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Phytochemical screening of various species of cola nut extracts for antifungal activity against phytopathogenic fungi

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Abstract

The antifungal activities of aqueous and methanol nut extracts of *C. nitida* and *C. acuminata* against phytopathogenic fungi namely; *Aspergillus niger*, *Aspergillus fumigatus, Rhizopus oryzae*, and *Mucor racemosus* were investigated using agar incorporation method. A significant difference ($p<0.05$) in extracts concentration activities was observed at varying concentrations (mg/ml) of 40, 60, 80 and 100. The highest percentage inhibition and extract sensitivity was recorded 82.96 in *Rhizopus oryzae* at 100mg/ml of aqueous extract of *C. nitida* and 81.2 for aqueous extract of *C. acuminata* at 100mg/ml respectively. In methanol extract *Rhizopus oryzae* shows a percentage growth inhibition of 72.76 in *C. nitida* and 71.85 in *C. acuminata* at 100ml/mg respectively. For *Mucor racemosus*, the percentage growth inhibition was 73.70 and 72.40 for the aqueous extracts of *C. nitida* and *C. acuminata* at 100mg/ml, while the methanol extracts was 66.66 and 66.29 at 100mg/ml for *C. nitida* and *C. acuminata* respectively. *Aspergillus fumigatus* shows 29.25 and 31.60 percentage inhibition for the aqueous extracts of *C. nitida* and *C. acuminata* at 100mg/ml, while in 100mg/ml of methanol extracts, the percentage inhibition was 40.88 and 38.88 for *C. nitida* and *C. acuminata* respectively, and there is no inhibition at 60 and 40mg/ml of the aqueous and methanol extracts of *C. nitida* and *C. acuminata* respectively. For *Aspergillus niger* at 100mg/ml of aqueous extact, the percentage growth inhibition was 69.62 and 65.52 for *C. nitida* and *C. acuminata*, *while* in 100mg/ml of methanol extracts, the percentage growth inhibition was 65.18 and 65.10 for *C. nitida* and *C. acuminata* respectively, and there is no inhibition at 60 and 40mg/ml of the aqueous and methanol extracts of *C. nitida* and *C. acuminata* respectively. In all the extracts, the percentage growth inhibition increases with increase in concentration of extracts in all the test organisms. However, from the two extracts used, they have shown a closely related percentage growth inhibition against all the isolate organism. Finally, the finding from this study reveals a rich source of bioactive compounds essentials in diseases treatment, and suggest the use of the extracts in herbal curl remedies.

Keywords

*C*ola *Nitida*, *Cola Acuminata,* Anitfungal Activy

1. Introduction

Cola is one of the largest genera in the family Sterculiaceae and is related to the South American genus *Theobroma*. It comprises of evergreen moderately sized trees often growing to a height of 20m with glossy ovoid

leaves up to 30cm long. *Cola* species are found mostly in the relatively dry parts of the rain forest, although *Cola millenii* and *Cola gigantea* are widely distributed in wet and dry forest environments (Kuoame and Sacande, 2006). The mature fruit of *Cola* species is a nut known as kola nut (Duke, 2001). It is chewed in many West African cultures. It is often used ceremonially, presented to tribal chiefs or to guests. Chewing kola nut can ease hunger pangs. Kola nuts are used mainly for their stimulant and euphoriant qualities. They have effects similar to other xanthine containing herbs like cocoa, and tea. However, the effects are distinctively different, producing a stronger state of euphoria and wellbeing (Benjamin *et al*., 1991). They have stimulant effects on the central nervous system and heart. Kola nuts are used as a source of alkaloids in pharmaceutical preparations (Newall *et al*., 1996).

Kola nut generally contains 2- 3% caffeine and smaller amount of theobromine and kolanin which tend to dispel sleep thirst and hunger. The nut contains 10% protein, 1.35% fats and 4% starch. Kola nuts are often used to treat whooping cough and asthma. The caffeine present acts as a bronchodilator, expanding the bronchial air passages (Jayeola, 2001). Benjamin *et al*., (1991) reported that the leaves of *Cola millenii* are used in the treatment of ringworm, scabies, gonorrhoea, dysentery and opthalmia.

