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PHYTOCHEMICAL SCREENING, ANTIOXIDANT AND CYTOPROTECTIVE ACTIVITIES OF FRUITS PULP EXTRACTS FROM *ADANSONIA DIGITATA* L. (BOMBACACEAE) AND *ZIZIPHUS MAURITIANA* LAM. (RHAMNACEAE)

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ABSTRACT: This current investigation was designed to assess *in-vitro* the ability of the extract to prevent free radical-induced oxidative cell damages. Fibroblasts were exposed to hydrogen peroxide and treated with gradual concentrations of ethanol and dichloromethane fruits pulp extracts from *Adansonia digitata* and *Ziziphus mauritiana*. The cell viability was measured after 24h of treatment by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium (MTT) assay. Moreover, the capacity of extracts to scavenge free radicals was evaluated by using the DPPH and ABTS models. The polyphenol contents of extracts were also quantified by spectrophotometry. All extracts in a concentration between 100 and 400 µg/mL didn't exhibit any cytotoxic effects on fibroblasts compared to the vehicle ($p > 0.05$). However, extracts showed interesting cytoprotective activity against the hydrogen peroxide-induced oxidative cell damage. The cytotoxic effects of the hydrogen peroxide were completely suppressed by adding the extracts of *A. digitata.* The ethanol extract of *A. digitata*s showed the best free radical quenching activities on DPPH radical (IC_{50} = 28.78 ± 4.97 µg/mL) and ABTS radical (IC_{50} =25.29 ± 2.27 µg/mL) as well as the highest phenolic content (11 728.07 ± 194.57mg GAE/100 g). The dichloromethane extract of *A. digitata showed* the highest flavonoid content (196.32 ± 15.49mg QE/100 g). All these findings suggested that the consumption of fruits of *A. digitata* and *Z. mauritiana* could prevent oxidative stress related-chronic diseases, probably due to their high polyphenol contents.

INTRODUCTION: Aerobic cells require the oxidation of organic molecules by oxygen for their vital function. This oxidation takes place at the level of the mitochondrial respiratory chain [1]. During oxidation, many parts of the oxygen escape to the reduction process and generate reactive oxygen species [2].

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These ROSs are hydroxyl radical, superoxide anion, hydrogen peroxide, nitric oxide radical, and other derivatives such as peroxyl and alkoxyl radicals [3].

Although these free radicals are important in certain cell functions, including inflammation, phagocytosis, and intercellular communication, they are involved in the oxidative degradation of biological molecules such as nucleic acids, membrane phospholipids, and proteins [4]. Many previous studies have shown the involvement of free radicals in many physiopathology of certain diseases such as cancer, hypertension, diabetes, Alzheimer's disease and obesity [5-7].

The cell possesses an endogenous enzymatic (catalases, superoxide dismutase, glutathione peroxidase) and non-enzymatic (glutathione, ascorbic acid, vitamin E) antioxidant system responsible for counteracting the harmful effects of free radicals [8, 9]. Exogenous antioxidants often supplement this endogenous antioxidant system in the fight against oxidative stress. Fruits and vegetables are the main sources of these antioxidant compounds.

Adansonia digitata belongs to the Bombacaceae family and is native to Africa [10]. The fruit pulp is enclosed in a strong capsule and is divided into small powdery slices **Fig. 1**. The fruit pulp is eaten and is well known for its high vitamin C content. The pharmacological properties of the fruit pulp include antioxidant [11, 12], anticancer [13], anti-inflammatory [10], antihypertension [14], antidiabetic [15, 16], antiviral [17] and hepato-protector [18]. Previous phytochemical investigations demonstrated that the fruit pulp contains bioactive compounds such as flavonoids (procyanidins, kaempferol-3-O-glucoside, quercetin-3-O-glucoside, catechin and epicatechin), phenolic acids (chlorogenic acid, caffeic acid and *p*-hydroxycinnamic acid) and saponins (triterpene and steroid glycosides) [19].

