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Antibacterial and antifungal activity of *Combretum farinosum* Kunth and *Combretum igneiflorum* Rendón & R. Delgad. extracts

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Abstract

Infectious diseases like bacterial, fungal, viral are the top killers of a third of the world population. Limited access to comprehensive treatment forces people to rely on herbal concoctions for treatment. *Combretum farinosum* Kunth and *C. igneiflorum* Rendón & R. Delgad. are two similar vine plant species that have insufficient scientific investigation. The purpose of the study is to determine the *in vitro* antimicrobial activity of crude extracts of different parts of *Combretum igneiflorum* (roots, stem, and leaves) and *Combretum farinosum* (roots, fruits, leaves, and stem) using petroleum ether, acetone, and ethanol-water. The crude extract was tested against food-borne pathogens. Twenty-seven crude extracts were prepared from *C. igneiflorum* (roots, stem, and leaves) and *C. farinosum* (roots, stem, leaves, and fruits) and screened for their antimicrobial activity against four Gram-positive bacteria (*Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus aureus* methicillin resistant, *Bacillus subtilis*), four Gram-negative bacteria (*Salmonella enteritidis*, *Pseudomonas aeruginosa*, *Shigella flexneri*, and *Escherichia coli* B), and one fungus species (*Candida albicans*) using agar disc-diffusion, and microbroth dilution assays. Results show that crude extracts of both plants tested had broad antimicrobial activity. The minimum inhibitory concentration (MIC) of *C. farinosum* extract against tested bacteria ranged from 0.32 to 100 mg/ml, whereas *C. igneiflorum* extracts that showed antibacterial activity ranged from 2.5 to 75 mg/ml. Gram-positive bacteria tested were more susceptible to the extracts than gram-negative bacteria. Crude extracts of *C. igneiflorum* and *C. farinosum* have broad antimicrobial activity against the microbes tested.

Keywords: *Combretum farinosum Kunth, Combretum igneiflorum Rendón & R. Delgad*. MTT, Minimum Inhibitory Concentration (MIC), Antifungal, Antibacterial.

INTRODUCTION

Infectious diseases are one of the primary causes of death in underdeveloped and developing countries worldwide, and beyond the scope of high morbidity there is also economic losses due to impediment of activity and medical expenses [1]. Infectious diseases thus negatively impact health, life expectancy, and day to day life. Lack of access to adequate healthcare has caused about 80% of the cultures around the world to depend on native plants as medication (herbal medicine) [2]. Herbal medicines are attractive because of their affordability and the generational knowledge, usually through oral history, that makes them accessible [3]. Folklore ethnobotany is still an inspiration for novel drugs development derived from natural products. The United States National Cancer Institute has, for example, launched an extensive program to explore folklore ethnobotany in discovering novel compounds that have therapeutic use [4,5]. This paves the path for the search of different antibiotics to combat antimicrobial resistance. The World Health Organization declared antimicrobial resistance as one of the top 10 global public health threats [6]. For instance, the Global Antimicrobial Resistance and Use Surveillance System (GLASS) found ciprofloxacin resistance for 8.4 to 92.9% of *E. coli* from urinary tract infections and a higher likelihood (64% more likely) for people to die from a methicillin-resistant *Staphylococcus aureus* (MRSA) infection than those with antibiotic-sensitive infections [7]. Antimicrobial resistance is a particularly problematic issue for underdeveloped and developing countries. It is imperative to look for alternate sources of compounds that could serve as future antibiotics.

Natural products have played a major role in the intellectual and experimental development of organic chemistry [8,9]. Natural products are metabolites with diverse structures, and they play important roles in plant growth, development, and response to environmental cues. These metabolites can be classified as primary (essential in plant growth and development) or secondary (not essential but critical for plants to survive external stresses [10,11]. The structures of primary metabolites are highly conserved whereas that of secondary metabolites are diverse across plant phyla and even within a family. Such diverse, complicated, and descriptive chemistry exhibited by natural products has formed an important part in the development of organic chemistry [11]. The growth of natural products databases has led to an increase in our understanding of synthetic processes. Such understanding serves as background on which future reactions can be predicted or explained [10]. Natural products typically have higher rigidity, structural complexity, molecular

