



Biological Effects of Propolis on Cancer

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<p><i>Review Article</i></p> <p>Received : 06/09/2019 Accepted : 08/01/2020</p> <p>Keywords: Anticancer Antitumor Extraction Flavonoid Propolis</p>	<p>Propolis is a special resin and wax material collected from the leaves and shells of trees, buds and shoots of plants by honey bees (<i>Apis mellifera L.</i>). In recent years, many researchers have studied the chemical composition, biological activity and pharmacological properties of propolis. The colour, odour and pharmacological properties of the propolis composition also vary as the composition changes depending on the plant, region, season and bee colony. Flavonoids, aromatic acids, phenolic acids and esters are the main compounds responsible for the biological activity of propolis. A number of studies have been conducted on the use of propolis or its active ingredients in the treatment of cancer. It has been observed that the use of propolis did not cause side effects according to <i>in-vitro</i> and <i>in-vivo</i> studies. Propolis should be extracted with different compounds for use in biological assays. The most commonly used compounds for extraction are ethanol, methanol, oil and water. A number of studies have been carried out showing the antitumor effect of propolis in cell culture and animal tests. The search for new drugs derived from natural products, which may function as chemotherapeutic agents and have low side effects, has increased in recent years. Combination with antioxidant therapy may improve the side effects of chemotherapy on leukocytes, liver and kidney, thus increasing the effect of chemotherapy with dose increase.</p>

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Kanserde Propolisin Biyolojik Etkileri

MAKALE BİLGİSİ	ÖZ
<p><i>Derleme Makale</i></p> <p>Geliş : 06/09/2019 Kabul : 08/01/2020</p> <p>Anahtar Kelimeler: Antikanser Antitumor Ekstraksiyon Flavonoid Propolis</p>	<p>Propolis, bal arıları (<i>Apis mellifera L.</i>) tarafından ağaçların yaprak ve kabuklarından, bitkilerin tomurcuk ve filizlerinden toplanan, özel reçine ve mumsu maddedir. Son yıllarda birçok araştırmacı propolisin kimyasal kompozisyonu, biyolojik aktivitesi ve farmakolojik özellikleri üzerine çalışmışlardır. Propolisin bileşimi bitkiye, bölgeye, mevsime ve arı kolonisine bağlı olarak değiştiğinden dolayı rengi, kokusu ve farmakolojik özellikleri de farklılık göstermektedir. Flavonoidler, aromatik asitler, fenolik asitler ve esterleri propolisin biyolojik aktivitelerinden sorumlu başlıca bileşiklerdir. Propolis veya aktif bileşenlerinin kanser tedavisinde kullanımıyla ilgili olarak çok sayıda çalışma yapılmıştır. <i>In-vitro</i> ve <i>in-vivo</i> yapılan çalışmalarda propolis kullanımının yan etkiye yol açmadığı gözlenmiştir. Biyolojik analizlerde kullanılmak üzere propolis farklı bileşikler ile ekstrakte edilmelidir. Ekstraksiyon işlemi için en çok kullanılan bileşikler etanol, metanol, yağ ve sudur. Hücre kültür ve hayvan testlerinde propolisin antitumor etkisini gösteren çok sayıda çalışma yapılmıştır. Kemoterapötik ajanlar olarak işlev görebilecek ve yan etkileri düşük olan özellikle de doğal ürünlerden türetilmiş yeni ilaç arayışı son yıllarda artmıştır. Antioksidan terapiyle kombinasyon lökositlerin, karaciğer ve böbreğin üzerine kemoterapinin yan etkilerini iyileştirebilir ve böylelikle doz artırımı ile kemoterapinin etkisi arttırılabilir.</p>

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Introduction

What is The Propolis?

In general terms propolis, which its content may be varied depending on vegetation and is obtained with modification as biochemical with saliva enzymes of honeybee of resin and waxy substances collected from leaves, shells, buds and shoots of plants by honeybees; it is used for many purposes (such as covering the inner surface of the hive, closing the crevices and cracks, hardening and repairing the edges of the combs, connecting the frames at the end of the summer, shrinking the hive entrance hole, and polishing the honeycomb eyes before laying the queen) by honeybees (Kumazawa et al., 2004; Kutluca et al., 2006; Silici and Güçlü, 2010; Doğan et al., 2014). Propolis, which has been widely used in medicine to treat and reduce the effects of various diseases since ancient times and praised by Hippocrates, Herodotus, Aristotle and other ancient scholar (Bogdanov, 2016).

