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Research Paper

Antibio-Deterioration Activity of Selected Plant Extracts on Chrysophylum Africanum A. DC Fruits

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The effect of the environment and microorganisms on the biodeterioration of plants cannot be underestimated with respect to reduction in the nutrient constituents of the host plant. This study was aimed at investigating the effect of extracts obtained from selected plants having antimicrobial properties on the biodeterioration of Chrysophylum africanum A. DC, (Sapotaceae). The fruit is indigenous to Southern Nigeria and is susceptible to biodeteriogens within 5 days of storage. Rotten and healthy fruits were obtained from Bodija and Oja-Oba markets in Ibadan, south western part of Nigeria. The plants used are Nauclea diderrichii. Treculia africana, Cassia fistula, Terminalia catappa, and Jatropha multifida. Decoctions from the plant leaves were obtained using distilled water and methanol and fungi were isolated from the rotten fruit samples of Chrysophylum africanum. The isolated fungi were cultured on sterile PDA plates containing different concentrations (15-50%) of the leaf decoctions. The isolated fungi were Aspergillus niger, Rhizopus nigricans, and Aspergillus flavus. The percentage growth inhibition (PGI) of Aspergillus flavus showed that at 30% concentration, distilled water and methanol decoctions of Nauclea diderrichii and Cassia fistula has the same percentage growth inhibition (PGI). The PGI for Aspergillus niger ranged from 3.9 to 43.4%. The methanol extracts were observed to suppress the growth of the isolated fungi than the aqueous extracts. However, percentage growth inhibition of the respective aqueous and methanol extracts vary depending on the botanicals used for the extraction.

Keywords: Chrysophylum africanum fruit, antibio-deterioration activity, plant extracts

INTRODUCTION

Chrysophyllum africanum A. DC known as the African star apple belongs to the family Sapotaceae which has up to 800 species and make up almost half of the order Ebernales (Ehiagbonare et al., 2008). It is primarily a forest tree species and its natural occurrence has been reported in Nigeria. The plant often attains a height of 36.5m though it may be smaller (Bada, 1997). The juice from the fruit is a potential source of soft drinks, and can be fermented for production of wine and alcohol (Opeke, 1982). The seeds have been studied to serve as feed ingredient for livestock following its utilization at 5% inclusion level by rats, thus contributing to reduce the ever increasing cost of livestock feeds (Madubuike and Okereke, 2003). Ogbe et al., (1992) reported that the fruit contains 8.8% protein, 17.5% oil, 21% sugar, 11% starch. The fruit also contain high content of ascorbic acid, 90 % anacardic acid used in protecting wood and as a source of resin (Adewusi, 1997) while other component of the tree including the roots and leaves are used for medicinal purposes (Bada, 1997). As a medicinal plant, it is

commonly used as antimicrobial, antiinflammatory and antioxidant agent (Idowu et al., 2006). Chrysophyllum africanum mucilage has been studied and evaluated as a binding agent in Paracetamol tablet formulations in comparison with methylcellulose (Ajala et al., 2016).

The fruits of *Chrysophyllum africanum* are not usually harvested from the tree but are left to drop naturally to the ground where they are picked. The fruits which could take up to about 10 days after harvesting to get to the markets in major cities, however, become bad within 5 days of storage resulting into huge losses due to biodeterioration (Adebisi, 1997). The observed susceptibility of the African star apple fruit to bio-deterioration disorder has been reported to be mediated by microbial infection insects thereby suggesting the disinfestations and fungicide treatment prior to storage (Adindu et al., 2003). For example, eight fungi were isolated from the deteriorating African star apple fruits and Aspergillus niger was the most prevalent of all the

isolates (Amusa *et al.*, 2003). Therefore, a possible method of controlling post-harvest disease could be the use of extracts from locally available medicinal plants which have long being successfully used against human disease (Sofowora, 1984). Biological control had attained importance in modern agriculture to control the hazards of intensive use of chemicals for pest and disease control (Tauber and Baker, 1988).

