Evaluation of Antioxidant Capacity, Antimicrobial, Anticancer Activities, and Phytochemical Investigation of Male Papaya Leaves (*Carica papaya* L.)

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Abstract

Cancer is one of the most dangerous diseases in humans and there has been a scientific discovery of anticancer agents from natural products. Several bioassays such as antimicrobial, antioxidant, and anti-cancer activities were determined. The main aim of the present research was to evaluate the biological properties of male Papaya leaves. The preliminary phytochemical tests indicated that various types of compounds such as alkaloids, glycosides, phenolic compounds, flavonoids, tannins, steroids, terpenoids, saponins, starch, carbohydrates, and α-amino acids were found to be present but cyanogenic glycosides and reducing sugars were not detected in *Carica papaya* L. leaves. The total phenols content of ethanol extract (7.92 µg GAE/mg) was higher than that of watery extract (4.654 µg GAE/mg) by the FCR Method. The total flavonoid content of ethanol extract (12.35 µg GAE/mg) was higher than that of watery extract (7.6 µg GAE/mg) by Aluminium Chloride Colorimetric Assay. The antioxidant capacity of the ethanol and watery extracts from leaves was studied by using the DPPH free radical scavenging assay. The antioxidant activity of ethanol extract (IC_{50} 18.52 µg/mL) was found to be higher than that of watery extract (IC_{50} 19.93 µg/mL). The antimicrobial activity of various crude extracts was screened by the Agar Disc Diffusion method. Watery extract (15-40 mm) showed antimicrobial activity against *Agrobacterium tumefaciens, Bacillus subtilis, Candida albicans, Escherichia coli,* and *Pseudomonas fluorescence*. In the case of *Pseudomonas fluorescence*, the antimicrobial activity of PE extract (59.5 mm) was inhibited more than standard medicine *chloramphenicol* (42.2 mm). *In vitro* antiproliferative activities of ethanol and watery extracts of the *C. papaya* L. leaves were investigated against human lung cancer and human cervix cancer by the CCK-8 assay. The IC_{50} values of ethanol and watery extracts for human lung cancer and human cervix cancer were observed to be greater than 200 µg/mL. Therefore, male Papaya leaves (*C. papaya* L.) may be used for preventing diseases caused by bacteria and also as an antioxidant agent.

Keywords: *Carica papaya* L., antioxidant, anticancer, antimicrobial

Introduction

Plants have a significant role in maintaining human health and improving the quality of human life. The World Health Organization (WHO) estimates that 80 % of people rely on traditional medicine. The medicinal plant, *Carica papaya* L. belongs to the family, Caricaceae is commonly known as "Thin-baw" in Myanmar. Papaya has been widely used in traditional medicine for many ailments: the juices for warts, corns, cancer, tumors, hemorrhoids, diuretics, improving skin condition, relief from asthma, healing from burns and scalding in combination with *Aloe vera*, etc. Daily consumption of Papaya leaves as greens and herbal infusions is common in some parts of Indonesia as a means of preventing malaria. Researchers have now become more interested in phytochemicals from edible plants than natural products of higher plants due to novel sources of antimicrobial agents (Ayoola, 2006). The aqueous extract of papaya leaves has also been shown to significantly decrease blood glucose levels in diabetic rats and lower blood pressure. Papaya leaves also contain high amounts of vitamins A, C, E, K, and B and minerals like calcium, magnesium, sodium magnesium, and iron (Samuel, 2017). The anti-inflammatory properties of Papaya leaves could also help reduce inflammation and chemotherapy side effects. Papaya plants grow in three sexes: male, female, and

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hermaphrodite. The male produces only pollen, never fruit. The female produces small, inedible fruits unless pollinated. The hermaphrodite can self-pollinate since its flowers contain both male stamens and female ovaries (Suhas *et al*., 2014). Scientific investigation on antimicrobial activity and the anticancer activity of male Papaya leaves is still lacking. In the present research work, male Papaya (Thin-baw) leaves were selected for the investigation of phytoconstituents, antioxidant activity, antimicrobial activity, and antiproliferative activity.