mass, and scaffold diversity than synthesized molecules [12-14]. These features account for why natural products bind selectively to certain receptors [14]. Even though natural products have been used as lead medicinal agents in almost all therapeutic areas, they have been more useful in the search for anti-cancer and anti-infectives [15-17]. They play roles in the drug discovery process in the following way: (1) Direct Natural Products: in this case, the natural product possesses all the potency, selectivity and pharmacokinetic traits needed to make it a clinically useful drug agent. Examples are Aspirin, Taxol, etc. (2) Indirect Natural Products: in most cases, natural products do not in themselves serve as lead agents. They provide the structural basis for the chemist to simplify or elaborate to yield a therapeutically important compound. Such compounds are classified as natural product-derived compounds. They help to build a library of analogs to find the most potent compounds. (3) Natural Product-Inspired: in this instance, natural products serve as an inspiration in developing more therapeutically potent compounds. Natural products offer insights into molecular processes in a particular disease and thereby can inspire one to create and synthesize compounds which may not bear structural semblance to the natural products but may be potent therapeutic agents [11,18,19].

Combretum farinosum Kunth and *C. igneiflorum* Rendón & R. Delgad. are two similar vine plant species whose medicinal capabilities have not been sufficiently studied. Our research group recently published the cytotoxicity studies of *C. farinosum* [20]. In that study, root petroleum ether extracts of *C. farinosum* were more cytotoxic than Doxorubicin™. When tested against normal cell lines, BJ and LL47 IC_{50} values of root petroleum ether extract were 0.504 mg/mL and 0.608 mg/mL respectively, and 0.642 mg/ml against cancer cell line H69. Studies to isolate and determine the identity of compound(s) responsible for the observed cytotoxicity are ongoing. Currently, no knowledge of the bactericidal and antifungal properties of these plants has been documented. *Combretum farinosum* Kunth (Combretaceae) is popularly known in Spanish as *bejuco de carape, peinecillo*, or *escobetillo*. In addition, the following local names have also been used: abacamiel, abamiel, amarguilla, angarilla, bejuco de cepillo, bejuco de chuparrosa, bejuco de cortes, bejuco de escobetillo, carape, cepillito, cepillo, chupamiel, chupamirto, chuparrosa, coamecate de agua, complo, empanada, flor de cepillo, juan viejo, palo de cepillo, papamiel, peine de chango, peine de mico, peine del diablo, peineta, peinetilla, peinetitas, *quie-tzine*. It is typically found in different types of habitats in the dry woodlands from Mexico (Campeche, Chiapas, Colima, Durango, Guerrero, Jalisco, México, Michoacán, Morelos, Nayarit, Oaxaca, Puebla, Quintana Roo, Sinaloa, Tabasco, Tamaulipas, Veracruz, and Yucatán), Central America (Guatemala, Honduras, El Salvador, Nicaragua, and Costa Rica) to South America (Perú and Bolivia) [21]. Its height reaches ca. 20 meters. Different parts of the plant are prescribed for treatment depending on the severity of an ailment. For instance, tea from the entire plant is prescribed for the treatment of severe coughing. The leaves are usually boiled in water to aid with kidney, lung, and heart afflictions. For diabetes, the patient sips the sap directly from the stems or vines or in the case of eye infections the sap is applied ophthalmologically. Its medicinal use has been documented for different ailments; the leaves are used to relieve headaches. It is said that the sap, obtained by distillation or sucked directly, is used to alleviate diabetes and some lung and kidney conditions, for which a decoction is prepared with the leaves taken as water for use; the sap is also used to cure eye infections [21].

C. igneiflorum Rendón & R. Delgad. is a species of *Combretum* discovered in 2011 and endemic to the Pacific Western Coast of Mexico (Jalisco, Colima, Nayarit, and Oaxaca states) [22]. There are no known ethnopharmacological uses of this plant because it was recently discovered.

