

# Investigation of Inhibitory Potential of Monkey Cola (*Cola Milleni*) against some Microorganisms

Olabinjo, Oyebola Odunayo<sup>1</sup> and Ganiyu, Foluso Hassan<sup>2</sup>

<sup>1</sup>Department of Agricultural Engineering, Federal University of Technology, Akure, Nigeria

<sup>2</sup>Department of Crop, Soil & Pest management, Federal University of Technology, Akure, Nigeria

Corresponding Author's

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**Abstract**— Microorganisms are used in processing bulky, perishable and inedible raw materials into safe, shelf- stable and palatable foods. The microbial activity of aqueous and ethanol extracts of pods and seeds of Monkey cola was evaluated for potential antimicrobial activity against important bacterial and fungal strains common to plants and animals. The antibacterial and antifungal activities of extracts from the seeds and pods of Monkey cola were tested against five bacterial strains — *Escherichia coli*, *Erwinia carotovora*, *Xanthomonas axonopodis*, *Staphylococcus aureus*, and *Pseudomonas solanacearum*, and four fungal strain; *Colletotrichum gloesporioides*, *Phytophthora megacarrion*, *Colletotrichum acutatum* and *Fusarium oxysporium* using standard methods. The zone of inhibition of the extracts were compared with standards of amoxicillin ciprofloxacin, for antibacterial and kocide for antifungal activity. The results showed remarkable inhibition (13-33mm) of the bacterial growth against the tested organisms. The antifungal activity of seed ethanoic extract result showed maximum mycelial growth of 100 percent against tested fungal organisms. Hence, the plant is recommended as bioactive natural product that may serve as leads in the development of new pharmaceuticals products against microorganisms in both plants and animals.

**Keywords**— Monkey cola, microorganisms, inhibition zone, antibacterial activity, antifungal activity.

## I. INTRODUCTION

Microorganisms constitute the largest group of living things or organisms on earth and with only a small portion of microbial species been identified. It is also called microbes and may be unicellular or multicellular, based on the number of cells. Microorganisms are one of the most diverse organisms and they include bacteria, fungi, archaea, protozoa, algae, and viruses.

Microorganisms are ubiquitous in the environment, where they have a variety of essential functions. Some microorganisms are Pathogenic; cause infections or intoxications, Saprophytic; play a role in biodegradation and cause food spoilage and Cultured; like probiotic bacteria that are essential in food processing (FAO 2009). Bacteria are the most important microorganisms to the food processor to produce several compounds (enzymes, flavors, fragrances etc.) either specifically for application as food additives or in situ as a part of food fermentation processes

(Longo and Sanromán, 2006). Most are harmless, many are highly beneficial, some indicate the probable presence of filth, disease organisms, spoilage and a few cause diseases. There are thousands of species of bacteria, but all are single-celled and fall into three basic shapes: spherical, straight rods, and spiral rods with high rate of production. Microorganisms determine the characteristics of the fermented food, e.g., acidity, flavour and texture, as well as health benefits that go beyond simple nutrition (Vogel *et al.*, 2011). Lipases of different microbial origin have been used for refining rice flavour, modifying soybean milk and for improving the aroma and accelerating the fermentation of apple wine (Hasan *et al.*, 2006; Treichel *et al.*, 2010; Sangeetha *et al.*, 2011).

Africa is endowed with humongous biodiversity resources (Kuate, 2013) and it has an estimation of about 45,000 species of plant out of which 2,000 species or more are used for medicinal purpose (Muanya, 2015). Africa is located

within the tropical and subtropical climate and plants in this region accumulate important secondary metabolites as a natural means of sustenance in their hostile environment (Bourgaudet *et al.*, 2001; Vardhini and Anjum, 2015). Based on her tropical conditions, Africa has a strong ultraviolet ray of the tropical sunlight and numerous pathogenic microbes, including several species of bacteria, fungi, and viruses, suggesting that African plants could accumulate chemo preventive substances more than plants from the northern hemisphere (Mahomoodally, 2013).