Infectious diseases of both plants and animals by fungi account for higher proportion of health problems in developing countries including Nigeria. Microorganisms have developed resistance to many synthetic fungicides and antibiotic. The resistance of the microorganisms increases due indiscriminate use of commercial antifungal agents. This situation force scientists and researchers to search for new antifungal substances from various sources including medicinal plants (Ali-Shtayeh, 1999). The perceived negative effect of the synthetic fungicide, bacteriocide and pesticides used on agricultural land and water increases public awareness of risk involved in synthetic chemicals. Much attention is being focus on alternative method of pathogen control (Amusa and Odubaku, 2007).

Plant extracts provide attractive alternative to currently used synthetic fungicides as regards control and management of pathogenic fungi since they constitute a rich source of bioactive molecules (Wink, 1993). They are often active against a limited number of specific target pest, biodegradable, and nontoxic products. Therefore, recent efforts have been directed toward the development of plant secondary metabolites as useful products for commercial fungicide or lead compound (Kim *et al*., 2003). The use of many synthetic fungicides has now been restricted because of undesirable toxicity that is persistence in nature and their property of biological accumulation in the food chain and their indiscriminate power to destroy both useful and harmful pest. It is therefore necessary to look for new cheap, effective biodegradable substance of biological origin to control micro-organisms. Green plants are reservoir of effective motherapeutants and can provide useful pesticide which is largely non phyto-toxic more systemic and easily biodegradable. Some of the products of higher plants have been shown to be effective source of chemotherapeutic agent and provide a renewable source anti-microbial of bio-degradable nature which is devoid of side effect (Farombi, 2003).

The presence of antifungal compounds in higher plants has long been recognized as an important factor in disease resistance. Such compounds been selective in their toxicity are considered valuable for controlling some plant diseases. In addition, plant extracts might have inhibitors to enzymes from invading pathogens and the effect of different phenolic compounds on the germination and growth of many fungal pathogens have been reported. Therefore, there is need to carry out scientific investigation to ascertain the authenticity of the claims on medicinal properties of cola nut species.

The aim of this research is to evaluate antifungal activities of water and methanol extracts of *Cola nitida* and *Cola acuminate* through the following objectives:

1 Evaluate the antifungal activity of water and methanol extracts, on the growth Aspergillus niger, Aspergillus fumigatus, Mucor racemosus, Rhizopus oryzae of C. nitida and C. acuminata
2 Compare and evaluate the highest percentage of growth inhibition at varying concentrations of the extracts.

2. Materials and Methods

2.1. Study Site

The experiments were conducted in the Mycology Laboratory, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto for the *in vitro* test of efficacy. Sokoto is located in a dry - sub humid agro ecological zone (lat. 13^0 N, North. 5^0 east, and 350 meters above sea level). The annual rainfall ranges from 250 to 500mm unimodal rainfall pattern. The rainy season starts and establishes between mid May to early June and reaches its peak in August. Dry season starts in the mid October and end in late April. The hottest months are March to May while coldest months are November to January which are characterized by dry harmattan Period (that is between March and May). The mean annual temperature is 27.8^0C (Ojanuga, 2006).

2.2. Collection of Samples

Five kilograms (5kg) each of *C. nitida* and *C. acuminata* were purchased at Sokoto Central market. The nuts were identified and authenticated by the curator of herbarium in the Department Biological Sciences, Botany unit of the Usmanu Danfodiyo University, Sokoto, where the voucher specimens were deposited.

The nuts were washed with tap water and air dried under the shade. Dried materials were grounded to fine powder using pestle and mortar and kept in a sterile plastic bag at temperature of 25± 2^0C for further use (Evans, 1999).

