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GRADUATE RESEARCH ARTICLE

# Effectiveness of Coconut (*Cocos nucifera*) Extracts as an Inhibitor Against Aspergillosis

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## ABSTRACT

The study of the Effectiveness of Coconut (*Cocos nucifera*) Extract Against Aspergilosis revealed that, the testing of some brown coconut extracts, coconut water, positive control and negative control on *Aspergillus* sp fungi showed clear zone formed around the agar well, ethanolic extract showed 26.00 mm, 25.33 mm and 20.00 mm, for *Aspergillus fumigatus, Aspergillus flavus* and *Aspergillus brasiliensis* respectively, donystatin antifungal drug was 22.67 mm, 17.67 mm and 20.83 mm for *Aspergillus brasiliensis, Aspergillus fumigatus* and *Aspergillus flavus* respectively, methanolic, aqueous, coconut water and distilled water showed no zone of inhibition. The ethanolic extract of brown coconut inhibited the growth/expansion of *Aspergillus* sp. The best inhibitor was acquired from the administration of ethanolic extract with an average diameter of 26.00 mm diameter of the inhibition zone. The nutritional and health implication of coconut fruit consumption should be encouraged because of its' potency which can form the basis to exploit *Cocos nucifera* for therapeutic benefits.

**Keywords:** Donystatin; Aspergillosis, Brown coconut

## 1 Introduction

Aspergillosis is an infection caused by a type of mold (fungus). The mold *Aspergillus* that causes these illnesses, is present everywhere, both indoors and outdoors. Although not every strain of this mold is harmful. *Aspergillos*is occurs in chronic or acute forms which are clinically very distinct. Most cases of acute Aspesrgillosis occur in people with severely compromised immune systems. Chronic colonization or infection can cause complications in people with underlying respiratory illnesses, like asthma [1], cystic fibrosis, [2], sarcoidosis, [3] , tuberculosis, or chronic obstructive pulmonary disease [4]. Most commonly, aspergillosis occurs in the form of Chronic Pulmonary Aspergillosis (CPA), aspergilloma, or Allergic

Broncho-Pulmonary Aspergillosis (ABPA) [5]. Some forms are intertwined; for instance, ABPA and aspergilloma can progress to CPA. Other, non-invasive manifestations include fungal sinusitis (both allergic in nature and with established fungal balls), otomycosis (ear infection), keratitis (eye infection), and onychomycosis (nail infection). In most instances, these are less severe, and curable with effective antifungal treatment.

The occasionally identified pathogens are *Aspergillus fumigatus* and *Aspergillus flavus*, are widespread organisms capable of living under ample environmental stress. Most people are thought to inhale thousands of *Aspergillus* spores daily but without effect due to an efficient immune response. Taken together, the major chronic, invasive, and allergic forms of aspergillosis account for around 600,000 deaths annually worldwide. [6], [3], [7], [8]. Aspergillosis is thought to affect more than 14 million people worldwide, [9] with Allergic Broncho-Pulmonary Aspergillosis (ABPA, >4 million), severe asthma with fungal sensitization (>6.5 million), and Chronic Pulmonary Aspergillosis (CPA, ~3 million) being considerably more prevalent than Invasive Aspergillosis (IA, >300,000). Other common conditions include *Aspergillus bronchitis, Aspergillus rhinosinusitis* (many millions), *Otitis externa*, and *Aspergillus onychomycosis* (10 million) [10]. Alterations in the composition and function of the lung microbiome and microbiome have been associated with an increasing number of chronic pulmonary diseases such as COPD, cystic fibrosis, chronic rhinosinusitis and asthma, [11]but a few can cause serious illnesses when people with weakened immune systems, underlying lung disease or asthma inhale their fungal spores. In some people, the spores trigger an allergic reaction. Other people develop mild to serious lung infections. The most serious form of aspergillosis; invasive aspergillosis occurs when the infection spreads to blood vessels and beyond. Depending on the type of aspergillosis, treatment may involve observation, antifungal medications or, in rare cases, surgery. Coconut (*Cocos nucifera*) belongs to the family *Arecaceae* and the subfamily *Cocoideae* is an important monocotyledon plant widely grown in the tropic and sub-tropics. The nut is the most marketable part, the inner part of the nut (endosperm) has two edible parts: a white kernel and a clear liquid: coconut water [12] . The cavity within the kernel contains coconut water, this part begins to form as a gel when the coconut is 5 to 6 months old, becomes harder and whiter as coconut matures, and the inside is filled with coconut water. At full maturity (12 months) coconut water represents between 15% and 30% of the weight of the nut. The amount of coconut water that can be harvested from each nut is about 300ml but depends to a great extent on the stage of maturity and on the variety of coconut. The coconut palm is sometimes referred to as 'great nut of India' owing to the use of all of its parts in some way or the other in coconut growing areas. Apart from the use of coconut water, the natural sterile liquid from the young immature coconuts as a beverage; the kernel is used as the source of food and oil. Of the more than 93 countries growing coconut Worldwide, India ranks 3rd by producing about 14.19 million metric tons of coconut annually, which contributes to about 19.3% of total coconut production of the World. The fact that plant secondary metabolites including polyphenolic compounds being diverse, different classes of compounds are soluble only in specific solvent system, [13], and the extraction yields depends on methods adopted, nature of plant materials and presence of various compounds [14]. Therefore, in the present study, secondary metabolites from Coconut Water (CW) and of pulverized endosperm were extracted using organic solvents (methanolic, ethanolic acid) and purified water, these extracts will be used to investigate the antimicrobial activity of *Cocos nucifera.*

