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# Assessment of the Anti-Fungal Activity of Green Coconut Water *(Cocos nucifera l.)* on *Candida albicans* - An *in-vitro* study

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**Abstract**

**Aim:** The aim of this study was to assess the anti‑fungal activity of green coconut water (Cocos nucifera L.) extract on Candida albicans.
**Materials and Methods:** Extract from (Cocos nucifera L.) was lyophilized and its anti-fungal activity against C. albicans was tested in vitro at various concentrations including 1000 µg, 500 µg, 250 µg and 100 µg respectively. The inhibitory effect of Cocos nucifera L extract on C. albicans was determined by agar culture and applied broth dilution test.
**Results:** Cocos nucifera L.extract at 1000 µg/ml concentration effectively inhibited the growth of C. albicans (14 ± 0.3) compared with the positive control ‑amphotericin B (15 ± 0.3) in a dose-dependent manner.
**Conclusion:** Tender coconut water (Cocos nucifera L.) extract had a strong dose-dependent anti‑fu ngal effect on C. albicans.

**Key words:** Anti-fungal activity, coconut water, Candida albicans

## INTRODUCTION

Candidiasis is a widespread oral and perioral opportunistic infection that usually results from overgrowth of endogenous Candida fungal microorganisms. Though many species of Candida exists, C. albicans is the most frequently (>80%) encountered organism in the ambulatory general practice dental patient. It is clinically presented in various forms such as angular chelitis, denture stomatitis, hyperplastic (candidial leukoplakia), chronic atrophic (erythematous), median rhomboid glossitis, inflammatory papillary hyperplasia, mucocutaneous, and pseudomembranous [1,2]. The increasing prevalence of acquired immune deficiency syndrome and other immuno-compromised patient groups in the society, the amplified usage of broad-spectrum antibiotics and corticosteroids, diabetes mellitus, and severe nutritional deficiencies have resulted in the revival of oral candidiasis as a relatively common affliction in patients [3]. Due to the increased resistance and adverse drug reactions to the currently marketed over the counter anti-fungal agents, there is a constant demand for a safe and competent alternative option for the prevention and treatment of these infections. Hence, phytochemicals derived from plants and its products used as traditional medicines are considered as an ideal choice.

The green coconut water (*Cocos nucifera* L.), a refreshing and nutritious beverage is widely consumed due to its commendable health benefits from control of hypertension to protection against myocardial infarction. [4]. It is assumed that coconut water could be used as an important alternative for oral rehydration and even so for intravenous hydration of patients in remote regions [4]. Besides its nutritional role, it is also extensively used in the plant tissue culture industry as a growth-promoting component and also seems to have growth regulatory properties, e.g., cytokinin-type activity [5]. Some of the most significant and useful components in coconut water are the cytokinins, which are a class of phytohormones (e.g., kinetin and *trans*-zeatin) showed significant anti-ageing, anti-carcinogenic, and anti-thrombotic effects [6,7]. Furthermore, micronutrients such as inorganic ions and vitamins in green coconut water (GCW) play a vital role in aiding the human body antioxidant system. Other components found include sugars, sugar alcohols, lipids, amino acids, nitrogenous compounds, organic acids and enzymes [8]. Thus the multitude of compounds, both known and unknown, present in coconut water, makes it a multidimensional liquid with immense significance. As per the available literature, the use of coconut oil, its wound healing nature, anti-allergic properties have only been considered and reported elaborately whereas the antifungal activity of the GCW has not been documented adequately. With this background, this study was conducted to assess the in-vitro anti-fungal effect of GCW extract on C. albicans.

## MATERIALS AND METHODS

### Plant material

#### *Collection and preparation of extract*

Ripe coconuts (*Cocos nucifera*) were bought from a local market at Chennai, Tamil Nadu. The coconuts were broken open and juice/water was collected. The juice was centrifuged and later lyophilized to dryness to obtain a powder extract. Serial dilutions of extract were prepared (solvent-water) and final concentrations of 1000, 500, 250, 100 µg/ml were obtained for further testing.

***Preparation of inoculum***

The microbial strain (C. albicans, MTCC 3958) used for this study was obtained from the Institute of Microbial Technology, Chandigarh. Stock cultures were maintained at 4°C on the slant of nutrient agar. Active cultures for experiments were prepared by transferring a loop full of cells from the stock cultures to test tubes of nutrient broth for fungi that were incubated for 24 h at 37°C. The assay was performed by disc diffusion method.

***Disk diffusion method***

Anti-fungal activity of the given sample was evaluated by disk diffusion method on Muller Hinton agar (MHA) medium. The MHA medium is poured in to the petri plate. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moisture with the bacterial suspension. The disks were placed on MHA plate with the help of sterile forceps and different concentration (1000 µg, 500 µg, 250 µg and 100 µg) of each samples were loaded on disks. Blank disc impregnated with solvent dimethyl sulfoxide followed by drying off was used as a negative control and amphotericin B (10 µg/disc) used as a positive control. The plates were incubated for 24 h, at 37°C. Then the microbial growth was determined by measuring the diameter of zone of inhibition.

