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The Effect of Botanical Tinctures and Essential Oils on the Growth and Morphogenesis of *Candida albicans*

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Abstract

Objective: *Candida albicans* is an opportunistic and polymorphic fungal pathogen that affects mucosal membranes and squamous epithelia as well as being part of the normal human flora. Historically, there have been many botanical-based remedies used to treat fungal conditions, including *C. albicans*. This study examined the efficacy of both botanical tinctures and essential oils on the growth and morphological differentiation of *C. albicans*.

Methods: The *in vitro* growth and differentiation of *C. albicans* was monitored following treatment with ethanol-based tinctures and essential oils prepared from several commonly used botanicals.

Results: Results demonstrated that all ethanol-based botanical tinctures tested did not inhibit the growth of *C. albicans*, but several tinctures, including *Marsdenia condurango*, *Juglans nigra (*Black walnut*), Anemopsis californica* (Yerba mansa) and *Piper cubeba (*Cubeb berry*)*, significantly reduced the morphological differentiation of the yeast into the invasive hyphae form. Alternatively, several botanical essential oils, including those from *Thymus vulgaris* (Thyme), *Rosmarinus officinalis* (Rosemary) and *Cymbopogon citratus* (Lemon grass) had a dramatic effect on inhibiting the growth of *C. albicans*.

Conclusions: These results suggest that botanical tinctures commonly used in the treatment of *C. albicans* infections may act by blocking the differentiation of the yeast into a more virulent hyphal form while not affecting the growth rate. In comparison, therapeutic essential oils may target both the differentiation and growth rate of *C. albicans*. The results support that different active constituents are present in botanical tinctures as compared to oils thereby contributing to our understanding of how these botanicals may be effective therapeutics in the treatment of *C. albicans* infections.

Keywords: *Candida albicans*; Fungi; Yeast; Hyphae; Virulence; Botanical; Tincture; Essential oils

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Introduction

Candida albicans is a commensal organism in the human cutaneous and mucosal flora, but can become a major human fungal pathogen given the proper conditions allowing for hyphal differentiation, biofilm formation, and overgrowth. Some of the most common areas for candidiasis include the oral and gastrointestinal tract, vaginal canal and topically on the skin [1]. In patients experiencing vulvovaginal candidiasis, common signs include burning, itching and redness. This condition affects up to 75% of women at least once in their lifetime [2]. Skin infections are common in burn victims and neonates due to their depressed or underdeveloped immune system [2]. In the AIDS patient population, there is an increased likelihood of developing oral and esophageal candidiasis, due to decreased immune function [2]. In the general population, *Candida* overgrowth in the gastrointestinal tract is exacerbated by high-sugar diets or medications including steroids and antibiotics. In these cases, people will present with digestive discomfort, gas or bloating. More seriously, *C. albicans*

can become systemic and cause an infection of the bloodstream known as candida emia. This infection can then further spread into organs, causing conditions such as hepatosplenic candidiasis or *Candida* peritonitis [3]. *C. albicans* has become the fourth-leading cause of hospital-acquired blood infections in the world and mortality rates for the more severe infections remain high at about 50% [1,4-6].

During growth, *C. albicans* may undergo a reversible morphological transition from single-celled yeast; round budding spores (also known as blastospores), to filamentous pseudohyphae and finally, multicellular, elongated tubal cells attached end-to-end known as true hyphae [1,2,6]. This transition, which is induced upon exposure of *C. albicans* to a number of host conditions, is required for increased virulence. The most common host conditions required for differentiation include the presence of serum, the environmental temperature, and pH levels [2]. In studying mucosal infections, the hyphal form of *C. albicans* has been found embedded and overgrown in tissue leading to the conclusion that in its multicellular form is when *C. albicans* is at its most invasive and infectious stage [7].

Currently, there are a large variety of anti-*Candida* treatments dependent on the anatomical location of the yeast overgrowth. Pharmacological oral agents such as fluconazole and amphotericin B have been used in cases of internal or invasive *Candida* overgrowth [8]. Topical agents such as nystatin and clotrimazole have been used to treat cutaneous candidiasis as well as certain oral candida overgrowths. Miconazole and tioconazole are more commonly used topically in the vaginal canal for treatment of vaginal yeast infections [3,8]. Due to *Candida* being a eukaryotic pathogen, many of the antifungal agents available interact negatively with human cells. These anti-fungal treatments have many adverse effects including hepatotoxicity as the most common and concerning [2]. Invasive and systemic forms of candidiasis are more difficult to treat using these medications because of their high side effect profile, particularly in patients who are already immunocompromised [3].

In this study, we examined the efficacy of botanical tinctures and essential oils to inhibit both the growth and morphological transformation of *C. albicans*.

Materials and Methods

Botanical tincture and essential oil preparation

Plant material was obtained from reputable sources with documentation of authenticity. All plant material was subsequently verified by qualified botanical specialists using herbal pharmacopoeia monographs and reference keys. A voucher specimen of all plant material was deposited in a repository. For tincture preparation, the dried botanicals were ground to a fine powder, resuspended in extraction solution and incubated for 3 days at room temperature. The extract was centrifuged at 3000 × g for 10 min to remove cell debris and the extraction solution filtered through a 0.2 µM filter. For standardization and comparison, all botanical extracts had an average non-volatile constituent concentration averaging 32.1 mg/ml (ranging from 22.4 - 37.2 mg/ml). This concentration (mg/ml) is based on the weight of non-volatile constituents present in the extract per ml of aqueous liquid. The source, extraction solution (percent ethanol:water), and plant dry to extraction solution ratio for each of the botanicals used in this study is shown in **Table 1**. The essential oils used in this study were prepared by steam distillation or cold pressed extraction. The source and preparation method for each essential oil used in this study is shown in **Table 2**.

Yeast growth

Media and the yeast culture *Candida albicans* ATCC 10231 were obtained from Hardy Diagnostics (Santa Monica, CA). For growth studies, 18-hour cultures (ranging from 3-8 × 10^7 colony forming units (CFU)/ml) grown at 30°C in YPD broth were diluted into media (1:1,000 dilution; yeast peptone dextrose broth (YPD)) followed by the addition of indicated concentrations of each botanical extract, essential oil, or control solution. For single-celled yeast growth, the cultures were incubated in YPD broth at 30°C with aeration (by continuous rotation) for a maximum of 24 hours. At 0, 4, 8, and 12 hrs, samples were removed and the cell concentration determined by serial dilutions on Sabdex agar (CFU/ml media). For hyphal growth, the cultures were incubated in YPD broth with 5% fetal bovine serum at 37°C with aeration (by continuous rotation) for a maximum of 24 hours. Determination of CFU/ml was done as above. For hyphal reversal studies, the 18-hour starter cultures were grown at 37°C in YPD broth plus 5% fetal bovine serum.

