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Research Article

Phytochemical, Antifungal and Antioxidant Potential of Cashew (*Anacardium Occidental* L.) Leaf Methanolic Extract Grown Under Controlled Field for Medicinal Uses

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Abstract

The Cashew nut (*Anacardium occidental* L.) tree is recognized for its medicinal importance and is widely used in many products for its therapeutic potential. However, the plant's distribution is limited to tropical climates due to its sensitivity to frost. The aim of this study was to grow cashew nuts under a controlled environment and evaluate their potential for medicinal applications. Cashew nut seeds were germinated at 35°C and 55% humidity in a glasshouse. Fresh leaf of four-month-old cashew seedlings were collected, air-dried, and reduced to fine powder. The extraction was carried out by five-day maceration process using the powdered leaf into methanol. After five days, the solution was filtrated, and filtrate was stored at 4°C until utilization. Phytochemical screening was performed to characterize the extract. The antifungal potential was assayed against *Aspergillus flavus* and *Candida albicans* using the agar well diffusion method, while the antioxidant activity was evaluated through a 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. It was found that the extract contained Alkaloids, Flavonoids, Phenols, Steroids, and Terpenoids. The cashew nut leaf extract demonstrated antifungal activity against *A. flavus* and *C. albicans*, with mean ± standard deviation values of 1.106 ± 0.03 cm and 1.17 ± 0.15 cm respectively. The extract exhibited concentration-dependent inhibition of DPPH radicals with high antioxidant activity (93.28%) at 100 µg/ml while 82.88% at 20 µg/ml. The finding suggests that cashew nuts cultivated in a controlled environment (glasshouse) can be used for medicinal purposes and further pharmaceutical applications.

Keywords

Anacardium occidental L., Phytochemical, Antifungal Activity, Antioxidant Activity, Cashew Seedling, Medicinal Plant

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1. Introduction

Cashew nut (*Anacardium occidental* L.) from the *Anacardiaceae* family comprises 60 to 74 genera and 400 to 600 species [1]. This nut having tremendous commercial and therapeutic significance, is native to Brazil. European explorers brought it to Africa and Asia in the sixteenth century [2]. Initially cultivated for afforestation and soil erosion control, the cashew nut has become a significant source of foreign exchange earnings due to its extensive economical, biological, medicinal, and bioenergy usage (Cashew Nutshell Liquid) has made it the top crop of all nuts [3-5]. In the modern day, it is one of the most widely grown nuts, especially in Benin, Indonesia, Nigeria, Brazil, Tanzania, Vietnam, India, Philippines, Sri Lanka, Guinea-Bissau, and Ivory Coast [6]. Its leaf, stems, bark, buds, and roots are used traditionally to treat many diseases, including asthma, diarrhea, fever, skin rashes, teeth caries, worms, cough, stomach pain, tooth decay, hypertension, and malaria [7-9]. In Benin, cashew nut bark is also used for allergies, yellow fever, eye pains, external and internal wounds, teeth bleeding, hypertension, diabetes, hemorrhoids and sexual weakness [9]. Studies on *Anacardium occidentale* L. have reported variety biological properties namely antioxidant, anti-inflammatory, antimicrobial, antibiofilm, antidepressant, anticonvulsant, antifungal, and analgesic [10-12]. The abundance of secondary metabolites found in *A. occidentale* is thought to contribute to its biological characteristics. For example, anacardic acid, sitosterol, and stigmasterol from cashew nuts have been shown to have antioxidant activity [13, 14], while gallic acid is an antifungal molecule [14]. The ethanolic bark extract of cashew trees has been proven to be an excellent option for use in cancer and fungal infection drug development [15]. Consequently, *A. occidental* is thought to be a possible source of naturally occurring bioactive chemicals for the creation of new medications.

Extraction and separation are crucial processes in studying plant materials that lead to the isolation of bioactive compounds. Several techniques, including maceration, Soxhlet, and supercritical CO_2, have produced extracts from various *A. occidentale* parts [16, 17].

