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Full Length Research Paper

Phytonutrients content and biological activities of fruits of *Diospyros mespiliformis* Hoschst ex A.DC (Ebenaceae) used in the treatment of gastroenteritis and gastroduodenal ulcer in Burkina Faso

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Abstract

Digestive disorders occupy the second place of the major health problems in Burkina Faso. The fruits of *Diospyros mespiliformis* are used in recipes to treat gastroenteritis and gastroduodenal ulcer. Phytonutrients content was evaluated by specific reagents and biological activities by antiradical and antibacterial test. The fruit seed of *Diospyros mespiliformis* had the best antioxidant activity (IC_{50} = 0.04 mg/mL) due to its content of total phenolic compounds (5.75 ± 0.6 mg GAE/g), flavonoids (0.78 ± 0.27 mg RE/g), hydrolyzable and condensed tannins (3.56 ± 0.03 mg TAE/g; 1.28 ± 0.42 mg CE/g) compared to the pulp whose content were 3.36 ± 0.12 mg GAE/g ; 0.46 ± 0.26 mg RE/g; 1.78 ± 0.35 mg TAE/g and 0.56 ± 0.11 mg CE/g respectively for total phenolic, flavonoids, hydrolysable and condensed tannins. The fruit seed had the best content of macronutrients (Na, K, Mg) and showed important antibacterial activity against *E. coli*. The fruit pulp had the best content of vitamin C, β-carotene and vitamin E, being 22.0 ± 0.01mg/100g; 0.007 ± 0.03 mg/100g and 0.119 ± 0.02 mg/100g respectively. The results constitute scientific prerequisites for the development of new functional foods and phytomedicines in the management of digestive disorders.

Key words: Digestive diseases, medicinal plant, radical scavenging, mineral elements, nutraceutical.

INTRODUCTION

Gastroenteritis also called gastro, is an inflammation of the wall of stomach and intestine, which can be caused either by bacteria (*Salmonella, Shigella, E. coli, Vibrio cholera*, etc.) or by a parasite (*Giardia, Cryptosporidium, amoebas*...) or by a virus. This disease affects both adults and children. Indeed, at least 800 000 people die each year from gastroenteritis in the world, including 500 000 children under the age of five (Markkula et al., 2017; Troeger et al., 2018). It is transmitted mainly by dirty hands, water and food and is manifested by acute diarrhea of sudden onset which maybe accompanied by nausea and / or vomiting, abdominal pain (cramps), or moderate fever. Gastric or duodenal ulcers, on the other hand,

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are sores that form on the inner lining of the stomach or duodenum. One of the causes of ulcer is the increase and accumulation of acid in the stomach conducive to the development of an aggressive bacterium responsible for the formation of gastric ulcers, *Helicobacter pylori* (Lynch et al., 2019). Other factors such as excessive consumption of alcohol, tea, coffee, spices or certain medication ssuch as non-steroidal anti-inflammatory drugs or even stress can cause an ulcer or make it worse (Delerue et al., 2020). Gastroscopy is the fundamental examination in the diagnosis of stomach or duodenal ulcer in health facilities. In Burkina Faso, digestive disorders rank second among major health problems after infections and infestations and gastric or duodenal ulcer perforations were estimated at 46.12% (Kambire et al., 2018; Ramde-Tiendrebeogo et al., 2019b). If gastrointestinal bleeding is reported as the most encountered complication of gastroduodenal ulcer disease, peritonitis by perforation is also common in sub-Saharan Africa (Sambo et al., 2017). Other complications such as stenosis or shrinkage of part of the stomach and cancerization can occur (Fougere, 2019). Drug treatment uses antacids to reduce acidity in the stomach, along with antibiotics to clear the *Helicobacter pylori* infection. Unfortunately, the treatment of certain diseases, including those of food origin, is becoming more and more difficult due to the phenomenon of antibiotic resistance, which constitutes one of the most serious threats to global health and food security. Previous works showed that plant-based diets and plant-rich diets could reduce mortality from diet-related diseases by 6% to 10% (Springmann et al., 2016). Indeed, some food plants contain active ingredients endowed with various medicinal properties that can intervene in the prevention and treatment of many diseases. They are rich in mineral elements, vitamins, carbohydrates, lipids, proteins, fibers, resin and gum which allow them to ensure good health and prevent various chronic diseases (Nahar et al., 2020; Ramde-Tiendrebeogo et al., 2019a). In Burkina Faso, the fruit pulp of *Diospyros mespiliformis* called Gaakain local Moore language is used in the diet of populations and its seed for the treatment of gastroenteritis and gastroduodenal ulcer in traditional medicine. However, there are not enough reliable data that scientifically explain the traditional use of the different parts of fruits of *Diospyros mespiliformis* in the treatment of digestive diseases. It is therefore important to promote alternative and local therapy based on food plants claiming virtues for health. The objective of this study was to compare phytonutrient content, antioxidant and antibacterial activity of the pulp and seed of *Diospyros mespiliformis* in order to provide scientific prerequisites for the development of new functional foods and phytomedicines from local plant in the management of digestive diseases.

