

# Pharmacological Activities of Bioactive Compounds from *Crescentia cujete* L. Plant – A Review

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**Abstract:** *Crescentia cujete* is widely known as a medicinal plant with broad indigenous ethnomedical uses such as anti-inflammatory, antibacterial, antivenom, antidiabetic, and antioxidant. Despite these known indigenous uses, the benefits obtained from *C. cujete* are far from fully used due to the underwhelming studies on its pharmacological potential, bioactive compounds, and mechanism of action that keep the pharmacological and new drug discovery progress slowly. The information in this review was gathered from major and trusted scientific databases and journal publications, books, and dissertations. In this review, available studies on ethnobotanical, general, and medical use, as well as phytochemical findings and known pharmacological applications of the plant, were collated and established. The most commonly investigated as well as the grey areas realized from this review regarding the pharmacological potentials of the plant consequently suggest the incorporation of *in silico* in-depth studies to understand the mechanism of action of bioactive compounds, as well as more pharmacological potential investigations of the compounds found in the least studied parts, such as the seeds, bark, and flowers of the plant. This will ensure continuous and fast-moving advances in the pharmacological application of *C. cujete*.

**Keywords:** pharmacological; phenols; bioactive compounds; *Crescentia cujete*; phytochemicals.

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## 1. Introduction

Medicinal plants are widely known as the source of many essential drugs currently being used in the modern world. Most of these indigenous medicinal plants are also used as spices, served fresh or cooked as food themselves, while other inedible parts of these plants serve as a foundation for making indigenous daily items such as bowls, utensils, etc. [1,2]. Interestingly, different parts of these medicinal plants are made up of multiple nutrients and phytochemicals deemed essential to combat many diseases, making them an essential source and focus for the advancement of pharmacological and medical studies. *Crescentia cujete* (*C. cujete*), commonly known as the calabash tree, is one of these medicinal plants worldwide, especially in tropical countries where it is highly cultivated for its numerous medicinal properties. Its fruit is globular in shape with a green, smooth, but complex and woody exterior [3]. The preparation and use of this plant for medicinal and traditional purposes vary by culture. Typically, fruit pulp and leaves are used to make concoctions to treat different ailments, such as colds and asthma, to function as a remedy for diabetes, internal abscesses, and snake bites

[2]. The flesh is also highly regarded in some countries as an effective cleansing and healing agent for cuts and other wounds [4].

In certain parts of the Philippines, *C. cujete* is regarded as the "Miracle fruit", as locals claim to have observed some significant health effects of the intake of extracts from its fruit and leave, specifically: antimicrobial, antibacterial, anthelmintic, anticholesterol, antidiabetic, antioxidant, and anti-inflammatory [5-7]. Due to these medicinal claims regarding this plant worldwide, a couple of studies focusing on the phytochemical properties, chemical constituents, and mineral composition determination of *C. cujete* were done in the past [3,8,9]. Although several studies have been conducted to determine its phytochemical properties and chemical constituents, information about secondary metabolites found in the Philippine *C. cujete* [3] and complementary *in vitro*, *in vivo*, and *in silico* tests is quite scarce and are still unabundant to further support ethnomedicinal claims about it. This paper highlights the different phytochemicals and pharmacological properties of *C. cujete* L. Exploring ethnomedicinal claims of *C. cujete* from the previous study provides potential future research ideas for agriculture, food, and pharmaceuticals.

## 2. Ethnobotanical, General, Nutritional and Medical Use.

Known as a beneficial medicinal plant, the entire plant of *C. cujete* is used in various applications worldwide. The most common is the use of different parts of the plant for decoctions and infusions to remedy various illnesses (Table 1). These decoctions and infusions are ingested orally, locally applied, externally applied, or used as a bath by people worldwide as a means of ethnomedical treatment. An example is the external/internal administration of the fruit pulp to accelerate bruise and sprain healing [10]. Another is the use of homemade drops of sap extracted from the plant as a remedy for respiratory ailments [10]. As tested by generations from different regions worldwide, each part of the plant is considered to have specific healing properties (Table 2).

**Table 1.** Medicinal uses of *C. cujete* (Bignoniaceae) [11-13].

S/N	Plant Part	Preparation and Way of Use	Specific Illness/Application
1	Fruit	Decoction, Maceration; Oral ingestion	Pneumonia, catarrh, tuberculosis
2	Flowers	Frying; Topical application	Earache
3	Fruit and Roots	Decoction; Oral ingestion	Venereal diseases, Gal'on
4	Fruit	Decoction, Juice Extraction, Maceration; Oral ingestion	Catarrh
5	Fruit	Decoction; Oral ingestion	Coolness (frialdad) in the uterus, menstrual irregularity
6	Leaves	Sap Extraction, Maceration; Oral ingestion	Cicatrization, Ulcers
7	Leaves	Infusion; Oral ingestion	Palpitations, hypertension, flu, pneumonia, coughs, catarrh, diarrhea
8	Leaves	Decoction; Oral ingestion	Diarrhea, diabetes, indigestion, palpitations, nervous irritations
9	Leaves, flowers and twigs	Infusion; Bath	Vaginal bath
10	Leaves	Sap Extraction; Local application	Pimples, freckles
11	Leaves	Teeth maceration; Oral ingestion	Toothache
12	Cortex	Decoction; Oral ingestion	Easing of child birth
13	Cortex	Decoction, Maceration; Local application	Haemorrhoids, prurigo of the genitals, vaginal colic, whooping cough
14	Inner bark	Juice Extraction; Local application	Bruises of the eyes
15	Fruit	Decoction; Oral ingestion	Respiratory irritations, bronchitis asthma, gastrointestinal irritations (colic, constipation, hepatitis), phthisis, inflammations, urethritis
16	Fruit	Sap Extraction, Decoction; Oral ingestion	Menstruation stimulant

S/N	Plant Part	Preparation and Way of Use	Specific Illness/Application
17	Fruit	Juice Extraction; Oral ingestion, Local application	Birth induction, placenta/dead fetus ejection
18	Fruit	Cooked; Oral ingestion	Anti-diarrheic, anti-inflammatory
19	Fruit	Raw; Oral ingestion	Purgative

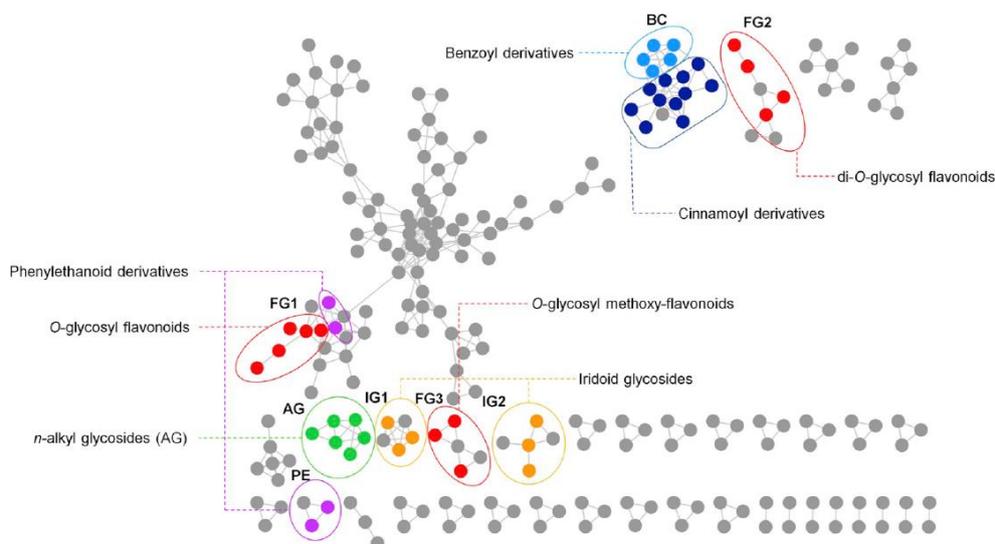
**Table 2.** *C. cujete* (Bignoniaceae) plant parts and their healing properties [11,12].