Cashew nut distribution is limited to ecological conditions due to its sensitivity to frost [18]. These conditions prevent countries with unsuitable climates from benefiting from the advantages of this plant. Therefore, considering all these facts, this study is designed to grow cashew nuts under a controlled environment and test their potential for medicinal usages through phytochemical screening, antioxidant and antifungal tests.

2. Materials and Methods

2.1. Cashew Nut Seeds Collection

The cashew nut (*Anacardium occidentale L.*) seeds used in this study were obtained from Sèmèrè, the northwest region of the Benin Republic in West Africa. These seeds were carefully selected from healthy and high-yielding cashew nut trees during the harvesting season in February 2022. They were then sun-dried for seven (7) days before they were finally brought to and identified by the Medicinal Plants Research Laboratory at Atta Ur-Rahman School of Applied Biosciences (ASAB), National University of Science and Technology (NUST) Islamabad, Pakistan.

2.2. Seeds Germination

Thirty (30) cashew nut (*Anacardium occidentale L.*) seeds were germinated in the glass house at 35 ℃ temperature and 55% humidity. After germination, seedlings (Figure 1) were handled for up to four months and then used for the experiment.

Figure 1. *Cashew nut Seedling obtained from germination under glasshouse conditions.*

2.3. Leaf Collection, Drying and Grinding

The fresh leaf of cashew nut (*Anacardium occidentale L.*) were collected from four-month-old seedlings obtained by germination in the glass house. After drying at room temperature (20 ℃ to 25 ℃) for five days, leaf were reduced into powder using porcelain mortar.

2.4. Plant Extract Preparation

Cashew nut dried leaf powder was dissolved into methanol in a 1: 10 ratio, and the mixture was kept in the dark for five days of maceration. After that, the solution was filtered using Whitman N°1 filter paper. The filtrate was stored at 4 ℃ until use.

2.5. Phytochemical Screening

The phytochemical screenings were performed based on the standard procedure described by Jones & Kinghorn [19].

2.6. Antifungal Activity Assay

The antimicrobial activity of cashew nut methanolic leaf extract was assayed against *Aspergillus flavus* and *Candida albicans* using the well diffusion method described by Michael & John [20] with slight modification. Wells of 8 mm in diameter were bored on inoculated Potato Dextrose Agar (PDA) plates with sterile borer. Amphotericin B [21] and methanol [22] were used as positive and negative control respectively. All experiments were done in triplicate and 100 µl of extract and controls were introduced into the wells. Plates were then incubated at 27 ℃ for *Aspergillus flavus* and 31 ℃ for *Candida albicans*.

2.7. Antioxidant Activity Assay

The antioxidant activity of cashew nut methanolic leaf extract was assayed based on modified method [23] using 2, 2-di-phenyl-1-picrylhydrazyl (DPPH) radical scavenging assay with Ascorbic acid as standard. Different concentrations adopted for extract and standard were 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and, 100 µg/ml, where DPPH was at 4 mg/ml. Each dilution was mixed with 3 ml of DPPH solution. Then mixtures were incubated in the dark for 30 minutes. After that, UV/VIS spectrophotometer T80+ (PG Instruments, UK) measured the absorbance at 517nm. All solutes were dissolved into methanol which was also used as blank.

2.8. Statistical Analysis

Data from antifungal activities were statistically analyzed for significance using the Microsoft Excel one-way test at a 5% significance level.

3. Results

3.1. Preliminary Phytochemical Tests

The phytochemical screening revealed the presence of Alkaloids, Flavonoids, Phenols, Steroids and Terpenoids in cashew nut methanolic leaf extract as shown in Table 1.