2.3. Preparation of Extracts

One hundred (100g) of each Cola *nitida* and Cola *acuminata* were separately placed in a round bottom flask containing one litre each of water and methanol. This was followed by mixing and agitation for six hours and it was allowed to stand for 24hours. The mixtures were filtered using muslin cloth and concentrated into powder by subjecting to heat using hot plate, the powdered dry extracts were scrapped off using spatula. The dried extracts obtained were used directly for the determination of *in vitro* antifungal activity (Evans, 1999).

2.4. Sample of Test Organisms

The pure samples of test organisms were collected from the Mycology laboratory of Department of Biological Sciences, Usmanu Danfodiyo University Sokoto.

2.5. Sterilization of Materials, Inoculation Room and Chamber

The glass ware were adequately washed with detergent and sufficiently rinsed with tap water and distilled water and then air dried. They were later sterilized in hot air oven at 100^0C for an hour, while conical flasks are autoclaved. Sterilization of inoculation room and chambers were done by boiling 4% formalin solution for 30 minute in the inoculation room 12 hours before use. The inoculation chamber and table were surface sterilized with cotton wool soaked in absolute alcohol prior to use (Evans, 1999).

2.6. Media Preparation

The basalum medium used was sarboroud dextrose agar (SDA) and was prepared according to manufacturer's instructions. Sixty two grams powder of SDA were weighted and dissolved in 1 litre of distilled water separately. Streptomycin solvent was added to inhibit bacteria growth. The conical flasks containing the media were then plugged with cotton wool, capped with aluminium foil, and then sterilized by using autoclave at 121^0C for 15 minute. The media was made to cool down to 45^0C before pouring in to sterile plates and the plates were kept at room temperature to set (Evans, 1999).

2.7. Working Concentration of the Extracts

For testing the antifungal effects, water and methanol extracts of nuts of *C. nitida* and *C. acuminata of* four different concentrations (40mg/ml, 60mg/ml, 80mg/ml, and 100mg/ml) were prepared for each extracts (Evans, 1999).

2.8. Negative Control Sample

Twenty mills (20ml) each of the plane SDA media solution was autoclaved in conical flasks then transferred when cool in to the petri dishes. After solidification each fungal isolates was transferred on the SDA surface with an inoculation needle (Evans, 1999).

2.9. Positive Control

Ketoconozole was measured from pulverized 500mg tablets. 5ml of water solution of Ketoconozole at concentration 40 and 100mg/ml were aseptically mixed with 15ml of SDA. After cooling and solidification of the medium the seeding was carried out by inoculating needle of the fungal isolates in the middle of petri-dishes. The treated and control petri-dishes were incubated at ambient laboratory condition for 72hours. Three replicates for each concentration were prepared and then the growth was observed (Evans, 1999).

3.8. Antifungal Efficacy of Cola Nut Extracts

Agar incorporation method was used to determine the antifungal effect of Cola nuts extract. Conical flasks of 100mls size were used in this experiment for each of the different extract. In each of the flask, 15ml of the SDA media was poured, plugged with a cotton wool and capped with aluminium foil before sterilization.

Five (5) ml of each of the varying concentrations of the two Kola nuts extracts were singly incorporated into each flask containing 15mls of the media, then poured in to the pre-sterilized Petri dishes and kept at room temperature (28^0C). For 3-7 days an actively growing culture of test isolates respectively were punched with a sterile cork borer and impregnation were made with the aid of sterile inoculating needles and then directly deposited at the centre of the Petri dishes containing varying concentrations three replication and maintained for each test isolates (Evans, 1999).

