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## **RESEARCH ARTICLE**

# IN VITRO EVALUATION OF ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF CARICA PAPAYA L. SEEDS GROWN IN THE REPUBLIC OF YEMEN

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## Abstract

This study aimed to investigate the phytochemical, antimicrobial, and antioxidant activities of Carica papaya L. seeds extracts collected from Al Hudaydah city, Yemen (Latitude: 14°47′52″ N Longitude: 42°57′16″ E) during the months of January and February 2021.

The seed was extracted with different solvents by the cold percolation method. The disk diffusion method was employed to assess the antibacterial activity of the seed extract against six bacterial and four fungal strains. Spectrometric methods were employed to calculate the total alkaloids, anthocyanin, flavonoid, phenolic, quinones, saponin, steroids, terpenoid, tannin, and phenols contents, as well as the antioxidant activities.

Antibacterial and antifungal activity tests exhibited that the selected microorganisms are highly sensitive to the ethanolic and methanolic extracts of C. papaya L. seeds, followed by chloroform, water, and n-hexane extracts. The samples also demonstrated a significant DPPH, FRAP, and APTS radical scavenging activity. Additionally, the preliminary phytochemical analysis revealed the presence of flavonoid, terpenoid, saponin, alkaloids, steroids, quinones, anthocyanin, tannin, and phenols, all of which potentially contribute to the antimicrobial activities of C. papaya L. seeds.

**Keywords:** Carica papaya seeds, Al- Hudaydah, Yemen, Antimicrobial, Antioxidant, Total phenolic, Phytochemical screening.

## 1. Introduction:

Medicinal plants have been a principal source of medicines for decades. Yemen has a long and illustrious history of awareness and knowledge about plant-based medicines for both preventive and curative medicine. About 88% of the people around the globe depends directly or indirectly on plant-based medicines and consume them as their first line of defense against a variety of diseases for maintaining health conditions [1]. Yemen has a large phytobiome diversity, but only a few have been exploited medicinally. The majority of these plants are used as a conventional medicines with proven efficacy and are passed down through generations [1]. There is dearth of scientific literature that entails the chemical composition of medicinal plants. Papaya is one of the most widely used medicinal plant especially its seeds [2]. Papaya (Carica papaya L.) is a traditionally important, nutritionally invaluable and medicinal plant that is a member of Carica genus (family Caricaceae). It

grows abundantly in tropical areas such as Yemen, Africa and Nigeria, which collectively produce the majority of the world's papaya [1]. It is a rapidly growing arborescent tree with a short lifespan, with a single straight, or sometimes branched, stem reaching a height of 2-10 meter. The standard papaya fruit is composed of the following components: pulp (79.5%), skin (12%), and seed (8.5%), and its fruit is consumed fresh or juiced. Papaya seeds are dark (blackish) in colour and are found embedded in the fruit pulp; they are considered as a byproduct. The year-round availability and low economic value of the papaya seeds have prompted nutritionists to explore them as a protein-rich source food ingredients, as well as functional therapy to increase blood platelets counts, treat stomach ulcers, diarrhea, HIV patients, organ transplant patients, cancer chemotherapy patients, malaria, dengue, jaundice, and immunomodulatory. In addition, other papaya seeds' properties include antibacterial, antifungal, antiviral and antispasmodic activity [3,4]. The papaya seeds, fruit pulp and leaves

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possess a variety of active components that boost antioxidant activity in the blood and lower lipid peroxidation levels. Among these compounds are ascorbic acid, chymopapain, cyanogenic glucosides, cystatin, flavonoids, papain, tocopherol, and glucosinolates [5,6]. Papaya fruit has all the macronutrients, in which carbohydrates are the most abundant; vitamins such as vitamin A, vitamin C, and vitamin E, flavonoids, polyphenols, vitamin B12, and folic acid. These compounds performed their antioxidative function by supplicating electrons to ROS. Papaya seeds have also shown to harbor certain minerals such as calcium, chlorine, potassium, magnesium and sodium [7,8].

In this study papaya seeds were evaluated for their potential role in folk medicine and to unravel their active extraction principle by isolating and characterizing their active ingredients.

# 2. Material and Method

## Scheme of conducting the current study

The flow chart illustrates the protocol for screening the phytochemical composition and antimicrobial activity of C. papaya L. seeds (Fig 1).



Fig 1. Scheme of conducting the current study

### **Collection of plant material**

During the months of January and February 2021, papaya fruits were collected from papaya trees located throughout Al Hudaydah district, Yemen (Latitude: 14°47′52″ N Longitude: 42°57′16″ E), described as a

yellow stripe near the fruit apex (70–80% of the yellow surface) (Figure 2).

The collected papaya fruits were splitted to extract the seeds, subsequently the seeds were grinded, air-dried under the shade, powdered, finally stored in air-tight plastic container until used.



Fig 2. Carica papaya L. tree (A), fruits and seeds of the C. papaya (B).

