Technical Report

Significance of Pau D'arco in CanXida Remove (Formula RMV)

Bioactive compounds from pau d'arco provide a full spectrum antimicrobial activity against bacteria, fungi, and protozoans as well as encourage the growth and restoration of the normal probiotic microbiome of the digestive system.

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Executive Summary

The inner bark and heartwood of pau d'arco are rich in bioactive compounds including potent antimicrobials such as lapachol, anthraquinones, naphthoquinones, and 1,4-naphthoquinones. Bioactive compounds from pau d'arco have a full-spectrum antimicrobial activity against a variety of pathogens such as *Staphylococcus aureus*, *Clostridium* species, and *Candida albicans** to mention a few. The bactericidal activity of bioactive compounds of pau d'arco does not affect the beneficial probiotic species of *Lactobacillus and Bifidobacterium*.

Pau d'arco in CanXida Remove (Formula RMV) works synergistically with other ingredients to effectively eliminate pathogens and restore the normal microbiome of the digestive tract. It is classified as GRAS (generally recognized as safe) and FDA permits its oral use as a dietary supplement. Its efficacy against drug-resistant *Helicobacter pylori* and *S. aureus* makes it an essential ingredient of CanXida Remove (Formula RMV) to combat bacterial overgrowth in the digestive tract and restore the normal probiotic microbiota*.

** 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

Pau D'arco (botanically known as *Tabebuia impetiginosa*) has been a hot topic in ethnobotany (the study of plants used by native and aboriginal people). It is also known as *Ipe roxo, lapacho morado, purple lapacho, red lapacho*, and *taheebo* among users across the Amazonia and tropical Americas (Jones 1995). Its inner bark and heartwood contain bioactive compounds with effective and potent antimicrobial activity against diverse species of bacteria, fungi, and protozoans*.

Pau D'arco has been an essential component of many alternative therapies for combating gastrointestinal infections caused by *Candida*, stomach ulcers caused by *H. pylori*, and dysentery caused by *Entamoeba*. Its antimicrobial activity is owing to its potent bioactive compounds such as quinones, naphthoquinones, furano-naphthoquinones, phenolic glycosides, benzoic acid, cyclopentene dialdehydes, ß-lapachone, and iridoids (Castellanos et al 2009). FDA has registered pau d'arco as a dietary supplement since 1999 (FDA, 1999). It is non-toxic when taken orally in recommended doses generally present in food products. Several research studies have reported that pau d'arco is classified as a GRAS (generally recognized as safe) product by the FDA (Castellanos, et al 2009; Mowrey 2001). In Brazil, the native place of this plant, it is used to treat a wide range of ailments of digestive system, mouth, skin, and other systemic infectious, without any reported side effects.

Pau d'arco is particularly effective against gastrointestinal pathogens, especially those drug-resistant ones. Intestinal bacteria and fungi often develop drug resistance by

Pau D'arco

Product Type:	Herbal, Natural Bioactives Substances
Source:	Cambium of Pau D'arco (inner bark) & Heartwood
Composition:	Quinones, Phenolic glycosides, Iridoids, Cyclopenetens, Naphthoquinones, and Minerals.
Bioactivity:	Antifungal, Antibacterial, & Anti-protozoans
FDA Status:	Approved (Dietary Supplement)
CFR category:	GRAS
Toxicity:	Non-toxic
Classification:	Food Additive, Food Supplement, Natural Antimicrobial

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exchanging genetic information with pathogens coming with food or developing resistance against antibiotics after repeated exposure. Pau d'arco has the potential to counteract drug-resistant pathogens due to its multiple antimicrobial bioactive compounds and also by stimulating the immune system. Bioactive compounds from pau d'arco possess both growth-inhibitory and antimicrobial activities for bacterial and fungal pathogens. Lapachol, quinones, and beta-lapachone are extensively studied bioactive compounds of pau d'arco.

