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Phytochemical Screening and Evaluation of the Anti Ulcer Activity of *Dacryodes edulis* (Burseraceae) Leaf Extracts in Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Authors NH, TEF and CF designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors NH, BH, NNB and MW managed the analyses of the study, data mining, performed the statistical analysis and the specified literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Peptic ulcer disease is a chronic condition of the gastrointestinal tract that results from an imbalance between protective and aggressive factors of the gastric mucosa. Many synthetic drugs are used for the treatment of this disease, but they are costly and exhibit limited efficacy, and could cause adverse effects. Hence, the present study aimed to screen the phytochemicals and evaluate the anti-ulcer activities of *Dacryodes edulis* (G. Don) H.J. Lam leaf extracts in Wistar rats. Four leaf

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extracts obtained by aqueous cold maceration, hydroethanol maceration, infusion and decoction respectively were used. Qualitative and quantitative phytochemical screening was carried out and an ulceration model based on HCl/ethanol mixture (150mM/60%) was used to evaluate the gastroprotective activity *in vivo* at a dose of 500mg/kg. The physiological and biochemical parameters of the homogenate were evaluated. Qualitative analysis showed the presence of total polyphenols, tannins, flavonoids, flavonols, saponosides, coumarins, anthraquinones, cardiac glycosides and ascorbic acid. Quantitative analysis showed a predominance of flavonoids followed by flavonols, polyphenols and tannins. The extracts obtained by hydro-ethanolic, and aqueous maceration showed the best gastroprotective activity with percentages of 81.61% and 75% respectively. The aqueous extracts obtained by infusion and decoction showed a low activity (52% and 26% respectively). The biochemical analysis of the gastric juice content showed a significant increase in the activity of antioxidant enzymes (Catalase, SOD, GSH) and a pronounced decrease in MDA in all groups. We concluded that *D. edulis* leaf extracts showed potential bioactive metabolites predorminantly of flavonoids, flavonols, polyphenols and tanins, numerous natural antioxidants that was responsible for the observed gastroprotective activity and this plant could be a promising source of a more effective and safer antiulcer drug. This study also confirm the use of *D. edulis* as a category 1 improved traditional medicine, while in development for a category 2 phytomedicine.

Keywords: Dacryodes edulis; antiulcer; phytochemicals; antioxidant; wistar rat.

1. INTRODUCTION

Peptic ulcer is one of the most common diseases of the gastrointestinal tract, with an increasing incidence and prevalence [1]. Indeed, it can lead to serious complications and even death in some cases [2]. It affects about 10% of the world's population with an estimated mortality rate of 15,000 deaths per year [3]. This condition is characterised by an imbalance between factors that damage and those that protect the integrity of the gastric mucosa [4]. It is multi factor, but the highest incidence is usually seen in the context of *Helicobacter pilori* infection, and the use of non-steroidal anti-inflammatory drugs (NSAIDs) [1].

Conventional therapies are based on the use of antacids, proton pump inhibitors (PPIs), anticholinergics, histamine H2 antagonists and antibiotics [4]. However, many previous studies have shown that these molecules can have limited efficacy and, above all, several adverse effects that are more or less tolerated by the subject. However, the usual use of PPIs exposes their users to some even more serious effects, including the development of Vitamin B12 deficiency and iron deficiency anaemia, alteration of vascular homeostasis and interaction with the metabolism of other drugs in the cytochrome P450 system [5-7].

The use of medicinal plants to cure many diseases is as old as mankind. Due to the various adverse effects associated with the use of conventional drugs, herbal medicines are considered to be the best alternative, and have been shown to give promising results in the treatment of peptic ulcers [8]. *D. edulis (G. Don)* HJ Lam [9], one of these plants, has been used for many years by local populations in West and Central Africa for its nutritive values and by tradi-practitioners of complementary medicine to cure many diseases [10], including: leprosy, dysentery, anaemia, diabetes, stiffness, tonsillitis, skin diseases, ear infections, fever, headache, malaria, snakebite treatment, oral care and wound healing [10-12].

This study therefore investigated the gastroprotective properties of *D. edulis* leaves in the Yaoundé locality of Cameroon. It also explored the phytochemical characterization of the leaf extracts, which could potentially present biological and pharmacological activities for the treatment, and prevention of peptic ulcers.

2. MATERIALS AND METHODS

2.1 Sample Collection and Identification

Fresh leaves were purchased from Yaounde locality, Cameroon. The plant was botanically identified and authenticated at the national herbarium under the voucher identification number: 66954.

2.2 Preparation of Plant Extracts

After harvesting, the leaves were dried in the shade at temperature for a fortnight. They were then finely pulverized with a mechanical grinder

and four extracts were prepared from this powder.