#### Microbial organisms used in the study

The bacterial pathogens, including Gram-positive bacterial strains (*Staphylococcus aureus* ATCC 6538 and *Bacillus cereus* ATCC 6059) and Gram-negative strains (*Aeromonas hydrophila* ATCC 7966, *Escherichia coli* O157:H7, *Shigella dysenteriae* NCTC 14131, and *Salmonella Typhimurium* ATCC 14028), along with fungal strains (*Aspergillus niger* MCC 98003, *Aspergillus flavus* AS3.35540, *Candida albicans* CMCC 98001, and *Candida tropicalis*) were obtained from the Department of Food Science, College of Agriculture, Sana'a University, Yemen. The microorganisms were cultured on nutrient agar. Tables 1 and 2 detail the tests, selected organisms, and antibiotics used.

#### **Preparation of papaya seed extracts**

Cold percolation method was employed to prepare papaya seed extracts after following the procedure demonstrated by *Rosenthaler* [9]. The powdered papaya seeds were soaked in 1:10 mixture of ethanol, methanol, chloroform, water and n-hexane for 24 hours (h) at room temperature (RT) with shaking (150 rpm). The filtrates of the extracts were dried at 40<sup>o</sup>C and powdered further and then re-suspended in 10% Dimethyl sulfoxide (DMSO), a universal solvent, to a concentration of 100 mg/mL.

# Determination of antimicrobial and antifungal activity of papaya seed extracts

The effect of *C. papaya* seed L. extracts on the pathogenic bacterial and fungal strains was determined using agar diffusion tests and zones of inhibition (mm) assays and results were noted after 24 h of incubation [9,10].

# Determination of minimum inhibitory concentration (MIC)

The MIC of the papaya seed extracts was determined after following the National Committee for Clinical Laboratory Standards (2010) recommended protocol [7]. Serial dilutions of the DMSO extracts were added in microtiter plates containing Mueller Hinton broth (MHB), to evaluate the MIC for bacterial strains and Sabouraud dextrose broth (SDB), to assess the MIC for fungal strains. The plates were then placed in a refrigerator (4°C) for 24 h to ensures uniform diffusion of the extracts, subsequently, plates were dried for 2 h at 37°C prior to inoculation with microorganisms. A loopful (ca. 3 mm in diameter) of overnight grown culture of each of the tested bacterial strains (S. aureus, Bacillus subtilis, E. coli S. Typhimuriu, Aeromonas hydrophila, and S. dysenteriae) and fungal strains was diluted to 10<sup>-6</sup> CFU/mL and evenly spread on MH agar. Plates harbouring bacterial strains were incubated at 37°C/24 h, while the fungal plates for 72 h. The MIC was described as the minimum concentration of seed extracts that did not result in visible growth of microorganisms [10,11].

# Phytochemical screening and total phenol content determination

To achieve a light-coloured suspension, C. papaya L. seeds powder was diluted to 1:10 ratio with sterilized distilled water and subjected to qualitative phytochemical analysis of flavonoids, terpenoids, saponins, alkaloids, steroids, quinones anthocyanin, tannins, and phenols [11,12]. Folin-Ciocalteu reagent was used to assess the total phenolic content (TPC), as described by [11]. 0.5 mL dry weight of grounded seeds (1 mg/mL) were mixed with 2 mL of Na<sub>2</sub>CO<sub>3</sub> (7%) and 0.5 mL Folin Ciocalteu reagent. The resultant mixtures were placed at 25°C/2 h, and the absorbance was measured via a UV/Vis spectrophotometer at 760 nm. The standard curve for catechol (g/mL) was constructed, and the TPC values were shown in catechol equivalents (µg/mg of dry mass), a commonly used reference compound.

#### **Determination of antioxidant activities**

The antioxidant activity of papaya seed extracts was measured using the 2, 2-diphenyl-1,1-picrylhydrazyl (DPPH) free radical scavenging test, as demonstrated earlier but with slight modifications [12,13]. Each essential oil concentration was added individually to a 1 mL methanol solution containing a 0.1 mmol/L DPPH solution in ethanol. After vigorous shaking, the reaction mixture was incubated in the dark for 25 minutes at RT, and the absorbance was determined at 517 nm using ascorbic acid (1 mmol/L) as a reference compound. Three technical repeats of each experiment was conducted and averaged. The following equation was used to measure the percent scavenging activity relative to control:

Scavenging activity  $\% = (1-\text{Absorbance sample/Absorbance control}) \times 100.$ 

The ferric reducing antioxidant power (FRAP) assay [11], and 2, 20-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay [14] were also conducted to assess the radical scavenging activity of papaya seed extracts. For FRAP assay, a standard curve was generated using Trolox and results mentioned in mmol/g of extract, whereas, and the ABTS results were shown in mmol TE/100 g of extract.

#### **Statistical Analysis**

The mean  $\pm$  SD of triplicate measurements was used to calculate the results. SPSS (version 19) was used to assess the significance of variations between different comparisons by performing one-way ANOVA followed by a Duncan test with P value of 0.05.

