Investigation of Phytoconstituents and Antioxidant Activity in the Male and Female Leaves of *Carica papaya* Linn.

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Abstract

The leaves of *Carica papaya* have been known to use in medicinal plants for the treatment of some diseases such as jaundice, heart diseases, asthma, tumor and fever due to its biological activities. This research aims to investigate the phytochemical constituents, phenolic content, flavonoid content and antioxidant activity *C. papaya* leaves. The phytochemical analyses revealed the presence of alkaloids, α-amino acids, flavonoids, organic acids, phenolic compounds, saponins, glycosides and tannins, however, carbohydrates and reducing sugars were not detected in selected samples. The phenolic content was determined by Folin-Ciocalteu's method while flavonoid content by aluminium trichloride. The antioxidant activity was determined by DPPH radical scavenging method. The highest values of total phenolic content (49.82 ± 0.02 µg GAE/mg) and total flavonoid content (37.22 ± 0.04 µg QE/mg) were found in ethanol extract of male *C. papaya* leaves. Moreover, IC_{50} values of ethanol and watery extracts possessed 16.53 µg/mL and 22.20 µg/mL in the male leaves and 34.11 µg/mL and 21.68 µg/mL in the female leaves of *C. papaya*. Therefore, the crude extracts of *C. papaya* leaves may be used as a good source of natural antioxidant.

Keywords: *Carica papaya* Linn., phytoconstituents, total phenolic content, total flavonoid content, antioxidant activity

Introduction

Natural products from medicinal plants have gained huge interests from researchers around the world for new drugs because of their potential bioactivity effects. The plant extracts and its phytoconstituents are proven for its biological activities such as antidiabetic, free radical scavenging, antihyperlipidemic and anti-inflammatory activities. *Carica papaya* belonging to the family of *Caricaceae*, is cultivated almost all over tropical and subtropical countries of the world particularly in India, South America, West India and widespread throughout the world (Kress *et al*., 2003). All parts of the papaya plant such as leaves, fruits, seeds and flowers can be used as medicine. The leaves of *C. papaya* have been shown to contain many active components that can increase the total antioxidant ability and reduce lipid peroxidation level. They have been used as an aboriginal remedy for various disorders including cancer and infectious diseases (Adebiyi *et al*., 2002).

The major groups of phytochemicals that have been suggested as a natural source of antioxidants may contribute to the total antioxidant activity of plant materials including polyphenols carotenoid and antioxidant vitamins such as vitamin C and E. So, the plant constituents containing greater amount of phenols and flavonoids are able to exert protective effects in biological system against oxidative stress. Several studies showed that phenolic compounds are the major bioactive phytochemicals with human health benefits. The bioefficacy of *Carica papaya* is owed to its main phytoconstituents such as alkaloids (carpaine, pseudocarpaine), glycosides (anthracene derivatives), saponins, flavonoids (kaempferol, quercetin, rutin), phenolic acids (ferulic acid, chlorogenic acid, vanillic acid) along with carotenoids namely β-carotene and cryptoflavin, papain an enzyme, vitamins and

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minerals. The main active constituents in *C. papaya* leaves are the macro cyclic lactone carpaine (Otsuki *et al*., 2010).

Scientific Classification of Selected Medicinal Plants

Kingdom : Plantae
Family : Caricaceae
Genus : *Carica*
Species : *Carica papaya*
Botanical name : *Carica papaya* Linn.
Common name : Papaya
Myanmar name : Thinbaw
Part Used : leaves

Figure 1. The plant of male and female *Carica papaya* Linn.

Materials and Methods

Sample Collection

The experimental works were performed in the Chemistry Laboratory of Banmaw University (BMU). The samples were collected from the Banmaw University Campus, Banmaw Township, in November, 2022. Then, the samples were identified at Botany Department, BMU.

Sample Preparation

The fresh leaves were cleaned by washing with water and air–dried at room temprature. Dry leaves samples were ground into powder and stored in air-tight container to prevent contaminations and kept for their phytochemicals and biological activities. The following instruments and materials were used for biological activities: electric balance, shaker, GENESYS-30 Visible Spectrophotometer, quartz cuvette (4 mL), micropipette (1 mL), measuring cylinder and beakers were obtained from Department of Chemistry, BMU.