Monkey cola with botanical name *Cola millenii* is known as “atewo-edun” (Yoruba) or “achiokokoro” (Igbo) and belongs to the family Sterculiaceae (Ratsch, 2005). The tree grows up to about 15meters or more in height with a low crown of arching branches. Monkey cola leaves are reported to be used in the treatment of ring worm, scabies, gonorrhea, dysentery and ophthalmic conditions (Odugbemi, 2006). The fruit is bright red in a stellate clustern covered with a felted fibrous coal and has an edible kernel/seed (Orisakeye and Ojo, 2013). The wood is white and very resilient which is used in Nigeria and Liberia for rat traps and bows. The phytochemical, proximate, mineral element compositions and antioxidant effects of leaves have been reported (Adeniyi *et al.*, 2004; Ibironke and Olusola 2013; Orisakeye and Ojo, 2013).

Communicable diseases are the most important global problem (Nair *et al.*, 2017), it's the major source of death (Vu *et al.*, 2015), and cause the death of almost 50,000 people's deaths per day (Namita and Mukesh, 2012) which mainly occur due to food poisoning. The World Health Organization (WHO) reported that about 80 percent of the world's population depend on traditional medicine for their primary healthcare needs (Nair *et al.*, 2005). The rural dwellers which had above 60 percent of the World population are still forced to practice traditional medicines for their common day ailments, most of these people form

the poorest link in the trade of medicinal plants, due to poverty, ignorance and unavailability of modern health facilities. It was reported by Monier and Abd, (2016) that 25 percent of the medical drugs used in developed countries are based on plants and their derivatives.

In appraising new antimicrobials or antibiotics, evaluation of biological activity is essential for the assessment of susceptibility of pathogens to the antimicrobial agent. Antimicrobial sensitivity or susceptibility testing is used in pathology to determine the resistance of certain microbial strains to different antimicrobials and in pharmacology research it is used to determine the efficacy of new antimicrobials from biological extracts against different microorganisms (Das *et al.*, 2010). Microbial growth or its inhibition can be measured in a number of ways, such as viable counts, direct microscopic counts, turbidity measurement, bioluminescence and fluorimetry (Grare *et al.*, 2008). The current exploratory study was designed to evaluate the microbial and antifungal property of medicinal plant Monkey cola on disease causing pathogens common to both plant and animal.

## II. MATERIAL AND METHODS

### 2.1 Collection, extraction and formulation of plant materials:

Fresh matured disease free of Monkey cola fruits were collected during the early raining season (April, 2019) from a village called Imafon in Akure Ondo state, Nigeria. It lies between longitude 5°06"E to 5°38"E and between latitude 7°07"N to 7°37"N in the Southwestern Nigeria. The collected fruits were washed thoroughly several times with running tap water, rinsed with sterile distilled water. The fruits were identified and authenticated by a botanist. The fruits were separated into seeds, seeds coats and pods (Figure 1).



Fig.1; Seed (A), seed coats (B) and pods (C) of Monkey cola

The fine powdered samples were divided into two, extracted in aqueous and ethanol at room temperature with continuous stirring on an electric shaker. They were filtered through

double layers of muslin cloth, centrifuged at 9000 rpm for 10 min and the solvents were removed in vacuum using rotary evaporator at 40°C. The dried extracts of the plant

materials were then stored in air-tight jars at 4°C for microbial analysis. The formulation of the extracts was made by dissolving the ethanoic and aqueous extract of each fruit materials in distilled water to a final concentration of 100 mg/ml, to obtain Monkey cola seed aqueous extract (A), Monkey cola seed ethanol extract (B), Monkey cola pods aqueous extract (C), and Monkey cola pods ethanol extract (D).

## 2.2 Studied Microorganisms

The clinical isolates strains were used in this study. Five bacterial strains, namely: *Escherichia coli*, (*E. coli*, (AA), *Erwinia carotovora*, (*E. carotovora* (AB), *Xanthomonas axonopodis*, (*X. axonopodis* (AC)), *Staphylococcus aureus*, (*S. aureus* (AD)) and *Pseudomonas solanacearum*, (*P. solanacearum* (AE)) were used as test organisms. Four fungi strains, namely: *Colletotrichum gloesporioides*, (*C. gloesporioides* (BA)), *Phytophthora megacarrar*, (*P. megacarrar* (BB)), *Colletotrichum umacutatum*, (*C. acutatum* (BC)) and *Fusarium oxysporium*, (*F. oxysporium* (BD)). These microorganisms were selected based on their potential to cause food poisoning as well as infections in both plants and animals.

