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Research Article

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Evaluation of antibacterial, antioxidant and cytotoxic activities of the combination of the leaves and bark extracts of *Dacryodes edulis* G. Don (Burseraceae)

Edouka B. Angelica[1], Ngoupayo Joseph[2,*], Chelea Machawe[3], Fotsing Réné[1], Kouamouo Jonas[1], Fekam Fabrice[4]

[1]Université des Montagnes, Bagangté, Cameroon
[2]Faculty of Medicine and Biomedical Sciences, University of Yaounde 1, Cameroon
[3]Institute of Medical Research and Medicinal Plants (IMPM), Yaoundé, Cameroon
[4]Faculty of Sciences, University of Yaoundé 1, Cameroon
*Corresponding author: Ngoupayo Joseph, FMSB, University of Yaounde I, ngoupayo@gmail.com

Abstract According to the World Health Organization, 80% of the African population use herbal remedies for their primary health care and have encouraged the production of improved traditional medicines. Since traditional medicine consists often ofa combination of plants or parts of plants, our work focused on the evaluation of the antibacterial, antioxidant and cytotoxic activity of the combination of leaves and bark of *Dacryodes edulis*. The leaves and bark of *D. edulis* were harvested, cut up, dried and crushed separately. The extraction was carried out by macerating the various powders in ethanol / water solvent (70/30).The extracts obtained were then subjected to phytochemical analysis by colorimetric reactions followed by the evaluation of the antibacterial potential of the combination of our extracts by the Checkerboard method. The antioxidant properties of the extracts were evaluated by the DPPH radical scavenging method and Ferric ion reduction and the cytotoxicity test on the Vero cell line by the colorimetric method with resazurin. The bark and leaf extraction yields were 8.1 and 9%, respectively. The phytochemical study revealed the presence of alkaloids, polyphenols, flavonoids, tannins, saponosides and terpenoids. All our extracts were non-cytotoxic with CC_{50}rangingfrom 88.685 to 110.85 ± 5 µg / ml. The combined synergistic effect (FICI) of extracts against *Klebsiella pneumoniae* ATCC 700603 was 0.16. The extracts had DPPH scavenging IC_{50s} ranging from 48 to >500 ± 3 µg / mL. The reducing power (OD) of the extracts on ferric ions ranged from 2.20 to 2.25 at a concentration of 125 µg / ml. The activities of *D. edulis* extracts suggest that the latter would be a potential source of active principle.

Keywords *Dacryodes edulis*, antibacterial combination, antioxidant, cytotoxicity

1. Introduction

An infectious disease results from the interaction between an infectious agent (virus, bacteria, parasites or fungi), its host and environmental factors [1]. Despite the advancement of science and technology that allowed the discovery of many natural and synthetic drugs, infectious diseases continue to be the leading cause of morbidity and mortality throughout the world, especially in developing countries [2]. According to WHO, infectious diseases are responsible for 50% of deaths in tropical countries and 90% of infections are caused by bacteria. The highest death rate in

Cameroon is due to infectious diseases [3]. WHO in 2017 also estimated that the top six infectious diseases responsible for 90% of deaths are: respiratory diseases (3.9 million / year), AIDS (1.5 million / year), diarrheal diseases (2.2 million / year), tuberculosis (1.8 million / year), malaria (1 million / year), and measles (0.11 million / year) [4].

The advent of antibiotics caused a tremendous advance in therapy. Their use has significantly reduced the morbidity associated with bacterial infections [5]. Very early on, the bacteria began to develop resistance to clinically used antibiotics, leading to frequent treatment failures [6, 7].

In addition to the burden of infectious diseases and associated high mortality rate, the world is increasingly confronted with new emerging diseases such as oxidative stress-related diseases. In the biological systems, oxidative stress is the consequence of an imbalance between the antioxidant defense system and the production of reactive oxygen species (ROS), which leads to the biochemical damage to the cells due to alterations in proteins, lipids, breaks in deoxyribonucleic acid (DNA) molecules, or damage to the integrity of the cell membrane [8] . Aggregation, glycation and oxidation are the main types of protein modifications frequently observed in many diseases such as diabetes, arteriosclerosis or neurodegenerative diseases including Parkinson's disease and Alzheimer's disease [10].

The use of synthetic antioxidants in the food, cosmetics and pharmaceutical sectors, known to address the health problem associated with oxidative stress, is currently being challenged by its potential toxicological risks. Indeed, a long-term use of synthetic antioxidants is considered to be teratogenic, mutagenic and carcinogenic [11]

Given the increased antibiotic resistance, which is currently a global public health problem [8] and the emergence of oxidative stress-related diseases, exploring natural resources specifically, plants appears to be one of the most promising. According to the World Health Organization, 80% of the African population use herbal remedies for their primary health care and have encouraged the production of improved traditional medicines [3]. This justifies the realization of several research studies on the therapeutic properties of indigenous plants [12, 13].

Our choice fell on *Dacryodes edulis* (Burseraceae) because its past use in traditional medicine as antibacterial [12], antioxidant [13], anti-malarial [14], anti-diabetic agents [15]. Since combination of plants or parts of plants is common in traditional medicine [16], in our search for new active compounds, the antibacterial and antioxidant activities of the combination of the leaf and bark extracts of *D. edulis* will be assessed. The general objective of this work was to contribute in enhancing Cameroonian medicinal plants towards the discovery of potential alternative drugs to be used as anti-infectious and antioxidant agents to reduce the prevalence of infectious and oxidative stress-related diseases.

2. Methodology

2.1. Drying and grinding of leaves and bark

The plant material (leaves and bark) was dried under sun and light free environment for two weeks, then powdered. The powders obtained were used for the preparation of the extracts.