Microscopy differentiation analysis

At 24 hours post-treatment, light microscopy was done to evaluate the morphological state of the *C. albicans*. Each broth culture was stained with Lactophenol Cotton Blue and visualized by light microscopy. A minimum of 100 cells were counted and the percent of single cells verses hyphal/pseudohyphal cells determined.

Results

In this study, several botanical tinctures and oils were utilized based on their historical and/or suggested therapeutic efficacy in the treatment of *C. albicans* infections. Upon testing tinctures for growth inhibitory effects on *C. albicans*, little or no effect was observed. **Figure 1** illustrates the lack of inhibitory growth with *Olea europea, Origanum vulgare, Thymus vulgaris* and *Cymbopogon citratus* on *C. albicans.* Each tincture was tested at concentrations of 0 µl/ml (no herbal treatment), 10 µl/ml and 30 µl/ml. At both 37°C with serum and 30°C without serum, all of the tinctures had no significant effect on the growth of *C. albicans*. Similar results were observed with all other ethanol tinctures tested including *Marsdenia condurango*, *Cinnamomum zeylanicum, Juglans nigra, Chilopsis linearis, Anemopsis californica, Arctium lappa, Tabebuia avellanedae, Alpinia officinarum, Carum carvi, Arcostaphylos uva-ursi, Nepeta cataria, Allium sativum, Cuminum cyminum* and *Artemisia absinthium* (data not shown). Vehicle control samples treated with 60% ethanol also showed no inhibitory effect on growth (data not shown).

Since morphological transformation of *C. albicans* to hyphal form is a key virulence factor, the herbal tinctures were tested for their ability to inhibit this transformation process. **Figure 2**

Table 1 The source, extraction solution (percent ethanol:water), and plant dry to extraction solution ratio for each of the botanicals.

Botanical name	Common name	Part	Origin	Company source	Extraction Soln (Ethanol:Water)	Extract ratio (Dry:liquid)
Marsdenia condurango	Condurango	Vine	Equador	Raintree Health	45%:55%	1:08
Cinnamomum zeylanicum	Cinnamon	Bark	United States	Starwest Botanicals	65%:35%	1:08
Juglans nigra	Black Walnut	Hull	United States	Starwest Botanicals	40%:60%	1:08
Chilopsis linearis	Desert Willow	Leaf	Sri Lanka	Starwest Botanicals	45%:55%	1:08
Anemopsis californica	Yerba Mansa	Root	United States	Starwest Botanicals	60%:40%	1:08
Arctium lappa	Burdock	Root	China	Starwest Botanicals	60%:40%	1:08
Tabebuia avellanedae	Pau D'Arco	Bark	Brazil	Starwest Botanicals	60%:40%	1:08
Alpinia officinarum	Lesser Galangal	Root	India	Starwest Botanicals	25%:75%	1:08
Carum carvi	Caraway	Seed	Egypt	Starwest Botanicals	60%:40%	1:08
Arctostaphylos uva ursi	Uva Ursi	Leaf	Croatia	Starwest Botanicals	60%:40%	1:08
Nepeta cataria	Catnip	Leaf	Canada	Starwest Botanicals	50%:50%	1:08
Allium sativum	Garlic	Bulb	China	Starwest Botanicals	0%:100%	1:08
Cuminum cyminum	Cumin	Seed	India	Starwest Botanicals	60%:40%	1:08
Artemisia absinthium	Wormwood	Aerial	Croatia	Starwest Botanicals	60%:40%	1:08
Origanum vulgare	Oregano	Leaf	Turkey	Starwest Botanicals	40%:60%	1:08
Piper cubeba	Cubeb	Berries	India	Starwest Botanicals	60%:40%	1:08
Olea europaea	Olive	Leaf	Egypt	Starwest Botanicals	60%:40%	1:08
Thymus vulgaris	Thyme	Leaf	Egypt	Starwest Botanicals	0%:100%	1:08
Cymbopogon citratus	Lemon Grass	Aerial	Egypt	Starwest Botanicals	0%:100%	1:08

Table 2 The source and preparation method for each essential oil.

Botanical	Common name	Origin	Company source	Method
Copaifera officinalis	Copaifera	Brazil	Mountain Rose Herbs	Steam distillation of the balsam
Oenothera biennis	Evening Primrose	N/A	Starwest Botanicals	Cold pressed extraction of the seed
Cannabis sativa	Hemp	N/A	Starwest Botanicals	Cold pressed extraction of the seed
Thymus vulgaris	Thyme	Spain	Starwest Botanicals	Steam distillation of the leaf
Cymbopogon citratus	Lemon Grass	India	Esoteric Oils	Steam distillation of the leaf
Rosmarinus officinalis	Rosemary	N/A	Starwest Botanicals	Cold pressed extraction of the leaf
Melaleuca alternifolia	Tea Tree	Australia	Starwest Botanicals	Steam distillation of the leaf and aerials
Mentha balsamea	Peppermint	United States	Starwest Botanicals	Steam distillation of the flowering herb
Origanum vulgare	Oregano	Turkey	Starwest Botanicals	Steam distillation of the leaf
Lavandula officinalis	Lavender	India	Starwest Botanicals	Steam distillation of the flowering top
Cymbopogon martini	Palmarosa	India	Starwest Botanicals	Steam distillation of the grass

represents the percent hyphae formation of *C. albicans* cultures treated with herbal tinctures for 24 hours. As shown, when *C. albicans* cultures were grown at 37°C with the addition of serum, 80-100% of the culture formed hyphae, whereas when grown at 30°C without serum, typically only 10% of the culture formed hyphae with 90% as single celled form. Upon the addition of *Marsdenia condurango* tincture at 10 µl/ml at 37°C with serum, decreased hyphae formation was observed to about 15% of the population and further decreased to 5% of the population at 30 µl/ml. This was the strongest acting tincture upon hyphae inhibition with *Piper cubeba, Juglans nigra, Anemopsis californica* and *Cinnamomum zeylanicum* also being significantly potent with only 10-25% hyphae growth at 30 µl/ml. At 10 µl/ml these botanicals provided between 50-75% hyphal growth.

