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RESEARCH ARTICLE

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Evaluation of Antifungal Susceptibility Pattern of Selected Filamentous Fungi to Methanol Extracts of *Psidium guajava*, *Cassia alata* Linn, *Mitracarpus villosus* and Some Conventional Agents

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Abstract

Fungal contamination in poultry farms poses significant risks to animal health and food safety. The widespread use of conventional antimicrobial agents including antifungal agents, has led to the emergence of antifungal resistance, reducing their efficacy. Moreover, conventional antifungal agents often exhibit toxicity, have a narrow spectrum of activity, and can be costly. These limitations highlight the need for alternative control measures. Medicinal plants with antifungal properties offer a promising avenue for exploration. This study investigates the antifungal activity of methanolic extracts of selected medicinal plants against fungal isolates from poultry farms, comparing their efficacy with conventional antifungal agents. This study evaluated the antifungal activity of methanolic extracts from *Mitrocarpus villosus, Psidium guajava,* and *Cassia alata* against 15 fungal isolates from poultry farms using disc diffusion, minimum inhibitory concentration (MIC), and minimum fungicidal concentration (MFC) assays. Ketoconazole and fluconazole served as positive controls, while 2% dimethyl sulphoxide (DMSO) was used as a negative control. The extracts exhibited varying degrees of inhibition, with *Mitrocarpus villosus* showing the most potent activity against fungal isolates such as *Paecillomyces varioti* (30.0 mm zone of inhibition at 20 mg/disc). The MIC and MFC ranged from 25-100 mg/mL. The positive controls demonstrated significant antifungal activity, while the negative control showed no inhibition. The study suggests that these plant extracts have potential as natural antifungal agents, with *Mitrocarpus villosus* exhibiting the most promising activity. The findings indicate that plant extracts like *Mitrocarpus villosus*, *Psidium guajava,* and *Cassia alata* could be used as promising alternatives or complementary therapies to conventional antifungals for controlling fungal contaminants in poultry environments. Further research is needed to determine their efficacy and safety.

KEYWORDS
Mitrocarpus villosus; *Psidium guajava*; *Cassia alata*;Poultry farms; Antifungal activity.

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1 | INTRODUCTION

Each year, over 6.5 million people contract life-threatening fungal infections, resulting in 2.5 million attributable deaths (Denning, 2024). Fungi inhabit every corner of our environment, with a staggering biodiversity estimated between 2–11 million species, many of which remain unidentified. They are ubiquitous and cause infections which

may be trivial or more deep seated and severe infections associated with mortality (Iyalla, 2014). One major environment that harbors these organisms is the poultry industry. Within poultry houses, fungi may be present in settled dust, bioaerosols, derived from soil, dust, droppings, moldy feed, and, to a lesser extent, from the birds themselves (Ngajilo, 2014; Mohammed and Abdel-latef, 2021). Birds can become infected during hatching by inhaling fungal spores from contaminated hatchery machines or litter (Plewa and Lonc, 2011; Jacquie, 2015). Infection can spread through direct contact with other birds or workers, influenced by factors such as building characteristics, task type, litter type, presence of organic matter, poultry production type, breeding method, feed distribution method, and airflow velocity (Sowiak *et al*., 2012; Mba *et al.,* 2020).

While many fungi are benign and contribute positively to ecosystems, others can cause serious diseases in humans, plants, and animals. The concern intensifies as both known and newly discovered species develop resistance to antifungal medicines. Recent research has shown an increase in resistance to currently used antifungal drugs (Kumar *et al*., 2020). Although synthetic chemicals have increased protection, they come with considerable limitations. Unfortunately, producing antifungal drugs suitable for use in both birds and humans is challenging, and the few available options often have significant side effects (Ekwealor *et al*., 2024). These compounds not only target fungal pathogens but also act on targets found in mammalian cells, potentially resulting in toxicity or adverse drug interactions (De Lucca *et al*., 1999). Hence, there is a growing need for alternative sources of antifungal agents. Recent studies have reported that a large proportion of antimicrobials used today are derived from natural products (Vanreppelen *et al*., 2023; Ekwealor *et al*., 2024). Exploring plant-based materials with antifungal properties presents a promising approach to addressing the challenge of antimicrobial resistance.

Healing with medicinal plants is as old as mankind itself (Ally-Charles *et al*., 2024). The connection between humans and their search for drugs in nature dates back to ancient times, with ample evidence from written documents, preserved monuments, and original plant-based remedies (Petrovska, 2012). Medicinal plants have been used in both traditional and conventional medicine to treat fungal infections in humans and animals (Ekwealor *et al*., 2024). In traditional medicine, various plant species are valued for their therapeutic properties.

