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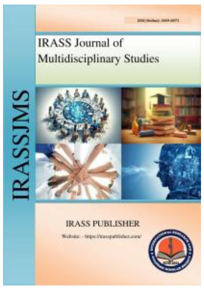

Evaluation of Antimicrobial Sensitivity of Essential oil from *Citrus Sinensis* Fruit Peelings and *Cupressocyparis Leylandii* Leaves on Some Skin Pathogens

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Abstract: INTRODUCTION: In the majority of African nations, where the health care system has been gradually collapsing due to rising disease rates, conflicts, poverty, starvation, and overall environmental degradation, health care-associated and community infections continue to be an issue. Since the 60s since Cameroon had its independence, the country has not been blessed with a single Drug discovery owned by the state. Purchase of pharmaceutical imported drugs lead to a heavy loss of state revenue, and the development policy has not been focused on available local resources (mainly medicinal plants)

OBJECTIVE: To evaluate the antimicrobial sensitivity of essential oil extract from *Citrus sinensis* and *Cupressocyparis leylandii* on some bacterial and fungal skin pathogens.

MATERIALS AND METHODS: We carried out an experimental study that included all the material and reagents: Disposable petri dishes, Wireloop, Incubator, speculum, Commercially prepared powder of nutrient agar, sabouraud dextrose agar and Muller Hinton agar, Alkaline peptone powder, Brain-heart effusion broth, An autoclave, distiller, blender, Dry test tubes, Pasteur pipette.

RESULTS: We isolated pure cultures of *Staphylococcus aureus* and candida spp for the study. Phytochemical screening results revealed that Oil extract of *Cupressocyparis leylandii* was highly positive for flavonoids, resins and tannins. It also contained a small amount of glycosides but was negative for alkaloids and saponins. Oil extract of *Citrus sinensis* was positive for flavonoids, alkaloids, resins, tannins, saponins but lacked glycosides. Essential oil of orange peels was sensitive against *Staphylococcus aureus* with a diameter of inhibition of **7 mm**. Candida spp showed resistance to the extract. Essential oil from cypress was sensitive against Staphylococcus aureus with a diameter of inhibition of **25mm** but Candida spp showed resistance

CONCLUSION: This study has proven that the fresh peels of *Citrus sinensis* and *Cupressocyparis leylandii* possess chemical compounds that are active against bacteria and it is believed to be a promising source for new antimicrobial agents for cosmetic purposes.

Keywords: Phytochemicals, antimicrobial sensitivity, skin bacterial pathogens, *Citrus sinensis, Cupressocyparis leylandii*

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1.1 Background

In order to cure, diagnose, and prevent illnesses or to maintain well-being, traditional medicine refers to health practices, approaches, knowledge, and beliefs that include manual techniques, exercises, spiritual therapies, and medications derived from plants, animals, and minerals. These methods can be used alone or in combination. In Cameroon, traditional medicine has gained a lot of popularity in the past ten years, in part because of the nation's protracted unsustainable economic position.According to estimates from the World Health Organization (WHO), 80% of people in underdeveloped nations get their primary medical care from traditional medicine that uses medicinal plants. Since medicine in Cameroon is still disorganized, it is unsuccessful when included into the healthcare system (Nkongmeneck et al., 2007). This interest has increased over the years because of the effort the state has put into natural product research through the University post-graduate research programmes and the Ministry of Research and Innovation's natural products research and development (CERUT, 1999; Nkuinkeu, 2000; Kuete et al., 2007).

1.2 Problem Statement

In the majority of African nations, where the health care system has been gradually collapsing due to rising disease rates, conflicts, poverty, starvation, and overall environmental degradation, health care-associated and community infections continue to be an issue. Cameroon has not been blessed with a single state-owned drug discovery since the 1960s, when the country gained its independence. State money is heavily lost when pharmaceuticals are imported, and local resources—primarily medicinal plants—have not received enough attention in development policies (Human and Weerdenburg, 1985; Jiofack and Ayissi, 2006). The current trend of government policy in Cameroon to pay for the health care services shows the inability of government to ensure provision of quality services at an affordable price to everyone and especially to the most vulnerable groups (Mbile et al., 2003 Cheek et al., 2004).

It might occasionally take days to locate the closest pharmacy, dispensary, or medical facility for consultation in Cameroon's remote districts. The high expense of medication must be taken into account in addition to lost working days and transportation costs. Cameroon has been increasingly interested in promoting the use of traditional remedies in recent years, joining the majority of developing nations in realizing that they lack the resources to provide comprehensive health care like some industrialized nations (Duncan 1989; Sunderland et al., 2002; Jiofack et al., 2007).

Many health-oriented ministries are now promoting the use of local medicinal plants for illness treatment in an effort to partially address the issue of medicine shortages or excessive costs. To carry out this TM policy, the Cameroonian Ministry of Health has set up traditional pharmacopeia units inside the ministerial organization. Ministries of Education, Forestry and Wildlife, Research, and Innovation have begun to incorporate medicinal plant conservation and biodiversity into their curricula and to raise public awareness (Nkongmeneck et al., 1996; Nguenang et al., 2005).

The absence of health care systems in rural areas forces residents to take care of themselves, either by using herbal remedies, purchasing expensive medications from rural markets, or, worse, purchasing inexpensive over-the-counter medications, putting them at risk for health problems due to the unidentified source of these subpar drugs (Ndhlala et al., 2009). People in rural areas generally start by taking care of themselves before visiting a contemporary doctor or a traditional practitioner. Medicinal plants are inexpensive, administered early in the course of the illness, and a practical alternative to the careless use of over-the-counter medications. Horan et al. (2003), Oyama et al. (2009), and Fokunang et al. (2000).