Table 1. *Phytochemicals screening of cashew nut methanolic leaf extract.*

Sr. Nº	Family of compounds	Type of test	Results
1	Alkaloids	Wagner's	+
2	Flavonoids	Lead Acetate	+
3	Phenols	$FeCl_3$	+
4	Steroids	Salkowski's	+
5	Terpenoids	cH_2SO_4	+

+= Presence of phytochemical in the extract

3.2. Antifungal Activity of Tested Extract

After 24h of incubation, the mean zone of inhibition for cashew nut methanolic leaf extract as presented in Figure 2, was 1.11 ±0.03 cm and 1.17 ±0.15 cm for *Aspergillus flavus* and *Candida albicans* plates, respectively. Whereas Amphotericin B mean zone for *Aspergillus flavus* and *Candida albicans* were 1.4 ± 0.1 cm and 2.3 ± 0.3 cm, respectively. The representative plates for this activity are depicted in Figure 3 and Figure 4. No zone of inhibition was observed from negative control. Results were statistically significant with p-value of 0.008 and 0.004 respectively for *A. flavus* and *C. albicans* activity.

3.3. Antioxidant Activity of Tested Extract

The cashew nut methanolic leaf extract showed antioxidant activity with the percentage of DPPH radical inhibition proportional to the concentration (Figure 4 and Table 2). It shows that the extract's percentage of DPPH radical inhibition at the lowest concentration (20 µg/ml) was 82.88% while at the highest concentration (100 µg/ml), it was 93.28%. However, the antioxidant activity of plant extract was relatively low (82.88%) and even constant at low concentrations (20 to 40 µg/ml) when compared to the standard (Figure 5).

Table 2. *Percentage inhibition of DPPH radicals.*

Sr. Nº	Concentration, µg/ml	Inhibition rate,%	
		Ascorbic acid	Cashew extract
1	20	90	82.88
2	40	95	82.88
3	60	96.07	85.36

Sr. Nº	Concentration, µg/ml	Inhibition rate,%	
		Ascorbic acid	Cashew extract
4	80	96.57	86.36
5	100	96.35	93.28

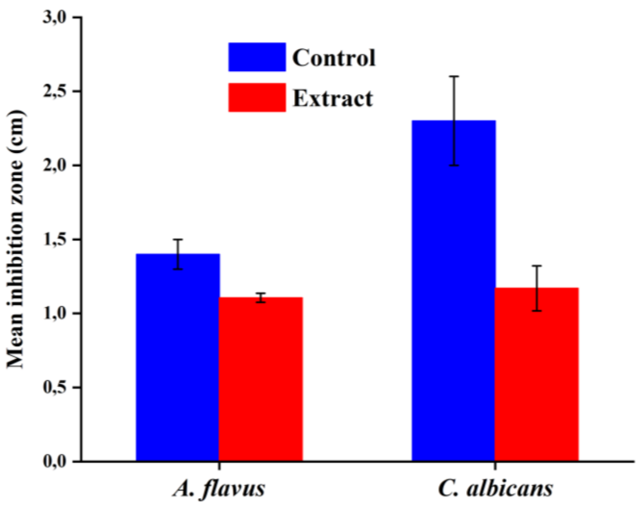

Figure 2. *Mean inhibition zone of the cashew nut methanolic leaf extract against A. flavus and C. albicans. The means are from triplicates (n = 3) and vertical bars show standard deviation.*

Figure 3. *Antifungal activity of cashew nut methanolic leaf extract against Aspergellus flavus.*

Figure 4. *Antifungal activity of cashew nut methanolic leaf extract against Candida albicans.*

