

Technical Report

Significance of Black Walnut Hull Extract in CanXida Remove (Formula RMV)

Black Walnut Hull Extract contains natural antimicrobial bioactive compounds along with anti-inflammatory and antioxidant properties which work synergistically with other ingredients in CanXida Remove (Formula RMV) for an effective pathogen clearance.

Contents

Executive Summary	1
1. Introduction	2
2. Bioactive Compounds of Black Walnut Hull Extract	3
2.1. Tannins	3
2.2. Naphthoquinones and Derivatives	3
2.3. α -Tetralones	3
2.4. Hydroxybenzoic Acids	3
2.5. Cinnamic Acids	3
2.6. Flavonoids	3
2.7. Other Bioactive Compounds	6
3. Health Benefits of Black Walnut Hull Extract	6
3.1. Antimicrobial Properties	6
3.2. Anti-Nematode Properties	7
3.3. Antioxidant and Anti-Inflammatory Effects	7
4. Biosafety profile	8
5. Effective Targets	9
6. Significance of Black Walnut Hull Extract in CanXida Remove	9
7. References	10

Executive Summary

Black walnut hull extract is a natural antimicrobial with antiparasitic properties ranging from unicellular bacterial and fungal pathogens to multicellular nematodes. It is prepared using a simple process involving water-based solvents producing a non-toxic and relatively pure substance rich in bioactive compounds. The extract contains naphthoquinones and their derivative compounds which in combination with tetralones provide full-spectrum antiparasitic properties. It is classified as a GRAS substance in the FDA CFR, and it is permissible for use in dietary supplements in quantities normally found in the food.

Black walnut extract in CanXida Remove (Formula RMV) works synergistically with other ingredients to ensure full-range clearance of pathogens, reduce inflammation, promote healing, and encourage restoration of normal probiotic flora. Its range of bioactive compounds includes flavonoids, hydroxybenzoic acids, phenols, cinnamic acids, terpenes, sterols, ceramides, minerals, vitamins, and antioxidants. Its inclusion in CanXida Remove formulation ensures effective clearance of unicellular pathogens and multicellular parasites effectively*.

** These statements have not been evaluated by Food and Drug Administration. This product is not intended to diagnose, treat, cure or prevent any disease.*

1. Introduction

Black walnut (scientific name: *Juglans nigra*) is an appreciated nut that is cultivated around the globe due to its edible kernel. It consists of four major parts, the kernel, skin, shell, and hull. Recent trends in research for naturally derived bioactive compounds for natural antioxidants and antimicrobials have provided plenty of evidence that black walnut hull is rich in natural bioactive compounds with wide-ranging health impacts. The walnut hull is a byproduct of the crop and an inexpensive raw material for the production of bioactive compounds such as phenols, flavonoids, quercetin, and tannins (Akbari et al 2012; Fernández-Agulló et al 2013).

Production of walnut hull extract is one of the cheapest and most environmentally friendly

methods. The majority of the bioactive compounds are soluble in an aqueous solution or mixture of water and ethanol. It not only provides the simplest method of extraction but also ensures that the extract is free from harmful chemicals. The extract is rich in a variety of antioxidant and antimicrobial compounds. Major antioxidants include flavonoids, hydroxy-tannins, and hydroxycinnamic acids. Prominent antimicrobial compounds include naphthoquinones and its derivatives. Recent research has shown that black walnut hull extract has proven antimicrobial efficacy against *Candida albicans*, *Staphylococcus aureus*, *Bacillus subtilis*, and *E. coli*.*

Black walnut hull extract is classified as a GRAS (generally recognized as safe) substance by WHO and FDA and can be used

Black Walnut Hull Extract

Extract Type:	Purified Natural Substances
Composition:	Flavonoids, Phenolic Acids, Naphthoquinones, Minerals.
Bioactivity:	Antioxidant & Antimicrobial
Source:	Agricultural by-products, Black Walnut Hull
FDA Status:	Approved
Last Reviewed:	June 2023
CFR category:	GRAS
CFR Ref Code:	21CFR172.510
Drug Bank	
Accession Number:	DB14306
Toxicity:	Non-toxic
Classification:	Food Additive, Food Supplement, Natural Antimicrobial

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as a food supplement and natural antimicrobial. The latest review of the extract by FDA CFR (Code of Federal Regulation) in June 2023 has retained its status as a food supplement and GRAS substance. It is available as an active ingredient in many over-the-counter food supplements. The presence of high-quality antioxidants in significant quantities in the extract makes it an attractive choice for nutraceuticals.

