EXTRACTION OF BIOACTIVE COMPOUNDS FROM LEAVES AND FRUIT OF PAPAYA

Sobia Khadam[1] , Hina Altaf [1] Sara Zahid , Anam AbdulRehman [2]

1. Department of Biochemistry and Biotechnology, PMAS Arid Agriculture University Rawalpindi, Pakistan.
2. Must, AJK

ABSTRACTS.

Carica papaya, A fruit-bearing tree growing in different parts of the world. Fruit , leaves, seeds and lotus of papaya are being use in folk medicines since centuries . Fruit contains secondary metabolites those help to minimize many human disorders. Located in the papaya leaf and lotus, papain is a commercially significant enzyme. Under the peptidase C1 family, papain is a cysteine protease. In order for the enzyme to function, papain needs a sulfhydryl group, which is present in a single polypeptide chain with three disulfide bridges. Define Unit: For every minute at pH 6.2 and 25 °C, one unit may hydrolyze 1.0 µmole of N-α-benzoyl-L-arginine ethyl ester (BAEE).

The results of the current experiments assessed the antioxidant and antibacterial properties of Carica papaya fruit and leaf extracts. According to the results, when DPPH, H2O2, ABTS, and reducing power tests were performed and compared with standards, leaves and fruit extracts demonstrated exceptional antioxidant properties. A notable minimum inhibitory dose for ethanolic leaf extracts was discovered, and the leaves and fruit extracts demonstrated significant growth inhibition against four bacterial and fungal species. Total phenols, flavonoids, and other secondary metabolites were found to be present in considerable amounts when extracts were analyzed for phytochemicals using a UV/visible spectrophotometer and high performance liquid chromatography. This suggests that these extracts may be a useful source of pharmaceutical ingredients needed by the pharmaceutical industry to prepare a range of medications for human use.

Key words: *Carica papaya*, Antioxidants, Antimicrobial, Secondary metabolites.

INTRODUCTION

Many papaya leaf products, including teas, extracts, pills, and juices, are frequently used to cure ailments and boost health in various ways, despite the paucity of human studies on the subject. In experiments conducted on animals and in test tubes, the distinct plant components found in papaya leaves have shown extensive pharmacological potential.

Therefore, information concerning the qualities, safety, and effectiveness of plants must be shared with the general public in order to promote appropriate decision-making regarding their usage for health purposes (Ellof, 1998).Literature-available data indicates that customers' interests are now focused on using natural antioxidant goods rather than synthetic ones and avoiding the usage of medications that may cause cancer after intake (Velioglu et al., 1998).

Significant antioxidant capacity was gained by plant-based nutrition, and this capacity is associated with lower death rates from a variety of human illnesses (Anderson et al., 2001).

Chen (1992) argues that the use of synthetic antibiotics to treat microorganisms is ineffective because many bacteria have the genetic capacity to develop resistance to these drugs after a few years. This has resulted in a new situation where hospitalized patients have suppressed immunity. As a result, new infections may occur in hospitalized patients, which could worsen their condition and increase mortality rates (Chem et al., 2017).

Many substances found in fruits and vegetables are thought to be anti-cytotoxic, anti-tumor toxic, and capable of lowering the occurrence of cancers.Thus, it's critical to comprehend the possible hazards and/or health advantages of these plants.

The brine shrimp assay is a widely used and simple technique for determining the cytotoxicity of plant materials. This bioassay is frequently used to assess the toxicity of many substances, including natural plant extracts, insecticides, heavy metals, and medications. As stated by Pisutthanan et al. (2004) and Mclaughlin et al. (1991).

The tropical and subtropical regions are home to the widely widespread Carica papaya L., which is a member of the Caricaceae family.This fruit, which resembles a berry, develops from a superior ovary and is parietal in placentation (Kochhar, 1986). Papaya leaves and fruits were rich in vital vitamins and minerals, carotenoids, flavonoids, and phenols (Rahmat et al., 2012).Papain, chymopapain, and caricain, three cysteine endopeptidases, were present in the Carica latex along with chitinase and glutaminly cyclase. Fruit pulp contains linalose, as do alkaloids such as carpaine, pseduocarpaine, and dehydrocarpaine I and II (Lim, 2012). Alternative remedies have historically employed various components of the papaya plant, such as the fruits, leaves, stems, seeds, and roots. The antioxidants included in C. papaya fruits are extremely beneficial for protecting the body against reactive oxygen species. In the human body, lipid oxidation produces these harmful byproducts. Nowadays, a number of human illnesses are thought to have their origins in reactive oxygen species (ROS) (Repetto and Llesuy, 2002; Rahmat et al., 2004). Dengue fever, which is brought on by viruses from the Flaviviridae family, is a widespread problem in many tropical and subtropical nations. Several findings in the literature suggest that using papaya leaf extracts can help persons who have dengue fever recover. Scientific evidence, however, on the effectiveness of papaya leaf extracts in treating dengue fever is

currently lacking.Thus, the current study was created to evaluate the antioxidant, antibacterial, and cytotoxic effects of leaf and fruit extracts of Carica papaya, taking into account the advantageous benefits of the plant's leaves and fruit (Rahmatet al., 2004).Dengue is a widespread tropical and subtropical illness that is brought on by viruses from the Flaviviridae family. Numerous evidence in the literature suggest that using papaya leaf extracts can help persons who have dengue fever recover. There is currently no scientific evidence supporting the use of papaya leaf extracts to treat dengue fever.Thus, the current study was aimed to evaluate the antioxidant, antibacterial, and cytotoxic properties of leaf and fruit extracts of C. papaya, taking into consideration the positive benefits of the fruit and leaves of the plant.