Nauclea diderrichii (De Wild. and T. Durand) Merrill is a large African forest tree yielding a strong hard yellow to golden brown lumber. It belongs to the family Rubiaceae, which is a widely distributed family of mostly tropical trees, shrubs and herbs (Dupuy and Mile 1993; Formecu, 1999). Nauclea diderrichii (N. diderrichii) was one of the dominant plantations in Nigeria (FAO, 1981). Some of its native names are: Opepe in Nigeria, Badi in Ivory Coast, Kusia in Ghana and Bilinga in Gabon. The root bark extract has been studied for its antimicrobial activities and wound healing effects (Akunne et al., 2017). Antimalarial and antiplasmodial activities of Nauclea diderrichii have also been reported (Lamidi et al., 1996; Mustofa et al., 2007)

Treculia africana (Decne. ex Trec) belongs to the family Moraceae and it is commonly known as African breadfruit and locally called 'ukwa' in Igbo vernacular. It is a tree, rarely shrub, 5–20 m tall, and a highly valued medicinal plant (Enibe, 2006). The crude extracts from the bark of the plant has been studied as an antibacterial agent in the treatment of lower abdominal pains caused by some bacterial pathogens of gastrointestinal tract (Ogbonnia et al., 2008). The phytochemical properties of the stem bark of Treculia africana include alkaloids, flavonoids, saponins and anthraquinone.

Cassia fistula (Linn) belongs to the family leguminosae-ceasalpinoidea. Ethno medicinal uses of Cassia fistula (C. fistula) plant include as antidiarrhorea antidysenterial agent (Manandhar, 1989), antidiabetic and in the treatment of skin diseases (Chopra et al., 1992). In addition, its antifungal properties have been recognised (Duraipandiyan et al., 2012). Terminalia catappa (T. catappa) (L.) belongs to the family of Combretaceae and commonly known as the tropical almond. It is a medium sized tropical trees. Extracts of the leaves and barks of the plant have been reported to antioxidant. hepatoprotective. anticancer. antiinflammatory, antihepatitis and aphrodisiac properties (Ahmed et al., 2005). The ethanol extract of the leaves has been reported to inhibit osmotically-induced haemolysis of human erythrocytes in a dose-dependent manner (Chen et al., 2000).

Jatropha multifida (J. multifida) Linn is a species of the genus Jatropha belonging to the family Euphorbiaceae and is commonly known as coral bush (Dehgan, 1982). In Nigeria, the leaves have been reported to be useful in the treatment of oral thrush, constipation and fever (Kayode and Omotoyinbo, 2009). The methanol, ethyl acetate, chloroform and n-hexane extract of the roots have been

shown to have antibacterial activity particularly against *Bacillus subtilis* and *Staphylococcus aureus* (Aiyelaagbe, 2001). This research was therefore embarked upon to investigate the effect of the leaf extracts of *N. diderrichii, T. Africana, C. fistula, T. catappa and J. multifida* on the bio-deterioration of *Chrysophylum africanum* A. DC.

The overall intent is to use medicinal plants to serve as protective means for *C. africanum* fruits against biodeterioration. This will reduce wastage of the fruits, boost economic output for farmers and preserve the nutrients in the fruits for the advancement of human health.

MATERIAL AND METHODS

Sources and collection of materials

African Star apple (rotten and healthy ones) were obtained from Bodija Market 7° 26' 6.216" N 51.512" E and Oja-Oba market 7° 23' 47.299" N 3° 55' 0.674" E. Ibadan in south western part of Nigeria during its season in March 2010. Fresh samples of T. catappa and C. fistula leaves were collected from the nursery of the Department of Botany University of Ibadan, Ibadan while T. africana, N. diderrichii and J. multifida were obtained from the Forestry Research Institute of Nigeria, Jericho hill, Ibadan. The plants were identified and authenticated at the Forestry Research Institute of Nigeria (FRIN) herbarium and the Department of Botany Herbarium. Plants were collected in sterile poly bags and labelled accordingly; and taken to the Pathology Laboratory of Department of Botany, University of Ibadan for the experiments.

