IIUC Studies, Vol.-19, Issue-1, Dec. 2022
DOI: https://doi.org/10.3329/iiucs.v19i1.69041

A review of β-caryophyllene oxide for its pharmacological properties supported by in silico findings

A.T.M. Mostafa Kamal
Department of Pharmacy
International Islamic University Chittagong (IIUC), Bangladesh

Abstract

Bicyclic sesquiterpene, β-caryophyllene oxide (BCO) is one of the major components of essential oil derived from aromatic plants. It is reported to possess several interesting pharmacological prospects. BCO's ability to suppress a few signaling cascades and down regulating various gene products responsible for cancer development against several cancer cell lines was seen along with some analgesic properties. Besides, its interaction with enzymes responsible for metabolism of the drugs performed *in-vitro* and *in-vivo* was found to show diminishing and enhancing effects on the enzymes. All the possible relevant published articles on BCO were collected using some popular search engines such as Google Scholar, Pubmed, ScienceDirect etc. The molecular docking was carried out with the help of Schrodinger Suites-Maestro 2017-1. In addition, the in-silico findings also provided strong evidence in favor of BCO with the ability to be a prospective therapeutic agent. However, from all the information gathered on this compound and clinical trials, this compound may yield beneficial results novel drug discovery.

Keywords β-caryophyllene oxide (BCO), Anti-cancer, Cytostatic drugs, BCO enzyme interactions, Genotoxicity.

Paper type Research paper

1. Introduction

Biologically active compounds obtained from natural products have been utilized widely for producing desired therapeutic drugs and found to be risk-free and inexpensive (Newman & Cragg, 2012). Essential oils, compounds gathered from aromatic plants are a prominent source of potential natural agents. From the numerous compositions of essential oils, sesquiterpenes have drawn mighty attention for their broad extent of biological functions. These sesquiterpenes are found to possess some pharmacological properties such as antibacterial, antifungal, anti-inflammatory, antirheumatic, antioxidant etc. Besides, their ability to induce apoptosis, modulation of DNA repair mechanism, cell cycle arrest, metastasis and proliferation are

IIUC Studies
Vol.-19, Issue-1, Dec. 2022
pp. 87-120
© IIUC
ISSN 2408-8544

noteworthy anticancer strategies (Astani, Reichling, & Schnitzler, 2011; Lněničková, Svobodová, Skálová, Ambrož, Novák, & Matoušková, 2018).

Cancer is a term to define the physiological condition of a patient bearing cancer cells characterized by loss of specialized characteristics resulting in the loss of normal regulatory mechanisms which control cell growth and multiplication. This remains one of the worlds most feared diseases as one in three person is affected by it and was recorded to cause a quarter of all deaths in the year 2000. Cancer should be the major cause of death in every nation of the world in the 21st century and is projected to be the most significant obstacle to a growing life expectancy. In 2015, cancer was the world leader before 70 years within 91 of 172 countries and holds third or fourth ranks in another 22 countries according to 2015 World Health Organization (WHO) statistics. Until 2018, lung cancer was the most diagnosed cancer for both sexes (11.6 percent of global cases) and the leading etiology of cancer mortality (18.4 percent of global cancer deaths), followed closely by breast cancer (11.6 percent), colorectal cancer (10.2 percent) and prostate cancer (7.1 percent). By sex, lung cancer is the leading cause of male cancer death, accompanied by the occurrence of prostate and colorectal carcinoma and mortality of the liver and stomach. In total, approximately 65% of currently diagnosed cancer cases and deaths account for the top 10 cancer types (Bray, Ferlay, Soerjomataram, Siegel, Torre, & Jemal, 2018; Patrick, 2013).

A variety of cellular disorders, such as improper signaling pathways, insensitivity to inhibitory growth signals, endless cell division, cell cycle regulatory irregularities, and so on, are linked to genetic flaws. Most of these conditions are to be accomplished if a potential abnormal cell has to become cancerous. This is why cancer takes a while to appear but is difficult to treat once it is diagnosed. Genes involved in coding proteins for controlling cell division and differentiation are termed proto-oncogenes. Mutation of these hampers normal functions and the cell turns cancerous. Some cellular proteins are responsible for detecting DNA damage in a cell and thus blocking DNA replication which makes it possible for the cell to repair the damaged portion prior to upcoming cell division. If this does not seem possible, leads to the cell committing apoptosis. The growth and division of a normal cell is reliable on different signals it receives from hormones called growth factors. The receptors then trigger a signal transduction pathway which ultimately reaches the nucleus and commands transcription of proteins necessary for cell growth and division. In most cancers, defect in this signaling process leads to the cell being commanded to multiply constantly. There are also cancer cells that are capable of dividing and

growing without external growth factors by producing growth factors themselves and releasing them to stimulate their own receptors by auto-phosphorylation.

Apoptosis is generally initiated by several pathways in different forms. In the extrinsic route, there could be a down regulation in growth factor or binding of death activator proteins to cell membrane proteins called tumor necrosis factor receptors (TNF-R) triggering a signaling process or production of T-lymphocytes perforating damaged cells to produce an enzyme called granzyme all of which initiate apoptosis. The intrinsic pathway is activated due to DNA damage by chemicals or ROS (Reactive oxygen species) which triggers excess production of tumor suppressor p53 protein resulting in apoptosis initiation. All of the signals mentioned above act on the mitochondria containing proteins with apoptotic ability mainly cytochrome c. A series of events then follows to activate several enzymes and ultimately caspases which are protease enzymes with catalytic activity destroying cellular proteins leading to cell destruction. This process is upregulated by proteins known as Bad and Bax and down regulated by proteins B-cell lymphoma 2 (Bcl-2) and B-cell lymphoma-extra-large (Bcl-xL). This is why Bcl-2 and Bcl-xL are known as apoptosis suppressors which are found to be overexpressed in many tumor cells. A number of proteins like cyclin dependent kinases (CDKs) regulates the cell cycle of cell division process. About 15 types of cyclin and 9 types of CDKs are known for their specific roles at various stages of cell cycle. When a cyclin is bound with its associated kinase leads to enzyme activation which serves the cell cycle to move from one phase to the next. This process of progression of cell cycle can be down-regulated by CDK inhibitors. The alterations of cyclins, CDKs and CDK inhibitors by oncogenes have been found out in 90% of human cancers. So, excessive cyclin or CDK production or insufficient CDK inhibitor production can result in disruption of normal cellular regulation and lead to cancer.

For pharmaceutical research and for the development of patient care, the discoveries of new anti-cancer drugs are important. One way to respond to the current medicines that could be unintended consequences as possible candidates is to accomplish this important aim. Systematic study of effective anticancer drugs may provide useful insight into patterns in the discovery of drugs that can help to identify new anti-cancer compounds systematically (Sun, Wei, Zhou, Wang, Liu, & Xu, 2017). The overcoming of MDR is one of the most difficult approaches to cancer treatment. A modified apoptosis and over-expression of multi-drug transporters are the main mechanisms for cancer cell resistance to drugs. The co-prescription of chemo-sensitive and

chemotherapeutic agents is an effective method for disrupting MDR and overcoming the harmful effects of anticancer drugs. One of the leading players in MDR is the efflux of a few anticancer drugs by the p-glycoprotein (P-gp), an efflux transporter encoded by the MDR-1 gene, which is an adenosine triphosphate (ATP). Over-expression of P-gp contributes to decreased effectiveness of the drugs vinblastine, paclitaxel, vinblastine, doxorubicin, etc. in liver, pancreas, gastrointestinal and ovarian cancer. In the MDR-dependent apoptotic pathway, apoptotic reactions to drugs dampen the atomic factor Kappa-B (NF-kB) or BCL-2 by dysregulation. To monitor MDRs in cancer, the combination of drug with MDR cytotoxic, modulating agents and chemo-sensitizers is included in several nanoparticles (Pramanik et al., 2012) developed the NanoDoxCurc (NDC) nanoparticle doxorubicin (DOX)-curcumin composite formulation to overcome DOX resistance. Its effectiveness as an impaired MDR phenotype tested for several DOX-resistant models of cancer (Prostate and Ovarian, Acute Leukemia, Multiple myeloma) in athymic nude mice (DOX resistant). In this course of therapy, which was subject to the reduction of the oxidative stress caused by DOX, decreased heart adversity and bone marrow suppression has additionally been indicated (Batra, Pawar, & Bahl, 2019).

Conventional anticancer drugs tend to be very toxic and exert their effects against various cellular targets via different mechanisms. Due to their high toxicity, they also affect the normal cells and can produce toxic effects. That is why their dose should be as high as to affect the tumor and also safe for the patient. For this reason, in the past few years anticancer medications are being prepared to keep their selectivity in mind to target specific abnormalities with reduced side effects. But keeping in mind the number of anomalies in a cancer cell, it seems that not one agent will be sufficient to overcome the threat and the use of drugs in combination possessing varied mechanism of action can be very fruitful (Patrick, 2013).

Doxorubicin (Figure 1) is one of the most potent anti-cancer medications used in the treatment of breast cancer due to its potential to inhibit pro-growth signaling. But it is accompanied by severe side effects (predominantly cardio-toxicity) and generation of multi drug resistance (MDR) which restricts its clinical use. This is why many approaches are being undertaken by researchers to limit the toxicity of doxorubicin on human health. Among these approaches, the combination with other drugs or with natural bioactive agents is looked at carefully (Hanušová, Boušová, & Skálová, 2011). Fluorouracil (5-FU) (Figure 2) is also another cytostatic medication which acts by entering the cell via a carrier-mediated transport system being converted into 5-fluorodeoxyuridine monophosphate and

resulting in the inhibition of thymidylate synthetase required for DNA synthesis and cell growth (Whalen, Finkel, & Panavelil, 2015). Oxaliplatin is also an anti-cancer drug used in combination with 5-FU in colorectal and early cancer which generally acts by forming cross link in the DNA strands preventing replication of DNA and causing the cell to die (Ehrsson, Wallin, & Yachnin, 2002; Graham, Muhsin, & Kirkpatrick, 2004).