Mitracarpus villosus, a member of the Rubiaceae family, is widely used across tropical and West Africa for various medicinal purposes (Jegede *et al*., 2005). It is locally known as “Irawo Ile” in Yoruba, “Obuobwa” in Igbo, and “Gududal” in Fulani. This versatile plant grows as a weed in tropical countries, including Nigeria. One of its notable applications is the treatment of fungal infections, which are prevalent health issues in many tropical regions. Traditionally, it has been used to manage skin conditions such as ringworm and eczema, often caused by fungal pathogens (Abere *et al*., 2007).

Studies have identified bioactive compounds in *Mitracarpus villosus*, including flavonoids and phenolic compounds, which contribute to its antifungal properties (Makambila-Koubemba *et al*., 2011). These compounds have demonstrated potential in inhibiting the growth of various fungal pathogens, making the plant a valuable resource in traditional medicine. Extracts from *Mitracarpus villosus* have also been used to treat bacterial infections, such as those caused by *Dermatophilus congolensis*, which can lead to skin infections in cattle (Gbaguidi *et al*., 2005). Further research is needed to fully explore its potential and develop effective treatments.

Psidium guajava, commonly known as guava, has also been extensively studied for its antifungal properties. Guava leaf extracts have shown significant activity against various fungal pathogens (Bezerra *et al*., 2018), including *Candida albicans*, sometimes outperforming current antifungal agents such as ketoconazole and fluconazole (Ally-Charles *et al*., 2024). In addition, these extracts have demonstrated inhibitory effects against *Cryptococcus neoformans* and have been effective against *Aspergillus niger* when combined with silver nanoparticles (Khan *et al*., 2018). Guava leaf extracts are also effective against dermatophytic fungi, such as *Trichophyton rubrum* and *Microsporum canis*. The antifungal properties of *Psidium guajava* are attributed to its rich content of bioactive compounds (Huynh *et al*., 2025), including flavonoids like quercetin, phenolic compounds, and potentially essential oils, which inhibit fungal growth and morphological transitions.

Recent studies have further confirmed the antifungal potential of *Psidium guajava*. A 2024 study published in the *Journal of Complementary and Alternative Medical Research* reported significant activity against *Candida albicans* and *Cryptococcus neoformans* (Ally-Charles *et al*., 2024). Another study published in 2025 demonstrated that silver nanoparticles synthesized from guava leaf extracts were effective against resistant strains of *Colletotrichum capsici* (Mazhar *et al*., 2025). Earlier research in 2014 explored the antifungal activity of secondary metabolites in guava leaves against dermatophytes (Perera *et al*., 2024).

Cassia alata is another plant with notable medicinal value. Originating from Argentina, it is an annual or occasionally biennial herb belonging to the family Fabaceae (or Leguminosae). It is a tropical ornamental shrub that thrives at low and medium altitudes. Commonly referred to as candle brush or candlestick (Senna alata), it is locally known as “Okoneyo” by the Annang, “Adaiyaokon” by the Ibibio, “Ogala” by the Igbo, and “Asunwo” by the Yoruba (Ogba *et al*., 2023). *Cassia alata* has traditionally been used to treat a variety of ailments, including constipation, intestinal worms, fungal infections, and skin diseases (Fatmawati *et al*., 2020). It is rich in polyphenols and

anthraquinones and also contains phenols, tannins, saponins, and flavonoids. These bioactive compounds contribute to its therapeutic effects (Idu *et al*., 2007). The leaves are particularly known for their potent antifungal and antibacterial activities and have been widely used to treat ringworm, eczema, skin infections, respiratory tract infections, burns, wounds, and constipation (Ogba *et al*., 2023).

Additionally, various parts of the plant such as the leaves, bark, and flowers, have been used to treat conditions such as convulsions, venereal diseases, fever, asthma, and snake bites (Anon, 2011). With its long history of traditional use and scientifically documented benefits, *Cassia alata* remains a valuable species for further exploration and therapeutic development. Fungal infections pose significant health risks to humans, animals, and plants. Traditional medicine offers promising alternatives, with plants such as *Mitracarpus villosus*, *Psidium guajava*, and *Cassia alata* demonstrating potent antifungal properties. Further research is essential to unlock their full therapeutic potential and to develop effective treatments. These natural products may provide valuable solutions in the face of growing antimicrobial resistance.

