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Phytochemical Analysis, Antioxidant and Antibacterial Capacities of a Congolese Traditional Recipe and Medicinal Plants Commonly Used in Prostate Diseases

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Abstract

Root barks of *Morinda lucida*, *Psorospermum febrifugum*, and trunk barks of *Alstonia congensis* are the three medicinal plants that make up a traditional recipe (*JK recipe*) used to treat prostate diseases in the Democratic Republic of Congo. This study aimed to evaluate the antioxidant, antibacterial and anti-inflammatory activities of the *JK recipe* as well as extracts from the selected parts of these three herbal medicines. Additionally, to better characterize the different parts of plants investigated, their microscopic histological features and their phytochemical fingerprints were determined. Powdered micrographic examination showed specific microscopic features for each botanical that constitute the database useful for the identification and authentication of these plants. The phytochemical study also focused on the reconstituted formulation. The TLC phytochemical analysis revealed the presence of various compounds, including coumarins, terpenoids, tannins, phenolic acids, and

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flavonoids, in both the reconstituted formulation and the individual plants. All three plants displayed important ABTS and DPPH radical-scavenging activities connected with their IC_{50} values with *P. febrifugum* as the most potent (IC_{50} = 18.410 ± 2.920 µg/mL for ABTS and IC_{50} = 92.5 ± 4.25 µg/mL for DPPH). Root barks of *M. lucida* exhibited a strong effect on the germ mostly involved in bacterial prostatitis, *Escherichia coli* (MIC = 250 µg/mL) followed by trunk barks of *A. congensis* (MIC = 500 µg/mL). The traditional formulation exhibited moderate antioxidant activity (IC_{50} = 50.8 ± 2.8 µg/mL for ABTS and IC_{50} = 107.2 ± 7.9 µg/mL for DPPH) and excellent anti-inflammatory activity. The antioxidant and antibacterial capacities of the three studied species and the anti-inflammatory activity of the formulation may have potential therapeutic interest and could justify their utilization in traditional medicine for the treatment of prostate diseases, but further studies are needed, especially *in vitro* and *in vivo* anti-cancer activities to demonstrate their efficacy on prostate cancer.

Keywords

A. congensis, *M. lucida*, *P. febrifugum*, Prostate, Prostatitis, Prostate Cancer, Benign Prostatic Hyperplasia, Prostatic Adenoma, Traditional Recipe

1. Introduction

Prostatitis, Benign Prostatic Hyperplasia (BPH) and prostate cancer are the three major ailments recognized as diseases of the prostate gland [1]. Prostatitis is a clinically significant entity with a prevalence ranging from 2.2% to 9.7% in adult males and is a relatively common condition in men under fifty [1] whereas prostatic adenoma and prostate cancer are mostly found in elderly men. With age and ageing, some significant symptoms of prostate disorders appear and become a source of anxiety [2]. Prostatic adenoma (Benign Prostatic Hyperplasia), disabling but not cancerous, affects half of the population of men aged seventy, while prostate cancer is the third most common cancer in men [3]. Nowadays, medicinal plants are widely used in several disease affections, including prostate diseases [4].

The virtues of the plants are recognized in the whole world, the medicinal plants being the object of more than 5000 studies published each year worldwide [5] and the WHO highlights the proper utilization of natural products and marked plant-based medicines as prime study candidates [6]. In DRC (Democratic Republic of the Congo), traditional medicine is considered the only effective medicine for certain segments of the population [7]. Easily accessible, traditional practitioners and their remedies, generally based on plants, are considered the last resort for illnesses for which the treatment offered by modern medicine is ineffective or too costly, such as prostate cancer [8] [9]. The modern treatment for prostate diseases is essentially based on the utilization of antibiotics, analgesics, anti-inflammatories, hormonal and surgical treatments, and even cancer chemotherapy [10]. How-

ever, the cost of the medication is too high, making it less accessible to low-income patients [11]. Additionally, most of the population is still tied to traditional medicine and relies on medicinal plants and remedies from traditional healers. In DRC, a survey conducted in Kinshasa, the capital of the country, revealed that about a hundred percent of participants acknowledged having visited a traditional healer for natural remedies, and eighty percent declared that the care offered by traditional healers was effective. The use of traditional medicines in DRC can be justified by the effectiveness of care, the type of diseases, as well as the affordability and proximity of remedies [7].

Herbal medicinal plants are reputed to be a source of a wide variety of bioactive molecules—"secondary metabolites" that can actually be useful against many diseases [12]. Several traditional remedies have been shown to be very much effective. However, due to a lack of data or studies highlighting their potential, it is difficult to demonstrate their therapeutic benefits in treating human diseases. Thus, it is necessary to assess and guarantee the efficacy of these herbal traditional medicines using appropriate methods so they can serve the entire population for healthcare [13]-[15]. The aim of this study was to compare the efficacy of a traditional recipe with its component through the assessment of their antioxidant, anti-inflammatory and antibacterial activities. Additionally, to better characterize the different parts of plants investigated (trunk bark of *A. congensis* and root barks of *M. lucida* and *P. febrifugum)*, their microscopical features and their phytochemical fingerprints were determined.

2. Material and Methods

2.1. Plant Material

Figure 1. Description of plant material—(A) *A. congensis* plant (1) and harvest; (B) harvesting of *M. lucida* (1) and roots of the plant (2) and (C) *P. febrifugum* plant (1) and the root of the plant (2).

