

Antioxidant and Antimicrobial Activities of Various Extracts from Safou (*Dacryodes edulis*) Cultivated in Côte d'Ivoire

ABSTRACT

Safou (*Dacryodes edulis*) is a fruit with very interesting nutritional and pharmacological properties. However, this fruit remains rather unknown to the Ivorian population.

Aims: The objective of this study was to highlight the antioxidant and antimicrobial potential of the seed and pulp of Safou (*Dacryodes edulis*) cultivated in Côte d'Ivoire for its valorization.

Study design: Microbiological and biochemical studies

Place and Duration of Study: Laboratory of Biotechnology and Food Microbiology, Abidjan, Côte d'Ivoire, between September 2020 and February 2021.

Methodology: Aqueous, ethanolic and methanolic extracts of the pulp and seed of Safou were prepared. Moisture content, pH, yield, total polyphenols, diphényl-2-picrylhydrazyle (DPPH) radical scavenging test of the different extracts were determined. Aqueous, ethanolic and methanolic extracts of the seed and pulp at varying concentrations of 200 mg/mL, 100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL and 6.25 mg/mL were tested against human pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Aspergillus fumigatus* and *Penicillium chrysogenum*.

Results: pH, moisture content and yield ranged from 3.16 to 4.74; 9 to 18.30%; and 10.79 to 18.78%, respectively. Total polyphenol content ranged from 1.45 mgEAG/g to 4.56 mgEAG/g. The highest levels of total polyphenols were observed for the methanolic extracts of pulp. The different extracts strongly scavenged the DPPH radical with percentages of anti-free radical activity that varied from 50.76 to 64.43%. The antimicrobial susceptibility results of the methanolic extracts of *D. edulis* seed showed highest zones of inhibition to the microbial isolates tested. The microorganisms were resistant to the aqueous extracts of pulp and seed.

Conclusion: The presence of bioactive compounds and the antimicrobial activity of Safou extracts could justify its use in the food and pharmaceutical fields.

Keywords: Safou (*Dacryodes edulis*), total polyphenols, antioxidant activity, antimicrobial activity.

1. INTRODUCTION

Plants are used in developing and developed countries for both dietary and medicinal reasons. According to a World Health Organization (WHO) report, 80% of the world's populations rely primarily on traditional therapies that involve the use of plant extracts or their active substances [1]. Among these plants, there is the safoutier (*Dacryodes edulis* (G. Don) H. J. Lam, Burseraceae) native to the countries of Central Africa and the Gulf of Guinea (Cameroon, Congo, Democratic Republic of Congo, Gabon, Equatorial Guinea and Nigeria) [2]. The different parts of this plant are used in many parts of Africa in the treatment of several diseases. The leaves are reportedly used to cure ringworm, scabies, and skin rashes [3]. The bark is said to be used to treat dysentery and anemia [4]. *D. edulis* fruit (Safou) has shown excellent nutritional qualities and interesting agro-industrial properties [5]. Its pulp and seed are rich in fatty acids and amino acids [6]. Nwokonkwo [7] in her study reported that the seed extracts of *Dacryodes edulis* and its secondary metabolites possessed potential antibacterial activity against some human pathogens in varying degrees. Antibacterial effect of the essential oil as well as the organic extracts of *D. edulis* plant has been reported [8,3]. It was indicated that the leaf extract of *Dacryodes edulis* possess broad spectrum antibacterial activity in a recent study [9].

In Côte d'Ivoire, the first Safou (*D. edulis*) trees were introduced before 1960s but disappeared [10]. The second introduction of Safou trees comes from Cameroon Safou seeds in 1980 [11]. However, few scientific works have been published either on their nutritional and therapeutic potential. This study investigated the antioxidant and antimicrobial potential of the seed and pulp of Safou (*Dacryodes edulis*) cultivated in Côte d'Ivoire necessary for their wider use.

2. MATERIAL AND METHODS

2.1 Sample Collection

Dacryodes edulis fruits (Safou) were harvested in the south of Côte d'Ivoire (Azaguié-blida, Agboville) from September to December 2020. The botanical authentication of this plant was done by the herbarium of National Floristic Center of University FELIX HOUPHOUET BOIGNY (Abidjan, Côte d'Ivoire), where a voucher specimen was conserved with reference number UCJ018940. The fruits were transported in net bags to Biocatalysis and Bioprocessing laboratory of NANGUI ABROGOUA University.

