

Article

Evaluation of Antioxidant and Antifungal Activity of *Chamaecyparis obtuse* Extract for Use in Oral Herbal Medicine

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Abstract: The aim of this study was to investigate the antifungal and antioxidant activities of *Chamaecyparis obtusa* (*C. obtusa*) extract (COE) against *Candida albicans* (*C. albicans*). Methods: The antioxidant activity was determined using three methods based on 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) scavenging, total polyphenol measurement, and total flavonoid measurement. In addition, the survival rate of human keratinocytes (HaCaT) was checked to confirm their stability. A one-way ANOVA also confirmed the antifungal activity of COE against *C. albicans* and its proliferative effect on HaCaT cells. In addition, to confirm the difference between each group, the Tukey HSD test was performed as a post hoc analysis, and the significance level was set at 0.05 as a two-tailed test. Results: COE was found to contain 424.150 mg GAE/g of polyphenols, 127.566 mg CE/g of flavonoids, and 83.594% of radical scavenging activity. Furthermore, 30 mg/mL of this extract exhibited complete antifungal activity. In addition, a noticeable killing effect on *C. albicans* was observed as the concentration of the extract increased. For instance, at a 2.5 mg/mL dosage of COE, HaCaT cells were safe, but *C. albicans* showed a distinct antifungal effect. Conclusions: COE has antioxidant and antifungal activity, so it has a high potential as an effective natural antifungal agent. Therefore, oral gargle containing COE is expected to be a safe and effective treatment in oral candidiasis in clinical practice.

Keywords: antioxidant; antifungal effect; *Candida albicans*; *Chamaecyparis obtuse*; toxicity



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1. Introduction

One of the most prevalent infections in humans, oral candidiasis, is caused by the overgrowth of *Candida* species [1]. In the 1980s, there was a clear surge of interest in oral candida infections largely due to the increased incidence of oral candida because of the escalation in the acquired immune deficiency syndrome (AIDS) epidemic, and, to date, oral candidiasis remains the most common oral opportunistic infection in human immunodeficiency virus (HIV)-positive individuals and in individuals with weakened immune systems [2]. These include *Candida albicans* (*C. albicans*), *C. tropicalis*, *C. glabrata*, *C. pseudotropicalis*, *C. guilliermondii*, *C. krusei*, *C. lusitaniae*, *C. parapsilosis*, and *C. stellatoidea* [3], and among them, *C. albicans* is the most common species [4]. With an organic relationship with *S. aureus*, *C. albicans* enhances bacterial colonization and biofilm formation, which resists antibiotic treatment and infects or destroys mucosal tissues [5]. The *C. albicans* biofilm matrix is largely composed of the polysaccharides β -1,3-glucan, β -1,6-glucan, and mannans, which form the mannan–glucan complex (MGCx) [6]. In the oral cavity, hyphae formation and the adherence to oral epithelial cells and other abiotic surfaces such as dentures promote the development of monomicrobial and polymicrobial biofilms [7]. Once a biofilm is established, the expression of *Candida* virulence factors increases, and

susceptibility to antimicrobials and phagocytosis decreases drastically [8]. Importantly, in addition to *Candida*'s pathogenic factors and interactions with the host immune system, it is now acknowledged that the bacterial component of the oral microbiome plays an important role in the development and exacerbation of oral candidiasis [9]. Among the oral bacterial flora, streptococci are important for the colonization of *C. albicans*. Therefore, the interaction between *C. albicans* and streptococci is known to show a synergistic effect as an oral fungus–bacteria interaction. Specifically, interactions with *Streptococcus oralis*, *Streptococcus mitis*, *Streptococcus gordonii*, and *Streptococcus mutans* have been reported to adhere to the hyphae of *C. albicans* [10]. By using the lactic acid produced by streptococci, *C. albicans* lowers the partial pressure of oxygen to a level favorable to conditional streptococci [11]. In an experimental study, it was reported that *C. albicans* caused occlusal caries because, similar to *S. mutans*, *C. albicans* has the ability to produce and withstand acid. Therefore, the potential role of *C. albicans* in caries development through physical and metabolic interactions with *S. mutans* has been confirmed through several lines of evidence [12]. Additionally, exoenzyme glucosyltransferase B (gtfB) produced by *S. mutans* has been shown to be deposited on the surface of *C. albicans* hyphae, helping the fungus attach to oral surfaces [13]. In this respect, *C. albicans* can interact with oral bacteria and cause more serious diseases.