#### **3. Results**

#### In vitro antimicrobial assay

The results of antimicrobial and antifungal activities of C. papaya L. seeds powder solvent extract are presented in Table 1 and Figure 3. The ethanol and methanol extracts of C. papaya L. seeds were found to be most effective against all the tested pathogens and to possess the most potent antibacterial activity. The ethanol and methanol extracts of the powdered seeds revealed the highest activity against S. aureus ATCC 6538 (23.0+ 2.15 and 20.0 + 2.68 mm, respectively), B. subtilis (22.0±0.33 and 22.0±1.00 mm, respectively), E. coli O157:H7 (16.0 + 2.12 and 18.0 + 2.48 mm, respectively),S. Typhimurium (15.7+ 1.40 and 14.65  $\pm$  0.38) A. hydrophila (16.50 ± 0.30 and 15.90 ± 0.85 mm, respectively) and S. dysenteriae ( $15.15 \pm 0.62$  and 15.22 $\pm$  0.52 mm, respectively). The chloroform, water and nhexane extracts shown moderate antibacterial activity against all the tested bacteria except chloroform on B. *subtilis* (17.0±0.9 mm).

The effective concentrations of the papaya seed extracts exhibited against the tested fungal strains (C. tropicalis, C. albicans, A. flavus, and A. niger) are mentioned in the table 2 and figure 4. The ethanol and methanol extracts of the powdered papaya seeds showed the highest activity against C. tropicalis (19.0  $\pm$  0.59 and 19.5  $\pm$  0.31 mm, respectively), C. albicans (20.5  $\pm$  0.30 and 20.0±0.12mm, A. niger (22.0±1.8 and 18.0±1.6 mm, respectively) and A. flavus (22.0±1.8 and 3.00±0.12 mm, respectively), A.flavus ( $3.05 \pm 0.15$  and  $3.17\pm0.14$  mm, respectively). The chloroform and water showed moderate inhibition on C. tropicalis (11.22  $\pm$  0.18 and 10.33  $\pm$  0.22 mm, respectively), C. albicans (11.22  $\pm$ 0.18 mm), C. albicans  $(19.13 \pm 0.13 \text{ mm and } 19.33 \pm 0.60 \text{ mm})$ respectively). The chloroform and water solutions demonstrated a low inhibition against A. niger (3.03  $\pm$ 0.60 and 3.35  $\pm$  0.23 mm, respectively) and A. flavus  $(2.10 \pm 0.15 \text{ mm and } 2.10 \pm 0.15 \text{ mm, respectively}).$ 

Table 1 Antibactorial activities exhibited b	v different solvent extracts of C	<i>papaya</i> seeds by agar-well diffusion method.
Table 1. Antibacterial activities exhibited b	y unicient solvent extracts of C.	<i>pupuyu</i> seeds by agai-well diffusion method.

Concentrations= 100 mg/ml						
S. aureus	B. subtilis	E. coli	S. Typhimurium	A. hydrophila	S. dysenteriae	
23.0+2.15	22.0±0.33	16.0 + 2.12	15.7+ 1.40*	$16.50\pm0.30$	$15.15\pm0.62$	
20.0 + 2.68	22.0±1.00	18.0 + 2.48	$14.65\pm0.38$	$15.90\pm0.85$	$15.22\pm0.52$	
12.0 + 2.12	17.0±0.9	11.0+ 3.56	$10.15\pm0.62$	$8.32\pm0.22$	$10.23\pm0.18$	
$10.17\pm0.50$	11.0 + 1.50	9.00+1.00	$6.11\pm0.48$	$7.32\pm0.50$	$7.36\pm0.13$	
$11.03\pm0.87$	$8.22\pm0.540$	$8.22\pm0.14$	$8.28\pm0.15$	$7.11\pm0.15$	$7.22\pm0.50$	
Positive control						
$25.5\pm0.67$	26.0+ 2.15	$20.5\pm0.30$	$20.0\pm0.15$	$19.0\pm0.59$	$19.0\pm0.15$	
	$23.0+2.15$ $20.0+2.68$ $12.0+2.12$ $10.17 \pm 0.50$ $11.03 \pm 0.87$	S. aureus         B. subtilis $23.0 \pm 2.15$ $22.0 \pm 0.33$ $20.0 \pm 2.68$ $22.0 \pm 1.00$ $12.0 \pm 2.12$ $17.0 \pm 0.9$ $10.17 \pm 0.50$ $11.0 \pm 1.50$ $11.03 \pm 0.87$ $8.22 \pm 0.540$	S. aureusB. subtilisE. coli $23.0+2.15$ $22.0\pm0.33$ $16.0+2.12$ $20.0+2.68$ $22.0\pm1.00$ $18.0+2.48$ $12.0+2.12$ $17.0\pm0.9$ $11.0+3.56$ $10.17\pm0.50$ $11.0+1.50$ $9.00+1.00$ $11.03\pm0.87$ $8.22\pm0.540$ $8.22\pm0.14$ Positive cor	S. aureus         B. subtilis         E. coli         S. Typhimurium $23.0 \pm 2.15$ $22.0 \pm 0.33$ $16.0 \pm 2.12$ $15.7 \pm 1.40^*$ $20.0 \pm 2.68$ $22.0 \pm 1.00$ $18.0 \pm 2.48$ $14.65 \pm 0.38$ $12.0 \pm 2.12$ $17.0 \pm 0.9$ $11.0 \pm 3.56$ $10.15 \pm 0.62$ $10.17 \pm 0.50$ $11.0 \pm 1.50$ $9.00 \pm 1.00$ $6.11 \pm 0.48$ $11.03 \pm 0.87$ $8.22 \pm 0.540$ $8.22 \pm 0.14$ $8.28 \pm 0.15$	S. aureus         B. subtilis         E. coli         S. Typhimurium         A. hydrophila $23.0 \pm 2.15$ $22.0 \pm 0.33$ $16.0 \pm 2.12$ $15.7 \pm 1.40^*$ $16.50 \pm 0.30$ $20.0 \pm 2.68$ $22.0 \pm 1.00$ $18.0 \pm 2.48$ $14.65 \pm 0.38$ $15.90 \pm 0.85$ $12.0 \pm 2.12$ $17.0 \pm 0.9$ $11.0 \pm 3.56$ $10.15 \pm 0.62$ $8.32 \pm 0.22$ $10.17 \pm 0.50$ $11.0 \pm 1.50$ $9.00 \pm 1.00$ $6.11 \pm 0.48$ $7.32 \pm 0.50$ $11.03 \pm 0.87$ $8.22 \pm 0.540$ $8.22 \pm 0.14$ $8.28 \pm 0.15$ $7.11 \pm 0.15$	