2.2. Preparation of the hydro-alcoholic plant extract

One thousand grams (1000 g) of each powder was macerated separately in the mixture of ethanol / distilled water (70/30) solvents at ambient temperature for 72 hours. The extracts were subsequently filtered through Whatman No. 2 filter paper, and then subjected to evaporation under vacuum at 40 ° C on a rotary evaporator. The two resulting solutions were dried in an oven (40 ° C) for 24 hours. The dry residues obtained were stored at 4 ° C until the tests were carried out. The extraction yield was calculated using the following formula:

Yield = (Mass of extract obtained (g)) / (Mass of powdered plant material in g)) * 100

2.3. Phytochemical screening

A phytochemical screening of the various extracts obtained previously was carried out in order to get an idea of the different groups of secondary metabolites contained in them. As a result, we searched for polyphenols, flavonoids, alkaloids, tannins, saponins, steroids, polyterpenes, coumarins and anthocyanins on the basis of the protocols described by Harborne (1976), Odebeyi and Sofowara (1978), Trease and Evans (1989), Sofowora (1993) with some modifications [17,18,19].

2.4. Presence of alkaloids by Mayer test

Five milliliters (5mL) of 10% H_2SO_4 were added to 1g of powder (plant extract). After 24 hours, the macerate was filtered and washed with water. Five drops of Mayer's reagent were added to 1ml of plant extract and left on for 15 minutes. The presence of alkaloid resulted in the appearance of a white-yellow or light yellow precipitate.

2.5. Polyphenols identification test

In a test tube containing 1 mL of ethanol, dissolve 5 mg of the plant extract; then add 3 drops of 10% Iron III Chloride. The appearance of a blue-violet or greenish color indicated the presence of polyphenols.

2.6. Flavonoids identification by Shinoda's test

In a test tube containing 1mL methanol, dissolve 5 mg of the plant extract. To this solution, add a few magnesium chips and 3 drops of concentrated H_2SO_4. Flavonoids were evidenced by the appearance of the following colourings: yellow for flavones, red for flavonols and pink for flavonones.

2.7. Tannin assay using Stiany's reagent

To 0.5 mg of extract, add 2 mL of distilled water, then 15 mL of Stiasny's reagent, after 30 minutes of standing in a water bath at 80 ° C. The above solution was filtered and the filtrate was saturated with sodium acetate. After adding 3 drops of iron chloride, the appearance of a blue-black tint would indicate the presence of gallic tannins.

2.8. Presence of saponosides by foam test

In a test tube containing 5 mL of distilled water, 5 mg of dry extract was added and the whole brought to boiling for 5 min. After cooling, the content of the tube was stirred vertically for 15 seconds, then left to stand. The appearance of persistent foam with a height of more than one centimeter indicated the presence of saponosides.

2.9. Presence of triterpenes and sterols: Liebermann - Burchard test

In a test tube containing 1 mL of methanol, dissolve 5 mg of plant extract and add 0.2 mL of each of the following reagents: chloroform, glacial acetic acid, concentrated H_2SO_4. The appearance of a purple or greenish colour indicated the presence of triterpenes and sterols.

2.10. *In vitro* evaluation of antibacterial activity

The antibacterial activity was evaluated using the micro dilution method in liquid medium and according to the Clinical Laboratory Standard Institute (CLSI) [20]. In a 96-well microplate, under the hood and near a bunsen burner, 100 µl of sterile Muller-Hinton broth (MHB) medium was introduced into all the wells. 100 µl of the stock solutions of extracts (hydroethanolic extracts from the leaves, bark of *Dacryodes edulis)* and ciprofloxacin were introduced into the first wells. From these wells, a series of geometric dilutions of order 2 was carried out. Then 100 µl of an inoculum solution titrated to 5×10^6 CFU / ml was added to all the wells in which there was the extract. Wells serving as negative control and sterility control were included in the test. The microplates were incubated at 37 ºC for 24 hours. At the end of the incubation time (24 hours), the MIC will be the smallest concentration showing no visible bacteria growth, materialized by the absence of deposits and turbidity of the medium. The positive control consisted of MHB, ciprofloxacin at 64 µg / ml and microorganism: absolute absence of microbial growth should be observed in the wells. The negative control consisted of MHB and microorganism: presence of microbial growth should be observed in the wells. The extract sterility check consisted of extract and MHB: no microbial growth was to be observed in the wells. The sterility control of the medium should not allow any microbial growth for the test to be valid.

2.11. Determination of CMBs

It is defined as the lowest concentration of an antibacterial agent capable of killing at least 99.99% of the starting bacterial inoculum after an incubation time of 18-24 hours at 37 ºC. It was determined from the MIC plates by the method used by Ngoupayo *et al* after subculture of the wells of the microplates used during the determination of the MIC [20]. The test was carried out on a 96-well microplate, 150 µl of the MBH medium were introduced into all the wells, 50 µl of the content of the wells corresponding to the MICs of the wells that precede them were reseeded into the microplate. It was then incubated at 37 ºC for 18-24 hours. The lowest concentration at which no visible growth was observed was considered the minimum bactericidal concentration.

2.12. Evaluation of the antimicrobial activity of the combination of leaf and bark extracts of *D. edulis*

The antimicrobial potential of the combination of extracts from the leaves and bark of *Dacryodes edulis* was carried out by microdilution using the Checkerboard method described by Johnson *et al,* 2014 [21] followed by some modifications. In a 96-well intermediate plate, 50 µl of culture medium (MHB) were introduced into all the wells of column 1 to 8. Then 50 µl of the hydro-ethanolic extracts of the leaves (DeFH) concentrated at 25 mg / ml (2CMI) were introduced into the wells of the first line (A). A series of eight dilutions of order 2 was carried out by transferring 50 µl from the first well to the second to the eighth well in order to obtain a concentration series of 25 mg / mL to 0.195 mg / mL. The dilution series of the hydro-ethanolic extracts of the bark (DeEH) was carried out in an intermediate plate in order to obtain the concentrations ranging from 25 mg / mL (line 1) to 0.195 mg / mL (line 8).