MATERIAL AND METHODS

Material

Plant material

The fruits of *Diospyros mespiliformis* were collected in December 2020 in the village of Dapelogo, located about 35 km north of the capital Ouagadougou of Burkina Faso (GPS coordinates: Lat : 12,24078 and Long:-1,72182). After identification by the botany team of Joseph KI-ZERBO University, a specimen was deposited in the herbarium of the Training and Research Unit in Life and Earth Sciences (UFR/SVT) under code 18006. The seed was separated from the pulp by hand. After cleaning them, each part of the fruit was dried separately under laboratory conditions. Then pulverized using a mechanical grinder and a sieve with 2 mm diameter pores. The powders thus obtained were packaged in transparent sachets, labeled and then stored at room temperature. Figure 1 presents the fruits of *Diospyros mespiliformis* and its different parts.

Biological material

It consisted of American Type Culture Collection (ATCC) reference strains of *Escherichia coli* (*E. coli* ATCC 25922) and *Staphylococcus aureus* (*S. aureus* ATCC 43300) obtained from the bacteriology laboratory of the Muraz Center in Bobo Dioulasso, which is a public institute for research on health in Burkina Faso.

Chemical material

The mainchemicals products were: 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma, St Louis, MO, USA), 6-hydroxy 2,5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox) and Folin-Ciocalteu Reagent (Sigma Chemical Company, Steinheim, Germany), NEU Reagent (Natural Products - Poly Ethylene Glycol), NaOH, Na_2CO_3, HPLC grade methanol (VWR, France), Analytical methanol, Dichloromethane, Ethylacetate, Ethanol absolute (Carlo Erba, France). Sulfuricacid, Aluminum trichloride and Aceticacid (Labosi, France), n-Hexane (SDS, France), Phosphoric acid,Gallicacid, Rutin, Tannicacid, Catechin, p-iodonitrotetrazolium (INT) (Sigma-Aldrich, Germany) and $AlCl_3$ (FlukaChemika, Switzerland).

Methods

Extraction and phytochemical screening

5 g of vegetable powder from each part of the fruit (pulp or seed) were extracted by maceration at low temperature (4°C) with 50 mL of dichloromethane for 24 hours. After filtration, a second maceration was carried out on the residual mark for 24 hours using a 70% ethanolic solution, then filtration. The two extracts obtained were concentrated by half under reduced pressure using a rotary evaporator (type BUCHI Heating bath B-490). The dichloromethane extract was used for the research of lipophilic compounds while the hydroalcoholic extract for the research of heterosidic substances. Phytochemical screening of extracts was carried out by following the method on HPTLC plates according to the analytical technique used by Kavit et al. (2013). Plates with aluminum support Silica Gel 60 F254 were used. The spots of extracts were deposited by using the system of Linomat 5 (Camag, Muttez; Switzerland) spray on automated instrument for HPTLC. Eluent system (Ethyl acetate: Formic acid: Water, 80: 10: 10, v/v/v) was used for the migration of flavonoids and phenolic acids.