Visually, **Figure 3A** shows microscopic images of the *C. albicans* grown at 0 µl/ml, 10 µl/ml and 30 µl/ml of *Marsdenia condurango* and *Piper cubeba* tinctures. At 30°C without serum at each concentration, there was mostly only single-celled yeast present for both tinctures. At 37°C plus serum without any herbal treatment large, hyphal structures were observed throughout the culture. At 10 µl/ml for both herbs, large hyphae were not detected, but pseudohyphae were present. At 30 µl/ml, the *M. condurango* treatment resulted with nearly only single-celled yeast being present upon observation of the broth culture. For

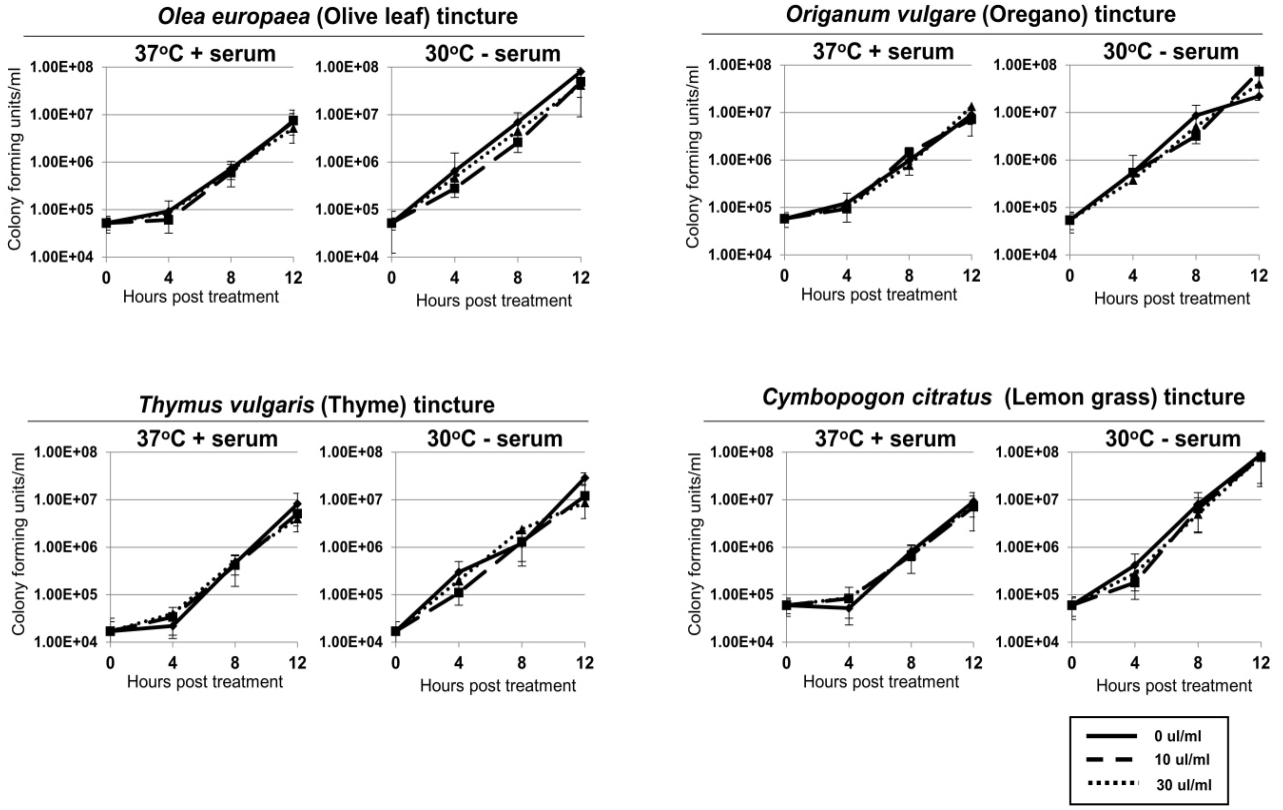

Figure 1 Effect of botanical tinctures on *C. albicans* growth rate. A starter culture of *C. albicans* was grown at 30°C in YPD. Diluted (1,000-fold) *C. albicans* cultures were left untreated (solid line), or treated with the indicated botanical extracts in the amount of 10 or 30 µl/ml at 0 hours (dashed lines). The cultures were incubated at 37°C in YPD plus serum or 30°C in YPD without serum with continuous aeration (by rotation) and CFU/ml determined every 4 hours over a period of 12 hours. Assays were repeated in triplicate.

P. cubeba at 30 µl/ml, pseudohyphae were still prevalent, but as shorter structures. These results agree with **Figure 1** where the growth rate of *C. albicans* was not affected by the herbs, but rather the morphological state of the cells was affected.

The effect of these tinctures on *C. albicans* differentiation can also be observed at a gross level with the broth cultures. **Figure 3B** shows *M. condurango* treatment of *C. albicans* cultures at 0 µl/ml, 10 µl/ml and 30 µl/ml at 37°C plus serum and 30°C without serum. At 30°C without serum, the broth cultures were turbid at every herb concentration due to the *C. albicans* growing in a single-celled form. When grown at 37°C with serum and no herb, the culture appeared flocculent due to large, hyphae formation (**Figure 3B**). This then decreased to a less flocculent and more turbid appearance at 10 µl herb/ml and a completely turbid appearance (similar to 30°C without serum) at 30 µl herb/ml.

For other herbs tested, intermediate acting herbs showed a significant effect on the morphological growth of *C. albicans* only at the higher doses of 30 µl/ml and were able to inhibit hyphal growth to 40-60% of the population (**Figure 2**). These herbs included *Chilopsis linearis, Alpinia officinarum* and *Carum carvi.* More minor effects on growth, with 75-80% of the culture still forming hyphae was seen with *Olea europea, Thymus vulgaris* and *Cymbopogon citratus* at the highest (30 µl/ml) concentrations of each tincture. Tinctures which proved to have no effect on hyphae growth were *Artemisia absinthium, Arcostaphylos uva-ursi, Nepeta cataria, Allium sativum, Cuminum cyminum, Arctium lappa, Tabebuia avellanedae* and *Origanum vulgare* (data not shown). In addition, vehicle control samples treated with 60% ethanol also showed no effect on hyphae growth or differentiation (data not shown).