The roots of *P. febrifugum* (Mukuta mutshi), *M. lucida* (Mulala mbwa), and the trunk bark of *A. congensis* (Muimu mutshi) were collected in Mont-Ngafula, as shown in **Figure 1**, in Kinshasa, by Jeanne KIABU, a traditional practitioner. These samples were air-dried at room temperature. The plants were identified by Boniface Nlandu of the Institut National d'Etudes et de Recherches en Agronomie (INERA), University of Kinshasa, Kinshasa, Democratic Republic of Congo. A specimen was deposited for each plant in the herbarium of the Faculty of Sciences (*A.*

congensis: BL1; *M. lucida*: BL2; *P. febrifugum*: BL3) at the University of Kinshasa. Prior to extraction, the plant materials were ground and stored in brown-covered glass bottles. The reconstituted formulation (*JK Recipe*), a decoction made from a mixture of the three plants, was also obtained from the traditional healer and stored at 4˚C. All biological tests were conducted within two weeks of their preparation.

2.2. Reagents and Chemicals

The solvents used were HPLC analytical grade. They were purchased from Merck VWR (Leuven, Belgium) as well as 2,2'-azino-bis-(3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS). 2-Aminoethyl diphenylborinate, anisaldehyde, and potassium persulfate were purchased from Sigma (Bornem, Belgium). Caffeic acid, chlorogenic acid (purity: 95%), and gallic acid (purity: 97%) were purchased from Sigma-Aldrich (Saint-Louis, United States). Rutin (purity: 99%), isoquercitrin (purity: 99%), and quercetin (purity: 98.5%) were HPLC quality and were purchased from Extrasynthese (Genay, France).

2.3. Microscopic Analysis

The microscopic analysis of the powders was carried out using the Steinmetz reagent (lactic acid reagent from the European Pharmacopoeia). The microscopic observation was made with a Novex BBPPH 86.375 microscope and the photos were captured using an iPad (8^{th} generation) model MYL92LL/A.

2.4. Preparation of Extracts

The organic extracts were prepared by a 48-hour percolation of 10 g of each plant's powder in 100 ml of a methanol-dichloromethane (1:1) mixture, followed by solvent evaporation. Aqueous extracts were prepared by decoction, using 10 g of sample powder in 100 ml of water. The decoction was cooled to room temperature before filtration, and the solvent was evaporated. The extracts, along with the formulation obtained from the traditional healer, were then weighed and stored in dark, hermetically sealed flasks at 4˚C.

2.5. Identification of Secondary Metabolites by TLC

Analytical TLC of 10 µL of solution for 1 g/5 mL of methanolic and ethyl acetate extracts was carried out on normal phase Silicagel 60 F_{254} plates (Merck), using different eluents for the identification of secondary metabolites [16].

2.6. Determination of Secondary Metabolites by UV-Visible Spectroscopy

2.6.1. Determination of Total Polyphenols

The total phenolic content of methanolic extracts (Methanol 80%) was determined according to the Folin-Ciocalteu method [17]. A calibration curve of gallic acid (0.025 - 0.4 mg/mL) was prepared, and phenolic contents were determined

in triplicate from the linear regression equation of this curve. The results were expressed as milligrams of gallic acid equivalent per gram of dried drug (mgGAE/gDD).

2.6.2. Determination of Total Flavonoids

The flavonoid content of the extracts was determined by UV-Vis spectrophotometry. Results are expressed in mg of quercetin equivalent per gramme of dry vegetal material (mgQE/gDD) [17].

2.7. Cell-Free Antioxidant Assay

The dried recipe and plant extracts were solubilized in methanol, and their effects were compared to a control test containing methanol alone. Antioxidant activity was assessed using spectrophotometric ABTS and DPPH assays, performed according to the method described by Kapepula *et al.* [18].

2.8. Antibacterial Activity

The antibacterial activity of the organic extracts of the studied parts of the plants was evaluated on three germs implied in bacterial prostatitis (*E. coli*, *S. aureus* and *P. aeruginosa*) by the method of microdilution under the conditions and the process described by Elaka *et al.* [19].

2.9. Acute Anti-Inflammatory Test Using the Formalin-Induced Mouse Paw Oedema Method

2.9.1. Principle

Inflammation was induced by injecting a 1% formalin solution into the aponeurosis of the mouse's foot sole. The edema caused by this phlogogenic agent was evaluated by measuring the foot perimeter with a caliper, allowing for the monitoring of the progression of the inflammatory response [20] [21].

2.9.2. Procedure

For the acute anti-inflammatory activity test, we used 4 batches of mice, which were fasted for 17 hours before the test.

The different treatments were administered by gavage as follows, with the different batches of mice outlined in Table 1.

Table 1. Different batches of mice.

	Number of mice	Treatment administered	Dosage
Batch 1: Untreated	5	-	-
Batch 2: Positive control	5	Ibuprofen	150 mg/kg body weight
Test Batch A	5	Sample	150 mg/kg
Test Batch B	5	Sample	300 mg/kg

– Untreated batch: Mice in this batch received an injection of formalin (75 µL;

1%) into the arch of the right paw

- Positive control batch: Mice in this batch were treated orally with ibuprofen, 30 min before formalin injection. Ibuprofen was administered at a dose of 150 mg/kg body weight.
- Test batch A: The extract to be tested (*JK* Recipe) was administered to mice orally at a dose of 150 mg/kg; 30 min before formalin injection.
- Test batch B: The extract to be tested (*JK* Recipe) was administered to mice orally at a dose of 300 mg/kg; 30 min before formalin injection.

