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IN VITRO AND IN VIVO EVALUATION OF AQUEOUS AND ETHANOLIC EXTRACTS OF *KHAYA SENEGALENSIS* AGAINST *COLLETOTRICHUM FALCATUM* IN SUGARCANE (*SACCHARUM OFFICINARUM*)

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Abstract
This study investigated the efficacy of *Khaya senegalensis* (Mahogany) extracts (leaves, stem, roots, and oil) as bio-control agents against sugarcane stem red rot (*Colletotrichum falcatum*) in Mubi North, Adamawa State. Phytochemical analysis confirmed the presence of alkaloids, flavonoids, tannins, and saponins in the ethanolic and aqueous extracts and oil. *In vitro* and *in vivo* experiments, using concentrations of 20%, 60%, and 100%, revealed that while *Khaya senegalensis* oil achieved a 33.00 mm zone of inhibition at 100% concentration, the synthetic positive control, Cyperforce, significantly outperformed it with a 99.67 mm (or 98.69 mm in a later mention) zone of inhibition. The *Khaya senegalensis* extracts showed a maximum zone of inhibition of 27.75 mm at 100% concentration. However, *in vivo* trials indicated that the mahogany root extract, particularly the aqueous form at concentrations below 100%, exhibited a pronounced effect and higher inhibition compared to ethanolic extracts. This suggests that *Khaya senegalensis* extracts show promise in suppressing *Colletotrichum falcatum* in both laboratory and field conditions, with the root and oil extracts being particularly noteworthy. Despite the positive control's superior performance, the presence of bioactive compounds in mahogany warrants further research into optimizing extraction methods, concentrations, and potential synergistic combinations for developing effective and cost-efficient alternatives for farmers.

Keywords: Phytochemicals, Antifungal activity, aqueous extract, ethanolic extracts, Disease incidence.

Introduction

Sugarcane (*Saccharum officinarum*) is a vital global commodity, crucial for economies in tropical and subtropical regions. It's not just for sugar; it's also a key source for ethanol and various industrial products. Globally, it produces between 1.7 and 1.9 billion tonnes annually, with Brazil, India, China, and the United States being major producers (USDA, 2022; Mario et al., 2020). In 2024, over 1.9 billion metric tonnes were produced on about 26 million hectares, with Brazil and India alone contributing over half (FAOSTAT (2024),). Sugarcane plants are tall (2–6 m) with thick stalks (2.5–8.0 cm) and can accumulate 10–15% sucrose. In Nigeria, it's important for rural jobs and local agro-industries (Gana, 2010).

Sugarcane productivity is severely hampered by diseases, particularly red rot, caused by the fungus *Colletotrichum falcatum*. This disease infects the stalk internally, creating red lesions and leading

to significant losses: up to 29% in cane weight, 31% in sugar yield, and 75% in sucrose content (Misra et al., 2022). Severe red rot outbreaks can reduce cane yield by 30–70% and juice purity by over 40%, especially in warm and humid conditions (25°C–35°C) that favor the pathogen. In extreme cases, juice quality degradation can exceed 90%. The management of *C. falcatum* is further challenged by its high variability in virulence, with reported levels ranging from 21.3% to 97.9% depending on the sugarcane cultivar's susceptibility (Viswanathan & Rao, 2021).

Managing red rot (caused by *Colletotrichum falcatum*) is difficult due to the fungus's varied virulence, which can cause losses from 21.3% to 97.9% depending on the sugarcane's susceptibility (Viswanathan et al., 2011). Current control methods, like synthetic fungicides and resistant varieties, have drawbacks. Fungicides, though effective in the lab, pose risks due to environmental persistence, toxicity, and the development of fungal resistance. They are also expensive for small farmers. Similarly, genetically resistant varieties become outdated as new pathogen strains emerge, typically within 8–10 years (Hossain et al., 2021; Mahata and Sardar, 2022).

Due to the limitations of current methods, there is a need for sustainable, plant-based solutions (phytomedicines) to control plant diseases. Botanical extracts, rich in compounds like alkaloids, flavonoids, and terpenoids, are a promising alternative. These substances can harm fungi by damaging their cell structures and interfering with their metabolism (Egubagi et al., 2019). For example, flavonoids and alkaloids can disrupt fungal membranes, inhibit the production of essential compounds like ergosterol, and prevent spore germination (Bonifacio et al., 2019; Armengol et al., 2021). Unlike synthetic chemicals, these plant-derived compounds are biodegradable, low-toxicity, and affordable for rural farmers.

