

Available online on 15.02.2024 at <http://jddtonline.info>

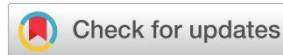
Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Copyright © 2024 The Author(s): This is an open-access article distributed under the terms of the CC BY-NC 4.0 which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited



Open Access Full Text Article



Research Article

Phytochemicals properties and antimicrobial activities of *Carica papaya* and *Balanites aegyptiaca* seeds aqueous infusion and decoction extracts

OUEDRAOGO Assétou ^{1,2} , NIKIEMA Philippe Augustin ^{1*} , NIKIEMA Marguerite Edith Malatala ¹ , YAMEOGO Gérard Josias ^{1,3}, SOURABIE Pane Bernadette ², BASSAVE Belinda Ramata Hafou ² , NIKIEMA Ouego ² , GOUMBRI Wendinmi Bertrand Florent ² , BARRO Nicolas ¹

¹Laboratoire de Biologie moléculaire, d'Epidémiologie et de Surveillance des bactéries et virus Transmissibles par les Aliments (LaBESTA), Université Joseph KI-ZERBO, Ouagadougou 03, Burkina Faso

²Agence Nationale pour la Sécurité Sanitaire de l'Environnement, de l'Alimentation, du Travail et des produits de santé (ANSSEAT)

³Laboratoire du Développement du Médicament (LADME), Centre de Formation de Recherche et d'Expertises en Sciences du Médicament (CEA-CFOREM), Université Joseph KI-ZERBO, Ouagadougou 03 BP 7021, Burkina Faso

Article Info:



Article History:

Received 19 Nov 2023
Reviewed 02 Jan 2024
Accepted 25 Jan 2024
Published 15 Feb 2024

Cite this article as:

Ouedraogo A, Nikiema PA, Nikiema MEM, Yameogo GJ, Sourabie PB, Bassave BRH, Nikiema O, Gombri WBF, Barro N, Phytochemicals properties and antimicrobial activities of *Carica papaya* and *Balanites aegyptiaca* seeds aqueous infusion and decoction extracts, Journal of Drug Delivery and Therapeutics. 2024; 14(2):46-52

DOI: <http://dx.doi.org/10.22270/jddt.v14i2.6393>

*Address for Correspondence:

NIKIEMA Philippe Augustin, Laboratoire de Biologie moléculaire, d'Epidémiologie et de Surveillance des bactéries et virus Transmissibles par les Aliments (LaBESTA), Université Joseph KI-ZERBO, Ouagadougou 03, Burkina Faso

Abstract

Carica papaya and *Balanites aegyptiaca* seeds extract have several uses, from food to traditional medicine. This study aims to determine their phytochemicals compound and appreciate their antimicrobial properties against clinical strains. Polyphenolic content was determined with standard methods while DPPH radical scavenging activity is used to appreciate the antioxidant properties, using ascorbic acid as standard. Antimicrobial activities were performed according to the inhibition diameters, using agar diffusion method. Minimum inhibitory concentrations and Minimum fungicidal concentrations were established based on broth dilution methods. The extraction yield is ranged between 6.3 and 20.3%. The results shown polyphenolic content varying from 1256.13±21.09 to 2315.81±53.52 µg GAE/100 mL for *Carica papaya* and from 303.98±0.93 to 948.06±3.23 µg GAE/100 mL for *Balanites aegyptiaca* while the antioxidant activity is ranged between 31.76±2.35 and 58.47±4.24 µg AEAC/100 mL for *Carica papaya* and from 29.80±0.32 to 78.44±2.81 µg AEAC/100 mL for *Balanites aegyptiaca*. Infusion's extracts always have the highest values of polyphenols content and antioxidant activity than decoction extracts, be it papaya or balanites seeds. Neither flavonoids content nor antibacterial activity was found. The inhibition diameter varies from 14.5±0.5 to 40.5±0.5 mm for papaya seeds extracts and from 16.5±0.5 to 21.0±0.5 mm for balanites seeds extracts without a significative effect of mixture for both extracts. MICs vary from 11.71±0.1 to 75.0±0.0 mg. mL⁻¹ for *Carica papaya* seeds extracts with 11.72±0.2 to 37.30±0.2 mg. mL⁻¹ and 18.74±0.1 to 100±0.0 mg. mL⁻¹ for *Balanites aegyptiaca*. Mixture extracts presented intermédiaire values. Decoction extracts have lowest MICs values and infusion reveals itself to be the sweetable aqueous extraction techniques but the appropriate infusion time need to be highlight.

Keywords: *Carica papaya*, *Balanites aegyptiaca*, Seeds extracts, Phytochemical, antifungal

INTRODUCTION

Carica papaya Lin and *Balanites aegyptiaca* are useful two widely promote around the world. Different parts of both *Carica papaya* and *Balanites aegyptiaca* are use as food, medicine and bio pesticides in agriculture and crop preservation¹⁻⁴. They play significant role in local economy⁵. *Balanites aegyptiaca* is believed indigenous to all dry lands in south of the Sahara, extending southward to Malawi in the Rift Valley, and to the Arabian Peninsula, introduced into cultivation in Latin America and India^{6,7} while *Carica papaya* is a tropical plant, spread to India, Oceania, Africa, and widely distributed throughout the tropics and subtropical areas⁸⁻¹¹. They both showed appreciate nutritional values including macronutrients, minerals, vitamins, amino acids, fatty acids bioactive compounds etc¹²⁻¹⁵. In traditional medicine, infusion, decoction and maceration are the main extraction techniques used for these herbal teas according to the chemical nature of their bioactive compounds. The chemical compound nature also required specific solvent for extraction. Solvents usually