Phytochemical Screening

Phytochemical constituents such as alkaloids, flavonoids, phenolic compounds, organic acids, α-amino acids, carbohydrates, saponins, glycosides, tannins and reducing sugars were determined using standard procedures as described by Marini–Bettolo *et al*., 1981.

Determination of Total Phenolic Contents in Crude Extracts by FCR method

The total phenolic content (TPC) in each crude extracts was determined spectrophotometrically (GENESYS-30) according to Folin-Ciocalteu method as described by Song *et al*., 2010. Total phenolic content was estimated as microgram gallic acid equivalents per milligram of crude extracts (µg GAE/mg).

Determination of Total Flavonoid Contents in Crude Extracts by Aluminium Chloride Colorimetric Assay (ACC)

The total flavonoid content (TFC) in each crude extract was estimated spectrophotometrically (GENESYS-30) according to aluminium chloride colorimetric assay (Zhishen *et al*.,1999). Total flavonoid contents (TFC) in the plant sample was expressed as microgram quercetin equivalent (QE) per milligram of crude extracts (µg QE/mg).

Determination of Antioxidant Activity of Crude Extracts by DPPH Free Radical Scavenging Assay

The antioxidant activity of crude extracts from male and female *C. papaya* L. leaves was determined by spectrophotometrically (GENESYS-30) according to DPPH free radical scavenging assay (Marinova and Batchvarov, 2011). The antioxidant power (IC_{50}) is expressed as the test substances concentration (µg/mL) that result in a 50 % oxidative inhibition of the substance.

Results and Discussions

Table 1. Phytochemicals Results in Male and Female Leaves of *Carica papaya*

No.	Phytochemicals	Extracts	Test Reagents	Observation	Results male	Results female
1.	Alkaloids	1 % HCl	Dragendorff's Mayer's	Orange ppt White ppt	+ +	+ +
2.	Flavonoids	EtOH	Conc: HCl Mg turning	Pink color	+	+
3.	Phenolic compounds	EtOH	10 % $FeCl_3$ solution	Dark blue color	+	+
4.	Organic acids	H_2O	Bromocresol green indicator	Yellow color	+	+
5.	α-amino acids	H_2O	Ninhydrin	Purple spot	+	+
6.	Carbohydrates	H_2O	10 % α-naphthol Conc: H_2SO_4	No red ring	-	-
7.	Saponins	H_2O	-	Frothing	+	+
8.	Glycosides	H_2O	10 % lead acetate	White ppt	+	+
9.	Reducing sugars	H_2O	Benedict's solution	Brick red ppt	-	-
10.	Tannins	EtOH	1 % Gelatin	Reddish brown	+	+

From the phytochemical tests, alkaloids, α-amino acids, flavonoids, organic acids, phenolic compounds, saponins, glycosides and tannins were present in selected samples. However, carbohydrates and reducing sugars were not detected in these samples.

Total Phenolic Contents of Crude Extracts in Leaves of *C. papaya*

In this study, the total phenolic contents of crude extracts in male and female leaves of *C. papaya* were estimated by Folin-Ciocalteu method. Gallic acid was used to construct standard calibration curve for total phenolic content estimation as shown in Table (2) and Figure (2).

Table 2. Absorbance of Standard Gallic Acid Solution at 765 nm

Concentration (µg/mL)	Absorbance
6.25	0.152
12.50	0.270
25.00	0.449
50.00	0.824
100.00	1.540

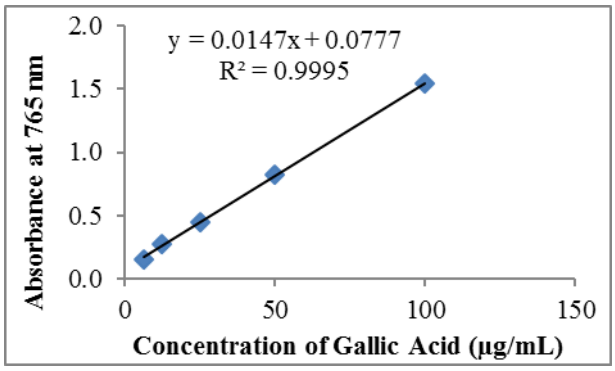

Figure 2. Gallic acid standard curve at 765 nm

According to the results in Table (3) and Figure (3), the total phenolic content (TPC) (µg GAE/mg) of ethanol and watery extracts in male leaves of *C. papaya* were 49.82 ± 0.02 and 38.93 ± 0.01 µg GAE/mg and that of 43.69 ± 0.02 and 30.05 ± 0.02 µg GAE/mg in female *C. papaya* leaves, respectively. From these results, it was found that ethanol extracts showed higher total phenolic content than watery extracts in these samples. So, ethanol extracts are more effective than watery extracts. The greater the total phenolic content showed the higher antioxidant activity.