2. METERIAL AND METHOD

2.1. Experimental Organisms

Fifteen (15) fungal isolates (*Aspergillus chevalieri Mangiin, Aspergillus conicus, Aspergillus fumigatus, Aspergillus flavus, Aspergillus tubingenses, Aureobasidium pullulans, Cunninghamella bertholletiae, Curvullaria verruculosa, Fusarium oxysporum, Lichtheimia corymbifera, Penicillium citrinum, Paecillomyces varioti, Syncephalis aggregate, Scopulariopsis brevicaulis* and *Trichoderma erinaceum*) isolated from birds, feeds and poultry workers in six poultry farms in Anambra State, Nigeria which were identified based on detailed studies of their macroscopic, microscopic and genetic features were used for the study.

2.2. Collection and identification of plants

Leaves of *Psidium guajava*, *Cassia alata* and *Mitrocarpus villosus* were obtained from matured plants in the morning hours from UNIZIK, Umunya in Anambra State and Nenwe in Ani Nri LGA, Enugu state, Nigeria respectively. They were identified and authenticated in the Department of Botany, Nnamdi Azikiwe University, Awka. Anambra State, Nigeria.

2.3. Preparation / extraction of crude extracts.

Soxhlet extraction of the plant extract was carried out according to methods described by (Ekwealor and Oyeka, 2015; Ugwu *et al.,* 2019; Ekwealor *et al*., 2024), using methanol as a solvent. Healthy leaves of the medicinal plants were washed with distilled water, dried in an oven at 40°C and ground into fine powder. Fifty grams (50 g) of the powder was used for the extraction with 500 mL methanol using a Soxhlet extractor. This was followed by the removal of solvent with a rotary evaporator apparatus. The crude extracts were stored in a freezer, at -4°C, for fungal susceptibility studies.

2.4. Inoculum preparation and antifungal activity of plants extracts.

The inoculum was prepared based on the method described by Ekwealor *et al*., (2024). This was done by growing a four-day old fungal isolate on SDA. The isolate was aseptically scrapped and transferred into a tube containing 10 mL sterile water, vigorously shaken and diluted Ten-fold (Espinel-Ingroff *et al*.,1998). In vitro antifungal activity of the extracts was evaluated by the disc diffusion method. The concentrated methanol extract (20mg) was dissolved in 1mL of 2% dimethyl sulphoxide (DMSO) and serially diluted two fold in sterile water. The different concentrations (20 mg, 10 mg, 5 mg and 2.5 mg) were impregnated on 6 mm diameter paper discs.

A 0.1 mL of 10^{-6} dilution of the inoculum suspension (equivalent to 1.5×10^6 sfu/mL) was inoculated on Sabouraud Dextrose Agar (SDA) plates using the spread plate method and allowed to dry. Discs which had been impregnated with methanol extract were then placed gently on the surface of the SDA using a pair of sterile forceps. The sensitivity test was done in duplicate plates. Disc impregnated with 2% dimethyl sulphoxide (DMSO) and another impregnated with 1.25 mg/mL (Ekwealor *et al*., 2024), Ketoconazole and fluconazole (Ally-Charles *et al*., 2024), served as negative and positive controls respectively. The petri dishes were incubated at 25°C for 48 hours and diameter of the zone of inhibition measured in millimeters using a calibrated meter rule.

2.5. Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of methanol extracts

Minimum inhibitory concentrations (MIC) of the different plant extracts were determined by broth dilution method

described by McGinnis, 1980 and Ekwealor *et al*., (2024). 200 mg of the Methanol extracts were dissolved in 1mL of 2% dimethylsulfoxide (DMSO) and serially diluted two fold to concentrations of 100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL (Ally-Charles *et al*., 2024) and 6.25 mg/mL (Ekwealor *et al*., 2024). Standardized suspension, 0.1 mL (1.5×10^6 sfu/mL dilution) of the test organism was aseptically inoculated into each of the dilution tubes and incubated at 25°C for seven days. Tubes with 2% dimethyl sulphoxide (DMSO) and another with 1.25 mg Ketoconazole and fluconazole served as negative and positive controls respectively. The tubes were observed for growth and MIC recorded as the lowest concentration of the plant extract in the tube that failed to show any visible fungal growth. Minimum fungicidal concentration (MFC) of the plant extract was determined by plating-out a loopful from tubes of MIC without visible fungal growth onto sterile plates of Sabouraud Dextrose Agar. The plates were incubated for seven days at 25°C and observed for growth. The MFC was taken as the lowest dilution of the extract that showed no visible growth on SDA plate (Ekwealor and Oyeka, 2015; Cheesbrough, 2006 and Ekwealor *et al*., (2024).