used included water, ethanol, methanol, n-hexan, acetone, ethyl acetone... The origins of the plants^[15] as well as the solvents and extraction methods used have an impact on the nutritional value, phytochemicals content and antimicrobial activities^{16,17}. The antimicrobial activity of both plants seeds extracts included bacterial strains like *Salmonella enteritidis*, *Salmonella tphi*, *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Citrobacter amalonaticus*, *Sporolacto bacillus*, *Proteus mirabilis*, *Proteus vulgaris* some virus as *Vibrio vulnificus*, *Vibrio cholerae*, human pathogen fungus like *Candida albicans*, *Aspergillus flavus*, *Penicillium citrinum*, *Aspergillus flavus*, phytopathogenic fungi like *Rhizopus stolonifer*, *Fusarium spp.* and *Colletotrichum gloeosporioides*^{3,15,18,19} Previous surveys and studies also highlight an important and several uses of both *C. papaya* and *B. aegyptiaca* in Burkina Faso^{20,21} but, their seeds phytochemicals content and antimicrobial analyses remain investigated^{20,21}. This study then aims to appreciate phytochemicals content of local *Carica papaya* and *Balanites aegyptiaca* varieties seeds as well as the impact of extraction

method used on the antimicrobial activity, using clinical strains for traditional medicine improvement purpose.

MATERIAL AND METHODS

1. Seeds sampling and microbial strains collection

Carica papaya and *Balanites aegyptiaca* seeds are purchased in local markets, mainly in Ouagadougou and Ziniaré respectively (Burkina Faso). Seeds have been dried under shade. The clinical bacteria and fungi strains were from human feces, collected at Schiphra Protestant Hospital and Charles de Gaulle Pediatric Hospital. It included *Candida albicans*, *Candida* spp, *Escherichia coli*, *Salmonella* spp, *Shigella* spp, and *Staphylococcus aureus*.

2. Seeds aqueous extraction techniques

Extraction is conducted as it is performed in practice in traditional medicine. The infusion extraction method involved using a boiling water. 500 mL of boiling demineralized water was poured into a jar containing 50 g of *Carica papaya* or *Balanites aegyptiaca* powder. The mixture is stirred and left to cool for 15 minutes. After cooling, the mixture is filtered. The residue is returned to the jar for a second filtration. The filtrate is transferred to a flask and placed in freezer for freeze-drying.

The decoction extraction method also used water as solvent. In a jar containing 500 ml of demineralized water on a hot plate, 50 g of *Carica papaya* or *Balanites aegyptiaca* seed powder is added. The mixture is boiled for 15 minutes. After cooling, the mixture is filtered. The residue is returned to the jar for a second filtration. The filtrate is then packed in a flask and placed in the freezer for freeze-drying.

3. Determination of polyphenolic and flavonoid contents

Total polyphenols content of *Carica papaya* and *Balanites aegyptiaca* seeds were analyzed with gallic acid as standard according to Salla et al.²². Then, 12.5 μ L of Folin-Ciocalteu's reagent was added to 12.5 μ L of extracts and 50 μ L of distilled water. After 5 min, 125 μ L of sodium carbonate 7% was added and the absorbance is measured at 750 nm after 90 min of incubation at room temperature. Flavonoid content was performed according to Ribarova et al.²³ method. About 25 μ L of extracts and 7.5 μ L of sodium nitrite 7.5 % were firstly measured. Then, 15 μ L of aluminum chloride 10%, 50 μ L of NaOH 1 M and 40 μ L of water were added. The mixture was incubated for 5 min, and the absorbance was measured at 520 nm.

4. Antioxidant properties

The DPPH radical scavenging activity was used to assess the antioxidant activity. Lamien-Meda et al.²⁴ and Konaré et al.¹⁹ method was optimized to determine the DPPH assay. About 210 μ L of DPPH (0.1 mM) was added to 40 μ L of papaya or Balanites seeds and to the blank. After 90 min of incubation, the absorbance was recorded at 517 nm at 30 min interval. The results were expressed as μ g of ascorbic acid equivalent antioxidant content (AEAC) per 100 mL of papaya or Balanites seeds extracts¹⁹.

5. Microbial strains and culture media

Main microbials responsible of gastroenteritis were the target ones. The standard strains included *Escherichia coli* ATCC 8739, *Salmonella*. Typhi ATCC 14028, and *Staphylococcus aureus* ATCC6538. The clinical strains from human feces, were collected at Schiphra Protestant Hospital and Charles de Gaulle Pediatric Hospital and included *Candida albicans*, *Candida* spp, *Escherichia coli*, *Salmonella* spp, *Shigella* spp, *Staphylococcus aureus*. All clinical strains

were previously characterized on biochemical level using API 20E and human plasma only for *Candida*. Strains were stored at -18°C. Before used, bacterial strains were streaked on plates containing Mueller-Hinton agar (MH) and yeasts, on Sabouraud dextrose agar (SDA) plates and incubated for 18 up to 24 hours at 37°C for bacterial and 27°C for yeasts. The operation is repeated twice to get viable and purified strains. The turbidity was adjusted in saline solution to 0.5 according to Mac Farland standard.

6. The antimicrobial activity

The antifungal and antibacterial activity of papaya and Balanites seeds extracts were performed based on the inhibition diameters using agar diffusion method²⁵. The microbial suspension was prepared in a saline solution and standardized to a turbidity of 0.5 on the McFarland scale. Papaya and Balanites seeds extract dilution are prepared to get appropriate concentration. A volume of 75 μ L of extracts is used to fill wells in the plate previously performed and containing infectious microbials. Plates were incubated for 24-48 h at 27 and 37°C respectively for fungi and bacterial. The evaluation of the antimicrobial activity was made by measuring zones of inhibition around wells. Experiments were carried out in triplicate.

