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Research Article

Evaluation of the Antibacterial Effect of Taiwan's Endemic Hinoki Hydroso on Candida Albicans

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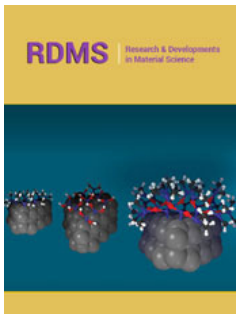
ISSN: 2576-8840



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Submission: October 24, 2024
Published: November 06, 2024

Volume 21 - Issue 2

How to cite this article: Chang Lung Yen*, Meng-Shiu Lee, Jian-Hung Chen, Hung-Yu Chien, Jen-Son Cheng, Meng-Shiu Lee, Chen-Cheng Huang and Yueh-Ying Wang. Evaluation of the Antibacterial Effect of Taiwan's Endemic Hinoki Hydroso on Candida Albicans. Res Dev Material Sci. 21(2). RDMS. 001006. 2024.
DOI: 10.31031/RDMS.2024.21.001006

Abstract

This study probed into the potential application of Taiwan's endemic plant, Hinoki, and the by-product distilled from Hinoki, Hinoki hydroso, to inhibiting Candida albicans. Candida albicans is a polymorphic fungus. It has become a common pathogenic bacteria causing nosocomial infections. Hinoki hydroso is a plant fungicide. It is a volatile substance released from plants to protect them against pathogens and insects. It has known insecticidal, antimicrobial, and antifungal properties.

In the experiment, the Hinoki hydroso was diluted in different concentrations. Partial inhibition of Candida albicans was observed at a concentration of 25%, and a greater inhibition was observed at 50%. Its growth was completely inhibited at a concentration of 75%. However, they grew at 12.5%, 25%, 50%, and 62.5% concentrations. No growth was observed, only at 75% and 100% concentration. According to the experimental results, the Minimum Inhibitory Concentration of Taiwan's endemic Hinoki hydroso extracted in this study for Candida albicans is 75%. The minimum bactericidal concentration of 25% partially inhibits the growth of Candida albicans.

This study provided important insights into Taiwan's endemic plants' conservation and sustainable utilization. It opened a new direction for the potential application of Hinoki hydroso for antifungal treatment.

Keywords: Candida albicans; Hydrolat; Minimum inhibitory concentration; Minimum bactericidal concentration

Introduction

Background and motives

Taiwan's Hinoki refers to Chamaecyparis formosensis and Chamaecyparis [1]. After 1950, Hinoki played a significant role in growing Taiwan's economy [2]. Red cypress and Chamaecyparis are the most essential coniferous trees in Taiwan. They are ideal building materials that are very useful in preventing insect damage. However, these valuable and precious trees are becoming extinct for various reasons [3]. The utilization of these precious trees-initiated research on chemical compounds of the hinokitiol found in Taiwanese cypress [4]. For half a century, most researchers probed into the productive land, physiological ecology, morphological characteristics, chemical composition, and bioactivity of Chamaecyparis formosensis and Chamaecyparis [5]. Quintain et al. [6] performed steam heat treatment of the outer layer of Hinoki bark and qualitatively studied the cypress to explore the possibility of using residual forest biomass to produce valuable chemicals [6].

The polymorphic fungus Candida albicans is found in human microbiome. In most people, Candida albicans is a lifelong and harmless symbiont. However, in some special circumstances,

it may cause various infections, ranging from superficial infections on the human skin to life-threatening systemic infections [7]. Since 1989, Candida gradually evolved into one of the reasons of nosocomial infections [8]. Candida is the fourth leading cause of nosocomial bloodstream infections in the United States [9]. Candida albicans is a fungal pathogen, mainly distributed on the mucosal epidermis of the gastrointestinal and urinary tracts. It is a normal symbiotic species. However, it often causes epidermal and systemic diseases in patients with insufficient or deficient immunity and even causes death [10]. Candida albicans is generally a benign member of the mucosal flora. It often causes mucosal diseases with a high incidence and can lead to life-threatening bloodstream infections in vulnerable patients [11]. In humans, Candida albicans can grow in at least three different forms: yeast, pseudohyphae, and hyphae [12].

Research purposes

This study aims to discuss the probability of using pure Hinoki hydroso to inhibit Candida albicans. Hinoki hydroso is a byproduct distilled from the Hinoki plant. The research purposes are as follows:

This study explored Hinoki hydroso after genetically confirming it. Its composition is analyzed using the GC-MS. This study explored the relationship between Hinoki hydroso and periodontal disease bacteria and whether the Hinoki hydroso can inhibit Candida albicans. The focus is on the inhibitory effect of Hinoki hydroso on periodontal disease bacteria.

