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Antimicrobial effects of the chloroform and ethanolic leaf extracts of *Dacryodes Edulis* (G. Don) H.J. Lam, *Garcinia Kola* Heckel and *Chrysophyllum Albidum* G. Don on some test isolates

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ABSTRACT

Dacryodes edulis, *Garcinia kola*, and *Chrysophyllum albidum* are well known plants used for traditional medicine in the treatment of various ailments and diseases. The crude extracts of these three plants were screened for their antimicrobial effects against some selected microorganisms; bacteria and fungi using an agar-diffusion method. The screened extracts of the plants showed substantial antimicrobial activities. The test isolates used in this study were *Escherichi coli, pseudomonas aeruginosa, staphylococcus aureus, Bacillis subilis, Aspergillus niger, Penicillum notatum*, *Mucor mucedo* and *Candida albicans*. The antimicrobial activity of the ethanolic leaf extracts of the plants ranged from 0.00± 0.00 to 21.00±0.08 for *Chrysophyllum albidum*, 6.67 ± 1.01 to 26.00±0.41 and 4.33 ± 0.16 to 22.67±0.00 for *Dacryodes edulis* and *Garcinia kola*. While the chloroform leaf extract of the plants on test isolates ranged from 8.67± 1.03 to 30.33± 0.09, 6.67 ± 0.54 to 32.67± 0.04 and 7.33 ± 0.10 for *Dacryodes edulis*, *Garcinia kola* and *Chrysophyllum albidum* respectively.MIC of ethanol leaf extract ranged from 6.25-100.0 mg/ml while MIC of chloroform leaf extract ranged from3.125-100.0 mg/ml for all three plants. Phytochemical analysis shows the presence of Alkaloids, Flavonoids, Saponins, Tannins, and Steroids. All the test bacterial isolates were resistant against commonly used antibiotics such as streptomycin, septrin, and ampicillin. This suggests that leaf extracts of the investigated plants contain active ingredients that qualify them for medicinal use. The results of this study could also be of commercial interest to both pharmaceutical companies and research institutes in the production of new drugs as the need for further studies cannot be over emphasized.

Keywords: *Chrysophyllum albidum, Dacryodes edulis, Garcinia kola*, Ethanolic and Chloroform extracts, Antimicrobial activity.

Abbreviations: CLSI - Clinical and Laboratory Standard Institute; SD - Standard deviation; MIC - Minimum Inhibitory Concentration

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1. INTRODUCTION

Traditional medicine is an important part of the health-care system in most countries of the world including Africa. About 80-90% of the populations in African countries are dependent on traditional medicine for their primary health care (Hostettman et al. 2000). For example, in Sudan, traditional medicine plays an important role for health care, since access to hospitals and other medical facilities are limited and a higher percentage of the population are nomads (Elegami et al. 2002). In Tanzania, over 60% of the health seeking populations has a traditional healer as their first point of contact (Hedberg et al. 1982). In South Africa, it is estimated that about 27millionpeople depend on herbal medicine for their primary healthcare (Meyer et al. 1996; Mander, 1998). In Nigeria, traditional medicine is well acknowledged and established as a viable profession (Kafaru, 1994), and almost all plants seem to have some kind of application in traditional medicine (Babayi et al. 2004).

Dacryodes edulis (G.don) H. J. Lam (Burseraceae) commonly known as 'African palm', 'native pear' or 'African plum' is a dioecious shade loving species of non-flooded forest in the humid tropical zone (Leakey, 1999; Leakey et al. 2002; Waruhiu et al. 2004; Anegbeh et al. 2005). It is characterized with fruits which contains seed surrounded by a pulpy butyraceous pericap, which is edible (consumed raw or cooked) and an excellent source of oils, proteins, minerals and vitamins (Sofowora, 1982). Besides the nutritional potentials of the fruits, it possesses medicinal properties and is used especially in herbal medicine to remedy diseased conditions (Stray, 1998) which include hypertension, diabetes, malaria, infertility, menstrual problems, eczema, ring worm, typhoid, etc. *Garcinia kola* Heckel (Guttiferaceae or Clusiaceae) commonly known as 'bitter kola' or 'male kola' because of its claimed aphrodisiac activity and its potential to cause nervous alertness as well as induce insomnia (Uko et al. 2001) is a forest tree. The roots of the plant are used as bitter chew- sticks, the nuts are chewed as a masticatory, the stem bark is used as a purgative, the latex is externally applied to fresh wounds to prevent sepsis, thereby assisting in wound healing, the fruit pulp is used in treatment of jaundice (Uko et al. 2001; Igbozulike and Aremu, 2009). The seeds are used in the treatment of bronchitis, throat infections, colics, headaches, chest colds, coughs, diarrhea, hepatitis, asthma and dysmenorrheal/menstrual cramps (Iwu, 1993; Okojie et al. 2009). The plant has been shown to possess anti-inflammatory, antimicrobial, antioxidant, antiviral,

