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Comparative Analysis on the Antimycotic Effect of *Azadiracta indica* and *Jatropha curcas* on Curvularia Species

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study was carried out to determine the phytochemical properties and the antimycotic effect of *Jatropha curcas* and *Azadiracta indica* leaves on Curvularia species. Isolation and identification of the fungal isolates were carried out using standard conventional method. Maceration technique was employed for crude extraction from the plant. The phytochemical tests were carried out using standard conventional methods. Agar well diffusion method was used to determine the susceptibility of the isolates. Phytochemical tests of A. indica revealed the presence of flavonoids, phenols, saponins, tannins, phenols, and glycosides. *Jatropha curcas* showed the presence of glycoside, tannins, saponin steroids and phenols. The antifungal activity of *Azadiracta indica* and *Jatropha curcas* on Curvularia species, the ethanol extract of *Azadiracta indica* showed more antifungal activity with highest percentage inhibition of 50.56 ± 0.84^{c} at 20% extract concentration, and Aqueous extract was 40.00 ± 0.55^{b} at 20%. The aqueous extract of *Jatropha curcas* showed more antifungal activity of 50.55 ± 0.05^{c} at 20% while that of ethanol extract was 35.55 ± 0.54^{a} at 20% extract concentration. The synergestic antifungal activity of the plants revealed that ethanolic extract had more antifungal effect of 57.54 ± 0.56^{c} at 20% concentration and that of the aqeous

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extract showed more activity of 45.89±0.50^{b} at 20%. There was significant difference (P<0.05) between the ethanoic and aqueous extracts of the plants. The Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of *Jatropha curcas* on Curvularia Species showed the MIC at 250 mg/ml extract concentration for both ethanol and aqueous extracts against Curvularia Species. The ethanol and aqueous extract of *Jatropha curcas* occurred has no fungicidal effect against Curvularia Species.

Keywords: Azadiracta indica; Jatropha curcas; curvularia species antimycotic; phytochemical.

1. INTRODUCTION

Plants are important sources of medicine, compounds derived from plants are referred to as Phytochemicals, and they attract much interest as natural alternatives to synthetic compounds [1]. Microbiologist have employed the used of phytochemicals against microorganisms because they have been found to be degradable and safe for human health [2]. Various diseases have been treated with extracts of medicinal plants like *Jatropha curcas, Zingiber officinale, Allium sativum*, *Azardirachta indica, Garcinia kola* etc all forming the basis for all traditional systems of medicine. The treatment and control of diseases by the use of available medicinal plants in a locality will continue to play significant roles in healthcare systems in developing countries [3].

Jatropha plant belongs to the family *Euphorbiaceae*, it is commonly known as Physic nut with a local name by the Yorubas as 'botuje' [4]. *Jatropha* can be defined as a large shrub or a small perennial tree that attain a height of three to ten meters. This plant is distributed in tropical and subtropical regions of Southeast Africa, Central and Latin America, Asia and India [5]. *Jatropha curcas* is a species that is able to grow in dry and hot conditions, as, for instance, in fringe areas of semi-arid regions, where many species do not survive [6]. Commonly, the plant is grown as hedges and fences around gardens and households in Northern Nigeria [7]. *Jatropha* has 172 species which shows significant antimicrobial, antioxidant, anticancer and pesticidal activities [8]. All part of this plant have been used in traditional human medicine and for veterinary purposes for a long time (Shukla *et al.,* 2015). *Jatropha curcas* has been reported for the treatment of bacterial and fungal infection including being used as an antiseptic during child birth [9].

Azadirachta indica, 'Neem' is also referred to as 'dogon yaro' by the Hausas and Yorubas. It belongs to the family of Meliaceae and has been used in ayurvedic treatment for more than 4000 years [10] and its usage was recorded around 4500 years ago (Khatkar *et al*., 2013). The plant is regarded as a village dispensary in India [11]. Neem is native to east India, Burma, South East Asia (SEA) and West Africa, Pakistan, Peninsular Malaysia, Singapore, Philippines, Australia [12]. The plant is about 12-18 meters in height with a circumference of 1.8-2.4m.The leaves are dark green with about 30cm in length. It has 3 lobed stigmata and seeded drupes [13].