Nowadays, a large number of Cameroonians, particularly the impoverished in the cities and the countryside, turn to herbal medicine when they are sick. Indeed, traditional herbal medicine is the primary, and sometimes the only, source of healthcare in many rural African communities (Bloom et al., 2002; Derita et al., 2009). Therefore, the effectiveness and acceptance of herbal remedies in African civilization cannot be questioned. Alternative traditional medicine has emerged as a viable option for a coordinated search for new chemical entities (NCEs) due to the high cost of medications and the growing resistance to the majority of antibiotics and antifungal medications. The spectrum of skin diseases in a rural setting in Cameroon (sub-Saharan Africa) reveals that 30.06% of skin diseases in Cameroon is of bacterial and fungal origin (Anne-cecile et al., 2012) .

1.3 Rationale / Justification

The outcome of this study will provide an alternative locally available and affordable home treatment for skin infections. This research will also solve the problem of the resistance of microorganisms to most antibiotics and antifungal agents by providing a source for new chemical entities (NCEs)

1.4 Research Question

What is the antimicrobial effect of essential oil from Citrus sinensis pillings and Cupressocyparis leylandii leaves on some skin pathegens?

1.5 Research Objectives

1.5.1 General Objective:

To determine the antimicrobial effect of essential oil from Citrus sinensis pillings and Cupressocyparis leylandii leaves on bacterial and fungal skin pathogens

1.5.2 Specific Objectives:

- To determine the sensitivity of essential oil from Citrus sinensis pillings and Cupressocyparis leylandii leaves on some bacterial and fungal skin pathogens
- To assess the phytochemical content of Citrus sinensis pillings and Cupressocyparis leylandii leaves.

1.6 Research Hypothesis

This study will be verifying the following hypothesis:

- Essential oil from Citrus sinensis pillings and Cupressocyparis leylandii leaves are sensitive against bacterial and fungal skin pathogens

2.0 Literature Review

2.1 SKIN FLORA

The bacteria that live on the skin, usually human skin, are referred to as skin flora (also known as skin microbiota).Numerous of them are bacteria, of which there are approximately 1000 species from 19 phyla on human skin. The majority are located in the top regions of hair follicles and the outermost layers of the epidermis.The majority of skin flora are either mutualistic (provide a benefit to their host) or commensal (do not harm their host). By competing for nutrition, releasing substances that inhibit them, or boosting the skin's immune system, bacteria can help prevent temporary harmful organisms from invading the skin's surface. Kong HH, Conlan S, and Grice EA (2009). However, resident bacteria can lead to skin conditions and blood system infections, which can be fatal, especially for immunocompromised individuals. Batrachochytrium dendrobatidis, a chytrid and non-hyphal zoosporic fungus that causes chytridiomycosis, an infectious disease believed to be the cause of the collapse in amphibian populations, is a significant non-human skin flora (Pappas, 2009).

2.1.1 Relationship To Host

There are three types of skin microbiota: pathogens, mutualistic, and commensals. Depending on how strong an individual's immune system is, they can frequently be all three. Microflora supports the development of immunity, according to studies on the immune system in the lungs and gut (Krausse et al., 2005). One example of a mutualistic bacterium that can become a pathogen and cause illness is Pseudomonas aeruginosa, which can cause infections in the respiratory, gastrointestinal, joint, and bone systems if it enters the bloodstream. Dermatitis may also result from it. Nevertheless, Pseudomonas aeruginosa generates antibiotic compounds like pseudomonic acid, which are used commercially as Mupirocin. This combats illnesses caused by streptococci and staphylococci. Additionally, Pseudomonas aeruginosa produces compounds that prevent the growth of Aspergillus fumigatus, Torulopsis glabrata, Saccharomyces cerevisiae, Candida krusei, and Candida albicans (Kerr, 1994). Additionally, it can stop Helicobacter pylori from growing. According to one study, "removing P. aeruginosa from the skin, through use of oral or topical antibiotics, may inversely allow for aberrant yeast colonization and infection." This indicates how significant its antimicrobial properties are. The production of bodily odor is another feature of bacteria. Sweat has no smell, but certain bacteria can devour it and produce byproducts that humans may find repulsive (unlike flies, for instance, who can find them desirable). Here are a few examples: Adolescent and adult sebaceous glands contain protonibacteria that can convert their amino acids into propionic acid. Sweat is broken down by Staphylococcus epidermidis into isovaleric acid (3-methyl butanoic acid), which produces body odor. Foot odor caused by Bacillus subtilis is intense.

2.1.2 Bacterial Skin Infections

One of the approximately 1,000 bacterial species that live on human skin is Staphylococcus epidermidis. While it is typically not harmful, it can cause skin infections and potentially fatal conditions in immunocompromised individuals.The use of 16S ribosomal RNA to identify bacterial species present on skin samples directly from their genetic material has drastically altered the estimation of the number of species present on skin bacteria. In the past, this type of identification was dependent on microbiological culture, which prevented the growth of many bacterial species and hence kept them secret from research (Grice EA, Kong HH, Conlan S (2009)). According to culturally based studies, Staphylococcus aureus and Staphylococcus epidermidis were considered to be dominant. Nevertheless, studies using 16S ribosomal RNA reveal that although these species are widespread, they only account for 5% of skin bacteria. Nonetheless, the diversity of skin offers microorganisms a rich and varied environment. Actinobacteria (51.8%), Firmicutes (24.4%), Proteobacteria (16.5%), and Bacteroidetes (6.3%) comprise the four phyla that comprise the majority.(EA Grice and others, 2008)

2.1.2.1 Acne vulgaris

According to Fitz-Gibbon et al. (2013), acne vulgaris is a common skin disorder that is characterized by inflammation of the skin and excessive sebum production by the pilosebaceous unit. Propionibacterium acnes, a commensal microbiota member even in acne-free individuals, usually colonizes affected areas. Although only some strains of P. acnes are significantly linked to acne, while others are linked to good skin, high populations of P. acnes are linked to acne vulgaris. People with and without acne have similar relative populations of P. acnes. Topical and systemic antibacterial medications are part of the current treatment, which reduces P. acnes colonization and/or activity. Using Staphylococcus epidermidis to stop P. acnes growth is one possible probiotic therapy. It has been demonstrated that the succinic acid produced by S. epidermidis inhibits the growth of P. acnes. When administered topically, Lactobacillus plantarum has also been demonstrated to enhance the skin's antibacterial qualities and have anti-inflammatory effects. It has also been demonstrated to be successful in decreasing the size of acne lesions. (Grice and others, 2013).