Since essential oils are also historically used in treating *C. albicans* infections, the effect of various essential oils on the growth and differentiation of *C. albicans* was also examined. **Figure 4** shows that certain essential oils were able to inhibit the replication of *C. albicans* at both 30°C without serum and 37°C with serum. Although *Thymus vulgaris* and *Cymbopogon citratus* as tinctures showed no effect on the replication rate of *C. albicans*, the essential oils inhibited growth approximately 1000-fold at 1 µl/ml and over 10,000-fold at doses of 3 µl/ml or higher. This appeared to be a fungicidal effect since levels of viable cells decreased over 2-logs below the initial concentration. For other essential oils, including those from *Rosmarinus officinalis, Melaleuca alternifolia, Mentha balsamea, Organum vulgare, Lavandula officinalis,* and *Cymbopogon martini,* a more dose dependent inhibition in growth was observed (**Figure 4**). These herbs generally inhibited growth approximately 10-fold at 1 µl/ml, 100-1,000-fold at 3 µl/ml, and greater than 10,000-fold at 10 µl/ml and higher. Not all essential oils tested inhibited *C. albicans*

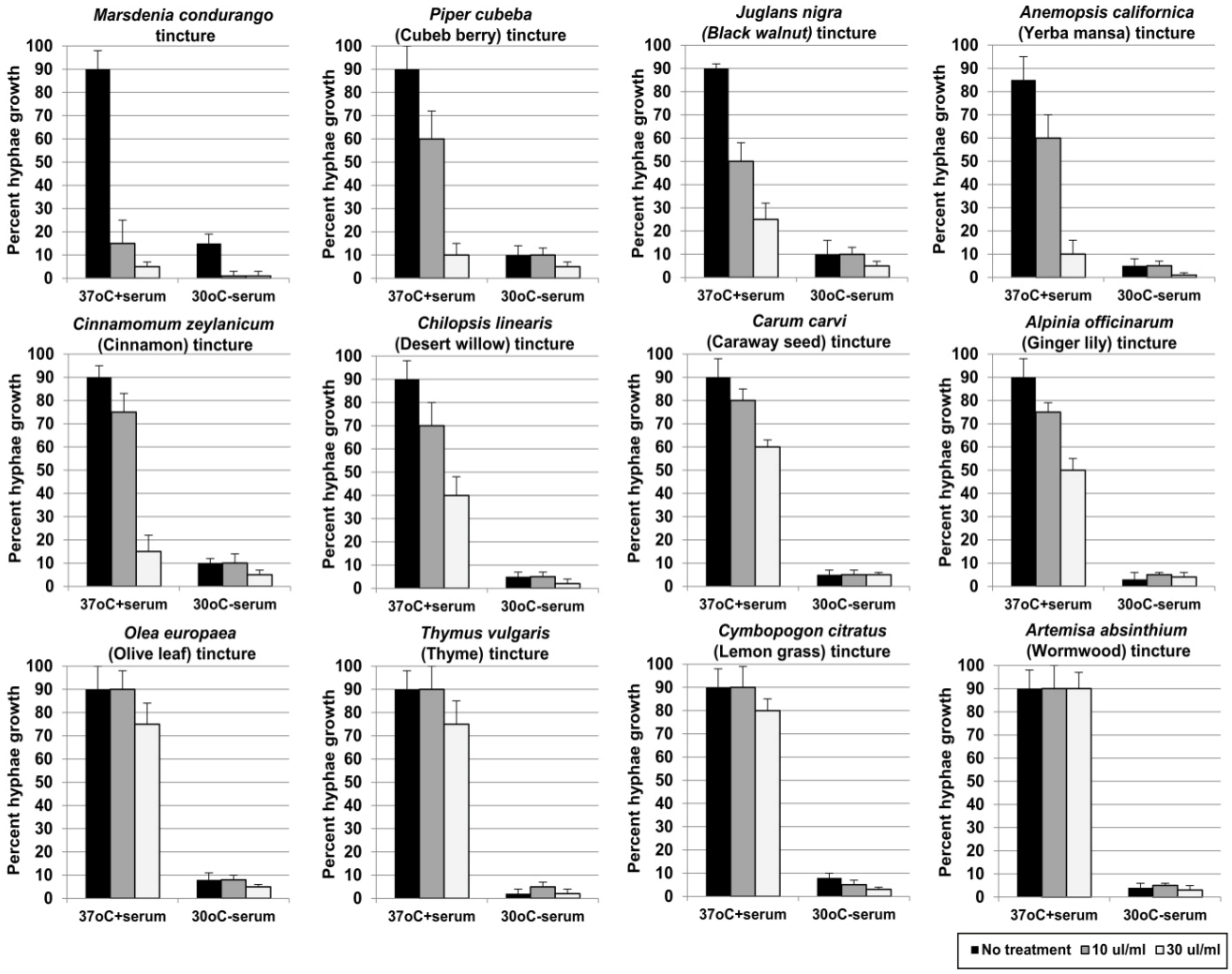

Figure 2 Effect of botanical tinctures on *C. albicans* morphological differentiation, A starter culture of *C. albicans* was grown at 30°C in YPD. Diluted (1,000-fold) *C. albicans* cultures were left untreated (solid bar), or treated with the indicated botanical extracts in the amount of 10 or 30 µl/ml at 0 hours (dotted bars). The cultures were incubated at 37°C in YPD plus serum or 30°C in YPD without serum with continuous aeration (by rotation) for 24 hours followed by observation by light microscopy. The percent of single cells versus hyphal/pseudohyphal cells was determined. Assays were repeated in triplicate.

growth. *Palmarosa* oil showed no activity on the growth of the *C. albicans* even at the higher concentrations tested (**Figure 4**). Similar non-inhibitory effects were observed with essential oils from *Copaifera officinalis, Oenothera biennis* and *Cannabis sativa* (data not shown).