Curvularia is a dematiaceous fungus found ubiquitously in soil, it was first described as *Acrothecium lunata* by Wakker and Went in 1898, *Curvularia* was not documented as a humanpathogen until 1959, when it was isolated from a mycetoma. Since that time, *Curvularia* has been reported as a cause of disease in the upper respiratory tract, lower respiratory tract, skin, endocardium, and cornea [14]. The genusconsists of species which are both plant and human pathogens worldwide, they are reported from the air, fresh water and soil. Currently there are 133 species of the genus Curvularia (Manamgoda *et al*., 2015), however, *Curvularia* lunata (teleomorph sexual state-Cochliobolus*lunatus*) is the lead causal agent of diseases in both plants and human within the genera [15], followed by *Curvularia geniculata* [16].

A fundamental problem associated with *Curvularia* infections is the risk of transferring a genetically evolved isolate from farms (where azole fungicides are used) to humans. Such transferability through human-plant interaction or intake of contaminated air could cause resistance to antifungal drugs since field isolates suffer from fungicide selective pressure and undergo virulence differentiation to adapt to adverse conditions [15]. Both immunocompetent and immunosuppressed persons could be infected by Curvularia species, infection occur in tropical and subtropical areas. Known clinical manifestations include cutaneous and subcutaneous infections, keretomycosis, cerebral

phaeohyphomycosis, allergic bronchopulmonary mycosis and endophthalmitis [15]. Diagnostic tests to confirm infection by *curvularia* species include gram and/or Calcofluor-White stains for hyphal elements, Gomori Methenamine silver stain for fungal biomass, Fontana-Masson silver stain for melanin, and panfungal polymerase chain reaction assay for rapid diagnosis [17].

2. MATERIALS AND METHODS

2.1 Sample Collection and Authentication

Freshly identified leaves of *Jatropha curcas* and *Azardirachta indica* of 20kg weight were collected from the botanical garden, and was identified in the herbarium section of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Kaduna State, with the voucher numbers (900239) and (908765) for *J. curcas* and *A. indica* respectively, they were packaged in a clean polythene bags and transported to the Microbiology laboratory of Kaduna State University for further analysis. Skin scrap from 50 immunocompromised patients were collected from National Veterinary Research Institute (NVRI) VOM, Jos, Plateau State using sterile blade into clean McCartney bottles. These were transported to the Medical Laboratory Department of Microbiology, Kaduna State University in an ice pack cooler (4 ^{0}C) for further investigation.

2.2 Media Preparation

Thirteen gram (13g) of Saboraud Dextrose Agar media was suspended in 200ml of distilled water, the medium was boiled for 2minutes to dissolve the medium completely and was sterilized by autoclaving at 121°C for 15 minutes, it was then cooled to 45-50°C and pour into Petri dishes. Sterility check was done by incubating a sterile prepared media plate without inoculation at 37^{o}C for 24hours.

2.3 Isolation of *Curvularia* Species

One gram (1g) of the skin scrap was suspended unto 9ml of sterile normal saline, it was shake thoroughly and allowed to stand for 10min on the laboratory bench. 1ml from the suspension was inoculated onto prepared saboraud dextrose agar plates using spread plate method [18]. This was incubated in an inverted order for 72hr at 37^{0}C. The pH of the medium was maintained at 5.5 being optimal for the growth and sporulation.

2.4 Preparation of Pure Culture of *Curvularia* Species

Pure culture of *Curvularia* species were prepared using the streak plate method [18]. A suspected colony was picked from a mixed culture plate of saboraud dextrose agar using a sterilized wire loop, this was streaked on a surface of freshly prepared saboraud dextrose agar medium and was incubated at 37^{o}C for 72hours, and discrete colonies were then inoculated into a sterile fresh saboraud dextrose agar slants to form the pure culture of *Curvularia* species respectively [18].

2.5 Identification of the Fungal Isolates

Identification of the isolates were carried out using standard mycological laboratory procedures to determine the fungal morphology, this involve both the macroscopy and microscopic examinations to determine their nature of sporulation, formation of chlamydospores, hyphae, length and width of the colony, the presence or absence of aerial mycelium of the isolates on Saboraud Dextrose Agar (SDA). Slide or micro culture was done to study the morphology of microconidia and other special structures such as spirals and pectinate.