2. Bioactive Compounds of Black Walnut Hull Extract

Black walnut hull is rich in different types of bioactive compounds. Major groups of bioactive compounds are as follows:

2.1. Tannins

Black walnut hull extract contains water-soluble and digestible tannins including ellagic acid and tannic acid. These compounds are antioxidants.

2.2. Naphthoquinones and Derivatives

Naphthoquinones are a common group of antimicrobial phytochemicals in black walnut hull extract. Around 27 different types of naphthoquinones and derivatives have been identified and characterized for health benefits. Some of the common naphthoquinones are juglone, 2-methoxy juglone, engelharquinone, and 1,4-naphthoquinone (Maleita et al 2017).

2.3. α -Tetralones

Tetralones are used to synthesize many antibiotics and have been used for centuries in ancient medicines. Black walnut hull extract is rich in tetralones. 5 different types of α -tetralones have been identified and characterized in the black walnut hull extract, excluding its derivatives which are generally formed by the combination of quinones.

Examples of tetralones are isosclerone, (4S)-4-hydroxy- α -tetralone, juglanone A&B, and regiolone (Tsasi et al 2016).

2.4. Hydroxybenzoic Acids

Hydroxybenzoic acids have been recognized as natural antioxidants and antimicrobials. Gallic acid, photocatechuic acid, vanillic acid, and syringic acid are some of the most abundant hydroxybenzoic acids found in the black walnut hull extract. Other medicinally important hydroxybenzoic are phthalic acid, dimethyl phenol, and dibutyl phthalate. Hydroxybenzoic acids are well-established antimicrobials and have been used in food products as natural antimicrobials.

2.5. Cinnamic Acids

Hydroxycinnamic acids such as caffeic acid are well known for their anti-inflammatory effect and for modulating the immune system. Black walnut hull extract contains more than 16 hydroxycinnamic acids including caffeic acid, ferulic acid, coumaric acid, sinapic acid, rosmarinic acid, and chlorogenic acid. Cinnamic acids are phenols or phenolic derivatives.

2.6. Flavonoids

Flavonoids are one of the most studied bioactive compounds in medicinal chemistry. Flavonoids are generally known for their antioxidant behavior; however, they are potent anti-inflammatory compounds. Although flavonoids don't have direct antimicrobial activity, they play crucial roles in combating drug-resistant microbes. Black walnut extract is rich in bioflavonoids including rutin, quercetin, myricetin, catechin, epicatechin, cirsilinoleol, and sudachitin. Some of the major bioactive compounds are listed in Table 1.

Table 1: List of different bioactive compounds found in black walnut hull extract. Source: Jahanban-Esfahlan et al (2019).

Phytochemical Group	Bioactive Compounds
Hydrolysable tannins	Ellagic acid
	Tannic acid
Naphthoquinones	Juglone
	2-Methoxy juglone
	3-Methoxyjuglone
	2-Ethoxy juglone
	3-Ethoxy juglone
	1-Naphthol
	8-Hydroxyquinoline
	1,4-Naphthoquinone
	5,8-Dihydroxy-1,4-naphthoquinone
	2-Hydroxy-1,4-naphthoquinone
	2,5-Dihydroxy-1,4-naphthoquinone
	3,5-Dihydroxy-1,4-naphthoquinone
	5-Methoxy-1,4-naphthoquinone
	1,2-Naphatalenediol
Engelharquinone	
α -Tetralones	Regiolone
	(S)-Regiolone
	5,8-Dihydroxy-4-methoxy- α -tetralone
	4,5-Dihydroxy- α -tetralone
	(4S)-(+)-Isosclerone
	Sclerone
	(4S)-4-Hydroxy- α -tetralone
	5-Hydroxy-4-methoxy- α -tetralone
(4S)-5-Hydroxy-4-methoxy- α -tetralone	

Table 1 continued: List of different bioactive compounds found in black walnut hull extract. Source: Jahanban-Esfahlan et al (2019).