Materials and Methods

Plant materials

The leaves of *C. papaya* L. were procured from Hlegu, Yangon Region. Leaves were left in the open air till they were completely dried. The dried sample was ground in a grinding machine. The drug powders were then stored in an airtight container.

Chemicals

Chemicals were procured from the BDH and E.Merck.

Phytochemical test for classification of compounds

The phytochemical examination of leaves is one of the important experiments because it provides a classification of the groups of chemical substances present in them. The preliminary phytochemical examination by test tube method indicates the chemical constituents of the *C. papaya* L. ((M-Tin Wa *et al*., 1972).

Preparation of crude extracts

Dried powders of leaves were extracted with 70 % ethanol and water using the Soxhlet apparatus. The extraction time with each solvent was six hours. After removing each solvent by rotary evaporator, the crude extract was dried and kept in a desiccator.

Determination of total phenolic content of the samples by the FCR method

The total phenol content (TPC) in each crude extract was stimulated by the Folin-Ciocalteu reagent method (Marizura *et al*., 2011). First, 0.5 mL of prepared extract solution was mixed with 5 mL of FCR reagent (1:10) and incubated for 30 minutes. 4 mL of 1 M sodium carbonate solution was added to each tube and the tubes were kept at room temperature for 15 min. The UV absorbance reaction mixture was read at λ_{max} 760 nm. The blank solution was prepared by using distilled water instead of the sample solution. Total phenol content was estimated as microgram gallic acid equivalents per milligram of different extracts (µg GAE / mg).

Determination of total flavonoid contents of the samples by aluminium chloride colorimetric assay

The total flavonoid contents of the samples were determined by UV spectrometry according to the aluminium Chloride Colorimetric Assay Method (Lee *et al*., 2015). Firstly, 0.5 mL of different concentrations of quercetin solution were mixed with 1.5 mL of methanol, 0.1 mL of 1 % aluminium chloride solution, 2.8 mL of distilled water, and 0.1 mL of 1 M potassium acetate. The resultant mixture was allowed to stand for 40 min at room temperature. The absorbance of the resulting yellow solution was measured against the reagent blank at 415 nm using a UV spectrophotometer. All samples were measured in triplicate. A standard curve was prepared by plotting the absorbance against concentrations of quercetin. This standard curve of quercetin solutions (ranging from 3.125 µg/mL to 100 µg/mL) was used for calibration.

Determination of antioxidant activity by the DPPH Assay method

The DPPH radical scavenging activity of 70 % ethanol and watery extracts of male Papaya leaves was determined by a UV-visible spectrophotometer. The control solution was prepared by mixing 1.5 mL of 0.002 % DPPH solution and 1.5 mL of ethanol in the brown bottle. The sample solution was also prepared by mixing 1.5 mL of 0.002 % DPPH solution with 1.5 mL of the tested sample solution. These bottles were shaken on a shaker for 30 min and incubated at room temperature. After incubation, these solutions were measured at 517 nm and the percentage of radical scavenging activity was calculated. The antioxidant power (IC_{50}) (50 % inhibition concentration) values were calculated by the linear regressive excel program (Marinova, 2011).

Antimicrobial screening by the agar disc diffusion method

Antimicrobial screening of crude extracts of male Papaya leaves was carried out by the agar disc diffusion method. The extracts were tested on six species of microorganisms such as *Agrobacterium tumefaciens, Bacillus subtilis, Candida albicans, Escherichia coli, Pseudomonas fluorescens,* and *Staphylococcus aureus.* The discs containing crude extract of Papaya leaves were placed in a clockwise position on the prepared agar plate. The solvent was used as a control to indicate that the antimicrobial activity of male Papaya leaves was not due to the effect of the solvent used. The antimicrobial activity was compared with the antibiotic disc (chloramphenicol) as a control (San San Aye, 2002).

Determination of antiproliferative activity by using the MTT assay

In vitro antiproliferative activity of the ethanol and watery extracts of the male Papaya leaves was determined against two human cancer cell lines A 549 (human lung cancer) and Hela (human cervix cancer) (Fatma *et al*., 2015). These tests were done at the Department of Natural Products Chemistry, the Institute of Natural Medicine, and the University of Toyama, Japan (Figure 1).