Gram-positive, gram-negative, and fungi are important pathogens present in developing and underdeveloped countries, making it important to look at their response to novel treatments. The selected gram-positive bacteria in this study were the following: (1) *Enterococcus faecalis* (EF) which causes 85-90% of enterococci infections in humans, making it vital to combat its growing antimicrobial resistance [23]. (2) *Staphylococcus aureus* (SA) and (3) *Staphylococcus aureus* methicillin resistant (MRSA) that are two extremely common bacteria; the impact of methicillin resistance has increased hospitalization costs over time, making it fundamental for underdeveloped countries to combat MRSA with other antibiotics [24]. (4) *Bacillus subtilis* (BS) which is an opportunistic bacterium that could affect vulnerable population in such countries. The selected gram-negative bacteria were the following: (1) *Salmonella enteritidis* (SE) is involved in a third of the cases of diarrheal disease worldwide and has led to typhoid fevers in some cases (Popa and Papa, 2021); (2) *Pseudomonas aeruginosa* (PA), a bacterium commonly found in water and can lead to pneumonia; it is constantly increasing its antibiotic resistance (CDC); (3) *Shigella flexneri's* (SF*)* increasing antibiotic resistance is closely linked with the increasing mortality rates [25-26]. (4) *Escherichia coli* B (EC) is a very common bacterium worldwide and serves as a model gram-negative bacterium. The fungus designated for the antimycotic assays was *Candida albicans* (CA). Selection of these organisms was based mainly on the role these agents play in foodborne illnesses and human disease. The purpose of the study was to extract and test the different crude extracts from both plants against Gram-positive, Gram-negative, and fungal organisms. This study will also report the phytochemical analysis of the crude extracts. Results from this study will serve as preliminary data for further bio-guided phytochemical screening of these plants to isolate and discover novel antimicrobial compounds.

2 MATERIALS AND METHODOLOGY

2.1 Plant Samples

The two plant species of *Combretum* were acquired by the collaboration of Francisco Javier Rendón Sandoval who gathered and shipped the samples from the State of Jalisco, México. *Bejuco de carape* (*C. farinosum* Kunth) and *peineta (C. igneiflorum* Rendón & R. Delgad.) samples were collected from the local roads between Cruz de Loreto and Mayto, Tomatlán, Jalisco, México.

2.2 Preparation of Plant Samples

Plant samples (separated into roots, stems, fruits, and leaves) were sanitized by spraying 10% ethanol solution, left to dry, then placed on clean, sanitized aluminum oven trays, labeled accordingly, and oven-dried at 60 ˚C to remove any excess moisture for 24 hours. The dried plants were ground into a fine/coarse powder utilizing a kitchen blender. Plant materials were stored in a plastic bag in a dark, cool place at room temperature.

2.3 Preparation of Plant Extracts

The procedure for the extraction process was adopted from Williams et al [20]. About 50 g of the powdered material was wrapped on individual cheesecloth pieces, placed in the Soxhlet chamber, and extracted sequentially using petroleum ether (P), acetone (A) and 90% ethanol in water (E). After the extraction process, each solvent was evaporated utilizing a rotary evaporator, redissolved in DI water, frozen, and lyophilized. The plant extract was labeled and stored in previously autoclaved amber vials and stored in a dark place.

The extracts were weighed to prepare stock solutions in DMSO from which appropriate dilutions were made for bioassay.

2.4 Bacterial and Fungal Strains and Cultivation

Four strains of gram-positive bacteria, four strains of gram-negative bacteria, and one fungus were selected as testing agents for the antimicrobial assays. The selected gram-positive bacteria were the following: *Enterococcus faecalis* (EF) (Presque Isle Cultures +522B), *Staphylococcus aureus* (SA) (Presque Isle Cultures+4651), *Staphylococcus aureus* methicillin resistant (MRSA) (Presque Isle Cultures +4656), and *Bacillus subtilis* (BS) (Presque Isle Cultures 620). The selected gram-negative bacteria were the following: *Salmonella enteritidis* (SE) (Presque Isle Cultures +371), *Pseudomonas aeruginosa* (PA) (Presque Isle Cultures +99), *Shigella flexneri* (Presque Isle Cultures +387), and *Escherichia coli* B (EC) (Presque Isle Cultures 337). The fungus designated for the antimycotic assays was *Candida albicans* (CA) (Presque Isle Cultures +925). Selection of these organisms was based mainly on the role these agents play in foodborne illnesses and human disease. All the bacterial strains were streaked onto Muller Hinton Agar (MHA) utilizing the four-quadrant streak method using aseptic techniques to obtain pure isolated bacterial colonies. Fungal strains were plated onto Potato Dextrose Agar using the four-quadrant method. Streak plates were incubated at 37°C for 18 h.