2.1.2.2 Atopic dermatitis

Staphylococcus aureus populations have been found to be higher in both lesional and nonlesional skin in people with atopic dermatitis (Grice, 2014).Due to S. aureus colonization, atopic dermatitis flares are linked to low bacterial diversity; however, after receiving standard treatment, bacterial diversity has been observed to rise.Corticosteroids, topical or systemic antibiotics, and diluted bleach baths are some of the current treatments. Using S. epidermidis, a commensal skin bacterium, to stop S. aureus growth is one possible probiotic treatment. It has been demonstrated that S. epidermidis populations rise in an effort to manage S. aureus populations during flare-ups of atopic dermatitis.Babies that have low gut microbial diversity are more likely to develop atopic dermatitis.Firmicutes are more prevalent in infants with atopic eczema than Bacteroides. According to Kong et al. (2013), Bacteroides' anti-inflammatory qualities are crucial in the fight against dermatitis.

2.1.2.3 Psoriasis vulgaris

Dryer skin areas like the elbows and knees are usually affected by psoriasis vulgaris. Compared to sebaceous sites, dry skin regions typically exhibit lower populations and a higher diversity of microorganisms (Hannigan et al., 2013). According to a study that used swab sampling techniques, psoriasis is linked to regions that are abundant in Firmicutes (mostly Streptococcus and Staphylococcus) and Actinobacteria (primarily Corynebacterium and Propionibacterium) (Alekseyenko et al., 2013). In contrast, a another study that used biopsies linked healthy skin to higher amounts of firmicutes and Actinobacteria. Nonetheless, the majority of research indicates that psoriasis patients have less microbial diversity in their afflicted areas.Phototherapy, systemic medications, and topical medicines are used to treat psoriasis. There are currently no viable probiotic treatments for psoriasis due to the inconsistent nature of the research on the skin microbiota's role in the condition (Fahlén et al., 2012).

2.1.2.4 Rosacea

Rosacea is usually associated with the skin's sebaceous sites. The skin mite Demodex folliculorum has a strong attraction for sebaceous skin locations because it produces lipases that enable it to consume sebum as sustenance. Despite being a component of the commensal skin microbiota, rosacea patients have higher levels of Demodex folliculorum than healthy people, which may indicate pathogenicity (Casas et al., 2012). Usually absent from the commensal skin microbiota, Bacillus oleronius, a Demodex-associated bacteria, starts inflammatory pathways that resemble those of rosacea patients. Additionally, S. epidermidis populations have been isolated from rosacea patients' pustules. However, as Demodex has been found to move germs about treatment, it's possible that they were transported to locations that favor growth. Laser therapy and oral and topical antibiotics are examples of current treatments. There are no viable probiotic therapies for rosacea because current research has not yet demonstrated a clear mechanism for Demodex's impact (Grice, 2014).

2.1.2.5 Cellulitis

A painful, red infection that is typically warm to the touch is the result of cellulitis.Although it can develop anywhere on the body, cellulitis most frequently affects the legs.

2.1.2.6 Folliculitis

An infection of the hair follicles called folliculitis results in red, swollen pimple-like lesions. Bacteria that cause folliculitis can be found in improperly handled hot tubs or pools.

2.1.2.7 Impetigo

It typically affects preschool-aged children and results in gushing sores. While the non-bullous form of impetigo appears yellow and crusty, the bullous variant produces enormous blisters.Hair follicles are the initial site of boils, which are deep skin infections. Firm, red, and sensitive, boils develop into pus-filled pimples beneath the skin. Lumenlearning.com

Cellulitis, folliculitis and impetigo are caused by Staphylococcus and streptococcus spp

2.1.3 Fungal skin infections

Mycoses, another name for fungal diseases, can be categorized into classes according on how invasive they are. Cutaneous mycoses are those that result in superficial infections of the skin, hair, and nails. Subcutaneous mycoses are those that infect deeper tissues by penetrating the epidermis and dermis. Systemic mycoses are mycoses that spread throughout the body.

- **Tineas**

Dermatophytes, fungal molds that cause a form of cutaneous mycoses called tineas, require keratin, a protein found in skin, hair, and nails, to thrive. The three dermatophyte genera that can cause cutaneous mycoses are Trichophyton, Epidermophyton, and Microsporum. While ringworm is the generic term for tineas on most regions of the body, specific locations may have their own names and symptoms (see Table 1). Remember that these Latinized names describe to places on the body, not to the creatures that cause them. Different dermatophytes can cause tineas in most parts of the body. Lumenlearning.com

Table 1: A Few Typical Tineas and Where They Occur

Ringworm, or tinea corporis	Body
Ringworm (tinea capitis)	Scalp
Pedis tinea (athlete's foot)	Feet
Tinea barbae, sometimes known as barbersitch	Beard
Jockitch, or tinea cruris	Groin
Unguium tinea (onychomycosis)	Toenails, fingernails