2.2 Preparation of Extract

The fruits were de-fleshed to separate the seed from the pulp. The fresh seed and pulp Safou plant were dried in an oven 45°C/ 72h. The seed and pulp of the plant were then grind to fine powder with the aid of a mechanical grinder. Ethanol, methanol and water were the extracting solvents used in extracting the phytochemicals from *D. edulis* [12] modified. Aqueous extract was obtained by maceration extraction method. Fifty grams (50 g) of each sample was macerated in 500 mL of distilled water at room temperature in the dark for 24 hours. The macerates obtained were then filtered successively twice on absorbent cotton and once on Whatman N°1 filter paper. The filtrates obtained were oven dried at 50°C for 4 days to obtain the aqueous extract.

Solvent extraction method has been used for ethanolic and methanolic extract. Fifty gram each of the powdered seed and pulp were soaked in 500 ml of the solvents (ethanol and methanol) in separate beakers and the set up allowed to run for 24 hours. After 24 hours of extraction, the suspensions were vigorously shaken and filtered with Whatmann No 1 filter paper. Ethanolic and methanolic solvents were evaporated at 40°C using a rotary evaporator. The concentrated extracts were stored in airtight bottles and labelled.

2.3 Analysis of physico-chemical constants

2.3.1 Extraction yield

Extraction yield was expressed as a percentage of the initial mass of the fruit powder subjected to extraction [13]. The extraction yield is calculated according to the following formula:

$$\text{Extraction yield} = (\text{mass of extract} / \text{mass of fruit powder}) \times 100$$

2.3.2 Moisture content

The moisture content was determined by differential weighing after oven drying according to AOAC [14]. Five (5) grams of each sample was weighed (m_1) and placed in a crucible. The crucible containing the sample (m_2) was placed in the oven at 105°C for 24 hours and weighed after cooling in a dry place (m_3). The moisture content expressed as a percentage was determined according to the following formula:

$$\text{Moisture content} = [(m_2 - m_3) / m_1] \times 100$$

2.3.3 pH

pH was determined according to [15]. One (1) gram of each extract of Safou was added to 50 mL of distilled water and homogenized. pH was read directly using a pH meter.

2.3.4 Color and texture

The color and texture of the different Safou extracts were determined by visual inspection.

2.4 Antioxidant Assay

2.4.1 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The free radical scavenging activities of the crude extracts of *D. edulis* were evaluated using the DPPH (2, 2-diphenyl- 1-picrylhydrazyl) assay method as described by [16]. Fifty (50) μL of each methanolic solution of the extracts at different concentrations (from 0.01 to 0.2 mg/mL) is added to 1.95 mL of the DPPH methanolic solution (0.025 g/L). At the same time, a negative control is prepared by mixing 50 μL of methanol with 1.95 mL of the DPPH methanolic solution. The absorbance reading is taken against a blank prepared for each concentration at 517 nm after 30 min of incubation in the dark at room temperature. Ascorbic acid (Vitamin C) was used as control. Each assay was done in triplicate and the results, recorded as the mean \pm SD of the three findings, and were illustrated in a tabular form. The radical scavenging activity (RSA, %) was calculated as follows: $\text{RSA} (\%) = [(A_{\text{DPPH}} - A_{\text{sample}}) / A_{\text{DPPH}}] \times 100$ (where A = Absorbance). The radical scavenging percentages were plotted against the logarithmic values of the concentration of test samples and a linear regression curve was established in order to calculate IC_{50} , which is the amount of sample necessary to inhibit 50% of free radical DPPH.

2.4.2 Total phenols contents (TPC)

TPC of different extracts of Safou was determined using the Folin-Ciocalteu reagent method described by [17]. A volume of two hundred (200) μL of each extract was mixed with 1 mL of Folin-Ciocalteu reagent. The mixture was thoroughly homogenized by manual shaking. After 3 min, a volume of 1 mL of 20% aqueous sodium carbonate solution was added and the volume was adjusted to 10 mL with distilled water. The mixture was placed in the dark for 30 min and the absorbance was measured at 765 nm with a spectrophotometer against the blank. A calibration curve was established with a concentration range of gallic acid solution from 0 to 0.1 mg/mL. The total phenol content was expressed as milligram equivalent of gallic acid per gram of extract (mgGAE/g).

2.5 Determination of the antimicrobial activity of different Safou extracts

2.5.1 Microbial strains

Escherichia coli, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiellapneumoniae*, *Proteus mirabilis* and *Aspergillus fumigatus*, *Penicilliumchrysogenum*were collected from Pasteur Institute of Côte d'Ivoire and Laboratory of Biotechnology and Food Microbiology of NANGUI ABROGOUA University respectively.