Table 3. Total Phenolic Contents of Extracts in Male and Female Leaves of *Carica papaya*

No.	Extracts	TPC (µg GAE/mg ± SD)
1	Ethanol (Male)	49.82 ± 0.02
2	Watery (Male)	38.93 ± 0.01
3	Ethanol (Female)	43.69 ± 0.02
4	Watery (Female)	30.05 ± 0.02

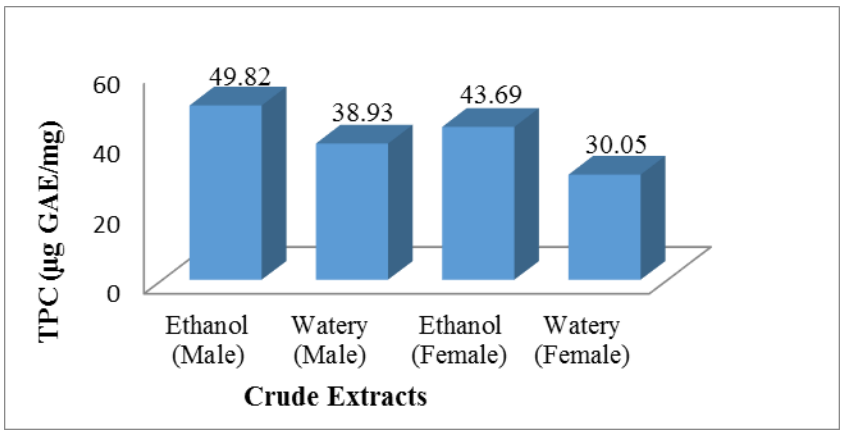

Figure 3. A bar graph of total phenolic contents of ethanol and watery extracts in male and female *C. papaya* leaves

Total Flavonoid Contents of Crude Extracts in Leaves of *Carica papaya*

From the study, the total flavonoid contents of crude extracts in male and female leaves of *Carica papaya* were estimated by aluminium chloride colorimetric method. Quercetin was used as standard compound for the quantification of standard calibration curve for total flavonoid estimation as shown in Table (4) and Figure (4).

Table 4. Absorbance of Quercetin Standard Solution at 415 nm

Concentration (µg/mL)	Absorbance
6.25	0.019
12.50	0.032
25.00	0.056
50.00	0.119
100.00	0.230

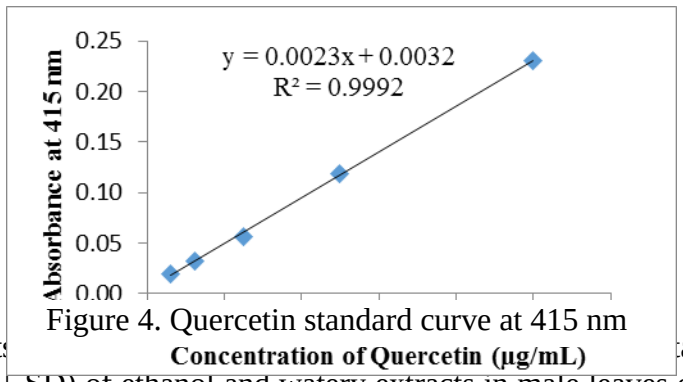

Figure 4. Quercetin standard curve at 415 nm

According to the results, total flavonoid contents (µg QE/mg ± SD) of ethanol and watery extracts in male leaves of *C. papaya* were 37.22 ± 0.04 and 25.04 ± 0.02 µg QE/mg and that of 32.00 ± 0.03 and 21.56 ± 0.01 µg QE/mg in female *C. papaya* leaves, respectively. From these results, it was found that ethanol extracts showed significantly higher total flavonoid contents (TFC) than watery extracts in these samples. The greater the total flavonoid contents, the higher the antioxidant activity might occur.