Since the herbal tinctures had an effect on the morphological differentiation of *C. albicans*, similar assays were done using the essential oils. As shown in **Figure 5**, *Lavandula officinalis, Rosmarinus officinalis, Mentha balsamea* and *Melaleuca alternifolia* oils were able to inhibit hyphal formation at concentrations below that which inhibited replication. For example, *L. officinalis* reduced hyphal formation to 5-15% at 10 and 1 µl/ml, respectively, but did not significantly affect the replication rate of *C. albicans* until 30 µl/ml. For *Thymus vulgaris, Cymbopogon citratus* and *Origanum vulgare* oils there was no significant effect on hyphal formation at doses that did not lead to growth inhibition (**Figure 5**). These results may suggest that herbs like *R. officinalus*, may have separate constituents which affect both growth and morphological differentiation of *C. albicans*, whereas herbs like *T. vulgaris*, only a constituent which affects only the growth rate. *Cymbopogon martini* essential oil showed no inhibition of hyphal differentiation at even the highest concentration of the essential oil (**Figure 5**). Other essential oils tested with no effect on hyphal growth include *Copaifera officinalis, Oenothera biennis* and *Cannabis sativa* (data not shown).

The previous results demonstrated that several tinctures and oils could inhibit the differentiation of *C. albicans* from a single-celled yeast to a hyphal form. In order to test if these herbs could also reverse differentiation from a hyphal form to a yeast form, pre-existing hyphal *C. albicans* was maintained under growth conditions of 37°C with serum and subsequently treated with various herbs and examined for morphological changes. These studies had the goal of indirectly assessing if an already

established invasive *C. albicans* infection could be reversed to the less invasive and more benign state of single-celled yeast. Based on earlier results, select tinctures and oils were chosen for these assays including *Marsdenia condurango* and *Piper cubeba* tinctures and *Melaleuca alternifolia, Rosmarinus officinalis, and Lavandula officinalis* oils. As shown in **Figure 6A and 6B**, with *M. condurango,* the initial culture started out with approximately 90% hyphae population. At 24 hours with no treatment, the hyphal growth remained unchanged. At 10 µl tincture/ml, a significant reduction to pseudohyphae and single cells was observed at 24 hours post treatment. At 30 µl tincture/ml, hyphae formation was decreased to 95% single-celled yeast following 24 hours post-treatment. **Figure 6B** shows similar results for the other tinctures and oils tested with *Piper cubeba* tincture, *Melaleuca alternifolia* oil, *Rosmarinus officinalis* oil and *Lavandula officinalis* oil all significantly reversing hyphal growth to a single-celled yeast form.

Discussion

Historically, many botanical treatments have been used as therapeutic agents for fungal infections. In this study, botanical tinctures and essential oils were tested individually for their efficacy towards both inhibiting the growth and preventing differentiation of *C. albicans*. *Juglans nigra* has been used to treat oral infections and has been shown to have inhibitory compounds towards *C. albicans* [9,10]. *Piper cubeba* has reported constituents with bacteriostatic, fungistatic and fungicidal properties that have been found useful historically in treating oral pathogens [11-14]. *Anemopsis californica* has reported very strong antifungal properties making it a potentially effective topical treatment for fungal infections [15]. Some herbs, such as *Marsdenia condurango* are commonly used by practitioners without laboratory-based studies or evaluations. Similarly, essential oils have a long history of therapeutic use for fungal infections. *Thymus vulgaris*, as an essential oil, has been suggested to inhibit the growth of *C. albicans* intracellularly [16-18]; *Rosmarinus officinalis* was demonstrated to inhibit germ tube formation as an essential oil [19,20]; and *Cymbopogon citratus* has been suggested to inhibite mycelial formation as well as yeast bud formation [21,22].

The botanical tinctures used in this study were prepared as ethanol-based herbal extractions. Unexpectedly, based on historical use, no herbal tincture was able to inhibit the growth rate of *C. albicans*. However, several tinctures inhibited the morphological differentiation of *C. albicans* to a hyphal form. These results may suggest that therapeutic herbal tinctures may act by inhibiting the progression of single-celled yeast into the more invasive hyphae form. The strongest herbs with this mechanism of action included *Marsdenia condurango, Piper cubeba, Juglans nigra, Anemopsis californica* and *Cinnamomum zeylanicum. C. albicans* is typically a serious problem only when it differentiates into a hyphal form, therefore, it may be therapeutically valuable to limit differentiation of *C. albicans* instead of completely inhibiting growth and maintaining the yeast form as part of the stable flora of the microbiome in the human body.

There have been very limited previous studies done on herbs and their constituents that effectively act on inhibiting hyphal growth. Gymnemic acid is found in the herb *Gymnema sylvestre* and has been traditionally used in the treatment of diabetes. Similar to the herbs tested in this study, gymnemic acid did not have an effect on the growth of *C. albicans*, but did inhibit differentiation of the single-celled yeast to hyphae. In this case, the effective constituent was isolated and tested in hyphae-inducing conditions, which supports the future possibility of isolating novel compounds from various botanicals with which effect hyphal growth and virulence of *C. albicans* [5].

To further study the original goal of the experiment, which was to evaluate the fungicidal effects of herbs, essential oils were also evaluated. Essential oils are the steam-extracted, volatile aroma compounds of plants. They typically have different constituents and a higher concentration of active constituents requiring a small dose for a therapeutic effect. In this study, several essential oil-based botanicals were able to completely block hyphae formation as well as inhibiting the replication of *C. albicans*. *Thymus vulgaris* and *Cymbopogon citratus* were the most effective essential oils, although they had no fungicidal effect as botanical tinctures. These two herbs as tinctures only had an effect on inhibiting differentiation of *C. albicans*. This suggests that the same herb extracted into either oil or tincture may contain different active constituents.

Since *C. albicans* in patients with severe infections is commonly differentiated into the hyphal form, several tinctures and oils were also tested for their ability to reverse the hyphal form to a single-celled yeast form. *M. condurango* was the most effective tincture against preventing differentiation and it, along with the other active tinctures and oils were shown to be highly effective in the reversal trial. These results support the possibility for these tinctures and oils to be used in active *C. albicans* infections.

Tinctures are appropriate to treat both oral and topical infections, whereas essential oils are more suitable to treat topical infections due to their highly concentrated and caustic nature. Most of the botanical tinctures and oils used in this study have a low side-effect profile when taken internally or applied topically, respectively, when compared to conventional anti-fungal treatments [3]. There is a potential for both the tinctures and the oils to be combined in a synergistic blend that could be more efficacious in the overall treatment of a *C. albicans* overgrowth by providing different mechanisms of action.