Values represented as mean  $\pm$  SD of three replications. A-B: Ampicillin B (100 mg/mL); C-E: Gentamicin(100) mg/mL: Diameter of the inhibitory zones(mm).



In Vitro Evaluation of Antimicrobial and Antioxidant Activity of Carica Papaya L. Seeds Grown in the Republic of Yemen



**Fig 3.** Effect of different extracts of *C. papaya* L. seed against various pathogens of [A1, B1, C1, D1, E1] :ethanol (A), methanol (B), chloroform (C), water (D and n-hexane extracts respectively]. ethanol (A), methanol (B), chloroform (C), water (D), n-hexane (E) extracts, Ampicillin (F). eth: ethanol; meth: Methanol; chlo : chloroform; wa: water; n-hex: n-hexane.

#### **Determination of MIC values**

The MIC values of seed extracts of *C. papaya* L. were measured using the two-fold broth micro-dilution method. The seed extract (SE) was chosen for MIC50 determination because it displayed the broadest

inhibitory activity against the bacteria and fungi strains studied. The MIC from all tested bacteria ranges from 125-1000  $\mu$ g/mL of all extracts of *C. papaya* L. seeds as mentioned in **Table 3**. While in fungi, MIC ranges from 500-1000  $\mu$ g/mL with the extracts (ethanol, methanol, chloroform and water) as indicated in **Table 3**.

Table 2. Antifungal activity of extracts of C. papaya seed on various pathogenic strains

Concentrations= 100 mg/ml							
	C. tropicalis	C. albicans	A. niger	A.flavus			
Plant extracts							
Ethanol	$19.0\pm0.59$	$20.5\pm0.30$	22.0±1.8	$3.05\pm0.15$			
Methanol	$19.5\pm0.31$	20.0±0.12	3.00±0.12	3.17±0.14			
Chloroform	$11.22\pm0.18$	19.13 ± 0.13	$3.03\pm0.60$	$2.10\pm0.15$			
Water	$10.33\pm0.22$	$19.33\pm0.60$	$3.35\pm0.23$	$2.10\pm0.15$			
	Positive	control (Nystatin 100 mg	/ml)				
	22.93±0.66	23.83±0.95	24±0.3	22.97±0.15			

Table 3. Minimal inhibitory concentration (MIC) of the seed of C. papaya against bacteria and fungi.

Selected plant	MIC (µg/mL of bacteria						
extract	S. aureus	B. subtilis	E. coli	S. Typhimurium	S. dysenteriae		
Ethanol	125	125	250	250	250		
Methanol	125	125	250	250	250		
Chloroform	500	500	500	500	500		
Water	500	500	500	500	500		
	MIC (µg/mL of fungi						
	C. albicans	C. tropicalis	A. niger	A. A.flavu			
Ethanol	500>	500>	500>	500>			
Methanol	500	500	1000>	1000>			
Chloroform	1000>	1000>	1000>	1000>			
Water	1000	1000	1000	1000			





**Fig 4**. Effect of different extracts of *C. papaya* seed against various pathogens of [A1, B1, C1 and D1 (ethanol (A), methanol (B), chloroform (C), water (D) extracts respectively]. Nystatin (E). eth: ethanol; meth: Methanol; chlo : chloroform; wa: water.

# Phytochemical screening and determination of total phenol content

The contents of flavonoid, terpenoid, monomeric, anthocyanin, saponin, alkaloids, steroids, quinones, and tannins in methanol, ethanol, chloroform, n-hexane and water extracts of Carica papaya seeds were determined separately and are presented in Table 4. Table 4 shows that anthocyanin and tannin were detected in all extracts. Flavonoid compounds were also found in all five extracts. All the solvents contained significant amounts of flavonoid, anthocyanin and tannin, but none contained quinones. Methanol, ethanol, benzene and chloroform extracts, on the other hand, possessed all the tested phytochemicals except Quinones. The TPC of selected extracts (methanol, ethanol, chloroform, and water) was performed based on the antimicrobial activity findings, and the resulting data is summarized in Figure 5. TPC content was found to be highest in ethanol extracts (96 g/mg), followed by methanol (90 g/mg), chloroform (75 g/mg), and water (40 g/mg) extracts. Due to the high TPC content of the ethanol, methanol, and methanol extracts, these extracts were subsequently subjected to antioxidant assays (Figure 5).