Oedema progression was monitored by measuring the paw diameter of mice in the treated group ($D(t)$) and the untreated group ($D(nt)$) at 0, 30, 60, 120, and 180 minutes after formalin injection. The anti-inflammatory activity of the tested products and its progression were assessed by calculating the average percentage of oedema inhibition, using the following formula: Inhibition Percentage (%) = $\frac{D(nt)-D(t)}{D(nt)}\times 100$, with $D(nt)$ = mean diameter of the oedematous leg of the untreated group, $D(t)$ = Mean diameter of the oedematous leg of the treated group, t: the moment of measurement in minutes past after formalin injection.

2.10. Statistical Analysis

Each assay was performed at three replicates and results were expressed as mean values ± standard deviation (SD). Statistical analysis was performed with GraphPad Prism 9.3.1 (GraphPad Software, San Diego California, USA). Two-way analysis (ANOVA) and paired Student's t-test were used and the level of statistical significance was set at $p < 0.05$. The IC_{50} values were calculated with GraphPad Prism 9.3.1 under the application of the function "log (inhibitor) vs normalized response-variable slope" after converting the concentrations to their respective decimal logarithms.

3. Results and Discussion

3.1. Microscopic Histological Characteristics

The microscopic examination carried out by bringing 2 to 3 drops of Steinmetz's reagent into contact on a microscope slide with a small quantity of bark powder from each plant showed the following microscopic elements (**Figure 2**). The interest in the micrographic characterization of plant powders lies in the fact that during the mechanical grinding of samples of plant origin (barks, leaves, fruits, barks, roots, etc.), the microscopic histological elements only undergo a simple swipe. They can, therefore, be observed under a microscope and thus facilitate the identification not only of the plant but also of the part of the latter whose grinding provided the powder thus studied. Crude powdered drugs can be identified based on the shape, presence or absence of different cell types based on their cytomorphological characters, e.g., parenchyma, collenchyma, fibers, stone cells, vessels, trichomes, secretory cells, and epidermal cells [22]. Indeed, vegetable powders of

economic or medical interest are subject to numerous falsifications making one pass for the other of greater value, of identical appearance but not having the same biological properties. Adulteration in plant samples is a serious problem that involves the deliberate or inadvertent mixing of one plant species with other lower-value species, plants of other genera, or even toxic materials [23]. So far, very few studies have described the micrographic characteristics of these three plants, in particular concerning the parts which have been the subject of our study, which means the stem bark of *A. congensis* as well as the roots bark of *M. lucida* and *P. febrifugum*.

A Previous work [24] studied the leaves of *M. lucida* demonstrated straight epidermal cells with a bundle of scalariform xylem vessels, oil droplets, and lignified fibers. There is also the presence of unicellular, diamond-shaped clothing trichomes and calcium oxalate prisms. This study that we have carried out thus provides a database making it possible to certify the identity of the vegetable powders obtained from the studied plants' parts. However, in-depth micrographic examinations will be welcome mainly to determine the dimensions of certain characteristic elements such as the size of the starch grains (highlighted in the root barks of *M. lucida* and *P. febrifugum* and tree trunk bark of *A. congensis*), calcium oxalate prisms, and the length of fibers and sclerides [23].

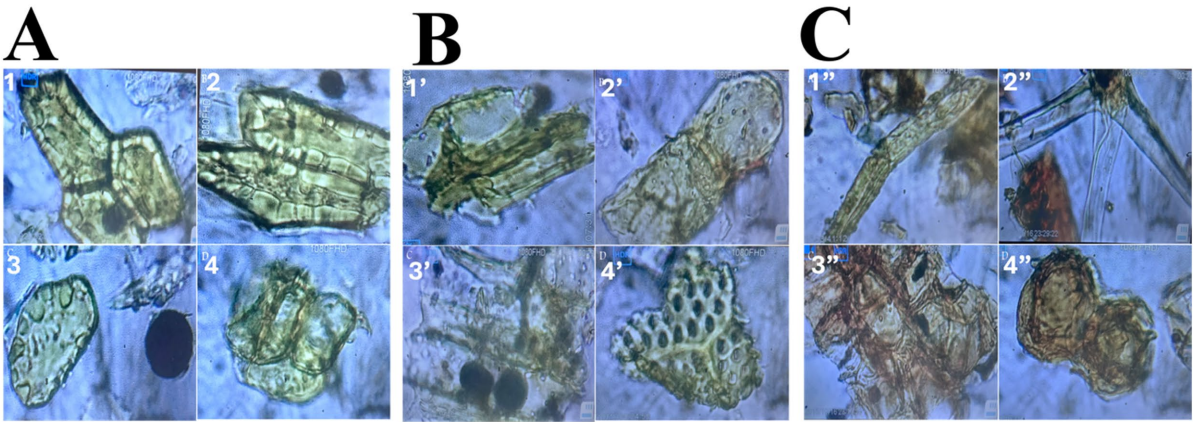

Figure 2. Illustration of histological features of (A) *A. congensis*—grouped sclereids of various shapes (1, 2), isolated sclereids and spherical starch grain (3), and tracheids (4); (B) *M. lucida*—parenchyma fragments (1'), tracheids (2', 3'), and a fragment of punctate vessels (4'); (C) *P. febrifugum*—fragment of sclerotic fiber (1"), Hair (2"), Fragment of suber (cork) studded with crystals (3") and Cells from the cortex (4").