3 RESULTS

The methanolic extracts of *Mitrocarpus villosus, Psidium guajava,* and *Cassia alata* showed varying degrees of antifungal activity against the tested fungal isolates (Tables 1-3). *Mitrocarpus villosus* exhibited the most potent activity, with significant zones of inhibition against several isolates, including *Paecillomyces varioti* (30.0 mm at 20 mg/disc) and *Syncephalis aggregata* (25.0 mm at 20 mg/disc). The MIC and MFC values ranged from 25-100 mg/mL, indicating varying levels of antifungal activity among the plant extracts (Tables 4a and 4b). *Mitrocarpusvillosus* showed the lowest MIC value (25 mg/mL) against *Paecillomyces varioti*. In comparison to the positive controls, ketoconazole and fluconazole, demonstrated significant antifungal activity against most fungal isolates (Tables 5 and 6), although some isolates showed resistance to fluconazole. The cold water extracts of the medicinal plants showed limited antifungal activity compared to the methanolic extracts (Table 7). These results suggest that the methanolic extracts of these plants have potential as natural antifungal agents.

Table 1: Susceptibility test of fungal isolates to methanolic extract of *Mitrocarpus villosus*

Species of Fungi	20 mg/mL	10 mg/mL	5 mg/mL	2.5 mg/mL
A. chevalieri Mangin	+	+	+	+
Aspergillus conicus	+	+	+	+
Aspergillus fumigatus	19.5	17.0	15.0	12.0
Aspergillus flavus	12.0	10.0	8.0	7.0
Aspergillus tubingensis	+	+	+	+
Aureobasidium pullulans	+	+	+	+
Cunninghamella bertholletiae	+	+	+	+
Curvularia verruculosa	+	+	+	+
Fusarium oxysporum	+	+	+	+
Lichtheimia corymbifera	20.0	17.0	15.0	12.0
Penicillium citrinum	20.0	18.0	15.0	12.0
Paecilomyces varioti	30.0	27.0	24.0	20.0
Syncephalis aggregate	25.0	23.0	20.0	16.0
Scopulariopsis brevicaulis	19.0	17.0	14.2	11.0
Trichoderma erinaceum	8.0	+	+	+

Note. '+' indicates no zone of inhibition (i.e., no antifungal activity).

Table 2: Susceptibility test of fungal isolates to Methanolic extract of *Psidium guajava*

Species of Fungi	20 mg/mL	10 mg/mL	5 mg/mL	2.5 mg/mL
A. chevalieri Mangin	+	+	+	+
Aspergillus conicus	+	+	+	+
Aspergillus fumigatus	15.0	12.0	11.2	9.0
Aspergillus flavus	+	+	+	+
Aspergillus tubingensis	+	+	+	+
Aureobasidium pullulans	+	+	+	+
Cunninghamella bertholletiae	+	+	+	+
Curvularia verruculosa	+	+	+	+
Fusarium oxysporum	+	+	+	+
Lichtheimia corymbifera	+	+	+	+
Penicillium citrinum	+	+	+	+
Paecilomyces varioti	+	+	+	+
Syncephalis aggregate	12.0	10.0	7.0	+
Scopulariopsis brevicaulis	+	+	+	+
Trichoderma erinaceum	+	+	+	+

Note. '+' indicates no zone of inhibition (i.e., no antifungal activity). (Space between tables 2 and 3 is too small)

Table 3: Susceptibility test of fungal isolates to methanolic extract of *Cassia alata*

Species of Fungi	20 mg/mL	10 mg/mL	5 mg/mL	2.5 mg/mL
A. chevalieri Mangin	+	+	+	+
Aspergillus conicus	+	+	+	+
Aspergillus fumigatus	19.0	16.0	14.0	12.5
Aspergillus flavus	15.0	12.0	10.0	8.0
Aspergillus tubingensis	+	+	+	+
Aureobasidium pullulans	+	+	+	+
Cunninghamella bertholletiae	14.0	10.5	+	+
Curvularia verruculosa	+	+	+	+
Fusarium oxysporum	18.0	16.0	14.0	10.0
Lichtheimia corymbifera	+	+	+	+
Penicillium citrinum	14.0	12.5	10.0	8.0
Paecilomyces varioti	+	+	+	+
Syncephalis aggregate	18.0	15.0	12.0	10.0
Scopulariopsis brevicaulis	+	+	+	+
Trichoderma erinaceum	+	+	+	+

Note. '+' indicates no zone of inhibition (i.e., no antifungal activity).