Oral candidiasis does not cause problems in healthy populations [1], but it primarily affects infants and the elderly and causes opportunistic infections in individuals with prolonged antibiotic use or immunocompromised status [14]. Therefore, antibiotics should not be used for a long period of time for the purpose of preventing oral infectious diseases or continuously suppressing oral pathogens. Accordingly, natural extracts, instead of synthetic chemical ingredients, are recommended in mouthwashes to control pathogens while maintaining oral microflora [15]. Thus, natural extracts have grown in popularity in recent years [16] as more research has been conducted.

Chamaecyparis obtusa (*C. obtusa*) is an evergreen conifer native to East Asia, including Japan and Taiwan, and a member of the *Cupressaceae* family. It produces a large amount of *phytoncide* with a distinct fragrance, which is known to be effective against pathogens in the oral cavity [17]. *Phytoncides* are volatile oils derived from the flowers, leaves, stems, roots, and resins of plants and are known to have antifungal effects against bacteria and fungi [18]. Specifically, *phytoncide* from cypress is known to be effective against Gram-positive bacteria (*Staphylococcus epidermidis*), Gram-negative bacteria (*Vibrio parahaemolyticus*, *Pseudomonas aeruginosa*, and *Pseudomonas putida*), yeast (*C. albicans*), and filamentous fungi (*Aspergillus nidulans*, *Alternaria mali*, and *Fusarium oxysporum*) [19]. Kang et al. [20] reported the antifungal effect of the *phytoncide* essential oil extracted from *C. obtusa* against *C. albicans*, which causes oral candidiasis. As a secondary photosynthetic metabolite, *phytoncide* is produced during stress responses in plants and microorganisms, including fungi [21]. This phenomenon, which directly and indirectly stresses the plants, is called allelopathy [22]. Allelochemicals are biochemical substances, and they can be refined in the form of essential oils through various methods such as steaming, compression, and extraction. The antimicrobial volatile (aromatic) organic chemicals contained in essential oils are called phytoncides. Representatively, the main components of *phytoncides* are terpenes (terpenoids), phenolics, alkaloids, phenylpropanes, and acetogenins [23]. *Phytoncide* essential oil from *C. obtusa* contains a complex mixture of compounds belonging to different groups with various functions, and the scent and flavor work individually or synergistically to have various effects such as calming effects in mind and body, blood circulation, anti-inflammatory, decongestive, immunomodulatory, antifungal, expectorant, antioxidant, psychotic, analgesic, and insecticidal effects. Toxicity and side effects, along with the purpose, dose, and route, need to be reviewed to confirm its safety and efficacy for clinical use [24]. For safety verification, human keratinocytes (HaCaT) extracted from adult skin are used due to their rapid cell proliferation and massive in vitro differentiation [25].

Therefore, in vitro study aimed to determine the antifungal and antioxidant effects of *C. obtusa* extract (COE) against oral *C. albicans*. In addition, the safety of COE as an oral

candidiasis treatment to be used as basic data for clinical application was confirmed by observing the survival rate of HaCaT.

2. Materials and Methods

2.1. Sample Extraction

C. obtusa used in this study was grown in Gyeongsang, Gyeongsangbuk, South Korea, and was purchased from Cheongmyeong Co., Ltd. (Paju, Republic Of Korea) COE was obtained using a rotary vacuum evaporator (N-1300E.V.S., Rikakikai Co., Ltd. (EYELA Co.), Tokyo, Japan) after filtration by adding 70% ethanol to 100 g of pulverized RVS at 60 °C for 12 h. *C. obtusa* was lyophilized using the freeze-drying process (Ilshin Lab Co., Ltd., Seoul, Republic of Korea). The COE was prepared in powder and stored in a sealed amber vial at −20 °C until used.