Data were analyzed using independent t-test. The level of significance was set at 0.05.

## RESULTS

GCW extract at 1000 µg/ml concentration effectively inhibited the growth of C. albicans (14 ± 0.3) compared with the positive control-amphotericin B (15 ± 0.3) [Figure 1]. Coconut water showed maximum inhibition at 1000 µg/ml, followed by 500 µg/ml (9 ± 0.2), 250 µg/ml (7 ± 0.3), and nil effect at 100 µg/ml and 50 µg/ml respectively. The inhibition pattern was seen to increase with the increase in concentration of the extract [Table 1]. Thus, it was a dose-dependent reaction. An independent t-test was done to compare the action of GCW extract and amphotericin B against C. albicans. The P value of ≤0.05 was found to be significant, and is concluded that there is no significant difference in the mean values between GCW extracts and amphotericin B against C. albicans hence proving that they have comparable efficacy with the conventional allopathic medication.

**Figure 1:** Anti-microbial activity of green coconut water extract against *Candida albicans** clockwise from top: 1000, 500, 250, 100 µg/disc and solvent (dimethyl sulfoxide) control center: Amphotericin B (100 µg/disc) control

| Nutritional value per 100 g (3.5 oz) | | |
|---|---|---|
| **Energy** | 63 kJ (15 kcal) | |
| **Carbohydrates** | 3.71 g | |
| Sugars | 2.61 g | |
| Dietary fibre | 1.1 g | |
| **Fat** | 0.2 g | |
| **Protein** | 0.72 g | |
| **Vitamins** | | |
| Vitamin A equiv. | 0 µg | (0%) |
| beta-carotene | 0 µg | (0%) |
| lutein zeaxanthin | 0 µg | |
| Thiamine ($B_1$) | 0.03 mg | (3%) |
| Riboflavin ($B_2$) | 0.057 mg | (5%) |
| Niacin ($B_3$) | 0.08 mg | (1%) |
| Pantothenic acid ($B_5$) | 0.043 mg | (1%) |
| Vitamin $B_6$ | 0.032 mg | (2%) |
| Folate ($B_9$) | 3 µg | (1%) |
| Vitamin C | 2.4 mg | (3%) |
| Vitamin E | 0 mg | (0%) |
| Vitamin K | 0 µg | (0%) |
| **Trace metals** | | |
| Calcium | 24 mg | (2%) |
| Iron | 0.29 mg | (2%) |
| Magnesium | 25 mg | (7%) |
| Phosphorus | 20 mg | (3%) |
| Potassium | 250 mg | (5%) |
| Zinc | 0.1 mg | (1%) |
| **Other constituents** | | |
| Water | 94.99 g | |

Units

µg = micrograms • mg = milligrams

IU = International units

Percentages are roughly approximated using US recommendations for adults.

Source: USDA Nutrient Database

**Figure 2:** Composition of green coconut water (USDA nutreint database)

## DISCUSSION

C. albicans in the oral cavity has been reported at the rate of 45% in neonates, 45-65% in healthy children, 30-45% in healthy adults, 50-65% in people wearing removable dentures, 65-88% in people living in acute and long‑term care facilities, 90% in patients with acute leukemia who are undergoing chemotherapy, and 95% in patients with HIV infection.[9] Given the incidence of oral diseases, adverse drug reactions of some antifungal agents currently used in dentistry and financial concerns in developing countries, there is a need to develop natural, safe and effective alternative treatment options. Consequently, our aim was to find an herbal anti‑fungal agent, which would effectively replace the commercially available antifungal agent and so, *Cocos nucifera* L. was chosen because of its bounteous availability and well‑known antioxidant, anti-ageing, anti-allergic, antimicrobial, anti- fungal and anti-carcinogenic potential.

**Table 1:**

| S.No | Microorganism | 1000µg | 500µg | 250µg | 100µg | 50µg | DMSO | Ampothericin B |
|---|---|---|---|---|---|---|---|---|
| 1. | *Candida albicans* | 14±0.3 | 9±0.2 | 7±0.3 | - | - | Not used | 15±0.3 |

T test t value =3.784525
P value =0.05(significant)

The edible part of the coconut fruit (coconut meat and coconut water) is an endosperm tissue. Endosperm tissues undergoes one of the three key modes of development, which includes the nuclear, cellular and helobial modes [10] out of which, the coconut endosperm belongs to the nuclear mode. Unlike the endosperms of other plants (e.g., wheat and corn), the cellularization process in a coconut fruit does not fill up the entire embryo sac cavity, but instead leaves the cavity filled with a solution identified as coconut water that is of a cytoplasmic origin [11]. Nutrients from GCW are obtained from the seed apoplasm (surrounding cell wall) and are transported symplasmically into the endosperm [12].