2.5 Disc Diffusion Antibacterial Assay

Initial antimicrobial activity was screened via disc diffusion assays [27]. Briefly, a crude extract with concentration 100 mg/ml was prepared with dimethyl sulfoxide (DMSO, VWR, USA) and filtered with 0.45 um sterilized filter. A single colony of each bacterial strain was selected and inoculated into a sterile test tube with 2 mL of Difco™ LB Broth (Miller) using a sterile toothpick. Two positive control tubes (with bacteria) and one negative control tube (without bacteria) were made for each bacterial strain. The test tubes were then incubated in a shaker bath at 37 ˚C at 250 rpm for 16-18 hours. The turbidity of bacterial cultures was determined using a Spectro 23RS spectrophotometer (Labomed, Inc., Culver City, California) at 625 nm. Sterile DI water was used as a blank for calibration. Each culture was standardized to an absorbance of 0.132 (equivalent to a 0.5 McFarland standard) using sterile DI water. A 100-µL aliquot of standardized bacterial suspension was aseptically spread plated onto MHA agar and evenly spread using sterile, disposable L-shaped spreaders (COPAN Diagnostics, Murrieta, California).

Sterile filter disks (6 mm diameter) were impregnated with 20 µL of the negative control (DMSO) or crude extract. As a positive control, different antibiotic disks were used depending on the type of bacteria employed in the experiments (see Figure 1 for sample set up). Positive controls for the gram-positive bacteria were as follows: Chloramphenicol 30 µg (BD BBL™ Sensi-Disc); Penicillin 10 µg (BD BBL™ Sensi-Disc); Vancomycin 30 µg (BD BBL™ Sensi-Disc); Amoxicillin/Clavulaic acid 20/10 µg (BD BBL™ Sensi-Disc); Ciprofloxacin 5 µg (BD BBL™ Sensi-Disc); Tetracycline 30 µg (BD BBL™ Sensi-Disc). For the gram-negative bacteria, positive controls were as follows: Chloramphenicol 30 µg (BD BBL™ Sensi-Disc); Vancomycin 30 µg (BD BBL™ Sensi-Disc); Amoxicillin/Clavulaic acid 20/10 µg (BD BBL™ Sensi-Disc); Ciprofloxacin 5 µg (BD BBL™ Sensi-Disc); Tetracycline 30 µg (BD BBL™ Sensi-Disc). The prepared plates were then incubated at 37˚C for 24 hours. After incubation, zones of inhibitions (ZOI) were measured in mm with a Pittsburg 4" Digital caliper (± 0.02 mm) to determine the effectiveness of the plant extracts against each bacterial organism. The experiment was performed in triplicates; three trials per extract solvent were performed for each microbe tested. The mean ZOI in mm ± the standard error of the means (SEM) was recorded.

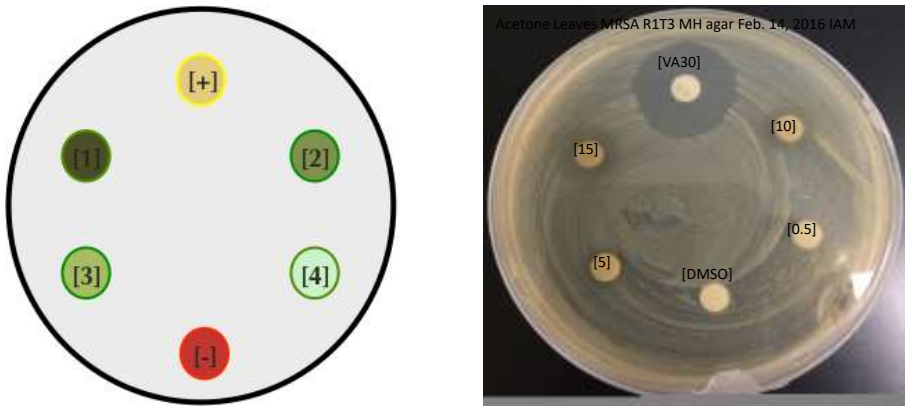

Figure 1: Visual representation of disk placement.

Note: (a) Experimental set up of disc diffusion assay. [+]: Positive Control; [-]: Negative Control; Crude extract concentrations 1 > 2 > 3 > 4. (b) Antimicrobial disc diffusion assay results of *C. farinosum* leaves extract against MRSA. On plate, 5, 10, 15 mg/ml of extract, APC (30 ug vancomycin) and DMSO.