3.2. Phytochemicals

Phytochemical analysis revealed the presence of phenolic acids, flavonoids, iridoids, tannins and terpenes. TLC fingerprints of different parts were different and characteristic. They showed the presence of flavonoids by green and yellow fluorescence spots and phenolic acids as main compounds for trunk bark of *A. congensis* the root bark of *M. lucida* and *P. febrifugum* as well as in the *JK Recipe* (**Figure 3(A)** and **Figure 3(B)**).

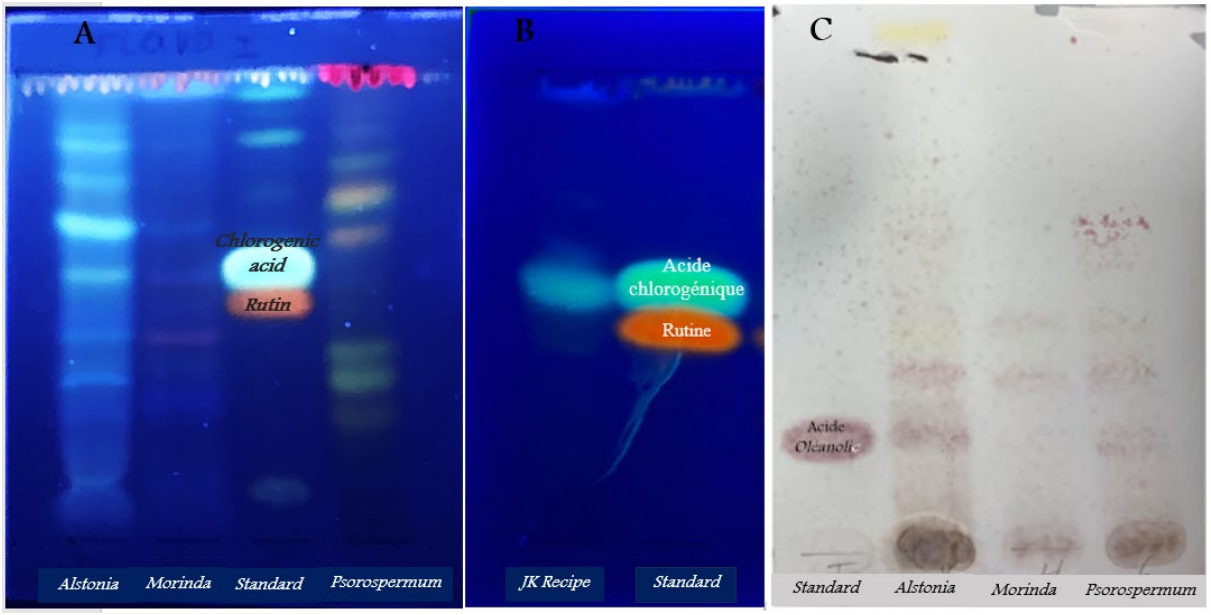

Figure 3. TLC chromatograms of (A) the methanol extracts of the plants studied in the system: SP: Silicagel F_{254}; MP: Dichloromethane/Acetic acid/Acetone (100:11:11:26) in the presence of controls. Flavonoids appear as yellow, yellow-orange, orange or green spots and phenol acids as blue fluorescent spots at 366 nm with Neu's reagent. (B) TLC chromatograms of the methanol extracts of the dried *JK Recipe* studied in the system: SP: Silicagel F254; MP: Dichloromethane/Acetic acid/Acetone (100:11:11:26) in the presence of standards. Flavonoids appear as yellow, yellow-orange, orange or green spots and phenol acids as blue fluorescent spots at 366 nm with Neu's reagent. (C) TLC chromatograms of the ethyl acetate extracts of the plants studied in the system: SP: Silicagel F254; MP: Toluene/Ethyl acetate (9:1). Terpenes appear as purple spots in the visible with sulfuric anisaldehyde.

These results corroborate those of Agbogba *et al.* [25] who detected the presence of saponins, polyphenols, gallic tannins, catechic tannins and anthocyanins in the root bark of *P. febrifugum*. Regarding *A. congensis*, the trunk barks are rich in flavonoids. Lumpu *et al.* [26] detected in the leaves of *A. congensis* the presence of alkaloids, flavonoids, saponins, anthraquinones, terpenoids and/or steroids, tannins, and anthocyanins while Cimanga *et al.* [27] demonstrated and isolated alkaloids in root barks.

Terpenes were highlighted in all the extracts and oleanolic acid was present in *A. congensis* and *P. febrifugum* (**Figure 3(C)**). Anthony *et al.* [28] also detected terpenes and coumarins, but also gallic and catechic tannins in the extracts of *A. congensis*.

As it concerns *M. lucida*, the methanolic extract studied contains phenolic compounds and flavonoids also highlighted by Osuntokun *et al.* [29] while the ethyl acetate extract is rich in terpenoids and coumarins.

3.3. Polyphenols and Flavonoids Content

The flavonoid and total polyphenol contents of the trunk barks of *A. congensis* and root barks of *M. lucida* and of *P. febrifugum* were determined from the linear regression equations of the calibration curves (**Figure 4**), plotted using quercetin and gallic acid respectively as standards for flavonoids and polyphenols.