2.2.1 Extracts obtained by Aqueous Maceration (AM) and Hydro-Ethanolic Maceration (HEM)

They were obtained by dissolving respectively 100g of powder in 1000 ml of distilled water and 1000 ml of a water/ethanol absolute mixture (40/60 proportion). After 48h at room temperature, the mixtures were filtered with a Whatman No 1 filter paper and the collected filtrates were evaporated in an oven at 50°C and the residue was recovered and weighed.

2.2.2 Infusion extract (INF)

A 100g of powder was dissolved in 1000 ml of distilled water previously boiled in a water bath. The mixture was then filtered and the filtrate collected was evaporated in an oven at 50°C and the residue was recovered and weighed.

2.2.3 Decoction extract (DEC)

A 100g of powder was dissolved in 1000ml of distilled water and boiled in a water bath at 100^0C for 30 minutes. The mixture was then filtered while hot and the filtrate obtained was evaporated in an oven at 50°C. The residue was recovered and weighed. The Extraction Yields (R) were determined using the following formula:

$$\mathrm{R}(\%) = \frac{\text{Weight of the residue obtained (g)}}{\text{Initial weight of powder used (g)}} \times 100$$

2.2.4 Phytochemicals screening of the extracts and identification of phytochemicals

The secondary metabolites that were investigated in this study included alkaloids, total polyphenols, alkaloids, flavonoids, tannins, saponosides, steroids, quinones, coumarins, cardiac glycosides, Betacyanins and ascorbic acid. They were identified using Sofowora's methods [13] Harbour [14] and Saeed et al*,* [15]. For identification tests, 1% of the extract solutions were used.

2.2.4.1 Alkaloids

Meyer's test: 2 ml of 1% extract was introduced into a test tube followed by 4 drops of Meyer's reagent (1.36 g $HgCl_2$ + 5g KI for a final volume of 100ml). The formation of a creamy white or white-yellow precipitate indicated the presence of alkaloids [16].

Wagener's test: 2 ml of 1% extract was introduced into a test tube followed by 4 drops of Wagner's reagent (1.27 g I_2 + 2g KI in a final volume of 100 ml). The formation of a creamy white precipitate indicated the presence of alkaloids [17].

2.2.4.2 Total polyphenols

Iron perchloride test: 2 drops of a 5% $FeCl_3$ solution were added to 2 ml of extract contained in several test tubes. A greenish precipitate indicated the presence of polyphenols.

Lead acetate test: 2 ml of extract was introduced into test tubes followed by 3 drops of lead acetate. The formation of a white precipitate indicated the presence of polyphenols.

2.2.4.3 Flavonoids

Test with NaOH: 2 ml of the 1% extract was introduced into test tubes, followed by 1 ml of 2N NaOH. The formation of a yellow-orange coloration indicated the presence of flavonoids.

Test with H_2SO_4: 2 ml of 1% extract was introduced into test tubes followed by a few drops of concentrated H_2SO_4. The formation of an orange coloration indicated the presence of flavonoids.

2.2.4.4 Tannins

Catechic tannins: in 2 ml of extract contained in test tubes, 3 drops of Stiasny's reagent (formalin 40% + HCl: V/V) were added and the whole was boiled in a water bath for 15 minutes. A beige colour indicated the presence of catechic tannins.

Gallic tannins: The solution used for the identification of catechic tannins was filtered. 3 drops of 2% $FeCl_3$ were then added to the filtrate and the appearance of a blue-black coloration indicated the presence of gallic tannins.

2.2.4.5 Saponins

Foam test: 10 ml of the extract was introduced into several test tubes. These were then shaken for 15 seconds and left to stand for 15 minutes. A persistent foam height greater than 1cm indicated the presence of saponin.

2.2.4.6 Steroids

Salkowsti test: 5 drops of concentrated H_2SO_4 were added to 1 ml of the extract. Red coloration would indicate the presence of steroids.

2.2.4.7 The quinones

2ml of concentrated H_2SO_4 was added to 2ml of extract in a test tube. The formation of a red coloration indicated the presence of Quinones.

2.2.4.8 Coumarins

3 drops of 10% $FeCl_3$ were added to a tube containing 1 ml of extract and 1 ml of distilled water. The appearance of a green or blue coloration turning to yellow by addition of HNO_3 indicated the presence of coumarins.

2.2.4.9 Anthocyanins

5 ml of 10% H_2SO_4 and 5 ml of ½ strength NH_4OH were added to 5 ml of the extract. The blue violet colour change in basic medium indicated the presence of anthocyanins.

2.2.4.10 Cardiac glycosides

2 ml of acetic acid, a few drops of $FeCl_3$, 5% and 1 ml of concentrated H_2SO_4 were added to 500 µl of the extract. The presence of a greenish or brown ring indicated the presence of cardiac glycosides.

2.2.4.11 Betacyanins

2 ml of 2N NaOH was added to 2 ml of the extract in a test tube. The tube was heated in a water bath for 5 minutes. The appearance of a yellow coloration indicated the presence of betacyanins.