Table 1
The effect (zone of inhibition in mm) of Ethanolic leaf extract of three indigenous plants on test isolates

Test isolates	Concentration of extract (100 mg/ml)		
	Dacryodes edulis	*Garcinia kola*	*Chrysophyllum albidum*
Escherichia coli	26.00 ± 0.41	22.67 ± 0.00	21.00 ± 0.08
Pseudomonas aeruginosa	17.67 ± 1.03	14.00 ± 0.04	18.00 ± 0.01
Staphylococcus aureus	20.67 ± 0.14	19.00 ± 0.07	14.33 ± 0.38
Bacillus subilis	11.67 ± 0.57	4.33 ± 0.16	8.33 ± 1.40
Aspergillus niger	10.33 ± 1.46	10.33 ± 0.11	0.00 ± 0.0
Penicillium notatum	7.67 ± 0.33	5.33± 1.02	7.33 ± 1.28
Mucor mucedo	6.6 7 ± 1.01	0.00 ± 0.00	5.67 ± 1.04
Candida albicans	15.33 ± 0.70	14.33 ± 0.20	11.33 ± 0.52

***NB**: The values are mean ± Standard deviation of triplicate determinations*

Table 2
The effect of 100 mg/ml of chloroform leaf extract of three indigenous medicinal plants

Test isolates	Zone of Inhibition (mm)		
	Dacryodes edulis	*Garcinia kola*	*Chrysophyllum albidum*
Escherichia coli	36.00 ± 0.14	27.33 ± 0.06	28.67 ± 0.09
Pseudomonas aeruginosa	28.33 ± 0.20	32. 67 ± 0.04	22.33 ± 1.46
Staphylococcus aureus	34.00 ± 0.30	23.33 ± 0.07	24.67 ± 0.07
Bacillus subilis	30.33 ± 0.09	19.67 ± 0.04	12.67 ± 0.05
Aspergillus niger	11.33 ± 1.06	10.33 ± 0.60	0.00 ± 0.00
Penicillium notatum	8.67 ± 1.03	5.33 ± 1.30	7.33 ± 0.10
Mucor mucedo	9.33 ± 1.08	6.67 ± 0.54	7.33 ± 0.10
Candida albicans	16.00 ± 1.26	14.33 ± 0.28	13.33 ± 0.01

***NB**: Values are means ± standard deviation of triplicate determinations*

Table 3
Minimum Inhibitory Concentrations (MICs) of the Ethanolic extract of the leaves of *Dacryodes edulis, Garcinia kola* and *Chrysophyllum albidum* against the test isolates

Test isolates	MIC(mg/ml)		
	Dacryodes edulis	*Garcinia kola*	*Chrysophyllum albidum*
Escherichia coli	6.25	6.25	25.0
Pseudomonas aeruginosa	25.0	25.0	50.0
Staphylococcus aureus	6.25	25.0	25.0
Bacillus subilis	50.0	50.0	50.0
Aspergillus niger	100.0	NIL	NIL
Penicillium notatum	100.0	100.0	100.0
Mucor mucedo	100.0	NIL	100.0
Candida albicans	50.0	100.0	100.0

Table 4
Minimum Inhibitory Concentrations (MIC) of the chloroform leaf extracts of *Dacryodes edulis, Garcinia kola* and *Chrysophyllum albidum* against the test isolates