Isolation of fungal pathogens from the African star apple

Fresh fruits were rinsed in distilled water and surfacesterilized.

They were put inside a white polythene bag and tightened after a moist cotton wool has been placed inside it. This provided a moisturized environment for the fruit while disallowing the entrance of microorganisms to hasten its biodeterioration. Rotten fruits were surface-sterilized with cotton wool soaked with 70% ethanol solution.

The innocula were taken from the rotten part with a sterile spatula and aseptically transferred onto solidified sterile Potato Dextrose Agar (PDA) medium in Petri dishes. About four sliced parts were placed at regular distance from one another on the medium surface and the 5th piece at the centre of the plate. The plates were incubated at 27 °C for 2-5 days and were examined daily. After 5 days of incubation, fungal growths were examined.

Determination of percentage incidence and selection of dominant fungal strains

The percentage incidence of the isolated fungal strains from the fruits was determined using equation 1 according to Whipp, (1987):

Incidence (%) =
$$(F_X/F_0) \times 100$$

Where

F_X = Number of isolated fungal species

 F_0 = Total number of fungal species isolated

Test for pathogenicity of the isolated fungal strains

To establish that the fungi isolated are the causative organism of the rotten fruits, pathogenicity tests on a fresh healthy fruit were carried out. A healthy African star apple fruit was surface-sterilized by cleaning with 70% ethanol solution with a cotton wool and a hole was made on the surface with the aid of a sterile cork-borer; which made a hole on the fruit. An inoculum from an established pure culture plate was placed inside the hole made on the test fruit sample. The hole was covered with the fleshy part removed earlier and covered with sterile Vaseline. This was allowed to incubate at 27°C with each preparation observed daily for signs of rottenness.

Processing and preparation of plant extracts

The plant leaves used were processed by washing samples with sterile-water and closely examined for the presence of dried and deteriorated leaves which were removed alongside extraneous materials. They were later air dried. The extraction was done by maceration using sterile distilled water and methanol. Approximately about 100 g of the plant processed leaves was weighed in a 1000 mL conical flask with 500 mL of sterilized distilled water and methanol respectively for 48 h. The extract was filtered with a filter paper and the marc discarded. The extracts were stored in air tight container from which the concentration used were prepared.

Direct medium treatment

The PDA/crude extract medium was prepared by spreading 1 mL of the extract separately on the surface of the solidified PDA in the Petri dishes. With the aid of sterile cork-borer, 3 mm diameter discs of five-day old of the test fungi isolate were inoculated and the plates examined for growths for a period of 5 days when the control mycelia had reached the edge of the Petri dish. Four concentrations (15, 25, 30 and 50 %^V/_V) of the plant

extracts were used. The growth measurement of the mycelia was taken after 5 days of incubation and the average diameter of the mycelia growth in triplicate plates for each concentration was calculated and recorded. The potency of the test plants was measured by their inhibition of the fungal growth while the percentage inhibition of mycelia growth was calculated as shown in equation 2:

%growth inhibition=(D0-Dt)/D0×100

2

Where

 D_0 = Diameter of mycelia growth in the control D_t = Diameter of mycelia growth in the treatment.