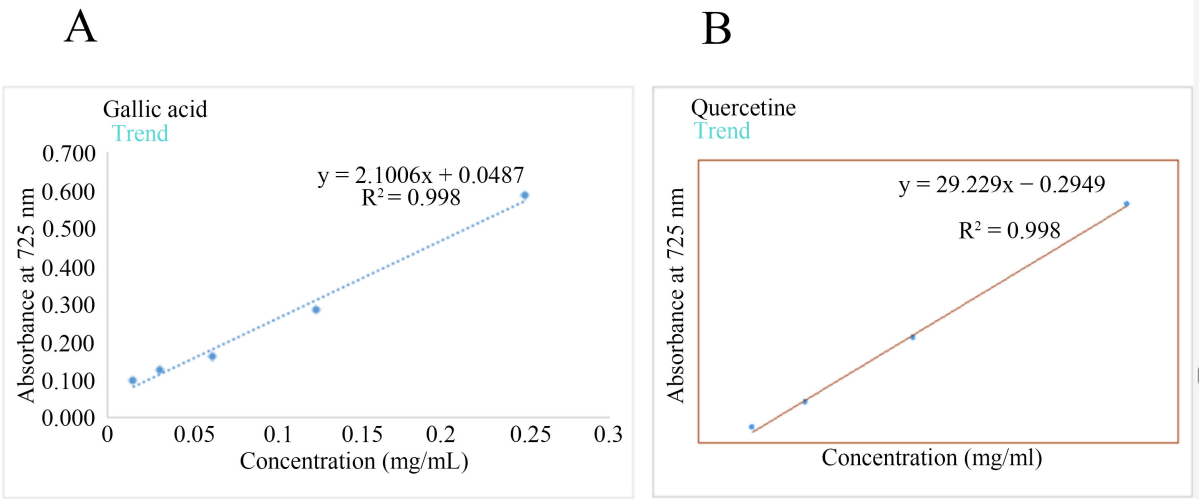

Figure 4. Trend lines of (A) Quercetin and (B) Gallic acid.

The values of the total flavonoid and polyphenol contents are given in the table (Table 2) below.

Table 2. Results of the determination of flavonoids and polyphenols.

Plants	Studied parts	Flavonoids (mgQE/1g DD)	Polyphenols (mgGAE/1g DD)
A. congensis	Trunk bark	22.0 ± 0.4	78.91 ± 1.2
M. lucida	Root bark	24.7 ± 0.1	40.4 ± 2.0
P. febrifugum	Root bark	56.7 ± 0.2	63.7 ± 2.7

Note: mgQE, mg Quercetin Equivalent; mgGAE, mg Gallic Acid Equivalent; DD, Dry Drug.

The root barks of *P. febrifugum* are richer in flavonoids than the root bark of *M. lucida* and trunk bark of *A. congensis*. This last one would however contain more polyphenols than *M. lucida* and *P. febrifugum*.

3.4. Antioxidant Activities

ABTS and DPPH assays showed that extracts of *A. congensis*, *M. lucida* and *P. febrifugum* parts can scavenge free radicals connected with their IC_{50} values below. Table 3 and Figure 5 indicate that all the extracts have good antiradical activity, which would be dependent on the secondary metabolites identified in the plants.

ABTS reacts simultaneously with the hydrophilic and lipophilic compounds of the matrix, which explains the values of IC_{50} that are weaker compared to DPPH which reacts only with the hydrophilic compounds of the analyzed matrix [18]. The aqueous extract and the organic extract of the root barks of *M. lucida* have the same inhibitory activity of the ABTS radical because the difference in their IC_{50} is not statistically significant (P = 0.3522, NS).

By the DPPH test, the antioxidant activity of *M. lucida* was also highlighted by

Osuntokun *et al.* (2016) [29]. Its extracts are however less active than those of *A. congensis* and *P. febrifugum* which inhibit the formation of the ABTS radical at lower concentrations. It should be noted that the difference in activities is not significant between the aqueous extract and organic extract of *A. congensis* either for the inhibition of the ABTS radical (P = 0.2091; NS) or for the DPPH radical (P = 0.2959; NS). As for *P. febrifugum*, the aqueous extract proved to be more active than the organic extract for the inhibition of ABTS ($P < 0.05$, statistically significant difference), but also of DPPH ($P < 0.05$, statistically significant difference). The aqueous extract of *P. febrifugum* is, therefore, the one that most effectively inhibits the formation of the ABTS radical (IC_{50} = 18.410 ± 2.920 µg/mL) but also that of DPPH (IC_{50} = 92.5 ± 4.25 µg/mL).

To the best of our knowledge, no study has so far assessed the antioxidant activity of the root barks of *P. febrifugum*. Other parts of the plant have certainly already been the subject of numerous studies, in particular those that demonstrated the antioxidant activity of the trunk bark of *P. febrifugum* [30] and those which assessed the antioxidant activity of leaves and trunk bark [31]. The antioxidant activities are identical for the aqueous and organic extracts of *M. lucida* and *A. congensis*. As for *P. febrifugum*, activity is greater for the aqueous extract than for its organic extract. Thus, the decoction which constitutes the form of preparation of the traditional recipe has very good antioxidant activity. Moreover, these data reveal that heat does not alter the antioxidant activity of the said plants, it even seems to increase that of the bark of the roots of *P. febrifugum*. Moreover, Anthony *et al.* [28] demonstrated greater antioxidant activity for the aqueous extract of *A. congensis* than for its organic extracts, with IC_{50} values weaker for the inhibition of ABTS but also of DPPH, which makes water a better extraction solvent for this activity. The antioxidant activities of the plants in the recipe would help fight against oxidative stress associated with prostate diseases.

