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ORIGINAL ARTICLE

ANTI-INFLAMMATORY AND ANTICANDIDAL ACTIVITY OF *CORIANDRUM SATIVUM* SEED EXTRACTS AGAINST *CANDIDA ALBICANS* AND *CANDIDA TROPICALIS*

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ABSTRACT

Background: Candidiasis in not uncommon among hospitalized patients. The fungal virulence and antifungal drug resistance make the host-parasite interactions complicated. The threat of azole drug resistance along with scarcity of antifungal therapies prompted the necessity of novel drug development from various natural sources. The objective of this study was to evaluate the anticandidal activity of *Coriandrum sativum* seed extracts against *Candida albicans* and *Candida tropilcalis*.

Materials & Methods: This study was conducted at Department of Basic Medical Sciences, College of Medicine, Prince Sattam Bin Abdulaziz University, Al-Kharj, Saudi Arabia from July 20, 2021 to September 26, 2021. The study analyzed anti-inflammatory assays via inhibition of albumin denaturation and hemolysis inhibition and anticandidal activity of the extract against *Candida albicans* and *Candida tropilcalis* by well diffusion method. The minimum inhibitory concentration (MIC) of the extract was also determined against both the fungi.

Results: Anti-inflammatory activity of *Coriandrum sativum* methanolic seed extracts were evaluated at five different concentrations from 100 μg to 1000 μg. The highest concentration showed 55.31±0.77% of inhibition of albumin denaturation, whereas the aspirin, the standard used, showed 80.33±0.32%. *Coriandrum sativum* seed extracts at 250 mg/ml, 500 mg/ml and 1000 mg/ml produced zones of growth inhibition with diameters 12.66±0.57, 17.66±0.57 and 24.33±1 mm against *Candida albicans*, and 10.33±0.57, 16.66±0.57 and 21.66±0.57 mm against *Candida tropicalis*. The MICs of the *Coriandrum sativum* seed extracts against *Candida albicans* was 1.95 mg and *Candida tropicalis* was 3.9 mg.

Conclusion: Phytochemical compounds in methanolic seed extracts of *Coriandrum sativum* showed significant anticandidal activity against *Candida albicans* and *Candida tropicalis* with highest zone of inhibition at 1000 μg extract concentration. The lower MICs of *Coriandrum sativum* seed extracts against *Candida albicans* and *Candida tropicalis* were also significant. Considering all these findings along with the antioxidant activities, it could be suggested that *Coriandrum sativum* seed extracts can further be investigated for development of novel anticandidal drugs.

KEY WORDS: Invasive Candidiasis; **C**andidiasis; Candidemia; Fungi; *Candida albicans; Candida tropicalis;* Antifungal Drug Resistance; *Coriandrum sativum;* Minimum Inhibitory Concentration; Antibiotics.

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1. INTRODUCTION

1.1 Background: In humans, the most common causative organisms for candidiasis are *Candida albicans* and *Candida topicalis*. Candida can be problematic if its growth or virulence becomes out of control or spreads deep into the body; bloodstream or visceral organs, including heart, brain or kidney). Candidiasis is distinguished by the location of infection in the body.[1] Vaginal candidiasis is characterized by itching or soreness in the vaginal area and symptoms get complicated with severity.[2] Invasive candidiasis, a pathologically serious condition, affects many of the tissues and vital organs like brain, heart, bones, blood, eyes etc. Candidemia, a bloodstream infection caused by Candida spp., is a general condition among hospitalized patients, especially in ICUs[3] with symptoms varying from fever and chills to eyes,

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heart, brain, joints or bones infections.[4] Oropharyngeal candidiasis, sometimes recognized as thrush affects the mouth and throat. Candida esophagitis is a sort of candidiasis that affects the esophagus and is one of the most common infections among HIV/AIDS patients.[5,6] All the above mentioned types are common in ICU patients. In various countries, the frequencies of the candidiasis per 1,000 ICU admissions have shown variations between 0.24 and 34.3, and the 30-day crude (44.7%) and attributable (19.6%) mortality rates among patients with Candidemia were found to be higher.[7]