MATERIALS AND METHODS

Collection and preparation of samples.

For future reference, samples of C. papaya fruit and leaves were gathered from several locations in Rawalpindi, verified by a specialist, and registered as a specimen (voucher number. 142). The fruit and leaf samples were first sun-dried, then shadow-dried, and then baked at 60 degrees Celsius. The dehydrated specimens were pulverized into an 80 mesh powder and preserved in delicate plastic bags for further applications.

Phytochemical analysis

200 g of the C. papaya fruit and leaf samples were divided among four 400 ml round bottle flasks. n-hexane, chloroform, methanol, and ethanol were used to macerate and extract the materials. Every technique was created at room temperature. Utilizing techniques described by Sofidiya et al. (2006), Harbone (1973), and Trease and Evans (1983), the leaves and fruit extracts were subjected to qualitative and quantitative examination of secondary metabolites.Different C. papaya extracts were investigated

for their total phenolic content (Kimet al., 2003).On the other hand, the flavonoid concentrations of fruit extracts and leaves were measured using a technique described by Hussain et al (2012).

Chromatographic analysis

The ethanolic fruit extracts that had demonstrated greater levels of total flavonoids were further quantified using higher performance liquid chromatography. This involved using isocratic elution, a 70:30 methanol and water mixture, a flow rate of one milliliter per minute, a retention period of twenty minutes, and 20 µl of sample to a C18 column at 30 degrees Celsius.Quercetin (a flavonoid) was measured using a UV/visible detector set at a wavelength of 368 nm..

Isolation and purification of papain enzyme from fruit and lotus of papaya. Papain is also known as papaya proteinase

Collection of sample

The samples of fresh leaves papaya were taken from locally grown

around Rawalpindi areas and shifted to laboratory

Extraction enzymes from papaya leaf

Extraction by grinding assisted

After being chopped, the fresh papaya leaves were cleaned with distilled water. For eight days, ambient air in the laboratory would be used to dry it. We used a grinder to ground the leaves. Five grams of ground-up papaya powder were precisely weighed out, and twenty milliliters of distilled water were added in a 1:5 ratio. Filter paper was used to filter the water-papaya combination (8, 15).

Extraction by ultrasound-assisted

Using stainless scissors, the sample was cut into cubes of 3 cm in length and breadth. Pretreatment of the samples involved three different temperatures for extraction (50, 60, and 70°C) and ultra sonication times (25, 30, and 60 minutes).

Two-step salt precipitation

to employ papain purification using young leaves. Pretreated crude enzyme was ground using ultrasonic technology, combined with 40 millimeters of cysteine at a 3:1 (w/v) ratio. The suspension was then brought to pH 5.6 using 6 milliliters of HCL, and agitated for 15 minutes at 40 degrees Celsius [15]. After filtering the mixture, 9M NaOH was used to raise the pH of the filtrate to 9.0. Centrifugation at 9000xg for 30min at 4o C was used to extract the insoluble material. With (NH4)2SO4 at 45% saturation, the supernatant was precipitated. For 30 minutes, the salt-enriched solution was incubated at 4 0 c. Centrifugation was used as before to collect the precipitate, and 20 mM cysteine was used to dissolve it. Prior to adding sodium chloride (10% w/v), the solution was maintained at 40 c.

Identification of papain

10 milliliters of 20% powdered skim milk (pH 5.5) with three drops of papain extract added was incubated at 37 degrees Celsius.

Determination of protein content

Using the Bradford technique, the quantity of protein in the samples was ascertained during purification.

Protease activity determination

After making minor modifications, the prolytic activity of the enzyme was assessed using Arnon's methodology. A pH of 8.0, 700µL of 50 Mm Tris-HCl buffers, 1000µl of enzyme solutions, and 200µl of 50mM casein and 20mM EDTA (disodium salt) were included in the reaction mixture. Three milliliters of 50% (v/v) trichloro acetic acid (TCA) were added to the mixture, which was then incubated at 37 0c for five minutes before cooling for one hour. The absorbance of the supernatant was measured at 275 nm after the reaction mixture was centrifuged.A blank to which the enzyme was added after the TCA was introduced resulted in a corrected reading.

Antioxidant activities of leaves and fruit extracts of C. papaya

As stated by Yildirim et al. (2001), the ability of the fruit and leaf extracts to convert Fe+3 ions into Fe+2 ions was evaluated.On the other hand, Moon and Shibamoto (2009) stated that the DPPH test was used to assess the scavenging capacity. The ABTS assay was used to test the extracts' capacity to scavenge free radicals (Ashafa et al., 2010).The Ruch et al. (1989) technique was used to evaluate the extracts' ability to scavenge H2O2.

Antibacterial and antifungal activities of extracts

Agar well diffusion experiment was used to evaluate plant substances for antibacterial activity against four bacterial strains: Bacillus subtilis (ATCC6633), Escherichia coli (ATCC15242), Klebsiella pneumonia (MTCC618), and Staphylococcus auresu (ATCC 6538). Using a spectrophotometer, absorbance was measured at 420 nm for standard antibiotics (Roxithromycin and Cefixime) as a comparative measure (Boyed, 1980). Based on the extracts' lowest concentration that prevented the bacteria from growing during a 24-hour incubation period, the minimum inhibitory concentration

was calculated. Using the Agar tube dilution technique, the antifungal activity of C. papaya extracts was measured against four strains of fungus (*Aspergillus niger* ,0198;*Aspergillus flavus* ,0064; *Aspergillus fumigates* ,66 and *Fusarium solani*, 0291)as reported earlier through Ettebong and Nwafor (2009)