2.3 Quantitative Determination of Phytochemicals

2.3.1 Total phenolic content estimation

The total phenolic content was determined according to the method described by Singleton and Makkar [18]. 200 µl of each extract was added to test tubes, followed by 1000 µl of 10-fold diluted Folin-ciocalteu reagent and 800 µl of 7.5% sodium carbonate solution. The tubes were shaken and kept in the dark for 120 minutes. For the Blank, the extract was substituted by distilled water. A spectrophotometric measurement was carried out at 765 nm based on a calibration curve carried out under the same conditions using gallic acid in the range of 0 to 1.100g/ml. The measurement was made in three replicates. The quantity of phenolic compounds was expressed as mg gallic acid equivalent per g dry matter extract (mgGAE/gMS).

2.3.2 Estimation of total flavonoids

In a test tube, 1000 µl of extracts, 150 µl of 5% $NaNO_2$ and 150 µl of 10% were mixed. After 5 minutes, 1000 µl of 4% NaOH was added. The solution was well homogenised and a spectrophotometric measurement was performed at 510 nm based on a calibration curve using a standard quercetin solution (0-100g/l) [19].

2.3.3 Estimation of total flavonols

It was done according to the method of Miliauska et al. [20]. In a tube, 1ml of the extract, 1ml of 2% $AlCl_3$ $6H_2O$ and 600l of 50g/l Sodium acetate were mixed to a final volume of 3ml. The solution was then incubated at room temperature for 150 minutes. Spectrophotometric measurement was performed at 440 nm based on a calibration using a standard quercetin solution (0 - 200 g/l). The flavonoles content was expressed as mg Quercetin equivalents / gram dry material.

2.3.4 Estimation of total tannins

The determination of tannins was done by the Folin-Ciocalteu method which is based on the reduction of phosphomolybdic and tungstic acid in alkaline medium [21]. In a test tube, 1 ml of the extract, 200µl of the folin reagent diluted to 1/10, 1ml of Na_2CO_3 35% was mixed. Spectrophotometric measurement was carried out based on a calibration using a standard solution of tannic acid (0-500g/ml). The total tannin content was estimated in mg tannic acid equivalents/g dry matter (E tannic acid/gMS).

2.3.5 Estimation of the amount of total protein

This was done according to the Lowry method [22]. In a test tube, 1ml of the extract and 2ml of Lowry D reagent were mixed. This solution was then left at room temperature for 10 minutes, 100µl of 1:10 diluted folin-Ciocalteu reagent was added. After 30 minutes of incubation at room temperature, a spectrophotometric measurement was carried out at 650 nm based on a calibration using a standard BSA solution. The protein

content was estimated in g of BSA/g of dry material (gBSA/gMS).

2.3.6 Estimation of carbohydrates

This was done based on the reducing properties of sugars to reduce picric acid to picramic acid. In a test tube, 100 ml of the extract, 1 ml of 13% picric acid and 1 ml of 4% NaOH. The mixture was boiled in a water bath for 10 minutes. A 570 nm spectrophotometric measurement was carried out on the basis of a calibration using a glucose solution expressed as glucose equivalent / g of dry plant extract (EG /gMS).

2.4 Assessment of Anti-Ulcer Activity *in vitro*

2.4.1 FDA test

According to the FDA, a molecule is considered as an antacid when it contributes to 25% of the total neutralisation of the product.

The FDA test was carried out in the following steps:

List 1. Results of FDA test

Leaf extracts	0,25 g
HCl 0,5N	2,5ml
Distilled water	100ml
Stirring for 10 minutes	
pH measurement (its value must be between 3 and 5)	

List 2. Acid Neutralising Capacity (ANC) test

Leaf extracts	1 g
Distilled water	10ml
Stirring and pH measurement after one minute	
30 mL of HCL 1,0N and homogenization for 15 minutes	
Titration with 0.5N NaOH	

The number of milli-equivalents was obtained using the following formula:

$$Meq = (30 \times Na) - (Vb \times Nb)$$

Na: HCl normality
Vb: volume of NaOH obtained
Nb: NaOH normality

2.4.2 Buffer capacity test

It was done according to the recommended method of Holber et al. [23].

List 3. Buffer capacity test

Leaf extracts	0,5g
HCl 0,1N	25 ml
Ph measurement at 0,5; 2; 4; 6; 8 et 10 minutes	
Replacement of 5 ml of this solution with 5 ml of 0.1N HCl to a pH below 2.75	

2.5 Assessment of Anti-Ulcer Activity *in vivo*

2.5.1 Preparation of the working solutions

2.5.1.1 The extract solution

The volumes of extracts administered to the animals were obtained based on the OECD rules.