Table 4. Phytochemical constituents of C. papaya seeds

Phytochemical analysis of C. papaya seed extracts							
	Methanol	water	Ethanol	N- hexane	Chloroform		
Flavonoid	+	+	+	+	+		
Terpenoid	+	-	+	+	+		
Saponin	+	+	+	+	-		
Alkaloids	+	-	-	-	+		
Steroids	+	-	+	-	+		
Quinones	-	-	-	-	-		
Anthocyanin	+	+	+	+	+		
Tannin	+	+	+	+	+		
Phenols	+	-	+	-	+		
	Kay - Present - Abcent						

Key: + = Present, - = Absent



Fig 5. TPC of selected plant extract of *C. papaya*. The results are the catechol equivalents TPC ( $\mu$ g /mg) of a sample. n=3, means S.



Fig 6. Antioxidant activity exhibited by various solvent extracts of *C. papaya* seeds

#### Antioxidant activities

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The antioxidant activities of papaya seeds were determined by the DPPH, ABTS, and FRAP assays (Figure 6). The ethanolic extract of papaya seed exhibited the highest DPPH scavenging activity of around 5500.44  $\mu$ mol TE/100 g compared to methanol, chloroform and water of around 4600.12, 2400.23 and 1300.11  $\mu$ mol TE/100 g, respectively. However, ethanol seed extract demonstrated the highest levels of activity (2700.23 and 2300.11  $\mu$ mol TE/100 g, respectively) as determined by the FRAP and ABTS assays, as compared to methanol, chloroform and water (Figure 6).

## 4. Discussion

Yemen has approximately 7000 flowering plants species, 2000 of which have medicinal properties [1]. Yemen has a rich and diverse range of vegetation that is consumed by the locals to treat and prevent diseases [10,11]. Today, there is widespread interest in maximizing the potential of plant-derived medicines. The aim of this study was to assess the antimicrobial and antioxidant abilities of in the midst of quest for unique therapeutic compounds. Plants are consumed across the globe either directly or indirectly to treat diseases, and drugs are being developed as a result of research on these medicinally important plants. The C. papaya L. seed extracts inhibited the growth of fungal and bacterial strains. However, a varying level of effectiveness was witnessed against these microbes. It has been previously demonstrated that different types and parts of medicinal plants exhibit varying degrees of inhibitory activity against various microbes, and that medicinal plants typically exhibit greater inhibitory activity against grampositive bacterial strains than gram-negative [10,15]. This may likely be due to the cell-wall composition of gram-positive bacteria, which shared peptidoglycan as a principal component. Gram-negative bacteria have a more complex cell wall, with an outer membrane composed of lipopolysaccharides (~80%) and an inner membrane composed of peptidoglycans (~20%). The present study relieved that the five tested C. papaya L. seeds extract have potential antibacterial activity against all tested bacteria and fungi. The ethanol extract of C. papaya L. showed significant activity against S. aureus

and *subtilis* of around  $23.0\pm2.15$  and  $22.0\pm0.33$  mm, respectively (Table 1) and MIC values around of 125 µg/ml, respectively Table 3. The chloroform extract of *C. papaya* exhibited had the lowest antibacterial activity on most of the tested bacteria, except on *B. subtilis* (17.0±0.9 mm) with MIC values of 500 µg/ml of *B. subtilis* and lowest in all bacteria (Table 3). Our findings corroborate previous findings that ethanol and methanol are superior solvents for antimicrobial material extraction when compared to chloroform, water, and n-hexane [16,17]. We demonstrated that the seed extract of *C. papaya* L. possesses antibacterial activities. Similar findings have demonstrated the efficacious antibacterial activity of papaya fruit extracts against certain Gramnegative and Gram-positive bacterial strains [18,19].

Nonetheless, chloroform, n-hexane, and water extracts of papaya seed demonstrated a mild inhibitory effect on all bacteria examined. Similar findings have been made about the antimicrobial function of aqueous extracts [20]. A. Seed extracts inhibited hydrophila, which is already proven to be multidrug resistant. These findings are important since A. hydrophila is capable of producing a variety of enterotoxins that can result in dysenteric gastroenteritis [21]. The fungal strains exhibited a higher sensitivity to the seed extract based on ethanol and methanol compared to the chloroform and water extracts [21]. Similarly, the effects of papaya on seed germination and seedling emergence of African yam bean (Sphenostylis stenocarpa) was appraised after exposing them to seed-borne fungi, A. niger, A. flavus, B. theobromae, and F. moniliforme [22]. The results of the current study demonstrates the efficacy of the papaya seeds as a medicinal plant and as a forum for the development of novel drugs, such as phytomedicine. A substantial amount of carbohydrates, alkaloids, flavonoids, steroids, and tannins were screened in the seed extract of C. papaya L. Thus, antimicrobial activity of papaya seeds can be accredited to tannins and saponins, as both of these metabolites contribute significantly to antimicrobial activity [20]. A number of phytochemical ingredients were detected during the phytochemical screening. TPC and phytochemical bioactive compounds present in three C. papaya L. seed extracts (ethanol, methanol and chloroform) exhibited antimicrobial and antioxidant activities. However, previous phytochemical studies of C. papaya L. also isolated a number of flavonoids, bioflavonoid, triflavonoid, triterpenoids and sterols [23,24,25]. The findings of this study suggested that ethanol and methanol are both highly effective solvents for extracting phenolic compounds owing to their high polarity and solubility [26,27]. Comparing these findings to the already published literature, the obtained TPC values were substantially higher than those previously recorded for jackfruit (29.0 mg of GAE/100 g), pineapple (38.1 mg of GAE/100 g), and sapodilla (13.5 mg of GAE/100 g) [27,28. The pulp contains a higher concentration of tetramethylammonium chloride (TMAC) than pineapple (11.62 mg/100 g), cashew apple (7.32 mg/100 g), guava (7.62 mg/100 g), papaya (1.87 mg/100 g), and tamarind (2.92 mg/100 g) (Figure 4 and Table 4) [28]. Ethanolic

