ANTIMICROBIAL ACTIVITY OF *COLA MILLENII* (MONKEY KOLA) EXTRACTS AGAINST SELECTED CLINICAL BACTERIAL ISOLATES

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Abstract
In this study, stem bark, leaf and pulp of Cola millenii were extracted, using ethanol and then subjected to in vitro antimicrobial activity screening against clinical isolates of Staphyloccocus aureus, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae and Candida albicans. The agar well diffusion method was adopted for the antimicrobial activity test while the double dilution broth method was used for the minimum inhibitory concentration determination. The results showed that Staphylococcus aureus a Gram-positive organism showed the highest susceptibility to the extracts at all the concentrations used recording 9.33, 18.33 and 11.33 mm zones of inhibition against stem bark, leaf and pulp extracts respectively at 50 mg/ml. Furthermore, only S. aureus was susceptible to stem bark extract at the lowest concentration used (10 mg/ml), while all the test bacterial species were susceptible to the leaf extract at 10 mg/ml, whereas the test yeast (C. albicans) was not susceptible to any of the extracts at 10 mg/ml. The lowest MIC obtained in this research was 2.50 mg/ml of leaf extract against S. aureus while the highest (50 mg/ml) was obtained against C. albicans with stem bark extract. S. aureus generally showed the lowest MIC to all the plant extracts which suggest that the organism may be very sensitive to C. millenii. The outcome of this study has shown that the leaf, pulp and stem bark extracts of C. millenii possess some level of antimicrobial activity. Therefore, it may be exploited for the development of alternative therapy to the synthetic antibiotics which have a plethora of adverse effects.
Keywords: Pathogens, *Cola millenii*, Antimicrobial, Susceptibility, MIC.

Introduction

Traditional/ indigenous medicine covers the health practices, approaches, knowledge and beliefs incorporating plants, animal and mineral based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illness or maintain well-being (Opawale, Oyetayo and Agbaje, 2015). Traditional medicine has been used since time immemorial with great contributions made by practitioners to human health, particularly as primary health care providers at the community level. Countries in Africa, Asia, and Latin America use traditional medicine to help meet some of their primary healthcare needs. In Nigeria, for example, herbal medicine is the first line of treatment for 60% of children with high fever from malaria, while 85 % of Nigerians use traditional medicine for health care, social and psychological benefits (Akinnibosun and Adewumi, 2018).

Herbal remedies have a therapeutic effect and are acceptable interventions for diseases and symptoms. Interestingly, demand for medicinal plants is progressively rising in industrialized nations as it is in developing countries (Adeshina, Kunle, Onaolapo, Ehinmidu and Odama, 2011). Medicinal plants have enormous therapeutic potentials to heal many infectious diseases, by avoiding many side effects. The plant kingdom has been the best source of remedies for curing a variety of diseases. This is why medicinal plants have played a key role in the worldwide maintenance of health. Natural products of higher plants are an important source of therapeutic agents; therefore, many research groups are currently screening the different activities of plants (Nwankwo, 2017). Similarly, many plants have known efficacy for control of diseases and disease vectors. This information has been passed down from generation to generation and offers an effective, low cost, sustainable and environmentally benign pest management strategy. Africa is endowed with rich biodiversity and indigenous knowledge system about bioactive plants, whose beneficial values have not been fully exploited in tropical medicine and agriculture (Efferth and Kaina, 2011).

Cola millenii K. Schum is a member of the genus Cola Schott & Endl of the family Sterculiaceae (Reid, 2005). Cola is one of the most popular genera in this family and it is related to the genus Theobroma. *C. milleni* is a tree that grows to 12 m high, occasionally to 20 m with a low crown of

arching branches; deciduous, of closed and transition forest, tending toward the drier parts, in Ivory Coast to Southern Nigeria. It is called "Atewo-edun" by the Yoruba in the southwestern Nigeria, while the Igbo of Eastern part call it "achi okokoro". The fruit is bright red in a stellate cluster; its seed is covered with a felted fibrous coal. The kernel is edible. The wood is white and very resilient. It is used in Nigeria for the stock of the cross bow and in Liberia for rat traps and bows (Akinnibosun and Adewumi, 2019). The leaf and stem bark of the plant are reportedly used in the treatment of ringworm, ringworm, scabies, gonorrhoea, dysentery and ophthalmic (Odugbemi and Akinsulire, 2006). In spite of the popularity of the plant in traditional application, there is a dearth of scientific explanation on the therapeutic potential of the various parts of this plant. Therefore, this study was designed to investigate the antimicrobial activity of various parts of this plant.