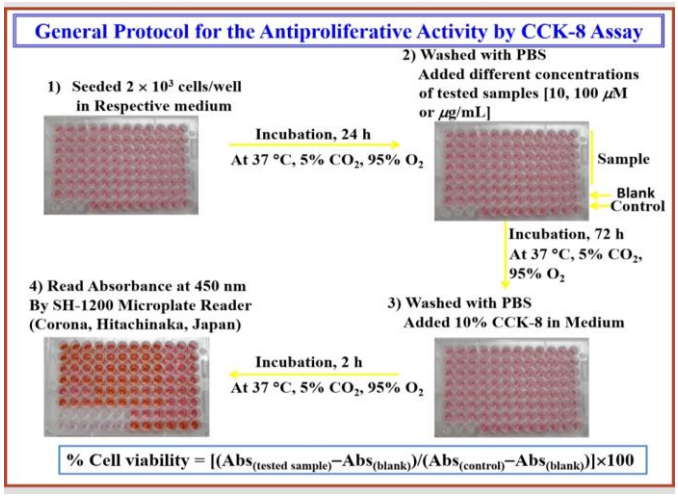


Figure 1 Procedure for the antiproliferative activity by MTT assay

Results and Discussion

Phytochemical investigation

The phytochemical constituents present in the *C. papaya* L. leaves (Thin-baw) were investigated by the test tube method. The phytochemical tests revealed that alkaloids, saponins, starch, phenolic compounds, glycosides, carbohydrates, tannins, flavonoids, steroids, terpenoids, and α-amino acids were present in the tested sample. However, reducing sugars and cyanogenic glycosides were not detected in *C. papaya* L. leaves (Table 1).

Total phenols content

Phenolic compounds possess one or more aromatic rings with one or more hydroxyl groups. They are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants. The presence of phenolic compounds in the medicinal plant is responsible for the antioxidant and anti-inflammatory activities of these species, allowing them to be used as potential chemo-preventives. Phenolic compounds have antioxidant properties that protect against degenerative diseases like heart disease and cancer.

The total phenolic contents (TPC) of the ethanol and watery extracts of *C. papaya* L. leaves were evaluated with a spectrophotometric method using a Folin-Ciocalteu reagent. The principle of this method is the reduced ability of the phenol functional group. Phenols react with an oxidizing agent phosphomolybdate in the Folin-Ciocalteu Reagent under alkaline conditions and resulting in the formation of the blue-coloured complex. The formation of the complex will increase when the extract contains more phenolic compounds. Thus, the colour will be darker and the absorbance will be higher. The absorbance can be measured as UV 760 nm. Gallic acid (3,4,5-trihydroxy benzoic acid) was used to construct a standard calibration curve.

In this study, a significant linear correlation was observed between the values for the total phenolic content and antioxidant activity. The high content of phenolic compounds indicates that these compounds contribute to antioxidant activity. According to the results, a higher TPC (µg GAE/mg) was detected in the ethanol extract (7.92 µg GAE/mg) than in the watery extract (4.65 µg GAE/mg). This means that phenolic compounds were more soluble in ethanol extract (Table 2).

Total flavonoid content

As a basis for the quantitative determination, the flavonoid content of ethanol and watery extracts of Thin-baw were determined using aluminium chloride in a colorimetric method. The results were derived from the calibration curve ($y = 0.021x + 0.1627$, $R^2 = 0.9918$) of quercetin (3.125-100 µg/mL) and ethanol and watery extracts of Thin-baw leaves were found to be (12.35 µg QE/mg) and (7.6 µg QE/mg). In this experiment, ethanol extract possessed higher flavonoid content than watery extract (Tables 3).