In the test plate, 50 µl of DeFH and DeEH were introduced into the wells. The direction of dilution was from line A to H for DeEH and 1 to 8 for DeFH. Then 100 µl of the inoculum solution prepared at 1.5×10^8 CFU / ml were introduced into all the wells for a final volume of 200 µl. The wells of column 9-12 were used to confirm the MICs of our two extracts. The H9-H12 wells contained only the culture medium and served as a sterility control. While the G9-G12 wells served as negative control. The microplates were incubated at 37 ^oC for 24 hours.

At the end of incubation time, growth inhibition in wells containing combinations of DeFH and DeEH was observed by deposition in the wells compared to the negative control and the medium sterility control. The smallest concentrations of combinations which did not allow visible growth of bacteria were used for the analysis of the effect of the combinations on antibacterial activity.

The measure of synergy by Checkerboard analysis was used to measure the impact on the potency of the combination of plant parts relative to their individual activities. This comparison was then represented as the value of the fractional inhibitory concentration index abbreviated FIC. The FIC index value takes into account the combination of extracts that produces the greatest change from the MIC of each antibiotic. To quantify the interactions between the antibiotics tested, the following equation was used;

$$FICI= A/CMI_A + B/CMI_B = FIC_A + FIC_B$$

Where A and B: are the MICs of the extracts from the leaves and bark in combination in a single well, CMIA and CMIB: are the MICs of extracts from the leaves and bark individually. The value of FIC was then used to categorise the interaction of the two extracts tested [21]. The interpretation of the FICI is done as follows: FIC value: Synergy <0.5, antagonism> 4, Additivity 0.5 - 4

2.13. Antioxidant activity

The evaluation of the antioxidant activity of hydro-ethanolic extracts from the leaves and bark of *D. edulis* was carried out by appropriate tests.

2.13.1. DPPH method

Determination of anti-free radical activity on DPPH

The DPPH radical was used as a reference free radical to determine the ability of a plant extract to donate protons to stabilisea free radical. The protocol that used for this method was that described by Bassene (2012), with some modifications.

In a 96-well microplate, introduce 100µl of distilled water into all the wells except that of line 1. Then introduce 200µl of our different extracts into the wells of line 1 then remove 100µl of each extract from line 1 and do second order dilutions of concentrations ranging from (500 to 15.625 µg / mL) from line 2 to 7 then discard the content of the tip. Half of the wells in row 8 contained the negative control consisting of DPPH (without extract) and the other half constituted the positive control (ascorbic acid) at concentrations of 25 µg / mL. Each extract dilution (25µl) was added to a new microplate and 75µl of 0.01% DPPH solution was added. The optical densities at 517 nm were read after 30 min incubation in the dark and at room temperature. The tests were carried out in duplicates. The percentages of inhibition were calculated from the optical densities according to the following formula:

PI (%) = [(Ac-Ae) / Ac] * 100

With: PI: percentage inhibition; Ac: absorbance of negative control; Ae: absorbance of the extract after 30min of incubation

The study of the variation of the anti-radical activity according to the concentration of the extracts madeit possible to determine the concentration which corresponded to 50% inhibition (IC_{50}), CE_{50} and PA.

IC_{50} (SC_{50}): anti-radical concentration allowing to trap 50% of free radicals.

The effective concentration 50 (EC_{50}) is the concentration of extract required to trap ½ mole of DPPH. It is calculated by the following formula: $CE_{50} = SC_{50} / ([DPPH])$

[DPPH]: concentration of DPPH

The anti-radical power (AP) is the reverse of the effective concentration the higher it is, the more the anti-radical power of the extract is good. It is given by the following formula:

$$PA = 1 / (CE_{50})$$

With: PA: anti-radical power

2.13.2. Reducing activity of Fe^{3+} ions

The Fe^{3+} reduction test was performed according to the modified protocol described by Path Canada (1994).

In a 96-well microplate, 100µL of solvent (ethanol) were introduced into all the wells except those of the first line 1. Then 200µL of our extracts were introduced into the wells of line 1, a series of dilution of order 2 was carried out up to line 6. Each extract dilution (25µl) was introduced into a new microplate and 25 µl of the Fe^{3+} solution at 1.2 mg / ml were added, the mixture was incubated for 15 min. After this incubation, 50 µL of 0.2% orthoPhenantroline solution was added and the plate was re-incubated for 20 min, still at room temperature. At the end of this incubation, the optical density of the contents of the wells was read at 505nm with a plate reader (TECAN Infinite). The test was performed in duplicate. The negative control which corresponded to 0% reduction consisted of solvent, Fe^{3+} and orthophenantroline. The results were expressed in reducing power (OD) as a function of the extract concentration.

2.14. Evaluation of cytotoxic activity

The cytotoxicity of active products was assessed according to the protocol described by Bowling *et al*., 2012[22]**.** African green monkey normal kidney Vero cells (ATCC CRL 1586) were maintained in T-25 flasks using complete Dulbecco's Modified Eagle's Medium, supplemented with, 10% Fetal Bovine Serum, 0.2% sodium bicarbonate (w/v) and 1% (v/v) penicillin-streptomycin. Cells were kept at 37°C for 72 hours in 5% CO_2 incubator, the medium was renewed each 72 h and the cell density was monitored under the inverted microscope Etaluma 520 until the formation of a monolayer. Confluent culture (nearly 90%) was trypsinised (0.05% Trypsin-EDTA), then centrifuged at 1,800 rpm for 5 minutes and the resulting pellet was re-suspended in culture medium. Cells at 10^4cells per well were seeded (100µL) in 96-well culture plates (Costar, USA) and incubated overnight to allow cell adhesion. Thereafter, 10 µL of serially diluted extracts, fractions (≤500 or ≤100 µg/mL), and compounds (≤50 µg/mL) were added to plate wells in duplicate. The plates were incubated in a humidified and 5% CO_2 atmosphere at 37°C for 48 h. Podophyllotoxin at 20µM was added as positive control and wells containing untreated cells was included as 100% growth control. Ten microliters of a stock solution of Resazurin (0.15 mg/mL in sterile PBS), were added to each well, and incubated for an additional 4 h. Fluorescence was then read using a Magelan Infinite M200 fluorescence multi-well plate reader (Tecan) with an excitation and an emission wave lengths of 530 and 590 nm, respectively. Cell viability (%) was calculated with regard to the negative control, and subsequently used to determine CC_{50} by non-linear regression GraphPad Prism 5.0 software (San Diego, California) using the criterion defined by the American National Cancer Institute (NCI).