Materials and Methods

Collection and Preparation of Plant Samples

The leaf, stem bark and fruits of monkey kola (*C. millenii*) were harvested fresh from the wild in a forest at Iyere, Owo Local Government, Ondo State. The plant materials were then authenticated at the Environmental Biology Unit of Science Laboratory Technology Departtment, Rufus Giwa Polytechnic, Owo and voucher specimens (CMP112IF-*C. millenii* fruit, CMP1122B- *C. millenii* stem bark and CMP1124L- *C. millenii* leaf) were deposited at the Department of Forestry Resources Technology, of the same Institution. The seed and pulp were removed from the fruit manually using laboratory knife. Thereafter, the plant materials were washed thoroughly in distilled water and air dried for four weeks in the laboratory. The dried samples were then ground into powder with the aid of a mechanical grinder and were stored in clean air- tight containers, and kept in a cool, dry place until required for use.

Extraction Procedure

One hundred gram (100g) portion of the powdered samples was soaked in 300ml of solvent (ethanol) for 48hrs with intermittent stirring using sterile spatula. Thereafter, extracts were filtered through filter paper into sterile containers and then dried using rotary evaporator at 50^0C (Ganiyat *et al*., 2010). Different concentrations of the extracts were prepared by diluting 0.10g, 0.20g, and 0.50g of the extracts in 100ml of 0.01% Tween-20 to obtain concentrations of 10mg/ml, 20mg/ml and 50mg/ml respectively (Opawale *et al*., 2015).

Collections of Test Microorganisms

The microorganisms employed in the study were *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus* and *Candida albicans* and were obtained at the Federal Medical Center, Owo.

Media Preparation

Malt extract broth, Malt extract agar, Mueller Hinton agar and Mueller Hinton broth were separately prepared according to the manufacturer's specification in sterile conical flasks. The mixtures were homogenized on a hot plate with magnetic stirrer (Eka 200P) until uniform solution was obtained. Thereafter, all the culture media were autoclaved at 121 ^{0}C for 15minutes and allowed to cool to about 50 ^{o}C before use.

Standardization of Microbial Culture

A loopful of test organism was aseptically inoculated into Mueller Hinton broth (MHB) and incubated for 24h at 37^{o}C. A portion of 0.2 ml from the 24h culture of the organisms was dispensed into 20ml sterile Muller Hinton broth and incubated for 3-5h to standardize the culture to 0.5 McFarland standards (1.0 x10^6 cfu/ml) before use, according to the method of Akinnibosun and Oyetayo (2018).

In vitro Antimicrobial Susceptibility Test

The extracts obtained from the test plants were screened against the test organisms by agar well diffusion method. A 25ml aliquot of Mueller-Hinton agar (bacteria) and Malt extract agar (yeast) was poured into each Petri plate. When the agar solidified, the pathogenic test organisms were inoculated on the surface of the plates (1×10^6 *cfu*/ml), using a sterile glass spreader and allowed to sink properly. Subsequently, the surface of the agar was punched with 6mm diameter cork borer into wells and a portion of 50µl of each of the extract concentrations was filled into the wells. Control wells containing the same volume of Chloramphenicol and ketoconazole were used as positive controls for the bacterial and yeast plates, respectively, and the plates were incubated at 37^0C for 24 h. Each experiment was carried out in triplicate and the diameter of the zones of inhibition was then measured in millimeters.

