

RESEARCH ARTICLE

PHYTOCHEMISTRY AND IN VITRO ANTIOXIDANT ACTIVITIES OF FOUR CONSUMED PICKING PRODUCTS IN SIKASSO, MALI

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..... Manuscript Info

Abstract

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..... The aim of this work was to study the phytochemistry and to evaluate the in vitro antioxidant activities of four fruits harvested consumed in Sikasso, Mali: Balanites aegyptiaca, Zizyphus mauritiana, Raphia sudanica and Saba senegalensis. A phytochemical screening has been performed using tube reactions. Total polyphenol and flavonoid were determined by the spectrophotometric method and in vitro antioxidant activities by DPPH test. The tests revealed in all the fruits the presence of many phytochemical groups. The mucilages have been found in the fruits of Raphia and Zizyphus. Fruits of Zizyphus had the highest polyphenolic content (1.72±0.07 mg GAE/100 mg) and Saba the lowest $(0.99\pm0.07 \text{ mg GAE} / 100 \text{ mg})$. The highest flavonoid content has been recorded with Raphia fruit (0.35±0.04 mg CE/100 mg) while Balanites fruit had the lowest content (0.16±0.034 mg CE/100 mg). As well the contents of polyphenols and flavonoids, the highest in vitro antioxidant activity has varied 30.66±2.16 % for Saba to 70.26±2.11 % for Raphia. These results have shown that these fruits are sources of many metabolites especially polyphenols and flavonoids and are endowed with antioxidant activities, therefore they could be used more in food to improve food ration and to fight against oxidative stress.

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Introduction:-

In sub-Saharan Africa, thousands of spontaneous plants are used daily by the majority of populations (Aké Assi, 2014). Several studies have shown the importance of wild edible plants in improving food intake and reducing poverty in different countries and at different levels (Diarra, 2012; Diarra et al., 2016; Sène et al., 2018).

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In Uganda, a study has been shown that wild edible plants can significantly complement cultivated exotic plants to reduce vitamin A deficiency in populations (Musinguzi et al., 2007). In Sahelian countries such as Mali, Burkina Faso and Senegal, edible wild plants are used as soldering food (Koné et al., 2009; Kouyaté et al., 2009; Thiombiano et al., 2014). Widely consumed, they improve the daily food intake as an energy source and through their micronutrient content (Makalao et al., 2015; Diarra et al., 2019). In 2016, Diarra et al. showed that the Malian flora is very rich in wild food plants and that fruits were the most consumed organs (62.2 %) by the population. Despite this floristic richness, statistics on malnutrition are worrying in Mali. The results of the ENSAN surveys in Mali showed that chronic food insecurity affects nearly a quarter (24%) of Malian households (ENSAN, 2016). As for the results of SMART Mali, the nutritional situation is considered precarious in several regions and particularly in Sikasso with prevalence's ranging from 5% to 10% of global acute malnutrition (SMART, 2016). Within the strategic framework of sustainable food security and in the context of poverty alleviation, the ways in which local populations exploit natural resources do not allow them to make the most benefits of these resources (Sène et al., 2018).

Studies have shown that many edible wild plants have a high level of antioxidant activity (Adjdir et al., 2018). Several metabolites are involved in the antioxidant and antimicrobial activities of plant extracts (Fatima et al., 2015; Bagewadi et al., 2019; Togola et al., 2019a). Among these metabolites, total polyphenol and flavonoid are predominant. It is why wild fruits are currently of great interest not only for the fight against malnutrition but also and above all for the treatment and/or prevention of some chronic diseases such as diabetes, cancer and high blood pressure. This craze for wild fruits began especially after several epidemiological studies showed that cancer rates were low among people who consume a lot of fruit rich in polyphenols and flavonoids and therefore in antioxidants (Riel, 1999); (Kabine et al., 2015).