Brine shrimp cytotoxicity bioassay

Shrimp hatching

In a shallow rectangular dish measuring 22 by 30 cm, brine shrimp eggs were incubated. The fake seawater was created by mixing commercial salt combination with double-distilled water. Two uneven sections were created in the dish by clamping a plastic separator with 2 mm holes. The bigger compartment was filled with 50 mg of eggs and was darkened, while the smaller compartment was left lit. The phototropic napulii were isolated from their shells by the separator and collected by pipette from the lit side after 48 hours. Using a pipette, twenty shrimp were placed into each sample vial, and then 5 milliliters of fake seawater were added. Against a bright background, the napulii in the pipette stem may be counted. Each vial was fed with a drop of dry yeast solution (3 mg in 6 mL of artificial sea water). The vials were kept under lighted conditions. Using three magnifying glasses, the survivors were tallied, and after a 24-hour period, the percentage of deaths at each dose and control was established. The statistics were adjusted in each instance when control deaths transpired by applying Abbott's methodology:

$$\% \text{ Death} = [\text{ Test} - \text{Control}] / \text{Control} \times 100$$

STATISTICAL ANALYSIS

The values were represented as means± SD after statistical analysis

RESULTS AND DISCUSSION

To investigate the pharmacological potential of any plant extracts and their fractions, phytochemical analysis is a necessity.Fruit and leaf extracts of C. papaya included

alkaloids, flavonoids, phenols, tannins, cardiac glycosides, saponins, and terpenes, according to study results (Table 1).

Bioactive substances such as flavonids and phenolics were quantified from fruit and leaf extracts (Table 2).

Table 1. Assessment of various phytochemicals fromleaves andfruit extracts of *C.papaya*

Extracts	Alkaloids	Flavonoids	Polyphenols	Tannins	Cardiac glycosides	Saponins	Terpenes
Ethanolicleaves extracts	++	+	+	++	+	-	+
Ethanolicfruit extracts	+	+++	++	+	++	+	++

+; present ; - absent

Table 2. Assessment of Flavonoidsand Phenoliccontents of leaves andfruit extracts of*C. papaya*

Extracts	**Total Flavonoids (mg rutin equivalent/g)**	**Total phenols (mg GAE/g**	**Yield of extracts (%)**
Ethanolicleaves extracts	47.12 ± 2.15 *	47.22 ± 2.14*	4.6 ± 0.7
Methanolic leavesextract	23.11 ± 0.51	25.63 ± 1.32	2.5 ± 0.4
n-hexane leaves extracts	15.15± 1.36	18.54± 1.71	2.4 ± 0.5
Ethanolic fruit extract	46.27± 1.23*	46.71 ± 1.32*	1.3 ± 0.6
Methanolic fruit extracts	21.15 ± 4.1	18.54 ± 2.93	1.5 ±0.7
n-hexane fruit extracts	15.110 ± 0.51	15.66 ± 1.3	2.4± 0.4

Values are represent as mean +SD (n=3); *significantly higher value ($p<0.05$)