The color of propolis, an adhesive and resinous substance, can vary from yellow-green to dark brown depending on the source from which it is obtained and the storage time (Silici, 2003; Silici and Güçlü, 2010). The color differences of propolis may vary depending on the concentration of phenolic compounds it contains. It has been reported that the propolis contains flavonoids, essential oils, cinnamyl alcohol, cinnamic acid and trace amounts of vanillin that give wax color (Şahinler, 2000). Propolis, which can darken during storage and lose its elasticity by the effect of sun rays, becomes brittle which can harden and break easily when the temperature of the environment is below 15°C. Propolis, which has a melting temperature of 80°C to 105°C, generally soluble in alcohol (ethanol, methanol); dissolves in water and hydrocarbons in a small amount, but completely soluble in ether or chloroform (Schmidt, 1997). In most of the studies, ethanolic extract of propolis has been used for a long time. Although extraction of propolis with alcohol and its derivatives is seen as a simple and effective method, it has a sharp resinous taste and limitations in its use in the cosmetic and pharmaceutical industries. However, the medical use of propolis alcohol extraction is not considered appropriate due to some disadvantages such as lead to harmful reactions and alcohol intolerance in some people (Konishi et al., 2004). In water extraction of propolis, it is reported that phenolic compounds are 10 times lower than ethanolic extraction due to the low water solubility of the active components of propolis (Mello et al., 2010; Moura et al., 2009). For this reason, researchers have started to obtain high activity propolis extract by using non-chemical natural solvents other than ethyl alcohol, propylene glycol and glycerol. Therefore, the dissolution of propolis with vegetable oils and the biological activities of oil-soluble phenolic compounds are of interest.

The composition of propolis, which is highly complex in chemical composition, varies in color, odor and pharmacological properties as it varies depending on the plant, region, season and colony. Propolis, which may have more than three hundred components; It contains 50% resin and balsam, 30% wax, 10% essential and aromatic oils, 5% pollen and 5% other organic matter (Castaldo and Capasso, 2002). The components of propolis soluble in solvents such as alcohol contain medically important components. Basic

chemicals determined in propolis; polyphenols (flavonoid aglycones, phenolic acids) and their esters, phenolic aldehydes, alcohols and ketones, sesquiterpene quinones, coumarins, steroids, amino acids and inorganic compounds. In the content of propolis, phenolic acids such as pinosembrine, acacetin, chrisin, routine, catesin, naringenin, galangin, luteolin, camferol, apigenin, mirsetin, quarcetin, phenolic acids such as caffeic acid and cinnamic acid, Mg, Ca, I, K, Na, Cu, Zn, Mn and Fe elements, vitamins such as B1, B2, B6, A, C, E, niacin, pantothenic acid (Özan, 2006; Cantarelli et al., 2011), enzymes such as succinic dehydrogenase, glucose-6-phosphatase, adenosine triphosphatase, acid phosphatase and a plurality of fatty acids (Table 1) (Bankova et al., 2000; Kutluca et al., 2006; Silici, 2003; Marcucci et al., 2001; Moreno et al., 2000). In addition, proteins, amides, amines and amino acids (0.7%; aspartic acid, glutamic acid, tryptophan, phenylalanine, leucine, cysteine, methionine, valine, serine, histidine, arginine, proline, tyrosine, threonine, alanine and lysine) are present in the propolis as the nitrogen source (Güney and Yılmaz, 2013). Besides these contents, it also contains prenylated p-coumaric acids, acetophenone derivatives, lignans, diterpenic acids, triterpenes, volatile components, monoterpenes, sesquiterpenes, aromatic components (xylose, galactose, mannose, glucuronic acid, lactose, maltose, melibiozyl, erythritol, etc.) (Bankova et al., 2000).

In recent years, many researchers have focused on the chemical composition, biological activity, pharmacological and therapeutic properties of propolis.

What are the Biological Properties of Propolis?

