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Antioxidant, Antifungal and Antitermite Activities of Residues from Hydrodistillation of *Cymbopogon citratus, Eucalyptus camaldulensis* and *Mentha piperita*

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Abstract

Aromatic plant distillation produces residues rich in phenolic compounds known to be bioactive. In this context, residues from the hydrodistillation of *Mentha piperita* L., *Cymbopogon citratus* Stapf, and *Eucalyptus camaldulensis* Dehnh were chemically and biologically analyzed. The ethanol percentages (70%, 50%, and 30%) were evaluated by determining antioxidant activity using 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging, total phenolic content, and total flavonoid content. Antifungal activity was evaluated "*in vitro*" against *Fusarium oxysporum, Alternaria alternata, Trametes versicolor,* and *Coniophora puteana* using the diffusion method in malt-agar medium. Antitermite activity was also assessed against *Reticulitermes flavipes* using the most active extract with low ethanol concentrations (50% and 30%). The optimum ethanol concentration to extract phenolic compounds is 70% for TFC and 30% for TPC, and the antioxidant activity was 0.45 ± 0.01 mg/mL (IC_{50}). The most active extracts were the ethanolic ones, particularly for *Eucalyptus camaldulensis,* presenting 100% inhibition against the mycelial growth of *Coniophora puteana* and *Alternaria alternata* at 1 mg/mL and 0.5 mg/mL, respectively. For antitermite activity, EE50 was the most effective, with a 24% termite survival rate and a degraded food supply surface of 28%. These data suggest the potential use of hydrodistillation residues for biopesticide development.

Keywords

Biopesticide, Polyphenols, Flavonoids, Antiradical Capacity, Antifungal, Antitermite

1. Introduction

Organic farming, considered a sustainable agricultural practice, is promoted worldwide. Plant-based formulations are interesting alternatives to synthetic chemical pesticides for phytosanitary treatment of crops [1]. Among pesticidal plants, aromatic plants are widely cited as candidates for phytosanitary applications [2]. These plants are mainly valued for their essential oils, used in perfumes, food flavorings, cosmetics, spirits, aromatherapy, and as botanical pesticides. However, the hydrodistillation process to obtain essential oils generates a considerable quantity of residues that still contain bioactive molecules, such as polyphenols, including flavonoids, known for many biological activities [3] [4]. Polyphenols constitute an important class of bioactive molecules with antioxidant, antibacterial, and pesticidal properties [5] [6].

Among the aromatic plant species widely available in Burkina Faso, *Cymbopogon citratus* (Stapf, 1906) (Poales: Poaceae) DC, *Eucalyptus camaldulensis* (Dehnh, 1832) (Myrtales: Myrtaceae), and *Mentha piperita* (Linné, 1753) (Lamiales: Lamiaceae), commonly known as lemongrass, eucalyptus, and peppermint respectively, are of interest for their biological activities, which justify their use in folk medicine or as biopesticides. Extracts from *C. citratus* exhibit antibacterial, insecticidal, and antifungal activities [7] [8]. Flavonoid-enriched extracts from *M. piperita* leaves reduced the *in vitro* the mycelium growth of *Phoma sorghina* and *Fusarium moniliforme* [9]. *E. camaldulensis* leaves are rich in phenolic acids, especially gallic acid, and flavonoid glycosides such as quercetin 3-*O*-rutinoside, quercetin 3-*O*-glucoside, quercetin 3-*O*-glucuronide, and kaempferol 3-*O*-glucuronide [10] [11]. Some studies have highlighted the biological properties and applications of *E. camaldulensis* [12] [13]. A previous study showed that a 70% ethanolic extract of *E. camaldulensis* leaves is effective against the termite *Reticulitermes flavipes* [3]. However, to date, the residues of these three aromatic plants, obtained after hydrodistillation and rich in polyphenols, particularly flavonoids, have not yet been assessed against *Fusarium oxysporum* (*F. oxysporum*), *Alternaria alternata* (*A. alternata*), *Trametes versicolor* (*T. versicolor*), and *Coniophora puteana* (*C. puteana*), which are major threats to agricultural production and/or wood in service.

In this work, the antifungal activities of the residues of three aromatic plants were evaluated, and the mineral composition of the plants was determined to assess their effect as macronutrients for fungi. Additionally, the chemical profile, the influence of solvent concentration on phytochemical contents, and the antioxidant, antitermite, and antifungal activities of the active extract were assessed.

Bioethanol is an environmentally friendly solvent like water but is costly. To reduce the amount of ethanol in the hydroalcoholic solvent used for extraction, three different ethanol concentrations (70%, 50%, and 30%) were screened for their phytochemical profile and biological activity.

The study aimed to evaluate the biopesticide potential of residues from three aromatic plants to incorporate them into a circular economy system.

2. Materials and Methods

2.1. Chemicals and Reagents

In addition to distilled water, the following chemicals, reagents, and solvents used were of analytical grade: Butan-1-ol (Sigma Aldrich), glacial acetic acid (VWR Chemicals), dimethyl sulfoxide (DMSO) (Sigma Aldrich), bioethanol 70%, methanol (HPLC-MS grade, CHEM-LAB, 99.99%), quercetin (Merck), gallic acid (Sigma Aldrich), diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich), aluminum chloride hexahydrate $AlCl_3 \cdot 6H_2O$ (Carlo Erba), sodium carbonate (Sigma-Aldrich, Steinheim, Germany), Folin-Ciocalteu reagent (Fluka Analytical), and formic acid (CARLO ERBA).