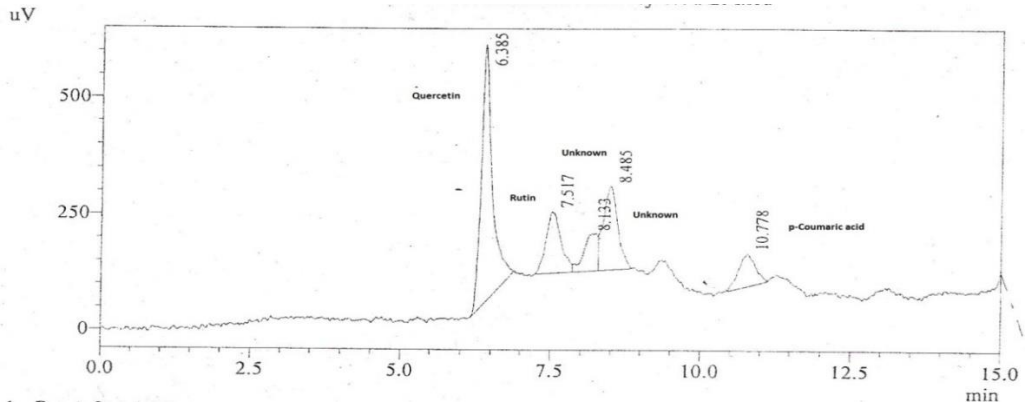

Figure 1.HPLC analysis of ethanolic fruit extractsof *C. papaya*

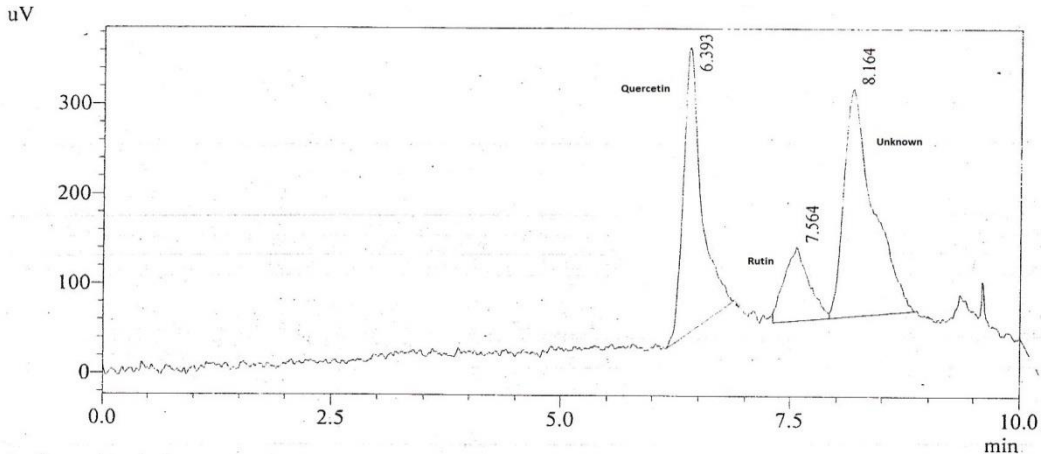

Figure 2.HPLC analysis of ethanolic leaves extracts of *C. papaya*

After analyzing many C. papaya extracts using high-performance liquid chromatography (HPLC), it was shown that ethanolic leaf extracts had a greater concentration of quercetin than ethanolic fruit extracts. Canini et al. (2007) and Yap et al., 2020) discovered quercetin in C. papaya samples, but the amounts they reported were less than the amounts of quercetin discovered in the current investigation (Figs. 1 and 2).

Extraction and purification of papin

In order to extract this enzyme, the whole samples that coagulated after being incubated at 37°C were examined, as seen in Figure 1. Between 0.055 and 0.003 mg/ml was the extract papain concentration. Papain enzyme was extracted from ground papaya leaves in greater amounts than from sonicated leaves; this notable difference may have resulted from the leaf being ground to remove the outer layer that encloses the cytoplasm [15]. The sample that was ground demonstrated the mean value, whereas the sample that was sonicated at 50C for 25 minutes had a minimal mean concentration of 0.003. Papain enzyme isolated from the ground leaf was more crude, which may have been contaminated by the ground particles inside the cellular compartments. However, the efficiency of the papain enzyme was higher in the sonicated leaf sample, indicating that relatively pure enzyme was isolated from the sonicated samples. As seen in Table 1, the minimal mean concentration that was obtained from the sonicated sample had various values that are noticeably comparable. Both the sonication duration and temperature were gradually raised along with the papain enzyme content. This demonstrated that heat promoted the destruction of undesirable cell components and that sonication duration was a crucial pretreatment requirement for the enzyme's separation. Enzyme activity is influenced by a variety of factors, including pH, temperature, and duration. The ideal temperature range for each kind of enzyme varies, and for the isolated papain enzyme, it was around 60 degrees Celsius. Water is therefore used as the extraction medium while removing the papain enzyme from papaya leaves. This is due to the fact that, in contrast to an organic solvent, water has excellent extractive qualities for polar molecules and can preserve the structural integrity of papain.

Table1: The concentration of extracted papain from papaya leaves

Table the concentration of extracted papain from papaya leaves

Pretreatment	Mean Concentration (mg/ml ± Standard Deviation
Grind	
	Sonicated
Time	Temp oC
25 min	50
	60
	70
30	50
	60
	70
1 hr	50
	60
	70
Total	

Proteolytic experiments were performed utilizing casein as the substrate, as indicated in Table 1 as the disclosed outcome of this treatment. At 275 nm, the supernatant's absorbance was examined, and the extract papain's mean absorbance ranged from 0.057 to 0.013. The sonicated sample at 600 c for 60 minutes had the largest mean value, whereas the ground sample showed the lowest mean absorbance. But compared to earlier times, there has been some progress in understanding the reasons for a little adjustment to the papain enzyme identification methodology.

Table 2. The activity of extracted papin from papaya leaves

Pretreatment	Mean Concentration (mg/ml ± Standard Deviation
Grind	
	Sonicated
Time	Temp oC
25 min	50
	60
	70
30	50
	60
	70
1 hr	50
	60
	70

Total	

The quantity of papain enzyme extracted from the leaf during grinding, avoiding the cytoplasm-enclosed exterior regions of the leaf. A significantly shorter processing time than the lengthy processing time (12) was required to generate highly pure papain. Although this research supports increased This is consistent with the findings of the current study, which showed that the sonicated leaf sample had a higher papain enzyme efficiency. This suggested that the enzyme was isolated from the sonicated samples relatively pure, whereas the papain enzyme isolated from the ground leaf was coarser, possibly due to contamination from the ground particles and cellular compartments. However, a little variation in their sonicated time of 25 minutes might indicate a different source due to the weather. The temperature and duration of sonication were used to gradually increase the quantity of this papain enzyme (15).

I concur with the findings of the present study

Ascorbic acid and rutin were utilized as standards in this work, and the results of five distinct assays—DPPH, H2O2, ABTS Phosphomolybdate, and Reducing Power—were used to assess the antioxidant capacity of C. papaya leaf and fruit extracts. While antioxidant activity has been demonstrated by all solvent extracts, ethanolic leaf and fruit extracts have demonstrated a greater degree of antioxidant potential than extracts from other solvents. The readings occasionally fell short of both the normal rutin and the positive control.

Comparing ethanol leaves and fruit extracts to all other extracts, cefixime, and DMSO, they demonstrated active inhibitory activity against strains of S. aureus, E. coli, K. pneumonia, and B. subtilis; however, lesser zones of inhibition were seen when compared to roxithromycin.

The findings obtained from determining the minimum inhibitory concentration (MIC) of several extracts and antibiotics are displayed in Table 5, which suggests that these extracts have high antibacterial activity.