## 2 Research Methodology

### 2.1 Collection of Isolates

The fungal isolates (*Aspergillus flavus, Aspergillus brasiliensis* and *Aspergillus fumigatus*) were collected from Biotechnology Extension Services Department (B.E.S.D) Laboratory, National Biotechnology Development Agency (NABDA), Abuja. They were cultured on PDA and incubated at 37$^{o}$c for 3 hours prior to usage.

## 2.2 Preparation of Plant Material

Some nuts of coconut were obtained from General market, Zuba. The coconut fruits were first washed with distilled water, then surface sterilized using 70% ethanol and opened at one of the eyes on the apical region using a sterile knife. The coconut water (liquid endosperm) was aseptically collected into a sterile glass bijou bottle, the nuts were further broken to release the solid endosperm (kernel). The endosperm was washed, grated and dried (oven drying at 50$^{o}$C). The dried samples were pulverized using a 500W blender.

## 2.3 Extraction of Plant Material

Powdered coconut (50g) was soaked in 150ml of each solvent (methanolic, ethanolic and aqueous). The colloids were concentrated by heating in the incubator shaker at 45$^{o}$C and 200rpm for 24hrs and filtered.

## 2.4 Antimicrobial Assay

The fungal test pathogens such as *Aspergillus brasiliensis, Aspergilllus fumigatus* and *Aspergillus flavus* were obtained from Biotechnology Extension Services Department (B.E.S.D) Laboratory, National Biotechnology Development Agency (NABDA). Antimicrobial assay was fulfilled by agar well dilution method in Potato Dextrose Agar [PDA] plates for fungi. The overnight grown fungal cultures were diluted with sterile saline [0.85% NaCl]. About 100 µl of these microbial suspensions were spread on to the entire surface of respective plates. Then, a well [6 - 8 mm] was made with a sterile tip and 100µl, 200µl and 300µl (T1, T2 and T3 respectively) concentration of different solvent extracts and standard antifungal drug such as Donystatin were introduced into these wells at different concentrations. All these activities were carried out in aseptic condition in a laminar air hood. Following this, the plates were incubated at 28˚C for 48 hours and the zone of inhibition was measured in mm.

## 3 Results

The screening results for antimicrobial activity of different solvent extracts against three fungal strains; *Aspergillus brasiliensis, Aspergillus fumigatus* and *Aspergillus flavus* presented in Table 1. The results indicated that ethanolic fractions of coconut endosperm and donystatin had antifungal activity against all tested pathogens with a maximum zone of inhibition obtained for the ethanolic fraction. Ethanolic extract showed antifungal activity against *Aspergillus brasiliensis, Aspergillus fumigatus* and *Aspergillus flavus* with a maximum zone of inhibition [30.00 mm] which is highest compared to all other solvent extracts, methanolic and aqueous fractions did not exhibit antimicrobial activity against the test pathogens at any concentration. The results of the average diameter in inhibition zone of each treatment were as follows for *Aspergillus brasiliensis*; ethanolic at 20.00±5.00 mm, methanolic at 0 mm, and aqueous at 0 mm. The average diameter of inhibition zone for positive control in *Aspergillus brasiliensis* (donystatin antifungal drug) was 22.67±2.52 mm, negative control (distilled water) was 0 mm and coconut water was 0 mm (Table 1). The results of the average diameter in inhibition zone of each treatment were as follows for *Aspergillus fumigatus*; ethanolic at 26.00±3.61 mm, methanolic at 0 mm, and aqueous at 0 mm. The average diameter of inhibition zone for positive control in *Aspergillus fumigatus* (donystatin antifungal drug) was 17.67±2.52 mm, negative control (distilled water) was 0 mm and coconut water was 20.0 mm (Table 2). The results of the average diameter in inhibition zone of each treatment were as follows for *Aspergillus flavus*; ethanolic at 25.33±5.03 mm, methanolic at 0 mm, and aqueous at 0 mm. The average diameter of inhibition zone for positive control in *Aspergillus flavus* (donystatin antifungal drug) was 20.83±3.82 mm (Table 3). The results indicated that the treatment of methanolic, aqueous, negative, and coconut water presented no difference in affecting/inhibiting the growth of *Aspergillus* sp. The treatment of ethanolic extract illustrated significant difference and the diameter of the inhibition zone was wider than that of antifungal drug (donystatin). It could be seen from the extent of the inhibition zone created.