Collection of plant material: Fresh aerial parts of *Cymbopogon citratus, Mentha piperita,* and leaves from *Eucalyptus camaldulensis* Lenhn aerial parts were collected at Ouagadougou, Burkina Faso. Identification of the three plants was conducted at the Laboratory of Botanical Biology and Ecology (LABEV) at the University Joseph KI-ZERBO in Ouagadougou, Burkina Faso. Numbers (N˚ID) were attributed as follows: 17,048, 6878 for *Cymbopogon citratus* (Stapf, 1906) (Poales: Poaceae), 17,047, 6877 for *Mentha piperita* (Linné, 1753) (Lamiales: Lamiaceae), and 17,046, 6876 for *Eucalyptus camaldulensis* (Dehnh, 1832) (Myrtales: Myrtaceae).

2.2. Extraction Procedures for Aqueous and Ethanolic Extracts

A sample (100 g) of air-dried plant powder was hydrodistilled using a Clevenger-type apparatus. The resulting crude mixture was filtered to obtain an aqueous solution and a solid residue. The aqueous solution was frozen and lyophilized with a LABCONCO FreeZone 2.5 plus apparatus to obtain the aqueous extract. The solid residue was dried for 72 hours at room temperature and then macerated under continuous magnetic stirring with 1 L of 70% ethanol for 24 hours at room temperature. The solvent was evaporated using a BUCHI R-100 rotavapor at 40˚C under 175 mbar to obtain the ethanolic extract. From the three plants, six extracts were obtained and named: *Cymbopogon citratus* Aqueous and Ethanolic extracts (CA and CE, respectively); *Eucalyptus camaldulensis* Aqueous and Ethanolic extracts (EA and EE, respectively); *Mentha piperita* Aqueous and Ethanolic extracts (MA and ME, respectively). The extraction capacity was evaluated using three ethanol concentrations: 70%, 50%, and 30% ethanol/water (v/v). The extracts from 50% and 30% ethanol were prepared similarly to the 70% ethanol extract and named EE70, EE50, and EE30 [3].

2.3. Mineralogical Analysis by Atomic Absorption Spectrometry (AAS)

The sample preparation method, based on calcination with silica elimination, involved weighing 1 g of air-dried plant powder in a platinum capsule. The capsule was placed in an oven, and the temperature was gradually increased to 450˚C, where it was maintained for 2 hours. The resulting ashes were allowed to cool to ambient temperature, then moistened with a few milliliters of demineralized water and dissolved in 3 mL of nitric acid and 1 mL of concentrated hydrofluoric acid. The solvent was gently evaporated to dryness using a hot plate. This process was repeated twice, each time adding 2 mL of nitric acid and 1 mL of hydrofluoric acid. The residue obtained is taken up in 3 mL of nitric acid and left to dissolve for 15 minutes before adding 20 mL of demineralized water. The solution is slightly heated and then transferred to a beaker. The capsule containing the solution is rinsed with hot demineralized water and boiled. After cooling, the solution volume is adjusted to 100 mL in a volumetric flask. The solution is then sent for AAS measurement for the following elements: Calcium (Ca), Potassium (K), Sodium (Na), Magnesium (Mg), Iron (Fe), Copper (Cu), Zinc (Zn), Manganese (Mn), Nickel (Ni), Cadmium (Cd), Chromium (Cr), and Lead (Pb) using appropriate cathode lamps for the element of interest [14].

2.4. Total Phenolic Content Measurement

Total Phenolic Content (TPC) of each extract was determined using the Folin-Ciocalteu colorimetric method [15]. The standard calibration curve (0.00048-0.03125 mg/mL) was prepared from gallic acid, and the total phenolic content was calculated as the average of triplicate data for each sample in milligram equivalents of gallic acid per gram of dry extract (mg GAE/g). A linear regression analysis equation ($y = 0.015x + 0.026$, $R^2 = 0.9994$) was applied to calculate TPC, where y is the absorbance at 415 nm, and x is the amount of gallic acid equivalent (GAE) in mg per gram of extract.

2.5. Total Flavonoid Content Measurement

The total flavonoid content (TFC) of the extracts from each plant was determined using the aluminum chloride ($AlCl_3$) method with slight modifications [16]. Two mL of a 2% AlCl3 methanolic solution was added to 2 mL of a methanolic plant extract solution. The mixture was shaken and incubated at room temperature for 40 minutes. The absorbance of the reaction mixture was measured at 420 nm with a UV/visible spectrophotometer, BMG Labtech SPECTROstar Nano, against a blank (methanol). A calibration curve was established (0.03 - 0.001 mg/mL) using a series of quercetin solutions as a reference. All preparations and analyses were carried out in triplicate. The TFC was calculated and expressed as milligrams per gram.

The total flavonoid content of each extract was calculated and expressed as milligrams of quercetin equivalents per gram of dry weight of extract (mg QE/g), using a calibration curve range with quercetin (0.03 - 0.001 mg/mL) as a standard ($y = 37.721x + 0.0039$; $R^2 = 0.9993$).