Phytochemical Group	Bioactive Compounds
Hydroxybenzoic Acids	Benzoic acid
	2,6-Dimethyl phenol
	Gallic acid
	Vanillic acid
	Syringol
	Syringic acid
	Salicylic acid
	Protocatechuic acid
	p-Hydroxybenzoic acid
	Dibutyl phthalate
	Phthalic acid
	2,3-Dihydroxybenzoic acid
	Tyrosol
	3-Hydroxy-1-(4-hydroxy-phenyl)-1-propanone
Flavonoids	(+)-Catechin
	(-)-Epicatechin
	Myricetin
	Quercetin
	Sudachitin
	Cirsilineol
	5,6,4'-Trihydroxy-7,3'-dimethoxy-flavone
	Eriodictyol
	Apigenin
	Apigenin 7-O- β -D-glucuronide
	Rutin
	Kaempferol

2.7. Other Bioactive Compounds

Black walnut hull extract also contains significant quantities of vitamins such as tocopherol and ascorbic acid. Other prominent compounds are sterols such as stigmasterol, campesterol, and oleanolic acid as well as ceramides and terpenes. Bioactive prominent terpenes include blumenol A & B, ursolic acid, epikatonic acid, and derivatives of oleanolic acid.

3. Health Benefits of Black Walnut Hull Extract

Black walnut husk extract contains a wide range of bioactive phytochemicals that provide health impacts as antioxidants, antimicrobials, antifungal, and anti-inflammatory compounds. It has the ability to destabilize biofilms of fungal and bacterial pathogens which contributes to its

antimicrobial effect. Some of the bioactive phenolic compounds are specifically bactericidal and fungicidal, while others provide a helping hand to the immune system by exposing the bacterial and fungal colonies*.

3.1. Antimicrobial Properties

Black walnut husk extract contains phenolic compounds that are responsible for its antimicrobial properties. The latest study by Arslan and colleagues (Arslan et al 2023) was published in the journal Springer Nature that demonstrated phenolic components from black walnut hull extract were effective against bacterial and fungal pathogens (Table 2). A comparative pre-clinical study between commercial antifungal agents and 4% black walnut hull extract demonstrated comparable antifungal effects against *Candida albicans* (Abedi et al 2017).

Table 2: Effect of phenolic compounds and minimum concentration to inhibit the pathogenic bacteria and fungi. Source: Arslan et al (2023)

Microorganisms	Minimum Inhibitory Concentration (mg/mL)	
	Extract	Concentrate
<i>Escherichia coli</i>	16	8
<i>Pseudomonas aeruginosa</i>	64	16
<i>Legionella pneumophila</i>	128	32
<i>Enterococcus hirae</i>	128	32
<i>Enterococcus fecalis</i>	128	64
<i>Staphylococcus aureus</i>	128	64
<i>Candida parapsilosis</i>	256	64
<i>Candida tropicalis</i>	256	128

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The extract is effective against a wide variety of Gram-positive and Gram-negative bacteria including *Bacilli*, *Staphylococcus*, *E. coli*, and *Klebsiella*. It is also effective against intestinal pathogens *Enterococcus* bacteria which are not only responsible for chronic intestinal infections but also oral and vaginal infections. The black walnut hull extract was effective in concentrations as low as 0.1 mg/mL (Oliveira et al 2008). Similar results were reported in other research studies as well, further confirming the antimicrobial effects of black walnut hull extract (Arslan et al 2023). The antimicrobial properties are mainly credited to naphthoquinones. However, for biofilm-forming drug-resistant microbes like *Candida albicans*, the flavonoids, and phenolic substances (cinnamic acids and some hydroxybenzoic acid) also play a crucial role in disintegrating the biofilms and exposing pathogens to the immune system.

3.2. Anti-Nematode Properties

Nematodes, also known as roundworms, are multicellular intestinal pathogens. Generally, these pathogens are too small in size (50 microns thickness and 2 mm length, on average), to be seen by the naked eye unless noticed by symptoms related to the infestation like intense itching, fatigue, and anemia. A report published in the American Chemical Society journal claimed that naphthoquinones, especially juglone, 1,4-naphthoquinone, and plumbagin show anti-nematode properties (Maleita et al 2017).

Nematode infestation is often associated with the absorption of digested food in the small intestine. As a result, the affected person develops chronic malnutrition although eating a healthy and well-balanced diet. The black walnut hull extract in the CanXida

Remove (Formula RMV) provides an added anti-parasitic advantage by discouraging the growth of nematodes.

3.3. Antioxidant and Anti-Inflammatory Effects

Salejda and colleagues from Wroclaw University of Environmental and Life Sciences, Poland, demonstrated that black walnut hull extract possesses strong antioxidant properties (Salejda et al 2016). Flavonoids are the chief contributors to antioxidant activity. Quercetin and Rutin are well-established anti-inflammatory bioflavonoids that are found abundantly in the black walnut hull extract.

