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Phytochemical screening and antimicrobial activities of almonds extracts from kernel of two mango (*Mangifera indica L.*) varieties grown in Korhogo (North of Ivory Coast)

Pascal Ahoussi BONI [1], Abdoulaye TOURE [1, 3, *], Landry Claude Ahmont KABLAN [1], Sophie Monnou GUEHI [1], Rodrigue Kouamé N'GUESSAN [1], Fabrice Armel ZORO [1], Naka TOURE [1, 4], Faustin Aka KABRAN [2] and Adama COULIBALY [3]

[1] *Laboratory of Biotechnology and Valorization of Agroresources and Natural Substances, Training and Research Unit of Biological Sciences, Peleforo GON COULIBALY University, Po.Box: 1328 Korhogo, Ivory Coast.*

[2] *Laboratory of Constitution and Reaction of Matter, Training and Research Unit of Sciences of Structure, Matter and Technology, Félix HOUPHOUËT-BOIGNY University, Po.Box: 582 Abidjan 22, Ivory Coast.*

[3] *Laboratory of Biochemical Pharmacodynamy, Training and Research Unit of Biosciences, Félix HOUPHOUËT-BOIGNY University, Po.Box: 582 Abidjan 22, Ivory Coast.*

[4] *Laboratoire of Biocatalysis and Bioprocess, Training and Research Unit of Food Science and Technology, NANGUI ABROGOUA University, Po.Box. 801 Abidjan 02, Ivory Coast.*

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Abstract

Mango (*Mangifera indica L.*) is a fruit marketed worldwide. It is a third fruit exported by Côte d'Ivoire with 10000-15000 tons for annual production among 150000 tons. The by-products of mango such as kernel almonds are not pratically valued. Therefore, this work aims to determine phytochemical compound and evaluate antimicrobial activity of extracts from kernel almonds of mango varieties Kent and Keitt. Aqueous and hydroethanolic extracts were prepared with almonds flours of Kent (EtaqKE and EethKE) and Keitt (EtaqKI and EethKI) varieties. Phytochemical screening of the four extracts was performed by tube tests and thin layer chromatography. Antimicrobial activity of these extracts was evaluated *in vitro* with agar broth dilution method against three bacteria (*Escheichia coli, Staphylococcus aureus* and *Pseudomans aeruginosa*) and three fungi (*Candida albicans, Trichophyton rubrum* and *Trichophyton mentagrophytes*) involved in skin infections. Phytochemical screening revealed presence of polyphenols, sterols, terpenes, flavonoids, anthraquinones and saponins in all the four almonds extracts. Antimicrobial tests showed that, extracts EethKE and EethKI are the most active with MIC and MBC ranging respectively from 0.39 to 12.5 mg/mL and 12.5 to 50 mg/mL for bacteria. The extract EtaqKI is more active on bacteria and has a bactericidal action on *P. aeruginosa* and *E. coli* strains with ratio CMB/CMI of 4. The four almonds extracts have any activity on fungi strains tested. The presence of secondary metabolites and antibacterial activities of extracts of almond from kernels of mango Kent and Keitt varieties demonstrate that these extracts could have therapeutic and cosmetic applications.

Keywords: *Mangifera indica*; Kernel almonds; Kent and Keitt; Phytochemical; Antimicrobial; Korhogo

1. Introduction

The ivorian flora contains many plants that contribute to nutritional, medicinal and cosmetic needs and economic activity of the populations [1]. The study of plants with potentially valuable properties in medicinal and food fields is a current topic. Moreover, mango is a much consumed fruit in Côte d'Ivoire. The main mango production region is located in the north, with Korhogo city, located about 650 km north of Abidjan. Many varieties of mangoes are produced. Among them, the most commercialized are Amelie and Kent. Harvests begin in March and last until July [2]. Mango is mainly

* Corresponding author: Abdoulaye Touré

consumed or traded fresh and does not keep for very long after ripening. Despite nutritional importance of mango and dietary interest of the populations, it is subject to huge post-harvest losses. The work of [3] have globally quantified the post-harvest losses of mangoes in the world at about 80 %. The mango kernel represents the largest part of these post-harvest losses because it is not consumed. Indeed, only the pulp is consumed. It appears that the kernel almond of mango is a by-product rich in nutritional compounds, such as lipids, proteins, carbohydrates and amino acids [4]. Therefore, it would be interesting to valorize the kernel almond of mango in medicinal and cosmetic fields. The objective of present study is to determine phytochemical compounds and evaluate antimicrobial activites of aqueous and hydroethanolic extracts from kernel almond of two mango varieties, Kent and Keitt, cultivated in Korhogo (northern Côte d'Ivoire).

