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Comparison of Antimicrobial Activity and Phytochemical Screening of Seeds and Testas of *Dacryodes edulis* and *Garcinia kola*

R. U. B. Ebana[1], U. O. Edet[1*], U. M. Ekanemesang[2], G. M. Ikon[1], J. F. Ekpenyong[1], N. W. Ntukidem[1] and N. U. Brown[1]

[1]Deparment of Microbiology, Faculty of Natural and Applied Sciences, Obong University, Obong Ntak, Etim Ekpo L.G.A., Akwa Ibom State, Nigeria.
[2]Department of Biochemistry, Faculty of Natural and Applied Sciences, Obong University, Obong Ntak, Etim Ekpo L.G.A., Akwa Ibom State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors RUBE, UOE, NUB and NWN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors UOE, GMI, UME and JFE managed the analyses of the study. Authors UOE, RUBE and NUB managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The aim of this study was to evaluate the seeds and testas of *Dacryodes eludis* and *Garcinia kola* for antimicrobial activity against bacterial and fungal isolates in addition to their screening for phytochemicals and quantification. Phytochemical screening with aqueous and ethanolic extracts, crude quantification of phytochemicals and antimicrobial sensitivity assay were all carried using standard techniques. Resulting replicate readings were subjected to statistical analysis to test for significance. The results of the phytochemical screening indicates the presence alkaloids, glycosides, saponins, tannins, flavonoids, reducing compounds and polyphenols but not phlobatannins, anthraquinones and hydroxymethyl anthraquinones in the aqueous and ethanolic

**Corresponding author: E-mail: uwemedet27@gmail.com, uwemedet@yahoo.com;*

extracts of the seeds and testas of *Dacryodes eludis*. However, in addition, phlobatannins was found in the testa and seeds of *G. kola.* Following quantification of the phytochemicals, the most abundant were polyphenol and tannins in the testa and seeds of *D. eludis* and *G. kola*, respectively. Analysis of variance showed significance ($p < 0.05$). The extracts showed varying levels of inhibition against the bacterial and fungal isolates. Consistently, the methanolic extract of *G. kola* seed gave the highest zones of inhibition against the bacterial isolates while its ethanolic extract also showed consistent antifungal activity. This is an urgent need for further studies aimed at further exploiting these phytochemicals.

Keywords: Medicinal plants; African pear; bitter kola; phytochemical; antimicrobial.

1. INTRODUCTION

The discovery and eventual introduction of antibiotics towards the middle of the 20th century brought about tremendous hope and decrease in the mortality associated with infectious diseases worldwide. It was even thought that infectious diseases would be eradicated by the end of the century. Sadly, it this has not happened. Rather, microorganisms have been developing resistance to almost every class of available antimicrobial agent at such an alarming rate that it is now a global health issue [1,2,3]. Moreover, since the early 1970's very few antibiotics have made it to the market around the world despite advances in drug design, pharmacogenomics and genomics [1-3]. As alternatives, medicinal plants are currently been exploited for bioactive (phytochemicals) components with antimicrobial activity [4,5,6].

Medicinal plants contribute a lot to health care of the third world for a number of reasons. These reasons include their low cost, perceived effectiveness, availability and cultural heritage of the people. It is estimated that about 80% of the third world still rely on medicinal plants even when orthodox medicine may be available [7,8,9]. Ibanga and Ekpa [10] described *D. eludis* as a plant with enormous potentials which is still very underutilized. Studies have shown that *G. kola and D. eludis* abounds with nutrients such as carbohydrates, proteins, fat, fibre, ash and moisture. Furthermore, they have been shown to contain minerals such as calcium, iron, nitrogen, phosphorus, and potassium and even amino acids, and vitamins [11,12].

D. eludis and *G. kola* seeds have been shown to contain a number of phytochemicals such as alkaloids, saponins, terpenoids, tannins, flavonoids, polyphenols, and so on [13,14,15,16]. Both plants have been shown to have antimicrobial activity against pathogens such as bacteria and fungi [15,16,17,18]. In a recent study, the boiled and raw seeds of *D. eludis* obtained from Calabar in Southern Nigeria were shown to contain different phytochemicals in both aqueous and ethanolic extracts [13]. John et al. [16] have shown that locally derived chewing sticks made from *Garcinia kola, Garcinia epunctata* and *Acacia kamerunensis* plants stems have antimicrobial activity as well as anti-oxidant activity. Despite these potentials, few studies still exist on the antibacterial and antifungal activity of the seeds and testa of *D. eludis* and *G. kola*. Therefore, the aim of this study was to screen the seeds and testa of *D. eludis* and *G. kola* for phytochemicals, quantification of the phytochemicals, antibacterial and antifungal activities of their aqueous, ethanolic and methanolic extracts.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Freshly harvested samples used in this study were purchased locally from dealer in Etim Ekpo Local Government Area of Akwa Ibom State and identified as *Dacryodes eludis* and *Garcinia kola*. The seeds and testas were then obtained from both plants and stored separately at room temperature for further analysis.