Serial Number	Plant Part	Healing properties
1	Leaves	Anti-inflammatory, antibacterial, abortive, emollient
2	Cortex	Calmative, antimicrobial
3	Fruit	Purgative, laxative, emetic, emollient, pectoral, antidiarrhoeic, anti-inflammatory, antibacterial, antiseptic, calmative, tonic, antidiabetic, reconstituent, sudorific, vulnerary, analgesic, emmenagogic, hemostatic aperitive, child birth accelerant
4	Seeds	Calmative, abortive

Different minerals (Ca, Mg, Na, K, Fe, Mn, Cu, Zn, and PO<sub>4</sub>), nutritive, and non-nutritive compounds (crude fiber, ash, crude protein, and carbohydrate: riboflavin, niacin, and thiamine) are also found abundant in dry pulps of *C. cujete* which are essential in the maintenance of electrolyte balance in the body as well as the maintenance of healthy dietary nutrients [14,15]. Hence, it is not only considered useful for medicinal remedies. Parts of this plant are also considered a delicacy or snack in some countries. The young seeds of *C. cujete*, commonly cooked, fried, or toasted to be eaten as a snack, contain approximately 2.6% sugars, 8% proteins, and 37% oil (oleic acid, linolic acid, and saturated oleic acids). Their abundance of oleic and linolic acid, which are best known for their health benefits: heart health, blood sugar control, joint and bone health, mood and mental health, and skin health, makes the young seeds of this plant a healthy alternative to snacking on [16,17].

### 3. Phytochemicals and Bioactive Compounds

Phytochemical and bioactive compound studies on *C. cujete* revealed numerous secondary metabolites that are considered contributors to its known medicinal and pharmacological applications (Figure 1).



**Figure 1.** UPLC-MS/MS-based molecular networking of *C. cujete*. The following phytochemical classes were observed in negative ionization mode and were grouped according to their substituent similarities: AG: alkyl glycosides, BC: benzoyl and cinnamoyl derivatives, FG1-3: flavonoid glucosides, PE: phenylethanoid derivatives, IG1-2: iridoids glycosides with their corresponding node text corresponding to their parent ion and node color corresponding to their chemical group (green: n-alkyl sugars, sky blue: benzoyl derivatives, dark blue: cinnamoyl derivatives, red: flavonoids glycosides, purple: phenylpropanoids derivatives, and gold: iridoid glycosides) [30].

The most abundant compounds in the entire plant are flavonoids, phytosterols, glycosides, and terpenoids [5,18,19]. Specific compounds were also determined in various parts of the plant. For example, essential oil from plant leaves was found to contain volatile compounds such as hexadecane, 1,1-dimethyl-3-hexyl-cyclopentane, 4-methyl-2-heptanone, *trans*-pinane, selina-4(15),6-diene, *allo*-aromadendrene, globulol, neophytadiene, hexadecanal, kaur-16-ene, phytol, and (z)-9,17-octadecadienal [20]. On the other hand, different extracts of the fruit were found to contain phytosterols, cardiac glycosides, terpenoids [9], crescentic acid, tartaric acid, citric and tannic acids [21], iridoids and their glycosides [21,22,23], saponins, alkaloids, anthraquinones, and cardenolides [8]. Stem bark extracts also showed evidence of the presence of glycosides, terpenes, and flavonoids [24]. Proximate composition analysis was also performed on the plant seeds, which also showed evidence of an abundant amount of phytate, tannin, saponins, and flavonoids [25]. Other plant parts were also studied, producing various chemical moieties (Table 3) [23,26-32].

**Table 3.** Isolated compounds with undetermined pharmacological potentials.

S/N	Compound names	Functional class	References
1	Catalpol	Iridoid	[23]
2	3-Hydroxymethylfuro [3,2-b]naphtho [2,3-β]furan-5,10-dione	Furanonaphthoquinones	[26]
3	Ajugol	Iridoid glycoside	[26,29]
4	6-O-p-hydroxybenzoylajugol	Iridoid	[27]
5	Aucubin	Iridoid glucoside	[26, 29]
6	6-O-p-hydroxybenzoyl-6-epiaucubin	Phenolic	[27]
7	Agnuside	Iridoid glucoside	[27]
8	Ningpogenin	Iridoid glucoside	[27]
9	5,7-Bisdeoxycynanchoside	Iridoid glucoside	[27]
10	Crescentin I	Iridoid	[27]
11	Crescentin II	Iridoid	[27]
12	Crescentin III	Iridoid	[27]
13	Crescentin IV	Iridoid	[27]
14	Crescentin V	Iridoid	[27]
15	Crescentoside A	Iridoid glucoside	[27]
16	Crescentoside B	Iridoid glucoside	[27]
17	Crescentoside C	Iridoid glucoside	[27]
18	Acanthoside D	Glucoside	[27]
19	β-D-Glucopyranosyl benzoate	Glucoside	[28]
20	(R)-1-O- β-D-Glucopyranosyl-1,3-octanediol	Glucoside	[28]
21	β-D-Fructofuranosyl 6-O-(p-hydroxybenzoyl) α-D-glucopyranoside	Glucoside	[28]
22	(2R,4S)-2-O- β-D-Glucopyranosyl-2,4-pentanediol	Glucoside	[28]
23	(2R,4S)-2-O-β-D-Glucopyranosyl-(1→6)-β-D-glucopyranosyl-2,4-pentanediol [C <sub>17</sub> H <sub>32</sub> O <sub>12</sub> ]	Glucoside	[26, 27]
24	(2R,4S)-2-O-β-D-Xylopyranose -(1→6)-→-D--- glucopyranosyl-2,4-pentanediol [C <sub>16</sub> H <sub>30</sub> O <sub>11</sub> ]	Glucoside	[28]
25	(R)-4-O-β-D-Glucopyranosyl-4-hydroxy-2-pentanone [C <sub>11</sub> H <sub>20</sub> O <sub>7</sub> ]	Glucoside	[28]
26	(R)-4-O-β-D-Glucopyranosyl-(1→6)-β-D-glucopyranosyl-4-hydroxy-2-pentanone [C <sub>17</sub> H <sub>30</sub> O <sub>12</sub> ]	Glucoside	[28]
27	(R)- 1-O-β-D-Apiofuranosyl-(1→2)-β-D-glucopyranosyl-1,3-octanediol [C <sub>17</sub> H <sub>32</sub> O <sub>12</sub> ]	Glucoside	[28]
28	(R)- 1-O-β-D-Glucopyranosyl-(1→6)-β-D-glucopyranosyl-1,3-octanediol [C <sub>20</sub> H <sub>38</sub> O <sub>12</sub> ]	Glucoside	[28]
29	6-O-(p-hydroxybenzoyl)-D-glucose	Glucoside	[28]
30	(2R,4S)-2-O-β-D-xylopyranosyl-(1→6)-β-Dglucopyranosyl-2,4-pentanediol	N-Alkyl	[28]
31	6-Epi-aucubin	Iridoid glycosides	[30]
32	Aucubin	Iridoid glycosides	[30]
33	Epi-eranthemoside	Iridoid glycosides	[30]

S/N	Compound names	Functional class	References
34	Crescentiol A	Iridoid glycosides	[30]
35	Crescentiol B	Iridoid glycosides	[30]
36	Sibirioside A	Phenols	[30]
37	1-O-trans-Cinnamoyl-β-D-glucopyranose	Phenols	[30]
38	Plumieride	Iridoid	[31]
39	Asperuloside	Iridoid	[31]
40	Ningpogenin	Iridoid	[31]
41	Quercetin 3-O-a-L-rhamnopyranosyl-(1-.6)-b-D-glucopyranoside	Flavonoids	[32]
42	Kaempferol 3-O-a-L-rhamnopyranosyl-(1-.6)-b-D-glucopyranoside	Flavonoids	[32]
43	Kaempferol	Flavonoids	[32]