Table 3. Antioxidant potential of various extractsof *C. papaya* at concentration of 100 µg/ml andIC 50 values (µg/ml) of radical scavenging when absorbance was measured at 700 nm

Extracts	**DPPH**	H_2O_2	ABTS	Reducing Power assays	Ascorbic acid	Rutin
Leaves extracts						
Ethanol	0.114±0.01	2.132±0.05	1.323±0.08	0.135±0.01	1.214±0.81	1.772±1.83
Methanol	0.251±0.03	2.251± 0.04	2.252±0.07	0.154±0.01	1.252 ±0.34	2.424±0.84
n-hexane	0.311±0.01	2.653± 0.05	2.656±0.07	0.532±0.06	1.545 ±0.32	1.551±0.75
Chloroform	0.023±0.01	1.924±0.71	7.655± 0.15	0.163±0.01	1.825±0.45	1.662±0.52
Aqueous	3.081±0.01	8.132±0.26	9.134±0.26	0.184±0.05	1.751±0.52	1.874±0.35
Fruit extracts	0.275±0.03	2.215±0.04	2.353±0.07	0.156±0.01	1.432±0.33	1.773±0.81
Ethanol	0.123±0.00	0.851±0.01	0.382±0.19	0.113±0.01	0.435±0.82	0.956±0.25
Methanol	0.254±0.03	2.352±0.04	2.252±0.07	0.135±0.01	1.225 ±0.34	1.434±0.83
n-hexane	0.125±0.01	1.193±0.81	1.911±0.52	0.196±0.05	1.362±0.55	1.125±0.35
Chloroform	0.223±0.01	1.254±0.93	1.653±0.74	0.091±0.06	1.554±0.36	1.435±0.54
Aqueous	0.318±0.01	1.435±0.75	1.864±0.43	0.182±0.01	1.635 ±0.72	2.254±0.36

Results are Means ± SD, (n = 3).

Table 4 Antibacterial activities of various extracts *C. papaya*; Zone of inhibition in mm.

Extracts	*S. aureus*	*E. coli*	*K. pneumonia*	*B. subtilis*	Roxithromycin	Cefixime	DMSO
Leaves extracts							
Ethanol	24.6±0.6	25.4±0.9	29.5±0.3	25.6±0.4	24.3±0.9	13.5±0.3	0.0
Methanol	21.6±0.8	22.6±0.6	26.6±0.7	24.6±0.6	22.6±0.7	11.6±0.5	0.0
n-hexane	17.2 ±0.5	17.3±0.8	17.6±0.4	16.5±0.7	12.3±0.9	11.4±0.8	0.0
Chloroform	18.3±0.6	19.2±0.3	14.2±0.4	15.8±0.5	13.6±0.8	12.7±0.9	0.0

Aqueous	12.4±0.8	15.6±0.6	11.3±0.5	11.6±0.3	10.2±0.7	9.4±0.8	0.0
Fruit extracts							
Ethanol	23.5±0.3	24.8±0.4	23.5±0.7	16.8±0.6	14.3±0.7	15.8±0.9	0.0
Methanol	22.6±0.9	23.5±0.7	22.6±0.8	13.4±0.5	11.9±0.3	12.6±0.5	0.0
n-hexane	19.6±0.5	19.2±0.9	19.2±0.6	14.5±0.5	14.5±0.7	15.3±0.8	0.0
Chloroform	13.4±0.4	15.7±0.8	21.6±0.5	15.7±0.9	13.6±0.7	14.8±0.5	0.0
Aqueous	11.5±0.7	13.2±0.7	13.5±0.4	11.2±0.7	9.7 ±0.8	8.2±0.3	0.0

Results mean± S D after triplicate analysis (n=3).

Table 5. Minimum inhibitory concentration (µg/ml) of *C. papaya*extracts for various bacterial strains.

Extracts	*S. aureus*	*E. coli*	*K. pneumonia*	*B. subtilis*	Roxithromycin	Cefixime	DMSO
Leaves extracts							
Ethanol	0.1±0.6	1.2±0.7	0.8±0.5	1.4±0.6	1.3±0.8	1.5±0.5	0.0
Methanol	0.5±0.2	2.2±0.3	1.1±0.3	2.1± 0.4	1.9±0.5	2.2 ±0.6	0.0
n-hexane	1.7 ±0.1	1.6±0.9	0.7±0.4	1.6±0.7	1.2 ±0.3	1.1±0.8	0.0
Chloroform	1.8±0.9	1.9±0.5	1.2.2±0.4	1.5±0.5	1.3±0.5	1.2±0.5	0.0
Aqueous	2.9±0.3	2.6±0.7	2.7±0.5	2.6±0.3	2.2±0.6	2.4±0.3	0.0
Fruit extracts							
Ethanol	0.5±0.6	0.8±0.1	0.7±0.9	1.1±0.4	0.2±0.7	0.8±0.3	0.0
Methanol	0.8 ±0.2	1.3± 0.4	1.1±0.2	1.6±0.6	0.7 ±0.1	1.4 ±0.1	0.0
n-hexane	1.6±0.5	1.9±0.8	1.9±0.5	1.5±0.5	1.5±0.9	1.1±0.8	0.0
Chloroform	1.4±0.4	1.5±0.9	1.6±0.7	1.4±0.6	1.6±0.4	0.4±0.6	0.0
Aqueous	1.5±0.7	1.3±0.7	1.8±0.4	1.9±0.3	1.7 ±0.8	2.2±0.3	0.0

Results are Means ± SD, (n = 3)

A greater fungal activity was represented by ethanolic leaf extracts (25.8±0.5mm), followed by ethanolic fruit extracts (24.5±0.1mm) and methanolic leaf extracts (23.4±0.3). The data in Table 6 illustrates the antifungal activity of plant extracts. Conversely, the aqueous leaf extract (10.5±0.6 mm) exhibited the lowest activity. The present study's results (Table 6) are consistent with previous reports of similar extracts by Fawole et al. (2008), Parekh and Chanda (2007), and Wijesooriya et al. (2019), who found that water extracts showed no or poor fungus toxicity compared to organic solvents after conducting an experiment. Extracts prepared in organic solvents have demonstrated reliable antifungal activities.