Biochemical tests with urea hydrolysis, 1% peptone agar, and SDA with 5% NaCl was used for species identification. Inoculating needle was flamed over the burning bunsen burner, using the needle, a small portion of the growth colony on the culture plate was transferred into a drop of lacto phenol in cotton blue on the slide. The specimen was teased carefully using inoculating wire lops to avoid squashing and over-crowding of the mycelium and was observed under the microscope at x40 magnification.

2.6 Preparation *Jatropha curcas* and *Azardirachta indica*

The leaves of *Jatropha curcas* and *Azardiracta indica* were washed using sterile water and dried at room temperature for 30 days, the dried leaves were pounded using ceramics mortar and pestle then sieved with 0.5mm sieve to obtained fine powder. The fine powder was packed in clean McCartney bottles and stored at 8^{0}C for extraction purposes [19].

2.7 Crude Extraction of *Jatropha curcas* and *Azardirachta indica*

Fifty grams (50g) of fine powder of the *Jatropha curcas* and *Azadiracta indica* leaves were

measured using electronic digital weighing machine and were suspended in 250ml solvents (95% ethanol) in a conical flask and refluxed at 50°C for 60 min [19]. The extracts were filtered through Whatman No. 1 filter paper and the extracts were evaporated to dryness using a hot air oven at a reduced temperature of 28^0C. The crude extracts were stored in beakers covered with foil papers and labeled appropriately [20].

2.8 Phytochemical Screening of *Jatropha curcas* and *Azardiracta indica*

The presence of tannins and saponins were determined according to the method described by Sofowora [21], while the method described by Harborne [22] was used for flavonoids, alkaloids, glycosides and steroids.

2.9 Determination of the Antifungal Activity of *Jatropha curcas* and *Azardiracta indica*

The invitro antifungal activities of each of the extract at different concentrations were evaluated in accordance to Sharma *et al* 2017 by the poisoned food technique (PFT). Known volumes of the extract and a commercial fungicide was incorporated into potato dextrose agar (PDA). The following amounts of the extracts for both ethanol and aqeous were prepared in milligram per mil. Four different concentrations; 500mg/ml (20%), 250mg/ml (10%), 125mg/ml (5%), 62.5mg/ml (2.5%) for both plants were prepared from the ethanolic extract and aqeous extract of both Jatropha *curcas* and *Azardiracta indica* (Sharma *et al 2017)*. Econazole was incorporated too into potato Dextrose Agar (PDA) as positive control. Furthermore, Plates with no treatment at all was also used as a plain control for this study. The activities carried out to determine the antifungal activity of *Azadiracta indica* and *Jatropha curcas* include; Preparation of Mueller Hinton agar, preparation of 0.5 Mcfarland standard, sensitivity testing of the isolates on the extracts, minimum inhibitory concentration of the extracts, minimum fugicidal concentration of the extract. The plates were incubated at 37^oC for 72 hours to 120 hours. The efficacy of each extract as an antifungal agent was evaluated by measuring fungal growth diameter using a centimeter scale. Percentage inhibition of the radial growth with different extract concentrations was compared to the control and was calculated using the following formula:

Percentage mycelial inhibition, MI (%) = (dc−dt)/dc×100,

Where,

dc = the mean colony diameter for the control set
dt = the mean colony diameter for the extract set.

2.10 Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration was determined using the broth dilution method as described by Lino and Deogracious, [23]. Two (2ml) of Mueller Hinton broth medium were dispense into five test tubes, 0.1ml of the respective inoculums and 0.1ml of each extract concentrations were inoculated into the broth respectively and was mixed, the initial absorbance of each broth tube was determined using spectrophotometer at 650nm wavelength. The tubes were incubated at 37^0C for 72hrs, and the final absorbance was taken after incubation. The least concentration of the leaf extract that gives a reduction in absorbance was termed the MIC of the fungal isolates.

2.11 Determination of Minimum Fungicidal Concentration (MFC)

The MFC was carried out using spread plate method as described by Pelzar and Chan, [12]. From each MIC tube that showed reduction in the final absorbance, a mil was spread onto Mueller-Hinton agar plate, it was incubated at 37^oC for 72hrs to 120hrs. The plates were observed for presence or absence of growth after 72hrs of incubation. The MFC was considered to be the lowest concentration that demonstrates the fungicidal activity of the isolates.