Antifungal drug resistance (ADR) and mycotic virulence are the key host-pathogen interaction problems in candida infections. Despite the fact that the azoles, particularly fluconazole (FLC) are still the extensively used antifungal medication, their broad usage in clinical practice for both therapy and prophylaxis has aided resistant strain development. The threat of azole drug resistance, together with a deficiency of antifungal therapeutics demanded the development of novel drugs.[8] Antibiotic resistance is a serious problem in contemporary medicine. Despite some encouraging signs, current resistance is proving difficult to overcome, and new resistances are forming and spreading across the globe and hence, new antibiotics are still in high demand.[9]

Plants and phytochemicals have always been contributing to the search for new natural anti-microbial agents. *Coriandrum sativum* under the family *Umbeliferae* is a thin, glabrous, branching plant that is widely cultivated in various part of the world. Even though all parts of this plant are eatable, fresh leaves and dried seeds are the most commonly used ingredients in cookery.[10] The major biologically active components of *Coriandrum sativum* are tocol, fatty acids, carotenoids, essential and oil sterol, and the chemical compositions and yields affected by the genotype, variety, ecotype, planting condition, harvesting time, growth stage, planting season, plant part, extracting process and other variables.[11] The methanolic extract of *Coriandrum sativum* proved to have seven bioactive phytochemical components 7aH-cyclopenta[a] cyclopropa[f] cycloundecene; ascorbic acid 2,6-dihexadecanoate; 9,10-secocholesta-5,7,10(19)-triene-3,24,25-triol; 1,6- octadien-3-ol, 3,7-dimethyl; geranyl vinyl ether; 1,6-octadien-3-ol,3,7-dimethyl; and 2-aminobenzoate, bicyclo[2.2.1] heptan-2- one,1,7,7-trimethyl.[12] Numerous recent comprehensive studies on coriander, focused on the bioactive components in general and on the phenolic compounds and their various pharmacological characteristics in particular have been published.[13] Even though there were few studies on anti-fungal activities of the *Coriandrum sativum* leaves and essential oil, no studies were reported on anticandidal activity of methanolic seed extracts.

1.2 Research Objectives (ROs)

RO 1: To evaluate the anticandidal activity of the *Coriandrum sativum* seed extracts against *Candida albicans* and *Candida tropilcalis*.

RO 2: To find out the minimum inhibitory concentrations (MICs) of the *Coriandrum sativum* seed extracts against *Candida albicans* and *Candida tropilcalis*.

RO 3: To analyze the anti-inflammatory assays via inhibition of albumin denaturation and hemolysis inhibition.

2. MATERIALS AND METHODS

2.1 Design, settings & duration: This study was conducted at the Department of Basic Medical Sciences, College of Medicine, Prince Sattam Bin Abdulaziz University, Al-Kharj, Saudi Arabia from July 20, 2021 to September 26, 2021.

2.2 Collection of *Coriandrum sativum*: The dry seeds of *Coriandrum sativum* were procured from local market and stored at room temperature (Figure 2.2). The pods were dried in shade at room temperature, purely grounded to powders and stored in sterile containers for extraction.

Figure 2.2: Collection of Coriandrum sativum seeds

2.3 Extraction of bioactive compounds using Soxhlet apparatus: Powders of *Coriandrum sativum* seeds were positioned in a porous cellulose paper bag which was placed in thimble chamber of the Soxhlet apparatus. The extractor was filled with solvent solution of methanol and a temperature of 60°C was set and left for 6 hours. The solvent was evaporated and the collected extracts were kept in sterile containers.

2.4 Anti-inflammatory activity

2.4.1 Albumin denaturation assay: The estimation of anti-inflammatory effect of *Coriandrum sativum* seed extracts was performed against egg albumin denaturation. 5 ml of the reaction mixture which contained egg albumin: 0.2 ml, phosphate saline buffer (PBS, pH 6.4): 2.8 ml and *Coriandrum sativum*

seed extract (with different concentrations as 100 μg/ml, 250 μg/ml, 500 μg/ml, 750 μg/ml and 1000 μg/ml): 2 ml - was prepared. The control was prepared with an equal volume of sterile distilled water and was incubated at 37.2 °C for 15 min. Following the incubation, it was heated to 70°C for 5 min. Then, at 660 nm, the absorbance was calculated. For reference aspirin at the same concentrations were used at the same absorbance.