One such promising plant is *Khaya Senegalensis* (African mahogany). This tall tree (15–30 m) from tropical Africa has a history of traditional use for treating infections. Phytochemical analysis confirms the presence of bioactive alkaloids and terpenoids in this species, showing significant antifungal properties (Kutawa et al., 2022; Mukaila et al., 2021).

The purpose of this paper is to evaluate the phytochemicals, antifungal activities of aqueous and ethanolic extracts of *Khaya senegalensis in vitro* and *in vivo* against *Colletotrichum falcatum* responsible for red of sugarcane (*Saccharum officinarum*).

MATERIALS AND METHODS

Study Area

The study was conducted in Mubi North Local Government Area (10°16'–10°30'N; 13°15'–13°30'E), located within the Sudan savanna agro-ecological zone. The area is characterized by an annual rainfall of 900–1,100 mm, temperatures ranging from 25–35°C, and relative humidity that often exceeds 70% during the rainy season (Adebayo, et al., 2020)

Source of Plant Samples and Reagents

Sugarcane stems exhibiting red rot symptoms were collected from sugar cane farms in Muchalla, Mubi-by-pass, and Wuro Gude using a quadrant sampling method. Leaves, stem bark, roots, and seeds of *Khaya senegalensis* were collected from healthy trees in the Botanical garden of the Department of Botany, Adamawa State University, Mubi. The solvents and all other chemicals were of standard purity and purchased from an accredited dealer- The Northen Scientific Yola, Adamawa state.

Isolation, and Identification of Pathogen

Infected sugarcane tissues were aseptically excised into approximately 5 mm^2 sections as described by Smith and Onion (1983). The table in the inoculation chamber was sterilized using 95% ethanol and the UV light was switched on for 30 minutes, while inoculation loop, needles and scissors were sterilized by flaming over a Bunsen burner . The excised tissues were cultured on Potato Dextrose Agar (PDA) prepared at 39 g L^{-1}, autoclaved at 121°C for 15 minutes (10 lbs), and 0.1% chloramphenicol (6 ml L^{-1}) was added to inhibit bacterial growth. The inoculated plates were incubated at 25°C for 5 days

to obtain pure cultures of the pathogen, following established procedures (Channya and Asama, 2019; Umar et al., 2023). Pure cultures were subcultured repeatedly until uniform colony morphology was obtained. Identification of *C. falcatum* was based on colony characteristics as described by Viswanathan et al. (2018).

Preparation of Plant Extracts

Leaves, stem bark, roots, and seeds of *Khaya senegalensis* collected were washed, and shade-dried at 27 ± 2°C for 7–10 days to preserve bioactive compounds (Egubagi et al., 2019). Additional plant materials were also air-dried for up to two weeks before grinding into fine powder (Channya et al., 2018). Mahogany oil was extracted from the seeds through kneading and heating of the seed paste in approximately 1 L of water with the addition of rock salt. For solvent extraction, 100 g of powdered material was soaked in 1,000 mL of distilled water for aqueous extraction (24 hours) and in 1,000 mL of 95% ethanol for ethanolic extraction for 48 hours (Navoda and Anupama, 2022; Rizvi et al., 2022). The mixtures were agitated, filtered using muslin cloth followed by Whatman No. 1 filter paper, and concentrated at room temperature. The extracts were stored at 4°C until use. Working concentrations of 20%, 60%, and 100% (v/v) were prepared to evaluate dose-dependent antifungal activity, as concentration is known to influence efficacy in plant extracts (Rizvi et al., 2022).

Phytochemical Screening

Phytochemical screening of the plant extracts was conducted using standard qualitative procedures to identify major bioactive compounds, following Zakawa et al. (2020). Saponins were detected using the foam test, flavonoids using the sodium hydroxide (NaOH) test, alkaloids using hydrochloric acid (HCl) followed by Mayer's reagent, and steroids using the sulfuric acid (H_2SO_4) test. These phytochemicals are associated with antifungal activity and were assessed to establish the bioactive potential of the extracts.