Commonly occurring in soils and the environment, dermatophytes are often spread to the skin by coming into contact with animals or other people. Additionally, fungus spores can spread through hair. Dark, wet conditions are ideal for the growth of many dermatophytes. For instance, athlete's foot, or tinea pedis, is frequently transmitted in public showers, and the fungus that cause it thrive in the dank, gloomy environment of perspiring socks and shoes. Similarly, jock itch, or tinea cruris, is prone to spreading in shared living spaces and grows best in warm, damp underwear.Lesions from tinea corporis, or body tineas, frequently develop radially and heal in the center. As a result, a red ring forms, giving ringworm its deceptive name. This is similar to the Clinical Focus case that began in Unicellular Eukaryotic Parasites. Tineas can be diagnosed in a number of ways. Commonly used is AWood's lamp, sometimes referred to as a black lamp, has a wavelength of 365 nanometers. When exposed to the UV light from the Wood's lamp, the hyphae and spores that make up a tinea shine. Using a microscope to directly examine samples of skin scrapings, hair, or nails can also reveal fungi. These specimens are frequently prepared in a wet mount using a potassium hydroxide solution (10%–20% aqueous KOH), which dissolves the keratin in skin, hair, and nail cells, allowing the hyphae and fungal cells to be seen. The samples can be grown on Sabouraud dextrose CC (chloramphenicol/cyclohexamide), a selective agar that inhibits the development of bacteria and saprophytic fungi and encourages the growth of dermatophytes. Macroscopic colony morphology is often used to identify the genus of the dermatophyte; microscopic morphology can be examined using a slide culture or a lactophenol cotton blue-stained sticky tape preparation to confirm identification.A number of antifungal drugs are beneficial in treating tineas. Terbinafine is a common ingredient in allylamine ointments; miconazole and clotrimazole are topical therapeutic alternatives; and griseofulvin is an oral treatment option. Lumenlearning.com

- **Cutaneous Aspergillosis**

The mold genus Aspergillus contains a wide variety of species, some of which can lead to aspergillosis. Another cause of cutaneous mycoses is Aspergillus. Primary cutaneous aspergillosis is an ailment that begins in the skin, however it is rare. The illness begins in the respiratory system and spreads throughout the body in secondary cutaneous aspergillosis, which is more common. Both primary and secondary cutaneous aspergillosis cause distinctive eschars to form at the site or locations of infection.Primary cutaneous aspergillosis usually occurs at the site of an injury and is most commonly caused by Aspergillus fumigatus or Aspergillus flavus. It is usually reported by patients who have been injured while working in an agricultural or outdoor environment. However, opportunistic infections can also occur in medical settings, often at the site of burns, surgical wounds, occlusive dressings, venipuncture wounds, and intravenous catheters. Immunocompromised people who are more vulnerable to opportunistic infections are often affected by aspergillosis, the second most common hospital-acquired fungal infection after candidiasis.

A skin biopsy, culture, and patient history are used to diagnose cutaneous aspergillosis. Antifungal drugs such voriconazole (which is recommended for invasive aspergillosis), itraconazole, and amphotericin B are used as treatments. If itraconazole is ineffective, amphotericin B is used. Medication may be administered, and immunotherapy or surgery may be required for burn patients or immunocompromised people. Lumenlearning.com

- **Candidiasis of the Skin and Nails**

Skin infections brought on by Candida albicans and other yeasts of the genus Candida are referred to as cutaneous candidiasis.Localized skin rashes, like intertrigo, a general term for a rash that appears in a skin fold, can occasionally be caused by Candida species. Candida can also infect the nails, stiffening and turning them yellow. The opportunistic skin infection known as cutaneous candidiasis, which presents as a red, itchy rash, is caused by the yeast Candida albicans. Fungal infections of the nail (tinea unguium) can be caused by dermatophytes or Candida species. The nail becomes yellow, brittle, and more prone to breaking. Among adults, this disorder is somewhat prevalent. Clinical observation, culture, Gram stain, and KOH wet mounts are used to diagnose skin and nail candidiasis. Antifungal agent susceptibility testing is another option. Topical or systemic azole antifungal drugs can be used to treat cutaneous candidiasis. Patients with HIV/AIDS, cancer, or other immune-compromising diseases may benefit from prophylactic treatment for candidiasis since it can become invasive. Treatment options include nystatin, terbinafine, andnaftifine; azoles, including nystatin; clotrimazole, econazole, fluconazole, ketoconazole, and miconazole. Chronic infections may require long-term therapy with drugs like ketoconazole or itraconazole. Although recurrent infections are common, this risk can be decreased by closely adhering to treatment guidelines, avoiding excessive wetness, staying healthy, maintaining proper cleanliness, and wearing appropriate clothing, (including shoes). (Lumenlearning.com)

- **Sporotrichosis**

Subcutaneous mycoses have the ability to penetrate deeper tissues than cutaneous mycoses, which are limited to the skin's visibility. Sporothrix schenkii is the fungus that causes sporotrichosis, often known as rose gardener's disease or rose thorn disease (remember "Every Rose Has Its Thorn" in Physical Defenses). It is the most common subcutaneous mycosis in temperate areas. Sporotrichosis is commonly contracted after handling dirt, plants, or wood because the fungus can enter through a tiny wound such as a splinter or thorn prick. Sporotrichosis can be avoided by gardening using gloves and protective clothing, as well as by promptly cleaning and disinfecting any cuts sustained while outdoors.Although the fungus can occasionally spread to the lymphatic system, small skin ulcers are the main symptom of sporotric infections. Nodules develop, becoming necrotic, and may even ulcerate as the infection spreads. Abscesses and ulcerations may spread across a greater area (often one arm or hand) as more lymph nodes are impacted. Although it is comparatively rare, in severe situations, It's possible for the illness to spread more widely throughout the body. Histologic analysis of the afflicted tissue can be used to diagnose sporothrix infection. The mold can be cultured on potato dextrose agar to view its macroscopic morphology, and a slide culture stained with lactophenol cotton blue can be used to observe its microscopic morphology. Itraconazole treatment is typically advised. (Lumenlearning.com)

- **Mycoses of the Skin**

Usually opportunistic, cutaneous mycoses only have the ability to spread infection when a wound breaches the skin's protective layer. The exception is tineas, which is caused by dermatophytes that can develop on skin, hair, and nails, particularly when the environment is damp. The majority of cutaneous mycoses can be prevented with appropriate wound care and hygiene. Antifungal drugs are necessary for treatment.

