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# Antifungal Effects of Different Extracts of *Cola nitida* on Selected Organisms

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## Abstract

**Background:** *Cola nitida* has been consumed as fruit and also play a significant role in the daily tradition and custom of the Nigerian people. Kola nut has also been used in folk medicine as an aphrodisiac, appetite suppressant, treatment for migraine, indigestion and vomiting control as well as for treatment of wounds, tooth infections, urinary tract infections and other numerous microbial infections. This study was carried out to evaluate the antifungal activities of *Cola nitida* extracts from different localities: Udi, Enugu state, Ado-Ekiti, Ekiti state and Ikom, Cross River State, Nigeria.

**Methods:** Three fungi which included *Candida albicans*, *Aspergillus flavus* and *Aspergillus niger* were used for the study. Agar diffusion and agar dilution assays respectively were carried out for fungi studies. Screening of the methanol and acetone extracts for their antifungal profile was done at varying concentrations.

**Results:** Findings showed statistically significant difference ($p<0.05$) between the extracts *Cola nitida* for the organisms. When the mycelial radial growth diameter for test organisms (P1) was compared, a statistically significant difference was recorded for all the fungi at all concentrations and when the mycelial growth inhibitions (M%) were compared *Aspergillus niger* and *Aspergillus flavus* showed statistically significant difference ($P<0.05$) with result ranging from 80% to 92% for both methanol and acetone extract of *Cola nitida* from all the regions.

**Conclusion:** The study identified kola nut extracts to be antifungal. This suggests that kola nut extracts can be used as alternative medicine in the treatment of fungal infections.

**Keywords:** Antifungal, Extracts, *Cola nitida,* Selected Organisms



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## Introduction

According to Iyere (1), kola nuts have been used by the African natives from immemorial as a necessity and a luxury. Before the emergence of Western civilization and religion, kola nuts played a very significant role in the daily life of the people of Nigeria in West Africa. Nothing was considered complete without kola nut. Kola is offered to a very important guest as a mark of respect and reconciliation, agreements are never considered sealed without kola nuts shared and the gods cannot be appeased without kola nuts. Similarly, marital rites are not complete without kola nuts being presented. Shrines are adorned with kola nuts as a means of sacrifice (2). It figured in compacts of friendship and mark of hospitality as it was readily served to visitors, especially among Igbo tribe in Nigeria as a sign of peace and acceptance of visitors. Some studies have revealed that the social significance of the use of the kola nut may be because it contains substances that stimulate the central nervous system (CNS). In addition, the nut has been revealed to contain caffeine which may help in relieving migraine, also, contains theobromine which acts as a cerebral vasodilator and thought to relive pain and neuralgia (3).

However, herbalists have used kola nuts for the treatment of microbial infections including typhoid fever, eye infections, urinary tract infections, and many other bacterial and fungal infections (4). Many people in Nigeria habitually eat kolanuts which they believe protects, strengthens and heals the teeth from all dental problems and ailments. These plant derivatives are considered safe and more affordable for majority of the developing world. There are reports of an increase in the use of these plant produce in the developed world (5), and more so with the emergence of difficult to treat infections which are resistant to modern synthetic antibiotics, however, kola nuts have been proven to have antimicrobial effects on multiple resistant fungal pathogens. (1, 3, 6, 7, 8, 9, 10, 11, 12). This study is therefore carried out to evaluate the antifungal activities of *Cola nitida* on selected fungi (*Aspergillus flavus, Aspergillus niger and Candida albicans*) on extracts from different localities: Udi, Enugu state, Ado-Ekiti, Ekiti state and Ikom, Cross River State, Nigeria.

## Methods

### Sample Collection

The sample collection was carried out between June and September, 2021, and January and June, 2023. The seeds of the kola nut were obtained from farmers in Udi, Ikom and Ado-Ekiti in Nigeria. The *Cola nitida* were identified by a botanist in Herbalium in Department of Plant and Biotechnology University of Nigeria Nsukka. The sample collection was carried out between June and September, 2021, and January and April, 2023.