Botanicals offer a therapeutic effect on many aspects of a fungal or microbial imbalance. Future research can explore the potential risk of antifungal resistance to these herbs, which may support the use of combined botanical preparations as opposed to a single-herb therapy and provide support for which herbs may work synergistically in order to best control *C. albicans* overgrowth in the human body.

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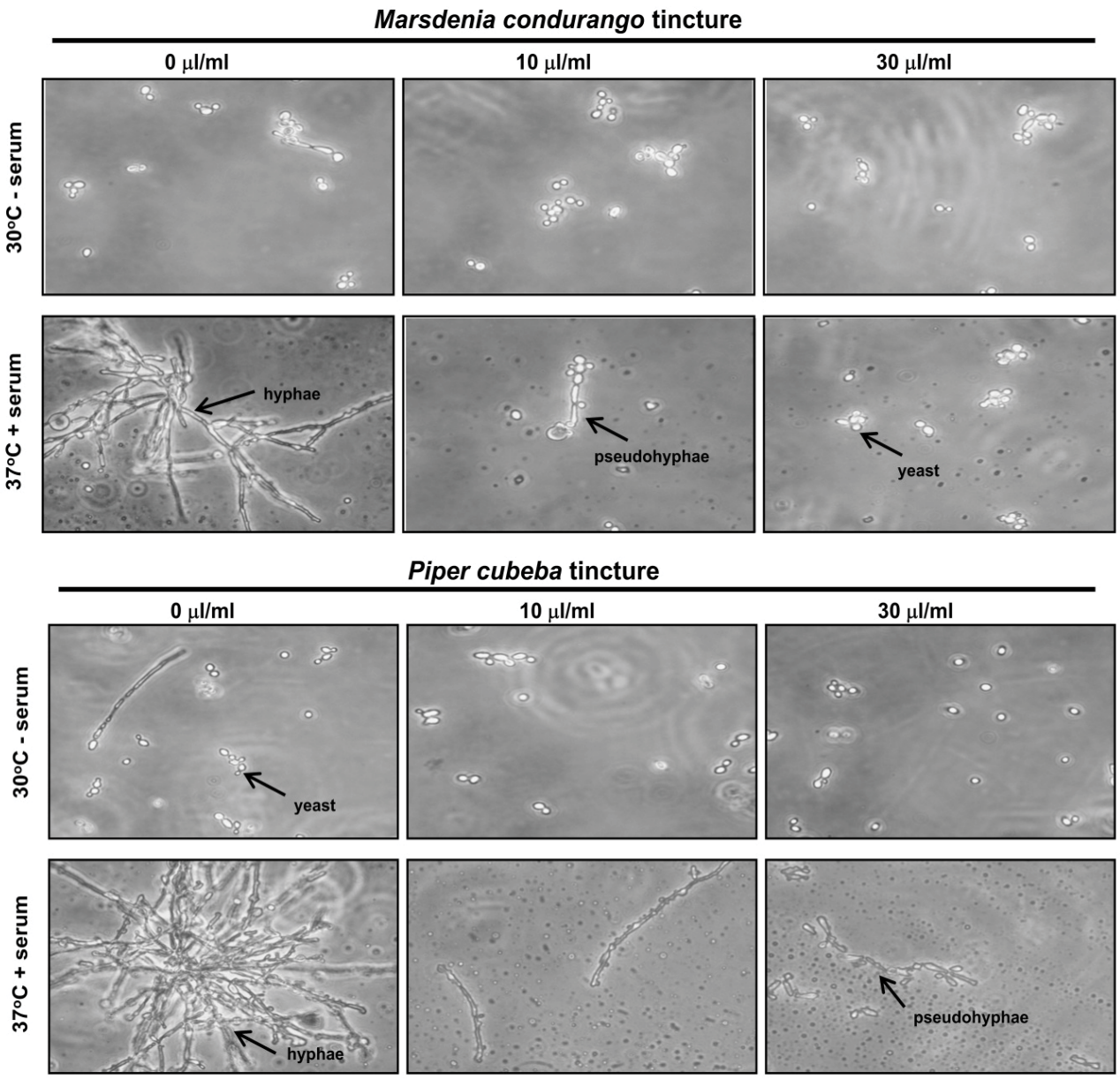

Figure 3a Visual representation of the effect of botanical tinctures on *C. albicans* morphological differentiation Microscopic images of *C. albicans* cultures treated with *M. condurango* or *P. cubeba* tinctures under conditions described in Figure 2. Examples of hyphae, pseudohyphae and single celled yeast cells are shown.

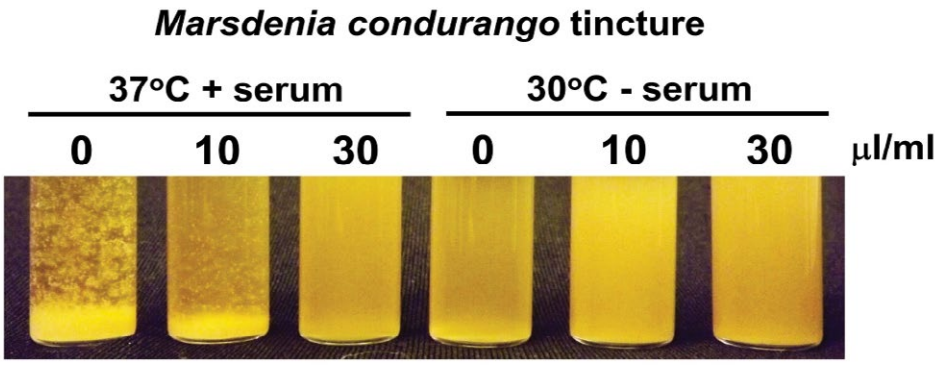

Figure 3b Visual representation of the effect of botanical tinctures on *C. albicans* morphological differentiation Image of culture characteristics of *C. albicans* cultures treated with *M. condurango* tincture.