2.9. Linear Measurement Method

The result was measured in millimetres (mm) by accessing the fungal growth from two lines, vertical and horizontal and mean taken. The mean of the 3 replicates served as the result of each of the varying concentrations. The antifungal activities in terms of percentage inhibition of the mycelia growth were calculated using the formula (Evans, 1999).

$$\text{Percentage inbibition} = \frac{C-T}{C} \; X \; 100$$

Where

C = average increase in mycelia growth in control plates.
T= average increase in mycelia growth in treated plates

2.10. Statistical Analysis

Data obtained from the studies were subjected to statistical analysis using Statistical Package for social sciences (SPSS) version 16.0. Analysis of variance (ANOVA) was carried on the data and means were separated using Duncan Multiple Range Test (DMRT).

3. Results and Discussion

3.1. Results

Table 1. *Effect of aqueous cola nuts extracts on the growth of phytopathogenic fungi.*

Cola nut	Conc (mg/ml)	Fungi/ percentage growth inhibition. Rhizopus oryzae.	Mucor recemosus.	Aspergillus niger.	Aspergillus fumigatus
C.nitida	40	23.14±0.92[a]	33.70 ± 3.22[a]	0.00 ± 0.00[a]	35.92 ± 1.16[a]
	60	51.48 ± 3.03[b]	45.00 ± 3.15[b]	0.00 ± 0.00[a]	43.70 ± 1.61[a]
	80	60.48 ± 3.21[c]	60.37 ± 1.64[c]	18.51 ± 0.72[b]	54.44 ± 3. 78[b]
	100	82.96 ± 1.64[d]	73.70 ± 3.74[d]	29.25 ± 1.76[c]	69.62 ± 2.25[c]
Keto conozole	40	75.85 ± 0.76[e]	54.52 ± 0.[9e1]	52.37 ± 0.19[e]	52.37 ± 6.01[d]
(positive control)	100	98.22 ± 0.45[f]	92.52 ± 0.42[f]	87.26 ± 0.19[f]	90.11 ± 0.56[e]
SDA (-ve) control		0.00 ± 0.00[g]	0.00 ± 0.00[g]	0.00 ± 0.00[g]	0.00 ± 0.00[f]
C. acuminata	40	19.62 ± 0.927[a]	36.29 ± 2.25[a]	0.00 ± 0.00[a]	35.00 ± 1.60[a]
	60	50.92 + 2.98[b]	44.44 ± 3.20[b]	0.00 ± 0.00[a]	40.00 ± 1.28[a]
	80	63.43 ± 3.37[c\]	59.60 _ 1.72[c]	19.25 ± 1.12[b]	51.11 ± 2.93[b]
	100	81.29 ± 3.08[d]	72.40 ± 2.08[d]	31.60 ± 0.84[c]	65.52 ± 1.93[c]
Keto conozole	40	76.84 ± 0.77[e]	55.56 ± 3.10[e]	55.36 ± 0.19[d]	53.36 ± 6.13[e]
(positive control)	100	98.23 ± 0.32[f]	91.52 ± 0,81[f]	86.26 ± 0,19[e]	88.11 ± 0.56[f]
SDA (-ve) control		0.00 ± 0.00[g]	0.00 ± 0.00[g]	0.00 ± 0.00[f]	0.00 ± 0.00[g]

Value are mean ± standard error of 3 replication mean in a column with different superscripts are significantly different (P<0.05).

3.2. Discussion

Table (1) shows the result of the effect of aqueous extracts of *C. nitida* and *C. acuminata* on the growth of phytopathogenic fungi with a significance difference (P< 0.05) on all the fungal isolate at varying concentration.

Rhizopus oryzae had the highest percentage of 82.96 growth inhibition in *C. nitida* and 81.29 in C. acuminate at 100mg/ml respectively. The lowest percentage 19.62 growth inhibition was recorded at 40mg/ml in *C. acuminata* with 23.14 in *C. nitida* respectively. However the positive control (Ketoconozole) at 40mg/ml in *C. acuminata* had 76.34% and *C. nitida* had 75.85% inhibitory activity which was lower than the 100mg/ml concentration, and at 100mg/ml it recorded the highest percentage growth inhibition of 98.23 in *C. acuminata* and 98.21 *C. nitida* respectively.