Eluent system (Hexane: ethyl acetate, 20:4, v/v) was used for the migration of sterols and triterpenes. Another system (Ethyl acetate: Methanol: Water: Trichloromethane, 18: 2, 4: 2, 1: 6, v/v/v/v) was used for the migration of tannins. Flavonoids and phenolic acids were revealed with Neu's reagent in the presence of UV light (366 nm), sterols and triterpenes were revealed by 3% H_2SO_4 in EtOH (96%) and tannins by $FeCl_3$2%.

Determination of total phenolics content

Total phenolics content of each part of the fruit was estimated using the Folin-Ciocalteu Reagent (FCR) as described by Koala et al. (2021).In brief, 1 mL of extract and Gallic acid solution was mixed with 1mL of Folin-Ciocalteu Reagent previously diluted ten times with distilled water. After vortexing, the mixture was incubated for 8 min at room temperature, and 2 mL of 7.5% saturated sodium carbonate solution was added. The combinations were set at 37 °C for 30 min in the dark. The absorbance of the resulting blue color were read at 760 nm with a spectrophotometer (Shimadzu UV-Vis). The phenolic content of plant extracts was determined using the equation of the calibration curve ($y=10.459x + 0.0335$; $R^2 = 0.9993$) and the result was expressed in milligrams of Gallic Acid Equivalents per gram of dry material (mg GAE/g). All determinations were performed in triplicate (n=3)

Determination of flavonoids content

Total flavonoids of the extracts was determined according to the method of Ouédraogo et al. (2018) with slight modifications. In a tube containing 1mL of extract; 2.4 mL of bidistilled water and 0.3 mL of $NaNO_2$ (0.05 g/mL in water) were introduced. After 5 min of incubation, 0.3 mL of $AlCl_3$ was added. Then 6 minutes later, 2mL of NaOH was added. The resulting mixture was incubated at room temperature for 30 min. 2 mL of this mixture were introduced into tanks for reading the absorbance at 510 nm against a blank made up of distilled water. Rutin was used as standard, and the quantification was expressed by reporting the absorbance in the calibration curve of the Rutin ($2.5608x + 0.0034$, $R^2 = 0.9995$). The total flavonoid content was expressed in milligrams of Rutin Equivalents per gram of dry material (mg RE/g). All determinations were performed in triplicate (n=3)

Determination of hydrolyzable tannins content

The hydrolysable tannins content of each part of the fruit was determined according to the method described by Çam and Hışıl (2010) with minor modifications. 5 mL of $NaIO_3$ were introduced into a tube containing 1 mL of extract. The mixture was vortexed for 10 seconds and incubated for 2 min. Then 2 mL of the mixture were introduced into tanks for reading the absorbance at 550 nm against a blank made up of distilled water. The hydrolysable tannins contents was evaluated using a standard calibration curve with Tannic acid as a reference substance ($y = 0.4158x + 0.0235$; $R^2 = 0.9981$). The content was expressed in milligrams of Tannic Acid Equivalents per gram of dry material (mg TAE/g). All determinations were performed in triplicate (n=3)

Determination of condensed tannins content

Condensed tannins content of each part of the fruit was determined according to the method described by Heimler et al. (2006) with minor modifications. In a tube containing 0.4 mL of the extract, 3 mL of the 4% vanillin solution in methanol and 1.5 mL of concentrated HCl were added. The resulting mixture was incubated at 37°C for 20 min. Then, 2 mL of this mixture were introduced into tanks for reading the absorbance at 500 nm. Condensed tannins content was evaluated using a standard calibration curve with Catechin as reference substance ($y = 2.9549x - 0.007$; $R^2 = 0.999$).
The content was expressed in milligrams of Catechin Equivalents per gram of dry material (mg CE/g). All determinations were performed in triplicate (n=3)

Determination off at-soluble vitamins content

The fat-soluble vitamins content was determined according to the method described by Kini et al. (2008). 10 mL of 10% KOH solution in methanol-water (1:1 v/v) was added to 0.5 g of the sample (pulp or seed). The mixture was then brought to reflux in a water bath at 70° C for 30 min. After allowing it to cool, the mixture was extracted with 3 x 5 mL of hexane. The hexane phases were combined and dried over anhydrous sodium sulphate then evaporated to dryness. The residue obtained was taken up in methanol for HPLC analysis. The evaluation of fat-soluble vitamins content was made by HPLC coupled to a UV-Visible detector. The analysis was done in isocratic mode on an Alumina-C18 column. The mobile phase was an acetonitrile/methanol mixture (80:20 v/v) with a flow rate of 1 mL/min. The detection of β-carotene (provitamin A) was done at λ = 455 nm; that of vitamin E at λ = 295 nm.

