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Gastroprotective, Antioxidant and Antibacterial Properties of the Aqueous Root Bark Extract of *Cassia arereh* Del. (Caesalpiniaceae) in a Wistar Rat Model

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MC and NC designed the study and wrote the protocol. Authors MC, AAP and DP managed the gastric ulcer tests. Authors MC, NC and LB managed biochemical analysis. Authors NC and FJM managed the bacterial tests. Authors NE and FJM did the phytochemical analysis. Authors MC and NOG did the literature search and statistical analysis. Authors MC and NOG wrote the first draft. Author TPV supervised the study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The objective of this study is to evaluate gastric cytoprotective, antioxidant and antibacterial properties of aqueous root bark extract of *Cassia arereh* (AECA).

Place and Duration of Study: Department of Biological Sciences (Animal Physiology Laboratory, Biochemistry Laboratory), Higher Teachers' Training College and Department of Organic Chemistry (Laboratory of medicinal chemistry), Faculty of Science, University of Yaoundé I. Between August 2015 and December 2016.

Materials and Methods: Phytochemical screening of AECA was carried out. Experimental Wistar rats were used to evaluate the antiulcerogenic effects by four methods: HCl/ethanol; indometacin - HCl/ethanol; indomethacin and pylorus-ligated. The gastric ulcerations, mucus production, pH, volume and acidity of the gastric juice were measured. Some parameters of oxidative stress (SOD and MDA) were measured in stomach samples obtained from the animals in the indomethacin model. The inhibition parameters of the bacterial growth to the extract were determined by the micro-dilution assay.

Results: The phytochemical screening revealed that the AECA contained bioactive substances such as triterpenes, sterols, flavonoids, alkaloids, phenols, saponins, tannins and lipids. AECA (100, 200 and 400 mg/kg) dose-dependently prevented ulcer formation by HCl/ethanol (11.13%, 23.51% and 55.26% of inhibition), indomethacin/HCl-ethanol (14.78% and 41.20% for 200 mg/kg and 400 mg/kg, respectively), indomethacin (10.39%, 40.26 and 87.01%) and pylorus ligature (2.25%, 28.60% and 40.99%). The inhibitory effect of the extract against HCl/ethanol induced ulcer was decreased by the pre-treatment with indomethacin (30 mg/kg, *i.p*.). ECO reduced Shay-ligated gastric acid secretion from 81.80±02.39 mEq/l in the controls to 56.00±06.49, 53.70±04.76 and 42.75±07.49 mEq/l for the extract doses 100, 200 and 400 mg/kg, respectively. AECA is bactericidal on all strains tested with MIC = 250 µg / ml and MBC / MIC ratio = 2. The prophylactic action of AECA was associated with significant increases in gastric mucus production. The levels of SOD were improved and the levels of MDA were decreased in rats treated with the extract.

Conclusion: The antiulcer activity of AECA was attributed to its ability to reduce acid secretion, to enhance mucosal defense, to improve *in vivo* antioxidant status and for its antibacterial properties.

Keywords: Cassia arareh; *gastric ulcer; Caesalpiniaceae; antioxidant; antibacterial activity.*

1. INTRODUCTION

Plants offer an alternative source to treat many diseases because they contain a range of bioactive chemicals, many of which are selective and have little or no harmful effect on non-target organisms and the environment [1]. The plant *Cassia arereh* Del. is a wild small tree, 2–5 m high that belongs to the family of Caesalpiniaceae. In Nigeria, it is locally called Marga, Maleduwa, Mihuski or Dandarazo in Hausa; Cabbi or Jutihi in Fulfulde; Mihuski in Gwari; kurnggilang in Babur-Bura and Maraguwa in Kare-kare languages [2]. Also, in the area of north Cameroon, the populations gave the names which are in connection with its uses: "*Binlay*" in Toupouri, referring to its employment to restore the fertility among women; "*Saheri*" in Fulfulde, for its anti-ulcerous effect and relieving congestion [3].

Almost all parts of the plant are used locally as medicine. The root and the stem-bark are used to treat disease conditions such as diarrhoea, dysentery, stomach ache, ascites, headache, cough, rheumatism, back pain, wound healing, weakness, avian plague, yellow fever and malaria. The fruit pulp is used as laxative while the leaves are used as diuretic, antipyretic, analgesic and in the treatment of pleurisy and burns. The seed is used for treatment of pneumonia and for magico-religious purposes [4]. The plant is reported to show different biological activities such as antimicrobial, *in vitro* antitrypanosomial, larvicidal, antiplasmodial, and cytotoxic properties. The phytochemical screening of the root and stem bark of *Cassia arereh* revealed that the root contains alkaloids, flavonoids, phlobatanins, saponins, steroids while the stem back contains alkaloids, anthraquinones, saponins, steroids and tannins [5]. Many studies have shown that natural antioxidants can reduce Deoxyribonucleic acid (DNA) damage, mutagenesis, carcinogenesis, and inhibit growth of pathogenic bacteria [6].