2.6 Anti-fungal Disc Diffusion assay

Antimycotic disk diffusion assay was employed in the initial screening of antifungal activity [28]. A single colony of *C. albicans* was selected using a sterile toothpick and incubated in 2 mL of Potato Dextrose Broth with two positive control tubes (with fungi) and one negative control tube (without fungi). All culture tubes were incubated in a warm shaker bath at 37°C for 16-18 hours. The turbidity of the *C. albicans* was adjusted to a 0.5 McFarland as above (OD_{625} of 0.132) and standardized using sterile DI water. A 100-µL aliquot of the suspension was inoculated onto Potato Dextrose Agar (PDA) agar plates and evenly spread using sterile, disposable L-shaped spreaders (COPAN Diagnostics, Murrieta, California, USA).

Sterile 6-mm diameter filter disks were impregnated with 20

µL of a negative control (DMSO) or crude extract and set up as in Figure 1. For a positive control, 20 mg/mL of miconazole from a 99% stock solution was prepared and stored at 4˚C. Inoculated plates were then incubated at 27˚C for 24 hours and analyzed similarly to the bacterial disc diffusion assays. The experiment was performed in triplicate; three trials per extract solvent were performed for each microbe tested and the mean ZOI in mm ± the standard error of the means (SEM) was recorded.

2.7 Minimum Inhibitory Concentration Determination Assay (MIC)

The minimum inhibitory concentration (MIC) is the smallest concentration of an antimicrobial agent which inhibits microbial growth after the determined incubation period [29]. MIC assays were performed for organisms that demonstrated sensitivity to crude plant compounds based on the presence of zones of inhibition in the antimicrobial or antimycotic disk diffusion assays. The bacteria susceptibility guideline for disc diffusion assay which is (≤9 mm), moderately sensitive (10-11 mm), or sensitive (≥12 mm) was used to select the highest concentration for each extract [30]. The MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5 diphenyltetrazolium bromide] serial microdilution assay was employed in this study because of its simplicity and the color reaction produced [31-33]. Viable cells or microorganisms reduce the yellow tetrazole to purple formazan and thus provide an indication if microorganisms are actively growing or not. MTT solution at a concentration of 5 mg/mL was prepared in PBS, filter- sterilized (0.45-µm filter) and stored. Microorganisms were prepared into suspensions as described in the antimicrobial and antifungal assays and used within 30 minutes. A 96-well microplate was used for the assay. Rows A to E were left for controls (MTT, DMSO + microbes without test agents, crude extract only, sterile media, and microbes with positive controls). Rows F-H had microbes, media, and crude extracts. Negative control wells consisted of bacteria or fungi only in their respective medium. The lowest crude extract concentration that was active against a microbe was used as the working solution. Two-fold serial dilutions of plant extracts were prepared using DMSO as a solvent. Using a multichannel pipette, 100 µl of extract from column 1 was transferred to column 2, 100 µl of DMSO added and the contents of the wells were mixed well with a pipet. The serial dilution process was continued through column 12 by picking 100 µl from column 2 into column 3 and diluted with 100 µl of DMSO as a solvent and incubated for 16-20 hours. The positive controls were the following: Chloramphenicol, 99% (Thermo Fisher Scientifc, Waltham, MA, USA), Penicillin G sodium (Thermo Fisher Scientific, Waltham, MA, USA) Vancomycin hydrochloride (Thermo Fisher Scientific, Waltham, MA, USA) Amoxicillin sodium salt, 89% (Thermo Fisher Scientific, Waltham, MA, USA), Clavulaic acid K salt (Crescent Chemical Co. Inc.), Ciprofloxacin, 95% (Thermo Fisher Scientific, Waltham, MA, USA), and Tetracycline hydrochloride (Biomedicals Antibiotics). At the end of the incubation time, 25 µl of thiazolyl blue tetrazolium bromide (MTT) indicator solution was added to each well, and the plate was incubated for 30 min at 37°C. The MIC was defined as the lowest concentration at which no growth was observed (blue colored) after incubation. DMSO had no inhibition effect.

