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PHYTOCHEMICAL COMPOSITION, ANTIMICROBIAL EFFECT OF *AZADIRACHTA INDICA* AND *CARICA PAPAYA* EXTRACTS ON FUNGI ISOLATED FROM *GMELINA ARBOREA* SEEDLINGS

[a]Umana E. Johnson, [a]Patrick I. Akwaji*, [a]Markson Aniedi-Abasi, [b]Udo S. Effiong, [a]Orok E. Effiom
[a] *Department of Botany, University of Calabar, Calabar, Nigeria.*
[b] *Department of Biological Sciences, Cross River University of Technology, Calabar, Nigeria.*

A B S T R A C T

Gmelina arborea Roxb has important economic value in Nigeria and worldwide. It has been used as timber, for pulp and paper, furniture, plywood and for particle board. Due to the menace caused by fungal diseases in nurseries and sites where the seedlings are raised and the observed disease severity at the *Awi Gmelina* Forestry Project Nursery in Cross River State, Nigeria. It became necessary to provide a viable environmentally friendly measure to curb the diseases, hence, a trial on the antifungal effects of leaf extracts of *Carica papaya* and *Azadirachta indica in-vitro* using different extract solvents on some pathogenic fungi isolated from *Gmelina arborea* seedlings. The isolated fungi were *Trichoderma viride* (from stem and leaf) and *Mucor mucedo* (stem and leaf). The solvents used were ethanol, methanol, propanol, butanol, acetone, chloroform and distilled water. Phytochemical screening of extracts of C. papaya from the different solvents showed that there was no tannin and hydroxymethyl anthraquinine. Flavonoids and polyphenols were in excess in acetone and methanol extracts respectively. For A. indica, polyphenols were only found in excess in ethanol and methanol extracts. Application of the extracts at different percentages of 0% (control), 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100% showed that Carica papaya extracted with butanol was more potent on *Trichoderma viride* and *Mucor mucedo* at 60%, 70%, 80% and 100% while the growth of *Trichoderma viride* and Mucor mucedo was checked by butanol extracts of A. indica at 70%, 80%, 90% and 100%. Conclusively, butanol extracts of C. papaya and A. indica at higher concentrations is recommended for use as spray to control the diseases.

Keywords: Plant extracts, extract solvents, concentration, antimicrobial effect, *Gmelina arborea* seedlings.

INTRODUCTION

Extract solvents have some effect on the potency of plant extracts. They either act by increasing or suppressing the toxicity of this point extracts (Onifade, 2000). Some of the extracts used in the extraction of plants are mainly organic solvents such as: chloroform, hexane, ethanol, butanol, petrol ether, acetone and water being the only inorganic solvent. *Carica papaya* and *Azadirachta indica* have medicinal properties which can be used in the control of some disease pathogens in both plants and animals. For instance, *Carica papaya* contains some phytochemical components such as alkaloids, glycosides, flavonoids and polyphenols in fruits and leaves which has antifungal and microbial effects (Mbadianya *et al.,* 2013).

* Corresponding Author:
Email: akwajiisnever@yahoo.com

Azadirachta indica (neem plant) is referred to as a miracle tree because of the many goods and services it has provided mankind over thousands of years. It has many beneficial and insecticidal properties. The seed oil of this plant is used to treat skin diseases, tuberculosis and even used as hair tonic (Lauridsen and Kjaer, 2002).
Gmelina arborea belongs to the family Verbanaceae. It was first introduced to Nigeria from Asia in 1917. Since then, it has remained important amongst the few tree species that are found in pure stand plantations in the country (Adegbehin *et al.,* 1988, Epinoza, 2003). The wood of Gmelina is soft, light (though strong for its weight) and the color often is pale yellow to cream colored or plukish. It has been discovered to be good for pulp and paper and also for furniture, joinery, plywood and particle board production (Dvorak, 2003). There have been reports on attack of Gmelina plants by fungal

pathogens (Duke, 2002). Most times, attack at the mature stage does not cause serious harm. Heavy damage has been reported at the seedling stage especially, under wet and hot weather. Because of the menace caused by fungal diseases in nurseries and sites where they are raised and the observed disease incidence/severity at the Awi Gmelina Forestry Project, and in view of the role played by this plant in the economy of Cross River State, Nigeria. It became necessary to isolate and identify the causative agents of the infection, to find out a better control measure that is environmentally friendly such as the use of plant extracts to control the infection.