2. Bioactive Compounds in Pau D'arco

Dr. Rainer Bussmann from the Department of Ethnobotany, Ilia State University, Georgia published a report depicting bioactive compounds across different pau d'arco species. The major bioactive compounds in pau d'arco are aromatic aldehydes (21.4%), aromatic compounds (7.3%), simple carbohydrates (11%), terpenoids (8.3%), quinones 6%), phenolic compounds (6.85%), flavonoids (6.1%), and alkanes (6.35%) (Ramalakshm & Muthuchelian 2011). Some of the medically significant bioactive compounds include alpha lapachol, anisic acid, cinnamaldehyde, vanillic acid, anthraquinones, quercetin, menaquinones, naphthoquinones, and vanillin to mention the least. A comprehensive list of well-studied bioactive compounds is provided in Table 1 (Bussmann, 2018).

2.1. Anthraquinones and naphthoquinones

Anthraquinones and naphthoquinones are aromatic phytochemicals with potent antimicrobial activity. Anthraquinones and their derivatives, quinone glycosides, are digested in the colon where they modulate the peristaltic activity of the intestine and improve bowel movements. Naphthoquinones, on the other hand, are active antimicrobials. Naphthoquinones, especially 1,4-naphthoquinones and derivative compounds are effective against Gram-positive and Gram-negative bacteria. These compounds have multiple targets for the biosynthetic machinery of bacteria including protein synthesis, genetic material, and cell wall synthesis (Ravichandiran et al 2005). These results have also been validated by recent research on naphthoquinones by Liu et al (2023) and Song et al (2020)

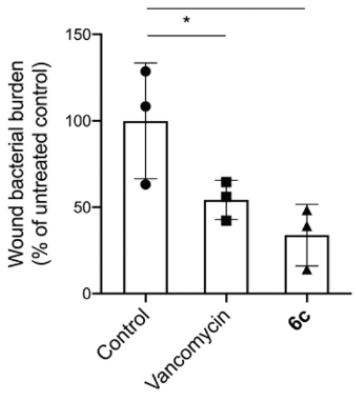

***Figure 1**: The bactericidal activity of naphthoquinone derivative 6c on drug-resistant bacteria S. aureus. Graph borrowed from Song et al (2020).*

2.2. Lapachol

Lapachol ($C_{15}H_{14}O_3$) is the most abundant compound found in the pau d'arco. It has been known since 1858 (Thomson, 1971). It belongs to the naphthoquinone family of bioactive compounds. It has growth-inhibitory activity of pathogens by interacting with energy production. It has been found effective against a variety of protozoans, especially *Leishmania* and *Toxoplasma* (Martínez, & Benito, 2005).

***Table 1**: Bioactive compounds found in pau d'arco. Source:* Bussmann, (2018) & Castellanos, et al (2009).

Bioactive Compound	Localization
Lapachol	Heartwood, inner bark
Dehydro--lapachone	Heartwood, inner bark
α-Lapachone	Heartwood
β-Lapachone	Heartwood
Lapachol methyl ether	Heartwood
Menaquinone-1	Heartwood
Desoxy lapachol	Heartwood
1-Hydroxyanthraquinone	Heartwood
1-Methoxyanthraquinone	Heartwood
2-Methylanthraquinone	Heartwood
2-Hydroxymethylanthraquinone	Heartwood
2-Acetoxymethylanthraquinone	Heartwood
Anthraquinone-2-carboxylic acid	Heartwood
Lapachenol	Heartwood
2-Acetyl-furanonaphtaquinone	Inner bark
2-Hydroxyethyl-furanonaphtoquinone	Inner bark
8-Hydroxy-2-acetyl-furanonaphtoquinone	Inner bark
8-Hydroxy-2-hydroxyethyl-furanonaphtoquinone	Inner bark
2-Ethyl-furanonaphtoquinone	Inner bark
2-Isopropyl-furanonaphtoquinone	Inner bark
2,3-Dihydro-2-(2-methyl phenyl)-furan naphthoquinone	Inner bark