Test isolates	MIC(mg/ml)		
	Dacryodes edulis	*Garcinia kola*	*Chrysophyllum albidum*
Escherichia coli	3.125	3.125	12.5
Pseudomonas aeruginosa	12.5	12.5	25.0
Staphylococcus aureus	3.125	12.5	25.0
Bacillus subilis	25.0	50.0	25.0
Aspergillus niger	100.0	100.0	100.0
Penicillium notatum	100.0	100.0	100.0
Mucor mucedo	100.0	100.0	100.0
Candida albicans	50.0	50.0	100.0

Table 5
Quantitative analysis of the phytochemicals of the different plants

Plants	Akaloids (%)	Flavonoid (%)	Saponins (%)	Tanins (%)	Terpenoids (%)	Steroids (%)
Dacryodes edulis	0.21	0.15	4.07	2.06	0.00	1.14
Garcinia kola	0.45	1.20	3.14	1.74	0.00	0.01
Chrysophyllum albidum	0.11	0.23	0.83	1.48	0.00	0.00

Table 6
Antifungal susceptibility test (Positive control)

Fungal isolates	Nystatin (100mg/ml)
Aspergillus niger	5.0mm
Penicillum notatum	8.0mm
Mucor spp.	11.0mm
Candida albicans	21.0mm

antidiabetic and anti-hepatotoxic activities (Iwu, 1993; Okwu, 2005). The seeds have shown a broad spectrum of antibacterial activities (Ezeifeka et al. 2004; Sibanda and Okoh, 2008; Okigbo and Mmeka, 2008).

Chrysophyllum albidum G.don (Sapotaceae) commonly reffered to as 'white star apple' or 'mululu' is a tropical forest tree whose natural occurrences have been reported in diverse ecozones in Nigeria, Uganda, Niger republic, Cameroon and cote d' Ivoire (Bada, 1997). The fruit of *C. albidum* is common in both urban and rural centres especially during the months of December to April. The fruits are not usually harvested from the trees, but left to drop naturally to the forest floor where they are picked up (Adewusi, 1997). Its seeds are a source of oils, which is used for diverse purposes (Ugbogu and Akukwe, 2009). The leaves are used as emollients and for the treatment of skin eruptions, diarrhoea and stomach ache which are as a result of infections and inflammatory reactions (Adisa, 2000). Studies have been carried out on seeds of *C. albidum*, for example, seed storage and its food value, physical properties of the seed, use of the shell of seeds for the removal of metal ions and antimicrobial effect of oil from its seeds against local clinical bacteria isolates (Amusa et al. 2003; Oyelade et al. 2005; Ugbegu and Akukwe, 2009; Oboh et al. 2009). This study is aimed at collecting and screening plant extracts of three indigenous medicinal plants found in Benin city metropolis for *in vitro* antimicrobial activities against different microorganisms as well as report the minimal inhibitory concentration of the screened medicinal plant extracts using a selected antimicrobial assay, thereby adding information and knowledge to the scientific research done on these indigenous medicinal plants used traditionally in Nigeria especially in Benin city.

2. MATERIALS AND METHODS

Collection of samples: Fresh leaves of *Dacryodes edulis*, *Garcinia kola* and *Chrysophyllum albidum* were collected from different areas within Ugbowo, Benin City. They were identified by the Herbarium team in the Department of Plant Biology and Biotechnology, University of Benin, Benin city, Nigeria.

2.1. Preparation and Extraction of plant materials

The fresh leaves of all three plants samples were air dried and transferred to the oven set at 40˚C for 5 – 10 minutes separately. The plant materials were then reduced to fine powder with the aid of a mechanical grinder. Ethanol and chloroform were chosen as the extraction solvents. Twenty grams (20g) each of the powdered leaf were macerated in 200 ml of ethanol and chloroform in separate beakers and allowed to stand for 24 hrs after which the resultant mixture was filtered using Whatmann No. 1 filter paper. Supernatant of each plant extract was transferred into pre-weighed beakers. The pre-weighed beakers were allowed to dry completely to obtain a solvent-free dried extract residue using water bath evaporator.

