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ASSESSMENT OF ANTIMICROBIAL ACTIVITY OF *Cola milleni* SEED AND PULP EXTRACTS AGAINST *Staphylococcus aureus, Escherichia coli* and *Penicillium notatum*

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ABSTRACT
All over the world, hundreds of plants have been identified based on researchers and experimental evidence as good sources of medicinal agents. The bioactive components (phytochemicals) of both the seeds and pulp of Cola milleni were extracted using ethanol as solvent. The bioactive components detected were alkaloids, tanins, saponins, cardiac glycosides, carbohydrates, sterols, resins and terpenes while Flavonoids, anthraquinones, anthracyanides and phenol were not detected for both the seed and pulps. Antimicrobial activity of the ethanol extract (Seed and pulp) against Staphylococcus aureus, Escherichia coli and Penicillium notatum was carried out using standard techniques. Staphylococcus aureus had the highest zone of inhibition for pulp having a range of 9.7mm±0.58mm - 19.7mm±2.52mm while Penicllium notatum had the least with 0.00mm. S.aureus also had the highest zone of inhibition range of 14.3mm±2.08mm - 21.3mm±1.53mm for the seed extract while penicillium had the least inhibition range of 5.0mm±1.00mm - 5.7mm±0.58. E.coli showed the highest minimum inhibitory concentration with ethanol extract of the pulp (160mg/ml) while penicillium notatum was not reactive. The minimum inhibitory concentration of seed against penillium notatum was the highest (160mg/ml) while staphylococcus aureus showed the lowest of 40mg/ml. The antimicrobial activity is as a result of the presence of phytochemicals detected, which suggest the use of the plant for the treatment of diseases caused by these organisms.
Key words: Cola millenii, Phytochemical, Antimicrobial activity, Bacteria, Fungi

INTRODUCTION

Plants are identified as good sources of medicinal agents. They are used in traditional medicine for different treatment of bacterial and fungal infection (Obafemi *et al*., 2009). Most of these plants have been reported to possess antimicrobial activity against a wide range of microorganisms. Studies have also revealed that the plant *Cola millenii* is one of such, which posses these properties (Narendra *et al*., 2013).

Cola millenii is a wild plant of tropical and sub-tropical countries whose place of origin is unknown, but probably from Indonesia. *C. millenii* belong to the Sterculiaceae family, genus cola and species millenii. *Cola millenii* is edible fruits of varying characteristics sweetness. It is widely distributed throughout tropical Africa, from Senegal to Cameroon. It was common in the southern Nigeria were they are eaten as edible fruits by the peasant farmers during the peak season (Adejo *et al*., 2014).

In the last few decades, most of pathogenic bacteria developed resistance to many antibiotics and this is a major threat to human health, medicinal plants are sources of diverse molecules, many of which display antimicrobial and antioxidant properties which protect human body from both pathogens and cellular oxidation. Thus, it is important to characterize different types of medicinal plants for their antioxidant and antimicrobial potential (Rahman et al., 2015).

The ethno-pharmacological uses of plants are also featured strongly among Nigerian people. It has been added that plants continued to play a prominent role in primary health-care of about 80% of the world population (Giwa *et al*., 2012). Over the years, there have been very alarming reports of multiple drug resistance in medically important strains of bacteria and fungi (Akinyemi *et al*., 2005).

The persistant increase in the incidence of such antibiotic resistance strains of organisms have lead to the development of more potent antibiotics such as the 3rd and 4th generation of cephalosporins by pharmaceutical companies (Odugbemi, 2006).

However, this has long given rise to recent studies pertaining the use of plants in folk medicine for the remedy in treating such cases

of diseases where the causal agent (organism) seem to pose resistance to the antibiotic drugs used (Adeneye *et al*., 2006).

Research programmes are ongoing in many countries including Nigeria to screen traditional medical preparations for their potential activities (Ahmad and Beg, 2011). In early findings, tropical Africa sub-region has been discovered to be home to many valuable fruit species whose potentials have not been fully realized. A good number of this fruit species are not yet domesticated. Nevertheless, tangible economic produce are been harvested from their wild (Abitogun *et al*., 2010).

Cola millenii (being one of this group of fruit plants) has been chosen as case study because of its rampant growth and produce in the wild and its consumption by most mammals especially monkeys and invariably humans. Thus, this gives rise to the view/objectives of this study in evaluating the antimicrobial effect of the fruit (pulp and seed) of the plant (monkey kola).