2.2. Measurement of Antioxidant Activity

2.2.1. Total Polyphenol Content

The Folin–Ciocalteu and Folin–Denis methods were used to measure the total polyphenol content [26]. CO was dissolved in distilled water to a concentration of 1 mg/mL; then, 0.15 mL of Folin–Ciocalteu phenol reagent (Sigma-Aldrich, St. Louis, MO, USA) was mixed. After 0.3 mL of 20% Na₂CO₃ (Daejung Chemicals & Metals Co., Ltd., Gyeonggi, Republic of Korea) was added before leaving it for two hours in a dark room for the reaction, the absorbance of the supernatant was measured at 725 nm. Gallic acid (Sigma-Aldrich Co., St. Louis, MO, USA) was used as a standard, which was analyzed with the same method as the sample, and the polyphenol content was measured based on the calibration curve obtained: mg gallic acid equivalent (GAE; dry basis) per gram of the extract. Experiments were performed in triplicate.

2.2.2. Total Flavonoid Content

A modified Davis [27] method was used to measure total flavonoid content. Diethylene glycol (0.7 mL) was initially added to 0.1 mL of the sample, and then 0.1 mL of 1 N NaOH solution was further added. After a 1 h reaction in a dark room, the absorbance was measured at 420 nm. The calibration curve was measured with (+)-catechin hydrate (CE; dry basis) as a standard, and the total flavonoid content of the extract was shown as mg (+)-catechin hydrate (CE; dry basis). Experiments were performed in triplicate.

2.2.3. DPPH Scavenging Activity

The radical scavenging activity was measured using 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) (Sigma Chemical Co., St. Louis, MO, USA) [28]. COE (0.2 mL) was mixed with 0.2 mM DPPH solution (0.8 mL), and the absorbance was measured at 517 nm after incubation in a dark room at room temperature for 30 min. The antioxidant was measured by calculating the electron-donating ability (%) using the formula $[1 - (\text{absorbance of the sample with additives} / \text{absorbance of sample without additives})] \times 100$. Distilled water was added to the control group, and gallic acid was used as a positive control. The radical scavenging activity was expressed in percentage (%). Experiments were performed in triplicate.

2.3. Antifungal Assays

2.3.1. Bacterial Cultures

C. albicans (KCTC 7965/ATCC 10231) was incubated in the yeast mold (YM) broth (YM, Difco, Detroit, MI, USA) and cultivated at 37 °C under microaerophilic conditions for 24 h. The bacteria were diluted to a concentration of 1×10^5 colony-forming units per milliliter (CFU/mL).

2.3.2. Agar Disc Diffusion Assay

Paper discs (8.0 mm, Advantec, Toyo Roshi Kaisha, Ltd., Tokyo, Japan) were placed on top of YM agar plates, and 100 μ L of different concentrations of each was dropped onto sterile 8 mm in diameter empty paper discs. The concentrations were used at concentrations of 1.25, 2.5, 5, 10, and 20 mg/mL and incubated anaerobically at 37 °C for 24 h. The size of the clear zone was measured around the soaked discs using a ruler.

2.3.3. Antimicrobial Test

To measure bacterial proliferation, COE was prepared at 1.25, 2.5, 5, 10, 20, and 30 mg/mL concentrations with *C. albicans*-containing medium into a 1.5 mL tube. After keeping them at a temperature of 37 °C for 24 h, the mixtures were diluted, and accurate amounts (1 mL) were uniformly spread onto YM agar plate. In order to check the antimicrobial activity of COE, the CFU were counted after a 24 h incubation. Experiments were performed in triplicate.