**Table 4.** Isolated compounds with documented pharmacological potentials.

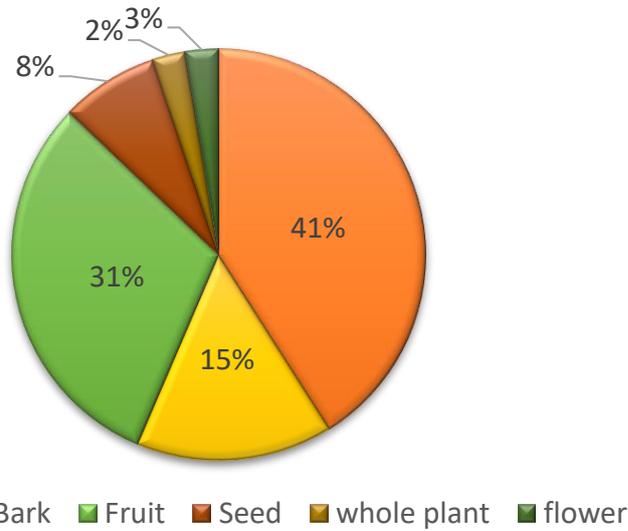
S/N	Compound names	Functional class	Pharmacological potentials	References
1	(2S,3S)-3-hydroxy-5,6-dimethoxydehydroiso-α-lapachone	Furanonaphthoquinones	DNA damaging agent	[29]
2	(2R)-5,6-dimethoxydehydroiso-α-lapachone	Furanonaphthoquinones	DNA damaging agent	[29]
3	(2R)-5-methoxydehydroiso-α-lapachone	Furanonaphthoquinones	DNA damaging agent	[29]
4	2-(1-Hydroxyethyl)naphtho[2,3-β]furan-4,9-dione	Furanonaphthoquinones	Cytotoxic and DNA damaging agent	[29]
5	5-Hydroxy-2-(1-hydroxyethyl)naphtho[2,3-β]furan-4,9-dione	Furanonaphthoquinones	Cytotoxic and DNA damaging agent	[29]
6	2-Isopropenylnaphtho[2,3-β]furan-4,9-dione	Furanonaphthoquinones	DNA damaging agent	[29]
7	5-Hydroxydehydroiso-α-lapachone	Furanonaphthoquinones	DNA damaging agent	[29]
8	trans-Cinnamic acid	Phenols	Acaricidal	[33]
9	Benzoic acid	Carboxylic	Acaricidal	[33]
10	Hexadecanoic acid	Fatty acids	Acaricidal	[33]

However, it should be noted that despite the numerous studies on the determination of the phytochemicals and bioactive compounds present in the plant, only a few dealt with an in-depth analysis of these constituents in terms of their pharmacological potentials. Recently, only a few of the constituents or compounds mentioned above (Table 4) were documented to have pharmacological potentials that are cytotoxicity and acaricidal activity.

#### 4. Pharmacological Applications of *C. kujete*.

*C. kujete* varieties from different parts of the world were studied for its pharmacological properties which includes: antioxidant [5,23,33-37], anti-diabetic [6,37,38] and anti-cholesterol [5,38], anti-inflammatory [23,31], anthelmintic [5,39], antibacterial [7], [23,34-35,40,41] and anti-mycobacterial [42,43], anti-venom [44,45,46], wound healing [4], cytotoxic [5,31,33,37,44], acaricidal [47], neurodegenerative disease protection [47,48], and antiangiogenic [50], smooth muscle contraction-inducing [51], and maternal-fetal development and growth [52]. Different parts of the plant were used as a focus for various studies exploring its pharmacological potential (Figure 2). A separate study was also conducted in which four endophytic fungi isolates (*Nigrospora sphaerica*, *Fusarium oxysporum*, *Gibberella moniliformis*, and *Beauveria bassiana*) from *C. kujete* plant were observed for their antioxidant, antibacterial, and anticancer activities [53]. These pharmacological activities studies are further discussed below.

## *Crescentia cujete* plant parts previously studied



**Figure 2.** Percentage of published studies focused on the pharmacological potentials of specific parts of the *C. cujete* plant from the year 1947-2021.

### 4.1. Antidiabetic activity.

In general, the presence of reducing sugars in any plant may be attributed to lower amylase activities [54]. Based on the phytochemical screening test of Billacura and Laciapag in the *C. cujete* plant [5], the reduced sugar content of different extracts of the plant makes it susceptible to  $\alpha$ -amylase inhibition activity. Therefore, the determination of the hypoglycemic and protective potentials of different *C. cujete* fruit extracts were made using an *in vitro* ( $\alpha$ -amylase inhibition activity) [55] and an *in vivo* screening assay (*Mus musculus*/albino mice as test organism) to observe the antidiabetic activity of the plant in one study [39]. Alloxan was used to effectively raise the blood glucose level of the *Mus musculus* test organisms, while Metformin was used as the positive control. Blood analysis was performed before and after eight days of treatment, in which the glucose level of the test organisms showed that all fruit extracts (fresh, hexane, aqueous, and crude ethanol) possess hypoglycemic potential due to the significant decrease in glucose level observed in all tested organisms treated, although all extracts exhibited a lower hypoglycemic effect compared to the positive control. The  $\alpha$ -amylase inhibition activity result supports the *in vivo* finding, which revealed the hexane extract followed by the aqueous and the crude ethanolic extracts as the ones with the highest activity inhibition of the enzyme. The *in vivo* assay also demonstrated all fruit extracts possess the potential to maintain the test organisms' glucose level before the occurrence of the metabolic disorder diabetes mellitus. Results in the colorimetric glucose determination also showed that the fresh and decocted fruit extracts contain a relatively high glucose concentration. All extracts showed concentration-dependent activity in terms of the hypoglycemic potential of the fruit.

Different preparation methods of fruit extracts affect hypoglycemic activity in male diabetic white mice [6]. The test organisms were also subjected to alloxan treatment to induce an increase in glucose levels and Metformin as a positive control. Taking into account the individual weight and blood glucose level to analyze the hypoglycemic effect of the extracts,

it was observed that the fresh pulp extract (36.53% reduction) performed better in lowering the blood glucose level of the test organisms than the fruit pulp decoction (16.15% reduction). The study also showed concentration-dependent activity without dwelling on the plant's probable toxic and mutative properties.

The potency of ultrasonic-assisted extracted plant extracts (petroleum ether, chloroform, methanol and water) to ameliorate hyperglycemia and oxidative stress in diabetes in adult Swiss albino mice (normoglycemic mice) [38] showed a decrease in blood glucose levels after administration of the different extracts of the plant. An *in vitro* Resazurin assay and a glucose uptake assay on HepG2 cells reflected antihyperglycemic LSAF and elevation in the 2-NDBG uptake of the cell cultures, respectively. The evaluated LSAF showed that the administered 50 µg/mL concentration is safe for intake due to the higher viability percentage (65.107%) compared to 100 µg/mL at (41.632%), while the significant elevation in the uptake of 2-NDBG is observed to be higher compared to the positive control (Metformin-treated) and control untreated. This reveals a metformin imitator mechanism action for *C. cujete* antidiabetic activity. An additional phytochemical study using LC-MS/MS also showed the presence of luteolin-7-glucoside and protocatechuic acid, which are both components with significant roles in the antidiabetic potency LSAF as recorded in the activity of the detected constituents in literature [55,56].

#### 4.2. Anthelmintic activity.

Tannins, a secondary plant metabolite, have been reported to have a direct and indirect antiparasitic effect. Known to be closely associated with plant defense mechanisms against insects and herbivores [57,58], tannin extraction to be used for anthelmintic applications was done to demonstrate its potential against numerous parasitic populations [60]. One study aimed to investigate the possible direct anthelmintic activity of extracted condensed tannins from the plant on different gastrointestinal parasites of sheep [40]. The capacity of tannins to bind to proteins and operate via several mechanisms, such as binding to the intestinal mucosa and to the cuticle of larvae, were presumed to be the cause of the nutrient availability reduction, larvae development inhibition, and death.

Due to the abundance of tannins found in *C. cujete*, specifically in ethyl acetate fruit extracts, a study [5] used *Eudrilus eugenia* as test organisms with Levamisole. It distilled water as control to test the anthelmintic potential of *C. cujete* extracts. Being the extract with the highest number of tannins, the 20,000 ppm ethyl acetate extract also showed the best anthelmintic activity. It has the least paralysis time recorded, having an average of 1.39 minutes. This exceeded the activity of the positive control, 20,000ppm Levamisole (Latigo-50), which has an average paralysis time of 2.93 minutes. On the other hand, the recorded death time of the extracts showed the ethyl acetate to be the most effective in inducing death onto the test organisms with a 2.59 min death time, beating the 6.69 min death time Levamisole. Despite the longer paralysis and death times of the other extracts, all except the hexane extract demonstrated a promising anthelmintic activity compared to the control. The lack of anthelmintic property of the hexane extract was explained due to the absence of tannins in the extract during the phytochemical analysis. Although the study concluded *C. cujete* fruit is a promising natural source of anthelmintic constituents effective against the *E. eugenia* worms *in vitro*, further *in vivo* experimental models and safety profiles are needed to support the claim further.