MATERIALS AND METHODS

Sources of Materials: Diseased parts of *Gmelina arborea* seedlings (stem and leaves) were obtained from the Awi Gmelina Forestry Project in Akamkpa local Government Area of Cross River State, Nigeria and wrapped in sterile cellophane bags and transported to the Laboratory. *Carica papaya and Azadirachta indica* leaves were obtained from the Botanic Garden of the Department of Botany, University of Calabar, Calabar, Cross River state, Nigeria.

Source of Fungal Pathogens and Morphological Identification: The fungal pathogens used in this research work were isolated from diseased leaves and stems of *Gmelina arborea* seedlings collected from the Awi Gmelina Forestry Project at Akamkpa Local Government Area of Cross River State, Nigeria. Cut sections of the diseased assay plants were surface sterilized with 70% sodium hypochlorite (bleach) solution for 1min and rinsed quickly in 3 changes of sterile distilled water, blotted dry on Whatman's No. 1filter paper and placed on Potato Dextrose Agar (PDA) in Petri dishes. Four (4) sections were inoculated per Petri dish. The plates were incubated at 28 ± 1°C until fungal growth was noticed. After 5 days, the different isolates were subcultured on freshly prepared PDA to obtain their pure culture. Isolated fungi were microscopically (Olympus optical, Phillipines) identified as far as possible using the identification guides of the International Mycological Institute, Kew and of Barnett and Hunter (1998), Alexopolous and Mins (1989). Stock cultures of these fungi were stored in agar slant bottles for subsequent use.

Preparation of Extracts: Leaves of *Carica papaya* and *Azadirachta indica* obtained were washed with distilled water and oven dried at a temperature of 80°C for 24 hours, grounded into fine powder and extracted separately using 100ml of 95% concentration of ethanol, methanol, acetone, chloroform, propanol, butanol and distilled water.

Susceptibility Test: The extracts percentage concentrations were prepared at 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100% with different solvents of ethanol, methanol, acetone, chloroform, propanol, butanol and distilled water.

Dilution Test Procedure: 1ml of each concentration was first poured into different Petri dishes using sterile syringes. The sterilized Potato Dextrose Agar (PDA) was also poured into the plates containing the solvent extracts after which the plates were gently swirled to ensure mixing. The media was allowed to solidify and with a sterilized No.2 cork borer of 5.5mm in diameter, a disc of the matured culture was punched out, inoculated at the centre of plates and incubated at room temperature of 28 ±1°C. As a control, the dishes were inoculated in distilled water-agar mix instead of solvent extracts-agar mix. Two (2) control plates were prepared for each solvent extracts. For positive control, no solvent extracts-agar mix or distilled water-agar mix was introduced into the plates. Growth measurement of the mycelia in diameter was done daily for seven days (Udo *et al.,* 2006).

Phytochemical Screening of Plant Extracts: Phytochemical screening of the plant extracts was carried out at the Department of Biochemistry, University of Calabar, Nigeria using the methods of Sofowora (1984), Trease and Evans (1973) and Culei (1982).

RESULTS

Identification of Pathogens: The fungal pathogens isolated and identified from this study and used were *Mucor mucedo* and *Trichoderma viride*.

Phytochemical Screening: Phytochemical screening of *Carica papaya* and *Azadirachta indica* leaves in this study showed that they contain secondary metabolites like alkaloids, glycosides, flavonoids and polyphenols as presented in (Tables 1 and 2).