7. Determination of MICs, MBCs and MFCs

The minimum inhibitory concentrations (MICs) were determined using the broth dilution method according to the CLSI M27-A3 guidelines (Clinical and Laboratory Standards Institute²⁶). The minimum bactericidal concentrations (MBCs) and the minimum fungicidal concentrations (MFCs) of both papaya and Balanites seeds extracts were recorded according to broth dilution method. The tests were performed in sterile 96-microwell plates, into which 100 μ L of the RPMI-1640 medium (pH 7.0) was added for each well. Before inoculum, 100 μ L of the extracts was added to the first well and a serially dilution from the first well is performed by taking 100 μ L into the next. This twofold dilution was continued until the 10th column of the plate was reached. The wells in the 11th column of the plate were reserved for the negative control (without inoculation) and the wells in the 12th column were reserved for the positive control (with FLZ). The final concentrations of extracts and FLZ in the wells were in the ranges of 256–0.5 μ g ml⁻¹ and 64–0.125 μ g ml⁻¹ respectively. The microbial colonies were suspended in the RPMI 1640 medium and the concentration was adjusted to 1–5 \times 10³ CFU per ml. The solution (100 μ L) was added to each well except those in the 11th column, which was supplemented with 100 μ L of the RPMI 1640 medium. The 96-microwell plates were incubated at 35°C for 48 h. Finally, the optic density values were measured at 630 nm by enzyme-linked immunosorbent assay (ELISA, Biotek Synergy HTX, Vermont, MA, USA). The MICs were determined as the lowest concentration of the test substances that caused 50% inhibition.

After the 96-well plates were incubated at 35°C for another 24 h, 20 μ L of solution from each test well was streaked onto an SDA plate or on Mueller-Hinton agar (MH), followed by incubation at 35°C for 48 h. The minimum fungicidal concentrations (MFCs) and minimum bactericidal concentrations (MBCs) were determined as the minimum drug.

8. Statistical Analysis

The data collection was performed with Excel version 2010. Analysis of variance (ANOVA), Principal component analysis (PCA) and average were performed using XLSTAT 2014.5.03.

RESULTS

1. Extraction yield

The extraction yield or efficiency of both *Carica papaya* or *Balanites aegyptiaca* seeds varied from 6.3 to 20.3 (Table I). The

important extraction yield is observe with *Balanites aegyptiaca* seeds. Values are ranged from 6.3 to 8.8 for papaya seeds extracts and from 17.9 to 20.3 for balanites's one. The highest yield values are observe with lipid extracts.

Table I: Extraction yield of papaya and balanites seeds

Types of extracts	Extraction yield (%)
Delipidated papaya seeds	6.3
Lipid papaya seeds	8.8
Lipid balanites seeds	20.3
Delipidated balanites seeds	17.9

2. Phytochemical properties

Carica papaya and *Balanites aegyptiaca*' seeds extracts have important polyphenols content and antioxidant activity presented in Table II. But flavonoids were not detected. The polyphenols content varies from 1256.13±21.09 to 2315.81±53.52 µg GAE/100 mL for *Carica papaya* and from 303.98±0.93 to 948.06±3.23 µg GAE/100 mL for *Balanites aegyptiaca*. Delipidated samples have high content of polyphenolic content than lipid ones for both papaya and Balanites seeds extracts. The antioxidant activity varies from 31.76±2.35 to 58.47±4.24 µg AEAC /100 mL for *Carica papaya* seeds and from 29.80±0.32 to 78.44±2.81 µg AEAC /100 mL for *Balanites aegyptiaca* seeds. Delipidated papaya of both decoction and infusion presented not antioxidant activity.

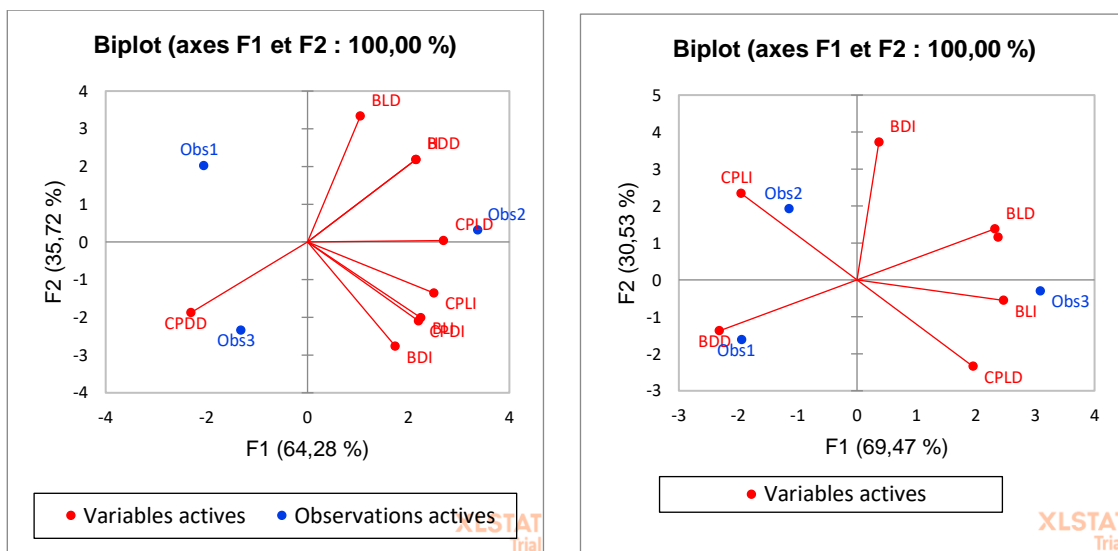
Table II: Phytochemicals properties (µg GAE /100 mL) and antioxydant activity (µg AEAC /100 mL)

		Types of extracts			
		Lipid infusion	Lipid decoction	Delipidated infusion	Delipidated infusion
<i>C. papaya</i>	TPC	1708.82±61.30 ^b	1256.13±21.09 ^c	2262.58±104.69 ^a	2315.81±53.52 ^a
	AA	31.76±2.35 ^d	58.47±4.24 ^{bc}	-	-
<i>B. aegyptiaca</i>	TPC	751.29±7.39 ^h	303.98±0.93 ^g	691.61±15.39 ^e	948.06±3.23 ^d
	AA	29.80±0.32 ^e	78.44±2.81 ^a	52.59±3.89 ^c	61.66±2.07 ^b

The values in the same column with different letters are significantly different (p-value <0.05, Newman-Keuls test).