2.12 Statistical Analysis

All data collected for this research were subjected to simple analysis of variance(ANOVA), using one-way classification, least significant difference (LSD) test was carried out at ($P<0.05$) to determine if there was significant difference between the means.

2.13 Significance of the Study

This study has demonstrated the antimycotic effect of *Jatropha curcas* and *Azadiracta indica* leaves on *Curvularia sp*. Hence, it provides information that *Jatropha curcas* and *Azadiracta indica* have the antifungal potential that can

adversely be used in treating diseases or infections associated with *Curvularia species*. Molecular studies on the isolates should be carried out in order to find out the possible gene that confers resistance in the case of *Curvularia species.*

3. RESULTS

Table 1. Phytochemical constituent of *Azardirachta indica*

Phytochemical	Aqueous Extract	Ethanol Extract
Alkaloids	-	-
Flavonoids	+	+
Steroids	-	+
Tannins	_	+
Phenols	+	+
Saponins	-	+
Glycosides	-	+

Key: + = Detected, - = Not detected

Table 2. Phytochemical constituent of *Jatropha curcas*

Phytochemical	Aqueous Extract	Ethanol Extract
Alkaloids	-	-
Flavonoids	-	-
Steroids	+	+
Tannins	+	-
Phenols	-	+
Saponins	+	+
Glycosides	+	-

Key: + = Detected, - = Not detected

Table 3. Cultural, morphological and biochemical characteristics of *Curvularia species*

Organism	Shape	Hyphae	Conidia	Hair Perforation Test	SDA	Urease hydrolysis
Trichophyton mentagrophyte	Hairy expanded colonies	Unbranched three septate, ovalaceous to ellipsoidal	Smooth walled conidia (21-31x 8.5-12um	Positive	Grossly, dark brown to black hairy expanded colonies.	Positive

Table 4. Antifungal activity of ethanol and aqueous extract of *Azadiracta indica* and *Jatropha curcas* on *Curvularia Species*

	Percentage (%) Concentration of Extract (Inhibition)			
Extracts	**20%**	**10%**	**5%**	**2.5%**
Aq. *A. indica*	40.00±0.55^{b}	37.52±0.43^{b}	25.45±0.43^{b}	0.00±0.00^{a}
EtoH. *A. indica*	50.56±0.84^{c}	42.50±0.53^{b}	37.52±0.54^{c}	25.00±0.50^{b}
Aq. *J. curcas*	50.55±0.05^{c}	37.55±0.23^{a}	25.00±0.00^{b}	17.38±0.43^{ab}
EtoH. *J. curcas*	35.55±0.54^{a}	25.56±0.45^{a}	10.89±0.67^{a}	0.00±0.00^{a}
EtoH. *A. indica* + EtoH *J. curcas*	57.54±0.56^{c}	50.50±0.26^{c}	40.34±0.35^{cd}	37.52±0.50^{b}
Aq. *A. indica* + Aq *J. curcas*	45.89±0.50^{b}	45.56±0.78^{d}	30.56±0.33^{b}	25.43±0.34^{a}

Key: Aq =Aqueuos, EtoH = Ethanol; Values are mean ± SEM of 3 determinant

Table 5. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of *Azadiracta indica* on *Curvularia* Species

Organism	MIC (mg/ml)	MFC (mg/ml)
	Aq EtoH	Aq EtoH
Curvularia sp	187 187	250 250

KEY: Aq = Aqueous Extract, EtoH= Ethanol Extract.

Table 6 Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of *Jatropha curcas* on *Curvularia* species

Organism	MIC (mg/ml)	MFC (mg/ml)
	Aq EtoH	AqEtoH
Curvularia sp	250 250	*

*KEY: Aq = Aqueous Extract, EtoH= Ethanol Extract, * = No MFC*

4. DISCUSSION

4.1 Phytochemical Constituent of *Jatropha curcas* and *Azardiracta indica*

The qualitative phytochemical analysis of ethanoic and aqueous extracts of *Jatropha curcas* and *Azardiracta indica* (Table 2) revealed the presence of glycoside, tannins, saponins, steroids and phenols in *Jatropha curcas.* Similarly, phenols, flavonoids, saponins, tannins, phenols, and glycosides were present in *Azardiracta indica* extracts. Mondali *et al.*, [24], Keta *et al.*, (2019), reported similar findings. The presence and absence of these phytochemicals on aqueous and ethanol extracts could be as a result of solvent polarity and nature for better solubility of active agent. Mule *et al.,* [25] reported that solvent used for extraction has an effect on the nature of the phytochemicals extracted. This clearly means that polarity of solvents plays crucial role on the types of bioactive compound that can be extracted from plant parts.