Antifungal Effect of *Carica papaya* and *Azadirachta indica* Extracts on Mycelia Growth of the Fungal Pathogens at the Different Concentrations: Results from percentage inhibition of the plant extracts on each fungus from this study showed that, at 10% and 20% concentrations, *Carica papaya* and *Azadirachta indica* extracts of methanol, ethanol, propanol, butanol,

chloroform, acetone and the control distilled water had no significant effect on the mycelia growth of *Trichoderma viride* and *Mucor mucedo* after seven days observation period as shown in (Figures 1 -14). However, *Carica papaya* and *Azadirachta indica* extracts of butanol showed the most inhibition. At 30% concentration, *Carica papaya* and *Azadirachta indica* extracts of ethanol and butanol had a slight significant effect on *Mucor mucedo* and *Trichoderma viride*. While at 40% concentration, *Carica papaya* extracts of propanol and butanol had a significant effect on *Mucor mucedo*. A. *indica* extract of butanol had the highest significant effect on *Mucor mucedo* and T. *viride* as shown in (Figures 1, 2, 8 and 9).

Table 1. Phytochemical components of *Carica papaya* leaves.

S/N	Chemical constituent	Ethanol	Methanol	Acetone	Chloroform	Water
1.	Alkaloids	++	+	+	+	+
2.	Glycosides	+	+	+	+	+
3.	Saponins	+	-	+	+	+
4.	Tannins	-	+	-	-	-
5.	Flavonoids	+	++	+++	+	+
6.	Reducing compounds	+	++	-	-	+
7.	Polyphenols	++	+++	+	+	++
8.	Phlobatanins	+	+	+	-	-
9.	Anthra Quinine	-	+	+	-	-
10.	Hydroxymethyl Anthraquinine	-	-	+	-	-

Note: +++ (strongly present) ++ (Moderately present) + (Present) – (Absent)

Table 2. Phytochemical components of *Azadirachta indica* leaves.

S/N	Chemical constituent	Ethanol	Methanol	Acetone	Chloroform	Water
1.	Alkaloids	+	+	+	+	+
2.	Glycosides	+	+	+	+	+
3.	Saponins	-	-	+	-	+
4.	Tannins	-	+	-	-	-
5.	Flavonoids	+	+	++	+	+
6.	Reducing compounds	+	++	+	-	+
7.	Polyphenols	+++	+++	++	+	+
8.	Phlobatanins	-	-	-	+	+
9.	Anthra Quinine	+	-	+	-	-
10.	Hydroxymethyl Anthraquinine	-	+	+	-	-

Note: +++ (strongly present) ++ (Moderately present) + (Present) – (Absent)

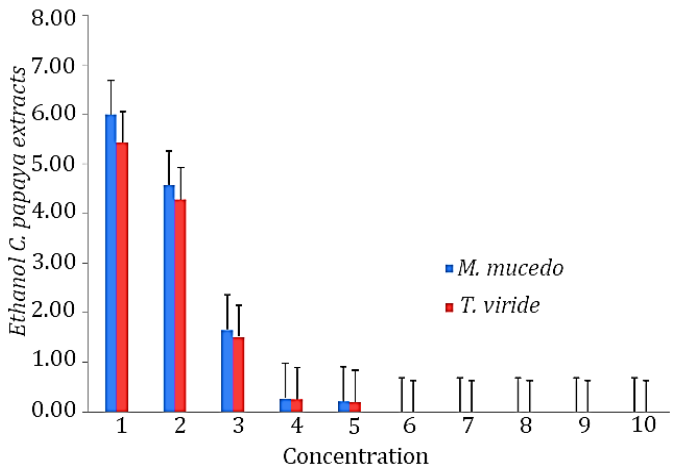

Figure 1. Effect of *Carica papaya* (ethanol) extract on mycelia growth of *M. mucedo* and *T. viride* at the different concentrations.