2. Material and methods

2.1. Materials

2.1.1. Biological material

Two mango varieties, Kent and Keitt, were harvested in ochards of GNINNAGNON Cooperative society from Korhogo city (North of Ivory Coast) during the 2021 season.

2.1.2. Microorganisms strains

The microorganisms used in this study consist of three fungal strains including (*Candida albicans*, *Trichophyton rubrum* and *Trichophyton mentagrophytes*). Three (03) bacterial strains (*Escherichia coli*, *Staphylococcus aureus* and *Pseudomanas aerugionsa*) were also used. All these strains were provided by Laboratory of Microbiology of Floristic National Center of Félix HOUPHOUËT-BOIGNY University (Abidjan, Ivory Coast).

2.1.3. Culture media

Sabouraud agar glucosed with chloramphenicol (references 01-366-500503081; Lot 50308) is a culture media used to perform antifungal test. As for antibacterial test, Mueller-Hinton agar (Code: M39; Lot B001196) and Mueller-Hinton broth (AFSSAPS n°Z2638 O lot/ch-B: 1L0242) were used.

2.1.4. Chemicals and reagent

The chemicals used consisted of: 100% distilled water, 70% ethanol and 30% distilled water were used as extraction solvents. 100% methanol (MeOH), 100% dichloromethane (DCM), 100% ethyl acetate (AcOet), 100% hexane, 100% ethanol, a tertiary mixture of methanol (15%), ethyl acetate (5%) with dichloromethane (80%), Dragendorff's reagent, iron chloride ($FeCl_3$), vanillin sulfur, potassium hydroxide (KOH), sodium hydroxide (NaOH), gallic acid ($C_7H_6O_5$), aluminum chloride ($AlCl_3$) and sodium chloride (NaCl) were used.

2.1.5. Technical equipment

The technical equipment used for realization of this work consists of a balance of precision balance (420 g ± 0.001 g), a mechanical grinder type Ikamag, petri dishes, capillary tubes, an autoclave, an incubator, a hot plate, an oven (BIOBASE 50 °C), a filtration device (funnels and absorbent cotton), an extractor hood (ASEM EN 14175, Italy), aluminium foil, tweezers, a spectrophotometer (JENWAY 7315), silica gel 60 F254 chromatographic plates on aluminium support (Merck KGaA, 64271 Darmstadt, Germany).

2.2. Methods

2.2.1. Preparation of almonds extracts from kernel of mango varieties Kent and Keitt

The preparation of aqueous extracts was carried out according to the method described below: 100 g of each flour (Kent and keitt) was mixed with 1000 mL of distilled water and subjected to a reflux decoction for 30 min. Then, the solution was let cool down before it double filtration on a white cloth and on absorbent cotton. The filtrates were collected in a jar and then steamed at 50 °C for one week to obtain the dry extracts of flours. The hydro-ethanol extracts were prepared from 100 g of powder of each flour in 1000 mL of ethanol/water mixture (70 mL ethanol + 30 mL water) for 30 min. After filtration, the filtrates were combined in a jar and then steamed at 50 °C for one week to obtain a dry hydroalcoholic extract of each flour.

The yields were calculated using the following formula (1):

$$R\ (\%) = (m\ /M) \times 100 \qquad (1)$$

M: mass of mango flour (100 g); m: mass of crude extract (g).

2.2.2. Phytochemical screening in tube of almonds extracts from mango Kent and Keitt varieties

Alkaloids test

Alkaloids were investigated according to the method described by [5] and [6]. These compounds were detected by Dragendorff (iodobismuthate depotassium), Valser-Mayer (potassium tetraiodomercurate) and Bouchardat (iodide depotassium) reagents. The principle of alkaloid research is based on the property of precipitating as salts in the presence of heavy metals such as bismuth, iodine, mercury, and tungsten. For this purpose, 1g of each dry extract of the two varieties (Kent or Keitt kernel) was placed in 10 mL of 90 % methanol. The mixture was stirred for 15 min. The extract was filtered and placed in each tube. Then, 1.5 mL of each stock solution was put into each tube. Subsequently, 1.5 mL of methanol was added and a few drops of Dragendorff's reagent. The appearance of orange coloration or precipitate indicates the presence of alkaloids.