Flavonoids, aromatic acids, phenolic acids and esters are the main compounds responsible for the biological activities of propolis. Propolis, which was discovered by Greeks for the first time in ancient times and used as a natural antibiotic, is used in medicine, apiterapy and biocosmetic fields due to its antibacterial, antiviral, antifungal, antiparasitic, antioxidant, immunostimulatory and liver protective effects (Silici and Güçlü, 2010; Doğan et al., 2014).

As a source of the antimicrobial properties of propolis; flavonoids, pinosembrin, galangin and pinobanksin might be included. Pinosembrin has also antifungal properties (Marcucci, 1995). As a result of research, the content of the antibacterial properties of propolis from prenylated p-coumaric acids; 3,5-diprenyl-4-hydroxycinnamic acid, 3-prenyl-4-dihydro-cinamoyloxycinnamic acid, 2,2, -dimethyl-6-carboxyethenyl-2H-1-benzopyran, lignanes; 3-acetoxymethyl-5 -[(E)-2-formylethen-1-yl]-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-2,3-dihydrobenzofuran, Sesamine, Ascantin, Sesartenin and diterpenic acids; 15-oxo-3,13-Z-colavadiene-17-oic acid and its E-isomer, kummuric acid, imbrikatoloic acid, isocupressic acid (Bankova et al., 2000).

It has been found that compounds may exhibit cytotoxic activity; some of these compounds Flavonoids such as aromadendrine-4'methyl ether, 3,5,7-trihydroxy-6,4'-dimethoxyflavone, Prenylated p-coumaric acids such as 3,5-diprenyl-4-hydroxycinnamic acid (artepillin C) and 9-E-2-dimethyl -6-carboxyethylene -8-prenyl-2H-1-benzopyran

and lignanes such as 1-(4-hydroxy-3-methoxyphenyl)-1,2-bis-[4-[(E)-3-acetoxypenten-1-yl]-2-methoxyphenoxy]-propan-3-ol acetate] and Yangambin and Ent-17-hydroxy-3,13-Z-klerodadiene-15-oic acid, a diterpeneic acid (Bankova et al., 2000).

Propolis components that stimulate the immune system are 3-caffeoylquinic (chlorogenic) acid, 4-caffeoylquinic acid, 5-caffeoylquinic acid, 3,5-dicafeoylquinic acid and 4,5-dicafeoylquinic acid methyl ester from the caffeulquinic acids (Bankova et al., 2000).

Propolis extract, which may act in role against various DNA and RNA viruses (Burdock, 1998), might also be curative against viruses-induced infections on plants, animals and humans (Silici and Kutluca 2005; Wieckiewicz et al., 2013).

What is the Importance of Propolis in Cancer Studies?

There are many studies on the use of propolis or its active components in the treatment of cancer and demonstrating the potential of these biological components in the development of new anti-cancer agents. Several studies conducted *in vivo* and *in vitro*, propolis showed antitumor properties and was used during treatment; It has been concluded that by increasing the effect of chemotherapy, it enables the elimination of side effects of chemotherapy and allows high dose application (Bogdanov, 2016; Lee et al., 2005; Sforcin, 2007). Propolis has been used in many studies in cancer research. As a result of these studies, the most studied two propolis species have been identified several compounds that can show anticancer activity.

Active compounds of poplar propolis; CAPE (caffeic acid phenethyl ester), caffeic acid, apigenin, quercetin, genistein, rutin, p-coumaric acid, ferulic acid, campferol, naringenin (Table 1) (Abubakar et al., 2014; Bogdanov, 2016).

Active compounds of Baccharis (Brazil) propolis; Artepillin C, baccharin, drupanin, cinnamic acid derivatives, prenylated p-coumaric acids, klerodone terpenes, benzofurans (Table 1) (Abubakar et al., 2014; Bogdanov, 2016).