The results for cytoxicity are interpreted as follows: an extract is cytotoxic if its CC_{50} on the host cells is less than 30 µg / ml [23].

3. Results

3.1. Extraction yield and characteristics of the extracts obtained

Table 1 below shows the extraction yields and characteristics of the leaves and barks of *D. eludis*. The extraction yields of DeFH and DeEH of *Dacryodes edulis* ranged from 8.1to 9% and with the leaves having slightly the highest yield. Results in Table1 also indicate that though both extracts had similar consistency (elastic and pastry), they differed greatly based on colour. The leaf extract was greenish black while bark extracts were brown.

Table 1: Extraction yield and characteristics of the extracts obtained

	Mass (g)	yield %	Physical characteristics	
			Colour	**Aspect**
DeFH	90	9%	Greenish black	Elastic and pasty
DeEH	81	8.1%	Brown	Elastic and pasty

DeFH: hydroethanolic extract of the leaves of *Dacryodes edulis* DeEH: hydroethanolic extract of the bark of *Dacryodes edulis*

3.2. Identification of secondary metabolites

Table 2 below describes the quality of secondary metabolites present in our extracts. The leaf extracts (DeFH) contained all the secondary metabolites except saponosides whereas terpenoids and steroids were absent in the bark extracts (DeEH). These results reflect the difference in the chemical profile between DeFH and DeEH.

Table 2: Identification of secondary metabolites

Chemical groups	*De*FH	*De*EH
Alkaloids	+	+
Phenolic compound	+	+
Flavonoids	+	+
Tannins	+	+
Saponosides	-	+
Terpernoids	+	-
Steroids	+	-

DeFH: hydroethanolic extract of the leaves of *D. edulis*; DeEH: hydroethanolic extract of the bark of *D. edulis*; (+): presence of metabolites; (-): absence of metabolites.

3.3. MICs and MBC of hydroethanolic extracts from the leaves and bark of *D. edulis*

The results of MICs and MBC of our extracts on six bacterial pathogenes are summarised in table 3 below. Both extracts were active on all the tested bacteria with MICs ranging from 25-12.5 mg / mL. However with less activity on *Staphylococcus aureus (*with an MIC of 25 mg / Ml).

Table 3: MIC of hydroethanolic of leaf and bark extracts of *D. edulis*

Bacterial Strains	Antibacterial activity (MIC in mg/mL)		
	*De*FHs	*De*EH	**Ciprofloxacinµg/mL**
			MIC
Staphylococcus aureus ATCC 46003	25±0	25±0	0.234
Klebsiellapneumoniae ATCC 700603	12.5±0	12.5±0	0.234
Salmonella enterica NR-4294	12.5±0	12.5±0	0.234
Escherichia coli ATCC 25922	12.5±0	12.5±0	0.234
Salmonella typhi CPC	12.5±0	12.5±0	0.468
Shigellaflexneri NR-518	12.5±0	12.5±0	0.468

3.4. Antibacterial activity of the combination of extracts from the leaves and bark of *D. edulis*

Table 4 below shows the antibacterial potential of the MIC in combination of the hydroethanolic extracts of the leaves and barks of *D. edulis* on *Klebsiella pneumoniae* ATCC 700603.The MICs in combination of hydroethanolic extracts of the leaves and barks ranged from 1.562 to 0.195 mg/ml with the percentage reduction in MIC varying between 87.50 and 98.44% respectively. Both FIC_{DeFH} and FIC_{DeEH} values ranged from 0.015 mg/mL to 0.124 mg/mL with 0.124 mg/ mL being the most frequent FICI value. The mean FICI combination of DeFH and DeEH

was 0.16 indicating the synergistic effect of the combined leaf and bark extracts on *Klebsiella pneumoniae* ATCC 700603.

Table 4: FIC and FICI incombination of DeFH and DeEH on *K. pneumoniae* ATCC 700603

	Comb MIC of *De*FH (mg/mL)	% MIC reduction of *De*FH	FIC_{DeFH} (mg/mL)	comb MIC of *De*EH (mg/mL)	% MIC reduction of *De*EH	FIC_{DeEH} (mg/mL)	FICI (FIC_{DeFH}/FIC_{DeEH})	Interpretation
8E	0.195	98.44	0.015	1.562	87.50	0.124	0.139	Synergy
7E	0.390	96.88	0.031	1.562	87.50	0.124	0.155	Synergy
6E	0.781	93.75	0.062	1.562	87.50	0.124	0.186	Synergy
5F	1 .562	87.50	0.124	0.781	93.75	0.062	0.186	Synergy
5G	1.562	87.50	0.124	0.390	96.88	0.031	0.155	Synergy
5H	1.562	87.50	0.124	0.195	98.44	0.015	0.139	Synergy
Mean FICI							**0.16**	**Synergy**

Comb MIC: minimum inhibitory concentration in combination; DeFH: hydroethanolic extract of the leaves; DeEH: hydroethanolic extract of the bark; FIC: fractional inhibitory concentrations; FICI: fractional inhibitory concentration index

3.5. Antioxidant activity based on DPPH radical trapping

The figure 1 below shows the DPPH scavenging potential of hydroethanolic extracts of leaves and barks of *D. edulis.*

The percent inhibition ranged from 81.77% to 23.99% and from 7.98% to 3.86% for DeEFa nd DeEH respectively. The percentage of DPPH inhibition increases as a function of the extract and vitamin C concentrations.