Polyphenols test

Polyphenols were detected according to the procedure described by [7]. The principle of the research of phenolic compounds takes advantage of the formation of colored precipitate with a solution of ferric chloride (FeCl3). The appearance of a blue-blackish or green coloration indicates the presence of polyphenolic compound. The appreciation of this coloration is made in comparison with a control test with a reference phenol. For the detection of polyphenols, 1 g of dry extract (Kéitt variety or Kent variety) was placed in 10 mL of distilled water for 10 min. After shaking, the extract was filtered and placed in a tube. A drop of 2 % alcoholic ferric chloride solution (FeCl3) was then added. The appearance of a more or less dark blue-black or green coloration indicates the presence of phenolic compound.

Saponins test

Saponosides or saponins are steroidal or triterpenic heterosides with surface-active properties. They have been identified according to the procedure described by [8] and [9]. The principle consists in determining the persistent foam index during a 15 min period of a 2 % aqueous decoction. After cooling and filtration, the volume was readjusted to 100 mL. In a series of 4 test tubes numbered 1 to 4, introduce successively 1g of each extract. Adjust the volume of each tube to 10 mL with distilled water. Each tube is shaken horizontally for 15 seconds and then left to stand for 15 min. The height of the persistent foam in cm was then recorded. If it is less than 1cm in all tubs, the index is less than 100. The dilution in the tube where the height of the foam is equal to 1cm represents the desired index, indicating the presence of saponins.

Flavonoids test

Flavonoids are universal pigments of plants responsible for the coloration of fruits, flowers and sometimes leaves. To 2 mg of extracts diluted in 2 mL of ethanol, are added a few drops of a 1 % aluminum trichloride (AlCl3) solution. The appearance of a yellow-green color indicates the presence of flavonoids in the extract [10].

Anthraquinones test

The anthraquinone test was performed according to method of [11]. Two (2) mg of extracts to be analyzed (aqueous or hydroethanolic) were introduced in a hemolysis tube 2 mg. Then, 2 drops of NaOH are added and shaken. The pink-purple or red coloration indicates the presence of free anthraquinones.

Tannins test

Successively, 2 mg of extract, 2 mL of ethanol and 1 mL of Stiasny's reagent were introduced in test tubes. After homogenization, the tubes were placed in a water bath at 80°C for 30 min. The precipitate formation in tubes reveals the presence of gall tannins [12].

2.2.3. Phytochemical screening on thin layer chromatography plate

This test was performed following the method of [11] with some modifications. An aluminum thin layer chromatography **(**TLC) plate containing silica gel (stationary phase) on which we drew a start line (deposit) and an end

line was used. Using a capillary tube, a few drops of the different extracts were deposited on the plate. The drops were then air-dried. Afterwards, we prepared the mobile phase (eluent) using 3 solvents: methanol (1.5 mL), ethyl acetate (0.5 mL) and dichloromethane (8 mL). These solvents were put into a chromatography tank. Then, the TLC plates were put into the tank. The tank containing in the TLC plate is subsequently closed to avoid evaporation of the solvent. Once the eluent reached the front line, we removed the TLC plate and it was air dried. The separation spots are circled with a pencil and observed under visible light with the developers. The chromatograms are then examined under UV (visible) and the observed bands were circled. The color of the bands under UV was noted and the front will be used to calculate the front ratio (Rf). The detection of the spots was done thanks to the reagent developer. The front ratio Rf is calculated using the following equation (2):

$$Rf = \frac{d}{D} \qquad (2)$$

d: distance travelled by the substance ;
D: distance travelled by the solvent front

Alkaloid test

Different extracts were ¨spotted¨ on the plate. The particles of the different extracts migrate in an elution mixture. The plate is then air-dried. It is impregnated in Dragendorff's reagent and observed with the naked eye. The presence of orange stains in the presence of Dragendorff indicates the presence of alkaloids in the extract [11].

Polyphenol test

The method used for the polyphenol test is that of [13] with some modifications. Extracts are spotted on the plate. The particles of the different extracts migrate in a mixture of elution, then dried in the open air. On the plate, iron chloride (2 % to 2 mL) is used as a developer. The appearance of more or less dark blue-black or green coloration indicates the presence of phenolic compound.

Sterols and terpenes test

To perform the sterols and terpenes test the method followed is that of [14] with some modifications. The different extracts were spot tested. After migration of the particles into a glass jar containing elution solvents, the plate is air dried. Then, it is heated on a hot plate protected with aluminum foil after being immersed in sulfuric vanillin. The appearance of purple and green stains indicates the presence of sterols and terpenes.