2.6. Determination of Antioxidant Activity

The antioxidant activity was determined using the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) method as a free radical. The inhibition assay used the Brand-Williams method modified by Miliauskas *et al.* [17]. To conduct this experiment, a methanolic solution of the DPPH radical (6×10^{-5} M) was prepared by dissolving 2.36 mg of DPPH in 100 mL of methanol. The absorbance (AR) of this solution was then determined.

To 150 µL of methanol solution of each plant extract, 4.5 mL of DPPH solution from the stock solution was added to a tube. The mixture was incubated at 28˚C in the dark for 20 minutes before measuring the absorbance at 515 nm. Absorbances were measured with a BMG Labtech SPECTROstar Nano UV/visible spectrophotometer against a blank (methanol). Percent inhibition was calculated according to equation (5). IC_{50} values (concentration that inhibits 50% of the DPPH radical) were determined by plotting the percent DPPH radical inhibition versus extract concentration using linear regression analysis. All preparations and analyses were performed in triplicate.

DPPH percentage inhibition was calculated as the average of triplicate data for each sample concentration using the following formula:

$$\%\text{inhibition} = \left(AB - AE\right) / AB \times 100, \tag{1}$$

where AB is the blank absorbance of the DPPH radical and AE is the absorbance of the extract. The percentage of inhibition was plotted against sample concentration, and the IC_{50} value in mg/mL was calculated using linear regression.

2.7. High Performance Liquid Chromatography (HPLC) Analysis

The extracts obtained from three different aqueous ethanol grades—70%, 50%, and 30% (*i.e.*, EE70, EE50, and EE30)—were submitted to HPLC analysis.

The prepared samples (1 mg/ml in HPLC-grade methanol) were solubilized in an ultrasonic bath for 2 minutes and then filtered through a 0.22 µm Millipore filter membrane before analysis. Individual phenolic compounds were analyzed using a U-HPLC 3000 liquid chromatography system equipped with a degasser, binary gradient pump, and multi-wavelength UV detector (DAD - 3000 RS and MWD - 3000 RS). A reversed-phase analytical column was used for separation (Hypersil BDS C18, 150 x 4.6 mm, 5 µm). The working temperature was 30˚C. The mobile phases consisted of Milli-Q water (A) with 0.1% formic acid and HPLC-grade methanol (B) with 0.1% formic acid. The solvent gradient was as follows: 0 min, 3% B; 10 min, 9% B; 20 min, 16% B; 50-55 min, 50% isocratic; 60 min, 3% B. The flow rate was 0.5 ml/min, and the injection volume was 20 µl. Data analysis was performed using Chromeleon software v.6.80 (Dionex, Thermo Fisher Scientific). Chromatographic peaks corresponding to each phenolic compound were identified by comparing their retention times and UV spectra with those of the phenolic standards. The quantification was based on calibration curves obtained from the phenolic standards. HPLC was carried out at a wavelength of 254 nm, which is characteristic of phenolic compounds.

2.8. Antifungal Essay

One isolate from each fungal species was tested using the diffusion method in malt-agar medium. Aqueous and ethanol solutions at different concentrations were used to prepare malt-agar medium (malt 40 g/L, agar 20 g/L) sterilized at 121˚C for 20 minutes in an autoclave. The ethanol extracts were dissolved in 10% DMSO. After cooling to 40˚C, the medium was distributed in Petri dishes 9 cm in diameter. Five (5) mm diameter mycelial explants of each fungus were taken using a punch, placed in the center of a Petri dish containing the culture medium, and then sealed with Parafilm®. The Petri dishes were placed in the incubation room at 25˚C ± 2˚C under 12-hour alternating cycles of near-ultraviolet light (NUV) and darkness for *Alternaria alternata* and *Fusarium oxysporum*, or at 22˚C and 70% relative humidity (RH) in the dark for *Trametes versicolor* and *Coniophora puteana*, for 7 days. A control with malt-agar (TE) was prepared. The diameter of the colonies was measured in each dish every two days after incubation of the explants. The experiments were conducted in three repetitions, and the mycelial growth inhibition rates were calculated using the formula in equation 1.

Inhibition rate (%) = $[(A - B)/A] \times 100$ equation 1;

Inhibition rate (%) = inhibition percentage of the mycelial growth;

A = mean colony diameter of the negative control (cm);

B = mean colony diameter of each treatment (cm).

2.9. Antitermite Assay

The antitermite activity of eucalyptus was evaluated using extracts from low concentrations of ethanol (50% and 30%), as a previous study showed the ethanol (70%) extract to be active [3].

The test was performed using the direct contact method described by Ohtani *et al.* [18] with slight modifications. Briefly, pure cellulose papers (Joseph paper) of 1 cm^2 were impregnated with 90 μL of aqueous and ethanolic solutions of extracts at different concentrations (10%, 5%, 2.5% m/m). The papers impregnated were dried at 20˚C for 2 hours. The tests involved placing 15 g of wet Fontainebleau sand (1 volume of water for 4 volumes of sand) on the periphery of petri dishes. The treated papers were placed on a grid in the middle of the petri dishes to avoid excessive humidity. Then, 20 termite (*Reticulitermes flavipes*) workers were spread over the sand. The Petri dishes containing the termites were stored in a dark room at 27˚C with a relative humidity of 75% for 28 days and were regularly monitored throughout the test. Two controls were used: an untreated paper and a paper impregnated with ethanol 70% (as a solvent control). The tests were conducted in three repetitions. At the end of the test, termite survival rates and paper degradation rates were determined.

