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EVALUATION OF PHENOLIC COMPOUNDS AND ANTIDIABETIC ACTIVITY OF ALMOND EXTRACTS FROM KERNELS OF TWO MANGO VARIETIES GROWN IN NORTHERN CÔTE D'IVOIRE

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ABSTRACT

Of all the mangoes, fruit of mango tree (*Mangifera indica* L.) produced in North of Côte d'Ivoire, only less than half is exported. The rest of these mangoes are intended for local consumption and processing of pulp, when they have not deteriorated. Thus, kernels resulting from mangoes processing and deterioration constitute a real problem of environnemental pollution. Therefore, this study aims to explore a way of therapeutic valorization of almond from mango kernel of varieties Keitt and Kent. For this, phenolic compound of aqueous and hydroethanolic extracts of flours from almonds of mango kernel were analyzed by spectrophotometric assay. Then, antidiabetic activity of hydroethanolic extracts of at 100 mg/kg body weight was determined on alloxanic Wistar rats compared with glibenclamide for 7 days. The highest polyphenol contents were obtained with extracts from flour of Kent variety with values of 34 ± 00 mg EAG/g and 32.67 ± 0.02 mg EAG/g respectively for aqueous and hydroethanolic extracts. This variety also had the highest tannin content, with 23620 ± 0.66 mg EAT/g and 23043 ± 0.44 mg EAT/g respectively for aqueous and hydroethanol extracts. Extracts from flour of Keitt variety recorded the highest flavonoid content with 11 ± 0.02 mg EQ/g for aqueous extract and 10.5 ± 0.01 mg EQ/g for hydroethanolic extract. Moreover, the hydroethanolic extracts of both varieties showed strong hypoglycemic potential in rats compared with glibenclamide. Particulary, hydroethanol extract of Keitt variety showed greater hypoglycemic activity with reducing blood glucose levels by 44 ± 0%. This research work highlighted that extracts of almonds from kernel of mango varieties Kent and Keitt contain high concentration of phenolic compound and exerts a strong antidiabetic activity.

KEYWORDS: Mango, kernel, almond, aqueous extract, hydroethanolic extract, phenolic compound, antidiabetic activity, Côte d'Ivoire.

INTRODUCTION

Mango, fruit of the mango tree (*Mangifera indica L*), now has more than 1,000 different cultivars[1] and is the world's fifth most cultivated tropical fruit, after bananas, apples, grapes and citrus fruits.[2] It is mainly processed into juices and nectars, canned cheeks and pieces with added syrup. It is also processed into fruit pastes, chutneys and sauces, as well as frozen purées and pulps.[3] Mango is a fruit with a high nutritional value, known for its richness in antioxidants, provitamin A and vitamin C, with around 27 mg/100 g of fresh matter. With an energy content of 56 Kcals per 100 g of fruit, mango is one of the medium-calorie fruits.[4] Annual production is estimated at over 50 million tonnes in 2018, with India the main producer, accounting for 40% of world production, followed by China (11%).[5] However, despite its consumption and industrial processing, this agricultural sector experiences huge post-harvest losses.[6] These losses are generally considered as waste, and mainly concern consumption by-products, such as skin and kernel.[7] Work carried out by[8] estimates that between 35% and 60% of waste is generated during mango processing alone. All this waste poses enormous environmental pollution problems.[9] In view of all these losses incurred by farmers and industrialists, and the ecological consequences of this waste, several authors have carried out research to find solutions to this phenomenon. Their results show that the

almond present in mango kernels is a by-product rich in nutritional compounds such as lipids, proteins, carbohydrates and amino acids.[10] It also contains powerful antioxidant activity, with relatively high phenolic contents.[11]

In view of these intersting characteristics, it is necessary to investigate almonds from mango kernels in purpose to therapeutic use. Therefore, the present study aims to evaluate the phenolic compounds and antidiabetic activity of aqueous and hydroethanolic extracts of almonds from kernels of two mango varieties (Kent and Keitt) grown mainly in northern Côte d'Ivoire.

MATERIALS AND METHODS

Plant material

The pits of mango varieties Keitt and Kent were collected respectively from orchards and dried mango factory of Cooperative "Gninnangnon" in Korhogo (Northern Côte d'Ivoire) in May 2021. After collection, almonds from kernel of mango varieties Keitt and Kent were removed, washed and sun-dried at 32°-35°C for 10 hours a day during 8 days, before shelled. After drying, the almonds were removed, cut into small pieces **(figure 1)** and ground to produce flours from kernels of mango varieties Keitt and Kent **(figure 2)**. The flours were used for preparation of aqueous and hydroethanolic extracts of mango varieties Keitt and Kent.