The role of free radicals and active oxygen is becoming increasingly recognized in the pathogenesis of the many human diseases, including cancer, aging and gastric ulcer [7]. Free

radicals can also cause lipid peroxidation in foods that leads to their deterioration. There is no information in literature about the antioxidant activity of *Cassia arereh in vivo*. So, the determination of natural sources of antioxidants and the antioxidant potential of plants is important. This study investigated the cytoprotectives, antioxydant and antibacterial effects of the aqueous root bark extract of *Cassia arereh.*

2. MATERIALS AND METHODS

2.1 Plant Collection, Identification and Extract Preparation

Fresh root-bark were collected from Poli locality in Faro Division (North Cameroon region), in august 2015 and identified botanically at the Cameroon National Herbarium by comparison with existing voucher specimen (No. 39932 /HNC) by Fulbert TADJOUTEU. The root barks of *C. arereh* were cut into small pieces using a sharp knife and then dried in the laboratory under room temperature. The dried samples were then crushed. 500 g of powdered plant were dissolved in 7000 ml of distilled water boiled for 15 minutes. After filtration through Whatman filter paper n° 2, the filtrate was lyophilized. The extract (powder) obtained (24.6 g, 4.92% yield) was stored at room temperature for subsequent experiments

2.2 Animals

Male Wistar rats (175 ± 25 g; 12 ± 1 weeks) were used for the experiments. The animals were raised in the animal house of the Higher Teachers' Training College, University of Yaounde 1. They were fed a standard laboratory diet: Protein 44%, fat 13%, cellulose 09%, mineral matter 12%, moisture 22% (NAAPCAM SARL, P.O.Box: 4113 Mvog Ada-Yaoundé, Cameroon) and given fresh water *ad libitum*. Prior authorization for the use of laboratory animals in this study has been obtained from Cameroon National Ethics Committee (Reg. N. FWA-IRB 00001954). The use, handling and care of animals were done in adherence to the European convention for the protection of vertebrate animals used for experimental and other purposes.

2.3 Phytochemical Tests

The aqueous root bark extract of *Cassia arereh* Del was subjected to qualitative chemical test for the identification of different phytoconstituents such as tannins, triterpenes, sterols, flavonoïds, alkaloids, saponins, phenols and lipids [8,9].

2.4 Anti-ulcer Test

2.4.1 HCl/ethanol-induced gastric lesions

Twenty five male rats fasted for 48 h before administration of extract. The HCl/ethanol solution was used to induce ulcers in the gastric mucosa according to the method of Hara and Okabe in 1985 [10]. The animals received the plant extract (100, 200 and 400 mg/kg) by oral route, one hour before they were given the necrotizing solution. Positive and negative control rats received, respectively sucralfate (Ulcar 1g, Lot 1045, Sanofiaventis, France) (100 mg/kg) and distilled water (1 ml) in place of the extract. One hour after, animals were sacrificed using ether, the abdomen of the animals was opened and the stomach was removed. The ulcers produced in the glandular region of each stomach were measured and scored as earlier described by Tan *et al* [11] and the ulcer index (UI), percentage of inhibition (% I) and percentage of ulcerated surface (%US) were calculated.

2.4.2 HCl/ethanol-induced gastric lesions in rats pre-treated with indomethacin

The effect of pre-treatment with indomethacin on the preventive effect of the extract on HCl/ethanol-induced gastric lesions was studied following the method described by Sun *et al* [12]. All rats received indomethacin (30 mg/kg) by intra-peritoneal route. 1h later, the test rats received the plant extract (100, 200 and 400 mg/kg) while the positive and negative control rats received sucralfate (100 mg/kg) and distilled water (1ml) respectively by oral route. An hour later, all the animals were given orally 1 ml of HCl/ethanol solution. The rats were then sacrificed after 1h using ether and the stomach examined for gastric lesions.

