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Original Research

Phytochemicals of *Chrysophyllum albidum, Dacryodes edulis, Garcinia kola* chloroform and ethanolic root extracts and their antimicrobial properties

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Summary
Aim: The phytochemistry and antimicrobial qualities of the chloroform and ethanolic root extracts of *Chrysophyllum albidum* G. Don Holl, *Dacryodes edulis* H.J. Lam and *Garcinia kola* H were investigated.
Method: Routine methods were used. The test isolates utilized were; *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Aspergillus niger*, *Penicillium notatum*, *Mucor mucedo* and *Candida albicans*.
Results: Phytochemical screening revealed the presence of alkaloids, flavonoids, saponins and tannins. Both extracts showed broad spectrum of antimicrobial activities but chloroform extracts gave higher zones of inhibition compared to corresponding concentrations of ethanol extracts. Maximal inhibitory zones were shown by *P. aeruginosa*; 30.7 mm ± 0.01 against *G. kola* chloroform root extract. Chloroform root extracts of *G. kola* were comparatively more potent against the test isolates than *C. albidum* and *D. edulis* root extracts. All the respective root extracts exhibited a greater antibacterial activity in comparison with the antifungal attributes.
Conclusion: The presence of bioactive antimicrobial compounds in the examined extracts of the medicinal plants particularly *G. kola* could indicate the possibility of obtaining potentially valuable antimicrobial phytochemicals from the plants.

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INTRODUCTION

Man since ancient time has been dependent on plants for food, drinks, shelter, clothing, equipment, dental care and medicine [1]. Infections due to pathogenic bacteria and fungi represent a critical problem to human health [2]. Over 25% of the prescribed medicines in industrialized countries are derived directly from plants [3]. Benefits derived from using medicine obtained from plants are that they are relatively safer than synthetic alternative by offering profound therapeutic benefits and more affordable treatment [4]. Plants contain phytochemicals, which are natural bioactive compounds [5] and possess antimicrobial, anti-oxidant and physiological activities [6].

Chrysophyllum albidum G. Don Holl (Sapotaceae) tree is common throughout the tropical Central, East and West Africa region and is valued for its edible fruits and ethno-medical uses [7-8]. *C. albidum* fruits (known as African star apple) are widely eaten in southern Nigeria [9]. The bark is used as a remedy for yellow fever and malaria, while the leaves are used as emollients and for the treatment of skin eruptions, diarrhea and stomach ache, resulting from infections and inflammatory reactions [10]. *Dacryodes edulis* H.J. Lam (Burseraceae) commonly called African pear is a fruit tree native to Africa. The fruit tree is an ellipsoidal drupe which varies in length from 4-12 cm. The fruit of *D. edulis* can be eaten raw, cooked, boiled or roasted [11]. The bark or leaf decoction is used to treat toothache, gum problem, tonsillitis and earache [12].

The roots are known to treat beri-beri and rickets when boiled with other herbs and administered orally [11]. *Garcinia kola* H. (Guttiferaceae) is a tree that grows in rain forests and swamps of West Africa and grows as a medium sized tree up to a height of about 12 m high [5]. *G. kola* is believed to be an important source of new chemical substances with potential therapeutic benefits. From its roots to its leaves, the plant is known to contain several phytochemicals noted for their medicinal importance [13]. *G. kola* is also used in the treatment of liver disease and diarrhea [13-14].

This study was done to investigate the phytochemical constituents and anti-microbial activity of *Chrysophyllum albidum*, *Dacryodes edulis* and *Garcinia kola* chloroform and ethanolic root extracts with a view of validating their folk use in treating some microbial ailments.

MATERIALS AND METHODS

Plant collection, preparation and extraction

The root samples were collected from the Forestry Department; Faculty of Agriculture, University of Benin, Benin City, Edo State, Nigeria in April 2012. The plants (trees) were identified by Dr. G.Emelue of the same Department. Samples of all three plants were kept in our institutions herbarium with voucher numbers UBHs0257, UBHb0291, UBHg0281 respectively. The materials were washed and cut into small pieces and air dried before grinding. Then 50 g of each powdered plant was soaked separately in 500 ml chloroform and 95% ethanol at room temperature for 24 h. The extracts were then filtered through cheesecloth for 30 minutes. The filtrates were collected, filtered through Whatman No 1 filter paper and evaporated in water bath at 40^{o}C. The extracts were collected and kept at 4^{o}C for further investigation.

Phytochemistry

Qualitative tests for alkaloids, tannins, saponin, flavonoids, steroids and terpenoids were conducted using standard methods such as Mayer's reagent test for determination of alkaloids, frothing test for saponin determination and flavonoid determination [15-16]. Quantitative phytochemical constituents of the ethanolic extracts were determined using methods described by [16-19].