Table 5. Total Flavonoid Contents of Extracts in Male and Female *Carica papaya* Leaves

Extracts	TFC (µg QE/mg ± SD)
Ethanol (Male)	37.22 ± 0.04
Watery (Male)	25.04 ± 0.02
Ethanol (Female)	32.00 ± 0.03
Watery (Female)	21.56 ± 0.01

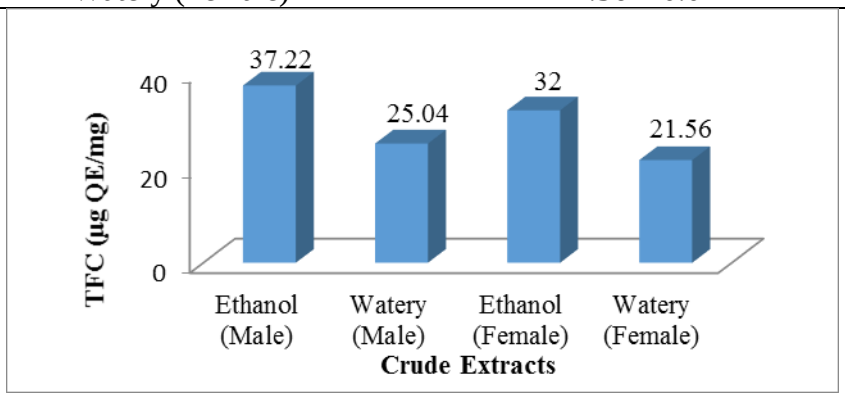

Figure 5. A bar graph of total flavonoid contents of ethanol and watery extracts in male and female *C. papaya* leaves

Antioxidant Activity of Crude Extracts in Male and Female Leaves of *C. papaya* L.

The results of percent oxidative inhibition values of crude extracts are summarized in Table (6) and Figures (6) and (7). From the experimental results, percent RSA values of ethanol and water extracts (100 µg/mL) were found to be 73.42 ± 0.01 % and 71.78 ± 0.00 % in the male leaves of *C. papaya* as well as 63.28 ± 0.02 % and 67.67 ± 0.01 % from the female leaves, respectively. Therefore, these crude extracts in *C. papaya* leaves had significant antioxidant activities. The IC_{50} values of crude extracts (ethanol and watery) were found to be 16.53 µg/mL and 22.20 µg/mL in male *C. papaya* leaves and 34.11 µg/mL and 21.68 µg/mL in female *C. papaya* leaves. Among these extracts, the lower IC_{50} showed the higher free radical scavenging activity, ethanol extract in male *C. papaya* leaves was found to be more potent than other crude extracts in free radical scavenging activity.

Table 6. Percentage of Radical Scavenging Activity (% RSA) of Standard Ascorbic Acid and Crude Extracts in Male and Female of *C. papaya* Leaves

Samples	% RSA ± SD of different concentrations (µg/mL)							IC_{50} (µg/mL)
	1.5625	3.125	6.25	12.50	25.00	50.00	100.00	
Ascorbic Acid	46.30 ± 0.02	55.34 ± 0.02	62.46 ± 0.02	69.58 ± 0.02	78.08 ± 0.03	81.64 ± 0.03	90.68 ± 0.01	2.20
EtOH (M)	18.90 ± 0.02	24.65 ± 0.01	30.41 ± 0.03	43.83 ± 0.03	51.78 ± 0.02	64.10 ± 0.02	73.42 ± 0.01	16.53
Watery (M)	14.24 ± 0.00	25.75 ± 0.01	31.78 ± 0.00	45.75 ± 0.01	58.90 ± 0.00	64.10 ± 0.00	71.78 ± 0.00	22.20
EtOH (F)	8.21 ± 0.02	20.54 ± 0.01	25.47 ± 0.01	37.53 ± 0.01	43.56 ± 0.02	61.23 ± 0.02	63.28 ± 0.02	34.11
Watery (F)	8.49 ± 0.02	15.61 ± 0.01	27.94 ± 0.01	36.71 ± 0.01	54.79 ± 0.02	61.09 ± 0.01	67.67 ± 0.01	21.68

