-116 cells with increasing concentrations via the induction of apoptosis. At the same time, the 48 hrs. post treated HCT-116 cells with B.C. gum resin showed many morphological changes such as cell shrinkage, membrane blebbing, degeneration of organelles and appearance of multiple vacuoles. These morphological changes confirmed the anti-proliferative impact of B.C. via apoptosis induction (Liu *et al.,* 2002).

Apoptotic-inducing effect of B.C. via caspase-3 activation

Apoptosis presents a crucial defense mechanism against cancer, which leads to the death of potentially harmful cells. Induction of apoptosis is a major cytotoxic mechanism of anticancer therapies (Liu *et al.,* 2017). Caspases are cysteine proteases, crucially involved in the morphological and biochemical changes occurring during apoptosis (Ouyang *et al.,* 2012). Caspases present a proteolytic network within the cell in which upstream initiator caspases are activated early in the apoptotic process (caspase-9), leading to the activation of downstream caspases (caspase-3). Caspases-3 targets the structural substrates leading to cell disassembly and DNA fragmentation (John *et al.,* 2015). Hence, the apoptosis induction target is a potentially promising approach for cancer therapy. The inducing-apoptotic ability of the B.C. gum resin via activating the caspase-3 level in human cancer cell line was evaluated. The concentrations of B.C. (1.7 µg/ml and 5 µg/ml) used throughout the study, exhibited a significant increase with a *p value = 0.03 in the caspase-3 activity (0.95 and 1.26 ng/mg protein), indicating its potent apoptosis-inducing effect against the HCT-116 colon cancer cells. The results obtained in this study are in agreement with Liu *et al.,*2002 who previously reported that boswellic acid induced apoptosis in both colon and liver cancer cells by a pathway dependent on caspase-8 and caspase-3 activation but independent of Fas/Fas ligand interaction. The pro-apoptotic effects and the cell differentiation induction of B.C. gum resin at low concentrations in HL-60 cells have also been reported.

Evaluation of B.C. apoptotic-inducing effect by flow cytometry

The flow cytometric assay confirms the apoptotic-inducing effect of B.C. As shown in figure 5b; 78% and 84% total apoptotic cells were revealed on HCT-116 cells treated for 48 hrs with 1.7 µg/ml and 5 µg/ml of B.C. respectively, compared with the untreated cells. These obtained results are obviously promising ones, since treatment with low concentrations of B.C. induce apoptosis. On the other hand, 5-FU, targeting the thymidylate synthase, one of the key enzymes

controlling DNA replication, was previously shown to induce apoptosis of HCT-116 colorectal cancer cells; however, it requires very high doses and a long treatment time (Marsh, 2005).While, a very low dose of 5-FU was not found to have an apoptotic effect on the HCT-116 cells (Subramanian *et al.,* 2016).

Conclusion

In conclusion, there is a serious need for natural-based agent to treat the colorectal cancer.B.C. gum resin showedgreat cytotoxic and anti-proliferative impact on the HCT-116 colon cancer cell line. It has the ability to induce apoptosismore than the chemotherapy-based drug, 5-FU.These were confirmed by the observed morphological changes; such as enlargement of cell, reduction of nucleus and formation of many vacuoles. The apoptotic-inducing effect of B.C. gum resin on the HCT-116 cells is linked to the caspase-3 activation in a concentration dependent manner. These results boost the promising role of B.C. gum resin as an anticancer natural agent against CRC. The potent anticancer effect of B.C. gum resin was thought to be due to its main active constituents of terpenes, Ca and Se.

References

Abraha AM, Ketema EB. 2016. Apoptotic pathways as a therapeutic target for colorectal cancer treatment. World Journal of Gastrointestinal Oncology**8 (8),** 583-591.
https://dx.doi.org/10.4251%2Fwjgo.v8.i8.583.