**Table 1.** *The result of inhibiting zone diameter in ethanolic, methanolic, and aqueous extracts, coconut water, donystatin antifungal drug and distilled water toward* Aspergillus brasiliensis

| Intervention | Inhibiting zone diameter of *Aspergillus brasiliensis*. (mm) | | | Average diameter of inhibiting zone (mm) |
|---|---|---|---|---|
| | T1 (100 µg/ml) | T2 (200 µg/ml) | T3 (300 µg/ml) | |
| Ethanolic | 15.0 | 20.0 | 25.0 | 20.00±5.00[a] |
| Methanolic | 0.0 | 0.0 | 0.0 | 0.0[x] |
| Aqueous | 0.0 | 0.0 | 0.0 | 0.0[x] |
| Coconut water | 0.0 | 0.0 | 0.0 | 0.0[x] |
| Donystatin antifungal drug | 25.0 | 23.0 | 20.0 | 22.67±2.52[a] |
| Distilled Water | 0.0 | 0.0 | 0.0 | 0.0[x] |

T= test, a = strong inhibiting, b = moderate inhibiting, c = weak inhibiting, x = no inhibition

**Table 2.** *The result of inhibiting zone diameter in ethanolic, methanolic, and aqueous extracts, coconut water, donystatin antifungal drug and distilled water toward* Aspergillus fumigatus

| Intervention | Inhibiting zone diameter of *Aspergillus fumigatus*. (mm) | | | Average diameter of inhibiting zone (mm) |
|---|---|---|---|---|
| | T1 (100 µg/ml) | T2 (200 µg/ml) | T3 (300 µg/ml) | |
| Ethanolic | 30.0 | 23.0 | 25.0 | 26.00±3.61[a] |
| Methanolic | 0.0 | 0.0 | 0.0 | 0.0[x] |
| Aqueous | 0.0 | 0.0 | 0.0 | 0.0[x] |
| Coconut water | 0.0 | 0.0 | 0.0 | 0.0[x] |
| Donystatin antifungal drug | 15.0 | 18.0 | 20.0 | 17.67±2.52[b] |
| Distilled Water | 0.0 | 0.0 | 0.0 | 0.0[x] |

T= test, a = strong inhibiting, b = moderate inhibiting, c = weak inhibiting, x = no inhibition

**Table 3.** *The result of inhibiting zone diameter in ethanolic, methanolic, and aqueous extracts, coconut water, donystatin antifungal drug and distilled water toward* Aspergillus flavus

| Intervention | Inhibiting zone diameter of *Aspergillus flavus*. (mm) | | | Average diameter of inhibiting zone (mm) |
|---|---|---|---|---|
| | T1 (100 µg/ml) | T2 (200 µg/ml) | T3 (300 µg/ml) | |
| Ethanolic | 30.0 | 26.0 | 20.0 | 25.33±5.03[a] |
| Methanolic | 0.0 | 0.0 | 0.0 | 0.0[x] |
| Aqueous | 0.0 | 0.0 | 0.0 | 0.0[x] |
| Coconut water | 0.0 | 0.0 | 0.0 | 0.0[x] |
| Donystatin antifungal drug | 17.5 | 20.0 | 25.0 | 20.83±3.82[b] |
| Distilled Water | 0.0 | 0.0 | 0.0 | 0.0[x] |

T= test, a = strong inhibiting, b = moderate inhibiting, c = weak inhibiting, x = no inhibition