## 2.3 Evaluation of Antibacterial assay of the samples

The bacterial isolates used for this assay were obtained from Crop, Soil and Pest Management Department of Federal University of Technology, Akure, They Organisms (AA, AB, AC, AD, and AE) were cultured aerobically at 37°C for 24hrs on peptone water and antibacterial test was carried out in the Mueller Hinton Agar plates using Agar well diffusion method (Aibinu *et al.*, 2007). Pure isolates of each peptone cultured bacterium were seeded on nutrient agar plates for about 30mins. Sterile cork borer of 10mm diameter was used to make wells on the solidified Agar into which 0.5ml of extract of the samples were aseptically introduced. The plates were incubated at 37°C for 24hrs. Antibacterial activity was determined by measurement of Inhibition Zone diameter around the wells using digital Vernier caliper to the nearest millimeter (mm). Results were quoted as radii (mm) of the zone of inhibition around the well (subtracting the radii/diameter of the cork borer). Control plates were also set up using standard antibiotics Amoxicillin Clavulanic acid at 0.1g/ml (w/v). The tests were performed in triplicate for each bacterial strain evaluated and the final results were expressed as arithmetic mean.

## 2.4 Evaluation of Antifungal assay of the samples

The selected fungi of choice used for this experiment are; *Colletotrichum gloesporioides* (BA), *Phytophthora megacarrar* (BB), *Colletotrichum acutatum* (BC) and *Fusarium oxysporium* (BD).

The Antifungal evaluation was performed using poisoned food techniques of Mohana and Raveesha (2007). 5ml of each reconstituted sample were aseptically mixed with 20ml of sterile molten potato Dextrose agar (PDA) that have been cooled to 45°C before been poured and allowed to solidify at ambient temperature. The 48hrs old cultured of each fungus were inoculated at the center of the PDA plate with the aid of 4mm cork borer and sterile inoculating needle. Kocide, a standard antifungal agent was used as a positive control at 100mg/ml. A negative control plates (NTR) without any treatment were also set up. All the plates were incubated at 27°C for 72hrs. Mycelial growth were measured with the aid of digital Vernier caliper to the nearest millimeter (mm). Mycelial growth inhibition was calculated in percentage using the formula (1) by Vinesh and Devendra, (2013).

$$\text{Mycelin growth inhibition} = \frac{NTR-TR}{NTR} * 100 \quad (1)$$

Where NTR- Average diameter of mycelial growth (fungal colony) in control

TR- average diameter of mycelial growth (fungal colony) in treatment

The tests were performed in triplicate for each fungal strain evaluated and the final results were expressed as arithmetic mean.

## 2.5 Statistical Analysis

All statistical analyses were performed using Minitab Statistical Software, version 18 (Minitab Inc., USA). Data were expressed as standard error of mean  $\pm$  (SEM). Statistical analysis was performed by one-way analyses of variance (ANOVA) test and the means were separated using Tukey test at significant level value of  $p < 0.05$ .

## III. RESULTS AND DISCUSSION

### Antimicrobial Activities of Crude Monkey Cola Extract of Pods and Seeds

The microbial study in the research is antibacterial and antifungal activities of selected bacteria and fungus strains that cause food poisoning as well as infections in both plants and animals.

#### 3.1 Antibacterial activities of crude Monkey cola extract of pods and seeds.

The present study investigated the in vitro antibacterial activity of two different solvents (aqueous (AQ) and ethanoic (ET)) of pods and seeds of Monkey cola extracts against the five bacterial strains represent as AA-AE. The results of antibacterial activities revealed that (AQ) and (ET) extracts of Monkey cola showed significantly higher inhibitory activity (between 20-33mm) against (AA) and