2.5.1.2 The ulcerogenic solution

It was a mixture of HCl (150mM) and ethanol (60%): EtOH/HCl

2.5.2 EtOH/HCl-induced ulcer

After 48 hours of fasting and under ideal acclimatization conditions, 30 female Wistar rats were randomly divided into 6 groups of 5 animals each. Group 1 (negative control) received only the vehicle (water), groups 2, 3, 4 and 5 received 500mg/Kg of the aqueous, hydro-ethanolic, infusion and decoction extracts respectively. And group 6 (positive control) received 20mg/kg of the standard drug (Omeprazole). One hour later, the ulcerogenic solution was administered to each group. After 2 hours of treatment, an analysis of the physiological parameters of the gastric juice content was performed while the gastric walls allowed to calculate the percentages of inhibition (%I) using the following formula [24]:

$$\%I = \frac{(SUc - SUt)}{SUc} \times 100$$

SUc = negative control ulcer surface
SUt = Ulcer area of the treated group.

These walls were then crushed and preserved in a 10% KCl solution for the determination of biochemical parameters (Protein, GSH, MDA, Catalase).

2.6 Measurement of Physiological and Biochemical Parameters

2.6.1 Pepsin

100µl of gastric juice was added to 1ml of BSA buffer. After 20 minutes of incubation at room

temperature, 2 ml of Trichloroacetic acid (TCA) was added and the whole was heated at 100°C for 5 minutes and then centrifuged. 1 ml of supernatant was taken and 400L of 2.5N NaOH + 500L of Folin-ciocalteu reagent was added. A spectrophotometric measurement was performed at 660 nm [25].

2.6.2 Catalase

Catalase activity was determined according to the method described by Prabhakar et al. [26], based on the rate of H_2O_2 decomposition by Catalase. The decrease in absorbance was monitored at 240 nm for one minute and the activity of catalase was expressed as µmol/mg protein.

2.6.3 Superoxide dismutase: SOD

The determination of SOD was done using the method described by Misra et al., which is based on the inhibition of the auto-oxidation of adrenaline to adrenochrome.

2.6.4 Lipid peroxidation: MDA

It was done following the method described by Varshney et al, and was calculated by the following formula:

$$\text{MDA (mg/of proteins)} = \frac{\text{Abs} \times \text{Homogenate volume}}{\text{E532 nm} \times \text{Sample vol} \times \text{mg of proteins}}$$

2.6.5 Glutathione: GSH

The colorimetric method using Ellman's reagent was used for this determination [27].

The method is based on cutting the 5,5'-dithios-2-nitrobenzoic acid (DTNB) molecule with GSH, which leads to the release of thionitrobenzoic acid (TNB) which absorbs at 412 nm. The result was expressed in µmol/ml.

2.7 Statistical Analysis

Statistical analysis was performed using Graph Pad Prism version 7.0. All data were expressed as mean ± SEM. Group means were compared by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test (when comparing group data with a control group). P <0.05 was considered statistically significant.

3. RESULTS

3.1 Extraction Yields

The different extraction yields obtained for the different extracts are presented in Table 1. The highest yield was recorded with the HEE (15%) and the least shown in DEC (6.8%), which was not significantly different from extracts AE and INF.

3.2 Phytochemical Composition

Table 2 shows the qualitative phytochemical components. These results show that the different extracts have nearly the same phytochemical composition. Moreover, we observed the absence of alkaloids (a very common metabolite in the plant world) and steroids in the different extracts.

3.3 Quantitive Analysis of Extracts

Figs. 1 to 6 show the quantitative estimation of the main metabolites in the different extracts

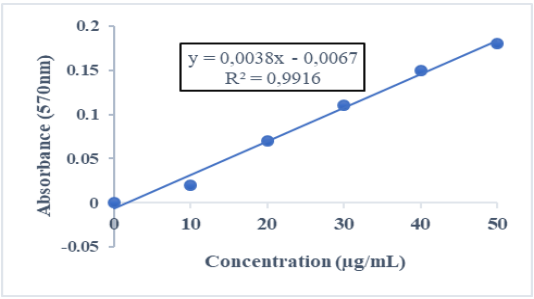


Fig. 1a. Glucose calibration curve

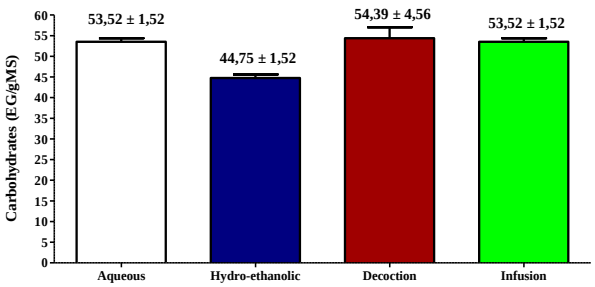


Fig. 1b. Concentration of carbohydrates in extract

Fig. 1. Quantitative assessment of carbohydrates

Table 1. Extraction yields

Extracts	HEE	AE	INF	DEC
Weighed mass (g)	100	100	100	100
Weight of the extract (g)	15,0	7,2	7,7	6,8
Yield (%)	15	7,2	7,7	6,8