Although studies in humans and mice have shown that the use of propolis does not cause side effects, its use for cancer treatment may also be relatively inexpensive. Ethanolic extract of propolis (EEP) and its phenolic compounds have shown biological activities such as immunomodulator, chemopreventive and antitumor (Sforcin, 2007). However, due to the possible negative effects of ethylalcohol, research on natural solvents and their effects on cancer has been concentrated. In fact, the anticarcinogenic effect of CAPE obtained by oil extractions is known (Artajo, 2006). The dissolution of propolis in vegetable oils by suitable methods is

very important for health because of its usefulness in different industries and the additional benefits of the solvent used. Biological activities of propolis extracted in different vegetable oils, such as antimicrobial, antitumoral, anxiolytic and antidepressant, have been scientifically proven. For example, Ramanauskienė and Inkenienė (2011) showed that ferulic acid, coumaric acid and vanillin compounds were dominant in the propolis olive oil extract of Lithuania, and that the extract had antimicrobial effect, especially against *C. albicans* and *B. cereus*. The efficacy of the Brazilian propolis oil extract against *C. albicans* has also been demonstrated (Finger et al., 2013). In a study showing that Brazilian propolis fat extract has antitumoral properties *in vitro* and *in vivo*, it has been proved that fat extract is as effective as alcohol extract in preventing tumor growth. In the study where 4 phenolic acids and 3 flavonoids were identified in the oil extract of propolis, it was stated that the extract provided the inhibition of tumor cells in the best way (Carvalho et al., 2011). In a similar study, the antiproliferative effect of fat and ethanolic extracts against HL-60, MDA/MB-435 and SF-295 cells was demonstrated (Buriol et al., 2009). In another research; Propolis oil extract (OEP) has a stimulatory, anxiolytic and antidepressant effect on the central nervous system and therefore OEP can be used as a therapeutic agent for the treatment of anxiety and depression (Reis et al., 2014). In a clinical study of olive oil extract of propolis, it was shown that topical application of honey-propolis olive oil extract and beeswax was effective on oral mucositis (Abdulrhman et al., 2012). In a study conducted in Iran, it has been proved that propolis accelerates wound healing in STX-induced diabetic rats by adding olive oil extract, honey and royal jelly (Rashidi et al., 2016).

Cytotoxic effects of propolis extracts in many cell lines have been studied since 1979 with different *in vitro* methods (Ghisalberti, 1979). Hladon et al. (1980) studied the cytotoxic activities of propolis extracts on human KB (nasopharynx carcinoma) and HeLa (human cervical carcinoma) cell lines. The etheric propolis fraction (DEEP) exhibited strong cytotoxicity. Ethyl acetate and butanol fractions of DEEP showed good activity, while CHCl₃/DEEP showed moderate activity.

The killing activity of propolis on HeLa cells was tested by Ban et al. (1983). A concentration of 10 µg/mL of propolis inhibited 50% of the colony's ability to form. In addition to the lethal effect of propolis, flavonoids have also been tested. HeLa cells are more susceptible to quercetin and ramnetine, but less sensitive to galangine. Grunberger et al. (1988) described caffeic acid phenethyl ester as the compound which is partly responsible for the cytotoxic properties of propolis. The effect of CAPE on human cancer cell lines was determined in cultured breast carcinoma (MCF-7) and melanoma (SK-MEL-28 and SK-MEL-170) cell lines.

Table 1 Biologically active ingredients in poplar and Baccharis propolis (Abubakar et al., 2014).

Active ingredients Anticancer and antitumor	
Baccharis propolis artepillin C, baccharin, drupanin, cinnamic acid derivatives, prenylated p-coumaric acids, clerodane diterpenes, benzofurans, and caffeoylquinic acid derivatives.	Poplar propolis caffeic acid, caffeic acid phenylethyl ester, apigenin, quercetin, genistein, galangin, luteolin, fisetin, ferulic acid, chrysin, acacetin, p-hydroxy-benzoic acid, p-methoxy-benzoic acid, gallic acid, and vanillic acid.

A dose of 10 µg/ml of CAPE inhibited the addition of [³H] thymidine to DNA in breast cancer. More dramatic effects have been observed in melanoma, colon (HT 29) and renal carcinoma cell lines, but the effect of CAPE on normal melanocytes and fibroblasts was significantly lower. As the cytotoxic effect of CAPE is more effective in transformed cells, we can say that propolis is responsible for the carcinostatic properties. Antitumor activity of caffeic acid derivatives such as methyl ferulate, methyl acetyl ferulate, methyl acetyl isoferulate and methyl diacetyl caffeate has been reported (Inayama et al., 1984). The cytotoxic effect of propolis *in vitro* against ovarian cancer cell lines created in Chinese hamster experimental animals was stated to be due to the naphthalene residues contained in propolis (Ross, 1990). Brazilian propolis extracts obtained from an *Apis mellifera* species on human hepatocellular carcinoma, KB and HeLa cell lines have been reported to show cytotoxic effects caused by propolis quercetin, caffeic acid and phenyl ester components by *in vitro* tests (Matsuno, 1992).