Table 4: Susceptibility test of fungal isolates to Ketoconazole

Species of Fungi	10.0 mg/mL	5.0 mg/mL	2.5 mg/mL	1.25 mg/mL	0.63 mg/mL	0.31 mg/mL
Aspergillus chevalieri Mangin	18.0	16.0	14.5	12.2	11.0	10.0
Aspergillus conicus	20.0	28.0	26.0	24.5	22.0	20.0
Aspergillus fumigatus	22.0	19.0	16.0	14.0	12.5	10.5
Aspergillus flavus	28.0	25.0	22.0	20.0	18.0	15.0
Aspergillus tubingensis	25.0	20.0	18.0	15.0	13.0	11.0
Aureobasidium pullulans	+	+	+	+	+	+
Cunninghamella bertholletiae	30.0	28.0	26.0	24.5	22.0	20.0
Curvularia verruculosa	+	+	+	+	+	+
Fusarium oxysporum	27.0	25.0	22.0	18.0	15.0	12.0
Lichtheimia corymbifera	22.0	18.0	16.5	14.0	12.0	11.5
Penicillium citrinum	30.0	28.0	25.0	22.0	19.0	14.0
Paecilomyces varioti	32.0	27.0	24.0	22.0	20.0	18.0
Syncephalis aggregate	38.0	33.0	28.0	25.0	20.0	18.0
Scopulariopsis brevicaulis	27.0	23.0	20.0	17.0	14.0	11.0
Trichoderma erinaceum	30.0	25.0	20.0	18.0	15.0	12.0

Note. '+' indicates no zone of inhibition (i.e., no antifungal activity).

Table 5: Susceptibility test of fungal isolates to Fluconazole

Species of Fungi	10.0 mg/mL	5.0 mg/mL	2.5 mg/mL	1.25 mg/mL	0.63 mg/mL	0.3 mg/mL
Aspergillus chevalieri Mangin	15.0	12.5	10.0	8.0	+	+
Aspergillus conicus	13.0	11.0	9.0	7.5	+	+
Aspergillus fumigatus	14.0	12.9	9.0	7.0	+	+
Aspergillus flavus	32.0	29.0	25.0	21.0	17.0	15.0
Aspergillus tubingenses	30.0	28.0	25.0	20.0	15.0	9.0
Aureobasidium pullulans	+	+	+	+	+	+
Cunninghamella bertholletiae	CC	CC	CC	35.0	30.0	25.0
Curvullaria verruculosa	25.0	20.0	12.0	11.0	+	+
Fusarium oxysporum	+	+	+	+	+	+
Lichtheimia corymbifera	15.0	13.0	+	+	+	+
Penicillium citrinum	9.0	7.0	+	+	+	+
Paecilomyces varioti	9.5	8.5	8.0	7.0	+	+
Syncephalis aggregate	28.0	20.0	18.0	+	+	+
Scopulariopsis brevicaulis	30.0	27.0	24.0	20.0	16.0	13.0
Trichoderma erinaceum	22.0	18.0	+	+	+	+

Note. '+' indicates no zone of inhibition (i.e., no antifungal activity).

4 | DISCUSSION

Antifungal resistance is a major concern both globally and regionally (Ally-Charles *et al.,* 2024). In recent years, fungal infections have emerged as a world-wide health care (Lobna and Abdel, 2014) especially in the poultry industry, causing direct harm to the workers as well as high morbidity, mortality and production losses (Sajid *et al.,* 2006; Kuldeep *et al*. 2013; Mba *et al*., 2020). The harm and losses could be either due to mycotoxins production or their zoonotic nature (Ekwealor *et al*., 2024). Over the last few years, increasing attention has been directed toward plants as a source for the discovery and development of new antibiotics, as

numerous studies have demonstrated that plant-derived compounds possess significant and potentially distinct antimicrobial properties compared to those of microbial origin (Huang *et al*., 2022). From available literature, many studies have been carried out on the antifungal activities of *Mitrocarpus villosus, Psidium guajava* and *Cassia alata* against *Candida* spp. and dermatophytes but not much on non-dermatophyte moulds (Ekwealor *et al*., 2014). The results from this current study revealed the scientific basis of the traditional usage of *Mitrocarpus villosus, Psidium guajava* and *Cassia alata* as therapeutic agents.