Statistical analysis of data

The data collected were categorized and analyzed using SPSS version 20. The means were separated with Duncan Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

The dominant fungi isolated from most the biodeterioration of C. africanum fruit based on the frequency of occurrence of the isolate fungi from rotten C. africanum collected at the two locations (Table 1) were identified to be Aspergillus niger and Aspergillus flavus as shown in (Figure 1). Other isolated biodeteriorating fungi include Rhizopus nigricans, Fusarium compactum and Fusarium oxysporum which were all tested to be pathogenic to C. africanum fruit as presented in (Table 2). The stored fruits were found to become infected from the third day of storage while biodeterioration in the fruit became manifested after seven days. This must have been because the infected fruits were not separated from the non-infected ones as reported in a past study (Adebisi, 1997). Separation of infected fruits from the others would definitely reduce the spread of the infection. However for a fruit farmer or seller, the task of separation could be herculean especially when larger quantities are handled. This is why the use of antibio-deteriorating agents are necessary.

The result obtained from the bioassay of the plants on the fruits showed an inhibitory effect on the growth of the two fungi isolated for study, but at different degrees. This could be attributed to the different phytochemical constituents and antifungal properties of the respective plants. The highest percentage growth inhibition of *Aspergillus flavus* was at 50 %^V/_v concentration of methanol extract *T. catappa* as shown in (Figure 2a) while the least percentage growth inhibition occurred at 15 %^V/_v aqueous extract of *N. diderrichii* as presented in (Figure 2b). Generally, from the all the figures, the plant

Table 1. Frequency of occurrence of the isolated fungi from *Chrysophylum africanum* fruit collected at the two locations.

Fungal Isolates	Incidence of fungal isolates in	Incidence of fungal isolates in		
	C. africanum fruit obtained from Bodija market (%)	C. africanum fruit obtained from Oja-Oba market (%)		
A. niger	68	71		
A. flavus	54	60		
Rhizopus nigricans	24	22		
F. compactum	22	15		
F. oxysporum	18	14		

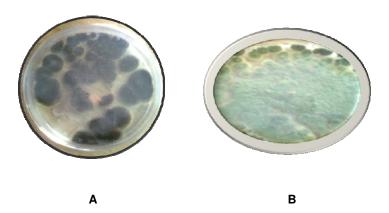


Figure 1. The macroscopical appearance of the isolated fungi responsible for the biodeterioration of *C. africanum* fruits: A-Aspergillus niger; B-Aspergillus flavus.

Table 2. Pathogenicity of isolated fungi from Chrysophylum africanum fruit collected from the two locations.

Fungal Isolates	Pathogenicity of fungi isolated from <i>C. africanum</i> fruit obtained from Bodija Market	Pathogenicity of fungi isolated from <i>C. africanum</i> fruit obtained from Oja Oba Market
A. niger	+	+
A. flavus	+	+
Rhizopus nigricans	+	+
F.compactum	+	+
F. oxysporum	+	+

extract demonstrated a concentration-dependent activity against the organisms responsible for biodeterioration of *C. africanum* fruits. In (Figure 3b), the methanol extract (50 % $^{\text{V}}$ / $_{\text{V}}$) of *Cassia fistula* demonstrated the highest activity in inhibiting the mycelial growth of *A. niger* while as shown in (Figure 3a), the aqueous extract of *N. diderrichii* (15 % $^{\text{V}}$ / $_{\text{V}}$) offered the least mycelial growth inhibition on the same organism. As shown in (Figure 2a), the percentage growth inhibition of *Aspergillus flavus* reveals that 30 % $^{\text{V}}$ / $_{\text{V}}$ aqueous extract of *N. diderrichii* and *C. fistula* has the same percentage growth inhibition.

The methanol extract of *N. diderrichi* on the mycelial growth of *A. flavus* was significant at 50 % v / $_{v}$ concentration of the extracts to the control, this could not be said of the aqueous extract of *N. diderrichi* which showed no significant difference (p >0.05) compared to the control at all concentrations tested (Table 3). Table 4

showed that the effect of 50 % $^{V}/_{V}$ methanol extracts of J. multifida was significantly different (p \leq 0.05) compared to the control and other concentration levels on the mycelial growth of both A. flavus and A. niger. Another trend observed from the result of the bio assay showed that the percentage growth inhibition of Aspergillus niger increased in comparison with the mycelial growth inhibition of Aspergillus flavus for all the plant extracts and concentrations used. The significant increase in the percentage inhibition of the studied fungi as the concentration increased indicate the ability of the solvents used for extraction to be able to remove the biologically active compound from the plants used required to inhibit the growth of the studied fungi at higher concentrations. The organic (methanol) extracts possessed a higher inhibitory potential on the test isolate compared to distilled water extract, this could be as a