2.8 Phytochemical Screening

The crude extracts of the plant samples were tested for the presence of flavonoids, terpenoids, alkaloids, terpenes, saponosides, quinines, tannins, cardiac glycosides, free anthracene derivatives, and reducing compounds using standard procedures [34-36]. The qualitative results are (+) for the presence and (-) for the absence of phytochemicals.

3.0 RESULTS AND DISCUSSION

3.1 Preliminary Screening via Disc Diffusion Assay

Initial antimicrobial activity screening was done via disc diffusion assay. The results of the disc diffusion assay are represented in Table 1. The negative control (DMSO) in all assays performed did not exhibit any inhibition. The susceptibility of bacteria to an extract was indicated by an inhibition zone diameter of 9 mm or more around the disc [30,37].

Antimicrobial Screening of *C igneiflorum* Extracts

All petroleum ether extracts for all *C igneiflorum* parts (root, stem, and leaves) studied were not active against the tested pathogens. Acetone and ethanol extracts of *C igneiflorum* parts studied were all active against *Candida albicans.* This is significant in that fungal colonization, especially from *C albicans,* are known to complicate wound care [38]. Therefore, traditional practices of preparing poultices for wound care would be comprehensive and effective. Acetone and ethanol extracts of *C igneiflorum* were active against all gram-positive bacteria tested except MRSA and EF which were resistant to the acetone of the root and leaves. Overall, polar extracts (from ethanol and acetone) demonstrated greater activity against the strains tested than the less polar extracts (petroleum ether). *C igneiflorum* extracts were not very active against gram-negative bacteria strains tested, except *Salmonella enteritidis*, which was susceptible to all the acetone and ethanol extracts. The selective activity of extracts against *Salmonella enteritidis* makes this plant worth studying. Infections caused by *Salmonella enteritidis* affect about 17 million people with an annual mortality of about 1%, the majority of which are in low and middle-income countries [39-41]. The ethanol extracts of *C igneiflorum* were all active against another gram-negative bacterium *Shigella flexneri*. With the increase in cases of *S flexneri* being resistant to current antibiotic cocktails, discovering novel antibiotics or lead compound(s) is very imperative [42].

Preliminary phytochemical characteristic tests (Table 2) show the presence of flavonoids, terpenoids, quinines, tannins, and cardiac glycosides in *C. igneiflorum* extracts which have been known to be sources of compounds that have both antifungal and antibacterial activities [43]. Tannins for instance is known to enhance cell proliferation and tissue regeneration for wound healing [44]. Their antibacterial activity is due to binding of proteins that inhibits bacterial metabolism [45,46]. Their ability to permeate through the cell wall makes them more effective against Gram-positive bacteria than Gram-negative bacteria as observed in *C. igneiflorum* and *C. farinosum* extract in this study [44].

Table 1: Initial Crude Extracts screening against bacterial strains using Disc Diffusion Assay

Samples			**Gram-Positive Bacteria**			**Gram-Negative Bacteria**					**Fungus**
Plant Species	Used Parts	Extracts	S.A.	MR-S.A.	B.S.	E.F.	P.A.	E.C.	S.F.	S.E.	C.A.
Dimethyl Sulfoxide (DMSO)			-	-	-	-	-	-	-	-	-
APC			+	+	+	+	+	+	+	+	+
C. igneiflorum	Root	Ethanol	+	+	+	+	-	+	+	+	+
		Acetone	+	-	+	-	-	-	-	+	+
	Stem	Ethanol	+	+	+	+	-	+	+	+	+
		Acetone	+	+	+	+	+	-	-	+	+
	Leaves	Ethanol	+	+	+	+	-	-	+	+	+
		Acetone	+	+	+	-	-	-	-	+	+
C. farinosum	Root	Ethanol	+	+	+	+	+	-	+	+	-
		Acetone	+	+	+	+	-	-	+	+	+
		Petroleum	-	-	-	-	-	-	-	+	-
	Stem	Ethanol	+	+	+	+	+	-	+	+	-
		Acetone	+	+	+	+	+	-	+	+	-
	Leaves	Ethanol	+	+	+	+	+	-	+	+	-
		Acetone	+	+	+	-	-	-	-	+	+
	Fruit	Ethanol	+	+	+	+	+	-	+	+	-
		Acetone	+	+	-	-	-	-	+	+	+
		Petroleum	-	-	-	-	-	-	-	+	-

Notes: (+) inhibition; (-) no inhibition.