Antioxidant capacity

According to the result of antioxidant activity by the DPPH assay method, the IC_{50} values of ethanol and watery extracts were found to be 18.52 µg/mL and 19.93 µg/mL, respectively. The lower the IC_{50} value, the higher the antioxidant activity of the extract. So, the antioxidant activity of ethanol extract possessed higher potency than that of water. Their antioxidant activity is compared with standard ascorbic acid (IC_{50} = 1.17 µg/mL) (Table 4).

Antimicrobial screening

The various crude extracts of male Papaya leaves (petroleum ether, ethyl acetate, 70 % ethanol, and watery) were tested on six species of microorganisms. The agar disc diameter is 6 mm. The inhibition zone diameter including agar disc diameter was measured. The larger the disc diameter, the greater the antimicrobial activity. In the case of *Pseudomonas fluorescens*, petroleum ether extract (59.5 mm) showed more potent antimicrobial activity than the standard medicine, chloramphenicol (42.2 mm). Moreover, ethanol and watery extracts of leaves were inhibited (14.5-18.5 mm) against *Escherichia coli* but standard medicine, chloramphenicol did not show (Figure 2 and Table 5).

Antiproliferative activity

The antiproliferative activity of ethanol and watery extracts of male Papaya leaves against A549 (human lung cancer) and Hela (human cervical cancer) was evaluated by using an MTT assay. The anticancer effect was expressed as IC_{50} values (50 % inhibitory concentration). Both extracts of male Papaya leaves were found to possess antiproliferative activity against human lung cancer and human cervix cancer cell lines (IC_{50} values > 200 µg/mL (Table 6).

Table 1 Results of Phytochemical Investigation of *C. papaya* L. Leaves

No.	Test	Extract	Reagent	Observation	Results
1	Alkaloids	1 % HCl	Mayer's Dragendorff's Wagner's Sodium picrate	Yellow ppt. Brown sol; White ppt. White ppt.	+
2	α-Amino acids	H_2O	Ninhydrin reagent	Violet ppt.	+
3	Carbohydrates	H_2O	10 % α-Naphthol and Conc.H_2SO_4	Red ring	+
4	Flavonoids	EtOH	Mg ribbon and Conc.HCl	Pink	+
5	Cyanogenic glycosides	H_2O	Conc.H_2SO_4, Sodium picrate	No brick red color	-
6	Glycosides	H_2O	10 % Lead acetate	White ppt	+
7	Phenolic compounds	H_2O	5 % $FeCl_3$	Dark green	+
8	Reducing sugars	H_2O	Benedict's solution	Frothing	-
9	Saponins	H_2O	Distilled water	Green	+
10	Starch	H_2O	Iodine solution	Greenish blue	+
11	Tannins	H_2O	2 % NaCl, Gelatin	Yellowish-brown	+
12	Steroids	PE	Acetic anhydrite & Conc. H_2SO_4	Green	+
13	Terpenoids	PE	Acetic anhydrite & Conc. H_2SO_4	Pink	+

(+) Presence, (-) Absence

Table 2 Total Phenol Content (TPC) of Crude Extracts

No.	Extracts	TPC (μg GAE/mg of extract)
1	Ethanol	7.92
2	Watery	4.65

Table 3 Total Flavonoid Content (TFC) of Crude Extracts

No.	Extracts	TFC (μg QE/mg of extract)
1	Ethanol	12.35
2	Watery	7.6

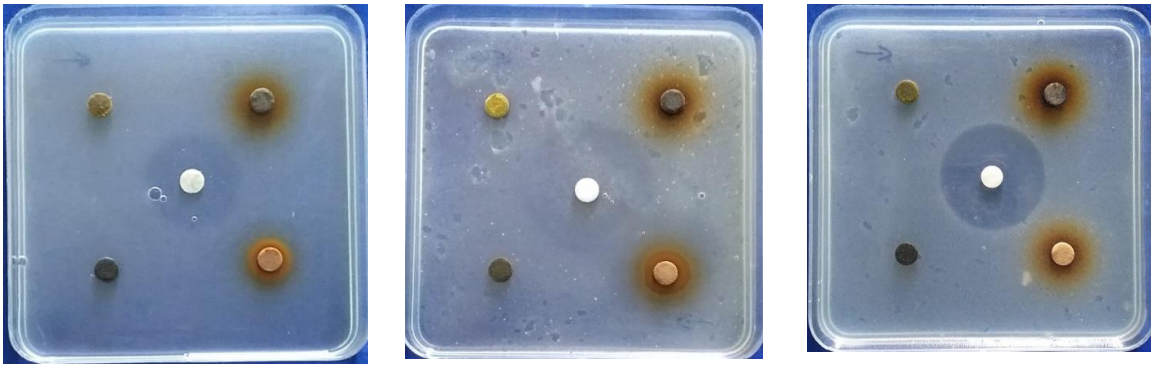
Table 4 Percent Radical Scavenging Activity (% RSA) of EtOH and Watery Extracts from Male Papaya Leaves (*C. papaya* L.)