Regarding this situation, it is necessary to optimise the level of knowledge on wild food plants in order to contribute to rational exploitation. This study was initiated within the overall framework of the valorisation of wild woody species of food interest. The aim is to contribute to the phytochemical study and evaluation of in vitro antioxidant activities of fruit extracts of four (04) harvested species consumed in Sikasso, Mali: Balanites aegyptiaca L. (Del.), Zizyphus mauritiana Lam., Raphia sudanica A. Chev., Saba senegalensis (A. DC.) Pichon. which are very consumed by local populations in Sikasso.

Material and Methods:-Material:-

The plant material used was the pulp of the fruits of Balanites aegyptiaca L. (Del.), Zizyphus mauritiana Lam., Raphia sudanica A. Chev., Saba senegalensis (A. DC.). After an ethnobotanical survey, these fruits (Figure 1) have been selected and bought (between October 2016 and June 2017) in the markets of Sikasso in Mali.



Raphia sudanicaSaba senegalensisFigure 1:- Fruits of the four wild food species studied.

Methods:-

Phytochemical screening:-

Phytochemical screening has been performed using colour and tube precipitation reactions. The analytical techniques used are summarized in table 1 below according to the protocols described by Konaré et al., (2019); Traoré et al. (2019).

Chemical groups	Reagents	Positive results
	1. Dragendorff (aqueous solution of	Orange to red precipitate at the bottom of
Alkaloids	potassium iodobismuthite)	the tube
	2. Mayer (aqueous solution of potassium	White to yellowish precipitate at the bottom
	mercuri-iodide)	of the tube
Tannins	Ferric chloride (FeCl ₃)	Dark blue, black or green colouring
Flavonoids	Shinoda (cyanidine reaction)	Orange (flavones), red (flavonols) or violet
		(flavanones) colouring
Steroid terpenes	Libermann-Burchard (Acetic anhydride	Purple, blue or green colouring
	$-H_2SO_4:50:1)$	
	Reaction to ammonia (NH4OH at 25%)	Appearance of intense fluorescence in the
Coumarins		tube under a UV lamp (WFH 201) at 366
		nm
	Reaction with sulphuric acid (H_2SO_4) in	The presence of a coloration that increases
Anthocyans	a basic medium	by acidification and then turns blue-violet
		in a basic environment.
Mucilages	Precipitation with ethyl alcohol	Appearance of a flaky precipitate
Saponins	Foam test	A persistent foam height greater than or
		equal to 1 cm
Reducing sugars	Reduction with Fehling liquor	Red brick precipitate after heating in the
		Grant Ser water bath N°1746047

Table 1:- S	necific reagents	and phytoc	hemical scre	ening reactions	2
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Sources: (Konaré et al., 2019; Traoré et al., 2019).

Total polyphenol and flavonoid extraction:-

The extraction of polyphenols and flavonoids from the samples has been assisted by an ultrasonic bath. Ten grams (10 g) of vegetable pulp (weighed using a nahita Blue analytical balance series 5134 EX) have been extracted with 100 mL of methanol (AnalaR NORMAPUR UN1230 from VWR) diluted to 80 % with distilled water (80: 20; v/v) for 20 min. After filtration, the residue has been rinsed with 100 mL of methanol. This residue has been extracted again under the same conditions and filtered. The two filtrates were added and dry evaporated using a rotary evaporator (BUCHI). The resulting residue has been dissolved in 50 mL of methanol and the volume has been completed to 100 mL with distilled water. The resulting mixture was centrifuged using a centrifuge (SIGMA 1-6p), then made up to 100 mL with 50 % methanol and stored cold (0° C) before analysis (Koné et al., 2012).