### Preparation of the Methanol and Acetone Extracts

Kola nuts extracts were shield dried, manually cut to smaller sizes and was grinded in laboratory blender. 100 g of kola nuts were soaked in a 300ml container with methanol and acetone and stirred at intervals for 24 hours and were sieved through Whatman filter paper then was filtered into a beaker. The extract obtained was allowed to dry up by pouring it on a clean, dry stainless-steel tray and allowing the methanol and acetone to evaporate completely, in two days. The extract weighed 2g was dissolved in 5mls of dimthyl sulphur oxide (DMSO) solution. The extract was preserved for use in the refrigerator.

**Sample Size:** Three fungal isolates: *Aspergillus flavus, Aspergillus niger* and *Candida albicans* were used for this study.
The organisms were isolated from food sources e.g Pork meat, yorghurts, maize, cashew nuts, beans and lots more. The fungi were identified by standard mycological method (13, 14).

### Agar Dilution Method:

Serial dilutions of the extract were carried out using test tubes from 1.5 ml (30%), 1.0 ml (20%), 0.5 ml (10%), 0.25 (5%) dilutions were made. Sabourand agar was used for the culture of the fungi, then, the growth of the organism from day 1 to day 7 was measured.
Each dilution is inoculated with standard inoculums of the test organism. After appropriate incubation, the inhibitory activities of antimicrobial agents are determined by measuring the mean radial growth of the organism and the minimum inhibitory concentration (MIC) (15, 16).

### Diffusion Test

In diffusion test, pure cultures of the test organisms are streaked evenly on the surface of solid agar plates. Wells of about 6mm are aseptically bored on the agar plate; serial dilutions of antimicrobial agents are then introduced into the wells. The plates are incubated and after the incubation, the zones of inhibitions diameter (ZID) by the antimicrobial agents are measured and the MIC (Minimum inhibitory concentration of the extract (15).

### Identification of *Aspergillus flavus*

### Lactophenol Cotton Blue test:

A drop of Lacto-phenol Cotton Blue stain was placed on a clean grease free slide. With the aid of a sterile teasing needle, a part of the fungal isolate from maize which was



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cultured accordingly in a Sabourand agar plate was placed on the stain and gently teased. Cover slip was placed on the teased sample and viewed using X10 and X40 objective lenses showed thick-walled conidiophores which have colourless stalks and were smooth with a round vesicle, phialides and metulae covering the entire surface.

**Identification of *Aspergillus niger***

**Lactophenol Cotton Blue test:**

A drop of Lacto-phenol Cotton Blue stain was placed on a clean grease free slide. With the aid of a sterile teasing needle, a part of the fungal isolate from cashew nut which was cultured accordingly was placed on the stain and gently teased. Cover slip was placed on the teased sample and viewed using X10 and X40 objective lenses. It stains the hyphae and fruiting structures a delicate blue with a pale blue background.

**Preparation of Different Stock Solutions of the Extracts**

Two grams of the methanol extracts of dried sample were dissolved in 3 ml of dimethyl sulphur oxide (DMSO) inside sterile bottle differently and then later top up with 4.5 ml of water to make it up to 7.5 ml in total

**Concentration of Methanol Extract of *Cola nitida:***

The concentrations were made into 100%, 50%, 25%, 12.5% and 6.25%. The 100% extract was the normal methanol concentrated stock without dilution. However, 50%, 25%, 12.5% and 6.25% concentrations of the extract were obtained by passing 1ml of distilled water into 4 different clean test tubes each. (serial dilution).

**Antifungal Activity**

The antimicrobial studies of the fungal organism to methanol extract of *Cola nitida* was evaluated using the agar broth dilution method (15).