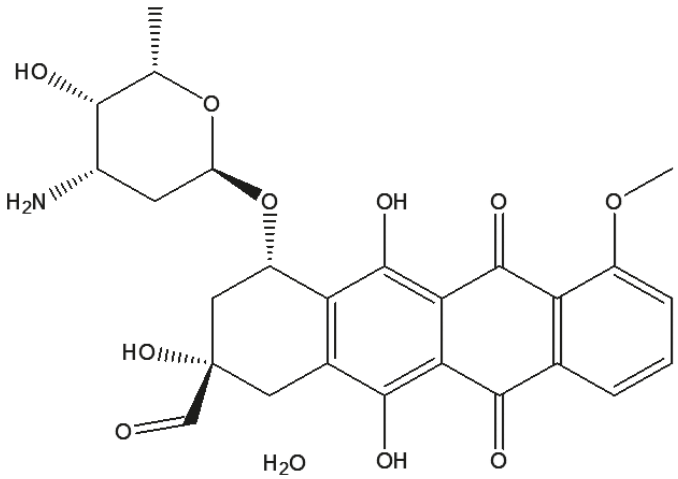

Figure 1
Doxorubicin

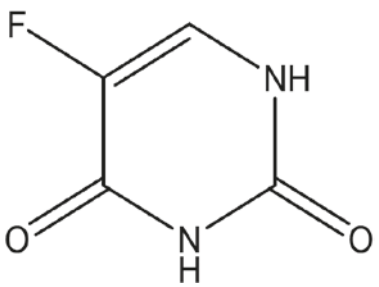

Figure 2
5-Fluorouracil (5-FU)

The unpleasant sensation of pain originates because of sensitization of specialized neurons which responds to external stimuli. This is a health burden as it hampers the normal way of living and leads to financial loss to the patients (Phillips, 2009). The cyclooxygenase 1 and 2 is oenzymes, COX-1 and COX-2, catalyze arachidonic acid (AA) conversion to

prostaglandin H2, which is the rate determining step in the biological synthesis of multiple prostaglandins (PG) and thromboxanes (TX). Different prostaglandin syntheses converting PGH2 to PGE2, PGI2, PGF2alpha, PGD2 and TXA2 can accomplish the following unique phase. Prostaglandin E2 (PGE2) plays an essential part in the signaling of pain (Figure 3) (Tegeder, 2013).

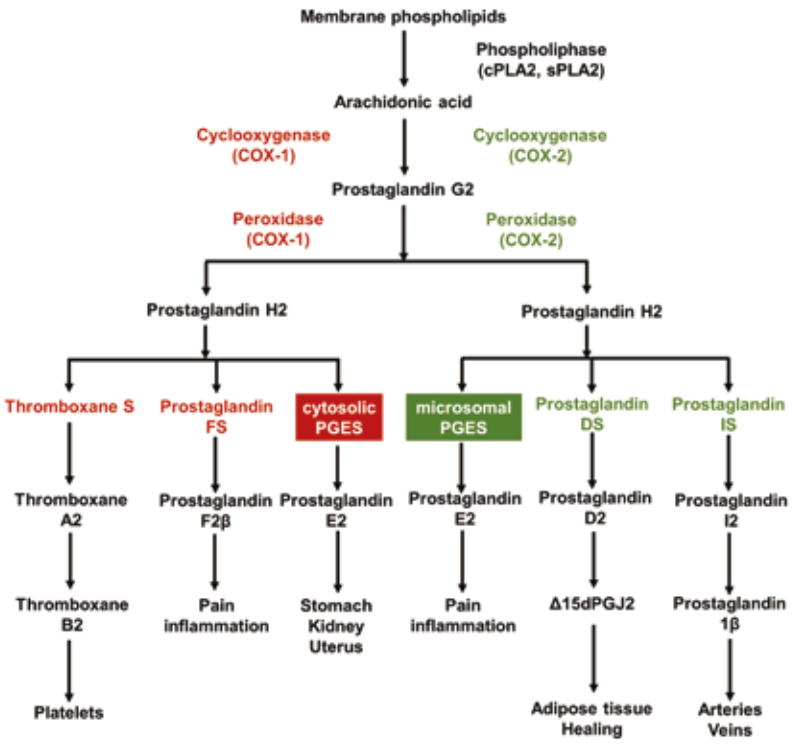

Figure 3
Pain signaling pathway

Pain related to cancer is the most difficult one to treat. Worldwide, about 1. 5 billion people suffer sometime in their lives from chronic pain. The American Pain Society estimated a rise in health care costs of between 560 billion and $635 billion annually because of pain. Various types of drugs have been used for pain management procedures in the form of analgesics, invasive/non-invasive stimuli and conventional Chinese medicine. However, an accessible and convenient device is still important that can be used to make homes comfortable, reduce care costs and improve quality of life.

The gate theory of pain is the most widely known theory of pain. The

theory says that nerve signals migrate from peripheral nerves to CNS and a gate regulates the flow of nerve pulses, minimizing the feeling of pain by "close condition". Nociceptors or pain receptors are natural, biological, chemical, thermal, mechanical or chemical sensation endings of the nerves. In general, for the transmission of pain pulses via the Dorsal Root gangly (DRG), which transmit harmful information to a spinal cord, two types of fibers, large fibers of A delta (fast pain) and C fibers (slow to chronic pain) are used. The spinal cord transmits information to the thalamus where it is perceived. The pyramidal neurons in the cortex at Layer V are stimulated and display increased activity, which is associated with higher pain perception. The ascending and descending paths include the illusion that pain is triggered by noxious stimuli and by pain control. Nerve activity of these neurons stimulates the release from the neuronal membranes of various neurotransmitters (endorphin, serotonin, GABA), which help to adjust the membrane action potential. This modulates the neuronal function of pyramidal neurons in the cortex, which indicates a lower sense of pain (Pradhan & Zheng, 2016).

Many factors are linked to cancer related pain such as infections related to cancer treatment of other cancers etc. which is why it is difficult to treat (Keefe, Abernethy, & Campbell, 2005). As a result, a large portion of cancer patients seem to overuse analgesics which can lead to a number of health complications and can even result in drug addiction. To overcome this issue natural bioactive compounds with potent analgesic activity and reduced toxic effects have been looked for (Fidyt, Fiedorowicz, Strządała, & Szumny, 2016).

Systemic administration of analgesic medications is the most effective way of treating pain. Analgesic drugs interfere with the production of negative affective reactions in the central nervous system and thereby reduce or suppress the integrity of the pain episode. These effects arise without unconsciousness inherently. Drug of other classes can decrease fear, anxiety and apprehension; triggers sleep, reverse psychotic pain, or antagonize depression, particularly depression exacerbated by chronic use of sedative-hypnotic medicines or opiates, individually or in combination with analgesics. Due to their palliative nature, analgesic medications are usually administered concurrently, with precise therapeutic action. If correctly administered, analgesics are very efficient and have major advantages over many other procedures in ease of administration and low cost. Unfortunately, the inappropriate application of these desirable qualities is also responsible for the medications being overused or underused, being incorrectly selected and the incorrect dose is selected.

Aspirin is the analgesic, antipyretic and anti-inflammatory drug most used and widely used. It is as effective as and less expensive than patented medicines and has a relatively low occurrence of secondary effects within the standard dosage range. This prototype is a standard guide for clinical trials that compare and test this type of agent to other members of its class. Physicians should consider using this new medication only if the performance of the medication matches or is greater than the analgesic effects of aspirin, while preserving the low incidence of adverse effects of aspirin. Regulated pain tests in patients with different causes have been replicated. It has been shown that aspirin-produced analgesia at 0.3 to 0.6 gmdosesevery four hours, which is superior to placebo-produced analgesia. Doses between 0.6 and 1.0 gm have recently been documented to produce an elevation in peak analgesia, with delayed analgesic activity in some case and no real increase in side effects observed (Halpern, 1977).

β-caryophylleneoxide, structurally a bicyclic sesquiterpene is commonly found in lemon balm *(Melissa officinalis)*, and in the eucalyptus, Melaleucastypheloids, whose essential oil contains about 43.8% (Farag, Shalaby, El-Baroty, Ibrahim, Ali, & Hassan 2004). It is also reported to be available in the essential oils obtained from plants such as black pepper *(Piper nigrumL.)*, oregano *(OriganumvulgareL.)*, clove *(Eugenia caryophyllata)*, cinnamon *(Cinnamomum spp.)* and guava *(Psidiumguajava)* (Kubo et al., 1996; Zheng, Kenney, & Lam, 1992). It is found to have a strong wooden odor and is also being used as a food additive and cosmetic. It is certified as a flavoring by the European Food Safety Authority (EFSA) and by the Food and Drug Administration (FDA) having identification number FL no: 16.043 (Sarpietro, Di Sotto, Accolla, & Castelli, 2015). Recently, this sesquiterpene has been studied and found to have significant anti-cancer potential with some analgesic properties as well (Fidyt, Fiedorowicz, Strządała, & Szumny, 2016; Kim, Cho, Kim, et al. 2014; Park et al., 2011). Besides, it has been reported to influencethe efficacy of several classical anti-cancer agents and main drug metabolizing enzymes (Di Giacomo, Di Sotto, Mazzanti, & Wink, 2017; Hanušová et al., 2017; Astani, Reichling, & Schnitzler, 2011; Lněničková, Svobodová, Skálová, Ambrož, Novák, & Matoušková, 2018; Martin et al., 2019; Nguyen et al., 2017). The aim of the present review is to compile all the relevant information of β-caryophyllene oxide as a potential therapeutic agent along with its toxicological profile which will guide the future scientific investigations on this compound.

2. Materials and methods

2.1. Literature review

With an aim to compile a review on BCO, all the possible relevant published articles on BCO were collected using some popular search engines such as Google Scholar, PubMed, ScienceDirect etc. All the references included were added with a well-known referencing tool named EndNote X7.

2.2. In-silicoStudy

2.2.1. Molecular Docking Analysis

The molecular docking was carried out with the help of Schrodinger Suites-Maestro 2017-1. In order to predict the best binder and probable drug ability, the Pockdrug online server was used. For the visualization discovery studio (v4. 1) was used.

2.2.2. Ligand preparation

Chemical structures of BCO (PubChem ID: 6604672) were exported from the PubChem library (https:/pubchem. ncbi. nlm. nih. gov/). The ligand has been developed using LigPrep, incorporated into the Schrödinger Suite-Maestro v11. 1, with the following parameters used for minimization: pH 7. 0 ± 2. 0 with Epik 2. 2, neutralized with intensity field OPLS3.

2.2.3. Receptor/Enzyme preparation

A protein Data Bank RCSB PDB: human inhibitor-binding CYP3A4 (PDB: 6DA5), human estrogen receptor (PPB: 3ERT), cycloo-xygènase-1 (PD B: 2OYE), cyclooxygenase-2 (PDB: 6COX) has been derived from three-dimensional structures of the enzyme / receptor. The Protein Preparation Wizard was used to preprocess, optimize and minimize the processes. This is included in the suit-maestro (v11. 1) of Schrodinger. At pH 7. 0, water molecules of less than three H-bounds were eliminated to nonwater. The structure was optimally optimized. The minimization was limited where the heavy atoms converged to 0. 30 Å RMSD in the OPLS3 force field implemented. After choosing the best binding sites, online PockDrug tools were used to create recipient grids.

2.2.4. Glide ligand molecular docking

The molecular docking was done to pick a better ligand to research in comparison with the traditional anti-cancer and anti-nociceptive drugs. The docking was achieved using the Schrodinger Suite Maestro (v11. 1) option by ligand docking (Adnan, Chy, Kamal, Chowdhury, Rahman, et al., 2020). The tablets and structures were then exported for further analysis. Discovery

Studio (v 4.1) software was used to view 3D receptor-ligand binding interaction.