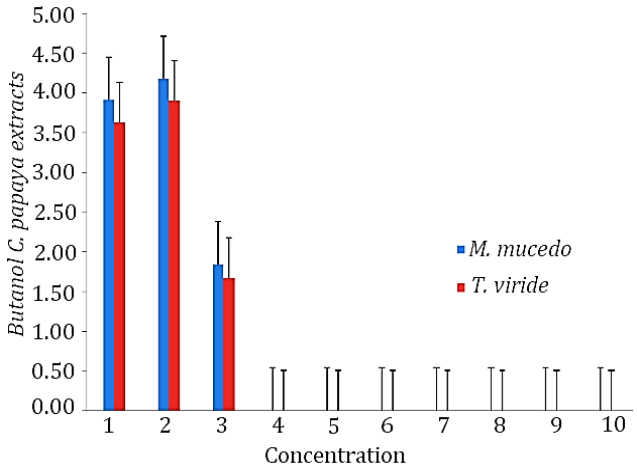

Figure 2. Effect of *Carica papaya* (butanol) extract on mycelia growth of *M. mucedo* and *T. viride* at the different concentrations.

At 50% concentration, C. *papaya* extracts of propanol and butanol had a significant effect on M. *mucedo*, while a slight effect was observed with C. *papaya* extracts of ethanol and chloroform. C. *papaya* extracts of acetone and ethanol had a slight effect on T. *viride*. The control distilled water also had a slight effect on T. *viride*. A. *indica* extracts of butanol, methanol and ethanol had a slight effect on M. *mucedo* and T. *viride* as shown in (Figure 1, 2, 3, 4, 6, 7, 8, 9 and 12).

At 60% concentration, C. *papaya* extracts of ethanol, propanol and butanol had a significant effect on M. *mucedo* and T. *viride*. The control distilled water and C. *papaya* extract of acetone had a slight effect on M. *mucedo* and T. *viride*. C. *papaya* extract of chloroform had no effect on the pathogens. While A. *indica* extracts of methanol and ethanol had a significant effect on T. *viride*, but a slight effect on M. *mucedo*. At 70% concentration, A. *indica* extracts of methanol, butanol, and acetone had a significant effect on M. *mucedo* and T. *viride*. While C. *papaya* extracts of ethanol, propanol and butanol had a significant effect on M. *mucedo* and T. *viride*. C. *papaya* extract of methanol had a significant effect on M. *mucedo* but not on T. *viride* as shown in (Figures 1, 2, 3, 4, 6, 7, 8, 9, 10 and 12).

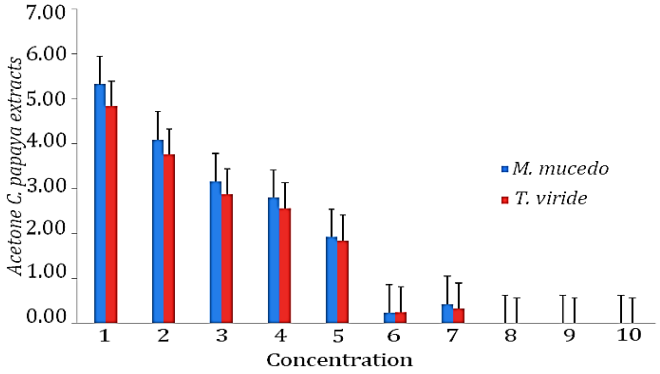

Figure 3. Effect of *Carica papaya* (Acetone) extract on mycelia growth of *M. mucedo* and *T. viride* at the different concentrations.