Table 2. Phytochemical content

Secondary metabolites	Extracts			
	HEE	AE	DEC	INF
Polyphenols	+	+	+	+
Tanins	+	+	+	+
Coumarins	+	+	+	+
Saponosides	+	+	+	+
Alcaloïds	-	-	-	-
Flavonoïds	+	+	+	+
Flavonols / Flavons	+	+	+	+
Betacyans	+	+	+	+
Quinons	+	+	+	+
Anthraquinone	+	+	+	+
Cardiac glycosyde	+	+	+	+
Steroïds	-	-	-	-
Vitamin C	+	-	+	+

(+) : Detected (-) : No detected

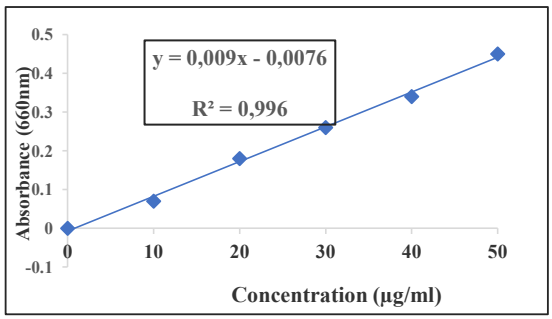


Fig. 2a. Albumin bovin calibration curve

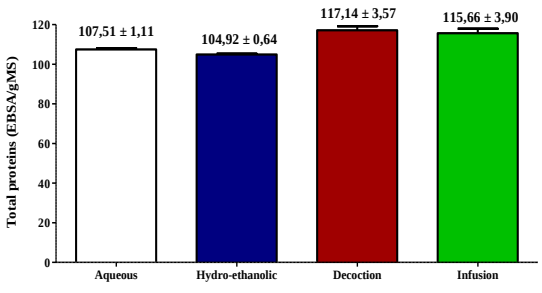


Fig. 2b. Concentration of total proteins

Fig. 2. Quantitative assessment of total proteins

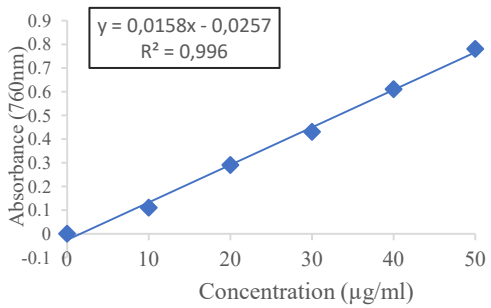


Fig. 3a. Calibration curve for gallic acid

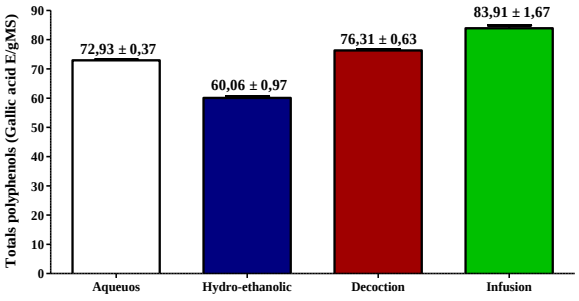
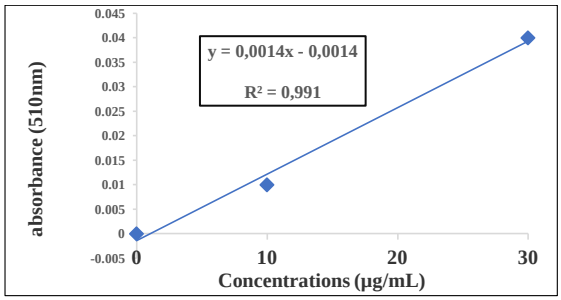


Fig. 3b. Concentration of total polyphenols

Fig. 3. Quantitative assessment of total polyphenols



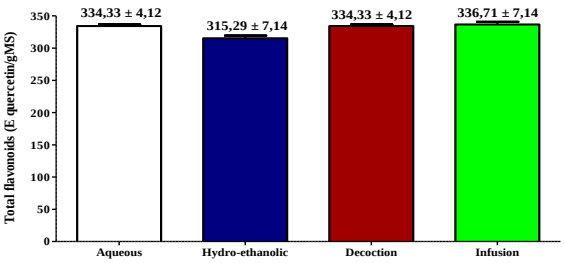
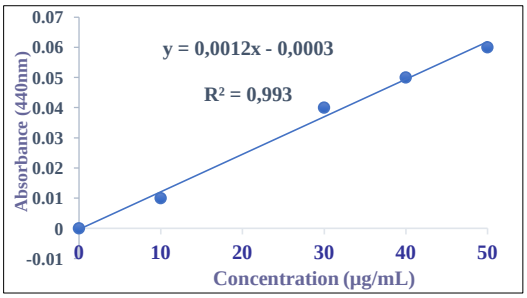