3. Results

3.1. Inorganic Elements in Aqueous Extracts

AAS analysis results of aqueous extracts CA, EA, and MA are reported in **Table 1**.

Table 1. Mineralogical composition of aqueous extracts.

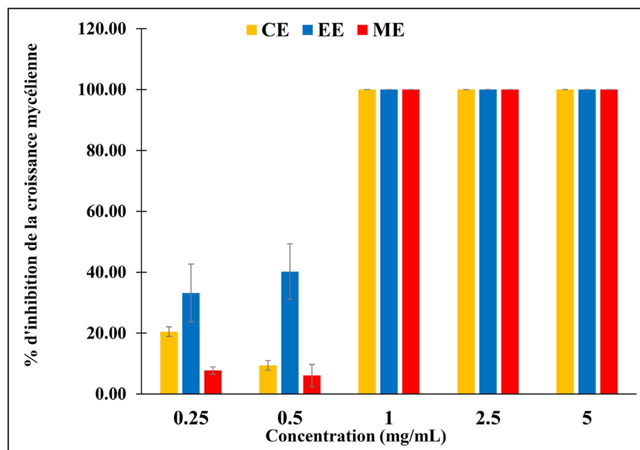
		Extracts		
		CA	EA	MA
Element content (ppm)	Ca	25404.02	215.42	638.79
	Mg	11387.46	4072.63	17971.11
	Na	2834.66	1465.42	758.26
	K	97413.71	3713.74	102182.25
	Mn	64.9	503.05	38.95
	Fe	692.17	450.67	71.53
	Zn	39.4	51.14	71.67
	Cu	10.42	7.32	19.39
	Ni	7.58	12.43	12.25
	Cr	0.31	0.68	1.48
	Cd	0.48	0.14	0.46
	Pb	1.46	2.13	1.1
	Total	137856.57	10494.77	122767.24

CA: lemongrass aqueous extract; EA: eucalyptus aqueous extract; MA: mentha aqueous extract.

The highest values of inorganic elements as macronutrients are Ca (Calcium), Mg (Magnesium), and K (Potassium). The inorganic element contents of CA and MA are the same, while eucalyptus has the lowest value in total inorganic elements.

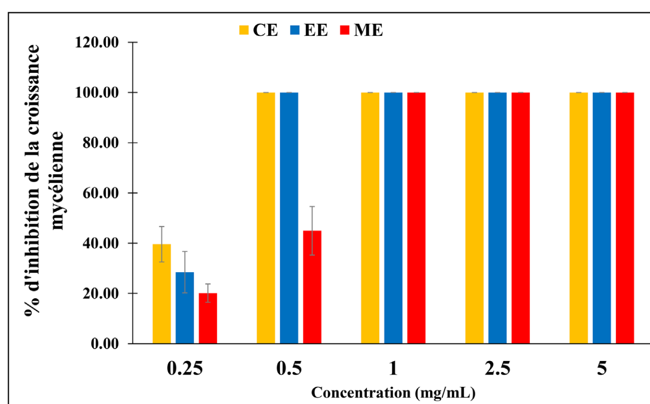
3.2. Antifungal and Antitermite Properties

Results on the antifungal activities of ethanolic extracts at different concentrations on the fungi *Fusarium oxysporum* (*F. oxysporum*) and *Alternaria alternata* (*A. alternata*) are presented as histograms in **Figure 1** and **Figure 2**. These data suggest that the three ethanolic extracts completely (100%) inhibited the mycelial growth of both pathogenic fungi at high concentrations of 5, 2, and 1 mg/mL. At low concentrations, the inhibition percentages were less than 50% for *F. oxysporum* (**Figure 1**). However, the ethanolic extract of eucalyptus (EE) was the most effective, displaying an inhibition percentage of approximately 40%, higher than the other two, which showed inhibition of less than 20%. On *A. alternata* (**Figure 2**), only the ethanolic extracts of citronella (CE) and eucalyptus (EE) were effective against the fungus, achieving complete inhibition (100%) of mycelial growth at a concentration of 0.5 mg/mL.



Ethanolic extracts: CE (lemongrass); EE (eucalyptus); ME (mentha).

Figure 1. Percentage inhibition of mycelial growth by ethanolic extracts on *Fusarium oxysporum*.



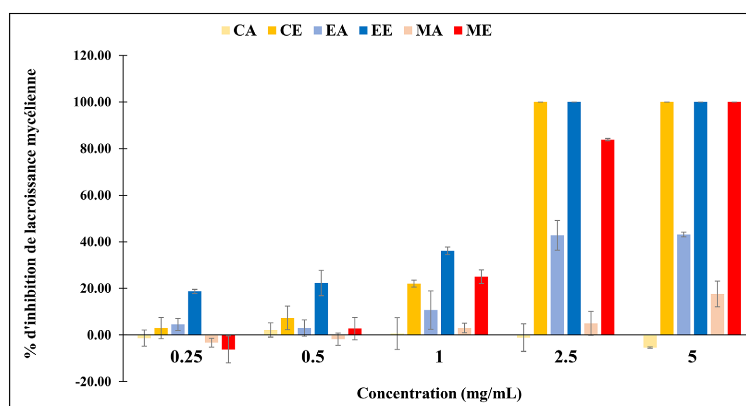
Ethanolic extracts: CE (lemongrass); EE (eucalyptus); ME (mentha).