3. Chemistry

β-Caryophylleneoxide (BCO) or β-Caryophyllene epoxide with formal name (1R, 6R, 10S)-4R, 12, 12-trimethyl-9-methylene-5-oxatricyclon [8.2.0.$0^{4,6}$] dodecane is a bicyclic sesquiterpene and a metabolite and epoxide analogue of β-caryophyllene. The major distinguishing factor between the two is the inability of BCO to interact with cannabinoid receptor CB2 (Fidyt, Fiedorowicz, Strządała, & Szumny, 2016; Gertsch et al., 2008). The structure of BCO consists of methylene and an exocyclic epoxide functional group which enables it to interact with amino and sulfhydryl groups of DNA bases and proteins by covalent bonds revealing its signal modulating capability in cancer cells (Park et al., 2011). Actually the epoxide group being electrophilic in nature can bind with nucleophiliccentres on DNA and proteins bringing about toxic effects (Di Sotto, Maffei, Hrelia, Castelli, Sarpietro, & Mazzanti, 2013). The reactive epoxide group is linked to the nature of the compound to alkylate and block transporter proteins. Besides, due to its hydrophobic nature it has been found to bind nicely to phospholipid bilayer and penetrate slowly across it (Sarpietro, Di Sotto, Accolla, & Castelli, 2015).

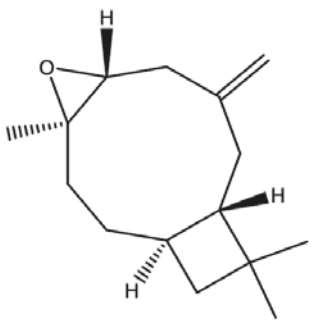

Figure 4
β-caryophylleneoxide.

4. Isolation

The method of isolation of BCO from leaf samples of Jejuguava (P. cattleianum) involves air drying, chopping and three times extraction by 80% methanol for fourteen days at room temperature. The resultant solvent was subjected to evaporation for drying at a temperature less than 40°C with the help of a rotary evaporator to attain the crude extract. This was followed by a part of the crude extract to be suspended in water and fractioned with

several organic solvents such as hexane followed by chloroform and butanol to obtain the hexane, chloroform, butanol and water soluble fractions. The hexane fraction was then taken to perform column chromatography to generate several fractions. The second fraction among them was executed to yet another chromatogram using a solvent system of n-hexane and diethyl ether with ranges reducing from 100:1 to 14:1 followed by evaporation to gain pure β-caryophyllene oxide (Jun et al., 2011).

5. Pharmacological properties

5.1. BCO as an anticancer agent

Studies revealed that two human prostate neoplasm (DU145 and LNCaP cells) have been demonstrated in studies to impede the growth of guava leaves, one of the main sources of BCO (Park et al., 2011). Recently Chen et al. 2010 have also found out that guava leaves are able to bring about apoptosis by the inactivation of AKT with activation of phospho-p38 and phospho-ERK in human prostrateLNCaP cells (Chen et al., 2010). Cytotoxic activity of BCO isolated from Jeju guava *(Psidiumcattleianum Sabine)* has been found out against many types of cancer cell lines. Among these,HeLa,AGS,SNU-1and SNU-16 are mentionable (Jun et al., 2011). In another study it was found out that, BCO isolated from the extracts of *Cinnamomumtamala* leaf showed moderate cytotoxic effect against human ovarian cancer cell line,A-2780 (Shahwar, Ullah, Khan, Ahmad, Saeed, & Ullah, 2015).

The effects of BCO on P13K/AKT/mTOR/S6K pathways in breast cancer and prostate cancer cells was studied by Park et al. (2011) where they reported the constitutive suppression of P13K/AKT/mTOR/S6K activation and induction of ROS-mediated MAPKs activation in human breast and prostate cancer cells. The induction of apoptosis through targeting the P13K/AKT/mTOR/S6K and MAPK signaling pathways was achieved through functionalization of caspase-3 and release of chrome c in tumor cells. Similar research also revealed the gene product down-regulating potential of BCO, which is responsible for anti-apoptosis, cell proliferation and metastasis. The suppressive capability of BCO of constitutive mTOR activation in PC-3 prostrate and MCF-7 breast cancer cells was also reported for the very first time which could be very interesting as previous reports confirms the functionalization of mTOR to be intimately linked with tumorigenesis (Park et al., 2011). Since some mTOR inhibitors recently have shown good promise for the ailment of a number of tumors (Campone et al., 2009), the potential of BCO to target mTOR signaling pathway could be very vital for preventing and treating cancer (Park et al., 2011). Research

revealed two components of essential oils, thymoquinone and isointermedeol induced apoptosis by generating ROSin tumor cells (El-Najjar et al., 2010; Kumar et al., 2008). Again, chemical agents which are able to induce ROS generation subsequently turn on MAPK pathways in different cell types (Hur, Hyun, Lim, Lee, & Kim, 2009; McCubrey, 2006; Torres & Forman, 2003; Xiao, Powolny, & Singh, 2008). In the earlier mentioned study, Park et al. (2011) demonstrated the ROS generating capacity of BCO which aids in the induction of MAPK activation and is very likely to be utilized in retarding tumorigenesis in tumor cell treatment (Park et al., 2011).

It was found that, BCO is capable of suppressing the expression of gene products responsible in tumor genesis and development. These gene products are B-cell lymphoma 2 (bcl-2), cyclin D1, survivin, Inhibitor of apoptosis 1 (IAP-1), B-cell lymphoma extra-large (bcl-xL) and Cyclooxygenase 2 (COX-2). AKT signaling via mTOR is a vital step ofoncogenesis that can safeguard neoplasms from apoptosis (Wendel et al., 2004). If the activation ofP13K/AKT/mTOR/S6K is suppressed by BCO,it could bring about down regulation of many cell survival genes and facilitate apoptosis in abnormal cells. The COX-2 and VEGF (vascular endothelial growth factor) suppressive potential of BCO might well be the desired link for the inhibition metastatic, angiogenesis and invasion. As bcl-2 and bcl-xL safeguard human prostate cancer cells in a differential manner from apoptosis being induced, down regulating them could lead to the potential of BCO to generate apoptosis in cancer cells (Park et al., 2011). BCO was also successful in retarding the proliferation and development of cyclin D1 (Park et al., 2011),which is essential along with p70S6K in the growth of cell and progression of G1 cell cycle in tumor cells (Matsushime, Roussel, Ashmun, & Sherr, 1991; Pullen & Thomas, 1997). Proteins p53 and p21 which are known to suppress tumor by interrupting the cell cycle at G1 phase are also proved to be up-regulated following treatment with BCO. In addition, this sesquiterpene is also able to enhance the apoptotic activity of some AKT inhibitors and this enhancement might well be guided by down regulation of anti-apoptotic gene residues controlled by P13K/AKT/mTOR/S6K signaling (8). BCO also focuses on STAT-3 (Signal transducer and Activator of Transcription)signaling cascades, which is responsible for invasion, angiogenesis, survival and metastasis of cancer and was proved to be very potent in a number of human tumors (Darnell, 1997). The decreased activity of STAT-3 transcription factor after being treated by BCO was seen by Kim, Cho, Kapoor et al. 2014 inprostate and breast cancer cell lines. Suppression of this STAT-3 pathway by BCO was achieved via activation of SHP-1

protein tyrosine phosphatase. Besides, BCO was successful in blocking the activation of STAT-3 induced by IL-6 and the upstream components of STAT-3 pathway, such as c-Src, JAK 1 etc. (Kim, Cho, Kapoor, et al., 2014)

Another postulated potential of BCO is its ability to trigger the apoptotic activities of TNFα. Interestingly, having the potential to induce significant apoptosis alone, BCO was found to increase the TNFα induced apoptosis from 22 to 48%. To be absolutely sure about its potentiating activity, tests were carried out which confronted CPO up-regulating TNFα induced early apoptosis and late apoptosis. It is very much possible that suppression of several anti-apoptotic gene products by CPO could have sensitized the cells to exhibit the apoptotic activity of TNFα. Again, BCO suppressed NF-ϰB activation which was achieved by inhibiting IϰBα kinase activation leading to suppression of IϰBαdegradation, inhibition of p65 phosphorylation and translocation to nucleus. Actually, BCO blocks NF-ϰB activation without hampering the ability of NF-ϰB to bind with DNA. Likewise, BCO also inhibited gene products that have been known to be linked in angiogenesis and invasion. So, inhibition of TNFα mediated invasion of tumor cells might be due to suppression of MMP-9, ICAM-1 and VEGF (Kim, Cho, Kim, et al., 2014).

5.1.1. BCO with Doxorubicin

BCO has been earlier reported to improve the efficacy of conventional cytostatic drugs when adminstered as a combination in several cancer cell lines. In one such instance, BCO improved the anticancer potential of paclitaxel and doxorubicin (DOX) in a number of human cancer cell lines (Kim, Cho, Kim, et al., 2014). Ambroz et al. 2015 in their research have proved, BCO in combination with valencene has the potential to enhance the pro-oxidative activity of doxorubicin in Caco2 cells. Besides, BCO in combination with trans-nerolidol has the potential to increase the accumulation of doxorubicin inside cancer cells (Ambrož et al., 2015). When investigated for anti-proliferative activity of doxorubicin in combination with BCO in MDA-MB-231 human breast cancer cell line, the combination expressed a strong synergistic effect. In the same study, the intracellular distribution and accumulation of doxorubicin combined with BCO was researched in MDA-MB-231 cell line, where an increased concentration of doxorubicin was seen in cells when treated with the combination rather than being treated alone with doxorubicin only (Hanušová et al., 2017). Having seen all these fine results in in vitro tests, experiment was carried out to observe the effects in vivo since only a limited number of study have been carried out to check the anti-cancer activity of sesquiterpenes in combination

with classical drugs in animal models in vivo (Fidyt, Fiedorowicz, Strządała, & Szumny, 2016; Hanušová et al., 2017). In vivo test was performed on mice having solid form of malignant tumor where comparable decrease in tumor weight was seen in the group treated with doxorubicin in combination with BCO (100mg/kg) (Hanušová et al., 2017).

5.1.2. BCO with 5-Fluorouracil and Oxaliplatin

Sesquiterpenes isolated from the essesntial oil of Myrica rubra leaves demonstrateda promising antiproliferative effect against a number of intestinal cancer cell lines. Some of these conventtional anti-cancer drugs if employed in combination (Legault & Pichette, 2007; Tauler & Baraza, 2015) BCO, being one of them was reported to increase the antiproliferative potential of DOX in Caco-2 cells in previous studies (Ambrož et al., 2015). These findings prompted the researchers to check out the impact of BCO on the potential of 5-Fluorouracil (5FU) and Oxaliplatin (OxPt) against Caco-2 colon cancer cells and metastatic SW-620 cancer cells, and also the mechanism by which they produce the effect. Results from the experiments carried out showed that, at elevated concentrations BCO in combination with OxPt was effective in enhancing anti-proliferative activity in Caco-2 cells rather than oxaliplatin tested singly. Furthermore, BCO in combination with 5FU displayed profound anti-proliferative activity at any concentration rather than fluorouracil tested alone. As in case of SW-620 cells, BCO potentiated the anti-proliferative activity of both fluorouracil and oxaliplatin significantly when BCO is treated in combination with either of them rather than the classical drugs used separately. Thus, BCO was found to be the most prolific sesquiterpene in cancer cell killing when used in combination with either fluorouracil or oxaliplatin but the mechanism by which this takes place was not clearly established (Ambrož et al., 2019).