and methanolic extract of Papaya seeds exhibited higher DPPH, FRAP, and ABTS values than chloroform and water extracts. Additionally, Pearson correlation tests showed a positive correlation among antioxidant activities (DPPH, ABTS, FRAP), phenolic content and total tannin in papaya seed extracts (r = 0.999, P 0.01), indicating that TTC concentrations increased as total phenolic and antioxidant activity concentrations increased. [29] also identified significant associations between total phenolic and antioxidant activities. Our results reinforce the ones previously reported by [30], which revealed a higher FRAP value in the pulp of sea buckthorn (Hippophae rhamnoides L.) than in seed. By contrast, papaya seed extracts were found to be a more potent source of antioxidants by ABTS method than tamarind (8.32 µmol TE/g), pineapple (3.78 µmol TE/g), murici (Byrsonima crassifolia) (15.73 µmol TE/g) and mangaba (10.84 µmol TE/g) as reported by [31,32,33]. The antioxidant potential of papaya seed was found to be higher by DPPH assay than that of some exotic fruits, such as tamarind pulp (2.04mol TE/g), murici (B. crassifolia) pulp (6.46mol TE/g), and mangaba pulps (5.27mol TE/g) [29,30]. In conclusion, our findings established a strong and important correlation between antioxidant activity and phenolic compounds (Figure 6).

#### 5. Conclusion

To summarize, ethanol and methanol shared a higher extractability than other used solvents. These extracts are unmatched in their ability to be opted to treat bacterial and fungal infections. The safety of medicinal plants is not complete because their use in general exposes people to other dangers. Therefore, further research is needed on papaya seeds from various habitats to determine the active component and its precise amount to inhibit microbial growth and could eventually play a significant role in drug development programs in the pharmaceutical industry.

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### References

- [1] (FAO). (2020). Food and Agriculture Organization Nations Statistics, Roma. of the United DOI: 10.4060/ca9229en
- [2] H. Aggie (2002). Home Fruit Production--Paw paw. Texas Agricultural Extension Service. Extension Horticulture Information Resource

- [3] O. Kadiri, B. Olawoye, O.S. Fawale and O.A. Adalumo, "Nutraceutical and antioxidant properties of the seeds, leaves and fruits of Carica papava: Potential relevance to humans diet, the food industry and the pharmaceutical industry-a review". Turk. J. Agric. Food Sci. Technol., 4: 1039-1052. DOI: 10.24925/turjaf.v4i12.1039-1052.569
- [4] W. Siriwattanametanon, W. Kanchanarach, R. Thiwthong and J. L. Dodgson, "Culture filtrates from laboratory grown Phellinus mushrooms for use as antibacterial agents". Chiang Mai J Sci. 41: 243-247. http://epg.science.cmu.ac.th/ejournal/
- [5] S. S. Hasson, Al-Balushi, M. S. Sallam, T. A. Idris, M. A. Habbal and A. A. Al-Jabri, "In vitro antibacterial activity of three medicinal plants-Boswellia (Luban) species". Asian Pac. J. Trop. Biomed., 1: S178-S182. DOI: 10.1016/S2221-1691(11)60151-2
- [6] D.S. Seigler, G.F. Pauli, A. Nahrstedt and R. Leen, "Cyanogenic allosides and glucosides from Passiflora edulis and Carica papaya". Phytochemistry, 60: 873-882. DOI: 10.1016/S0031-9422(02)00170-X/
- [7] E. M. Ervin, and J. K. Kariuki, "Demonstrating the minimal impact of cultivation conditions on antioxidants in fruits and vegetables by differential pulse voltammetry". DOI: 10.1080/10942912.2015.1048354
- [8] T. Puangsri, S. M. Abdulkarim, and H. M. Ghazali, "Properties of Carica papaya L.(papaya) seed oil following extractions using solvent and aqueous enzymatic methods". J. food lipids, 12: 62-76. DOI: 10.1111/j.1745-4522.2005.00006.x
- [9] A. K. Khan, R. Rashid, G. Murtaza, and A. J. T. R. "Gold nanoparticles: synthesis Zahra, and applications in drug delivery". Trop. J. Pharm. Res. 13: 1169-1177. DOI: 10.4314/tjpr.v13i7.23
- [10] N. Q. M. Al-Hajj, H. X. Wang, C. Ma, Z., Lou. M. Bashari, and R. Thabit, "Antimicrobial and antioxidant activities of the essential oils of some aromatic medicinal plants (Pulicaria inuloides-Asteraceae and Ocimum forskolei-Lamiaceae) ". Trop. J. Pharm. Res. 13: 1287-1293. \*DOI: <u>10.4314/tjpr.v13i8.13</u>
- [11] N. Q. M. Al-Hajj, M. N. Algabr, H. Raza, R. Thabi, A.F. Ammar, W, Aboshora, and H. Wang, "Antibacterial activities of the essential oils of some aromatic medicinal plants to control pathogenic bacteria and extend the shelf-life of seafood". TURK J FISH. 17: 181-191. DOI: 10.4194/1303-2712-v17 1 20