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentrations (MICs) were determined as the lowest concentration of oil or extract inhibiting the visible growth of each organism on the agar plate. The MIC of the plants extracts were determined by double dilution broth methods of Obafemi, Akinpelu, Taiwo and Adeloye (2006). Twofold serial dilutions of the extracts and oils were prepared in Mueller-Hilton broth and Malt extract both for bacteria and yeast, respectively, to achieve decreasing concentrations, ranging from the least concentration that produced clear zone of inhibition (10mg/ml to 0.156mg/ml). All tubes with the controls were labeled accordingly. Each dilution was seeded with 1ml of standardized inoculums (1.0×10^6 *cfu*/ml) incubated at 37^0C for 24 hr. A tube containing only seeded broth (i.e. without plant extract) was used as the positive control, while the un-inoculated tube was used as negative control. The lowest concentration of each extract and oil sample that showed a clear zone of inhibition, when compared with the controls, was considered as the MIC (Adeniyi, Groves and Gangadharam, 2004).

Data Analysis

Data were presented as Mean Standard Error (SE). Significance difference between different groups was tested, using two-way analysis of variance (ANOVA) and treatment means were compared with Duncan's New Multiple Range Test (DNMRT) using SSPS window 7 version 17.0 software. The significance was determined at the level of $p< 0.05$.

Results and Discussion

Many infectious diseases are known to be treated with herbal remedies throughout the history of mankind and cola species have been reported to possess antimicrobial properties (Odugbemi, 2006). Results from this work as presented in Table 1 and 2 support this position. The ethanol extracts of the stem bark, leaf and pulp extracts of *Cola millenii,* which were investigated for their possible antimicrobial activities, shows various degrees of activities against the selected pathogenic organisms used. *Staphylococcus aureus,* a gram positive organism, showed the highest susceptibility to the extracts at all the concentrations used, recording 9.33, 18.33 and 11.33 mm zones of inhibition against stem bark, leaf and pulp extracts, respectively at 50 mg/ml. This is in line with the previous reports on the species of Cola (Reid *et al*., 2005). Further, only *S. aureus* was susceptible to stem bark extract at the lowest concentration used (10 mg/ml), while all the test bacterial species were susceptible to the leaf extract at 10 mg/ml, whereas the test yeast was not susceptible to any of the extracts at 10mg/ml

Table 1: Comparative antimicrobial activity of different parts of *C. millenii* against some organisms

Concentration	**10**			**20**			**50**			**Control**
Organism	SBE	LFE	PPE	SBE	LFE	PPE	SBE	LFE	PPE	
S. aureus	2.00±0.58	8.33±0.05	4.33±0.08	5.00±0.01	12.00±0.01	8.67±0.03	9.33	18.33	11.33	24.33
B. subtilis	0.00±0.00	5.67±0.01	0.00±0.00	3.00±0.02	10.33±0.03	4.50±0.01	6.67	13.67	8.00	21.67
E. coli	0.00±0.00	6.67±0.10	4.00±0.02	4.67±0.00	10.00±0.05	6.67±0.03	7.50	16.33	10.00	19.33
K. pneumoniae	0.00±0.00	7.00±0.15	3.67±0.03	5.00±0.07	11.33±0.00	6.33±0.01	9.33	17.67	8.67	22.67
C. albicans	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.01	5.67±0.20	2.33±0.00	3.33	8.00	5.33	15.33

Key: SBE= stem bark extract, LFE= leaf extract, PPE= pulp extract, Values are Mean±S.E.M (mm), Values followed by different alphabet along the rows are significantly different at p≤0.05,

Table 2: Comparative MIC of different parts of C. millenii against some organisms (mg/ml)

Organism	SBE	LFE	PPE
S. aureus	10	2.5	7.5
B. subtilis	20	5	20
E. coli	15	6.25	10
K. pneumonia	15	3.125	10
C. albicans	50	15	20

Key: SBE= stem bark extract, LFE= leaf extract, PPE= pulp extract.

Moreover, the antimicrobial activity of the plant material followed a concentration dependent pattern, as wider zones of inhibition were observed at higher concentration of the extracts. Also, the leaf extract showed a better activity against the test organisms at all the concentrations, followed by the pulp extract, while stem bark extract showed the least activity. Compared with the control, only the leaf extract had significant activity against the test organisms.