5.2. BCO as an analgesic

Though BCO does not express itself as a prominent pain modulator,it is thought to have antinociceptive activity. This is due to the research conducted by Chavan et al. where they postulated BCO isolated from the bark extract of Annona squamosa having analgesic potential both centrally and peripherally throughinvivo model (Chavan, Wakte, & Shinde, 2010). This analgesic effect is thought to be achieved by suppression of central pain receptors and inhibiting release of pain mediators such as COX-2, PGE-2, IL-1 BETA etc. (Fidyt, Fiedorowicz, Strządała, & Szumny, 2016).

5.3. BCO as a chemosensitizing agent

Doxorubicin,being a potent anti-cancer agent has a limited clinical application due to its toxicity and genesis of multidrug resistance (MDR) (Zhao et al., 2016). Among the reasons of doxorubicin mediated MDR,decreased anti-cancer drug accumulation is a major one. Doxorubicin being hydrophobic in nature is easily passively diffused within cells and its efflux is mediated by a number of proteins such as P-glycoprotein (P-gp), multidrug resistance protein 1 (MRP-1), ATP-binding cassette (ABC) transporters and so on. Doxorubicin-mediated MDR has been linked to excessive expression of these proteins in cancer cells (Kathawala, Gupta, Ashby Jr, & Chen, 2015; Kunjachan, Rychlik, Storm, Kiessling, & Lammers, 2013; van de Ven et al., 2009). So interrupting these proteins could bring about fruitful results in enhancing doxorubicin concentration in cancer cells (Chou, 2006). Of late, some bioactive compounds have been experimented with due to their cancer MDR cell resensitizing property when used in combination with common anti-cancer medications at low doses (Nobili, Landini, Mazzei, & Mini, 2012). BCO being one of them, was tested by Silvia et al. and was reported to possess chemosensitizing property when used in amalgamation with doxorubicin to control MDR phenomenon. This was demonstrated to be achieved by BCO altering cell membrane permeability and function of transmembrane proteins along with ABC-transporters (Di Giacomo, Di Sotto, Mazzanti, & Wink, 2017).

6. Interaction of BCO with enzymes responsible for drug metabolism *(in vitro, in vivo)*

Sesquiterpenes,often being elements of dietary food and beverages come into human contact more often than not. Keeping this in mind, their possible effect on enzymes responsible for drug metabolism was studied using subcellular fractions of human and rat liver invitro. Among other experimented sesquiterpenes which are structurally related, BCO yielded the most promising results. BCO inhibited Ethoxyresorufin-O-deethylase (EROD) and Benzyloxyresorufin (BROD) activities in both human and aminal liver tissue-specific fractions. In thecase of EROD activity, which is mainly related to CYP1A2 inhibition in human microsomes were weaker than in rat microsomes and the inhibition was competitive in human liver but non-competitive in rat liver microsomes. And in BROD activity, which is concerned mainly with CYP3A inhibition was slightly lesser than in rat liver microsomes. The results concluded that, BCO possesses strong ability to minimize CYP3A functions in human liver but other CYPs, conjugation

enzymes (UGTs, SULT, GSTs) and carbonyl-reducing enzymes (CBR1, AKRs) were not seriously affected (Nguyen et al., 2017).

In a very contrasting study performed in mice models invivo, activity of several drug metabolizing enzymes (mainly cytochromes) were found to be enhanced profoundly by BCO. BCO isolated from the essential oil of Myrica rubra was chosen and checked for its modulating effect on a number of Cytochromes (CYP1A1/2, CYP2B, CYP3A), reducing enzymes (CBR1/3, AKR1A1, AKR1C, NQO1) and conjugating (UGT, SULT, GST) enzymes. BCO exerted the most significant effect on CYPs present both in the liver and small intestine. Cytochromes present in the small intestine were greatly activated rather than those in the liver due higher intestinal concentration than hepatic concentration post oral adminstration. CYP1A1 and CYP1A2 remained unchanged in their activity in liver and was not found in small intestine. But in liver, BCO enhanced the activity of CYP2B 2.5 times and that of CYP3A twice in relation to control group 6hrs post oral adminstration. After 24hrs, the enhancement in activity by BCO was even greater. Among reducing enzymes, AKR1A1 and AKR1C were unaffected both in the liver and intestine until 6hrs of treatment. But NQO1's activity seemed to reduce at both organs after 6- and 24-hrs of treatment. Only carbonyl-reducing CBR1/3 enzyme showed an elevated activity post 24-hour of oral adminstration. The tested conjugating enzymes, generally known for their catalyzing role in phase 2 of drug metabolism were rather unchanged both in liver and small intestine (Lněničková, Astani, Reichling, & Schnitzler, 2011; Lněničková, Svobodová, Skálová, Ambrož, Novák, & Matoušková, 2018).

7. Genotoxicity evaluation

To assess BCO's safety profile as a food additive, invitro tests were carried out on bacterial and mammalian cells focusing on point mutations and chromosomal degradation respectively. This was proceeded through the Ames test (for point mutations) and micronucleus assay (for chromosomal degradation), where the evaluation was done at various levels of the bacterial and mammalian cell genomes to reveal possible genotoxins an carcinogens. No mutagenic effects were seen for BCO in several bacterial strains sensitive to base substitution and frameshift mutations. Besides, lack of mutagenicity in the presence of metabolic activator confirmed that themetabolic derivatives produced by the compound itselfare not genotoxic. In addition, the micronucleus assay revealed no genotoxic change at the chromosomal level.

BCO, although having a potential risky epoxide function in its structure was proven incapable of producing DNA-degradation as the epoxide function is the lone reactive site within an inflexible formation hindering its reactivity. Furthermore, the above-stated explanation in addition to the finding that BCO possesses the ability to penetrate the bilayered cell membrane reinforces the fact that it is devoid of genotoxic properties at chromosomal as well as gene level as a base-substitution orframeshift mutagen (Di Sotto, Maffei, Hrelia, Castelli, Sarpietro, & Mazzanti, 2013; Yang, Lederer, McDaniel, & Deinzer, 1993).

8. In-silico study

8.1. PASS prediction

Table I

PASS Prediction of BCO

Activity	Pa	Pi
Antineoplastic	0. 950	0. 004
Antineoplastic (lung cancer)	0. 791	0. 004
Antineoplastic (ovarian cancer)	0. 626	0. 005
Antineoplastic (brain cancer)	0. 522	0. 004
Antineoplastic (pancreatic cancer)	0. 501	0. 005
Antineoplastic (breast cancer)	0. 494	0. 019
Prostate cancer treatment	0. 427	0. 018
Antineoplastic (liver cancer)	0. 370	0. 006
Antineoplastic (colorectal cancer)	0. 366	0. 026
Antineoplastic (colon cancer)	0. 343	0. 027
Antineoplastic (renal cancer)	0. 323	0. 009
Antineoplastic (thyroid cancer)	0. 289	0. 007
Antineoplastic (endocrine cancer)	0. 253	0. 024
Antineoplastic (cervical cancer)	0. 186	0. 046
Antineoplastic (non-small cell lung cancer)	0. 170	0. 123
Arachidonic acid antagonist	0. 225	0. 027

8.2. Molecular docking

Table II

Docking score of BCO and VS

Compounds	Docking Score	(Kcal/mol)
	6DAF	3ERTR
BCO	−5. 568	−7. 848
VS	−7. 913	−4. 896

VS- Vincristine sulfate, Lung cancer protein (PDB: 6DA5), Breast cancer protein (PDB:3ERTR)

Table III

Docking score of BCO and DS

Compounds	Docking Score (Kcal/mol)	
	2OYE	6COX
BCO	−7. 116	−6. 733
DS	−6. 917	−7. 545

DS- Diclofenac Sodium, COX-1 enzyme (PDB: 2OYE), COX-2 enzyme (PDB: 6COX)

8.3. ADME properties

Table IV

ADME Properties of BCO

Rules	β-caryophyllene oxide (BCO)
Molecular Weight (<500 g/mol)	220. 35
Hydrogen Bond Acceptor (10)	1
Hydrogen Bond Donor (5)	0
Log P (≤5)	3. 67
Number of rotatable bond	0
Topological polar surface area	12. 53
Lipinski's Variations (≤1)	0