Table 6. Antifungal activities of variousextracts of *C. papaya*; Zone of inhibition in mm.

Extracts	*Aspergillus Niger*	*Fusarium salani*	*Aspergillus Flavous*	*Aspergillus Fumigates*	Terbinafine	DMSO
Leaves extracts						
Ethanol	25.8±0.5	24.8±0.4	18.3±0.3	21.5±0.4	18.4±0.3	0.0
Methanol	23.4 ±0.3	22.5±0.7	16.5 ±0.6	19.5 ±0.2	16.3±0.8	0.0
n-hexane	18.3 ±0.5	17.2±0.5	17.8±0.4	15.4±0.8	15.3±0.5	0.0
Chloroform	16.3±0.4	18.2±0.3	16.3±0.4	15.8±0.5	14.6±0.3	0.0
Aqueous	11.4±0.2	9.6±0.3	11.6±0.2	11.6±0.3	10.1±0.6	0.0
Fruit extracts						
Ethanol	24.5±0.1	19.8±0.3	21.1±0.5	19.6±0.3	16.3±0.6	0.0
Methanol	22.6±0.5	18.2±0.7	19.3±0.8	16.5±0.5	14.8±0.4	0.0
n-hexane	18.6±0.3	17.2±0.9	17.2±0.9	18.5±0.6	15.5±0.4	0.0
Chloroform	16.4±0.2	16.1±0.9	16.6±0.3	15.7±0.9	14.6±0.7	0.0
Aqueous	10.5±0.6	11.2±0.4	11.5±0.4	9.2±0.5	9.7 ±0.3	0.0

Results are Means ± SD, (n = 3).

Cytotoxiciy assessment of pinus needles leaves

Assessment of cytotoxic behavior of medicines pants used for drugs development are important which indicates that plant extracts is how much toxic to any cell. Results of brine shrimp cytotoxicity assay is given in table 3.12.

Table 3.12.Cytotoxicity screening of methanolic*P. Roxburghi* pine needles extracts (µg/ml)

Concentration (ug/ml)	**Total nupuli**	**Live after 24 hours**	**Death after 24 hours**	**% of death**
10	18	17	1	5.56
100	20	14	6	30.0
300	20	12	8	40.0
600	19	10	9	47.37

Values are Mean±SD ,(n=3) and significantly different ($P<0.05$); positive control are saline sea salt

DISCUSSION

The potential of phytochemicals derived from plants, vegetables, and fruits to positively impact human metabolism is being investigated extensively. The goal of the study was to give scientific evidence for the safe and advantageous eating of the leaves and fruit of C. papaya, drawing on traditional knowledge about the plant's uses.Compared to other extracts examined in this experiment, the ethanolic fruit and leaf extracts of C. papaya produced larger amounts of total phenol and flavonoid contents (Table 2, Figures 1 and 2).It highlights the value of C. papaya fruit and leaves and recommends using them for human health. The phytonutrient results from

this investigation were similar to those previously published by Kim et al. (2003) and other authors. Phenolic compounds are naturally occurring secondary metabolites found in a broad variety of plants. They have the ability to scavenge free radicals and are thought to have a significant influence on human health as possible antioxidant agents (Jayaprakash et al., 2001).Phenolic chemicals are very significant antioxidants that have a variety of uses, including as hydrogen donors, reducing agents, oxygen quenchers, free radical scavengers, and cell savers. Omotade et al. (2011) reported a lower concentration of flavonoids in C. papaya than what the current investigation showed. In addition to having antiviral, antifungal, and antibacterial properties, flavonoids shield the stomach and liver from a variety of illnesses (Okeniyiet al., 2007). A flavonoid called quercertin is present in C. papaya fruit and leaf extracts. stops the body from producing histamines, which would otherwise trigger an allergic reaction. In addition, quercetin inhibits atherosclerosis, or the formation of plaque in the arteries, which can result in obesity, heart attacks, and strokes (Chirumbolo, 2012).

Plant extracts including isoflavones, flavonoids, anthocyanin, coumarin lignans, catechins, and isocatechins have been shown by Aqil et al. (2006) to always have a strong potential for antioxidant activities.

According to Rathee et al. (2008), an analysis using high-performance liquid chromatography (HPLC) on C. papaya extracts showed a consistent amount of quercetin (Figs. 1 and 2). It is likely that this flavonoid, together with other secondary metabolites, was responsible for the bulk of the extracts' biological activity.Conversely, Devasagayam et al. (2004) discovered that medications with strong antioxidant capability are beneficial in treating complicated infections in humans, such as cancer, diabetes, Alzheimer's disease, atherosclerosis, and stroke.

The results of the study indicate that plant extracts containing antimicrobial components, such as flavonoids, have the potential to treat infectious disorders caused by highly resistant pathogenic microorganisms. The extracts demonstrated remarkable antibacterial potential against strains of both Gram-ve and Gram-ve bacteria as well as fungal strains. The results presented here, with a few minor modifications, are consistent with those published by other writers (Ettebong and Nwafor, 2009).Plant-based medications have been used for a long time to treat infectious diseases worldwide. They have a great potential to cure antibiotic-resistant infections, which is a growing global public health problem (Fawoleet al., 2009 :Parekh and Chanda, 2009).The present study's findings revealed fluctuations in the inhibitory concentrations of every extract for certain bacteria. Because plant components are soluble in pertinent solvents and the ethanol extracts had the lowest MIC (Table 5), this might be explained. The current investigation's findings were consistent with those reported by Guessan et al. (2007) and other writers.