Matsuno (1995) isolated klerodone diterpenoid from propolis and showed that the inhibition of human hepatocellular carcinoma cells (HuH 13) at a dose of 10 µg/mL and lethality at a dose of 20 µg/mL, resulting in a lethal effect on human lung carcinoma (HLC-2). It has also been showed that primary rabbit kidney cells had a survival rate of 75% at a dose of 100 µg/mL. The susceptibility of cells to CAPE-related cell death can be determined by reduced regulation of normal redox status in transformed cells (Chiao et al., 1995).

The aqueous extract of Japanese propolis (AEP) inhibited the growth of S-180 mouse sarcoma *in vitro*. In addition, administration of AEP to the mouse significantly inhibited the growth of transplanted tumor cells (Inoue et al., 2008).

Orsolic (2010) states that the chemopreventive activity of propolis in animal models and cell cultures is due to its ability to inhibit DNA synthesis in tumor cells, to induce tumor cells to apoptosis, and to activate macrophages that produce factors that regulate the functions of B-, T- and NK-cells.

Propolis and some isolated polyphenolic substances (caffeic acid, caffeic acid phenyl ester and quercetin) reduced the number of tumor nodules in the lung (Ozkul et al., 2005). Although most polyphenols have anti-metastatic effect, caffeic acid phenyl ester from poplar propolis and Artepilin C compound from Baccharis propolis have been identified as the most potent antitumor agents. Regular use of propolis as a food supplement provides a protective effect against cancer-causing mutations in humans (Bogdanov, 2016).

Turkish propolis has also been reported to exhibit anticarcinogenic properties (Piredda et al., 2015; Catchpole et al., 2015) and explained the inhibition of leucine, uridine and thymidine addition in carcinoma cells and thus inhibition of DNA synthesis by the antitumor activity of flavonoids.

Numerous studies have been demonstrated the effect of propolis on cell culture and animal testing (Awale et al., 2008; Orsolic, 2010; Catchpole et al., 2015). In other words, several studies indicating that propolis can be consumed with regular anticancer effect. Regular consumption of propolis as a food supplement can be

protective against cancer caused by mutations in humans (Riberio and Salvadori, 2003). Human papilloma virus (HPV), which is the most common cause of cervical cancer in women especially in developed countries, is a great threat for women. There is a study showing that HPV infections can be eliminated as a result of local treatment with propolis for six months (Iljazovic et al., 2006). In a similar study, 76% PAP smear development was prevented by using propolis (Imhof et al., 2005).

The water-miscible extract of CAPE-rich propolis has nearly been completely suppressed the growth of human NF1 cancer and human NF2 Schwannoma tumor, called MPNST (malignant peripheral nerve sheath tumor) (Demestre et al., 2009).

Controlled clinical studies have shown that propolis inhibits oral mucositis in breast cancer patients (Piredda et al., 2015).

The antitumor effects of phenolic components of propolis include pinosembrine, pinobanksin-3-O-acetate, tectocrizine, dimethylallyl caffeate, 3-methyl-3-butenyl caffeate, benzyl ferulate and benzyl isoferulate were investigated onto the gastro-intestinal cell lines; HCT-116 showed good activity in anti-proliferative analyzes against colon carcinoma, KYSE-30 esophageal squamous carcinoma and NCI-N87 gastric carcinoma (Catchpole et al., 2015). Chinese and Brazilian EEP showed anticancer activity in 4 human colon carcinoma cell lines (CaCo, HCT116, HT-29 and SW480) and increased dose-dependent inhibition in both extracts. Chinese propolis induces apoptosis and caused a dose-dependent increase in cellular mRNA levels of proteins associated with tumor suppression (p21CIP1 and p53) in HCT116 cell lines (Ishihara et al., 2009). The active compounds of the Netherlands propolis, benzyl, phenethyl and cinnamyl caffeates, have been determined to show antiproliferative activities on the colon 26-L5 carcinoma (Banskota et al., 2002). Chrysin has acting the TNFα signaling pathway and in this way markedly sensitizes human cancer cells to TNFα-induced apoptosis (Li et al., 2010). Galangin, another component of propolis, has been shown to induce apoptosis and suppress proliferation *in vitro* tests against the gastric cancer cell line SNU-484 (Kim et al., 2012). Pinosembrine has shown activity against human adenocarcinoma HCT 116 cell line. The mechanism of action is triggered by Bax-dependent mitochondrial apoptosis but non-Bax-expressing HCT 116 cells have been shown to be immune to pinosembrine (Kumar et al., 2007).