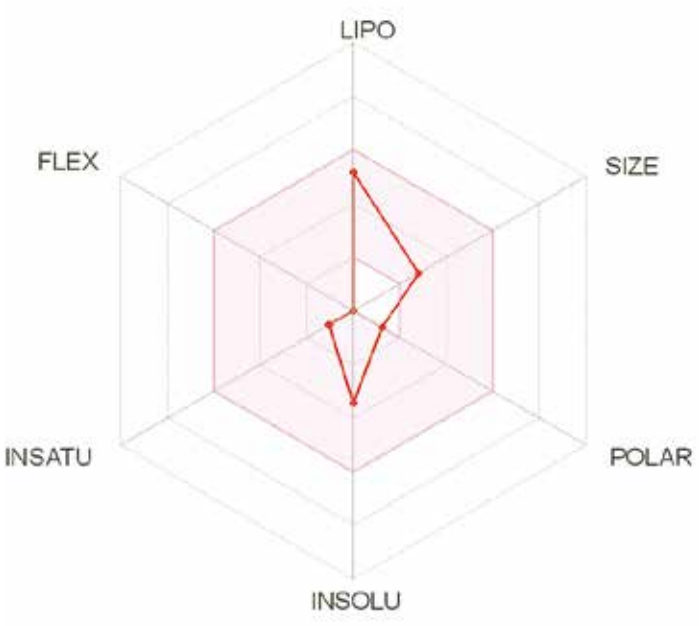

Figure 5
ADME properties of BCO

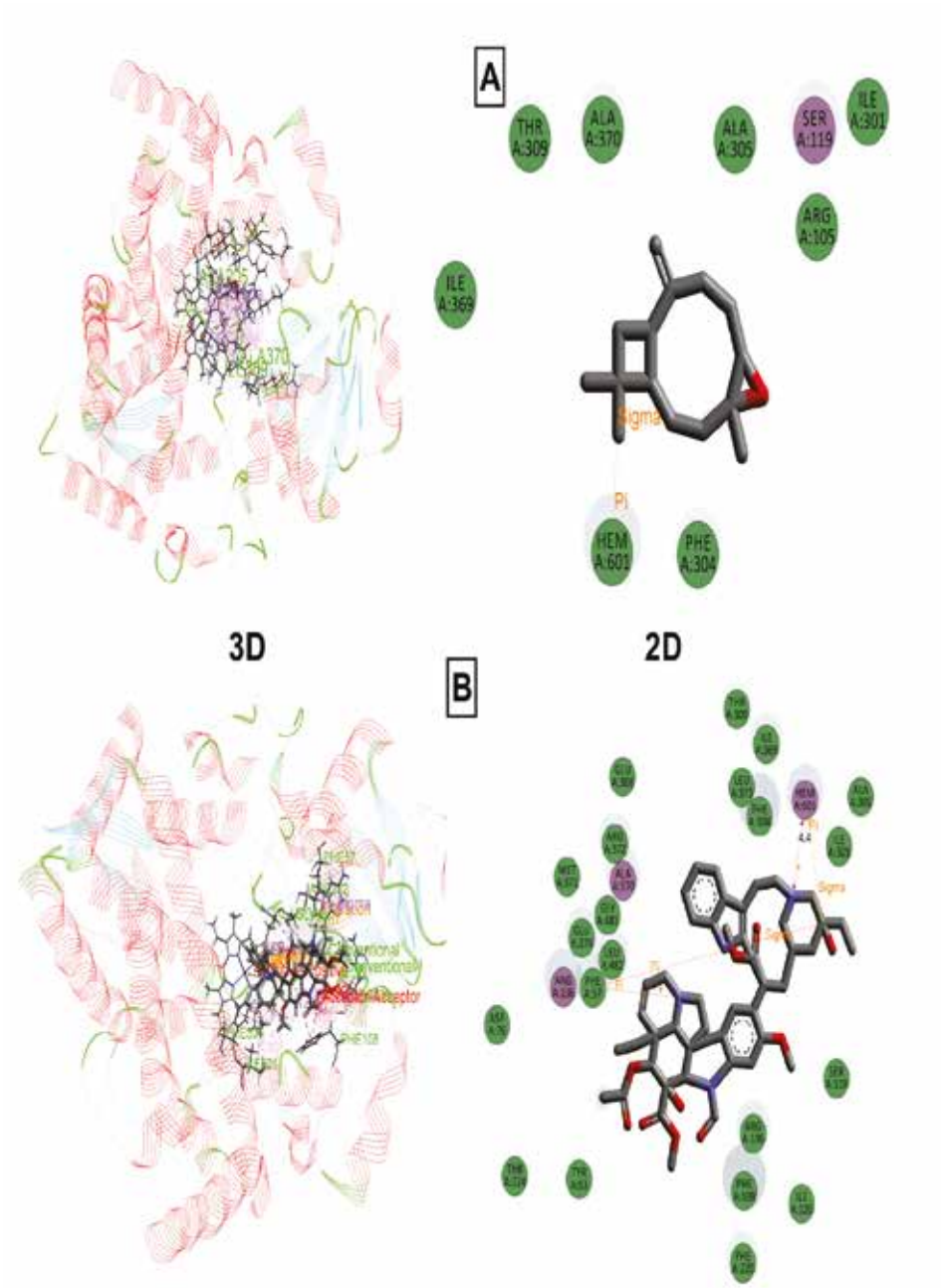

Figure 6

3D and 2D interactions of BCO (A) and vincristine sulfate (B) with human CYP3A4 bound to an inhibitor (PDB: 6DA5) for anticancer activity.

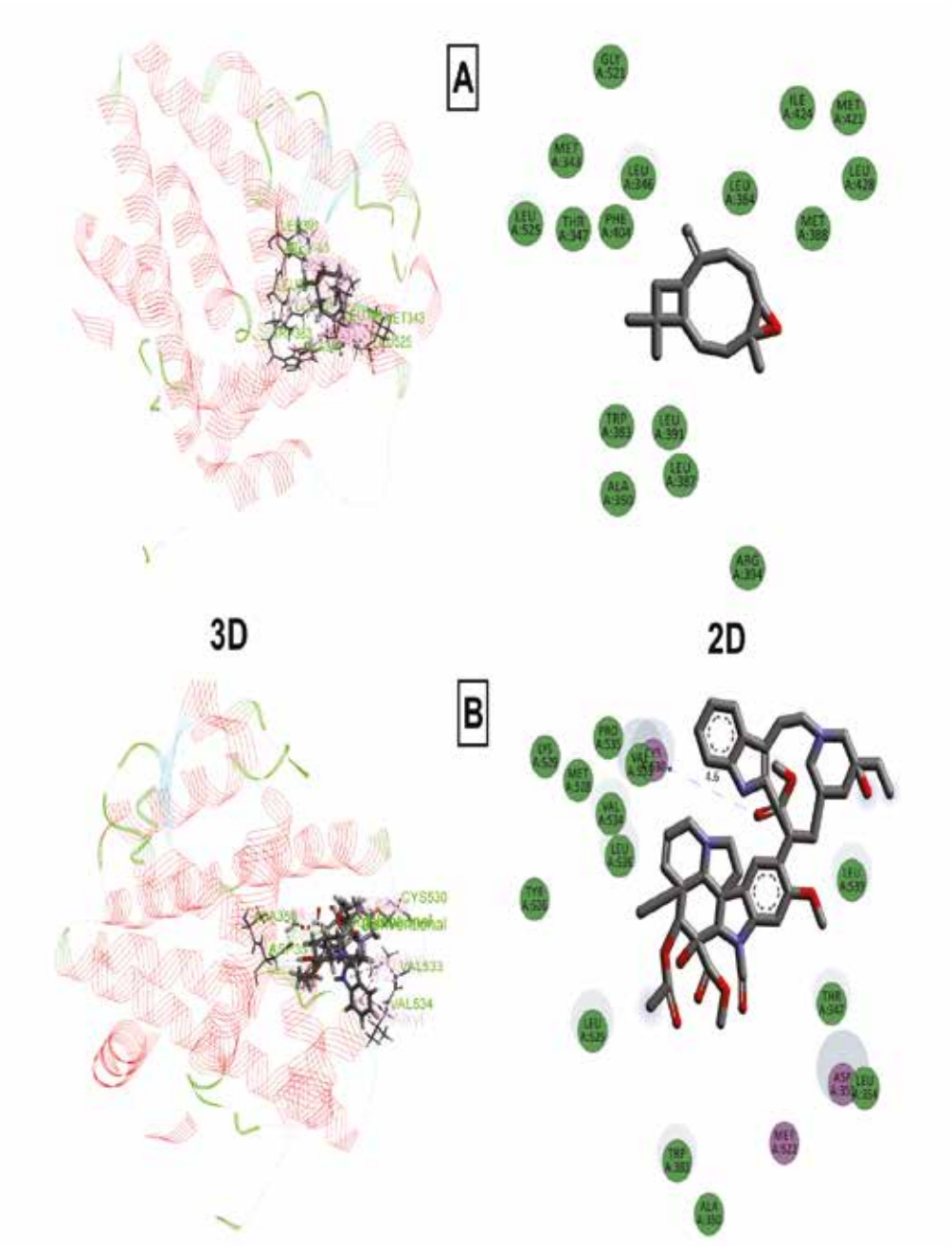

Figure 7
3D and 2D interactions of BCO (A) and vincristine sulfate (B) with human estrogen receptor (PDB: 3ERTR) for anticancer activity

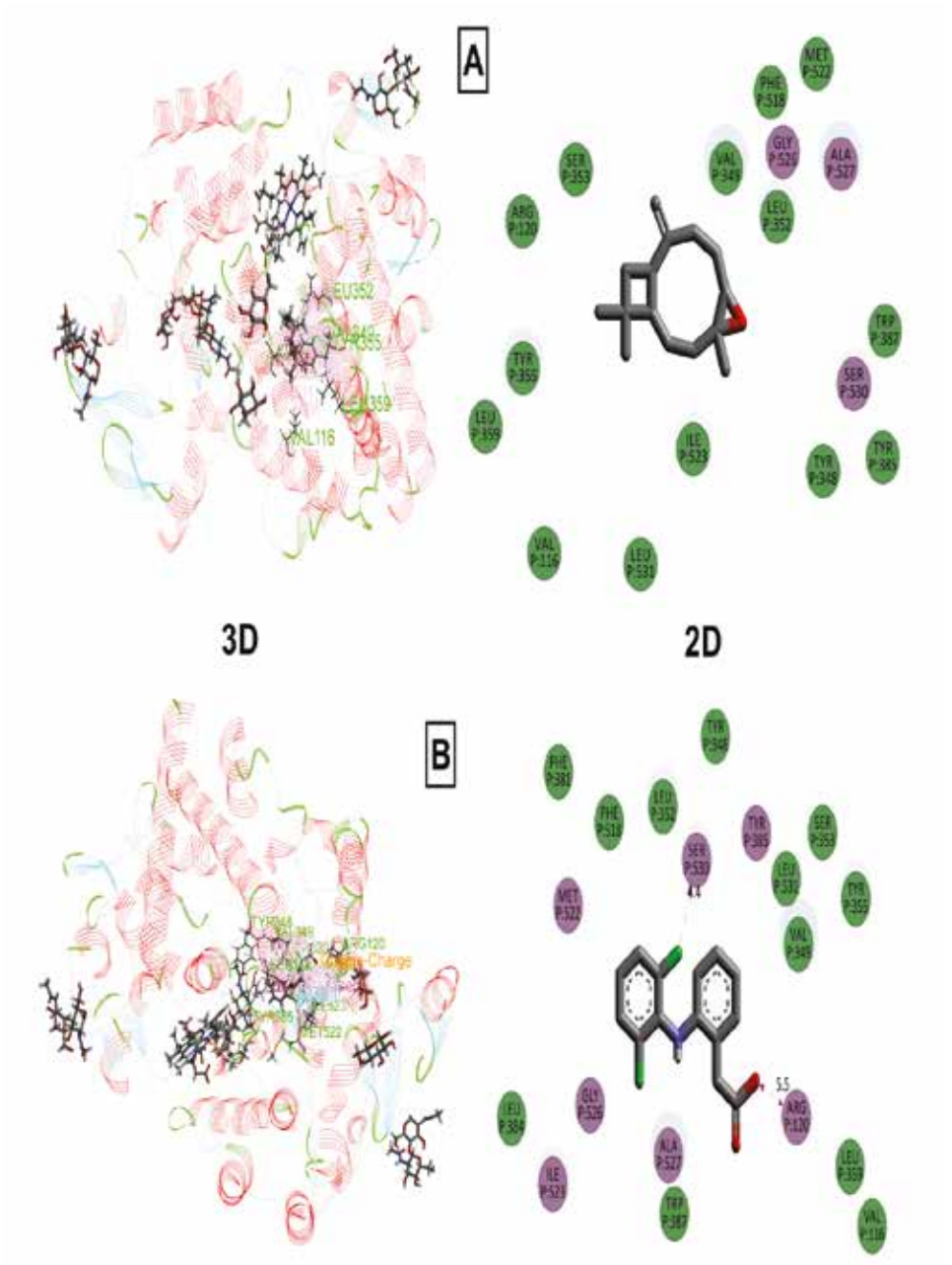

Figure 8

3D and 2D interactions of BCO (A) and diclofenac sodium (B) with Cyclooxygenase-1 (PDB: 2OYE) for analgesic activity