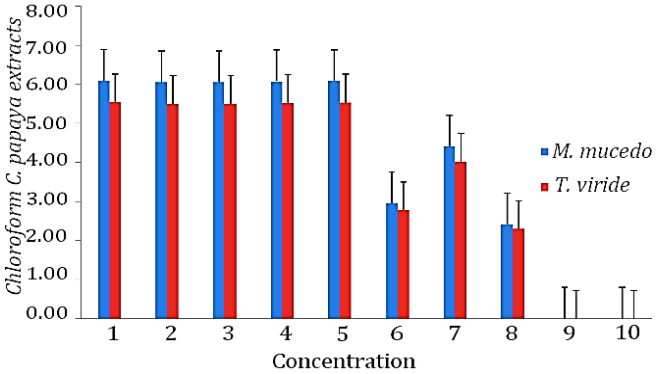

Figure 4. Effect of *Carica papaya* (Chloroform) extract on mycelia growth of *M. mucedo* and *T. viride* at the different concentrations.

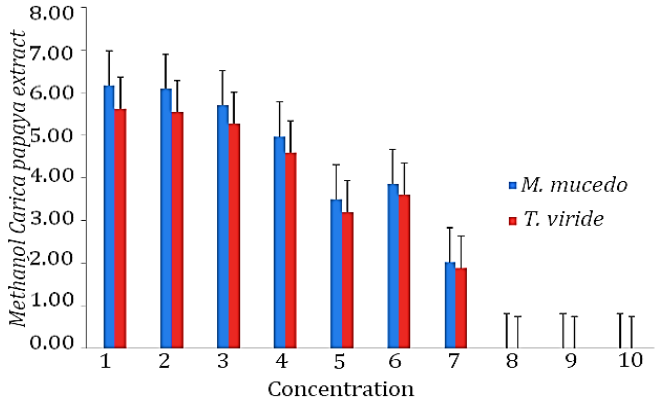

Figure 5. Effect of *Carica papaya* (Methanol) extract on mycelia growth of *M. mucedo* and *T. viride* at the different concentrations.

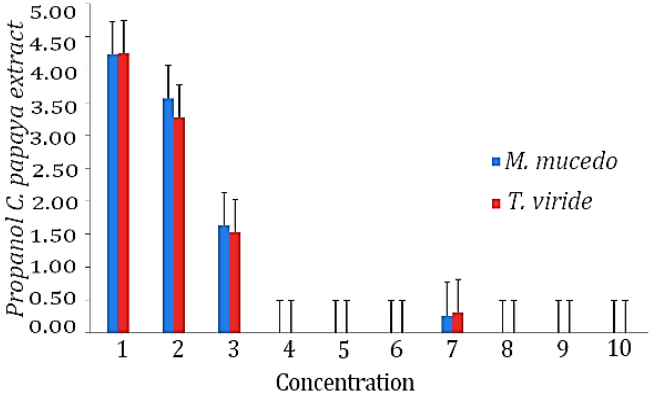

Figure 6. Effect of *Carica papaya* (Propanol) extract on mycelia growth of *M. mucedo* and *T. viride* at the different concentrations.

At 80 and 90% concentrations, C. *papaya* and A. *indica* extracts of methanol, ethanol, butanol, propanol, acetone and the control distilled water had a significant effect on M. *mucedo* and T. *viride* except at 80% concentration of C. *papaya* extracts of methanol and acetone which had a slight effect on T. *viride*. Also A. *indica* extract of propanol had a slight effect on T. *viride* as shown in (Figures 3, 5, 8, 9, 10, 12, 13 and 14).

At 100% concentration, C. *papaya* extracts of the different solvents had a significant effect on M. *mucedo* and T. *viride*. The control distilled water had a significant effect on M. *mucedo* but not on T. *viride*. While A. *indica* extracts of methanol, ethanol, propanol, butanol and acetone had a significant effect on the fungal pathogens except for chloroform and the control distilled water as shown in (Figure 1-14).