Table 6: Susceptibility test of cold water extract of medicinal plants against fungal isolates

Species of Fungi	***Mitrocarpus villosus***	***Psidium guajava***	***Cassia alata***
A. chevalieri Mangin	8.60	+	8.20
Aspergillus conicus	+	+	+
Aspergillus fumigatus	+	+	+
Aspergillus flavus	12.0	+	+
Aspergillus tubingenses	+	+	10.0
Aureobasidium pullulans	+	+	+
Cunninghamella bertholletiae	+	+	8.0
Curvularia verruculosa	+	+	+
Fusarium oxysporum	+	15.0	30.0
Lichtheimia corymbifera	+	+	+
Penicillium citrinum	+	+	12.0
Paecilomyces varioti	+	+	12.5
Syncephalis aggregate	10.0	+	+
Scopulariopsis brevicaulis	12.0	+	+
Trichoderma erinaceum	+	15.0	+

Note. '+' indicates no zone of inhibition (i.e., no antifungal activity).

Table 7a: Minimum Inhibitory Concentration of Methanolic extract against fungal isolates

Species of Fungi	***Mitrocarpus villosus***	***Psidium guajava***	***Cassia alata***
Aspergillus conicus	ND	ND	ND
Aspergillus fumigatus	50.0	50.0	50.0
Aspergillus flavus	50.0	ND	50.0
C. bertholletiae	ND	ND	50.0
Curvularia verruculosa	ND	ND	ND
Fusarium oxysporum	ND	ND	50.0
Lichtheimia corymbifera	50.0	ND	ND
Penicillium citrinum	50.0	ND	50.0
Paecilomyces varioti	25.0	ND	ND
Syncephalis aggregate	50.0	50.0	50.0
Scopulariopsis brevicaulis	50.0	ND	ND
Trichoderma erinaceum	50.0	ND	ND

Note. '+' indicates no zone of inhibition (i.e., no antifungal activity); Note. ND = Not determined.

Table 7b: Minimum Fungicidal Concentration of Methanolic extract against fungal isolates

Species of Fungi	***Mitrocarpus villosus***	***Psidium guajava***	***Cassia alata***
Aspergillus conicus	ND	ND	ND
Aspergillus fumigatus	100	100	100
Aspergillus flavus	100	ND	100
C. bertholletiae	ND	ND	100
Curvularia verruculosa	ND	ND	ND
Fusarium oxysporum	ND	ND	100
Lichtheimia corymbifera	100	ND	ND
Penicillium citrinum	100	ND	100
Paecilomyces varioti	50.0	ND	ND
Syncephalis aggregate	100	100	100
Scopulariopsis brevicaulis	100	ND	ND
Trichoderma erinaceum	100	ND	ND

Note. '+' indicates no zone of inhibition (i.e., no antifungal activity); Note. ND = Not determined.

Antifungal susceptibility of *Mitrocarpus villosus, Psidium guajava* and *Cassia alata* against 15 filamentous fungal isolates from 10 poultry farms was investigated and the results from this current study revealed the scientific

basis of the traditional usage of *Mitrocarpus villosus, Psidium guajava* and *Cassia alata* as therapeutic agents. The susceptibility tests of the fungal isolates to methanolic extracts of the plants are presented in Tables 1-3. As shown in table 1, methanol extract of *Mitrocarpus villosus* inhibited 7 out of 15 fungal isolates, with the zones of inhibition ranging between 7.0mm at 2.5mg/mL (against *A. flavus*) to 30.0mm at 20 mg/mL (against *Paecilomyces varioti*). Zones of inhibition measuring 7.7 mm and 9.4 mm were reported by Ekwealor *et al.* (2012) against *Scopulariopsis brevicaulis* and *Fusarium oxysporum*, respectively. Similarly, as observed in Table 1 of our study, *Scopulariopsis brevicaulis* was susceptible to the methanolic extract of *Mitrocarpus villosus* at all tested concentrations, with the highest inhibition zone of 19.0 mm recorded at 20 mg/mL. However, contrary to our findings, Ekwealor *et al*. (2012) reported antifungal activity of the extract against *Fusarium oxysporum*, while no activity was observed against this organism in our study. This discrepancy also contrasts with the report by Irobi and Daramola (1993), who similarly observed susceptibility of *Fusarium oxysporum* to the plant extract.