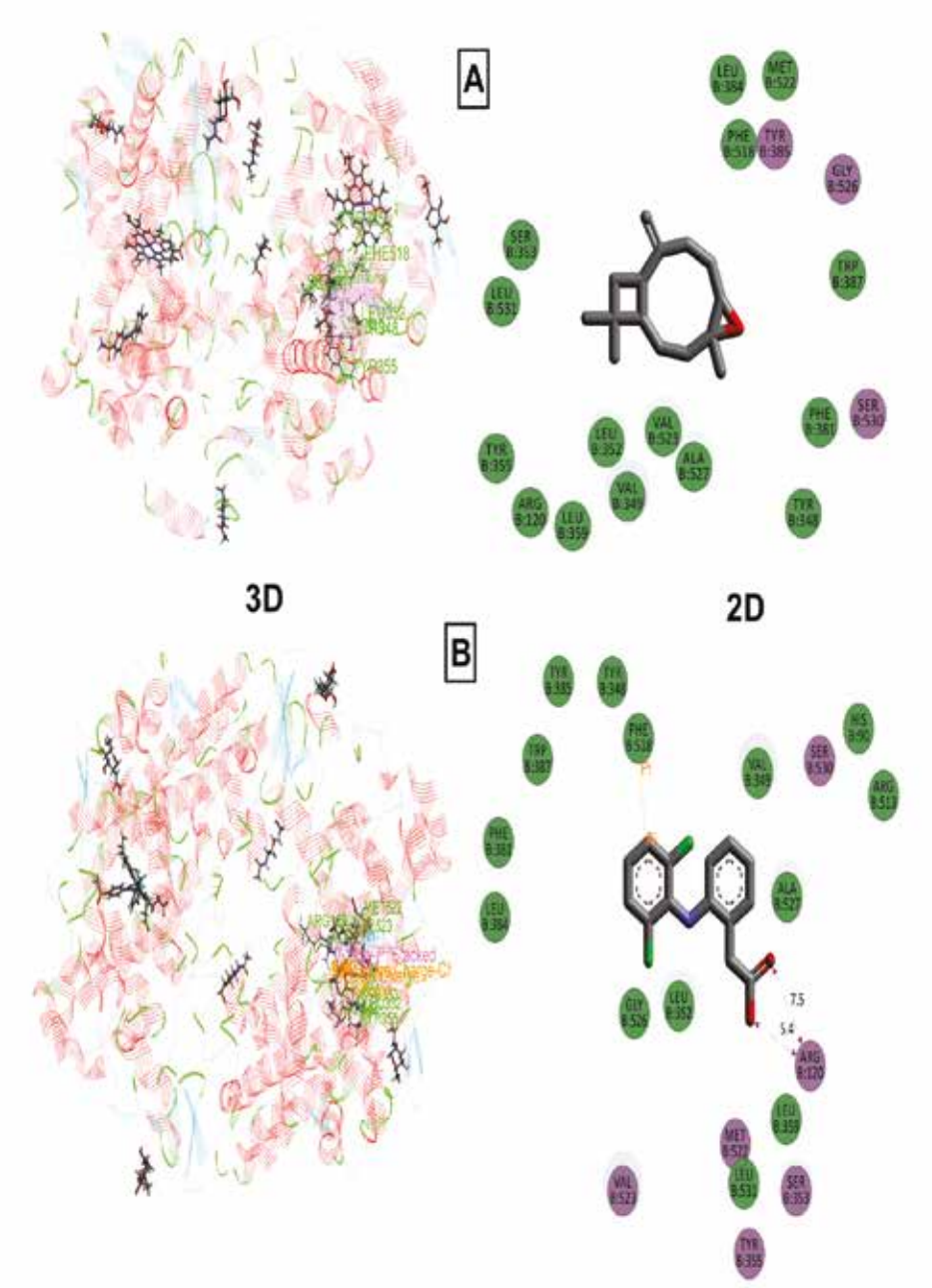

Figure 9

3D and 2D interactions of BCO (A) and diclofenac sodium (B) with Cyclooxygenase-2 (PDB: 6COX) for analgesic activity

9. Results and discussion

Cancer is a word used to describe disorders in which abnormal cells divide out of control and invade surrounding tissues. Cancer cells can potentially move through the blood and lymph systems to other regions of the body. According to the minimal evidence available, the majority of human cancers are produced by genetic transpositions rather than conventional mutagens. Although the molecular biology of transposition is becoming clearer, the extrinsic factors that determine its frequency have yet to be thoroughly investigated (Cairns, 1981). The number of people undergoing chemotherapy has risen dramatically in recent decades (Nussbaumer, Bonnabry, Veuthey, & Fleury-Souverain, 2011). This means there is a greater need of anti-cancer medications than before with least possible side effects. BCO's ability to suppress a number of signaling cascades and down regulating various gene products responsible for cancer development shows an interesting pathway for BCO to act as an anticancer agent (Park et al., 2011). To further analyze this, in silico studies were carried out, starting with PASS prediction. It is based on the probability of activity (Pa) and probability of inactivity (Pi) values. It is preferable that the Pa value is much higher compared to the Pi value. The probability of activity (Pa) must be greater than the probability of inactivity (Pi), with Pa greater than 0.7 being regarded a pharmacologically promising substance (Adnan, Chy, Kamal, Chowdhury, Islam, et al., 2020b). In the current study multiple Pa values for anti-cancer activities in PASS Prediction such as- Antineoplastic (0.950) and Antineoplastic (lung cancer) (0.791) were found to have values of 0.7 or more, with Pi values as low as 0.004, which demonstrates the potential of BCO to be a prospective anti-cancer medication (Table I).

In structural biomolecular study and computer-assisted drug development, molecular docking is a critical tool. This tool aids in the prediction of active chemical binding modes to relevant proteins (Khan et al., 2019). The goal of ligand–protein docking is to use a scoring function to predict the most common binding forms of a ligand with a protein having a known three-dimensional structure (Morris & Lim-Wilby, 2008). The ligand-protein interaction process would be more spontaneous if the binding energy score (binding affinity) was lower or more negative. BCO exhibited a binding affinity of -7. 848 kcal/molagainst the breast cancer protein (PDB: 3ERTR) whilethe reference standard drug for anti-cancer activity, Vincristine sulfate (VS) showed a binding affinity of −4.896 Kcal/mol against the same protein. Since the binding affinity score of BCO (−7.848 Kcal/mol) was lesser against breast cancer protein (PDB:3ERTR), than the reference standard Vincristine sulfate (VS) (−4.896 Kcal/mol), it further adds to the

potential of BCO as an anti-cancer agent with better activity (Table II). Similarly for analgesic activity, BCO exhibited slightly lower binding affinity (−7.116 Kcal/mol) against COX-1 enzyme (PDB: 2OYE) than that of currently existing standard drug for analgesic activity, Diclofenac sodium (DS) with a docking value of (−6.917 Kcal/mol) demonstrating its promising analgesic activity (Table III).

When developing bioactive compounds as medicinal treatments, high oral bioavailability is frequently a factor to consider. As a result, gaining a sufficient understanding of the molecular features that limit oral bioavailability is an important goal for drug research in order to aid the development of feasible new therapeutic candidates (Veber, Johnson, Cheng, Smith, Ward, & Kopple, 2002). Lipinski and colleagues looked at drugs that made it through Phase I and into Phase II clinical trials, and they found a link between their computed physicochemical parameters and their aqueous solubility, absorption and oral bioavailability. The authors came up with the "Rule of Five" as a mnemonic tool for medicinal chemists to utilize during the drug development and optimization process to swiftly assess compounds' potential of having good solubility and permeability characteristics (Pollastri, 2010).

Understanding the range of key compound qualities that best corresponded with compound survival or failure in clinical trials was experimented to develop a set of computational techniques that would best predict the physicochemical properties of compound. To that purpose, the authors looked at all of the drugs that have passed Phase I clinical trials before moving on to Phase II. Because poorly absorbed compounds are unlikely to get beyond the first phases of human evaluation due to a lack of systemic subjection or other formulation difficulties, this chemical collection should demonstrate which compounds are orally bio available. Since most drug companies registered for these names at the entry stage of Phase II, a subset of the -50,000 compounds in the World Drug Index (WDI) was filtered for compounds with a United States Adopted Name (USAN) also known International Non-proprietary Name (INN). Following the removal of compounds that were not small drug molecule (nucleotides, peptides, and polymers), a list of 2300 drug compounds emerged for further investigation, which became known as the USAN Library (Pollastri, 2010). It was seen that increased molecular weight is linked to decreased permeability in the intestines and into the central nervous system (Pardridge, 1995). In lipid bilayers, increasing molecular weight was previously linked to a lower penetration rate (Cohen & Bangham, 1972). When comparing the USAN Library to the original WDI list of 50,000 compounds, only 11% of the

USAN drug compounds had molecular weights more than 500g/mol, compared to 22% that of the WDI list. Because the USAN Library in 1997 only included compounds having oral activity, it's reasonable to assume that the compound shaving larger molecular weight are less likely to be active after oral administration than lower molecular weight compounds (Pollastri, 2010). According to this, BCO with lower molecular weight than 500g/mol is more likely to have oral bioavailability.

Lipophilicity, or a molecule's capacity to partition into octanol compared to water, is a physicochemical feature that is usually thought to be very important to absorption rate (Testa, Carrupt, Gaillard, Billois, & Weber, 1996). The logarithmic ratio of drug that divides into organic phase compared with aqueous phase, or log P, is commonly used to express lipophilicity. Only 10 percent of the chemicals in the USAN library subset chosen for Lipinski's investigation had a Log P greater than 5. To put it another way, substances with a Log P of less than 5 were 90 % more likely to be orally accessible (Pollastri, 2010).

Increasing the hydrogen-bond donor groups in a compound can diminish a molecule's capacity to permeate a membrane bilayer, in addition to its molecular weight and lipophilicity (Paterson, Conradi, Hilgers, Vidmar, & Burton, 1994). Compounds with a significant number of hydrogen-bond donors exhibit partition into a strongly hydrogen-bonding solvent (such as water) unlikely the lipophilic environment found in a cellular membrane, as one might expect. Hydrogen-bond donor features that correlate to poor physicochemical qualities can be well described by a simple accounting of O-H and N-H bonds in a molecule. Surprisingly, the USAN library contains 92 % of compounds with five or fewer hydrogen-bond donors (Pardridge, 1995). Hydrogen-bond acceptors increase permeability by reacting favorably with highly hydrogen bonding solvents like water, in the same way that hydrogen-bond donors diminish the permeability of molecules into lipophilic environments. Lipinski and team found that merely adding up the quantity of nitrogen and oxygen atoms in a molecule can be used as a surrogate for oral bioavailability. Indeed, the USAN library contains 88 % of molecules with less than 10 nitrogen and oxygen atoms (Pollastri, 2010).

Veber, Johnson, Cheng, Smith, Ward, and Kopple (2002) analyzed the oral bioavailability of approximately 1100 potential compounds from Smith-Klein and French, and found a relationship between permeability and molecular flexibility in the compounds. The number of rotatable bonds can be used to characterize flexibility. Surprisingly, assessing the amount of rotatable bonds influences permeability features without taking molecular weight into account, implying that molecules with more than ten rotatable

bonds will have poor permeability. Veber also related a large polar surface area (more than 140 Å2) to lower permeability (Veber, Johnson, Cheng, Smith, Ward, & Kopple, 2002). Results of the current study in ADME analysis demonstrated that BCO with molecular weight 220.35g/mol, Log P value 3.67, no hydrogen bond donor, 1 hydrogen bond acceptor complies with Lipinski's rule and with no rotatable bond and topological polar surface area of 12.53 Å2 also complies with Veber's rule as a possible lead compound having promising oral bioavailability (Table IV).