(AD). All extracts of Monkey cola showed inhibiting activity (between 13-25mm) against (AB) and (20-32mm) against (AE) (Table1). The present investigation has shown that the (AQ) and (ET) extracts of Monkey cola have active phytochemical, which can inhibit the growth of the studied pathogenic bacteria. All crude plant extracts of Monkey cola showed antibacterial activities against both Gram positive and Gram-negative bacteria strains tested. The potency of the extracts was assessed quantitatively by determining inhibition zones as given in Table 1 for all the extracts. The average inhibition of the pods (AQ) extract was highest against the growth of AA ( $33.00 \pm 1.00$ ) and the lowest against AB ( $21.33 \pm 0.58$ ) while pods (ET) extract were highest for AD ( $33.00 \pm 1.00$ ) and lowest for AE ( $24.67 \pm 1.53$ ). The average inhibition of the seeds (AQ) extract was highest against the growth of AA ( $25.00 \pm 0.00$ )

and the lowest against AB ( $13.33 \pm 0.58$ ) while seeds (ET) extract was highest for AA ( $32.67 \pm 1.53$ ) and lowest for AB ( $13.00 \pm 0.58$ ). The pods (AQ) had the highest inhibition zone while seeds (ET) had the lowest value.

It was found that ethanoic and aqueous extracts of Monkey cola from pods showed significantly higher zone of inhibition (21-33mm) against all the strains of bacteria studied and comparable higher than the standard antibiotic amoxicillin clavulanic acid of inhibition zone (18-20mm) as shown in Table 1. The result shows that both extracts from the pods performed extremely higher than the synthetic antibiotics for all the studied bacteria strains. In the study some bacterial shown a stronger effect than aqueous extract which could be explained by the differences in the compounds between these two extracts.

Table 1. Inhibition effect of Antibacterial assay (diameter Zones of inhibition) of the pods and seed extracts of Monkey cola.

Treatments	Inhibition zones in mm				
	Gram Positive Bacteria		Gram Negative Bacteria		
	(AB)	(AA)	(AC)	(AD)	(AE)
Pods (AQ)	$21.33 \pm 0.58^B$	$33.00 \pm 1.00^A$	$24.00 \pm 2.00^A$	$22.00 \pm 1.00^{BC}$	$32.00 \pm 1.00^A$
Pods (ET)	$25.00 \pm 0.00^A$	$25.33 \pm 2.52^B$	$24.00 \pm 2.00^A$	$33.00 \pm 1.00^A$	$24.67 \pm 1.53^B$
Seeds (AQ)	$13.33 \pm 0.58^D$	$25.00 \pm 0.00^B$	$15.00 \pm 1.00^B$	$24.00 \pm 1.00^B$	$23.00 \pm 1.00^{BC}$
Seeds (ET)	$13.00 \pm 0.58^D$	$32.67 \pm 1.53^A$	$14.00 \pm 2.00^B$	$22.67 \pm 2.00^{BC}$	$20.00 \pm 2.00^C$
Control	$20.00 \pm 0.00^C$	$20.00 \pm 0.00^C$	$18.00 \pm 0.00^B$	$20.00 \pm 0.00^C$	$20.00 \pm 0.00^C$

All values represent a mean of three replicate tests and the standard error of the mean (SEM) has been calculated. The mean score in a column with different superscript letters are significantly different at  $p < 0.05$ .

The result of antibacterial showed that all the extracts of (AQ) and (ET) of Monkey cola with inhibition zones against AA (23-33mm), AD (22-33mm) and AE (21-32mm) inhibit better than synthetic antibiotics of inhibition zone (20mm) for these three strains of bacteria. It was found that ethanoic solvent extracts showed significantly higher zone of inhibition of (25-33mm) against AA which is lower than the ethanoic extract of Marigold (48mm) and higher than Tamarid ethanoic extract of 15mm against AA as reported by Alcasid *et al.*, (2016). The extracts of aqueous and ethanoic solvents of Monkey cola showed a higher zone of inhibition of 25-30mm and lower than extracts of chloroform, butanol, ethyl acetate and n-hexane extract of Medicago falcate of inhibition zone of 17-19mm as reported by Javid *et al.*, (2015).