Research structure

This paper discusses the possibility of using Hinoki hydroso to inhibit Candida albicans. It develops the theory of using Hinoki hydroso to inhibit Candida albicans.

Section 1: Introduction, including research motivation, research methods, research process, research purpose, and research framework.

Section 2: Literature Review, describing research concerning Hinoki hydroso and Candida albicans.

Section 3: Analysis of Taiwan's mouthwash industry and development process of the mouthwash industry.

Section 4: Research methods and experimental analysis are discussed.

Section 5: Conclusion and suggestions for future development and discussing the future development of Hinoki hydroso in oral hygiene.

Research methods

Plant fungicides are volatile substances released from plants to protect plants against pathogens and insects, and their insecticidal, antimicrobial, and antifungal activities are known [13]. The oil extracted from leaves and branches has been used as functional additives or spices in soaps, toothpaste, and cosmetics [14]. The Hinoki extract contains terpenoids with high antibacterial, insect prevention, and antiseptic properties and is antihypertensive and anticancer [15].

Different bacterial species grow in the oral cavity after cleaning with different mouthwashes. Therefore, this study examined the changes in the bacterial species of oral periodontal disease after using the Hinoki hydroso for oral cleaning. This study also explored the influence of Hinoki hydroso on the bacterial species of oral periodontal disease. It will help enterprises develop oral mouthwashes in the future.

Research process

According to the purpose of this study, the research flow chart is as follows (Figure 1).

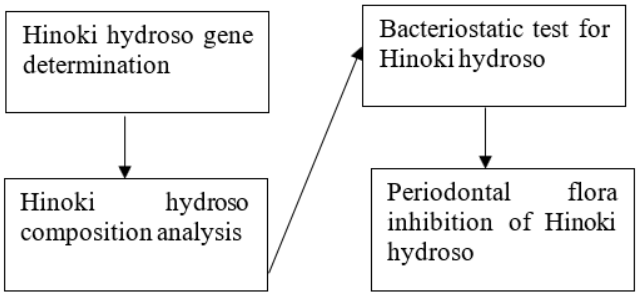

Figure 1: Research process.

Literature Review

A Hydrolat is a condensed liquid soluble in water after a high-temperature distillation of logs, plants, or plant flowers. The crude terpene glycosides are derived from traditional Hinoki leaves steaming extract [16]. The antibacterial effect of Hinoki hydroso is mainly induced by the volatile oil and non-volatile substances in the bark, while the neutral and acidic substances are highly active against pathogens [17]. In addition to a small amount of essential oils, the Hydrolat contains water-soluble substances from all logs, plants, or flowers [18]. Ancient Chinese traditional medicine attached great importance to plants' therapeutic effects and the use of rare aromatics [19]. Hydrolat is a biopesticide produced from natural products [20].

Hinoki has been said to be antibacterial since ancient times. Housewives of the older generation put their leftovers in vegetable baskets made of Hinoki to preserve them for longer. The essential oil of Hinoki can also be used for air purification, and fruit and house flies show strong repellent behavioral responses to the essential oil [13].

Gas chromatography-mass spectrometry (GC-MS) combines the characteristics of Gas Chromatography Gas Chromatography and Mass Spectrometry to identify different substances [21]. The Gas Chromatography-Mass Spectrophotometer (GC-MS) analyzes the main components [22]. It is widely accepted as a gold standard analytical method for measuring organic substances, including extractives in aqueous matrices [23]. It analyzes the composition and content of soluble organic matter [24]. It performs qualitative

and quantitative analyses of aromatic odor molecules adsorbed on textile materials [25]. In this study, the GC-MS was used to determine the composition percentage of Hinoki hydroso. Previous studies examined essential oils extraction using water distillation. They used it to test and analyze the chemical composition of three Pogostemon cablin Benth essential oils of different months [26]. The essential oil and Hydrolat were analyzed using GC/MS. The conclusions were drawn based on the chromatograms of the essential oil and Hydrolat. The two extracts comprised monoterpenes and phenylpropane derivatives [27]. Scholars also studied the essential oil components of the leaves of Hinoki and Hinoki seedlings. The factor and cluster analyses were used to determine the essential oil composition by GC-MS [28]. The partial essential oil of Hinoki was obtained by hydrodistillation and was analyzed using GC-MS and C-NMR spectra methods [29]. The GC-MS was used to analyze the main components of Hydrolat [29].