4.3. Antibacterial activity.

The continuous widespread use and misuse of antibiotics led to the emergence of multidrug-resistant (MDR) mutations of pathogenic bacteria. After decades since discovering antibiotics, many pathogenic bacteria resistant to the current antimicrobial drugs or antibiotics have been considered a complex global public health challenge. This led to numerous studies, especially focusing on medicinal plants in the hopes of discovering new natural antimicrobial drugs and methods to maintain the effectiveness of existing drugs to deal with the current shortage of effective antibiotics [61]. Being one of the most studied pharmacological activities, the antibacterial activity of plants was attributed to the abundance of these plants of bioactive extracts containing complex mixtures of compounds with synergistic actions that can produce an enhanced effect (34). Major classes of the extracted and identified antimicrobial compounds (Table 5) showed that most of these compounds are also abundantly present in the plant *C. cujete*.

**Table 5.** Major classes of identified plant-sourced antimicrobial compounds.

Classes	Subclass	Mechanism	Reference
Phenolics	Simple Phenols	Efflux pump inhibition, Substrate deprivation	[62,63]
	Phenolic acids	Efflux pump inhibition, Membrane disruption	[63,64]
	Quinones	Efflux pump inhibition, Adhesin-binding, cell wall complex, enzyme inactivation	[63,65]
	Flavonoids	Efflux pump inhibition, Adhesin-binding	[63,66,67]
	Flavones	Efflux pump inhibition, Cell wall complex, enzyme inactivation. HIV reverse transcriptase inhibition	[63,68,69]
	Flavonols	Efflux pump inhibition	[63]
Coumarins	Tannins	Efflux pump inhibition, Protein-binding, adhesin-binding, enzyme inhibition, substrate deprivation, cell wall complex, membrane disruption, metal ion complexation	[63,70-75]
		DNA gyrase inhibition, Efflux pump inhibition, Eukaryotic DNA interaction (antiviral activity)	[63,76,77]
Terpenoids		Membrane disruption, ATPase inhibition, cellular yeast transformation inhibition, membrane disruption, metabolic activity disruption	[63,78]
Alkaloids		Cell wall/DNA intercalation, Efflux pump inhibition, cell division inhibition, protein and DNA synthesis inhibition	[63,77,79-82]
Lectins, polypeptides and polyacetylenes		Viral fusion block or adsorption, disulfide bridges formation	[83]
Organosulfur		Sulfhydryl-dependent enzyme inhibition, DNA and protein synthesis inhibition, bacterial membrane destruction, ATP synthase inhibition	[63,84]

The possible produce preservation ability of *C. cujete* against *E. coli* contamination was investigated by using an *E. coli*-infected lettuce [7]. Using both leaf and fruit extracts to observe the *in vitro* antibacterial effect of *C. cujete* leaf and fruit extracts showed a decrease in the lesion size on the *E. coli*-affected lettuce, which is attributed to the successful inhibition of the bacteria growth, which confirms the antibacterial activity present in both extracts. The effectiveness of the extracts was also found to be concentration-dependent, and the higher concentration extracts showed a smaller lesion size after the treatment.

The crude ethanol extracts of the leaf and stem bark of *C. cujete* and the chloroform fractions evaluated for their antimicrobial activity against *Staphylococcus aureus* (Gram-positive) and *E. coli* (Gram-negative) using the *in vitro* disc diffusion method [24] showed that the leaf chloroform fraction exhibited the most antibacterial activity with an *E. coli* zone inhibition of 29 mm and 10 mm, and *S. aureus* zone inhibition of 15 mm and 14 mm for the 200 µg/disc and 100 µg/disc concentrations, respectively with the higher concentration's activity equivalent with the positive (Kanamycin K, 30 µg/disc) control's. All extracts exhibited

antibacterial activity against *E. coli*, with the crude ethanolic stem bark extract having the least activity with only 5 mm and 8 mm for the 100 µg/disc and 200 µg/disc concentrations, respectively. On the other hand, the crude ethanolic stem bark extract showed no sensitivity against *S. aureus* due to the insufficient antibacterial bioactive compounds present in the extract. The difference between the inhibitory activity of the extracts in *E. coli* and *S. aureus* was attributed to the difference in the gram-stain of each bacterium. Gram-positive bacteria have thick peptidoglycan layers than Gram-negative bacteria [85], making it difficult to disrupt their membrane.

Two complementing studies explored the potency of the ethanolic extracts and different fractions of the plant's stem bark [35], fruit rind, and flesh extracts also used the disc diffusion method [36] along with the following methods to evaluate the antibacterial activity: DPPH method, TLC-bioautographic and agar diffusion methods [35], measurement of optical density (OD), and total plate count (TPC) against cultures of *E. coli* and *S. aureus* bacteria [36]. The ethanolic fruit rind extract showed inhibition zones less than 10 mm (3.0 mm and 4.2 mm for 80% (w/v) and 100% (w/v), respectively), while the other concentrations showed no inhibition zone at all for both bacteria cultures, thus classified as no inhibition. The MIC test performed showed that the fruit rind showed a minimal antibacterial potential against *S. aureus* with clear zone diameters of 3.38 mm (65% w/v), 3.75 mm (70% w/v), and 3.75 mm (75% w/v). On the other hand, the dichloromethane and ethyl acetate fractions of the stem bark extract showed inhibition activity against *S. aureus* (3.67 mm and 2.72 mm inhibition zones, respectively), while only the aqueous stem bark extract showed inhibition against *E. coli* (2.36 mm inhibition zone). Despite the lack of inhibition of the fruit rind extracts for *S. aureus* and *E. coli*, a previous study reported that the fruit flesh has promising antibacterial activity against *Aeromonas salmonicida*, a bacteria found in freshwater fish [86], and *Vibrio alginolyticus*, a bacteria found in grouper fish [87]. This confirms the antimicrobial activity of the plant, which was supported by the presence of antimicrobial bioactive compounds present in the extracts, and the less potentiality of the fruit rinds and fruit flesh for antibacterial activity was attributed to being caused by the age, region, and time of sampling the plants, which resulted in the sub-optimum extraction of the secondary metabolites [88].

The ethanolic leaf extracts of *C. cujete* were found to be an effective antimicrobial agent against *Shigella dysenteriae*, *Bacillus cereus*, *Bacillus subtilis*, *Bacillus megaterium*, and *S. aureus* was also confirmed in one study [42]. The sensitivity of the ethanolic leaf extract to *S. dysenteriae* was found to be equivalent with the highest inhibition zone (23.35 mm) to the positive control, Doxycycline hydrochloride. The minimum inhibitory and minimum bactericidal concentrations (MIC and MBC) using the broth microdilution method showed the lowest MIC (2500 µg/ml) and MBC (2000 µg/ml) were against *B. subtilis*. A study about numerous medicinal plants from Suriname [89] supported these findings as the ethanolic leaf extracts showed one of the most promising sensitivities against *B. subtilis* and *S. aureus*, which only needed 5 mg/ml concentration to compete with other plants with higher concentrations (zone of inhibition > 15 mm). Complementing the findings of this study, a study focusing on the inhibitory activity of ethanolic fruit extracts against *S. aureus* and *E. coli* using the disc diffusion method revealed the insensitivity of the fruit extracts against the two strains of bacteria [90].

Tested against *M. tuberculosis* H37Rv as control, multi-drug resistant isolates DKU-156, JAL-1236, and mycobacterial pathogen *M. fortuitum* in Lowenstein Jensen (L-J) medium and Middlebrook 7H9 broth in BacT/ALERT 3D system [43], a promising antitubercular

potential of the aqueous and ethanolic stem bark and leaf extracts was observed which was attributed to the significant decrease of colony-forming units (CFU) of all extracts against the mycobacterium tuberculosis strains.