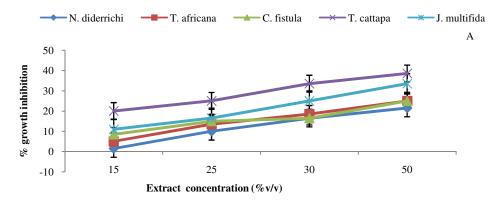


Figure 2a. The effect of aqueous extract concentration on the mycelia growth inhibition of *Aspergillus flavus*.

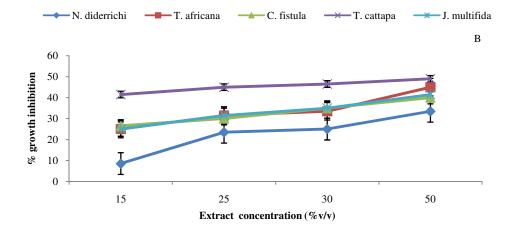


Figure 2b. The effect of methanol extract concentration on the mycelia growth inhibition of *Aspergillus flavus*.

result of the extraction potential of the solvent used which conformed with the report of Preethi (2010).

Generally, as shown in (Table 3), the aqueous extracts demonstrated a concentration-dependent activity on the mycelia growth of *A. flavus*. The ranking of plant extract activity was *T. africana >J. multifida > C. fistula >T. catappa >N. diderrichi* at the highest concentration while the order changed at the lowest concentration to *T. catappa> C. fistula> J. multifida> T. Africana>> N. diderrichi*. This indicates that *N. diderrichi* aqueous extract has lower activity in comparison with the other plants irrespective of concentration of use. Comparing the activity of the extracts to the control (distilled water), the difference was statistically significant (p<0.05), thus the effect seen in the extracts could be regarded as real and may be attributed to the presence of bioactive metabolites.

The mean effect of the methanol extracts on the mycelia

growth of *A.flavus* is presented in (Table 4.)

The methanol extracts showed a slightly higher activity than the aqueous extracts on the mycelia growth of A. The activity on the organism was also concentration-dependent for all the plants. Methanol as a solvent is expected to pull both polar and non-polar compounds out from the leaves during maceration. The higher activity observed from the methanol extracts in comparison to the aqueous extracts could therefore be due to other metabolites not present in the aqueous extracts. The ranking of plant extract activity at lower concentration was J. multifida > C. fistula> T. catappa> T. Africana> N. diderrichi while the order at highest concentration was T. Africana> C. fistula> T. catappa= J. multifida> N. diderrichi. In both types of extract, N. diderrichi had the least effect on the mycelia growth. Table 5 shows the mean effect of the aqueous extracts on the mycelia growth of Aspergillus niger. Generally, the

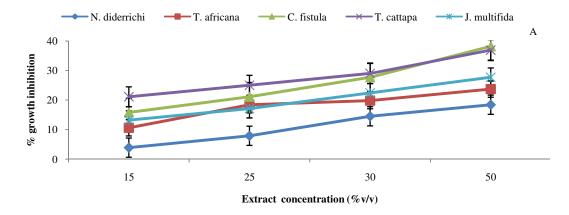


Figure 3a. The effect of aqueous extract concentration on the mycelia growth inhibition of Aspergillus niger.