Table 2: Summarizes the traits of a few prevalent cutaneous fungal diseases.

Diseases	**Pathogens**	**Signs and symptoms**	**Transmission**	**Antimicrobialdrugs**
Aspergillosis	*Aspergillus fumigatis, Aspergillus Flavus*	Differential eschars at the site or sites	It is frequently a hospital-acqu infection that enters through tl (cutaneous primary aspergillo respiratory system (secondary cutaneous aspergillosis).	Itraconazole, voriconazoe, Amphoterici
Candidiasis (cutaneous)	*Candida albicans*	Intertrigo, localised rash, yellowing of nails	Opportunistics infections in immunocom patients	Azoles
Sporotrichosis (rose Gardener's disease)	*Sporotrichosis Schenkii*	Abscesses and subcutaneo may spread via a thornpric wound across a wide area.	Direct contact with infected people, bite of animals	Itraconazole
Tineas	*Trichophyton spp, Epidermophyton spp, microsporum spp*	Itchy ring-likelesions at the site of infection	Direct contact with skin or nails lesions of infected people	Azoles

2.1.4 Skin defences

- Antimicrobial peptides

Cathelicidins and other antimicrobial peptides produced by the skin regulate the growth of skin microorganisms. In addition to directly lowering the quantity of microorganisms, cathelicidins also trigger the production of cytokines, which promote angiogenesis, inflammation, and reepithelialization. The decrease of cathelicidin synthesis has been connected to conditions like atopic dermatitis.

Inflammation in rosacea is caused by aberrant cathelicidin processing.Self-DNA produced from cathelicidin peptides, which results in autoinflammation, has been connected to psoriasis. Vitamin D3 plays a key role in cathelicidin regulation. Gallo and Schuuber (2008).

- **Acidity**

Because sweat and skin bacteria produce lactic acid, the skin's outermost layers are inherently acidic (pH 4-4.5). Mutualistic flora like Staphylococci, Micrococci, Corynebacterium, and Propionibacteria thrive at this pH, whereas transitory bacteria like Gram-positive Staphylococcus aureus and Gram-negative Escherichia and Pseudomonas do not. The fact that acidic environments boost the antimicrobial compounds the skin secretes is another factor influencing the growth of pathogenic microorganisms. Bacteria are more easily excreted and no longer adhere to the skin in alkaline environments. It has also been noted that in alkaline environments, the skin swells and opens up, enabling movement to the surface. In 2006, Lampers et al.

- **Immune system**

Cell-mediated immunity against microorganisms like dermatophytes (skin fungi) is produced by the skin's immune system when it is activated. Increasing stratum corneum turnover is one way to get rid of the fungus on the skin's surface. Trichophyton rubrum and other skin fungi have developed compounds that suppress the immune system's reaction to them. One common way to manage the accumulation of flora on the skin's surface is through skin shedding. (Dahl and others, 1993)

2.1.5 Hygiene

It is noteworthy that the human skin harbors a multitude of bacterial and fungal species, the majority of which have not been thoroughly studied. Some of these species are known to be hazardous, while others are known to be beneficial. Bacterial and fungal populations that are resistant to the chemicals used will unavoidably arise from the use of bactericidal and fungicidal soaps. (Dahl and others, 1993). The prevalence of skin disorders can be decreased by practicing good skin hygiene, whereas the haphazard use of antiseptic soaps can cause ecological imbalance and increase the risk of developing many skin infections.

2.2 Citrus sinensis

2.2.1Taxonomy

- Phylum: Tracheophyta Kingdom: Plantae
- Magnoliopsida class
- The Rosidae subclass
- Rutanae is the subordinate.
- Sapindales Order; Rutaceae Family
- The Aurantioideae subfamily
- Aurantieae is the tribe.
- Citrinae is the subtribe.
- Genus: Citrus
- Sinensis species
- Citrus sinensis is its botanical name (Zipcode Zoo, 2012).

2.2.2 Morphology

The orange tree is a tiny, prickly tree with a compact crown that usually grows to 7.5 meters, though it can occasionally grow to 15 meters. The evergreen, leathery leaves are elliptical, oblong, or oval, 6.5–15 cm long, and 2.5–9.5 cm wide. They frequently have narrow wings and petioles (leaf stems). The fragrant white flowers have five petals, twenty to twenty-five yellow stamens, and are about five centimeters across. They can be produced alone or in clusters of up to six. The fruit, which ripens to orange or yellow, can be globose or oval and is normally 6.5 to 9.5 cm wide. The fruit skin (rind or peel) contains numerous small oil glands. The flesh or pulp of the fruit is typically juicy and sweet, divided into 10-14 segments (although there are seedless varieties) and ranges in color from yellow to orange to red (eol.org).

2.2.3 Antiperoxidative, Antithyroidal, Antihyperglycemic and cardioprotective role

The effectiveness of a citrus sinensis (CS) peel extract in reducing tissue lipid peroxidation (LPO), hyperthyroidism, and hyperglycemia in mice produced by L-thyroxine (L-T4) was assessed. The most efficacious and antiperoxidative of the three doses of CS peel extract (12.5 mg/kg, 25 mg/kg, and 50.0 mg/kg) was determined to be 25 mg/kg, but 50.0 mg/kg was shown to be hepatotoxic in the initial experiment. Thus, in the pilot study, the effects of 25 mg/kg/day of CS for 10 days were examined in mice with hyperthyroidism produced by L-T (4). Thyroxine (T4) and triiodothyronine (T3) levels rose in response to L-T(4) (500µg/kg/day for 10 days), which also caused an increase in serum glucose, α-amylase activity, heart/body weight ratio (HW/BW), kidney/body weight (KW/BW), and cardiac and hepatic LPO. Nonetheless, it reduced the levels of several serum lipids, including triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), and total cholesterol (TC). Most of these findings were reversed when CS extract (25 mg/kg/day) was administered to hyperthyroid rats, indicating that CS extracts may be able to mitigate the negative consequences of hyperthyroidism. The test extract's antithyroidal, HDL-C-stimulating, and antioxidant/free radical-scavenging qualities seem to be its main mechanisms of action (Parmar et al., 2008).