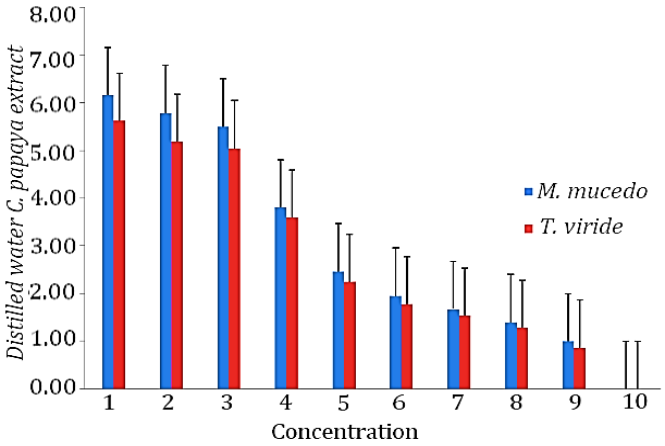

Figure 7. Effect of *Carica papaya* (Distilled water) extract on mycelia growth of *M. mucedo* and *T. viride* at the different concentrations.

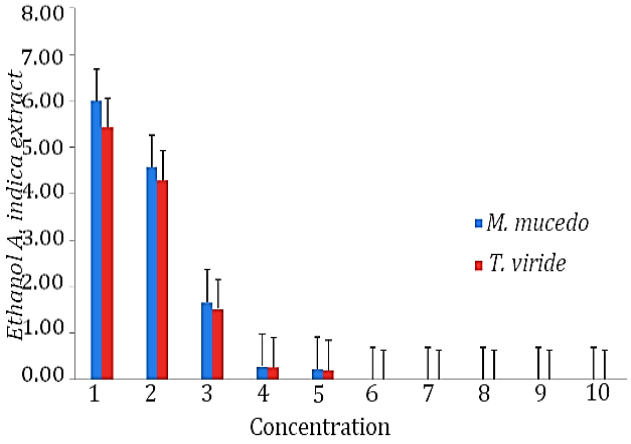

Figure 8. Effect of *Azadirachta indica* (Ethanol) extract on mycelia growth of *M. mucedo* and *T. viride* at the different concentrations.

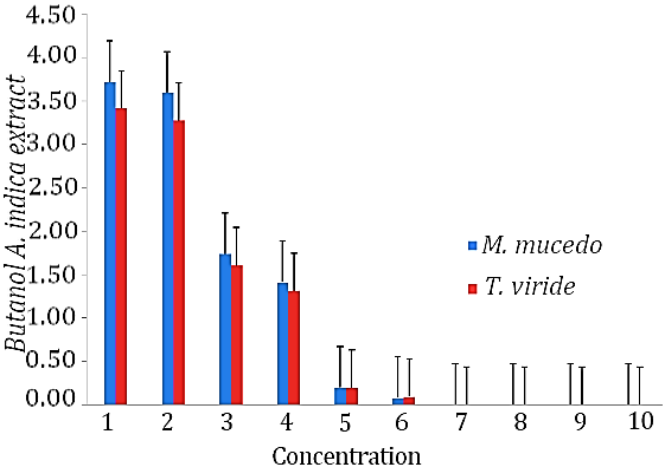

Figure 9: Effect of *Azadirachta indica* (Butanol) extract on mycelia growth of *M. mucedo* and *T. viride* at the different concentrations.

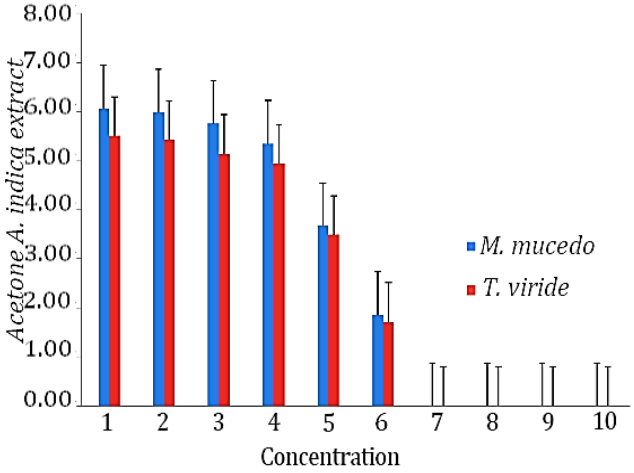

Figure 10: Effect of *Azadirachta indica* (Acetone) extract on mycelia growth of *M. mucedo* and *T. viride* at the different concentrations.