The use of the agar-well diffusion method to focus on the investigation of the antibacterial activity of the oils extracted from the plant's seeds [91] showed that the seed oil is sensitive to *S. dysenteriae* (2 mm inhibition zone at 2 ml and 22 mm inhibition zone at 4 ml) while no inhibitory activity was observed against *E. coli*, and *S. typhi*. This finding is consistent with a previous study regarding suppressed oils which may result in the inactivation of some of the potentials of the oil extract. Using a different oil extraction method, it was observed that the oil is now sensitive to all bacteria tested [92].

**Table 6.** *In vivo/in vitro* antibacterial assay applications of different *C. cujete* plant extracts.

Plant part	Mode of Extraction/Solvent	<i>In vitro/In vivo</i>	References
Leaf, fruit	Crude	<i>E. coli</i> -affected lettuce	[7]
Leaf, stem bark	Crude ethanol, chloroform	Disc diffusion method against <i>S. aureus</i> and <i>E. coli</i>	[24]
Stem bark	Ethanol	DPPH, TLC-bioautographic, agar diffusion methods against <i>E. coli</i> and <i>S. aureus</i>	[35]
Fruit rind, flesh	Ethanol	Optical density measurement, total plate count against <i>S. aureus</i> and <i>E. coli</i>	[36]
Leaf	Water	Gold nanoparticle synthesis, MTT cell viability assay against <i>E. coli</i> , <i>P. aeruginosa</i> , <i>V. cholerae</i> , <i>Salmonella typhi</i> , <i>S. flexneri</i> , and <i>B. subtilis</i>	[41]
Leaf	Ethanol	MIC and MBC assays against <i>E. coli</i> , <i>S. typhi</i> , <i>S. partyphi-A</i> , <i>S. lutea</i> , <i>S. dysenteriae</i> , <i>B. cereus</i> , <i>B. subtilis</i> , <i>B. megaterium</i> , <i>S. aureus</i>	[42]
Stem bark, leaf	Water, Ethanol	Against <i>M. tuberculosis H37Rv</i> , <i>DKU-156</i> , <i>JAL-1236</i> , and <i>M. fortuitum</i>	[43]
Leaf	Ethanol	Against <i>S. aureus</i> and <i>E. coli</i>	[90]
Leaf	Ethanol	Against <i>S. aureus</i> and <i>B. subtilis</i>	[91]

#### 4.4. Antivenom activity.

Snake envenomation is known to cause several pathophysiological changes in a victim's body, such as alterations in the blood coagulation system, inflammation, edema, hemorrhage, and necrosis before causing death. Antivenom activity-possessing compounds were required by WHO to be tested for their *in-vivo* biological effects neutralization capacity before being considered as a potential source of antivenom serum development [93]. Several studies to find a suitable neutralizing drug and snake venom antagonizing drug was carried out throughout the years, and these studies found that the following plant components are considered promising active ingredients in antivenom serum: alkaloids, acids, steroids, flavonoids, coumestans, pterocarpan, terpenoids, phenols, and tannins [94].

Due to most components' abundant presence, *C. cujete*'s ethanolic fruit extract was used to investigate its *in vitro* and *in vivo* lethality and hemorrhagic lesion neutralization capacity [45]. Westar albino rats and Swiss albino mice test organisms with I.P. administration after oral administration of 2LD<sub>50</sub> and 3LD<sub>50</sub> (*in vivo*). Higher dose levels (200 and 400 mg/kg) of the extract showed effective inhibition of the *Vipera russeli* venom-induced *in vitro* lethality with a survival rate of 83.33% and 100% against 2LD<sub>50</sub>, respectively, while lower survival rates against 3LD<sub>50</sub> with 50% and 83.33% for the dose 200 and 400 mg/kg, respectively. For the *in vivo* neutralization potency of the extracts, 400 mg/kg showed significant inhibition of the venom-induced *in-vivo* lethality with a survival rate of 66% and 50% against 2LD<sub>50</sub> and 3LD<sub>50</sub>, respectively.

*C. kujete* fruits, along with other medicinal plants in the northern region of Colombia, have a neutralization potential of the hemorrhagic effect against the venom of *Bothrops atrox* [46]. Mixtures containing the *B. atrox* venom with doses of the unripe ethanolic fruit extracts were pre-incubated with the venom before injection into Swiss Webster mice and were observed to have neutralized 21-72% of the hemorrhagic effect at doses up to 4 mg/mouse. Although this neutralization activity is below the activities of other plants tested in the same study, it demonstrated the potential of the plant's fruit extract as a natural source of antivenom serum constituents.

#### 4.5. Wound healing.

Being used as a common wound-healing remedy by traditional healers, a study investigating the wound healing potential of the ethanolic and ethyl acetate leaf extracts of *C. kujete* was done [4]. Healthy male Wistar albino rats were subjected to excisional wounds that were treated with leaf extracts, ointment base (control), and 5% povidone-iodine (positive) every 24 h for a span of 15 days. The percentage wound closure or wound contraction measurement was used to evaluate the study results, which demonstrated 15% relative wound closure induced in the ethanolic and ethyl acetate leaf extract-treated test organisms just after day 3 of treatment. 50% and 65% wound closure were observed after day 9 for both the organisms treated with ethanolic and ethyl acetate leaf extract, respectively, which were significantly different from the negative control with only 35% wound closure. On the last day, all treated test organisms except for the negative control displayed a 95-100% wound closure which confirms the promising wound healing properties of the ethanol and ethyl acetate leaf extracts of *C. kujete*.

#### 4.6. Cytotoxicity and safety profiles.

Defined as the toxicity resulting from the action of chemotherapeutic agents on living cells, the determination of the cytotoxicity and safety profiles of compounds with potential pharmacological or medicinal applications is essential to identify methods to specifically enhance or inhibit their potential for biomedical use [95].

The extracted furofuranonaphthoquinones: 3-Hydroxymethylfuro[3-2b]naphtho[2-3d]furan-5,10-dione 1 and 9-Hydroxy-3-hydroxymethylfuro[3-2b]naphtho[2-3d]furan-5,10-dione 2 were found to possess cytotoxic activity with the furofuranonaphthoquinone 1 having a selective DNA-damaging activity against yeast [29]. The same compounds were tested for cytotoxicity against Vero cells. Both compounds showed an order of magnitude greater activity (IC<sub>50</sub> of 0.41 µg/ml and 0.21 µg/ml, respectively) than its cytotoxicity to KB cells reported in the previous study [96]. The isolated compounds are all planar, suggesting that DNA intercalation may be one of the mechanisms of action involved in DNA damage. [45]

The rine shrimp eggs and *Artemia salina* lethality tests against the ethanolic and aqueous leaves, bark, and fruit extracts of *C. kujete* showed that all parts of the plant extracted with all solvents were bioactive and toxic [34]. Using *Artemia salina* and its eggs as test organisms and referring to Meyer's toxicity index, all extracts exceeding the LC<sub>50</sub> of 1000 µg/mL were considered toxic, while those below that threshold were considered nontoxic [97]. After 24 h of incubation with the extracts, it was observed that the leaves are the most toxic part of the plant, followed by the bark, making the fruit the least toxic. The effect of different extract solvents on the potency of each of the plant parts was also evident. Aqueous fruit extract

showed a higher toxicity activity compared to the 50% ethanolic and 100% ethanolic extract ( $LC_{50} = 38.74 \pm 1.35 \mu\text{g/mL}$ ,  $133.15 \pm 4.50 \mu\text{g/mL}$ , and  $292.17 \pm 0.00 \mu\text{g/mL}$ , respectively) with the leaf extracts following similar trend of toxicity (aqueous  $LC_{50} = 5.49 \pm 0.32 \mu\text{g/mL}$ , 50% ethanol  $LC_{50} = 4.84 \pm 0.32 \mu\text{g/mL}$ , 100% ethanol  $LC_{50} = 11.83 \pm 0.33 \mu\text{g/mL}$ ). On the other hand, a different trend was observed with the bark extracts. No significant differences were observed for both the aqueous and 50% ethanolic extracts ( $LC_{50} = 25.74 \pm 1.35 \mu\text{g/mL}$  and  $26.07 \pm 4.50 \mu\text{g/mL}$ , respectively), with the 100% ethanolic extract exhibiting the highest toxicity activity with the lowest  $LC_{50}$ ,  $14.04 \pm 0.00 \mu\text{g/mL}$ . This difference in toxicity behavior with different extracts is attributed to the presence of other bioactive compounds in the bark that are not present in the fruits and leaves of the plant.