2.5.2 Preparation of the microbial suspensions

The density of selected organisms was adjusted equal to that of the 0.5 McFarland standards (1.5×10^8 CFU/mL) by adding them to nutrient broth for bacteria and Sabouraud broth for molds. A 24 hold culture was used for the preparation of microbial suspension. McFarland standards were used as a reference to adjust the turbidity of microbial suspension so that the number of microorganisms would be within a given rang.

2.5.3 Antimicrobial susceptibility test

The National Committee for Clinical Laboratory Standards was the reference in determining antimicrobial susceptibility through the Well Diffusion Method. The different extracts of Safou were tested using standard Mueller Hinton II plates to detect the antimicrobial activity of these testing solutions [18]. The plates with inoculated microorganisms were provided with 100 μ L aliquots in 6 mm diameter punched wells. The surface of the agar plate was streaked over the entire sterile agar surface rotating the plate to ensure an even distribution of inoculum with a final swab around the rim. All of the plates were placed in the incubator set at a temperature of 37°C/ 24h. The antibacterial and antifungal activities of methanolic, ethanolic and aqueous extract of Safou were measured using a ruler by determining the zone of inhibition. This was carried out in at least an average of three parallel independent trials. A substance is said to be ineffective if the diameter of inhibition is less than 8 mm while it is said to be effective if the diameter is between 9 and 14 mm. It is considered very effective when the diameter is between 15 and 19 mm and then extremely effective if the diameter is greater than 20 mm.

2.5.4 Determination of the Minimum Inhibitory Concentration (MIC)

MIC determination was performed with a series of concentrations of the different extracts: 200 mg/mL, 100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL and 6.25 mg/mL. The plates with inoculated microorganisms were provided with 100 μ L aliquots in 6 mm diameter punched wells. The MIC was obtained from the lowest concentration of the plant extract for which no bacterial and fungal colonies were obtained [19].

2.6 Statistical analysis

The one-way analysis of variances (ANOVA) was carried out with the XLSTAT software to compare the variables measured on the different hydroalcoholic products. This software was used to calculate the means and standard deviations of the analysis parameters

3. RESULTS

3.1 Physicochemical properties of Safou (*Dacryodes edulis*) extracts

The texture and color of the different extracts vary according to the type of extract. The aqueous extracts of pulp and seed have a black pasty texture. While the methanolic and ethanolic extracts of the pulp are pasty and green and those of the seed show a brown pasty texture. The yields of methanolic, ethanolic and aqueous extracts of Safou pulp are 10.79%, 17.39% and 18.8% respectively. Those of the seed are 10.79%, 10.89% and 10.91% respectively. The moisture contents of the pulp extracts (methanolic, ethanolic and aqueous) are 9.22%, 9.40% and 19.00% respectively. Those of the seed are 18.30%, 13.09% and 15.34% respectively. pH of the methanolic, ethanolic and aqueous extracts of the pulp and seed of Safou is acidic. pH is 3.43, 3.16 and 4.24 for the methanolic, ethanolic and aqueous extracts of the pulp; and 3.68, 3.57 and 4.74 for those of the seed respectively (Table 1).

Table 1: Physico-chemical parameters of Safou (*Dacryodes edulis*) extracts

Extract	Texture	Color	Yields (%)	pH	Moisture content (%)
MEP	pasty	green	17.07±0.61 ^a	3.43±0.12 ^a	9.22±1.53 ^a
MES	pasty	brown	10.79±0.58 ^b	3.68±0.01 ^a	18.30±1.55 ^b
EEP	pasty	green	17.39±1.06 ^a	3.16±0.06 ^a	9.40±1.27 ^a
EES	pasty	brown	10.89±2.11 ^b	3.57±0.04 ^a	13.09±1.40 ^c
AEP	pasty	black	18.78±2.23 ^c	4.24±0.01 ^b	9.00±1.98 ^a
AES	pasty	black	10.91±0.78 ^b	4.74±0.03 ^b	15.34±1.27 ^d

In column, the averages affected by the same letter are not significantly different at the 5% threshold according to the Newmann-Keuls test. EEP= ethanolic extract pulp; MEP: methanolic extract pulp; AEP: aqueous extract pulp; EES: ethanolic extract seed; MES: methanolic extract seed; AES: aqueous extract seed

3.2 Antioxidant activity of Safou (*Dacryodes edulis*) extracts

The table 2 showed antioxidant activity of methanolic, ethanolic and aqueous extracts of Safou. The highest levels of total polyphenol were observed for the methanolic extracts of the pulp with 4.56 mgGAE/g and of the seed with 4.03 mgGAE/g followed by the ethanolic extracts of the seed with 3.93 mg GAE/g and of the pulp with 2.93 mgGAE/g). The lowest contents of total polyphenols were obtained with the aqueous extracts of pulp with 1.98 mg GAE/g and of seed with 1.45 mg GAE/g.