Mucor recemosus shows significance differences (p< 0.05) with the highest percentage growth inhibition of 73.70 in *C. nitida* and 72.40 in *C. acuminata* at 100mg/ml respectively, the lowest percentage growth inhibition was recorded at 40mg/ml which indicated 33.70 in *C. nitida* and 36.29, in *C. acuminata.* However the positive control at 40mg/lm recorded lower percentage (%) inhibition as compared with highest concentration 100mg/ml, furthermore, positive control at 100mg/ml recorded the highest percentage growth inhibition of 92.52 in *C. nitida* and 91.52 in *C. acuminata* respectively.

The aqueous extracts of *C. nitida* and *C. acuminata* did not show any activity at 40mg/ml and 60mg/ml respectively, in *A. niger,* the activity started at 80mg/ml with 19.25% in *C. acuminata* and 18.51% in C. nitida. The highest percentage growth inhibition was at 100mg/ml with 29.25 in *C. nitida* and 31.60 in *C.* acuminate, the activity strength increase with increasing concentration of the extracts. The positive control at 40mg/ml and 100mg/ml recorded the highest inhibition compared to the test isolates.

Finally, the aqueous extracts of *Aspergillus fumigatus* show significance difference of (P< 0.05) at various concentrations, the highest percentage growth inhibition was at 100mg/ml and recorded 69.62% in *C. nitida* and 65.72% for *C. Acuminata* respectively; the lowest inhibition was recorded at 40mg/ml, that is 35.0% in *C. acuminata* and 35.92% in *C. nitida.* By comparison, positive control at 100mg/ml reveals greater percentage growth inhibition in *C. nitida* with 90.11% and 88.11% in *C. acuminata* respectively.

From fig. (1 and 3) it can be seen that, positive control at 100mg/ml of the aqueous extract on *Rhyzopus aryzae* shows significance difference (P< 0.05) of antifungal activity against fungal isolate at various concentration. Considering this result, the percentage inhibition was positively and significantly obtained from all fungal isolate used, wth the positive control showing more inhibitory activity at various concentration then the tested isolates.

Table (2), contains the effect of methanol extracts of *C. nitida* and *C. acuminata* on the growth of phytopathogenic fungi, it shows a significance difference (P< 0.05) on all the fungal isolate at varying concentration. The percentage fungal growth inhibition at varying concentrations of 40, 60, 80 and 100mg/ml range from 14.62 - 72.96% against *Rhizopus oryzae,* 21.29 - 66.66% against *Mucor recemosus*, 0.00 - 40.88% against *Aspergillus niger,* 26.11 - 65.18% *against Aspergillus fumigates* for C. *nitida*, while for *C. acuminata* the range was 13.73 - 71.85% against *Rhizopus oryzae*, 17.77 - 66.29% against *Mucor recemosus*, 0.00 - 38.88% against *Aspergillus niger*, 24.07 - 65.18 against *Aspergillus fumigates.* The highest percentage growth inhibition was recorded at 100mg/ml in all the test isolate but *Aspergillus niger* shows lower percentage growth inhibition even at 100mg/ml (P< 0.05). The positive control (Ketoconozole) at 100mg/ml concentration recorded the highest activity against all the fungal isolate which was significantly higher than that of the test isolates as clearly stated in fig.(2 and 4).

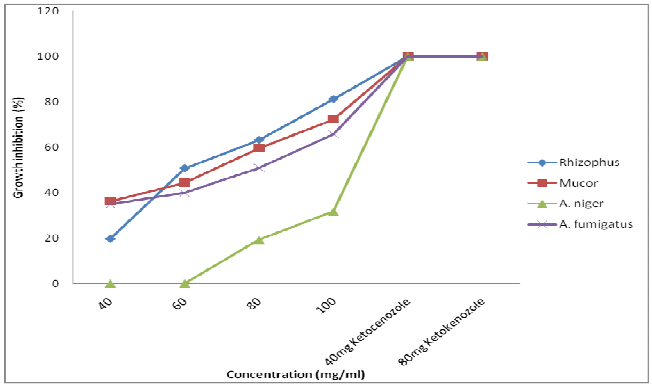

Fig 1. *Percentage inhibition of different concentration of water extract of C. acuminata nut on R. oryzae, M. recemosus, A. niger and A. fumigatus.*