Fig. 4a. Calibration curve for quercetin

Fig. 4b. Concentration of total flavonoids

Fig. 4. Quantitative assessment of total flavonoids



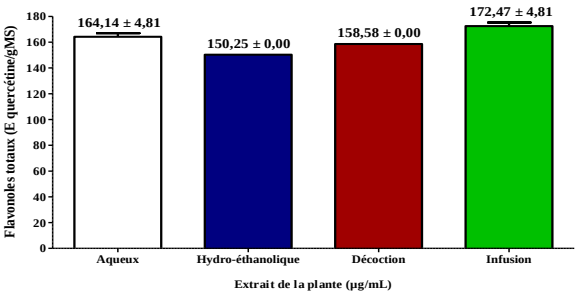
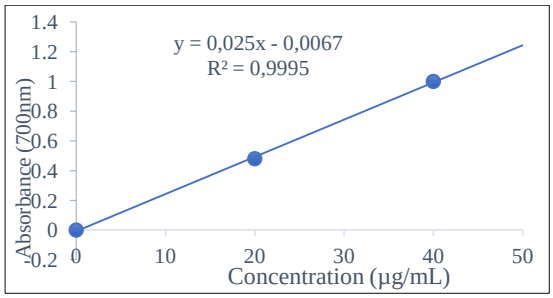


Fig. 5a. Calibration curve for quercetin

Fig. 5b. Concentration of total flavonols

Fig. 5. Quantitative assessment of total flavonols



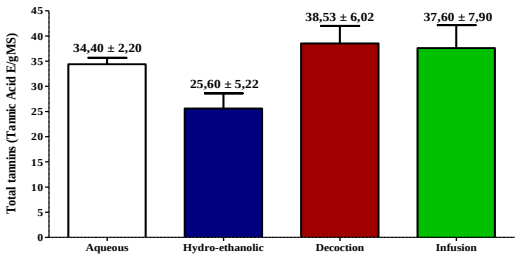


Fig. 6a. Calibration curve for tannic acid

Fig. 6b. Concentration of total tannins.

Fig. 6. Quantitative assessment of total tanins

3.4 Antacid Properties of Extracts *in vitro*

In vitro tests were performed to determine whether the plant extracts had antacid properties compared to antacids commonly used in gastric ulcer therapy (Table 3). As a product can only be considered as an antacid if its pH in the preliminary test, (FDA test) is above three. The results showed that the standard antacids (Bicarbonate, Maalox and Rennie) had pH values above three. As for the extracts, the decoction and the aqueous extract exhibited an antacid property because they also presented pH values higher than three as shown in Table 3.

3.5 *In Vivo* Anti-Ulcer Activity

3.5.1 Ulcer index

Administration of the ulcerogenic solution caused the production of ROS and an imbalance in the protection of the gastric mucosa resulting in the production of ulcers (Fig. 7A). The pretreatment of the animals with the plant extracts and the standard drug therefore protected the gastric mucosa (Fig. 7B, 7C, 7D). Thus, protection percentages of 93.03% for the reference drug; 81.61% for the hydroethanolic extract; 75.22% for the aqueous extract; 52.26%

for the infusion and 26.14% for the decoction were obtained.

3.6 Physiological and Biochemical Parameters

The results show that physiological parameters (Table 4) such as pH and total acidity were not improved following the administration of the extracts, unlike the standard drug where they were significantly improved (p-value< 0.001). For the biochemical parameters (Table 4), administration of the extracts showed a non-significant increase (p-value > 0.05) in Catalase activity in all test groups with higher values in the aqueous extract and the gold standard. Superoxide dismutase (SOD) activity was significantly increased (p-value < 0.05) in all test groups compared to the negative control group. Furthermore, GSH activity was significantly increased in the infusion group with a p-value < 0.001 and non-significantly in the other test groups with a p-value > 0.05.

The administration of the extracts decreased lipid peroxidation, symbolised by the biochemical marker MDA. This decrease was much more significant in the aqueous extract with a p-value > 0.05 and significant with a p-value < 0.01 in the positive control group.

4. DISCUSSION

This study investigated the gastroprotective activity of *D. edulis* in Wistar rats. A mixture of HCl/EtOH was used as a model for ulcer induction. Indeed, this model was chosen because it closely characterises the development of ulcers in humans [28]. Here, HCl induces damage in the mucosa making it vulnerable, while ethanol causes severe injury by suppressing mucus and enzyme production, resulting in oxidative stress which alters mucosal permeability and consequently haemorrhagic injury, mucosal friability, oedema, inflammatory cell infiltration and loss of cells characteristic of the wound [24,25].