The adhesion of Candida albicans and oral bacteria is critical for colonizing the oral cavity. Regarding interactions, Candida albicans and Pseudomonas aeruginosa are described as competitive and antagonistic. Another interesting interaction is between Staphylococcus aureus and Candida albicans, which is not fully characterized but initially appears synergistic [30]. The prevalence of oral carriers of Candida albicans in healthy-toothed adult subjects is 44.4%. Females were more frequent carriers than males. They had a lower pH of tongue surface saliva. Their age, DMF index, Russell periodontal index, plaque index, and intraoral temperature do not affect the carriage rate. Candida is not evenly distributed in the oral cavity. The tongue is the main reservoir of the fungi. However, a certain proportion may exist in the oral mucosa, plaque-covered surface of teeth, and saliva [31]. Bacteria and fungi exist together in countless environments, especially in biofilms, where the attached species interact with each other through different signaling mechanisms. These microorganisms have co-existed over billion years. However, the research on fungal-bacterial interactions is still in its infancy, particularly in the context of polymicrobial infections. However, research on interactions between the fungal pathogen Candida albicans and various bacterial pathogens is growing. One example of a mutually beneficial interaction is co-aggregation. This phenomenon occurs in oral biofilms, where the adhesion of Candida albicans and oral bacteria is critical for their colonization in the oral cavity [30].

Candida albicans is an opportunistic fungal pathogen that can cause various human diseases, such as oral thrush and disseminated candidiasis [32]. Its ability to transition between symbiosis and pathogenesis is the key focus of many ongoing studies [33]. Its survival in the oral cavity is dependent on a stable yeast population. The microorganisms are continuously removed from the oral cavity through the host clearance mechanism. So, to survive this ecosystem, Candida albicans cells must adhere and replicate [34]. They can be separated from the healthy individuals' oropharynx, gastrointestinal tract, and vagina. However, when local or systemic host defense mechanism is damaged, Candida can cause oropharyngeal, esophageal, or vulvovaginal candidiasis. In susceptible hosts, the microorganisms can penetrate the gastrointestinal mucosa and enter the blood, causing hematogenous disseminated candidiasis [35].

There is an opportunity to develop high-value products if this study can control the quality of the distilled hydroso. This is also the concept of a circular economy that cherishes the environment and resources today.

Research Method

Research design

The oral microbiome is critical to health as it contributes to oral and systemic diseases. It resides within the mouth's biofilm, forming an ecosystem that balances health. However, imbalances within this balanced state can cause pathogens and diseases [36]. The human oral cavity is home to the most diversified microflora in the body, including viruses, fungi, protozoa, archaea, and bacteria [37].

The MIC is defined as the lowest concentration of an antimicrobial agent inhibiting the visible growth of microorganisms after an overnight incubation. The MBC is defined as the lowest concentration of an antimicrobial agent that prevents the growth of microorganisms after overnight incubation [38].

Bacillus DSM 4312, Enterococcus ATCC 29212, Salmonella ATCC 14028, Staphylococcus ATCC 25923/ATCC 12228, and Candida ATTC 10231 exist in the oral microbiome [39]. Therefore, these bacteria are used as experimental species in our research design.

The experimental method is used to clarify the relationship between causal variables in social sciences [40]. The experimental sequence for this study is as follows:

1. First, it is required to confirm whether the source of the product is Taiwan Hinoki wood.

2. Then, the Hinoki hydroso is distilled from the confirmed sample, and the Hinoki hydroso is entrusted to an impartial organization to measure its composition by GC-MS.

3. The Hinoki hydroso is tested based on the stock solution 100% sample.

The Hinoki hydroso is further explored in this study, hoping to determine the relationship between Hinoki hydroso and periodontal disease bacteria. The focus is on the inhibitory effect of Hinoki hydroso on periodontal disease bacteria.

Material verification

This study is based on the "Fruit of the Poisonous Tree" [41]. Taiwan has many kinds of wood, with as many as 451 species of endemic woody plants [42]. There is a certain risk in determining the Taiwan Hinoki hydroso merely according to the rule of thumb and wood smell. The source of Taiwan Hinoki is confirmed by this study. In case of non-confirmation, the research data of Taiwan Hinoki is used.

This study must determine the wood type order to avoid the Fruit of the Poisonous Tree. The Taiwan Hinoki wood sample

identified by the rule of thumb and wood smell is verified by the government unit - Taiwan Forestry Research Institute. The official report confirms that the commissioned test sample is Taiwan Hinoki [43]; (Figure 2).