Figure 1: Pieces of mango kernel almond.

Figure 2: Flour of mango kernel almond.

Animal material

Twenty five (25) male and female Wistar rats of three (3) months and weighing 150 ± 0.1 g on average, were used to study the antidiabetic activity of aqueous and hydroethanolic extracts of almonds from kernel of mango varieties Keitt and Kent. These animals were supplied in August 2023 by the Pharmacology Laboratory of Pharmaceutical and Biological Sciences Unit of Training and Research at University Felix HOUPHOUET-BOIGNY (Abidjan, Côte d'Ivoire).

Preparation of extracts from almonds of mango kernel

The aqueous extracts of mango varietes Keitt (KIaq) and Kent (KEaq) were prepared by macerating 100 g of flour in 1 L of distilled water under magnetic stirrer for 24 h at room temperature. After homogenization, mixture was filtered through absorbent cotton and filtrate was concentrated in an oven at 50°C until total evaporation of solvent, to obtain the aqueous extracts. The hydroethanolic extracts of mango varieties Keitt (KIhe) and Kent (KEhe) were prepared under the same conditions with as extraction solvent, a mixture composed of ethanol/water (70/30) (v/v).

Determination of phenolic compounds in almond extracts

Total polyphenols

The method of[12] was used for the determination of total polyphenols. A volume of 2.5 mL of diluted (1/10) Folin-Ciocalteu reagent was added to 30 µL of extract. The mixture was kept for 2 minutes in the dark at room temperature, then 2 mL of sodium carbonate solution (75 g/L) was added. The mixture was then placed in a water bath at 50°C for 15 minutes before rapidly cooled. Absorbance was measured at 760 nm, using distilled water as the blank. A calibration line was made with gallic acid at different concentrations. Polyphenol concentration was expressed in milligram extract gallic acid equivalent (mg EAG/g extract) from a gallic acid calibration line (Y = 1.645 X + 0.0113, R2= 0.9945).

Flavonoids

Total flavonoids were determined using the method described in.[13] In flask of 25 mL, 0.75 mL sodium nitrite (NaNO2) at 5% (w/v) was added to 2.5 mL extract. The mixture was supplemented with 0.75 mL of aluminum chloride (AlCl3) at 10% (w/v) and then incubated for 6 minutes in the dark. After incubation, 5 mL of sodium hydroxide (NaOH, 1N) was added and the volume made up to 25 mL. The mixture was shaken

vigorously before being determined by UV spectrophotometer at 510 nm. A quercetin stock solution was prepared under the same conditions as the assay and used as a reference standard. Flavonoid contents of extracts were expressed in milligrams of quercetin equivalent per gram of dry extract (mg EQ/g extract) from a quercetin calibration line (Y= 6.2 X+ 0.0067, R2 = 0.994).

Total tannins

Total tannin content was determined according to the method described by.[14] 50 mL of each extract was added to 1500 µL of vanillin solution (4%) in methanol. The resulting mixture was vigorously shaken and 750 µL of concentrated hydrochloric acid added. The resulting mixture was left to react at room temperature for 20 minutes. Absorbance was measured at a wavelength of 550 nm against a blank consisting of the vanillin solution (4%) in methanol. A stock solution of tannic acid was used as a reference standard to establish the calibration curve and quantify condensed tannin content, expressed in milligram equivalents of tannic acid per gram of dry matter (mg EAT/g dry matter). The condensed tannin content of extracts was determined from the tannic acid calibration line ($Y = 0.0009X + 0.0129$, $R^2 = 0.9994$).

Determination of antidiabetic activity of almond extracts

The antidiabetic activity was determined using hydroethanol extracts of kernel kernels of the two mango varieties using the method described by.[15] Wistar rats were treated by subcutaneous injection of alloxane monohydrate (120 mg/kg bw) dissolved in saline to establish permanent diabetes (hyperglycemia). Blood was drawn from the tail vein of the animals to determine normal blood glucose levels using a glucometer prior to alloxane injection (D-2). Then, 48 hours after alloxane administration (D0), the rats' glycosuria was determined using test strips (Kéto-diastix). Twelve (12) animals showing frank glycosuria were selected for experimentation and divided into four (4) batches of three (3) rats each, treated as follows:

- Batch 1 (control): physiological water at 10 mL/Kg;
- Batch 2: Glibenclamide 0.3mg/kg, an oral antidiabetic of sulfonamide hypoglycemic family;
- Batch 3: hydroethanolic extract of almond from Kent kernels (100 mg/Kg).
- Batch 4: hydroethanolic extract of almond from Keitt kernels (100 mg/Kg).