### 3.2 Antifungal activities of crude Monkey cola extract of pods and seeds

The Aqueous (AQ) and ethanoic (ET) extracts of Monkey cola for both seeds and pods were evaluated for antifungal activity against Four fungi strains labelled; BA, BB, BC and BD. All crude plant extracts of Monkey cola showed antifungal activities against fungal strains tested as tabulated in Table 2. The results revealed that aqueous and ethanoic extract of Monkey cola showed significantly at  $p < 0.05$  higher inhibitory activity (3.69-100) against BA and (2.14-100) against BB. All extracts of Monkey cola showed activity (39.92-100) against BC and (0.100) against BD (Table2). The present investigation has shown that the aqueous and ethanolic extract of Monkey cola have active phytochemical, which can inhibit the growth of the studied pathogenic fungi. All crude plant extracts of Monkey cola showed antifungal activities except seed aqueous extract against BD. Plate 3 showed the picture of the fungal strain and the extracts.

Table 2. Inhibition effect of Antifungal assay (percentage Zones of inhibition) of the pods and seed extracts of Monkey cola.

Treatments	Inhibition Zones in percentage			
	BA	BB	BC	BD
Pods (AQ)	83.03 ± 0.08 <sup>B</sup>	83.53 ± 0.15 <sup>B</sup>	39.00 ± 0.42 <sup>D</sup>	73.05 ± 0.06 <sup>C</sup>
Pods (ET)	42.65 ± 0.05 <sup>C</sup>	43.49 ± 0.04 <sup>C</sup>	39.92 ± 0.48 <sup>D</sup>	42.70 ± 0.01 <sup>D</sup>
Seeds (AQ)	3.69 ± 0.04 <sup>D</sup>	2.14 ± 0.04 <sup>D</sup>	42.40 ± 0.02 <sup>C</sup>	0.00 ± 0.00 <sup>E</sup>
Seeds (ET)	100.00 ± 0.0 <sup>A</sup>	100.00 ± 0.00 <sup>A</sup>	100.00 ± 0.00 <sup>A</sup>	100.00 ± 0.00 <sup>A</sup>
Control	100.00 ± 0.00 <sup>A</sup>	100.00 ± 0.00 <sup>C</sup>	60.57 ± 0.04 <sup>B</sup>	89.90 ± 0.10 <sup>B</sup>

Mean score in a column with different superscript letters are significantly different at  $p < 0.05$  with SEM-standard error of mean

The evaluation of antifungal rates of two different solvents (aqueous and ethanoic) of pods and seeds of Monkey cola extracts were studied. The potency of the extracts was assessed quantitatively by determining % mycelial growth inhibition (Table 2). The average inhibition of the monkey cola seeds ethanoic extract was highest against the growth of all the fungus studied (100 %) and it showed a higher value than standard antifungal (Kocide) with 60.57 % and 89.90 % against BC and BD respectively. The ethanoic extracts of the seeds of Monkey cola showed prominent antifungal activity treatment of pathogenic diseases associated with the infection of these four pathogens. and could inhibit all the pathogens of the studied fungus. The average inhibition of the aqueous extract of pods against BA, (83.03 %), BB (83.53 %), BC (39 %) and BD (73.05) inhibit with higher value than pods ethanoic extracts of (42.65 %), (43.49 %), (39.92 %) and (42.70 %) for BA, BB, BC and BD respectively. The lowest was recorded against BD (0.00 ± 0.00) with seeds aqueous extract while 3.69 ± 1.00 %, 2.14 % and 42.40 % were recorded by BA, (83.03 %), BB (83.53 %), and BC respectively.

#### IV. CONCLUSION

The study had clearly revealed the antibacterial and antifungal potential of Monkey cola pods and seeds against common bacterial and fungal. The ethanoic and aqueous extracts of the parts of Monkey cola showed prominent antibacterial and antifungal activity treatment of pathogenic diseases associated with the infection of these studied strains pathogen both in plants and animals. The Monkey cola plant extracts which is eco-friendly control measure can be used as source of antibacterial, antifungal and in preventing food borne disease or as preservative in pre- and post-harvesting processing of food and bio-materials. The synthetic fungicides and antibiotic can pose harmful effects to environment and living organisms including humans and also increase the cost of production.

The other parts of the plants should be evaluated for antimicrobial properties. Also, other solvents could be used to extract the Monkey cola and minimum inhibitory concentrations to evaluate the potency of the extract.

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