Flavonoid test

The performance of the flavonoid assay follows the method described by [14], with modifications. The presence of flavonoids was performed on TLC for our different extracts. The extracts are spot on the plate. The particles of our different extracts migrate in an elution mixture, then air-dried. The plate is revealed thanks to the aluminum chloride reagent. The appearance of yellow, orange and pink stains indicates the presence of flavonoids.

Anthraquinone test

The method of [13] with some modifications was used to perform the anthraquinone test. Extracts are spotted on the plates. The particles of the different extracts migrate in a mixture of elution, then dried in the open air. On the plates, sodium hydroxide and potassium hydroxide are used as developers. The appearance of red or yellow coloration indicates the presence of anthraquinone.

2.2.4. Evaluation of antimicrobial activities of almonds extracts from kernel of mango varieties Kent and Keitt.

In this study, antifungal and antibacterial activities aqueous and hydroethanolic extracts of almonds from mango Kent and Keitt varieties were evaluated by the solid state dilution method.

2.2.5. Antifungal activities of mango almonds extracts by dilution method in solid medium

The antifungal activity was carried out by the dilution method in solid medium in order to study efficiency of extracts on fungi and to determine the Minimum Inhibitory Concentration (MIC) and Minimum Fungal Concentration (MFC). Sabouraud agar was prepared according to manufacturer's instructions. A series of ten (10) test tubs were used to test each extract. Each set consists of eight (8) test tubes and two (2) control tubes, one without extract, used as a control

for fungal growth (T) and the other, without germs or extract serving as a control for sterility (TS) of the culture medium [15, 16]. The test tubs contain a range of decreasing extract concentration that varies from 50 mg/mL to 0.39 mg/mL describing a geometric sequence of reason 1/2. To perform the double dilution, the incorporation of the extracts to the agar was done, homogenizing in the beaker 2g of each extract in 40 mL of Sabouraud. Half the volume of the mixture in the beaker was transferred to the first tube containing 20 mL of agar. The mixture was homogenized. This operation was repeated successively for the other tubes until tube 8 (C8), with the lowest concentration (0.39 mg/mL). For this last tube, half of the volume of the mixture is discarded. The tubes thus prepared were sterilized at 121 °C in an autoclave for 15 min before being aseptically poured into sterile Petri dishes or the marking of the fungi is done by lines on the lids of the dishes and at laboratory temperature to allow cooling and solidification of the agar [17, 18].

The fungal inoculum was prepared before inoculation of culture media on the petri dishes with old colonies obtained on Sabouraud agar during 2 to 10 days (2 to 3 days for *Candida albicans*, 3 to 5 days for *Trichophyton rubrum* and 5 to 10 days for *Trichophyton mentagrophytes).* Indeed, a perfectly isolated colony is rigorously shaken in 10 mL of sterile distilled water to give an estimated inoculum of 10^6 cfu/mL [19, 20]. After solidification of agar and preparation of inoculum, all petri dishes (C1 to C8 and the growth control) were inoculated with estimated at 10^5 cfu/mL, approximately 10^3 microbial cells, except for the sterility control. The inoculated plates were incubated at 30°C for 3 days for *Candida albicans* and 7 days for *Trichophyton rubrum* and *Trichophyton mentagrophytes*.) The experiment was performed three (3) times to reduce the risk of errors. The growth of fungi is read by a simple visible observation through a coloration or not of the sown line. After incubation, the minimum inhibitory concentration (MIC), minimum concentration for which there is no growth visible to the naked eye, is determined [19, 21]. The minimum fungal concentration (MFC) is the lowest concentration of extract in petri dishes that gives at least 99.99 % inhibition compared to the growth control dish or conversely, it is the dish that leaves a 0.01 % survival compared to the growth control dish. In practice, it is determined by a sterility test of the boxes without visible germ growth. In fact, a subculture of samples taken on the surface of the inhibitory concentrations from the MIC is carried out. These subcultures are made on a new agar without extracts and incubated for 3 to 7 days. The MFC will be the concentration from which the subculture shows no visible germ growth [22, 23].