Moreover, the IC_{50s} varied between 48 µg / ml and 2024.2 µg / ml depending on the extract (Figure 2). With the DeFH extract having the best IC_{50} (48 µg / ml. Similarly, the hydroethanolic extract of the leaves of *D. edulis* had better EC_{50} (EC_{50} = 2.4) compared to bark extract (EC_{50} = 101.21) as seen in Table 5.

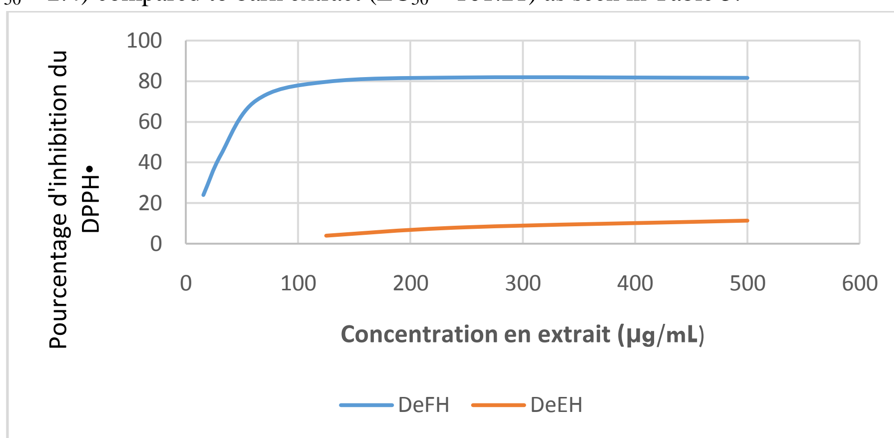

Figure 1: DPPH scavenging potential of hydroethanolic extracts of leaves and barks of D. edulis

Figure 2 below indicate that the absorbance of the Fe^{2+}increased proportionally with the concentration of extracts. At the concentration of 125 µg / ml, DeEH and DeFH recorded optical densities of 2.5 and 2.2 respectively. The highest optical densities for DeEH and DeFH were obtained at the concentration of 500µg / ml (3.01 and 2.71).

Table 5: DPPH reduction parameters by *D. edulis* extracts

Extract	IC_{50} (µg/ml)	CE_{50} x 10^3	PA x 10^{-5}
***De*FH**	48±3	2.4	4.1
***De*FH**	2024.2±3	101.21	98.1
Vitamin C	10.7	0.5	1.8

Reducing activity of Fe^{3+} ions to Fe^{2+}

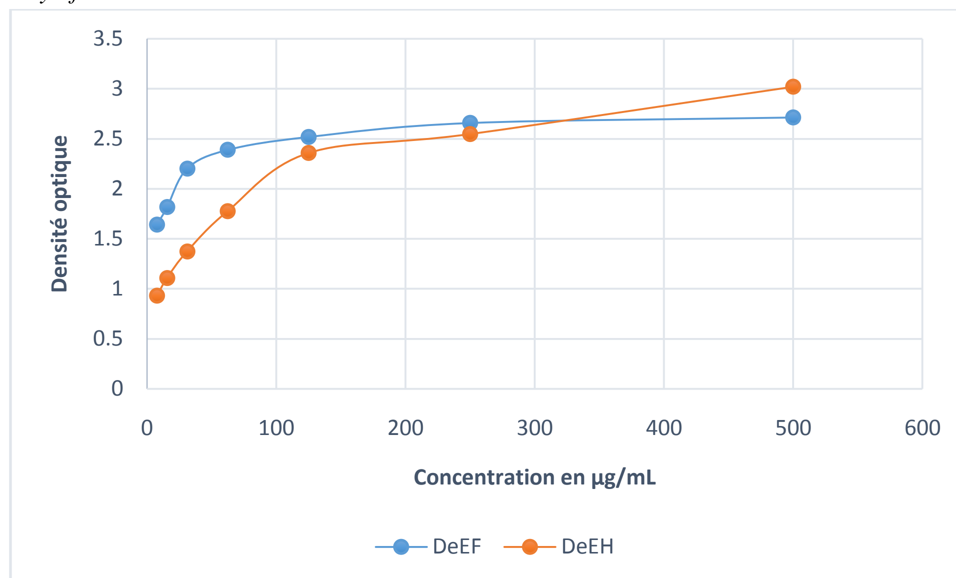

Figure 2: Reducing power of ferric to ferrous iron as a function of extract concentrations

DeFH: hydroethanolic extract of the leaves of *D. edulis*; DeEH: hydroethanolic extract of the bark of *D. edulis.*

3.6. Cytotoxic activity

Results in Table 6 indicated that the CC_{50} of DeFH and DeEH extracts ranged from 110.85 ± 5 and 88.685 ± 5µg/mL thereby demonstrating that all the extracts were non-cytotoxic.

Table 6: CC_{50} of *De*FH and *De*EH

Extract	CC_{50} en µg/ml	Interpretation
***De*FH**	110.85±5	Non cytotoxic
***De*EH**	88.685±5	Non cytotoxic

DeFH: hydroethanolic extract of the leaves of *D. edulis*; DeEH: hydroethanolic extract of the bark of *D. edulis*; CC_{50}: cytotoxic concentration 50.