2.4.3 Indomethacin-induced gastric lesions

Gastric mucosal lesions were induced by the method describe by Pillai and Santhakumari [13]. The test rats were administered the plant extract (100, 200 and 400mg/kg) *per os* while the negative control and normal rats received distilled water (1ml). Those of 5th group (positive control) received by oral route 60 mg/kg of

omeprazole (Prazol 20 mg, Lot B2159-2, bottus.a, Casablanca, Maroc) (a reference drug). 1h later, all the animals, except normal rats, received the indomethacin solution, 50 mg/kg (Indocid 25 mg, Lot 3400930525692, HAC Pharma, 1400 Caen, France) by oral route. After 4h, under light ether anesthesia, the abdomen of each rat was opened and the stomach removed. The ulcers produced in the glandular region of each stomach were measured. Scores were attributed to the different ulcerated surfaces based on the scale proposed by par Martin et al. [14]. A sample of each stomach was cut and stored frozen for the subsequent antioxidant tests.

2.4.4 Pylorus-ligated gastric secretion and ulceration

The method of Shay et al. [15] was used to study the ability of extract to reduce gastric acid secretion as well as prevent gastric ulceration resulting from auto digestion by stomach secretions. The test rats received the plant extract (100, 200 and 400 mg/kg), controls received the distilled water (1 ml) and those of the 5th group received omeprazole (60 mg/kg) by oral route one hour before the experiment. The pylorus of each rat was tied under light ether anesthesia and the abdominal incisions were closed. The rats were sacrificed six hours later and the gastric juice produced by each was collected, centrifuged (6000 r/min) and the volume measured. Ulcers produced in the glandular region of the stomachs were measured and scored: score 0 (no ulcer); score 1 (dilation of vessels and small dots of ulcer); score 2.5 (ulcer ≤ 4 mm long) and score 5 (ulcer ≥ 5 mm long).

2.4.4.1 Measurement of gastric acidity

Samples of gastric contents (1ml) were analyzed for hydrogen ion concentration by pH-metric titration with 0.1N NaOH solution using a digital pH-meter. The acid content was expressed as mEq/l.

2.4.4.2 Measurement of mucus production

The mucus covering of each stomach was gently scraped using a glass slide and the mucus was weighed carefully using a sensitive digital electronic balance. The same researcher performed this exercise each time.

2.5 Measurement of *in vivo* Anti-oxidant Capacity

Superoxide dismutase (SOD) activity was measured using a standard method [16] and expressed in U/mg of protein, while catalase was determined and expressed as mM of H_2O_2/min/mg of protein [17]. Tissue protein was measured using the Biuret method of protein assay. Lipid peroxidation was assessed by measuring the levels of malondialdehyde (MDA) [18]. Quantification of MDA was done using an extinction coefficient of $\varepsilon = 1{,}56 \times 10^5\ cm^2.mmol^{-1}$. Tissue protein was measured using the Biuret method of protein assay [19].

2.6 Antibacterial Test

2.6.1 Source of microorganisms

The organisms were collected from Yaoundé Molecular Research Center. The bacterial species include *Enterobacter aerogene, Escherichia coli, Klebsiella pneumoniae, Enterobacter cloacea* and *Providencia stuartii*.

2.6.2 Preparation of agar medium

7.6 g of nutrient agar and 13.65 g of nutrient broth were added to 200 ml of distilled water in a 650 ml sterilized conical flask. The suspension was heated to dissolve the nutrient agar and broth. After complete dissolution of the media, the mouth of the conical flask was closed tightly with aluminum foil. The media were then sterilized using autoclave at 121℃ for 30 and 15 minutes respectively.

2.6.3 Preparation of stock

The stock solution of extract was prepared by dissolving 18.0 mg of crude extracts in 225 µl of DMSO to give a concentration of 80000 µg/ml and was diluted in the MHB in order to obtain a concentration of 8000 µg/ml. Concentration of 2000 µg/ml of Ciprofloxacine was prepared in sterile distilled water and was used fresh as the standard antibiotic.

2.6.4 Determination of antibacterial activity using microdilution method

To determine MIC and CMB of the extract, the microdilution method described by Newton et al. [20] was used. Using a micropipette, 100 µl of culture broth were introduced into each of the 96 wells of the microdilution plate. 100 µl of extract

solution were added to the first wells of each column, successive dilutions varying according to a progression of factor 2 were carried out. The same procedure was used for the reference antibiotic (Ciprofloxacin). Some wells containing the strain and culture medium served as a negative control. In each well, there was a final volume of 200 µl with a final inoculum concentration of 1 × 10 6 CFU / ml. The coated plates were placed in the incubator (New Brunswick Scientific) for 18 hours. MIC values were determined. To determine the MBC, 100 µl of the contents of the wells in which the bacterial strains were actively inhibited were pipetted and introduced into wells of a new plate containing 100 µl of MHB per well and then reincubated for 18 h. In wells with a low MIC, 20 µl of rezasurine was introduced and reincubated for 5 h.