Determination ofwater-solublevitamin content

The water-soluble vitamin content(vitamin C) was determined according to the method described byNoba et al. (2020). 5 g of the sample (pulp or seed)were extracted with 15 mL of 5% metaphosphoric acid solution for 15 min at room temperature. After filtration, the residue was mixed with 10 mL of 5% metaphosphoric acid solution for two successive extractions. The three filtrates were combined and centrifuged for 10 min at 4,000 g and 5°C. The supernatant was collected and made up to 40 mL and then filtered with a 0.2 mm Advantec filter for HPLC analysis. The mobile phase was acidified with 0.1% phosphoric acid in distilled water (solvent A) and acetonitrile (solvent B), performed at the ratio 25:75. The flow rate was 1 mL/min, and the injection volume was 20 µL. HPLC-Diode Array Detector (HPLC-DAD) was used, and L-ascorbic acid was

detected at 245 nm by High-Performance Liquid Chromatography (SHIMADZU LC 20A) with the Shodex Asahipark NH2-NP column (5 µm, 250 × 4.6 nm from Showa Denko K.K. USA) at 40°C

Determination of minerals content

The minerals were determined by wet way using an atomic absorption spectrophotometer (Perkin-ElmerModel3110, Connecticut, USA)according to the method used by Makalao et al. (2015) with slight modifications. For mineralization 0.2g of each sample was dissolved in a test tube containing 5 mL of concentrated nitric acid (HNO_3). The solution thus obtained was placed in a mineralizer integrated in the absorption spectrophotometer for 2h30 min to ensure digestion. After cooling, the content of the tube was inserted into a 25 mL volumetric flask, then topped up with distilled water to the gauge mark. This mixture was filtered through a 0.45 µm Wattman filter paper. Each mineral is dosed according to its wavelength and the content was expressed following the formula

T= CxVxFD/Pe

T: Mineral content; C: Concentration; V: Volume; DF: Dilution factor;Pe: Test portion.

Antiradical activity by the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) test

The antioxidant activity of the samples was determined by the DPPH antiradical test according to the method used by Yabalak et al. (2020) with slight modifications. Ten numbered tubes (1-10) were prepared. The DPPH radical was dissolved in methanol (2 mg/50 mL). Then 0.5 mL of the extract was put in tube 1 to which 2 mL of methanolic solution of the DPPH radical (0.04 mg/mL) was added. The range of extract concentrations was prepared by cascade dilution. After 10 min of incubation at 37°C in the dark, the residual DPPH absorbance was read at 490 nm using a spectrophotometer (BIO-RAD Model 680). The antiradical activity of each sample was evaluated by determining the concentration (mg/mL) necessary to reduce the DPPH radical by 50% (IC_{50}).

Antibacterial activity

The antibacterial activity was determined according to the method used by Guinoiseau et al. (2010) with minor modifications.

Preparation of extracts and inoculum

250 mg of each fruit sample was dissolved in 0.5 mL of 10% DMSO in centrifuge tubes. The resulting solution was homogenized using the sonicator. Each tube was properly labeled and stored in the refrigerator at a temperature of 4°C. After preparing the test solutions, the inoculum was prepared under sterile conditions. Thus, 2 mL of liquid LB medium (composed of Tryptone, yeast extract and Nacl) was inoculated with 0.4 mL of bacterial strain in sterile tubes at 37° C with continuous stirring (100 rpm) for 18 hours. Then, the bacterial culture was centrifuged at 3200 G and 24°C for 5 minutes. The supernatant was removed and the bacterial pellet was washed twice as follows: first the pellet was taken up in 2 mL of liquid LB medium, and the bacterial suspension was gently homogenized using a micropipette of 1mL then centrifuged at 3200 G at 20°C for 5 minutes. The bacterial pellet obtained was taken up in 1 mL of liquid LB. The concentration was obtained by diluting 5 mL of the bacterial solution so that the optical density at 600 nm of 100 µL of this dilution + 1900 µL of liquid LB medium was between 0.02 and 0.03.

