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Thymus vulgaris, Syzygium aromaticum, and Cinnamomum verum Aqueous Extracts' In Vitro Antifungal Activity Against Cat-Isolated *Candida albicans*

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***Abstract*—** The effectiveness of aqueous extracts of thyme (Thymus vulgaris), clove (Syzygium aromaticum), and cinnamon (Cinnamomum zeylanicum) against Candida albicans isolated from cat oral cavities was assessed in vitro. The opportunistic yeast Candida albicans is frequently found in animals, such as dogs and cats, and infections brought on by this organism can be difficult to treat with traditional antifungal medications.Each plant's aqueous extracts were made at 0.5%, 1.5%, 3%, and 5% (v/v) concentrations and added to Sabouraud dextrose agar medium. Each medium was inoculated with a standardized C. albicans inoculum, and radial fungal growth was assessed following the incubation period.concentration-dependent way. When compared to the control group, all extracts showed partial suppression of fungal growth at lower concentrations (0.5% and 1.5%). There was a noticeable decrease in colony diameter when the concentration rose to 3% and 5%. Thymus vulgaris aqueous extract decreased the mean colony diameter from 80 mm in the control group to 42.67 ± 2.08 mm at the maximum concentration (5%). By contrast, the mean colony diameter was reduced to 24.00 ± 1.00 mm by the aqueous extract of Syzygium aromaticum and to 27.33 ± 2.08 mm by the extract of Cinnamomum zeylanicum.Syzygium aromaticum and Cinnamomum zeylanicum had the highest antifungal activity among the plant extracts that were examined, while Thymus vulgaris had the lowest but nevertheless noteworthy inhibitory effect. These results are in line with the antibacterial qualities of these medicinal plants that have been previously documented.The study's findings lend credence to the possibility of using aqueous plant extracts as substitute antifungal medications in veterinary care. To assess their therapeutic efficacy and safety in clinical settings, more in vivo research is advised.

***Keywords* —** Thymus vulgaris, Syzygium aromaticum, Cinnamomum zeylanicum , C. albicans and essential oil .

INTRODUCTION

Candida varieties are common in the upper respiratory, lower urogenital, and digesting systems of humans as well as pets. Although human infections are linked to immune suppression, antibiotic-triggered proliferation, and alteration for mucosal protective layer, primary infections as well as subsequent consequences with cats and dogs are uncommon. (1,2) Although the processes that allow yeast strains attach and then colonized the surfaces of saliva are currently thoroughly documented, typical host defense mechanisms must be compromised for illness to become generated. The previously predatory fungal Candida albicans is frequently present throughout the everyday mucous microbe of livestock, particularly cats (3), yet when the host's immune systems are compromised, it may lead to widespread or localized illnesses. Although less common when compared to individuals, Candida is being linked to mouth, superficial, but chronic diseases among animal companions and is becoming more frequently recorded in veterinarian clinical specimens (4,5). Although the azoles, polyenes, especially echinocandins are still the primary mode of treatment, negative effects, failures of treatment, especially the evolution of resistant or tolerant strains underscore the demand for new or supplemental antimicrobial approaches. (6,7) Considering associated numerous modes for metabolism, wide-ranging biological activity, including past effectiveness with antifungal purposes, biological products—especially derived from plants essential oils and their main components—have become appealing substitutes (8). The essential oils of thyme (Thymus vulgaris), clove (Syzygium aromaticum), and cinnamon (Cinnamomum spp.) comprise naturally occurring phenolic components which possess fungicide properties regarding various kinds of Candida in vitro. These elements include the compounds thymol and carvacrol in thyme, the chemical eugenol in clove, and cinnamonaldehyde in cinnamon. Those chemicals work by disrupting cell envelopes including membranes, interfering alongside the production about biofilms, as well as modifying fungus virulence indicators. According to certain research, they have been shown to work in concert with traditional antimicrobial agents (9).

Thus, the purpose using this research is to assess the in vitro antifungal efficacy of thyme, clove, and cinnamon essential oil extracts versus cat-derived Candida albicans. The findings might aid within the creation of natural antimicrobial substitutes which might prove useful in animal healthcare and lessen the need for artificial antifungal medicines.

MATERIALS AND MTHODS

Sample Collection and Candida Isolation. Pet cats that were clinically suspected of having oral fungal infections had their mouth swab specimens taken. The samples were delivered to the lab in an aseptic environment after being appropriately stored. After being inoculated onto Sabouraud dextrose agar (SDA), each specimen was incubated for 48 to 72 hours at 37 °C.