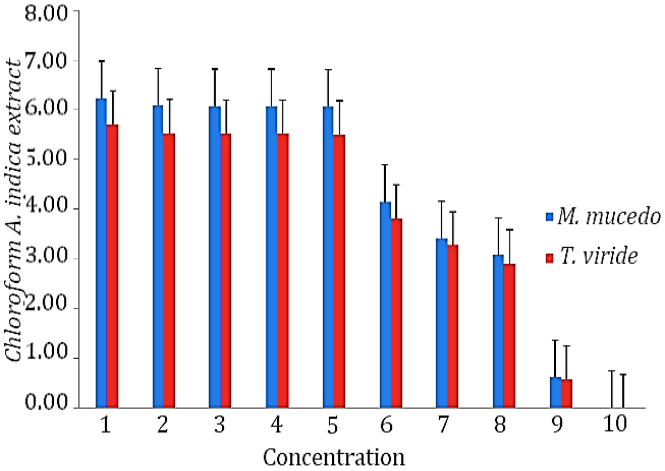

Figure 11: Effect of *Azadirachta indica* (Chloroform) extract on mycelia growth of *M. mucedo* and *T. viride* at the different concentrations.

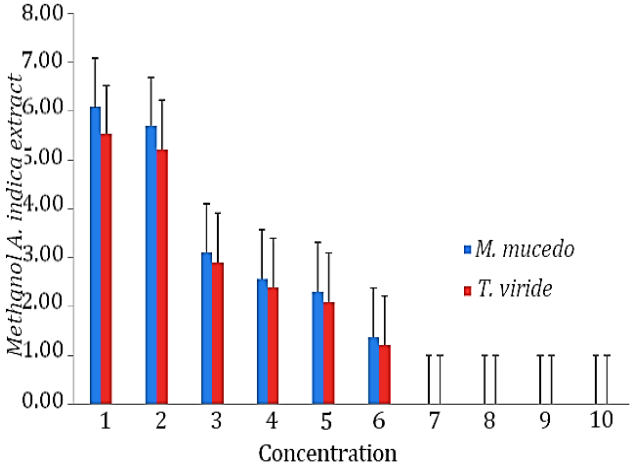

Figure 12: Effect of *Azadirachta indica* (Methanol) extract on mycelia growth of *M. mucedo* and *T. viride* at the different concentrations.

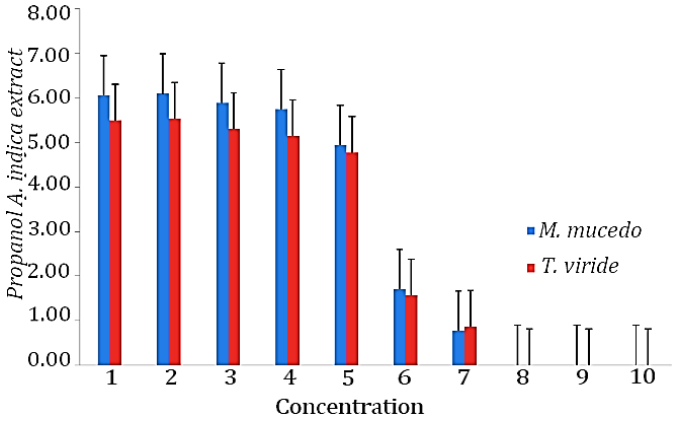

Figure 13: Effect of *Azadirachta indica* (Propanol) extract on mycelia growth of *M. mucedo* and *T. viride* at the different concentrations.