The following formula was used to estimate the protein denaturation inhibition:

% inhibition of egg albumin denaturation = $100\times(1-OD2-OD1OD3-OD1)$

Where, OD_1 = Unheated test sample; OD_2 = heated test sample and OD_3 = heated control sample.

2.4.2 Hemolysis inhibition: Blood samples from the veins of healthy adult individuals were collected into glass centrifuge tube covering a known amount of anticoagulant. The supernatant was discarded after centrifuging for 10 minutes at 1500 rpm. The resulting pellet was rinsed with phosphate buffered saline (0.1M) to get buff colored cells called packed RBCs. To study H_2O_2 induced oxidative damage, 0.1 mL of 0.1 mM $FeCl_3$, 0.1 mL of 1 mM H_2O_2 and 100 μL of plant extracts were incubated for 15 minutes. Then 500 μL of packed RBC was added and incubated at 37^{0}C for 1 hr. Aspirin at different concentrations was used as positive control. Thereafter, the content of the tube was centrifuged at 1500 rpm for 10 minutes and the absorbance was calculated at 540 nm using a spectrophotometer. Further, the extent of erythrocyte damage due to lipid peroxidation of cell membrane was measured in terms of malondialdehyde content.

2.5 Anticandidal activity using well diffusion method: The anticandidal potentials of the plant extract against the clinical pathogens was assessed by well diffusion method. Overnight cultures of test pathogens were grown and 0.1% of culture solution of each of the test organisms was streaked onto the Muller-Hinton agar. 6 mm bore wells were prepared on the agar surface and about 100 μl of the samples were filled into the well and the plates were incubated for 48 h at 37ºC. The anticandidal activity was determined based on diameters of the growth inhibitory zones which were measured in millimeters (mm).

2.6 Evaluating of minimum inhibitory concentrations (MICs) of the plant extracts: The plant extracts were diluted into several concentrations (1.95 mg/ml, 3.9 mg/ml, 7.8 mg/ml, 62.5 mg/ml, 15.6 mg/ml, 31.5 mg/ml, 125 mg/ml and 250 mg/ml, in 1 ml of sterile nutrient broth) in test tubes. A 100 μl of *Candida albicans* culture at 0.5 McFarland standards was inoculated to the tubes. Similarly, this was repeated for *Candida tropicali* also. The tubes were incubated at 37°C for 24 h and observed for the growth or turbidity with unaided eye.

3. RESULTS

3.1 Anti-inflammatory activity

3.1.1 Albumin denaturation assay: Protein denaturation causes inflammatory and arthritic disorders. So, phytochemiclas having potential inhibition properties on protein denaturation can be utilized in developing anti-inflammatory drugs. The anti-inflammatory activity of *Coriandrum sativum* seed extracts were evaluated at five different concentrations (100 μg, 250 μg, 500 μg, 750 μg and 1000 μg) and aspirin was used as the standard. 100 μg, 250 μg, 500 μg, 750 μg and 1000 μg concentrations of the plant extracts showed 8.57±1.25%, 14.28±1.12%, 34.28±1.54%, 37.14±0.89% and 55.31±0.77% of inhibition of albumin denaturation, whereas the aspirin showed 23.54±0.93%, 42.22±0.55%, 61.78±1.04%, 65.41±0.46% and 80.33±0.32% of inhibition. (Figure 3.1.1)

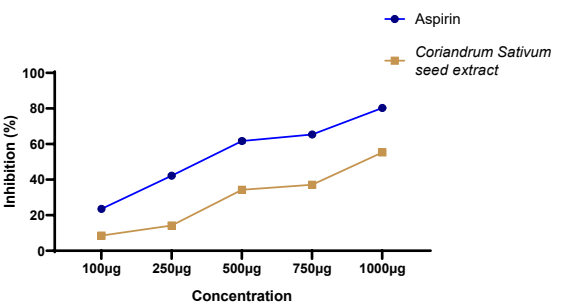

Figure 3.1.1: Inhibition of albumin denaturation assay of *Coriandrum sativum* seed extracts vs. aspirin