Ethanol extract of Brazilian red propolis has been reported to exhibit 100% cytotoxic activity against the human pancreatic cancer PANC-1 cell line (Awale et al., 2008). In this study, 43 compounds (3 of which were new) were isolated. All isolated compounds were tested against PANC-1 cells under nutrient-free conditions. A component of propolis [(6aR, 11aR) -3,8-dihydroxy-9-methoxypterocarpan] showed cytotoxicity (100%) over time in a non-apoptotic manner that did not lead to DNA fragmentation, but accompanied by necrotic type morphological changes (Awale et al., 2008).

In studies on the anticancer and anti-inflammatory activity of propolis, it is thought that polyphenols found in poplar propolis are responsible especially for the chrysin and caffeic acid phenethyl ester (CAPE) (Araújo et al., 2011; Chan et al., 2013; Jaganathan and Mandal, 2009; Sawicka et al., 2012).

The search for new drugs that can function as chemotherapeutic agents and have low side effects, especially derived from natural products, have been increased in recent years (Watanabe et al., 2011; Franchi et al., 2012). Propolis also induced the apoptosis in a B-lymphoma cancer cell line. This apoptotic effect of propolis leads to loss of mitochondrial membrane potential and this pathway is activated by caspase signaling (Figure 1) (Alday-Provencio et al., 2015; Sawicka et al., 2012). Campos et al. (2014) reported that the ethanolic extract of propolis from *M. orbignyi* (EEP) showed the cytotoxic activity against the human K562 erythro leukemia cell line and that despite the fact that the resistance to certain chemotherapeutic agents, the number of these cells decreased with the application of EEP.

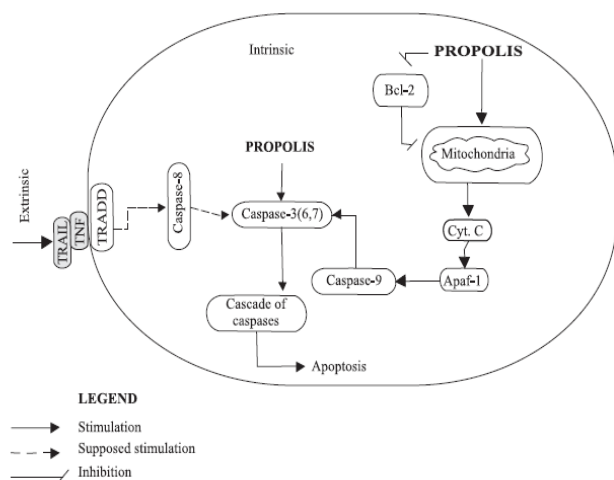


Figure 1. Effect of propolis on apoptosis pathways in cancer cells (Sawicka et al., 2012)

The antitumor effect of propolis is due to the inclusion of flavonoids such as terpenes and caffeic acid phenethyl ester, which is thought to be responsible for the synergy between these substances (Watanabe et al., 2011; Valente et al., 2011). The synergy between propolis and anticancer agents is quite interesting. Therefore, propolis flavonoids act a protective role against the toxicity of radiation and chemotherapeutic agents especially on mice. It would also suggest that it might have similar activity in humans. Combination with antioxidant therapy might be improve the side effects of chemotherapy on leukocytes, liver and kidney, and thereby increase the effect of chemotherapy on dose dependent.

According to the studies, the use of propolis as a preventive and supportive will shed light on the future synergistic effects of pharmaceutical drugs.

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