Test organisms

Several clinical test isolates were used in this study; *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Candida albicans*, *Penicillium notatum*, *Mucor mucedo* and *Aspergillus niger*. The bacterial and yeast pure cultures were sourced from the Medical microbiology laboratory, University of Benin Teaching Hospital. The filamentous fungal isolates were obtained from Edo Environmental Consults and Laboratory, Benin City. The identities of the bacterial pure cultures were confirmed by conducting several routine morphological and biochemical tests as described by [20] and [21]. The results of the tests were compared with reference tables as stated by [22].

Standardization of the microbial inocula

The procedures as described by [23] and [24] were adapted in the standardization of the microbial test pure cultures All the test bacterial and fungal isolates were sub-cultured on freshly prepared Nutrient agar and Sabouraud Dextrose agar plates and incubated for 24 h and 48 h respectively. Portions of the streaked bacterial and fungal colonies were transferred into test tubes containing 8ml of sterile nutrient broth and incubated for 12h and 48 h at 37 ^{0}C. The growth of bacterial and fungal suspension obtained was compared to that of freshly prepared Barium sulphate opacity standard {0.5 ml of 1% Barium in Chloride to 99.5 ml of 1% H_2SO_4 (0.36 Normal).The turbidity was adjusted by adding more sterile nutrient broth to match 0.5 McFarland standards (10^6 cfu/ml and 10^6 spores /ml).

Determination of antimicrobial activity

The agar-well diffusion assay as described by [25] was used to ascertain the inhibitory effects of the respective root extracts on the test isolates. The tests were carried out using a stock concentration of 100 mg/ ml prepared by dissolving 0.01 g of the respective ethanolic and chloroform root extracts into 10 ml of distilled water. Prepared and labeled Nutrient agar (NA) and Sabouraud Dextrose agar (SDA) plates were seeded with 2ml of standardized bacterial and fungal broth cultures. The microbial lawn was done using a sterile glass rod. The seeded plates were allowed to dry. A 4 mm sterile cork borer was used to punch 2 equidistant holes in the middle of the labeled inoculated agar plates. The holes were filled with 0.2 ml of the differing concentrations of the seed extracts. The bored agar plates were left at room temperature for 10 min, allowing the diffusion of the extracts into the agar. Then the plates inoculated with bacterial and yeast isolates were incubated at 35 ^{0}C for 24 h. The plates with the filamentous fungal cultures were kept at room temperature for 72 h. At the end of the incubation period, the plates were observed. The antibacterial activity of the seed extracts was assessed by an inhibition zone surrounding the well. The mean zones of inhibition was measured and expressed in millimeters. The process was repeated in the case of fungi however, inoculated plates were stored at room temperature for 72 h after which the zones of inhibition

were measured using a meter rule. However, commercially available antibiotic discs (Pefloxacin and Nystatin) were used as positive control for the bacterial and fungal test cultures.

Minimum Inhibitory Concentration (MIC)

The MIC of both the chloroform and ethanolic root extracts were determined by the broth dilution method [26]. The plant extracts were prepared to the highest concentration of 100 mg/ ml (stock concentration) in sterile distilled water and serially diluted to give concentrations ranging from 50 mg /ml to 3.125 mg/ ml. Zero point one (0.1) ml of the standardized microbial broth cultures were inoculated into the labeled tubes containing the diluted extracts. The tubes were incubated at room temperature for 24 h for bacteria and at room temperature for 48 h for fungi [27]. The least concentration of the extract which inhibited the growth of the inoculums was considered as the minimum inhibitory concentration.

Statistical analysis

Results are expressed as mean ± standard error of mean using SPSS 16.0 computer software package. The level of significance was determined at 0.05.

RESULTS AND DISCUSSION

Alkaloids, flavonoids, saponins and tannins were present in all the respective ethanolic root extracts (Table 1) whilst terpenoids and steroids were not detected. These bioactive compounds have been known to show medicinal activities as well as exhibited physiological activities useful to man [28]. Saponins have been reported to have antifungal properties while tannins prevent the development of microorganisms by precipitating microbial protein and making nutritional proteins unavailable to them [29]. The saponin and tannin content of the extracts ranged from 0.57% and 1.25% for *D. edulis* and *G. kola* to 2.34% and 1.97% for *G. kola* and *C. albidum* respectively (Table 2). The alkaloid content of the respective root ethanolic extracts ranged from 0.22% for *D. edulis* for 0.57% for *G. kola* (Table 2). Flavonoids have been shown to have a wide range of biological and pharmacological activities in in vitro studies. Examples include anti-allergic,[30] anti-inflammatory,[30,31] antioxidant,[31] anti-microbial (antibacterial, [32, 33] antifungal,[34, 35] and antiviral[34, 35]), anti-cancer,[36][31] and anti-diarrheal activities[37]. Flavonoids have also been shown to inhibit topoisomerase enzymes [38, 39] and to induce DNA mutations in the mixed-lineage leukemia (MLL) gene in vitro studies[40]. The flavonoid value varied from 0.29% for *C. albidum* to 1.86% for *G. kola* (Table 2). The presence of plant secondary metabolites has been implicated for most plants therapeutic activities [41]. Also, plants containing these metabolites (alkaloids, flavonoids, tannin, saponins etc) usually demonstrate stronger antimicrobial properties than others [42]. Ethno botanical reports by [11] showed that root extracts of *D. edulis* are used in the treatment of leprosy, hypertension, beri-beri and rickets. The stems resin exudate is applied to ecto-parasitic infestations and infections as well as dressing cuts, bruises and wounds [7, 43, and 44].The phytochemicals identified in the ethanolic extract of *C. albidum* (Table 1 and 2) are similar to the report of [45] . The results obtained for the quantitative phytochemical screening of *G. kola* are similar to a report by [5].