Asssessment of disease incidence and severity

Disease incidence was determined as the percentage of infected plants, while disease severity was assessed using a 1–5 scale corresponding to infection levels of 1–10% to 31–40%, following the procedure of Zakari et al. (2024)

Experimental Design and Statistical Analysis

Completely Randomized Design (CRD) with three replicates was used. Treatments consisted of two extract types (aqueous and ethanolic), three concentration levels (20%, 60%, and 100% v/v), and a synthetic fungicide (Cyperforce) used as a positive control (Chanya et al., 2019). The data obtained were subjected to analysis of variance (ANOVA) at a 5% level of significance ($p \leq 0.05$) using SPSS version 20., and mean separation was conducted using Duncan Multiple Range Test (DMRT) at $p \leq 0.05$

RESULTS AND DISCUSSION

Phytochemical Basis of Antifungal Activity of *Khaya senegalensis*

Table 1 presents the qualitative phytochemical constituents of *Khaya Senegalensis*in Mubi-North Local Government Area. The phytochemical results show that *Khaya Senegalensis* contains important secondary metabolites such as alkaloids and flavonoids in all plant parts, while tannins and saponins were absent in the stem bark but present in the leaf, root, and oil. The antifungal activities observed in this study may be attributed to limonoids, tannins, alkaloids, flavonoids, and terpenoids previously reported in *K. senegalensis*. Kutawa et al. (2022) reported inhibition zones ranging from 18.00–32.50 mm against fungal isolates using methanolic leaf extracts of *K. senegalensis*, while Bonifacio et al. (2019) observed that hydroethanolic plant extracts containing flavonoids produced inhibition zones above 30 mm against pathogenic fungi**.** The presence of alkaloids and flavonoids in all the investigated plant parts (namely, stem bark, leaf, root, and oil) are consistent with findings from earlier studies (Egubagi et al., 2019; Jiya, 2021).

Plant Part	Alkaloids	Flavonoids	Tannins	Saponins
Stem bark	+	+	−	−
Leaf	+	+	+	+
Root	+	+	+	+
Oil	+	+	+	+

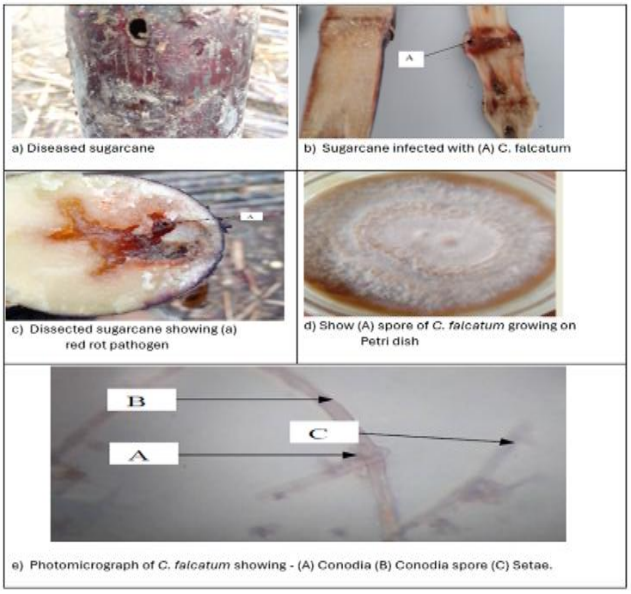
Key: + = Present, − = Absent

Table 1. Qualitative Phytochemical constituent of Khaya senegalensis

Table 2 presents incidence and severity of sugarcane red rot in Mubi North Local Government Area and it reveals that, even as the level of infestation may be low but *Colletotrichum falcatum* has infected sugarcane farms in the sampled areas of Mubi North Local Government Area. The sugarcane farms in Muchalla area recorded the highest incidence at 10%, followed by Muvur and Mubi by-pass with 7.5% each, while Wuro-Gude had the lowest incidence at 5%. The comparably low incidence and absence of the *Colletotrichum falcatum* suggest that even when the pathogen is present, its effect on the crop is limited under the prevailing conditions. This study agrees with report of Olahan et al (2020) that the percentage incidence of the red rot of disease in Bida LGA range from 5.0 to 6.5, it ranges from 6.1 to 12.0 in Gbako L.G.A while it ranges between 5.4 and 6.3 in Lavul L.G.A this result collaborated with the fact that red rot disease exist in all sugarcane growing towns and villages in the world.