Antimicrobial Screening of *C farinosum* Extracts

All petroleum extracts were inactive against the tested microorganism except the root and fruit that showed activity against SE. The antifungal activity of *C. farinosum* extracts was not as pronounced as *C. igneiflorum*. Only acetone extracts prepared from the roots, leaves, and fruits of *C. farinosum* exhibited activity against *C. albicans*. The antibacterial activity of *C. farinosum* was like *C. igneiflorum*. All *C. farinosum* extracts were active against SE, a gram-negative bacterium that is a known food pathogen. Except for the acetone extract of the leaves, all acetone and ethanolic extracts of the roots, stem, leaves, and fruits were active against SF. EC was resistant to all *C. farinosum* extracts that were prepared. *C. farinosum* showed greater activity against gram-positive bacteria than gram-negative bacteria tested. Preliminary phytochemical screening of *C. farinosum* ethanol and acetone extracts (Table 2) shows the presence of flavonoids, tannins, alkaloids, terpenoids, anthraquinone, and quinines which have antibacterial activities [43].

Overall, the disc diffusion assay gave us a quick way to screen active crude extracts, but as widely observed, this method has the potential of producing false negatives especially for nonpolar extracts. It is therefore not surprising that except for root, and fruit of *C. farinosum* all ether extracts of both plants were not active against microbes tested. Our previous cytotoxic studies showed the petroleum ether extracts as the most cytotoxic [20]. The lack of antimicrobial activity observed could be attributed to the inability of the hydrophobic compounds in the crude ether extracts to diffuse in the polar media to inhibit bacterial growth.

Table 2: Preliminary Phytochemical Analysis of Crude Extract of CF and CI

Phytochemical Test		***C. ignefloreum***			***C. farinosum***		
		Ethanol	Acetone	Petroleum Ether	Ethanol	Acetone	Petroleum Ether
Flavonoids		+	+	-	+	+	+
Tannins		+	+	+	+	+	-
Alkaloid Tests	Mayer's Reagent	-	-	+	+	+	+
	Wagner's Reagent	-	-	+	+	+	+
Saponins		+	-	-	-	+	-
Terpenoids		+	+	+	+	+	+
Terpenes		N/A	N/A	N/A	-	-	+
Cardiac Glycosides		+	+	+	+	+	+
Anthraquinone		N/A	N/A	N/A	+	+	-
Quinine		+	+	+	+	+	-
Reducing Compounds		-	+	+	+	+	-
Free Anthracene Derivatives		-	-	-	N/A	N/A	N/A

Notes: (+) presence of phytochemical; (-) absence of phytochemical; (N/A) Not tested

3.2 Minimum Inhibitory Concentration (MIC)

MIC studies were performed on crude extracts of *C. igneiflorum* and *C. farinosum* against the tested pathogens and the results summarized in Table 3. All acetone and ethanol extracts of *C. igneiflorum* exhibited antifungal activity against *C albicans* with MIC range of 4.5-12.8 mg/ml whereas only two extracts from *C. farinosum* (acetone extracts of leaves and fruits) high antifungal activity against C albicans with MIC of 0.6 and 1.2 mg/ml. EC, a gram-negative bacteria was resistant to all the *C. farinosum* extracts except two *C. igneiflorum* ethanol extracts from the root and stem which exhibited weak activity with MICs of 50 and 75 mg/ml. Overall, MICs ranged from 0.91-60 mg/ml for gram-positive bacteria tested and 2.5-100 mg/ml for gram-negative bacteria. These results indicate that gram-positive bacteria are more sensitive to *C. igneiflorum* and *C. farinosum* than gram-negative bacteria. *C. igneiflorum* and *C. farinosum* and were very active against *C. albicans* with low MICs ranging from 0.6 -12.5 mg/ml.