FIG. 1: *ADANSONIA DIGITATA* FRUIT

Ziziphus mauritiana is commonly found in subtropical countries. The fruit is a globular drupe, oblong to ovoid in shape **Fig. 2**. HPLC analysis of methanol extract of its fruit pulp showed the presence of predominant flavonoids such as rutin, myricetin, and apigenin [20]. Non-volatile compounds were identified in the methanol extract of fruit by GC-MS, the most abundant was5-hydroxymethylfurfural [21]. The fruit pulp of *Z. mauritiana* is several pharmacological properties, including antioxidant [22, 23] anticancer [24], anti-inflammatory [24], anti-bacterial [25] and anti-diarrhoeal [26]. In this study, the property of fruit pulp of *A. digitata* and *Z. mauritiana* to protect human fibroblasts against ROS-induced oxidative cell damages was investigated.

FIG. 2: *ZIZIPHUS MAURITIANA* FRUITS

MATERIAL AND METHODS:

Chemicals: 2, 2-Diphenyl-1-picrylhydrazyl 2, 2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis-3-ethyl-ethylbenzothiazoline-6-sulphonate (ABTS), dimethyl sulfoxide (DMSO), Gallic acid, Quercetin, Ascorbic acid, Hydrogen peroxide, Sodium carbonate, Folin-Ciocalteu reagent, Aluminum trichloride, Potassium persulfate, Penicillin-Streptomycin, Fetal bovine serum, L-glutamine, Trypsin-EDTA, Dulbecco's Eagle Modified Medium (DEMM), Phosphate buffer saline (PBS) and bromure de 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium (MTT)were purchased from Sigma Aldrich, Germany. Ethanol, Dichloromethane were purchased from Prolabo (Paris, France). All reagents were analytical grades.

Plant Material and Extraction: The fruits of *Z. mauritiana* and *A. digitata* were purchased at the Zogona and Tintoulou markets, respectively (Ouagadougou, Burkina Faso) during the month of August 2020. The fruits were identified by Dr. Evelyne PARE, associate researcher at the Plant Biology and Ecology laboratory of the UFR / SVT, Joseph KI-ZERBO University. For the extraction, pulps of fruits were scraped and powdered in a mortar. The powder of each pulp was soaked successively in ethanol and dichloromethane (24 h, 25°C, continuous stirring) to obtain the ethanol extract and the dichloromethane extract respectively. Each extract was filtrated, concentrated to dryness, and kept at 4 °C for further investigations.

Total Phenolics and total Flavonoids Determination: The total flavonoid content of fruit pulps extracts was assessed by spectrophotometry at 415 nm as described previously [27]. Quercetin was used to generate a calibration curve ($y = 0.0397x + 0.0523$; $R^2 = 0.99$, $p<0.0001$). The amount of flavonoids was expressed as mg equivalent of quercetin/100g of extract. The total phenolic content was quantified at 760 nm using the Folin-Ciocalteucolorimetric assay [28].

Gallic acid was used to generate the standard curve ($y = 0.057x + 0.172\,5$; $R^2 = 0.99$, $p<0.0001$) and results were expressed as mg equivalent of gallic acid/100 g of extract.

Free Radical Scavenging Activity: Extracts' free radical quenching activity was assessed by evaluating their ability to scavenge DPPH radical and $ABTS^{\bullet+}$. The capacity of serial diluted concentrations of each extract to quench the DPPH radical was measured at 517 nm as described previously [29].

Results were expressed as concentration (µg/mL) quenching 50% of free radicals (IC_{50}). The flavonoid quercetin and the phenol gallic acid were used as standard antioxidant compounds. The ability of extracts to scavenge $ABTS^{\bullet+}$ radical was assessed at 734 nm by using the method described previously [30].

Data were expressed as concentration (µg/mL) quenching 50% of free radicals (IC_{50}). Ascorbic acid was used as a reference.