Table 3. IC_{50} values (µg/mL) of the aqueous and organic extracts of the three plants for the tests at ABTS and DPPH (mean ± standard deviation, n = 3).

Plants	Studied parts	Types of extracts	ABTS (IC_{50} in µg/mL)	DPPH (IC_{50} in µg/mL)
A. congensis	Trunk bark	Aqueous	35.9 ± 7.1	99.3 ± 9.9
		Organic	27.9 ± 5.8	114 ±18.7
M. lucida	Root bark	Aqueous	91.4 ± 13.8	131 ±12.3
		Organic	102.1 ± 10.9	144 ±16.0
P. febrifugum	Root bark	Aqueous	18.4 ± 2.9	92.5 ± 4.3
		Organic	41.0 ± 4.7	130.3 ± 8.8
	JK Recipe		50.8 ± 2.8	107.2 ± 7.9
	Quercetin		**3.57 ± 0.18**	**7.05 ± 0.10**

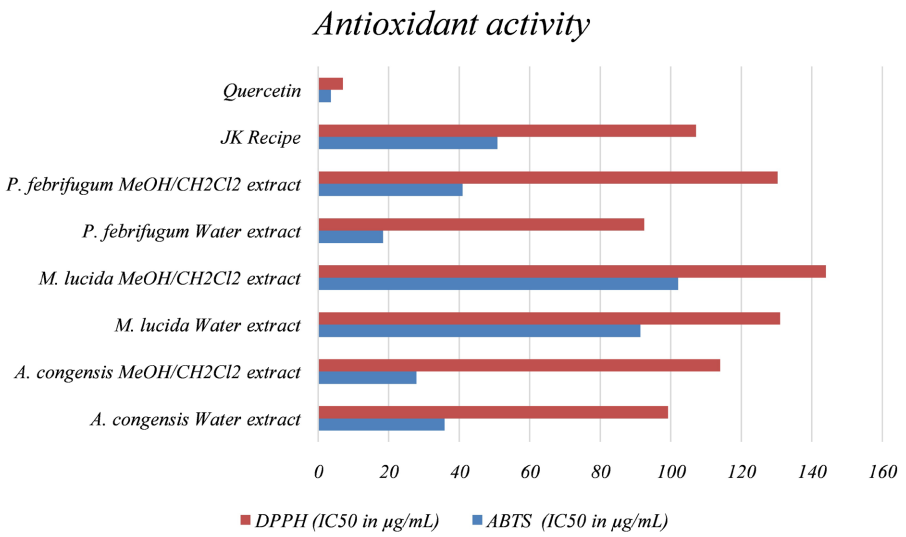

Figure 5. Antioxidant activity of the recipe and the aqueous and organic extracts of the three plants for the tests at ABTS and DPPH.

3.5. Antibacterial Activities

The antimicrobial activity is weak for the extract of the root bark powder of *P. febrifugum* against *S. aureus* while it is null against *E. coli* and *P. aeruginosa* with a minimum inhibitory concentration greater than 4000 µg/mL. In the literature, studies that have evaluated the antibacterial activity of *P. febrifugum* concerned other parts of the said plant, in particular Namukobe *et al.* [30] who assessed the antimicrobial efficacy of stem bark from *P. febrifugum* on *E. coli* with good activity observed for the methanol and ethyl acetate extracts, while the aqueous extract was found to be inactive. Tamokou *et al.* [32] demonstrated the antibacterial activity of the trunk bark of *P. febrifugum* on *S. typhi*, *E. coli*, *L. monocytogenes* and *S. aureus*. The results of the evaluation of the antibacterial activity by the microdilution method are recorded in **Table 4** below.

Regarding *M. lucida*, the root bark powder extract is inactive on the *P. aeruginosa* and weakly active against *S. aureus* while active on *E. coli* (MIC = 250 µg/mL), which corroborates the result obtained by Fakoya *et al.* [33] who demonstrated the antibacterial activity of the bark of *M. lucida* on *E. coli* with efficacy comparable to Ciprofloxacin. Furthermore, the work of Adomi [34] highlighted the activity of the leaves of *M. lucida* on *E. coli*, *P. aeruginosa*, *S. typhi*, *S. aureus*, *K. pneumoniae* et *B. subtilis*. As for *A. congensis*, the extract of trunk bark is inactive on *S. aureus* (MIC = 2000 µg/ml) and on *P. aeruginosa* (MIC > 4000 µg/ml), while it is active on *E. coli* whose growth it inhibits with an MIC = 500 µg/mL, which corroborates previous results [35] who demonstrated significant antibacterial activity of the trunk bark of *A. congensis* on *E. coli*. Furthermore, Lumpu *et al.* [26] showed that the leaves of *A. congensis* were active against germs responsible for diarrhea such as *B. cereus*, *E. coli*, *S. typhimurium*, *S. flexneri*, *S. sonnei*, *S. dysenteria* and *S. aureus*. The *JK Recipe* showed no activity against the tested germs. In sum, *M. lucida* the sample that showed the best efficiency on *E. coli*, the germ

most involved in bacterial prostatitis with an MIC = 250 µg/mL, followed by *A. congensis* whose trunk bark extract exerts an inhibitory activity on *E. coli* with a minimum concentration equal to 500 µg/mL.