Table 3. *In vitro* anti-ulcer activity of extracts

Extracts and standard drugs	FDA Test	ANC (mEq/ml)	Bufer capacity (min)
Bicarbonate	8,44 ± 0,19	7,45 ± 0,26	100
Maalox	4,26 ± 0,13	12,27 ± 0,24	140
Rennie	6,32 ± 1,02	9,68 ± 0,28	110
INF	2,92 ± 0,13	4,17 ± 0,12	10
DEC	3,18 ± 0,27	4,25 ± 0,22	10
AE	3,24 ± 0,19	4,40 ± 0,35	20
HEE	1,78 ± 0,16	4,88 ± 0,16	0

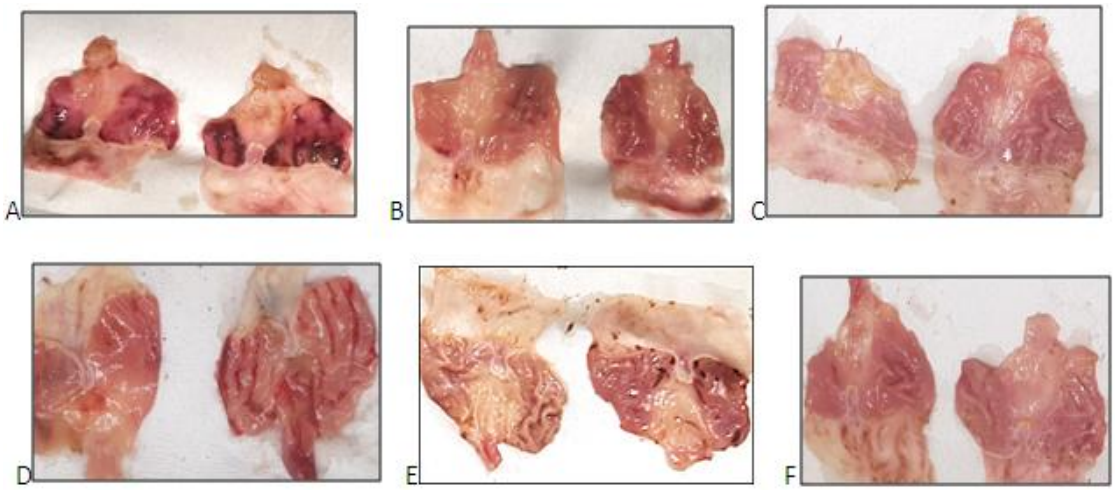


Fig. 7. Morphology of the gastric walls after treatment with the ulcerogenic solution EtOH/HCl
A = Negative control (water + ulcerogenic solution), B= Positive control at 100mg/kg (Omeprazol + EtOH/HCl), C = Test group 1 (HEE+ EtOH/HCl) at 500mg/kg, D = Test group 2 (AE + EtOH/HCl) at 500mg/kg, E = Test group 3 (INF + EtOH/HCl) at 500mg/kg, F = Test group 4 (DEC + HCl) at 500mg/kg

Table 4. Physiological and biochemical parameters of homogenates

Parameters	Negative control	AE	HEE	DEC	INF	Omeprazole
Areas of ulcers (mm^2)	10,33 ± 2,99	2,56 ± 0,44***	1,90 ± 0,98***	7,63 ± 1,79	3,38 ± 1,71***	0,72 ± 0,13***
Gastric juice volume (ml)	5,56 ± 1,66	4,09 ± 1,19	3,05 ± 1,14*	3,15 ± 1,22*	3,35 ± 1,04*	2,90 ± 0,80*
pH	2,40 ± 1,15	1,74 ± 0,68	1,66 ± 0,29	1,54 ± 0,21	1,68 ± 0,31	6,54 ± 0,58***
Mucus weight (g)	0,93 ± 0,50	0,33 ± 0,15***	0,30 ± 0,07***	0,32 ± 0,06***	0,34 ± 0,06***	0,26 ± 0,03***
Total acidity (meq/ml)	97,80 ± 38,24	81,80 ± 26,48	71,80 ± 30,87	91,00 ± 17,26	82,80 ± 23,37	11,40 ± 2,30***
Pepsin (µmol/ml)	1259,45 ± 307,09	854,73 ± 463,92	1006,00 ± 290,92	1055,94 ± 652,11	1096,00 ± 460,34	99,31 ± 48,73**
Carbohydrates (mg/l)	515,38 ± 186,57	301,97 ± 102,83	347,13 ± 151,14	306,66 ± 74,82	327,34 ± 143,62	247,13 ± 93,66
Proteins in gastric juic (mg/dl)	304,18 ± 78,39	257,60 ± 41,52	280,60 ± 9,34	241,44 ± 54,73	265,80 ± 37,53	90,93 ± 15,22
Catalase (µmol/min/ml)	56,18 ± 20,16	72,18 ± 10,95	56,39 ± 21,96	73,04 ± 15,67	53,25 ± 10,36	82,11 ± 8,05
SOD (µmol/min/mg of proteins)	819,81 ± 13,62	906,77 ± 58,67*	903,86 ± 44,02	909,99 ± 33,65*	853,25 ± 44,38	907,97 ± 65,07*
Proteins in homogenat (mg/dl)	111,56 ± 14,66	116,49 ± 7,69	114,11 ± 5,53	110,82 ± 4,56	120,87 ± 5,98	129,00 ± 16,35
MDA (µmol/min/ml)	0,96 ± 0,46	0,42 ± 0,29	0,66 ± 0,38	0,46 ± 0,32	0,46 ± 0,25	0,22 ± 0,03**
GSH (µmol/ml)	0,44 ± 0,25	0,87 ± 0,08	0,81 ± 0,19	0,77 ± 0,44	1,17 ± 0,16***	0,76 ± 0,16