3.1.2 Haemolysis Inhibition Assay: The haemolysis inhibition analysis of *Coriandrum sativum* seed extracts was evaluated at five different concentrations (100 μg, 250 μg, 500 μg, 750 μg and 1000 μg) and aspirin was used as the standard. 100 μg, 250 μg, 500 μg, 750 μg and 1000 μg concentrations of the plant extract showed 11.2±1.47%, 28.13±1.33%, 43.24±0.65%, 51.35±1.18% and 60.5±1.43% of inhibition of albumin denaturation, whereas aspirin showed 26.88±0.88%, 30.56±0.63%, 57.66±0.47%, 64.73±0.81% and 85.42±0.55% of inhibition. (Figure 3.1.2)

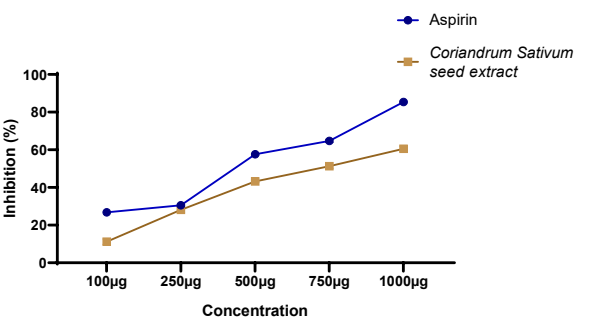

Figure 3.1.2: Hemolysis inhibition assay of *Coriandrum sativum* seed extracts vs. aspirin

3.2 Anticandidal activity: *Coriandrum sativum* seed extracts at 250 mg/ml, 500 mg/ml and 1000 mg/ml showed zones of growth inhibition with diameters 12.66±0.57 mm, 17.66±0.57 mm and 24.33±1 mm against *Candida albicans*; and 10.33±0.57 mm, 16.66±0.57 mm and 21.66±0.57 mm against *Candida tropicalis.* (Figure 3.2.1 & 3.2.2). No inhibitory zones were observed for 100 mg/ml and 125 mg/ml concentration (Figure 3.2.3).

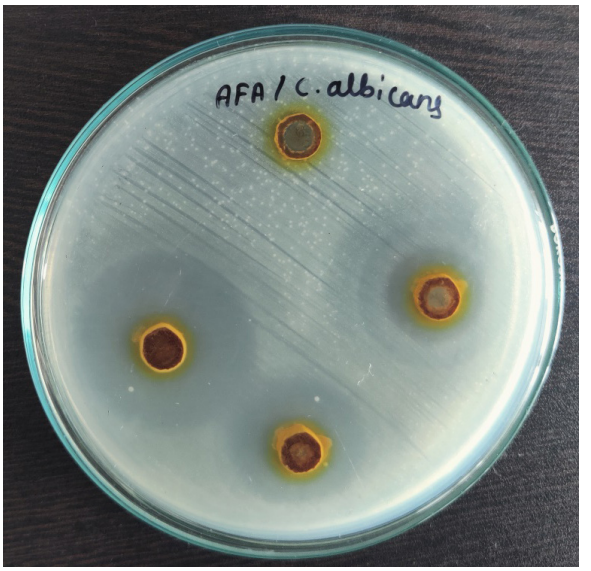

Figure 3.2.1: Anticandidal activity of the *Coriandrum sativum* seed extracts against *Candida albicans*

Figure 3.2.2: Anticandidal activity of *Coriandrum sativum* seed extracts *Candida tropicalis*

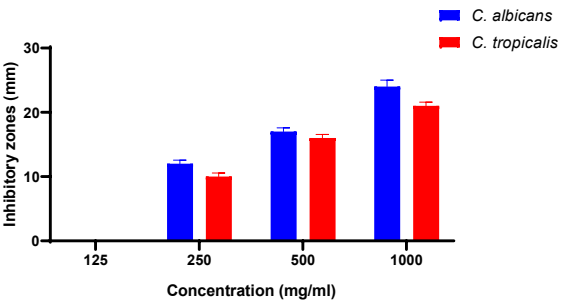

Figure 2.3.3: Schematic representation of anticandidal activity of the *Coriandrum sativum* seed extracts

3.3 Minimum inhibitory concentrations (MICs) analysis: The fungal growth was observed in naked eyes and the concentration showing no growth of the test fungus was defined as the MIC. The MICs of the *Coriandrum sativum* seed extracts against *Candida albicans* was found to be 1.95 mg and *Candida tropicalis* was 3.9 mg. (Figure 3.3.1 & 3.3.2)

Figure 3.3.1: MIC evaluation *Coriandrum sativum* seed extracts against *Candida albicans*

Figure 3.3.2: MIC evaluation *Coriandrum sativum* seed extracts against *Candida tropicalis*

4. DISCUSSION

Candida albicans is a fungal opportunistic pathogen responsible for various kinds of candidiasis like the thrush.[14] *Candida tropicalis* is a fungus that is harmful in neutropenic hosts and spreads to peripheral organs via the circulatory system.