Table 4. MIC of extracts in different germs.

Methanol/Ethyl acetate extracts		MIC in µg/mL		
Plants	Used parts	*Staphylococcus aureus* ATCC25923	*Escherichia coli* ATCC25922	*Pseudomonas aeruginosa* ATC 257783
A. congensis	Trunk bark	2000	500	>4000
M. lucida	Root bark	1000	250	>4000
P. febrifugum	Root bark	500	>4000	>4000
JK Recipe		>4000	2000	>4000

Note: MIC values below 500 µg/mL suggest good antibacterial activity, while extracts with MICs between 500 and 1000 µg/mL have low antibacterial activity and those with MICs above 1000 µg/mL are considered inactive [38].

3.6. Anti-Inflammatory Activity

The preclinical trials conducted to evaluate the anti-inflammatory activity of the studied formulation, compared to ibuprofen used as a control, revealed the following results at the specified time points post-formalin injection (30 minutes, 60 minutes, 120 minutes, and 180 minutes) as summarized in **Table 5**, which presents the changes in mouse paw diameter before and after administration of a 1% formalin solution. Data represent the mean paw diameter in millimeters:

- The percentage inhibition of mouse paw diameter increase by ibuprofen at these time points was 7.02%, 11.36%, 29.49%, and 23.44%, respectively, with the peak anti-inflammatory activity observed at 120 minutes.
- At a dose of 150 mg of the formulation per kg, the percentage inhibition of mouse paw diameter increase was 0.83%, 1.36%, 12.44%, and 3.13%, respectively.
- For the formulation at a dose of 300 mg/kg, the percentages of inhibition were 2.07%, 2.27%, 23.96%, and 13.54%, respectively.

Thus, the formulation demonstrates significant anti-inflammatory activity, with a maximum peak observed at 120 minutes, at which point the anti-inflammatory effect of the formulation (23.96% inhibition) approaches that of ibuprofen (29.49% inhibition) administered at a dose of 150 mg/kg. However, the onset of action for the formulation is notably longer than that of ibuprofen, which exhibits significant activity as early as 30 minutes post-administration at the same dosage per kg of mouse body weight. These findings are summarized in **Table 6**, which presents the percentages of inhibition of mouse paw diameter increase.

To the best of our knowledge, this study is the first to investigate the anti-inflammatory activity of the formulation derived from the bark of *A. congensis*, as well as the root bark of *M. lucida* and *P. febrifugum*. Furthermore, there are limited studies on the acute anti-inflammatory activity of *A. congensis*, *M. lucida*, and *P. febrifugum* evaluated in isolation.

Table 5. Changes in mouse paw diameter before and after administration of a 1% formalin solution. data represent the mean paw diameter in millimetres.

Samples	At the time of injection of 1% formol	30 min	60 min	120 min	180 min
No treatment	1.91 ± 0.55	2.42 ± 0.41	2.20 ± 0.49	2.17 ± 0.21	1.92 ± 0.25
Ibuprofen 150 mg/kg	1.53 ± 0.35	2.25 ± 0.52	1.95 ± 0.50	1.53 ± 0.47	1.47 ± 0.30
JK Recipe (150 mg/kg)	1.84 ± 0.22	2.40 ± 0.31	2.17 ± 0.17	1.90 ± 0.23	1.86 ± 0.42
JK Recipe (300 mg/kg)	1.60 ± 0.12	2.37 ± 0.22	2.15 ± 0.33	1.65 ± 0.18	1.66 ± 0.29

Table 6. Percentages of inhibition of mouse paw diameter increase.

Samples	30 min	60 min	120 min	180 min
Ibuprofen 150 mg/kg	7.02	11.36	29.49	23.44
JK Recipe (150 mg/kg)	0.83	1.36	12.44	3.13
JK Recipe (300 mg/kg)	2.07	2.27	23.96	13.54

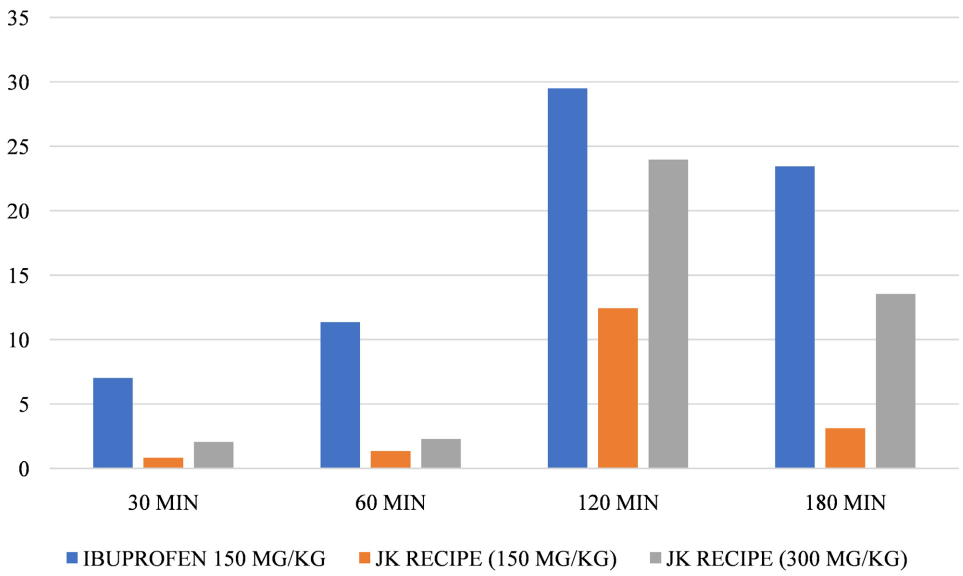

Figure 6. Anti-inflammatory activity of the *JK Recipe* and the positive standard (Ibuprofen).