Al-Harrasi A, Al-Saidi S. 2008. Phytochemical Analysis of the Essential Oil from Oleo gum Resin of Boswellia sacra (Omani Luban). Molecules **13(9)**, 2181-2189. PMID:18830149.

Alvarez-Cabellos R, Garcia-Carbonero R, Garcia-Lacalle C, Gomez P, Tercero A, Sanchez D, Paz-Ares L. 2007. Fluorouracil-based chemotherapy in patients with gastrointestinal malignancies: influence of nutritional folate status on toxicity. Journal of Chemotherapy**19**, 744–749.
https://doi.org/10.1179/joc.2007.19.6.744.

Aman M, Abraha, Ketema EB. 2016. Apoptotic pathways as a therapeutic target for colorectal cancer treatment. World Journal of Gastrointestinal Oncology **8(8)**, 583-591.
https://dx.doi.org/10.4251/wjgo.v8.i8.583.

Badria FA, Abu-Karam M, Mikhaeil BR, Maatooq GT, Amer MM. 2003. Anti-Herpes activity of isolated compounds from Frankincense. Biosciences, Biotechnology Research Asia **1(1)**, 01-10.

Barbara P, Kumar R, Naccarati A, Novotny J, Prasad RB, Forsti A, Hemminki K, VodickaP and Bermejo JL. 2011. 5-Fluorouracil based chemotherapy for colorectal cancer and MTHFR/MTRR genotypes. British journal of clinical pharmacology **72(1)**, 162-163.
https://dx.doi.org/10.1111%2Fj.13652125.2010.03892.x.

Chudzik M, Szlacheta IK, Król W. 2015. Triterpenes as Potentially Cytotoxic Compounds. Molecules **20**, 1610-1625.
https://doi.org/10.3390/molecules20011610.

Coppi A, Cecchi L, Selvi F, Raffaelli M. 2010. The Frankincense tree (Boswellia sacra, Burseraceae) from Oman: ITS and ISSR analyses of genetic diversity and implications for conservation. Genetic Resources and Crop Evolution **57,** 1041–1052.
http://dx.doi.org/10.1007/s10722-010-9546-8.

Dai Q, Shrubsole MJ, Ness RM, Schlundt D, Cai Q, Smalley WE, Li M, Shyr Y, Zheng W. 2007. The relation of magnesium and calcium intakes and a genetic polymorphism in the magnesium transporter to colorectal neoplasia risk. American Journal of Clinical Nutrition **86**, 743–751.
https://doi.org/10.1093/ajcn/86.3.743.

Fitzer CJ, O'Brian CA, Guillem JG, Weinstein IB. 1987.The regulation of protein kinase C by chenodeoxycholate, deox-ycholate and several structurally related bile acids. Carcinogenesis **8**, 217-220.

https://doi.org/10.1093/carcin/8.2.217.

Flanagan L, Meyer M, Fay J, Curry S, Bacon O, Duessmann H, John K, Boland KC, McNamara DA, Kay EW, Bantel H, Schulze-Bergkamen H, Prehn GHM. 2016. Low levels of Caspase-3 predict favourable response to 5FU-based chemotherapy in advanced colorectal cancer: Caspase-3 inhibition as a therapeutic approach. Cell death disease **7(2),** 1-11.
https://doi.org/10.1038/cddis.2016.7.

Garland C, Shekelle RB, Barrett-Connor E, Criqui MH, Rossof AH, Paul O. 1985. Dietary vitamin D and calcium and risk of colorectal cancer: a 19-year prospective study in men. Lancet **1,** 307-309.
https://doi.org/10.1016/S0140-6736(85)91082-7.

Gomha SM, Riyadh SM, Mahmmoud EA, and Elaasser MM. 2015. Synthesis and Anticancer Activities of Thiazoles, 1,3-Thiazines, and Thiazolidine Using Chitosan-Grafted-Poly (vinylpyridine) as Basic Catalyst. Heterocycles **91(6)**, 1227-1243.