行政院農業委員會林業試驗所

樹木及林產物檢定報告書

申請單位(人)：鼎右企業有限公司　　發文日期：中華民國 109 年 12 月 22 日

申請日期：109 年 12 月 03 日　　發文字號：農林試利字第 1092251632 號

送(取)驗單位(人)：顏昌隆

聯絡人：顏昌隆　　聯絡電話：(04)2275-7722

工程(計畫)名稱：無

檢定結果：如下表

序號	項　目	結　果	備　註
1.	木材組織辨識	*Chamaecyparis obtusa* var. *formosana* (台灣扁柏)	Cupressaceae 柏科
~以下空白。			

Figure 2: Taiwan Forestry Research Institute's report on wood genes [43].

Experimental method

The test results are marked with Hinoki hydroso, an endemic species of Taiwan. The sample reaches "the concentration (included) or above", which is undetectable bacterial growth, i.e., the Minimum Inhibitory Concentration (MIC).

The MIC is defined as the lowest concentration of an antimicrobial agent that inhibits the visible growth of microorganisms after overnight incubation, and the MBC will inhibit the growth of microorganisms after subculture on the antibiotic-free medium. The MIC is mainly used to confirm drug resistance in diagnostic laboratories but is most commonly used as a research tool to determine the in vitro activity of new antimicrobial agents. The research data have been used to determine MIC breakpoints [38].

Test material samples

The Gas Chromatography-Mass Spectrophotometer (GC-MS) is efficient and universal analytical technology with numerous scientific applications that can meet the needs of applied science and technology fields [44]. It identified 90 main components of Tarchonanthus camphoratus [45]. It is an excellent technique for analyzing carbohydrates. Different functional groups can be found, and the diversity of samples requires specific methods [46,47].

After the source of Taiwan Hinoki was confirmed by the Taiwan Forestry Research Institute's report on wood genes, traditional distillation was used for treatment. The Taiwan Hinoki hydroso was obtained, which was entrusted to the government agency - Taiwan Forestry Research Institute to test its components using the GC-MS, and an analysis report on the composition of Taiwan Hinoki hydroso was obtained [43] (Figures 3 & 4).

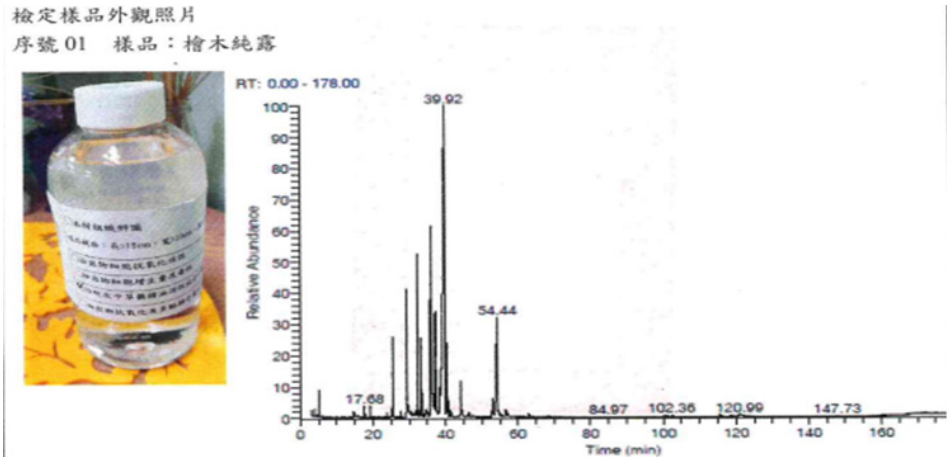

Figure 3: GC-MS Taiwan Hinoki hydroso spectrum [43].

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申請單位(人)： 鼎右企業有限公司　　發文日期：中華民國 109 年11月22日

申請日期： 109 年 11 月 23 日　　發文字號：農林試化字第 1092242467 號

送(取)驗單位(人)：鼎右企業有限公司

聯絡人：顏昌隆　　聯絡電話：04-22757722

檢定結果：如下表

序號	項　目	結　果	備　註
01	檜木純露成分分析	α-Terpineol (松油醇)-35.05%	RT-39.92(防蚊成分)
		Borneol (龍腦)-19.92 %	RT-36.27(防蚊成分)
		(-)-Camphor ((-)-樟腦)-8.55 %	RT-32.42(防蚊成分)
		Fenchyl Alcohol (葑醇)-6.92 %	RT-29.44(防蚊成分)
		Terpinen-4-ol (4-萜烯醇)-5.59 %	RT-37.23(防蚊成分)
		Dihydroterpineol (氫化松油醇)-4.25 %	RT-33.33(防蚊成分)
		Fenchone (葑酮)-3.06 %	RT-25.47(防蚊成分)

Figure 4: GC-MS Taiwan Hinoki hydroso composition [43].