2.2.4 Antimicrobial Effects

Using the agar well diffusion method, Shetty evaluated the in vitro antimicrobial activity of aqueous and ethanol (cold and hot) extracts made from Citrus sinensis peels against Streptococcus mutans and Lactobaccilus acidophilus. The MIC values for both test organisms were ascertained. The hot ethanolic extract of citrus sinensis peel suppressed dental caries bacteria the greatest, followed by the cold ethanolic extract. Even at extremely high concentrations, aqueous extracts worked well. Citrus sinensis peel ethanolic extracts, both hot and cold, have MICs of 12–15 mg/ml against both dental caries bacteria. According to Shetty et al. (2016), it was discovered that the extract from citrus sinensis peels was efficient against dental caries bacteria.

2.3 Cupressocyparis leylandii

2.3.1Taxonomy

- Kingdom: Plantae
- Subkingdom: Viridiplantae
- Superdivision: Embryophyta
- Division: Tracheophyta
- Subdivision: Spermatophytina
- Class: Pinopsida
- Subclass: Pinidae
- Order: Pinales
- Family: Cupressaceae
- Genus: Cuprocyparis
- Species: leylandii (ITIS, 2018)

2.3.2 Morphology

Cupressocyparis is an intergenetic hybrid of Alaska yellow cedar (Chamaecyparisnootkatensis), and Monterey cypress (Cupressusmacrocarpa) (Liu, 2009).

2.4 Phytochemicals

Medicinal plants are useful for treating and curing human illnesses because they contain phytochemical components. Non-nutritive substances with protective or illness-preventive qualities are called phytochemicals (Cannatelli et al., 2000). These are non-essential nutrients found in the rear of trees, leaves, roots, fruits, and vegetables. They have defense mechanisms and are protected from a variety of ailments, according to recent studies. According to Bono et al. (2007), there are over a thousand known phytochemicals that can be categorized as primary compounds (such as proteins, chlorophyll, and common sugar) or secondary compounds (such as terpenoid, alkaloids, phenolic, and others). The body uses phytochemicals for a variety of purposes.

2.4.1 Function of Phytochemical in the body

According to research, phytochemicals can boost the immune system, stop harmful substances in the diet from turning into cancer, lower inflammation, stop DNA damage and help repair it, lessen oxidative cell damage, slow the growth of cancer cells, prompt damaged cells to self-destruct (apoptosis) before they can proliferate, help control intracellular hormone signaling and gene expression, and activate insulin receptors.(Hanhineva et al., 2010; WCRF/AIFCR et al., 2007).The antioxidant properties of phytochemicals have been the subject of several laboratory studies. But throughout metabolism, their antioxidant action is diminished in the body, and their levels in blood and tissue are short-lived and very low (Gordon et al., 2012; Tsao et al., 2010). Even at low concentrations of phytochemicals in plasma and tissues, many of the phytochemicals in diet have antioxidant effects on cell signaling and gene expression that may be more significant for health benefits than direct antioxidant action (Gordon et al., 2012).Flavonoids, saponins, polysaccharides, anthraquinones, tannins, and cardiac glycosides are among the chemical components found in the oil extract from citrus sinensis and cupressocyparisleylandii.

2.4.2 A quick examination of a few secondary metabolites

- **Alkaloids**

The amine function, which gives its constituents chemical traits associated with significant toxicity, defines alkaloids as complex molecules that are basic in nature. According to Bouayad et al. (2011), alkaloids that are part of the beta-carboline group have antibacterial, anti-HIV, and anti-parasitic properties. Numerous investigations have shown the antibacterial properties of isolated chemicals and plant extracts high in alkaloids (Bruneton et al., 1999).Additionally, they function as detoxicating agents for plants, protective compounds against animals and insects, and proteins (Prashant et al., 2011).

- **Flavonoids**

The pigments that give most flowers, fruits, and seeds their color are called flavonoids. Plants contain these secondary metabolites in large quantities (Winkel-Shirley, 2001). Strong antioxidants with anti-inflammatory and immune-boosting properties are flavonoids.

- **Glycosides**

A molecule known as a glycoside is created when a sugar forms a glycosidic connection with another functional group. Glycosides are involved in many vital processes in living things. Inactive glycosides are a common way for plants to retain chemicals. Enzyme hydrolysis can activate them, breaking off the sugar portion and releasing the chemical for application (Brito-Arias, 2007). These substances become available for use when the enzymes activate them. Numerous plant glycosides have medicinal applications. Glycosides play a number of vital roles in all living things and are soluble in both alcohol and water. Glycosides are categorized by their aglycone, glycone, and type of glycosidic bond. The non-sugar component of the glycoside is referred to as the aglycone or genin portion, whereas the sugar group is called the glycone. One sugar group (monosaccharide) or many sugar groups (oligosaccharide) can make up the glycone.The French scientists Pierre Robiquet and Antoine Boutron-Charlard discovered amygdalin in 1830, making it the first glycoside ever discovered (Robiquet et al., 1930).