2.2 Collection and Identification of Bacterial Isolates

The clinical isolates used in this study were obtained from Microbiology Department, University of Uyo Teaching Hospital. The bacterial isolates were characterized using morphological and biochemical tests as previously described [19,20]. The probable isolates were *Pseudomonas aeruginosa, Staphylococcus aureus* and *Bacillus sp*.

2.3 Preparation of Seeds and Testas Extracts

This was done as previously described [21,22]. Briefly, the seeds and testas of *D. eludis* and *G. kola* were oven dried for 1 hour at 100℃. After drying, the seeds and testas were separately ground using a mortar and pestle into powder, and stored in universal bottle for extraction. Separately, about 20 g of each samples were weighed out and dissolved in 100 ml of distilled water and ethanol, respectively. These were then allowed to stand for 72 hours before heating in a water bath at 70℃ to allow the solvents to evaporate. The slurry left behind were then stored at 4℃ for further analysis.

2.4 Phytochemical Screening of Plant Material

Both plants parts used for this study were also screened for phytochemicals. The phytochemicals screened for were alkaloids, tannins, saponins, polyphenols, anthraquinones, glycosides, flavonoids, reducing compounds, phlobatannins, and hydroxymethyl anthraquinones. All screenings were carried out following procedure already described [21,22].

2.5 Crude Quantification of the Phytochemicals

The phytochemicals that tested positive were then further subjected to quantification using methods already described [21,22].

2.6 Collection and Characterisation of Fungi

The fungi isolates were collected from decaying plants materials from Obong University and identified using cultural characterisation and microscopic examination. Briefly, the fungal samples were inoculated on sabouraud dextrose agar and the colonies morphologies of the isolates recorded and compared with descriptive features. Procedures were carried out as previously described [23,24].

2.7 Antimicrobial Sensitivity

Antimicrobial assay was carried out as previously described [22,25,26]. Disks of exactly 5 mm in diameter were wrapped with aluminum foil and sterilized in the hot air oven at 40℃ for 30 minutes. Colonies of each test isolates were then sub-cultured on nutrient agar. The sterilized filter paper disks were soaked in the respective test extracts and then placed on the plates aseptically. The plates were incubated at 37℃ for 24 hours. After incubation, the zones of inhibition were determined in triplicates and the mean values determined and recorded. The antifungal activity was carried out using sabouraud dextrose liquid medium. For all the extracts, exactly 100 mg/ml were prepared and used for the sensitivity testing.

2.8 Statistical Analysis

Replicate readings obtained from quantification of phytochemicals were then analyzed for significance using Analysis of Variance (ANOVA) at 95% significance level. All the analyses were done using Microsoft Excel 2007 Version.

3. RESULTS

The results of the study are presented in Tables 1 to 6. The results of the phytochemical screening of the seeds and testas of *D. eludis* and *G. kola* are presented in Table 1. The results indicates that the ethanolic and aqueous extracts of the seed and testa of *D. eludis* contains alkaloids, glycosides, saponins, tannins, flavonoids, reducing compounds and polyphenols but not phlobatannins, anthraquinones and hydroxymethyl anthraquinones. In addition, phlobatannins was found in the testa and seeds of *G. kola.* The most abundant phytochemicals were reducing compounds and polyphenols in *D. eludis*. However, in G. kola, glycosides, reducing compounds and polyphenols were most abundant.

On quantification of the crude phytochemicals, the most abundant phytochemical examined was polyphenol and tannins in the testa and seeds of *D. eludis* and *G. kola*, respectively. This was closely followed by flavonoids. The least abundant was tannins in the testa and seed of *D eludis*. Analysis of variance showed significance ($p < 0.05$) and this is presented in Table 2.

Using similar concentration of 100 mg/ml, aqueous, ethanolic and methanolic extracts of the seeds and testa of both studied plants showed varying degrees of inhibition as shown in Tables 3 and 4. The highest zone of inhibition of 18.67 mm was shown by ethanolic extract of the seed of the *D. eludis* against *Bacillus species* while the least was shown by the aqueous

extract of the seed and testa 10.00 mm against *Pseudomonas aeruginosa*. Almost similar zones were seen with the extracts of the seed and testa of *G. kola* against *P. aeruginosa.* Methanolic extract of the seed of *G. kola* gave the overall highest inhibition of 19.00 mm against *S. aureus*. Consistently, the methanolic extract of *G. kola* seed gave the highest zones against the study isolates.