Bioactive Compound	Localization
Anisaldehyde	Inner bark
Anisic acid	Inner bark
Benzo[B]furan-6-Carboxaldehyde	Inner bark
Dehydro-alpha-isolapachone	Inner bark
Kigelinone	Inner bark
Vanillic acid	Inner bark
Vanillin	Inner bark
Veratric acid	Inner bark
Veratric aldehyde	Inner bark
Xyloidone	Inner bark
Dehydro-alpha-lapachone	Heartwood, inner bark
4-Hydroxy-benzoic-acid	Heartwood, inner bark
Anthraquinone-2-Aldehyde	Heartwood
Anthraquinone-2-Carboxylic-acid	Heartwood
2,3-Dimethyl-1,4-Naphthoquinone	Heartwood
O-hydroxybenzoic-acid	Heartwood
Phthiolol	Heartwood
Quercetin	Heartwood
Tabebuin	Heartwood
Tectoquinone	Heartwood
P-hydroxy-benzoic-acid	Heartwood
Cyanidin-3-O-beta-d-rutinoside	Flower
Peonidin-3-Cinnamyl-sophoroside	Flower

2.3. Anisic Acid

Anisic acid and its derivative anisaldehyde are found in significant quantities. Anisic acid is a powerful antifungal agent with fungicidal activity.

2.4. Menaquinone-1

Menaquinone-1, also known as vitamin K2, is a powerful antioxidant and a co-factor of enzymes of many probiotic bacteria. It is an essential component of energy biosynthesis of anaerobic (living without oxygen) bacteria found in the intestinal tract.

2.5. Kigelinone

Kigelinone possesses antibacterial and antifungal properties. Its bacterial targets include *Pseudomonas, Bacillus subtilis,* and *S. aureus*.

2.6. Other Bioactive Compounds

Pau d'arco is rich in a variety of bioactive compounds including powerful antioxidants such as quercetin, α-lapachone, β-lapachone, and xyloidone. Some compounds such as vanillin and vanillic acid support other bioactive compounds during antimicrobial activity.

3. Health Benefits of Pau D'arco

Pau D'arco has been used by native Americans in Brazil, Argentina, Paraguay, Bolivia, and Peru for thousands of years for the treatment of skin and gastrointestinal microbial diseases. Bioactive compounds of pau d'arco containing quinone ring are potent antimicrobials because of the reactive nature of the ring which interferes with the biosynthetic machinery of bacteria or induces cellular stress by producing reactive oxygen species (ROS) inside the pathogen cells. A plethora of antimicrobial compounds in the pau d'arco can also counter drug-resistant and multi-drug-resistant bacterial and fungal strains found in the gut*.

3.1. Antibacterial Properties and Gut Microbiome

The human digestive system is a complex ecosystem with resident microorganisms including bacteria. These bacteria, such as *Bifidobacterium and Lactobacillus*, contribute to maintaining a healthy digestive system as well as regulating immune function. On the other hand, some pathogenic bacteria such as *Clostridium* and *Escherichia coli.*

Park and colleagues from Seol National University, South Korea, published research findings that bioactive compounds of pau d'arco have a growth inhibitory effect on pathogenic bacteria while promoting the growth of beneficial microbiota in the intestine (Park et al 2005). Pau d'arco bioactive compounds did not affect or weekly inhibit the growth of probiotic *Bifidobacterium adolescentis, Bifidobacterium bifidum, Bifidobacterium infantis, Lactobacillus acidophilus*, and *Lactobacillus casei* while strongly inhibiting the growth of *Clostridium paraputrificum, Clostridium perfringens,* and *E. coli* at the same time. These selective growth inhibitory properties of pau d'arco are uniquely significant in restoring the microbiota of a depleted gut microbiome*.

A detailed overview of the effect of bioactive compounds from pau d'arco on the probiotic and pathogenic bacterial strains is provided in Tables 2 and 3, respectively.