All the methanolic, ethanolic and aqueous extracts of Safou have high antioxidant power. Methanolic extracts of pulp (64.43), ethanolic extracts of seed (62.68), methanolic extracts of seed (60.03) and ethanolic extracts of pulp (58.96) of Safou had the highest activities.

The median inhibitory concentration (IC₅₀) values of Safou extract range from 12.5 to 21 µg/mL. The highest values were obtained with extracts from Safou seeds.

Table 2: Antioxidant activity of Safou (*Dacryodes edulis*) extracts

Extract	Total polyphenol (mgGAE/g)	Percentage of DPPH radical inhibition (%)	IC ₅₀
MEP	4.56 ±0.08 ^a	64.43±0.40 ^a	13.5 ^a
MES	4.03 ± 0.07 ^a	60.03±0.51 ^b	21 ^b
EEP	2.93± 0.23 ^b	58.96±0.91 ^c	14.6 ^a

EES	3.93 ± 0.04 ^c	62.68±0.22 ^a	19.5 ^b
AEP	1.98 ± 0.09 ^d	53.95±0.30 ^d	12.5 ^a
AES	1.45±0.18 ^d	50.76±0.12 ^e	18.7 ^b
Vit C	-	90.02	6

In column, the averages affected by the same letter are not significantly different at the 5% threshold according to the Newmann-Keuls test. EEP= ethanolic extract pulp; MEP: methanolic extract pulp; AEP: aqueous extract pulp; EES: ethanolic extract seed; MES: methanolic extract seed; AES: aqueous extract seed

3.3 Antimicrobial activity of Safou (*Dacryodes edulis*) extract

The results showed that methanolic and ethanolic extract of *D. edulis* showed good, noticeable, and remarkable activity against the test organisms as compared with aqueous extract while no zone of inhibition was observed on *Pseudomonas aeruginosa* (Table 3). Methanolic extracts showed highest zone of inhibition with *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Proteus mirabilis*. The aqueous extracts of Safou showed no zone of inhibition when tested against all the collected microorganisms isolates.

Table 3: Antimicrobial activity of Safou (*Dacryodes edulis*) extract: diameter of zones of inhibition (mm)

Extract	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Klebsiella pneumoniae</i>	<i>Proteus mirabilis</i>	<i>Aspergillus fumigatus</i>	<i>Penicillium chrysogenum</i>
MEP	17 ± 0.4	16 ± 0.2	-	12 ± 0.5	9 ± 0.4	4 ± 0.2	5 ± 0.2
MES	20 ± 0.6	18 ± 0.2	-	16 ± 0.3	10 ± 0.3	5 ± 0.1	6 ± 0.1
EEP	10 ± 0.5	12 ± 0.4	-	8 ± 0.3	4 ± 0.1	-	-
EES	14 ± 0.4	15 ± 0.4	-	10 ± 0.3	5 ± 0.1	-	-
AEP	-	-	-	-	-	-	-
AES	-	-	-	-	-	-	-

EEP= ethanolic extract pulp; MEP: methanolic extract pulp; AEP: aqueous extract pulp; EES: ethanolic extract seed; MES: methanolic extract seed; AES: aqueous extract seed

3.4 Minimum Inhibitory Concentrations (MICs) of Safou (*Dacryodes edulis*) extracts

The methanolic extract gave the lowest MIC value compared to the ethanolic extract. The MIC values ranged from 12.5 - 100 mg/ml for methanolic extract and 50–200 mg/ml for ethanolic extract as depicted in table 4.