The results of the antifungal activity of *G. kola* and *D. eludis* are presented in Tables 5 and 6. It shows that the aqueous extract of *D. eludis* showed antifungal activity against the *Penicillum sp, Aspergillus sp* and *Aspergillus niger* used in this study while the methanolic extract of *D. eludis* showed no antifungal activity. Ethanolic extract of *G. kola* also showed consistent antifungal activity.

4. DISCUSSION

Phytochemical screening and quantification was performed on *D. eludis* and *G. kola* seeds and testa extracts in addition to their antimicrobial activity. The results indicate that the presence of phytochemicals such as saponins, alkaloids, tannins, reducing compounds, polyphenols, flavonoids and glycosides in both seeds and testa of *D. eludis* and *G. kola*. In addition, phlobatannins was also found in the seed and testa of *G. kola*. In a study by Dah-Nouvlessounon [17], they also found the presence of alkaloids, tannins, flavonoids and reducing compounds, in addition to other phytochemicals such as tritepenoids, coumarins and mucilags. In another study, phytochemicals in the various stages of the fruits maturation of edible fruits of *D. eludis*, were flavonoids, alkaloids, saponins, tannins and cyanogenic glycosides which were all found in our study. In another study in Cameroon, the leaves have been shown to have similar phytochemicals found in the seed and testa in our study [18]. In another study in Ghana [16], using thin layer chromatography, they showed the presence of alkaloids and saponins in *G. kola,* saponins in *G. epunctata,* and saponins and flavonoids in *Acacia kamerunensis* in their aqueous extracts which were also recorded in our studied plants. A more recent study on both the boiled and raw seeds of *D. eludis* harvested from Southern Nigeria showed an abundance of phytochemicals that differed slightly with the different treatments. When compared to our study, the phytochemicals obtained were similar however, it was negative for phlobatannins. The aqueous extract of the boiled seeds also tested negative to phlobatannins, alkaloids, and saponins [13].

Elsewhere, similar results was also obtained by Dike and Asuquo [11] who reported the presence of phytochemicals such as alkaloids, flavonoids, saponins, tannins and phenol in *G. kola* which were all consistent with our finding. Furthermore, on quantification, they found that the highest phytochemical to be alkaloid 3.61% which when compared with the alkaloids in our study was slightly higher than our findings for the testa of both plants and the seeds of *D. eludis* but lower than the 5.60% recorded for the seeds of *G. kola*. Other components in our study were far greater than those they reported. Similar results for phytochemicals were recorded in an earlier study with the pods and seeds of *Cola nitida* and *C rostada* [27].

Recent studies have shown that *D. eludis* and *G. kola* both have antimicrobial activity [13,14,16]. Amise et al. [13] showed that both the pulp and the seeds extracts can inhibit an array of microorganisms such as *Klebsiella pneumonia, Proteus vulgaris, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and not *Enterococcus faecalis.* In their study, the highest zone recorded was 21 mm against *P. vulgaris* by the ethanolic extract used in their study. Although the highest zone of inhibition recorded in our study was 18.67 mm, our findings for all the test extracts were more consistent. John et al [16] using broth dilutions showed that G. kola stem aqueous extract was most consistent inhibitor of their test isolates across concentrations of 128 to 8 mg/ml. In an earlier study, the essential oil of *D. eludis* was reported to have a consistent antibacterial and antifungal activity of ≥16mm against thirteen bacteria species including *S. aureus, E. coli, P aeruginosa* and *Bacillus cereus*, and three different strains of *Candida albicans* [26]. When compared to our findings, it was higher than the zones exhibited by testa and seeds extracts of *G. kola* against *Bacillus spp* and *P. aeruginosa*, and also those of seed and testa of *D. eludis* against *S. aureus* and *P. aeruginosa*. Our findings were also lower than those reported earlier for *Cola rostada* and *C. nitida* against clinical isolates [27]. Our findings were more agreeable to those of an earlier study where they reported inhibition zones ranging from 6.00 to 18.00 at 20.00 mg/ml of aqueous extract of *G. kola*. The antimicrobial activity observed by the seeds and testas of the studied plants have been explained by the presence of alkaloids and saponins [28,29].