2.7 Statistical Analysis

Values in tables are given as arithmetic means ± standard error of the mean (SEM). The significance of differences between groups was analyzed by means of Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison tests using Graph Pad In Stat. P values less than 0.05 were considered as significant.

3. RESULTS

3.1 Phytochemical Characteristics of Extract

Preliminary phytochemical screening: The result of preliminary phytochemical analysis shows that the aqueous extract possess tannins, triterpenes, sterols, flavonoids, alkaloids, saponins phenols and lipids (Table 1).

3.2 HCl/EtOH- induced Gastric Lesions

The effects of aqueous extract of *C. arareh* on gastric lesion induced in albino rats are shown in table 2. HCl/ethanol solution was administered orally to control induced gastric ulcers. Pre-treatment of rats with the aqueous extract at doses employed (100, 200 and 400 mg/kg) produced 11.13%, 23.51% and 55.26% protection against gastric mucosal damage, while the standard drug, sucralfate 100 mg/kg, exhibited 56.70% protection under the same condition. The plant source provides increased mucosal resistance and regulate mucosal repair (Table 2).

3.3 HCl/ethanol-induced Gastric Lesions in Rats Pre-treated with Indomethacin

The effect of pre-treatment with indomethacin on the protective effect of the aqueous extract of *C. arareh* against HCl/ethanol-induced lesions is shown in Table 3. This procedure had the effect of reducing the protective effect of the extract at the dose of 200 and 400 mg/kg. Thus, the prevention of lesion formation reduced from 55.26% to 41.20% at 400 mg/kg extract due to indomethacin pre-treatment (Table 3). This was accompanied by a reduction in mucus production for all treated groups.

3.4 Indomethacin - Induced Gastric Lesions

The root bark aqueous extract of *Cassia arereh* (100, 200 and 400 mg/kg) dose-dependently offered significant cytoprotection (10.39%, 40.26% and 83.82% inhibition) to the stomach mucosa of rats against the indomethacin solution compared with the controls. The mean ulcer index score was reduced from 3.85±0.18 in controls to 0.50±0.50 for the rats receiving 400 mg/kg of extract. The ulcerated area was reduced from 30.40 mm^2 in controls to 0.20 mm^2 for the rats receiving 400 mg/kg of extract. This significant, dose-dependent, reduction of ulcerated area was accompanied by a significant ($p < 0.05$) increase of mucus production in *C. arereh* extract treated rats (Table 3).

3.5 Pylorus-ligated Induced Gastric Lesions

Table 5 shows the effect of *C. arareh* on pH, volume of gastric fluid, gastric acidity, mucus content, ulcer index, % ulcerated surface and % Inhibition. Ulcer-induced rats pretreated with *C. arareh* showed significant decrease in gastric acidity when compared to that of ulcerogen-treated control animals. The animals pretreated with the test drug showed a significant reduction in pH and gastric acidity when compared to the control. The mucus content was found to be increase significantly in ulcer-induced control animals.

3.6 *In vivo* Antioxidant Effect of *C. arareh* Extract

In order to explore the effects of antioxidant defenses on the process of ulceration, in all stomach tissues, the antioxidant levels were

evaluated. The level of superoxide dismutase (SOD) and malondialdehyde (MDA) were evaluated in normal, ulcer control and drug treated groups. Stress produced depletion of SOD and increased the level of MDA in control group compared with the normal rats. In drug treated groups, pretreatment with *C. arereh* significantly decreased in a dose-dependent manner the levels of MDA, and increased the level of SOD in gastric tissue compared with the control group (Tables 6).

3.7 Antibacterial Tests

The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) values of *C. arareh* extract against tested microbes : *Enterobacter aerogen* (CM64), *Enterobacter cloacae* (BM64), *Escherichia coli* (ATCC8739), *Providencia stuartii* (PS 299645), *Klebsiella pneumoniae* (ATCC 11296) were 250 µg/ml and 500 µg/ml, respectively. The CMB/CMI ratio is equal to 2 (Table 7).