2.3.4. Minimum Inhibitory and Bactericidal Concentrations

The minimum inhibitory concentration (MIC) was determined via the broth dilution method [29]. The minimum bactericidal concentration (MBC) was determined by spreading from each test tube containing different concentrations on the plate. The MBC was described as the lowest concentration resulting in no bacterial growth [30]. The sample was prepared by dissolving COE in an aqueous solution containing dimethyl sulfoxide (DMSO). COE was made in 7 sterile test tubes containing 900 μ L of different concentrations (1.25, 2.5, 5, 10, and 20 mg/mL) and 100 μ L of *C. albicans* inoculum containing 1×10^5 CFU/mL to yield a final volume of 1 mL per each tube. After 24 h, the aliquots of 1 mL from all the tubes were uniformly smeared in an YM agar medium and then cultured at 37 °C for 24 h to check the MIC and MBC. Experiments were repeated three times to observe the reproducibility of the measurements.

2.4. Cell Proliferation Assay

The quantification of the effect of COE on cell growth rate was measured using water-soluble tetrazolium salt-1 (WST-1) analysis [31]. HaCaT cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (USA) containing 10% (*v/v*) heat-inactivated fetal bovine serum (FBS; Gibco, Grand Island, NY, USA) and 1% penicillin/streptomycin (100 μ L/mL; Gibco, Grand Island, NY, USA) at 37 °C and 5% CO₂ in a humid chamber. Experiments were performed in 96-well plates containing a final volume of 100 μ L of medium per well. HaCaT cells were seeded at an initial density of 1×10^5 cells/cm² and incubated at 37 °C for 24 h. Then, the medium was changed to serum-free DMEM containing 1.25, 2.5, 5, 10, 20, and 30 mg/mL concentrations of COE. After 3 h, the incubation medium was removed, and the WST-1 solution was added. The plates were incubated at 37 °C for 2 hours, and the optical density (OD) of the sample was measured at 450 nm using an ELISA reader (Multiskan FC, Thermo Fisher Scientific, Waltham, MA, USA). Cell viability was repeated independently at least three times.

2.5. Statistical Analysis

The antioxidant effect was confirmed using statistical software (SPSS v. 24.0, SPSS Inc., Chicago, IL, USA). In addition, ANOVA analysis was performed to confirm the bactericidal effect of *C. albicans* according to the dose of COE. To confirm the safety of COE, the viability of HaCaT cells was analyzed according to the dose, and the Tukey HSD test was performed as a post hoc analysis to confirm the difference according to each dose. The significance level was set to 0.05 in a two-tailed test.

3. Results

3.1. Antioxidant Activity

Table 1 displays the polyphenol content, flavonoid content, and DPPH radical scavenging activity of COE. The polyphenol content showed 424.150 mg GAE/g, the flavonoid content showed 127.566 mg CE/g, and the radical scavenging activity indicated 83.594%, indicating a high level of activity.

Table 1. Effect of total phenolic and flavonoid contents and DPPH radical scavenging activity of the *Chamaecyparis obtusa* extract.

Sample	Total Polyphenol (mg GAE/g)	Total Flavonoid (mg CE/g)	DPPH (%)
<i>Chamaecyparis obtusa</i> extract	424.150 ± 4.543	127.566 ± 9.745	83.594 ± 0.903

3.2. Clear Zone of Inhibition against *C. albicans*

The measured diameters by the disk diffusion method are presented in Figure 1. Growth inhibition showed a clear zone with diameters of 12, 14, and 15 mm at 5, 10, and 20 mg/mL, respectively. It was confirmed that the diameters increased as the concentrations of COE increased.