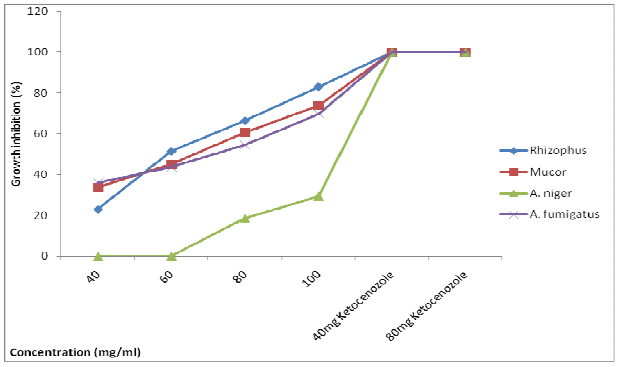

Fig 3. *Percentage inhibition of different concentration of water extract of C. nitida nut on R. oryzae, M. recemosus, A. niger and A. fumigates.*

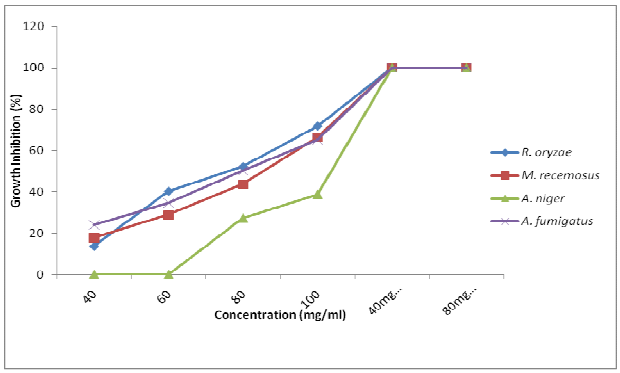

Fig 2. *Percentage inhibition of different concentration of methanol extract of C. acuminata nut on R. oryzae, M. recemosus, A. niger and A. fumigates.*

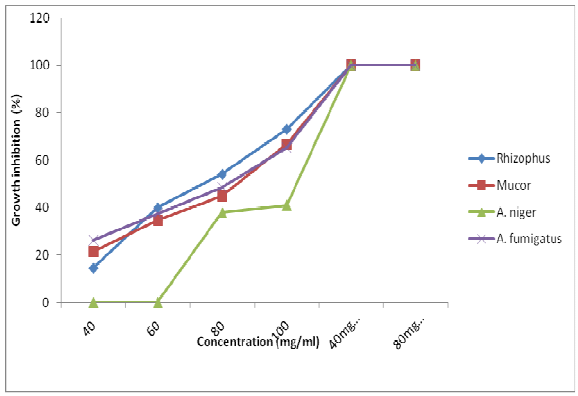

Fig 4. *Percentage inhibition of different concentration of methanol extract of C. nitida nut on R. oryzae, M. recemosus, A. niger and A. fumigatus.*

Table 2. *Effect of methanol cola nuts extracts on the growth of phytopathogenic fungi.*