Sandeep et al. (2009) published results on brine shrimp lethality that were similar to those of the current investigation.Any plant extract's bioactivity that might be suitably linked to cytotoxic and antitumor qualities can be learned using the brine shrimp assay.

Conclusion: Important bioactive secondary metabolites (polyphenols and flavonoids) were found in the C. papaya fruit and leaf extracts. These extracts' ability to scavenge free radicals and stop the development of harmful bacterial and fungal strains has been demonstrated. It is anticipated that using suitable dosages of C. papaya leaf and fruit extracts may assist to alleviate a variety of human illnesses, particularly those involving antibiotic resistance or a decrease in a person's blood platelet counts. Because of these pharmacological qualities, papaya fruit and leaves can be consumed by humans to reduce the amount of food that is consumed. To isolate the lead

chemical that the pharmaceutical industry may need to produce medications to treat different liver conditions, more research is necessary.

Conclusion

As a result of our research, we now know that the papain enzyme may be used for a variety of purposes by employing distinct applications. Thus, it can be argued that further research is necessary and that it also supports the economic aspects of emerging nations.

REFERENCES

Anderson KJ, Teuber SS, Gobeille A, Cremin P, Waterhouse AL and Steinberg FM (2001). Walnut polyphenolics inhibit in vitro human plasma and LDL oxidation. Biochemical and molecular action of nutrients. J. Nut. (131): 2837–2842.

Aqil F, Ahmed I and Mehmood Z (2006). Antioxidant and free radical scavenging Properties of twelve traditionally used Indian medicinal plants. Turk. J. Biol. (30): 177-183.

Ashafa AOT, Grierson DS and Afolayan AJ (2010). *In vitro* antioxidant activity of extracts from the leaves of feliciamuricatathunb. and underutilize medicinal plant in the eastern cape province, South Africa. Afr. J. Trad. Compl. Altern. Med., **7**(4):296-302.

Canini A, Alesiani D, D'Arcangelo G and Tagliatesta P (2007). Gas chromatography- mass spectrometry analysis of phenolic compounds from *Carica papaya* L. leaf. J. Food Compos. Anal. (20): 584-590.

Chen L., Deng H., Cui H., Fang J., Zuo Z., Deng J., Li Y., Wang X and Zhao L (2017). Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget.* (9):7204–7218.

Chirumbolo S (2010). Role of quercetin , flavonoes and flavonoes in modulating and inflammatory cell function. Infamm allerg drug targ 9(4): 263-85.Cohen ML (1992). Epidemiology of drug resistance: implications for a post- antimicrobial era. Science 257 : 1050-1055.

Devasagayam TPA, Tilak JC and Boloor KK et al.(2004). Review: Free radical and antioxidants in human health. Curr Stat Fut Pros JAPI 53: 794-804.

Ellof JN (1998). Which extractant should be used for the screening and isolation of antimicrobial components from plants? J. Ethnopharmacol. (60) : 1-6.

Ettebong E and Nwafor P (2009). *In vitro* antimicrobial activities of extracts of *carpolobia lutea* root. Pak J Pharm Sci. 22(3):335–338.

Fawole OA, Finnie JF and Van Staden J (2009). Antimicrobial activity and mutagenic effects of twelve traditional medicinal plants used to treat aliments related to treat gastrointestinal tract in south Africa. South Afr. J. Bot . (75): 356-362.

Harborne J B (1973). Phytochemical methods London Chapman and Hall Ltd. 49-168.

Hossain AM, Ferdous T, Salehuddin SM and Das AK. (2009). *In vitro* cytotoxicity (LC_{50}) of extracts obtained from the seeds of *Zea mays*. Asian J Food Ag-Ind. 2(3):336–344.

Hussain A I, Chutta SAS, Noor S, Khan ZA, Arshad MU and Rathore H A *et al*.(2012). Effect of extraction techniques and solvent systems on the extraction of antioxidants compounds from peanunt (*Arachis hypogaea* L.) hulls. Food. Anal. Methods. (5):890-896.

Jayaprakasha G, Singh R and Sakariah K (2001). Antioxidant of grape seed (*Vitisvinifera*) extracts on peroxidation models in vitro. Food Chem. (37): 285- 290.

Kim D, Jeond S and Lee Ch (2003). Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. Food Chem. (81) : 321–326.

Kochhar SL (1986). Pawpaw fruit. In: Tropical crops, a textbook of economic Botany (ed) Kochhar SL New Delhi Macmillan company publishers: India, 263-264.

Lim TK (2012). *Carica papaya*. In: Edible Medicinal and Non-Medicinal Plants. *Springer* sci & Business Media Netherlands (1):693-717.

McLaughlin JL, Chang CJ, and Smith DL (1991).Benchtop bioassays the discovery of bioactive natural products: an update. Nat Prod Chem (.9) : 383- 397.

Moon J K and Shibamoto T (2009) .Antioxidant assays for plant and food components. *J. agric*. Food Chemist. 57(5) : 1655-1666.

N'guessan JD, Dinzedi MR, Guessennd N, Coulibaly A, Dosso M, Djaman AJ and Guede-Guina F (2007) Antibacterial activity of the aqueous extract of *Thonningia sanguinea* against extended-spectrum-β-lactamases (ESBL) producing *Escherichia coli* and *Klebsiella pneumoniae* strains. Trop J Pharm Res. 6(3):779–783.

Okeniyi JA, Ogunlesi TA, Oyelami QA and Adeyemi LA (2007). Effectiveness of dried *Carica papaya* seeds against human intestinal parasitosis. a pilot study. J Med Food (10):194-196.