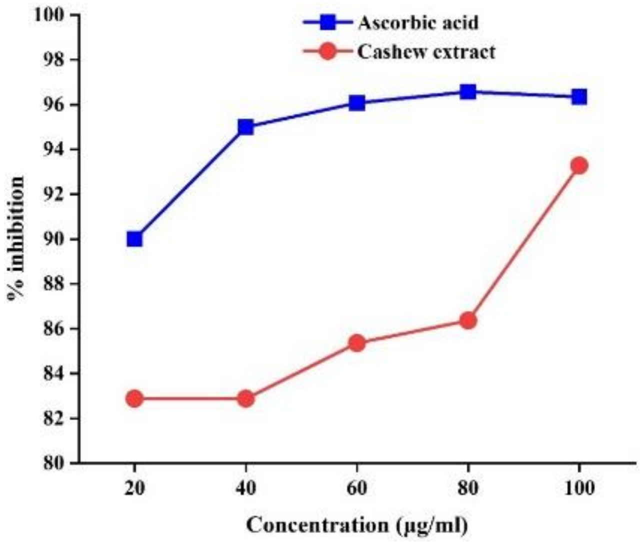

Figure 5. *Antioxidant activity of cashew nut methanolic leaf extract.*

4. Discussion

In this work, we have studied glasshouse-grown cashew leaf for their potential to produce high-quality secondary metabolites with biological activity, offering alternative medicinal applications compared to naturally grown plants. Glasshouse cultivation enhances consistency, quality control, and sustainability while reducing the need for chemical pesticides and conserving natural populations. This method also supports scalability, economic viability, and climate resilience, ensuring a stable and continuous supply for pharmaceutical industries. Overall, this research contributes to advancements in plant-based medicine, sustainability, and economic development.

The ability of plants to produce secondary metabolites is related to their medicinal and biological properties. Terpenoids, alkaloids, and phenolics are the three main categories into which the secondary metabolites derived from plants are categorized. They contain numerous organic compounds with intriguing pharmacological properties [24, 25].

The phytochemicals found on cashew nut leaf in this study are similar by the findings of Philip et al. [26] who reported that the aqueous extracts of *Anacardium occidentale* leaf and nuts contained Resin, Flavonoids, Phenols, Carbohydrates, Alkaloids, and Terpenoids. In addition, the plant's aqueous leaf extract contained Steroids, Tannins, Saponins and Phlobatanins. Thus, the production of these significant classes of secondary metabolites by glasshouse-grown cashew nut seedlings may serve as a valuable indicator of their potential medicinal application.

The findings of this study regarding the antifungal properties of cashew nuts align with those of several other research studies. For example, Oladele and Ishola [27] investigated the effects of pawpaw (Carica papaya L.) and cashew nut leaf extract on the growth of Aspergillus species mycelia sourced from decomposed cashew fruit. Additionally, Da Silva et al. [12] explored the antimicrobial and antioxidant activities of Anacardium occidentale L. flowers in comparison to extracts from the bark and leaf. This antifungal potential could be linked to acid, alkaloid and phenolic compounds in the extract.

The results of this study indicate the antioxidant activity of cashew nut leaf. This outcome is consistent with Tan & Chan [28] who investigated the antibacterial, antityrosinase and antioxidant properties of fresh and processed *Anacardium occidentale* and *Piper betle* leaf. They observed that fresh cashew leaf had the highest antioxidant potential when compared to other herbs like marjoram, thyme, and rosemary.

5. Conclusion

This study revealed that cashew nuts (*Anacardium occidental* L.) grown in a glasshouse produce secondary metabolites and have antifungal and antioxidant potential. This implies that cashew nuts can be grown under controlled conditions for pharmaceutical and medical applications in the scenario where a suitable environment is not available for growth of this plant. It is also important to keep in mind that cashew nuts cultivated in these conditions or in their early stages may produce different phytocompounds from those found in trees in their native habitat. However, this study did not evaluate that matter. Therefore, further comprehensive research is warranted to substantiate these findings and assess the quality and quantity of produced compounds and the toxicity of cashew leaf grown under controlled conditions compared to those grown in nature. This additional research is crucial to ensure the safety and efficacy of using glasshouse-grown cashew leaf for medicinal purposes.

Abbreviations

DPPH	2, 2-diphenyl-1-picrylhydrazyl
PDA	Potato Dextrose Agar
UV/VIS	Ultraviolet/Visible

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Author Contributions

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Conflicts of Interest

The authors declare no conflict of interest.

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