- **Tannins**

Higher herbaceous and woody plants frequently contain tannins, which are water-soluble polyphenols that are mostly found in the tissues of the leaves, buds, seeds, roots, and stems of both gymnosperms and angiosperms (Scarbelt, 1991). They fall into two groups: those that are hydrolyzable and those that are not (condensed). Catechin is a member of the non-hydrolyzable class of gallotannins, whereas tannic acid is a significant member of the hydrolyzable class.Esters of phenolic acids and a polyol, typically glucose, are known as hydrolyzable tannins (Chung et al., 1998). According to reports, food-borne bacteria, aquatic bacteria, and microorganisms that produce bad flavors were inhibited by tannic acid and propyl gallate but not by gallic acid. The hydrolysis of the ester bond between gallic acid and the polyols that are hydrolyzed when many edible fruits develop appears to be linked to their antibacterial activities. Therefore, the tannins in these fruits act as a natural defense against microbial diseases. Numerous bacteria, viruses, yeasts, and fungi are inhibited in their growth by them (Chung et al., 1998).Additionally, they may also act as insecticides, defend against predators, and regulate plant growth (Katie et al., 2006).

- **Resins**

Resin is classified as a "solid or highly viscous substance," and it may usually polymerize. These viscous materials can be manmade or produced from plants. Frequently, they are blends of organic substances. Terpenes and their derivatives make up the majority of the resin that most plants produce (Jean et al., 2003). In general, the majority of resins have antibacterial properties and aid in wound healing in both plants and animals. It has been demonstrated that applying pine resin topically to burns and wounds promotes squamous epithelization, normalizes wound hemodynamics, and boosts local immunological activity. Beyond this, the effects of resins derived from various plants can be very diverse. For instance, it is widely known that frankincense, the gum resin of Boswelliaserrata, is an effective inflammation modulator that helps with ulcerative colitis and asthma. This is due in part to the fact that it inhibits 5-lipoxygenase. According to medicinal plants (2011), the gum resin of commiphoramolmol, or myrrh, has antiparasitic, analgesic, and antitumor properties.

- **Saponin**

Polycyclic aglycones joined to one or more sugar side chains form saponins, which are glucosides having foaming properties. According to Hostettmann et al. (1995), the aglycone portion, commonly known as sapogenin, is either a triterpene (C30) or a steroid (C27). The combination of a hydrophilic (water-soluble) sugar component and a hydrophobic (fat-soluble) sapogenin gives saponins their foaming capacity. The majority of vegetables, legumes, and herbs contain saponins, which are compounds with a bitter flavor. Peas, soybeans, and certain herbs with names that suggest they have foamy qualities are the most well-known sources of saponins. The health benefits of saponins are numerous. Research has demonstrated the positive impacts on immune system stimulation, cancer, blood cholesterol levels, and bone health.

3.0 Materials and Methods

3.1 MateriaL and Reagents

- Disposable petri dishes
- A Wireloop
- An incubator
- A speculum
- Commercially prepared powder of nutrient agar,sabouraud dextrose agar and Muller Hinton agar.
- Alkaline peptone powder
- Brain-heart effusion broth
- An autoclave
- A distiller
- A blender
- Dry test tubes
- A Pasteur pipette

3.2 Study Design

An experimental design was used in this work.

3.3 Study Duration

This work was carried out from the 5th of April to the 5th of May 2019.

3.4 Sample Collection

3.4.1 Collection of Plants and Essential Oil Extraction

- **Extraction of oil from orange peelings**

Women selling oranges at the regional hospital Bamenda gate provided fresh citrus sinensis peelings, which were then thoroughly cleaned with water, dried, and processed into powder using a dry, clean grinding stone. After that, the powder was kept in a container with a label. Two liters of water were used to combine and put the remaining amount in a distiller. Following distillation, a layer of oil was seen floating on the distillate's surface. The oil was collected from the water's surface using a Pasteur pipette to prevent it from combining with the water.

- **Extraction of oil from Cypress**

Fresh leaves of cypress were collected, whashed, a portion blended and transferred in a distiller for oil extraction. Another portion was also dried and powdered.

- **Staphylococcus aureus isolation and purification**

A specimen of pus was first collected on an infected wound, innoculated on nutrient agar and incubated for 18 hours. After gram staining the colonies after growth, a distinct colony of gram positive cocci was picked and subcultured on nutrient agar for purity. Catalase and coagulase tests were then performed for specie identification.

- **Candida spp isolation and purification**

A specimen of vaginal swab from a patient presenting symptoms of vaginal mycoses was innoculated on sabouraud dextrose agar. After 18hours of growth, distinct colonies of yeast cells were picked and subcultured in another SDA for purity.

3.5 Preparation of Innoculum for Sensitivity

Few colonies from the pure culture of yeast were picked and transferred in 10ml of brain heart infusion broth, then incubated for 24hours at 37°c.

Pure colonies of *Staphylococcus aureus* were also transferred in 10ml of alkaline peptone water and incubated at 37°C for 24hrs.

3.6 sensitivity Testing

Sensitivity testing was done using the well agar diffusion method.

3.6.1 Sensitivity testing against staphylococcus aureus

The innoculum of *Staphylococcus aureus* in alkaline peptone water was poured on two plates of Muller Hinton agar, the excess remove aseptically and the surface allow to dry. Three Wells were dug on each plates with a sterile radio antenna of about 8mm; in the first plate,one well was filled with the oil extract from orange peels, the second with a solution of 1mg/ml of doxycycline (positive control) and the third well was left empty (negative control)

In the second plate, the first well was filled with the oil extract from Cypress leaves, the second well filled with a 1mg/ml doxycycline solution (positive control) then the third well was left empty as negative control.

For twenty-four hours, the plates were incubated at 37°C, then the antimicrobial sensitivity was evaluated.

3.6.2 Sensitivity testing against Candida Spp

The innoculum of yeast in brain heart infusion broth was poured on two plates of Muller Hinton agar, the excess remove aseptically and the surface allow to dry. Three Wells were dug on each plates with a sterile radio antenna of about 4mm ; in the first plate,one well was filled with the oil extract from orange peels, the second with a solution of 1mg/ml of fluconazole (positive control) and the third well was left empty (negative control)

In the second plate, the first well was filled with the oil extract from Cypress leaves, the second well filled with a 1mg/ml fluconazole solution (positive control) then the third well was left empty as negative control. The plates incubated at 37°C for 24hours, then the sensitivity was evaluated.