Gastrointestinal cells are under constant chemical and microbial insults coming with food and ingested materials. Sometimes, it leads to an aggressive immune response to food substances, producing inflammatory substances in the digestive system. This results in an overall reduction of digestive efficiency, poor immune response to pathogens, and an encouraging environment for pathogenic microbes. Antioxidants and anti-inflammatory bioactive compounds found in the black walnut hull extract modulate the immune system and provide a more targeted and efficient approach. Major antioxidants and anti-inflammatory compounds in the black walnut extract are caffeic acid, gallic acid, vanillic acid, and cinnamic acid derivatives. Tannic acid and ellagic acid are hydrolyzable bioactive compounds with antioxidant, anti-inflammatory, as well as antimicrobial properties. Some naphthoquinones also provide anti-inflammatory and antimicrobial properties simultaneously.

4. Biosafety Profile

Black walnut hull extract is a non-toxic substance classified as GRAS by the FDA and is approved by WHO to be used as a food additive, dietary supplement, and natural antimicrobial in dietary products. Its biosafety has been reviewed recently (June 2023) and the FDA finds it safe to be used in quantities generally found in the food. It is generally safe if taken up to 20 – 30 mg/day.

Being a completely natural product, its different components are also found in other foods of routine use. For example, tannins are also found in berries, apples, and bananas while cinnamic acids are also found in citrus fruits, grapes, tea, cocoa, spinach, and celery. It has been used as an over-the-counter dietary supplement and a natural antimicrobial suitable for oral use. NIH dietary supplement database contains over 30,000 registered labels of dietary supplements and nutraceuticals containing black walnut hull extract which are currently in the market.

The bioactive compounds in the black walnut hull extract are often soluble in water. Solubility in water makes it easy for the body to metabolize and excrete it through the urinary system. Most bioactive compounds such as tannins and ellagic acid are hydrolyzed in the digestive system or metabolized within the liver into simpler compounds which are later excreted through urine. Water compatibility of different bioactive compounds also contributes to short retention time within the body when absorbed, further minimizing any side effects.

Table 3 shows different bioactive compounds found in the black walnut hull extract and their retention time.

Table 3: bioactive compounds in black walnut hull extract with retention time in the body.

Compound Name	Retention Time (minutes)
Chlorogenic acid	28.90
Caffeic acid	30.95
p-Coumaric acid	45.95
Ferulic acid	49.53
Trans-ferulic acid	68.63
Sinapic acid	51.63
Cilicone b	24.3
Catechin	18.42
Epicatechin	40.39
Myricetin	70.68
Quercetin	17.7
Sudachitin	29.1
Cirsilineol	35
Eriodictyol	94.2
Apigenin	20.4
Rutin	34.55

As the extraction process does not involve any toxic solvents, the purified extract is free of any toxic impurities. Furthermore, black walnut hull extract has been used for centuries in traditional remedies for skin infections, digestive problems, and wound healing.

5. Effective Targets

Black walnut hull extract is effective against Gram-positive and Gram-negative bacteria, commensal pathogens of the intestine, oral and vaginal pathogens (Enterococcus, Candida), and biofilm-producing pathogens. It also targets the inflammatory sites of the intestine. An added benefit of black walnut hull extract is its anti-nematode activity which helps in combating the roundworms in the gastrointestinal tract.

Enterococcus Bacteria: *E. hirae* and *E. faecalis* are the common pathogenic species belonging to the enterococcus group. These bacteria are often resident pathogens of the intestine but can also cause infection in the oral cavity and vagina. These bacteria are often associated with other infections such as those of candida or other bacteria.

Nematodes: Nematodes are intestinal pathogens usually infesting the small intestine where digested food is absorbed. Black walnut hull extract has shown promising effects against nematodes and roundworms.

Klebsiella and Foodborne Pathogens: Black walnut hull extract is generally employed to limit the growth of foodborne pathogens such as *Klebsiella* and *Shigella*. It is also effective against hemolytic *E.coli*. These bacterial pathogens produce enterotoxins which alter the chemistry of the intestinal wall, producing ulceration, inflammation, and chronic bleeding.

Fungal Pathogens: Black walnut hull extract has been widely studied for its antifungal activity against Candida species including *C. albicans*, *C. parapsilosis*, and *C. tropicalis*. Candida species are the most common pathogens of the gastrointestinal and urinary

tract. Other fungal pathogens susceptible to the antifungal effects of black walnut hull extract include *Cryptococcus neoformans*, a yeast that often co-infects with candida.