Total polyphenol and flavonoid assays:-

Total polyphenol:-

The total polyphenol contents were determined by the Folin-Ciocalteu method described by Fofié et al. (2017). One hundred microliters (100 μ L) of properly diluted extract were introduced into a test tube. Then 100 μ L of Folin Ciocalteu reagent were added to the mixture and stirred. After 5 min, 1 mL of a 7 % sodium bicarbonate (Na₂CO₃) solution was added with stirring and the final volume was immediately increased to 2.5 mL with distilled water and vigorously stirred. After a 90 minute incubation at room temperature (30-35° C), the absorbances were read at 760 nm against a blank prepared with distilled water with a spectrophotometer (Zuzi 4010/1). The polyphenol levels were determined using a gallic acid standard curve (Pancreac 99 % PS series 152830.1610) at different concentrations according to the linear regression equation y = 0.1015x + 0.0026; R² = 0.99 (figure 2). The polyphenol levels, expressed as mg gallic acid equivalent per 100 mg of extract (mg GAE/100 mg extract), have been calculated according to the following formula (Fofié et al., 2017):

$$T = \frac{Cs \ x \ D}{Ci} \ x \ 100$$

T = Polyphenol content of the sample expressed as mg GAE/100 mg extract;

Cs: Concentration of the sample read from the calibration curve;

Ci: Initial concentration of the mother solution (mg/L); D: Dilution factor.

NB: All measurements have been repeated three times.

Flavonoids:-

The quantification of flavonoids was carried out by the colorimetric method described by Koné et al. (2012). This method uses aluminium trichloride which forms a yellow complex with flavonoids and sodium nitrite which forms a pink complex which absorbs in the visible at 510 nm. 100 μ L of each properly diluted extract and 30 μ L of a 5 % sodium nitrite (NaNO₂) solution were introduced into a test tube. After 5 minutes, 30 μ L of 10 % aluminium chloride were added to the mixture. And 6 minutes later, 2 mL of 1 M soda were added and the mixture was stirred. The final volume was filled up to 700 μ L with distilled water and vigorously stirred. The absorbances were read at 510 nm with a spectrophotometer (Zuzi 4010/1) against a blank prepared under the same conditions with distilled water. A standard curve has been established using a catechin solution (C1251-5G), powder; Sigma Aldrich) at different concentrations (figure 3). The flavonoid contents of the extracts (average of three trials), expressed as mg catechin equivalent per 100 mg of extract (mg CE/100 mg extract), were determined from the following formula:

 $T = \frac{Cs \times D}{Ci} \times 100$ (Fofié et al., 2017)

T = Polyphenol content of the sample expressed as mg CE / 100 mg extract;

Cs: Concentration of the sample read from the calibration curve;

Ci: Initial concentration of the mother solution (mg/L);

D: Dilution factor.

NB: All measurements are repeated three times.

Screening for in vitro antioxidant activity:-

Thin layer chromatography (TLC) has been used to investigate the *in vitro* antioxidant activities of our extracts. The system composed of butanol - acetic acid - water (70- 10-20: v/v/v) was used as eluent. 20 µL of each extract was deposited on a silica plate (Polygram SIL® G REF 805013). The revelation was made with a methanolic solution of 1mg/mL DPPH (from Sigma Aldrich: D9132-6G) (Traoré et al., 2019).

Evaluation of in vitro potential antioxidant:-

The trapping activity of the radical DPPH was measured according to the protocol described by Togola et al. (2019a). From a solution of 100 μ g/mL extract, a calibration range was established. Fifty microliters (50 μ L) of each extract at different concentrations were added to 1.95 mL of DPPH methanolic solution (0.024 g/L). At the same time, a negative control was prepared by mixing 50 μ L of methanol with 1.95 mL of the methanol solution of DPPH. After 30 minutes of incubation in darkness and at room temperature (30-35° C), the absorbances were read against a blank prepared at 515 nm using a UV-VIS spectrophotometer. Ascorbic acid was used as a standard antioxidant (positive control).

The radical-scanning activity of DPPH (or percentage of free radical inhibition) was calculated as follows: Percentage of free radical inhibition (I %) = $[1 - \frac{\text{Sample Absorbance}}{\text{Negative Control Absorbance}}] \times 100$ (Togola et al., 2019a)

Data analysis:-

The data obtained were processed using an Excel® spreadsheet version 2013. The ANOVA was chosen to compare the mean total polyphenol and flavonoid contents at the 0.05 threshold using Minitab 18.1 software.