Yeast-like colonies were inspected both macroscopically and microscopically after incubation. Colony morphology on SDA and microscopic analysis using Gram staining, which showed Gram-positive oval budding yeast cells typical of Candida species, were used for preliminary identification (10, 11).

The germ tube test in human serum was used to confirm suspected Candida isolates as Candida albicans. A positive result was defined as the production of germ tubes following two to three hours of incubation at 37 °C. Another confirming test for species identification was the development of chlamydospores on maize meal agar (10,12).

The study only included isolates that met all of the phenotypic identification requirements for Candida albicans. To ensure reproducibility, a uniform C. albicans inoculum was created and used consistently in all experimental experiments.

A reliable botanical supplier provided dried clove buds (Syzygium aromaticum), thyme leaves (Thymus vulgaris), and cinnamon bark (Cinnamomum zeylanicum). With a few minor adjustments, a traditional decoction procedure was used to prepare the aqueous extracts (13,14).

In short, 100 mL of distilled water was combined with 20 g of finely crushed dried plant material from each species. To obtain sterile aqueous extracts, the mixtures were boiled for 15 minutes, cooled to room temperature, and then filtered through sterile gauze and a 0.22 μm membrane filter. The final concentrations of 0.5%, 1.5%, 3%, and 5% (v/v) were obtained by diluting the resultant stock extracts with sterile distilled water.

Antifungal Assay. An agar incorporation technique has been employed to test each substance's antifungal property. To get the correct amount, Sabouraud dextrose agar (HiMedia Laboratories Pvt. Ltd., Mumbai, India), boiled to about 45°C, and then combined with the correct amount from every extraction. There was no extraction on the counterpart plates. A 5-mm agarose plug from a C. albicans colony which had been developing rapidly was used to properly inoculate every plate. For the duration of 48 hours, the plates had been incubated at 37 °C Following the incubation process, Two opposing directions were used to measure the fungal colony growth width (in millimeters), and the mean value was computed. Four duplicate plates were utilized for the control group and each aqueous extract (AE) concentration (15).

Data Analysis. The mean fungal colony diameter for each treatment group was determined and reported as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to statistically examine differences between the tested aqueous extract concentrations and the control group. Tukey's post hoc multiple comparison test was used to find statistically significant pairwise differences between specific treatment groups when a significant overall difference was found.

A p-value of less than 0.05 was considered statistically significant. The study made it possible to identify which concentrations differed significantly from the control and from one another, as well as to determine the concentration-dependent effects of the aqueous extracts. Standard statistical software was used for all statistical analyses.

RESULT AND DISCUSSION

The findings showed that thyme aqueous extract reduced Candida development through a dose-dependent way. The group serving as a control displayed the greatest mean score of eighty, indicating uninhibited, typical development of fungi. The mean dropped to 67.67 ± 1.53 when 0.5% thyme aqueous extract was used, suggesting a slight antifungal impact. Greater inhibition was demonstrated by lowering the mean to 60.33 ± 1.53 when the dose was increased to 1.5%. The greatest concentration (5%) showed the greatest amount of noticeable inhibition with a mean of 42.67 ± 2.08, whereas the mean value decreased to 51 ± 1.00 at 3%. (Table 1 , Figure 1)

The findings demonstrated that clove gradually inhibited Candida infection at higher doses. The comparison group showed the highest mean score of eighty, suggesting unconstrained regular development of fungi. The mean dropped to 53.33 ± 0.58 at a dosage of 0.5%, indicating a considerable antimicrobial impact. The mean was decreased further to 39.33 ± 1.15 when the amount present was increased to 1.5%, suggesting greater inhibition. The maximum concentration (five percent) produced the lowest mean (24 ± 1.00), indicating the greatest antifungal action, whereas the average value decreased to 34 ± 1.00 at 3%.(Table 1 , Figure 2).

According to the findings, the growth of Candida was clearly inhibited by cinnamon and this impact grew in a direct relationship with the A E concentration. The group serving as the control had the highest mean value of eighty, indicating uninhibited, typical fungal development. The standard deviation dropped at an amount of 0.5%, signifying the start of the antifungal effect. The mean values gradually decreased as concentrations rose to 1.5% and 3%, indicating a more potent inhibitory impact on fungal growth. The strongest antifungal impact was seen at the greatest dosage (five percent), which also produced the lowest mean value, indicating maximum suppression of Candida growth.(Table 1 , Figure 3).