Bacillus subtilis, a gram positive organism, showed the least susceptibility to the extracts at the highest concentration, recording 6.67 mm (stem bark extract), 13.67 mm (leaf extract) and 8.00 mm (pulp extract), respectively. The susceptibility of *S. aureus, E. coli and K. pneumoniae* was comparable at all the concentrations suggesting that *Cola millenii* may be active against both gram negative and gram positive bacteria. This correlates with the findings of Sonibare, Soladoye, Esan and Sonibare (2009) who reported antibacterial activity of *Cola millenii* extracts against *S. aureus* and *E. coli*. However, this observation was at variance with the earlier report of Indabaw and Arzai (2011) who reported antibacterial activity of *Cola millenii* extracts against *Pseudomonas aeruginosa* and *Bacillus subtilis* but no activity against *E. coli* and *S. aureus*. The higher activity of the leaf extract against the test organisms compared to the stem bark and pulp extracts suggests that the leaf extract may contain more bioactive phytochemicals than the other parts screened. There are reports that *Cola millenii* possesses as phytochemicals, such as alkaloid, tannin, saponin, steroids, terpenes and glycosides; some of these are known to exhibit inhibitory activity on microorganisms (Manikandan, Senthikumar, Rajesh and Suresh, 2006).

The various plant extracts showed low activity against *C. albicans.* This is at variance with the work of Tiew, Ioset, Kokpol, Chavasiri and Hostettman (2003) where the antifungal properties of members of the Sterculiaceae family were reported. Also, it contradicts the report of Giwa, Onileke, Adesina and Adebote (2012) who reported antifungal activity of *C. millenii* seed and pulp against *Aspergillus niger* and *Aspergillus flavus*. This has also been observed earlier by Sonibare et al. (2009) in a report of the antimicrobial activity of leaf extracts of cola species. The difference in the antifungal activity recorded and those reported may be due to the time of harvest and the difference in the geographical location of the plants.

Generally, the inhibitory activity showed by the stem bark and pulp extracts were low, compared to the controls. A possible explanation for this may be the lower amount of bioactive components in the stem bark and pulp extracts compared with the leaf. The minimum inhibitory concentration of an antimicrobial agent is the minimum concentration of the agent that prevents visible growth of the test organism in a broth culture. The lowest MIC obtained in this research was 2.50 mg/ml of leaf extract against *S. aureus,* while the highest (50 mg/ml) was obtained against *C. albicans* where stem bark extract. *S. aureus* generally showed the lowest MIC to all the plant extracts which suggests that the organism may be very sensitive to *C. millenii*. This is of particular interest, since *S. aureus* is prone to antibiotic resistance. Therefore, active principles may be obtained from *C. millenii* to combat the menace of this virile organism. The plant extracts appeared to be bacteriostatic and fungistatic since none of the concentrations used successfully killed the test bacteria, even at the high concentrations greater than 100 mg/ml. This is an indication that the extract may not be useful as curative remedy for the infections caused by these organisms. The outcome of this study has shown that the leaf, pulp and stem bark extracts of *Cola millenii* possess antimicrobial activity. Therefore, it may be exploited for the

development of alternative therapy to the synthetic antibiotics, with a plethora of adverse effects.

Conclusion

Based on the results obtained in this study, it can be safely concluded that *Cola millenii* extracts possess appreciable antimicrobial activity against the test organisms. Further, the leaf extract possesses more inhibitory activity against the test isolates than the stem bark and pulp extract. The extracts were more potent as antibacterial than antifungal and this explains the folkloric use in the management of infectious diseases.

References

Adeniyi, B.A., Groves, M.J. & Gangadharam, P.R.J. (2004). *Invitro* anti-mycobacterial activities of three species of Cola plant extracts (Sterculiaceae). *Phytotherapy Research* 18 (5): 414-418.

Adeshina, G.O., Kunle, O.F., Onaolapo, J.A., Ehinmidu, J.O. & Odama, L.E. (2011). Evaluation of antimicrobial potentials of methanolic extract of *Alchornea cordifolia* leaf. *European Journal of Scientific Research,* 49(3): 433-441.