4. Discussion

The present work was aimed at determining the chemical potential and the cytotoxic, anti-hemolytic and antioxidant properties of leaf and bark extracts of *D. edulis*. The extraction yields of DeFH and DeEH of *D. edulis* ranged from 8.1to 9% and with the leaves having slightly the highest yield. Results in Table1 also indicate that though both extracts had similar consistency (elastic and pastry), they differed greatly based on colour. The leaf extract was greenish black while bark extracts were brown. Unlike the bark, the plant leaves are the site of photosynthesis in the plant and therefore the privileged place of synthesis of plant metabolites [13]. This may explain the relatively higher extraction yield for the leaves of *D. edulis*. Two previous studies agreed with the extraction yield of our leaves by reporting 8.6 and 8 % for the methanoli cand ethanolic extraction of the leaves of *D. edulis* in Nigeria *(*16, 13). In this case, the extraction yield of leaves of *D. edulis* was not influenced by the extraction and methods nor by geographical location.

The phytochemical screening revealed that except the saponosides, all the tested secondary metabolites were present in e leaf extracts (DeFH); however, terpenoids and steroids were absent in the bark extracts (DeEH). These results reflect the difference in the chemical profile between DeFH and DeEH. It is interesting to note the presence of polyphenols (flavonoids, tannins) in both extracts, With the exception of the absence of saponins in the bark extracts of our plant, these results are in agreement with the study carried out by Oyetunji and Adeniyi in 2017 [14] which revealed the presence of polyphenols (flavonoids, tannins) alkaloids, in the methanolic extracts of the leaves of *D. edulis.*

The antibacterial activity of the leaf and bark extracts of *Dacryodes edulis* was determined against six microorganism comprising of one Gram positive (*Staphyloccocus aureus* ATCC 46003) and five Gram-negative bacteria (*Shigella flexneri* NR-518, *Klebsiella pneumoniae* 700603, *Salmonella typhi* CPC, *Salmonella enteric* NR-4294, *Escherichia coli* ATCC 25922). Both extracts were active on both tested Gram positive and Gram-negative organisms with more antibacterial activities against the five Gram negative bacteria at a concentration of 12.5 mg / mL. Apart from the fact that they were not tested against *Salmonella* species, the methanolic extracts of the leaves of *D. edulis* were found to be more active against *Shigella, Klebsiella pneumoniae, S. aureus* and *E. coli* in the experiments of Oyetunji and Adeniyi in 2017 [14] than in our study. Moreover, though, inactive against *E. coli*, the essential oil from the leaves of *D. Edulis* in the study carried out by Riwom and teammates [24] had almost similar performance compared with our results against *S. typhi*, *Shigella* and *S. aureus* with MIC =18.75 mg/mL.

The antibacterial activities of our extracts could be attributed to the presence of phenolic compounds, flavonoids and tannins. These compounds are recognized for their ability to destabilize the membrane of microorganisms thus causing cell death. According to the work of Cushnie *et al* (2011) flavonoids are endowed with great antibacterial power, they act by various mechanisms such as: inhibition of the synthesis of topoisomerases; inhibition of energy metabolism; inhibition of the synthesis of the bacterial wall and inhibition by perforation of the plasma membrane [25]. Moreover Ngoupayo *et al* (2016) reported that tannins inhibit bacterial growth by complexing enzymes (permeases) and proteins of the outer membrane of bacteria; inhibition of these enzymes would disrupt the vital functions of microorganisms [20].

However, the combination of hydroethanolic leaf and bark extracts of *D. Edulis* underlines the synergistic interactions of individual extracts. The MICs in combination of hydroethanolic extracts of the leaves and barks ranged from 1.562 to 0.195 mg/ml with the percentage reduction in MIC varying between 87.50 and 98.44% respectively. Both FIC_{DeFH} and FIC_{DeEH} values ranged from 0.015 mg/mL to 0.124 mg/mL with 0.124 mg/ mL being the most frequent FICI value. The mean FICI combination of DeFH and DeEH was 0.16 indicating the synergistic effect of the combined leaf and bark extracts on *Klebsiella pneumoniae* ATCC 700603. This reduction in the MIC reflects a reduction in the dose of the extracts tested individually, this characterizes the synergistic effect which could be due to the combined action of the components of each extract or by the fact that certain compounds present in the two extracts act on different targets to inhibit bacterial growth. The combined inhibitory effects of terpenoids and steroids present only in the leaf extract and the saponosides present only in the bark extracts together with remaining secondary metabolites contained in both extracts which antibacterial activity was demonstrated by Ajaiyeoba in 2002 [26] explain the effectiveness of the combination.

The antioxidant power of the DeFH and DeEH extracts was evaluated by the method of trapping the DPPH radical and the reduction of Fe^{3+}.

The DPPH scavenging potential of hydroethanolic extracts of leaves and barks of *D. edulis* (with the percent inhibition ranging from 81.77% to 23.99% and from 7.98% to 3.86% for DeEF and DeEH respectively) increases as a function of the extract and vitamin C concentrations. This is an indication that this extract is rich in flavonoids which is an anti-stress. Moreover, the IC_{50}s varied between 48 µg / mL and 2024.2 µg / mL depending on the extract with DeFH extract having the best IC50 (48 µg /ml. Similarly, the hydroethanolic extract of the leaves of *D. edulis* had better EC_{50} (EC_{50} = 2.4) compared to bark extract (EC_{50} = 101.21). The IC_{50} (48µg /ml) value of the leaf extract in the present study is far greater than that was recorded by the methanolic leaf extract (IC_{50}s of 10.45 µg / ml) of *D. Edulis* as reported by Oyetunji and colleagues. This difference could be due to differences in extraction method and geographical location *D. Edulis* [14].