Extracts	% Inhibition (mean ± SD) in different concentrations (μg/ mL)							IC_{50} (μg/mL)
	6.25	12.5	25	50	100	200	400	
Ethanol	45.62 ± 1.95	48.12 ± 0.65	52.02 ± 1.05	58.07 ± 0.31	65.97 ± 2.95	69.66 ± 2.29	75.41 ± 1.69	18.52
Watery	39.67 ± 1.26	45.27 ± 5.66	53.22 ± 2.13	59.92 ± 6.89	65.21 ± 6.49	72.66 ± 2.95	78.25 ± 1.62	19.93

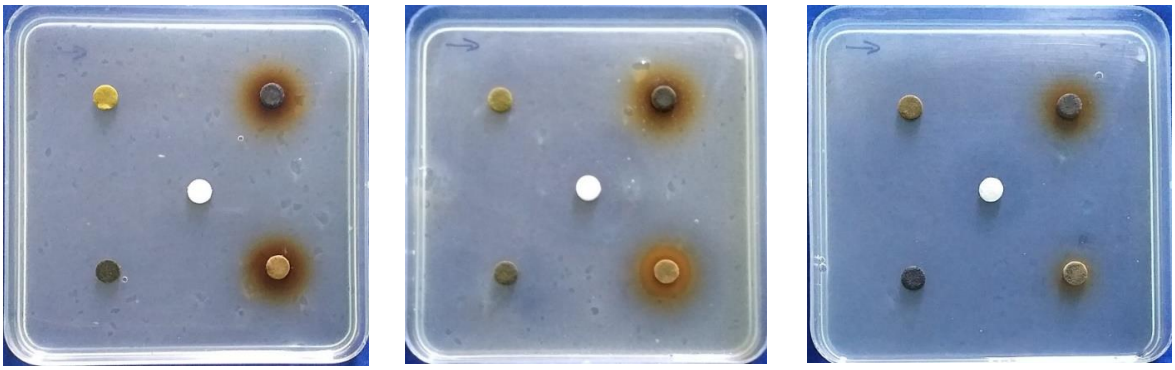
Table 5 Antimicrobial Screening of Male Papaya Leaves (*C. papaya* L.)

No.	Tested microorganisms	Inhibition zone diameter (mm)				
		PE	EtOAc	EtOH	H_2O	Chloramphenicol
1	*Agrobacterium tumefaciens*	-	-	20.0	29.8	32.0
2	*Bacillus subtilis*	-	-	-	35.0	45.0
3	*Candida albicans*	-	-	-	15.0	39.0
4	*Escherichia coli*	-	-	14.5	18.5	-
5	*Pseudomonas fluorescens*	59.5	38.5	13.2	40.0	42.2
6	*Staphylococcus aureus*	28.2	-	27.0		29.0

Agar disc diameter: 6 mm, (-) No activity



Agrobacterium tumefaciens *Bacillus subtilis* *Candida albicans*



Escherichia coli *Pseudomonas fluroescens* *Staphylococcus aureus*

Figure 2 Antimicrobial screening of crude extracts from male *C. papaya* L. leaves