Table 2. Incidence and Severity of Sugarcane Red Rot in Mubi North

Farm location	**Incidence (%)**	**Severity**
Muchalla	10	Not severe
Muvur	7.5	Not severe
Mubi by-pass	7.5	Not severe
Wuro Gude	5	Not severe



In vitro antifungal activity of *Khaya senegalensis*: effects of concentration, extract type, plant part.

Table 3 shows the in-vitro antifungal activity of *K. senegalensis* extracts against *C. falcatum*. The 60% ethanolic leaf extract (24.67 mm) significantly ($p \leq 0.05$) inhibited growth more than the 100% aqueous leaf extract (21.67 mm). Ethanolic root extracts at both 20% (23.67 mm) and 60% (22.33 mm) were more effective than their aqueous counterparts. Leaf extracts exhibited higher inhibition at a lower 20% ethanolic concentration (22 mm) compared to 60% (17 mm). However, aqueous root extracts showed increasing inhibition with concentration, with 60% (19 mm) being more effective than 20% (14.67 mm).

In contrast to root extraction, the 100% ethanolic extract was more potent on leaves (24.67 mm) than aqueous extracts (21.67 mm), though leaf extracts did not exhibit a clear preference for either method. Stem activity, however, was significantly higher with the 100% ethanolic extract (26.33 mm) compared to the aqueous extract (19.67 mm), suggesting ethanol's suitability for extracting less polar, dominant compounds from stems. This indicates ethanol is optimal for stems, but less so for roots and oils, highlighting how the differential solubility of active metabolites influences extraction efficiency. Ethanol typically targets moderately polar compounds, while aqueous solvents extract polar constituents like tannins and glycosides. The highest inhibition zones were achieved by the 100% aqueous oil (39.67 mm) and root (36.67 mm) extracts. Notably, ethanolic extracts of stem and oil, even at 100% concentration, resulted in identical and lower inhibition zones (26.33 mm). The superior inhibition from oil and root extracts may be due to higher levels of lipophilic antifungal compounds, such as limonoids and essential oils, a finding supported by Alagnon et al. (2024).

Furthermore, the highest inhibition zones were recorded for the oil (39.67 mm) and root (36.67 mm) when extracted using aqueous solvents at 100% concentration. In contrast, ethanolic extracts of stem and oil, even at the maximum concentration of 100%, exhibited identical and reduced inhibition zones (26.33 mm). The comparatively greater inhibition zones associated with oil and root extracts suggest a higher concentration of lipophilic antifungal compounds, such as limonoids and essential oils, within these plant tissues. This is consistent with the findings of Alagnon et al. (2024), who reported strong antifungal activity in oil-rich botanical extracts, with inhibition zones exceeding 35 mm against fungal pathogens.

Table 3. Effect of the *K. senegalensis* extracts and oil on the inhibition of *C.falcatum*

Plant part	Treatment concentration (%)	Inhibition zone (mm) Ethanolic Extract	Aqueous Extract
Leaf	20	22.00^{cg}	14.67^{gh}
Leaf	60	17.00^{eh}	19.00^{dh}
Leaf	100	24.67^{cd}	21.67^{cg}
Leaf	Control (Cyperforce)	99.00^{a}	99.67^{a}
Stem	20	13.33^{h}	16.67^{eh}
Stem	60	19.00^{dh}	17.00^{eh}
Stem	100	26.33^{cd}	19.67^{ch}
Stem	Control (Cyperforce)	99.33^{a}	100.00^{a}
Root	20	23.67^{ce}	16.33^{eh}
Root	60	22.33^{cf}	20.67^{ch}
Root	100	27.00^{c}	36.67^{b}
Root	Control (Cyperforce)	99.00^{a}	96.00^{a}
Oil	20	15.67^{fh}	16.33^{eh}
Oil	60	17.00^{eh}	25.33^{cd}
Oil	100	26.33^{cd}	39.67^{b}
Oil	Control (Cyperforce)	97.33^{a}	99.00^{a}

Means with the same superscripts in the same column are not significant different at $p \leq 0.05$ according the DMRT