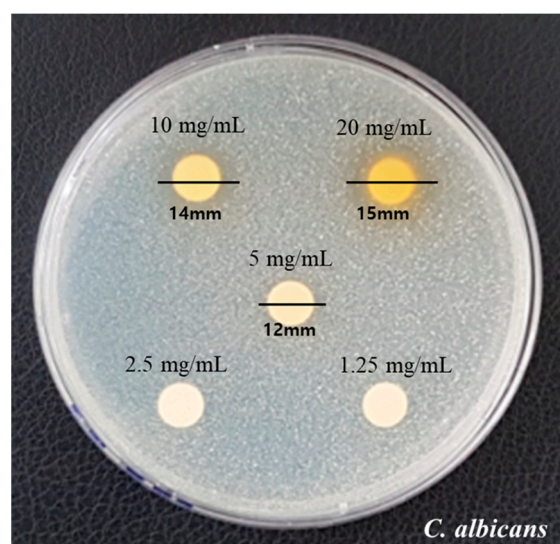


Figure 1. Clear zone formation by different concentrates (1.25, 2.5, 5, 10, 20, and 30 mg/mL) of COE on *C. albicans*.

3.3. Antifungal Effects

Antifungal activity against *C. albicans* by COE was observed with no bacterial growth at 30 mg/mL (Figure 2). Table 2 shows the results of the statistical analysis of changes in CFU. As the concentration of COE increased, a clear inhibitory effect on *C. albicans* was observed. However, there was no statistically significant difference in the concentration of COE from 2.5 mg/mL to 30 mg/mL ($p > 0.05$).

3.4. MIC and MBC Determination

In concentrations of 10 mg/mL, the inhibition of bacterial growth was exhibited. The concentrations of 30 mg/mL were observed to show no growth of bacteria, hence confirming these concentrations as bactericidal. The MIC values of COE that prevented visible growth of *C. albicans* were 10 mg/mL, whereas the MBC values were 30 mg/mL for *C. albicans*, as presented in Figure 3.

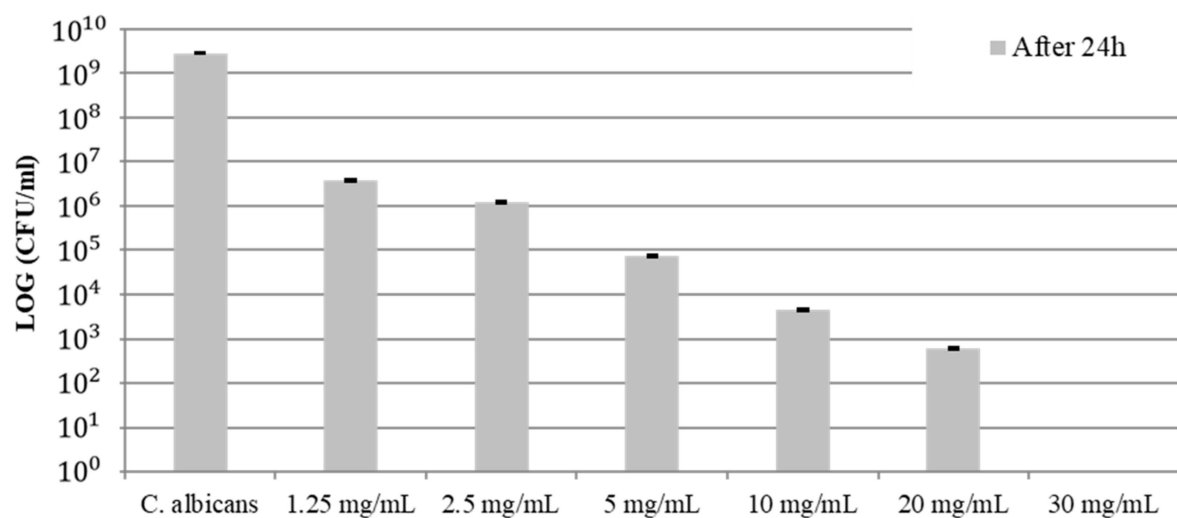


Figure 2. *C. albicans* growth inhibition of COE at different concentrations.

Table 2. Comparison of the CFU at different concentrations of COE.