Results and Discussion:-

Phytochemical screening:-

The results of the phytochemical screening are recorded in table 2 below. The phytochemical study has revealed the presence of flavonoids, anthocyanins, steroid terpenes, reducing sugars in the fruits of the four species studied. The presence of these secondary metabolites could be responsible for the anti-inflammatory, analgesic, antioxidant activities of these fruit extracts; which would justify their use in traditional medicine (Haidara et al., 2016; Keita et al., 2018; Traoré et al., 2019). The mucilages were detected in the fruits of Raphia sudanica and Zizyphus mauritiana. However the alkaloids have not been detected in the four fruits studied. Similarly, tannins, coumarins and saponins have not been found in the fruits of Saba senegalensis. Yougbaré-Ziébrou et al. (2015) have highlighted saponins in the leaf stems of Saba senegalensis.

Our findings match the results of previous studies (Kini et al., 2012; Keita et al., 2018). Indeed, these authors have shown the presence of the same metabolites in the fruits of Balanites and Zizyphus. Kini et al. (2012) have also reported the absence of alkaloids, tannins, coumarins and saponins and the presence of anthocyanins, terpenes and flavonoids in the fruits of Saba senegalensis.

	Samples				
Chemical groups	Balanites aegyptiaca	Raphia sudanica	Saba senegalensis	Zizyphus mauritiana	
Alkaloids	-	-	-	-	
Flavonoids	+	+	+	+	
Tannins	+	+	-	+	
Coumarins	+	+	-	+	
Saponins	+	-	-	-	
Anthocyans	+	+	+	+	
Mucilages	-	+	-	+	
Steroid terpenes	+	+	+	+	
Reducing sugars	+	+	+	+	

Table 2:- Characterization of chemical groups.

* Presence (+); Absence (-).

Total polyphenol and flavonoid assays:-

Many studies have shown that several metabolites are involved in the antioxidant activities of plant extracts. Among these metabolites, total polyphenols and flavonoids play an important role (Fatima et al., 2015; Bagewadi et al., 2019; Togola et al., 2019a). That is the reason why we were interested in evaluating their contents in our extracts. The calibration curves obtained from the absorbances of the different concentrations are shown in figures 2 and 3 respectively for gallic acid and catechin.



Figure 2:- Calibration curve of gallic acid.



Figure 3:- Calibration curve of catechin.

The contents of polyphenols (mg GAE/100 mg extract) and flavonoids (mg CE/100 mg extract) obtained are shown in figure 4.



Figure 4:- Total polyphenol and flavonoid contents.

The figure 4 showed that the total polyphenol (p-value of 0.00031 < 0.05) and flavonoid (p-value of 0.0032 < 0.05) contents have been varied from one fruit to another.

The highest polyphenol content has been observed in the fruits of Zizyphus mauritiana $(1.72 \pm 0.07 \text{ mg GAE}/100 \text{ mg})$ followed by those of Raphia sudanica $(1.57 \pm 0.04 \text{ mg GAE}/100 \text{ mg})$; Balanites aegyptiaca $(1.44 \pm 0.08 \text{ mg GAE}/100 \text{ mg})$ and finally Saba senegalensis $(0.99 \pm 0.07 \text{ mg GAE}/100 \text{ mg})$. The highest amount of Flavonoids is found in Raphia sudanica fruits with $0.35 \pm 0.04 \text{ mg CE}/100 \text{ mg}$ whereas the lowest content is observed in Balanites