Cytotoxicity and Cytoprotection Activity: Human gingival fibroblasts provided by the university hospital center Bogodogo (Ouagadougou, Burkina Faso) were exponentially grown in a Dulbecco's eagle modified medium supplemented with 10% of fetal bovine serum, 20 mM of L-glutamine and 1% of penicillin-streptomycin in a humidified incubator (5% CO_2, 37°C) until cells were reached confluence 80%. Cells were harvested with a trypsin-EDTA solution and seeded in a 96-wells plate (5000 cells/well) for the cytotoxic and cytoprotective study.

To assess the cytotoxic activity of extracts, cells were put in contact with different concentrations (100, 200,400 µg/mL) of each extract for 24 h and cell viability was recorded by using the standard MTT assay [31].

To assess the cytoprotective activity of extracts, cells were concomitantly treated with 100 mM of hydrogen peroxide and different concentrations (100, 200,400 µg/mL) of each extract for 24h and the MTT assay was used to measure the cell viability. Cells treated with hydrogen peroxide alone were used as positive control and cells treated with the DMSO 1% in a culture medium were used as vehicle.

Statistical Analysis: All experiments were performed in triplicate (n=3) and data were expressed as mean value ± standard deviation. The one-way ANOVA followed by the Newman-keuls posted-test was used to verify the impact of the treatment on the cell viability. A significant difference was considered at $p<0.05$.

RESULTS AND DISCUSSION:

Total Phenolic and Total Flavonoids Contents: Total phenolic and total flavonoids of fruits pulps extracts from *A. digitata* and *Z. mauritiana* were investigated and data were showed in **Table 1**.

The ethanol extract of *A. digitata* fruits pulps presented the highest total phenolic content (11 728.07 ± 194.57mg GAE/100 g) while its dichloromethane extract exhibited the highest total flavonoids content (196.32 ± 15.49mg QE/100 g). In general, phenolic compounds of fruits pulps are more extractible by ethanol than dichloromethane

TABLE 1: TOTAL PHENOLICS AND TOTAL FLAVONOIDS CONTENTS OF FRUITS PULPS EXTRACTS

Plants	Extracts	Phenolics contents (mg GAE/100 g)	Flavonoids contents (mg QE/100 g)
Adansonia digitata L.	Ethanol	11 728.07 ± 194.57 [a]	101.11 ± 1.55 [b]
	Dichloromethane	1321.05 ± 88.07 [b]	196.32 ± 15.49 [a]
Ziziphus mauritiana Lam.	Ethanol	225.15 ± 10.13 [c]	101.91 ± 4.27 [b]
	Dichloromethane	118.42 ± 8.77 [d]	48.33 ± 7.24 [c]

Values are expressed as Mean ± S.D. (n=3). Data in each column with different superscript letters [(a,b,c,d)] were statistically different ($P < 0.05$). mgGAE/g: mg gallic acid equivalent/gram; mgQE/g: mg quercetin equivalent/gram.

Antioxidant Activity of Fruits Pulps Extracts: The antioxidant potentiality of fruit pulps extracts was evaluated by measuring their ability to scavenge free radicals such as DPPH and $ABTS^{\bullet+}$. Data were showed in **Table 2**. The ethanol extract of *A. digitata*'s fruits pulps showed the strongest DPPH radical and $ABTS^{\bullet+}$ radical scavenging power with IC_{50} values ranging respectively from 28.78 ± 4.97µg/mL and 25.29 ± 2.27µg/mL. However, all fruits pulps extracts were less active than the reference compounds.