The composition of the aqueous extract of GCW is enlisted in a number of data bases primarily the USDA nutrient database [refer figure 2][8], highlighting a wide array of imperative health promoting constituents, including vitamin B, nicotinic acid (B3, 0.64 mg/mL), pantothenic acid (B5, 0.52 mg/mL), biotin (0.02 mg/mL), riboflavin (B2, o0.01 ng/mL), folic acid (0.003 mg/mL), with trace quantities of vitamins B1, B6, and C, pyridoxine, thiamine, folic acid, amino acids, L-arginine, plant hormones (auxin, 1,3-diphenylurea, cytokinin), enzymes (acid phosphatase, catalase, dehydrogenase, diastase, peroxidase, RNA polymerases), and growth-promoting factors [13,14].
In studies with crude extract and five TLC fractions (I-V) of fiber mesocarp of C. nucifera fruit, in vitro antimicrobial activity was seen in all trial strains of S. aureus tested with fractions II-V [15]. Antifungal activity was revealed as growth inhibition of Candida albicans, Cryptococcus neoformans or Fonsecaea pedrosoi. The antifungal, antimicrobial, and antiviral effects were attributed to the condensed tannins and catechins present in the crude extract and fractions II-V, especially fraction II, which had a higher concentration of these compounds.
Coconut water has prophylactic action against nephrolithiasis in an experimental Wistar rat model (16). Myocardial infarction induced by isoproterenol in rats (17), were protected by the intake of this water. GCW could also reduce total cholesterol, very-low density lipoprotein, low density lipoprotein, and triglyceride levels in serum (18). Administering coconut water (4 mL/100 g body weight) in male rats counteracted the increases in these substances promoted by cholesterol feeding. The hepatoprotective effect of coconut water was investigated in carbon tetrachloride (CCl4)-intoxicated female rats. GCW also increased insulin levels and liver glycogen concentrations and reduced glycated hemoglobin levels in diabetic rats. In addition, elevated levels of enzymes markers like alkaline phosphatase, serum glutamate oxaloacetate transaminase, and serum glutamate pyruvate transaminase in diabetic rats were significantly reduced upon treatment with mature coconut water [19].
With the animal trials proving beyond doubt the health benefits of Cocos nucifera , the results of our study indicate that GCW extract shows anti-fungal effects on C. albicans and the inhibitory effect varies with its concentration. GCW extract at 1000 µg/ml concentration maximally inhibited the growth of C. albicans (14 ± 0.3) compared with the positive control- amphotericin B (15 ± 0.3) [Figure 1], followed by 500 µg/ml (9 ± 0.2), 250 µg/ml (7 ± 0.3), and no effect at 100 µg/ml and 50 µg/ml respectively, thus suggesting a dose-dependent reaction. Therefore, oral therapeutic anti-fungal preparations can be formulated from higher concentrations of GCW extracts to treat oral candidiasis. Reports also show extensive consumption of the fruit with no toxic effects in several parts of the world for over a number of generations.[20] Hence additional studies are required to test higher concentrations of GCW against C.albicans for enhanced benefits.
Of most opportunistic infections, C.ablicans seems to be a potential threat in terms of treatment, due to its frequent recurrence. Hence in order to contain the problem of reoccurrence herbal medication and natural products are frequently preferred. Though, antifungal activity of GCW (Cocos nucifera) was comparatively higher in the present study when compared with other medicinal herbal agents like Noni (*Morinda citrifolia*) extracts [21] and aloe vera (*Aloe indica* Royle) [22], the argument about the usage of GCW as an anti fungal medication in the field of dentistry needs to be further warranted. As Cocos nucifera is well grown in various parts of India, it would be a viable and cost effective option that could possibly replace the currently available drug options.
As with other studies, this study also has its circumspections as it is an in-vitro study. Further in vivo studies have to be conducted to check the safety, tolerance, and cost-effectiveness of the extract. Also, geographical and seasonal variations among countries and regions can influence the chemical composition of the studied material. Therefore, standardized procedures for collecting samples and quantifying compounds should be used to assure the reproducibility of results. Other important factor that needs to be analyzed is that, GCW is a natural beverage and the active ingredients might not always be constant and the exact mechanism of action against Candida is also not explained. Thus, further bioassay-guided fractionation and isolation of specific molecules are highly supported so that the chemical moiety responsible for the activity can be identified and its method of action established alongside the dosages and formulations as an oral anti-fungal agent.

## CONCLUSION

Cocos nucifera is a widely dispersed plant that has important pharmacological effects with low toxicity. Furthermore, medicinal use of C. nucifera has an environmental appeal, since this plant is widely used in the food industry and use of discarded plant parts will reduce waste and pollution. The pharmacological effects of the plant differ according to the part of the plant or fruit used. Antioxidant activity predominated in the constituents of the endocarp and coconut water. Within the limitations of the study, green coconut water proved to be a valuable antifungal agent against Candida albicans

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