aegyptiaca fruits with 0.16 ± 0.03 mg CE/100 mg. Aiche & Ait Aissa (2017) obtained lower levels of polyphenols with seeds (1.3 g GAE/100 g) and higher levels with leaves (8.4 g GAE/100 g) with hydromethanolic extracts of Zizyphus mauritiana. For flavonoids, he obtained 0.97 g quercetin equivalent/100g for seed and 8.3 g quercetin equivalent/100g for leaf. Halimi (2016) obtained higher levels than ours with the delipidated fruits of Zizyphus lotus, 3.5 % equivalent gallic acid of polyphenols against 3 % equivalent catechin of flavonoids. In 2007, a team of researchers from the Centre for International Cooperation in Agronomic Research for Development (CIRAD) measured the quantities of polyphenols from 24 fruits consumed in Europe (CIRAD, 2007). The maximum contents that they have recorded (in % GAE) in strawberries (0.26 %), lychee (0.22 %) and grapes (0.20 %); were lower than polyphenol contents that we have observed with our results. Studies have shown that the preventive properties of food and medicinal plants are due to the presence of polyphenols and flavonoids, among others, which are molecules with antioxidant power, hence their importance in regulating oxidative stress (Chaouche, 2014; Fagbohoun, 2014).

In scientific literature, it has been reported that the consumption of fruits rich in flavonoids reduces the risk of coronary thrombosis and myocardial infarction through their platelet inhibitory effect (Chira et al., 2008). The same study showed that phenolic compounds in red grapes (20-170 mg/kg at the pulp level) prevented the development of early atherosclerosis induced by atherogenic feeding. In these studies, the consumption of grapes (Hamburg Muscat variety at a dose equivalent to 600 g/day for a 70 kg man) or their juice (dose equivalent to 500 mL/day for a 70 kg man) reduced the surface area of lipid deposits on the aortic stick by 78 % and 93 % respectively. The physiological effects obtained for the nutritional consumption of grape polyphenol extract on atherosclerosis, diabetes or hypertension show an *in vivo* prevention of these pathologies. Grape polyphenols can therefore play a preventive nutritional role (Chira et al., 2008). The antimicrobial and antioxidant effects of these compounds have also been reported by many authors (Fofié et al., 2017; Seggani & Boukehil, 2017; Keita et al., 2018; Togola et al., 2019b).

So our fruits with their polyphenol and flavonoid contents could serve as potential candidates in the fight against these diseases and malnutrition.



The figure 5 illustrates the chromatogram of the in vitro antioxidant activity of fruit extracts revealed by DPPH.



Figure 5:- Chromatogram of in vitro antioxidant activity.

This chromatogram showed that all extracts have revealed yellow patches on a purple background, indicating that the extracts have reduced the free radical DPPH. This activity could be due to the polyphenolic constituents present in these extracts. Many studies have attributed the antioxidant effect of plant extracts to polyphenols and flavonoids (Nesrine, 2014; Bruneton, 2016; Seggani & Boukehil, 2017). Therefore, the relatively high levels of polyphenols and flavonoids show that our fruits are said to be rich in antioxidants, which are currently the subject of many studies.

Evaluation of in vitro potential antioxidant:-

The antiradical potential of the extracts was evaluated by the DPPH method. The results obtained were expressed as percentages of inhibition of free radicals in DPPH (table 3).

Concentrations (µg/mL)	25	50	75	100	125
Extracts	DPPH free radical inhibition rate (%)				
Balanites	$6,80\pm2,18^{\circ}$	$15,84\pm2,29^{\circ}$	$25,64\pm2,63^{bc}$	36,25±1,53 ^c	48,06±6,13 ^b
Saba	16,03±0,79 ^b	20,94±0,50 ^b	$22,60\pm1,18^{\circ}$	$26,37\pm0,42^{d}$	$30,66\pm2,16^{\circ}$
Zizyphus	9,72±2,02 ^c	20,93±2,30 ^b	30,42±3,01 ^b	42,97±4,37 ^b	52,17±2,85 ^b
Raphia	36,09±3,17 ^a	$44,06\pm0,89^{a}$	52,43±4,34 ^a	57,82±2,41 ^a	70,26±2,11 ^a
P-value	0,01E-4	0,15E-6	0,08E-4	0,03E-4	0,09E-4

Table 3:- DPPH free radical inhibition rate (%) by extracts

*The difference has been significant when p-value < 0.05.