This study provides information on the antimicrobial activities of *C. igneiflorum* and *C. farinosum*, two plants which have not been studied for their antimicrobial activity. Both *C igneiflorum* and *C farinosum* exhibited higher activity against gram-positive bacteria than gram-negative. The observed resistance of gram-negative bacteria to extracts of *C. farinosum* and *igneiflorum* can be attributed to the presence of their outer lipid membrane surrounding the cell wall, which hinders the diffusion of hydrophobic compounds [29]. Differences in the polarity of solvent used for chemical extraction showed great effect on the overall efficiency of the plant extract on the different bacteria, with petroleum ether not being a suitable solvent for extracting antibiotics. This was shown in its inability to provide an inhibitory effect on most of the microbes in the experiment. In contrast, ethanol worked as a sufficient extraction solution that had the greatest effect on the different microbial species due to its high polarity index and thus being capable of extracting polar antimicrobial chemicals more efficiently. However, using acetone as an extract for *C. farinosum* fruit performed excellent against *S. aureus*, methicillin-resistant *S. aureus*, *S. enteritidis*, *S. flexneri*, and *C. albicans*, though ineffective against the other microbes. *C. farinosum* in general was also very effective against every microbe, except for *E. coli*. The observed high bioactivity of extracts prepared from polar solvents (acetone and ethanol) could be due to the limitations of the disc diffusion assay. This phenomenon has been observed in our previous studies and confirmed by other studies [27,31,47].

Table 3: Average MICs (mg/ml) of *C. igneiflorum* and *C. farinosum* crude extracts against tested pathogens

Plant Species	Used Parts	Extracts	Gram-Positive Bacteria			Gram-Negative Bacteria					Fungus
			S.A.	MR-S.A.	B.S.	E.F.	P.A.	E.C.	S.F.	S.E.	C.A.
C. igneiflorum	Root	E	6.6	4.9	12.5	12.5	NS	75.0	12.5	4.8	4.5
		A	17.5	NS	1.8	NS	NS	NS	NS	12.5	12.8
	Stem	E	6.1	4.4	4.4	45	NS	50.0	30.0	6.4	5.5
		A	6.2	4.5	6.9	7.7	100	NS	NS	4.8	5.5
	Leaves	E	29.4	25.0	36.3	75	NS	NS	12.5	5.6	11.2
		A	8.4	10	3.1	25	NS	NS	NS	2.5	5
C. farinosum	Root	E	3.3	1.9	4.6	5.10	50	NS	3.9	0.32	NS
		A	NS	NS	NS	NS	NS	NS	NS	NS	NS
		P	NS	NS	NS	NS	NS	NS	NS	1.0	NS
	Stem	E	5.9	5.4	8.3	6.2	35.7	NS	4.8	9.1	NS
		A	26.0	25.2	24.8	30.2	100	NS	31	32.0	NS
	Leaves	E	4.3	4.1	60.0	37.5	30.5	NS	2.7	4.9	NS
		A	1.1	1.2	10.0	NS	NS	NS	NS	1.2	1.2
	Fruit	E	2.2	2.4	7.5	4.1	6.4	NS	2.6	1.8	NS
		A	1.1	0.91	NS	NS	NS	NS	3.5	2.5	0.6
		P	NS	NS	NS	NS	NS	NS	NS	30.0	NS

Notes: A: Acetone; E: Ethanol/water; P: Petroleum ether; NS: Not studied

CONCLUSION

Overall, extracts of both plants (except for the petroleum ether extracts) showed moderate to high antimicrobial activity against clinically important microbes tested. These findings therefore justify the use of C. farinosum traditionally for treating infections. Gram-positive bacteria (SA, MRSA, EF, and BS) were more susceptible to the extracts of *C igneiflorum and C farinosum* than gram-negative bacteria (EC, PA, SF, and SE). Acetone and ethanol extracts of both plants studied also exhibited antifungal activity by inhibiting *C. albicans*. *C. igneiflorum* and *C. farinosum* have exhibited broad antibacterial and antifungal activities and therefore warrant further bio-guided isolation of compounds in the plants. Phytochemical screening revealed the presence of classes of compounds with known antibacterial activities. *C. igneiflorum* and *C. farinosum* have the potential to serve as lead compounds for designing novel antibiotics.

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Competing interests/Conflicts of interest:

The authors declare no competing interests or conflict of interests.

Author Contributions

Alfred Addo-Mensah and Monica O. Mendez designed the project, Fransico Javier Rendon Sandoval collected and identified the plant species, Irma Andrea Maldonado, Cristobal Lopez, and Andrea Alarcon carried out the experiments. All authors helped with data analysis, discussion and manuscript edit & review.

Ethical Approval: N/A

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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