TABLE 2: ANTIOXIDANT ACTIVITY OF FRUITS PULPS EXTRACTS

Plants	Extracts	DPPH radical quenching activity IC_{50} (µg/mL)	$ABTS^{\bullet+}$ radical quenching activity IC_{50}(µg/mL)
Adansonia digitata L.	Ethanol	28.78 ± 4.97 [c]	25.29 ± 2.27 [b]
	Dichloromethane	102.72 ± 5.02 [d]	209.72 ± 8.04 [d]
Ziziphus mauritiana Lam.	Ethanol	1207. 50 ± 88.19 [e]	128.55 ± 11.79[c]
	Dichloromethane	Non-active	526.52 ± 29.54 [e]
Standards	Quercetin Gallic acid Ascorbic acid	1.38 ± 0.07 [b], 0.38 ± 0.01 [a] Nd	Nd, Nd 10.52 ± 1.82 [a]

Values are expressed as Mean ± S.D. (n = 3). Data in each column with different superscript letters [(a,b,c,d,e)] were statistically different ($P < 0.05$). IC_{50}: Inhibitory concentration of extract quenching 50% of radical; Nd: Not determined.

Cytotoxic and Cytoprotective Activity: To assess the cytotoxic property of fruits pulps extracts, cells were treated with different concentrations of extracts and the cell viability was compared to the DMSO 1% treatment (vehicle) . Data were shown in **Fig. 1**.

The percentage of viable cells in all tested concentrations of the different extracts was more than 80%. Moreover, there was no significant difference in the percentage of viable cells when cells were treated with extracts or with vehicles ($p>0.05$).

This finding suggested that fruit pulps extracts (100-400 µg/mL) weren't cytotoxic on human fibroblasts.

To assess the cytoprotective property of fruit pulps extracts, cell death was induced by hydrogen peroxide and the percentage of viable cells was compared to those that the concomitant treatment of hydrogen peroxide and extracts.

As expected, the hydrogen peroxide treatment observed significant cell death compared to the vehicle ($p<0.05$).

However, the concomitant treatment with extracts suppressed the cytotoxic effects of the hydrogen peroxide. *A digitata* fruit pulps exhibited more cytoprotective activity than *Z. mauritiana* fruit pulp.

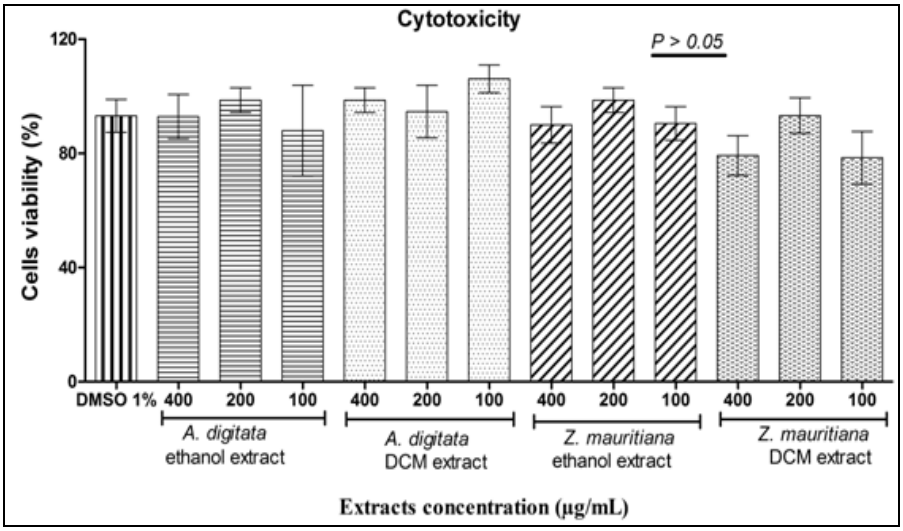

FIG. 3: CYTOTOXIC ACTIVITY OF FRUITS PULPS EXTRACTS. Cells were in contact with extracts for 24 h, and the standard MTT assay recorded the cells' viability. Data were expressed as the mean value ±std of three repetitive independent experiments. No significant difference was observed from the vehicle DMSO 1% ($p>0.05$). DCM: Dichloro-methane.