In-vivo* antifungal activity of *Khaya senegalensis* parts and extraction methods on the growth of *C. falcatum

The results of the antifungal activities of extracts of different parts *Khaya senegalensis* on *C. falcatum* is shown in Table 4. The result indicated the effects of concentrations, extract type, plant part, the inhibition zone observed for the *K. Senegalensis* extracted via the aqueous solvent was significantly higher (46.50 mm) than the inhibition zone observed using any plant part and extraction method ($p \leq 0.05$). Overall, ethanolic extracts consistently produced relatively higher inhibition zones across most plant parts, except for the root where the aqueous extract performed better, highlighting the importance of both extraction method and plant part in influencing antifungal effectiveness. The superior performance of aqueous root extracts under *in vivo* conditions may be associated with enhanced diffusion and bioavailability of water-soluble phytochemicals within plant tissues. Amadioha (2000) similarly observed that aqueous botanical extracts reduced fungal infection by more than 65% under field conditions

Table 4. Effects of *Khaya senegalensis* Plant part and Extraction method on the inhibition zone of *C. falcatum*

Plant part	Zone of Inhibition (mm) Ethanolic Extract	Aqueous Extract
Leaf	42.00^{b}	38.50^{b}
Stem	40.67^{b}	38.67^{b}
Root	40.25^{b}	46.50^{a}

Oil	41.00[b]	40.50[b]

Means with the same superscripts in the same column are not significant different at p ≤ 0.05 according the DMRT

The effect of different concentrations and extraction methods on the inhibition zone of *C. falcatum*

Table 5 shows the results of the effect of different concentrations and extraction methods on the inhibition zone of *C. falcatum* revealed distinct patterns. Ethanolic extracts demonstrated a dose-dependent increase in inhibition zone, escalating from 17.67 mm at 20% to 27 mm at 100%. In contrast, aqueous extracts achieved their maximum inhibitory effect of 38.67 mm at a 60% concentration, with lower zones observed at 20% (21 mm) and 100% (27 mm). However, in all cases, the plant extracts' inhibitory zones were significantly outmatched by the control, Cyperforce. While less potent in this context, the plant extracts offer a compelling alternative due to their biodegradability and reduced environmental impact, unlike synthetic fungicides which, despite their high efficacy (often exceeding 90 mm zones), can lead to environmental contamination and pathogen resistance (Chanya et al., 2019).

Table 5. The effect of different concentrations and extraction methods on the inhibition zone of *C. falcatum*

	Zone of inhibition (mm)	
Treatment concentration (%)	**Ethanolic Extract**	**Aqueous Extract**
20	17.67[d]	21.00[c]
60	20.67[d]	38.67[b]
100	27.00[c]	27.00[c]
Cyperforce	95.67[a]	99.33[c]

Means with the same superscripts in the same column are not significant different at p ≤ 0.05 according the DMRT.

Conclusion

Plant extracts demonstrated antifungal activity, with treatment concentration being a key driver of inhibition zone size. This effect was stronger in vitro than in vivo. The extraction method also played a role, with aqueous extracts generally showing better efficacy than ethanolic ones, especially at moderate concentrations. Different plant parts exhibited varying levels of antifungal activity, with some consistently showing higher potency.

In vitro experiments yielded larger and more distinct inhibition zones, suggesting that controlled laboratory conditions optimize the expression of the plant's antifungal potential. In contrast, in vivo results were less pronounced, likely due to environmental factors and reduced bioavailability of active compounds in a living organism.

Despite their effectiveness, the plant extracts were significantly less potent than the synthetic control (Cyperforce), which produced considerably larger inhibition zones.

Recommendations

Based on the findings, it is recommended to employ the 60% aqueous extract as the optimal concentration (38.67 mm inhibition zone) to achieve enhanced efficiency and cost-effectiveness, obviating the need for higher concentrations. Prioritization of the most active plant part utilizing aqueous extraction is also advised, given its consistent superior inhibition compared to ethanolic extracts. Future investigations should endeavor to improve the *in vivo* performance of these extracts to sustain the observed *in vitro* efficacy under field conditions. Furthermore, comprehensive analysis via

gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) is warranted to elucidate the identity of the responsible secondary metabolites

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