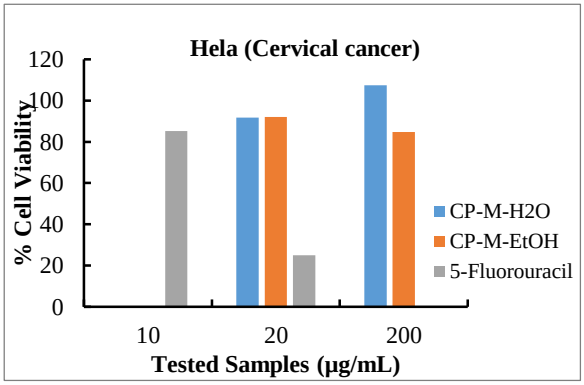


Figure 3 Cytotoxicity of crude extracts of *C. papaya* leaves against human cervical cancer cell

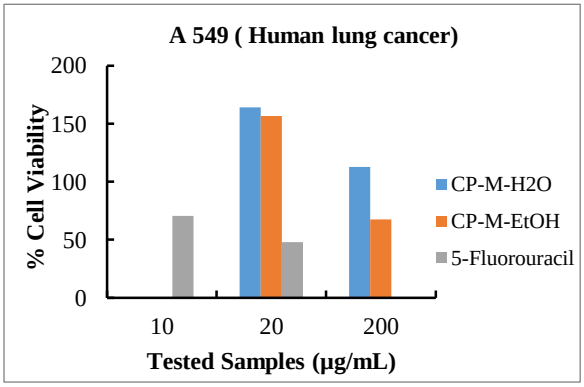


Figure 4 Cytotoxicity of crude extracts of *C. papaya* leaves against human lung cancer cell

Table 6 Antiproliferative Activity of the Male Papaya Leaves (*C. papaya* L.)

Extracts	IC_{50} (µg/mL)	
	Human lung cancer	Human cervical cancer
EtOH	>200	>200
Watery	>200	>200
5-fluorouracil	19.06	15.84

Conclusion

From the present research work on "Evaluation of Antioxidant Capacity, Antimicrobial, Anticancer Activities and Phytochemical Investigation of Male Papaya Leaves (*Carica papaya* L.)", the following conclusions can be drawn.

The preliminary phytochemical investigation of the selected sample indicated that alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, steroids, saponins, starch, tannins, and terpenoids were found to be present but cyanogenic glycosides and reducing sugars were not detected in male Papaya leaves (*C. papaya* L.).

The total phenolic content of ethanol extract (7.92 μg GAE/mg) was higher than that of watery extract (4.65 μg GAE/mg). The greater the total phenolic content, the higher the antioxidant activity.

The total flavonoid content of ethanol extract (12.35 μg QE/mg) was found to be greater than watery extract (7.6 μg QE/mg). The greater the total flavonoid content, the higher the antioxidant activity.

Investigation of the antioxidant potential of ethanol and watery extracts was performed by the DPPH assay. According to the experiment, the IC_{50} values of the ethanol and watery extracts were observed to be 18.52 μg/mL and 19.93 μg/mL, respectively. The lower the IC_{50} value, the higher the antioxidant activity of the extract. The antioxidant activity of the extract was positively associated with the total phenolic content and total flavonoid content of the extract. Therefore, the antioxidant capacity of ethanol extract was higher than that of watery extract.

The antimicrobial activity of the polar and nonpolar extracts was screened by using the Agar Disc Diffusion method on six microorganisms. The ethanol and watery extracts

(14-18 mm) showed antimicrobial activity against *Escherichia coli* but medicine (chloramphenicol) did not inhibit it. In the case of *Pseudomonas fluorescence*, petroleum ether extract (59.5 mm) has more potent antimicrobial activity than standard medicine chloramphenicol (42.2 mm).

Antiproliferative activity of the ethanol and watery extracts (IC_{50} >200 μg/mL) was found to be lower than that of standard 5-fluorouracil (IC_{50} < 20 μg/mL) against two anticancer cell lines (Human lung cancer and Human cervix cancer).

In conclusion, the results of the present study indicated that the male Papaya leaves may be used as an antimicrobial agent, anticancer, and neutral source of antioxidants to prevent the progression of many diseases.

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