**Inoculation of Fungi Test Organisms**

Petri dishes, control with different concentration of plant extract (%) were prepared with molten Sabouraud Dextrose Nutrient Agar (Biotec) as shown on Table 1 above. The prepared petri dishes were divided into four equal portions and colonies, 5/4mm in diameter of the organisms were inoculated and labeled properly. The inoculation was done using sterile scalpel blade to cut the required diameter and a ruler to accurately measure the size. This was done under aseptic condition in sterile, empty, clean, grease free Petri dishes. The colony was picked up with a sterilized teasing needle unto the appropriate segments of the agar which are appropriately labeled with the names of the organism for easy identification. The set ups were incubated at room temperature and observed for 7 days. The set up containing no plant (0 % concentration) extract was used as control.

**Determination of Percentage Inhibition of Fungi**

The colony growth was measured daily for seven consecutive days and observed for growth variation, the diameter of the growth was measured horizontally, vertically and diagonally, and the mean value of the figure were recorded. The measurement was carefully done with a well calibrated ruler.

Fungal growth rate (colony diameter) was measured and percentage inhibition calculated (13).

Percentage Inhibition = Zi - Pi/Zi ×100/1

Percentage growth = Pi/Zi × 100/1

Were

Li = Radial (colony) growth in control plate

Pi = Radial (colony) growth in antimicrobial plate

**Result**

**Table 1.** Percentage inhibition of fungi

| Days | *Aspergillus flavus* | | *Aspergillus niger* | |
|---|---|---|---|---|
| | **Methanol** | **Acetone** | **Methanol** | **Acetone** |
| | | 5% conc | | |
| **Day 1** | 0 | 0 | 0 | 0 |
| **Day 2** | 75 | 76.9 | 85 | 85 |
| **Day 3** | 79.2 | 80.6 | 77.3 | 78.3 |
| **Day 4** | 76.5 | 81.2 | 71.6 | 71.6 |
| **Day 5** | 76.3 | 80.6 | 66.7 | 82.1 |
| **Day 6** | 73.8 | 80.8 | 66.0 | 81.6 |
| **Day 7** | 70.1 | 80.2 | 65.7 | 80.2 |
| *P-values* | ***0.035*** | | ***0.180*** | |
| | | 10% conc | | |
| **Day 1** | 0 | 0 | 0 | 0 |
| **Day 2** | 82.7 | 82.7 | 85 | 85 |
| **Day 3** | 84.7 | 87.5 | 86.4 | 86.4 |
| **Day 4** | 82.4 | 89.4 | 85.1 | 85.1 |
| **Day 5** | 81.7 | 90.3 | 82.1 | 89.3 |
| **Day 6** | 81.6 | 91.3 | 81.6 | 91.3 |
| **Day 7** | 79.2 | 89.7 | 79.6 | 89.6 |
| *P-value* | ***0.041*** | | ***0.097*** | |
| | | 20% conc | | |
| **Day 1** | 0 | 0 | 0 | 0 |
| **Day 2** | 82.7 | 82.7 | 85 | 85 |
| **Day 3** | 87.5 | 87.5 | 86.4 | 86.4 |
| **Day 4** | 89.4 | 89.4 | 87.8 | 87.8 |
| **Day 5** | 90.3 | 90.3 | 89.3 | 89.3 |
| **Day 6** | 92.0 | 91.3 | 91.3 | 91.3 |
| **Day 7** | 91.6 | 91.6 | 91.7 | 91.5 |
| *P-value* | ***0.898*** | | ***1*** | |
| | | 30% conc | | |



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| Days | *Aspergillus flavus* | | *Aspergillus niger* | |
|---|---|---|---|---|
| | **Methanol** | **Acetone** | **Methanol** | **Acetone** |
| **Day 1** | 0 | 0 | 0 | 0 |
| **Day 2** | 82.7 | 82.7 | 85 | 85 |
| **Day 3** | 87.5 | 87.5 | 86.4 | 86.4 |
| **Day 4** | 89.4 | 89.4 | 87.8 | 87.8 |
| **Day 5** | 90.3 | 90.3 | 89.3 | 89.3 |
| **Day 6** | 92.0 | 91.3 | 91.3 | 91.3 |
| **Day 7** | 91.6 | 91.6 | 91.7 | 91.5 |
| *P-value* | ***1*** | | ***1*** | |