2.2.6. Antibacterial activities of mango almonds extracts by the solid-state dilution method

Antibacterial tests of extracts were carried out by the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) by the solid-state dilution method. A serie of 10 tests tubes was used with eight (8) tubes numbered from C1 to C8 and two (2) control tubes, one without extract for growth control (TC) and the other without bacteria or extract for a sterility control of the culture medium (TS). Extracts were incorporated into agar by homogenizing 2 g of each extract in 40 mL of Mueller Hinton Agar (MHA) in beaker. Half of the volume of mixture in beaker was transferred to tube C1 containing 20 mL of MHA and the mixture was homogenized. This operation was repeated for tube C2 and so on by double dilution until tube C8. We thus obtain for a given extract, the concentration range from 50 mg/mL to 0.39 mg/mL (50 mg/ml; 25 mg/mL; 12.5 mg/mL; 6.25 mg/mL; 3.125 mg/mL; 1.562 mg/mL; 0.78 mg/mL; 0.39 mg/mL). In the TC tube in place of extract, he received 20 mL of MHA and the TS tube without bacteria or extract containing only 20 mL of MHA. The media thus prepared were sterilized at 121 °C for 15 minutes. They then maintained in supercooling at 47 °C before being spread aseptically in sterile petri dishes where the marking of bacteria is done by lines on lids of dishes. After solidification at room temperature, the media were oven-dried for 15 minutes at 37 °C before use. The bacteria inoculum was prepared at the time of seeding of the culture media of petri dishes, from colonies aged 18 to 24 hours obtained on MHA of each germ (*Eschericia coli*, *Staphylococcus aureus* and *Pseudomanas aeruginosa*). Indeed, a perfectly isolated colony is rigorously shaken in 10 mL of sterile distilled water to give an inoculum estimated at 10^6 cfu/mL [19, 20]. After solidification of agar and preparation of inoculum, all the petri dishes (C1 to C8 and the growth control) were inoculated with inoculum estimated at 10^5 cfu/mL, approximately 10^3 microbial cells, except for the sterility control. The inoculated plates were incubated at 37 °C for 24 hours for each germ. The experiment was performed three (3) times to reduce the risk of errors. After 24 hours of incubation, the growth and the microbial resistance were observed by staining or not the identifying features of the germs. The MIC is the lowest concentration of extract with any visible germ growth observed with the naked eye on culture medium after an incubation time of 18 to 24 hours [21, 23, 24]. The MBC is lowest concentration of extract in petri dishes that gives at least 99.99 % inhibition compared to growth control dish or conversely, it is the dish that leaves a 0.01 % survival compared to growth control dish. In practice, it is determined by a sterility test of the boxes without visible germ growth. In fact, a subculture of samples taken on surface of inhibitory concentrations from the MIC is carried out. These subcultures are made on a new agar without extracts and incubated at 37 °C for 24 hours. The MBC will be the concentration for which the subculture shows no visible germ growth [22, 24].

3. Results

3.1. Yields of different extractions of almonds from kernels of mango Kent and Keitt varieties

The aqueous extract of Kent variety prepared using distilled water gave a good yield of 10.30±0.70 % compared to the aqueous extract of Keitt variety which has a yield of 6.55±0.94 % (Table 1).The hydro-ethanol extract prepared from a distilled water/ethanol mixture gave a higher extraction yield for Kent variety 39.21±1.09 % compared to that of Keitt variety which is 20.07±0.82 %. The Kent variety has a good yield in different extractions.

Table 1 Yields of almond meal extracts of mango Kent and Keitt varieties

Extraction process	Types of almond meals	Yields %.
Aqueous extracts	Kent variety	10.30±0.70
	Keitt variety	6.55±0.94
Hydroethanolic extracts	Kent variety	39.21±1.09
	Keitt variety	20.07±0.82

NB: Values are expressed as mean and standard deviation (n = 3).

3.2. Characterization of extracts in tubes

The phytochemical tests in tube of the mango kernel extracts were carried out. The results of the preliminary phytochemical analysis of the extracts lead to the identification of several secondary metabolites. Table 2 shows that polyphenols and tannins are present in both varieties. While, saponins exist with a moderate concentration. However, anthraquinones and flavonoids are fairly present in both varieties. The absence of alkaloids in both varieties studied was observed.

Table 2 Secondary metabolites constituents of almond meal extracts of mango Kent and Keitt varieties in tube test

	Extracts of mango almonds			
Secondary metabolites	**EtaqKE**	**EethKE**	**EtaqKI**	**EethKI**
Alkaloids	-	-	-	-
Anthraquinone	+/-	+/-	+/-	+/-
Flavonoids	+/-	+/-	+/-	+/-
Polyphenols	+++	+++	+++	+++
Saponins	++	++	++	++
Tannins	+++	+++	+++	+++

(-) Absent; (+/-) present; (++) present at moderate concentration; (+++) present at high concentration; EtaqKE : Total aqueous extract of Kent almond; EethKE : Hydro-ethanolic extract of Kent almond; EtaqKI : Total aqueous extract of Kent almond; EethKI : Hydro-ethanolic extract of Kent almond.