Table 1. Qualitative phytochemical composition of the aqueous root bark extract of *C. arareh*

Triterpenes	Sterols	Flavonoids	Alkaloids	Phenols	Saponins	Tannins	Lipids
+	+	+	+	+	+	+	+

+: presence

Table 2. Effect of *C. arareh* extract on HCl/ethanol-induced gastric lesions in rats

Treatment	Dose (mg/kg)	N	Mucus production (mg)	Ulcerated area (mm^2)	Ulcer index	% ulcerated surface	Inhibition (%)
Control	-	5	71.55 ± 6.90	94.50 ±11.07	4.85 ± 0.43	14.00	-
C. arareh	100	5	100.86 ±2.75**	57.00 ±10.13	4.31 ± 0.22	8.44	11.13
C. *arareh*	200	5	106.26 ±5.44**	35.90 ± 8.13	3.71 ± 0.40	5.32	23.51
C. *arareh*	400	5	137.12±13.49***	23.15 ±10.24*	2.17 ± 0.82*	3.43	55.26
Sucralfate	100	5	98.43 ±7.14	9.60 ± 4.52*	2.10 ± 0.60**	1.42	56.70

*Statistically different relative to control; *P=0.05; **P=0.01; ***P=0.001; N, number of rats. The values are expressed as mean ± SEM*

Table 3. Effect of *C. arareh* extract on HCl/ethanol-induced gastric lesions in rats pre-treated with indomethacin

Treatment	Dose (mg/kg)	N	Mucus production (mg)	Ulcerated area (mm^2)	Ulcer index	% ulcerated surface	Inhibition (%)
Control	-	5	54.87 ± 7.45	114.50 ± 11.27	6.02 ± 1.01	16.89	-
C. arareh	200	5	64.84 ± 6.81	66.80 ±13.80**	5.13 ± 0.31	9.90	14.78
C. *arareh*	400	5	83.49±14.44**	54.40 ±11.11**	3.54 ±0.15**	8.06	41.20
Sucralfate	100	5	75.62 ± 7.45*	48.00 ±13.07**	3.72 ± 0.46*	7.11	38.21

*Statistically different relative to control; *P=0.05; **P=0.01; N, number of rats. The values are expressed as mean ± SEM*

Table 4. Effect of *C. arareh* extract on indomethacin induced gastric lesions in rats

Treatment	Dose (mg/kg)	N	Mucus production (mg)	Ulcerated area (mm^2)	Ulcer index	% ulcerated surface	Inhibition (%)
Control	-	5	35.68 ± 5.93	30.40 ± 5.27	3.85 ± 0.18	4.50	-
C. arareh	100	5	49.83 ± 8.59	15.40 ± 3.72*	3.45 ± 0.53	2.28	10.39
C. *arareh*	200	5	59.32 ± 3.99*	2.10 ±1.36***	2.30 ± 0.60	0.32	40.26
C. *arareh*	400	5	76.41 ±4.56**	0.20 ±0.20***	0.50 ± 0.50***	0.03	87.01
Omeprazol	60	5	42.94 ± 4.96	0.40 ±0.29***	0.95 ± 0.42***	0.06	75.32

*Statistically different relative to control; *P=0.05; ***P=0.001; N, number of rats. The values are expressed as mean ± SEM*

Table 5. Effect of *C. arareh* extract on pylorus-ligated gastric ulceration in rats

Treatment	Dose (mg/kg)	N	mucus (mg)	Ulcerated area (mm2)	% ulcerated surface	% Inhibition	Volume of gastric juice (ml)	pH	Acidity of gastric juice (mEq/l)
Control	-	5	101.13±19.78	21.30±4.85	3.16	-	3.21±0.61	2.64±0.15	81.80±02.39
C. arareh	100	5	131.53±5.94	20.15±8.93	2.99	2.25	3.40±0.73	3.76±0.34	56.60±06.49
C. arareh	200	5	139.38±2.11*	8.40±2.90***	1.24	28.60	3.56±0.44	3.52±0.16	53.70±04.76*
C. arareh	400	5	160.28±4.21***	6.50±2.15***	0.96	40.99	3.34±0.73	4.03±0.58	42.75±07.49**
Omeprazol	60	5	118.66±29.57	2.10±1.55***	0.31	66.22	2.66±0.57	5.17±0.40**	32.50±11.59***

*Statistically different relative to control; *P=0.05; **P=0.01; ***P=0.001; N, number of rats.*

Table 6. Effect of *C. arareh* extract on SOD, MDA and total Protein in gastric ulcerate rats

Treatment	Dose (mg/kg)	N	Total protein (g/L)	MDA (nmol/g of tissue)	SOD (U/mg of protein)
Normal rats	-	5	19.95 ± 1.22	0.95 ± 0.11	63.74 ± 3.94
Control	-	5	28.20 ± 1.92	1.32 ± 0.08	45.23 ± 3.00
C. arereh	100	5	22.27 ± 2.04	0.88 ± 0.02 *	58.51 ± 6.23
C. arereh	200	5	21.05 ± 3.01	0.78 ± 0.10 **	63.63 ± 7.46
C. arereh	400	5	20.10 ± 1.88	0.56 ± 0.08 ***	64.40 ± 5.58
Omeprazol	60	5	20.79 ± 1.03	0.68 ± 0.02 ***	60.82 ± 3.03