Another brine shrimp lethality test against *Artemia nauplii* uses the following *C. cujete* extracts (fresh, decocted fruit, ethanol, hexane, and ethyl acetate) further confirm its bioactivity and toxicity [5]. After 24 h of incubation, all extracts except for the 10-ppm crude ethanolic extract and 10-ppm aqueous extract showed a 100% death rate or  $LC_{50} = 0$  ppm. Having an  $LC_{50} = 0$  ppm just after 6 h of incubation, the decoction and fresh extracts (3:1, 2:1, 1:1 ratio) were considered to be the most toxic among all the extracts tested, followed by the ethyl acetate extracts with an  $LC_{50}$  of 1.50 ppm after 6 h, the hexane extracts with  $LC_{50} = 2.49$  ppm, the crude ethanolic extracts with  $LC_{50} = 0.529$  ppm after 24 h, making the aqueous extracts are the least toxic with an  $LC_{50} = 4.64$  ppm.

Despite the known toxicity of certain *C. cujete* extracts against brine shrimp *A. salina* and *A. nauplii*, no significant cytotoxic effect was observed in the J774.A1 macrophage cell line when methanolic extracts were used [32]. After the optical density ( $OD_{620}$ ) comparison between the serial dilutions of the methanolic extract of *C. alata*, a similar specie of *C. cujete*, and its derived flavanols with the control or LPS-stimulated wells showed  $LC_{50}$  values greater than 1000  $\mu\text{g/mL}$  proving the absence of any toxicity and bioactivity against the cell line culture.

A resazurin reduction assay to evaluate the cytotoxic effect of the ultrasonic-assisted (chloroform, methanol, petroleum ether, and water) fruit extracts on HepG2 cells showed that a low concentration of the plant extract, 50  $\mu\text{g/mL}$ , is safe for consumption due to its higher viability percentage of 65.107% when compared to a higher concentration, 100  $\mu\text{g/mL}$  which has 41.632% viability percentage [38]. This was supported by the acute toxicity test results, which were done through the oral administration of the extracts (petroleum ether and chloroform reconstituted in 20% tween 80, methanol and aqueous reconstituted in normal saline) to female albino mice. No significant changes in terms of physical activity and general behavior of the test organisms were recorded. No mortality was recorded within the 24 h and 7-day observation periods, demonstrating that the doses of plant extracts used did not cause any adverse effect on the test organisms.

Due to the underdeveloped morphology and observed mortality of ducks (*Anas platyrhynchos* embryos), the methanolic fruit extracts of *C. cujete* are considered toxic [50]. Displaying an antiangiogenic property, it is not surprising to observe mortality and the test embryos having underdeveloped morphology compared to the untreated duck embryos. This was considered due to the abundant presence of some phytochemicals (alkaloids, tannins, and phenols) that, despite being beneficial, are also considered toxic and even corrosive at a definite level [98]. Therefore, lower concentrations of less than 50% or 0.24 g/mL were suggested to avoid observing any adverse effect on *A. platyrhynchos* embryos.

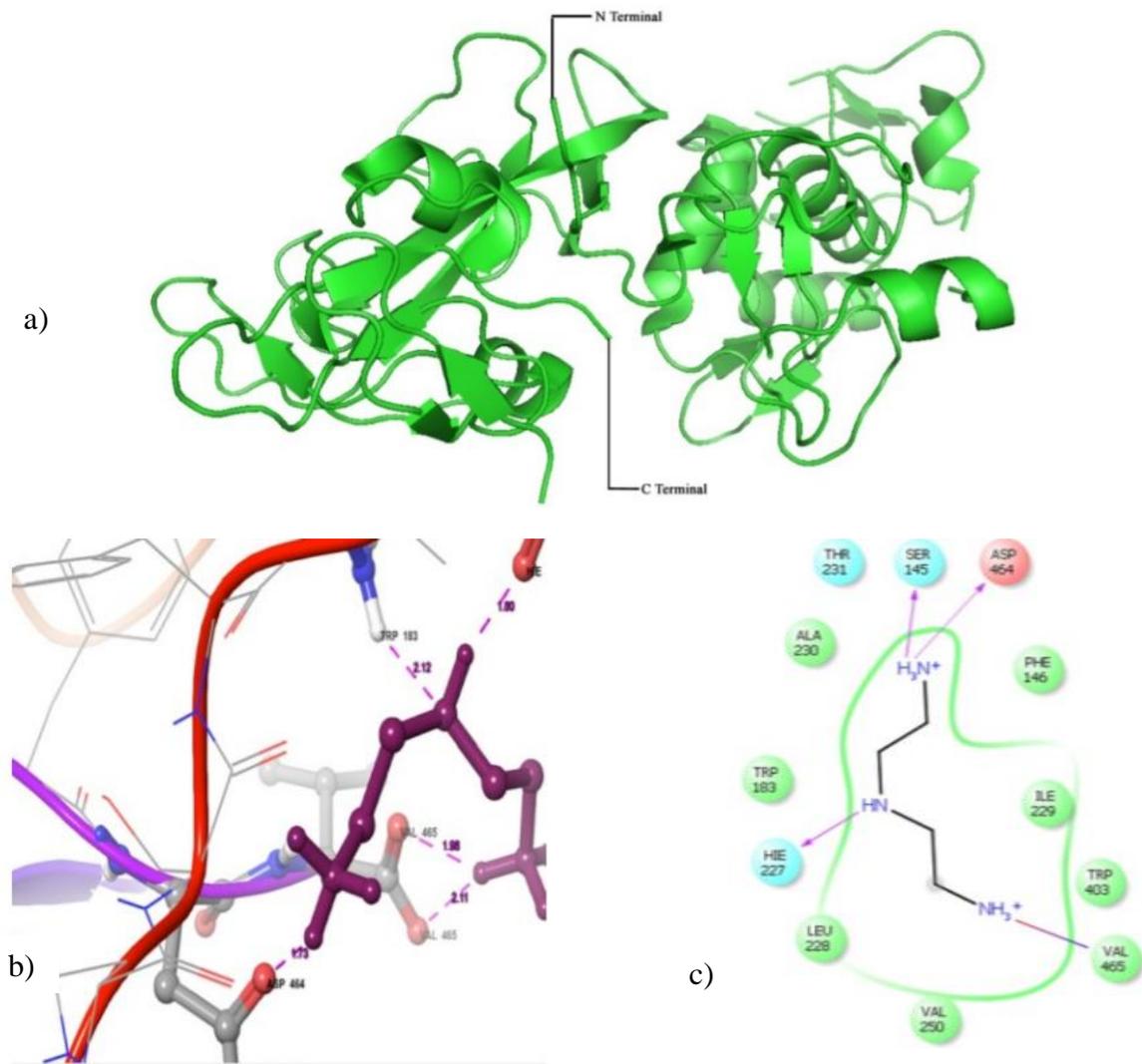
Oral administration of ethanolic fruit extracts of *C. cujete* to Sprague Dawley rats during the 6<sup>th</sup>-19<sup>th</sup> days of gestation showed a significant change in the maternal blood count and growth and development of the fetus and maternal organs [52]. Based on the maternal organ and fetal weight recorded for each test organism treated with different concentrations of fruit extract during the organogenetic period, 75% and 100% concentrations are considered fatal doses for both the fetus and the mother upon continuous consumption. The fetal weight is significantly lower compared to 25%, 75%, and the control with only 3.5625 g, while the blood counts of the 75% and 100% treated were also significantly lower than the expected range of 5.0-10.0 x 10<sup>9</sup>/L. Hence, it was suggested to intake any fruit extract of the plant with utmost caution during pregnancy.