3.3. Analysis of almonds extracts of mango Kent and Keitt varieties by thin layer chromatography (TLC)

TLC was used to confirm the results of the tube characterization reactions and to separate the chemical constituents contained in the extracts (Table 3). The plates were observed after being developed with 10 % sulfuric vanillin, iron chloride and Dragendorff's reagent.

These tests were performed with a mixture of solvents of increasing polarity with a ternary mixture consisting of dichloromethane (80 %), ethyl acetate (5 %) and methanol (15 %). The different spots observed on the chromatograms are labeled with respect to their frontal ratio. Each component is characterized by its retention factor. A high frontal ratio, characterizes the apolar compounds, value between 0.16 and 0.37. The medium polar compounds, value between 0.45 and 0.62 and the polar compounds between 0.64 and 0.87. The results of our different calculated frontal ratio are recorded in Table 4.

Table 3 Secondary metabolites of almonds extracts of mango Kent and Keitt varieties by thin layer chromatography

	Extracts			
Secondary metabolites	**EtaqKE**	**EethKE**	**EtaqKI**	**EethKI**
Alkaloids	-	-	-	-
Anthraquinone	+/-	+/-	+/-	+/-
Flavonoids	+/-	+/-	+/-	+/-
Polyphenols	+++	+++	+++	+++
Terpenes and sterols	+++	+++	+++	+++

(-) Absent; (+/-) present; (++) present at moderate concentration; (+++) present at high concentration; EtaqKE : Total aqueous extract of Kent almond; EethKE : Hydro-ethanolic extract of Kent almond; EtaqKI : Total aqueous extract of Kent almond; EethKI : Hydro-ethanolic extract of Kent almond.

Table 4 Results of frontal ratios of almonds extracts of mango Kent and Keitt varieties using eluent with 3 solvents: methanol (15 %) / ethyl acetate (80 %) / dichloromethane (5 %)

		Almond extracts of Kent		Almond extracts of Keitt	
Chemical compounds	**Revealers**	**Frontal ratios (Rf)**			
		EtaqKE	**EethKE**	**EtaqKI**	**EethKI**
Alkaloids	Dragendroff	-	-	-	-
Polyphenols	$FeCl_3$	0.58	0.52	0.64	0.87
Flavonoids	$AlCl_3$	0.45	0.41	0.17	0.48
Anthraquinone	KOH	0.79	0.62	0.57	0.87
Terpenes and Sterols	Vanillin	0.37	0.69	0.82	0.71

EtaqKE : Total aqueous extract of Kent almond; EethKE : Hydroethanolic extract of Kent almond; EtaqKI : Total aqueous extract of Kent almond; EethKI : Hydroethanolic extract of Kent almond; -: not determined.

3.4. Antimicrobial activities of extracts from kernel almond of mango on germ growth

Efficacy of extracts on the *in vitro* growth of fungi: Results of efficacy test of almonds extracts of mango Kent and Keitt varieties on *in vitro* growth of fungi in solid medium led were summarized in Table 5. These results showed that the extracts studied could not inhibit the *in vitro* growth of fungi (*C. albicans, T. rubrum* and *T. mentagrophytes*) up to the maximum concentration tested (50 mg/mL). Therefore, the minimum inhibitory concentration values obtained are considered higher than 50 mg/mL. The minimum fungal concentration was not determined because our extracts are not active on the studied fungi.

Table 5 Minimum inhibitory concentration values of aqueous and hydroethanolic almonds extracts mango Kent and Keitt varieties against fungi

		Extracts			
Fungi strains	**Antifungi parameters**	**EtaqKE**	**EethKE**	**EtaqKI**	**EethKI**
Candida albicans	MIC (mg/mL)	> 50	> 50	> 50	> 50
Trichophyton mentagrophytes	MIC (mg/mL)	> 50	> 50	> 50	> 50
Trichophyton rubrum	MIC (mg/mL)	> 50	> 50	> 50	> 50

Values are results of 3 tests carried out in solid medium. MIC: Minimum inhibitory concentration. EtaqKE: Total aqueous extract of Kent almond; EethKE: Hydroethanolic extract of Kent almond; EtaqKI: Total aqueous extract of Keitt almond; EethKI: Hydroethanolic extract of Keitt almond