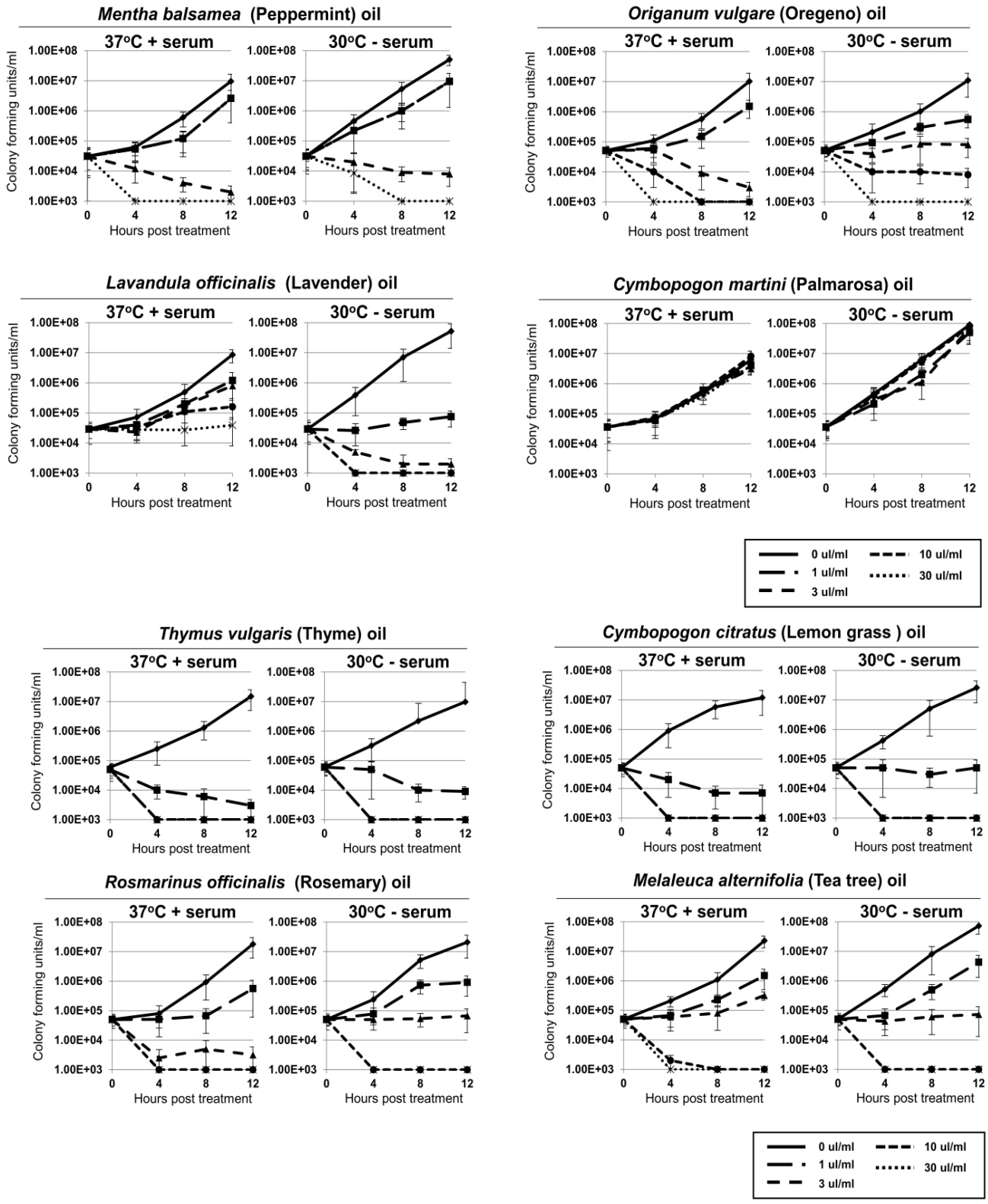

Figure 4 Effect of botanical essential oils on *C. albicans* growth rate, A starter culture of *C. albicans* was grown at 30°C in YPD. Diluted (1,000-fold) *C. albicans* cultures were left untreated (solid line), or treated with the indicated botanical oils in the amount of 1.0, 3.0, 10 or 30 µl/ml at 0 hours (dashed lines). The cultures were incubated at 37°C in YPD plus serum or 30°C in YPD without serum with continuous aeration (by rotation) and CFU/ml determined every 4 hours over a period of 12 hours. Assays were repeated in triplicate.