Treatment was carried out per os every other day for 7 days (D0; D2; D4; D6), during which blood samples were taken from the tail vein and after treatment was stopped until D10 to measure blood glucose levels using a glucometer.

Statistical analysis

Analysis were performed in triplicate, and results were expressed as means together with standard error of mean (Mean ± SEM) using Excel 2013 software. Means were compared using Student's t-test with GraphPad Prism 8.2.1. The difference was considered significant when $P<0.05$. Graphical representations of data were also produced using Graph Pad Prism 8.2.1 software.

RESULTS AND DISCUSSION

Phenolic compound content

Total polyphenols

The total polyphenol contents of mango kernel extracts are shown in **figure 3.** The highest contents were obtained with the aqueous or KEaq (34 ± 00 mg EAG/g) and hydroethanolic or KEhe (32.67 ± 0.02 mg EAG/g) extracts of the Kent variety, while the lowest contents were recorded with the aqueous (KIaq) and hydroethanolic (KIhe) extracts of Keitt respectively 31.83 ± 0.03 mg EAG/g and 23.33 ± 0.04 mg EAG/g. However, there was no significant difference ($P>0.05$) between the polyphenol contents of KEaq, KEhe and KIaq extracts. These results are superior to those of[16], who obtained polyphenol contents for aqueous extracts of Indian mango kernels of the Neelam and Banganpalle varieties of 9.98 ± 0.02 mg EAG/g and 7.49 ± 0.07 mg EAG/g respectively. This difference in values could be due to the mango varieties used, as well as to climatic and soil conditions.

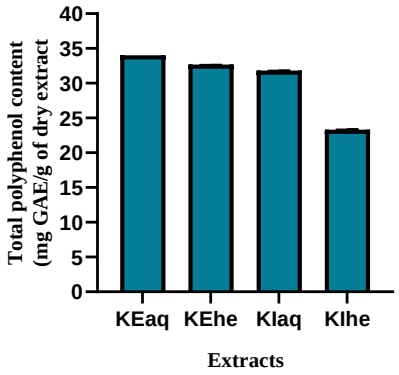

Figure 3: Total polyphenol content of mango kernel extracts.

KEaq: Kent aqueous extract; KEhe: Kent hydroethanol extract; KIaq: Keitt aqueous extract; KIeh: Keitt hydroethanol extract. Bars with different letters are significantly different (P < 0.05).

Flavonoid content

Figure 4 shows the flavonoid contents of kernel extracts from the two mango varieties. Keitt extracts recorded the highest contents of 11 ± 0.02 mg EQ/g for KIhe and 10.5 ± 0.01 mg EQ/g for KIaq. The lowest content (10 ± 0.00 mg EQ/g) was obtained with KEaq. However, there was no significant difference between these levels ($P>0.05$). Flavonoid levels in this study are lower than those in.[16] Indeed, in India, these authors recorded mean contents for aqueous and methanolic extracts of almonds of local varieties of 25 mg EQ/g and 93 mg EQ/g respectively. This variation in flavonoid content could be

due to the mango variety used, climatic and soil conditions, and the nature of the extraction solvent used.

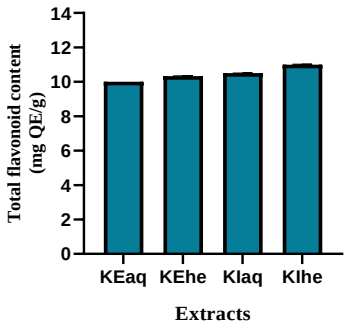

Figure 4: Total flavonoid content of mango kernel extracts.

KEaq: Kent aqueous extract; KEhe: Kent hydroethanol extract; KIaq: Keitt aqueous extract; KIeh: Keitt hydroethanol extract. Bars with different letters are significantly different (P < 0.05).

Total tannin content

The total tannin content of the different extracts is shown in **figure 5**. The results show that Kent kernels contain more condensed tannins than Keitt kernels. Indeed, total tannin contents were respectively 23620 ± 0.66 mg EAT/g dry extract for KEaq, 23043 ± 0.44 mg EAT/g for KEhe, 22586 ± 0.63 mg EAT/g for KIaq and 17614 ± 0.02 mg EAT/g for KIhe. Statistical analysis revealed a significant difference between extract contents (P < 0.05).

These results are considerably higher than those of[17], who obtained contents of 45.28 ± 0.26 mg EAT/g and 71.81 ± 0.33 mg EAT/g respectively for ethanolic extracts of mango kernels of the Opioro and Julie varieties grown in Nigeria. This variation in extract tannin content could also be due to the mango variety used, climatic and soil conditions, and the nature of the extraction solvent used.