Table 1. Phytochemical screening of seed and testa of African peer (*Dacryodes edulis*) using ethanol and aqueous extract

Phytochemicals	DES		DET		GKS		GKT	
	Ethanol extract	Aqueous extract	Ethanol extract	Aqueous extract	Ethanol extract	Aqueous extract	Ethanol extract	Aqueous extract
Alkaloids	++	+	++	+	++	+	+	+
Glycosides	++	+	++	+	+++	++	++	+
Saponins	+	+	+	++	++	+	+	+
Tannins	+	++	+	++	+	++	+	++
Flavonoids	++	++	+	++	+	+	+	+
Reducing compounds	++	+++	++	+++	+++	++	++	+
Polyphenols	++	+++	++	+++	+++	++	++	+
Phlobatannins	-	-	-	-	+	+	+	+
Anthraquinones	-	-	-	-	-	-	-	-
Hydroxymethyl Anthraquinones	-	-	-	-	-	-	-	-

Keys: + = present, ++ = present in excess, +++ = present in much excess and - = absent. DES = D. eludis seed, DET = D. eludis testa, GKS = G. kola seed and GKT = G. kola testa

Table 2. Quantitative estimation of crude phytochemical components of testa of *D. edulis* and *G. kola* (%)

Phytochemical components	*D. edulis* testa	*G. kola* testa	*D. edulis* seeds	*G. kola* seeds
Alkaloids	1.60 ±0.10[a]	2.40 ±0.20[b]	1.75 ± 0.02[b]	5.60 ± 0.10 [b]
Glycosides	1.25 ± 0.01	1.57 ± 0.01	1.25 ± 0.20	2.54 ± 0.01
Saponins	1.20 ± 0.10	3.20 ± 0.10	1.35 ± 0.02	3 .84 ± 0.02
Tannins	0.31 ± 0.01	20.81 ± 0.01	0.34 ± 0.02	21.20 ± 0.10
Flavonoids	4.50 + 0.10	8.22 ± 0.02	7.20 ± 0.10	9.17 ± 0.01
Reducing compounds	1.73 ± 0.01	2.40 ±0.20	1.63 ± 0.03	5.60 ± 0.10
Polyphenols	11.45 ±0.01	1.57 ± 0.01	12.36 ± 0.02	2.54 ± 0.01

[a,b]ANOVA of replicate readings gave significant mean±SD ($p < 0.05$)

Table 3. Microbial sensitivity to *D. eludis* seeds and testa (mm)

Organisms	AqS	MeS	EtS	AqT	MeT	EtT
S. aureus	11.00±1.00	12.33±1.52	11.67±1.52	11.00±1.00	13.00±1.00	13.33±1.53
Bacillus spp	15.33±2.51	14.67±2.08	18.67±4.73	13.33±1.53	17.67± 3.51	12.67±1.53
P. aeruginosa	10.00±0.00	10.00±0.00	11.00±1.00	11.00±1.00	12.00±1 .00	10.00±0.00

Key: AqS = seed aqueous, MeS = methanolic and EtS = ethanolic extracts and AqT = testa aqueous, MeT = methanolic and EtT = ethanolic extracts

Table 4. Microbial sensitivity to *G. kola* seeds and testa (mm)

Organisms	AqS	MeS	EtS	AqT	MeT	EtT
S. aureus	14.33±1.15	19.00±2.65	16.67±1.53	11.00±1.00	14. 00±2.65	15.00±3.00
Bacillus spp	12.50±0.50	15.33±2.52	12.00±1.00	12.67±2.52	11. 00±1.00	11.00±1.00
P. aeruginosa	10.33±0.57	12.00±1.00	11.00±1.00	11.00±1.00	10.00± 0.00	10.00±1.00

Key: AqS = seed aqueous, MeS = methanolic and EtS = ethanolic extracts and AqT = testa aqueous, MeT = methanolic and EtT = ethanolic extracts.

Table 5. Antifungal activity of the seeds and testas of *G. kola*

Organisms	AqS	MeS	EtS	AqT	MeT	EtT
Penicillum sp	+	+	+	+	-	-
Aspergillus sp	+	-	-	-	+	-
Aspergillus niger	-	-	+	-	-	-

Key: - = no growth, + = growth. AqS = seed aqueous, MeS = methanolic and EtS = ethanolic extracts and AqT = testa aqueous, MeT = methanolic and EtT = ethanolic extracts

Table 6. Antifungal activity of the seeds and testas of D. eludis

Organisms	AqS	MeS	EtS	AqT	MeT	EtT
Penicillum sp	_	+	_	_	+	+
Aspergillus sp	_	_	_	_	+	-
Aspergillus niger	_	+	+	_	+	-

Key: - = no growth, + = growth. AqS, MeS and EtS = seed aqueous, methanolic and ethanolic extracts and AqT, MeT and EtT = testa aqueous, methanolic and ethanolic extracts.

5. CONCLUSION

The results of the study indicate that the seeds and testas of *D. eludis* and *G. kola* abound with phytochemicals with potential antimicrobial activity. There is an urgent need therefore to further exploit the phytochemicals in these plant parts previously underexploited.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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