However, a study conducted by Nwokocha *et al.* [37] demonstrated that the aqueous extract of *A. congensis* bark exhibited significant anti-inflammatory activity in rats subjected to carrageenan-induced inflammation. The extract effectively reduced paw oedema and leukocyte migration into inflammatory tissues. While there are no studies specifically addressing the anti-inflammatory activity of the root bark of *M. lucida* and *P. febrifugum*, the results of Nwokocha *et al.* [37] support our findings, asserting that the trunk bark of *A. congensis*, one of the components of the formulation, possesses significant anti-inflammatory properties. These results, illustrated in **Figure 6** and **Figure 7** suggest that the observed anti-inflammatory effects may be attributed either to *A. congensis* alone or to a synergistic interaction among the three plant components.

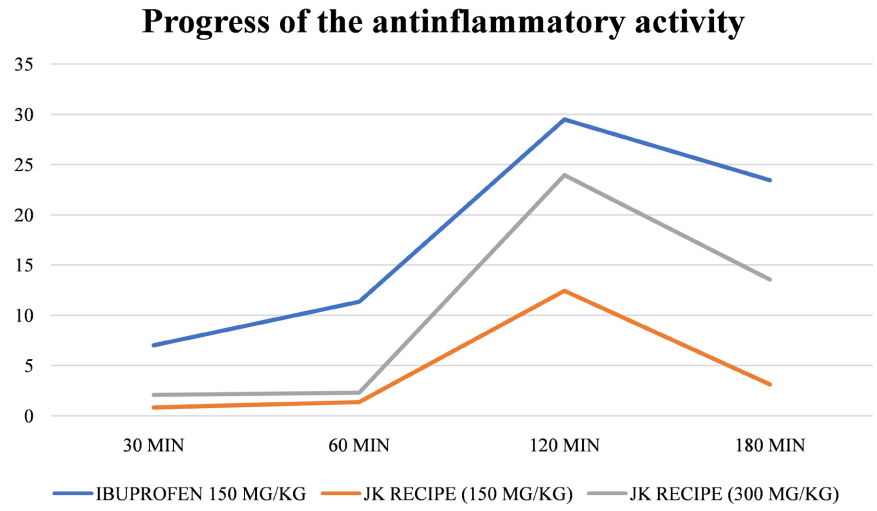

Figure 7. Progression of the anti-inflammatory activity of ibuprofen and the JK formulation from the time of 1% formalin injection (T = 0 min) to 180 minutes after treatment administration.

Regarding *M. lucida* and *P. febrifugum*, some studies have investigated other parts of these plants. Olukemi *et al.* [38] found that the ethanolic extract of *M. lucida* leaves exhibited significant anti-inflammatory activity in rats subjected to carrageenan-induced inflammation, effectively reducing paw oedema and leukocyte migration in inflamed tissues. Additionally, Asogwa *et al.* [39] highlighted the anti-inflammatory activity of the ethanolic extract of *P. febrifugum* leaves.

Given that the formulation demonstrates established anti-inflammatory activity, its use is warranted in the treatment of prostate diseases associated with inflammation of this organ within the male urogenital system. Nonetheless, further studies are necessary to evaluate the chronic anti-inflammatory activity of both the formulation and the individual plants.

4. Conclusions

In conclusion, the evaluation of the antioxidant and antibacterial capacities of the root barks of *M. lucida*, *P. febrifugum*, and the trunk bark of *A. congensis* has yielded promising results that support the potential application of these plant extracts in traditional recipes for managing prostate diseases. The identification and characterization of novel active compounds from this traditional recipe would significantly contribute to the scientific understanding of their therapeutic properties. The confirmed antioxidant and anti-inflammatory activities highlight the relevance of these species in traditional medicine.

Phytochemical analysis via thin-layer chromatography (TLC) of the dry extract of the formulation revealed the presence of secondary metabolites, including phenolic acids and terpenes, which may account for the anticipated biological activities. While the formulation demonstrated significant antioxidant and anti-inflammatory effects, it exhibited inactivity against the tested pathogens, which may limit its applicability in cases of bacterial prostatitis caused by *E. coli*. Nonetheless,

we hope this work serves as a foundational step toward developing an enhanced traditional medicine indicated for prostate-related diseases. Future research should encompass the evaluation of chronic anti-inflammatory, anticancer, and antibacterial activities against all pathogens capable of colonizing and infecting the prostate. We also advocate for this formulation's acute and chronic toxicity studies to ensure the safety of patients who may utilize it.

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Conflicts of Interest

The authors declare no conflict of interest regarding the publication of this paper.

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