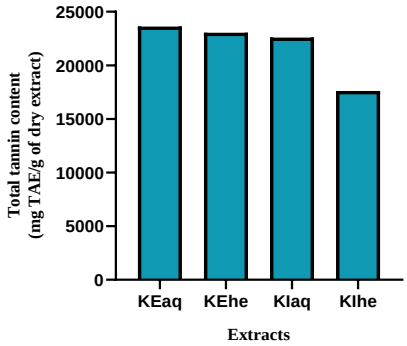

Figure 5: Condensed tannin content of mango kernel extracts.

KEaq: Kent aqueous extract; KEhe: Kent hydroethanol extract; KIaq: Keitt aqueous extract; KIeh: Keitt hydroethanol extract. Bars with different letters are significantly different (P < 0.05).

Antidiabetic activity of extracts

The results of the antidiabetic activity in alloxanic rats are presented in Table I. These results show that 48 hours after alloxane administration, hyperglycemia was observed in rats from different batches (1.88 g/L to 1.93 g/L). This hyperglycemia persisted throughout the 10-day experiment in control rats. However, blood glucose levels in glibenclamide-treated rats fell by half (51.53%) from D0 to D10, from 1.96 ± 1.11 g/L to 0.95 ± 2.89 g/L. In the case of rats treated with the extracts, blood glucose levels in batch 4 receiving the KIhe extract fell from 1.94 ± 2.67 g/L to 1.07 ± 2.89 g/L (44.84%). As for batch 3 treated with KEhe extract, blood glucose levels fell from 1.95 ± 1.78 g/L to 1.27 ± 6.67 g/L, or 34.87%. These results show that the different extracts showed appreciable antidiabetic activity, but less than that of glibenclamide, with a significant difference (P < 0.05). They are superior to those of[18], who evaluated the antidiabetic effect of ethanolic extract of Indian mango kernel at a dose of 100 mg/kg on alloxanic Wistar rats. In fact, these authors obtained blood glucose levels ranging from 255.57 ± 2.77 mg/dL to 195.93 ± 2.94 mg/dL in 14 days of treatment, with a 23.33% reduction in blood sugar levels. This difference could be due to the origin of the mango, climatic and soil conditions, and solvent used.

The antidiabetic activity of KIhe and KEhe extracts could be explained by the presence of phytochemicals in hydroethanol extracts tested. Indeed, phytochemical screening work carried out on these extracts by[19] revealed the presence of total polyphenols, flavonoids, tannins, terpenes and sterols. Moreover, the phytochemical assay carried out in the present study also showed the presence and high quantity of phenolic compounds such as total polyphenols, flavonoids and tannins. These compounds could therefore be responsible for the antidiabetic effect exerted by mango kernel extracts. In fact, according to several authors, these compounds are endowed with strong antioxidant powers, giving them antidiabetic properties.[20,21]

Table I: Blood glucose values (g/L) of alloxanic rats treated with glibenclamide and extracts of almond from mango kernels.

Batches	Time (days)							Reduction in blood glucose (%)
	D-2	D0	D2	D4	D6	D8	D10	
Batch 1 : Negative control	0.71 ± 6.00	1.87 ± 2.44	1.93 ± 4.00	1.88 ± 1.33	1.84 ± 0.89	1.87 ± 0.44	1.88 ± 1.33	00[a]
Batch 2 : Positive control (Glibenclamide)	0.72 ± 2.89	1.96 ± 1.11	1.89 ± 0.44	1.64 ± 6.22	1.36 ± 10.66	1.23 ± 7.55	0.95 ± 2,89	51.53[b]
Batch 3 : KEhe (100mg/kg bw)	0.73 ± 8.22	1.95 ± 1.78	1.88 ± 1.78	1.80 ± 1.11	1.65 ± 3.11	1.46 ± 0.89	1.27 ± 6.67	34.87[c]
Batch 5 : KIhe (100mg/kg bw)	0.75 ± 3.78	1.94 ± 2.67	1.93 ± 1.78	1.62 ± 2.89	1.42 ± 4.44	1.28 ± 3.33	1.07 ± 2.89	44.84[d]

CONCLUSION

This research work was conducted to evaluate composition in phenolic compounds and antidiabetic activity of almond extracts from kernel of mango Kent and Keitt varieties grown in northern Côte d'Ivoire. These investigations revealed that almond extracts from kernels of both mango varieties are very rich in total polyphenols, flavonoids and tannins. In addition, these extracts showed strong antidiabetic potential compared with the usual antidiabetic agent (glibenclamide). These results could provide a good basis for formulation of dietary supplement based on almond of mango kernels to fight against diabetes.

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