M = Male, F = Female

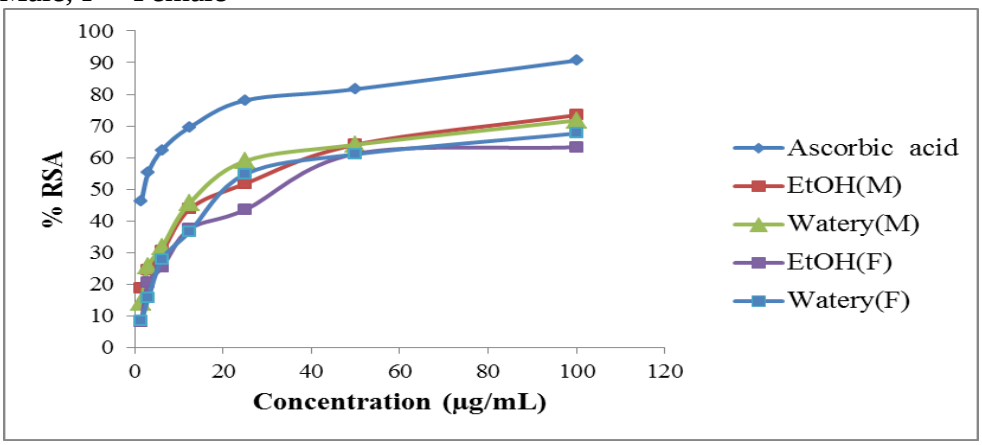

Figure 6. A plot of % radical scavenging activity vs concentration (µg/mL) of standard ascorbic acid and crude extracts in male and female *C. papaya* leaves

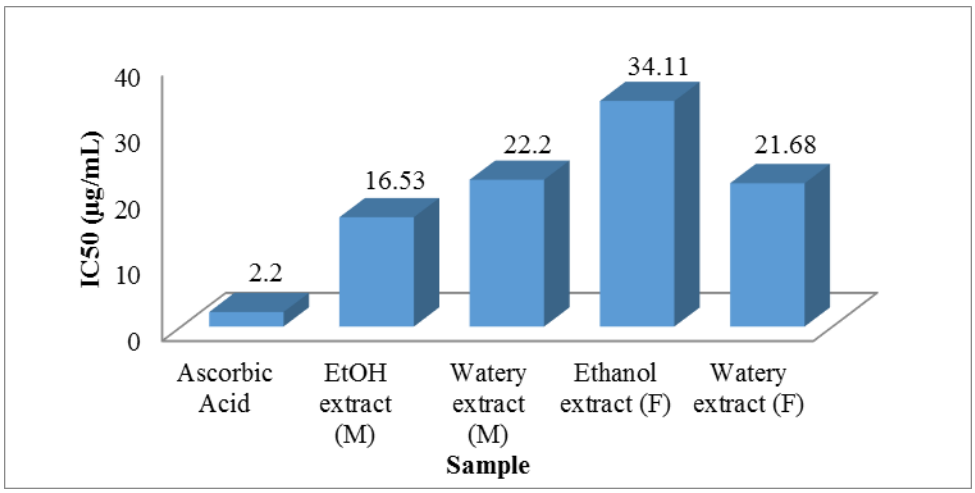

Figure 7. A bar graph of IC_{50} value of ascorbic acid and crude extracts in male and female *C. papaya* leaves

Conclusion

The following inferences could be deduced from the overall assessment of the chemical investigation in male and female *Carica papaya* leaves. Phytochemicals such as alkaloids, α-amino acids, flavonoids, organic compounds, phenolic compounds, saponins, glycosides and tannins were found to be present in selected medicinal plants. Total phenolic content and flavonoid content of ethanol crude extract in male leaves were more effective than that of other crude extracts in selected medicinal plants. The high contents of phenols and flavonoids constituents indicated that these samples contribute directly to their antioxidant action. According to experiments, the IC_{50} values of ethanol and watery extracts were observed at 16.53 µg/mL and 22.20 µg/mL in male leaves and then, 34.11 µg/mL and 21.68 µg/mL in female leaves. Therefore, crude extracts in male *C. papaya* leaves showed more potent antioxidant activity than the crude extracts in female leaves. However, antioxidant activity of these crude extracts were observed to be moderate potency than that of standard ascorbic acid (IC_{50}=2.20 µg/mL). The findings from the present work will contribute to the scientific development of Myanmar herbal medicine, sources of natural antioxidants and other bioactive compounds in food and pharmaceutical industries.

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