The absorbance of the Fe^{2+}increased proportionally with the concentration of extracts. At the concentration of 125 µg / ml, DeEH and DeFH recorded optical densities of 2.5 and 2.2 respectively. The highest optical densities for DeEH and DeFH were obtained at the concentration of 500 µg / ml (3.01 and 2.71). Results indicated that the CC_{50} of DeFH and DeEH extracts ranged from 110.85 ± 5 and 88.685 ± 5 µg/mL thereby demonstrating the non-cytotoxicity of both extracts. These results are similar to those of Inocha *et al*. (2011) who obtained CC_{50} of 116.6 µg / ml with the hexane fraction of the leaves of *Dacryodes edulis* on the cells of the Vero line, thus indicating its harmlessness [32].

Thus, a high absorbance would reflect the presence of a large amount of Fe^{2+} -orthophenantroline complex and therefore an antioxidant power. These results can be attributed to the richness of the extracts in polyphenolic compounds because the polyphenol antioxidant property is conferred on the hydroxyl groups that characterise them. Ferreira *et al* (2013) have demonstrated that the hydroxyl groups of polyphenols can behave as reducing agents that donate hydrogen and as both free radicals [27]. Similarly, Kolechar *et al* (2008) have shown that catechetical tannins are characterised by their richness in hydroxyl groups and known for their remarkable trapping of free radicals [28]. Equally, the free radical scavenging potential of flavonoids against reactive species of oxygen has been shown. As a result, they act as antioxidants by a free radical scavenging mechanism with the formation of less reactive flavonoid phenoxyl radicals; this ability can be explained by explained by their ability to donate a hydrogen atom from their hydroxyl group and thereby scavenge the free radicals [29]. Likewise, studies have revealed that the reducing activity of Fe^{3+} by tannins is attributed to the presence of phenolic groups in their chemical structure. These groups have the ability to form stable complexes with ferric ions [30]. In fact, the reducing capacity of iron in DeFH and DeEH extracts by forming complexes with ferric ions would have an advantage against bacteria as they may contribute to reducing the quantity of iron that can be used by these microorganisms and necessary for their metabolism [31].

Some plants used for their therapeutic effect can in high doses pose a threat to human health. This explains the relvance in studying the toxicity of the leaf and bark extracts of *D. edulis* through the evaluation of their cytotoxic activity to the Vero cell line.

Our results show that the CC_{50} was 110.85 and 88.785 µg / ml for the DeFH and DeEH extracts respectively. Therefore, all the extracts were found to be non-cytotoxic because the CC_{50}s are greater than 30 µg / ml according to the National Cancer Institute of America (NCI). These results are similar to those of Inocha *et al*. (2011) who obtained CC_{50} of 116.6 µg / ml with the hexane fraction of the leaves of *Dacryodes edulis* on the cells of the Vero line, thus testifying to its harmlessness [32].

5. Conclusion

The antibacterial, antioxidant and cytotoxic activities of hydroethanolic extracts of leaves and bark suggest that of *D. edulis* is a plant relatively rich in active metabolites with extraction yields ranging from 8 to 9%. Phytochemical profile revealed the presence of polyphenols (flavonoids, tannins), alkaloids, terpenoids and saponosides in all the extracts.

The hydroethanolic extracts of the leaves and bark of *D. edulis* were active Both extracts were active on both tested Gram positive (*S. aureus* ATCC 46003 at 25 mg / mL) and Gram-negative organisms (*S. Flexneri* NR-518, *K. pneumoniae* 700603, *S. Typhi* CPC, *S. Enteric* NR-4294, *E. coli* ATCC 25922) with more antibacterial activities against the five Gram negative bacteria at a concentration of 12.5 mg /mL.

The mean FICI value of 0.16 reveals a synergistic effect of the combined leaf and bark extracts on *K. pneumoniae* ATCC 700603.

The antioxidant activity based on DPPH radical scavenging power and iron chelating capacity demonstrated that our extracts could exhibit high antioxidant power. Moreover, the non-cytoxicity of leaf and bark extracts of *D. edulis* has been shown on Vero cells with CC_{50} values ranging 88.685 to 110.85 ± 5 µg / mL.

The presence of polyphenols (flavonoids, tannins) and alkaloids, terpenoids and saponosides in the extracts of *D. edulis* provides this plant with pharmacological properties thereby supporting its use in traditional medicine for the relief of various pathologies (typhoid fever, diarrhea, pneumonia, skin infections, urinary tract infections, and oxidative stress).

Conflict of interest statement

We declare that we have no conflict of interest.