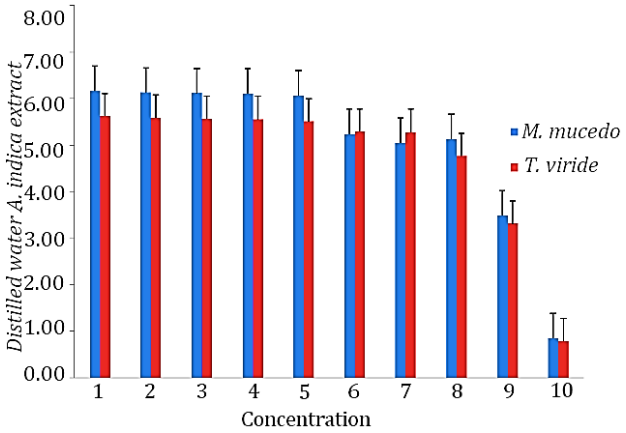

Figure 14: Effect of *Azadirachta indica* (Distilled water) extract on mycelia growth of *M. mucedo* and *T. viride* at the different concentrations.

DISCUSSION

Phytochemical screening of extracts of *C. papaya* from the different solvents showed that there was no tannin and hydroxymethyl anthraquinine. Flavonoids and polyphenols were in excess in acetone and methanol extracts respectively. For *A. indica*, polyphenols were only found in excess in ethanol and methanol extracts, these results are similar to those of Mbadianya *et al.,* (2013) who reported the presence of flavonoids, alkaloids, glycosides and polyphenols in methanolic and ethanolic leaf extracts of C. *papaya* and A. *indica*. In this study, a general trend of increased antifungal activities was observed with a corresponding increase in the concentrations of the extract solvents of *Azadirachta indica* and *Carica papaya.* The fungal pathogens were completely inhibited at 80%, 90% and 100% concentrations this disagrees with that of Olahan and Amadi (2006) who reported the fungicidal effect of aqueous and ethanolic leaf extracts of C. *papaya* against *Fusarium verticilliodes* at different concentrations of 10, 30 and 50%. Both extracts retarded the radial growth of F. *verticilliodes* with ethanolic extract having a greater fungistatic effect at the different concentrations tested. The antifungal effect of the plant extracts however declined as the concentration reduced to 10%, this is in conformity with the work of Onifade (2000) who reported a general trend of increased antifungal activity with a corresponding increase in concentration of aqueous A. *indica* extract on *Colletotrichum lindemuthianum.* In this study, we observed that C. *papaya* and A. *indica* extract of chloroform had the least effect on the potency of the plant extracts on the fungal pathogens, while C. *papaya* and A. *indica* extracts of butanol was the most effective. The potency of these plant extracts could be due to increase in concentration as proposed by Maragathavalli *et al.,* (2012) who observed that the percentage inhibition of an isolated fungus increased with a corresponding increase in the concentration of the extract. Also the inhibition of the growth of the fungus is as a result of the phytochemical contained in the extracts as reported by Udo *et al.,* (2006). We also observed that *Azadirachta indica* extracts was less effective in the control of the fungal pathogens as compared to *Carica papaya* extracts at lower concentrations; this may be due to the fact that the phytochemicals contained in C. *papaya* extracts were stronger and effective than those of A. *indica*. This finding agrees with that of Mahesh and Satish (2008) on antimicrobial activity of some medicinal plants on fungal pathogens.

CONCLUSION

This study showed that *Carica papaya* extracts was more effective in inhibiting the mycelia growth of both *Trichoderma viride* and *Mucor mucedo* than those of *Azadirachta indica*. The inhibition increased with the increase in concentrations of the extracts for both plants. C*. papaya* and A. *indica* extracts of butanol were found to be the most effective in inhibiting the growth of both fungal pathogens while chloroform was least effective. Therefore*, Carica papaya* and *Azadirachta indica* extracts of butanol at higher concentrations is highly recommended to be used as spray for the control of the fungal diseases of the *Gmelina arborea* seedlings.

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