Determination of the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC)

In a 96-well plate, were introduced 120 µL of LB medium and 130 µL of extracts diluted in 10% DMSO. Cascade dilutions in the wells made it possible to have the concentration series in each column of the plate. A 10 µL aliquot of bacterial suspension was added to each well of the plate. After incubation at 37°C for 18 hours with stirring at 150 rpm, 50 µL of p-iodonitrotetrazolium (INT) 0.2 mg/mL were added to each well. The plate was again incubated for 30 min. The appearance of a pink color indicates the presence of microbial growth in the wells. The Minimum Inhibitory Concentration (MIC) of each extract was determined from the wells having no pink coloration. For the determination of the Minimum Bactericidal Concentration (MBC), 100 µL of extract were taken from the well which made it possible to determine the MIC, then inoculated onto a plate of LB-agar. This operation was repeated with the other wells showing no pink coloration in the presence of INT. Petri dishes were incubated for 18 h at 37°C. The MBC corresponds to the lowest concentration that does not allow the development of bacteria.

Statistical analysis

The results were expressed as mean ± SEM (n = 3). Data were analyzed using analysis of variance (ANOVA) with XLSTAT software. Differences were considered statistically significant for a p value<0.05.

RESULTS

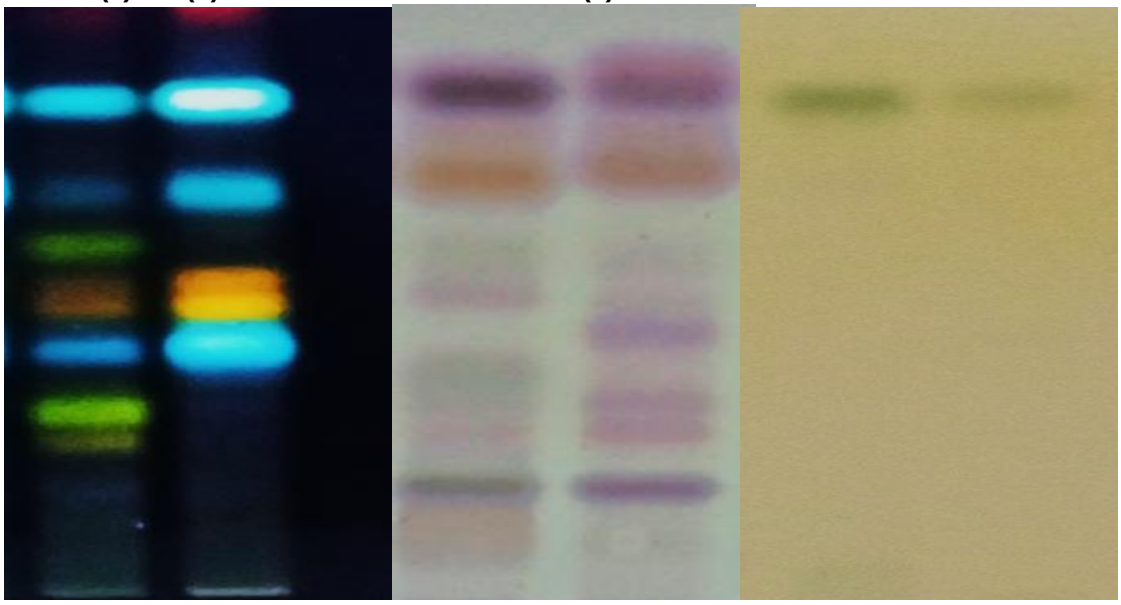
Phytochemical profile of fruit parts

The determination of constituents by HPTLC and the revelation of the spots were showed in Figure 2.

Flavonoids react positively to Neu reagent and were yellow-orange while phenolic acids were blue (a). Sterols and terpenes react to 3% H_2SO_4 in EtOH (96%). Terpenes were purple while sterols were brown (b).The presence of tannins was

a. Whole fruits b. Pulps c. Seeds

Figure 1: Whole fruits, pulps and seeds of *Diospyrosmespiliformis*(photo Bado D., 2020)



P,pulp; **S**, seed; **(a),** revelation of flavonoids by Neureagent in the presence of UV light (366 nm);**(b)**, revelation of sterols and triterpenes in ethanolic (70%) extracts; **(c)**,revelation of tannins by $FeCl_3$ 2%.