The methanolic extract of *Psidium guajava* (Table 2) exhibited relatively weak antifungal activity. Among the fungal isolates tested, only *Aspergillus fumigatus* and *Syncephalis aggregata* showed notable zones of inhibition, with maximum diameters of 15.0 mm and 12.0 mm, respectively, at the highest concentration tested (20 mg/mL). Other fungi, including *Aspergillus flavus*, *A. tubingensis*, and *Fusarium oxysporum*, showed no inhibition at any concentration, indicating limited antifungal efficacy of the methanolic extract. These findings is (are) in contrast with those of Ally-Charles *et al*. (2024), who reported significant antifungal activity of *P. guajava* methanolic extract against *Candida albicans* and *Cryptococcus neoformans*. *Psidium guajava* is known for various biological properties demonstrated through both in vitro and in vivo studies, including antifungal effects (Camacho-Hernández *et al.,* 2004;Huynh *et al*., 2025). Specifically, *Psidium guajava* L. holds considerable ethnomedicinal importance and is traditionally used to manage infections, particularly those caused by *Candida* species (Morais-Braga *et al*., 2016). The limited antifungal activity of *P. guajava* methanolic extract observed in this study may have resulted from a combination of factors, such as low phytochemical content in the plant material used, suboptimal extraction efficiency, intrinsic resistance of the fungal isolates tested, and potential antagonistic effects within the crude extract. Further investigations involving phytochemical profiling, alternative solvents, and broader isolate screening may provide deeper insight.

The results in Table 3 show that only six of the fungal isolates were sensitive to the methanolic extract of *Cassia alata*. The susceptibility of *Aspergillus fumigatus* and *Aspergillus flavus* to the plant extract contrasts with the findings of Alalor *et al*. (2012) and Ekwealor *et al*. (2012), who reported that the extract was not active against *Aspergillus niger* and *Aspergillus flavus*, respectively. The sensitivity of *Fusarium oxysporum* and *Penicillium* species to the methanolic extract of *Cassia alata*, as reported by Ekwealor *et al.* (2012), supports the observations made in this study (Table 3), in which *F. oxysporum* and *Penicillium citrinium* were inhibited by the plant extract. However, Alalor *et al*. (2011) did not record any inhibitory effect of the extract on *Penicillium* species.

The effectiveness of *C. alata* against both dermatophytes and non-dermatophyte molds (such as *Penicillium marneffei* and *A. flavus*), as well as yeasts, has been reported by several researchers (Phongpaichit *et al*., 2004; Makinde *et al.,* 2003). Phongpaichit *et al.* (2004) described *C. alata* as an excellent candidate for the treatment of penicilliosis. This observation aligns with the susceptibility of *A. flavus* and *Penicillium* species observed in this study. On the other hand, *A. flavus*, *A. fumigatus*, and *A. conicus* were found to be resistant to the cold water extract of *C. alata* (Table 6). This is consistent with the findings of Somchit *et al*. (2003), who also reported resistance of *Aspergillus fumigatus* to the methanolic leaf extract of *Cassia alata*

As presented in Table 4, all fungal isolates were found to be sensitive to Ketoconazole except *Aureobasidium pullulans* and *Curvularia verruculosa*. Messer *et al.* (2006), Azam *et al*. (2012) and Farrag *et al*. (2012) reported the efficacy of Ketoconazole against *Aspergillus* species. They support the findings in this study in which all *Aspergillus* species were observed to be sensitive to Ketoconazole. However, Moore *et al*. (2001) and Hsueh *et al*. (2005), noted the inactivity of Ketoconazole against *Aspergillus* species and a number of different species. The sensitivity of *Penicillium citrinum* to Ketoconazole is in line with the findings of Farrag *et al*. (2012). Who observed the efficacy of this antifungal drug to *Penicillium chrysogenum*

Aureobasidium pullulans was resistant to Fluconazole at all concentrations (Table 5). This observation agrees with the work of Java *et al.* (2014), who reported the resistance of *A. pullulans* to Fluconazole. However, Kileen *et al*. (2011), noted its susceptibility to Fluconazole at a concentration of 64mg/l, which is contrary to the findings in this study, Speeleveld *et al*. (1996), observed no activity of Fluconazole on *Fusarium oxysporum,* which supports the result obtained in this study (Table 5). As shown in Table 5, *Cunninghamella bertholletiae* was susceptible to Fluconazole at low concentrations and completely inhibited at higher concentrations. In contrast to this finding, Nikolaos *et al*. (2007) and Vitale*et al*. (2012) reported the resistance of *C. bertholletiae* to the Azoles. Riley *et al*. (2016), also noted that *Mucorales* are inherently resistant to most widely used antifungal agents.