*Statistically different relative to control; *P=0.05; **P=0.01; ***P=0.001; N, number of rats =5. The values are expressed as mean ± SEM*

Table 7. Inhibitory effect of *C. arareh* extract against CM64, BM64, ATCC8739, PS 299645, ATCC 11296

Parameters	Treatment	CM64	BM64	ATCC8739	PS 299645	ATCC 11296
MIC	*C. arereh*	250	250	250	250	250
MIC	Cyprofloxacine	0.37	<0.078	0.15	<0.078	0.37
MBC	*C. arereh*	500	500	500	500	500
MBC/MIC	*C. arereh*	2	2	2	2	2

Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC)

4. DISCUSSION

The results of phytochemical analysis show that aqueous root bark extract of *Cassia arereh* contains tannins, triterpenes, sterols, flavonoids, alkaloids, saponins, phenols and lipids (Table 1). These results confirm the richness of this extract in bioactive compounds because all the phytochemical tests carried out proved positive. In 2012, the phytochemical screening of *Cassia arereh* revealed that the root contains alkaloids, flavonoids, phlobatanins, saponins, steroids and while the stem bark contains alkaloids, anthraquinones, saponins, steroids and tannins [5]. These observed differences attributed to different plant parts, harvesting sites, harvest period and method of preparation of the extract.

Hydrochloric acid and alcohol are among the rare elements that the stomach absorbs. H^+ ions are potent inducers of gastric ulcers because they pass through the mucous membrane cells and reach the deep layers of the stomach [21]. The presence of alcohol causes stimulation of the pneumogastric which leads to an increase in the production of acetylcholine and also stimulates the secretion of gastrin by the G cells. The acetylcholine acts on the parietal and main cells while gastrin stimulates the production of histamine which causes the increase in gastric acid secretion [22]. In addition, alcohol has a triple inducing action of ulcers: it erodes the gastric mucosa, causes its congestion and cell necrosis [22,23]. H^+ ions and ethanol are potent inducers of ulcers, the HCl / Ethanol mixture significantly causes the induction of haemorrhagic and necrotic lesions on the gastric mucosa. Compared to the negative control batch, the aqueous extract of *Cassia arereh* inhibited the induction of gastric lesions in a dose-dependent manner with a significant decrease (P = .05) at 400 mg/kg, corresponding to a percentage inhibition of 55.26% (Table 2). Sucralfate (100 mg/kg) exhibited a slightly higher inhibition percentage (56.70%) than that of the extract. These results suggest that the aqueous root bark extract of *Cassia arereh* offer protection of the gastric mucosa against irritants which have a cytoprotective potential [24]. The aqueous extract of the *C. arereh* bark induced a significant (P =.05) increase in mucus secretion at the 400 mg/kg dose (137.12 ± 13.49 mg) compared to the negative control batch (71.55 ± 6.90 mg). The quantity of secreted mucus increased proportionally with the dose of the extract administered either by direct action on mucus cells or through endogenous prostaglandins.

Indomethacin-pretreated rats (30 mg/kg *ip*) had higher ulcer areas and ulcer indices than those receiving only the HCl/EtOH solution (Table 3). Indeed, indomethacin is a Non-Steroidal Anti-Inflammatory (NSAID) that inhibits the secretion of endogenous prostaglandins. Prostaglandins have the role of protecting the stomach against lesions by stimulating the secretion of bicarbonates and mucus, maintaining gastric microcirculation and regulating the repair of the stomach mucosa [25,26]. The inhibitory action of indomethacin exposes the gastric mucosa thus allowing the acid and alcohol to severely attack the gastric wall and generate ulcers. This explains why the lesions observed on the mucous membranes of rats subjected to indomethacin pretreatment are more violent than those of rats not pretreated with indomethacin (Table 3). A dose - dependent decrease was observed in the ulcerated surface and in the ulcer index compared to the negative control. A dose-dependent increase in mucus secretion was also observed in comparison to the negative control. *C. arereh* would not have a cytoprotective effect *via* the prostaglandin pathways since the extract would therefore contain one or more substances which would directly stimulate the secretion of the mucus without passing through the prostaglandin pathway. The aqueous root bark extract of *Cassia arereh* showed the presence of tannins and saponins [5]. The protective activity of saponins is due to the fact that they stimulate the mucus production factors in the mucosa [27]. It has also been shown that tannins can prevent the development of ulcer. They cause precipitation of proteins at the site of the ulcer. This precipitation of proteins (microprotein) forms an impermeable layer on the site of the ulcer; which protects the gastric mucosa against irritant substances [28,29]. The extract of *Cassia arereh* leads to an increase in mucus secretion compared to the control group, significant (P =.05) at the dose of 200 mg/kg (139.38 ± 2.11 mg) and highly significant (P = .001) at 400 mg/kg (160.28 ± 4.21 mg) (Table 3). This indicates that the *C. arereh* extract has cytoprotective activity by reinforcing the mucobicarbon barrier.