Figure 2. Determination of chemical compounds in the different parts of fruits of *Diospyros mespiliformis* by HPTLC

confirmed by the reactivity to $FeCl_3$ 2% (c).

Previous authors had indicated the presence of these secondary metabolites in the root, leaf and stem-bark extracts of the plant(Ebbo et al., 2014; Sara et al., 2018).

Phenolics content and antiradical activity

The content of phenolic compounds and the concentration inhibiting 50% of DPPH are regrouped inTable 1.

The fruit seed of *Diospyros mespiliformis* had the best antioxidant activity (IC_{50} = 0.04 mg/mL) due to its high contents ($p < 0.05$)of total phenolic compounds (5.75 ± 0.6 mg GAE/g), flavonoids (0.78 ± 0.27 mg RE /g), hydrolyzable tannins (3.56 ± 0.03 mg TAE/ g) and condensed tannins (1.28 ± 0.42 mg TAE/ g).Our results corroborate previous works (Lamien-Meda et al., 2008) regarding total phenolics content in an acetone extract which was 5.91 ± 0.36 mg/g. But the

Table 1. Phenolics content and antiradical activity by the DPPH test of fruits pulp and seed of *Diospyros mespiliformis*

Diospyrosmespiliformis			**Reference**
Samples	**Pulp**	**Seed**	**Trolox**
Total phenolics (mg GAE/*g*)	3.36 ± 012[a]	5.75 ± 0,6	
Flavonoids (mg RE/g*)*	0.46 ± 0.26	0.78 ± 0.27	
Hydrolyzable tannins (mg TAE/g)	1.78± 2.35	3.56 ± 0.03	
Condensed tannins (mg CE/g)	0.56 ± 0.11	1.28 ± 0.42	
Antiradical activity IC_{50} (mg/mL)	1.11	0.04[b]	0.012

Values are mean±SEM (n = 3);[a]p < 0.05 against seed of *Diospyros mespiliformis;*[b]p<0.05 against Trolox

flavonoids content was better than that expressed by this author in an acetone extract which was 0.27 ± 0.00 mg/g.The tannins content (hydrolyzable or condensed) was much higher than that determined in the fruit pulp by Nyambe et al. (2019), which was 0.03 mg/g

Water and fat soluble vitamins content

The content of water and fat soluble vitamins are presented in Table 2.

The fruit pulpof *Diospyros mespiliformis* had the best (p < 0.05)vitamins C, E andβ-carotene content.
The content of vitamin C (22.0 ± 0.01 mg/100g) and β-carotene (0.007 ± 0.03 mg/100g) were significantly lower than those obtained in the whole fruit byHegazy et al. (2019),whose values were 23.82 ± 3.12 mg/g and 709.52 ± 23.84 µg/100 mg respectively.

Mineral content

Mineral elements content are presented in Table 3.

The fruit seed had the best content of macroelements (Na, K, Mg) and the fruit pulp Ca content (0.73± 0.34 mg/100g) was slightly higher than that obtained by (Nyambe et al., 2019). All the contents expressed in micro and macro elements were significantly lower than those expressed in the whole fruit according to the work of (Hegazy et al., 2019).

Antibacterial activity

Antibacterial activity offruit parts (pulp and seed)of *Diospyros mespiliformis* is presented in Table 4.
All the CMB/CMI ratios as indicated in Table 4 were less than 4. Which would mean that the two parts of fruit (pulp and seed) are effective against infections caused by *E. coli* and *S. aureus.* Previous authors have shown that the ethanolic extract of *Diospyros. Mespiliformis* exerts a bactericidal effect on *S. aureus* (Yovo et al., 2020). But in our studies, the fruit seed was the most potent because it has the best minimum inhibitory concentration (MIC=3.12 mg/mL) and the best bactericidal concentration (MBC=1.56 mg/mL) on *E. coli.*