Reference

[1]. Cuzin L, Delpierre C. épidémiologie des maladies infectieuses. EMC maladies infectieuses.2005; 2(4): 287-9.
[2]. Barre-Sinoussi F. Globalization and infectious diseases: A challenge announced by the emergence of HIV/AIDS. Proceedings of the Solemn Meeting of the Academy of Science. Paris: Barre-Sinoussi F. 2009:1-5.
[3]. OMS | Rapport annuel des Statistiques sanitaires mondiales [en ligne]. WHO: https://www.who.int/gho/publications/world_health_statistics/fr/ [consulté 25 nov 2019]
[4]. WHO | World Health Organization [enligne]. WHO. http://www.who.int/gho/mortality_burden_disease/en/ [consulté le 25 nov 2019]
[5]. Profil Sanitaire Analytique du Cameroun 2016 | MINSANTE [en ligne]. https://www.minsante.cm/site/fr/content/profil-sanitaire-analytique-du-cameroun-2016 [consulté le 25 nov 2019]
[6]. Diallo O, Baron S, Jimeno M-T, Abat C, Dubourg G, Chaudet H, et al. Mortalité des bactériémies causées par des bactéries difficiles à traiter «Difficult to TreatResistance» DTR. Médecine Mal Infect. 2019;49(4):48.
[7]. Duval M, Cossart P. Un nouveau mécanisme de résistance aux antibiotiques - Le recyclage des ribosomes. médecine/sciences. 2019;35(8-9):613-5.
[8]. Radia L. Evaluation in-vitro des activités anti-oxydante et anti-inflammatoire des huiles essentielles d' Origanumglandulosumet Rosmarinusofficinalisseules et en combinaison. [Mémoire de Master]. Université Abderrahmane Mira de Béjaia, Algerie; 2014.
[9]. Farida C, Meriem C. Effet préventif de pathologies hémolytiques liées au stress oxydatif des extraits de l'écorce de Fraxinus angustifolia. [Mémoire] Master en biochimie physiopathologie. Université Abderrahmane Mira de Béjaia; 2017.
[10]. Rondeau P. Stress oxydant et glycation: relation structure et activités biologiques de l'albumine in vitro et in vivo dans le cadre de la pathologie diabétique [Thèse] Doctorat en biochimie. Université de la Réunion, France; 2009.
[11]. Amel MB. Etude de l'activité antioxydante de l'extrait hydroacetonique des racines de l'Arbutusunedo de la région de Tlemcen [Mémoire] Master en biologie. Université Abou BekrBelkaidde Tlemcen; 2016-2017; 66.
[12]. Bekakria O, Atta M. Prescription antibiotique et résistance bactérienne [Thesis]. Université de Béjaia, Algérie; 2019.
[13]. Ajibesin KK, Essien EE, Adesanya SA. Antibacterial constituents of the leaves of Dacryodes edulis.African Journal of Pharmacy and Pharmacology.2011; 5(15):1782–1786.
[14]. Oyetunji OO, Opeyemi AP. Antibacterial and Antioxidant Activities of Dacryodes edulis Methanolic Leaf Extract. Journal of Advances in Medical and Pharmaceutical Sciences. 2017; 1–11.
[15]. Conrad OA, Uche AI. Assessment of In vivo antioxidant properties of Dacryodes edulis and Ficusexasperata as anti-malaria plants.Asian Pacific Journal of Tropical Medecine. 2013;3(4):294–300.
[16]. Ononamadu CJ, Alhassan AJ, Ibrahim A, Imam AA, Ihegboro GO, Owolarafe TA, et al. Methanol-Extract/Fractions of Dacryodes edulis Leaves Ameliorate Hyperglycemia and Associated Oxidative Stress in Streptozotocin-Induced Diabetic Wistar Rats. Journal of Evidence Based Integrated Medecine.2019 ; 7(5):1-12.
[17]. Zintchem R, Njinkio B, Kamgang R, Fokunang C, Tsala DE BS. Antioxydative properties of Mallotusoppositifolum decoction leaves extract using in vitro models. International Journal of Biological and Chemical Sciences. 2013;7(6):2396-408.
[18]. Bruneton J. Pharmacognosie, phytochimie, plantes médicinales. Paris Tec Doc Ed. 1999; 7(2) 344-352.
[19]. MP W. Activité antifalcémiante et screening phytochimique de la fraction éthérométhanolique de BEAT-SS [Thèse]. PharmacieUniversité de Kinshasa, Congo. 2012.
[20]. Ngoupayo J, Assonfack RM, Matchawe C, Djiele P, Ndelo J. Evaluation of the antimicrobial activity of tanin extracted from the backs of Erythrophleum guineensis (Caesalpiniaceae). Journal of Pharmacognosy and Phytochemistry.2016; 5:287-291.
[21]. Fadeyi SA, Fadeyi OO, Adejumos AA, Okoro C, Myles El. In vitro anticancer screening of 24 locally used Nigerian medicinal plants. BMC complementary and Alternative Medicine Impact Journal.2013; 13:79.
[22]. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity tests. Journal of Immunological Methods.1983; 65:55-63.

[23]. Fadeyi SA, Fadeyi OO, Adejumos AA, Okoro C, Myles El. In vitro anticancer screening of 24 locally used Nigerian medicinal plants. BMC complementary and Alternative Medicine Impact Journal. 2013; 13:79.
[24]. Riwom H, Ndoye F, Nyegue M, Wanki R, Voundi O, Etoa F. chemical composition and in vitro antibacterial activity of the essential oil of leaves, resin and barks of Dacryodes edulis growing in Cameroon on diarrhea associated strains. Journal of Applied Pharmaceutical Sciences. 2015; 5(10):06-11.
[25]. Cushnie T. Lamb A. recent advances in understanding the antibacterial properties of flavonoids. International Journal of Antimicrobial Agents. 2011; 38(2):99-107.
[26]. Ajaiyeoba E. Phytochemical and antibacterial properties of Parkia bicolor leaf extracts. African Journal of Biomedecine Research. 2002; 35(5): 125-129.
[27]. Ferreira B, Baltisberger M. Medical ethnobotanics of Zopotecs of the Isthmus_sierra (Oxaca, Mexico). Document and assessment of indigenous uses. Journal of Ethnopharmacology 2013; 62:149-165.
[28]. Kolechar V, Kulbikova K, Kuca K, Jun D, Jahodar L. Condensed and hydrolysable tanins as antioxydants influencing the health. Mini-Review in Medicinal Chemistry. 2008; 8:436-447.
[29]. Amié D, Davidoé A, Beslo D, Trinajstié N. Structural radical scavenging activity scavenging activity relationships of flavonoids. Croatica Chemistry. 2003; 76(1):55-61.
[30]. Sheh-Hong L, Ibrahim D, Kassim J, Salaim S. Antimicrobial and antioxydant activities of condensed tanins from Rizophora apiculata barks. Journal of chemical and pharmaceutical research 2011; 3:436-444.
[31]. Haddouchi F, Chaouche T, Halla N. Screening phytochimique, activités antioxydantes et pouvoir hémolytique de quatres plantes sahariennes d'Algérie. Journal of pharmacognosy and phytochemistry.2016; 5:287-291.
[32]. Onocha P, Opasola O, Ganiyadat O. Cytotoxicity and free radical scavenging activities of hexane fractions of Nigeria specie of African pear (Dacryodes edulis). International journal of biological chemistry. 2011:5(2); 143-149.