*TPC: Total polyphenolic compounds; AA: Antioxidant activities.

The Principal component analysis (PCA) showed differences in both polyphenolic content and antioxidant activity according to the extraction process. Figure 1 presents the biplot of the PCA. Polyphenolic content and antioxidant activity don't have the same repartition.



a : PCA for polyphenolics content

b : PCA for antioxidant activities

CPLI : Lipid papaya infusion ; CPLD : Lipid papaya decoction ; CPDI :Delipidated papaya infusion ; CPDD : Delipidated papaya decoction BLI : Lipid balanites infusion ; BLD : Lipid balanites decoction ; BDI :Delipidated balanites infusion ; BDD : Delipidated balanites decoction

Figure 1: Principal component analysis of phytochemical content

3. Antimicrobial activity of Papaya and Balanites seeds extract

Any antibacterial activity was observed with both papaya and Balanites seeds aqueous extracts. The antifungal activity varies from 14.5±0.5 to 40.5±0.5 mm diameter for papaya seeds and 16.5±0.5 to 21.0±0.5 mm diameter for Balanites. Mixed extraction (Papaya and Balanites) has an antifungal activity range between 13.0±0.5 and 32.1±0.5 mm. Based on the extraction method used, the infusion gave an inhibition diameter varying from 14.5±0.5 to 39.5±0.5 mm for papaya

seeds extracts and from 19.0±0.5 to 21.0±0.5 mm for balanites. Concerning decoction, the inhibition diameters vary from 15.5±0.5 to 39.5±0.5 for papaya and from 16.5±0.5 to 18.5±0.5 for Balanites. The more sensitive fungal strains to papaya seeds extraction are respectively *Candida.ssp* 27 (39.5±0.5), *Candida.ssp* 103 (39.5±0.5) and *Candida.ssp* 1166 (39.5±0.5). The less sensitive ones are *Candida.ssp* 001 (14.5±0.5) and *Candida.ssp* 194 (15.5±0.5). The more important antifungal activity is obtained with infusion extract. The aquatuous extracts presented not an inhibition activity against pathogenic bacterial strains.

Table III: Antifungal activity of Papaya and balanites seeds extracts

	<i>Carica papaya</i>		<i>Balanites aegyptiaca</i>		Mixed solution	
	CPI (100mg/mL)	CPD (100mg/mL)	BEI (150mg/mL)	BED (150mg/mL)	BIPI (125mg/mL)	PDBD (125mg/mL)
<i>Candida.ssp</i> 103	38.5±0.5	39.5±0.5	-	-	-	-
<i>Candida.ssp</i> 194	-	-	18.0±1.0	15.5±0.5	13.0±0.0	13.5±0.5
<i>Candida.ssp</i> 1166	39.5±0.5	35.5±0.5	16.0±0.0	-	14.0±0.0	-
<i>Candida.ssp</i> 27	39.5±0.5	39.5±0.5	15.5±0.5	-	-	-
<i>Candida.ssp</i> 001	27.5±0.5	17.5±0.5	21.0±0.0	18.0±1	12.5±0.5	13.5±0.5
<i>Candida albicans</i> 10231	18.5±0.5	16.5±0.5	16.5±0.5	18.5±0.5	13.0±0.0	14.5±0.5
<i>Candida.ssp</i> 438	16.5±0.5	-	-	-	14.0±0.0	15.5±0.5

PLI : Lipid papaya infusion ; PLD : Lipid papaya decoction; PDI :Delipidated papaya infusion ; PDD : Delipidated papaya decoction BLI : Lipid balanites infusion ; BLD : Lipid balanites decoction; BDI :Delipidated balanites infusion ; BDD : Delipidated balanites decoction

The collected fungi strains were all sensitive to commercial antibiotics including nystatin, fluconazole and clotrimazole. The antifungigram is as presented in table IV. On average, nystatin (27,5 mm) is the most effective commercial antibiotics against fungi, followed by clotrimazole (24.5mm) and fluconazole (18mm).

Table IV: Antifungigram of collected fungi's strains

Fungi strains	Nystatin	Fluconazole	Clotrimazole
<i>Candida spp</i> 438	28±0.5	14	25
<i>Candida spp</i> 103	29±0.5	28	24
<i>Candida spp</i> 1	26±0.5	25	23
<i>Candida spp</i> 27	27±0.5	16	26
<i>Candida spp</i> 194	27±0.5	14	22
<i>Candida spp</i> 1166	28±0.5	14	27
Quality evaluation of antibiotics			
	Nystatin	Fluconazol	Clotrimazole
<i>C.Albicans</i> 10231	19/18	20/20	27/27

4. Minimum inhibitory concentrations (MIC)

The minimum inhibitory concentrations are presented in table V. The lowest value of inhibitory concentrations (11.71±0.1 mg. mL⁻¹) is obtained with papaya seeds extracts while the highest value (100±0.0 mg. mL⁻¹) is obtained with Balanites seeds extracts. Both infusion (CPI) and decoction (CPD) extracts of papaya seeds have the same minimum inhibitory concentrations against *Candida.ssp* 103, *Candida.ssp* 1166 and

Candida.ssp 27. But for *Candida.ssp* 001 and *Candida albicans* 10231, infusion (CPI), is more effective (11.71±0.0 mg. mL⁻¹) than decoction (75.0±0.0 mg.mL⁻¹). Balanites seeds decoction and infusion extracts have the same inhibitory concentrations for *Candida.ssp* 001 and *Candida albicans* 10231. But, for *Candida.ssp* 194, infusion extract (BEI) has lower concentration value (75.0±0.0) than decoction (100±0.0 mg. mL⁻¹). The minimum concentration values of mixed extracts are ranged between infusion and decoction.