BCO's ability to suppress a number of signaling cascades and down regulating various gene products responsible for cancer development shows an interesting pathway for BCO to act as an anticancer agent. Along with this, the cytotoxic activity of the compound against several cancer cell lines adds to its interesting prospect as a potential anticancer medication. Besides, BCO was found to inhibit pain both centrally and peripherally in in vivo models which could facilitate a path for the development of an anti-cancer drug with significant analgesic activity. Other interesting activities of this compound included its ability to brisk the efficacy of currently established anti-cancer drugs by increasing their concentrations in cancer cells. The insilico study was approached via PASS prediction, ADME properties and molecular docking respectively. Promising results of some activities (antineoplastic, arachidonic acid antagonist) from PASS prediction guided the research to be conducted for ADME properties which thereafter showed good values for oral bioavailability of the prospective drug. Then the molecular docking of the compound against several cancer proteins and cyclooxygenase enzymes was performed which yielded significant values of bonding energies with those proteins. Thus, the insilico findings provided strong evidences in favour of BCO with the ability to be a prospective therapeutic agent. Besides, the sound genotoxic profile of BCO is also a promising sign for its safety and tolerancy for human trial. Research on BCO's interaction with enzymes responsible for metabolism of drugs performed in vitro and in vivo were found to have diminishing and enhancing effects on the enzymes which remains a point of further research for better understanding to reach a conclusive decision.

10. Conclusion

The insilico findings provided strong evidences in favour of BCO with the ability to be a prospective therapeutic agent. Besides,the sound genotoxic profile of BCO is also a promising sign for its safety and tolerancy for human trial. With all these findings,there is still a significant research gap regarding the pharmacokinetic and pharmacodynamic profile of this compound as not

many research has been carried out as yet involving human subjects. Research on BCO's interaction with enzymes responsible for metabolism of drugs performed in vitro and in vivo were found to have diminishing and enhancing effects on the enzymes which remains a point of further research for better understanding to reach a conclusive decision. But, from all the information gathered about this compound, this remains a very hopeful compound to be used in the field of drug therapy. Clinical trials using this compound,if carried out properly may yield beneficial results in the field of novel drug discovery.

Funding: This work is organized with the individual funding of the author.
Institutional Review Board Statement: Not applicable.
Informed Consent Statement: Not applicable.
Data Availability Statement: Available data are presented in the manuscript.
Acknowledgments: The authors is thankful to the Department of Pharmacy, International Islamic University Chittagong (IIUC), Bangladesh.
Conflicts of Interest: Author declared that there is no conflict of interest.

References

Adnan, M., Chy, M. N. U., Kamal, A. M., Chowdhury, K. A. A., Rahman, M. A., Reza, A. A., ... & Cho, D. H. (2020). Intervention in neuropsychiatric disorders by suppressing inflammatory and oxidative stress signal and exploration of in silico studies for potential lead compounds from Holigarna caustica (Dennst.) Oken leaves. *Biomolecules, 10*(4), 561. doi:10.3390/biom10040561

Adnan, M., Chy, M. N. U., Kamal, A. M., Chowdhury, M. R., Islam, M. S., Hossain, M. A., ... & Cho, D. H. (2020). Unveiling pharmacological responses and potential targets insights of identified bioactive constituents of cuscuta reflexa roxb. Leaves through in vivo and in silico approaches. *Pharmaceuticals, 13*(3), 50. doi:10.3390/ph13030050

Ambrož, M., Boušová, I., Skarka, A., Hanušová, V., Králová, V., Matoušková, P., ... Skálová, L. (2015). The influence of sesquiterpenes from Myrica rubra on the antiproliferative and pro-oxidative effects of doxorubicin and its accumulation in cancer cells. *Molecules, 20*(8), 15343-15358. doi:10.3390/molecules200815343

Ambrož, M., Šmatová, M., Šadibolová, M., Pospíšilová, E., Hadravská, P., Kašparová, M., . . . Skálová, L. (2019). Sesquiterpenes α-humulene and β-caryophyllene oxide enhance the efficacy of 5-fluorouracil and oxaliplatin in colon cancer cells. *Acta Pharmaceutica, 69*(1), 121-128. doi:10.2478/acph-2019-0003

Astani, A., Reichling, J., & Schnitzler, P. (2011). Screening for antiviral activities of isolated compounds from essential oils. *Evidence-Based Complementary and Alternative Medicine. 2011* doi:10.1093/ecam/nep187

Batra, H., Pawar, S., & Bahl, D. (2019). Curcumin in combination with anti-cancer drugs: A nanomedicine review. *Pharmacological research, 139*, 91-105. doi: 10.1016/j.phrs.2018.11.005

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., & Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians, 68*(6), 394-424. doi: 10.3322/caac.21492.

Cairns, J. (1981). The origin of human cancers. *Nature, 289*(5796), 353-357.

Campone, M., Levy, V., Bourbouloux, E., Berton Rigaud, D., Bootle, D., Dutreix, C., . . . & Raymond, E. (2009). Safety and pharmacokinetics of paclitaxel and the oral mTOR inhibitor everolimus in advanced solid tumours. *British Journal of Cancer, 100*(2), 315-321. doi:10.1038/sj.bjc.6604851

Chavan, M. J., Wakte, P. S., & Shinde, D. B. (2010). Analgesic and anti-inflammatory activity of Caryophyllene oxide from Annona squamosa L. bark. *Phytomedicine, 17*(2), 149-151. doi:https://doi.org/10.1016/j.phymed.2009.05.016

Chen, K. C., Peng, C. C., Chiu, W. T., Cheng, Y. T., Huang, G. T., Hsieh, C. L., & Peng, R. Y. (2010). Action mechanism and signal pathways of Psidium guajava L. aqueous extract in killing prostate cancer LNCaP cells. *Nutrition and Cancer, 62*(2), 260-270. doi:10.1080/01635580903407130

Chou, T. C. (2006). Theoretical basis, experimental design, and computerized simulation of synergism and antagonism in drug combination studies. *Pharmacological reviews, 58*(3), 621-681.

Cohen, B., & Bangham, A. (1972). Diffusion of small non-electrolytes across liposome membranes. *Nature, 236*(5343), 173-174.

Darnell, J. E. (1997). STATs and gene regulation. *Science, 277*(5332), 1630. doi:10.1126/science.277.5332.1630

Di Giacomo, S., Di Sotto, A., Mazzanti, G., & Wink, M. (2017). Chemosensitizing properties of β-caryophyllene and β-caryophyllene oxide in combination with doxorubicin in human cancer cells. *Anticancer Research, 37*(3), 1191-1196. doi:10.21873/anticanres.11433

Di Sotto, A., Maffei, F., Hrelia, P., Castelli, F., Sarpietro, M. G., & Mazzanti, G. (2013). Genotoxicity assessment of β-caryophyllene oxide. *Regulatory Toxicology and Pharmacology, 66*(3), 264-268. doi:10.1016/j.yrtph.2013.04.006

Ehrsson, H., Wallin, I., & Yachnin, J. (2002). Pharmacokinetics of oxaliplatin in humans. *Medical Oncology, 19*(4), 261-265. doi:10.1385/MO:19:4:261

El-Najjar, N., Chatila, M., Moukadem, H., Vuorela, H., Ocker, M., Gandesiri, M., . . . Gali-Muhtasib, H. (2010). Reactive oxygen species mediate thymoquinone-induced apoptosis and activate ERK and JNK signaling. *Apoptosis, 15*(2), 183-195. doi:10.1007/s10495-009-0421-z

Farag, R. S., Shalaby, A. S., El-Baroty, G. A., Ibrahim, N. A., Ali, M. A., & Hassan, E. M. (2004). Chemical and biological evaluation of the essential oils of different Melaleuca species. *Phytotherapy Research, 18*(1), 30-35. doi:10.1002/ptr.1348

Fidyt, K., Fiedorowicz, A., Strządała, L., & Szumny, A. (2016). β-caryophyllene and β-caryophyllene oxide—natural compounds of anticancer and analgesic properties. *Cancer Medicine, 5(*10), 3007-3017. doi:10.1002/cam4.816

Gertsch, J., Leonti, M., Raduner, S., Racz, I., Chen, J.-Z., Xie, X.-Q., . . . Zimmer, A. (2008). Beta-caryophyllene is a dietary cannabinoid. *Proceedings of the National Academy of Sciences, 105*(26), 9099. doi:10.1073/pnas.0803601105

Graham, J., Muhsin, M., & Kirkpatrick, P. (2004). Oxaliplatin. *Nature Reviews Drug Discovery, 3(*1), 11-12. doi:10.1038/nrd1287

Halpern, L. M. (1977). Analgesic drugs in the management of pain. *Archives of Surgery, 112*(7), 861-869. doi:10.1001/archsurg.1977.01370070075013

Hanušová, V., Boušová, I., & Skálová, L. (2011). Possibilities to increase the effectiveness of doxorubicin in cancer cells killing. *Drug Metabolism Reviews, 43*(4), 540-557. doi:10.3109/03602532.2011.609174

Hanušová, V., Caltová, K., Svobodová, H., Ambrož, M., Skarka, A., Murínová, N., . . . Skálová, L. (2017). The effects of β-caryophyllene oxide and trans-nerolidol on the efficacy of doxorubicin in breast cancer cells and breast tumor-bearing mice. *Biomedicine & Pharmacotherapy, 95,* 828-836. doi:10.1016/j.biopha.2017.09.008

Hur, J. M., Hyun, M. S., Lim, S. Y., Lee, W. Y., & Kim, D. (2009). The combination of berberine and irradiation enhances anti-cancer effects via activation of p38 MAPK pathway and ROS generation in human

hepatoma cells. *Journal of cellular biochemistry, 107*(5), 955-964. doi:10.1002/jcb.22198

Jun, N. J., Mosaddik, A., Moon, J. Y., Ki-Chang, J., Dong-Sun, L., Ahn, K. S., & Cho, S. K. (2011). Cytotoxic activity of [beta]-caryophyllene oxide isolated from Jeju Guava (Psidium cattleianum Sabine) Leaf. *Records of Natural Products, 5*(3), 242-246.

Kathawala, R. J., Gupta, P., Ashby Jr, C. R., & Chen, Z. S. (2015). The modulation of ABC transporter-mediated multidrug resistance in cancer: A review of the past decade. *Drug resistance updates, 18*, 1-17. doi:10.1016/j.drup.2014.11.002

Keefe, F. J., Abernethy, A. P., & Campbell, L. C. (2005). Psychological approaches to understanding and treating disease-related pain. *Annu. Rev. Psychol., 56*, 601-630.