Group	<i>C. albicans</i>	1.25 mg/mL	2.5 mg/mL	5 mg/mL	10 mg/mL	20 mg/mL	30 mg/mL	ANOVA <i>p</i> -Value
24 h	$2.9 \pm 0.2 \times 10^9$ ^a	$3.8 \pm 0.2 \times 10^6$ ^b	$1.2 \pm 0.0 \times 10^6$ ^c	$7.5 \pm 0.0 \times 10^4$ ^c	$4.5 \pm 0.4 \times 10^3$ ^c	$6.1 \pm 0.2 \times 10^2$ ^c	0.0 ^c	0.000 *

* The statistically significant difference between groups in a one-way ANOVA. Different letters (a, b, and c) based on the significant post hoc Tukey HSD test result ($p < 0.05$).

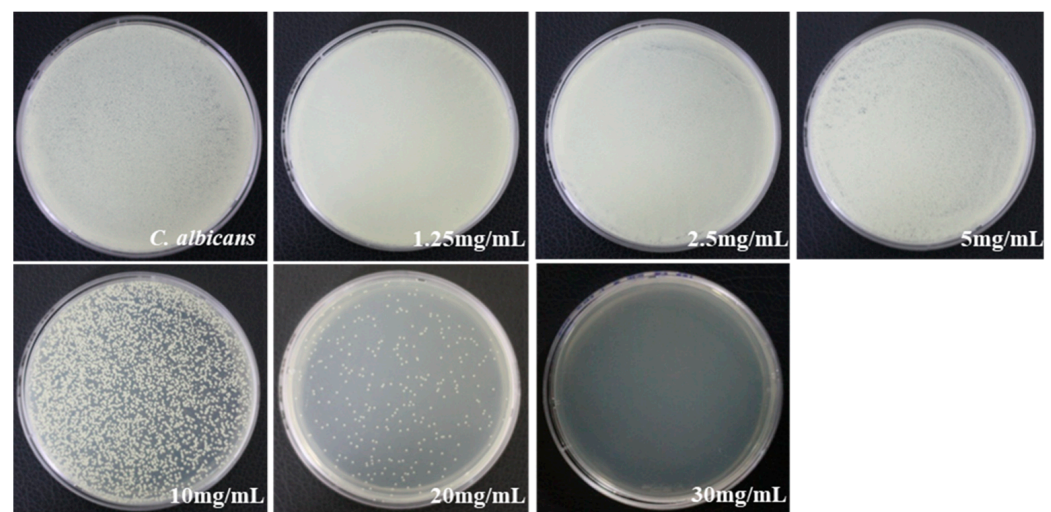


Figure 3. MIC and MBC values of COE on *C. albicans* determined using the agar culture method.

3.5. Cell Viability Determination of HaCaT Cells

The cell viability was measured using the WST-1 assay at approximately 77%, 61%, 20%, 15%, 3%, and 2% after applying 1.25, 2.5, 5, 10, 20, and 30 mg/mL concentrations of COE, respectively, for 3 h (Figure 4). As shown in Figure 4, the half-maximal inhibitory concentration (IC_{50}) was 2.5 g/mL, and the higher the COE concentration, the lower the survival rate, which indicated cytotoxicity.

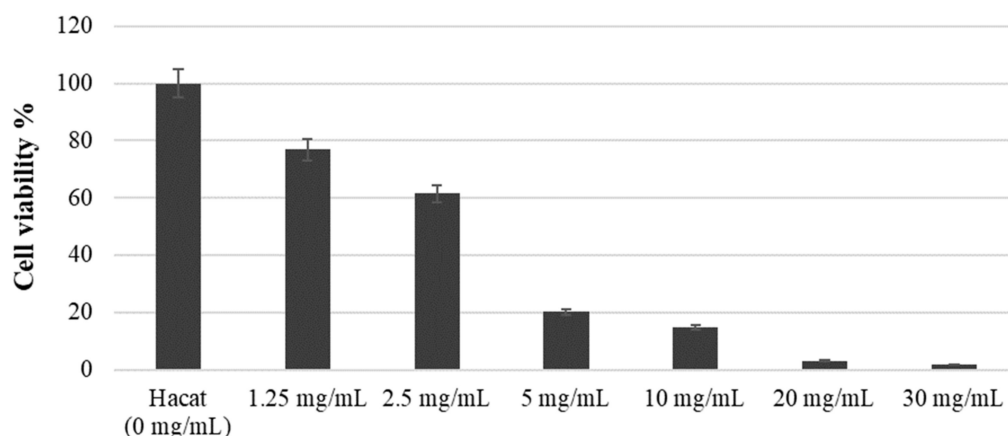


Figure 4. Survival rate of HaCaT cell growth treated with COE.