**Table II** Comparison of mycelial growth inhibition (m%) between the methanolic and acetone extracts of *Cola nitida* extracts for each fungus at different levels of concentrations for Udi, Enugu state.

| **Days** | **Aspergillus flavus** | | **Aspergillus niger** | |
|---|---|---|---|---|
| | **Methanol** | **Acetone** | **Methanol** | **Acetone** |
| | | 5% conc | | |
| Day 1 | 0 | 0 | 0 | 0 |
| Day 2 | 83.3 | 74.1 | 52.8 | 46.7 |
| Day 3 | 88.3 | 77.9 | 53.8 | 43.3 |
| Day 4 | 90.5 | 80 | 54.7 | 38.0 |
| Day 5 | 88.8 | 83.2 | 51.0 | 34.1 |
| Day 6 | 87.0 | 82.4 | 48.6 | 30 |
| Day 7 | 86.1 | 81.8 | 46.5 | 29.6 |
| P-values | 0.025 | | 0.035 | |
| | | 10% conc | | |
| Day 1 | 0 | 0 | 0 | 0 |
| Day 2 | 83.3 | 83.3 | 67.9 | 80 |
| Day 3 | 88.3 | 88.3 | 73.8 | 80.6 |
| Day 4 | 90.5 | 90.5 | 73.7 | 81.7 |
| Day 5 | 92.8 | 92.8 | 71.6 | 79.3 |
| Day 6 | 93.1 | 93.1 | 68.2 | 77 |
| Day 7 | 93.4 | 93.4 | 65.8 | 74.1 |
| P-value | 1 | | 0.025 | |
| | | 20% conc | | |
| Day 1 | 1 | 0 | 0 | 0 |
| Day 2 | 83.3 | 83.3 | 83.0 | 80 |
| Day 3 | 88.3 | 88.3 | 88.8 | 86.6 |
| Day 4 | 90.5 | 90.5 | 90.5 | 89.0 |
| Day 5 | 92.8 | 92.8 | 91.2 | 90.2 |
| Day 6 | 93.1 | 93.1 | 91.6 | 91 |
| Day 7 | 93.4 | 93.4 | 91.2 | 91.7 |
| P-value | 1 | | 0.655 | |
| | | 30% conc | | |
| Day 1 | 0 | 0 | 0 | 0 |
| Day 2 | 83.3 | 83.3 | 83.0 | 80 |
| Day 3 | 88.3 | 88.3 | 88.8 | 86.6 |
| Day 4 | 90.5 | 90.5 | 90.5 | 89.0 |
| Day 5 | 92.8 | 92.8 | 91.2 | 90.2 |
| Day 6 | 93.1 | 93.1 | 91.6 | 91 |
| Day 7 | 93.4 | 93.4 | 91.2 | 91.7 |
| P-value | 1 | | 0.655 | |



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From Table 2, the comparison for *A. flavus* at 5% conc shows that there is a statistically significant difference between them ($p<0.05$) while *A. niger* was not statistically significant ($p>0.05$). It also shows that there was no significant difference between the extracts for both fungi at 10%, 20% and 30% levels of concentrations ($p>0.05$) with the exception at 10% concentration where *A. flavus* was statistically significant ($p<0.05$).