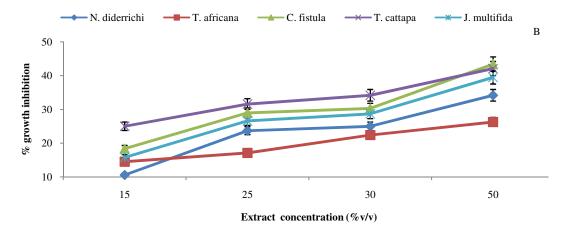


Figure 3b. The effect of methanol extract concentration on the mycelia growth inhibition of *Aspergillus niger*.

Table 3. The mean effect of the aqueous extracts on the mycelia growth of *Aspergillus flavus* (Mean ± SD; n=3).

Plant Extract	Concentration (% ^v / _v)				
	15	25	30	50	Control (without extracts)
N. diderrichi	1.29 ± 0.02 ^a	1.52 ± 0.02 ^a	1.73 ± 0.02 ^a		0.53 ± 0.01 ^a
T. africana	2.11± 0.02 ^a		2.42 ± 0.02^{b}		0.53 ± 0.01 ^a
C. fistula	2.32 ± 0.02^{a}		2.52 ± 0.02^{a}		0.53 ± 0.01 ^a
T. cattapa			2.44 ± 0.02^{a}		0.53 ± 0.01 ^a
J. multifida	2.15 ± 0.03 ^a	2.42 ± 0.02^{a}	2.44 ± 0.01 ^a	2.55 ± 0.02^{b}	0.53 ± 0.01^a

Means with the same letter are significantly (p \leq 0.05) different from one another.

aqueous extract showed a concentration-dependent activity on the mycelia growth of *A. niger* except for *T. catappa* extract whose activity reduced at the highest concentration. There were statistically significant differences (p<0.05) between the activity of the plant extracts and the control showing that the effects observed were due to the extracts and phyto-constituents within. The aqueous extracts also offered higher activity

against *A. niger* in comparison to *A. flavus* as shown in (Table 3). The ranking of plant extract activity against the organism at the lowest concentration was *T. catappa> C. fistula> J. multifida>T. Africana> N. diderrichi* while at higher concentration, it was *T. Africana> C. fistula> T. catappa> J. Multifida = N. diderrichi.*

As in the effect of methanol extract against the mycelia growth of *A. flavus*, the activity of the methanol extracts

Table 4. The mean effect of the methanol extracts on the mycelia growth of *Aspergillus flavus*. (Mean \pm SD; n=3).

Plant Extract	Concentration (% ^V / _v)				
	15	25	30	50	Control (without extracts)
N. diderrichi	1.42 ± 0.02 ^a	1.56 ± 0.03 ^a		2.43 ± 0.02^{ab}	0.53 ± 0.01 ^a
T. africana	2.13 ± 0.12 ^b	2.45 ± 0.01 ^{ab}	2.55 ± 0.02^{a}	2.65 ± 0.12^{a}	0.53 ± 0.01 ^a
C. fistula	2.44 ± 0.02^{a}	2.01 ± 0.02^{b}	2.55 ± 0.02^{a}	2.59 ± 0.02^{b}	0.53 ± 0.01 ^a
T. cattapa	2.43 ± 0.02^{a}	2.45 ± 0.02^{a}	2.45 ± 0.02^{a}	2.54 ± 0.02^{a}	0.53 ± 0.01 ^a
J. multifida	2.45 ± 0.03^{a}	2.51 ± 0.02^{a}	2.53 ± 0.02^{b}	2.54 ± 0.02^{ab}	0.53 ± 0.01 ^a

Means with the same letter are significantly (p \leq 0.05) different from one another.

Table 5. The mean effect of the aqueous extracts on the mycelia growth of *Aspergillus niger* (Mean \pm SD; n=3).