6. Significance of Black Walnut Hull Extract in CanXida Remove

CanXida Remove (Formula RMV) is a promising dietary supplement with broad-acting antimicrobial effects. The distinguished antimicrobial properties of Black walnut hull extract along with its anti-inflammatory and anti-nematode effect provide a full range of parasitic clearance. It works synergistically with the other ingredients present in the CanXida Remove formulation for a more effective antimicrobial effect in the whole digestive system. Its bioactive compounds modulate the immune system and recruit immune cells to eliminate pathogens including drug-resistant strains.

Anti-inflammatory components of black walnut hull extract are responsible for the complete regeneration of gut walls while antioxidants speed up the healing process. Controlled release of black walnut hull extract in CanXida Remove (Formula RMV) provides a sustained and consistent antimicrobial bioactivity, ensuring an effective clearance of pathogens.

In conclusion, black walnut hull extract in the CanXida Remove (Formula RMV) holds the promise of full-range antimicrobial and anti-inflammatory properties owing to its rich profile of bioactive compounds. Its added antiparasitic benefit against nematodes makes it a unique antimicrobial extract that can not only counter unicellular pathogens such as bacteria and yeast but also multicellular pathogens such as roundworms.

7. Reference

- Abedi, P., Yaralizadeh, M., Fatahinia, M., Namjoyan, F., Nezamivand-Chegini, S., & Yaralizadeh, M. (2018). Comparison of the Effects of *Juglans nigra* Green Husk and Clotrimazole on *Candida albicans* in Rats. *Jundishapur Journal of Microbiology*, 11(2).
- Akbari, V., Jamei, R., Heidari, R., & Esfahlan, A. J. (2012). Antiradical activity of different parts of Walnut (*Juglans regia* L.) fruit as a function of genotype. *Food chemistry*, 135(4), 2404-2410.
- Arslan, H., Ondul Koc, E., Ozay, Y., Canli, O., Ozdemir, S., Tollu, G., & Dizge, N. (2023). Antimicrobial and antioxidant activity of phenolic extracts from walnut (*Juglans regia* L.) green husk by using pressure-driven membrane process. *Journal of Food Science and Technology*, 60(1), 73-83.
- Fernández-Agulló, A., Pereira, E., Freire, M. S., Valentão, P., Andrade, P. B., González-Álvarez, J., & Pereira, J. A. (2013). Influence of solvent on the antioxidant and antimicrobial properties of walnut (*Juglans regia* L.) green husk extracts. *Industrial crops and products*, 42, 126-132.
- Gupta, A., Naraniwal, M., & Kothari, V. (2012). Modern extraction methods for preparation of bioactive plant extracts. *International journal of applied and natural sciences*, 1(1), 8-26.
- Jahanban-Esfahlan, A., Ostadrahimi, A., Tabibiazar, M., & Amarowicz, R. (2019). A comprehensive review on the chemical constituents and functional uses of walnut (*Juglans* spp.) husk. *International journal of molecular sciences*, 20(16), 3920.
- Maleita, C., Esteves, I., Chim, R., Fonseca, L., Braga, M. E., Abrantes, I., & de Sousa, H. C. (2017). Naphthoquinones from walnut husk residues show strong nematocidal activities against the root-knot nematode *Meloidogyne hispanica*. *ACS Sustainable Chemistry & Engineering*, 5(4), 3390-3398.
- Maleita, C., Esteves, I., Chim, R., Fonseca, L., Braga, M. E., Abrantes, I., & de Sousa, H. C. (2017). Naphthoquinones from walnut husk residues show strong nematocidal activities against the root-knot nematode *Meloidogyne hispanica*. *ACS Sustainable Chemistry & Engineering*, 5(4), 3390-3398.
- Oliveira, I., Sousa, A., Ferreira, I. C., Bento, A., Estevinho, L., & Pereira, J. A. (2008). Total phenols, antioxidant potential and antimicrobial activity of walnut (*Juglans regia* L.) green husks. *Food and chemical toxicology*, 46(7), 2326-2331.
- Salejda, A. M., Janiewicz, U., Korzeniowska, M., Kolniak-Ostek, J., & Krasnowska, G. (2016). Effect of walnut green husk addition on some quality properties of cooked sausages. *LWT-Food Science and Technology*, 65, 751-757.
- Tsasi, G., Milošević-Ifantis, T., & Skaltsa, H. (2016). Phytochemical study of *Juglans regia* L. pericarps from Greece with a chemotaxonomic approach. *Chemistry & Biodiversity*, 13(12), 1636-1640.