Figure 2. Percentage inhibition of mycelial growth by ethanolic extracts on *Alternaria alternata*.

Figure 3 and Figure 4 show the histograms of the percentage inhibition of mycelial growth of *Trametes versicolor* (*T. versicolor*) and *Coniophora puteana* (*C. puteana*). At a concentration of 2.5 mg/mL, CE and EE are effective against *T. versicolor*, with 100% inhibition and 83.84% for ME. However, at lower concentrations (1, 0.5, and 0.25 mg/mL), all extracts were less active, with inhibition percentages below 40% (Figure 3).

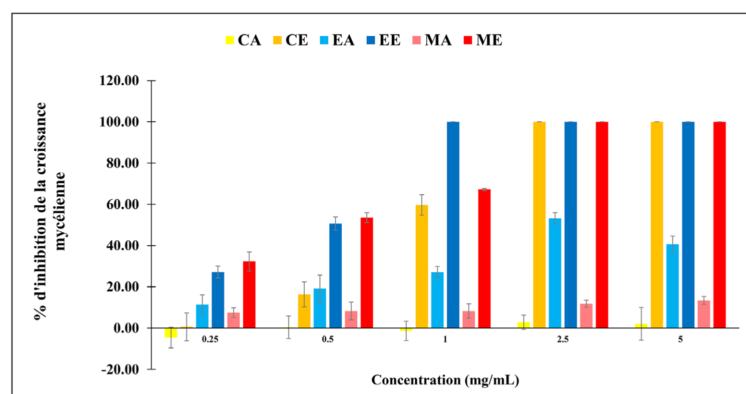
Concerning *C. puteana*, the three ethanolic extracts (CE, EE, and ME) at 5 and 2.5 mg/mL concentrations effectively inhibited 100% of the mycelial growth of the fungus. At a concentration of 1 mg/mL, only EE showed 100% inhibition of mycelial growth. At that concentration, the CE and ME extracts maintained good inhibition percentages at 59.67% and 67.20%, respectively. Extracts EE and ME exhibited significant inhibitions of 50.66% and 53.52%, respectively, at a dose of 0.5 mg/mL. At the concentration of 0.25 mg/mL, all extracts showed lower inhibition percentages below 35% (Figure 4).

Aqueous extracts stimulated fungal growth at the highest concentration (5 mg/mL) compared to controls. However, at 2.5 mg/mL, EA showed an average inhibition of 53.17% on *C. puteana* (**Figure 4**). In general, eucalyptus (aqueous and ethanolic extract) proved to be the most effective against all the studied fungi.



Ethanolic extracts: CE (lemongrass); EE (eucalyptus); ME (mentha); Aqueous extracts: CA (lemongrass); EA (eucalyptus); MA (mentha).

Figure 3. Percentage inhibition of mycelial growth of ethanolic extracts on *Trametes versicolor*.



Ethanolic extracts: CE (lemongrass); EE (eucalyptus); ME (mentha); Aqueous extracts: CA (lemongrass); EA (eucalyptus); MA (mentha).

Figure 4. Percentage inhibition of mycelial growth by ethanolic extracts on *Coniophora puteana*.

3.3. Ethanol Grade Effect on Chemical Profile and Pesticidal Activities of EE Extract

In previous research, aqueous ethanol 70% used as a solvent is known to avoid mineral elements [3]. Regarding the highest biopesticidal potential presented by EE, two additional concentrations with reduced quantities of ethanol were investigated: 50% and 30%.

3.4. Effect on Chemical Profile Variability

The chemical profile variability is based on TPC, TFC, and AA of the eucalyptus

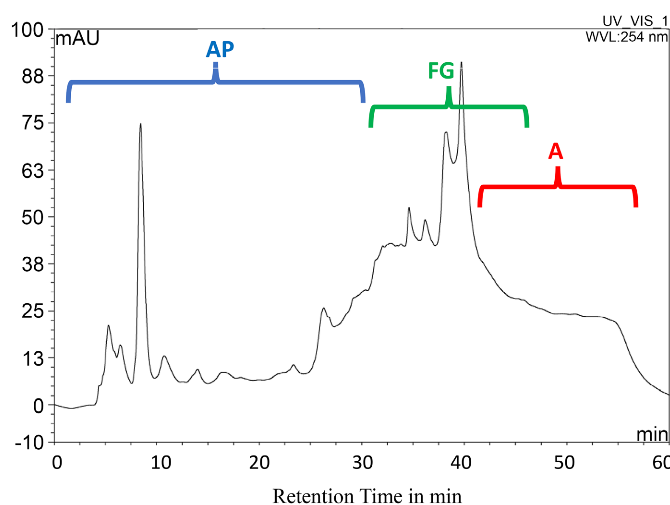
ethanolic extract (EE) with different ethanol concentrations. The contents obtained vary from 106.68 to 363.93 mg GAE/g for total polyphenols and from 17.89 to 48.33 mg QE/g for total flavonoids (Table 2). The extract with the lowest ethanol concentration (30%) has the highest content of polyphenols (363.93 ± 84.63 mg GAE/g) and the lowest content of total flavonoids (17.89 ± 2.54 mg QE/g). The highest total flavonoid content (48.33 ± 1.5 mg QE/g) is obtained with a 70% ethanol extract, which presents the lowest content of total polyphenols (106.68 ± 12.51 mg GAE/g). According to the results in Table 2, reducing the ethanol percentage is proportional to the total flavonoid content and inversely proportional to the total polyphenol content.