Plant Extract	Concentration (% ^v / _v)				
	15	25	30	50	Control (without extracts)
N. diderrichi	2.09 ± 0.02^{a}	2.49 ± 0.02^{a}	2.44 ± 0.02 ^b	2.51 ± 0.02 ^a	0.34 ± 0.01 ^a
T. africana			2.42 ± 0.02^{a}		0.34 ± 0.01 ^a
C. fistula	2.37 ± 0.03^{a}	2.48 ± 0.03^{a}	2.61 ± 0.02^{a}	2.71 ± 0.03^{ab}	0.34 ± 0.01 ^a
T. cattapa	2.43 ± 0.01^{a}	2.39 ± 0.12^{a}	2.83 ± 0.02^{a}	2.59 ± 0.02^{a}	0.34 ± 0.01 ^a
J. multifida	2.19 ± 0.02^{a}	2.41 ± 0.02^{a}	2.49 ± 0.02^{a}	2.51 ± 0.02^{b}	0.34 ± 0.01 ^a

Means with the same letter are significantly (p \leq 0.05) different from one another.

Table 6. The effect of the methanol extracts on the mycelia growth of Aspergillus niger (Mean ± SD; n=3).

Plant extract	Concentration (% ^V / _v)				
	15	25	30	50	Control (without extracts)
N. diderrichi		2.52 ± 0.01 ^a		3.12 ± 0.02^a	0.34 ± 0.01 ^a
T. africana	2.19 ± 0.02^{b}	2.28 ± 0.02^{b}	2.43 ± 0.02^{a}	2.88 ± 0.02^{a}	0.34 ± 0.01 ^a
C. fistula	2.44 ± 0.01^{b}	2.50 ± 0.02^{a}	2.65 ± 0.12^{a}	2.72 ± 0.03^{b}	0.34 ± 0.01 ^a
T. cattapa	2.47 ± 0.01^{a}	2.42 ± 0.02^{b}	2.89 ± 0.02^{a}	2.90 ± 0.02^{ab}	0.34 ± 0.01 ^a
J. multifida	2.22 ± 0.01^{a}	2.34 ± 0.01^{a}	2.36 ± 0.01^{a}	2.42 ± 0.01^{b}	0.34 ± 0.01^{a}

Means with the same letter are significantly ($p \le 0.05$) different from one another.

on A. niger was more pronounced than the aqueous extracts. In addition, the activity on A. niger is higher than on A. flavus meaning that the former is more susceptible to the metabolites in the extracts than the latter. There were significant differences (p<0.05) in the activity of the plant extracts in comparison with control showing that the effect observed were not due to chance and could be attributed to the extracts and the metabolites within them. The ranking of the methanol extract activity at lower concentration against A. niger was T. catappa> C. fistula> J. multifida> T. Africana> N. diderrichi while at higher concentration the order was N. diderrichi >T. catappa> T. Africana> C. fistula> J. multifida. The effect of N. diderrichi on this organism seem to be significant even at lower concentration both for aqueous and methanol extracts.

Conclusion

This study has shown the effectiveness of the extract of *T. cattapa* and *C. fistula* in inhibiting the microbial growth

of *A. flavus* and *A. niger* respectively. The efficacy of the extracts of *N. diderrichi*, *T. africana* and *J. multifida* was also observed to be significant at varying degrees. The methanol extract of *T. cattapa* and *C. fistula* showed the tendency to inhibit the growth of the biodeteriogens more than its aqueous extracts. Therefore, the use of the extracts from medicinal plants should be encouraged and preferred to the use of pesticides to help reduce cost and safeguard the environment against possible hazard that may arise as a result of the constant use of fungicides on fruits and crop species.

The preparation of extracts from medicinal plants for disease control is relatively cheap compared to the expensive conventional fungicides. Hence, the inhibitory effects of the studied plant extracts could be used as a progressive step for the *in vivo* biological control of phytopathogenic fungi, thereby reducing over reliance on chemical fungicides.

Further research should be encouraged to ascertain the active biological compound (secondary metabolites) which inhibited the growth of the studied phytopathogenic fungi.

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Authors' declaration

We declare that this study is an original research by our research team and we agree to publish it in the journal.

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