Coriander is proven to have a number of potential bioactive properties and has widely been used in various traditional branches of medicine to treat respiratory, digestive and urinary system diseases because it possesses diuretic, diaphoretic, carminative, and stimulating properties.[15,16] Coriander has also been shown to have a variety of pharmacological properties, including activities against oxidants, mutagens, diabetes, ulcer, lead toxicity etc. It also has anthelmentic activity, sedative-hypnotic activity, antifungal activity, cholesterol lowering activity, anxiolytic activity, anticonvulsant activity, diuretic activity, hepatoprotective activity, anticancer activity, post-coital anti-fertility, anti-protozoal activity and heavy metal detoxification.[17] Checking the other major biochemical properties, in a scavenging assay, with an IC50 of 5.84±1.17% and in a carotene bleaching test with an IC50 of 0.23±0.01 %, the commercial coriander essential oil with linalool as its main component shows scavenging characteristics on fair radicals and the ability to inhibit the lipid peroxidation, and thus it is supposed to be considered a potential agent for production of the anti-inflammatory drugs. [18,19]

In the present study, the *Coriandrum sativum* seed extracts at 250 mg/ml, 500 mg/ml and 1000 mg/ml showed zones of growth inhibition with diameters 12.66±0.57 mm, 17.66±0.57 mm and 24.33±1 mm against *Candida albicans*; and 10.33±0.57 mm, 16.66±0.57 mm and 21.66±0.57 mm against *Candida tropicalis*. Also, the minimum inhibitory concentrations (MICs) of the *Coriandrum sativum* seed extracts against *Candida albicans* was found to be 1.95 mg and *Candida tropicalis* was 3.9 mg. This, in fact, is accordance with the studies conducted by Wei, et al.[20], which found that the essential oil from coriander leaves had powerful antifungal properties, particularly against *Candida* spp. The ethanol extract, on the other hand, only inhibited the viability of *Candida albicans* (ATCC 1223) among all the fungal strains examined. Another report by Matasyoh, et al.[21] showed that the minimum inhibitory concentration of oil against Gram-negative bacteria was between 130 and 217 mg/ml, whereas the MIC against Gram-positive bacterial species was as low as 108 mg/ml. It is a fact that the antibacterial activity of the oil is frequently higher than antifungal activity.

This study found that the anti-inflammatory activities of the *Coriandrum sativum* has been challenging and this finding was underlined by an investigation by Tang, et al.21 who described that the *Coriandrum sativum* roots had the strongest antiproliferative activity (IC50 = 200.0, at a concentration of 2.6 g/mL) and the highest phenolic content, FRAP and DPPH scavenging capabilities.

5. CONCLUSION

In the present study, the methanolic extracts of coriander seeds showed evident anti-inflammatory potential at the highest tested concentration, even though it was lesser than the corresponding concentration of the standard taken, the aspirin. So, it is a promising finding that the bioactive agents from this natural source can be further investigated for its potential anti-inflammatory compounds. Also, the extract showed remarkable activity against both of the selected fungal pathogens, *viz., Candida albicans* and *Candida tropilcalis* and it was further determined that the minimal inhibitory concentrations (MICs) of the extract against both of the given fungi were comparatively lower. In the present scenario of extensive antifungal drug resistance (ADR) reports, these results encourage the development of potential drugs against infections by both of the selected fungal pathogens, by further purification of the extract and through detailed *in vivo* studies.

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CONFLICT OF INTEREST
Authors declare no conflict of interest.
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AUTHORS' CONTRIBUTION

The following authors have made substantial contributions to the manuscript as under:

Conception or Design:	MMP, AG
Acquisition, Analysis or Interpretation of Data:	MMP, AG, APM, KR
Manuscript Writing & Approval:	MMP, AG, APM, KR

All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.