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***Table 2**: Effect of selective bioactive compounds from pau d'arco on the probiotic strains. For comparison commercial antibiotics tetracycline and chloramphenicol are also provided as a positive control. Sing " – " shows no inhibitory effect while sing " + " shows a significant inhibitory effect. Data source: Park et al (2005).*

Compound	Dose (µg)	*B. Adolescentis*	*B. Bifidum*	*B. Infantis*	*L. Acidophilus*	*L. Casei*
Anthraquinone-2-carboxylic acid	1000	–	–	–	–	–
	200	–	–	–	–	–
	100	–	–	–	–	–
	10	–	–	–	–	–
	1	–	–	–	–	–
	0.1	–	–	–	–	–
Lapachol(2)	1000	–	–	–	–	–
	200	–	–	–	–	–
	100	–	–	–	–	–
	10	–	–	–	–	–
	1	–	–	–	–	–
	0.1	–	–	–	–	–
Tetracycline	1000	++++	–	–	++++	++++
	200	+++	–	–	++++	++++
	100	++	–	–	++++	+++
	10	+	–	–	++	++
	1	+	–	–	+	+
	0.1	–	–	–	–	–
Chloramphenicol	1000	++++	–	–	++++	++++
	200	+++	–	–	++++	++++
	100	+	–	–	++++	++++
	10	–	–	–	+++	+++
	1	–	–	–	+	+
	0.1	–	–	–	–	–

***Table 2**: Effect of selective bioactive compounds from pau d'arco on the pathogenic strains with comparable dose for probiotic bacteria. Sing " – " shows no inhibitory effect while sing " + " shows a significant inhibitory effect. Data source: Park et al (2005).*

Compound	Dose (µg)	*Clostridium paraputrificum*	*Clostridium perfringens*	*E. coli*
anthraquinone-2-carboxylic acid	1000	++++	++++	+++
	200	++++	+++	++
	100	++++	+	+
	10	++++	–	+
	1	++++	–	–
	0.1	+	–	–
Lapachol	1000	++++	++	+
	200	+++	+	+
	100	++	+	+
	10	+	+	–
	1	–	–	–
	0.1	–	–	–
Tetracycline	1000	++++	++++	++++
	200	++++	++++	++++
	100	++++	++++	++++
	10	+++	+++	+++
	1	+++	+++	+
	0.1	+++	+++	–
Chloramphenicol	1000	++++	++++	++++
	200	++++	++++	++++
	100	+++	+++	+++
	10	+++	+++	+++
	1	+	+	–
	0.1	–	–	–

3.2. Antifungal Properties

Lapachol and its derivatives such as α-lapachone and β-lapachone have broad-acting bioactivity against fungal pathogens. These compounds have demonstrated significant growth inhibitory activity against *Candida albicans*, and *Candida tropicalis* (Guiraud et al 1994; Hussain et al 2007).

Lapachol inhibits the growth of candida by interfering with its energy production processes (electron transport chain), its synthesis of cell walls, and its ability to proliferate. Its efficacy in inhibiting the growth of candida is as effective as amphotericin B, a commercial antifungal agent.

β-lapachone also possesses antifungal activity chiefly due to its ability to produce oxidative stress. Once inside the fungal cells, it creates free radicals and reactive oxygen species which then react with essential biomolecules such as proteins, lipids, and enzymes rendering them inactive. This restrains fungal growth and impedes its metabolic activity, producing a population of weakened pathogens which are then eliminated by the immune system.