Cola nut		Fungi/ percentage growth inhibition.			
	Conc. (mg/ml)	Rhizopus oryzae.	Mucor recemosus.	Aspergillus niger.	Aspergillus fumigatus
C. nitida	40	14.62 ± 2.80^{a}	21.29 ± 1.29^{a}	0.00 ± 0.00^{a}	26.11 ± 1.60^{a}
	60	40.00 ± 2.93^{b}	34.44 ± 1.69^{b}	0.00 ± 0.00^{a}	37.22 ± 2.42^{b}
	80	54.07 ± 3.22^{c}	44.81 ± 2.49^{c}	27.77 ± 1.28^{b}	48.33 ± 3. 64^{c}
	100	72.76 ± 0,67^{d}	66.66 ± 2.10^{d}	40.88 ± 1.89^{c}	65.18 ± 3.22 ^{d}
Ketoconozole	40	75.85 ± 0.57^{e}	74.54 ± 3.29^{e}	50.37 ± 0.81^{d}	55.38 ± 6.13^{e}
(positive control)	100	98.22 ± 0.32^{f}	97.33 ± 0.81^{f}	90.26 ± 0.81^{e}	92.28 ± 0.91^{f}
SDA (-ve) control		0.00 ± 0.00^{g}	0.00 ± 0.00^{g}	0.00 ± 0.00^{f}	0.00 ± 0.00^{g}
C. acuminata	40	13.7 ± 2. 67^{a}	17.77 ± 1.28^{a}	0.00 ± 0.00^{a}	24.07 ± 1.61^{a}
	60	40 37 ± 3.29^{b}	28.88 ± 2. 93^{b}	0.00 ± 0.00^{a}	34.62 ± 2.04^{b}
	80	52.22 ± 2.93^{c}	43.51 ± 3.88c	30.37 ± 1.95^{b}	50.18 ± 2.91^{c}
	100	71.85 ± 1.33^{d}	66.29 ± 2.25^{d}	38.88 ± 1.28^{c}	65.10 ± 2.25^{d}
Keto conozole	40	77. 85 ± 0.67^{e}	76.46 ± 2.88^{e}	54.0 ± 0.19^{d}	56.37 ± 5.13^{e}
(positive control)	100	96.23 ± 0.32^{f}	94. 52 ± 0.92^{f}	86.27 ± 0.19^{e}	88.11 ± 0.56f
SDA (-ve) control		0.00 ± 0.00^{g}	0.00 ± 0.00^{g}	0.00 ± 0.00^{f}	0.00 ± 0.00^{g}

Values are mean ± standard error of 3 replication mean in a column with different superscripts are significantly different (P< 0.05).

However from the two extracts used both aqueous and methanol indicated closely related percentage growth inhibition against all test isolates. The strong antifungal activity expressed by cola nut extracts used by this research also corroborates previous works of (Tiew *et al.,* 2003) where they reported the antifungal properties of other member of the family sterculiaceae.

The finding shows the effect of plant extracts on growth of fungi may vary depending on the cola species used, solvent of extraction, concentration of the extracts and

applied procedure of extraction.

A close study of percentage inhibition of *C. nitida and C. acuminata* revealed methanol extracts more sensitive against the tested fungi with moderate antifungal activity against *Aspergillus niger* at various concentration. Therefore the finding supports the uses of *C. nitida* and *C. acuminata* in herbal curl remedies.

4. Conclusion

From the analysis carried out in this research work, the result show scientific basis for the traditional uses of the plant extracts. The results also show varying degrees of antifungal activities on the tested fungi. The *C. nitida* and *C. acuminata* extracts show they are potential in the impairment of growth of test isolates by the tested organism and can serve as bio fungicide for agricultural application. This is a step forward for further evaluation of plant and animal protection strategies .The C .nitida and C. acuminata extracts reduces the mycelia growth of test isolates and impaired the growth of test isolates *Aspergillus niger, Aspergillus fumigates, Mucor recemosus, Rhizopus oryzae* and serve as botanical fungicides.

5. Recommendations

Based on the result of the study, the following recommendations are one ward advanced.

1 The phytochemical substances present in *C. nitida* and *C. acuminata* should be isolated and purified to obtain their maximum therapeutic potentials.
2 The accurate formulation and application as fungicide should be considered.
3 Further research should be done with a view of stabilizing the active ingredient so that they can be prevented from losing their potency.

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