- [12] M. Lu, B. Yuan, M. Zeng, and J. Chen, "Antioxidant capacity and major phenolic compounds of spices commonly consumed in China". Int. Food Res. J. 44 : 530-536. DOI: <u>10.1016/j.foodres.2010.10.055</u>
- [13] A. Adedapo, A, Jimoh, F. O. Afolayan, A. J. and P. J. Masika, "Antioxidant Properties of the Methanol Extracts of the Leaves and Stems of Celtis africana". Rec. Nat. Prod. 3: d 01/13/2009 EISSN:1307-6167
- [14] A. V. Khan, Ahmed, Q. U. Mir, M. R. Shukla, I. and A. A. Khan, "Antibacterial efficacy of the seed extracts of Melia azedarach against some hospital isolated human pathogenic bacterial strains". Asian Pac. J. Trop. Biomed. 1: 452-455. DOI: <u>10.1016/S2221-1691(11)60099-3</u>
- [15] E. M. Tekwu, A. C. Pieme, and V. P. Beng, "Investigations of antimicrobial activity of some Cameroonian medicinal plant extracts against bacteria and yeast with gastrointestinal relevance". Journal of ethnopharmacology, 142: 265-273, 2012. DOI: <u>10.1016/j.jep.2012.05.005</u>
- [16] G. Onivogui, R. Letsididi, M. DiabyL. Wang, and Y. Song, "Influence of extraction solvents on antioxidant and antimicrobial activities of the pulp and seed of Anisophyllea laurina R. Br. ex Sabine fruits". Asian Pac. J. Trop. Biomed. 6: 20-25. DOI: <u>10.1016/j.apjtb.2015.09.023</u>.
- [17] R. Naz, and A. Bano, "Antimicrobial potential of Ricinus communis leaf extracts in different solvents against pathogenic bacterial and fungal strains". Asian Pac. J. Trop. Biomed. 2: 944-947. DOI: <u>10.1016/S2221-1691(13)60004-0</u>
- [18] S. Saravanan, and T. Parimelazhagan, "In vitro antioxidant, antimicrobial and anti-diabetic properties of polyphenols of Passiflora ligularis Juss. fruit pulp". Food Sci. Hum. Wellness, 3: 56-64. DOI: <u>10.1016/j.fshw.2014.05.001</u>
- [19] R. khan Pathan, P. R. Gali, Pathan, P. Gowtham, T. and S. Pasupuleti, "In vitro antimicrobial activity of Citrus aurantifolia and its phytochemical screening". Asian Pacific Journal of Tropical Disease, 2, S328-S331. DOI: <u>10.1016/S2222-1808(12)60176-5</u>
- [20] E. O. Nwachukwu, and C. I. Umechuruba, "Antifungal activities of some leaf extracts on [] seed-borne fungi of African yam bean seeds, seed germination and seedling emergence". ASEM, 5: 10.4314/jasem.v5i1.54936
- [21] A. A. Ebbo, Mammam, M. Suleiman, M. M. Ahmed, A. and А. Bello, "Preliminary phytochemical screening of Diospyros mespiliformis". 4: 2161. Anat. Physiol, DOI: 10.4172/2161-0940.1000156