*Values are mean ± MSE (n=5). Data analysis done using the ANOVA test, followed by Dunnet's post hoc comparison test. Differences were considered significant for *p-value <0.05 and **p-value<0.01*

The phytochemical composition of the extracts showed numerous bioactive molecules, including total phenols and several other secondary metabolites. Similar studies using other screening methods (HPLC) have shown similar results [26-28]. On the other hand, alkaloids, a widely cited component of *D. edulis* in many other studies, were not detected. This result might be a particular characteristic of the local species used or related to the nature of the solvents and the extraction process used. The quantitative estimation of these primary metabolites showed a predominance of proteins followed by carbohydrates. That of secondary metabolites showed a predominance of total flavonoids, followed by total flavonols, total polyphenols and total tannins. The extracts obtained by decoction and infusion show higher concentrations of metabolites than the hydro-ethanolic and aqueous extracts. This could be explained by the presence of neoformed phenolic compounds called "Maillard reaction products: MRP" which form under the effect of the heat increase and also react with Folin-Ciocalteu reagent [29-32]. The main mechanisms for the regulation of gastric acidity include direct neutralisation of existing acidity and inhibition of acid secretion pathways. The tests of the anti-acid property of the extracts showed pH values below 3 for the ethanolic extract and the infusion. On the other hand, it was observed respectively for the aqueous extract and the decoction a pH higher than 3 (3.24 and 3.18). In this regard, these extracts would present an antacid property although the ANC did not reach the minimum value of 5 mEq/ml as recommended by the USP. It is in this logic that Enengedi et al. [33] and Nwaonukuru et al. [34], following similar work, have evoked an antioxidant activity and an increase in the production of PGE.

The investigation of the gastroprotective properties of *D. edulis* leaf extracts showed a 93.03% of protection against induced ulcer for the standard drug (Omeprazole). This drug has the ability to initiate mechanisms other than the cyclooxygenase pathway or PP inhibition that can protect the gastric mucosa, in particular the scavenging of the OH^- radical, which is one of the major players in oxidative damage [35]. In the test groups, the hydro-ethanolic and aqueous extracts showed the best percentages of protection (81.61% and 75% respectively); Nwaonukuru et al obtained similar results (64.75% for a dose of 200mg/kg) [34] on a methanolic extract, Odo also observed a strong decrease in the ulcer index with the ethanolic extract at 200mg/kg [36]. On the other hand, the extracts obtained by infusion and decoction did not protect significantly, and this could be explained by the phenomenon of thermal degradation that can occur during heat extraction of polyphenols, as underlined by Antony and Farid. The analysis of the physiological parameters of the gastric contents showed that none of the different extracts have an effect on acidity, whereas Omeprazole, as a PPI, strongly reduces acid secretion. These results demonstrate once again that this plant acts by a mechanism other than the regulation of acidity. Compared to the negative control, the results show an overall Globally, in all the groups including Omeprazole, a significant increase in the activity of antioxidant enzymes (Catalase, SOD, GSH), and a decrease in MDA which is a lipid peroxidation indicator. Indeed, one of the effects of ethanol on the gastric mucosa is oxidative stress and the production of reactive oxygen species (ROS). The ROS (superoxide anion (O^-), hydroxide radical (OH^-) and hydrogen peroxide (H_2O_2) can induce oxidative modifications on biological macromolecules, thus altering their function [37]. The increase in Catalase, SOD and GSH activity can therefore be explained by the scavenging and conversion of these free radicals by the antioxidants contained in the extracts. The decrease in MDA indicates an inhibition of lipid peroxidation. This antioxidant property is due to the presence of numerous phenolic compounds and also of vitamin C, which is a polyvalent antioxidant.

5. CONCLUSION

It appears from this study that *D. edulis* leaf extracts possess numerous bioactive compounds (total polyphenols, tannins, flavonoids, flavonols, saponosides, anthraquinones, cardiac glycosides, flavonols). In addition, the study also revealed that the ethanolic extract had significantly higher anti-ulcer activity, due to the presence of numerous phenolic compounds. This provides scientific evidence for the folk use of this plant in the management of gastric ulcers.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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