MATERIALS AND METHODS

Sample Collection and Authentification

The *Cola millenii* was collected fresh from the farm centre, owo ondo state and oja oba, sabo oke Illorin, kwara state. It was authenticated at federal University of Technology Akure (at herbarium section with voucher numbers CMP1121P-C and CMP1122S-C for pulp and seed respectively) before it was finally transferred to the laboratory for further processing and analysis.

Clinical isolates of the following test microorganisms *Staphylococcus aureus* (10), *Escherichia coli*(10) and *Penicillium notatum*(10) were obtained from Yusuf Dantsoho Memorial Hospital, Tudun Wada, Kaduna and National ear care Center, Unguwar Rimi Kaduna.

Processing and Drying of Sample

The freshly collected seed and pulp of *Cola millenii* were thoroughly washed with clean tap water followed by distilled water. They were then air dried for one week and grinded into fine powder. The grounded powder was separately passed through a sieve to collect refined powder which were packaged and labelled accordingly.

Plant Extraction/Preparation of Extracts

Ninety five percent (95%) ethanol was used for the phytochemical extraction of both parts of the plant. Twenty five (25g) of the powdered plant materials were separately dissolved in enough sterilized ethanol in sterilized conickal flasks to make 100ml of ethanol extract (25% w/v).The mixture were kept undisturbed at room temperature for 48hours in the sterile flask covered with aluminium foil to avoid evaporation. They were subjected to filtration through sterilized Whatman's N0.1 filter paper. After filtration, the extracts were evaporated in water bath until 25ml exctract was left in the container. The ethanol extracts thus obtained were immediately evaluated for antibacterial activity and antifungal activity using agar well diffusion method (Barreto *et al*., 2002).

Phytochemical Screening

The ethanol extract of both the seed and pulp of *Cola millenii* were tested for the presence of phytochemicals as described by Giwa *et al*. (2012).

Preparation of standard Inocula

Astandard inoculum was prepared using previously overnight broth culture of each of the test organism by diluting with sterile saline solution to march Mcfarland standard. The standardized inoculum was used for the antibacterial activity testing (Akinpelu and Onokoya, 2006).

Evaluation of Antimicrobial Activity

The antimicrobial activity of the crude ethanol extracts of both the seed and pulp of *Cola millenii* against the test isolates of both bacteria and fungi were evaluated using agar well diffusion method (Ahmad and Beg, 2011). Molten agar plates (NA and PDA) were respectively inoculated with 1ml of standardized inocula; wells of 5mm in diameter were bored with sterile cork borer into the agar plates containing the bacterial and fungal inocula. About 1.0ml of the plant extract was poured into a well of inoculated plates at varying concentrations (100mg/ml, 200mg/ml and 300mg/ml) respectively. Antibiotic Gentamycin (80mg/ml) was used as a positive control which was introduced in a well instead of plant extract. Solvent ethanol was used as a negative control which was introduced into a well instead of plant extracts. The plates prepared were left at room temperature for about ten minutes allowing the diffusion of the extracts and antibiotics into the agar (Giwa *et al*., 2012). The plates inoculated with the bacteria were incubated for 24 hours at 37^0C while those inoculated with fungi were incubated at room temperature (25^0C) for 72 hours. The plates were observed after the incubation periods.

The presence of antimicrobial activity was indicated by an inhibition zone sorrounding the well containing the plant extracts. The zones of inhibition were measured and expressed in millimeters (Maneemegalai and Naveen, 2010). The diameter of zone of inhibition was determined.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bacterial/Fungicidal Concentration (MBC/MFC) of pulp and seed ethanol Extracts against Bacterial Fungal Isolates.

MIC and MBC/MFC of the pulp and seed of the ethanol extracts were determined by macro-dillution broth methods (Giwa *et al.,* 2012). Macro-dillution broth method; a two-fold serial-dillution of the seed and pulp ethanol extracts were prepared in sterile nutrient broth to achieve a decreasing concentration ranging from 160 to 1.25mg/ml in eight sterile tubes labeled 1 to 8. Each dilution was seeded with 1ml of the standardized bacterial inocula. The inoculated culture tubes were incubated at 37^0C for 24 hours. A set of tubes containing only seeded broth was kept as control. The lower concentration that did not permit any visible growth which was compared with the control was considered as the MIC. The MBC/MFC is the lowest concentration of antimicrobial agent that will prevent the growth of an organism on a culture medium. In determining the MBC, 1ml from the tube that did not show visible turbidity was placed on NA plate (bacteria) and PDA (fungi) were spread over the plates. After incubation at 37^0C for 24 hours (bacteria) and 25^0C for 72 hours the plates were examined for the growth of bacterium to determine the concentration of the extract at which killing of the bacterial and fungal isolates was achieved (Irkin and Korukluogu, 2007).