4.2 Cultural, Morphological and Biochemical Characteristics of *Curvularia species*

The cultural, morphological and biochemical characteristics of *Curvularia species* (Table 3) revealed that *Curvularia species* appeared to be grossly dark brown to black hairy expanded colonies on Sabraouds Dextrose Agar. Microscopically, using Lactophenol cotton blue x 40, it appeared with erect conidiophores, unbranched, septate, flexuose in apical part with smooth walled conidia (21-31 x 8.5- 12um), three septate, ovalaceous to ellipsoidal with subterminal cell swollen and distinctly larger than remaining cells were observed

4.3 Antifungal Susceptibility of *Azadiracta indica* and *Jatropha curcas on Curvularia sp*

The phytochemical test revealed the presence of saponins, terpenoids, glycoside, phenols and tannins in *J. curcas*, whereas flavonoids, saponins, tannins, phenols, steroids and glycoside were found in *A. indica*. These phytochemicals possessed antimicrobial activity and were able to inhibit the growth of *Curvularia sp*. The antifungal activity of Ethanol and Aqueous Extract of *Azadiracta indica* and *Jatropha curcas* on *Curvularia Species* showed that at 20% concentration, ethanol extract of *Azadiracta indica* and aqueous extract of *Jatropha curcas* both were most effective (187mg/ml) against *Curvularia* species, while combined effect of the aqueous extract of *Azadiracta indica* and *Jatropha curcas* and ethanol extract of *Jatropha curcas* both demonstrated the least activity against the growth of *Curvularia* species at the same concentration. Ethanol extract of *Jatropha curcas* and aqueous extract of *Azadiracta indica* did not show any activity at 5%, however, the combined effect of ethanol extract of *Azadiracta indica*/*Jatropha curcas* at the same concentration was found to be most effective (250mg/ml) against *Curvularia* species. At the least concentration of the extract (2.5%), ethanol extract *Azadiracta indica*/*Jatropha curcas* with demonstrated the highest activity against *Curvularia* species.

4.4 Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of *A. indica* and *J. curcas* on *T. mentagrophyte*

The Minimum Inhibitory Concentration and Minimum Fungicidal Concentration of *A. indica* (Table 5) is lower than that of *J. curcas*, suggesting higher activity of *A. indica*. *Jatropha curcas* alone or in combination with *A. indica*, was not fungicidal to *Curvularia species.* The pattern of the MIC and MFC of the fungal species *Curvularia species* could be attributed to the fact that *Curvularia species* have been identified as a pathogen of both plant and humans [27], thereby adapting to the toxicity of the plant extract.

5. CONCLUSION

The phytochemical test revealed the presence of saponins, terpenoids, glycoside, phenols and

tannins in *J. curcas*, whereas flavonoids, saponins, tannins, phenols, steroids and glycoside were found in *A. indica*. Though the aqueous extract of *A. indica* and ethanol extract of *Jatropha curcas* at 2.5% could not inhibit the growth of *Curvularia species* in this study, all other concentration of all plant and extract used demonstrated inhibitory activity to the growth of *Curvularia species*. Combined effect of *Jatropha curcas* and *A. indica* against the *Curvularia species* was found to be most effective at 20% concentration using both aqeous and ethanolic extract. The relative activity of the individual plant extract against the isolate at different concentrations of the extract is an indication of the efficacy of the extracts against the *Curvularia* Specie, there is significant difference in the activity of the extracts against *Curvularia* species. The higher the extract concentration, the higher the activity against the test organism. The minimum inhibitory and fungicidal activity of *A. indica* and *Jatropha curcas* were found to be statistically different at 95% ($P<0.05$). *A. indica* demonstrated inhibitory and fungicidal activity to the isolate at 187.5mg/ml and 250mg/ml concentration but *J. curcas* had no fungicidal effect on *Curvularia* sp. Findings from this study indicates that *A. indica* has more effect than J. *curcas* extracts and can be further developed for new drug formulation meant for treatment of infections or diseases associated with *Curvullaria* specie.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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