Table V: Minimum inhibitory concentrations of Papaya and Balanites seeds extract

	CPI	CPD	BEI	BED	BIPI	PDBD
<i>Candida.ssp</i> 103	18.75±0.0	18.75±0.0	-	-	-	-
<i>Candida.ssp</i> 194	-	-	75.0±0.0	100±0.0	18.75±0.0	-
<i>Candida.ssp</i> 1166	18.75±0.0	18.75±0.0	18.74±0.1	-	75.0±0.0	-
<i>Candida.ssp</i> 27	11.71±0.1	11.7±0.0	18.75±0.1	-	-	-
<i>Candida.ssp</i> 001	11.71±0.0	75.0±0.0	75.0±0.0	75.0±0.0	75.0±0.0	75±0.5
<i>Candida albicans</i> 10231	11.72±0.2	75.0±0.0	75.0±0.0	75.0±0.0	75.0±0.0	18.75±0.5
<i>Candida.ssp</i> 438	37.30±0.2	-	-	-	37.2±0.0	37.2±0.5

PLI : Lipid papaya infusion ; PLD : Lipid papaya decoction; PDI :Delipidated papaya infusion ; PDD : Delipidated papaya decoction BLI : Lipid balanites infusion ; BLD : Lipid balanites decoction; BDI :Delipidated balanites infusion ; BDD : Delipidated balanites decoction

5. Minimum fungicidal concentrations (MFCs)

The minimum fungicidal concentrations (MFCs) are in the same order than the minimum inhibitory concentrations (MICs). The values are ranged from 23.4±0.0 to 150±0.5 for papaya seeds

extracts and from 37.2±0.5 to 150±0.0 for Balanites seeds extracts as showed in Table VI. The lowest values are mainly obtained with infusion extracts while highest values are observed with decoction extracts.

Table VI: Minimum fungicidal concentrations

	CPI	CPD	BEI	BED	BIPI	PDBD
<i>Candida.ssp</i> 103	37.2±0.0	37.2±0.0	-	-	-	-
<i>Candida.ssp</i> 194	-	-	150±0.0	150±0.0	37.2±0.0	150±0.0
<i>Candida.ssp</i> 1166	37.2±0.0	37.2±0.0	37.2±0.0	-	150±0.0	-
<i>Candida.ssp</i> 27	23.4±0.1	37.2±0.0	37.2±0.5	-	-	-
<i>Candida.ssp</i> 001	23.4±0.0	150±0.5	150±0.5	150±0.0	150±0.0	150±0.0
<i>Candida albicans</i> 10231	23.4±0.0	150±0.5	150±0.5	150±0.0	150±0.0	100±0.0
<i>Candida.ssp</i> 438	75.0±0.0	-	-	-	75.0±0.0	37.2±0.0

PLI : Lipid papaya infusion ; PLD : Lipid papaya decoction; PDI :Delipidated papaya infusion ; PDD : Delipidated papaya decoction BLI : Lipid balanites infusion ; BLD : Lipid balanites decoction; BDI :Delipidated balanites infusion ; BDD : Delipidated balanites decoction

DISCUSSION

The polyphenolic content vary from 1256.13±21.09 to 2315.81±53.52 µg GAE /100 mL for *Carica papaya* and from 303.98±0.93 to 948.06±3.23 µg GAE /100 mL for *Balanites aegyptiaca* while the antioxidant activity is ranged between 31.76±2.35 and 58.47±4.24 µg AEAC/100 mL for *Carica papaya* seeds extract and from 29.80±0.32 to 78.44±2.81 µg AEAC/100 mL for *Balanites aegyptiaca* seeds extract. The inhibition diameter varies from 14.5±0.5 to 40.5±0.5 mm for papaya seeds extracts and 16.5±0.5 to 21.0±0.5 mm for balanites one's without a significative effect of mixture of both extracts. The minimum inhibitory concentrations showed little value (11.71±0.1 mg. mL⁻¹) with papaya seeds extracts than balanites (100±0.0 mg. mL⁻¹). The minimum fungicidal concentrations are in the same order that the minimum inhibitory concentrations. Both papaya and balanites seeds presented neither a flavonoids content nor antibacterial activity.

The two traditional extraction process used, infusion and decoction lead to different phytochemical contents. The difference in the content in both polyphenolic and antioxidant activity is statistically significative between infusion and decoction extracts (Table II). This difference is highlight by the principal compound analyses (Figure 1). Infusion's extracts always have the highest values of polyphenolics content and antioxidant activity than decoction extracts, be it papaya whole seeds or balanites. The exception is observed with delipidated seeds extracts. Several studies reported variations in the phytochemical compound with different extraction techniques²⁷. Some like Acharya et al.²⁸, Gosh et al.²⁹ and Ennaifer et al.³⁰ showed a significant difference in phytochemicals content between infusion and decoction methods used. Acharya et al.²⁸ and Gosh et al.²⁹ found infusion extracts more enriched in phytochemicals with high content that decoction, which is the same case in this study. But the infusion time also have an impact on the phytochemicals content as put in evidence by Messaoud et al.³¹. The extraction solvent also has an impact on the phytochemicals content³². It's then necessary to select sweetable extraction method and time for an optimal phytochemicals content. Infusion then seemed to be more appropriate for papaya and balanites seeds aqueous extraction for an high phytochemicals content. But, the

appropriate infusioin time for both papaya and balanites needed to be determinated.