The table shows that the percentage of inhibition varies according to extracts and concentrations (p-value < 0.05). At the concentration of 100 µg/mL of extracts, the free radical inhibition rate has ranged from $16.07 \pm 2.08\%$ in S. senegalensis to 57.82 ± 2.41 in R. sudanica. At $125 \mu g/mL$, this rate has varied in the same order and at the same extract level from $21.38 \pm 0.26\%$ to $70.26 \pm 2.11\%$. This classification is consistent with the polyphenol and flavonoid levels obtained at the level of extracts, the highest of which have been recorded in R. sudanica extracts with 1.57 ± 0.04 mg GAE/100mg for polyphenols and 0.35 ± 0.04 mg GAE/100mg for polyphenols and 0.17 ± 0.01 mg EC/100mg for flavonoids (Figure 4). These results have revealed a strong capacity to reduce free radicals by extracts, which shows a strong antioxidant activity. However, all our extracts are less active than our positive control: ascorbic acid which at the concentration of 10 µg/mL induces a 94.45 % reduction.

Our results are similars to those reported by Abdoune & Yahiaoui (2015) which were 50.99 % inhibition rate with Zizyphus mauritiana fruit extracts. With different extracts (aqueous, methanolic and acetate) of Zizyphus fruit, Adjdir et al (2018) have also reported similar reduction capacities to ours with IC₅₀ ranging from 12 to 503.6 μ g/mL. On the other hand, the inhibition rates (93.98 %) have been reported by Kabine et al. (2015) with Raphia sudanica fruit extracts are higher than ours.

It appears from this study that the extracts of our different fruits have a good antiradical activity. Antiradical activity being a good indicator of antioxidant potential, our extracts could be a potential source of natural antioxidants, which are currently in high demand. Because in addition to a definite interest in the conservation of edible foodstuffs and against photophobia-oxidation or photodegradation, they could prove useful in the prophylaxis and treatment of diseases in which oxidative stress would be incriminated (Kouamé et al., 2009; Tounkara et al., 2014). For this reason, wild fruits are currently of great interest not only for the fight against malnutrition but also and above all for the treatment and/or prevention of some chronic diseases such as diabetes, cancer and high blood pressure, as shown by epidemiological studies that have shown that cancer rates are low among people who consume a lot of antioxidant-rich fruits (Riel, 1999; Kabine et al., 2015). Therefore, in addition to their nutritional potential, the fruits of B. aegyptiaca, Z. mauritiana, R. sudanica and S. senegalensis would be promising in the fight against antioxidant stress.

Correlation between in vitro antioxidant activity and polyphenol and flavonoid contents:-

To understand the involvement of polyphenols and flavonoids in the in vitro antioxidant activity of our extracts, we performed the main component analysis. The results of this analysis are shown in figure 6.

This diagram revealed a strong correlation between in vitro antioxidant activity and total polyphenol and flavonoid levels in extracts. However, this correlation is even stronger with flavonoids than with polyphenols. These

observations are in agreement with those of several authors (Nesrine, 2014; Bruneton, 2016; Seggani & Boukehil, 2017).



Figure 6:- Double projection diagram showing the correlations between *in vitro* antioxidant activity and polyphenol and flavonoid contents.

Conclusion:-

This study showed that the fruit pulp extracts from the four wild food species studied contain many metabolites, which would justify their food and medicinal uses. The results of the assay showed that these fruits would be a potential source of polyphenols, flavonoids and natural antioxidants. Therefore, in addition to their nutritional potential, the fruits of *B. aegyptiaca*, *Z. mauritiana*, *R. sudanica* and *S. senegalensis* would be promising in the fight against antioxidant stress.

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