Khan, S., Nazir, M., Raiz, N., Saleem, M., Zengin, G., Fazal, G., . . . Mahomoodally, F. M. (2019). Phytochemical profiling, in vitro biological properties and in silico studies on Caragana ambigua stocks (Fabaceae): A comprehensive approach. *Industrial Crops and Products, 131,* 117-124. doi:10.1016/j.indcrop.2019.01.044

Kim, C., Cho, S. K., Kapoor, S., Kumar, A., Vali, S., Abbasi, T., . . . Ahn, K. S. (2014). β-caryophyllene oxide inhibits constitutive and inducible STAT3 signaling pathway through induction of the SHP-1 protein tyrosine phosphatase. *Molecular Carcinogenesis, 53*(10), 793-806. doi:10.1002/mc.22035

Kim, C., Cho, S. K., Kim, K. D., Nam, D., Chung, W. S., Jang, H. J., ... Ahn, K. S. (2014). β-Caryophyllene oxide potentiates TNFα-induced apoptosis and inhibits invasion through down-modulation of NF-κ B-regulated gene products. *Apoptosis, 19*(4), 708-718. doi: 10.1007/s10495-013-0957-9

Kubo, I., Chaudhuri, S. K., Kubo, Y., Sanchez, Y., Ogura, T., Saito, T., . . . Haraguchi, H. (1996). Cytotoxic and antioxidative sesquiterpenoids from Heterotheca inuloides. *Planta Medica, 62*(5), 427-430.

Kumar, A., Malik, F., Bhushan, S., Sethi, V. K., Shahi, A. K., kaur, J., . . . Singh, J. (2008). An essential oil and its major constituent isointermedeol induce apoptosis by increased expression of mitochondrial cytochrome c and apical death receptors in human leukaemia HL-60 cells. *Chem Biol Interact, 171*(3), 332-347. doi: 10.1016/j.cbi.2007.10.003

Kunjachan, S., Rychlik, B., Storm, G., Kiessling, F., & Lammers, T. (2013). Multidrug resistance: Physiological principles and nanomedical solutions. *Advanced Drug Delivery Reviews, 65*(13), 1852-1865. doi:10.1016/j.addr.2013.09.018

Legault, J., & Pichette, A. (2007). Potentiating effect of beta-caryophyllene on anticancer activity of alpha-humulene, isocaryophyllene and paclitaxel. *The Journal of Pharmacy and Pharmacology, 59*(12), 1643-1647. doi:10.1211/jpp.59.12.0005

Lněničková, K., Svobodová, H., Skálová, L., Ambrož, M., Novák, F., & Matoušková, P. (2018). The impact of sesquiterpenes β-caryophyllene oxide and trans-nerolidol on xenobiotic-metabolizing enzymes in mice in vivo. *Xenobiotica, 48*(11), 1089-1097. doi:10.1080/00498254.2017.1398359

Martin, A., Markéta, Š., Michaela, Š., Eva, P., Pavlína, H., Michaela, K., . . . Lenka, S. (2019). Sesquiterpenes α-humulene and β-caryophyllene oxide enhance the efficacy of 5-fluorouracil and oxaliplatin in colon cancer cells. *Acta Pharmaceutica, 69*(1), 121-128. doi:https://doi.org/10.2478/acph-2019-0003

Matsushime, H., Roussel, M. F., Ashmun, R. A., & Sherr, C. J. (1991). Colony-stimulating factor 1 regulates novel cyclins during the G1 phase of the cell cycle. *Cell, 65*(4), 701-713. doi:10.1016/0092-8674(91)90101-4

McCubrey, J. A. (2006). Reactive oxygen species-induced activation of the MAP kinase signaling pathways. *Antioxid. Redox Signal., 8*, 1775-1789.

Morris, G. M., & Lim-Wilby, M. (2008). *Molecular docking. In Molecular modeling of proteins* (pp. 365-382). Springer: Humana Press.

Newman, D. J., & Cragg, G. M. (2012). Natural products as sources of new drugs over the 30 years from 1981 to 2010. *Journal of Natural Products, 75*(3), 311-335. doi:10.1021/np200906s

Nguyen, L. T., Myslivečková, Z., Szotáková, B., Špičáková, A., Lněničková, K., Ambrož, M., . . . Skálová, L. (2017). The inhibitory effects of β-caryophyllene, β-caryophyllene oxide and α-humulene on the activities of the main drug-metabolizing enzymes in rat and human liver in vitro. *Chem Biol Interact, 278*, 123-128. doi:10.1016/j.cbi.2017.10.021

Nobili, S., Landini, I., Mazzei, T., & Mini, E. (2012). Overcoming tumor multidrug resistance using drugs able to evade P-glycoprotein or to exploit its expression. *Medicinal Research Reviews, 32*(6), 1220-1262. doi:10.1002/med.20239

Nussbaumer, S., Bonnabry, P., Veuthey, J. L., & Fleury-Souverain, S. (2011). Analysis of anticancer drugs: A review. *Talanta, 85*(5), 2265-2289. doi:10.1016/j.talanta.2011.08.034

Pardridge, W. M. (1995). Transport of small molecules through the blood-brain barrier: biology and methodology. *Advanced Drug Delivery Reviews, 15*(1-3), 5-36.

Park, K. R., Nam, D., Yun, H. M., Lee, S. G., Jang, H. J., Sethi, G., ... & Ahn, K. S. (2011). β-Caryophyllene oxide inhibits growth and induces apoptosis through the suppression of PI3K/AKT/mTOR/S6K1 pathways and ROS-mediated MAPKs activation. *Cancer letters, 312*(2), 178-188. doi:10.1016/j.canlet.2011.08.001

Paterson, D. A., Conradi, R. A., Hilgers, A. R., Vidmar, T. J., & Burton, P. S. (1994). A non-aqueous partitioning system for predicting the oral absorption potential of peptides. *Quantitative structure-Activity Relationships, 13*(1), 4-10. doi: 10.1002/qsar.19940130103

Patrick, G. L. (2013). *An introduction to medicinal chemistry* (5th ed.). New York, USA: Oxford university press.

Phillips, C. J. (2009). The cost and burden of chronic pain. *Reviews in Pain, 3*(1), 2-5. doi:10.1177/204946370900300102

Pollastri, M. P. (2010). Overview on the rule of five. *Current protocols in pharmacology, 49*(1), 9-12. doi: 10.1002/0471141755.ph0912s49.

Pradhan, R., & Zheng, Y. (2016). A hierarchical neural stimulation model for pain relief by variation of coil design parameters. *arXiv preprint arXiv:1609.03730*. doi: 10.48550/arXiv.1609.03730

Pramanik, D., Campbell, N. R., Das, S., Gupta, S., Chenna, V., Bisht, S., . . . Steenbergen, C. (2012). A composite polymer nanoparticle overcomes multidrug resistance and ameliorates doxorubicin-associated cardiomyopathy. *Oncotarget, 3*(6), 640. doi: 10.18632/oncotarget.543

Pullen, N., & Thomas, G. (1997). The modular phosphorylation and activation of p70s6k. *FEBS Letters, 410*(1), 78-82. doi:10.1016/S0014-5793(97)00323-2

Sarpietro, M. G., Di Sotto, A., Accolla, M. L., & Castelli, F. (2015). Interaction of β-caryophyllene and β-caryophyllene oxide with phospholipid bilayers: Differential scanning calorimetry study. *Thermochimica Acta, 600*, 28-34. doi:10.1016/j.tca.2014.11.029

Shahwar, D., Ullah, S., Khan, M. A., Ahmad, N., Saeed, A., & Ullah, S. (2015). Anticancer activity of Cinnamon tamala leaf constituents towards human ovarian cancer cells. *Pakistan Journal of Pharmaceutical Sciences, 28*(3), 969-972.

Sun, J., Wei, Q., Zhou, Y., Wang, J., Liu, Q., & Xu, H. (2017). A systematic analysis of FDA-approved anticancer drugs. *BMC Systems Biology, 11*(5), 87. doi:10.1186/s12918-017-0464-7

Tauler, M., & Baraza, E. (2015). Improving the acclimatization and establishment of Arundo donax L. plantlets, a promising energy crop, using a mycorrhiza-based biofertilizer. *Industrial Crops and Products, 66*, 299-304. doi:10.1016/j.indcrop.2014.12.039

Tegeder, I. (2013). COX-1 and COX-2 in Pain. In G. F. Gebhart & R. F. Schmidt (Eds.), *Encyclopedia of Pain* (pp. 791-794). Berlin, Heidelberg: Springer Berlin Heidelberg.

Testa, B., Carrupt, P. A., Gaillard, P., Billois, F., & Weber, P. (1996). Lipophilicity in molecular modeling. *Pharmaceutical Research, 13*(3), 335-343.

Torres, M., & Forman, H. J. (2003). Redox signaling and the MAP kinase pathways. *BioFactors, 17*, 287-296.

van de Ven, R., Oerlemans, R., van der Heijden, J. W., Scheffer, G. L., de Gruijl, T. D., Jansen, G., & Scheper, R. J. (2009). ABC drug transporters and immunity: novel therapeutic targets in autoimmunity and cancer. *Journal of Leukocyte Biology, 86*(5), 1075-1087. doi:10.1189/jlb.0309147

Veber, D. F., Johnson, S. R., Cheng, H.-Y., Smith, B. R., Ward, K. W., & Kopple, K. D. (2002). Molecular properties that influence the oral bioavailability of drug candidates. *Journal of Medicinal Chemistry, 45*(12), 2615-2623. doi:10.1021/jm020017n

Wendel, H. G., Stanchina, E. d., Fridman, J. S., Malina, A., Ray, S., Kogan, S., . . . Lowe, S. W. (2004). Survival signalling by Akt and eIF4E in oncogenesis and cancer therapy. *Nature, 428*(6980), 332-337. doi:10.1038/nature02369

Whalen, K., Finkel, R., & Panavelil, T. A. (2015). *Lippincott illustrated reviews: Pharmacology* (6th ed.): Lippincott Williams & Wilkins.

Xiao, D., Powolny, A. A., & Singh, S. V. (2008). Benzyl isothiocyanate targets mitochondrial respiratory chain to trigger reactive oxygen species-dependent apoptosis in human breast cancer cells. *The Journal of Biological Chemistry, 283*(44), 30151-30163. doi:10.1074/jbc.m802529200

Yang, X., Lederer, C., McDaniel, M., & Deinzer, M. (1993). Hydrolysis products of caryophyllene oxide in hops and beer. *Journal of Agricultural and Food Chemistry, 41*(11), 2082-2085. doi:10.1021/jf00035a049

Zhao, Y., Huan, M. L., Liu, M., Cheng, Y., Sun, Y., Cui, H., ... & Zhou, S. Y. (2016). Doxorubicin and resveratrol co-delivery nanoparticle to overcome doxorubicin resistance. *Scientific Reports, 6*(1), 1-15. doi:10.1038/srep3526

Zheng, G. Q., Kenney, P. M., & Lam, L. K. T. (1992). Sesquiterpenes from clove (Eugenia caryophyllata) as potential anticarcinogenic agents. *Journal of Natural Products, 55*(7), 999-1003. doi:10.1021/np50085a029

Corresponding author
A.T.M. Mostafa Kamal can be contacted at: mostafakamal285@yahoo.com