Table 1. Qualitative phytochemical constituents of *C. albidum, D. edulis* and *G. kola* ethanolic root extracts

Phytochemical	*C. albidum*	*G. kola*	*D. edulis*
Alkaloids	+	+	+
Flavonoids	+	+	+
Saponins	+	+	+
Tannins	+	+	+
Terpenoids	ND	ND	ND
Steroids	ND	ND	ND

Legend: +: Present, ND: Not Detected

Table 2. Quantitative phytochemical constituents of *C. albidum, D. edulis* and *G. kola* ethanolic root extracts.

Phytochemical	*C. albidum* (%)	*G. kola* (%)	*D. edulis* (%)
Alkaloids	0.24	0.57	0.22
Flavonoids	0.29	1.86	0.60
Saponins	2.04	2.34	0.57
Tannins	1.97	1.25	1.32
Terpenoids	0.00	0.00	0.00
Steroids	0.00	0.00	0.00

The zone of inhibition due to the antimicrobial activities of the chloroform and ethanolic extracts of *C. albidum*, *D. edulis* and *G.kola* are presented in Tables 3 and 4. The inhibitory zones elaborated by the test isolates exposed to *C. albidum* ranged from 5.3 mm ± 0.07 for *Mucor* sp. to 18 mm ± 0.03 for *E. coli* (Table 3). The microbial cultures exposed to *D. edulis* ethanolic root extract showed inhibitory zones which varied from 1.67 mm ± 0.20 for *P. notatum* to 12 mm ± 0.04 recorded for *E. coli* (Table 3). Zones of inhibition exhibited by the exposed isolates to *G. kola* alcoholic extract ranged from 2.7 mm± 0.20 for *P. notatum* to 19.3 mm ± 0.01 for *E. coli* (Table 3). The inhibitory zones observed in the bacterial isolates exposed to the control antibiotic; Pefloxacin and Nystatin ranged from 10 mm ± 0.04 and 5 mm ± 0.00 for *P. aeruginosa* and *A. niger* to 19.00 mm ± 0.01and 21 mm ± 0.18 for *S. aureus* and *C. albicans* (Table 3).

Table 3. Antimicrobial activity of *C. albidum, D .edulis* and *G. kola* ethanolic root extracts

Test organisms	Zone of inhibition (mm)			Positive control	
	C. albidum	*D. edulis*	*G. kola*	Pefloxacin (10µg)	Nystatin (100mg/ml)
E. coli	18 ±0.03	12 ±0.04	19.3 ±0.01	14 ±0.00	ND
P. aeruginosa	15.3 ±0.03	6 ±0.08	10 ±0.04	10 ±0.04	ND
S. aureus	8 ±0.06	17 ±0.03	17 ±0.03	19 ±0.01	ND
B. subtilis	6 ±0.08	1.67±0.23	NZI	16 ±0.02	ND
A. niger	NZI	5 ±0.00	7.3 ±0.09	ND	5 ±0.00
P. notatum	7.00±0.00	5.67±0.07	2.7 ±0.20	ND	8 ±0.29
M.mucedo	5.3 ±0.07	5.67±0.07	NZI	ND	11 ±0.25
C. albicans	9.6 ±0.06	6.67±0.06	7 ±0.07	ND	21 ±0.18

Legend: Values are means ± Std. Error, ND: Not Determined, NZI: No Zone of Inhibition

The inhibitory zones displayed by the test isolates exposed to *C. albidum* chloroform root extract ranged from 9 mm± 0.05 for *B. subtilis* to 26 mm ± 0.02 for *E. coli* (Table 4). The microbial cultures exposed to *D. edulis* extract showed inhibitory zones which varied from 5.00 mm± 0.00 for *A. niger* to 21 mm ± 0.02 for *E. coli* (Table 4). Zones of inhibition elicited by the exposed isolates to *G. kola* chloroform root extract ranged from 2.7 mm± 0.20 for *P. notatum* to 30.7 mm ± 0.01 for *P. aeruginosa* (Table 4). The inhibitory zones elaborated by the bacterial isolates exposed to the control antibiotics; Pefloxacin and Nystatin varied from 10 mm± 0.03and 5 mm± 0.00 for *P. aeruginosa* and *A. niger* to 19 mm± 0.01 and 21 mm ± 0.18 for *S. aureus* and *C. albicans* respectively (Table 4).