These two extracts showed interesting antifungal activity but none antibacterial activity. There is then a correlation between phytochemicals content and the antifungal activity, for a difference is still observed between infusion and decoction extracts. In general, papaya seeds extracts were more effective than balanites. From papaya seeds extracts, the infusion one was the most effective, against six of the seven fungal strains while papaya seeds decoction extract was effective against five fungal strains. *Candida.ssp* 438 which was sensitive to papaya seeds infusion extract was not to the decoction one. Balanites infusion extract was effective against five of the seven fungal strains when decoction extract was sensitive to only three strains. As for the phytochemicals content, mixture did not neither amplified nor reduce the antifungal activity of extracts. It only allowed to adjust it. Some trains were sensitive to either papaya or balanites extracts and others to particular extraction way. The highest inhibition diameter (39.5±0.5 mm) is obtained with papaya extract on *Candida.ssp* 27, *Candida.ssp* 1166 and *Candida.ssp* 103. Little inhibition diameters are obtained with mixture extracts. *Candida.ssp* 103 which was very sensitive to papaya seeds extract was resistant to all balanites seeds extracts, included mixed extracts. And, *Candida.ssp* 194 which is resistant to papaya infusion and decoction extracts was sensitive to balanites ones. But, *Candida.ssp* 438, *Candida.ssp* 1166 and *Candida.ssp* 27 which were sensitive to infusion extrats were not to decoction extrats. The inhibition diameter found with these extracts are less than those obtained with papaya seeds essential oils³³ which was up to 60.0±0.0 mm diameter. Previous studies also put in evidence antifungal activity of papaya and balanites seeds extracts. Masfufatun et al.³⁴ found and anti-*Candida albicans* activity of *Carica papaya* seeds extracts with 14.5mm as inhibition diameter (lower compare to this result) while Varadarajan et al.³⁵ found up to 26±1.82 mm of inhibition against *Candida albicans* (higher compare to this result). The nature of the solvent used has an impact on the antibacterial and antifungal activity³⁶⁻³⁸. *Balanites aegyptiaca* seeds extracts also showed antifungal activity varying according to the solvent used. Abaka et al.³⁹ found 17 ± 0.69 mm of inhibition diameter against *candida albicans* while Khamis et al.¹⁵ found up to 29.42 ± 4 mm as inhibition diameter.

MICs vary from 11.71±0.1 to 75.0±0.0 mg. mL⁻¹ for *Carica papaya* seeds extracts with 11.72±0.2 to 37.30±0.2 mg. mL⁻¹ as infusion concentration and 11.71±0.0 to 75.0±0.0 mg. mL⁻¹ for decoction extracts. *Balanites aegyptiaca*'s MICs vary from 18.74±0.1 to 100±0.0 mg. mL⁻¹ with 18.74±0.1 to 75.0±0.0 mg. mL⁻¹ as infusion MICs and from 75.0±0.0 to 100±0.0 mg. mL⁻¹ for decoction one. Mixture extracts presented intermediaire values, varying from 18.75±0.0 to 75.0±0.0 mg. mL⁻¹ (Table IV). MFCs varie from 23.4±0.0 to 150±0.0 mg. mL⁻¹ (Table V) in the same order than MICs. The lower values of inhibition concentrations are obtained with *Carica papaya* infusion extract. Mixture helps to balance specific activity of both two plants seeds extracts and was expected to cover more fungal species. The mixture effect may be more effective with high extract volume or more concentration of *Carica papaya* extract than *Balanites aegyptiaca*. Based on these results, *Carica papaya* seeds infusion extract is the most effective extract. Previous results of Abaka et al.³⁹ showed MICs values against *Candida albicans*, varying between 6.25 and 12.5 mg/mL using respectively n-hexan and methanol as solvent. Masfufatun et al.³⁴ found 15% as MICs against *Candida albicans*. Based on these results, *Carica papaya* seeds infusion extract is the most indicated extract with need to determine the appropriate infusion time for optimal antifungal activity.

CONCLUSION

This study reveals that aqueous seeds extracts of *C. papaya* and *B. aegyptiaca* contain an appreciable phytochemical content and a potential activity related to medicinal properties. All extracts showed an antifungal activity against clinical *Candida* spp, and *Candida albicans* strains. But flavonoids and antibacterial activity were not recorded. Infusion reveals itself to be the sweetable aqueous extraction. The aqueous extractions techniques had an impact on both phytochemicals content and antifungal activity. Papaya and balanites seeds demonstrate themselves as potential promoting traditional improved medicine against pathogenic fungi, with infusion as the appropriate aqueous extraction.

Acknowledgment

The technical assistance of National Agency for Health Safety of the Environment, Food, Work and health products (ANSEAT) as whell as the financial support of National found for education and research (FONER) is gratefully acknowledged.

Conflict of Interest

The authors declare any conflict of interest.

Autor contribution statement

Ouedraogo Assétou, **BASSAVE Belinda Ramata Hafouo**, **NIKIEMA Oueogo**, **NIKIEMA Marguerite Edith**, **GOUMBRI Wendinmi Bertrand Florent** carried out the experiment. **Ouedraogo Assétou** wrote the manuscript with support from **NIKIEMA Philippe Augustin**. **Yameogo Gérard Josias**, **Sourabie Pane Bernadette** helped supervise the project. **NIKIEMA Philippe Augustin** and **Barro Nicolas** supervised the project. All authors discussed the results and contributed to the final manuscript

REFERENCES

- Koubala BB, Ange-Patrice TM, Djilè B, Aristide GSK, and Germain K. "Evaluation of Insecticide Properties of Ethanolic Extract from " *Balanites aegyptiaca*," " *Melia azedarach*" and " *Ocimum gratissimum*" leaves on" *Callosobruchus maculatus*" (Coleptera: Bruchidae): Asian Journal of Agricultural Sciences, 2013; 5: 93-101.
- Ogbonna CU, Nnaemeka JO, Edith NN, Lilian CE, Chioma OA, Irikannuk C, and Egbuche CM. "Carica papaya seed oil extract in the management of insect pest of cabbage plant both in the

laboratory and field: International Journal of Entomology Research, 2021; 6(2): 12-21.