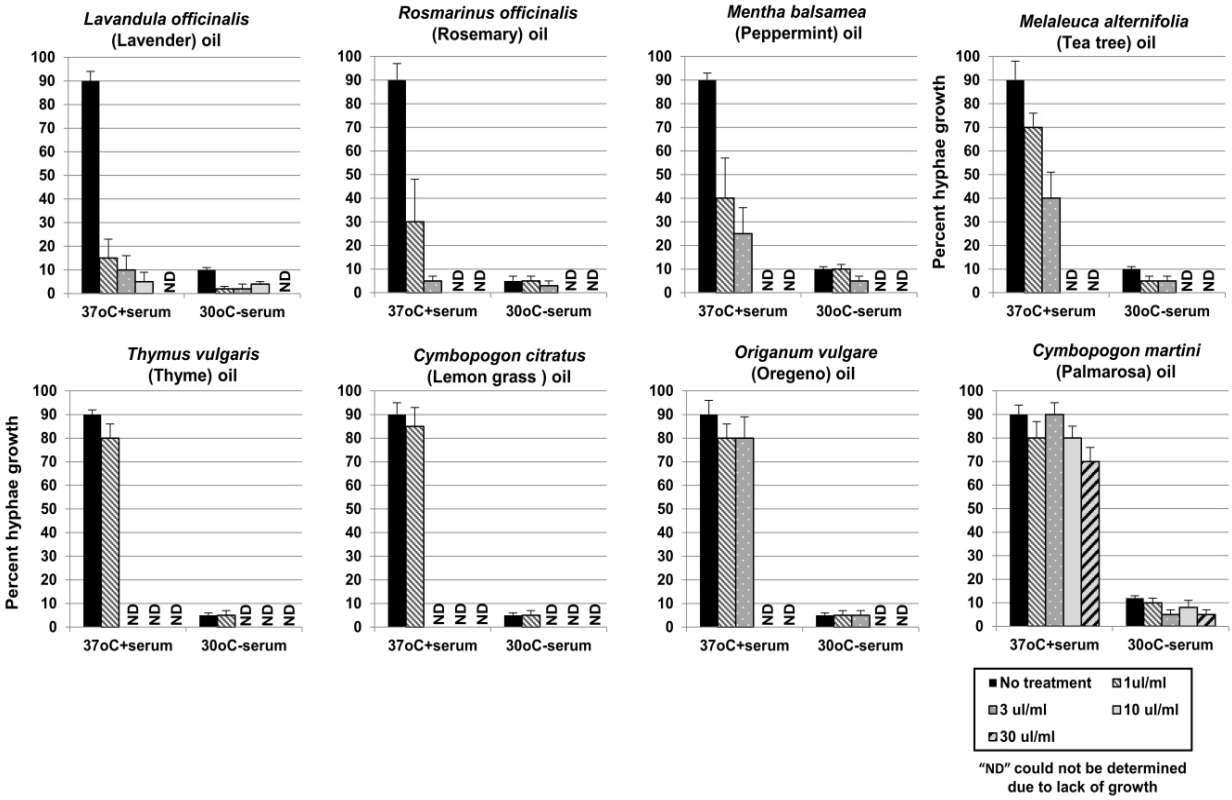

Figure 5 Effect of botanical essential oils on *C. albicans* morphological differentiation; A starter culture of *C. albicans* was grown at 30°C in YPD. Diluted (1,000-fold) *C. albicans* cultures were left untreated (solid bar), or treated with the indicated botanical oils in the amount of 1.0, 3.0, 10 or 30 µl/ml at 0 hours (dotted/dashed bars). The cultures were incubated at 37°C in YPD plus serum or 30°C in YPD without serum with continuous aeration (by rotation) for 24 hours followed by observation by light microscopy. The percent of single cells versus hyphal/pseudohyphal cells was determined. "ND" indicates samples which could not be evaluated due to a lack of growth. Assays were repeated in triplicate.

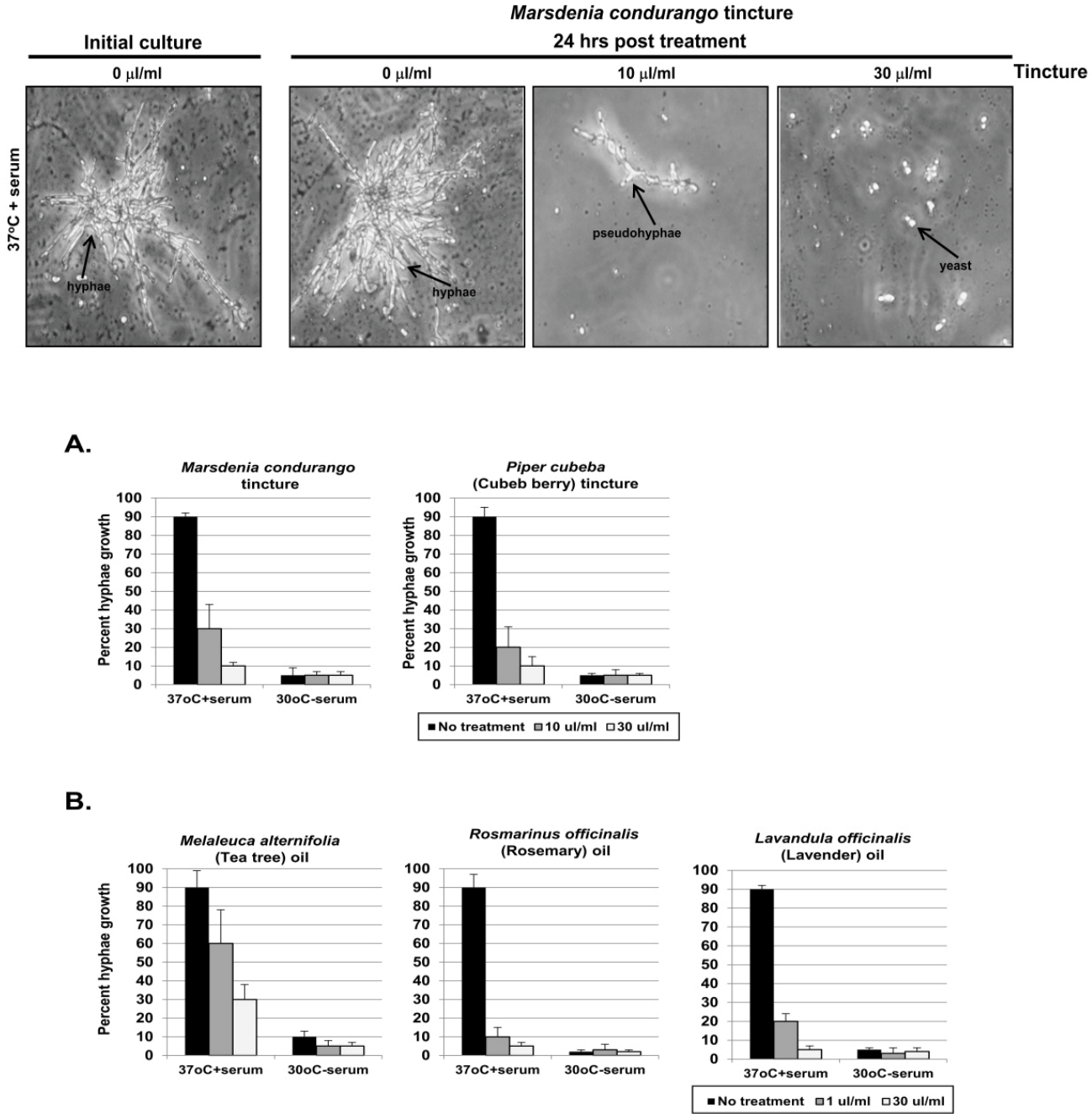

Figure 6 Ability of botanical tinctures and oils to reverse *C. albicans* hyphal formation, A starter culture of *C. albicans* was grown at 37°C in YPD plus serum. Diluted (1,000-fold) *C. albicans* cultures were left untreated (solid bar), or treated with the indicated botanical extracts/oils in the amount of 1.0, 10 or 30 µl/ml at 0 hours (dotted bars). The cultures were incubated at 37°C in YPD plus serum or 30°C in YPD without serum with continuous aeration (by rotation) for 24 hours followed by observation by light microscopy (microscopic image shown for *M. condurango* in Part A). The percent of single cells versus hyphal/pseudohyphal cells was determined and graphed in Part B. Assays were repeated in triplicate.

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