Table 2. Effect of ethanol concentration on the phytochemical contents and antioxidant activity of eucalyptus ethanolic extract (EE).

Concentration of ethanol (%)	TPC (mg GAE/g of dry extract)	TFC (mg QE/g of dry extract)	DPPH (IC_{50} mg/mL)
EE 70	106.80 ± 12.51	48.33 ± 1.50	0.64 ± 0.016
EE 50	259.53 ± 27.60	23.30 ± 1.36	0.47 ± 0.011
EE 30	363.93 ± 84.63	17.89 ± 2.54	0.41 ± 0.010

It can be concluded that 70% aqueous ethanol is the most effective extractive solvent for flavonoid extraction. It was found that reducing the ethanol percentage decreases flavonoid content, increases polyphenol content, and reduces the AA value.

The eucalyptus extracts analyzed through HPLC chromatographic profiling show a solvent effect on chemical sub-families: phenolic acids, aglycones, and glycosides (Figure 5 and Figure 6).



AP: phenolic acids; FG: glycosyled flavonoids; A: aglycons.

Figure 5. Zone of chemical groups in the chromatographic profile of eucalyptus aqueous extract.

Observations on different zones of the chromatogram indicate that the extract with 30% ethanol (EE 30), which has high water content, contains more glycosides and phenolic acids similar to the aqueous extract profile.

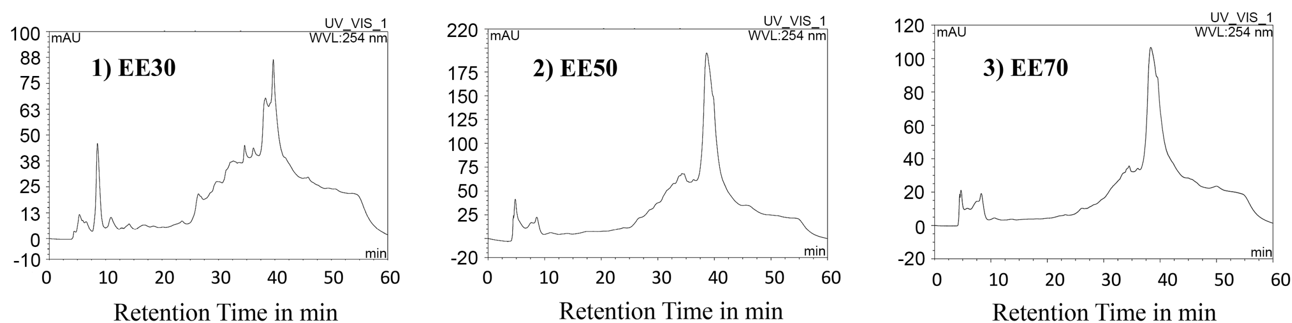
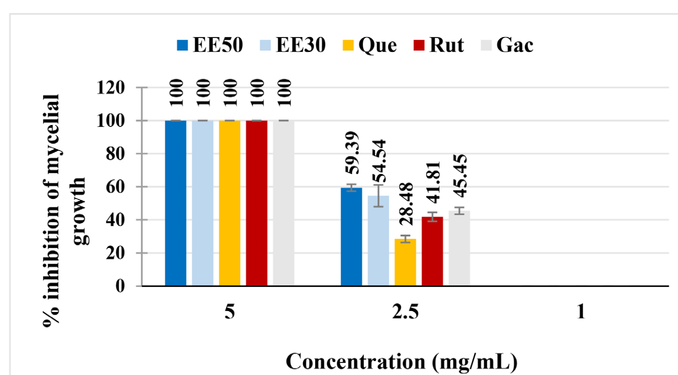


Figure 6. Chromatographic profiles of extracts 1) EE30, 2) EE50 and 3) EE70.

3.5. Effect on Antifungal and Antitermite Activities of EE Extract

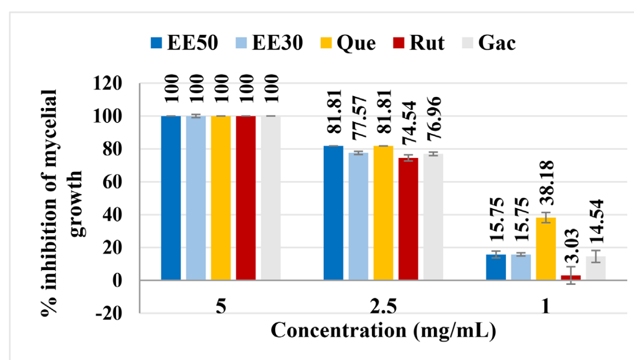
The antifungal and antitermite tests were conducted using extracts obtained with lower ethanol concentrations of 30% (EE30) and 50% (EE50) to evaluate the minimal effective concentration of ethanol needed for efficient extraction. Gallic acid, quercetin, and its glycosylated form, rutin, were also used in the tests to assess the structure-activity relationship of the extract's components.