In our study, Ketoconazole, a conventional antimicrobial agent, consistently exhibited the strongest and broadest antifungal activity, inhibiting nearly all species with large zones up to 38 mm (against *Syncephalis*

aggregate at 20mg/mL). Fluconazole was also highly effective, especially against *A. flavus*, *C. bertholletiae*, and *Scopulariopsis brevica. Mitrocarpus villosus* showed the strongest antifungal activity, with high inhibition zones against *P. varioti* (30 mm), *S. aggregata* (25 mm), and moderate activity against several others while *Psidium guajava* was the least effective, with inhibition observed only in a few species and at lower concentrations. A very threatening observation is the resistance of *Aurobasidium pullulans* and *Curvularia verrucullosa* to the plant extracts and ketoconazole and this calls for further studies on the susceptibility of these isolates to other antifungal substances. These observations agree with the work ofAlly-Charles *et al*. (2024) who reported that *Psidium guajava* leaf extracts demonstrated antifungal activity against some of the tested fungal isolates. However, ketoconazole and fluconazole generally showed greater efficacy, particularly ketoconazole, which was consistently more effective across most strains. Fluconazole had variable activity and was ineffective against some strains where the plant extracts showed inhibition. This is supported by studies with *C. albicans* ATCC, in which the plant extracts outperformed the standard drugs, suggesting potential for complementary or alternative use (Akwongo *et al*., 2024).

Cold water extract of *Mitrocarpus villosus* was observed to be active against only four fungal isolates (Table 6). The susceptibility of *Aspergillus chevalieri* and *Aspergillus flavus* to cold water extract of *Mitrocarpus villosus* is contrary to the findings of Irobi and Daramola (1993) who noted that the cold water extract of the plant does not have any activity against *Aspergillus* species and there by supporting our findings that the aqueous plant extract is inactive on *Fusarium* species. In variance with our findings Ekwealor *et al*., (2012) recorded resistance of *A. flavus* and *Scopulariopsis* species to cold water extract of *Mitrocarpus villosus* which were sensitive to the extract in this study.

Fusarium oxysporum and *Trichoderma erinaceum* are the only fungal isolates susceptible to the cold water extract of *Psidium guajava* (Table 6). *Aspergillus* species were observed to be resistant to the cold water extract of *Psidium guajava*. This result does not agree with the report of Amit and Shweta (2011), who noted the activity of the aqueous extract of *Psidium guajava* against *Aspergillus species*

The antifungal activity of cold water extract of *Cassia alata* against the fungal isolates (Table 6), showed that only six fungal isolates were susceptible. The Susceptibility of *Aspergillus* species to cold water extract of *Cassia alata* agrees with the result of Alalor *et al*. (2012) and Makinde, *et al*., (2003) who also noted the inhibitory effect of cold water extract of *Cassia alata* against *Aspergillus* species.

The MIC and MFC results in this study indicate that *Mitrocarpus villosus* exhibited the greatest antifungal activity among the three plants extracts, with the lowest MIC value of 25 mg*/*mL against *Paecillomyces varioti*, suggesting strong fungistatic potential (Tables 7a and 7b). *Cassia alata* demonstrated moderate activity, particularly against *Aspergillus fumigatus*, *A. flavus*, and *Penicillium citrinum*, with MIC values consistently at 50 mg/mL. *Psidium guajava*, in contrast, showed limited inhibitory and fungicidal effects, with activity observed only against *A. fumigatus* and *S. aggregata* at higher MIC and MFC values (50–100 mg/mL)**.** The MFC values were generally double the MICs**,** supporting the notion that higher concentrations are needed for fungicidal activity, as seen in *M. villosus* and *C. alata*, which required 100 mg/mL to kill most susceptible isolates. These results align with findings by Ekwealor *et al*. (2012) and Makambila-Koubemba *et al*. (2011)**,** who reported the efficacy of *M. villosus* against filamentous fungi at relatively low concentrations. The limited activity of *P. guajava* contradicts earlier reports by Ally-Charles *et al*. (2024)and Morais-Braga *et al*. (2016)**,** which showed strong antifungal effects, likely due to differences in extraction methods or fungal strains tested. The moderate efficacy of *C. alata* supports earlier observations by Alalor *et al*. (2012) and Phongpaichit *et al*. (2004)**,** confirming its potency in treating infections caused by *Aspergillus* and *Penicillium* species

5. Conclusion

While the tested plant extracts, especially *Mitrocarpus villosus***,** showed promising antifungal properties**,** they generally exhibited lower and narrower antifungal activity than standard drugs. However, they could serve as potential leads for new antifungal agents**,** especially in combination or in cases of resistance to conventional drugs.

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