- [22] E. M. Ervin, and J. K. Kariuki, "Demonstrating the minimal impact of cultivation conditions on antioxidants in fruits and vegetables by differential pulse voltammetry". DOI: <u>10.1080/10942912.2015.1048354</u>
- [23] A. H. M. E. D. Alwashli, M. Al-Sobarry, Y. Cherrah, and K. Alaoui, "Anti-Inflammatory and analgesic effects of ethanol extract of Dracaena Cinnabari Balf, as endemic plant in Yemen". Int J Pharm Bio Sci, 3, 96-106. DOI: <u>10.1016/0031-9422(94)00743-D</u>
- [24] O. Kadiri, Akanbi, C. T. Olawoye, B. T. and S. O. Gbadamosi, "Characterization and antioxidant evaluation of phenolic compounds extracted from the protein concentrate and protein isolate produced from pawpaw (Carica papaya Linn.) seeds". *Int. J. Food Prop*, 20: 2423-2436. DOI: 10.1080/10942912.2016.1230874
- [25] A. Fernández-Agulló, E. Pereira, Freire, M. S. Valentao, P. Andrade, P. B. González-Álvarez, J. and J. A. Pereira, "Influence of solvent on the antioxidant and antimicrobial properties of walnut (Juglans regia L.) green husk extracts". Ind Crops Prod, 42: 126-132. DOI: 10.1016/j.indcrop.2012.05.021
- [26] M. M. B. Almeida, Â. M. C. de Sousa, G. M. do Prado, C.E. de Carvalho Magalhães, G. A. Maia, and T. L. G. de Lemos, "Bioactive compounds and antioxidant activity of fresh exotic fruits from northeastern Brazil". Int. Food Res. J, 44: 2155-2159. DOI: <u>10.1016/j.foodres.2011.03.051</u>
- [27] L. M. R. da Silva, E. A. T. De Figueiredo, N. M. P. S. Ricardo, I. G. P. Vieira, R. W. De Figueiredo, I. M. Brasil, and C. L. Gomes, "Quantification of bioactive compounds in pulps and by-products of tropical fruits from Brazil". Food chemistry, 143: 398-404. DOI: <u>10.1016/j.foodchem.2013.08.001</u>
- [28] J. Contreras-Calderón, L. Calderón-Jaimes, E. Guerra-Hernández and B. García-Villanova, "Antioxidant capacity, phenolic content and vitamin C in pulp, peel and seed from 24 exotic fruits from Colombia". Int. Food Res. J, 44: 2047-2053. DOI: <u>10.1016/j.foodres.2010.11.003</u>
- [29] G. Korekar, Stobdan, T. Singh, H. Chaurasia, O. and S. Singh, "Phenolic content and antioxidant capacity of various solvent extracts from seabuckthorn (Hippophae rhamnoides L.) fruit pulp, seeds, leaves and stem bark". Acta Alimentaria, 40: 449-458. DOI: 10.1556/aalim.40.2011.4.4

- [30] A. H. M. E. D. Alwashli, M. Al-Sobarry, Y. Cherrah, and K. Alaoui, "Anti-Inflammatory and analgesic effects of ethanol extract of Dracaena Cinnabari Balf, as endemic plant in Yemen". Int J Pharm Bio Sci, 3, 96-106. DOI: 10.1016/0031-9422(94)00743-D
- [31] S. Gouveia, and P. C. Castilho, "Antioxidant potential of Artemisia argentea L'Hér alcoholic extract and its relation with the phenolic Dracaenacinnabari". Phytochemistry, 38: 795 796. DOI: 10.1016/0031-9422(94)00743-D
- Arunkumar, M. Muthuselvam, [32] S. and R. phytochemical Rajasekaran, "Analysis of constituents and antimicrobial activity of some Southern India medicinal plants". J. Pharm. Res, 3: 1841-1843. http://jpronline.info/.../1628
- [33] K. Thakur, L. Bala, D.M. Rajeshwer, and A.K. Bhatt, "Evaluation of effectiveness of biologically synthesized silver nanoparticles of Eucalyptus globules leaf extract against pathogenic and acneinducing bacteria". J Nanomed Nanotechnol. 8: 2. DOI: 10.4172/2157-7439.1000443

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## مقالة بحثبة

التقييم المختبري لنشاط مضادات الميكروبات ومضادات الأكسدة لبذور الباباي Carica papaya L. المزروعة في الجمهورية اليمنية

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# المُلخّص

أجريت هذه الدراسة على مستخلصات بذور الباباي Carica papaya L المقاومة للمضادات الميكروبية ومضادات الأكسدة خلال الفترة يناير حتى مايو 2021م.

تم جمع نبات الباباي Carica papaya L من مدينة الحديدة، الحسينية (Latitude: 14°47'52" N Longitude: 42°57'16" E)، اليمن خلال شهر يناير وشهر فبراير 2021م، وتم الاستخلاص بأربعة مذيبات عضوية وبطريقة الترشيح البارد، بعد ذلك تم اجراء اختبار النشاط المضاد للبكتيريا ضد ست سلالات بكتيرية وأربع سلالات فطرية بطريقة الانتشار القرصي، وأظهرت النتائج أن جميع البكتريا والفطريات قيد الدراسة كانت شديدة الحساسية لمستخلص الإيثانول والميثانول يليها مستخلص الكلوروفورم ثم مستخلص الماء وأخيرا مستخلص الهكسان .n-Hexane

بالإضافة إلى ذلك تم إجراء تقييم النشاط المضاد للأكسدة لمستخلصات نبات الباباي بطريقة DPPH، DPPH وFRAP، وأظهرت النتائج أن مستخلص الإيثانول كان الأفضل عند DPPH مقارنة مع.APTS and FRAP

وأخيراُ تم إجراء الفحص الكيميائي النباتي لتحديد المكونات الفينولية Phenolic في مستخلصات نبات الباباي وفقًا للإجراءات القياسية، وأظهرت النتائج أن جميع المذيبات المستخدمة في الدراسة احتوت على كميات كبيرة من Flavonoid, anthocyanin and tannin باستثناء الكينونات Ouinones.

الكلمات المفتاحية: بذور البابايا كاربكا، الحديدة، اليمن، مضادات الميكر وبات، مضادات الأكسدة، الفينول الكلي، الفحص الكيميائي النباتي.

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