Omotade O, Jeferson F, Daniel R, Joao R, Magareth A and Aline B (2011). Antioxidative Properties of Ethyl Acetate Fraction of Unripe Pulp of *Carica papaya* in Mice. J Micro Biotech Food Science (13): 409-425.

Parekh J and Chanda S (2009). In vitro antifungal activity of methanol extracts of some Indian medicinal plants against pathogenic yeast and mould . Afr. J. Biotech. 7(23):4349-4353

Pisutthanan S, Plianbangchang P, Pisutthanan N, Ruanruay S and Muanrit O (2004). Brine shrimp lethality activity of Thai medicinal plants in the family Meliaceae. Naresuan Univ. J.12(2): 13-18

Rahmat A K, Khan MR, Sahreen S and Ahmed M (2012). Evaluation of phenolic contents and antioxidant activity of various solvent extracts of Sonchusasper (L.) Hill. J Chem Central. 6:12.

Rahmat A, Abubaka MF, Faezah N and Hambali Z (2004). The effect of consumption of guava (Psidium guajaya) or *Carica papaya* on total antioxidant and lipid profile in normal male youth. J. Asia Pac. Clin . Nut. (13): 106.

Rajkiran RB, Veera BN and Pratap R K. (2015). Green synthesis and characterization of carica papaya leaf extract coated silver nanoparticles through X-ray diffraction ,electron microscopy and evaluation of bactericidal properties . S.J.Biol. Sci.22(5):637-644

Rathee D,Madhavi T, Reena A and Sheetal A (2008). Simultaneous Quantification o Bergenin, (+)-Catechin, Gallicin and Gallic acid; and quantification of using HPTLC fromBergenia ciliata (Haw.) Sternb. Forma ligulata Yeo (Pasanbheda). J. Pharm Anal Acta 1(10): 2153-2435.

Repetto MG and Llesuy SF (2002). Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. JBraz Med Biol Res (35): 523-534.

Ruch R.J, Cheng S J and Klauning JE (1989). Carcinogen, 10 pp. 1003–1008.

Ruch RJ, Cheng SJ, Klaunig JE (1989). Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis 10 : 1003-1008.

Sandeep BP, Nilofar SN and Chandrakant SM. 2009. Review on phytochemistry and pharmacological aspects of *Euphorbia hirta* Linn. J Pharma Res Health Care.1:113–133

Sofidiya M O, Odukoya OA, Familoni OB and Inya-Agha ST (2006). Free radicals scavenging activity of some Nigerian medicinal plant extracts. J Pak Biol Sci (9): 1438-1441.

Steel RGD and Torrie JH (1980) *Principles and Procedures of Statistics*, Second Edition, New York: McGraw-Hill.

Trease GE and Evans WC (1983). Pharmacognosy 12th Ed. Bailliere Tindal, London (UK).

Velioglu, YS, Mazza G, Gao L and Oomah BD (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products J Agri. Food and Chem 46: 4113–4117.

Wijesooriya A., Deraniyagala S and Hettiarachchi C (2019). Antioxidant, Anti-Inflammatory and Antibacterial Activities of the Seeds of A Sri Lankan Variety of *Carica papaya*. Biomed. Pharmacol. J. (12):539–547.

Yap J.Y., Hii C.L., Ong S.P., Lim K.H., Abas F and Pin K.Y (2020). Effects of drying on total polyphenols content and antioxidant properties of *Carica papaya* leaves. J. Sci. Food Agric. (100):2932–2937.

Yildirim A, Mavi A and Kara AA (2001. Determination of antioxidant and antimicrobial activities of Rumes crispus Leaf extracts. J Agr Food Chem. 49: 4083-4089.

References

1. Allan M, Aneja S, Armstrong F, Garica R, General biology. A laboratory manual for biology. Contemporary publishing Co. Raleigh, North Carolina, 1983, 265.

2. Banerjee A. Boerhaa viadiffusa Linn. A review on its phytochemical and pharmacological Profile. J Econ, 1986.

3. Bodansky M, Ros W. Comparative studies of Enzymes. Am. Jour. Pysiol. 1922; 62:473-482. Tax. Bot. 8: 271290.

4. Brocklehurst K, Baines B, Kierstan M. Papain and other constituents of Carica papaya. Enzyme Ferment. Biotechnol. 1981; 5:262-35.

5. Bradford M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem. 1976; 72:248-54.

6. Bouchut. Enzyme manual. Proteolytically active constituent in the latex of tropical papaya fruit, 1945. 7. Chaudhari R. Macroscopic and microscopic evaluation of Carica papaya l. Leaves. Herbal drug industry. Eastern publishers. New Delhi, 1996, 45-78.

8. David R. Gondar architecture and urbanism for Italy's fascist empire. J precipitation. Enzyme and Microbial Technology. 2011; 39:1103-1107.

Evans WW. Trease and Evans Pharmacognosy Saunders. 14th ed. London, 1997.

10. Johanson W. Effects of Elastase, Collagenase, and Papain on Structure and Function of rat lungs in vitro. J.C.I, 1972; 51:288-293.

11. Mahmood K, Sidiki Salmah. Architectural Historians. 2005; 70:492-511.

12. Sarote N, Rajni H, Pawinee K. Purification of papain from Carica papaya latex: Aqueous twophase extraction versus two-step salt precipitation. Enzyme and Microbial Technology. 2006; 39:1103-1107.

13. Pharmacognostic evaluation of Carica papaya. Int. J Mol. Med. Adv. Sci, 2006; 4:398-401.

14. Milind Parle, Gurditta. Basketful benefits of papaya. International Research Journal of Pharmacy. 2011; 2:612.

15. Nakasone H, Paul R. Post-harvest biology and Biotechnology. UK. Cab International. 1998; 7:199-254.