Pyloric ligation is one of the most widely used methods for studying the effects of drugs on gastric secretion. The ligation of the pylorus causes an accumulation of gastric acid in the

stomach. This leads to a decrease in gastric pH and activation of the pepsinogens. These events lead to a self-digestion of the gastric mucosa, exposing the underlying layers to the action of pepsin [15]. Omeprazole is an inhibitor of the proton pump of gastric parietal cells, its action leads to the reduction of acid secretion independently of the source of stimulation [30]. The aqueous extract of the *Cassia arereh* caused a significant decrease in the acidity of the gastric juice at a dose of 400 mg/kg (42.75 ± 07.49 mEq/L) compared to the control group (81.80 ± 02.39 mEq/L) (Table 5). This result suggests that the aqueous extract of *C. arereh* is either an antacid or an antisecretory. The solution of the aqueous extract of the *C. arereh* (100 mg/ml) used in the handling showed a pH of 6.98. This solution is a weak acid and can't directly neutralize the H^+ protons present in the gastric juice. According to this result, the aqueous extract of *C. arereh* would not be an antacid; it would rather exercise an anti-secretory activity.

In addition, phytochemical analysis of the aqueous root bark extract of *C. arereh* showed the presence of flavonoids (Table 1). It has been suggested that flavonoids protect the gastric mucosa against lesions. These phytochemicals reduce the secretion of histamine by ECL cells by inhibiting the activity of histidine decarboxylase, enzyme that catalyzes the conversion of histidine to histamine [31].

Induction method of gastric lesions by oral administration of indomethacin is rapid and reliable in the evaluation of the antiulcer properties of medicinal plant extracts [13]. The oral administration of indomethacin causes inhibition of endogenous prostaglandins secretion, which results in the reduction of gastric blood flow; decreased bicarbonate ions secretion; mucus and surface phospholipids [32,33]. In addition, indomethacin inactivates gastric peroxidases thereby inducing tissue necrosis by reactive oxygen species [34]. Indomethacin-induced oxidative stress is manifested by an increase in the lipid peroxidation of cell membranes and a decrease in thiol groups which react easily with oxygenated reactive species (EOA). Indomethacin therefore causes an increase in free radical hydroxides (OH-) and inactivation of gastric mucosal peroxidase; this causes an increase in endogenous H_2O_2 as well as its derivatives [34]. Indomethacin also reduces the activity of antioxidant enzymes such as catalase, SOD and glutathione S-transferase [35].

Omeprazole is a gastric anti-secretion which acts by inhibiting the enzymatic activity of H^+/K^+ ATPase in the gastric parietal cell, possibly reducing the secretion of gastric acid. Its antisecretory activity is powerful and prolonged [36]. Omeprazole has direct or indirect antioxidant activity by interacting with the hypochlorous acid which is the most abundant and toxic of the oxidants generated by the phagocytes. However, Omeprazole is a scavenger of the hydroxyl radicals but not of the superoxide anion [36,37].

Administration of the aqueous root bark extract of the *Cassia arereh* at a dose of 400 mg / kg induced a dose-dependent increase (P = .001) in mucus secretion (76.31 ± 4.56 mg) compared to the control group (35.68 ± 5.27 mg) (Table 4). The aqueous extract of *Cassia arereh* stimulates the secretion of the gastric mucus by direct action on the cells mucus. Non-steroidal anti-inflammatory drugs (NSAIDs) such as indomethacin inhibit cyclooxygenase. This results in an inhibition of prostaglandin synthesis. This is demonstrated by the very significant reduction (P =.001) dose-dependent on the ulcer index at the 400 mg/kg dose (0.5 ± 0.5) (Table 4). This reinforcement of the mucobicarbon barrier by *Cassia arereh* could be explained by the richness of this extract in some phytoconstituents such as tannins. Tannins stimulate the secretion of gastric mucus [38,39].