Table 4: Minimum Inhibitory Concentrations (MICs) of methanolic and ethanolic extract of Safou (*Dacryodes edulis*) against the test isolates

microorganisms	MIC (mg/ml)			
	MEP	MES	EEP	EES

<i>Escherichia coli</i>	12.5	12.5	25	25
<i>Staphylococcus aureus</i>	50	50	50	50
<i>Klebsiellapneumoniae</i>	100	100	200	200
<i>Proteus mirabilis</i>	-	100	-	-

EEP= ethanolic extract pulp; MEP: methanolic extract pulp; EES: ethanolic extract seed; MES: methanolic extract seed;

4. DISCUSSION

The results of this study showed that the yields vary depending on the part of the fruit and the type of extract. The difference in yield between extracts could be explained by the chemical composition which differs from one extract to another as well as by the type of solvent used. Indeed, several authors such as [20, 21] showed that the extraction efficiency was influenced by several parameters such as the nature of the phytochemicals, the method used for extraction, the temperature, the extraction time, the particle size as well as the solvent used. pH of the different extracts of Safou is acidic. According to [22] an acidic pH is recommended for food preservation as it would be harmful to bacteria. The high content of total polyphenols in the different extracts shows that Safou has chemical constituents that could be responsible for many pharmacological actions. Indeed, polyphenols play an important role in human health by preventing degenerative diseases such as cancers, cardiovascular diseases, osteoporosis, dyslipidemia, anxiety or constipation. The results of this study corroborate with those of [12, 23] who showed high levels of total polyphenols in safflower in Malaysia and Nigeria respectively. The methanolic extracts of pulp and seed showed the highest values. The extraction solvents have an effect on the extraction yield and the content of bioactive compounds, thus affecting the biological activity of the extract. The results of this study could be due to higher solubility of the compounds in methanol than the other solvents tested (ethanol and distilled water). Methanol is an efficient extractor for polyphenols. It is a solvent widely used in laboratories and industries. The results of this study are similar to those of [21] who obtained the higher levels of total polyphenols in methanolic extracts.

The antioxidant activity in Safou extracts is influenced by the extraction method and the part of the fruit studied. Antioxidants are defined as substances present in foods that act on human physiological functions by significantly reducing the harmful effects of reactive oxygen species, nitrogenous oxygen species, or both [25]. These results suggest that the extracts of Safou could be potential antioxidant agents to be incorporated in some drugs.

Antimicrobial properties of substances are useful and necessary weapons for microbiologists in the fight or eradication of (pathogenic) microorganisms most importantly in food industry (food spoilage) and in the treatment of infectious diseases, as their active components can inhibit the growth and interfere with the metabolism of microorganisms in a negative manner [24-25]. The result of this study show antimicrobial methanolic and ethanolic extracts of Safou (*Dacryodes edulis*) against microorganisms tested. These antimicrobial activities demonstrated could be dependent on the presence of secondary metabolites (steroid/triterpenes, saponins, tannins, alkaloids, cardiac glycosides, reducing sugars, flavonoids and Phenols). Methanolic extract of *D. edulis* had the highest activity against microorganisms tested. This result corroborates with investigations made by [26, 27] using methanol as solvents for the extraction. A previous study has indicated great potential for the plant in the battle against malaria and other diseases [12, 28].

5. CONCLUSION

This study highlighted the antioxidant and antimicrobial potential of the pulp and seed of Safou (*Dacryodes edulis*) grown in Côte d'Ivoire. The aqueous, ethanolic and methanolic extracts of the pulp and seed of Safou are rich in total polyphenols and showed high antioxidant activity. Only the methanolic and ethanolic extracts have an antibacterial activity

on the different microorganisms tested. Safou (*Dacryodes edulis*) could of immense benefit to pharmaceutical industries for the development of new antimicrobial or chemotherapeutic drugs to address unmet therapeutic and also can be used as natural preservatives in food against the well-known causal agents of foodborne diseases and food spoilage.

COMPETING INTERESTS DISCLAIMER:

AUTHORS HAVE DECLARED THAT NO COMPETING INTERESTS EXIST. THE PRODUCTS USED FOR THIS RESEARCH ARE COMMONLY AND PREDOMINANTLY USE PRODUCTS IN OUR AREA OF RESEARCH AND COUNTRY. THERE IS ABSOLUTELY NO CONFLICT OF INTEREST BETWEEN THE AUTHORS AND PRODUCERS OF THE PRODUCTS BECAUSE WE DO NOT INTEND TO USE THESE PRODUCTS AS AN AVENUE FOR ANY LITIGATION BUT FOR THE ADVANCEMENT OF KNOWLEDGE. ALSO, THE RESEARCH WAS NOT FUNDED BY THE PRODUCING COMPANY RATHER IT WAS FUNDED BY PERSONAL EFFORTS OF THE AUTHORS.

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