**Table III** Comparison of mycelial growth inhibition (m%) between the methanolic and acetone extracts of *Cola nitida* extracts for each fungus at different levels of concentrations for Ado Ekiti, Ekiti state.

| ***Days*** | ***Aspergillus flavus*** | | ***Aspergillus niger*** | |
|---|---|---|---|---|
| | **Methanol** | **Acetone** | **Methanol** | **Acetone** |
| | | *5% conc* | | |
| ***Day 1*** | 0 | 0 | 0 | **0** |
| ***Day 2*** | 76.9 | 82.7 | 85 | 85 |
| ***Day 3*** | 80.6 | 84.7 | 78.3 | 86.4 |
| ***Day 4*** | 80 | 81.2 | 82.4 | 87.8 |
| ***Day 5*** | 77.4 | 80.6 | 79.8 | 79.8 |
| ***Day 6*** | 75.7 | 76.7 | 72.8 | 79.8 |
| ***Day 7*** | 74.5 | 74.8 | 69.4 | 77.4 |
| *P-values* | ***0.307*** | | ***0.307*** | |
| | | *10% conc* | | |
| ***Day 1*** | 0 | 0 | 0 | 0 |
| ***Day 2*** | 82.7 | 82.7 | 85 | 85 |
| ***Day 3*** | 87.5 | 87.5 | 86.4 | 86.4 |
| ***Day 4*** | 89.4 | 89.4 | 87.5 | 87.8 |
| ***Day 5*** | 90.3 | 88.2 | 84.5 | 89.3 |
| ***Day 6*** | 92.0 | 87.3 | 81.6 | 89.3 |
| ***Day 7*** | 89.7 | 84.1 | 79.6 | 87.7 |
| *P-value* | ***0.250*** | | ***0.085*** | |
| | | *20% conc* | | |
| ***Day 1*** | 0 | 0 | 0 | 0 |
| ***Day 2*** | 82.7 | 82.7 | 85 | 85 |
| ***Day 3*** | 87.5 | 87.5 | 86.4 | 86.4 |
| ***Day 4*** | 89.4 | 89.4 | 87.8 | 87.8 |
| ***Day 5*** | 90.3 | 90.3 | 89.3 | 89.3 |
| ***Day 6*** | 92.0 | 92.0 | 91.3 | 91.3 |
| ***Day 7*** | 91.6 | 91.6 | 91.7 | 91.5 |
| *P-value* | ***1*** | | ***1*** | |
| | | *30% conc* | | |
| ***Day 1*** | 0 | 0 | 0 | 0 |
| ***Day 2*** | 82.7 | 82.7 | 85 | 85 |
| ***Day 3*** | 87.5 | 87.5 | 86.4 | 86.4 |
| ***Day 4*** | 89.4 | 89.4 | 87.8 | 87.8 |
| ***Day 5*** | 90.3 | 90.3 | 89.3 | 89.3 |
| ***Day 6*** | 92.0 | 92.0 | 91.3 | 91.3 |
| ***Day 7*** | 91.6 | 91.6 | 91.7 | 91.5 |
| *P-value* | ***1*** | | ***1*** | |

The comparison for *A. flavus* and *A. niger* at 5% conc shows statistically significant differences between the two organisms ($p<0.05$).

**Table IV** Comparison of mycelial growth inhibition (m%) between the methanolic and acetone extracts of *Cola nitida* extracts for each fungus at different levels of concentrations for Ikom, Cross River state.



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| ***Cola nitida* Udi Enugu** | | | | |
|---|---|---|---|---|
| Concentrations | **Clinical** | | **Environmental** | |
| | **Methanol** | **Acetone** | **Methanol** | **Acetone** |
| **3.125%** | 0 | 0 | 0 | 0 |
| **6.25%** | 0 | 9 | 0 | 0 |
| **12.5%** | 0 | 10 | 12 | 0 |
| **25%** | 0 | 13 | 14 | 0 |
| **50%** | 0 | 15 | 16 | 13 |
| **100%** | 0 | 18 | 18 | 15 |
| *P-value* | ***0.001*** | | ***0.298*** | |

The comparison for *A. flavus* and *A. niger* at 5%, 10%, 20% and 30% conc shows that there is no significant difference between them ($p>0.05$). The table shows a significant difference ($p<0.05$) between the methanolic, and acetone extracts of *Cola nitida* for the clinical *C. albicans*