Oxidative stress plays a very important role in the pathogenesis of several diseases, including gastric ulcers. The oxidative stress induced by oral administration of indomethacin is shown by an increase in lipid peroxidation in the cell membranes, a decrease in the thiol groups and a reduction in the activity of the antioxidant enzymes such as catalase, SOD and Glutathione S-transferase [35]. Administration of the aqueous roots bark extract of *Cassia arereh* induced a reduction in lipid peroxidation in the cell membranes. The *Cassia arereh* aqueous extract (200 mg/kg and 400 mg/kg) resulted in a significant (P = .001) dose-dependent decrease in MDA concentration in stomach tissue, and significant (P = .05) at the dose of 100 mg/kg (Table 6). This significant reduction in the concentration of MDA tissue shows that the extract significantly reduced the oxidative stress induced by indomethacin. Indeed, the antioxidant activity of the extract could be explained by the

presence of phytoconstituents having antioxidant properties. Phenolic compounds act as antioxidants by two mechanisms involving antiradical activity and metal chelation. Most true antioxidants are phenolic in nature. They have an ideal chemical structure for this antiradical activity. Their efficiency is explained by the delocalization of the single electron from oxygen with the aromatic nucleus. Polyphenols exhibit a broad spectrum of biological effects derived from their antioxidant properties [40]. The phytochemistry of the plant revealed the presence of flavonoïds which are powerful antioxidants capable of trapping the reactive oxygen species [41]. Flavonoïds are a group of polyphenols that are widely distributed in the world of plants. They deactivate singlet oxygen and inhibit several enzymes, including lipoxygenase [42]. The aqueous root bark extract of *Cassia arereh* resulted in a non-significant increase in SOD and a significant decrease in MDA. The aqueous extract of *Cassia arereh* would thus inhibit lipid peroxidation and trap free radicals resulting from oxidative stress (Table 6).

Phytochemical screening of the aqueous *Cassa arereh* extract indicated the presence of secondary metabolites such as triterpenes, flavonoids, alkaloids, phenols, saponins, tannins and lipids (Table 1). The flavonoids, alkaloids, phenols and saponins present in this extract are responsible for antibacterial activity on a wide range of strains [43]. The tannins present in the *Cassia arereh* extract possess an antimicrobial activity which is due to their ability to complex to the transport proteins [44]. Flavonoids have free radical scavenging actions and possess antimicrobial activity [45]. Previous studies show that organic and aqueous stem bark extracts of *Cassia arereh* have antibacterial activities [46]. On the other hand, the work of De and Kundu shows that the aqueous extracts of the root and stem barks of *C. arereh* did not produce any measurable antimicrobial activity against all the tested microorganisms [47]. It should be noted that the antibacterial activity of the extracts depends on the bioactive molecules present in the plant, the extraction mode, the type of solvent, the concentration of active ingredient and the bacterial strains tested [48,49].

Analysis of the results of the antibacterial activity of the aqueous extract revealed that all the five strains tested, namely CM64; BM64; ATCC8736; Ps 299645 and ATCC 11296 were very sensitive to the aqueous extract of *Cassia arereh*. This extract showed a stable and low MIC (250 µg / ml) on all the bacterial strains tested (Table 7). According to the classification of Aligianis et al. [50] any substance with a MIC less than 500 mg/ml has a strong bacterial inhibition. The extract of *C. arereh* would therefore be capable of inhibiting bacteria. The extract has a MIC greater than that of the reference antibiotic (Ciprofloxacin), so the sensitivity of these strains to this extract is lower than that of Ciprofloxacin. This may be explained by the low concentration of active compound/s is found in the extract since extract is a mixture of compounds and Ciprofloxacin is a pure compound.

When the CMB of an antibiotic on a strain is close to MIC (CMB / MIC = 1 or 2), this antibiotic has bactericidal activity. On the other hand, if the MIC and CMB have relatively distant values (4 < MBC / MIC < 16), the antibiotic is considered to be bacteriostatic. Finally, if MBC / CMI > 32, it's the case of "tolerance" of the microbial strain [51,52]. The aqueous extract of Cassia arereh is therefore bactericidal on all strains tested (CM64, BM64, ATCC8736, Ps 299645 and ATCC112960) because the ratio MBC / CMI = 2.

5. CONCLUSION

The present study shows that the aqueous root bark extract of *Cassia arereh* has gastro-cytoprotective, anti-secretory, antioxidant and bactericidal properties. These results would justify the use of the barks of *Cassia arereh* in the traditional treatment of gastroduodenal ulcers.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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