Action of almond extracts on in vitro growth of bacteria: The results of the action of mango kernel almond extracts of mango Kent and Keitt varieties on the *in vitro* growth of bacteria in solid medium are recorded in Table 6. This table 6 indicates that the plant extracts do not have the same inhibitory action on the bacteria studied. Moreover, it appears

from the table that hydroethanolic extracts of almonds inhibit the growth of *E. coli* and *P. aeruginosa* strains with the same minimum inhibitory concentration (MIC) of 0.39 mg/mL. Concerning the aqueous extracts of plants, the minimum inhibitory concentration obtained are from 0.78 to 6.25 mg/mL. The tested extracts significantly inhibit the *in vitro* growth of *E.coli* and *P. aeruginosa***,** according to a dose-effect relationship allowing to determine the minimum inhibitory concentration and the minimum bactericidal concentration (MBC). Almonds hydroethanolic extracts of Kent and Keitt varieties have identical minimum bactericidal concentration and minimum inhibitory concentration while the aqueous extracts these two varieties have MBC higher than MIC.

Table 6 Minimum Inhibitory Concentration and Minimum Bactericidal Concentrations values of aqueous and hydroethanolic almonds extracts of mango varieties Kent and Keitt on *in vitro* growth of bacteria

		Extracts			
Bacteria strains	**Antibacterial parameters**	**EtaqKE**	**EethKE**	**EtaqKIT**	**EethKIT**
Escherichia coli	MIC (mg/mL)	0,78	0,39	3,125	0,39
	MBC (mg/mL)	12,5	25	12,5	25
Pseudomonas aeruginosa	MIC (mg/mL)	1,56	0,39	6,25	0,39
	MBC (mg/mL)	12,5	50	25	50
Staphylococcus aureus	MIC (mg/mL)	3,125	1,56	1,56	0,78
	MBC (mg/mL)	6,25	12,5	3,125	50

Values are results of 3 tests carried out in solid medium. MBC: Minimum Bactericidal Concentration, EtaqKE: Total aqueous extract of Kent almond, EethKE: Hydroethanolic extract of Kent almond; EtaqKI: Total aqueous extract of Keitt almond; EethKI: Hydroethanolic extract of Keitt almond.

With respective minimum inhibitory concentrations (MIC) of 0.78 mg/mL to 6.25 mg/mL and minimum bactericidal concentrations (MBC) of 12.5 to 25 mg/mL. Aqueous extracts of almond of Kent variety (EtaqKE) has an identical MBC in *E.coli* and *P.aeruginosa* unlike aqueous extracts of almond of Keitt variety (EtaqKI) which has a different BMC in the two strains. The extracts (EtaqKE and EtaqKI) have better activities on *E.coli* and *P.aeruginosa*. Ethanolic extracts of Kent (EethKE) and Keitt (EethKI) varieties show intermediate activities with MBC = 25 mg/mL to 50 mg/mL and MIC = 0.39 mg/mL. The extracts EethKE and EethKI are less active on both strains.The extracts tested inhibit the *in vitro* growth of *Staphylococcus aureus*, according to a dose-effect relationship that allows the determination of the MBC) and the minimum inhibitory concentration (MIC) (Table 6). It can be seen that for extract EethKE, the MBC (12.5 mg/mL) is higher than the MIC. The MBC value of extract EtaqKE (6.25 mg/mL) is twice that of the MIC. The BMC of extract EtaqKI (3.125 mg/mL) is double the MIC while the MBC of extract EethKI (50 mg/mL) is well above the MIC. With the lowest MBC, extracts EtaqKE and EtaqKI were found to have the best activity on *S. aureus*. With respective MBC of 12.5 mg/mL and 50 mg/mL, extracts EethKE and EethKI showed the lowest activities against *S. aureus.* At the end of our investigations, it appears that the best activity against the 3 tested strains is revealed mainly by the extracts EtaqKE and EtaqKI.