4. Discussion

C. albicans colonizes mucous membranes and can act as an etiological factor in opportunistic infection, which can lead to life-threatening mucosal infections in immunosuppressed patients [32]. The local factors of oral candidiasis include oral cancer, dentures, decreased salivary gland function, and the use of steroids. The systemic factors include age, smoking, diabetes, Cushing syndrome, decreased immunity, malignant tumors, malnutrition, and antibiotics [19]. Topical local antifungal treatments, such as clotrimazole and nystatin, are used to treat *C. albicans*. However, clotrimazole has a risk of causing dental caries with its high sugar content, and in many cases with nystatin, the treatment is unsuccessful due to limitations such as oral structure (tongue, palate, gingiva, and teeth) and saliva dilution [33]. Among other things, the most important drawbacks of these antifungal agents are suboptimal selectivity, increased toxicity, and increased likelihood of developing resistance. In particular, although amphotericin B is considered the “standard” for antifungal therapy, it is intrinsically toxic because it lacks selectivity, given that fungi and mammalian cells are eukaryotic and share many similar biological processes [34]. The newest class of antifungals and the first fungi-specific antifungals are represented by echinocandins (caspofungins), which target key components of the fungal cell wall that are not present in mammalian cells. Unfortunately, the clinical use of echinocandins is limited to the treatment of systemic candidiasis, and the emergence of resistance in *C. glabrata* is of particular concern [35]. Therefore, natural mouthwash ingredients are actively researched to treat *C. albicans*. *C. obtusa* belongs to the Cupressaceae family and is native to Japan. It was introduced to South Korea in the early 20th century and is now naturalized in the central and southern regions of the country [36].

Antioxidant materials are extensively researched in various ways due to their safe and natural active-oxygen scavenging activity [37]. This study investigated the antioxidant activity of *C. obtusa*. As a result, the MIC value of COE preventing the visible growth of *C. albicans* was 10 mg/mL, while the MBC value was 30 mg/mL for *C. albicans*. According to Nagappan’s report, when 0.2% chlorhexidine mouthwash was used against *C. albicans*, the MIC was 12.5%, confirming that *C. obtusa* was more effective against *C. albicans* than chlorhexidine, which is widely used worldwide. Also, Arowash liquid mouthrinse containing three herbs (Cadila Pharmaceuticals, Cadila Pharmaceutical Ltd. Ahmedabad, Gujarat, India) also reported MBC at 50%, suggesting the possibility of using the COE-containing mouthwash used in this study [38].

Polyphenolic compounds are the secondary metabolites that are widely distributed in plants and contain two or more phenolic hydroxyl (–OH) groups in one molecule, which easily combine with various compounds. It has been reported that polyphenolic compounds have various physiological activities, including antioxidant, antimicrobial, anticancer, anti-inflammatory, and antidiabetic [39]. Furthermore, flavonoids are known to assist in antioxidant activity, platelet aggregation inhibition, and anti-inflammatory and

anticancer activities [40]. The higher the total amounts of polyphenols and flavonoids, the higher the antioxidant activity, indicating a positive correlation. In this study, the polyphenol and flavonoid contents in COE were measured as 424.150 mg GAE/g and 127.566 mg CE/g, respectively. Compared to the polyphenol contained in the ethanol-extracted sea buckthorn leaf, which is reported to be 285.64 mg GAE/g [41], the total polyphenol amount from COE measured in this study was 1.49 times higher. Furthermore, the total flavonoid amount in this study was 2.13 times higher than the other research result, which reported 58.84 ± 0.47 mg CE/g in ethanol-extracted artichoke extract [42]. Furthermore, the free radical scavenging effect was measured with DPPH. The characteristic indigo color disappears when a relatively stable radical converts to a nonradical with electrons or hydrogen donated from an antioxidant. Using this characteristic, DPPH is widely used to measure the antioxidant activity of natural products due to its high correlation with actual antioxidant activity [43]. Therefore, 83.59% DPPH radical scavenging activity was observed at 1 mg/mL COE concentration, showing similar activity to a control group, gallic acid (89%). This confirms the high antioxidant power of COE with its high DPPH radical scavenging function.