Akinnibosun, F.I. and Adewumi, L.B. (2018). Evaluation of phytochemical components of various parts of *Cola millenii* K. Schum. *Ovidius University Annals of Chemistry* 29 (1): 29-35.

Akinnibosun, F.I. & Adewumi, L.B. (2019). Properties and Potentials of *Cola milenii* K. Schum: A Review. *Nigerian Research Journal of Engineering and Environmental Sciences,* 4(1): 166-173.

Akinnibosun, F.I. & Oyetayo, A.M (2018). Preliminary study of phytochemical constituents and antimicrobial activity of two varieties of cashew apple grown in Nigeria. *Malaysian Journal of Microbiology,* 14(4): 624-631.

Efferth, T. & Kaina, B. (2011). Toxicities by herbal medicines with emphasis to traditional Chinese medicine. *Current Drug Metabolism,* 12(10):989- 96.

Ganiyat, K.O., Patricia, A.O., Sothka, J., Oguntokun, O. & Thoda, E. (2010). Phytochemical screening, antimicrobial and antioxidant activities of four Nigerian medicinal plants. *Annuals of Biological Research,* 1(2): 114-120.

Giwa, O.E., Onileke, F.O., Adesina, I.A. & Adebote, V.T. (2012). Phytochemicals and antimicrobial properties of seed and pulp of Monkey Cola (*Cola millenii*) on some selected slinical and food borne isolate. *International Journal of Applied Biology and Pharmaceutical Technology,* 3: 390-400.

Indabaw I.I. & Arzai A.H. (2011): Antibacterial Activity of Garcinia kola and Cola nitida Seed Extracts. *Bayero Journal of Pure and Applied Sciences,* 4 (1):379.

Manikandan, L., Senthilkumar, G.P, Rajesh, L.T. & Suresh, R. (2006): Cancer chemoprotective agents from medicinal plants. In: Trivedi PC (ed). Medicinal Plants: Ethnobotanical approach. Agrobios., India.

Nwankwo, C.E. (2017). Antimicrobial and spectroscopic analyses of *Cola millenii* K. Schum. ftSc dissertation in the Department of Biological Sciences, College of Natural Sciences submitted to the School of Postgraduate Studies, Redeemer's University, Ede, Nigeria.

Obafemi, C.A, Akinpelu D.A Taiwo O.O & Adeloye, A. (2006). Antimicrobial Activities of Solvent extracts of *Terminalia catapa* linn leaves. *Ife Journal of Science,* 8(1): 29-33.

Odugbemi, T. & Akinsulire, O. (2006). Medicinal plants by Species Name. In: Outlines and Pictures of Medicinal Plants from Nigeria. Odugbemi, T (Ed). Lagos: University of Lagos Press.

Opawale, B.O., Oyetayo, A.M. & Agbaje, R.B. (2015). Phytochemical Screening, Antifungal and Cytotoxic Activities of *Trichilia heudelotii* Planc (Harm). *International Journal of Sciences: Basic and Applied Research (IJSBAR),* 24(6): 267-276.

Reid, K.A., Jager, A.K., Light, M.E., Mulholland, D.A. & Staden, J.V. (2005). Phytochemical and Pharmacological Screening of Sterculiaceae species and Isolation of antibacterial Compounds. *Journal of Ethnophamacology,* 97 (2): 285-291.

Sonibare, M.A., Soladoye, M.O., Esan, O.O., & Sonibare, O.O. (2009). Phytochemical and antimicrobial studies of four Species of Cola Schott & Endl. (Sterculiaceae). *African Journal of Traditional and Complimentary Alternative Medicine,* 6 (4): 518 – 525.

Tiew, P., Ioset, J.R., Kokpol, U., Chavasiri, W. & Hostettman, K. (2003). Antifungal, Antitoxidant and Larvicidal Activities of Compounds Isolated from the Heartwood of *Mansonia gagei*. *Phytotherapy Research,* 17 (2): 190-193.