- Singh P, Ram LS, Neelam P, Pradeep KS, Manikant T, and Sukanta M. "Phytochemistry and nutraceutical properties of *Carica papaya* (Linn.): A review: Dietary Supplements and Nutraceuticals, 2022; 1(9): 1-15. <https://doi.org/10.31989/dsn.v1i9.991>
- Wiesman Z, and Bishnu PC. "Larvicidal activity of saponin containing extracts and fractions of fruit mesocarp of *Balanites aegyptiaca*." *Fitoterapia*, 2006; 77(6): 420-424. <https://doi.org/10.1016/j.fitote.2006.05.012> PMID:16814957
- Ouédraogo S, Oumarou O, Adjima T, and Joseph IB: The role of *Balanites aegyptiaca* (L) Delile in the livelihood and local economy in Sahelian and Sudano-Sahelian zones of Burkina Faso: basis for its conservation." *Environment, Development and Sustainability*, 2023; 25(2): 1420-1440. <https://doi.org/10.1007/s10668-021-02100-w>
- Chothani DL, and Vaghasiya HU. "A review on *Balanites aegyptiaca* Del (desert date): phytochemical constituents, traditional uses, and pharmacological activity: Pharmacognosy reviews, 2011; 5(9): 55. <https://doi.org/10.4103/0973-7847.79100> PMID:22096319 PMCID:PMC3210005
- Tesfaye A. "Balanites (*Balanites aegyptiaca*) Del., multipurpose tree: a prospective review: International Journal of Modern Chemistry and Applied Science, 2015; 2(3): 189-194.
- Chávez-Pesqueira M, and Juan NF. Domestication and genetics of papaya: a review: *Frontiers in Ecology and Evolution*, 2017; 5: 155. <https://doi.org/10.3389/fevo.2017.00155>
- Hillocks RJ, and Thresh JM, eds. *Cassava: biology, production and utilization*. CABI publishing, 2002. <https://doi.org/10.1079/9780851995243.0000> PMID:12400448
- Lim TK. "Medicinal and non medicinal plants. Fruits. Springer, 2012; 1: 1-3. https://doi.org/10.1007/978-94-007-4053-2_1
- Assétou O, Augustin PN, Bazoin SRB, Pane BS, Fulbert N, Assétou S, Marceline O, Aissama S, Nicolas B. Nutritional Values of *Carica papaya* L. and *Balanites aegyptiaca* Del. Seeds, Two Herbal Infusions in Traditional Medicine: *American Journal of Food and Nutrition*, 2023; 11(1): 1-6. <https://doi.org/10.12691/ajfn-11-1-1>
- Chai YH, Suzana Y, and Muhammad SHR. Review of bioactive compounds extracted from *Carica papaya* linn: *Current Nutrition & Food Science*, 2020; 16(9): 1287-1298. <https://doi.org/10.2174/1573401316999200727142506>
- Daagama AA, Orafa PN, and Igbua FZ. Nutritional potentials and uses of pawpaw (*Carica papaya*): A review: *European Journal of Nutrition & Food Safety*, 2020; 12(3): 52-66. <https://doi.org/10.9734/ejfnfs/2020/v12i330209>
- Khamis G, Ahmed MS, Talaat HH, Wael NH, Mohammed AMW, Jutta P, and Hamada A. Provenance effect on bioactive phytochemicals and nutritional and health benefits of the desert date *Balanites aegyptiaca*: *Journal of food biochemistry*, 2020; 44(6): e13229. <https://doi.org/10.1111/jfbc.13229> PMID:32250478
- Ayoola AA, Deji AE, Sulaiman BM, Mutiu AO, and Yusuf AA. The effects of extraction methods of *Mangifera indica* and *Azadirachta indica* bark on in vitro antimicrobial efficacy and performance of broiler chickens: *Journal of World's Poultry Research*, 2020; 10(1): 28-35. <https://doi.org/10.36380/jwpr.2020.4>
- Bhebhe M, Fuller TN, Chipurura B, Muchuweti M. Effect of solvent type on total phenolic content and free radical scavenging activity of black tea and herbal infusions, 2016. 9: 1060-1067. <https://doi.org/10.1007/s12161-015-0270-z>
- Chávez-Pesqueira M, and Juan NF. Domestication and genetics of papaya: a review: *Frontiers in Ecology and Evolution*, 2017; 5: 155. <https://doi.org/10.3389/fevo.2017.00155>
- Konaré MA, Singou K, Marius KS, Issiaka T, Nouhoum D, and Rokia S. Antibacterial Activities of Extracts from four Wild Food Fruits: *European Journal of Nutrition & Food Safety*, 2023; 15(1): 62-71. <https://doi.org/10.9734/ejfnfs/2023/v15i11289>
- Sawadogo M, Mindiédba JB, Bernice D, Armandine L, Hyacinthe MT, Beboila O, Hermann YO, and Georges AO. "Ethnobotanical