Table 1. Effect of aqueous extract on Candida Growth

Group	Mean	Std. Deviation
0 thyme	80	0
0.5 thyme	67.67	1.528
1.5 thyme	60.33	1.528
3 thyme	51	1
5 thyme	42.67	2.082
0 clove	80	0
0.5 clove	53.33	0.577
1.5 clove	39.33	1.155
3 clove	34	1
5 clove	24	1
0cinnamon	80	0
0.5 cinnamon	56.67	2.082
1.5 cinnamon	43.67	0.577
3 cinnamon	37.33	1.528
5 cinnamon	27.33	2.082

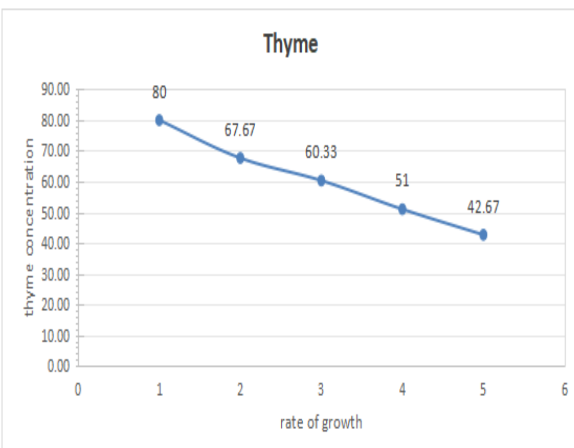

Figure 1. Antifungal Activity of thyme against Candida albicans

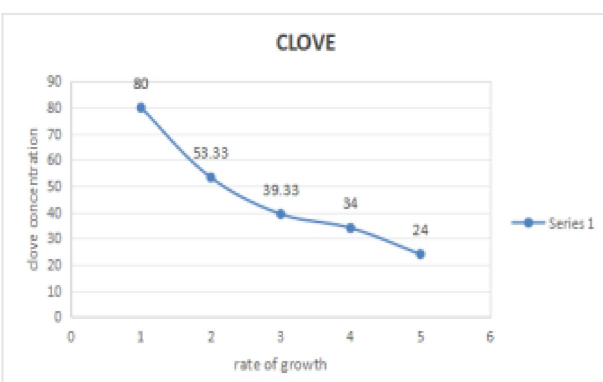

Figure 2. Antifungal Activity of clove against Candida albicans

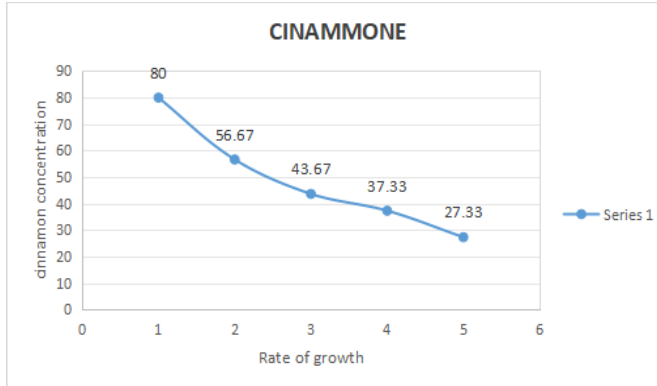

Figure 3. Antifungal Activity of cinnamon against Candida albicans

The current investigation demonstrated that thyme oil had strong in vitro antimicrobial action versus cat-isolated Candida albicans. Greater levels of oil were found to have a greater antimicrobial impact, suggesting a dose-dependent relationship between fungus suppression with thyme oil content. This outcome demonstrates that thyme oil has strong antifungal effects which may successfully inhibit Candida growth.

Thymol as well as carvacrol, two phenolic compounds with broad-ranging antibacterial qualities, were the main components of thyme oil that serve as the basis with its antimicrobial activity. These substances work by compromising the strength within the fungal cell membrane, which causes cell death and material leaking (16,17). Furthermore, the ergosterol production, a crucial part within the fungal cell membrane, is disrupted by thymol, changing the ability to leak underlying the outer layer and preventing fungal development (18).