When compared to the essential oils from *Pinus densiflora*, *Pinus koraiensis*, and *Chamaecyparis pisifera*, COE oil has one of the best antifungal properties. Specifically, it has been reported that yoshinol, isolated and purified from *C. obtusa* wood, has strong antifungal effects against *Escherichia coli*, *Mycobacterium chelonae*, *Pseudomonas aeruginosa*, and *C. albicans* [44]. Furthermore, COE oil inhibits mycelial growth of the two dermatophytes (*Microsporum canis* and *Trichophyton mentagrophytes*) at a concentration of 500 ppm and shows antifungal properties [36]. This study also confirmed the antifungal effects of COE on oral candidiasis. The higher the concentration of COE, the greater the diameter of the zone of inhibition, showing its devastating effect on *C. albicans*. However, no significant difference was observed between the concentrations ranging from 2.5 mg/mL to 30 mg/mL ($p > 0.05$). COE showed complete antifungal properties at a 30 mg/mL concentration. This result is consistent with the result of Lee et al. [19], which reported increased strain growth inhibition with increased COE concentration. Furthermore, Kang et al. [20] indicated the continuous growth inhibition of *C. albicans* when cultured with 0.25% *phytoncide*, decreasing the number of viable cells and resulting in the total elimination of *C. albicans* in 20 h. The growth-suppressing and lethal effects of *phytoncide* on *C. albicans* are consistent with the findings of this study. It has been reported that the antimicrobial mechanism of essential oils is due to *phytoncide* damaging the cell membranes of microorganisms and increasing their permeability to induce cytoplasmic liberation or affecting the bacterial respiratory metabolism [23,45].

Even though *phytoncide* shows antifungal effects against *C. albicans* and can kill cells through cell lysis, the level of cytotoxicity and cell viability depend on the concentration. After observing *C. albicans* with an electron microscope for 6 h after the *phytoncide* treatment, Kang et al. reported an increased number of atypical cell shapes, an increased electron density of the cytoplasm, increased high-density granules, and the division of intracellular organelles becoming more unclear in higher concentrations. Therefore, this study used HaCaT cells to confirm the safety of COE, which revealed a lower survival rate of HaCaT cells at higher concentrations, thus confirming its cytotoxicity. Overall, the COE concentration of 2.5 mg/mL indicated a clear antimicrobial effect without any observable cytotoxicity. Additional clinical studies are needed to actively utilize COE as a safe natural antibiotic. Therefore, it is necessary to confirm substantial changes in the patient's oral condition by applying COE-containing gargles to humans in the clinical field. In addition to these functional confirmations, additional research will be needed to confirm the applicability of COE-containing gargles by examining satisfaction with the use of gargles.

For further research, through research on the quantification of phytochemical compounds of COE that cause antifungal effects, it is necessary to carry out chemical evaluation through component analysis. Above all, research should be conducted to confirm the continued harmlessness to the human body by analyzing the safety test through more

specific and diverse experiments. In addition, the need to develop an antifungal agent class using various natural extracts should be emphasized by identifying the pathogenicity of *C. albicans* and the mechanism of the host immune response.

5. Conclusions

This study confirmed that COE at concentrations up to 2.5 mg/mL has the potential to be used as a safe and natural treatment for oral candidiasis due to its antioxidant and antifungal properties. Therefore, if oral gargles containing COE are applied in clinical settings, they will be used effectively and safely for the treatment of oral candidiasis.

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