**Table V** Comparison of difference between the ZID of methanol and acetone extracts of *Cola nitida* for both clinical and environmental *C. albicans* across all concentrations for Enugu state.

| ***Cola nitida* Ado-Ekiti, Ekiti State** | | | | |
|---|---|---|---|---|
| **Concentrations** | **Clinical** | | **Environmental** | |
| | **Methanol** | **Acetone** | **Methanol** | **Acetone** |
| **3.125%** | 0 | 10 | 0 | 0 |
| **6.25%** | 0 | 12 | 0 | 0 |
| **12.5%** | 0 | 14 | 0 | 0 |
| **25%** | 10 | 15 | 0 | 0 |
| **50%** | 12 | 18 | 12 | 10 |
| **100%** | 15 | 19 | 15 | 15 |
| *P-value* | ***0.055*** | | ***1*** | |

## Discussion

The findings from this study revealed that the methanolic and acetonic extracts of *Cola nitida* has strong antimycotic activity against *Candida albicans, Aspergillus flavus* and *Aspergillus niger*. It was observed that the effectiveness of the plant seed extract increased with increasing concentration, giving the highest activity with 30% concentration for the entire organisms. The study revealed that all fungal isolates were most susceptible to the extract being inhibited 100% all through from day one to day seven for 30% and 20% concentration. 10% concentration for all the days also showed a significant growth inhibition. *Aspergillus flavus* and *Aspergillus niger* showed almost the same percentage inhibition for all the days at 10% concentration of the extract which growth was increasing little by little daily, at 5% concentration *Aspergillus flavus* has the highest growth inhibition by the methanolic extract of *Cola nitida* from Ado-Ekiti. From the results of this study, methanolic and acetonic extracts of *Cola nitida* from all the zones has higher antimycotic inhibition against *Aspergillus flavus* when compared with *Aspergillus niger* which shows a statistically significant difference which supported similar reports (16,17).

*Cola nitida* (acetone extracts) from Ikom showed highest zone of inhibition of 20.0 mm against isolate of *Candida albicans,* it disagreed with other studies (14) which reported acetone and ethyl acetate extracts of both red and white *cola nitida* did not show any activity against all tested organism at all concentrations (0.00 mm), but concurred with (18,19), confirmed antimicrobial activity of the kola nuts on *Candida albicans* and other organisms.

## Conclusion

The study demonstrated that the methanolic and acetonic extract of *Cola nitida* showed a significant antifungal activity against the selected fungi. However, the antimicrobial studies showed that acetone extracts of *Cola nitida* from Ikom has the highest zone of inhibition (ZID) 20 mm against *Candida albicans*. Finally, this study showed that methanolic and acetonic extracts of *Cola nitida* from all the zones has higher antimycotic



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inhibition against *Aspergillus flavus* when compared with *Aspergillus niger*.