3.7 Phytochemical Screening

In accordance with the conventional protocols outlined by Trease and Evans (1989) and Faraz et al. (2005), the powder of our plants was utilized as samples for the qualitative phytochemical screening for resins, alkaloids, saponins, glycosides, flavonoids, and tannins.

3.7.1 Test for Flavonoids

1g of each sample was dissolved in 4mL of dilute NaOH solution. A few drops of conc.H_2SO_4 was then added. Disappearance of color suggested a positive test (Trease and Evans, 1989).

3.7.2 Test for Alkaloids

1g of each sample was stirred with 10mL of 2N HCl in a steam bath and were filtered.the filtrates were tested with few drops of Wagner's reagent. The formation of a precipitate was indicative of a positive test (Faraz *et al*., 2005)

3.7.3 Test for Saponins

In a test tube, 10 ml of sterile distilled water was added to 1g of each sample, and the mixture was then brought to a boil. The presence of saponins was suggested by the existence of froth that lasted after warning (Faraz et al., 2005).

3.7.4 Test for Glycosides

Ten milliliters of boiling distilled water were used to mix one gram of each sample. After filtering, a few drops of concentrated hydrochloric acid were used to hydrolyze two milliliters of the filtrates, and a few drops of ammonia solution were added to make the solution alkaline. After adding five drops of this solution, two milliliters of Fehling's qualitative reagent were brought to a boil. Glycosides were indicated by the appearance of a reddish-brown precipitate (Trease and Evans, 1989).

3.7.5 Test for Resins

1g of each sample, 4mL of boiling ethyl alcohol was added and filtered through whatmann No.1 filter paper. The filtrate was then diluted with 5mL of 1% aqeous HCL. Formation of a heavy resinous precipitate was suggestive of a positive test (Trease and Evans, 1989).

3.7.6Test for Tannins

Ten milliliters of boiling distilled water were used to mix one gram of each sample. This was filtered, and the filtrates were mixed with a few drops of 6% ferric chloride. The presence of tannins was suggested by the dark green coloring. A few drops of iodine solution were added to the second section of the filtrate. The presence of tannins was established by the appearance of a slight bluish coloring (Trease and Evans, 1989).

4.0 Results

Table1: Phytochemical analysis results

Phytochemicals / Plants	Flavonoids	Alkaloids	Saponins	Glycosides	Resins	Tannins
Cupresocyparis Leylandii	+++	-	-	+	+++	+++
Citrus sinensis	++	+	+	-	++	+++

Key:

- Absent

+ Moderate

++ Present

+++ Highly present

Oil extract of *Cupressocyparis leylandii* was highly positive for flavonoids,resins and tannins. It also contained a small amount of glycosides but was negative for alkaloids and saponins.

Oil extract of *Citrus sinensis* was positive for flavonoids, alkaloids, resins, tannins, saponins but lacked glycosides.

4.1 Antimicrobial Sensitivity Results

Essential oil of orange peels was sensitive against Staphylococcus aureus with a diameter of inhibition of **7 mm**. Candida spp showed resistance to the extract.Essential oil from cypress was sensitive against Staphylococcus aureus with a diameter of inhibition of **25mm** but Candida spp showed resistance. (See table below)

Table 2: Antimicrobial succeptibility results.

	Staphylococcus aureus	Candida spp
Control	Sensitive (Ø=21m)	Resistant
Citrus sinensis	Sensitive (Ø=7mm)	Resistant
Cupressocyparis leylandii	Sensitive (Ø=25mm)	Resistant

Ø=diameter of inhibition of growth

Figure3: **muller hinton agar showing clear zone of inhibition of growth by the oil extracts**

5.0 Discussion

The aim of this study was to determine and evaluate the antimicrobial Sensitivity of oil extract of citrus sinensis and cupressocyparis leylandii on Bacterial and fungal skin pathogens. The results are represented in tables. the two plants exhibited good bactericidal activities with various diameters of inhibition but *Candida albicans* showed resistance to both extracts

Phytochemical screening of *Citrus sinensis* peel showed the presence of tannins, flavonoids, resins, saponins and alkaloids which are similar to the work carried out by Mamta *et al*., (2013) in India working on phytochemical screening of Orange peel and Pulp using hexane extract.

Screening of *Cupressocyparis leylandii* revealed the presence of resins, flavonoids, glycosides and tannins**.**

Study Limitations and Strengths

Study limitations: our study has the following limitations

The sensitivity test was done on single specie of Bacterial skin pathogen, *Staphylococcus aureus* and a single specie of fungal skin pathogen, *Candida albicans.* Also, the minimum inhibitory concentration (MIC) could not be performed because of the lack of a suitable oil diluent**.**

Study strengths: Despite these limitations, our study has the following strengths

To the best of our knowledge this is one of the first studies in our country and in sub-Saharan Africa on phytochemicals for the search of new chemical entities.

6.0 Conclusions

This study has proven that the fresh peels of *Citrus sinensis* and *Cupressocyparis leylandii* possess chemical compounds that are active against bacteria and it is believed to be a promising source for new antimicrobial agents for cosmetic purposes.

6.1 Recommendations

We recommend:

- **To the research community:**

Researchers should carry out phytochemical screening and antimicrobial properties of extracts from many species of plants. Researchers should look at a ways to determine the minimum inhibitory concentrations and study the safety of these essential oils for use.

- **To the Government**

The government of Cameroon is concerned about the increase resistance of microorganisms to drugs and the unavailability of the drugs to patients due to the cost. For these reasons, the government should sponsor researchers and encourage them to make use of local plants develop local drugs. The government should encourage the use of plants as medicine because they are cheap, affordable, have little side effects as compared to synthetic ones who turn out to be expensive and can lead to drugs toxicity.

The Ministry of Forestry and Wild life should encourage the planting of medically important plants.

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