4. Discussion

The phytochemical screening carried out using both methods (tube reaction and TLC plates) allowed the identification of different chemical groups present in the two extracts (aqueous and hydro-ethanolic) of mango kernels: anthraquinones, flavonoids, terpenes and sterols, saponins and polyphenols. The study showed that mango kernel flours contain in variable proportions, anthraquinones, flavonoids, polyphenols, saponosides, terpenes and sterols. Alkaloids, flavonoids, polyphenols, saponosides, terpenes and sterols are present in all two (02) extracts (Etaq and Eeth) of mango. Our results are different from those obtained by [25] who during their studies on characterization of two mango varieties (Kent and Amelie), showed that tannins, saponins and anthraquinones are absent in both mango varieties. Furthermore, several studies conducted on these chemical compounds (anthraquinones, flavonoids, polyphenols, saponosides, terpenes and sterols). Alkaloids, flavonoids, polyphenols, saponosides, terpenes and sterols have revealed that they have antimicrobial properties [5, 23, 26, 27, 28, 29, 30, 31]. According to [5], alkaloids would intercalate in the cell wall and DNA of microorganisms, thus preventing protein synthesis. In addition, they would possess antioxidant effects and reduce the production of nitrate which is useful for protein synthesis, terpenes and sterols would play a detoxification role. During the phytochemical screening, we observed compound that were difficult to migrate. Studies carried out by a number of researchers, including [32], reveal that the polar molecules present in plant extracts contribute to the increase in anti-free radical activity. Chromatography on thin layer, confirm the richness

of extracts of mango kernel flour in polyphenolic substances like flavonoids, phenolic acids and tannins already identified by other authors in previous studies [33, 34, 35, 36, 37, 38].

After phytochemical screening of almonds extracts of mango Kent and Keitt varieties, their antimicrobial activities (antifungal and antibacterial activities) were studied. The comparison of efficacy of different mango almonds extracts on fungi (*C. albicans, T. rubrum* and *T. mentagrophytes*) and bacteria (*E. coli, S. aureus* and *P. aeruginosa*) was performed. The study showed that mango kernel almonds (Kent and Keitt) possess antimicrobial activities against fungi and bacteria tested. This result on antibacterial activity agrees with that of [39]. The work of these authors with methanol extracts of dried and finely pulverized mango kernels have highly significant antibacterial activity against *Enterococcus faecalis*, comparable to that of conventionally used 5% sodium hypochlorite (NaOCl). They concluded that mango kernel almond can be used as a natural antibacterial agent to destroy *Enterococcus faecalis* biofilm as promising results were obtained at a very low concentration. At the level of antifungal activity, our different extracts have no activity on fungi (*C. albicans, T. rubrum* and *T. mentagrophytes*). Our results obtained are different from those obtained by [40] who during their studies on the evaluation of the antifungal activity of *Morinda morindoides* (Baker) Milne-Redhead (*Rubiaceae*) extracts on fungi, showed that the hexanic extract manifested the strongest antifungal activity contrary to other extracts. This difference could be explained by the nature of plants studied. The study of the antibacterial activity of the aqueous and hydro-ethanolic extracts of mango kernel almond carried out in solid medium by the method of double dilutions led to determination of MIC and MBC. The aqueous extract of kernel almond is more active on bacteria and presents a bactericidal action on strains *P. aeruginosa* and *E. coli* with regard to its activity ratio (MBC/MIC) which is equal to 4. The *S. aureus* strain undergoes a bacteriostatic action of almond extracts of both mango varieties. This thesis is confirmed by [41] for whom, when activity ratio MBC/MIC of an antimicrobial substance is less than or equal to four (≤ 4) the latter qualified as a bactericidal substance. If this ratio is greater than four (> 4), then this substance had a bacteriostatic activity. In addition, it has been shown that antimicrobial effectiveness of extracts depends on their chemical compositions and nature of the microorganism [42].

5. Conclusion

This research work is a contribution to valorization of kernel almond flours of two mango varieties (Kent and Keitt) cultivated in Korhogo, northern Côte d'Ivoire for their use in medicine and cosmetics fields. The phytochemical screening and antimicrobial activity were carried out on the extracts of mango kernel almonds. Through the bibliographic review, the phytochemical screening and the antimicrobial activity of extracts kernel almond of mango varieties Kent and Keitt from Korhogo were carried out for the first time. Phytochemical screening revealed the presence of polyphenols, sterols and terpenes, anthraquinones, flavonoids, tannins and saponins. Aqueous extracts of mango kernel almond (EtaqKE and EtaqKI) are more active on the studied bacteria. The hydroethanolic extracts of almond (EethKE and EethKI) had a very moderate activity on the bacteria. The aqueous and hydroethanolic extracts of almond from both mango varieties were inactive on fungi of this study grow. The presence of secondary metabolites and antibacterial activities of extracts of almond from kernels of mango varieties Kent and Keitt demonstrate that these extracts could have therapeutic and cosmetic applications.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

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