RESULTS AND DISCUSSION

The result of the Phytochemical screening of the extracts of seed and pulp of *Cola millenii* revealed the presence of saponins, carbohydrate, resins, alkaloids, steroids, tannis and tarpenes. This is in line with the findings of Giwa *et al*. (2012). Various studies have shown that plants that are rich in alkaloids and tannin compounds possess antimicrobial activity usually against a wide range of microorganisms (Giwa *et al.,* 2012).

Table 1: Phytochemical Constituents of Seed and Pulp Extracts of *Cola millenii*

Phytochemicals	Seed	Pulp
Alkaloids	+	+
Saponins	+	+
Taninins	+	+
Flavonoids	-	-
Anthraquinones	-	-
Terpenes	+	+
Cardiac glycosides	+	+
Sterols	+	+
Resins	+	+
Anthracyanides	-	-
Carbohydrates	+	+
Phenol	-	-

Key: + = Detected, - = Not detected

Table 2: Antimicrobial Activity of Crude Ethanol Extracts of *Cola millenii* (Seed and Pulp) on Test Isolates.

Test Isolates	Concentration Mean Zone of Inhibition			
	Pulp			
	100mg/ml	200mg/ml	300mg/ml	Gentamycin/Fungisol
Staphylococcus aureus	9.7±0.58	13.0±2.00	19.7±2.52	28.0±0.00
Escherichia coli	10.3±1.53	13.3±1.75	16.3±1.15	25.7±1.15
Penicillium notatum	0.00±0.00	0.00±0.00	0.00±0.00	18.3±0.58
	Seed			
Staphylococcus aureus	14.3±2.08	18.3±1.15	21.3±1.53	29.0±0.00
Escherichia coli	14.3±0.53	18.0±0.00	20.7±0.58	27.0±0.00
Penicillium notatum	5.0 ±1.00	5.3±1.53	5.7±0.58	20.0±0.00

The result of the evaluation of antimicrobial activity also showed that *Cola milleni* extract can be used as a reliable source of antimicrobial agent. The ethanol extract from the seed showed higher effect on the tested isolates than the pulp ethanol extract. It had low effect against *pencillium notatum*. Contrary to the study carried out by Giwa *et al.* (2012), whos result showed that the extract demonstrated higher activity against both the Gram Positive and Gram Negative bacteria and high activity on the fungi.

The susceptibility of *Staphylococcus aureus* and *Escherichai coli* are comparable at all the concentration suggesting that *cola milleni* may be active against both Gram negative and Gram positive bacteria. This agrees with the findings of Sonibare *et al*. (2009). This does not agree with findings of Akindele, (2013) who reported that the pulp demonstrated more activity against the microorganisms studied. In 2009, Sonibare also reported that both the ethanol pulp and seed extracts were active against *Aspergillus niger.*

Similar work was carried out by Akindele (2013), reported that the aqueous extract of *Cola milleni* seed and pulp showed relatively low activities against the test organisms. *Klebsiella pneumonia* showed a rather very low sensitivity to these extracts as compared to *Escherichia* coli. The aqueous extract was reported to demonstrate no activity against *Pencillum notatum.*

The aqueous extraction carried out by Giwa *et al.* (2012) was also reported to demonstrate very low activity against seven (7) microorganisms, which support the fact that water is a less effective solvent for extraction of the bioactive compounds.

Table 3: Minimum inhibitory concentration (MIC) and Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) of pulp and Seed of Cola millenii Ethanol Extract against Test Isolates

Test Isolates	MBC/MFC (mg/ml)	
	Pulp	**Seed**
Escherichia coli	160 (ND)	80(160)
Staphylococcus aureus	80 (ND)	40(160)
Penicillium notatum	ND (ND)	160(ND)

Key: ND = Not detected

The MBC/MFC results from the extracts of the different plant parts are found to be higher than the MIC value. That is, the extracts are bacteriostatics at lower concentrations and bactericidal at higher concentrations. Although this may be attributed to the fact that the extracts are in their crude form and may contain very small amount of the bioactive compounds. This agrees with Akinyemi *et al.* (2006) which stated that MBC values obtained for the crude extracts against the pathogens are higher than MIC values.

CONCLUSION

This study revealed that the seed and pulp of *Cola milleni* possess antimicrobial properties. This is as a result of the bioactive components (Phytochemical) which were present in both the seed and pulp of the plant. The seed extract of *cola milleni* possess higher antimicrobial activity than the pulp extracts. The pulp and seed extracts are more active against bacteria than fungi.

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