DISCUSSION

Gastroenteritis and gastroduodenal ulcer disease are among the most worrying infectious pathologies in humans. If the treatment of *E. coli* gastroenteritis can use antidiarrheals, that of gastroduodenal ulcer was often based on triple therapy combining a proton pump inhibitor (PPI) and two antibiotics. However, this tritherapy has become obsolete nowadays, due to the increase in antibiotics resistance, but also because of the side effects caused by taking certain conventional drugs.
The use of medicinal plants for the treatment of diseases dates back to antiquity. It is the fruit of the combination of instinct, observation, taste and experience. Indeed, the man of antiquity learned by experience to distinguish between the parts of plants presenting beneficial effects and those which were either dangerous or ineffective.
The fact remains that stress is a triggering or accelerating factor for the disease. Indeed, at high levels, free radicals and oxidants released by cells generate oxidative stress, a deleterious process that can damage cellular structures, lipids, proteins and DNA (Bissinger et al., 2019).Thus, the oxidative effect of stress can cause an imbalance within the stomach, altering the vascularization of the stomach wall and increasing acidity. Stressed and anxious people are more affected by gastric and duodenal ulcers. A stressed person can more easily develop nervous gastritis which will promote attacks by the *Helicobacter* bacterium if it is present in the digestive tract of the person. Previous studies showed that reducing oxidative stress can greatly improve the health status of populations (Liguori et al., 2018). It is therefore important to evaluate the antioxidant potential of natural resources that can act in the prevention or treatment of gastroenteritis and gastroduodenal ulcer.
Results showed that the fruit pulp and seed of *Diospyros mespiliformis* had an antioxidant property due to their ability to trap free radical DPPH. However, the seed which had the best content of total phenolic compounds (5.75 ± 0.6 mg GAE/g), flavonoids (0.78 ± 0.27 mg RE/g), hydrolysable tannins (3 .56

Table 2. Water and fat soluble vitamins content (mg/100g) of fruits pulp and seed of *Diospyros mespiliformis*

Samples	Pulp	Seed
Vitamin C	22.0 ± 0.01	11.52 ± 0.01 [a]
β-carotene	0.007 ± 0.03	0.001 ± 0.02
Vitamin E	0.119 ± 0.02	0.08 ± 0.05

Values are mean±SEM (n = 3); [a]p< 0.05 against the pulp of *Diospyros mespiliformis.*

Table 3. Mineral content (mg/100g) of fruits pulp and seed of *Diospyros mespiliformis*

Samples	Pulp	Seed
Fe	0.01 ± 0.31	0.02 ± 0.59
Zn	0.000± 0.12	0.001 ± 0.05
Na	0.1± 0.02	0.26 ± 0.04
K	1.1± 0.36 [a]	3.19± 1.10
Ca	0.73± 0.34	0.69 ± 0.22
Mg	0.08± 0.15	0.14± 0.31

Fe, Iron ; Zn, Zinc ; K, Potassium ; Na, Sodium ; Ca, Calcium ; Mg, Magnesium; values are mean±SEM (n = 3), [a]p < 0.05 against the seed of*Diospyros mespiliformis*..

Table 4. Antibacterial activity of fruits pulp and seed of *Diospyros mespiliformis.*

Bacterialstrains	***E.coli***		***S.aureus***	
Samples	**Pulp**	**Seed**	**Pulp**	**Seed**
MIC (mg/mL)	12.5	3.12	25	25
MBC (mg/mL)	6.25	1.56	6.25	6.25
MBC/MIC	0.5	0.5	0.25	0.25

Escherichia coli (E. coli); *Staphylococcus aureus (S. aureus) ;* Controls: **Amoxicillin disk for antibiogram**(10µg/disk) and clarithromycin (15 µg/disk).

± 0.03 mg TAE/g)and condensed tannins (1.28 ± 0.42 mg CE/ g) had the best antioxidant activity with a 50% inhibitory concentration (IC_{50}) equal to 0.04 mg/mL. The present results are in agreement with previous studies which reported the antioxidant activity of some underutilized wild fruits and showed that this antioxidant activity is related to phenolics content (Lamien-Meda et al., 2008). Compared to the antiradical effect of the reference control Trolox (IC_{50} = 0.012 mg/mL), this result, obtained with a crude extract, shows significant antiradical activity of the active ingredients contained in the fruit seed of *Diospyros mespiliformis*. The antioxidant activity of the fruit pulp and seed would be partly due to the presence of certain chemical compounds including flavonoids and tannins in the extracts.