The results on antifungal activity showed that at 5 mg/mL, the extracts (EE50 and EE30), as well as the standards, completely inhibited the mycelial growth of the two fungi, *T. versicolor* and *C. puteana*. The decrease in ethanol concentration had no effect on the extract's efficacy against fungi at 5 mg/mL (Figure 7 and Figure 8). At 2.5 mg/mL, the extracts and standards were effective against *C. puteana*, with inhibition percentages ranging from 74.54% to 81.81%. However, on *T. versicolor*, only EE50 and EE30 were effective at 2.5 mg/mL, with inhibition percentages of 59.39% and 54.54%, respectively. EE50 was found to be more active than the standards, suggesting a synergistic action within the components of the extract.



EE50: 50% ethanolic eucalyptus extract; EE30: 30% ethanolic eucalyptus extract; Que: quercetin; Rut: rutin; Gac: gallic acid.

Figure 7. Effect of ethanol concentration on mycelial growth of *Trametes versicolor*.



EE50: 50% ethanolic eucalyptus extract; EE30: 30% ethanolic eucalyptus extract; Que: quercetin; Rut: rutin; Gac: gallic acid.

Figure 8. Effect of ethanol concentration on mycelial growth of *Coniophora puteana*.

At 1 mg/mL, extracts and standards showed weak activity on *C. puteana* and no activity on *T. versicolor*. However, when the ethanol concentration decreased by 20%, the inhibition percentage of *T. versicolor* decreased by nearly 40%, from EE (100%) to EE30 (54.54%) at a concentration of 2.5 mg/mL (Table 3). On *C. puteana* at the same concentration, the percentage inhibition decreased by nearly 20% from EE70 (100%) to EE30 (77.57%) (Table 3). Regarding the antitermite test, only the EE50 extract was effective against termites at 10%, with a survival rate of 24% and a paper degradation surface of 28% (Table 4).

Table 3. Effect of decreased solvent concentration of eucalyptus ethanolic extract on cth of *T. versicolor and C. puteana*.

	% Inhibition (*C. puteana*)			% Inhibition (*T. versicolor*)		
	Concentration (mg/mL)					
Extracts	5	2.5	1	5	2.5	1
EE50	100.00 ± 0.00	81.81 ± 0.00	15.75 ± 2.09	100.00 ± 0.00	59.39 ± 2.09	0.00 ± 00
EE30	100.00 ± 0.00	77.57 ± 1.04	15.75 ± 2.77	100.00 ± 0.00	54.54 ± 6.55	0.00 ± 00

Table 4. Antitermite test by treatment of cellulose paper with ethanolic extracts of eucalyptus.

	Concentration (% m/m)					
	5			10		
Extracts	DT (days)	TS	SD (%)	DT (days)	TS	SD (%)
EE50	6	90	100	27	24	28
EE30	6	90	100	15	55	100
T_0	27	0	x	x	x	x

Continued

Tcontrol	27	90	100	x	x	x
TEtOH	27	90	100	x	x	x

EE50: 50% ethanolic eucalyptus extract, EE30: 30% ethanolic eucalyptus extract; T_0: no control (termites exposed to no cellulose paper), Tcontrol: water control (termites exposed to cellulose paper soaked in water), TEtOH: ethanol control (termites exposed to cellulose paper soaked in ethanol); SD: degraded surface of the cellulose paper, TS: termite survival rate, DT: treatment duration.

4. Discussion

Regarding different results from *in vitro* antifungal tests, ethanolic extracts from the solid residues of hydrodistillation of three aromatic plants (*Cymbopogon citratus, Eucalyptus camaldulensis* and *Mentha piperita*) were shown to be more active (fungicides or fungistats). The effect varies with plant species and depends on the dose for the mycelial growth of *F. oxysporum, A. alternata, C. puteana* and *T. versicolor*. Similar observations were reported in previous studies [3]. The inhibitory effects of extracts from 13 plants on mycelial growth and conidial germination of *F. oxysporum* f. sp. *lycopercisi* and *P. infestans* showed that ethanolic extracts completely inhibited both at 25 mg/mL, whereas aqueous extracts required 50 mg/mL [19]. Jahan *et al.* [20] demonstrated that the methanolic extract of eucalyptus leaves was more effective against *E. coli* and *Bacillus subtilis* compared to ethyl acetate and aqueous extracts, with the aqueous extract being the least effective [20].

The ethanolic extracts and essential oils of three species of eucalyptus leaves, namely *E. camaldulensis*, *E. globulus* and *E. tereticornis*, were tested against *Colletotrichum gloeosporioides*. The ethanolic extracts were more effective than the essential oils. The ethanolic extract of *E. camaldulensis* inhibited fungal growth by 98% at 5000 mg/L, while *E. globulus* showed 50% inhibition at 500 mg/L [21]. Four extracts from the leaves of *E. camaldulensis* were effective against *A. pisi*, a pathogen of *Pisum sativum*. In particular, the alcoholic extract (butanol) was able to control the pathogen on all varieties of pepper tested [22]. A moderate inhibition of the eucalyptus aqueous extract was observed in this study. This extract contains bioactive compounds in low doses or competitive nutritive compounds for the fungus, reducing its efficiency against both *C. puteana* (53.17% at 2.5 mg/mL and 40.66% at 5 mg/mL) and *T. versicolor* (43.13% at 5 mg/mL and 42.78% at 2.5 mg/mL). Similar results showed that the aqueous extract of *C. citratus* was ineffective against *Bipolaris onyzae* and *Fusarium moniliforme*, either by stimulating the mycelial growth of the former or by showing no activity against the latter, compared to the water control [23]. Thus, the aqueous extract of eucalyptus, which showed the strongest antioxidant activity, was able to moderately inhibit the mycelial growth of *C. puteana*. This result aligns with the findings of Fernandez-Agullo *et al.* [24], who showed that the aqueous extract of wood residues from *E. globulus* moderately inhibited the growth of bacteria such as *S.*