2.2. Test organisms

The test organisms used were bacteria and fungi isolates. The bacterial isolates are *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli*. The fungi are *Aspergillus niger, Penicillum notatum, Mucor mucedo* and *Candida albicans.*

2.3. Antimicrobial Susceptibility Test

According to the standard operational procedures, antimicrobial susceptibility tests were done on Nutrient agar (Oxoid, using Kirby Bauer diffusion method) (Bauer et al. 1966). Morphologically identical 4-6 bacterial colonies from overnight culture were suspended in 5 ml normal saline and incubated for 4 hours at 37˚C. The surface of Mueller Hinton agar plate was evenly inoculated with the culture by surface inoculation. The antibiotic discs were applied to the surface of the inoculated agar. After 18-24 hours of incubation, the diameter of growth inhibition around the discs was measured. The antibiotics tested were tetracycline (30µg), nitrofurantoin (300µg), erythromycin (15µg), chloraphenicol (30µg), gentamicin (10µg), ciproflaxcin (5µg), cephalotin (30µg), zinnaef(2µg), streptomycin (30µg), roephin (25µg) septrin (30µg) and amoxicillin (30µg). Susceptibility data were interpreted according to Clinical and Laboratory Standard Institute (CLSI), (2006).

Clinical and Laboratory Standard Institute (CLSI): CLSI is setting the standard for quality in clinical laboratory testing around the world. A not-for-profit membership organization, the Clinical and Laboratory Standards Institute (CLSI) brings together the global laboratory community for a common cause: fostering excellence in laboratory medicine. Together, we guide the development and implementation of standards and guidelines that help all laboratories fulfill their responsibilities with efficiency, effectiveness, and global applicability (clsi.org).

2.4. Determination of MIC

The minimum inhibitory concentration (MIC) of the extracts was determined according to the method described by Kabir et al (2005). Extracts were diluted to concentrations ranging from 3.125-100 mg/ml to each dilution of plant extracts in nutrient broth tubes and were seeded with 0.1 ml of the standard bacterial inoculums. Negative control tubes with no bacterial inoculation, were simultaneously maintained. Tubes were incubated aerobically at 37˚C for 24 hrs. The lowest concentration of the extract that produced no visible bacterial growth (turbidity) was recorded as the MIC. Dilutions showing no visible growth for MIC was sub-cultured onto a fresh nutrient Agar plate and incubated at 37˚C for 24 hours. Statistical analysis: Results are expressed as mean ± standard deviation (S.D) using SPSS 10.0 computer software package.

3. RESULTS

The results of the antimicrobial activities of the chloroform and ethanol leaf extracts of *Garcinia kola, Chrysophyllum albidum* and *Dacryodes edulis* are presented in Table 1. There was a variation in the antimicrobial activity between different plants as well as between different extracts made from these medicinal plants. The diameter of the zone of inhibition depends on type of extracts, solvents and nature of the organisms. Extracts of the leaves of *Garcinia kola* were as effective as the leaf extract of *Dacryodes edulis* against the test microorganisms (Table 1 and 2).

3.1. Minimum Inhibitory Concentration (MIC) of the investigated plants leaf extracts

Minimum inhibitory concentration (MIC): Minimum inhibitory concentrations (MICs) are defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation, and minimum bactericidal concentrations (MBCs) as the lowest concentration of antimicrobial that will prevent the growth of an organism after subculture on to antibiotic-free media.

The chloroform extract gave the lowest MIC value compared to the ethanol extract. The MIC values ranged from 12.25mg/ml for chloroform extract and 50–100 mg/ml for ethanol extract as depicted in table 3. The MIC values of the ethanol extract ranged from 6.25-100 mg/ml for *D. edulis* and *G. kola* and 25-100 mg/ml for *C. albidum.* The MIC values of the chloroform extract ranged from 3.125-50 mg/ml for *D. edulis* and *G. kola,* and 12.5-100mg/ml for *C. albidum* as represented in the table 4.

3.2. Phytochemical analysis

The qualitative and quantitative phytochemicals of the respective plant materials are presented in table 5 and 6.