In this study, the composition of Taiwan Hinoki hydroso was tested by the Forestry Bureau of the Council of Agriculture, Executive Yuan, using the GC-MS. The obtained Taiwan Hinoki hydroso is composed of α-Terpineol 35.05%, Borneol 19.92%, Cornphor 8.55%, Fenchyl Alcohol 6.92%, Terpinen-4-ol 5.59%, Dihydroterpineol 4025%, and Fenchone 3.06% [43].

Data analysis

This study used the comparison method. In the first step, the GC-MS obtained all components of Taiwan's endemic Hinoki hydroso before experiments.

Test strains:

1. Candida albicans (BCRC 21538).
2. Conditions of action: Candida albicans: 32 °C ± 2 °C; 24 - 48 hours of action.
3. Culture conditions: Candida albicans: 32 °C ± 2 °C; 48 hours of culture.
4. Culture medium: Sabouraud Dextrose Agar.

Preparation: grouping-control group:

1. Negative control (expected to have no antibacterial effect), strain suspension without samples.
2. Blank (expected culture medium is clear): physiological experimental water.

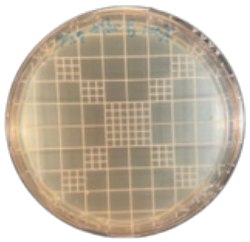
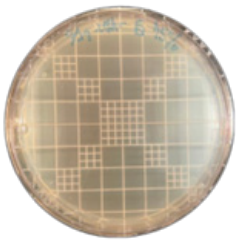
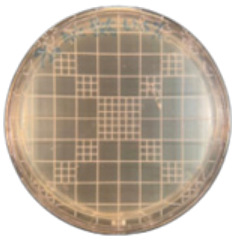
Grouping-sample experimental group:

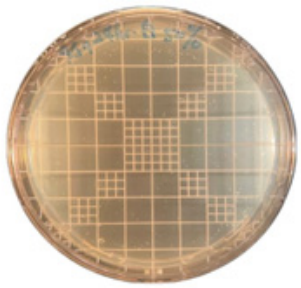
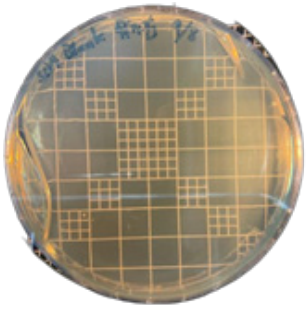
1. The 100% Hinoki hydroso stock solution was diluted with culture medium to 75%, 62.5%, 50%, 25%, and 12.5% concentrations. It was then tested with Candida albicans (BCRC 21538).
2. The initial bacterial load was adjusted to the expected bacterial count of 1.08 x 107cfu/ml for subsequent dilution.
3. The experimental, positive control, and negative control groups were individually inoculated with 106cfu/ml bacteria solution.
4. After inoculation, the experimental and the control groups acted at 32 °C ± 2 °C for 24 hours.
5. After the action, the samples of the experimental and the control groups were inoculated on appropriate culture media and cultured in the environment specified in the culture conditions. The growth conditions were observed and recorded.

Test results:

The partition results of Taiwan's endemic Hinoki hydroso at different experimental concentrations are shown in Figure 5. The experimental results show:

Figure 5: Results of Taiwan's endemic Hinoki hydroso at various test concentrations cultured in the medium partitioned into four zones with Candida albicans test liquid.

Test species: Candida albicans		
100% shows: no bacterial growth	75% shows: no bacterial growth	62.5% shows: tiny bacterial growth
		

50% shows: a little bacterial growth 	25% shows: considerable bacterial growth 	12.5% shows: considerable bacterial growth 
Blank shows: no bacterial growth 		Negative control shows: high bacterial growth 

Conclusion

The MIC is defined as the lowest concentration of an antimicrobial agent inhibiting the visible growth of microorganisms after overnight incubation. The MBC is defined as the lowest concentration of an antimicrobial agent that prevents the growth of microorganisms after overnight incubation (Andrews, 2001).

When Taiwan's endemic Hinoki hydroso was diluted to 25%, Candida albicans growth was partially inhibited and to a greater extent when diluted to 50%. A complete inhibition of the growth of Candida albicans was observed at 75% dilution.

Candida albicans grew when the concentration of Taiwan's endemic Hinoki hydroso was 12.5%, 25%, 50%, and 62.5%. There was no growth of Candida albicans at 75% and 100% concentrations. Therefore, the MIC of Taiwan's endemic Hinoki hydroso against Candida albicans is 75%. Regarding MBC, when Taiwan's endemic Hinoki hydroso was diluted to 25%, the growth of Candida albicans was partially inhibited.

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