## 4 Discussion

*Aspergillus* is the genus name for a group (over 185 species) of filamentous fungi or common molds, most of which occur in an asexual state, and reproduce by producing conidia (asexual spores or conidiophores) that can spread into many different environments, germinate, and then grow. *Aspergillus fumigatus* is the most common of the group, followed by *Aspergillus flavus* and *Aspergillus brasiliensis* (formerly termed niger) [15]. Given the risk of oral diseases and adverse drug reactions of some antifungal agents such as voriconazole currently used in dentistry, limited availability and financial concerns in developing countries, there arise the need to develop an effective alternative treatment option that tends to be more natural and safer. The aim of our research was to find an herbal anti-fungal agent, which would effectively replace the commercially available antifungal agents currently used in the treatment of Aspergillosis, *Cocos nucifera.* was chosen because of its bounteous availability and well-known antioxidant, antiageing, anti-allergic, antimicrobial, anti- fungal and anticarcinogenic potential. 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 [16] 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 [17]. Nutrients from coconut water are obtained from the seed apoplasm (surrounding cell wall) and are transported symplasmically into the endosperm [18]. The composition of the aqueous extract of *Cocos nucifera* is enlisted in a number of databases primarily the USDA nutrient database [19] the antimicrobial activity of various solvent fractions of coconut endosperm was assessed using three fungal pathogens (*Aspergillus brasiliensis, Aspergillus fumigatus* and *Aspergillus flavus*).

The results indicate that *C. nucifera* extract shows antifungal effects on *A. brasiliensis, A. fumigatus* and *A. flavus* and the inhibitory effect varies with its concentration. *Cocos nucifera* ethanolic extract at 100 µg/ml concentration maximally inhibited the growth in *A. fumigatus* (30.0mm) compared with the positive control- donystatin (15.0mm), followed by 200 µg/ml (23.0mm) compared with the positive control- donystatin (18.0mm), and 300 µg/ml (25.0mm) compared with the positive control- donystatin (20.0mm) [Table 2], also at 100 µg/ml concentration *Cocos nucifera* ethanolic extract maximally inhibited the growth in *A. flavus* (30.0mm) compared with the positive control- donystatin (17.5mm), followed by 200 µg/ml (26.0mm) compared with the positive control- donystatin (20.0mm), and 300 µg/ml (20.0mm) compared with the positive control- donystatin (25.0mm) [Table 3], and at 100 µg/ml concentration *Cocos nucifera* ethanolic extract maximally inhibited the growth in *A. brasiliensis* (15.0mm) compared with the positive control- donystatin (25.0mm), followed by 200 µg/ml (20.0mm) compared with the positive control- donystatin (23.0mm), and 300 µg/ml (25.0mm) compared with the positive control- donystatin (20.0mm) [Table 1] thus suggesting a dose-dependent reaction. Therefore, therapeutic antifungal preparations can be formulated based on corresponding concentrations of the extract to treat Aspergillosis. Previous studies have also shown that the extensive consumption of the fruit has no toxic effects in several parts of the world for over a number of generations [20]. Hence further studies are required to test higher concentrations of *Cocos nucifera* ethanolic extract against *Aspergillus* sp for enhanced benefits. 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 treatment. Standardized procedures for collecting samples and quantifying compounds should be used to assure the reproducibility of results. *Cocos nucifera* is a natural beverage and the active ingredients might not always be constant and the exact mechanism of action against *Aspergillus* sp is also not explained. Thus, further bioassay-guided fractionation and isolation of specific molecules are highly supported so that the chemical segment responsible for the activity can be identified and its method of action established alongside the dosages and formulations as an antifungal agent.

## 5 Conclusion

*Cocos nucifera* is a widely distributed plant that has important pharmacological effects with zero to no toxicity. Furthermore, the medicinal use of *Cocos. nucifera* has an environmental appeal, since this plant is widely used in the food industry. The pharmacological effects of the plant differ according to the part of the plant or fruit used. Within the limitations of this study, the ethanolic extract of *Cocos nucifera* proved to be a valuable antifungal agent against Aspergillosis, hence showing the potency which can form the basis to exploit *Cocos nucifera* for therapeutic benefits.

## 6 Declarations

### 6.1 Study Limitations

The number of active components and their chemical and physical nature in each extract are undetermined due to lacking equipment.

### 6.2 Competing Interests

There are no conflicts of interest that are relevant to this study for the authors of this manuscript.

### 6.3 Publisher's Note

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