3.3. Antibiotics susceptibility test

Antibiotics discs containing different antibiotics were used as positive control for the bacterial isolates. The antibiotics were pefloxacin, streptomycin, zinnacef, amoxicillin, ceporex, nalidixic acid, ofloxacin, augmentin, ampicilli. Nystatin (100mg/ml) was used as positive controls for the fungal isolates (Table 6). The results show that the zone of inhibition of the standard antibiotics was comparably lower than that of some plant extracts. Ciprofloxacin produced the highest zones of inhibition of 30 mm against *Escherichia coli.* All bacterial isolates were resistant to septrin, ampiclox and streptomycin. *Candida albicans* was most susceptible to mystatin with diameter of zone of inhibition of 21.0mm.

4. DISCUSSION

Antimicrobial properties of substances are desirable tools in the control of undesirable tools in the control of undesirable microorganisms especially in the treatment of infectious diseases and in food spoilage. The active components usually interfere with growth and metabolism of microorganisms in a negative manner (Aboaba et al. 2012). The antimicrobial activity of the ethanol and chloroform leaf extracts of *Garcinia kola, Chrysophyllum albidum and Dacryodes edulis* was reported in this study (Table 4). The result of the antimicrobial screening which showed that the test isolates were susceptible to ethanol and chloroform extracts of different plants. This indicates that some of the antimicrobial compounds in the investigated plants might be polar. The zones of inhibition of growth of the microorganism are function of relative antibacterial and antifungal activity of the extracts. The MIC of the leaf extracts of the plants against the test microorganism ranged from 3.125 to 100mg/ml. the effect of the plant extract on the MIC for the test microorganisms varied widely in the degree of their susceptibility (Elekwa et al., 2009). An antimicrobial activity of plant extracts with highly active antimicrobial agent gives a low MIC while a low activity against a microorganism has a high MIC.

The phytochemicals screening of different plants used in the analysis revealed the presence of all secondary metabolite analyzed which included saponin, flavonoids, alkaloids and tannins. Saponin, which is responsible for numerous pharmacological properties, was also tested positive in plants leaves extracts (Estrada et al. 2000). Saponins are considered a key ingredient in traditional Chinese medicine and are responsible for most of the observed biological effects (Liu and Henkel, 2002). Okwu (2005) reported that alkaloids are known to exhibit marked physiological activity when administered to animals. These antioxidants are compounds that reduce the formation of free radicals or react with and neutralize them. Thus, potentially protecting the cells from oxidative damage (Onyeche et al. 2010). Tannins and saponins are known for their astringency and bitterness respectively (Okwu, 2005). Saponins also display foaming capacity. This property distinguishes it from other phytochemicals. The astringent property of tannins and the forming capacity of saponins may make *D. edulis* fruits effective against wounds. Both tannins and saponins are sometimes employed as astringents in gastrointestinal tract, on skin abrasions, wounds etc. The presence of these phytochemicals in the investigated plants may contribute to the effects of plants as remedy for various diseases. These results suggest the presence of potent antibacterial activity of the leaves extracts of the investigated plants against the bacteria might be due to naturally occurring bioactive phytochemicals present in the plant materials. It could be concluded that detailed characterization of various compounds from leaves of plants under investigation is needed, so that structure activity relationship in terms of antimicrobial and antifungal activity could be investigated. The high degree of antimicrobial activity of some of the plants seems to confirm the folk therapy of infections and traditional therapeutic claims of these herbs. The antimicrobial activity of the leaf extracts of the different plants were compared with the commercially available antibiotics under the same laboratory conditions. The activity of some of the plant extracts was greater also than that of the standard antibiotics when they were investigated.

5. CONCLUSION

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The results obtained from the present study support the use of this plant parts in the traditional treatment of diseases in Nigeria. The result of this finding could be of very important to pharmaceutical industries for the development of new antimicrobial drugs to address unmet therapeutic needs. Such screening of various natural organic compounds and identification of active agents is the need of the hour for saving life and providing good health to humanity. There is need for further studies on the plant parts in order to isolate, identify, characterize and elucidate the structure of antimicrobial bioactive compounds.

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Bauer et al. (1966): The "Kirby-Bauer" antibiotic susceptibility test is another standard method that you should cover in microbiology class. The method involves getting a pure culture of the bacteria you want to treat, and then growing it in a petri dish. By putting paper disks soaked with various anti-bacterial substances, you can identify which ones are most effective at killing (or at least stopping) the bacteria (bigroom.org).