Gusella MA, Frigo CC, Bolzonella. 2009. Predictors of survival and toxicity in patients on adjuvant therapy with 5- fluorouracil for colorectal cancer. British Journal of Cancer **100(10)**, 549–1557.
http://dx.doi.org/10.1038%2Fsj.bjc.6605052.

John K, Rosner I, Keilholz U, Gauler T, Bantel H, Grunwald V. 2015. Baseline caspase activity predicts progression free survival of temsirolimus-treated head neck cancer patients. European Journal of Cancer **51**, 1596–1602.
https://doi.org/10.1016/j.ejca.2015.05.021.

Kuno T, Hatano Y, Tomita H, Hara A, Hirose Y, Hirata A, Mori H, Terasaki M, Masuda S,Tanaka T. 2013. Organomagnesium suppresses inflammation-associated colon carcinogenesis in male Crj: CD-1 mice. Carcinogenesis **34**, 361–9.
https://doi.org/10.1093/carcin/bgs348.

Lamprecht S, Lipkin M. 2003. Chemoprevention of colon cancer by calcium, vitamin D and folate: molecular mechanisms. Nature Review Cancer **3(8)**, 601-14.
https://dx.doi.org/10.1038/nrc1144.

Liu JJ, Duan RD. 2009. LY294002 enhances boswellic acid-induced apoptosis in colon cancer cells. Anticancer Research **29**, 2987–91.
https://dx.doi.org/10.1186/1476-511X-8-51.

Liu JJ, Nilsson A, Oredsson S, Badmaev V, Zhao WZ, Duan RD. 2002. Boswellic acids trigger apoptosis via a pathway dependent on caspase-8 activation but independent on Fas/Fas ligand interaction in colon cancer HT-29cells. Carcinogenesis **23(12)**, 2087-2093.
https://doi.org/10.1093/carcin/23.12.2087.

Liu PF, Hu YCh, Kang BH, Tseng YK, Wu PC, Liang, CC, Hou YY, Fu TY, Liou HH, Hsieh IC, Ger LP, Shu CW. 2017. Expression levels of cleaved caspase-3 and caspase-3 in tumorigenesis and prognosis of oral tongue squamous cell carcinoma. PLoS ONE **12(7)**, e0180620.
https://doi.org/10.1371/journal.pone.0180620.

Ma E, Sasazuki S, Inoue M, Iwasaki M, Sawada N, Takachi R, Tsugane S. 2010. High dietary intake of magnesium may decrease risk of colorectal cancer in Japanese men. Journal of Nutrition **140**, 779–785.
https://doi.org/10.3945/jn.109.117747.

Marsh S. 2005. Thymidylate synthase pharmacogenetics. Investigational New Drugs **23**, 533–537.
https://doi.org/10.1007/s10637-005-4021-7.

Meyerhardt JA, Mayer RJ. 2005.Drug therapy: systemic therapy for colorectal cancer. The New England Journal of Medicine **352 (5)**, 476–487.
https://doi.org/10.1056/NEJMra040958.

Mosmann T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunological Methods **65**, 55-63.
https://dx.doi.org/10.1.1.458.9709&rep=rep1.

Nagwa MA, Seham SH, Ahmed AM, Rehab AH, Tatsufumi O. 2013. Phytochemical Study of the Biologically Active Fractions of the Oleo-gum-resins of Boswellia carteri and Commiphora myrrha. Advances in Environmental Biology **7(9)**, 2573-2583.

Nishimura R, Kimura Y, Suzuki T. 2006. Anti-inflammatory activities of the triterpene acids from the resin of Boswellia carterii. Journal of Ethnopharmacology **107**. 249−253.
https://doi.org/10.1016/j.jep.2006.03.006.

Ogbazghi W, Rijkers T, Wessel M, Bongers F. 2006. Distribution of the frankincense tree Boswellia papyrifera in Eritrea: the role of environment and land use. Journal of Biogeography**33**, 524−535.
https://doi.org/10.1111/j.1365-2699.2005.01407.x.