Flavonoids are compounds capable of stimulating the production of prostaglandins in order to cause an increase in the secretion of bicarbonate which play a major role in the regulation of blood pH. They are the body's main buffer and neutralize excess acid. This promotes the production of mucus, thus protecting the stomach wall (Garg et al., 2014).Previous authors have shown that flavonoids have anti-ulcerogenic properties due to their ability to inhibit the growth

of *Helicobacter pylori,* the main germ responsible for chronic gastritis, duodenal ulcers and stomach cancers (Guerra-Valle et al., 2022). The presence of these compounds in the fruit seed of *Diospyros mespiliformis* could explain the interest of this plant in the management of gastroenteritis and gastroduodenal ulcer. In addition, Diospyrone is a Naphthoquinone isolated from Diospyros species by previous work and recognized for its bioactivity on certain germs(Uc-Cachón et al., 2014). So, the fruit seed of *Diospyros mespiliformis* which had the best flavonoid content (0.78 ± 0.27 mg RE/g), constitutes an important source of new natural compounds with antioxidant power in the management of certain digestive diseases including gastroenteritis and gastroduodenal ulcer.

All the CMB/CMI ratios as indicated in Table 4 are less than 4. According to some authors, if the CMB/CMI ratio ≤ 4, the substance tested is bactericidal, and if the CMB/CMI ratio is >4, the substance tested is bacteriostatic (Okou et al., 2018).Which would mean that the two parts of fruits of *Diospyros mespiliformis* are effective against infections caused by *E. coli* and *S.aureus.* But, the seed showed the best minimum inhibitory and bactericidal concentrations which were 3.12 mg/mL and 1.56 mg/mL on *E. coli* respectively. The presence of tannins in the extracts is a very important advantage and could explain the antibacterial activity. Indeed several works reported that tannins are antibacterial, antidiarrheal (Adji et al., 2022).

Indeed, by their astringent property, tannins are able to tighten the pores in order to reduce acid secretion or to precipitate proteins of the mucous membrane making it resistant to irritants. Tannins would therefore lead to a reduction in the acidity of the gastric contents (Zakaria et al., 2014).Symptoms such as abdominal pain could be relieved by the sterols and triterpenes present in the extracts. These compounds are endowed with anti-inflammatory activity and have a well-recognized analgesic potential (Sajid et al., 2017). This study shows that the fruit pulp of *Diospyros mespiliformis* which had the best content of β-carotene could be a source of provitamin A. Indeed, some authors report that β-carotene would contribute to the maintenance of tissues of the epidermis and mucous membranes and would play an important role in the prevention of cancer(Chen et al., 2021).

The results showed an important microelement (Fe) and macroelements (K, Ca) content which is an advantage in the management of deficiency diseases. Indeed, potassium (K) is sufficient to lower blood pressure level and decrease the risk of kidney stones and calcium (Ca) is required in the formation and physical strength of bones and teeth. Iron (Fe) is important in the formation of haemoglobin which is essential in transporting oxygen in blood. The non-negligible contents of other mineral elements highlight the nutritional and therapeutic potential of these fruit species.

Faced with new health problems linked to an unbalanced diet in the countries of Sub-Saharan Africa, it is very important to promote food plants with therapeutic virtues in order to increase the resilience of populations in the face of extreme climatic events.

CONCLUSION

The objective of the present study was to determine phytonutrients content, antioxidant and antibacterial activity of fruits of *Diospyros mespiliformis* used in the treatment of gastroenteritis and gastroduodenal ulcer. The results showed that the fruit seed had the best content of total phenolic compounds, tannins and flavonoids with antioxidant and antibacterial properties. Also, the presence of essential vitamins and minerals in the pulp makes the fruit of *Diospyros mespiliformis* an interesting source for the development and promotion of new natural foods and phytomedicines from local plant for the management of digestive diseases.

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