## References

1. Iyere IJ. The Socio-Religious Significance of Obi (kola nut) among the Igala people of kogi state. *Cross-Cultural Communication. 2011;* 7 (2):199-208.
2. Ratsch C. The encyclopedia of psychoactive plants: Ethno pharmacology and its application park street press, USA. 2005: 657.
3. Mboto CI,d Udoh P. Susceptibility of *Proteus mirabilis*, *Staphylococcus aureus* and *Candida albicans* to extracts of *Cola acuminata*. *Global Advanced Research Journal of Microbiology*. 2014; 3(5): 078-082.
4. Emeka P. M., Badger-Emeka L. I., and Fateru F. In vitro antimicrobial activities of acalypha ornate leaf extracts on bacterial and fungal clinical isolates. Journal of Herbal Medicine. 2012; 2(4) 136-142.
5. Badger–Emeka L., Haney, E., and Madu, E. Evaluation of different fractions of *Garcinia kola* Extracts against multidrug resistant clinical bacterial and fungal isolates *Pharmacognosy Journal*. 2018; 10(5): 1055-1060.
6. Alaribe, A. A. A., Ejezie, G. C., and Ezedinachi, E. N. U. The role of Kola Nut (*Cola nitida*) in the etiology of malaria morbidity. *Pharmaceutical Biology*. 2003; 41:6 (458-462).
7. Durand, D., Hubert, A., Nafan D., Haziz S., Pacome A., Farid B., Aldophe A., Joachim D. and Lamine B. Antimicrobal, Antioxidant, cytotoxic activities and phytochemical assessment of *Cola acuminata* used in Benin. *International Journal of Pharmacy and Pharmaceutical Sciences*. 2015; 1(1):1-12.
8. Efe, M. O., Stephen, A. J. and Asefon, O. A. The Phytochemical properties and antimicrobial potentials of Aqueous and methonalic seed Extract of *Cola nitida* (vents) and *Cola acuminata* (Beauvoir) grown in South West Nigeria. *Saudi Journal of Medical and Pharmaceutical Science*. 2016; 2 (12):354-363.
9. Indabawa II Arzai, AH. Antibacterial activity of *Garcinia kola* and *Cola nitida* seed extracts. *Bayero Journal Pure and Applied Science*. 2010; 4(1): 52-55.
10. Jackie, K.O. and Anthony, S. (2014). Antibacterial activity of methanolic extracts of *Cola nitida* seeds on selected pathogenic organisms. *International Journal of Current Microbiology and Applied Science*. 2014; 3(8): 999-1009.
11. John D, Okwubie L, Njemanze IO. Antimicrobial activity microorganisms located from the oral cavity. *International Journal of Pharmacognosy and Phytochemical Research*. 2018;10(4): 151-156.
12. Muhammad S Fatima A. Studies on phytochemical evaluation and anti-bacterial properties of two varieties of kola nut (cola nitida) in Nigeria *Journal of Bioscience and Medicine*. 2014; 2: 37-42.
13. Kwoong Chun J. Standard mycological methods. In C. W., Hesseltine, J. E., Smith, and J. W. Fell (Eds.), *Handbook of Food Spoilage Microorganisms*. 2009; (2nd ed., 1-22). Marcel Dekker.
14. Onyemelukwe, N. F., Ezekwesili-Ofili J. O., and Onyemelukwe AC. Effects of essential Oils and extract from some Nigerian medicinal plants and production of aflatoxin by *Aspergillus flavus*. Book of Abstracts-Mycored Africa. (IMMYT; international forum Capetown, South Africa. 2011; 125-126.
15. Ochei J, Kolhatkar A. Medical Laboratory Science: Theory and Practice. 6th Edition, Tata McGraw-Hill, New York. 2007; 730.
16. Adeniyi BA, Mebude OO, Lawal TO, Nwanekwu KE. Invitro Antifungal Activities of Cola nitida Schtt and Endl. (Stercculiacae) against five Candida species and four Dermatophytes." Research Journal International. 2016; 14(2):1-8.
17. Dah-Nouvlessounon D, Adoukonou-Sagbadja H, Diarrassouba N, Sina H, Adjanohoun A, Inoussa M, Akakpo D, Gbenou JD, Kotchoni SO, Dicko MH, Baba-Moussa L. Phytochemical analysis and biological activities of Cola nitida bark. Biochemistry research international. 2015;2015(1):493879.
18. Shama IY, Ahmed AN, Wala MM, Warda SA. Antimicrobial activity of the masticatory *Cola acuminata* Nut (Goro). *Current Research Journal of Biological Science*. 2011; 3(4): 357-362.
19. Sonibare M, Soladoye M, Esan O, Sonibare O. Phytochemical and antimicrobial studies of four species of Cola Schott & Endl. (Sterculiaceae). African Journal of Traditional, Complementary and Alternative Medicines. 2009;6(4).



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