Ouyang L, Shi Z, Zhao S, Wang FT, Zhou TT, Liu B, Bao JK. 2012. Programmed cell death pathways in cancer: a review of apoptosis, autophagy and programmed necrosis. Cell Proliferation**45**, 487-498.
https://doi.org/10.1111/j.1365-2184.2012.00845.x.

Poeckel D. and Werz O. 2006. Boswellic acids. Biological actions and molecular targets. Current Medicinal Chemistry **13(28)**, 3359-3369. PMID:17168710.

Stein A, Atanackovic D, Bokemeyer C. 2011. Current standards and new trends in the primary treatment of colorectal cancer. European Journal of Cancer **47(3),** S312-S314.
https://doi.org/10.1016/S0959-8049(11)70183-6.

Subramanian AP, Jaganathan SK, Mandal M, Supriyanto E, Muhamad II. 2016. Gallic acid induced apoptotic events in HCT-15 colon cancer cells. World Journal of Gastroenterology **22(15)**, 3952-3961.
https://dx.doi.org/10.3748%2Fwjg.v22.i15.3952.

Sunnichan VG, Mohan Ram HJ, Shivanna KR. 2005. Reproductive biology of Boswellia serrata, the source of salai guggal, an important gum- resin. The Journal of Linnean Society. Botany **147**, 73−82.

Takahashi M, SungB, Shen Y, Keun K, Link AC, Boland R, Bharat B, Aggarwal A, Goel A. 2012. Boswellic acid exerts antitumor effects in colorectal cancer cells by modulating expression of the let-7 and miR-200 microRNA family. Carcinogenesis **33 (12)**, 2441-2449.
https://doi.org/10.1093/carcin/bgs286.

Tebbutt NC, Cattell E, Midgley RD, Cunningham, Kerr D. 2002. Systemic treatment of colorectal cancer. European Journal of Cancer**38(7)**, 1000−1015.
https://doi.org/10.1016/S0959-8049(02)00062-X.

Valdiglesias V, Pasaro E, Mendez J, Laffon B. 2010.In vitro evaluation of selenium genotoxic, cytotoxic, and protective effects: A review. Archive of Toxicology **84,** 337−351.
https://doi.org/10.1007/s00204-009-0505-0.

Van Bergen PF, Peakman TM, Leigh-Firbank EC, Evershed RP Chemical evidence for archaeological frankincense. 1997. Boswellic acids and their derivatives in solvent soluble and insoluble fractions of resin-like materials. Tetrahedron Letters **38**, 8409−8412.
https://doi.org/10.1016/S0040-4039(97)10234-9.

Yadav VR, Prasad S, Sung B, Gelovani JG, Guha S, Krishnan S, Aggarwal BB. 2012. Boswellic acid inhibits growth and metastasis of human colorectal cancer in orthotopic mouse model by downregulating inflammatory, proliferative, invasive and angiogenic biomarkers. International Journal of Cancer **130**, 2176−2184.
https://doi.org/10.1002/ijc.26251.

Yeihseu Z, Chen ZS, Tsai CC, Tsui CC, Cheng SF, Liu CL, Lin HT. 2002.Digestion methods for total heavy metals in sediments and soils. Water, Air, and Soil Pollution **141**, 189–205.
http://nr.niu.edu.tw/per/

Zeng H. 2009. Selenium as an essential micronutrient: Roles in cell cycle and apoptosis. Molecules **14**, 1263-1278.

https://doi.org/10.3390/molecules14031263.

Zorzi D, Laurent A, Pawlik TM, Lauwers GY, Vauthey JN, Abdalla EK. 2007. Chemotherapy-associated hepatotoxicity and surgery for colorectal liver metastases. British journal of surgery **94**, 274-286.
https://doi.org/10.1002/bjs.5719.