- survey on medicinal plants (*Carica papaya* L. and *Agelanthus dodoneifolius* (DC.) Polhill & Wiens) used in the treatment of Hepatitis in Burkina Faso, phytochemistry and antioxidant activity: *World J. Adv. Pharm. Life Sci*, 2021; 1: 23-34. <https://doi.org/10.53346/wjapls.2021.1.1.0019>
21. Tapsoba H and Jean-Pierre D. Use of medicinal plants for the treatment of oral diseases in Burkina Faso: *Journal of ethnopharmacology*, 2006; 104(1-2): 68-78. <https://doi.org/10.1016/j.jep.2005.08.047> PMID:16214302
22. Salla S, Rajitha S, Simon O, Lloyd TW, and Martha V. Antioxidant activity of papaya seed extracts against H2O2 induced oxidative stress in HepG2 cells: *LWT-Food Science and Technology*, 2026; 66: 293-297. <https://doi.org/10.1016/j.lwt.2015.09.008>
23. Ribarova F, Maria A, Marinova D, Ribarova F, and Atanassova M. Total phenolics and flavonoids in Bulgarian fruits and vegetables: *JU Chem. Metal*, 2005; 40(3): 255-260.
24. Lamien-Meda A, Charles EL, Moussa MYC, Roland NTM, Martin K, Boukare Z, Jeanne FM and Odile GN. Polyphenol content and antioxidant activity of fourteen wild edible fruits from Burkina Faso: *Molecules*, 2008; 13(3): 581-594. <https://doi.org/10.3390/molecules13030581> PMID:18463567 PMCID:PMC6245336
25. Konaré MA, Nouhoum D, Cheickna C, Daouda AKT, Issiaka T, Aigna K, Rokia S, and ASO. Evaluation of the biological activities of leaf and bark extracts of *Ficus platiphylla* Delile, a medicinal plant used in Mali: *Journal of Medicinal Plants Research*, 2020; 14(3): 118-128. <https://doi.org/10.5897/JMPR2019.6874>
26. Watts JL, Shryock TR, Apley M, Bade DJ, Brown SD, Gray JT, Heine H. Clinical and laboratory standards institute. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals: approved standard. National Committee for Clinical Laboratory Standards (2008).
27. Dhanani T, Sonal S, Gajbhiye NA, and Satyanshu K. Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of *Withania somnifera*: *Arabian journal of chemistry*, 2017; 10: S1193-S1199. <https://doi.org/10.1016/j.arabjc.2013.02.015>
28. Acharya K, Sandipta G, and Rhituparna B. Total phenolic contents and antioxidant effects of infusion and decoction from *Lepista sordida* (Schumach.) Singer: *Fabad Journal of Pharmaceutical Sciences*, 2018; 43(1) : 17-24.
29. Ghosh S, Tribeni C, Anirban S, Ishita C, Arup B, Angana D, Akash M, and Krishnendu A. Antioxidant properties and phytochemical screening of infusion and decoction obtained from three cultivated *Pleurotus* species: A comparative study: *Jordan Journal of Pharmaceutical Sciences*, 2020; 13(2): 121-129.
30. Ennaifer M, Taroub B, Chokri M, and Moktar H. Phytochemicals, antioxidant, anti-acetyl-cholinesterase, and antimicrobial activities of decoction and infusion of *Pelargonium graveolens*: *Natural product research*, 202; 34(18): 2634-2638. <https://doi.org/10.1080/14786419.2018.1547299> PMID:30584784
31. Messaoud C, Abdelmonoem L, and Mohamed B. *Myrtus communis* L. infusions: the effect of infusion time on phytochemical composition, antioxidant, and antimicrobial activities: *Journal of food science*, 2012; 77(9): C941-C947. <https://doi.org/10.1111/j.1750-3841.2012.02849.x> PMID:22888790
32. Eke ON, Augustine AU, and Ibrahim H F. Qualitative analysis of phytochemicals and antibacterial screening of extracts of *Carica papaya* fruits and seeds: *International Journal of Modern Chemistry*, 2014; 6(1): 48-56.
33. Ouédraogo A, Philippe AN, Gérard JY, Pane BS, Belinda RHB, Soumaïla K, Marguerite EN, Oueogo N, Wendinmi BFG, and Nicolas B. Phytochemicals properties of *Carica papaya* Linn seeds' essential oil and their antifungal and antibacterial activities. *Journal of medicinal plants research*; Vol.17(11): 331-337. <https://doi.org/10.5897/JMPR2023.7321>
34. Masfufatun N, Yani PW, and Putri NPY K. Antimicrobial assay of papaya seed ethanol extract (*Carica papaya* Linn) and phytochemical analysis of its active compounds: *Journal of Physics: Conference Series*, 2019; 1277(1), 012-018. <https://doi.org/10.1088/1742-6596/1277/1/012018>
35. Varadarajan S, Malathi N, Malaiyandi M, and Chamundeeswari D. In vitro anti-mycotic activity of hydro alcoholic extracts of some indian medicinal plants against fluconazole resistant *Candida albicans*: *Journal of Clinical and Diagnostic Research*, 2015; 9(8): ZC07-ZC10.
36. Emmanuel OBOH, Daniel A, Peace O, and Ojei O. Investigating the Astonishing Antimicrobial Potential of Papaya seeds against *Salmonella* spp and *Candida albicans*. *International Journal of Traditional and Complementary Medicine Research*, 2023; 4(1): 26-30. <https://doi.org/10.53811/ijtcmr.1249215>
37. Peter JK, Yashab K, Priyanka P, and Harison M. Antibacterial activity of seed and leaf extract of *Carica Papaya* var. Pusa dwarf Linn: *IOSR Journal of Pharmacy and Biological sciences*, 2017; 9(2): 29-37. <https://doi.org/10.9790/3008-09272937>
38. Sundar S, Padmalatha K, Apsana SK, Himaja P, Nandini V, and Sirisha D. Antibacterial and Antifungal activity of *Carica papaya* L seed extracts: *Research Journal of Pharmacy and Technology*, 2021; 14(2): 1085-1090. <https://doi.org/10.5958/0974-360X.2021.00195.5>
39. Abaka AK, Gali AI, Aishatu H, and Bello PA. Phytochemicals screening and antifungal activity of *Balanites aegyptiaca* Seed and Callus Extract against *Candida albicans*: *Asian Plant Research Journal*, 2020; 4(4): 9-16. <https://doi.org/10.9734/aprj/2020/v4i430091>