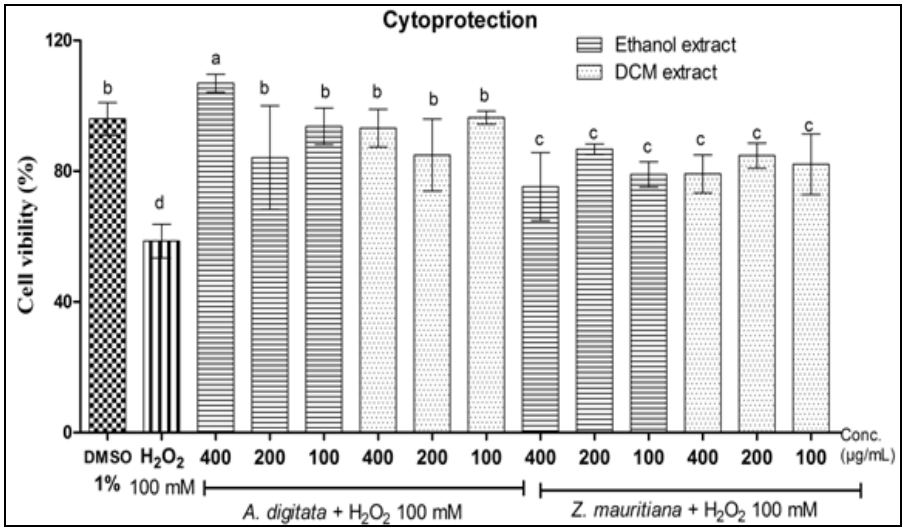

FIG. 4: CYTOPROTECTIVE ACTIVITY OF FRUITS PULPS EXTRACTS. Cells were concomitantly treated with different concentrations of extracts and a single concentration of hydrogen peroxide, and the cell viability was measured by the MTT assay. Data were expressed as the mean value ± std of three repetitive independent experiments. Histograms with different letter (a,b,c) were statically different at $p < 0.05$. DCM: Dichloromethane.

Fruits have long been used in human alimentation because of their sweet taste. Many previous studies demonstrated their high nutritional value [32, 33]. Indeed, fruits contain micronutrients (Ca, Na, P, Mg, Fe, K), proteins, fats, carbohydrates, and vitamins [34, 35]. In addition, the fruits contain bioactive secondary metabolites such as flavonoids, phenol acids, and terpenes [36]. Parallel to their food uses, fruits are also used as sources of medicaments. Epidemiological studies have established a link between the consumption of fruits rich in antioxidants and the reduction in the prevalence of chronic diseases such as cancer, diabetes, cardiovascular and neurodegenerative diseases [37, 38]. These health properties of fruits are due to their bioactive compounds that can directly or indirectly trap the free radicals involved in the development of these pathologies [39].

Indeed, the free radicals generated at the mitochondrial respiratory chain level can degrade cellular components such as membrane phospholipids, nucleic acids, and carbohydrates, leading to cell death or carcinogenesis. In this study, as in previous studies, the fruits of *A. digitata* and *Z. mauritiana* showed good DPPH and ABTS radical scavenging activities [15, 23]. These antioxidant activities are believed to be largely due to their polyphenol contents. Through their antioxidant activities, fruit extracts may protect cells against oxidative damage mediated by hydrogen peroxide. The extracts could intervene at several levels: reduction of hydrogen peroxide to water, neutralization of the hydroxyl radical resulting from the homolytic cleavage of hydrogen peroxide (Fenton reaction), protection of membrane phospholipids by preventing the initiation and propagation of lipid peroxidation, modification of membrane permeability or induction of antioxidant enzymes expression.

In previous studies, fruit pulp of *A. digitata* and *Z. mauritiana* showed a very strong anti-lipid peroxidation activity (inhibition percentage more than 96 %) and restored the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSHPx) activity that could justify their cytoprotective activity against the oxidative stress [40, 41].

CONCLUSION: Fruits pulp of *A. digitata* and *Z. mauriatiana* are potent sources of antioxidant compounds that can prevent oxidative cell damage. The daily intake of these fruits as food supplements could reduce the prevalence of oxidative stress-related diseases. Further phytochemical investigations are necessary to identify the bioactive compounds.

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