Significant antifungal effectiveness of thyme oil versus animal-derived Candida species has been additionally documented in earlier research. For instance, another study [19] discovered those thyme oil had a significant antimicrobial impact on isolates of Candida albicans which is equivalent with that of conventional antifungal medications. Additionally, (20,21) showed that thymol reduced the aggressiveness of Candida albicans through preventing its growth going between yeast to hyphal structure, a crucial stage that determines pathogenicity. These results support the current studies that demonstrate the beneficial effects of thyme oil in the treatment of microbial illnesses (22, 23).

Additionally, thyme had been demonstrated to prevent Candida albicans from forming colonies (24), which is therapeutically significant because colonies shield the pathogen coming from medicinal products . Thyme oil's effectiveness for an all-natural antimicrobial medication is increased by having the ability to enter and break up microbes. (25).

Additionally, the present research highlights the possibility of use of thyme oil in veterinary medicine for a herbal replacement for pharmaceutical antifungal medication, particularly for cat candidiasis. Essential oils like thyme provide a more secure, more affordable, and beneficial to the environment alternative than commercial antifungal medicines including azoles, which frequently struggle with cytotoxicity as well as tolerance. In general, our results imply that the oil of thyme may be a useful natural antifungal agent against cat-isolated Candida albicans. To assess the drug's security, effectiveness, and ideal dosage for veterinary therapeutic usage, additional in cat investigation and manufacturing trials are advised. The different antimicrobial characteristics of clove and cinnamon oils seem to originate from mostly determined based on their chemical differences. (26) Flavonoids and tannins, polyphenolic chemicals that are well known for possessing antibacterial qualities involving mechanisms with the value dissolving surfaces, dissolving components within cells, and disrupting microbe proteins and membranes. These substances are abundant within clove oil. Degradation of membrane function, interruption of metabolic flux, and ultimately cellular death are frequently the results from these exchanges. Cinnamon oil's relatively weak antimicrobial properties, especially at submaximal concentrations, may be explained by their absence. Eugenol, a lipophilic phenylpropanoid with a variety of antifungal properties, is a major active ingredient in clove oil. By inserting itself through the bilayers of lipids of fungi membranes it increases permeation as well as mobility, which facilitates the efflux of inside the cell substances and charged particles. The chemical also has beneficial antioxidant properties that increase oxidative damage in cells of fungi, that eventually results in mitochondria malfunction and mortality. These characteristics make clove oil active at significantly smaller amounts while establishing eugenol as a strong antifungal. (27,28)However, cinnamonaldehyde, an aromatic aldehyde that targets the enzymes found in mitochondria as well as modifies oxidative regulation, is primarily responsible behind the antimicrobial properties of cinnamon oil. Additionally, it prevents morphological flipping, which is a crucial factor in Candida albicans pathogenicity. nevertheless, at lower doses, the substance's accessibility could have been diminished by its extremely volatile nature, restricted absorption in water-based environments, and moderate cell membrane penetration. This is linked to research showing where, especially in resistance fungus varieties, overall antimicrobial action is more noticeable than its fungicide action. Interestingly, plant-based phytosterols have been identified throughout both oils as well as could function in concert either flavonoids or cinnamon aldehyde to impair ergosterol production, a process essential to the flexibility and organization of fungal membranes. (29)Consumption about ergosterol causes cell breakdown, inhibits the intake of nutrients, and makes the body more vulnerable to osmosis (30).

Clove oil's higher antioxidant framework makes the combination apparent. Clove oil is a viable option towards the creation of anti-fungal medications due to its strong medicinal properties at low concentrations, especially in surface medications, orally rinses, or contraceptives. However, problems including instability, possible mucosal inflammation, hosting inflammation, as well as formulations consistency still need to be carefully taken into account, making the transition between benches to clinic difficult. The durability and bioavailability associated with these chemical compounds may be improved by techniques like nanoencapsulation, fluid-based administration, or matrices of polymers. Even though requiring higher dosages, cinnamon oil may provide additional benefits avoidance or preventive uses wherein prolonged antimicrobial stress is beneficial. When combined, these oils offer useful scaffolding require antimicrobial treatments. Their unique yet complimentary molecular characteristics call for more pharmacological and toxicological research (31).

CONCLUSION

While both clove and cinnamon essential oils have significant antimicrobial action versus Candida albicans, the present research shows that clove oil has highly fungicidal effectiveness at lower concentrations, rendering it the preferred choice towards clinical research. These findings are consistent alongside recent investigations and encourage more investigation towards synergistic combinations including clinical studies

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