Comparatively, the chloroform root extracts were more potent against the test isolates than the ethanolic extracts (Tables 3 and 4). This could be indicative of the increased solubility of the phytochemicals in chloroform solvent. The observed antimicrobial activity of the respective root extracts might have been dependent on both the concentration as well as nature of the extraction solvent used. Comparatively, the *G. kola* chloroform root extract were more potent against the test isolates than the antibiotic control; Pefloxacin (Table 4). However the antifungal activities of the all the root extracts were lesser than that elicited by the antifungal drug; Nystatin (Table 3 and 4). The susceptibility of the test bacterial isolates to *G. kola* chloroform root extract might be reflective of the broad spectrum bactericidal activities of the respective phytochemicals present in the extract.

The highest MIC values were displayed by the ethanolic root extracts of *D. edulis* and *G. kola* (6.25 mg/ml) against *E. coli* (Table 5). *A. niger*, *B. subtilis* and *M. mucedo*. were resistant to the highest concentration (100 mg/ml) of *C. albidum*, *D. edulis* and *G. kola* ethanolic root extracts (Table 5).

Table 4. Antimicrobial activity of *C. albidum, D. edulis* and *G. kola* chloroform root extracts

Test organisms	Zone of inhibition(mm)			Positive control	
	C. albidum	*D. edulis*	*G. kola*	Pefloxacin (10µg)	Nystatin (100mg/ml)
E. coli	[a]26 ±0.02	21 ±0.02	23.7 ±0.02	14 ±0.00	ND
P. aeruginosa	20.3 ±0.02	25.3 ±0.02	30.7 ±0.01	10 ±0.03	ND
S. aureus	10.7 ±0.05	19.67±0.02	21 ±0.02	19 ±0.01	ND
B. subtilis	9 ±0.05	17.3 ±0.02	17 ±0.03	16 ±0.02	ND
A. niger	NZI	5 ±0.00	6.7 ±0.07	ND	5 ±0.00
P. notatum	7 ±0.00	5.7 ±0.07	2.7 ±0.20	ND	8 ±0.29
M.mucedo	5.7 ±0.07	5.7 ±0.07	3 ±0.16	ND	11 ±0.25
C. albicans	12 ±0.04	6.7 ±0.06	12.33±0.04	ND	21 ±0.18

Legend: Values are Means ± Std. Error, ND: Not Determined, NZI: No Zone of Inhibition

Table 5. Minimum Inhibitory Concentration (MIC) values of the ethanolic root extracts of *C. albidum, D. edulis* and *G. kola*.

Test Isolates	Concentration of extracts (mg/ml)		
	C. albidum	***D. edulis***	***G. kola***
E. coli	50	6.25	6.25
P. aeruginosa	50	50	50
S. aureus	100	50	50
B. subtilis	100	>100	>100
A. niger	>100	100	100
P. notatum	100	100	100
M. mucedo	100	>100	>100
C. albicans	100	100	100

Chloroform root extracts of *D. edulis* and *G. kola* elicited maximal MIC values against *E. coli* (3.125 mg/ml) (Table 6). *A. niger* exhibited the lowest MIC reading (>100 mg/ml) against *C. albidum* root extracts (Table 6).

Table 6. Minimum Inhibitory Concentration (MIC) of C*. albidum, D. edulis* and *G. kola* chloroform root extracts.

Test Isolates	Concentration of extracts (mg/ ml)		
	C. albidum	*D. edulis*	*G. kola*
E. coli	50	3.125	3.125
P. aeruginosa	50	12.5	12.5
S. aureus	50	25	25
B. subtilis	50	100	100
A. niger	>100	100	100
P. notatum	100	100	100
M. mucedo	100	100	100
C. albicans	100	50	50

CONCLUSION

Chloroform root extracts of *G. kola* were comparatively more potent against the test isolates than *C. albidum* and *D. edulis.* All the respective root extracts exhibited a greater antibacterial activity in comparison with the antifungal attributes. The presence of bioactive antimicrobial compounds in the examined alcoholic and chloroform extracts of the medicinal plants especially *G. kola* could indicate the possibility of obtaining potentially valuable antimicrobial phytochemicals from these plants. Further antimicrobial studies on the fractions of the solvent extracts of these plants are recommended.

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