#### 4.7. Acaricidal activity.

Mites such as cattle ticks are known as an important constraint against livestock, specifically in tropical and subtropical areas. The use of synthetic acaricides available in the market is the primary method to control ticks, but due to the continuous gene mutations and resistance of these mites against the existing acaricides, it is essential to continuously develop advancements to preserve the efficacy of these treatments [99-100]. One study focused on the acaricidal activity against *Rhipicephalus microplus* of the *C. cujete* fruit pulp extracted using the following solvents: ethanol, ethyl acetate, ethyl ether, methanol, and water [47]. All extracts except ethanol acetate and ethanol extract only caused a mortality rate of less than 20% in larvae of *R. microplus*. The ethanol acetate extract showed a promising acaricidal property with its 100% mortality against the *R. microplus* larvae at a concentration of only 10% (LC<sub>50</sub> of 5.9% and LC<sub>95</sub> between 5.6% to 6.2%), while the ethanol extract for all concentrations did not show acaricidal activity against the *R. microplus* larvae. These acaricidal activities were approximately four times greater compared to the acaricidal activity of *Chenopodium ambrosioides* extracts (Amaranthaceae) to control *R. microplus* [101].

#### 4.8. Protection from neurodegenerative diseases.

Parkinson's disease, the second most common neurodegenerative disorder, is characterized by its four main symptoms: muscle rigor, slowness of movement, involuntary and uncontrollable shaking, and loss of postural balance and/or secondary manifestations such as memory, language, problem-solving, and other thinking skills declination [102]. Research studies identified E3 ubiquitin (Ub) ligase that is found in Homo sapiens as the autoinhibited Parkin catalytic domain (PDB ID: 4BM9) [103], and mutations of this domain lead to the autosomal recessive Parkinson's disease [104]. An *in silico* docking study on the inhibition of the Parkin catalytic domain using phytochemicals from the ethanolic extract of *C. cujete* (Figure 3) showed an interaction of the lead ligand with the crystal structure of the autoinhibited Parkin catalytic domain (4BM9) suggesting an enhanced inhibitory activity of the domain [48]. This, along with the G-score for the rest of the five compounds and the docking features of the phytochemical 1,2-ethanediamine, N-(2-aminoethyl) with the target protein satisfying the Lipinski rule of 5 and ADMET properties, support the idea of the said compound as a candidate towards the design and development of an anti-Parkinson agent.



**Figure 3.** (a) 3D structure of the autoinhibited Parkin catalytic domain used from RSCB Protein Data Bank (PDB ID: 4BM9) <https://www.rcsb.org/structure/4BM9>; (b) Molecular interaction of 1,2-Ethanediamine, N-(2-aminoethyl) with the target protein (4BM9), purple dashed lines: hydrogen bond-linked atoms; (c) detailed 2D enzyme interaction [48].

A significant decrease in dopaminergic supply to the striatum causes an imbalance with essential neurotransmitters such as acetylcholine and dopamine, resulting in Parkinson's disease (PD) symptoms [105]. Cell toxicity mediated by 1-methyl-4-phenylpyridinium (MPP<sup>+</sup>), a known active metabolite of 1-methyl-4-phenyl-2,3,6-tetrahydropyridine (MPTP), is commonly used as an *in vitro* model of the disease. An *in vitro* study [49] investigated the effect of *C. cujete* ethanolic leaf extracts on neuroinflammation and intracellular ROS generation. This study focuses on the neuroprotective potential of *C. cujete* ethanolic leaf extracts against the toxicity in SH-SY5Y neuroblastoma cells using cell viability assays: 2,5-diphenyl-2H-tetrazolium bromide (MTT), sulforhodamine (SRB), trypan blue exclusion, and lactate dehydrogenase (LDH) for cell membrane damage, showed a concentration-dependent relationship with the level of inhibition and cell viability reduction when compared with the control. This demonstrated the ability of the extract to shield SH-SY5Y cells against the induced damage caused by 1-methyl-4-phenyl-2,3,6-tetrahydropyridine (MPTP), confirming its neuroprotective potential.

#### 4.9. Antiangiogenic Activity.

Angiogenesis, or the formation of new blood vessels from preexisting blood vessels, is a known mechanism essential for embryonic development, wound healing, and the menstrual cycle [106]. However, it is also known as a pathology precursor for diseases such as cancer. Chorioallantoic membrane (CAM) assay is considered one of the favorable systems for studying tumor angiogenesis and metastasis (10,6) due to the undeveloped and unestablished condition rejections of the immunocompetence system the embryo. In a study in which air-dried *C. cujete* fruit pulp methanolic extracts were topically applied to the surface of the chorioallantoic membrane of eight-day-old duck (*Anas platyrhynchos*) developing eggs [50], inhibition of angiogenesis was evident due to the significant reduction of the CAM vasculature in all membranes treated with extracts. The concentration-dependent activity was observed, as reduced CAM was more pronounced in the two extracts of higher concentration (75% and 100%), which showed almost no vasculature after treatment.

#### 4.10. Contraction of the smooth muscle of the uterine.

Linoleic acid is known as a precursor for prostaglandins, one of the known triggers for smooth muscle contraction [107], while saponins are known as cell membrane permeating agents due to their detergent-like properties [108]. The hydrophobic aglycone moieties found on saponins disrupt lipid bilayers, allowing the influx of Ca<sup>2+</sup> ions to trigger uterine contractile responses [109]. Both constituents were found in numerous phytochemical analyses of *C. cujete*. Hence, the plant's potential to contract uterine smooth muscle tissues to induce menses, birth, after birth, or abortions was explored. Aqueous seed and fruit pulp extracts were used to treat virgin female mice, *Mus musculus*, and isolated uterine horn tissues [51], which showed an increase and a slightly better response than the positive controls (treated with oxytocin and treated with acetylcholine) in the contractile frequency of the uterine tissues in both *in vitro* and *in vivo* assays.

In Brazil, an *in vivo* study focused on plant poisoning in ruminants was conducted wherein oral administration of the ripe fruits to pregnant goats [110-111]. It was observed that the pregnant goats orally administered with the ripe fruits of *C. cujete*, experienced abortion which also resulted in perinatal mortality. This supported the abortion-inducing and toxic potential of the ripe fruits of *C. cujete* when taken in large doses.

#### 4.11. Other Applications.

Ethyl acetate extract of isolated endophytic fungi from healthy leaf samples of *C. cujete* L. (*Beauveria bassiana*, *Fusarium oxysporum*, *Gibberella moniliformis*, and *Nigrospora sphaerica*) displayed promising pharmacological properties, including antibacterial, antioxidant, and anticancer activities [53]. The *B. bassiana* crude extract exhibited an effective antibacterial activity against all the human pathogens except *S. aureus*, while *F. oxysporum* showed strong inhibition against *S. typhi* and *Shigella sp.* Strains *N. sphaerica* and *G. moniliformis* exhibited moderate inhibition against all pathogens except *P. aeruginosa* and *S. aureus*. CC *F. oxysporum* 2 and *B. bassiana* are revealed to be the most prominent strains against human pathogens. The results of the DPPH scavenging assay showed that all fungal extracts exhibited a lower but effective scavenging activity (with the *B. bassiana* strain the highest with 13-46%) compared to standard BHT (17% to 70%). The MTT cell viability assay and AO/EB and Hoechst 33528 staining methods showed that the four isolates triggered HepG2

cell death by apoptosis, with the *B bassiana* strain displaying a nuclei abnormality of 56%, followed by *F. oxysporum* with 55%, and strains *N. sphaerica* and *G. moniliformis* with 49% and 48%, respectively.

The potential of aqueous *C. cujete* extract as a reducing agent to reduce the Au<sup>3+</sup> ions to synthesize biogenic gold nanoparticles gave rise to a promising enhancement for antibacterial nanomaterials [41]. Characterization tests such as Fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM), x-ray diffraction (XRD) and dynamic light scattering (DLS) confirmed the successful benign synthesis of anisotropic-shaped *C. cujete*-gold nanoparticles (CCAuNPs) with a mean size of 32.89 nm. These CCAuNPs were also investigated for their pharmacological activities using a 3-(4, 5-Dimethyl-2-thiazolyl)-2, 5-diphenyl-2H tetrazolium bromide (MTT) cell viability assay against the HeLa cell line, and it was observed that it has extraordinary antibacterial activity against cultures of *E. coli*, *P. aeruginosa*, *V. cholerae*, *Salmonella typhi*, *S. flexneri*, and *B. subtilis* bacteria compared to the positive control, Streptomycin. The CCAuNPs were more sensitive against *S. flexneri* with the greatest zone of inhibition, and due to the ROS-independent mechanism of action in pathogens of the synthesized AuNPs, the study suggests their low toxicity to mammalian cells. The cytotoxicity of the CCAuNPs to the HeLa cell line was also exhibited in the study, which was observed in a concentration-dependent pattern. The growth inhibition percentage was also increased with a higher concentration, which gave an inhibition concentration rate (IC<sub>50</sub>) of 316 µg/ml. The following morphological changes in the HeLa cell line upon exposure to CCAuNPs were observed using phase-contrast microscopy: cell burst, cell clumping, cell growth retardation, and loss of membrane stability. All of which were associated with different mechanisms, such as clathrin-dependent endocytosis and micropinocytosis, which led to programmed cell death activation [112].