3.3. Complementary Health Benefits

Bioactive compounds from pau d'arco provide physiological assistance to the immune system in combating pathogens. The antioxidant activity of pau d'arco also plays a pivotal role in reducing inflammation and the healing process. Some of the complementary health benefits are as follows:

i. Quercetin, a powerful antioxidant, is present in the pau d'arco and has an anti-inflammatory effect on the digestive system.

ii. Vanillic acid and vanillin are known as potentiators of the antimicrobial activity of bioactive compounds. These are not antimicrobials itself but can increase the activity of other antimicrobials for stronger effects.

iii. Some quinone compounds such as α-lapachone and lapachone have anti-protozoal properties against vaginal and intestinal parasites including *Entamoeba*, and *Trichomonas vaginalis*.

iv. Some of its bioactive compounds are digested and broken down into active compounds in the last part of the intestine, i.e., the colon, and modulate probiotic microorganisms. This provides pau d'arco with a full spectrum of bioactivity and antimicrobial activity across the digestive tract from the mouth to colon.

4. Biosafety Profile

Pau d'arco has been used for thousands of years by Amazonian native Americans and is one of the extensively studied plants among ethnobotanists. Its crude extracts, bark paste, tea, powder, and purified compounds have been researched for unique antimicrobial properties. FDA recognizes pau d'arco as a dietary product and it has been classified as GRAS (generally recognized as safe).

Most of its bioactive compounds like lapachol, lapachone, and some naphthoquinones stay for a prolonged period of time in the intestine for a potent and effective microbicidal effects. This also fits into the CanXida Remove (Formula RMA) bioactivity profile which is designed to

eliminate pathogens across the digestive system. There have been several purified pau d'arco products in the market for more than 10 years without any reported side effects.

5. Effective Targets

Pau d'arco antimicrobial activity is responsible for the growth inhibition of pathogenic bacteria while it also positively affects probiotic bacteria.

5.1. Pathogenic Microbes

Pau d'arco is effective against the following pathogenic bacteria and fungi:

Helicobacter pylori: *H. pylori* is a drug-resistant bacteria that commonly infects acidic parts of the digestive system, i.e., stomach and duodenum. It is often drug-resistant and is treated with combination therapy. This bacterium is responsible for stomach problems including ulcers and damage to the mucus wall. Pau d'arco is effective against this pathogenic bacterium.

Staphylococcus aureus: *S. aureus* is responsible for bacterial overgrowth in the intestine as well as symptoms associated with irritable bowel syndrome. It often develops drug resistance during overgrowth phases. Pau d'arco bioactive compounds are effective against this bacterium.

Clostridium: Infectious strains of clostridium such as *Clostridium paraputrificum* and *Clostridium perfringens* are responsible for damaging the intestinal mucus wall because of bacterial toxins released by these strains during infection. These are also susceptible to pau d'arco antimicrobial activity.

Candida: Pau d'arco has strong fungicidal effect against effective against *candida albicans* and *candida tropicalis.*

5.2. Probiotic Bacteria

Pau d'arco positively influences the lactic acid producing probiotic bacteria such as *Lactobacillus acidophilus*, and *Lactobacillus casei*. These bacteria are important for maintaining probiotic microbial flora and normal functioning of the immune system. On the other hand, *Bifidobacterium bifidum*, Bifidobacterium *Adolescentis, and Bifidobacterium infantis* are another group of probiotic bacteria that are responsible for nutrient absorption, digestion, and maintenance of probiotic balance.

6. Significance of Pau Darco in CanXida Remove

CanXida Remove (Formula RMV) is designed not only for the elimination of pathogenic bacteria and fungi but also to restore the normal microbial flora of the digestive tract. Pau d'arco provides comprehensive antimicrobial bioactivity and restores the growth of probiotic microbes simultaneously. This makes Pau d'arco a complete package with full spectrum health benefits.

Pau d'arco works synergistically with other ingredients of CanXida Remove (Formula RMV) in combating pathogens in the digestive system. Its thousand years of usage among different ancient cultures, and its proven antimicrobial properties demonstrated by modern research from the past 60 years makes it a worthwhile ingredient of CanXida Remove (Formula RMV). Its added benefits come with its positive influence on the probiotic bacteria while maintaining its antimicrobial activity for pathogens, making it uniquely suitable for CanXida Remove formulation for pathogen elimination and probiotic restoration at the same time.

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