epidermis, *S. aureus*, *B. cereus*, *B. subtilis*, *E. coli*, *P. aeroginosa*, and fungi such as *C. parapsilosis*, *C. krusei* and *C. glabrata*. The 50% ethanolic extract showed the strongest antioxidant activity in their study. The aqueous extract of *E. camaldulensis* was effective against four bacterial strains (*P. aeruginosa*, *E. coli*, *S. aureus* and *Bacillus subtilis*), with inhibition zones ranging from 9 to 14 mm [25]. The inactivity of the aqueous extracts can be explained by various mechanisms, including the non-diffusion of the extracts in the culture medium, the absence or low concentration of active molecules in the tested extracts, or the presence of nutrients [26] [27]. Macronutrients such as potassium and magnesium are used in significant amounts by fungi for growth. This could explain why aqueous extracts rich in potassium and magnesium are often found inactive, particularly for *M. piperita* and *C. citratus* aqueous extracts, as shown in this study.

On the other hand, we investigated the total phenolic content, total flavonoid content, and antioxidant activity using different extracts with varying solvent concentrations (70%, 50%, and 30% ethanol). These solvents were chosen for being environmentally friendly and less toxic to humans. According to the results, the EE30% aqueous ethanol extract showed the highest total phenolics content (363.93 ± 84.63 mg EAG/g) and antioxidant activity (0.41 ± 0.010 mg/mL). These results proved that solvents with low alcohol levels could efficiently extract polyphenols. However, EE70 shows the highest total flavonoid content, with fewer phenolic acids and glycosides by HPLC. According to Gullon *et al.* [28], alcohols like ethanol are commonly used solvents to extract flavonoids due to their strong hydrogen-bonding interactions with the hydroxyl and carbonyl groups of flavonoids [28]. A high percentage of water in ethanol favors the extraction of glycosides still present in the solid residue, while EE70 would contain more aglycones.

Decreasing the ethanol concentration showed that 50% and 30% ethanol extracts are equally effective as the 70% ethanol extract at 5 mg/mL. Thus, the 30% ethanolic extract of eucalyptus can be used at 5 mg/mL for *T. versicolor* and at 2.5 mg/mL for *C. puteana* in antifungal control. Since the extracts are generally more effective against fungi than the standards individually, this suggests their effectiveness is likely due to the combined action of these or other compounds.

The data on antitermite activity showed that the EE50 extract was more effective against termites than the EE30 extract. However, the EE extract (70% ethanol) remains the most effective against termites, as demonstrated in a previous study [3] and in antimicrobial and schistosomicidal assays [10]. In fact, this extract resulted in survival rates of 0% and degraded surfaces of 10% and 20% at concentrations of 10 mg/mL and 5 mg/mL, respectively.

In general, the bioactive potential of eucalyptus ethanolic extract is high, making it suitable for phytosanitary treatment. Additionally, some combinations of extracts could be planned to enhance biological activity through a synergistic effect, involving less active extracts from *C citratus* [29] [30].

5. Conclusions

Ethanolic hydrodistillation residues from three aromatic plants-*Cymbopogon*

citratus, Eucalyptus camaldulensis, and *Mentha piperita*-exhibited antifungal activities depending on fungus.

The eucalyptus ethanolic extract obtained with 70% ethanol (EE70) was the most active against fungi at a 5% concentration. This extract showed the highest total flavonoid content as aglycones and had fewer phenolic acids and glycosides, as determined by HPLC.

The three plants contain a non-negligible amount of minerals that can affect the efficacy of extracts. Using a reasonable quantity of ethanol for extraction can solubilize maximum biomolecules and reduce mineral co-extraction during biopesticide formulation preparation. For this purpose, 50% ethanol could be proposed for the extraction of *E. camaldulensis* hydrodistillation solid residues. Finally, results showed that hydrodistillation residues could be used as cheap and effective biofungicide formulations.

Authors' Contributions

The study was conceived by JCWO, MFT and YLBC. It was run in the *Laboratoire de Chimie Analytique, Environnementale et Bio-organique* (LCAEBiO) at Joseph KI-ZERBO University for chemical analysis. Antifungal test on *T. versicolor* and *C. puteana* and antitermite test were done at the laboratory BioWooEB of CIRAD at Montpellier, by AN under supervision of MTF. Antifungal test on *F. oxysporum* and *A. alternata* was done at Laboratoire de phytopathologie at Université Nazi Boni of Bobo-Dioulasso by AN under supervision of SB. The paper was drafted by AN and revised by all co-authors. All the authors approved the final manuscript.

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Conflicts of Interest

The other authors declare that they have no competing interests.

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