The presence of tannins in the FTIR analysis confirmed the successful synthesis of Au-NPs from the fresh fruit extract of *C. cujete* mediated by the synthesis of blue-green alga of Au-NPs [113]. Because of the presence of bioactive compounds, also known as effective reducing agents, such as tannins and phenols, the degradation of toxic chemicals produced during the reduction process was made possible. As reported by the FTIR analysis, the present tannins were identified as the primary bioreduction compound of the CCAuNPs. The Au<sub>3+</sub> ions of chloroauric acid were stabilized by tannins and phenols, which are the reduction agents and stabilizers present in the fruit extract, while the alga extract helps to degrade the toxic secondary metabolites [114] easily. The use of ultraviolet-visible spectroscopy, FTIR, and TEM characterized and confirmed the stability of the synthesized CCAuNPs. Plant or biosynthesis of nanoparticles is found to be more advantageous due to their natural pharmacological properties from the plant compounds such as bactericidal, fungicidal, nematocidal, mosquitocidal, and anticancer potential [115].

The influence of *C. cujete* leaves on the growth, antimicrobial, antioxidant, and meat quality in broiler chickens showed its potential as a good natural supplement for livestock [116]. *C. cujete* supplemented (CCL) one-day-old Ross 308 chicks showed a significant change in terms of body weight and carcass weight and lower serum LDL cholesterol and higher HDL cholesterol compared to the untreated birds. CCL birds also showed a higher ileal *Lactobacillus* count, Splenic IL-10, superoxide dismutase, glutathione peroxidase, catalase, and total antioxidant capacity, while promising repression of splenic tumor necrosis- $\alpha$  and immunoglobulin G. This proved that the supplementation of CCL is an effective and cost-efficient method to raise the quality of the livestock, specifically of the broiler chickens.

Traditional fermentation (TF) and gut-filtrated fermentation (GFTF) of *C. cujete* seeds enhanced its nutritional composition, which made it a promising natural source of aqua feedstuff [117]. Both fermented seeds showed a significant increase in all proximate compositions except for nitrogen-free extracts. A 26.15% decrease was observed in the traditionally fermented seeds and a 30.26% decrease in the gut-filtrated fermented seeds. A sudden decrease in alkaloid and phytate contents was also observed on both TF and GFTF with 21.56% and 75%, and 55.88% and 67.85%, respectively. The following proximate and anti-nutrient contents showed an increase for both fermented seeds: saponin and flavonoid TF (47.61 % and 95.44%, respectively) and GFTF (82.63% and 92.07%, respectively), crude protein (79.75% TF and 72.74% GFTF), and lipid (7.82% TF and 17.41% GFTF). The significant decrease in the alkaloid and phytate content in the fermented samples was attributed to the extended hours of soaking to remove the seed coat and the fermentation process [118] and the activity of the endogenous phytase enzyme [119]. On the other hand, the increase in the other contents was attributed to the increase in the number of microorganisms and proteolytic activities of enzymes during the fermentation process [120]. This study shows the potential of fermented *C. cujete* seeds as a good natural source of protein and energy for livestock and animals due to their improved nutritional composition.

Employed as a green, reducing agent to synthesize *C. cujete* AgNPs, a highly-stable antidermatophytic cream was made [121]. Nanoparticles are constantly incorporated into cosmetic formulations to stabilize active constituents such as vitamins, unsaturated fatty acids, and antioxidants, which in turn enhance their pharmacological potential. The presence of tannins and phenols in the leaves of *C. cujete* helped reduce the toxic secondary metabolites during the synthesis of the AgNPs while also stabilizing the other bioactive compounds present in the formulation. The tests showed no change in pH, no phase separation, homogeneous mixture, a stable thermal cycle, thermal change, and freezing and thawing temperature, demonstrating the successful stabilization of the antidermatophytic cream. Although stability was confirmed, further tests to determine its antibacterial or antidermatophytic activity must be performed.

*C. cujete* fruit juice was found to be an effective fungicide against *Trichophyton verrucosum* [122]. Physical administration of the crude ethanolic fruit extract directly onto the dermatomycosis-infected Rena breed calves showed that the 50% concentration (82% inhibition) is the most effective against the dermatomycosis pathogen, followed by the 100% (78%) with the control (5% Iodine at 42% inhibition) being the least effective.

The presence of flavonoids in the *C. cujete* plant is found to be useful as an attractant or repellent to certain pollinators or pests [123]. In Marajó, the green flesh was observed to be the most effective part as a fly repellent due to its abundance in the plant. Although it was considered to have pesticide activity, further tests against other pests should be done to evaluate its pesticide potential.

Response surface methodology and the use of the *C. cujete* fruit pulps to produce bioethanol from *Saccharomyces cerevisiae* [124] and *Cronobacter malonaticus* [125] proved that the use of a wider range of temperatures 25 – 40 °C and 28 – 32 °C for *S. cerevisiae* and *C. malonaticus*, respectively is possible which gives optimized bioethanol despite the concentration of reducing sugars found from the plant pulp. The following optimal parameters were also obtained from the use of *S. cerevisiae* and *C. malonaticus*: pH range of 5.0 – 6.5 and 5.95 – 6.5, inoculum volume of 5.5% v/v and 10% v/v, respectively, which gave the optimal bioethanol yield of 6.19% v/v (*S. cerevisiae*) and 5.08% v/v (*C. malonaticus*).

## 5. Conclusion and Research Prospective

Although numerous studies were already done exploring the pharmacological activities of *C. cujete*, other possible pharmacological activities are yet left unexplored. Some of these possible activities are the anti-fatigue and antinociceptive activities of the plant. Evident enhanced quality when used as a treatment and supplement to livestock and its neurological defense activity may also be attributed to its anti-fatigue and antinociceptive activities. This will be possible by finding the specific extract fractions and extraction methods that are suitable for each pharmacological activity to optimize the plant's potential as a natural source of bioactive compounds/phytochemicals. The presence of numerous and abundant secondary metabolites and nutritive components from the different parts of the plant (Figure 2) only proves the plant's wide possibilities for pharmacological and medical applications. In light of this review, future researchers are encouraged to explore the least explored areas related to *C. cujete* to understand better its full potential, such as a detailed analysis of the pharmacological activities of the plant's seeds, bark, and flower extracts. It should be noted that the commonly used method of preparation is primarily extraction with polar solvents (ethanol, methanol, and aqueous/decoction), which is the most common extraction method also used in indigenous medicine preparations with therapeutic claims confirmed mainly through experimental models *in vitro* and *in vivo* (Table 6). However, a thorough understanding of how these extracts work inside the body of an organism is not yet fully understood. Therefore, the purification and isolation of specific bioactive compounds responsible for the bioactivity of the extracts/fraction paired with *in silico* studies such as the involvement of '-omics' (proteomics, transcriptomics, genomics, and metabolomics studies) is recommended. This will help to understand the mechanism of action of the plant's bioactive compounds that will be beneficial in developing new pharmacological/drug design studies to maximize the economic benefits of the plant fully. All things considered, the review highlighted that most of the studies performed on the pharmacological potentials of the plant are preliminary (*in vitro*) with the use of crude alcoholic and aqueous extracts, and a wide range of reported high-risk diseases such as cancer, hypertension, and infertility/gynecological diseases have yet to be investigated. Hence, this review serves as a reference and inspiration for future researchers interested in the potential of *C. cujete* to focus on these gray areas that are left to be explored to maximize the benefits of the plant.

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### Conflicts of Interest

The authors declare no conflict of interest.

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